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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206-619
Supporting document/s:	001, 002, 003, 004
Applicant's letter date:	21 April 2014
CDER stamp date:	22 April 2014
Product:	Viekira Pak, consisting of: Dasabuvir (250 mg) and Paritaprevir (75 mg)/ Ritonavir (50 mg), and Ombitasvir (12.5 mg)
Indication:	The treatment of chronic HCV GT1 infection in adults, including those with compensated cirrhosis, who are either treatment-naïve or previously treated with pegylated interferon (pegIFN) and ribavirin.
Applicant:	AbbVie, Inc.
Review Division:	DAVP
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1 Executive Summary

1.1 Introduction

The applicant is seeking marketing approval for Viekira Pak, consisting of:
A non-nucleoside inhibitor of nonstructural protein 5B (NS5B) RNA polymerase (Dasabuvir), a nonstructural protein 3/4A (NS3/4A) protease inhibitor combined with ritonavir (Paritaprevir/r) to enhance systemic exposures, and a nonstructural protein 5A (NS5A) inhibitor (Ombitasvir). Viekira Pak would be marketed as a treatment of chronic HCV genotype 1 infection in adults, including those with compensated cirrhosis, who are either treatment-naïve or previously treated with pegylated interferon (pegIFN) and ribavirin. The proposed daily dosages for the components of Viekira Pak are Dasabuvir (500 mg), Paritaprevir/r (150 mg/100 mg), and Ombitasvir (25 mg). 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

Paritaprevir
The gallbladder was identified as a target organ of Paritaprevir (ABT-450) toxicity in repeat dose studies in mice and dogs. Findings include edema, mononuclear and mixed cell infiltration and epithelial cell necrosis with increased serum alkaline phosphatase, suggesting biliary effects.

In clinical trials, transient elevations in total bilirubin that peaked at Week 1 and declined thereafter were observed in subjects who received the combination of three DAAs present in Viekira Pak, and Ribavirin (RBV). The increase was driven by an elevation in the indirect bilirubin fraction. Paritaprevir is a known inhibitor of the organic anion transporting polypeptide 1B1 (OATP1B1) bilirubin transporter. In addition, RBV-induced hemolysis may have contributed to hyperbilirubinemia. There were no clinically significant increases in alkaline phosphatase.

Paritaprevir was positive in an *in vitro* human chromosome aberration test. Paritaprevir was negative in a bacterial mutation assay, and in two *in vivo* genetic toxicology assays (rat bone marrow micronucleus and rat liver Comet tests). Paritaprevir was not carcinogenic in nonclinical studies. Likewise, oral administration of paritaprevir to pregnant rats and mice did not result in teratogenicity at systemic exposures up to 8x (rats) and 98x (mice) the expected human exposure. Paritaprevir did not affect male or female fertility in rats at systemic exposures 2-5x the expected human exposure.

Ritonavir
In nonclinical toxicology studies with ritonavir, the main target organs of toxicity were the liver and the eyes. As stated on the Norvir label, hepatic transaminase elevations exceeding 5 times the upper limit of normal, clinical hepatitis, and jaundice have occurred in patients receiving NORVIR alone or in combination with other antiretroviral drugs. Retinal toxicity was observed in animals but this has not been seen in patients.

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Also, pancreatitis has been observed in patients receiving NORVIR therapy, including those who developed hypertriglyceridemia.

Ombitasvir

Ombitasvir (ABT-267) has minimal solubility in aqueous solutions (<0.1 µg/mL). In toxicology studies, (b) (4) solution formulations provided maximum feasible exposures at steady state in mice, rats and dogs. Mouse and dog were chosen as the nonclinical species for pivotal repeat dose studies based on the fact that higher systemic exposures were achieved in these species compared to rats and monkeys, and based on the similarity of the metabolite profile between mouse, dog and humans in hepatic *in vitro* systems. At maximum feasible doses and at exposure levels that reflect saturation of absorption, no toxicologically significant effects of ombitasvir were noted in nonclinical studies. In those studies, systemic exposures were approximately 20-40-fold higher than clinical exposures. Therefore, although no target organs of toxicity were identified, the nonclinical toxicology program is considered to be adequate to predict toxicity in the clinical setting.

Two metabolites of ombitasvir, M29 and M36 (A-1538855 and A-1548255, respectively), were identified as unique human metabolites (i.e., metabolites that were not present in nonclinical species), and were present in human plasma as 20% and 13%, respectively, of total drug-related exposures (AUC) at steady-state. The toxicological profile of each metabolite was assessed in genetic toxicology assays, repeat dose studies and reproductive toxicology studies. No significant toxicological effects were identified.

As noted above, no target organs were identified in nonclinical toxicology studies. Ombitasvir was not genotoxic, and was not carcinogenic in transgenic mice at exposures up to 26x the recommended clinical dose.

Reproductive toxicity studies were conducted in mice, rather than rats, based on the fact that higher systemic exposures were achieved in mice compared to rats, and pivotal general toxicology studies were conducted using mice. Ombitasvir did not affect male or female fertility in mice up to 29x exposures at the recommended clinical dose. Ombitasvir-related changes in male reproductive organ weights that were not considered to be toxicologically significant included increases weights of the prostate and seminal vesicles (without fluid) and decreases in the absolute and relative weights of the testes.

In mice, ombitasvir was not maternally toxic or teratogenic up to 29x exposures at the recommended clinical dose. In rabbits, ombitasvir was not maternally toxic or teratogenic at doses up to 4x exposures at the recommended clinical dose, with plasma drug levels measured in fetuses between 1% and 2% of those measured in females at the time of Caesarean-section.

Dasabuvir

No toxicologically significant effects of dasabuvir (ABT-333) were noted in nonclinical studies. In rats following six months of dosing, alveolar histiocytosis and granulomatous inflammation of the ileum at 800 mg/kg/day were test article-related changes, but were

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not associated with adverse clinical effects. In dogs dosed for nine months, moderately increased mean absolute liver weights were noted in males given 60 mg/kg/day. These moderate increases correlated with increased hepatocellular vacuolation likely due to increased glycogen in hepatocytes. The ultrastructure of hepatocytes was otherwise normal. Overall, systemic exposures in nonclinical studies ranged from approximately 10 to 120-fold higher than clinical exposures. Therefore, the nonclinical toxicology program is considered to be adequate to predict toxicity in the clinical setting.

No target organs were identified in nonclinical toxicology studies. Dasabuvir was not genotoxic, and was not carcinogenic in transgenic mice at exposures up to 39x exposures at the recommended clinical dose.

Dasabuvir did not affect male or female fertility in rats dosed up to 800 mg/kg, equivalent to approximately 33x exposures at the recommended clinical dose. In rats, dasabuvir was not maternally toxic or teratogenic at doses up to 800 mg/kg/day. Systemic exposures were equivalent to approximately 48x exposures at the recommended clinical dose. In rabbits, dasabuvir was not maternally toxic or teratogenic at doses up to 400 mg/kg/day, equivalent to approximately 12x exposures at the recommended clinical dose.

1.3 Recommendations

1.3.1 Approvability

It is recommended that Viekira Pak be approved.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended.

1.3.3 Labeling

The following labeling text is recommended by the reviewer.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Category B

<u>Risk Summary</u>

Adequate and well controlled studies with VIEKIRA PAK have not been conducted in pregnant women. In animal reproduction studies, no evidence of teratogenicity was observed with the administration of ombitasvir (mice and rabbits), paritaprevir/ritonavir (mice and rats), or dasabuvir (rats and rabbits) at exposures higher than the recommended clinical dose *[see Data]*. Because animal reproduction studies are not

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always predictive of human response, VIEKIRA PAK should be used during pregnancy only if clearly needed.

If VIEKIRA PAK is administered with ribavirin, it is contraindicated in pregnant women and in men whose female partners are pregnant. Refer to the ribavirin prescribing information for more information on use in pregnancy.

Data

Animal data

In animal reproduction studies, there was no evidence of teratogenicity in offspring born to animals treated throughout pregnancy with ombitasvir and its major inactive human metabolites (M29, M36), paritaprevir/ritonavir, or dasabuvir. For ombitasvir, the highest dose tested produced exposures approximately (b) (4)-fold (mouse) or 4-fold (rabbit) the exposures in humans at the recommended clinical dose. The highest doses of the major, inactive human metabolites similarly tested produced exposures approximately 26-fold the exposures in humans at the recommended clinical dose. For paritaprevir/ritonavir, the highest doses tested produced exposures approximately 98-fold (mouse) or 8-fold (rat) the exposures in humans at the recommended clinical dose. For dasabuvir, the highest dose tested produced exposures approximately 48-fold (rat) or 12-fold (rabbit) the exposures in humans at the recommended clinical dose.

8.3 Nursing Mothers

It is not known whether (b) (4) their metabolites are present in human milk. Unchanged ombitasvir, paritaprevir and its hydrolysis product M13, and dasabuvir were the predominant components observed in the milk of lactating rats, without effect on nursing pups.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Viekira Pak and any potential adverse effects on the breastfed child from Viekira Pak or from the underlying maternal condition.

If VIEKIRA PAK is administered with ribavirin, the nursing mothers information for ribavirin also applies to this combination regimen. (b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

If VIEKIRA PAK is administered with ribavirin, refer to the prescribing information for ribavirin for information on Carcinogenesis, and Mutagenesis.

Ombitasvir

Ombitasvir was not carcinogenic in a 6-month transgenic mouse study up to the highest dose tested (150 mg per kg per day).

The carcinogenicity study of ombitasvir in rats is ongoing.

Ombitasvir and its major inactive human metabolites (M29, M36) were not genotoxic in a battery of *in vitro* or *in vivo* assays, including bacterial mutagenicity, chromosome aberration using human peripheral blood lymphocytes and *in vivo* mouse micronucleus assays.

Paritaprevir/ritonavir

Paritaprevir/ritonavir was not carcinogenic in a 6-month transgenic mouse study up to the highest dose tested (300/30 mg per kg per day). Similarly, paritaprevir/ritonavir was not carcinogenic in a 2-year rat study up to the highest dose tested (300/30 mg per kg per day), resulting in paritaprevir exposures approximately 9-fold higher than those in humans at 150 mg.

Paritaprevir was positive in an *in vitro* human chromosome aberration test. Paritaprevir was negative in a bacterial mutation assay, and in two *in vivo* genetic toxicology assays (rat bone marrow micronucleus and rat liver Comet tests).

Dasabuvir

Dasabuvir was not carcinogenic in a 6-month transgenic mouse study up to the highest dose tested (2000 mg per kg per day).

The carcinogenicity study of dasabuvir in rats is ongoing.

Dasabuvir was not genotoxic in a battery of *in vitro* or *in vivo* assays, including bacterial mutagenicity, chromosome aberration using human peripheral blood lymphocytes and *in vivo* rat micronucleus assays.

<u>Impairment of Fertility</u>

If VIEKIRA PAK is administered with ribavirin, refer to the prescribing information for ribavirin for information on Impairment of Fertility.

Ombitasvir

Ombitasvir had no effects on embryo-fetal viability or on fertility when evaluated in mice up to the highest dose of 200 mg per kg per day. Ombitasvir exposures at this dose were approximately 25-fold the exposure in humans at the recommended clinical dose.

Paritaprevir/ritonavir

Paritaprevir/ritonavir had no effects on embryo-fetal viability or on fertility when evaluated in rats up to the highest dose of 300/30 mg per kg per day. Paritaprevir exposures at this dose were approximately 2- to 5-fold the exposure in humans at the recommended clinical dose.

Dasabuvir

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Dasabuvir had no effects on embryo-fetal viability or on fertility when evaluated in rats up to the highest dose of 800 mg per kg per day. Dasabuvir exposures at this dose were approximately 33-fold the exposure in humans at the recommended clinical dose.

2 Drug Information

2.1 Drug

Generic Name Paritaprevir

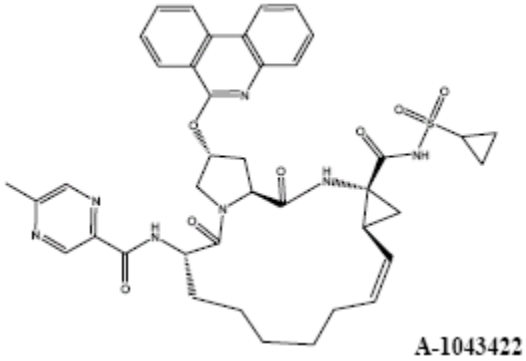
Code Name ABT-450; A-1043422

Chemical Name

(2R,6S,12Z,13aS,14aR,16aS)-N-(cyclopropylsulfonyl)-6-{[(5-methylpyrazin-2-yl)carbonyl]amino}-5,16-dioxo-2-(phenanthridin-6-yloxy)-1,2,3,6,7,8,9,10,11,13a,14,15,16,16a tetradecahydrocyclopropa[e]pyrrolo[1,2-a][1,4]diazacyclopentadecine-14a(5H)-carboxamide hydrate

Molecular Formula/Molecular Weight C40H43N7O7S (anhydrous); C40H43N7O7S•H2O (hydrate) 765.88 (anhydrous free acid); 783.89 (free acid hydrate)

Structure or Biochemical Description



Pharmacologic Class Nonstructural protein 3 (NS3) protease inhibitor

Generic Name Ombitasvir

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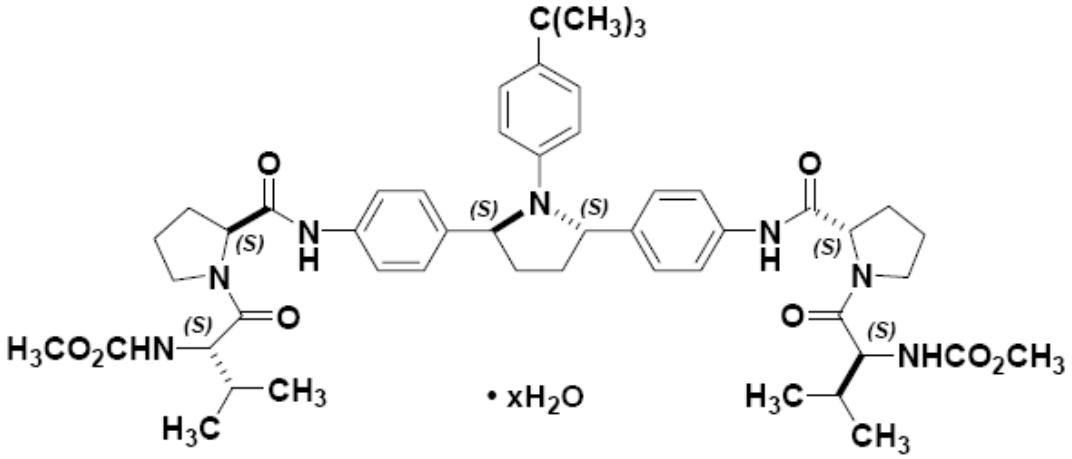
Code Name ABT-267; A-1233617

Chemical Name

Dimethyl ([(2*S*,5*S*)-1-(4-*tert*-butylphenyl) pyrrolidine-2,5-diyl]bis{benzene-4,1-diylcarbamoyl(2*S*)pyrrolidine-2,1-diyl[(2*S*)-3- methyl-1-oxobutane-1,2-diyl]})biscarbamate hydrate

Molecular Formula/Molecular Weight C50H67N7O8 •4.5H20 (hydrate)/ 894.11 (anhydrate); 975.20 (hydrate)

Structure or Biochemical Description



Pharmacologic Class Nonstructural protein 5A (NS5A) inhibitor.

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Generic Name Dasabuvir

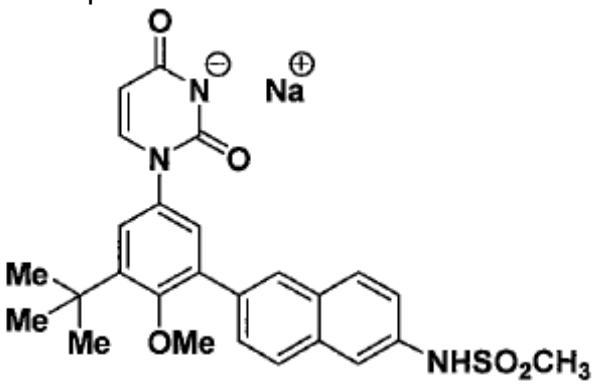
Code Name ABT-333; A-99821

Chemical Name

Methanesulfonamide, N- (6- (5 -(3 ,4-dihydro-2,4-dioxo-1 (2H)-pyrimidiny 1)- 3-(1,1-dimethy lethy 1)- 2- methoxypheny 1) - 2-naphthalenyl)-,monosodium salt

Molecular Formula/Molecular Weight $C_{26}H_{26}N_{30}O_5SNa$ (salt); (acid)/ 515.56 (salt); 493.57 (acid)

Structure or Biochemical Description



Pharmacologic Class Non-nucleoside inhibitor of nonstructural protein 5B (NS5B) RNA polymerase

2.2 Relevant INDs, NDAs, BLAs and DMFs

AbbVie evaluated direct acting agents (DAAs) for chronic hepatitis C under the following INDs: IND 103,526 (ABT-450), IND 101,636 (ABT-333), IND 108,434 (ABT-267)

Cross references are made to the Norvir (ritonavir) NDA, 22-417.

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2.3 Drug Formulation

The Ombitasvir/Paritaprevir/Ritonavir tablet [redacted] (b) (4) (b) (4) form the final tablet. [redacted]

Dasabuvir Film-Coated Tablets are immediate release tablets containing 250 mg of dasabuvir (free acid).

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

The sponsor's proposed specifications for impurities/degradants likely to be present in the drug product are considered acceptable from a pharmacology/toxicology perspective based on the results of general toxicology studies, empirical Ames assays, in vitro chromosomal aberration assays, and/or (quantitative) structure-activity relationship [(Q)SAR] predictions of mutagenicity. See complete review by Dr. Mark Powley in Appendix 5.

2.6 Proposed Clinical Population and Dosing Regimen

Paritaprevir /Ritonavir/Ombitasvir and Dasabuvir are indicated for the treatment of GT1 chronic hepatitis C infection, including in patients with cirrhosis. The recommended adult oral dose of Paritaprevir /r/Ombitasvir with Dasabuvir is two Paritaprevir /Ritonavir/Ombitasvir (75/50/12.5 mg) tablets QD (in the morning) and one Dasabuvir (250 mg) tablet BID (morning and evening) with food without regard to fat or calorie content. Duration of therapy and addition of RBV are dependent on the patient population:

- For patients with HCV GT1b without cirrhosis, Paritaprevir /Ritonavir/Ombitasvir and Dasabuvir for 12 weeks is recommended.
- For patients with HCV GT1a without cirrhosis, Paritaprevir /Ritonavir/Ombitasvir and Dasabuvir with RBV for 12 weeks is recommended. (b) (4) [redacted]

[redacted] (b) (4)

2.7 Regulatory Background

This NDA was reviewed under the PDUFA V program under Priority Review. These drugs were previously reviewed under INDs 103526, 108434 and 101636.

The combination of drugs was granted Breakthrough Therapy Designation on May 1, 2013 and the individual components were previously granted Fast Track status. The Agency granted the company's request for rolling review in December 2013, and the company submitted the agreed-upon rolling components in January, February and March 2014.

11. Integrated Summary and Safety Evaluation

Paritaprevir

Repeat dose studies to assess paritaprevir toxicity were performed in mice (up to 6 months), rats (up to 13 weeks), and dogs (up to 9 months). *In vitro* and *in vivo* genotoxicity studies were conducted. A six-month oral carcinogenicity study was conducted in transgenic mice and a two-year oral carcinogenicity study was conducted in rats. The reproductive and developmental toxicity studies included fertility studies in male and female rats, embryo-fetal developmental studies in mice and rats, and a peri- and postnatal developmental study in rats.

In early studies (b) (4), paritaprevir oral bioavailability values ranged from 0% in rats and monkeys to 40.9% in dogs. Co-administration with ritonavir increased plasma exposures of paritaprevir up to five fold following oral co-dosing in all species. (b) (4)

Also in dogs, plasma AUC values were greater than six fold higher in fasted compared with fed animals.

In clinical development, paritaprevir was co-administered with ritonavir to boost systemic exposures. Therefore, nonclinical studies have been performed with varying paritaprevir /ritonavir dose levels to determine pharmacokinetics and toxicity. Parent drug was the primary compound in plasma following paritaprevir /ritonavir co-administration. No metabolites were present at concentrations greater than 10% of parent. *In vitro* studies showed that paritaprevir is primarily metabolized by human CYP3A4/5. *In vivo*, following co-administration of paritaprevir /ritonavir, parent drug was the primary plasma component (~90% of drug related material). An oxidation product (M2) and hydrolysis product (M29) were the main metabolites in human plasma, representing ~5.6% and 2.5% of total drug related material, respectively. The main human metabolites, M2 and M29, were detected in mouse plasma and dog feces

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following co-administration of paritaprevir /ritonavir, indicating that these species were appropriate nonclinical models for toxicology studies.

Paritaprevir has been shown to inhibit several hepatic transporters (OATP1B1, OATP2B1, OATP1B3, MRP2 and Bile Salt Export Pump), renal transporters (OAT1, OAT3, and MATE2K (multidrug and toxin extrusion protein 2)), the primarily intestinal transporter BCRP, and the multi-tissue (hepatic, renal, intestinal) transporter P-gp.

Studies demonstrated that uptake transporters only affect the distribution behavior of paritaprevir between plasma and liver, without affecting the liver exposure of paritaprevir in general.

Doses of 100 mg/kg paritaprevir /15 mg/kg ritonavir and higher induced CNS excitation in rats (jumping, fear and reactivity to touch, chewing, head twitches, salivation, and slight mydriasis) at exposures approximately 5x the recommended clinical dose. No excitatory effects were noted in clinical trials.

In anesthetized dogs administered paritaprevir as consecutive 30 minute intravenous infusions, a statistically significant drop in arterial pressure was noted in the post-infusion monitoring period. Peak plasma concentrations were 65.72 ± 4.14 µg/mL. No effects on respiratory or gastrointestinal systems were noted following paritaprevir administration.

At concentrations of 6860 ± 399 and 19150 ± 970 ng/mL, respectively, paritaprevir reduced hERG channel tail current by 7.5 and 17.7%. For comparison, *in vitro* efficacy assays suggest an effective concentration (EC_{50}) of between 6.7 and 18 ng/mL for inhibition of HCV subgenomic replication. The effect of paritaprevir on the hERG channel is considered weak and not a safety concern. Any effect of plasma protein binding (~99% in human plasma *in vitro*) has not been considered.

The gallbladder was identified as a target organ of toxicity in repeat dose studies in mice and dogs. Findings include edema, mononuclear and mixed cell infiltration and epithelial cell necrosis with increased serum alkaline phosphatase, suggesting biliary effects.

In clinical trials, transient elevations in total bilirubin that peaked at Week 1 and declined thereafter were observed in subjects who received with paritaprevir/ritonavir, ombitasvir, dasabuvir and ribavirin (RBV). The increased bilirubin values were driven by an elevation in the indirect bilirubin fraction. Paritaprevir is a known inhibitor of the organic anion transporting polypeptide 1B1 (OATP1B1) bilirubin transporter. In addition, RBV-induced hemolysis may also contribute to hyperbilirubinemia. There were no clinically significant increases in alkaline phosphatase.

Paritaprevir was positive in an *in vitro* human chromosome aberration test. Paritaprevir was negative in a bacterial mutation assay, and in two *in vivo* genetic toxicology assays (rat bone marrow micronucleus and rat liver Comet tests).

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Paritaprevir/ritonavir carcinogenicity was assessed in Model 001178-T (Hemizygous) CBYB6F1-Tg(HRAS)2Jic mice and Sprague-Dawley rats. No neoplastic lesions were noted in mice following administration of dose levels of 6/30, 60/30, and 300/30 mg/kg/day for six months. The estimated mean AUC on Day 87 in high dose animals was 269 μg*hr/mL.

Likewise, no neoplastic lesions were noted in rats following administration of paritaprevir/ritonavir dose levels of 6/30, 60/30, or 300/30 mg/kg/day for up to 104 weeks. The highest estimated mean AUC on Day 87 was 65 μg*hr/mL in middle dose animals, corresponding to approximately 9x exposures at the recommended clinical dose.

Paritaprevir/ritonavir did not affect male or female fertility in rats at doses up to 300/30 mg/kg/day, corresponding to likely systemic exposures of approximately 12 (males) and 36 (females) μg*hr/mL. Oral administration of paritaprevir/ritonavir to pregnant rats at doses up to 450/45 mg/kg from days 6 to 17 of gestation did not elicit maternal toxicity or teratogenicity. The high dose corresponded to gestation day 17 exposures of approximately 59 μg•hr/mL (8x exposures at the recommended clinical dose). Oral administration of paritaprevir/ritonavir to pregnant mice at doses up to 300/30 mg/kg from days 6 to 15 of gestation did not elicit maternal toxicity, developmental toxicity, or teratogenicity. Systemic exposures on gestation day 15 (as determined by AUC) in a dose range finding study were 1340 μg•hr/mL. In the pivotal study, the high dose corresponded to gestation day 15 exposures of approximately 686 μg•hr/mL, equivalent to 98x exposures at the recommended clinical dose. Developmental studies were not conducted in rabbits due to insufficient systemic exposures following oral dosing.

The potential for adverse effects of paritaprevir/ritonavir on pregnant/lactating female rats and on the development of the conceptuses and offspring was assessed at doses up to 300/30 mg/kg/day. Paritaprevir/ritonavir was administered orally to mated female rats from gestation day 7 through lactation day 20, the days that correspond to the period from implantation to weaning of rats. No effects were noted on maternal health, reproductive health, or viability and development of offspring at maternal exposures of 116 ± 133 μg*hr/mL, equivalent to 17x exposures at the recommended clinical dose.

<u>Ritonavir</u>
In nonclinical toxicology studies with ritonavir (Norvir), the main target organs of toxicity were the liver and the eyes. According to the Norvir label, hepatic transaminase elevations exceeding five times the upper limit of normal, clinical hepatitis, and jaundice have occurred in patients receiving NORVIR alone or in combination with other antiretroviral drugs. Retinal toxicity was observed in nonclinical studies but this has not been seen in patients. Also, pancreatitis has been observed in patients receiving NORVIR therapy, including those who developed hypertriglyceridemia. In some cases fatalities have been observed.

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Paritaprevir: NOAELs, Systemic Exposures, and Margins of Exposure

Species	Study Type	NOAEL (mg/kg)	AUC µg*hr/mL	Margin (Ratio of Animal to Human Exposure)*
Mouse	4-wk Tg	450/30	324 (male) 517 (female) 418 (overall)	46 74 60
	13-week	300/30	272 (male) 561 (female) 417 (overall)	39 80 60
	6-month[1]	30/30	33	5
	Embryo-fetal	300/30	686	98
	Carci Tg	300/30	269	Not applicable
Rat	4-week	520	30	4
	13-week	450/45	91 ± 53	13
	Fertility	300/30	11.5 (males) 36 (females)	2 5
	Embryo-fetal	450/45	59 ± 47	8
	Pre-Postnatal	300/30	116±133	17
	Carcinog.	60/30	65	9
Dog	4-week[2]	10/5	389	56
	4-week[3]	10/5	264	38
	13-week	40/20	1130 (males) 737 (females)	161 105
	9-month[4]	20/10	615	88

*AUC in human: 7 µg.hr/ml at 150 mg/day ABT-450 with 50 mg/kg/day RTV.
[1] Histopathology effects noted in the gallbladder of the mid- and high-dose groups at 220 and 304 µg*hr/mL.
[2] At mean exposures of 816 µg*hr/mL, findings include gall bladder effects (edema, mononuclear and mixed cell infiltration and epithelial cell necrosis) with increased serum alkaline phosphatase, suggesting biliary effects. Increased liver weights were noted in females.
[3] Microscopic effects were noted in gall bladders in high dose animals (20/10 mg/kg, associated with AUCs of 645±260 µg*hr/mL).
[4] The NOAEL was defined as the middle dose (20/10 mg/kg day) based on histopathological findings in gallbladder at the high dose (80/20 mg/kg, associated with AUCs of 1490 µg*hr/mL).

Ombitasvir

Repeat dose studies to assess ombitasvir toxicity were performed in mice (up to 6 months), rats (up to 13 weeks), and dogs (up to 6 months). Mouse and dog were chosen as the nonclinical species for chronic repeat dose studies based on the fact that

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higher systemic exposures were achieved in these species compared to rats and monkeys, and based on the similarity of the metabolite profile between mouse, dog and humans in hepatic *in vitro* systems. *In vitro* and *in vivo* genotoxicity studies were conducted. A six-month oral carcinogenicity study was conducted in transgenic mice. The reproductive and developmental toxicity studies included fertility studies in male and female mice. Mice were selected as the appropriate rodent species based on the fact that higher systemic exposures were achieved in mice compared to rats, and chronic general toxicology studies were conducted using mice.

Ombitasvir has minimal solubility in aqueous solutions (<0.1 µg/mL). In toxicology studies, [redacted] (b) (4) solution formulations provided maximum feasible exposures at steady state in mice, rats and dogs. At maximum feasible doses and at exposure levels that reflect saturation of absorption, no toxicologically significant effects of ombitasvir were noted in nonclinical studies. In those studies, systemic exposures were approximately 20-40-fold higher than clinical exposures. Therefore, the nonclinical toxicology program is considered to be adequate to predict toxicity in the clinical setting.

Oral bioavailability values ranged from 24.8% in rat to 57.3% in dog. Plasma elimination half-lives after intravenous dosing which ranged from 4.4 hours in monkey to 11.4 hours in rat. [redacted] (b) (4)

Identification of ombitasvir metabolites in mouse, rat, rabbit and dog plasma was conducted at steady state, following oral dosing for eight days. At least eight metabolites were identified in mouse plasma, including the amide hydrolysis metabolites M6, M7 and M23, the oxidative metabolites M1, M2, M3, the hydration metabolite M9, and the tert-butyl hydroxyl metabolite, M26. In rat, M19 was the most significant metabolite in plasma, with at least eight additional metabolites identified. In rabbit, at least nine metabolites were identified, with M23 as the most abundant in plasma. In dog, six metabolites were identified in plasma, with the amide hydrolysis products M23 and M6, and the hydration product M9 as the most abundant metabolites.

In humans, nearly all of an administered radioactive dose of ombitasvir (90.2%) was recovered in feces, with limited radioactivity (1.91%) found in urine. Unchanged parent drug accounted for 8.85% of total radioactivity in plasma. A total of 13 metabolites were identified in human plasma; M23, M29, M36, and M37 were present as major circulating metabolites. The $AUC_{0\text{-}192hr}$ of M29, M36, M23 and M37 were about 31.2%, 21.4%, 15.0%, and 13.9 % of the total radiochemical plasma $AUC_{0\text{-}192hr}$, respectively. Other metabolites (M5, M25, M26, and M34) accounted for < 5% of drug-related radioactivity in circulation. The major human metabolism pathway involves hydrolysis of ombitasvir to dianiline (M23; A-1242846) which undergoes oxidation and C-demethylation of the tert-butyl group to form metabolites M29, M36 and M37.

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Two metabolites of ombitasvir, M29 and M36 (A-1538855 and A-1548255, respectively), were identified as unique human metabolites (i.e., metabolites that were not present in nonclinical species) and were present in human plasma as 20% and 13%, respectively, of total drug-related AUC at steady-state. The toxicological profile of each metabolite was assessed in genetic toxicology assays, repeat dose studies and reproductive toxicology studies. No significant toxicological effects were identified.

Incubations with human recombinant CYPs suggested that CYP3A4/5 and CYP2C8 have the potential to metabolize [3H]A-1233617. Further characterization of enzyme kinetics in rhCYP3A4 and rhCYP2C8 suggested CYP3A4 was the primary enzyme responsible for metabolizing [3H]A-1233617.

Ombitasvir does not appear to significantly inhibit OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1 or MATE2K, P-gp and BCRP. Inhibition of MRP2 was <30% at 100 µM. A-1233617 inhibited the ATP-dependent transport of taurocholic acid (TCA) by Bile Salt Export Protein in a concentration-dependent manner, with an apparent IC50 value of 95 µM (n=2).

In genetically-altered mice, the absence of both P-gp and BCRP resulted in a significantly higher area under the concentration–time curve in plasma, brain and liver after an intravenous dose, indicating P-gp or BCRP or both played important roles in the disposition and elimination of A-1233617. The AUCs in plasma, brain and liver increased more significantly after oral administration, indicating that P-gp or BCRP or both have an impact on the absorption of A-1233617, in addition to disposition and elimination. No exposure changes in Mrp2 knockout mouse were observed after either intravenous or oral dosing, indicating Mrp2 transporter is not involved in the absorption, disposition and elimination of A-1233617.

No effects on the central nervous system, cardiovascular system, respiratory or gastrointestinal systems were noted following Ombitasvir administration.

In mice, findings were primarily in male mice and were limited to liver effects in a two-week non-GLP study. None of the liver effects were noted in longer (1-, 3-, or 6-month) studies. No adverse effects were noted in repeat-dose studies with rats up to 3-months in duration.

Test article-related effects in the dog were limited to effects within the small intestine of animals administered 100 mg/kg/day in the 3-month study (minimal to mild focal dilatation of the lacteals within villi of the jejunum) and of animals administered 20 and 100 mg/kg/day in the 6-month study (minimal to mild vacuolation of small intestinal villi in duodenum and/or jejunum). No effects on food consumption or body weight were noted. Therefore, these findings were not considered to be adverse.

Ombitasvir was not mutagenic (Ames) or clastogenic in *in vitro* (human lymphocytes) assays and did not induce chromosomal aberrations *in vivo* (mice).

Ombitasvir carcinogenicity was assessed in Model 001178-T (Hemizygous) CBYB6F1-Tg(HRAS)2Jic mice. No neoplastic lesions were noted in mice following administration

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of dose levels of 2.5 (male), 5 (female), 10 (male), 20 (female), or 150 mg/kg/day mg/kg/day for six months. The estimated mean AUC on Day 87 in high dose animals was 37 µg*hr/mL.

Reproductive toxicity studies with ombitasvir were conducted in mice, rather than rats, based on the fact that higher systemic exposures were achieved in mice compared to rats, and pivotal general toxicology studies were conducted using mice. Ombitasvir did not affect male or female fertility in mice dosed up to 200 mg/kg, equivalent to approximately 29x exposures at the recommended clinical dose. Ombitasvir-related changes in male reproductive organ weights included: increases weights of the prostate at 20 and 200 mg/kg/day; the seminal vesicles (without fluid) at 200 mg/kg/day; and decreases in the absolute and relative weights of the testes at 200 mg/kg.

In mice, ombitasvir was not maternally toxic or teratogenic at doses up to 150 mg/kg/day. Saturating systemic exposures were achieved in mice at 50 mg/kg/day, equivalent to approximately 29x exposures at the recommended clinical dose. In rabbits, ombitasvir was not maternally toxic or teratogenic at doses up to 60 mg/kg/day, equivalent to approximately 4x exposures at the recommended clinical dose, with plasma drug levels measured in fetuses between 1% and 2% of those measured in females at the time of Caesarean-section.

Ombitasvir: NOAELs, Systemic Exposures, and Margins of Exposure

Species	Study Type	NOAEL (mg/kg)	AUC µg*hr/mL	Margin (Ratio of Animal to Human Exposure)*
Mouse	2-week (non-GLP)[1]	2.85	Not determined	___
	4-week	60 (male) 120 (female)	103 (male) 67 (female)	74 48
	13-week	400	63 (male) 37 (female)	45 26
	6-month	400	41 (male) 32 (female)	29 23
	Embryo-fetal	50	40	29
	Pre- and postnatal	200	40 (based on EFT)	29
	Carci Tg	150	37	Not applicable
Rat	13-week	300	22 (male) 35 (female)	16 25
Rabbit	Embryo-fetal[2]	60	6	4
Dog	4-week	60	49 (combined)	35
	13-week[3]	100	61 (male) 106 (female)	44 76
	27-week[4]	100	83 (combined)	59

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*AUC in human: 1.4 µg•hr/mL at 25 mg/day.
[1] Findings were primarily in male mice and were limited to liver effects in a two-week non-GLP study. Mice were dosed with 2.85, 28.5, or 95.1 mg/kg. Findings included increased transaminase levels (ALT: 2-10x and AST: 2-5x) at the middle dose with increased glutamate dehydrogenase at the high dose. Minimal to mild hepatocellular single-cell necrosis was noted in middle and high dose animals. Systemic exposures were only assessed at the high dose. At that dose systemic exposures were 127.3 and 63.9 µg*hr/mL in male and female mice, respectively. None of the liver effects were noted in longer (1-, 3-, or 6-month) studies.

[2]Two fetuses from two different litters in the 60 mg base/kg/day group were observed with external malformations and variations, and skeletal malformations. The incidence of these external malformations and variations were within historical control ranges for this laboratory, or in the case of craniorachischisis, were within historical ranges in rabbits in general, and considered unrelated to treatment. Visceral malformations and visceral variations were seen at a low incidence and were either similar to controls or within historical control ranges for this laboratory and considered unrelated to treatment. Based on these results, the no observed adverse effect level (NOAEL) for maternal or embryo-fetal toxicity was 60 mg base/kg/day, resulting in a systemic exposure (AUC0-24) of 6.26 µg•hr/mL.

[3] In high dose groups, males (3/4) and females (3/4) had minimal to mild focal dilatation of the lymphatic capillaries (lacteals) within villi of the small intestine. In one high dose female, diffuse, mild hepatocellular vacuolation which correlated with increased liver weight. Finally, in one high dose female, extrahepatic bile duct was inadvertently collected (attached to the pancreas sample), and on examination histopathology was noted. Mild subacute/chronic inflammation involving the tunica mucosa and muscularis was seen microscopically, without correlative findings in the biliary tree. This reviewer raises the possible connection between findings in lacteals and bile ducts, but acknowledges that none of the noted findings are considered adverse.

[4] Minimal to mild vacuolation within intestinal villi was present among most animals at greater than or equal to 20 mg/kg/day (middle dose), without dose-dependent effects on incidence or severity. The vacuoles were variably sized, smoothly rounded, clear spaces distinct from lacteals and not associated with a cellular reaction. Similar vacuoles were noted within the outer medulla and subcapsular sinuses of the mesenteric lymph node from a high-dose female. The intestinal villar vacuoles were considered not adverse, due to lack of an appreciable functional effect.

<u>Dasabuvir</u>

Repeat dose studies to assess dasabuvir toxicity were performed in mice (up to 13 weeks), rats (up to 6 months), and dogs (up to 9 months). *In vitro* and *in vivo* genotoxicity studies were conducted. A six-month oral carcinogenicity study was conducted in transgenic mice. The reproductive and developmental toxicity studies included fertility studies in male and female rats, embryo-fetal developmental studies in rats and rabbits, and a peri- and postnatal developmental study in rats.

Bioavailability from an oral dose was low in both monkey (4.5%) and rat (21.3%), but high in dog (95.9%). The ABT-333 plasma elimination half-life ($t_{1/2}$) was short in monkeys (2 hr), but averaged 3.6 hours in rats and 19.5 hours in dogs.

Reference ID: 3628623

In bile duct cannulated rats (5 mg/kg, IV), [^{3}H]ABT-333 underwent extensive oxidative metabolism (< 2% of the dose was recovered as intact parent) in the rat. The most significant product of metabolism was the M3 (proposed as the glucuronide conjugate of M1) representing ~50% of the dose, followed by M1 (A-1041392, *t*-butyl hydroxy) and M5 (A-1039710, *t*-butyl acid), representing 8 and 5% of the total dose, respectively. Other products of metabolism included a M2, a sulfate conjugate, M6, a glucuronide conjugate of the *t*-butyl acid M5 and M4, a proposed *t*-butyl aldehyde. Similar metabolite profiles were observed following a single 5 or 10 mg/kg dose.

Parent drug was the most significant radiolabeled component in plasma after a 5 mg/kg dose (~80 to 90% of the total plasma radioactivity), followed by M1 (A-1041392, *t*-butyl hydroxyl; ~10 to 20% of the total plasma radioactivity). M3 was detected in trace amounts (< 3% of the total plasma radioactivity).

In humans, nearly all of an administered radioactive dose (94%) was recovered in feces, with limited radioactivity (2%) found in urine. Unchanged parent drug accounted for 60% of total radioactivity in plasma. One metabolite, M1, was the only major metabolite, accounting for 22% of radioactivity. including a sulfate conjugate M2, a glucuronide conjugate M3, secondary oxidation products (M4 and M5), a glucuronide conjugate of M5 (M6) and a trace desmethyl metabolite M11, were considered minor metabolites as each accounted for less than 5% of radiochemical in plasma.

Incubations with human recombinant CYPs suggested that 2C8, 2B6, 3A4, and 2D6 have the potential to metabolize dasabuvir. Further characterization of enzyme kinetics indicated that CYP2C8 had the most significant contribution to metabolism followed by CYP3A4 and CYP2D6 (~60, 30, and 10%). There was no evidence of active efflux by transporters.

No effects on the central nervous system, cardiovascular system, respiratory or gastrointestinal systems were noted following dasabuvir administration.

No toxicologically significant effects of dasabuvir were noted in nonclinical studies. In rats following six months of dosing, alveolar histiocytosis and granulomatous inflammation of the ileum at 800 mg/kg/day were test article-related changes, but were not associated with adverse clinical effects. In dogs dosed for nine months, moderately increased mean absolute liver weights were noted in males given 60 mg/kg/day. These moderate increases correlated with increased hepatocellular vacuolation likely due to increased glycogen in hepatocytes. The ultrastructure of hepatocytes was otherwise normal.

Dasabuvir was not mutagenic (Ames) or clastogenic in *in vitro* (human lymphocytes) assays and did not induce chromosomal aberrations *in vivo* (mice). Dasabuvir carcinogenicity was assessed in Model 001178-T (Hemizygous) CBYB6F1-Tg(HRAS)2Jic mice. No neoplastic lesions were noted in mice following administration of dose levels of 200, 600, 2000 mg/kg/day for six months. The estimated mean AUC on Day 87 in high dose animals was 265 µg*hr/mL. A carcinogenicity study in rats is ongoing.

Reference ID: 3628623

Dasabuvir did not affect male or female fertility in rats dosed up to 800 mg/kg, corresponding to approximately 33x exposures at the recommended clinical dose. In rats, dasabuvir was not maternally toxic or teratogenic at doses up to 800 mg/kg/day. Systemic exposures were approximately 48x exposures at the recommended clinical dose. In rabbits, dasabuvir was not maternally toxic or teratogenic at doses up to 400 mg/kg/day, corresponding to approximately 12x exposures at the recommended clinical dose.

Dasabuvir: NOAELs, Systemic Exposures, and Margins of Exposure

Species	Study Type	NOAEL (mg/kg)	AUC µg*hr/mL	Margin (Ratio of Animal to Human Exposure)*
Mouse	4-week Tg	2000	396	58
	13-week	5000	197	29
	Carci Tg	2000	265	Not applicable
Rat	4-week	150	108	16
	13-week	200	34 male 94 female	5 14
	6-months	800	119 male 319 female	18 47
	Fertility	800	224	33
	Embryo-fetal	800	329 maternal	48
	Pre-, post-natal	800	302	44
Rabbit	Embryo-fetal	400	84 maternal	12
Dog	9-months	60	839	123

*AUC in human: 6.8 µg•hr/mL at 250 mg/day.

Appendix 1. Paritaprevir

Studies Reviewed

Secondary Pharmacology

Study RD081575. In Vitro Pharmacology High Throughput Profile Study of A-1043422.0

Safety Pharmacology

Study RD081479. A-1043422.5: CNS/Neurobehavioral Safety Pharmacology Profile in the Rat (P.O. Administration)

Study RD081203. A Neurobehavioral Safety Evaluation of Orally Administered A-1043422 (ABT-450) and A-84538 (Ritonavir) in Rats

Study RD081521. A-1043422: In Vitro Effects on hERG Current

Study RD12513. Effects of A-1043422 (ABT-450) on Cloned hERG Potassium Channels Expressed in Mammalian

Study RD081504. Effects of A-1043422 on Cardiovascular and Hemodynamic Function in the Anesthetized Dog

Study RD081205. A Cardiovascular Safety Evaluation of Orally Administered A-1043422 (ABT-450) in Beagle Dogs

Study RD081204. A Respiratory Safety Evaluation of Orally Administered A-1043422 (ABT-450) and A-84538 (Ritonavir) in Rats

Study RD081535. A-1043422: Effects on Ferret Emetic Liability and Rat Gastrointestinal Transit

Pharmacokinetics/ADME

A-1043422.0 (ABT-450) Preformulation Report (Updated in 2013): Physicochemical Properties of A-1043422.0

Study A-1043422 Drug Metabolism Memo No. 11. The In Vitro Permeability and Transport Characteristics of A-1043422 Across Human Caco−2 Cells

Study RD081582. Preclinical pharmacokinetic summary of A-1043422 in mouse, rat, rabbit, monkey and dog

Study RD13784. Integration of Pharmaceutics, Formulations and Pharmacokinetics for the Definition of Maximum Feasible Exposures in Preclinical Studies with A 1043422

Study A-1043422 Drug Metabolism Memo No. 13. A-1043422 Pharmacokinetics following Oral Dosing in Mouse

Study A-1043422 Drug Metabolism Memo No. 34. Plasma Concentrations of A-1043422, Ritonavir and A-1233617 after Oral Co-Dosing in CD-1 Mouse

Study A-1043422 Drug Metabolism Memo No. 06. A-1043422 Pharmacokinetics following Intravenous or Oral Dosing in Rat

Study RD081580. Disposition and Metabolism of [3H]A-1043422 in Male Rats

Study A-1043422 Drug Metabolism Memo No. 14. A-1043422 Pharmacokinetics following Intravenous, Subcutaneous, Intraperitoneal and Oral Dosing in Rabbit

Reference ID: 3628623

Study A-1043422 Drug Metabolism Memo No. 08. A-1043422 Pharmacokinetics following Intravenous or Oral Dosing in Dog

Study A-1043422 Drug Metabolism Memo No. 09. Liver and Plasma Concentrations of A-1043422 following Oral Dosing in Dog

Study A-1043422 Drug Metabolism Memo No. 10. Effect of Formulation on A-1043422 Bioavailability following Oral Dosing in Dog

Study A-1043422 Drug Metabolism Memo No. 07. A-1043422 Pharmacokinetics following Intravenous or Oral Dosing in Monkey

Study RD13054. Absorption, Distribution, Metabolism, and Excretion (ADME) Study of [14C] ABT-450/r in Healthy Male Subjects Following a Single Oral Dose Administration

Study RD13186. Determination of the Binding of A-1043422 to Human and Animal Plasma Proteins

Study RD13103. Determination of the Binding of A-1043422, A-1233617, A-998821, A-1041392 and A-84538 to Human Plasma Proteins from Subjects in Study M12-215

Study RD13814. Determination of the Binding of A-1043422, A-1233617, A-998821, A-1041392 and A-84538 to Human Plasma Proteins from Subjects in Study M12-193

Study RD13744. Determination of the Blood-to-Plasma Concentration Ratios Following Incubations of A-1043422 in Rat, Dog, Monkey and Human Whole Blood

Study RD13053. Quantitative Whole-Body Autoradiography of Pigmented Rats Following Oral Administration of 14C-ABT-450 and Ritonavir

Study RD13518. Placental Transfer, Lacteal Excretion, and Tissue Distribution of Radioactivity in Pregnant Female Sprague Dawley Rats Following Oral Administration of 14C-ABT-450 and Ritonavir

Study RD081579. In Vitro Metabolism of [3H]A-1043422

Study RD13743. Determination of the Metabolic Stability of A-1043422 in Liver Hepatocytes Across Species

Study RD13743. Determination of the Metabolic Stability of A-1043422 in Liver Hepatocytes Across Species

Study A-1043422 Drug Metabolism Memo No. 15. Assessment of Uptake and Inhibition of A-1043422 Mediated by OATP1B1

Study A-1043422 Drug Metabolism Memo No. 25. Uptake of A-1043422 by Organic Anion Transporting Polypeptide (OATP) 1B3

Study A-1043422 Drug Metabolism Memo No. 23. Assessment of A-1043422 Efflux Mediated by P glycoprotein (P-gp/MDR1) and Breast Cancer Resistance
Protein (BCRP)

Study A-1043422 Drug Metabolism Memo No. 17. Metabolite profiling of ABT-450 (A-1043422) in mouse and rat plasma samples from preclinical study, V10-
0101, TD09-193, TD09-138, TA09-018A
Study A-1043422 Drug Metabolism Memo No. 33. A-1043422 Metabolite Profiles in Rat Milk and Plasma after a 30 mg [14C]A-1043422/kg and a 15 mg Ritonavir/kg Oral Dose in Female Sprague Dawley Rats

Reference ID: 3628623

Study A-1043422 Drug Metabolism Memo No. 32. Metabolism and Disposition of [14C]A-1043422 (ABT 450) after a Single 1 mg/kg Oral Dose, Co-dosed with 5 mg/kg Ritonavir, in Beagle Dogs

Study A-1043422 Drug Metabolism Memo No. 18. Preliminary Metabolite Identification of A-1043422 (ABT-450) in Phase I Human Plasma Samples (M10-
7491 & M10-7492)

Study A-1043422 Drug Metabolism Memo No. 19. Plasma Concentrations of A-1231059 (M2) after Oral Dosing with A-1043422/ritonavir in Human

Study A-1043422 Drug Metabolism Memo No. 20. Structural Characterization of ABT-450 Metabolite M2 by NMR Spectroscopy

Study RD121024. Metabolism and Disposition of [14C]ABT-450 (A-1043422) in Male Subjects After a Single 200 mg Oral Dose with Co-Administered 100 mg Ritonavir

Study RD081581. Assessment of the Effects of A−1043422 on the Activity of Cytochrome P450 (CYP450) Isoforms in Human Liver Microsomes

Study RD13204. Assessment of CYP Time Dependent Inhibition Potential by A-1043422 in Human Liver Microsomes

Study RD121089. An In Vitro Investigation of Cytochrome P450 Induction by A-1043422 (ABT-450) in Cultured Human Hepatocytes

Study RD12914. Assessment of the Effect of A-1043422 on the Activity of UDP-Glucuronosyltransferases 1A1 (UGT1A1) Isoform in Human Liver Microsomes

Study A-1043422 Drug Metabolism Memo No. 22. The In Vitro Permeability and Transport Characteristics of A-1043422 Across Human Caco-2 Cells

Study A-1043422 Drug Metabolism Memo No. 24. Inhibitory Interaction of A-1043422 on P-glycoprotein (P-gp/MDR1) and Breast Cancer Resistance Protein
(BCRP)

Study A-1043422 Drug Metabolism Memo No. 28. Inhibitory Interaction of A-1043422 on Breast Cancer Resistance Protein (BCRP)

Study A-1043422 Drug Metabolism Memo No. 29. Inhibitory Interaction of A-1043422 on Multidrug Resistance Protein 2 (MRP2)

Study A-1043422 Drug Metabolism Memo No. 30. Inhibitory Interaction of A-1043422 on Bile Salt Export Pump (BSEP)

Study A-1043422 Drug Metabolism Memo No. 31. Inhibitory Interaction of A-1043422 on Organic Anion Transporting Polypeptide (OATP) 2B1

Study A-1043422 Drug Metabolism Memo No. 27. Inhibitory Interaction of A-1043422 on Organic Anion Transporting Polypeptide (OATP) 1B3, Organic Anion Transporters (OAT) 1, 3, Organic Cation Transporters (OCT) 1, 2, and Multidrug and Toxin Extrusion Proteins (MATE) 1 and 2K

ABT-450/Ritonavir/ABT-267/ABT-333: Pharmacokinetic Drug-Drug Interactions:
Metabolism and Transporters R&D/13/893

Reference ID: 3628623

Study A-1043422 Drug Metabolism Memo No. 26. A-1043422 Pharmacokinetics following Intravenous Dosing in Transporter Knockout Mouse

Single and repeat-dose Toxicology
Study RD081425. One-Day Oral Toxicity Study of A-1043422 in Sprague-Dawley Rats (with a Two-Week Recovery Period) TA08-272

Study RD081426. Single Dose Oral Capsule Toxicokinetics Study of A-1043422 (ABT-450) in Beagle Dogs (With A Two-Week Recovery Period) TB08-277

Study RD091295. 1-week oral dose range-finding study with A-1043422 and A-84538 in model 001178-w (wild type) CByB6F1-Tg(HRAS)2Jic Mice TD09-193

Study RD091434. Four-Week Oral Tolerability Study of Cremophor EL:PEG-400:Oleic Acid (10:10:80, w/w) in CD-1 Mice TD09-159

Study RD09895. Four-Week Oral Dose Range-Finding Study with A-1043422 and A-84538 in Mice TD09-138 RD09895

Study RD10126. 4-Week Oral Gavage Study with A-1043422 and A-84538 in Model 001178-W (Wild Type) CByB6F1-Tg(HRAS)2Jic Mice TD09-194

Study RD091208. Thirteen-Week Oral Maximum Tolerated Dose Toxicity Study of A-1043422 and A-84538 in Mice TD09-139

Study RD091381. 6-Month Oral Dose Toxicity Study with A-1043422 and A-84538 in Mice with a 1-Month Recovery Period TD09-225

Study RD081427. Four-Week Oral Dose Toxicity Study of A-1043422 and A-84538 (Ritonavir) in Sprague-Dawley Rats (With a Four-Week Recovery Period) TA08-270

Study RD09023. Four-Week Oral Toxicity Study of A-1043422 with Ritonavir (A-84538) in Sprague-Dawley Rats (with a Four-Week Recovery Period) TA08-430

Study RD09990. Thirteen-Week Oral Toxicity Study of A-1043422 with Ritonavir (A-84538) in Sprague-Dawley Rats TA09-018

Study RD081866. Amended Report Four-Week Oral (Gavage) Toxicity Study of A-1043422 and Ritonavir in Combination with Ribavirin in Sprague-Dawley Rats TA08-498

Study RD09525. Thirteen-Week Oral Toxicity Study of A-1043422 and A-84538 (Ritonavir) Co-Dosed with Ribavirin in Sprague-Dawley Rats Study TA09-036

Study RD08733. Five-Day Oral Capsule Tolerability Study of A-1043422 and A-84538 (Ritonavir) in Beagle Dogs TB08-191

Study RD081109. Four-Week Oral Capsule Dosage Range-Finding Toxicity Study of A-1043422 and A-84538 (Ritonavir) in Beagle Dogs TB08-192

Study RD081170. Four-Week Oral Capsule Toxicity Study of A 1043422 and A-84538 (Ritonavir) in Beagle Dogs (with a Four-Week Recovery Period) TB08-271

Study RD09053.Thirteen-Week Oral Capsule Toxicity Study of A-1043422 and A-84538 (Ritonavir) in Beagle Dogs

Reference ID: 3628623

Study RD091026. 9-Month Oral Capsule Dose Toxicity Study with A-1043422 and A-84538 in Beagle Dogs Followed by a 1-Month Recovery Period TB09-147

Genotoxicity/Carcinogenicity

Study RD081363. Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with A-1043422 TX08-205

Study RD081364. Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with A-1043422 TX08-206

Study RD081365. In Vivo Rat Micronucleus Assay with A-1043422 TA08-207

Study RD081786. Comet Assay in Rat Liver Cells Following Oral Administration of A-1043422 TA08-456

Study RD12179. 26-Week Oral Gavage Oncogenicity Study with A-1043422 and A-84538 in Model 001178-T (Hemizygous) CByB6F1-Tg(HRAS)2Jic Mice TD11-120

Study RD101434. 104-Week Oral Dose Carcinogenicity Study with A-1043422 and A-84538 in Rats TA10-013

Reproductive and Developmental Toxicology

Study RD101351. Amended report for Oral (Gavage) Fertility and General Reproduction Toxicity Study of A-1043422 and A-84538 in Rats, Including a Toxicokinetic Evaluation TA10-257

Study RD09891. A Range-Finding Oral (Gavage) Developmental Toxicity Study with A-1043422 and A-84538 in Mice, Including a Toxicokinetic Evaluation TD09-109

Study RD091178. Oral (Gavage) Developmental Toxicity Study of A-1043422 and A-84538 in Mice, Including a Toxicokinetic Evaluation TD09-113

Study RD09882. A Range-Finding Oral Developmental Toxicity Study with A-1043422 and A-84538 in the Rat, Including a Toxicokinetic Evaluation TA09-019

Study RD09847. An Oral Embryo-Fetal Developmental Toxicity Study with A-1043422 and A-84538 in the Rat, Including a Toxicokinetic Evaluation TA09-021

Study RD09599. An Exploratory Oral Toxicokinetic Range-Finding Study with A-1043422 and A-84538 in the Pregnant Rabbit TE09-020

Study RD12186. An Oral (Gavage) Pre- and Postnatal Study with A-1043422 in Combination with A-84538 in Rats, Including a Postnatal Assessment

Impurity Studies

Study RD101064. Four-Week Oral Gavage Toxicity Study of A-1043422 (with Impurities) and A-84538 in Sprague-Dawley Rats TA10-152l Behavioral/Functional Evaluation TA11-265

Study RD13392. Salmonella Escherichia Coli/Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with (b) (4) Free Form TX13-133

Study RD13484. Salmonella Escherichia Coli/Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with (b) (4) Free Form TX13-160

Study RD13684. Bacterial Reverse Mutation Assay with (b) (4) Free Form. Abbott Study TX13-179

Reference ID: 3628623

Pharmacology

Primary Pharmacology

Refer to Microbiology review.

Secondary Pharmacology

Significant binding (77% inhibition of a control substance) was noted at the opiod receptor δ_2(h) (DOP). Highest densities of the DOP receptor are found in the olfactory bulb, cerebral cortex, nucleus accumbens and the caudate putamen. Studies with DOP receptor knockout mice revealed that they display hyperlocomotor activity (McDonald and Lambert, 2005)[1], which is of interest given that rats exhibited increased excitation following treatment (see Safety Pharmacology section below). DOP receptor knockout mice also displayed anxiogenic and depressive-like responses, suggesting that the receptor may act to regulate mood (McDonald and Lambert, 2005). A distribution study of radiolabelled Paritaprevir without ritonavir in Sprague-Dawley rats suggests slight but quantifiable distribution of radioactivity to the brain. However, when radiolabelled Paritaprevir was co-administered with ritonavir in Long Evans rats, no measurable radioactivity was detected in brain. No excitatory effects were noted in clinical trials.

Weak to moderate binding was noted at the following receptors: sodium (Na+) channel (site 2), NK2(h), peripheral benzodiazepine receptor (PBR), vasopressin (V1a), and GABA-gated chloride channel receptors.

No significant effects related to secondary pharmacology were noted in nonclinical safety pharmacology and repeat dose toxicology studies, and the off-target binding identified in the receptor screen is not expected to be clinically relevant.

Safety Pharmacology

Doses of 100 mg/kg ABT-450/15 mg/kg ritonavir and higher induced CNS excitation in rats (jumping, fear and reactivity to touch, chewing, head twitches, salivation, and slight mydriasis). In anesthetized dogs administered ABT-450 as consecutive 30 minute intravenous infusions, a statistically significant drop in arterial pressure was noted in the post-infusion monitoring period. Peak plasma concentrations were 65.72 ± 4.14 µg/mL.

No effects on respiratory or gastrointestinal systems were noted following ABT-450 administration.

At concentrations of 6860 ± 399 and 19150 ± 970 ng/mL, respectively ABT-450 reduced hERG channel tail current by 7.5 and 17.7%. For comparison, *in vitro* efficacy assays suggest an effective concentration (EC_{50}) of between 6.7 and 18 ng/mL for inhibition of HCV subgenomic replication. The effect of ABT-450 on the hERG channel is considered weak and not a safety concern. Any effect of plasma protein binding (~99% in human plasma *in vitro*) has not been considered.

<u>Neurological effects</u>:

Study no. R&D/08/1203: A Neurobehavioral Safety Evaluation of Orally Administered A-1043422 (ABT-450) and A-84538 (Ritonavir) in Rats

[1] McDonald and Lambert, "Opiod receptors" in, Continuing Education in Anaesthesia, Critical Care & Pain, Volume 5 Number 1, 2005.

Reference ID: 3628623

In this screening test for behavioral effects, ABT-450 (A-1043422) was co-administered orally (gavage) with ritonavir (RTV; A-84538) to assess the neurobehavioral effects in rats.

Dose levels were as follows:
50 mg/kg A-1043422 / 15 mg/kg A-84538.0 (50/15)
150 mg/kg A-1043422 / 15 mg/kg A-84538.0 (150/15)
500 mg/kg A-1043422 / 15 mg/kg A-84538.0 (500/15)

The study included the functional observation battery (FOB; as modified by Moser), forelimb and hindlimb grip strength, hindlimb splay, and pain perception (thermal stimulus) performed at approximately three and six hours post-dose in eight female rats per group, along with plasma analysis for test article in three animals per group.

The 500/15 dose was associated with peak ABT-450 concentrations of 4.92 ± 2.38 µg/mL.

No effects were noted at the study time points (three and six hours post-dose).

Study no. R&D/08/1479 (ABT-08-356): A-1043422.5: CNS/Neurobehavioral Safety Pharmacology Profile in the Rat (P.O. Administration).

ABT-450 (A-1043422) was co-administered orally with ritonavir (RTV; A-84538) to assess the CNS/neurobehavioral effects in rats. This study differed from the above study primarily in the assessment time points, as parameters were measured as early as fifteen minutes post-dose (compared with 3 hours post dose in the earlier study).

Dose levels were as follows:
3 mg/kg A-1043422 / 15 mg/kg A-84538.0
10 mg/kg A-1043422 / 15 mg/kg A-84538.0
30 mg/kg A-1043422 / 15 mg/kg A-84538.0
100 mg/kg A-1043422 / 15 mg/kg A-84538.0
300 mg/kg A-1043422 / 15 mg/kg A-84538.0

The study included the following tests: Primary Observation (Irwin) Test in the rat; Activity Meter Test in the rat; Ethanol Interaction Test (sleep induction) in the rat; and the Pentylenetetrazole (PTZ) Seizure Test in the rat.

In the Primary Observation (Irwin) test, the vehicle induced slight excitation in 2 of 4 rats, increased sniffing and reactivity to touch in all four rats at 15 minutes. Compared to controls, ABT-450 at 3, 10, and 30 mg/kg, co-administered with RTV (15 mg/kg) did not induce changes. At 100 mg/kg ABT-450/15 mg/kg RTV, treatment induced slight excitation in all four rats, jumping in one rat, and fear and reactivity to touch from 15 to 120 minutes post-dose in all four rats. At 300 mg/kg ABT-450/15 mg/kg RTV, treatment induced the same effects as the previous dose, plus chewing, head twitches, salivation, and slight mydriasis (*see table below excerpted from sponsor*).

Reference ID: 3628623

TREATMENT (mg/kg p.o.)						
Vehicle	A-84538.0 (15)	A-1043422.5 (3) + A-84538.0 (15)	A-1043422.5 (10) + A-84538.0 (15)	A-1043422.5 (30) + A-84538.0 (15)	A-1043422.5 (100) + A-84538.0 (15)	A-1043422.5 (300) + A-84538.0 (15)
Excitation + (2/4) at 15' **Stereotypies (sniffing)** (4/4) → 15' **↑ Reactivity to touch** (4/4) at 15' (1/4) at 120'	**Stereotypies (sniffing)** (4/4) → 15' **↑ Reactivity to touch** (4/4) at 15'	**Stereotypies (sniffing)** (4/4) → 15' **↑ Reactivity to touch** (1/4) at 120'	**Stereotypies (sniffing)** (4/4) → 15'	**Stereotypies (sniffing)** (4/4) → 15' **↑ Reactivity to touch** (4/4) at 15'	**Excitation** + (4/4) at 15' **Jumping** (1/4) → 15' **Stereotypies (sniffing)** (4/4) → 15' **↑ Fear** (4/4) at 15' (4/4) at 120' **↑ Reactivity to touch** (4/4) 15' → 120'	**Excitation** + (4/4) 15' → 60' **Jumping** (1/4) 0 → 15' **Stereotypies (sniffing)** (4/4) → 15' (3/4) at 60' **Stereotypies (chewing)** (2/4) 0 → 15' (4/4) at 15' **Head twitches** (2/4) 0 → 15' (4/4) at 15' (1/4) 60' → 120' **↑ Fear** (4/4) 15' → 120' **↑ Reactivity to touch** (4/4) 15' → 120' **Salivation** (2/4) at 15' **Mydriasis** + at 15'

Treatment with ABT-450/RTV had no statistically significant effect on the Activity Meter Test, but appears to have affected activity from 20 to 40 minutes post-dose at 100 and 300 mg/kg levels, as evidenced by a 52% and 58% increase, respectively, in the number of crossings (*see table below excerpted from sponsor*).

TREATMENT (mg/kg) p.o. -180 min	TREATMENT (mg/kg) p.o. -60 min	NUMBER OF CROSSINGS 20 to 40 min			NUMBER OF REARS 20 to 40 min		
		mean ± s.e.m.	p value	% change from control	mean ± s.e.m.	p value	% change from control
Distilled water	Distilled water	13.3 ± 3.5	-	-	17.0 ± 3.9	-	-
Vehicle	Distilled water	8.4 ± 2.3 NS (a)	0.2564	-37% (a)	14.6 ± 5.0 NS (a)	0.7089	-14% (a)
A-84538.0 (15)	Distilled water	8.9 ± 3.6 NS (b)	0.9091	+6% (b)	11.7 ± 3.7 NS (b)	0.6466	-20% (b)
A-1043422.5 (10) + A-84538.0 (15)	Distilled water	9.1 ± 2.2 NS (b)	0.8293	+8% (b)	15.1 ± 4.8 NS (b)	0.9432	+3% (b)
A-1043422.5 (30) + A-84538.0 (15)	Distilled water	7.5 ± 2.6 NS (b)	0.8016	-11% (b)	7.9 ± 2.6 NS (b)	0.2519	-46% (b)
A-1043422.5 (100) + A-84538.0 (15)	Distilled water	12.8 ± 2.4 NS (b)	0.2047	+52% (b)	11.5 ± 2.3 NS (b)	0.5811	-21% (b)
A-1043422.5 (300) + A-84538.0 (15)	Distilled water	13.3 ± 2.9 NS (b)	0.2079	+58% (b)	15.1 ± 3.8 NS (b)	0.9372	+3% (b)
Distilled water	Caffeine (32)	28.0 ± 3.3 ** (a)	0.0063	+111% (a)	39.5 ± 6.0 ** (a)	0.0056	+132% (a)
Distilled water	Chlorpromazine (16)	0.9 ± 0.4 ** (a)	0.0022	-93% (a)	0.0 ± 0.0 *** (a)	0.0004	-100% (a)

Student's t test: NS = Not Significant; ** = $p < 0.01$; *** = $p < 0.001$

(a): compared with distilled water control.
(b): compared with vehicle control.

Vehicle = oleic acid, polyethylene glycol 400 and Cremophor® EL (80/10/10%: w/w/w).

Co-administration of ABT-450 with RTV did not influence the Ethanol Interaction Sleep Test, indicating no sedative/hypnotic activity of ABT-450. There was no clear proconvulsant or anticonvulsant activity related to a GABAergic mechanism, as assessed using the Pentylenetetrazole (PTZ) Seizure Test, although decreases in latency to clonic convulsion and latency to death approached statistical significance at 100/15 and 30/15.

Cardiovascular effects:

Study no. R&D/08/1521: A-1043422: *In Vitro* Effects on hERG Current

ABT-450 reduced hERG channel tail current by 7.5 and 17.7% at concentrations of 6860 ± 399 and 19150 ± 970 ng/mL, respectively (IC_{50} ~ 88,000 ± 1000 ng/mL; 115 ± 1 µM). For comparison, *in vitro* efficacy assays suggest an EC_{50} of between 8.7 – 23 nM (6.7 – 18 ng/mL) for inhibition of HCV subgenomic replication. Note: any effect of plasma protein binding (~99% in human plasma *in vitro*) has not been considered.

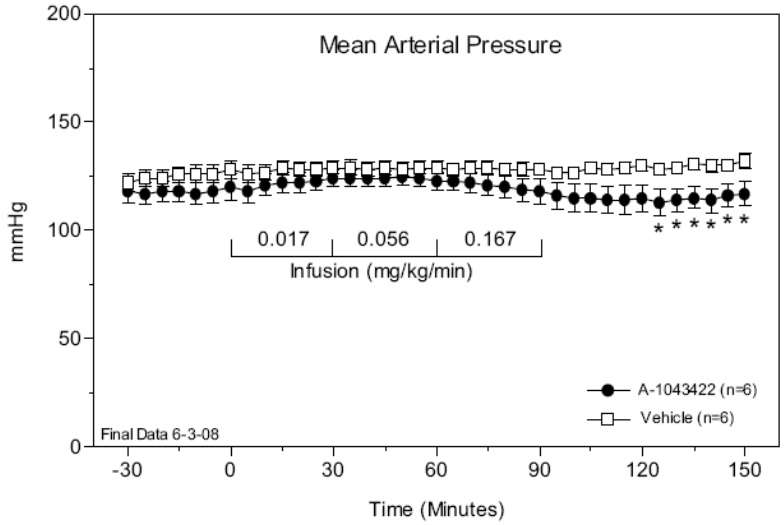
Study RD12513. Effects of A-1043422 (ABT-450) on Cloned hERG Potassium Channels Expressed in Mammalian Cells

A-1043422 at a measured concentration of 8.24 µg A-1043422.0/mL produced a 5.30 ± 1.242 percent inhibition in hERG-mediated potassium currents. Therefore, hERG channel inhibition is not expected to be a concern in the clinical setting.

Study no. R&D/08/1504: Effects of A-1043422 on Cardiovascular and Hemodynamic Function in the Anesthetized Dog

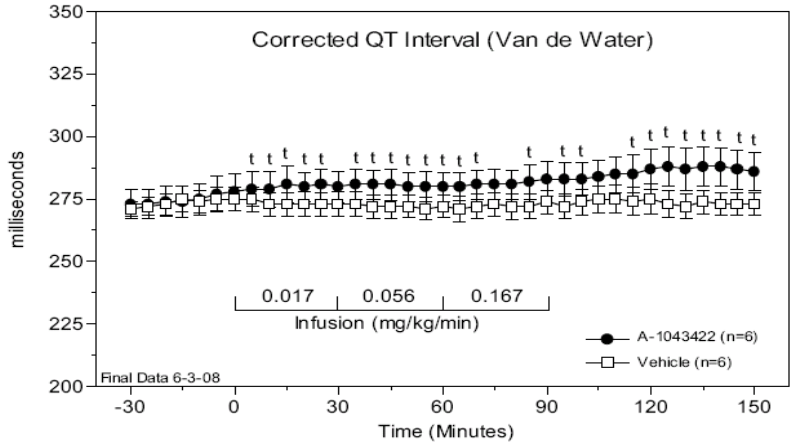
Male beagle dogs anesthetized with pentobarbital (n = 6) were administered ABT-450 as consecutive 30 minute intravenous infusions at 0.5, 1.67, and 5.01 mg/kg/30 minutes. Peak plasma concentrations following the low, middle and high doses were 2.62 ± 0.31, 17.49 ± 1.71, and 65.72 ± 4.14 µg/mL, respectively.

ABT-450 administration had no dose-related effect on hemodynamic parameters during the infusion period, but a statistically significant drop in arterial pressure was noted in the post-infusion monitoring period (see figure below *excerpted from sponsor*).



*p < 0.05 *vs.* vehicle
t p< 0.05 *vs.* Change from baseline, A-1043422 treatment relative to vehicle

ABT-450 produced small but statistically significant increases in the QT-interval corrected for heart rate (Van de Water; QTcV) with limited dose-dependency (4 ± 2, 5 ± 2 and 7 ± 4 ms; vehicle = -1 ± 1, -2 ± 1 and -1 ± 1 ms at the end of each dosing period, respectively). QTcV continued to increase and was 10 ± 4 ms above baseline 60 minutes post-infusion (vehicle = -1 ± 3ms) (see figure below, *excerpted from sponsor*).



*p < 0.05 *vs.* vehicle
t p< 0.05 *vs.* Change from baseline, A-1043422 treatment relative to vehicle

Study no. R&D/08/1205: A Cardiovascular Safety Evaluation of Orally Administered A-1043422 (ABT-450) in Beagle Dogs

The same six male beagle dogs were administered vehicle control or vehicle plus ABT-450 orally via capsules at doses of 10, 30, and 100 mg, with a 4 to 7 day washout period between each treatment. Animals were surgically instrumented with radio telemetry transmitters for measurement of body temperature, blood pressure, heart rate, and the electrocardiogram (ECG).

The 100 mg/kg dose was associated with plasma levels of 96.9 ± 22.5 µg/mL.

Sporadic statistically significant effects on body temperature, hemodynamics and ECG parameters did not show dose-dependency.

Pulmonary effects:

Study no. R&D/08/1204: A Respiratory Safety Evaluation of Orally Administered A-1043422 (ABT-450) and A-84538 (Ritonavir) in Rats

ABT-450 (A-1043422) with ritonavir (A-84538) was administered via oral gavage at a dose volume of 2 mL/kg according to the schedule below (*excerpted from sponsor*).

Experimental Design			
	Dose	Number of Animals	
Group Number	(mg A-1043422.0/ A-84538.0 kg)	Respiratory Evaluations	Plasma Evaluations
1	0	8	0
2	50/15	8	3
3	150/15	8	3
4	500/15	8	3

Respiratory function (respiratory rate, tidal volume, and minute volume) was monitored for approximately 1 hour prior to dosing (Day 1) to establish baseline and for approximately 4 hours post-dose. Blood samples were collected at approximately 3 hours (±10 minutes) post-dose for plasma analysis of test item.

No dose-related effects on respiratory parameters were noted.

500 mg/kg ABT-450, co-dosed with 15 mg RTV was associated with peak ABT-450 plasma levels of 0.705 ± 0.0780 µg/mL

Gastrointestinal effects:

Study no. R&D/08/1535: Effects on Ferret Emetic Liability and Rat Gastrointestinal Transit

Ferrets were administered ABT-450 at doses of 7.5, 25, and 75 mg/kg. After dosing, the number of emetic episodes and the presence of behaviors believed to correlate with nausea in ferrets were recorded for each animal over a period of one hundred eighty minutes.

Oral administration of ABT-450 at doses of 7.5, 25 and 75 mg/kg produced maximum mean plasma concentrations of 0.13 ± 0.04, 0.56 ± 0.14, and 8.15 ± 4.47 µg/mL, respectively, measured in samples obtained one hundred eighty minutes (Tmax) after dosing. The incidence of emesis was 0% (0/5) at 7.5 mg/kg, 0% (0/6) at 25 mg/kg and 17% (1/6) at 75 mg/kg.

Rats were administered ABT-450 at doses of 30,100 and 300 mg/kg via oral gavage.

Reference ID: 3628623

Oral administration of ABT-450 at doses of 30, 100 and 300 mg/kg produced peak mean ± SEM plasma concentrations of 0.042 ± 0.010, 0.196 ± 0.041, and 0.235 ± 0.082 µg/mL, respectively, 1.75 hours after dosing. ABT-450 had no significant effects on GI transit at any dose tested.

Pharmacokinetics/ADME/Toxicokinetics

PK/ADME

Pharmacokinetics

Plasma elimination half-lives after intravenous dosing with ABT-450 ranged from 0.4 hours in rat and monkey to 1.2 hours in dog. Volumes of distribution were low to moderate in all species, with values ranging from 0.15 L/kg in dog to 0.98 L/kg in rat. Plasma clearance values were high in rat (3.0 L/hr•kg) and monkey (1.9 L/hr•kg), but lower in dog (0.11 L/hr•kg). Clearance values were lower when ABT-450 was co-administered with ritonavir.

Pharmacokinetic parameters for ABT-450 are presented in the tables below. In general, ABT-450 has low bioavailability following oral administration in rats and monkeys. In dogs, oral bioavailability was calculated to be approximately 40% (see table below *(excerpted from sponsor)*.

Reference ID: 3628623

Intravenous Dose

Species	Dose (mg/kg)	$t_{1/2}$° (hr)	V_c (L/kg)	V_{ss} (L/kg)	V_p (L/kg)	$AUC_{0-\infty}$ (µg•hr/ml)	CL_p (L/hr•kg)	n
Rat	5	0.4	0.54	0.98	1.71	1.9 (0.8)	3.0 (1.1)	3
Monkey	2.5	0.4	0.25	0.52	1.24	1.4 (0.4)	1.9 (0.5)	3
Dog	2.5	1.2	0.10	0.15	0.19	22.8 (1.7)	0.1 (0.0)	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
Rat	5	-.-	0.00 (0.00)	-.- (-.-)	0.0 (0.0)	0.0 (0.0)	3
	5/5	-.-	0.03 (0.04)	1.3 (2.3)	0.1 (0.2)		3
Monkey	10	-.-	0.00 (0.00)	-.- (-.-)	0.0 (0.0)	0.0 (0.0)	3
	10/10	1.8	0.05 (0.05)	3.0 (1.4)	0.3 (0.2)		3
Dog	5	0.9	6.32 (1.81)	3.3 (0.6)	18.7 (1.6)	40.9 (3.4)	3
	5/5	1.8	22.68 (1.36)	3.7 (0.6)	84.8 (14.1)		3
	2.5/5[g]	5.0	2.56 (1.64)	0.8 (0.3)	7.9 (4.3)		3
	2.5/5[h]	2.2	15.11 (6.12)	3.7 (0.6)	51.0 (28.8)		3
	25/50 mg[a]	2.4	1.86 (1.34)	1.8 (0.8)	9.5 (7.4)		6
	25/50 mg[b]	2.5	3.45 (1.40)	1.4 (0.9)	19.4 (12.3)		6
	50/50 mg[c]	2.9	49.39 (11.39)	2.0 0.0	131.5 (31.4)		3
	50/50 mg[d]	3.0	4.87 (3.72)	2.7 (1.2)	10.6 (7.6)		3
	50/50 mg[e]	2.4	34.42 (14.02)	2.2 (0.8)	174.3 (77.6)		3
	50/50 mg[f]	2.1	33.28 (25.49)	3.0 (0.0)	134.8 (67.9)		3

Data provided as mean (SD); ° harmonic mean; dose in mg eq acid/kg A-1043422/ritonavir.

a. Suspension of the free acid administered to fasted dogs (food ~12 hr after dosing)

b. Suspension of the sodium salt administered to fasted dogs (food ~12 hr after dosing)

c. FIH lipid formulation of the sodium salt administered to fasted dogs (food ~4 hr after dosing)

d. Solid dosage formulation of the sodium salt administered to fasted dogs (food ~4 hr after dosing)

e. FIH lipid formulation of the free acid administered as 1 x 50 mg capsules to fasted dogs (food ~4 hr after dosing)

f. FIH lipid formulation of the free acid administered as ten 5 x 10 mg capsules to fasted dogs (food ~4 hr after dosing)

g. Oleic acid formulation of the free acid administered to non-fasted dogs

h. Oleic acid formulation of the free acid administered to fasted dogs (food ~4 hr after dosing)

In clinical development, ABT-450 will be co-administered with ritonavir to boost systemic exposures. Therefore, nonclinical studies have been performed in rats and dogs with varying ABT-450/ritonavir dose levels to determine pharmacokinetics and toxicity. Pharmacokinetic parameters are presented below. Of note are the low systemic exposures in rats, even with ritonavir boosting, and the variability in exposure concentrations observed in dogs.

Reference ID: 3628623

In mice, the highest AUC values obtained in multiple dose studies were with the single daily 300/30 mg/kg dose (541 µg•hr/mL).

In rats, at the limits of solubility and the maximum tolerated dose volume of 10 ml/kg, maximal exposures were obtained with a 1200/100 mg/kg A-1043422/ritonavir dose, which provided an A-1043422 AUC of 1012 µg•hr/ml and a ritonavir AUC of 25.8 µg•hr/ml. Pharmacokinetic parameters following multiple oral doses are presented in the table below (*excerpted from sponsor*).

Table: Pharmacokinetics of ABT-450 (A-1043422) and Ritonavir after Multiple Oral Co-dosing in Rat (*table excerpted from sponsor).*

A-1043422 Pharmacokinetics

Dose	Day	C_{max}	C_{max}/D	T_{max}	AUC	AUC/D
100	1	6.7 (3.6)	0.067 (0.036)	5.4 (2.4)	37.5 (20.1)	0.38 (0.20)
	28	5.5 (5.2)	0.055 (0.052)	4.0 (1.8)	30.9 (34.6)	0.31 (0.35)
300	1	6.6 (2.7)	0.022 (0.009)	4.8 (2.1)	41.3 (15.6)	0.14 (0.05)
	28	8.5 (8.2)	0.028 (0.027)	2.2 (1.0)	36.7 (29.0)	0.12 (0.10)
600	1	7.2 (3.6)	0.012 (0.006)	8.1 (6.0)	56.2 (26.8)	0.09 (0.04)
560	8	3.6 (2.4)	0.007 (0.005)	3.0 (1.8)	15.0 (8.7)	0.03 (0.02)
	28	6.3 (3.8)	0.012 (0.007)	2.9 (1.4)	31.0 (18.2)	0.06 (0.03)

Data provided as Mean (SD, n=10 per group); Units: dose (mg base/kg/day); C_{max} (µg/mL); C_{max}/D (µg/mL per mg/kg); T_{max} (hr); AUC (µg•hr/mL); AUC/D (µg•hr/mL per mg/kg).

Ritonavir Pharmacokinetics

Dose	Day	C_{max}	C_{max}/D	T_{max}	AUC	AUC/D
15	1	0.7 (0.3)	0.044 (0.018)	7.5 (2.1)	4.7 (2.5)	0.31 (0.17)
	28	1.7 (0.6)	0.111 (0.042)	7.2 (2.1)	12.8 (5.8)	0.85 (0.39)
15	1	0.4 (0.2)	0.025 (0.016)	7.8 (2.1)	2.9 (2.0)	0.19 (0.13)
	28	1.4 (0.6)	0.092 (0.040)	6.6 (1.9)	11.4 (3.2)	0.76 (0.21)
15	1	0.2 (0.1)	0.011 (0.008)	11.4 (4.6)	1.5 (1.1)	0.10 (0.07)
	8	1.0 (0.6)	0.069 (0.038)	6.6 (1.9)	7.4 (4.1)	0.49 (0.28)
	28	1.4 (0.9)	0.092 (0.060)	7.2 (1.5)	10.7 (7.2)	0.71 (0.48)

Data provided as Mean (SD, n=10 per group); Units: dose (mg base/kg/day); C_{max} (µg/mL); C_{max}/D (µg/mL per mg/kg); T_{max} (hr); AUC (µg•hr/mL); AUC/D (µg•hr/mL per mg/kg).

In rabbits, parenteral administration (intravenous, subcutaneous, intraperitoneal) did not provide tolerated options for multiple dosing nor decrease the observed variability. A−1043422 AUC values following oral co-dosing with ritonavir averaged only 3.92 µg•hr/mL, a value less than the 1x target from the clinical studies (6.5 µg•hr/mL from 200/100 mg doses of A−1043422/ritonavir).

In dogs, following a single 10/5, 30/15 or 50/25 mg/kg oral dose (A-1043422/ritonavir), peak plasma concentrations averaged 35.1/6.7, 76.1/15.1 or 74.5/14.8 µg/mL for A-1043422/ritonavir, respectively. A-1043422/ritonavir AUC values averaged 194.3/20.6, 748.8/59.4 or 648.2/60.8 µg•hr/mL, in the same treatment groups. Pharmacokinetic parameters following multiple oral doses are presented in the table below (*excerpted from sponsor*).

Table: Pharmacokinetics of ABT-450 (A-1043422) and Ritonavir after Multiple Oral Co-dosing in Dog (table excerpted from sponsor).

A-1043422 Pharmacokinetics

Dose	Day	C_{max}	C_{max}/D	T_{max}	AUC	AUC/D
5	1	20.7 (6.4)	4.1 (1.3)	3.0 (0.0)	90.4 (15.8)	18.1 (3.2)
	28	21.6 (8.9)	4.3 (1.8)	3.3 (0.9)	88.1 (32.3)	17.6 (6.5)
10	1	41.2 (19.8)	4.1 (2.0)	3.1 (1.2)	214.0 (115.0)	21.4 (11.5)
	28	48.4 (12.0)	4.8 (1.2)	3.3 (0.9)	264.0 (47.6)	26.4 (4.8)
20	1	72.7 (33.2)	3.6 (1.7)	4.2 (1.5)	540.0 (275.0)	27.0 (13.7)
	28	91.8 (27.5)	4.6 (1.4)	3.3 (0.9)	645.0 (260.0)	32.2 (13.0)

Data provided as Mean (SD, n=10 per group); Units: dose (mg base/kg/day); C_{max} (µg/mL); C_{max}/D (µg/mL per mg/kg); T_{max} (hr); AUC (µg•hr/mL); AUC/D (µg•hr/mL per mg/kg).

Ritonavir Pharmacokinetics

Dose	Day	C_{max}	C_{max}/D	T_{max}	AUC	AUC/D
5	1	3.3 (1.3)	0.65 (0.25)	3.0 (0.0)	9.0 (2.9)	1.80 (0.58)
	28	4.4 (2.4)	0.88 (0.48)	3.1 (1.2)	12.8 (7.3)	2.56 (1.46)
5	1	2.4 (1.6)	0.47 (0.32)	2.8 (0.6)	6.9 (4.9)	1.38 (0.97)
	28	4.0 (1.6)	0.81 (0.32)	3.0 (0.0)	12.4 (4.4)	2.49 (0.88)
10	1	5.8 (5.0)	0.58 (0.50)	2.8 (0.6)	20.6 (17.0)	2.06 (1.70)
	28	9.5 (5.2)	0.95 (0.52)	3.0 (0.0)	32.5 (19.1)	3.25 (1.91)

Data provided as Mean (SD, n=10 per group); Units: dose (mg base/kg/day); C_{max} (µg/mL); C_{max}/D (µg/mL per mg/kg); T_{max} (hr); AUC (µg•hr/mL); AUC/D (µg•hr/mL per mg/kg).

In monkeys, ABT-450 peak plasma concentrations following a single 30/15 or 50/25 mg/kg oral dose of ABT-450/ritonavir averaged 0.4 or 1.4 µg/mL, respectively, with AUC0-t values of 2.0 or 8.0 µg•hr/mL. Following multiple daily dosing of 50/25 mg/kg ABT-450/ritonavir in a separate group of monkeys, ABT-450 AUC values were lower following the fourth dose at 3.6 µg•hr/mL compared to 8.0 µg•hr/mL following a single dose. Multiple daily doses of 100/25 mg/kg provided higher mean AUC values, with a high degree of animal to animal variability (20.2 µg•hr/mL; range 3.0-45.9 µg•hr/mL).

Absorption

In vitro assays with heterogeneous human epithelial colorectal cells (Caco-2) indicated that ABT-450 could potentially be well absorbed following an oral dose. Permeability values ranged from 6.7–9.8 x 10-6 cm/sec at concentrations ≤10 µM and are in the moderate permeability range characteristic of compounds showing <70% absorption in humans.

Studies conducted in mouse, rat and dog demonstrated poor oral bioavailability from aqueous suspensions, with minimal improvements (b) (4) in most species. In early studies (b) (4), A-1043422 oral bioavailability values ranged from 0% in rat and monkey to 40.9% in dog. Co-administration with ritonavir increased plasma exposures of ABT-450 up to five fold following oral co-dosing in all species. (b) (4) Also in dogs, plasma AUC values were greater than six fold higher in fasted compared with fed animals.

Distribution

There was no species difference observed in plasma protein binding between human, rat and monkey and mouse. However species difference were observed between human and dog; and also between dog and other species tested. The compound was highly protein bound (>96%) for all species tested. In humans with hepatic or renal impairment, protein binding was not significantly different.

ABT-450 does not partition preferentially into the cellular compartment in any of the species studied.

Reference ID: 3628623

In dog, liver to plasma ratios ranged from 5.6:1 to 19.1:1 over a range of doses and time. The apparent elimination half-life in liver was comparable to the plasma.

Following oral administration of radiolabelled ABT-450 (without ritonavir) to Sprague-Dawley rats, highest concentrations of drug-related material were in small intestine at 1 hr (likely representing unabsorbed drug). Cmax for most tissues was 1 or 3 hours post dose. Tissue-to-plasma (t/p) ratios decreased markedly by six hours and there was no apparent retention or accumulation of [3H] ABT-450 or metabolites in tissues (see able below, excerpted from sponsor).

Table: ABT-450 tissue distribution in rats following oral administration (20 mg/kg; *table excerpted from sponsor*)

	Mean Concentration (µg eq./g) (n = 3)				Mean Tissue to Plasma Ratio (T/P) (n = 3)			
	1hr	3hr	6hr	24hr	T/P @1h	T/P @3h	T/P @6h	T/P @24h
Bone-Tibia	0.60	0.91	0.11	0.01	8.51	4.83	1.56	6.71
Pigmented Skin	0.11	0.04	0.02	0.01	1.32	0.32	0.28	20.0
Eyes	0.07	0.02	0.02	0.01	0.82	0.18	0.21	22.0
Brain	0.04	0.17	0.08	0.04	0.47	1.66	1.00	73.7
White Fat	1.25	0.07	0.17	0.05	17.8	0.47	2.18	69.4
Muscle-Skeletal	0.02	0.12	0.01	0.00	0.19	0.88	0.16	7.55
Heart	0.86	0.37	0.26	0.17	9.12	3.46	3.38	260
Lungs	19.1	0.24	0.17	0.10	240	2.45	2.05	122
Spleen	0.18	0.09	0.10	0.25	2.28	0.57	0.94	358
Liver	23.0	53.2	23.1	0.20	283	410	270	299
Kidneys	0.76	0.48	0.18	0.05	10.6	3.37	2.15	68.0
Small Intestine	49.8	96.4	28.3	0.64	643	827	335	875
Bone	0.28	0.18	0.02	0.02	3.54	0.98	0.21	29.3
Plasma	0.08	0.14	0.08	0.00	1.00	1.00	1.00	1.00

Following oral co-administration of ^{14}C-ABT-450 dihydrate and ritonavir to male Long Evans rats, radioactivity was poorly distributed to tissues of the pigmented rat. ^{14}C-ABT-450-derived radioactivity distributed to a limited number of tissues, with most of the radioactivity remaining within the GI contents throughout the course of this study. Maximum concentrations of radioactivity were observed at 4 hours postdose in blood, urine, and most tissues that had measurable levels of radioactivity. Levels of radioactivity in tissues declined rapidly after reaching C_{max} levels, and by 24 hours postdose, concentrations in all tissues were below measurable levels, except for cecum, liver, small intestine, urinary bladder, and urine. At 48 hours postdose, low concentrations of radioactivity remained only in liver (see table below, excerpted from sponsor).

Reference ID: 3628623

Concentrations of radioactivity in blood, plasma, and tissues as determined by quantitative whole-body autoradiography at specified times following a single oral administration of ^{14}C-ABT-450 and Ritonavir (Group 1, 30 and 15 mg/kg, respectively) or ABT-450 and Ritonavir (Group 2, 30 and 15 mg/kg, respectively) to male rats

	Concentration (ng Equivalents ^{14}C-ABT-450/g)										
	Animal Number (Sacrifice Time, Hours)										
	Group 1										Group 2
Tissue or Matrix	B30529 (0.5)	B30530 (1)	B30531 (2)	B30532 (4)	B30533 (8)	B30534 (24)	B30535 (48)	B30536 (96)	B30537 (168)	B30538 (192)	B30541 (24)
Adrenal gland(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Arterial wall	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	BLQ	444	607	1620	403	ND	ND	ND	ND	ND	ND
Bone	ND	ND	ND	BLQ	ND	ND	ND	ND	ND	ND	ND
Bone marrow	ND	ND	ND	468	ND	ND	ND	ND	ND	ND	ND
Brain cerebellum	ND	ND	ND	BLQ	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	ND	ND	ND	BLQ	ND	ND	ND	ND	ND	ND	ND
Brain medulla	ND	ND	ND	BLQ	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	ND	ND	ND	BLQ	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cecum	ND	2340	2850	3970	5640	1380	BLQ	ND	ND	ND	ND
Diaphragm	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Epididymis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Esophagus	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Exorbital lacrimal gland	ND	ND	ND	756	BLQ	ND	ND	ND	ND	ND	ND
Eye lens	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

BLQ Below the limit of quantitation (<278 ng equivalents ^{14}C-ABT-450/g).
ND Not detectable (sample shape not discernable from background or surrounding tissue).

In pregnant Sprague-Dawley rats, ^{14}C-ABT-450-derived radioactivity was secreted into milk for at least 24 hours postdose. Placental transfer of radioactivity was minimal, resulting in limited exposure to only the fetal liver.

Metabolism

ABT-450 showed no significant inhibition of CYP 1A2, 2B6, 2C9, 2C19, 2D6 or 3A4, but was a weak competitive inhibitor of CYP2C8 with an IC50 value of 13 μM. ABT-450 was a weak CYP3A4 time-dependent inhibitor in human liver microsomes.

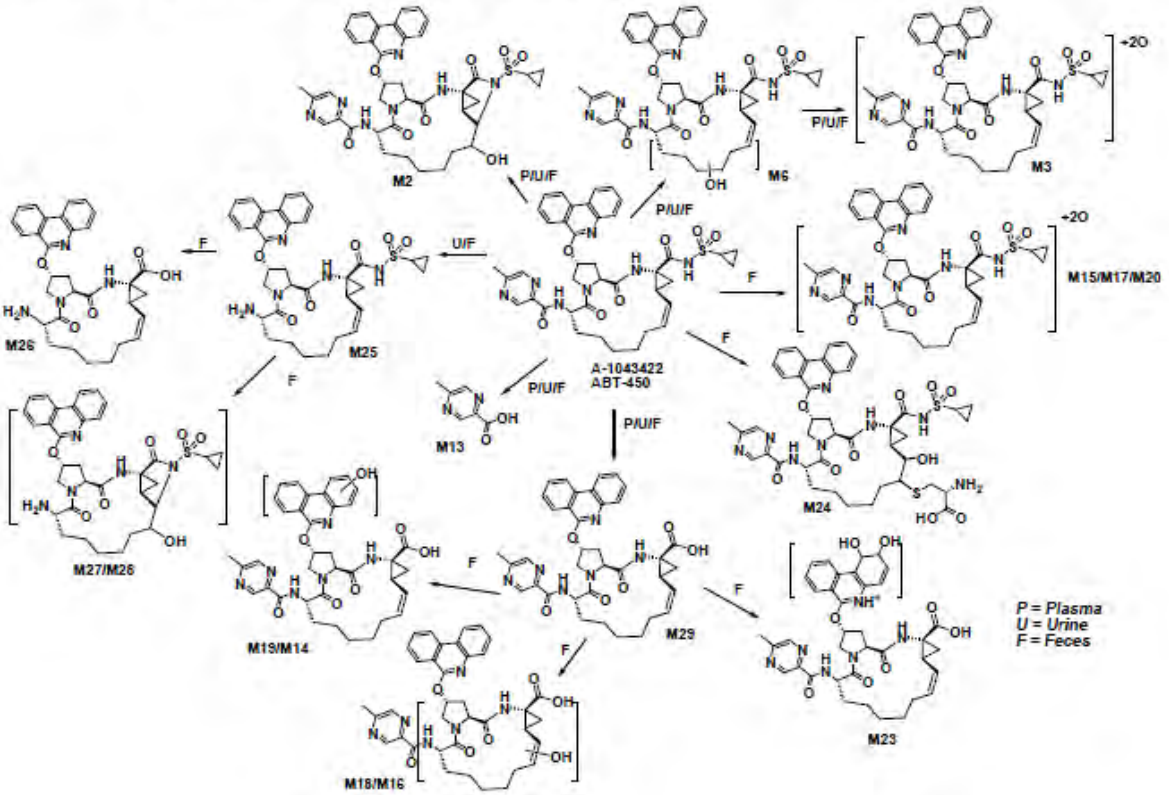
ABT-450 did not increase CYP1A2 and 2B6 mRNA expression up to 10 µM in the three donors. ABT-450 induced CYP3A4 mRNA at 10 µM with an average fold induction of 15 ±13 over vehicle control in three donors (5.4-, 11-, and 29-fold in each donor), which was about 31 ± 1.4% of rifampicin induction response, and therefore considered significant.

ABT-450 inhibited UGT1A1 with an IC50 of 3.62 µM.

The metabolic stability of [^{3}H]ABT-450 was examined in liver microsomes across species. Microsomal intrinsic clearance of ABT-450 was relatively high in monkey (94 µl/min/mg) and human (88 µl/min/mg), followed by rat (50 µl/min/mg) and dog (31 µl/min/mg). Identification of enzymes involved in metabolism of ABT-450 using recombinant human CYP enzymes (rhCYP) showed measurable metabolism primarily by CYP3A4/5.

The excretion and metabolism of A-1043422 (ABT-450) was studied in four healthy adult male subjects following a single 200 mg (100 µCi) oral dose of [14C]A-1043422, co-administered with 100 mg ritonavir. A mean of 87.8% (± 8.58%) of the administered radioactivity was excreted in feces up to 192 hours post-dose; 8.76% (± 8.69%) was excreted in urine. Radioactivity recovery in individual subjects ranged from 96.0% to 96.9%. Parent drug was the major component of drug related radiochemical material in plasma, with AUC0-∞ value of 4682 ng-eq•hr/g. Five metabolites were identified in plasma, including M2 (A-1231059); M29 (a hydrolysis product with a loss of cyclopropanesulfonamide); M3 (di-hydroxyl); M13 (a hydrolysis product, 5-methylpyrazine-2- carboxylic acid) and M6 (mono-hydroxyl). The main plasma metabolites were M2 and M29, with AUC0-∞ values of 391 and 175 ng-eq•hr/g, respectively. M2 represented ~5.6% of total drug related material AUC0-∞, and M29 represented about 2.5% of total AUC0-∞. The AUC0-t of M2 and M29 were about 7.8% and 3.2% of the total radiochemical AUC0-t, respectively. Metabolites M3, M13 and M6 were also detected at trace levels. In comparison to preclinical data, the metabolites identified in plasma in this study, including M2, M3, M6 and M29 were also observed in the preclinical safety species (rat, mouse, dog).

Proposed Metabolic Pathway for A-1043422 in Human



Seven oxidative metabolites were identified in mouse plasma (M1, M2, M3, M5, M6, M8 and M12). In the absence of ritonavir, the major metabolites in mouse plasma were M1, M2 and M3, representing an estimated 4%, 8% and 1%, respectively, exposure relative to parent drug; other metabolites were less than 1% exposure. When A−1043422 was co-administrated with ritonavir, metabolite concentrations were decreased >10-fold such that M1 and M2 were 0.2% or less of parent drug exposure.

Treatment of rats with A-1043422 in combination with ritonavir (TA09-018A) showed only one metabolite, M1, which was present at about 0.1% of parent drug exposure.

The metabolism and excretion of [^{14}C]A-1043422 was studied in male beagle dogs given a single 1 mg/kg oral dose, co-dosed with 5 mg/kg ritonavir (RTV). Mean total recovery of radioactivity from male dogs was 95.2 ± 2.3 % up to 72 hours post dose. Drug-related radioactivity was mainly recovered in feces, constituting 87.7 ± 2.7 % of the dose. Renal elimination was relatively minor (5.7 ± 1.7 % of dose). Unchanged parent drug A-1043422 was the major components identified in dog plasma, representing ~96.7% of total radioactivity in plasma. In addition to unchanged parent drug, hydrolysis metabolite M13 represented about 3.3% of total plasma radioactivity. In dog feces, unchanged parent drug was the major component. Oxidative metabolites M1, M2, and hydrolysis products M25 (no C-14 label) and M29 were detected in feces. In urine, M13 was detected.

Transporters

ABT-450 has been shown to inhibit several hepatic transporters (OATP1B1, OATP2B1, OATP1B3, MRP2 and Bile Salt Export Pump), renal transporters (OAT1, OAT3, and MATE2K (multidrug and toxin extrusion protein 2)), the primarily intestinal transporter BCRP, and the multi-tissue (hepatic, renal, intestinal) transporter P-gp.

ABT-450 is also a substrate for OATP1B1 and OATP1B3, as well as P-gp and BCRP.

The pharmacokinetics of ABT-450 were characterized following intravenous dosing in wild type or transporter gene knockout mice strains. Results suggest that multiple efflux transporters may contribute to the elimination of ABT-450 in mice. Changes in both plasma and liver AUC (2-3 fold) were observed in Mdr1a/b-Bcrp KO mice suggesting combined contribution from multiple efflux transporters to the elimination of ABT-450 in mice. These effects tended to diminish at a higher dose (1 mg/kg), reflecting the saturable nature of the active transport process. Mrp2 also appears to make some contribution to the elimination of ABT-450 in mice (<2 fold increase in liver AUC in Mrp2 KO mice) following intravenous dose at 0.1 mg/kg.

In contrast, uptake transporters like OATPs affect the distribution behavior of ABT-450 between plasma and liver, rather than the elimination process. Therefore, even though the liver:plasma AUC ratio decreased from greater than 300-fold in wild type FVB mice to less than 50-fold in Oatp1a/1b cluster KO mice, liver exposure of ABT-450 remained largely unchanged, supporting the conclusion that uptake transporters only affect the distribution behavior of ABT-450 between plasma and liver without affecting the liver exposure of ABT-450 in general.

General Toxicology

Single-Dose Toxicity

Study no. R&D/08/1425: One-Day Oral Toxicity Study of A-1043422 in Sprague-Dawley Rats with a Two-Week Recovery Period.

Reference ID: 3628623

Species/strain: Rats / Sprague-Dawley [Crl: CD® (SD)]
Number/sex: see table below (*excerpted from sponsor*)
Age: nine to ten weeks old at the start of the dosing period.
Weight: 282 to 355 g, and 193 to 238 g, in males and females, respectively.

Test Group	Test Material	Dosage (mg/kg)	Concentration (mg/ml)	Number of Rats[d]	
				Males	Females
1 (control)	Vehicle[a]	0	0[c]	5 (5)	5 (5)
2 (low)	A-1043422[b]	50	16.67	5 (5)	5 (5)
3 (mid)	A-1043422[b]	300	100	5 (5)	5 (5)
4 (high)	A-1043422[b]	600	200	5 (5)	5 (5)

a. Vehicle: Cremophor EL:PEG 400:Oleic acid (10:10:80, w/w/w)
b. A-1043422 free acid (A-1043422.0), Lot: 65801PP00; potency: 961 mcg A-1043422.0/mg of drug substance.
c. The dose volume was 3 mL/kg.
d. The last five rats, as numbered sequentially, per sex in Groups 1 through 4 (within parentheses) were designated recovery rats.

There were no statistically significant effects of ABT-450 on evaluated parameters, although apparent increases in alkaline phosphatase levels (all doses on day 2) and triglyceride (mid-dose and high-dose, day 2) were seen in males.

Results of toxicokinetic analyses following ABT-450 administration suggest that maximum systemic exposure was reached following the 300 mg/kg dose in males, but not females (see table below, *excerpted from sponsor*).

Mean Toxicokinetic Parameters for A-1043422

Collection Interval	Sex	A-1043422 Dosage (mg/kg/day) 50	300	600
		Mean Plasma AUC_{24} (µg•hr/ml) *		
Day 1	Males	5.09	14.8	8.89
	Females	13.6	38.4	62.6
		Mean Plasma C_{max} (µg/ml)		
Day 1	Males	0.538	3.98	1.82
	Females	3.29	5.66	27.9

* AUC calculated as AUC_{0-24}.

Study no. R&D/08/1426: Single Dose Oral Capsule Toxicokinetic Study of A-1043422 in Beagle Dogs with a Two-Week Recovery Period

Species/strain: Dogs/beagle
Number/sex: see table below (*excerpted from sponsor*)
Age: 10 months
Weight: 5.3 – 9.0 kg

Test Group	Dosage		Concentration (mg/ml) [b]	Number of Dogs	
	Test Material	(mg/kg)[a]		Males	Females
1 (control)	0[c]	0	0	3	3
2 (low dose)	A-1043422[d]	10	20	3	3
3 (mid dose)	A-1043422	30	60	3	3
4 (high dose)	A-1043422	100	200	3	3

a. Administered as liquid formulation placed in capsule immediately prior to dosing.
b. Dose volume: 0.5 mL/kg.
c. Cremophor EL:PEG 400:Oleic acid (10:10:80, w/w).
d. A-1043422 free acid (A-1043422.0), Lot 65801PP00; potency 961 µg A-1043422.0/mg of drug substance.

In early dose selection studies, peak plasma concentrations of ABT-450 did not increase when the dose administered to dogs was raised from 100 to 300 mg/kg. Given that result, it was concluded that 100 mg/kg was the dose at which maximum blood levels of ABT-450 can be achieved in dogs.

The plasma concentration data suggest that the systemic exposure, as characterized by plasma AUC0-24 and Cmax, was similar for both male and female dogs. The estimated mean (male and female) AUCs on Day 1 were 40.2, 117 and 285 µg•hr/mL in the low, middle and high dose groups respectively were approximately dose proportional given the individual variability between dogs. The average T_{max} occurred approximately three hours post dosing (see table below, *excerpted from sponsor*).

Collection Interval	A-1043422 Dosage (mg/kg/day)			
	Sex	10	30	100
		Mean Plasma AUC_{24} (µg•hr/ml) *		
Day 1	Males	44.7 ± 13.7	98.0 ± 10.4	243 ± 154
	Females	35.8 ± 10.5	136 ± 63.5	328 ± 57.1
	Overall	40.2 ± 12.0	117 ± 45.6	285 ± 114
		Mean Plasma C_{max} (µg/ml)		
Day 1	Males	11.0 ± 3.08	28.2 ± 3.71	48.1 ± 29.2
	Females	8.11 ± 2.63	31.6 ± 16.3	74.4 ± 14.7
	Overall	9.54 ± 3.00	29.9 ± 10.7	61.3 ± 25.2

AUC calculated as AUC_{0-24}.

There were no statistically significant effects of ABT-450 on evaluated parameters.

Repeat-Dose Toxicity

Non-pivotal studies (two dose range-finding studies and an oral tolerability study of the formulation vehicle) are briefly summarized below:

Study RD091295. 1-week oral dose range-finding study with A-1043422 and A-84538 in model 001178-w (wild type) CByB6F1-Tg(HRAS)2Jic Mice TD09-193

Male and female CByB6F1-Tg(HRAS)2Jic (Model 001178-W [wild type]) mice were assigned to four groups, and doses were administered once daily via oral gavage as indicated in the following table.

Reference ID: 3628623

Group[a]	Subgroup[b]	No. of Animals Male	No. of Animals Female	Dose Level A-1043422/RTV[c] (mg/kg/day)	Dose Concentration A-1043422/RTV[d] (mg/mL)
1 (Control)	1 (Toxicity)	5	5	0	0
2 (Low)	1 (Toxicity)	5	5	100/30	50/15
	2 (Toxicokinetic)	23	23	100/30	50/15
3 (Mid)	1 (Toxicity)	5	5	300/30	150/15
	2 (Toxicokinetic)	23	23	300/30	150/15
4 (High)	1 (Toxicity)	5	5	450/30	225/15
	2 (Toxicokinetic)	23	23	450/30	225/15

a Group 1 received vehicle control article (Cremophor EL:PEG [Polyethylene glycol] 400:oleic acid [10:10:80, w/w/w]) only.
b Toxicokinetic animals were included solely for the purpose of blood sample collections. Two animals/sex/group were included to serve as replacement animals but were not used.
c A-1043422 and RTV were co-formulated and dosed at a volume of 2 mL/kg.
d Concentrations were corrected for lot specific purity. Correction factors of 1.042 for A-1043422 (ABT-450) and 1.006 for RTV were used.

Assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, and clinical and limited anatomic pathology. Blood samples were collected for toxicokinetic evaluations.

Exposures were similar for the two highest dose groups (450/30 and 300/30 mg/kg/day) (see table below, excerpted from sponsor).

Mean Toxicokinetic Parameters for A-1043422

Dose A-1043422/A-84538 (mg/kg/day)	Day 7 C_{max} (µg/mL)	Day 7 T_{max} (hr)	Day 7 AUC (µg•hr/mL)
100/30	26.3	3.0	157
300/30	58.4	6.0	346
450/30	77.0	6.0	370

A-1043422/RTV in Cremophor EL:PEG 400:oleic acid (10:10:80, w/w/w) in CByB6F1-Tg(HRAS)2Jic (wild type) mice for 7 days was well-tolerated at dose levels up to 450/30 mg/kg/day. The no observed adverse effect level (NOAEL) is 450/30 mg/kg/day, corresponding to a Day 7 mean Cmax of 77 µg/mL and AUC0-24 of 370 µg·hr/mL for A-1043422.

Study RD09895. Four-Week Oral Dose Range-Finding Study with A-1043422 and A-84538 in Mice TD09-138

CD-1 mice were administered the test articles at dose levels of 100/10, 100/100, 300/30, and 300/100 mg/kg/day (A-1043422 / A-84538). Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Clinical observations were conducted twice weekly for main study animals. Body weights were measured and recorded twice weekly. Food consumption was measured and recorded twice weekly for main study animals. Ophthalmoscopic examinations were conducted pretest and prior to the scheduled terminal necropsy for main study animals. Blood samples for clinical pathology evaluations were collected from designated main study animals at the scheduled terminal necropsy. Blood samples for determination of the plasma concentrations of A-1043422 and A-84538 were collected from TK animals at designated time points on Days 1 and 28. After blood collection, the TK animals were euthanized and the carcasses were discarded. The toxicokinetic parameters were determined for the test articles. At study termination, necropsy examinations were performed, organ weights were recorded, and selected tissues were microscopically examined.

Mild increases (34 to 39%) in reticulocytes in high dose animals were not considered adverse as there were no correlative changes in RBC counts. No effects on red cell mass were noted.

Statistically significant organ weight changes were observed in the livers of males and females given 100/100, 300/30 and 300/100 mg/kg/day.

Test Article-Related Organ Weight Changes: Terminal Male and Female (Percent change relative to controls)						
Dose level (A-1043422/A-84538) mg/kg/day	100/100		300/30		300/100	
Sex	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10
Liver (g)	↑47[b]	↑45[b]	↑21[a]	↑12	↑48[b]	↑26[b]
Liver /BWt %	↑43[b]	↑35[b]	↑14[a]	↑10[a]	↑38[b]	↑22[b]

[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)
↑ - Increased. BWt – Body Weight

Increased liver weights appeared to correlate with increased dosage of A-84538 (ritonavir). Increased liver weights correlated with microscopic panlobular, hepatocellular hypertrophy in animals given 100/100 and 300/100 mg/kg/day. Hepatocellular hypertrophy was considered adaptive and not likely adverse. Presence of prominent cytoplasmic basophilic stippling appears consistent with smooth endoplasmic reticulum (SER) proliferation but could not be confirmed without electron microscopy. The hepatocellular hypertrophy was consistent with expected toxicological effects of ritonavir. The following description of ritonavir (Norvir) toxicology is from the European Medicines Agency:

"Repeated dose oral toxicity was studied in mice (with doses up to 1000 mg/kg/day), rats and dogs with treatment duration up to 6 months in rats and dogs. AUC and Cmax values were determined in all studies. The safety margin for ritonavir cannot be calculated because systemic exposure in different species, even at the highest dose was equal to or below human therapeutic exposure. In all three species the main target organs of toxicity were the liver and the eyes. The assumption that rodent liver and eye lesions (retina degeneration, retinal pigment epithelium hypertrophy) due to treatment were related to phospholipidosis (common phenomenon after administration of amphiphilic cationic compounds) was made even if several non-phospholipidosis associated lesions, in particular hepatocellular necrosis, pericholangitis and bile duct hyperplasia were observed in rodents."

Test Article-Related Liver Effects Terminal Male and Female										
Dose level (A-1043422/A-84538) mg/kg/day	0/0		100/10		100/100		300/30		300/100	
Sex	M	F	M	F	M	F	M	F	M	F
Number examined	10	10	10	10	10	10	10	10	10	10
Liver										
hypertrophy, hepatocyte, panlobular	0	0	0	0	9	8	0	0	10	2
-minimal	0	0	0	0	5	8	0	0	3	2
-mild	0	0	0	0	4	0	0	0	7	0

Mean A-1043422 and A-84538 toxicokinetic parameters are presented below (table excerpted from sponsor). Note that exposures at 300/30 were greater than exposures (AUC) at 300/100.

Reference ID: 3628623

	Day 1			Day 28		
			A-1043422			
Dose (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
100/10	20.6	3.0	106	17.8	1.0	77.4
100/100	11.5	6.0	119	21.3	1.0	109
300/30	73.9	6.0	490	66.3	6.0	484
300/100	66.0	1.0	377	34.3	3.0	218
			A-84538			
Dose (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
100/10	0.157	3.0	0.737	0.252	1.0	1.14
100/100	10.2	1.0	99.9	15.0	6.0	169
300/30	1.95	6.0	11.0	2.56	6.0	17.2
300/100	9.22	1.0	68.4	12.3	3.0	136

Based upon a lack of adverse findings at any dose level, the NOAEL was 300/30 mg/kg/day of A-1043422/A-84538, corresponding to an AUC of 484 µg·hr/mL.

Study RD091434. Four-Week Oral Tolerability Study of Cremophor EL:PEG-400:Oleic Acid (10:10:80, w/w/w) in CD-1 Mice (TD09-159)

The vehicle used in subsequent nonclinical toxicology studies, Cremophor EL:PEG-400:oleic acid (10:10:80, w/w), was tolerated by CD-1 mice when orally administered at volumes up to 10 mL/kg for four weeks. There were no deaths in this study or adverse clinical signs that were attributed to administration of Cremophor EL:PEG-400:oleic acid (10:10:80, w/w) at any volume. Body weight gain was similarly unaffected by test item administration, and there were no toxicologically significant effects on food consumption observed. There were no toxicologically relevant test item-related effects on hematology or clinical chemistry parameters, and no test item-related gross observations at necropsy.

Study title: 4-Week Oral Gavage Study with A-1 043422 and A-84538 in Model 001178-W (Wild Type) CByB6F1-Tg(HRAS)2Jic Mice

Key study findings:

- Based upon a lack of adverse findings attributed to ABT-450 administration, the NOAEL was defined as the high dose, 450/30 mg/kg/day of ABT-450/RTV. The corresponding systemic exposures (AUC) were 324 µg.hr/mL and 517 µg.hr/mL in males and females, respectively.

Study no.: Study no. R&D/10/126
Volume #, and page #: vol. 1 (SD-58; June 29, 2010)
Conducting laboratory and location: (b) (4)

Date of study initiation: 21 January 2010
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 74983PPO1, 99.7 % purity

Methods
Doses: 30/30, 100/30, 300/30, or 450/30 mg/kg ABT-450/RTV
Species/strain: Mouse/Model 0011 78-W (wild type), CByB6F1-Tg(HRAS)2Jic

Number/sex/group:	15/sex/dose
Route, formulation, volume:	Cremophor EL:PEG 400:oleic acid (10:10:80, w/w/w), 2 mL/kg/day
Age:	8.1 to 9.0 weeks
Weight:	21.3 to 30.5 g for males and 17.2 to 24.2 g for females

Results:

Mortality: Animals were checked twice daily for morbidity, mortality and/or signs of distress. Twenty-five animals (14 from toxicity groups and 11 from toxicokinetic groups) died or were sacrificed early during the study. Thirteen deaths were due to confirmed gavage error. Twelve deaths (one control-, four low-middle-, five middle-high- and two high- dose group animals) were undetermined as to cause, but aspiration of test article was considered likely. At least ten mice/sex/dose groups remained on study for four weeks.

Clinical signs: Once daily cage-side observations for clinical signs were performed during the dosing phase. Detailed observations were performed twice weekly. Noted clinical signs were related to morbidity of animals sacrificed early and were not related to ABT-450 administration.

Body weights: Body weights were recorded twice weekly during the dosing phase. There were no test article-related findings.

Food consumption: Food consumption was measured weekly during the dosing phase. There were no test article-related findings.

Clinical Pathology: Samples for hematology and clinical chemistry were collected at terminal sacrifice.

Hematology: There were no test article-related findings.

Clinical chemistry: Cholesterol was increased 20-40% across dose levels in male and female mice. Given the apparent lack of ABT-450 dose response, the finding was attributed to RTV administration. [Published studies show that in fasted mice, ritonavir increased serum glucose by 29%, cholesterol by 40%, and triglyceride by 99%.]

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

Organ weights: The following organs were weighed: brain, heart, kidney, liver with gallbladder, spleen, testis, and thymus. Liver weights were increased 15 to 26% across all doses in males and females. Due to the lack of a histopathologic correlate, the finding is not considered toxicologically significant.

Histopathology: Adequate Battery: yes
Peer review: yes

There were no significant microscopic findings.

Toxicokinetics
Samples for toxicokinetic assessment were taken prior to the start of dosing and approximately 1, 3, 6, 9, 12, and 24 hours postdose. Toxicokinetic parameters for ABT-450 and RTV are presented below (*tables excerpted from sponsor*).

Reference ID: 3628623

Mean Toxicokinetic Parameters for A-1043422

A-1043422/RTV Dose Level (mg/kg/day)	Sex	Day 28 C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24} (µg•hr/mL)
30/30	Male	6.46	3	30.0
	Female	19.9	3	72.0
	Overall	13.2	3	51.0
100/30	Male	20.9	6	126
	Female	32.4	3	136
	Overall	25.8	3	131
300/30	Male	52.8	3	249
	Female	71.1	6	478
	Overall	60.7	3	363
450/30	Male	47.3	6	324
	Female	84.6	3	517
	Overall	61.5	6	418

Mean Toxicokinetic Parameters for A-84538 (RTV)

A-1043422/RTV Dose Level (mg/kg/day)	Sex	Day 28 C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24} (µg•hr/mL)
30/30	Male	9.57	3	50.3
	Female	15.3	3	66.7
	Overall	12.4	3	58.4
100/30	Male	7.71	6	51.2
	Female	8.24	3	43.2
	Overall	7.29	3	47.2
300/30	Male	6.64	6	56.8
	Female	8.16	6	55.5
	Overall	7.40	6	56.1
450/30	Male	6.29	9	37.2
	Female	6.34	9	35.4
	Overall	6.32	9	36.3

Study title: Thirteen-week Oral Maximum Tolerated Dose Toxicity Study of A-1043422 and A-84538 in Mice

Key study findings:

- Hepatic findings were consistent with previously-seen induction of hepatic enzymes by RTV.
- Based upon a lack of adverse findings attributed to ABT-450 administration, the NOAEL was defined as the high dose, 300/30 mg/kg/day of ABT-450/RTV.
- The corresponding Day 90 systemic exposures (AUC) were 272 µg.hr/mL and 561 µg.hr/mL in males and females, respectively.

Study no.: Study no. R&D/09/1208:
Volume #, and page #: vol. 5, p.1 (SN 043)
Conducting laboratory: (b) (4)

Date of study initiation: September 16, 2009
GLP compliance: Yes
QA report: Yes

Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 74983PP01, 96% purity
ABT-538, Ritonavir, RTV; Lot No. 76164TL01; 99.4% purity

Methods

Doses: 0, 30/30, 100/30 and 300/30 mg/kg (A-1043422/ A-84538; see table below)
Species/strain: CD-I strain Cr1:CDI (j(ICR)) mice
Number/sex/group: See study design table below (excerpted from sponsor)
Route, formulation, volume: Liquid formulation in Cremophor™ EL:PEG 400:oleic acid (10: 10:80, w/w/w; 2.0 mL/kg/day
Age: ~ 6 weeks
Weight: males: 29.1 to 33.5 g
females: 23.3 to 26.6 g

Group Assignments

Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study			
1	0 A-1043422/0 A-84538	15	15
2	30 A-1043422/30 A-84538	15	15
3	100 A-1043422/30 A-84538	15	15
4	300 A-1043422/30 A-84538	15	15
Toxicokinetic			
5	0 A-1043422/0 A-84538	8[a]	8[a]
6	30 A-1043422/30 A-84538	38[a]	38[a]
7	100 A-1043422/30 A-84538	38[a]	38[a]
8	300 A-1043422/30 A-84538	38[a]	38[a]

[a]Two animals/sex included as replacement animals.

Results:

Mortality: Mice were checked twice daily for morbidity/mortality. Twenty main study and 9 toxicokinetic animals died or were sacrificed early. Eleven of the 29 deaths were from untreated control groups. In all but three cases, dosing error was suspected or confirmed histologically. In the remaining three cases, the cause of death was not determined.

Clinical signs: Mice were checked for signs of injury twice daily. Detailed clinical examinations were performed twice weekly. There were no remarkable findings.

Body weights: Body weights were recorded weekly during the study. There were no remarkable findings.

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

Ophthalmoscopic Examinations: Ophthalmoscopic examinations were conducted prior to the scheduled terminal necropsy. There were no remarkable findings.

Clinical Pathology: Samples for clinical pathology assessments were collected at the terminal necropsy.
Hematology: Slightly decreased hemoglobin in females at 30/30 and 300/30 mg/kg was not toxicologically significant.

Reference ID: 3628623

Clinical chemistry: Triglycerides were mildly to moderately increased in males and females at 30/30 mg/kg/day (45 and 41 %), 100/30 mg/kg/day (66 and 101 %), and 300/30 mg/kg/day (70 and 32%). Similarly, cholesterol was mildly increased in males and females at 30/30 mg/kg/day (15 and 23%), 100/30 mg/kg/day (13 and 34%), and 300/30 mg/kg/day (24 and 28%). The lack of dose-related increases suggests that the changes are due to the constant dose of RTV. Changes in alkaline phosphatase (ALP) activity, total bilirubin, and aspartate aminotransferase (AST) activity were not toxicologically significant.

Gross pathology: There were no remarkable findings.

Organ weights: There were no remarkable findings. It should be noted that liver weights were not recorded.

Histopathology: Adequate Battery: yes
Peer review: yes

Minimal centrilobular hypertrophy was observed in the livers of a small number of males in each test article-treated group. The hepatocellular hypertrophy was characterized by increased hepatocellular size with abundant eosinophilic cytoplasm with minimal basophilic stippling. The hepatocellular hypertrophy was considered related to administration of RTV and not directly related to ABT-450 administration.

Toxicokinetics: Samples for toxicokinetic analysis were collected from 2-3 animals/interval by cardiac puncture. Samples were collected from treated TK animals at 1, 3, 6, 9, 12, and 24 hours post-dose on Days 1 and 90. Mean toxicokinetic parameters are presented in the table below (*excerpted from sponsor*). In males, exposures decreased between Day 1 and Day 90, consistent with induction of hepatic enzymes by RTV.

Mean Toxicokinetic Parameters for A-1043422

A-1043422/A-84538 Dose Level (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 AUC (µg•hr/mL)	Day 90 C_{max} (µg/mL)	Day 90 T_{max} (hr)	Day 90 AUC (µg•hr/mL)
30/30	Male	2.98	3.0	13.8	1.45	3.0	7.19
	Female	4.49	3.0	15.4	11.3	3.0	36.6
	Overall	3.74	3.0	14.6	6.36	3.0	21.8
100/30	Male	34.0	3.0	186	40.6	3.0	177
	Female	25.6	1.0	160	31.8	3.0	285
	Overall	27.5	3.0	173	36.2	3.0	231
300/30	Male	51.5	3.0	359	43.0	3.0	272
	Female	47.9	6.0	389	87.4	3.0	561
	Overall	46.3	3.0	374	65.2	3.0	417

Study title: Study no. R&D/09/1381: 6-Month Oral Dose Toxicity Study with A-1043422 and A-84538 in Mice with a 1-Month Recovery Period

Key study findings:

- ABT-450 and ritonavir (ABT-450/r) were coformulated and administered via oral gavage to CD1 mice (40/sex/group) at dose levels of 30/30, 100/30 and 300/30 mg/kg/day ABT-450/r.
- In middle and high dose groups, microscopic findings of the gallbladder included focal erosion/ulceration, inflammation (both acute and chronic), and epithelial hypertrophy/hyperplasia in approximately 10-23% of mice. Corresponding ABT-450 exposures were approximately 220 µg*hr/mL.
- Changes in the gallbladders of the recovery animals were limited to epithelial hypertrophy/hyperplasia in one high dose male and acute inflammation in two high dose males.
- Based on the adverse histopathology effects noted in the gallbladder of the mid- and high-dose groups on this study, the No Observed Adverse Effect Level was the low-dose of 30/30 mg/kg/day of A-1043422/A-84538, corresponding to an AUC of 33.4 µg·hr/mL at Day 182.

Study no.: Study no. R&D/09/1381
Volume #, and page #: electronic (SN 088)
Conducting laboratory and location: (b) (4)

Date of study initiation: September 16, 2009
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 74983PP01, 96% purity
ABT-538, Ritonavir, RTV; Lot No. 76164TL01; 99.4% purity

Methods

Doses: 0, 30/30, 100/30 and 300/30 mg/kg (A-1043422/ A-84538; see table below)
Species/strain: CD-I strain Cr1:CDI (j(ICR)) mice
Number/sex/group: See study design table below (excerpted from sponsor)
Route, formulation, volume: Liquid formulation in Cremophor™ EL:PEG 400:oleic acid (10: 10:80, w/w/w); 2.0 mL/kg/day
Age: ~ 6 weeks
Weight: males: 27.4 to 34.4 g
females: 20.7 to 27.7 g

Group Assignments

Group Number	Dose Level (mg/kg/day (A-1043422/A-84538)	Number of Animals	
		Male	Female
	Main Study		
1[a]	0/0	50[b]	50[b]
2	30/30	40	40
3	100/30	40	40
4	300/30	50[b]	50[b]
	Toxicokinetic		
5[a]	0/0	10	10
6	30/30	48	48
7	100/30	48	48
8	300/30	48	48

[a]Animals in Groups 1 and 5 received the vehicle control article only.
[b]Seven, 9, or 10 animals/sex (10 males and 7 females for controls and 9 males and 7 females for high dose) were retained for a 4 week recovery period following 184 days of dosing.

Results:

Mortality: Mice were checked twice daily for morbidity/mortality. Sixteen main study and 13 toxicokinetic animals died or were sacrificed early. Five of the 29 deaths were from untreated control groups. In all but three cases, dosing error was suspected or confirmed histologically. In the remaining three cases, the cause of death was not determined.

Main Study Animal Mortality			
	A-1043422/A-84538	Number of Deaths	
Group	Dose Level (mg/kg/day)	Males	Females
1	0	0	3
2	30/30	2	2
3	100/30	4	1
4	300/30	1	3

Toxicokinetic Animal Mortality			
	A-1043422/A-84538	Number of Deaths	
Group	Dose Level (mg/kg/day)	Males	Females
5	0	0	2
6	30/30	3	2
7	100/30	1	2
8	300/30	0	3

Clinical signs: Mice were checked for signs of injury twice daily. Detailed clinical examinations were performed twice weekly. There were no remarkable findings.

Body weights: Body weights were recorded weekly during the study. There were no remarkable findings.

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

Ophthalmoscopic Examinations: Ophthalmoscopic examinations were conducted prior to the scheduled terminal necropsy. There were no remarkable findings.

Clinical Pathology: Samples for clinical pathology assessments were collected at the terminal necropsy.

Hematology: At recovery, neutrophils in the females at 300/30 (A-1043422/A-84538) remained higher (89%) than controls, but individual values remained within expected ranges and were not considered test article-related.

Clinical chemistry: Cholesterol was increased 23, 21, and 22% in females at 30/30 mg/kg/day (A-1043422/A-84538) 100/30 mg/kg/day (A-1043422/A-84538), and 300/30 mg/kg/day (A-1043422/A-84538) at termination. These moderate increases were not considered to be toxicologically significant.

Gross pathology: There were no remarkable findings.

Organ weights: A-1043422/A-84538-related organ weight changes were limited to the livers of terminal males and females at all dose levels. Minimal increases in absolute and relative liver weights persisted in recovery 300/30 mg/kg/day (A-1043422/A-84538) males; conversely, liver weight decreases were observed in recovery 300/30 mg/kg/day (A-1043422/30 A-84538) females. There were no microscopic correlates associated with the minimal liver weight increases at either the terminal or recovery phases. The increases in liver weights were considered to be related to RTV administration, as similar findings were noted in shorter term studies with RTV alone.

Reference ID: 3628623

Control Means (g) and Percentage Change from Control for Selected Absolute and Relative Liver Weights - Terminal								
Dose level: group	0/0		30/30		100/30		300/30	
Sex	M	F	M	F	M	F	M	F
Number Examined	40	40	37	38	35	39	40	40
Liver								
Absolute (g)	2.2	1.7	↑7.1[a]	↑5.0	↑10.4[b]	↑12.2[b]	↑7.9[a]	↑14.1[b]
Relative %	5.4	5.3	↑9.4[b]	↑5.9	↑9.2[b]	↑10.7[b]	↑7.8[b]	↑12.4[b]

Group 1 = 0/0 mg/kg/day (A-1043422/A-84538)
Group 2 = 30/30 mg/kg/day (A-1043422/A-84538)
Group 3 = 100/30 mg/kg/day (A-1043422/A-84538)
Group 4 = 300/30mg/kg/day (A-1043422/A-84538)

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

↑ - Increased
↓ - Decreased

M – Male
F - Female

Control Means (g) and Percentage Change from Control for Selected Absolute and Relative Liver Weights - Recovery				
Dose level: group	0/0		300/30	
Sex	M	F	M	F
Number Examined	10	7	9	7
Liver				
Absolute (g)	1.9	1.6	↑8.3	↓3.1
Relative %	4.7	5.1	↑8.9[a]	↓2.0

Group 1 = 0/0 mg/kg/day (A-1043422/A-84538)
Group 4 = 300/30mg/kg/day (A-1043422/A-84538)

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

↑ - Increased
↓ - Decreased

M – Male
F - Female

Histopathology: Adequate Battery: yes
Peer review: yes

Adverse A-1043422/A-84538-related microscopic findings were limited to the gallbladders of terminal males and females given 100/30 and 300/30 mg/kg/day (A-1043422/A-84538) and in recovery males and females given 300/30 mg/kg/day (A-1043422/A-84538). Some or all of the following changes were observed in the gallbladder: focal erosion/ulceration, chronic active inflammation, locally extensive gallbladder epithelial hypertrophy/hyperplasia and diffuse, acute inflammation.

Gall Bladder
Minimal to moderate mucosal erosion/ulceration was observed in 1 male and 2 females administered 100/30 mg/kg/day (A-1043422/A-84538) and 2 females administered 300/30 mg/kg/day (A-1043422/A-84538). Mucosal erosion/ulceration was characterized by focal necrosis and/or loss of the mucosal epithelial cells with penetration deeper into the underlying lamina propria, tunica muscularis and serosa. Transmural fibrosis and mixed inflammation accompanied this change. The mucosal epithelium adjacent to the mucosal erosion/ulcers was minimally to mildly hypertrophic and hyperplastic.

In a small number of animals (2 males and 2 females administered 100/30 mg/kg/day (A-1043422/A-84538) and 2 males and 2 females administered 300/30 mg/kg/day A-1043422/A-84538)) a similar change of focal minimal to mild transmural fibrosis accompanied by chronic active inflammation (consisting of neutrophils, lymphocytes, plasma cells and macrophages) were observed in the absence of mucosal erosion/ulceration. The finding of minimal to mild epithelial hypertrophy/hyperplasia was

observed in 3 males and 7 females administered 100/30 mg/kg/day (A-1043422/A-84538) and 4 males and 4 females administered 300/30 mg/kg/day (A-1043422/A-84538). This change was noted to be focal yet locally extensive and most prominent when adjacent to mucosal erosion/ulceration. Minimal to mild acute inflammation was observed in 5 male and 6 females administered 100/30 mg/kg/day (A-1043422/A-84538) and 4 males and 2 females administered 300/30 mg/kg/day (A-1043422/A-84538).

Changes in the gallbladders of the recovery animals were limited to epithelial hypertrophy/hyperplasia in 1 male and acute inflammation in 2 males administered 300/30 mg/kg/day (A-1043422/A-84538).

A-1043422/A-84538 Related Microscopic Changes - Terminal

Dose level: group	0/0		30/30		100/30		300/30	
Sex	M	F	M	F	M	F	M	F
Number Examined	40	43	39	40	40	40	41	43
Gallbladder								
erosion/ulcer, chronic active, focal	0	0	0	0	1	2	0	2
- minimal	0	0	0	0	0	1	0	0
- mild	0	0	0	0	1	1	0	1
- moderate	0	0	0	0	0	0	0	1
inflammation, chronic active, focal	0	0	0	0	2	2	2	2
- minimal	0	0	0	0	2	1	1	0
- mild	0	0	0	0	0	1	1	2
hypertrophy/hyperplasia, epithelium, locally extensive	0	0	0	0	3	7	4	4
- minimal	0	0	0	0	1	4	4	2
- mild	0	0	0	0	2	3	0	2
inflammation, acute	0	0	0	0	5	6	4	2
- minimal	0	0	0	0	4	6	3	1
- mild	0	0	0	0	1	0	1	1

Group 1 = 0/0 mg/kg/day (A-1043422/A-84538)
Group 2 = 30/30 mg/kg/day (A-1043422/A-84538)
Group 3 = 100/30 mg/kg/day (A-1043422/A-84538)
Group 4 = 300/30mg/kg/day (A-1043422/A-84538)

M - Male
F - Female

A-1043422/A-84538 Related Microscopic Changes - Recovery				
Dose level: group	0/0		300/30	
Sex	M	F	M	F
Number Examined	10	7	9	7
Gallbladder				
hypertrophy/hyperplasia, epithelium, locally extensive				
- minimal	0	0	1	0
inflammation, acute				
- minimal	0	0	2	0

Group 1 = 0/0 mg/kg/day (A-1043422/A-84538)
Group 2 = 30/30 mg/kg/day (A-1043422/A-84538)
Group 3 = 100/30 mg/kg/day (A-1043422/A-84538)
Group 4 = 300/30mg/kg/day (A-1043422/A-84538)

M - Male
F - Female

Toxicokinetics: Samples for toxicokinetic analysis were collected from 3 animals/interval by cardiac puncture. Samples were collected from animals at 0/0 mg/kg/day at 1 hour postdose and from animals at 30/30, 100/30, and 300/30 mg/kg/day (A-1043422 / A-84538) at 1, 3, 6, 9, 12, and 24 hours postdose on Days 92 and 182. Mean toxicokinetic parameters for ABT-450 are presented in the table below (*excerpted from sponsor*). In males, exposures decreased between Day 1 and Day 90, consistent with induction of hepatic enzymes by RTV.

Mean Toxicokinetic Parameters for A-1043422							
		Day 92			Day 182		
A-1043422/A-84538 Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
30/30	Male	3.49	3.0	15.5	6.90	3.0	26.0
	Female	12.8	3.0	40.3	7.49	3.0	40.9
	Overall	8.15	3.0	27.9	7.20	3.0	33.4
100/30	Male	32.1	3.0	132	45.2	3.0	162
	Female	50.1	3.0	305	48.2	1.0	278
	Overall	41.1	3.0	218	37.3	3.0	220
300/30	Male	128	1.0	402	41.4	3.0	261
	Female	86.2	1.0	370	68.4	1.0	348
	Overall	107	1.0	386	52.6	3.0	304

Study title: Four-week Oral Dose Toxicity Study of A-104322 and A-84538 (Ritonavir) in Sprague-Dawley Rats (With a Four-week Recovery Period)

Key study findings:

- Clinical signs (yellow hair staining, red hair staining and loss/thinning hair) and slight (<5%) decreases in female group mean body weights at all doses were considered to be related to administration of ABT-450/RTV, but are not considered to be adverse events.

- By Day 28 of dosing, mean systemic exposures were essentially equal across all dose groups ~30 µg.hr/mL, although within group values were highly variable.

Study no.: R&D/08/1427
Volume #, and page #: Vol. 10, p. 1
Conducting laboratory and location: Abbott Laboratories
Toxicology and Safety Pharmacology
100 Abbott Park Road,
Abbott, Park, IL
Date of study initiation: June 18, 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 65801PP00, 96.1% purity

Methods

Doses: see table below (*excerpted from sponsor*)
Species/strain: see table below (*excerpted from sponsor*)
Number/sex/group or time point (main study): see table below (*excerpted from sponsor*)
Route, formulation, volume: Gavage; 2.0 mL/kg/day
Age: 9 weeks
Weight: 192.6 – 331.6 grams
Sampling times: Samples of blood were collected from each surviving satellite rat at 1, 3, 6, 9, 12 and 24 hours (± 5 minutes to each time point) after dosing on Day 1, on Day 8 (from Group 4 satellite animals only) and Day 28

Test Group	Test Material	Dosage (mg /kg/day)	Concentration (mg /ml)	Number of Rats[g]	
				Males	Females
1 (control)	Vehicle[a]	0	0[d]	15 (5)	15 (5)
2 (low)	A-1043422[b]/A-84538[c]	100/15	50/7.5	15 (5)	15 (5)
3 (mid)	A-1043422[b]/A-84538[c]	300/15	150/7.5	15 (5)	15 (5)
4 (high)	A-1043422[b]/A-84538[c]	600/15[e] 520/15[f]	300/7.5[e] 260/7.5[f]	15 (5)	15 (5)

a. Vehicle: Cremophor EL:PEG 400:Oleic acid (10:10:80, w/w/w)
b. A-1043422 free acid (A-1043422.0), Lot number 65801PP0; potency 961 mg/g test item
c. A-84538 free acid (A-84538.0, RTV), Lot 56088TL; potency 994 mg/g of test item (see Section 16.0, Study Plan Deviation 2).
d. The dose volume was 2 mL/kg.
e. On Day 1 and 2.
f. Starting on Day 3.

Results:

Mortality: Each animal was observed at least twice each day of dosing for survival and general condition. Two rats from the 300/15 dose group were euthanized for humane reasons prior to their scheduled sacrifice. Microscopic evaluation revealed findings consistent with gavage errors.

Clinical signs: Detailed clinical observations were made between four and six hours post-dose on at least two days per week. Yellow hair staining, red hair staining and loss/thinning hair were observed in animals from all dose groups during dosing. These findings are considered to be related to administration of ABT-450/RTV, but are not considered to be adverse events.

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Body weights: Body weights were recorded twice weekly during dosing and recovery periods. Females had slight (<5%) decreases in group mean body weights at all doses. Those differences resolved by day 3 of the recovery period. Treated males tended to have higher group mean body weights (8 – 14%) than controls at the end of the recovery period, though the differences were not dose dependent. These findings were considered to be related to administration of ABT-450/RTV, but were not considered to be adverse events.

Food consumption: Food consumption was recorded twice weekly during dosing and recovery. Treated male rats tended to consume more food during the recovery period than did control males, leading to differences in body weight noted above.

Ophthalmoscopy: Ophthalmologic examinations were performed pre-dose and near the end of the dosing and recovery periods. No remarkable findings attributable to ABT-450 administration were noted.

Clinical Pathology: Blood samples for clinical pathology were collected at the end of dosing. In addition, samples for serum chemistry analysis were collected on days 10 and 21.

Hematology: No remarkable findings attributable to ABT-450 administration were noted.

Coagulation Parameters: No remarkable findings attributable to ABT-450 administration were noted.

Clinical chemistry: No remarkable findings attributable to ABT-450 administration were noted.

Urinalysis: Near the end of the dosing period and near the end of the recovery period, surviving animals were placed in metabolism cages for up to four hours for urine collection. No remarkable findings attributable to ABT-450 administration were noted.

Gross pathology: At the end of the dosing or recovery period, complete necropsies were performed on all surviving animals. No remarkable findings attributable to ABT-450 administration were noted.

Organ weights Adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus and thyroid with parathyroid glands from each rat were weighed at scheduled necropsies. No remarkable findings attributable to ABT-450 administration were noted.

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

Reference ID: 3628623

Organs Weighed and Tissues Preserved and Examined Microscopically

Tissue	Comprising[a]	Organ Weight	Microscopic Examination
Adrenal glands	Left and right	X	X
Aorta, thoracic			X
Bone	Included with left femoro-tibial joint		X
Bone marrow	Included with left femoro-tibial joint		X
Brain		X	X
Epididymides[b]	Left and right		X
Esophagus	Midsection		X
Eyes (with optic nerves)[b]	Left and right		X
Femoro-tibial joint	Left cartilage surfaces and capsule, physis		X
Gross lesions			X
Harderian gland[b]	Left and right		X
Heart		X	X
(Injection site, if parenteral route used)			X
Kidneys	Left and right	X	X
Large intestine	Colon, cecum		X
Larynx			X
Liver		X	X
Lung	Left and right caudal lobes, main bronchi		X
Lymph nodes	Left mandibular Left medial iliac Mesenteric Tracheobronchial		X X X X
Mammary gland	Left 2nd last caudal complex plus nipple		X
Nasal cavity	With turbinates		X
Nerve, peripheral	Left sciatic nerve		X
Ovaries	Left and right	X	X
Oviducts	Left and right		X
Pancreas	Left lobe of pancreas		X
Peyer's patch/GALT	Jejunum		X
Pituitary gland		X	X
Prostate gland	Ventral lobe	X	X
Salivary gland	Left mandibular Left sublingual Left parotid		X

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Tissue	Comprising[a]	Organ Weight	Microscopic Examination
Seminal vesicles	Left and right		X
Skeletal muscle	Left gastrocnemius muscle		X
Skin	Included with mammary gland		X
Small intestine	Duodenum, jejunum, ileum		X
Spinal cord	Cervical, thoracic, lumbar		X
Spleen		X	X
Stomach	Forestomach, cardia, fundus, pylorus		X
Testes[b]	Left and right	X	X
Thymus		X	X
Thyroid and parathyroid	Left and right glands	X	X
Tongue			X
Trachea	Midsection		X
Ureters	Left and right		X
Urinary bladder			X
Uterus	Corpus with cervix, left and right horns		X
Vagina			X

a. Entire organ was fixed in neutral buffered formalin, unless otherwise specified.
b. Preserved in modified Davidson's fixative.

No remarkable findings attributable to ABT-450 administration were noted.

Toxicokinetics: Systemic exposure to ABT-450 was consistent across dose groups (see table below, *excerpted from sponsor*).

Treatment Group	Dose (mg/kg/day)	Sex	Days 1 or 8[b] C_{max} (µg/mL)	T_{max} (hr)	AUC[c] (µg•hr/mL)	Day 28 C_{max} (µg/mL)	T_{max} (hr)	AUC[c] (µg•hr/mL)
2	100	male	4.69 (±1.37)	7.2 (±1.6)	29.1 (±8.01)	3.21 (±1.92)	4.4 (±2.3)	18.7 (±9.88)
		female	8.60 (±4.10)	3.6 (±1.3)	45.9 (±25.9)	7.88 (±6.61)	3.6 (±1.3)	43.1 (±47.2)
		overall	6.65 (±3.55)	5.4 (±2.4)	37.5 (±20.1)	5.54 (±5.21)	4.0 (±1.8)	30.9 (±34.6)
3	300	male	6.42 (±2.75)	6.0 (±2.1)	42.6 (±15.9)	5.03 (±3.05)	1.4 (±0.9)	28.4 (±14.0)
		female	6.74 (±3.03)	3.6 (±1.3)	39.9 (±17.2)	12.0 (±10.5)	3.0 (±0.0)	44.9 (±39.1)
		overall	6.58 (±2.73)	4.8 (±2.1)	41.3 (±15.6)	8.50 (±8.16)	2.2 (±1.0)	36.7 (±29.0)
4	600	male	5.35 (±4.19)	10.2 (±8.4)	44.6 (±24.3)			
		female	9.00 (±1.91)	6.0 (±0.0)	67.8 (±26.3)			
		overall	7.18 (±3.63)	8.1 (±6.0)	56.2 (±26.8)			
4[a]	520[a]	male	3.14 (±2.59)	2.2 (±1.1)	13.2 (±8.27)	6.69 (±5.12)	2.6 (±0.9)	33.1 (±24.9)
		female	3.98 (±2.50)	3.8 (±2.2)	16.7 (±9.79)	5.95 (±2.31)	3.2 (±1.8)	29.0 (±10.6)
		overall	3.56 (±2.44)	3.0 (±1.8)	15.0 (±8.74)	6.32 (±3.76)	2.9 (±1.4)	31.0 (±18.2)

a. The 600 mg/kg/day dose was lowered to 520 mg/kg/day beginning on Day 3.
b. Samples for the 520 mg/kg/day dose group were taken on Day 8.
c. Calculated as AUC_{0-24}.

Study title: Four-Week Oral Toxicity Study of A-1043422 with Ritonavir (A-84538) in Sprague-Dawley Rat (with a Four-Week Recovery Period)

Key study findings:

- No remarkable toxicities were attributable to ABT -450.
- Changes in clinical pathology parameters as well as in the liver and the thyroid gland were consistent with known hepatic enzyme-inducing effects of RTV in rats. Co-administration of ABT -450 with RTV did not exacerbate the RTV findings.
- The no-observed-adverse-effect-level (NOAEL) was 450/45 mg/kg/day ABT-450/RTV, corresponding to mean steady state (day 28) ABT-450 AUC values of 134 and 239 µg.hr/mL in males and females, respectively.

Study no.: Study no. R&D/09/023:
Volume #, and page #: vol. 1, p.1 (SDN 021)
Conducting laboratory and location: Abbott Laboratories
Toxicology and Safety Pharmacology
100 Abbott Park Road,
Abbott, Park, IL
Date of study initiation: 12 November 2008
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 85585-21, 95.9% purity
RTV, lot no. 57144TL, 99.4% purity

Methods

Doses: 0/0, 100/15, 300/30,or 450/45 mg/kg of ABT-450/RTV, or 45 mg/kg RTV
Species/strain: Rat/Sprague-Dawley [Crl: CD (SD)]
Number/sex/group: 15/sex/dose
Route, formulation, volume: Suspension in Cremophor EL:PEG 400:0 Acid (10:10:80, w/w/w); 2 mL/kg
Age: 9 weeks
Weight: 187.9-341. 7 grams

Test Group	Test Material	Dosage (mg /kg/day)	Concentration (mg /mL)	Number of Rats[c]	
				Males	Females
1 (vehicle control)	Vehicle[a]	0	0[b]	10 (5)	10 (5)
2 (RTV control)	RTV control[d]	0/45	0/22.5	10 (5)	10 (5)
3 (low)	A-1043422[e]/RTV[d]	100/15	50/7.5	10 (5)	10 (5)
4 (mid)	A-1043422[e]/RTV[d]	300/30	150/15	10 (5)	10 (5)
5 (high)	A-1043422[e]/RTV[d]	450/45	225/22.5	10 (5)	10 (5)

a. Vehicle: Cremophor EL:PEG 400:Oleic acid (10:10:80, w/w/w)
b. The dosage volume was 2 mL/kg for all groups.
c. The last five rats, as numbered sequentially, per sex in Groups 1-5 (within parentheses) were designated recovery rats.
d. A-84538 free base (A-84538.0, RTV), Lot 57144TL; assigned chemical potency 994 mg base/g of test item. (See Study Plan Deviation 4.)
e. A-1043422 free acid (A-1043422.0, ABT-450), Lot 85585-21; assigned chemical potency 959 mg base/g of test item.

Results:

Mortality: Animals were observed twice daily during the dosing period for morbidity/mortality. There were no early deaths.

Clinical signs: Detailed clinical signs were obtained twice per week during the dosing phase. Clinical signs were limited to red hair staining (both sexes; in RTV control and all dosages of ABT -450/RTV); and

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yellow hair staining of the ano-genital area, increased salivation, and hair matting, and were attributed to RTV administration.

Body weights: Body weights were collected twice per week during dosing and recovery. There were no remarkable findings.

Food consumption: Food consumption was recorded once weekly. There were no remarkable findings.

Ophthalmoscopic Examinations: Ophthalmoscopic examinations were conducted prior to the scheduled terminal necropsy. There were no remarkable findings.

Clinical Pathology:

Hematology: Mild increases in monocytes D29 (0/45, 300/30 and 450/45 mg/kg/day males and 450/45 mg/kg/day females) were attributed to RTV administration.

Clinical chemistry: Mild increase in cholesterol values D29 (0/45, 300/30 and 450/45 mg/kg/day females) were attributed to RTV administration.

Gross pathology: There were no remarkable macroscopic findings at necropsy.

Organ weights: Weights were obtained for adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus and thyroid with parathyroid glands. Histopathological findings below correlated with increased liver weights in these RTV-treated rats.

Histopathology: Adequate Battery: yes
Peer review: yes

Minimal hepatocellular hypertrophy was noted in RTV-treated females (0/45, 300/30 and 450/45 mg/kg/day males and 0/45 and 450/45 mg/kg/day). Additionally, minimal to mild multinucleated hepatocytes were noted in low numbers of males in the 0/45 and 450/45 mg/kg/day dose groups (see table below, *excerpted from sponsor*). These changes were partially resolved by the end of the recovery period.

Incidences of Selected Microscopic Observations in Liver

A-1043422/A-84538 mg/kg/day	0	0/45	100/15	300/30	450/45	0	0/45	100/15	300/30	450/45
	Males					Females				
Hypertrophy, hepatocellular	0	9	4	10	10	0	10	0	1	9
Minimal	0	9(1)	4(1)	10	10(3)	0	10(1)	0	1	9
Multinucleated, hepatocytes	0	1	0	0	1	0	0	0	0	0
Minimal	0	1(2)	0	0	0	0	0	0	0	0
Mild	0	0(1)	0	0	1	0	0	0	0	0

n=10, numbers in ( ) = incidence in recovery animals [n = 5]

Minimal hypertrophy of the thyroid gland follicular epithelial cells was observed in both sexes at 0/45 and 450/45 mg/kg/day and at lower incidences in males and females administered 300/30 mg/kg/day (see table below, *excerpted from sponsor*). These changes were completely resolved in females and partially resolved in males by the end of the recovery period.

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Incidences of Thyroid Gland Follicular Cell Hypertrophy

A-1043422/ A-84538 mg/kg/day	0	0/45	100/ 15	300/ 30	450/ 45	0	0/45	100/ 15	300/ 30	450/ 45
	Males					Females				
Hypertrophy, follicular cell(s)	0	10	NE	4	8	0	5	NE	1	7
Minimal	0	10(3)	NE	4(3)	8(2)	0	5	NE	1	7

n=10, numbers in ( ) = incidence in recovery animals [n = 5]. NE = Not examined

Toxicokinetics: Blood samples for toxicokinetic analysis were collected 1, 3, 6, 12, and 24 hours post-dose on days 1 and 28. Mean toxicokinetic parameters for ABT-450 are presented below (*table excerpted from sponsor*). By Day 28 of dosing, sex-related differences in systemic exposures are seen, with exposures in males remaining constant and exposures in females increasing approximately 2-4 fold.

Mean Toxicokinetic Parameters for ABT-450

Test Group	Dose (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 AUC (µg •hr/mL)	Day 28 C_{max} (µg/mL)	Day 28 T_{max} (hr)	Day 28 AUC (µg •hr/mL)
3	100	male	11.2	3.0	36.6	19.4	6.0	48.4
		female	13.1	3.0	39.2	17.3	3.0	67.8
		overall	12.2	3.0	37.9	18.4	4.5	58.1
4	300	male	21.3	3.0	79.4	11.8	3.0	73.5
		female	15.6	3.0	65.4	34.6	3.0	214
		overall	18.5	3.0	72.5	23.2	3.0	144
5	450	male	9.14	3.0	128	25.6	6.0	134
		female	13.3	3.0	65.0	75.2	6.0	239
		overall	11.2	3.0	96.7	50.4	6.0	187

Study title: Four-Week Oral (Gavage) Toxicity Study of A-1043422 and Ritonavir in Combination with Ribavirin in Sprague-Dawley Rats

Key study findings:

- Histopathological findings in liver and thyroid were attributed to RTV and/or RBV, and were not considered to be effects of ABT-450.
- The high dose used in this study, 450 mg/kg ABT-450 is the highest technically feasible ABT-450 concentration in the vehicle used (Cremophor EL:PEG 400:01eic acid (10:10:80, w/w/w)).

- The mean ABT-450 AUC values for animals administered 450/45/0 ABT-450/RTV/RBV were 60 µg.hr/mL and 21 µg.hr/mL in males and females, respectively.
- Co-administration of RBV with ABT-450 and/or RTV did not produce any novel toxicities, exacerbate compound-specific observations, nor remarkably alter compound-specific plasma exposures.

Study no.:	Study no. R&D/08/1866:
Volume #, and page #:	vol. 1, p.1 (SN019)
Conducting laboratory:	Abbott Laboratories Toxicology and Safety Pharmacology 100 Abbott Park Road, Abbott, Park, IL
Date of study initiation:	14 January 2009
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	ABT-450 (A-1043422), lot no. 85585-21; 95.9% purity RTV, Lot 69514TL; 99.3% purity RBV, Lot 71FP1; purity not noted

Methods

Doses:	0/0/0, 450/45/0, 0/0/60, 0/45/60, 100/15/60 and 450/45/60 mg/kg ABT-450/RTV/RBV
Species/strain:	Rat/Sprague-Dawley [Crl: CD (SD)]
Number/sex/group:	10/sex/dose
Route, formulation, volume:	Suspension in Cremophor EL:PEG 400:0 Acid (10:10:80, w/w/w); 2 mL/kg (1.5 mL/kg RBV)
Age:	Approx. 8 weeks
Weight:	males: 265.0 - 334.3 g females: 174.l - 221.8 g

		Dosage Concentration		Number of rats	
Test Group	Test Material	ABT-450/ RTV/RBV (mg/kg bw/day)	ABT-450/ RTV/RBV (mg/mL)[a]	Males	Females
01	vehicle[b]	0/0/0	0/0/0	10	10
02	ABT-450[c]/RTV[d]	450/45/0	225/22.5/0	10	10
03	Vehicle[b]/RBV[e]	0/0/60	0/0/40	20[f]	20[f]
04	RTV[d]/RBV[e]	0/45/60	0/22.5/40	20[f]	20[f]
05	ABT-450[c]/RTV[d]/RBV[e]	100/15/60	50/7.5/40	20[f]	20[f]
06	ABT-450[c]/RTV[d] /RBV[e]	450/45/60	225/22.5/40	20[f]	20[f]

a. The dose volume were 2 mL/kg bw/day for ABT-450/RTVor vehicle and 1.5 ml/kg bw/day for RBV
b. Vehicle: Cremophor EL:PEG 400:Oleic Acid (10:10:80, w/w/w)
c. A-1043422 free acid (A-1043422.0, Lot number 85585-21; potency 959 mg A-1043422.0/g drug substance)
d. A-84538 free base (A-84538.0, Lot number 69514TL; potency 993 mg A-84538.0/g drug substance).
e. Ribavirin (Rebetol®, 40 mg/ml); RBV were administered daily approximately three hours after the ABT-450/RTV dose.
f. Ten animals per sex in Groups 03, 04, 05 and 06 were designated satellite rats used for determination of RBV plasma test item concentrations.

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Results:

Mortality: Rats were observed twice daily for morbidity/mortality. One rat from the high dose group died early. Three (one middle dose male on day 7 and two high dose females on days 14 and 18) rats were sacrificed moribund. All early deaths were attributed to gavage error.

Clinical signs: Detailed clinical examinations were performed twice weekly. Clinical signs in surviving animals were limited to salivation and hair loss in all groups, including controls.

Body weights: Body weights were measured pre-dose and twice weekly during dosing. Lower body weight gain (30%) in males in the 450/45/60 dose group were apparently due to RBV effects, as animals dosed with 60 mg/kg RBV alone tended to gain less weight than untreated controls (30% and 21% less in males and females, respectively.)

Food consumption: Food consumption was measured pre-dose and once per week during dosing. Decreased food consumption in RBV-treated rats correlated with decreased body weight gain described above.

Ophthalmoscopic Examinations: Ophthalmoscopic examinations were performed pre-dosing to qualify animals for study. On Day 24 of dosing, rats in the untreated control group and the high dose group were examined. As there were no findings in the high dose group, examinations of animals in the remaining treated groups were not conducted.

Clinical Pathology: Blood samples were collected at necropsy.

Hematology: Effects on RBCs (counts, hematocrit, hemoglobin) were attributed to RBV.

Clinical chemistry: There were no remarkable effects.

Coagulation parameters: There were no remarkable effects.

Urinalysis: There were no remarkable effects.

Gross pathology: There were no remarkable effects.

Organ weights: Adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, thyroid and parathyroid glands were weighed. Rats treated with RTV had minimal to mild increases in liver weight and minimal increases in thyroid weight. Groups treated with RBV had minimal decreases in thyroid weights. Co-administration with ABT-450 did not exacerbate those effects.

Histopathology: Adequate Battery: yes
Peer review: yes

Histopathological examination was performed on main study rats of Groups 01and 06 as well as main study rats that died or were euthanized during the dosing period. Tissues (liver. thyroid. thymus) exhibiting potential test item-related lesions in the high dose group were also examined in rats in one or more of the lower/ the next lower dose group(s).

Minimal (mild in one high dose female) hepatocellular hypertrophy and minimal to mild thyroid follicular cell hypertrophy was noted in males and females dosed with RTV.

A decrease in lymphocytes was noted in thymus tissue from males and females treated with RBV.

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Toxicokinetics: Two rats from each group were bled at 1, 3, 6, 12, and 24 hours post-dose on days 1 and 28 for the determination of ABT-450 and RTV plasma levels. Two rats from RBV-treated groups were also bled on the same days at 4, 6, 9, l5, and 27 hours after administration of ABT-450 and RTV for the determination of RBV plasma levels.

Toxicokinetic values tended to be highly variable between the two rats bled at each time point. As seen in other rat studies, there was an apparent sex-related difference in systemic exposures. Also, exposure to ABT-450 appeared to decrease with repeated dosing, as seen previously in male rats. This is likely related to induction of hepatic enzymes by RTV (see macroscopic and microscopic changes in liver noted above). When ABT-450/RTV were co-administered with RBV, no change to systemic exposure was evident on Day 1 of dosing. By Day 28, ABT-450 exposures appeared to be decreased in males and increased in females, although the results from two values at each time point were variable. Mean toxicokinetic parameters for ABT-450, RTV, and RBV are presented below (*tables excerpted from sponsor*).

Mean Toxicokinetic Parameters for A-1043422

Collection Interval	Sex	Dosage (mg/kg bw/day) ABT-450/RTV/RBV 450/45/0	100/15/60	450/45/60
		Mean Plasma AUC_{0-24h} (μg•hr/ml)		
Day 1	Males	149	61.5	148
	Females	54.7	20.9	59.1
Day 28	Males	60.7	6.07	15.8
	Females	20.7	3.93	60.2
		Mean Plasma C_{max} (μg/ml)		
Day 1	Males	59.3	12.8	17.6
	Females	22.4	5.29	7.33
Day 28	Males	36.1	1.48	2.53
	Females	4.73	1.54	26.3

Reference ID: 3628623

Mean Toxicokinetic Parameters for RTV

Collection Interval	Sex	Dosage (mg/kg bw/day) ABT-450/RTV/RBV 450/45/0	0/45/60	100/15/60	450/45/60
		Mean Plasma AUC_{0-24h} (µg•hr/ml)			
Day 1	Males	5.78	43.3	4.21	5.19
	Females	18.1	50.0	7.20	22.2
Day 28	Males	16.4	34.6	6.64	21.1
	Females	59.9	54.1	6.49	51.5
		Mean Plasma C_{max} (µg/ml)			
Day 1	Males	1.79	5.14	0.387	0.557
	Females	1.65	4.60	0.915	1.85
Day 28	Males	2.00	5.67	1.10	3.36
	Females	4.56	5.25	1.34	3.39

Mean Toxicokinetic Parameters for RBV

Collection Interval	Sex	Dosage (mg/kg bw/day) ABT-450/RTV/RBV 0/0/60	0/45/60	100/15/60	450/45/60
		Mean Plasma AUC_{0-24h} (µg•hr/ml)			
Day 1	Males	9.05	10.0	10.3	10.8
	Females	8.99	7.18	7.80	7.22
Day 28	Males	11.7	12.2	14.1	12.5
	Females	11.6	12.3	11.5	12.9
		Mean Plasma C_{max} (µg/ml)			
Day 1	Males	0.924	0.849	0.828	1.16
	Females	1.02	1.07	0.856	0.595
Day 28	Males	0.904	0.987	1.08	0.863
	Females	1.13	1.36	0.988	1.22

2.6.6.3 Repeat-dose toxicity

Study title: Thirteen-week Oral Toxicity Study of A-1043422 with Ritonavir (A-84538) in Sprague-Dawley Rats

Key study findings:

- Rats were administered ABT-450 and/or ritonavir (RTV) alone at doses of 0/0, 0/15, 0/45, 100/15,300/30 or 450/45 mg/kg/day (ABT-450/RTV) for 13 weeks.

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- RTV administration resulted in enlarged livers and thyroid glands with histological correlates (cellular hypertrophy).
- Concurrent hepatocellular and thyroid follicular cell hypertrophy is suggestive of a rodent-specific mechanism by which induction of liver enzyme-mediated metabolism of thyroid hormones results in the release of thyroid-stimulating hormone and subsequent thyroid gland hypertrophy. This mechanism is not relevant to humans, such that the findings are not considered to be toxicologically significant.
- Therefore, the NOAEL is defined as the high dose, 450/45 mg/kg/day ABT-450/RTV, corresponding to (highly variable) systemic exposures of 91 ± 53 µg*hr/mL (males and females combined).

Study no.:	Study no. R&D/09/990:
Volume #, and page #:	vol. 1, p.2
Conducting lab and location:	Abbott Laboratories Toxicology and Safety Pharmacology 100 Abbott Park Road, Abbott, Park, IL
Date of study initiation:	21 April 2009
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	ABT-450 (A-1043422), lot no. 74983PPO1, 99.7 % purity

Methods

Doses:	0/0, 0/15, 0/45, 100/15,300/30 or 450/45 mg/kg/day ABT-450/RTV
Species/strain:	Rat/ Sprague-Dawley (Cd: CD® (SD))
Number/sex/group:	10/sex/dose
Route, formulation, volume:	Cremophor EL: PEG 400:Oleic Acid; 10: 10:80, w/w/w, 2 mL/kg/day
Age:	9 weeks
Weight:	275-332 grams for males and 194-231 grams for females

Results:

Mortality: Animals were checked twice daily for morbidity, mortality and/or signs of distress. Four animals were euthanized in moribund condition due to gavage errors.

Clinical signs: Once daily cage-side observations for clinical signs were performed during the dosing phase. Detailed observations were performed twice weekly. Clinical signs were noted in RTV control groups as well as ABT-450/RTV-treated animals, and were related to hair loss or poor grooming (e.g., rough or red-stained hair).

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

Food consumption: Food consumption was measured weekly during the dosing phase. There were no toxicologically-significant findings.

Ophthalmology: Ophthalmoscopic examinations were performed prior to dosing and on dosing day 81. There were no remarkable findings.

Clinical Pathology: Samples for hematology and clinical chemistry were collected at terminal sacrifice.

Reference ID: 3628623

Hematology: There were no ABT-450-related findings. Changes in RBC counts, hemoglobin, hematocrit and reticulocytes were noted previously in rats following treatment with RTV, and indicated a loss of RBC mass with a regenerative response (see table below, *excerpted from sponsor*).

Mean Values of Selected Hematologic Parameters

mg/kg/day A-1043422/Ritonavir	RBC (E12/L)	Hgb (g/dL)	Hct (%)	RDW (%)	Ret (E9/L)	LOWRET[b] (%)	HIGHRET[b] (%)
Males							
0/0	8.941	15.44	47.20	12.52	205.97	37.78	38.37
0/15[a]	9.077	15.38	46.90	12.74	215.27	36.34	37.69
0/45[a]	8.800	14.20*	43.52*	13.63*	318.08*	28.71	46.03
100/15	8.830	14.89	45.52	13.12	234.05	33.58	41.66
300/30	8.839	14.71	45.87	13.64*	261.48	28.49	48.72
450/45[a]	8.932	14.71	45.20	13.50*	266.41*	30.37	46.77
Females							
0/0	7.960	14.49	43.15	11.27	187.16	39.03	35.62
0/15	8.249	14.64	43.60	11.40	182.07	38.22	35.59
0/45	7.950	14.13	42.25	12.44*	238.28*	33.83	41.88
100/15[a]	8.066	14.40	43.21	11.62	209.49	38.02	36.02
300/30	7.979	14.25	42.85	12.09*	226.58	33.66	42.43
450/45[a]	7.918	14.31	42.39	12.08*	229.93	34.33	42.73

n= 10 per group, except where indicated.

a. n=9 per group.

b. No statistical analysis was performed.

* Indicates statistical significance relative to control data ($p<0.05$)

Coagulation parameters: There were no toxicologically-significant changes in coagulation parameters.

Clinical chemistry: Apparent increases in liver transaminases (ALT and AST) in male rats treated with RTV correlated with hepatic hypertrophy noted microscopically. Increased cholesterol in males and females was also attributed to treatment with RTV (see table below, *excerpted from sponsor*).

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Mean Values of Selected Clinical Chemistry Parameters

mg/kg/day A-1043422/Ritonavir	ALT (IU/L)	AST (IU/L)	CO2 (mmol/L)	P (mg/dL)	TP (g/dL)	Chol (mg/dL)
Males						
0/0	30.2	105.2	27.6	8.16	6.80	68.5
0/15	92.8*	216.7*	29.2*	7.41	6.64	73.0
0/45	87.0*	199.8*	28.5	7.17*	7.14*	104.1*
100/15	49.7	153.1	28.6	7.39	6.67	82.7
300/30	70.0*	179.1*	28.2	8.23	7.05	85.0
450/45	52.4	116.8	27.9	7.76	7.16*	95.3*
Females						
0/0	39.3	160.2	27.4	6.54	7.16	83.4
0/15	33.6	86.0*	26.9	6.57	7.59	104.3
0/45	27.8	75.8*	27.4	6.88	7.63	148.7*
100/15	24.9	91.0*	26*	6.73	7.22	108.0
300/30	30.6	94.6*	26.9	6.67	7.26	127.4*
450/45	38.1	115.1	26.7	6.89	7.47	128.0*

n= 10 per group, except 450/45 mg/kg/day A-1043422/Ritonavir males and females, n=9.
* Indicates statistical significance relative to control data ($p<0.05$)

Urinalysis: There were no remarkable findings.

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

Organ weights: The following organs were weighed: adrenals, brain, heart, kidney, liver with gallbladder, spleen, ovaries, pituitary gland, prostate gland, testes, thymus, and thyroid/parathyroid. Increased liver, thyroid and spleen weights were attributed to RTV treatment (see table below, *excerpted from sponsor*). Increased liver weights correlated with centrilobular hepatocellular hypertrophy. Increased thyroid weights correlated with hypertrophy of follicular lining cells. There was no histological correlate for increased spleen weights

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Selected Absolute Organ Weights:
Control Means (g) and Percent Differences from Control Mean

mg/kg/day A-1043422/Ritonavir	Terminal body weight	Liver	Thyroid glands	Spleen
Males				
0/0	529.77	13.0967	0.0173	0.8387
0/15	-4	0	7	-8
0/45	1	37*	29(*)	31*
100/15	2	14(*)	-7	11
300/30	3	32*	15	16
450/45	-2	36*	8	21(*)
Females				
0/0	315.54	7.7446	0.0143	0.5764
0/15	6	24*	17	6
0/45	0	46*	41*	34*
100/15	-2	13(*)	9	11
300/30	-1	29*	21	27*
450/45	-2	35*	10	29*

n= 10 per group, except n=9 for 450/45 mg/kg/day A-1043422/Ritonavir males and females.
* Indicates absolute and relative organ weights have statistical significance relative to controls ($p<0.05$).
(*) Indicates relative organ weights have statistical significance relative to controls ($p<0.05$).

Histopathology: Adequate Battery: yes
Peer review: yes

Microscopic findings in liver (centrilobular hepatocellular hypertrophy, periductal infiltration, multinucleation) and thyroid (follicular cell hypertrophy) were attributed to RTV administration (see table below for incidence, *excerpted from sponsor).*

Incidence of Ritonavir-Related Histopathologic Findings

		Males						Females					
	A-1043422	0	0	0	100	300	450	0	0	0	100	300	450
mg/kg/day	Ritonavir	0	15	45	15	30	45*	0	15	45	15	30	45*
Liver													
Hypertrophy, hepatocellular, centrilobular													
	minimal	0	2	4	0	6	5	0	1	5	4	5	7
	mild	0	0	6	0	3	5	0	0	5	0	0	0
Infiltration, mononuclear cell, periductular													
	minimal	0	0	2	0	0	2	0	2	1	0	0	0
	mild	0	1	4	0	0	0	0	0	0	0	0	0
	moderate	0	0	3	0	0	0	0	0	1	0	0	0
Multinucleation													
	minimal	0	1	1	0	5	5	0	0	1	0	0	0
	mild	0	0	5	0	1	2	0	0	0	0	0	0
Thyroid glands													
Hypertrophy, follicular cell													
	minimal	0	6	1	6	4	4	0	2	2	2	4	3
	mild	0	1	9	2	4	4	0	1	5	0	3	1

n= 10 per group, except males administered 100/15 mg/kg/day A-1043422/Ritonavir, thyroid glands, n=9 (due to unreadable tissue from 4017).

* 450/45 mg/kg/day A-1043422/Ritonavir groups include unscheduled deaths 6001 and 6020.

Toxicokinetics: Samples of blood were collected from each surviving satellite rat at approximately 1, 3, 6, 12, and 24 hours after dosing on Day 1, Day 28, and Day 87. Toxicokinetic parameters were highly variable among dose groups and between males and females, such that no difference was apparent between sexes (see table below, *excerpted from sponsor*). It is interesting to note that between Day 1 and 28 systemic exposures appear to decline by >50% (91 µg*hr/mL vs. 35 µg*hr/mL), but between Day 1 and Day 87 there is no apparent difference.

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Mean (±SD) Toxicokinetic Parameters for A-1043422

Test Group	Dose A-1043422/ Ritonavir (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 AUC (µg•hr/mL)	Day 28 C_{max} (µg/mL)	Day 28 T_{max} (hr)	Day 28 AUC (µg•hr/mL)	Day 87 C_{max} (µg/mL)	Day 87 T_{max} (hr)	Day 87 AUC (µg•hr/mL)
4	100/15	male	9.99	4.2	62.3	3.31	5.0	21.5	10.3	4.8	62.1
			±3.91	±1.6	±26.3	±0.891	±2.2	±6.39	±8.07	±1.6	±50.1
		female	8.87	4.4	63.4	8.09	3.0	38.6	14.1	3.0	85.6
			±7.00	±2.3	±48.4	±5.67	±0.0	±22.0	±8.59	±0.0	±58.9
		overall	9.43	4.3	62.9	5.70	4.0	30.1	12.2	3.9	73.9
			±5.37	±1.9	±36.8	±4.58	±1.8	±17.7	±8.11	±1.4	±53.0
5	300/30	male	14.4	5.4	105	13.3	3.2	70.1	11.9	4.2	89.7
			±10.9	±1.3	±79.5	±5.40	±1.8	±38.2	±6.30	±1.6	±54.5
		female	9.21	4.8	81.4	10.7	4.8	66.6	23.9	3.0	119
			±4.88	±1.6	±66.5	±7.81	±1.6	±59.3	±10.4	±0.0	±33.4
		overall	11.8	5.1	93.0	12.0	4.0	68.3	17.9	3.6	104
			±8.43	±1.4	±70.2	±6.46	±1.8	±47.1	±10.3	±1.3	±45.3
6	450/45	male	16.1	3.0	118	4.65	5.4	29.3	11.5	4.8	69.7
			±8.23	±0.0	±42.4	±3.50	±3.9	±12.3	±4.58	±1.6	±21.8
		female	8.60	3.6	64.5	9.08	3.0	41.3	24.2	3.0	113
			±2.13	±1.3	±20.0	±8.09	±0.0	±43.5	±16.7	±0.0	±68.0
		overall	12.3	3.3	91.5	6.86	4.2	35.3	17.8	3.9	91.1
			±6.90	±0.9	±42.3	±6.32	±2.9	±30.8	±13.3	±1.4	±52.7

Study title: Thirteen-week Oral Toxicity Study of A-1043422 and A-84538 (Ritonavir) Co-dosed with Ribavirin in Sprague-Dawley Rats

Key study findings:

- Rats were administered ABT-450/ritonavir (RTV) with and without ribavirin (RBV) at doses of 0/0/0, 450/45/0, 100/15/60 and 450/45/60 mg/kg ABT-450/RTV/RBV for 13 weeks.
- RTV administration resulted in enlarged livers and thyroid glands with histological correlates (cellular hypertrophy).
- Concurrent hepatocellular and thyroid follicular cell hypertrophy is suggestive of a rodent-specific effect.
- In the thymus, decreased lobule size and lymphocyte numbers is consistent with known RBV effects.
- The NOAEL is defined as the high dose, 450 mg/kg/day ABT-450, corresponding to (highly variable) systemic exposures of 34.6± 22.9 µg*hr/mL (males and females combined).

Study no.: Study no. R&D/09/525
Volume #, and page #: vol. 5, p.1 (SDN 027)
Conducting laboratory and location: Abbott Laboratories
Toxicology and Safety Pharmacology
100 Abbott Park Road,
Abbott, Park, IL
Date of study initiation: 20 April 2009 (initial data collection)
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 74983PP01; 96.6 % purity
RTV, Lot 69514TL; 99.3% purity
RBV, Lot 1JFP1; purity not noted

Methods

Doses:	0/0/0, 450/45/0, 100/15/60 and 450/45/60 mg/kg ABT-450/RTV/RBV
Species/strain:	Rat/Sprague-Dawley [Crl: CD (SD)]
Number/sex/group:	10/sex/dose
Route, formulation, volume:	Suspension in Cremophor EL:PEG 400:0 Acid (10:10:80, w/w/w); 2 mL/kg (1.5 mL/kg RBV)
Age:	9 weeks
Weight:	201.7 – 338.5 g

Results:

Mortality: Animals were checked twice daily for morbidity, mortality and/or signs of distress. There were seven early deaths due to gavage errors.

Clinical signs: Once daily cage-side observations for clinical signs were performed during the dosing phase. Detailed observations were performed twice weekly. Clinical signs were noted in vehicle and ABT-450/RTV control groups as well as ABT-450/RTV/RBV-treated animals, and were related to raspy, noisy or labored respiration, poor grooming (e.g., rough or red-stained hair) with or without sneezing and salivation. These findings were considered to be related to reflux or aspiration of the dosage formulation, and not test-article related.

Body weights: Body weights were recorded twice weekly during the dosing phase. Fluctuations in body weight and body weight gain were considered to be secondary to gavage-related stress. There were no test article-related findings.

Food consumption: Food consumption was measured weekly during the dosing phase. Changes in food consumption correlated with fluctuations in body weight and were attributed to gavage-related stress. There were no toxicologically-significant findings.

Ophthalmology: Ophthalmoscopic examinations were performed prior to dosing and on dosing day 85. There were no remarkable findings.

Clinical Pathology: Samples for hematology and clinical chemistry were collected at terminal sacrifice.

Hematology: There were no ABT-450-related findings. Changes in RBC counts, hemoglobin, hematocrit were noted previously in rats following treatment with RTV and/or RBV, and indicated a loss of RBC mass. An indication of a regenerative response (shift towards reticulocytes) is also consistent with known RTV/RBV effects.

Coagulation parameters: There were no toxicologically-significant changes in coagulation parameters.

Clinical chemistry: Apparent increases in liver transaminases (ALT and AST) in male rats treated with RTV correlated with hepatic hypertrophy noted microscopically.

Urinalysis: There were no remarkable findings.

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

Organ weights: The following organs were weighed: adrenals, brain, heart, kidney, liver with gallbladder, spleen, ovaries, pituitary gland, prostate gland, testes, thymus, and thyroid/parathyroid. Increased liver and thyroid weights were attributed to RTV treatment. Increased liver weights correlated with centrilobular hepatocellular hypertrophy. Increased thyroid weights correlated with hypertrophy of

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follicular lining cells. Decreased thymus weights were consistent with known RBV effects and were consistent with microscopic effects.

Histopathology: Adequate Battery: yes
Peer review: yes

Microscopic findings in liver (hepatocellular hypertrophy, periductal infiltration, multinucleation) and thyroid (follicular cell hypertrophy) were attributed to RTV administration. In the thymus, decreased lobule size and lymphocyte numbers is consistent with known RBV effects (see table below for incidence, *excerpted from sponsor).*

Incidence of Histopathologic Findings Attributed to Ritonavir or Ribavirin

Dose mg/kg/day	Males				Females			
A-1043422	0	450[a]	100	450	0	450[a]	100	450[a]
Ritonavir	0	45	15	45	0	45	15	45
Ribavirin	0	0	60	60	0	0	60	60
Liver								
Multinucleation, hepatocellular								
Minimal		2	1	4				2
Mild		6		4				
Hypertrophy, hepatocellular								
Minimal		5		4		2	1	1
Infiltration, periductular								
Minimal		3		1		1		
Mild				1				
Thyroid gland								
Hypertrophy, follicular cell								
Minimal		5	4	7		2	2	4
Mild		1						
Thymus								
Decreased number, lymphocytes, cortex								
Minimal		1		4	1	1	1	2
Mild	1	1	2	2				2
Moderate		1		2				1
Marked								1
Decreased size, lobules								
Minimal	1	2		2	3	2		1
Mild	2	2	5	5		2	6	1
Moderate	1	2	4	1			3	2

a. Data for early deaths included in table.

Toxicokinetics: Samples of blood were collected from each surviving satellite rat at approximately 1, 3, 6, 12, and 24 hours after dosing on Day 1, Day 29, and Day 91. Toxicokinetic parameters were highly variable among dose groups and between males and females, such that no difference was apparent between sexes (see table below, *excerpted from sponsor*).

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Mean (±SD) Toxicokinetic Parameters for A-1043422

Dose A-1043422/RTV/ RBV (mg/kg/day)	Sex	Day 1			Day 29			Day 91		
		C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
450/45/0	male	13.5 ± 6.91	4.0 ± 2.7	119 ± 67.9	2.66 ± 0.826	3.2 ± 1.8	20.8 ± 4.14	9.81 ± 6.67	2.0 ± 1.2	63.3 ± 40.1
	female	17.2 ± 3.20	3.0 ± 0.0	136 ± 64.0	3.83 ± 3.02	3.2 ± 1.8	27.9 ± 24.1	20.5 ± 12.3	2.5 ± 1.0	91.8 ± 77.2
	overall	15.4 ± 5.44	3.5 ± 1.9	127 ± 62.9	3.25 ± 2.18	3.2 ± 1.7	24.4 ± 16.7	15.2 ± 10.8	2.3 ± 1.0	77.5 ± 59.0
100/15/60	male	12.6 ± 9.34	4.8 ± 4.0	109 ± 86.8	2.37 ± 1.77	4.8 ± 1.6	13.6 ± 9.12	2.62 ± 1.46	2.5 ± 1.0	22.5 ± 15.6
	female	15.8 ± 5.98	5.4 ± 1.3	111 ± 37.8	11.7 ± 9.01	3.0 ± 0.0	56.6 ± 52.6	13.2 ± 7.54	3.0 ± 0.0	52.2 ± 36.1
	overall	14.2 ± 7.59	5.1 ± 2.8	110 ± 63.2	7.02 ± 7.84	3.9 ± 1.4	35.1 ± 42.2	8.47 ± 7.75	2.8 ± 0.7	39.0 ± 31.4
450/45/60	male	20.2 ± 6.75	5.0 ± 2.2	193 ± 76.0	3.18 ± 2.91	2.6 ± 0.9	17.3 ± 8.15	10.7 ± 8.98	3.2 ± 1.8	38.2 ± 20.8
	female	17.0 ± 4.52	5.6 ± 4.2	176 ± 63.2	3.75 ± 2.04	2.2 ± 1.1	19.9 ± 10.5	9.13 ± 9.30	2.2 ± 1.1	31.0 ± 26.9
	overall	18.6 ± 5.66	5.3 ± 3.2	184 ± 66.4	3.47 ± 2.39	2.4 ± 1.0	18.6 ± 8.97	9.91 ± 8.66	2.7 ± 1.5	34.6 ± 22.9

Study title: Five-Day Oral Capsule Tolerability Study of A-1043422 and A-84538 (Ritonavir) in Beagle Dogs

Key study findings:

- This was a five day tolerability study in dogs with no vehicle control group, so that the significance of parameter values other than clinical signs are difficult to assess.
- No histopathology was performed.
- Slight to moderate emesis was noted in animals from both dose groups.
- Toxicokinetic parameters were obtained.

Study no.: R&D/08/733:
Volume #, and page #: Vol. 8 p. 277 - 436
Conducting laboratory and location: Abbott Laboratories
Toxicology and Safety Pharmacology
100 Abbott Park Road,
Abbott, Park, IL
Date of study initiation: May 02, 2008 (first day of data collection)
GLP compliance: No
QA report: No
Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 1561563, 99.1% purity

Methods

Doses: 15/7.5 (ABT-450/RTV); 30/15
Species/strain: Dog/Beagle
Number/sex/group or time point (main study): 2/sex
Route, formulation, volume: Oral capsule; 0.5 mL/kg/day
Age: 3 – 4 years
Weight: 6 – 13 kg
Sampling times: Venous samples of blood were collected from each dog at approximately 1, 3, 6, 9, 12, and 24 hours after dosing on Days 1 and 5.

Test Group	Test Material[a]	Dosage (mg/kg/day)[b]	Concentration (mg/mL)	Number of Dogs Males	Number of Dogs Females
Group 1	A-1043422[c]/A-84538[d]	15/7.5	30/15	2	2
Group 2	A-1043422[c]/A-84538[d]	30/15	60/30	2	2

a. Test material is a combination dose of both A-1043424 free acid and A-84538 free base in a combined formulation.

b. The dosage is listed as a combined A-104342 free acid/A-84538 free base. The combined formulation was administered as a liquid formulation placed in capsules immediately prior to dose administration at a dose volume of 0.5 mL/kg.

c. A-1043422 free acid (A-1043422.0), Lot 1561563; potency 970 mg/g of test item.

d. A-84538 free base (A-84538.0, Ritonavir), Lot 37241TL; potency 994 mg/g of test item.

Results:

Mortality: All dogs were observed once each day during the pre-dosing period and twice each day during the dosing period for survival and general condition. All dogs survived the five-day dosing period.

Clinical signs: Detailed observations of physical condition and behavior were recorded daily approximately three to four hours after dosing during the dosing period. Slight to moderate emesis was noted in animals from both dose groups.

Toxicokinetics:

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A-1043422:

Collection Interval	Dosages (mg/kg/day) 15	30
	Mean Plasma AUC_{24} (µg•hr/ml)	
Day 1	472	968
Day 5	350	547
	Mean Plasma C_{max} (µg/ml)	
Day 1	63.3	109
Day 5	62.6	91.6

Ritonavir:

Collection Interval	Dosages (mg/kg/day) 7.5	15
	Mean Plasma AUC_{24} (µg•hr/ml)	
Day 1	18.9	32.0
Day 5	20.6	26.0
	Mean Plasma C_{max} (µg/ml)	
Day 1	4.5	6.1
Day 5	5.4	5.6

Study title: Four-Week Oral Capsule Dosage Range-Finding Toxicity Study of A-1043422 and A-84538 (Ritonavir) in Beagle Dogs

Key study findings:

- Beagle dogs were administered ABT-450 and ritonavir (RTV) at doses of 10/5, 20/10 and 30/15 (ABT-450 mg/kg/RTV mg/kg).
- Pharmacokinetic analysis revealed that systemic exposure to ABT-450, as estimated by AUC, was greatest at the 20/10 dose.
- At that dose, corresponding to mean systemic exposures (AUC) of 816 µg*hr/mL, findings include gall bladder effects (edema, mononuclear and mixed cell infiltration and epithelial cell necrosis) with increased serum alkaline phosphatase, suggesting biliary effects. Increased liver weights were noted in females.
- Based on the biliary effects, the NOAEL was defined as 10 mg/kg ABT-450/5 mg/kg RTV, corresponding to mean systemic exposures (AUC) of 389 µg*hr/mL.

Study no.: R&D/08/1109
Volume #, and page #: Vol. 9, p. 1
Conducting lab and location: Abbott Laboratories
Toxicology and Safety Pharmacology
100 Abbott Park Road,
Abbott, Park, IL
Date of study initiation: May 19, 2008
GLP compliance: No
QA report: No
Drug, lot #, and % purity: ABT-450 (A-1043422), lot no. 1561563, 99.1% purity

Methods

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Doses:	10/5, 20/10, 30/15 mg/kg (ABT-450/RTV).
Species/strain:	Dog/Beagle
Number/sex/group:	2/sex/dose
Route, formulation, volume:	Liquid formulation in oral capsule; 0.5 mL/kg/day
Age:	6- 8 months
Weight:	6.7 – 10.3 kg
Sampling times:	Venous samples of blood were collected from each dog at approximately 1, 3, 6, 9, 12, and 24 hours after dosing on Days 1 and 1, 3, 6, 12 and 24 hours near the end of dosing. A single sample was collected at necropsy.

Results:

Mortality: Dogs were observed twice each day during the dosing period. All dogs survived to necropsy.

Clinical signs: Observations of physical condition and behavior were recorded twice weekly approximately 3-4 hours after dosing. Increased post-dose emesis and loose/abnormal stool were noted in treated animals (see table below, *excerpted from sponsor*).

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Frequency of Post-Dose Emesis

Dosage Level Animal Number	Day(s) of Incidence	Dosage Level Animal Number	Day(s) of Incidence
Male		Female	
0 mg/kg/day		0 mg/kg/day	
1001	2	1002	–
1003	3,22	1004	–
10/5 mg/kg/day		10/5 mg/kg/day	
2001	–	2002	17
2003	–	2004	–
20/10 mg/kg/day		20/10 mg/kg/day	
3001	1,3	3002	3,10,15
3003	3,10,18	3004	3,8,16,19
30/15 mg/kg/day		30/15 mg/kg/day	
4001	1,3,4,4,5,7,8,10,15,17,18, 19,22,24	4002	1,2,3,10,18,23,24
4003	4,15,17,18,24	4004	3,10,10,18,22,28

– No incidence observed.
Days listed twice for each animal indicate observation was present during each session.

Frequency of Loose/Abnormal Stool

Dosage Level Animal Number	Day(s) of Incidence	Dosage Level Animal Number	Day(s) of Incidence
Male		Female	
0 mg/kg/day		0 mg/kg/day	
1001	22,	1002	3,
1003	3,7	1004	3,5
10/5 mg/kg/day		10/5 mg/kg/day	
2001	7	2002	25
2003	–	2004	–
20/10 mg/kg/day		20/10 mg/kg/day	
3001	2,15,18,23	3002	28
3003	4,5,7,10,18,	3004	18,22,25,28
30/15 mg/kg/day		30/15 mg/kg/day	
4001	8,11,18,22,24,25	4002	2,23,28
4003	1,2,3,5,24	4004	1,8,15,17,18,23,25, 28

– No incidence observed.

Body weights: Body weights were recorded pre-dose and twice weekly during dosing. No remarkable findings were noted.

Food consumption: Food consumption was recorded pre-dose and twice weekly during dosing. No remarkable findings were noted.

Clinical Pathology: Blood samples were taken once pre-dose, and on days 2, 8, 15, 22, and 28.
Hematology: No remarkable findings were noted.

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Clinical chemistry: Alkaline phosphatase levels were increased in MD and HD animals (see table below, *excerpted from sponsor*).

Percentage Change from Control Means of Serum Alkaline Phosphatase Throughout the Dosing Period

A-1043422 and A-84538 (Ritonavir)						
	Day 1	Dosing Phase				
mg/kg/day	(Baseline)	Day 2	Day 8	Day 15	Day 22	Day 28
Males						
0[a]	96.5	91.5	96.0	95.5	86.5	87.5
10/5	18	28	27	32	40	28
20/10	-3	30	71	119	134	110
30/15	1	5	5	4	15	16
Females						
0	97.0	94.0	96.5	90.0	87.0	84.0
10/5	29	34	47	74	86	76
20/10	31	61	159	163	162	146
30/15	4	18	18	33	43	45

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

Coagulation parameters: No remarkable findings were noted.

Urinalysis: A urine sample was collected at necropsy. No remarkable findings were noted.

Gross pathology: No remarkable findings were noted.

Organ weights: Adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary glands, prostate gland, spleen, testes, thymus, and thyroid with parathyroid glands were taken and weighed at necropsy.

Liver weights were elevated in the 20/10 and 30/15 mg/kg/day groups (see table below, *excerpted from sponsor*)

Percentage Change from Control Means of Liver Weights at the End of the Dosing Period

A-1043422 and A-84538 (Ritonavir)								
	Males				Females			
mg/kg/day	0[a]	10/5	20/10	30/15	0	10/5	20/10	30/15
Absolute (g)	304.69	10	8	7	237.62	-2	29	44
Relative (%)	3.0664	10	10	8	3.0431	-2	28	33

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

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

Reference ID: 3628623

Findings include minimal to mild gall bladder effects (edema, mononuclear and mixed cell infiltration and epithelial cell necrosis; see table below, *excerpted from sponsor*).

Incidence of Selected Microscopic Observations in Gall Bladder

A-1043422/A-84538	Male				Female			
Dose mg/kg/day	0	10/5	20/10	30/15	0	10/5	20/10	30/15
Edema	0	1	2	2	0	0	1	1
Minimal	0	1	1	2	0	0	1	0
Mild	0	0	1	0	0	0	0	1
Infiltration, mononuclear cell	0	0	1	0	0	0	0	1
Minimal	0	0	1	0	0	0	0	1
Infiltration, mixed cell	0	0	0	0	0	0	1	1
Minimal	0	0	0	0	0	0	1	1
Necrosis, focal, acute, epithelial cells	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Necrosis, multifocal, acute, epithelial cells	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1

Toxicokinetics:

As noted above, the mid-dose (20/10) resulted in the greatest systemic exposure. Pharmacokinetic parameters are summarized in the table below (excerpted from sponsor).

	Day 1			Day 28		
Dose (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
			A-1043422			
10/5	54.2	3.8	337.4	64.8	3.0	388.9
20/10	98.5	3.8	729.0	110.5	3.0	816.1
30/15	114.2	3.0	1101.7	44.4	3.0	449.2
			A-84538			
10/5	2.7	3.0	8.2	5.9	3.0	19.2
20/10	9.8	3.0	31.5	10.3	3.0	41.9
30/15	11.5	3.0	42.2	4.0	2.5	20.6

Study title: Four-Week Oral Capsule Toxicity Study of ABT-450 and RTV in Beagle Dogs with a Four-Week Recovery Period

Key study findings:

- Dogs were administered ABT-450/RTV at doses of 5/5, 10/5, and 20/10 mg/kg.

- At the end of the dosing period, absolute thymus and pituitary weights were decreased 50% at the high dose.
- Microscopic effects were noted in gall bladders in high dose animals (20/10 mg/kg, associated with AUCs of 645±260 µg*hr/mL).
- Slight but statistically significant decreases in activated partial thromboplastin time (APTT) were noted at all doses.
- Post-dose emesis and loose/abnormal stool were noted at all doses.
- The NOAEL was defined as 10/5 mg/kg ABT-450/RTV due to microscopic gall bladder effects. Corresponding exposures (AUC) were 264±48 µg*hr/mL.

Study no.:	R&D/08/1170
Volume #, and page #:	Vol. 14, p. 1
Conducting laboratory and location:	Abbott Laboratories Toxicology and Safety Pharmacology 100 Abbott Park Road, Abbott, Park, IL
Date of study initiation:	June 25, 2008
GLP compliance:	Yes
QA report:	Unaudited draft report (Final Report submitted April 9, 2009)
Drug, lot #, and % purity:	ABT-450 (A-1043422), lot no. 65801PP00, 96.1% purity

Methods

Doses:	5/5, 10/5, 20/10 mg/kg (ABT-450/RTV)
Species/strain:	Dog, beagle
Number/sex/group:	5/sex (2/recovery group)
Route, formulation, volume:	Oral capsule; 0.5 mL/kg/day
Age:	8 months
Weight:	5.9 – 10.3 kg
Sampling times:	Venous samples of blood were collected from each dog at approximately 1, 3, 6, 9, 12, and 24 hours after dosing on Day 1 and pre-dose 1, 3, 6, 12 and 24 hours after dosing on day 28 of dosing. A single sample was collected at necropsy.

Results:

Mortality: Dogs were observed for general condition and survival twice daily during dosing. No dogs were found moribund during the study.

Clinical signs: Detailed observations were conducted at least twice weekly approximately 3 – 5 hours after dosing. Post-dose emesis and loose/abnormal stool were noted (see tables below, excerpted from sponsor).

Reference ID: 3628623

Frequency of Post-Dose Emesis			
Dosage Level Animal Number	Day(s) of Incidence	Dosage Level Animal Number	Day(s) of Incidence
Male		Female	
0 mg base/kg/day		0 mg base/kg/day	
1001	–	1002	1
1003	–	1004	19
1005	–	1006	–
1007	–	1008	–
1009	–	1010	–
1011	18		
5/5 mg base/kg/day		5/5 mg base/kg/day	
2001	–	2002	–
2003	–	2004	16
2005	31	2006	20
2007	10	2008	2,15,22
2009	19	2010	–
10/5 mg base/kg/day		10/5 mg base/kg/day	
3001	–	3002	–
3003	15	3004	29,
3005	–	3006	1,7,20
3007	18	3008	–
3009	1	3010	6,15
20/10 mg base/kg/day		20/10 mg base/kg/day	
4001	1	4002	1,3,15,18,29
4003	1,2,8-11,29	4004	5,11
4005	11,15,21	4006	5,8
4007	1,2,4,8,9,11,13-16	4008	4,6,10,13,14,18,20,24
4009	1,4,15	4010	3,4,8,15,20

- No incidence observed.

Frequency of Loose/Abnormal Stool			
Dosage Level Animal Number	Day(s) of Incidence	Dosage Level Animal Number	Day(s) of Incidence
Male		Female	
0 mg base/kg/day		0 mg base/kg/day	
1001	–	1002	–
1003	–	1004	2,18
1005	4,17	1006	14,16
1007	–	1008	R21
1009	8	1010	18
1011	–		
5/5 mg base/kg/day		5/5 mg base/kg/day	
2001	1,11,16,18,22,25,28,29	2002	–
2003	–	2004	8,21,29
2005	4,8,22,28	2006	–
2007	–	2008	14, 21,29
2009	4,9,11,19,28,R17	2010	20
10/5 mg base/kg/day		10/5 mg base/kg/day	
3001	–	3002	–
3003	4,8,14	3004	23
3005	–	3006	29
3007	16,18	3008	–
3009	16,22	3010	–
20/10 mg base/kg/day		20/10 mg base/kg/day	
4001	–	4002	3
4003	22	4004	4,15,25
4005	22	4006	4,8
4007	4,8,13-15,18,21	4008	7,15,25,R3
4009	22,28,29	4010	15,21,23,28,29

– No incidence observed.

Body weights: Body weights were recorded pre-dose and twice weekly during dosing. No remarkable findings were noted.

Food consumption: Food consumption was recorded pre-dose and twice weekly during dosing. No remarkable findings were noted.

Ophthalmoscopy: Ophthalmologic examinations were performed pre-dose, near the end of dosing and near the end of the recovery period. No remarkable findings were noted.

EKG: Electrocardiograms were recorded pre-dose, near the end of the first week of dosing, near the end of dosing, and near the end of the recovery period. No remarkable findings were noted.

Clinical Pathology: Blood samples were taken once pre-dose, and on days 7, 14, and 21 after the initiation of dosing, and near the end of the dosing and recovery periods.

Hematology: No remarkable findings were noted.

Clinical chemistry: No remarkable findings were noted.

Coagulation parameters: Slight and at times statistically significant decreases in activated partial thromboplastin time (APTT) were noted at all doses (see table below, excerpted from sponsor).

Daily Dose (mg base/kg) (A-1043422/A-84538)	0 / 0 (Control)		5 / 5		10 / 5		20 / 10	
Number of Animals	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Hematology	-	-	-	-	-	-	-	-
Coagulation	-	-	-	-	-	-	-	-
Activated Partial Thromboplastin Time (APTT, sec)[a]	15.16	15.52	14.22	14.58*	13.92*	14.94	13.94*	14.04*
Clinical Chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-
Organ Weights[b]								
Thymus								
Absolute weight, g	8.3687	-	3.4977*	-	3.8720*	-	4.5017*	-
% difference from control	-	-	-58	-	-54	-	-46	-
Relative weight, %	0.08630	-	0.04134*	-	0.04317*	-	0.04839*	-
% difference from control	-	-	-52	-	-50	-	-44	-

- No noteworthy findings. * = p<0.05 with Dunnett's test (statistical significance determined relative to T0).

Urinalysis: An attempt was made to collect a urine sample at necropsy. No remarkable findings were noted in analyzed samples.

Gross pathology: Complete necropsies on all animals at the end of dosing or recovery periods. No remarkable findings were noted.

Organ weights: Adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary glands, prostate gland, spleen, testes, thymus, and thyroid with parathyroid glands were taken and weighed at necropsy.

All males treated with ABT-450 had decreased thymus weights at the end of dosing relative to controls (see table below, *excerpted from sponsor*).

Reference ID: 3628623

Dosing Group	Statistic	Spleen (g)		Thymus (g)		Thyroid (g)		Liver (g)	
		Absolute	% BW	Absolute	% BW	Absolute	% BW	Absolute	% BW
1: Control A-1043422/A-84538, 0/0 mg/kg/day	Mean	86.1630	0.88103	8.3687	0.08630	0.6540	0.00675	307.8143	3.20384
	SD	25.5101	0.15203	1.7784	0.01038	0.1267	0.00061	50.0481	0.53572
	Min	67.133	0.7665	6.916	0.0747	0.565	0.0061	250.481	2.7053
	Max	115.150	1.0535	10.352	0.0947	0.799	0.0073	342.764	3.7702
	N	3	3	3	3	3	3	3	3
2: Low A-1043422/A-84538, 5/5 mg/kg/day	Mean	46.5513	0.54551	3.4977	0.04134	0.6703	0.00790	331.4950	3.90550
	SD	20.9391	0.23939	1.0314	0.01287	0.0852	0.00115	14.2780	0.27039
	Min	28.659	0.3477	2.309	0.0266	0.572	0.0066	320.560	3.7392
	Max	69.581	0.8116	4.156	0.0504	0.720	0.0087	347.648	4.2175
	N	3	3	3	3	3	3	3	3
3: Mid A-1043422/A-84538, 10/5 mg/kg/day	Mean	50.4710	0.56374	3.8720	0.04317	0.7390	0.00825	283.9723	3.16632
	SD	7.4538	0.09047	0.9165	0.01000	0.2086	0.00234	16.8814	0.14265
	Min	42.589	0.4723	3.323	0.0366	0.502	0.0056	270.034	3.0728
	Max	57.406	0.6532	4.930	0.0547	0.895	0.0098	302.743	3.3305
	N	3	3	3	3	3	3	3	3
4: High A-1043422/A-84538, 20/10 mg/kg/day	Mean	66.0313	0.70350	4.5017	0.04839	0.7010	0.00749	334.1917	3.57312
	SD	3.8726	0.03910	1.8570	0.02124	0.0710	0.00097	23.3376	0.43223
	Min	61.652	0.6727	3.126	0.0313	0.658	0.0066	310.400	3.1052
	Max	69.004	0.7475	6.614	0.0722	0.783	0.0085	357.047	3.9575
	N	3	3	3	3	3	3	3	3
Results of Statistical Analysis:		NS	NS	2,3,4<1	2,3,4<1	NS	NS	NS	NS

Notes: (1) "NS" means that no statistically significant difference from Group 1 was found.
(2) The symbols "1", "2", "3" and "4" represent Group 1, Group 2, Group 3 and Group 4 in the presentation of the Results of Statistical Analysis.

Females in the 20/10 mg/kg dose group had increased thymus weights relative to controls. A dose dependent decrease in pituitary weight among females at the 20/10 mg/kg dose was noted.

Dosing Group	Statistic	Kidneys (g)		Adrenal (g)		Pituitary (g)		Spleen (g)	
		Absolute	% BW	Absolute	% BW	Absolute	% BW	Absolute	% BW
1: Control A-1043422/A-84538, 0/0 mg/kg/day	Mean	32.8930	0.46912	1.1167	0.01590	0.0627	0.00089	66.9183	0.95004
	SD	0.8460	0.02548	0.1386	0.00169	0.0090	0.00010	22.0181	0.28643
	Min	32.315	0.4419	1.003	0.0140	0.054	0.0008	47.356	0.6614
	Max	33.864	0.4925	1.271	0.0173	0.072	0.0010	90.763	1.2342
	N	3	3	3	3	3	3	3	3
2: Low A-1043422/A-84538, 5/5 mg/kg/day	Mean	31.4720	0.45875	1.1457	0.01664	0.0533	0.00078	65.5137	0.94790
	SD	1.5758	0.02602	0.2547	0.00334	0.0101	0.00017	43.6551	0.61236
	Min	29.841	0.4289	0.885	0.0134	0.044	0.0006	36.963	0.5313
	Max	32.986	0.4769	1.394	0.0200	0.064	0.0010	115.767	1.6510
	N	3	3	3	3	3	3	3	3
3: Mid A-1043422/A-84538, 10/5 mg/kg/day	Mean	28.1773	0.41709	1.1343	0.01695	0.0410	0.00062	84.7153	1.25675
	SD	2.0541	0.02746	0.1102	0.00334	0.0106	0.00020	30.3384	0.44861
	Min	25.889	0.3854	1.021	0.0148	0.029	0.0004	51.179	0.7407
	Max	29.862	0.4337	1.241	0.0208	0.049	0.0008	110.253	1.5533
	N	3	3	3	3	3	3	3	3
4: High A-1043422/A-84538, 20/10 mg/kg/day	Mean	30.7817	0.45738	1.0873	0.01614	0.0307	0.00046	48.5363	0.71802
	SD	1.5499	0.03532	0.0872	0.00135	0.0105	0.00017	11.0503	0.14541
	Min	28.992	0.4186	0.992	0.0146	0.020	0.0003	42.118	0.6194
	Max	31.677	0.4877	1.163	0.0170	0.041	0.0006	61.296	0.8850
	N	3	3	3	3	3	3	3	3
Results of Statistical Analysis:		3<1	NS	NS	NS	4<1	4<1	NS	NS

Notes: (1) "NS" means that no statistically significant difference from Group 1 was found.
(2) The symbols "1", "3" and "4" represent Group 1, Group 3 and Group 4 in the presentation of the Results of Statistical Analysis.

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

Changes in gall bladder histopathology were noted in high dose animals. Microscopic effects included epithelial cell degeneration accompanied by luminal aggregates of anucleate, eosinophilic, epithelial cell remnants. In HD females, edema of the lamina propria was associated with the mucosal changes. Once the gall bladder was identified as the target organ, no other tissues were examined microscopically at the end of recovery.

Toxicokinetics:

Reference ID: 3628623

Mean (± SD) Toxicokinetic Parameters for A-1043422

Test Group	Dose (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 AUC_{0-24} (µg•hr/mL)	Day 28 C_{max} (µg/mL)	Day 28 T_{max} (hr)	Day 28 AUC_{0-24} (µg•hr/mL)
2	5	male	18.9	3.0	86.7	15.8	3.0	70.9
			±6.04	±0.0	±13.3	±6.65	±0.0	±29.8
		female	22.5	3.0	94.1	27.3	3.6	105
			±6.97	±0.0	±18.8	±7.20	±1.3	±26.8
		overall	20.7	3.0	90.4	21.6	3.3	88.1
			±6.43	±0.0	±15.8	±8.92	±0.9	±32.3
3	10	male	40.6	3.6	231	44.8	3.6	261
			±19.8	±1.3	±131	±10.3	±1.3	±32.4
		female	41.7	2.6	197	52.0	3.0	268
			±22.2	±0.9	±108	±13.7	±0.0	±63.5
		overall	41.2	3.1	214	48.4	3.3	264
			±19.8	±1.2	±115	±12.0	±0.9	±47.6
4	20	male	93.5	4.8	719	88.3	3.0	635
			±16.0	±1.6	±145	±30.5	±0.0	±298
		female	51.9	3.6	360	95.3	3.6	654
			±33.7	±1.3	±261	±27.2	±1.3	±251
		overall	72.7	4.2	540	91.8	3.3	645
			±33.2	±1.5	±275	±27.5	±0.9	±260

Collection Interval	Tissue	A-1043422/A-84538 Dosage (mg/kg/day) 5/5	10/5	20/10
Dosing Phase	Plasma (µg/mL)[a]	0.041/0.00	0.106/0.00	0.647/0.00
	Liver (µg/g)[a]	0.24/0.00	1.49/0.00	5.27/0.72
	Liver:Plasma Ratio	5.9/NA	14.1/NA	8.1/NA
Recovery Phase	Plasma (µg/mL)[a]	0.00/0.00	0.00/0.00	0.00/0.00
	Liver (µg/g)[a]	0.00/0.00	0.00/0.00	0.00/0.00
	Liver:Plasma Ratio	NA/NA	NA/NA	NA/NA

a. Values represent combined sex mean; NA – not applicable

Study title: Thirteen-week Oral Capsule Toxicity Study of A-1043422 and A-84538 (Ritonavir) in Beagle Dogs

Key study findings:

- Beagle dogs were co-administered ABT-450 (A-1044322) and ritonavir (A-84538) for 13 weeks (91 to 94 days).
- There were no toxicologically significant findings.
- The NOAEL was defined as the highest dose tested (40/20 mg/kg day), corresponding to Day 91 mean systemic exposures (AUC) of 1130 µg*hr/mL and 737 µg*hr/mL in males and females, respectively.

Study no.: Study no. R&D/09/053 (TB08-433)
Volume #, and page #: vol. 7, p.1
Conducting laboratory and location: Abbott Laboratories
Toxicology and Safety Pharmacology
100 Abbott Park Road,
Abbott, Park, IL
Date of study initiation: 14 January 2009
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: ABT-450 (A-1043422)/RTV (A-84538), lot no. 85585-21/69514TL, 99.3% purity

Methods

Doses: 0/0, 5/5, 10/5, 20/10 (QD) or 40/20 (20/10 BID) mg/kg/day

Species/strain:	Dog/Beagle
Number/sex/group:	5/sex/dose
Route, formulation, volume:	Liquid formulation in oral capsule; 0.5 mL/kg/day
Age:	8- 10 months
Weight:	6.11 – 8.43 kg

Results:

Mortality: Animals were assessed twice daily for morbidity/mortality. There were no early deaths during the study.

Clinical signs: General clinical signs were assessed twice daily. Detailed observations were performed twice weekly. Clinical signs were limited to post-dose emesis, abnormal feces, and salivation that increased in incidence and severity with dose.

Body weights: Body weights were recorded twice weekly. There were no significant effects on body weight.

Food consumption: Food consumption was recorded twice weekly. There were no significant effects on food consumption.

Ophthalmologic Examination: Examinations were conducted prior to the start of dosing and near the end of the dosing period. No ocular abnormalities were noted.

Electrocardiogram Examinations: ECG tracings were obtained from animals pre-dose, during the first week of dosing, and near the end of the dosing period. There were no remarkable findings.

Clinical Pathology: Blood samples were collected once during the pre-dose period, and at approximately one and two months during the dosing period.

Hematology: Decreased red cell mass (decreases in RBCs, hemoglobin, and hematocrit) at the 40/20 and 20/10 doses were attributed to ritonavir, as those findings were previously reported in studies of ritonavir alone.

Clinical chemistry: Alkaline phosphatase activity was increased two- to five fold in 40/20 dose group animals, without microscopic correlate. The change is possibly due to ritonavir, as increased ALP has been observed when RTV is administered alone to dogs. However, increased ALP was noted in the 4-week study described above, where the gall bladder was identified as a target organ. One high dose (40/20) female had a two- to six-fold increase in ALT without microscopic correlate.

Coagulation parameters: There were no toxicologically significant effects of ABT-450/RTV administration.

Urinalysis: An attempt was made to collect urine from dogs at necropsy. There were no toxicologically significant effects of ABT-450/RTV administration.

Gross pathology: There were no toxicologically significant effects of ABT-450/RTV administration.

Organ weights: Weights were recorded for the following organs: adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, thyroid and parathyroid. There were no toxicologically significant effects of ABT-450/RTV administration.

Reference ID: 3628623

Histopathology: Adequate Battery: yes
Peer review: yes

There were no toxicologically significant effects of ABT-450/RTV administration.

Toxicokinetics
Venous samples of blood were collected from each dog at approximately 1, 3, 6, 9, 12, and 24 hours after dosing on Days 1 and week 4, 8 and near the end of dosing. Toxicokinetic parameters are included in the tables below (*excerpted from sponsor*). In contrast to rats, neither sex-related differences nor repeated-dose effects on exposures were noted.

Mean (±SD) Toxicokinetic Parameters for A-1043422

			Day 1			Day 91		
Test Group	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
2	5	male	24.6	3.0	98.2	11.9	3.0	51.3
		female	11.0	4.0	43.0	8.27	3.0	36.7
3	10	male	42.0	3.0	212	31.2	3.0	170
		female	56.0	3.0	292	53.1	3.0	242
4	20	male	87.9	4.0	638	91.3	4.0	769
		female	97.7	4.0	626	87.3	3.0	530
5	40	male	79.9	3.0	866*	86.1	2.3	1130
		female	81.3	4.0	1000*	64.1	2.3	737

Mean (±SD) Toxicokinetic Parameters for A-84538

			Day 1			Day 91		
Test Group	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
2	5	male	4.55	3.0	13.2	3.48	3.0	10.2
		female	0.73	4.0	2.2	0.97	3.0	3.4
3	5	male	3.73	3.0	11.0	3.36	3.0	10.8
		female	3.29	3.0	9.53	4.56	3.0	14.7
4	10	male	9.49	3.0	32.0	10.9	3.0	49.5
		female	9.84	3.0	37.3	10.9	3.0	37.0
5	20	male	7.14	3.0	46.9*	6.14	2.3	46.6
		female	7.74	3.0	49.7*	4.87	2.3	34.9

Study title: 9-Month Oral Capsule Dose Toxicity Study with A-1043422 and A-84538 in Beagle Dogs followed by a 1-Month Recovery Period

Key study findings:
- Beagle dogs were co-administered ABT-450 (A-1044322) and ritonavir (A-84538) for 9 months.
- The NOAEL was defined as the middle dose (20/10 mg/kg day) based on histopathological findings in gallbladder at the high dose, corresponding to Day 271 mean systemic exposures (AUC) of 615 µg*hr/mL (males and females combined).

Study no.: Study RD091026 (Sponsor study no. TB09-147)
Volume #, and page #: Electronic (SN0088)
Conducting lab and location: (b) (4)

Reference ID: 3628623

(b) (4)

Date of study initiation:	20 August 2009
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	ABT-450 (A-1043422), Lot No. 74983PP01, 96% purity /RTV (A-84538), Lot No. 76164TL01, 99% purity

Methods

Doses:	5/5 (QD), 20/10 (QD) and 40/10 (BID) mg/kg/day (A-1043422/A-84538)
Species/strain:	Dog/Beagle
Number/sex/group:	4/sex/dose (+2 control/high dose for recovery)
Route, formulation, volume:	Cremophor™EL:Polyethylene Glycol (PEG) 400:Oleic acid (10:10:80, w/w/w); gelatin capsules, 0.5 mL/kg/day
Age:	6 - 7 months
Weight:	males: 7.07 – 9.26 kg females: 6.55 – 7.68 kg

Note regarding high dose selection:
Exploratory pharmacokinetic studies indicate that maximum A-1043422 exposures via daily (QD) dosing are obtained at 40/10 mg (A-1043422/A-84538)/kg body weight. Computer modeling suggested that appreciable increases in A-1043422 exposures can be obtained via twice daily (BID) (12 hour) dosing of 40/10 mg/kg/dose (80/20 mg/kg/day). Based upon this information, the high dose of 80/20 mg/kg/day (40/10 mg/kg/dose BID 12 hours) was expected to produce maximum feasible exposures of A-1043422.

Results:

Mortality: Animals were assessed twice daily for morbidity/mortality. There were no early deaths.

Clinical signs: General clinical signs were assessed twice daily. Detailed observations were performed twice weekly. Clinical signs were primarily in high dose (80/20 mg/kg) animals and included increased incidences of abnormal (soft, mucoid, discolored, or watery) feces, salivation, and vomitus/emesis.

Body weights: Body weights were recorded weekly. Individual body weight loss of up to 24% was noted in high dose animals. The peak magnitude of decrease for mean body weights was Week 11 for males (11.4%) and Week 2 for females (14.7%) when compared to Week -1 body weight values. However, for individual animals, this magnitude exhibited considerable variation, ranging from 4.4% to 19.6% for males at Week 11 and from 7.0% to 24.0% for females at Week 2.

Food consumption: Food consumption was recorded weekly. Weight loss in high dose animals correlated with decreased food consumption of standard basal diet, resulting in the need to provide supplemental moist canned food (Hills Prescription Diet a/d) to these animals from Week 12 through the end of the study.

Ophthalmologic Examination: Examinations were conducted prior to the start of dosing and prior to necropsy. There were no remarkable findings.

Electrocardiogram Examinations: ECG tracings were obtained from animals pre-dose, during the first week of dosing, and near the end of the dosing period. There were no remarkable findings.

Clinical Pathology: Blood samples were collected pretest, and Day 14 (urine not collected), Week 13, Week 24, one day during the last week of dosing, and during the last week of recovery.

Reference ID: 3628623

Hematology: On Day 14, mean neutrophils were increased 52 and 85% in males and females, respectively, at 80/20 mg/kg/day (A-1043422/A-84538) relative to controls, but these were resolved by Week 13.

Mean platelets were increased 34 to 70% in males from Day 14 through Week 39 relative to controls. In the females at 80/20 mg/kg/day (A-1043422/A-84538) platelets were increased 36 to 74% relative to controls at Weeks 13 through 39, but were more variable and comparable to respective pretest counts. The effects were not seen in high dose animals at the end of the recovery period.

Clinical chemistry: Mean phosphorus was decreased 13, 24, 31, and 21% relative to controls in males from Day 14 through Week 39 at 80/20 mg/kg/day (A-1043422/A-84538). In females at 80/20 mg/kg/day (A-1043422/A-84538) the phosphorus was decreased 9 and 20% on Weeks 13 and 39. The effects were not seen in high dose animals at the end of the recovery period.

Coagulation parameters: The mean activated partial thromboplastin time (APTT) was shortened 8 and 7% in males at 20/10 mg/kg/day (A-1043422/A-84538) at Weeks 13 and 39, and 10, 9, and 11% at 80/20 mg/kg/day (A-1043422/A-84538) at Weeks 13, 24, and 39, relative to controls. In the females, APTT was shortened 10, 13, 11, and 11% at 5/5 mg/kg/day (A-1043422/A-84538), 9, 10, 11, and 10% at 20/10 mg/kg/day (A-1043422/A-84538) relative to controls from Day 14 through Week 39. At 80/20 mg/kg/day (A-1043422/A-84538) the APTT was shortened 9 % at Weeks 13 through 39. None of these changes was considered adverse due to the magnitude or the direction of change. The effects were not seen in high dose animals at the end of the recovery period.

Urinalysis: An attempt was made to collect urine from dogs at necropsy. There were no remarkable findings.

Gross pathology: There were no remarkable findings.

Organ weights: Weights were recorded for the following organs: adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, thyroid and parathyroid. Increased absolute and relative liver weights were observed in terminal 80/20 mg/kg/day (A-1043422/A-84538) males and females when compared to control (0/0 mg/kg/day (A-1043422/ 0 A-84538)) males and females. At recovery, there was a slight increase in absolute and relative liver weights in 80/20 mg/kg/day (A-1043422/A-84538) males and females when compared to controls. There were no microscopic correlates to account for the increased liver weights.

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Test Article-related Organ Weight Changes - Terminal Male and Female (Percent change relative to control) 1 vs 2, 3, 4						
Dose Level (mg/kg)[a]	5/5		20/10		80/20	
Sex	Male	Female	Male	Female	Male	Female
Number Examined	4	4	4	4	4	4
Liver (g)	↑7.3	↑14.4	↑18.2	↑10.3	↑31.8[c]	↑32.3[b]
Liver/Body Weight%	↑9.4	↑12.3	↑27.7[b]	↑27.8[b]	↑35.8[c]	↑30.1

[a]Dose level A-1042422/A-84538 (mg/kg/day) ↑ - Increased
[b]Significantly different from control; ($p<0.05$)
[c]Significantly different from control; ($p<0.01$)

Test Article-related Organ Weight Changes - Recovery Male and Female (Percent change relative to control) 1 vs 4		
Dose level (mg/kg)[a]	80/20	
Sex	Male	Female
Number Examined	2	2
Liver (g)	↑15.0	↑8.4
Liver/Body Weight%	↑23.7	↑13.6

[a]Dose level A-1042422/A-84538 (mg/kg/day) ↑ - Increased

Histopathology: Adequate Battery: yes
Peer review: yes

A-1043422/A-84538-related microscopic findings were limited to the small intestines (duodenum and/or jejunum) of terminal and recovery males and females administered ≥ 20/10 mg/kg/day (A-1043422/A-84538), gallbladders of terminal males and females administered 80/20 mg/kg/day (A-1043422/A-84538) and livers and kidneys of terminal and recovery males and females administered 80/20 mg/kg/day (A-1043422/A-84538).

In the small intestine (duodenum and/or jejunum), there was minimal to moderate lamina propria vacuolation in the villar tips confirmed as lipid with Oil Red O stain. The intestinal changes were considered non-adverse based on the minimal nature of the finding and the lack of significant clinical chemistry correlates. Minimal lamina propria lipid accumulation persisted in the recovery males and females administered 80/20 mg/kg/day (A-1043422/A-84538).

In the gallbladder, there was minimal edema in three terminal males and one terminal female administered 80/20 mg/kg/day (A-1043422/A-84538). In addition there was one terminal male administered 80/20 mg/kg/day (A-1043422/A-84538) with minimal multifocal necrosis of the gallbladder epithelium. In addition, one 80/20 mg/kg/day (A-1043422/A-84538) terminal female had mild calculi (mineral) accumulations within the gallbladder lumen, however, no indication of mucosal wall damage was observed. The gallbladder changes were considered non-adverse based on the minimal nature of the findings and the lack of clinical chemistry correlates. The gallbladder changes were not observed in recovery animals.

In the liver there was mild, diffuse intrasinusoidal cell vacuolation in males and females administered 80/20 mg/kg/day (A-1043422/A-84538) characterized by a single large vacuole. The vacuolation was not

considered adverse in the absence of associated hepatocellular changes. Mild intrasinusoidal vacuolation persisted in the recovery males and females administered 80/20 mg/kg/day (A-1043422/A-84538).

In the kidneys, there was minimal to mild, diffuse vacuolation of renal tubular epithelium in males and females administered 80/20 mg/kg/day (A-1043422/A-84538). This change was not considered adverse based on the minimal nature of the change and lack of significant clinical chemistry correlates. Minimal renal tubular epithelial vacuolation persisted in the recovery males and females administered 80/20 mg/kg/day (A-1043422/A-84538).

Toxicokinetics

Venous samples of blood were collected from each dog at approximately 1, 3, 6, 9, 12, and 24 hours after dosing on Days 1 and weeks 13 and 39. Toxicokinetic parameters are included in the tables below (*excerpted from sponsor*).

Mean Toxicokinetic Parameters for A-1043422

A-1043422/A-84538 Dose Level (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 AUC (µg•hr/mL)	Day 271 C_{max} (µg/mL)	Day 271 T_{max} (hr)	Day 271 AUC (µg•hr/mL)
5/5	Male	17.3	3.3	80.7	13.6	2.0	58.7
	Female	21.9	2.5	100	16.3	2.5	72.3
	Overall	19.6	2.9	90.6	15.0	2.3	65.5
20/10	Male	79.8	3.8	780	73.0	3.0	689
	Female	85.1	3.8	898	69.4	3.0	542
	Overall	82.4	3.8	839	71.2	3.0	615
80/20	Male	108	4.0	1860	106	2.7	1270
	Female	116	5.0	2000	118	3.2	1720
	Overall	112	4.5	1930	112	2.9	1490

Mean Toxicokinetic Parameters for A-84538

A-1043422/A-84538 Dose Level (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 AUC (µg•hr/mL)	Day 271 C_{max} (µg/mL)	Day 271 T_{max} (hr)	Day 271 AUC (µg•hr/mL)
5/5	Male	3.20	2.5	11.0	1.98	2.0	6.83
	Female	3.13	2.5	10.7	3.83	2.5	14.3
	Overall	3.16	2.5	10.8	2.91	2.3	10.5
20/10	Male	11.6	2.5	55.0	12.4	3.0	58.3
	Female	9.76	3.3	47.2	5.67	3.0	28.1
	Overall	10.7	2.9	51.1	9.03	3.0	43.2
80/20	Male	7.65	3.2	69.6	5.94	2.7	39.8
	Female	6.46	3.5	65.6	4.48	2.3	36.0
	Overall	7.05	3.3	67.6	5.21	2.5	38.0

Reference ID: 3628623

Genetic Toxicology

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

Study title: *Salmonella-Escherichia coli*/Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with ABT-450

Key findings: The results of this study indicate that, under the conditions tested, ABT-450 (A-1043422) did not cause a positive increase in the mean number of revertants per plate with any of the tester strains in the presence or absence of Aroclor™ 1254-induced rat liver S9, and is therefore considered negative in this assay.

Study no.: 6161-423 (Sponsor Report no. R&D/08/1363)

Volume #, and page #:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	June 27, 2008
GLP compliance:	yes
QA reports:	yes
Drug, lot #, and % purity:	ABT-450, Lot Number 65801PP00, 96.1%

Methods

Strains/species/cell line: *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2*uvr*A.

Doses used in definitive study: Doses tested in the confirmatory mutagenicity assay, using the preincubation method were 100, 333, 667, 1000, 3330 and 5000 µg/plate for all tester strains in the presence and absence of S9 mix.

Basis of dose selection: The concentrations tested in the initial and confirmatory mutagenicity assays were selected based on the results of a dose range-finding study using tester strains TA100 and WP2*uvr*A and ten concentrations of test article ranging from 6.67 to 5000 µg per plate, one plate per level, both in the presence and absence of S9 mix, using both the plate incorporation and pre-incubation plating methods.

Negative controls: dimethylsulfoxide (DMSO)

Positive controls: see table below (*excerpted from sponsor*)

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	Benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	Sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	Sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0*

* 0.4 µg/plate for the preincubation exposure method

Incubation and sampling times: 52 ± 4 hours at 37 ± 2°C.

Results

Reference ID: 3628623

Study validity: All validity criteria were met.

Study outcome: The results of this study indicate that, under the conditions tested, ABT-450 (A-1043422) did not cause a positive increase in the mean number of revertants per plate with any of the tester strains in the presence or absence of Aroclor™ 1254-induced rat liver S9, and is therefore considered negative in this assay.

In Vitro Assays in Mammalian Cells

Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with ABT-450

Key findings: The test article, A-1043422, was considered positive for inducing chromosomal aberrations in human lymphocytes and negative for inducing polyploidy or endoreduplication under conditions without and with metabolic activation.

Study no.: 6161-424 (Sponsor Report no. R&D/08/1364)

Volume #, and page #: 16, p. 84
Conducting laboratory and location: (b) (4)
Date of study initiation: June 27, 2008
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:

Test article	Lot No.	Storage	Potency	Retest Date
A-1043422 (A-1043422.0 (free acid form), A-1043422 free acid intermediate, ABT-450, Molecular Weight of A-1043422 is 765.88)	65801PP00	(b) (4)	96.1%	01 Dec 2008

Methods

Strains/species/cell line: cultured human peripheral blood lymphocytes without and with S9 for metabolic activation

Doses used in definitive study: In the chromosomal aberrations assay, concentrations of 100, 200, 300, 350, 400, 450, 500, 575, 650, and 800 µg/mL were tested without metabolic activation (3-hour treatment); 7.50, 15.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 100, and 125 µg/mL were tested without metabolic activation (~22-hour treatment); and 50.0, 80.0, 125, 160, 200, 250, 300, 350, 400, and 500 µg/mL were tested with metabolic activation (3-hour treatment).

All cultures were harvested ~22 hours from the initiation of treatment.

Basis of dose selection: Observed cytotoxicity in a dose range-finding study.

Negative controls: see table below (*excerpted from sponsor*)

Control Article	CAS No.	Supplier	Lot/Batch No.	Purity	Expiration Date
DMSO	67-68-5	Sigma-Aldrich	01533MH 00196JJ	99.99% 99.98%	30- Jul 2011 15 Aug 2011

Reference ID: 3628623

Positive controls: see table below (*excerpted from sponsor*)

Control Article	CAS No.	Supplier	Lot No.
Mitomycin C (MMC)	50-07-7	Sigma-Aldrich	018K1619
Cyclophosphamide (CP)	6055-19-2	Sigma-Aldrich	036K1225

Incubation and sampling times: 3-hour with and without metabolic activation; 22-hour without metabolic activation

Results

Study validity: All criteria for a valid study were met.

Study outcome: The test article, A-1043422, was considered positive for inducing chromosomal aberrations in human lymphocytes and negative for inducing polyploidy or endoreduplication under conditions without and with metabolic activation.

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

Study title: *In Vivo* Rat Micronucleus Assay with ABT-450

Key findings: The test article did not increase the micronucleus frequency in the PCEs at any test article doses examined (500, 1000, and 2000 mg/kg) at 24 and 48 hours. In addition, A-1043422 was not cytotoxic to the bone marrow (i.e., no statistically significant decreases in the PCE:NCE ratios) at any the test article dose examined.

Study no.: 6161-425 (Sponsor Report no. R&D/08/1365)

Volume #, and page #: 16, p. 170
Conducting laboratory and location: (b) (4)
Date of study initiation: June 26, 2008
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: ABT-450, Lot Number 65801PP00, 96.1%

Methods

Strains/species/cell line: Rat, CD®(SD) IGS BR

Doses used in definitive study: 500, 1000, 2000 mg/kg

Basis of dose selection: Lack of toxicity in dose range-finding study

Negative controls: Oleic Acid: Cremophor EL: PEG400 (80:10:10 w/w)

Positive controls: Cyclophospamide (60 mg/kg)

Incubation and sampling times: see table below (*excerpted from sponsor*)

Reference ID: 3628623

Dosing Scheme for the Micronucleus Assay

Target Dose Level (mg/kg)	Stock Concentration (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Animals/Harvest Timepoint 24 Hour Male	48 Hour Male	Pharmacokinetic Animals* Male
Positive Control, 60	6	10	Oral Gavage	5	-	-
Vehicle Control, 0	0	10	Oral Gavage	5	5	3
A-1043422, 500	50	10	Oral Gavage	5	-	6
A-1043422, 1000	100	10	Oral Gavage	5	-	6
A-1043422, 2000	200	10	Oral Gavage	5	5	6

Vehicle Control = Oleic Acid: Cremophor EL: PEG400 (80:10:10 w/w), Positive Control = Cyclophosphamide
*Three rats/dose group were bled for possible measurement of whole blood levels at approximately 1, 6, and 24 hours postdose and three rats/dose group were bled at approximately 0.5, 3, and 12 hours postdose. In addition, three rats were dosed with the vehicle control and bled approximately 1 hour postdose.

Results

Study validity: Bone marrow from five animals per dose group was analyzed. Micronucleus frequency was determined by analyzing at least 2000 polychromic erythrocytes from each animal. The results of toxicokinetic analysis confirmed the exposure of the bone marrow to A-1043422 and its metabolite in this study. The vehicle control group had less than the published value of approximately 0.4% micronucleated PCEs and the group mean was within the historical control range for males. The positive control group had a statistically significantly higher ($p < 0.01$) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.

Study outcome: A-1043422 was determined to be negative in the rat bone marrow micronucleus assay under the conditions of this assay.

Other Genetic Toxicity Studies

Study title: Comet Assay in Rat Liver Cells Following Oral Administration of A-1043422

Key findings: Following administration to rats on two consecutive days (500, 1000, and 2000 mg/kg/day), ABT-450 (A-1043422) was found not to induce DNA damage in liver cells and was considered negative in the Comet assay.

Study no.: Study RD081786 ((b) (4) study no. AC21LD.423.BTL)

Volume #, and page #: SN 004/vol 1, p. 1
Conducting laboratory and location: (b) (4)
Date of study initiation: 10 November 2008
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity: ABT-450, Lot Number 65801PP00, 96.1%

Methods

Strains/species/cell line: Rat/Sprague-Dawley (Hsd:SD)

Doses used in definitive study: 500, 1000, 2000 mg/kg/day

Basis of dose selection: The doses were selected based on the previous in vivo micronucleus assay in rats (Study no. TA08-207).

Reference ID: 3628623

Negative controls: 0.9% Sodium chloride for injection

Positive controls: Ethyl methanesulfonate

Incubation and sampling times: Animals were dosed orally (gavage) on two consecutive days. Animals were sacrificed and organs collected approximately 3 hours following the dose on day 2.

Results

Study validity: All validity criteria were met.

Study outcome: The results of this study indicate that, under the conditions tested at doses up to 2000 mg/kg/day, ABT-450 (A-1043422) did not induce DNA damage in liver cells and was considered negative in the Comet assay.

Carcinogenicity

Study title: 26 –Week Oral Gavage Oncogenicity Study with A-1043422 and A-84538 in Model 001178-T (Hemizygous) CBYB6F1-Tg(HRAS)2Jic mice

Key Study Findings

- There were no dose-related neoplastic findings.
- Non-neoplastic microscopic findings were limited to inflammation and hyperplasia effects in gall bladder.
- At the NOAEL of 300/30 mg/kg, the estimated mean AUC on Day 87 was 269 μg*hr/mL for A-1043422.

Study no.:	Study RD12179 (b) (4) Study Number 126-641 ABBVIE Study Number: TD11-120
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	31 January 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ABT-450: Lot No. 88310PP11, 92.4% (93.7% week 20) RTV: Lot No. 04301TL, 99.2%
CAC concurrence:	Yes

Adequacy of Carcinogenicity Study: Mice were exposed to adequate concentrations of test article, and assessments were sufficient to determine the carcinogenicity of test article.

Appropriateness of Test Models: The transgenic mouse species used here is an appropriate model in which to assess the carcinogenicity of chemical agents.

Reference ID: 3628623

Evaluation of Tumor Findings: There were no tumor findings associated with test article administration.

Methods

Doses:	A-1043422/A-84538) at dose levels of 6/30, 60/30, and 300/30 mg/kg/day
Frequency of dosing:	once a day
Dose volume:	2 mL
Route of administration:	oral gavage
Formulation/Vehicle:	Cremophor EL®:PED 400:oleic acid (10:10:80, w/w/w)],
Basis of dose selection:	1-month study (max. feasible exposures at high dose) and ECAC agreement
Species/Strain:	Model 00178-T (Hemizygous) CByB6F1-Tg(HRAS)2Jic mice
Number/Sex/Group:	35
Age:	7-9 weeks
Animal housing:	individually in solid bottom cages
Paradigm for dietary restriction:	none
Dual control employed:	Yes (water)
Interim sacrifice:	None
Satellite groups:	Positive controls, N-Nitroso-N-methylurea (NMU (15/sex) Sentinel animals for serological health screen purposes (10/sex) Toxicokinetic (Taconic model 001178-W (nontransgenic), CByB6F1-Tg(HRAS)2Jic: 6/sex controls or 21/sex treated
Deviation from study protocol:	Deviations noted are not expected to have influenced the conclusions of this study.

Study Design

Group	A-1043422/ A-84538 Dose Level (mg/kg/day)	A-1043422/ A-84538 Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals		Dose Duration (Weeks)
				Males	Females	
			Main Study			
1[a]	0	0	2	35	35	26
2[a]	0	0	2	35	35	26
3	6/30	3/15	2	35	35	26
4	60/30	30/15	2	35	35	26
5	300/30	150/15	2	35	35	26
6	Positive Control	NA	10	15	15	1[b]
			Sentinel			
7[c]	NA	NA	NA	10	10	NA
			Toxicokinetics			
8[a]	0	0	2	3+3[d]	3+3[d]	13
9[a]	0	0	2	3+3[d]	3+3[d]	13
10	6/30	3/15	2	18+3[d]	18+3[d]	13
11	60/30	30/15	2	18+3[d]	18+3[d]	13
12	300/30	150/15	2	18+3[d]	18+3[d]	13

[a]Animals in Group 1 and 8 received the water control only, while animals in Group 2 and 9 received the vehicle control only.
[b]Animals were administered the positive control once on Day 1.
[c]Animals in Group 7 served as sentinel animals for health screening purposes only.
[d]Three animals/sex/group were designated as TK replacements.
NA – Not applicable

Results

Reference ID: 3628623

Mortality: There were 32 unscheduled main study deaths which included: four males and one female at 300/30 mg/kg/day A-1043422/A-84538; seven males and two females at 60/30 mg/kg/day A-1043422/A-84538; two males and one female at 6/30 mg/kg/day A-1043422/A-84538; three males and three females at 0 mg/kg/day (vehicle control); one male and two females at 0 mg/kg/day (water control); one positive control (N-Nitroso-N-methylurea (NMU)) male and five positive control females.

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Unscheduled Deaths of Main Study Terminal Males and Females

Animal No.	Sex	Dose Level	Fate	Fate Day	COD
1015	M	0 mg/kg/day(WC)	EE	72	Undetermined
1061	M	0 mg/kg/day(VC)	EE	113	Undetermined
1063	M	0 mg/kg/day(VC)	EE	136	Lung tumor
1066	M	0 mg/kg/day(VC)	EE	122	Undetermined
1091	M	6/30 mg/kg/day	EE	93	Prob. dosing injury
1104	M	6/30 mg/kg/day	EE	98	Accidental injury
1109	M	60/30 mg/kg/day	EE	38	GI tympanites
1119	M	60/30 mg/kg/day	FD	111	Undetermined
1128r	M	60/30 mg/kg/day	EE	115	Undetermined
1129r	M	60/30 mg/kg/day	EE	77	Dosing injury
1130	M	60/30 mg/kg/day	EE	104	Undetermined
1134	M	60/30 mg/kg/day	EE	109	Undetermined
1140	M	60/30 mg/kg/day	EE	115	GI tympanites
1148	M	300/30 mg/kg/day	EE	114	Undetermined
1155	M	300/30 mg/kg/day	EE	94	Hemangiosarcoma/ hemangioma
1159	M	300/30 mg/kg/day	E	66	Accidental
1164	M	300/30 mg/kg/day	ED	30	Not examined
1184	M	Positive Control	EE	182	Sarcoma,Type Undetetermined(Head)
1301	F	0 mg/kg/day(WC)	FD	174	Hemangiosarcoma/ hemangioma
1304	F	0 mg/kg/day(WC)	FD	23	Undetermined
1329	F	0 mg/kg/day(VC)	EE	26	Accidental injury
1333	F	0 mg/kg/day(VC)	EE	169	Dosing injury
1335	F	0 mg/kg/day(VC)	EE	91	Hemangiosarcoma/ hemangioma
1358	F	6/30 mg/kg/day	FD	94	Accidental injury
1391	F	60/30 mg/kg/day	EE	169	Clitoral gland tumor

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1410	F	60/30 mg/kg/day	FD	120	Dosing injury
1450	F	300/30 mg/kg/day	FD	96	Dosing injury
1455	F	Positive Control	FD	181	Lymphoid tumor
1456	F	Positive Control	FD	135	Lymphoid tumor
1459	F	Positive Control	FD	140	Lymphoid tumor
1460	F	Positive Control	EE	163	Vagina tumor
1465	F	Positive Control	FD	135	Hemangiosarcoma/ hemangioma

M – Male
F - Female
FD – Found Dead
E – Euthanized
ED – Euthanized and Discarded
EE – Euthanized *in extremis*

The deaths were sporadic across all groups without dose dependency; the causes of death were variable, incidental and not considered directly related to A-1043422/A-84538 administration. The sponsor's table below describes survival among groups after 27 weeks of dosing.

Number of Animals Surviving to the Scheduled Terminal Necropsy (Week 27)*		
Dose Level	Male	Female
0 mg/kg/day (Water Control)	34/35	33/35
0 mg/kg/day (Vehicle Control)	32/35	32/35
6/30 mg/kg/day	33/35	34/35
60/30 mg/kg/day	28/35	33/35
300/30 mg/kg/day	31/35	34/35
Positive Control	14/15	10/15
*Number of survivors/Number on study		

Clinical Signs: Cageside observations were made twice daily. Detailed clinical examinations were conducted twice weekly during dosing. There were no test article related clinical observations.

Body Weights: No consistent, dose-related effects on body weight and/or body weight gain were noted.

Feed Consumption: No dose-related effects on feed consumption were noted.

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

Findings in animals from the positive-control (NMU) groups (increased incidence of skin masses and/or nodules) were anticipated.

Histopathology

Reference ID: 3628623

Peer Review: Yes.

Neoplastic: None of the statistical tests showed significant tumor incidence results.

Summary of Neoplastic Lesions of Tumor Bearing Animals: Male						
Dose level: group	1	2	3	4	5	6
Number Examined	35	35	35	35	34	15
Harderian gland						
adenoma	0	2	0	0	0	0
Lung						
adenoma, bronchiolar alveolar	3	1	3	3	1	3
carcinoma, bronchiolar alveolar	0	1	0	0	0	0
Multicentric neoplasm						
hemangiosarcoma	1	2	1	1	1	1
lymphoma	0	0	1	0	0	1
Skin						
papilloma, squamous cell	0	0	0	0	0	4
Skin, subcutis						
sarcoma, undifferentiated	0	0	0	0	0	1
Stomach, nonglandular						
papilloma, squamous cell	0	0	1	0	0	9
carcinoma, squamous cell	0	0	0	0	0	5

Group 1 - 0 mg/kg/day (Water Control)
Group 2 - 0 mg/kg/day (Vehicle Control)
Group 3 - 6/30 mg/kg/day
Group 4 - 60/30 mg/kg/day
Group 5 - 300/30 mg/kg/day
Group 6 - Positive Control

Summary of Neoplastic Lesions of Tumor Bearing Animals: Female						
Dose level: group	1	2	3	4	5	6
Number Examined	35	35	35	35	35	15
Clitoral gland						
carcinoma, squamous cell	0	0	0	1	0	0
Harderian gland						
adenoma	1	2	1	1	0	0
Lung						
adenoma, bronchiolar alveolar	2	2	2	0	2	6
carcinoma, bronchiolar alveolar	0	1	0	0	0	1
Mammary						
adenocarcinoma	0	0	0	0	1	0
Multicentric neoplasm						
hemangiosarcoma and hemangioma	1	3	2	0	0	2
lymphoma	0	0	0	0	0	3
Skin						
papilloma, squamous cell	0	0	0	0	0	1
Stomach, nonglandular						
papilloma, squamous cell	0	0	0	0	1	7
carcinoma, squamous cell	0	0	0	0	0	1
Uterus with cervix						
polyp	0	1	0	0	0	7
Vagina						
carcinoma, squamous cell	0	0	0	0	0	1

Group 1 - 0 mg/kg/day (Water Control)
Group 2 - 0 mg/kg/day (Vehicle Control)
Group 3 - 6/30 mg/kg/day
Group 4 - 60/30 mg/kg/day
Group 5 - 300/30 mg/kg/day
Group 6 - Positive Control

Non Neoplastic: Non-oncogenic A-1043422/A-84538-related microscopic findings were limited to the gallbladder in males and females from the middle and high dose animals (60 and 300 A-1043422/30 A-84538 mg/kg/day). Gall bladder histopathology included epithelium hypertrophy/hyperplasia, subacute/chronic inflammation, and/or focal erosion/ulceration.

Increased incidence and/or severities of the hypertrophy/hyperplasia and the subacute/chronic inflammation were observed in middle and high dose females.

Mucosal epithelium hypertrophy/hyperplasia was characterized by locally extensive increases in size and/or number of mucosal epithelial cells elongated and thickened villous-like projections. The hypertrophied/hyperplastic mucosal epithelium was frequently associated with variable thickening/hypertrophy of the underlying muscularis. The mucosal epithelial cells frequently exhibited abundant hypereosinophilic cytoplasm.

Increased incidence and/or magnitude of subacute/chronic inflammation consisted of variable numbers of mixed inflammatory cells (neutrophils, lymphocytes, plasma cells, and/or macrophages) infiltrating the mucosa and infrequently the underlying muscularis and/or serosa.

Mild mucosal erosion/ulceration was observed in a single male at 300/30 mg/kg/day A-1043422/A-84538.

A-1043422/A-84538-related Microscopic Findings

Dose level: group	1		2		3		4		5		6	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number Animals Examined	35	35	35	35	35	35	35	35	34	35	15	15
Number Gallbladders Examined	35	35	34	34	35	35	34	35	34	35	1	5
Gallbladder												
hypertrophy/hyperplasia, epithelium	0	0	0	0	0	0	2	28	7	30	0	0
- minimal	0	0	0	0	0	0	1	10	5	10	0	0
- mild	0	0	0	0	0	0	1	14	2	15	0	0
- moderate	0	0	0	0	0	0	0	4	0	5	0	0
inflammation, subacute/chronic	6	3	1	3	3	5	2	24	7	31	0	0
- minimal	4	3	1	3	3	5	2	21	7	26	0	0
- mild	2	0	0	0	0	0	0	3	0	5	0	0
erosion/ulcer												
- mild	0	0	0	0	0	0	0	0	1	0	0	0

Group 1 - 0 mg/kg/day (Water Control)
Group 2 - 0 mg/kg/day (Vehicle Control)
Group 3 - 6/30 mg/kg/day
Group 4 - 60/30 mg/kg/day
Group 5 - 300/30 mg/kg/day
Group 6 - Positive Control

M - Male
F - Female

Toxicokinetics

The estimated mean AUCs on Day 87 were 1.59, 121 and 269 μg*hr/mL in the low, mid and high dose groups of A-1043422 and 30 mg/kg/day of A-84538, respectively for A-1043422. The mean exposure (AUC) appeared not to be proportional to dose for both sexes at all three dose levels on Day 87. The average Tmax occurred approximately between 3 to 9 hours post dosing.

The plasma concentration data suggest that the exposure on Day 87, as characterized by AUC0-24 and Cmax, was similar for both male and female mice for A-84538. The estimated mean AUCs on Day 87 were 48.6, 41.5 and 35.2 μg*hr/mL in the low, mid and high dose groups of A-1043422 and 30 mg/kg/day of A-84538, respectively for A-84538. The average Tmax occurred approximately between 3 to 9 hours post dosing.

Reference ID: 3628623

Mean Toxicokinetic Parameters for A-1043422				
		Day 87		
A-1043422/A-84538 Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
6/30	Male	0.494	9.0	2.08
	Female	0.231	1.0	1.04
	Overall	0.263	9.0	1.59
60/30	Male	16.8	3.0	76.5
	Female	33.6	6.0	165
	Overall	21.9	6.0	121
300/30	Male	27.8	3.0	143
	Female	57.6	3.0	395
	Overall	42.7	3.0	269

Dosing Solution Analysis: Homogeneity and concentrations were confirmed.

Study title: 104-Week Oral Dose Carcinogenicity Study with A-1043422 and A-84538 in Rats TA10-013

Study no.: TA10-013
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: October 20, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-1043422 (also known as A-1043422.0, A-1043422 free acid, and ABT-450); 88310PP12; 92-94%
CAC concurrence: Yes

Key Study Findings

- There were no dose-related neoplastic findings.
- Multinucleated hepatocytes were seen: They have been noted previously in rats treated with Ritonavir.
- Highest systemic exposures (AUC = 65 µg.hr/mL for males and females combined) were noted in middle dose (60/30 mg/kg) animals.

Adequacy of Carcinogenicity Study: Rats were exposed to adequate concentrations of test article, and assessments were sufficient to determine the carcinogenicity of test article.

Appropriateness of Test Models: The rat species used here is an appropriate model in which to assess the carcinogenicity of chemical agents.

Evaluation of Tumor Findings: There were no tumor findings associated with test article administration.

Reference ID: 3628623

Methods

Doses:	0 (water), 0 (vehicle), 0 (vehicle), 6/30, 60/30, 300/30 mg/kg/day ABT-450/RTV
Frequency of dosing:	Once per day
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Cremophor EL®:PEG 400:Oleic acid (10:10:80, w/w/w)
Basis of dose selection:	3 month study and ECAC concurrence
Species/Strain:	CD® [Crl:CD®(SD)] rats
Number/Sex/Group:	80
Age:	6 weeks
Animal housing:	Adequate (individually housed in suspended, stainless steel, wire-mesh type cages in an environmentally controlled room)
Paradigm for dietary restriction:	Not applicable
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes (toxicokinetics)
Deviation from study protocol:	Listed deviations are not likely to impact study quality.

Results

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. Beginning with Week 53, a third mortality check in the evening was conducted. A detailed clinical examination was performed weekly during the study. The examinations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, atypical behavior, and the palpation of masses. The location, appearance, and size of the masses were documented beginning in Week 26.

Mortality: No test article-related or dose level-dependent changes in survival were observed.

Number of Animals Surviving to the Scheduled Terminal Necropsy (Week 105 for Males and Week 99 for Females)*		
A-1043422/A-84538 Dose Level (mg/kg/day)	Male (Week 105 Necropsy)	Female (Week 99 Necropsy)
0/0 (Water Control)	26/80	20/80
0/0 (Combined Vehicle Control)	46/160	48/160
6/30	22/80	20/80
60/30	19/80	25/80
300/30	23/80	30/80
*Number of Survivors/Number on Study		

Clinical Signs: There were no treatment related or dose related clinical observations, including the occurrences of masses.

Body Weights: Body weights for all animals were measured and recorded once weekly for Weeks 1 through 14, every 4 weeks thereafter, and then during Week 104.

Reference ID: 3628623

Decreases in mean body weight gain in males at all A-1043422/A-84538 doses and in females at the two highest A-1043422/A-84538 doses were not clearly dose related, were not substantial (did not exceed 11%), and were not considered adverse.

Feed Consumption: Food consumption for each main study animal was measured and recorded once weekly for Weeks 1 through 14, every 4 weeks thereafter, and then during Week 104 (for males).

In treated animals, food consumption was decreased compared with water controls, but increased (as much as 24%) compared with combined (water and vehicle) control groups. The changes were not clearly dose related, and not considered to be adverse.

Gross Pathology: No dose related macroscopic findings were noted. The only macroscopic observations related to treatment occurred in the groups receiving the Cremophor EL®:PEG 400:Oleic acid vehicle alone or combined with the test material. Tan or white focus/foci were noted in the lungs of these animals, with greater incidence in males than females. These foci corresponded to necrosis and/or inflammation and were the result of deposition or aspiration of the vehicle into the lungs.

Histopathology

Peer Review: Yes

Neoplastic: No test article-related increases in tumor incidence occurred in either sex. The most common tumor type was pituitary tumors in both males and females and mammary tumors in females. Tumors noted were typical of those seen rats of this strain and age and were considered incidental to test article administration.

Non Neoplastic: The only test article-related non-neoplastic findings occurred in the liver of both sexes and in the kidneys of females. In the liver multinucleated hepatocytes were present in all groups receiving the test material in both sexes with greater incidence in females than males (see sponsor's table below). Incidence and severity occurred without a dose response to the ABT-450 portion of the test material and multinucleated hepatocytes are known to occur in rats treated with Ritonavir. Other than the presence of the multinucleated hepatocytes, no deleterious effects were noted on the liver of affected animals.

Test Article-related Microscopic Observations - Terminal												
Dose level: mg/kg/day	Water		Vehicle		Vehicle		6/30		60/30		300/30	
	Control		Control 1		Control 2							
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number Examined	80	80	80	80	80	80	80	80	80	80	80	80
Liver												
Multinucleated, hepatocytes	0	0	0	0	0	0	1	18	4	6	5	8
-minimal	0	0	0	0	0	0	1	16	2	6	5	7
-mild	0	0	0	0	0	0	0	2	2	0	0	1

M - Male
F - Female

Chronic progressive nephropathy of the kidney was slightly increased in severity in females (but not males) of all groups receiving the test materials, without a dose response to the ABT-450 portion of the test material (see sponsor's table below). There was no corresponding impact on the incidence of renal tumors among females in this study.

Test Article-related Microscopic Observations - Terminal Female						
Dose level: mg/kg/day	Water Control	Vehicle Control 1	Vehicle Control 2	6/30	60/30	300/30
Number Examined	80	80	80	80	80	80
Kidneys						
Nephropathy, chronic progressive	62	52	49	70	57	65
-minimal	47	40	36	28	23	35
-mild	11	11	10	27	23	22
-moderate	4	1	2	13	11	5
-severe	0	0	1	2	0	3

Toxicokinetics

Samples from the treated TK animals were collected at 1, 3, 6, 9, 12, and 24 hours postdose on Days 92 and 182.

Mean A-1043422 toxicokinetic parameters for Day 182 are presented in the following table (*excerpted from sponsor*):

Mean Toxicokinetic Parameters for A-1043422				
		Day 182		
A-1043422/A-84538 Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
6/30	Male	0.113	6.0	0.928
	Female	0.0596	3.4	0.388
	Overall	0.0864	4.7	0.658
60/30	Male	7.62	6.0	49.3
	Female	14.8	5.4	80.7
	Overall	11.2	5.7	65.0
300/30	Male	11.2	4.2	60.1
	Female	11.0	3.6	46.2
	Overall	11.1	3.9	53.2

Dosing Solution Analysis: The Sponsor provided data indicating that A-1043422/A-84538 formulated in vehicle is stable for 31 to 43 days at concentrations that bracket the ones used in this study (b) (4)

Reference ID: 3628623

The results for A-1043422 and A-84538 concentrations ranged from 95 to 105% of theory. The relative standard deviation of A-1043422 and A-84538 concentrations ranged from 0.3 to 1.3%.

Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study title: Amended report for Oral (Gavage) Fertility and General Reproduction Toxicity Study of A-1043422 and A-84538 in Rats, Including a Toxicokinetic Evaluation

Study no.:	20006769/TA10-257
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted] USA
Date of study initiation:	12November2010 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1043422: 88310PP00/92.4% and A-84538: 82554TL01/99.4%

Key Study Findings

- Co-administration of A-1043422 and A-84538 did not affect fertility parameters in male or female rats.
- The NOAEL was defined as 300/30 mg/kg, corresponding to systemic exposures (AUC) of 11.5 (males) and 35.6 (females) at the end of the mating period.

Methods

Doses:	6/30, 60/30 and 300/30 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Cremophor® EL:PEG 400:Oleic Acid (10:10:80, w/w/w)
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25
Satellite groups:	Toxicokinetics (6/group)
Deviation from study protocol:	No significant deviations

Results

This study was performed to assess effects of co-administration with A-1043422 and A-84538 on the estrous cycle, tubal transport, implantation, and development of preimplantation stages of the embryos of female rats. The study design permits detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male rat reproductive organs. In addition, the toxicokinetic characteristics of A-1043422 and A-84538 were determined.

Viabilities, clinical observations, body weights and feed consumption values were recorded for all rats. Estrous cycling was recorded for female rats.
After completion of the cohabitation period, all surviving male rats were euthanized and a gross necropsy was performed. To assess the potential toxicity of the test article on the male reproductive system, reproductive organs were weighed and retained in neutral buffered 10% formalin.

Blood samples were collected from male and female rats assigned to toxicokinetic sample collection for determination of plasma concentration of A-1043422 and A-84538. Samples were collected from each male and female rat at the initiation of cohabitation (on the morning that the main study rats were placed into cohabitation).

Mortality: Two male rats (one each from middle dose and high dose) were euthanized early. Death was attributed to gavage errors. One female rat in the low dose group was euthanized early. Cause of death was gavage error.

Clinical Signs: Excessive salivation (moderate) was seen in high dose males and high dose and middle dose females. Also in those groups, perioral discoloring was noted. The findings were not considered to be toxicologically significant.

Body Weight: There were no treatment-related effects.

Feed Consumption: There were no treatment-related effects.

Toxicokinetics

Summary of Mean Toxicokinetic Parameters for A-1043422 and A-84538 in Male and Female Rats at the End of the Premating Dosage Period

Dosage Level (mg/kg/day)	Males (DS 28)		Females (DS 15)	
	C_{max} (µg/mL)	$AUC_{(0-t)}$ (µg*hr/mL)	C_{max} (µg/mL)	$AUC_{(0-t)}$ (µg*hr/mL)
A-1043422				
6	0.0608 ±0.0268	0.396 ±0.213	0.0388 ±0.0137	0.0752 ±0.0333
60	3.96 ±1.84	21.9 ±9.53	1.45 ±0.995	6.98 ±4.36
300	3.41 ±2.94	11.5 ±7.87	6.40 ±1.13	35.6±11.9
A-84538				
30	2.86 ±0.536	20.2±2.65	3.13 ±0.627	30.3 ±4.41
30	2.16±0.570	15.3±3.63	2.37 ±0.563	21.9±6.35
30	1.84±0.752	12.8±3.64	2.42 ±0.485	29.9±5.08

DS = Day of Study

Dosing Solution Analysis: Dosing solutions were analyzed and were found to meet acceptance criteria for homogeneity and concentration.

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

Co-administration of A-1043422 and A-84538 did not affect fertility parameters in male or female rats.

Embryonic Fetal Development

Study title: An Oral Embryo-fetal Developmental Toxicity Study with A-1043422 and A-84538 in the Rat, Including a Toxicokinetic Evaluation Study TA09021

Key study findings:

- Female rats were administered 30/15, 100/15, or 450/45 ABT-450/RTV mg/kg/day or the vehicle, Cremophor EL:Polyethylene Glycol (PEG) 400:oleic acid (10:10:80, w/w/w), via oral gavage once a day from Gestation Day 6 to 17.
- There were no significant treatment-related effects up to the highest dose tested (450/45 mg/kg ABT-450/RTV; AUC = 59 ± 47)

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Study no.: 126-432
Volume #, and page #: vol. 9, p159 (SN027)

Conducting laboratory and location: (b) (4)

Date of study initiation: July 8, 2009
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: ABT-450 (A-1043422); Lot 85585-21,

Methods

Doses:	30/15, 100/15, and 450/45 ABT-450/RTV mg/kg/day
Species/strain:	Cr1:CD® (SD) rats
Number/sex/group:	25
Route, formulation, volume, and infusion rate:	Cremophor EL:Polyethylene Glycol (PEG) 400:oleic acid (10:10:80, w/w/w), via oral gavage, 2 mL/kg
Satellite groups used for toxicokinetics:	4 (vehicle control) and 5 (treated)/group
Study design:	Female rats were dosed once a day from Gestation Day (GD) 6 to 17,
Parameters and endpoints evaluated:	gravid uterine weights, the total number of corpora lutea, implantations, early and late resorptions, sex and viabilty of fetuses, and malformations and developmental variations

Results

Mortality (dams): Animals were observed twice daily for signs of morbidity/mortality and injury. There were no early deaths.

Clinical signs (dams): Detailed clinical observations were performed once a day during the dosing period. There were no significant clinical signs.

Body weight (dams): Body weights were recorded on GD 0 and daily from GD 6 to 20. There were no significant treatment-related effects.

Food consumption (dams): Food consumption was recorded on GD 0 and daily from GD 6 to 20.There were no significant treatment-related effects.

Toxicokinetics: Samples were collected from the treated animals prior to dosing and at 1, 3, 6, 12, and 24 hours after dosing on GD 6 and 17. Toxicokinetic parameters for ABT-450 (A-1043422) are shown below (*table excerpted from sponsor*).

Mean (±SD) Toxicokinetic Parameters for A-1043422

		GD 6			GD 17		
Group	Dose A-1043422/RTV (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)
6	30/15	1.69 ±1.04	6.0 ±0.0	5.73 ±1.58	0.770 ±0.272	5.4 ±1.3	2.31 ±0.542
7	100/15	6.97 ±2.35	4.2 ±1.6	42.8 ±15.1	7.27 ±9.87	4.2 ±1.6	45.2 ±68.6
8	450/45	8.11 ±2.53	7.8 ±9.1	65.8 ±23.6	10.0 ±7.18	4.2 ±1.6	58.6 ±47.1

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): There were no significant treatment-related effects.

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Offspring (malformations, variations, etc.): There were no significant treatment-related effects.

Study title: A Range-Finding Oral (Gavage) Developmental Toxicity Study with A-1043422 and A-84538 in Mice, Including a Toxicokinetic Evaluation

Key study findings:

- In this dose range-finding study, mice were administered 100/10, 100/100, 300/30 or 300/100 mg/kg/day (A-1043422/RTV) via oral gavage from GD 6 – 15.
- There were no adverse effects on maternal health or fetal development.
- Maximum exposures were achieved at the 300/30 mg/kg dose (AUC of 1340 µg•hr/mL at GD 15).

Study no.: CHI00414 (Sponsor study no. R&D/09/891)
Volume #, and page #: Electronic (SN 068)
Conducting laboratory and location: (b) (4)

Date of study initiation: July 13, 2009
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: ABT-450, lot 74983PP01, 99.69%

Methods

Doses:	0, 100/10, 100/100, 300/30 and 300/100 mg/kg/day (A-1043422/RTV)
Species/strain:	Crl:CD1(ICR) mice
Number/sex/group:	6
Route, formulation, volume, and infusion rate:	Cremophor EL:Polyethylene Glycol (PEG) 400:oleic acid (10:10:80, w/w/w), via oral gavage, 2 mL/kg
Satellite groups used for toxicokinetics:	30/group
Study design:	Female mice were dosed once a day from Gestation Day (GD) 6 to 15,
Parameters and endpoints evaluated:	viability, clinical observations, body weights, toxicokinetics, necropsy observations, Caesarean-sectioning and litter observations, including fetal body weights, sex and fetal gross external alterations

Results

Mortality (dams): Mice were checked for morbidity/mortality at least twice a day. There were no early deaths in the toxicity group animals. There were four early deaths in the pharmacokinetic groups (2, 1, and 1 in the 100/100, 300/30 and 300/300 mg/kg/day ABT-450/RTV groups, respectively). Deaths were considered to be related to aspiration of dosing formulation.

Clinical signs (dams): Mice were observed for clinical signs at multiple time points from GD 6 through GD12, and once daily for the remainder of the dosing period. There were no remarkable observations.

Body weight (dams): Body weights were recorded daily during the dosing period and post-dosing period. There were no treatment-related effects on body weight.

Reference ID: 3628623

Toxicokinetics: Blood samples for toxicokinetic analysis were collected at approximately 1, 3, 6, 9, and 24 hours post-dose on GDs 6 and 15. Toxicokinetic parameters for ABT-450 (A-1043422) are shown below (*table excerpted from sponsor*).

Mean Toxicokinetic Parameters for A-1043422							
		GD 6			GD 15		
Dosage Group	Dose (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
II	100	54.2	6.0	527	87.0	3.0	559
III	100	39.9	9.0	516	29.5	6.0	218
IV	300	98.1	3.0	1410	127	3.0	1340
V	300	103	3.0	939	65.2	3.0	545

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): There were no significant treatment-related effects.

Offspring (malformations, variations, etc.):
Fetal body weights (combined, male and female) appeared to be slightly lower in the treated animals but values fell among historical control data at this facility. Observed variations were not unexpected based on historical control data for this mouse species. There were no toxicologically significant findings in this pilot study with 6 mice/dose group.

Study title: Oral (Gavage) Developmental Toxicity Study of A-1043422 and A-84538 in Mice, Including a Toxicokinetic Evaluation

Key study findings:
- Female mice were administered 30/30, 100/30 or 300/30 mg/kg/day (ABT-450/RTV) via oral gavage from GD 6 - 15.
- There were no adverse effects on maternal health or fetal development.
- Maximum exposures were achieved at the 300/30 mg/kg dose (AUC of 686 µg•hr/mL at GD 15).

Study no.: CHI00416 (Sponsor study no. R&D/09/1178)
Volume #, and page #: Vol. 2, p. 1 (SN 043)
Conducting laboratory and location: (b) (4)

Date of study initiation: September 28, 2009
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: ABT-450, lot 74983PP01, 99.69%

Methods
Doses: 0, 30/30, 100/30 or 300/30 mg/kg/day (ABT-450/RTV)
Species/strain: Crl:CD1(ICR) mice

Reference ID: 3628623

Number/sex/group:	25
Route, formulation, volume, and infusion rate:	Cremophor EL:Polyethylene Glycol (PEG) 400:oleic acid (10:10:80, w/w/w), via oral gavage, 2 mL/kg
Satellite groups used for toxicokinetics:	50/group
Study design:	Female mice were dosed once a day from Gestation Day (GD) 6 to 15
Parameters and endpoints evaluated:	viability, clinical observations, body weights, toxicokinetics, necropsy observations, Caesarean-sectioning and litter observations, including fetal body weights, sex and fetal gross, external, soft tissue and skeletal evaluations

Results

Mortality (dams): Mice were checked for morbidity/mortality at least twice a day. Early deaths are summarized in the table below (*excerpted from sponsor*). Given the number of deaths in the control group (5), early deaths were not attributed to test article. Deaths were considered to be related to aspiration of dosing formulation and/or gavage errors (i.e., esophageal perforations).

Summary of Mortality in the Main and Toxicokinetic Groups

	A-1043422/RTV Dose Level							
	0/0 mg/kg/day		30/30 mg/kg/day		100/30 mg/kg/day		300/30 mg/kg/day	
	Main	TK	Main	TK	Main	TK	Main	TK
No. Evaluated	25	24	25	50	25	50	25	50
No. Found Dead	1	0	1	0	0	0	1	1
No. Unscheduled Euthanasia	1	0	0	0	0	2	0	0
No. Aborted	1	0	1	0	0	0	0	0
No. Delivered Early	2	0	0	0	0	0	1	0
Total Mortality	5	0	2	0	0	2	2	1
No. Survived to Scheduled Euthanasia	20	24	23	50	25	48	23	49
No. Pregnant at Scheduled Euthanasia	11	21	17	43	22	43	20	47

RTV = A-84538
TK = Toxicokinetic

Clinical signs (dams): Mice were observed for clinical signs at multiple time points from GD 6 through GD12, and once daily for the remainder of the dosing period. Clinical signs related to aspiration of dosing solution were noted in controls and treated groups. There were no remarkable observations.

Body weight (dams): Body weights were recorded daily during the dosing period and post-dosing period. There were no treatment-related effects on body weight.

Toxicokinetics: Blood samples for toxicokinetic analysis were collected at approximately 1, 3, 6, 9, and 24 hours post-dose on GDs 6 and 15. Toxicokinetic parameters for ABT-450 (A-1043422) are shown below (*table excerpted from sponsor*).

Reference ID: 3628623

Mean Toxicokinetic Parameters for A-1043422						
A-1043422/ RTV Dose Level (mg/kg/day)	GD 6			GD 15		
	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
30/30	15.6	3.0	106	23.8	6.0	224
100/30	76.6	1.0	569	68.3	3.0	631
300/30	106	6.0	1260	99.1	3.0	686

GD = Gestation Day

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): There were no significant treatment-related effects.

Offspring (malformations, variations, etc.): There were no significant treatment-related effects.

Prenatal and Postnatal Development

Study title: An Oral (Gavage) Pre- and Postnatal Study with A-1043422 in Combination with A-84538 in Rats, Including a Postnatal Behavioral/Functional Evaluation

Key Study Findings

- No effects were noted on pre and postnatal development.
- The maternal and reproductive no-observable-adverse-effect-level (NOAEL) for co-administration of A-1043422 and A-84538 is the high dose of 300/30 (A-1043422/A-84538) mg/kg/day.
- Likewise, the NOAEL for viability and growth in the offspring is 300/30 (A-1043422/A-84538) mg/kg/day, the highest dose tested.

Study no.: 200021946/TA11-265
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 07February2012 (protocol signed)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-1043422: 88310PP00/92.9% and A-84538: 04301TL/97.7%

Reference ID: 3628623

Methods

Doses: 6/30, 30/30 and 300/30 mg/kg/day
Frequency of dosing: Once daily
Dose volume: 2 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: Cremophor® EL:PEG 400:Oleic Acid (10:10:80, w/w/w)
Species/Strain: Crl:CD(SD) rats
Number/Sex/Group: 25
Satellite groups: Toxicokinetics (5/group)
Study design:

Text Table 1
Experimental Design

Group No.	Test Material	A-1043422/ A-84538 Dose Level (mg/kg/day)	A-1043422/ A-84538 Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Main Study Rats	No. of Toxicokinetic Study Rats
1	Control Article	0	0	2	25	5
2	A-1043422/ A-84538	6/30	3/15	2	25	5
3	A-1043422/ A-84538	30/30	15/15	2	25	5
4	A-1043422/ A-84538	300/30	150/15	2	25	5

Deviation from study protocol: No significant deviations.

Mated F0 generation female Crl:CD(SD) rats were co-administered the test articles (A-1043422 and A-84538) and/or the control article formulations (Cremophor® EL:PEG 400:Oleic Acid [10:10:80, w/w/w]) once daily via oral gavage on Gestation Day (GD) 7 and continuing through either Lactation Day (LD) 14 (rats assigned to the toxicokinetic study), LD 20 (rats assigned to natural delivery that delivered a litter) or GD 24 (rats assigned to natural delivery that did not deliver a litter). F1 generation pups were not directly given the test articles and/or the control article formulations, but may have been exposed during maternal gestation (in utero exposure) or via maternal milk during the lactation period.

Results

Reference ID: 3628623

F_0 Dams

Survival: One rat in each treatment group was found dead. The death of the low dose female was considered to be due to complications at parturition. The death of the middle dose group animal was attributed to gavage error. The cause of death of the high dose rat could not be determined, and was considered to be a spontaneous event. This dam delivered 12 apparently healthy pups prior to her death.

Clinical signs: Excessive salivation was noted in dams treated with ≥ 30/30, and the incidence of urine-stained abdominal fur was increased in high dose animals.

Body weight: There were no treatment effects.

Feed consumption: There were no treatment effects.

Uterine content: Pregnancy was confirmed in 24, 24, 24 and 25 of the 25 mated female rats in the 0 (Control), 6/30, 30/30 and 300/30 (A-1043422/A-84538) mg/kg/day dose groups, respectively.

Necropsy observation: There were no treatment effects.

Toxicokinetics:

Summary of Mean Maternal Toxicokinetic Parameters for A-1043422/A-84538 and Pooled Pup Plasma Concentrations on Day 14/15 Postpartum

Group	Dose Level (mg/kg/day)	C_{max} (µg/mL)	AUC (µg*hr/mL)	T_{max} (hr)	Pooled Pup Plasma Concentrations on LD 15 (µg/mL)
A-1043422					
2	6	0.0835 ± 0.0306	0.345 ± 0.149	3.0 ± 2.7	0.00 ± 0.00 [n=5]
3	30	0.802 ± 0.604	5.36 ± 4.00	3.2 ± 1.8	0.00 ± 0.00 [n=5]
4	300	25.1 ± 27.5	116 ± 133	2.0 ± 1.2	0.0734 ± 0.0194 [n=4]
A-84538					
2	30	3.20 ± 0.231	18.1 ± 5.52	2.8 ± 2.1	0.00 ± 0.00 [n=5]
3	30	2.93 ± 0.709	20.2 ± 7.32	3.2 ± 1.8	0.00 ± 0.00 [n=5]
4	30	2.64 ± 1.6	17.0 ± 10.8	5.5 ± 4.8	0.00 ± 0.00 [n=4]

LD – Lactation Day

Dosing Solution Analysis

Dose preparations were assessed for homogeneity and concentration, acceptance criteria were met for both.

F_1 Generation

Survival: All F1 generation male and female rats survived to scheduled euthanasia.

Clinical signs: Observed clinical signs were not associated with test article, as evidenced by a lack of dose dependency and the transient nature of the effects.

Body weight: Body weights and weight gain rates were not affected by dose.

Feed consumption: Feed consumption was comparable among control and dose groups.

Physical development: Physical development was not affected by treatment.

Neurological assessment: No effects were noted in motor activity, startle reflex, or learning and memory as assessed using the water maze test.

Reproduction: Mating and fertility parameters were not affected by treatment.

Reference ID: 3628623

F_2 Generation

External evaluation: Fetal external abnormalities that were observed in the F2 generation fetuses were limited to one fetus in the 300/30 mg/kg/day dose group. These findings included no anal opening and a pedunculated tail. Both findings are known to occur in historical controls.

Other: The litter averages for corpora lutea, implantations, the percentage of preimplantation loss, litter sizes, live and dead fetuses, early and late resorptions, the percentage of postimplantation loss, fetal body weights, the percentage of dead or resorbed conceptuses, and the percentage of live male fetuses were comparable among dose groups.

Reference ID: 3628623

Appendix 2 Ombitasvir/ABT-267/A-1233617

Studies Reviewed

Secondary Pharmacology
Study RD10517. In Vitro Pharmacology: High-Throughput Profile - Study of A-1233617.0

Safety Pharmacology
Study RD10068. Effects of A-1233617.0 on Spontaneous Locomotor Activity in the Rat (P.O. Administration)

Study RD10053. A Neurobehavioral Safety Evaluation of Orally Administered A-1233617 (ABT-267) in Mice

Study RD10393. A-1233617: In Vitro Effects on hERG Current

Study RD12072. Effects of A-1233617 (ABT-267) on Cloned hERG Potassium Channels Expressed in Mammalian Cells

Study RD10391. Effects of A-1233617 on Cardiovascular and Hemodynamic Function in the Anesthetized Dog

Study RD10055. A Cardiovascular Safety Evaluation of Orally Administered A-1233617 (ABT-267) in Beagle Dogs

Study RD10054. A Respiratory Safety Evaluation of Orally Administered A-1233617 (ABT-267) in Mice

Study RD10410. A-1233617: Effects on Ferret Emetic Liability and Rat Gastrointestinal Transit

Pharmacokinetics/ADME
Study PTR-10-0007. A-1233617.0 (ABT-267) Preformulation Report: Physicochemical Properties of A-1233617.0

Study RD10174. Preclinical Pharmacokinetic Summary of A-1233617 in Mouse, Rat, Rabbit, Monkey and Dog

Study RD13724. Integration of Pharmaceutics, Formulations and Pharmacokinetics for the Definition of Maximum Feasible Exposures in Preclinical Studies with A-1233617

Study A-1233617 Drug Metabolism Memo No. 03. A-1233617 Pharmacokinetics following Intravenous or Oral Dosing in Mouse

Study A-1233617 Drug Metabolism Memo No. 04. A-1233617 Pharmacokinetics following Intravenous or Oral Dosing in Rat

Study RD10172. Absorption, Distribution, Metabolism and Excretion of [3H]A-1233617 in Male Sprague Dawley Rats

Study A-1233617 Drug Metabolism Memo No. 16. A-1233617 Pharmacokinetics following Single and Multiple Oral Dosing in Rabbit

Study A-1233617 Drug Metabolism Memo No. 06. A-1233617 Pharmacokinetics following Intravenous or Oral Dosing in Dog

Reference ID: 3628623

Study A-1233617 Drug Metabolism Memo No. 05. A-1233617 Pharmacokinetics following Intravenous or Oral Dosing in Monkey

Study RD13056. Absorption, Distribution, Metabolism, and Excretion (ADME) Study of [14C]ABT-267 in Healthy Male Subjects Following a Single Oral Dose Administration

Study RD13185. Determination of the Binding of A-1233617 to Human and Animal Plasma Proteins

Study A-1233617 Drug Metabolism Memo No. 29. Determination of the Binding of A-1538855 and A-1548255 to Human and Mouse Plasma Proteins

Study A-1233617 Drug Metabolism Memo No. 11. Determination of the Blood-to-Plasma Concentration Ratios Following Incubations of A-1233617 in Human, Monkey, Dog and Rat Whole Blood

Study RD13055. Quantitative Whole-Body Autoradiography of Pigmented Rats Following Oral Administration of 14C-ABT-267

Study RD13517. Placental Transfer, Lacteal Excretion, and Tissue Distribution of Radioactivity in Pregnant Female Sprague Dawley Rats Following Oral Administration of 14C ABT-267

Study RD10171. In Vitro Metabolism of A-1233617

Study RD13742. Determination of the Metabolic Stability of A-1233617 in Liver Hepatocytes Across Species

Study A-1233617 Drug Metabolism Memo No. 23. Uptake of A-1233617 by Organic Anion Transporter Polypeptide (OATP) 1B1 and 1B3

Study A-1233617 Drug Metabolism Memo No. 18. Assessment of A-1233617 Efflux Mediated by P-glycoprotein (P-gp/MDR1) and Breast Cancer Resistance
Protein (BCRP)

Study A-1233617 Drug Metabolism Memo No. 22. Preliminary Metabolite Identification of A-1242846 (A-1233617 M23 Metabolite) in Human Recombinant CYP2C8 Incubations

Study A-1233617 Drug Metabolism Memo No. 17. Metabolism and Disposition of [^{14}C]A-1233617 (ABT-267) after Single 30 mg/kg Oral Dose in Male CD-1 Mice

Study A-1233617 Drug Metabolism Memo No. 13. Preliminary Metabolite Identification of A-1233617 (ABT-267) in CByB6F1-Tg(HRAS)2Jic non-Transgenic Mice Plasma Samples (TD11-004)

Study A-1233617 Drug Metabolism Memo No. 15. Preliminary Metabolite Identification of A-1233617 in Sprague Dawley Rat Plasma (TA10-249)

Study A-1233617 Drug Metabolism Memo No. 32. Metabolite Profiles of [^{14}C]A-1233617 (ABT-267) in Milk and Plasma after a 5 mg/kg Oral Dose in Female
Sprague-Dawley Rats

Study A-1233617 Drug Metabolism Memo No. 25. Metabolism and Disposition of [^{14}C]A-1233617 (ABT-267) after a Single 30 mg/kg Oral Dose in Sprague- Dawley Rats

Study A-1233617 Drug Metabolism Memo No. 35. A-1233617 Metabolite Profiles in Monkey Plasma after Single 2.5 or 20 mg/kg Oral Dose

Study A-1233617 Drug Metabolism Memo No. 26. Metabolism and Disposition of [^{14}C]A-1233617 (ABT-267) after Single 1 mg/kg Oral Dose in Beagle Dogs

Reference ID: 3628623

Study A-1233617 Drug Metabolism Memo No. 34. A-1233617 Metabolite Profiles at Steady State after Multiple Oral Dosing in Mouse, Rat, Rabbit and Dog

Study A-1233617 Drug Metabolism Memo No. 14. Preliminary Metabolite Identification of A-1233617 (ABT-267) in Human Plasma Samples From Clinical Phase I Study M12-116

Study RD13057. Metabolism and Disposition of [^{14}C]ABT-267 (A-1233617) in Male Subjects after a Single 25 mg Oral Dose

Study A-1233617 Drug Metabolism Memo No. 20. Preliminary Metabolite Identification of A-1233617 (ABT-267) in Human Plasma Samples

Study A-1233617 Drug Metabolism Memo No. 21. Plasma Concentrations of Selected A-1233617 (ABT-267) Metabolites following Multiple Oral Dosing in Human

Study RD10173. Assessment of the Effect of A-1233617 on the Activity of Cytochrome P450 (CYP450) Isoforms in Human Liver Microsomes

Study A-1233617 Drug Metabolism Memo No. 09. Assessment of the Time Dependent CYP3A4 Inhibition by A-1233617 in Human Liver Microsomes

Study RD13205. Assessment of CYP Time Dependent Inhibition Potential by A-1233617 in Human Liver Microsomes

Study RD121091. An In Vitro Investigation of Cytochrome P450 Induction by A-1233617 (ABT-267) in Cultured Human Hepatocytes

Study RD13253. Assessment of the Effect of A-1233617 on the Activity of UDP-glucuronosyltransferases 1A1 (UGT1A1) Isoform in Human Liver Microsomes

Study A-1233617 Drug Metabolism Memo No. 19. Inhibitory Interaction of A-1233617 on P-glycoprotein (P-gp/MDR1) and Breast Cancer Resistance Protein (BCRP)

Study A-1233617 Drug Metabolism Memo No. 28. Inhibitory Interaction of A-1233617 on Bile Salt Export Pump (BSEP)

Study A-1233617 Drug Metabolism Memo No. 27. Inhibitory Interaction of A-1233617 on Multidrug Resistance Protein 2 (MRP2)

Study A-1233617 Drug Metabolism Memo No. 24. Inhibitory Interaction of A-1233617 on Organic Anion Transporting Polypeptides (OATP) 1B1, 1B3, Organic Anion Transporters (OAT) 1, 3, Organic Cation Transporters (OCT) 1, 2, and Multidrug and Toxin Extrusion Proteins (MATE) 1 and 2K

Study A-1233617 Drug Metabolism Memo No. 33. Disposition of [3H]A-1233617 in MDR1A/1B P Glycoprotein- and BCRP-deficient Triple Knockout Mice, and in Multidrug Resistance Protein MRP2 Knockout Mice

Study A-1233617 Drug Metabolism Memo No. 30. Pharmacokinetics and Metabolite Profile of A-1538855 (A-1233617 M29 Metabolite) following Intravenous or Oral Dosing in Mouse

Study A-1233617 Drug Metabolism Memo No. 31. Pharmacokinetics and Metabolite Profile of A-1548255 (A-1233617 M36 Metabolite) following Intravenous or Oral Dosing in Mouse

<u>Repeat-Dose Toxicity</u>

Reference ID: 3628623

Study RD11643. Five-Day Oral Toxicokinetic Study of A-1233617 in Male CByB6F1-Tg(HRAS)2Jic Non-Transgenic Mice TD11-143

Study RD10493.Two-Week Oral Dosing Study of A-1233617 in CD1 Mice CMET09-147 / CMET09-148

Study RD10003. Amended Report for Four-Week Oral Toxicity Study of A-1233617 Free Base in CD-1 Mice TD09-269

Study RD11096. Four-Week Oral Toxicity Study of A-1233617 in CByB6F1-Tg(HRAS)2Jic non-Transgenic Mice TD11-004

Four-Week Oral Safety Study of ABT-450/Ritonavir and ABT-267 Using Milled Fixed Dose Combination Tablets in CD-1 Mice TD13-238

Study RD10458. Thirteen-Week Oral Dose Toxicity Study with A-1233617 in Mice TD10-071

Study RD11561. Amended version of A 6-Month Oral Toxicity Study of A-1233617 in Mice with a 1-Month Recovery Period TD10-204

Study RD091241. Two-Week Oral Range-Finding Toxicity Study with A-1233617 in Rats (Non-GLP) TA09-188

Study RD101472. A Thirteen-Week Oral Maximum Tolerated Dose Toxicity Study of A-1233617 in Sprague-Dawley Rats TA10-249

Study RD091242. Two-Week Oral Range-Finding Toxicity Study of A-1233617 in Beagle Dogs (Non-GLP) TB09-189

Study RD091465. Amended Report for Four-Week Oral Capsule Toxicity Study of A-1233617 in Beagle Dogs TB09-270

Study RD10461. 3-Month Oral Dose Toxicity Study with A-1233617 in Beagle Dogs TB10-073

Study RD101371. Twenty-Seven Week Oral Capsule Toxicity Study of A-1233617 Free Form in Beagle Dogs TB10-205

Genotoxicity
Study RD10102. Salmonella-Escherichia coli / Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with A1233617 TX09-277

Study RD10111. Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with A-1233617 TX09-278

Study RD10369. In Vivo Mouse Bone Marrow Micronucleus Assay with A-1233617 TD09-279

Carcinogenicity
Study RD12574. 26-Week Oral Gavage Carcinogenicity Study with A-1233617 Free Form in Model 001178-T (Hemizygous)CByB6F1-Tg(HRAS)2Jic Mice TD12-080

Reproductive Toxicology
Study RD12279. An Oral (Gavage) Fertility and Early Embryonic Development Study of A-1233617 in Mice TD11-274

Study RD101462. A Dose Range-finding Embryo-fetal Development Study of A-1233617 by Oral Gavage in Mice TD10-221

Reference ID: 3628623

Study RD11369. An Embryo-Fetal Development Study of A-1233617 by Oral Gavage in Mice TD11-036

Study RD101491. A Range-Finding Oral Embryo-Fetal Developmental Toxicity Study with A-1233617 in Rabbit, Including a Toxicokinetic Evaluation TE10-274

Study RD11406. An Oral Developmental Toxicity Study with A-1233617 in Rabbits, Including a Toxicokinetic Evaluation TE11-037

Study RD12711. A Developmental and Perinatal/Postnatal Reproduction Study of A-1233617 by Oral (Gavage) in Mice, Including a Postnatal Behavioral/Functional Evaluation TD12-079

Other Toxicity Studies
Study RD13760. Four-Week Oral Toxicity Study of A-1538855 (b) (4) in CD-1 Mice (with a Four- Week Recovery Period) TD13-188

Study RD13701. Four-Week Oral Toxicity Study of A-1548255 (b) (4) in CD-1 Mice (with a Four- Week Recovery Period) TD13-184

Study RD13487. Salmonella-Escherichia coli / Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with A-1538855 and A- 1548255 TX13-149

Study RD13485. Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with A-1538855 and A-1548255 TX13-150

Study RD13483. Ten-Day Oral Dose Tolerability Study of A-1538855 and A-1548255 Free Base in Pregnant CD-1 Mice TD13-178.

Study RD13548. Oral (Gavage) Developmental Toxicity Study of A-1538855 in Mice, Including a Toxicokinetic Evaluation TD13-180

Study RD13549. Oral (Gavage) Developmental Toxicity Study of A-1548255 in Mice, Including a Toxicokinetic Evaluation– December report TD13-181

Study RD12570. Four-Week Oral Gavage Toxicity Study of A-1233617 (with Impurities) in CD-1 Mice TD12-112

Special toxicity Studies
Study RD11436. Repeat Dosage Phototoxicity Study of A-1233617 by Oral Gavage in Hairless Mice TD11-080

Pharmacology

Primary Pharmacology

Refer to Microbiology review.

Preliminary pharmacokinetic/pharmacodynamic modeling showed that A-1233617 (ABT-267) was projected to reduce plasma wild type HCV RNA by 3 to 4 log10 at steady state AUC values of 224 to 1090 ng•hr/mL. The predicted C_{max} values associated with these predicted AUC values are 13 to 69 ng/mL for a once daily dosing regimen.

Secondary Pharmacology

No significant secondary binding was noted in a high throughput assay.

Reference ID: 3628623

Safety Pharmacology

Neurological effects:

Study RD10068. Effects of A-1233617.0 on Spontaneous Locomotor Activity in the Rat (P.O. Administration)

Study RD10053. A Neurobehavioral Safety Evaluation of Orally Administered A-1233617 (ABT-267) in Mice

CNS and neurobehavioral effects of ABT-267 were assessed in an assay for spontaneous locomotor activity (Rj:Wistar (Han) rats) as well as in a mouse (CD1) modified Irwin Functional Observation Battery assay. There were no statistically significant effects on locomotor activity in rats at doses ranging from 0.3 to 30 mg/kg. Likewise, no effects on the Functional Observation Battery were noted in mice following administration of 10, 40, or 120 mg/kg ABT-267.

Cardiovascular effects:

Study RD10393. A-1233617: In Vitro Effects on hERG Current

Potential effects of ABT-267 on the QT repolarization interval were assessed in the in vitro hERG assay. At the limit of solubility (4560 ng/mL), hERG tail current was significantly reduced (14.3%). In the thorough QT clinical trial, the 3-DAA combination, at supratherapeutic exposures, did not result in QTc prolongation.

Study RD12512. Effects of A-1233617 (ABT-267) on Cloned hERG Potassium Channels Expressed in Mammalian Cells

A-1233617 at a measured concentration of 43.4 ng A-1233617.0/mL produced no meaningful change in hERG-mediated potassium currents.

Study RD10391. Effects of A-1233617 on Cardiovascular and Hemodynamic Function in the Anesthetized Dog

Study RD10055. A Cardiovascular Safety Evaluation of Orally Administered A-1233617 (ABT-267) in Beagle Dogs

In anesthetized dogs, ABT-267 had no effect on cardiovascular parameters through the highest infusion of 0.006 mg/kg/min (plasma concentration = 483 ng/mL). In awake, telemeterized dogs, oral doses of 2, 10, or 60 mg/kg ABT-267 had no effect on measured parameters. At 60 mg/kg the associated plasma concentration was 2620 ng/mL).

Pulmonary effects:

Study RD10054. A Respiratory Safety Evaluation of Orally Administered A-1233617 (ABT-267) in Mice

The effects of ABT-267 on respiratory function in mice were assessed following oral administration of 5, 20, or 60 mg/kg. There were no effects through 60 mg/kg.

Gastrointestinal effects:

Study RD10410. A-1233617: Effects on Ferret Emetic Liability and Rat Gastrointestinal Transit

Effects of ABT-267 (0.5, 1.5, 5, or 15 mg/kg) on gastrointestinal tolerability were assessed in fasted male ferrets. No dose-dependent emesis was observed.

Reference ID: 3628623

Effects of ABT-267 (1.5, 5, or 15 mg/kg) on gastrointestinal transit were assessed in male rats. No effect on gastrointestinal transit was seen.

Pharmacokinetics/ADME/Toxicokinetics

PK/ADME

Pharmacokinetics

The pharmacokinetic parameters of ABT-267 were determined in the CD-1 mouse, Sprague-Dawley rat, Cynomolgus monkey and beagle dog. The pharmacokinetic parameters included plasma elimination half-lives after intravenous dosing which ranged from 4.4 hours in monkey to 11.4 hours in rat. Volumes of distribution (Vss) were moderate to high in all species, with values approximately 1.5 to 1.8 L/kg for mouse, dog and monkey with higher values for rat (4.8 L/kg). Plasma clearance values were low in rat (0.46 L/hr•kg) and monkey (0.38 L/hr•kg), with even lower values in dog (0.18 L/hr•kg) and mouse (0.11 L/hr•kg).

The pharmacokinetics of ABT-267 metabolite M29 (A-1538855) in CD-1 mice were assessed. The pharmacokinetic profile of A-1538855 following a 1 mg/kg intravenous dose in male CD-1 mice is described by low plasma clearance (CLp = 0.37 L/hr•kg), with moderate volumes of distribution (Vss = 1.1 L/kg) and an apparent elimination half-life of 3.3 hours.

The pharmacokinetics of ABT-267 metabolite M36 (A-1548255) in CD-1 mice was evaluated. The pharmacokinetic profile of A-1548255 following a 1 mg/kg intravenous dose in male CD-1 mice is described by low plasma clearance (CLp = 0.95 L/hr•kg), with high volumes of distribution (Vss = 4.2 L/kg) and an apparent elimination half-life of 7.6 hours (range 5.4-9.7 hours).

Absorption

A-1233617 oral bioavailability values ranged from 24.8% in rat to 57.3% in dog. A-1233617 plasma concentrations following oral co-dosing with ritonavir in rat, monkey and dog were comparable to those obtained from an equivalent dose of A-1233617, administered alone.

In dog, AUC values following a 10 mg/kg oral dose were higher when administered under non-fasting conditions, although the food effect was minimal with lower doses.

(b) (4)

In rat, minimal differences in exposure were noted at single doses between 100-500 mg/kg, with maximal AUCs ~10 µg•hr/ml in a two week multiple dose study. In a 13-week rat study, AUCs at the end of the study averaged ~30 µg•hr/ml, with minimal differences between the 30 and 300 mg/kg/day treatment groups; apparent accumulation was noted in rats, with AUC values after multiple dosing higher than obtained on Day 1 in all rat studies.

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The maximal exposures obtained in pregnant New Zealand white rabbits were also low, similar to those noted in rats, averaging ~7-8 µg•hr/ml at 60 mg/kg/day; no accumulation was noted over the dosing interval in the embryo-fetal developmental toxicity study.

In CD-1 and CByB6F1-Tg(HRAS)2JIC non-transgenic littermates mice, maximal exposures were also obtained at ~100 mg/kg, with comparable exposures at doses up to the limits of solubility (500 mg/kg); maximal exposures in mice were ~5-fold higher than in rats, with values in the 40-60 µg•hr/ml range after multiple daily dosing. No apparent accumulation was noted in mice, with exposures after multiple daily dosing generally comparable to those observed on Day 1.

Within the limits of variability, ABT-267 AUCs obtained from single 100 mg/kg oral doses in beagle dogs were not different from those obtained at 60 mg/kg (~40 µg•hr/ml). However, accumulation in line with the half-life and daily dosing interval provided an ~2-fold increase in exposures, with AUCs of ~90 µg•hr/ml at the end of the 6-month dosing interval in dogs.

The 0.5 and 5 mg tablets used in initial clinical trials provided dose-linear increases in Cmax and AUC in dog, while plasma concentrations from the 25 mg tablet were less than would have been predicted from the two lower doses.

In four healthy males administered a single oral dose of 25 mg, the mean AUC was 30 µg•hr/ml.

Distribution

The extent of binding of ABT-267 to human and animal plasma proteins has been assessed at different concentrations (0.1-10 µM) using equilibrium dialysis. No concentration dependency of protein binding was seen in mouse, rat, rabbit, dog, monkey or human plasma over the concentration range tested. There was no species difference observed in plasma protein binding across species. The compound was highly protein bound (>99%) for all species tested.

The unbound fraction of A-1538855 and A-1548255 (metabolites of ABT-267) to human and mouse plasma was evaluated at different concentrations (0.1-10 µM) using equilibrium dialysis. No concentration dependency of protein binding was observed in mouse plasma in the concentration range of 0.1 to 10 µM for both A-1538855 and A-1548255. In human plasma, the individual assays and the overall combined data indicate concentration dependency in unbound fraction at concentration below 1 µM for both A-1538855 and A-1548255. The fraction unbound obtained for A-1538855 and A-1548255 in human and mouse were similar (within 3-fold difference) at all concentrations except 0.1 µM, where human plasma unbound fraction was 3- and 5-fold lower than mouse, respectively. The compounds were highly protein bound (>96%) in both species tested.

The distribution of [^{3}H] ABT-267 in blood was studied across four species at four concentrations (400, 80, 51 and 8 ng/mL). [3H] ABT-267 does not partition preferentially into the cellular compartment of blood in any of the species studied and the concentration effects appear to be insignificant.

Following oral administration of ^{14}C-ABT-267 to male Long Evans rats, drug-derived radioactivity was poorly distributed into tissues of the pigmented male rat at 0.5 and 1 hour postdose. By 2 hours postdose, most tissues evaluated had measurable levels of radioactivity, with peak concentrations occurring at either 4 or 8 hours postdose. Concentrations of radioactivity in most tissues then declined after reaching peak levels, and by 96 hours postdose, only the liver, Harderian gland, and preputial gland had measurable levels of ^{14}C-ABT-267-derived radioactivity. Elimination of radioactivity from all tissues was complete by 168 hours postdose.

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Radioactivity concentrations were below measurable levels in cerebellum, cerebrum, medulla, olfactory lobe, spinal cord, and the lens of the eye at all collection times throughout the study.

Study RD13517. Placental Transfer, Lacteal Excretion, and Tissue Distribution of Radioactivity in Pregnant Female Sprague Dawley Rats Following Oral Administration of ^{14}C ABT-267

In pregnant Sprague-Dawley rats, ^{14}C-ABT-267-derived radioactivity was secreted into milk for at least 24 hours postdose. Placental transfer of radioactivity was minimal, resulting in limited exposure to only the fetal liver. Radioactivity was eliminated from most maternal tissues by 72 hours postdose following a single oral gavage dose of ^{14}C-ABT-267. Accumulation of radioactivity was not observed in any maternal or fetal tissues, but was retained in abdominal fat, Harderian gland, and liver through the last collection time of 72 hours postdose.

Metabolism

The metabolism of [^{3}H]A- ABT-267 was investigated using hepatic microsomes, hepatocytes from several species and recombinant human enzymes. The metabolic stability of [^{3}H]A- ABT-267 was examined in liver microsomes across species. A-1233617 was stable in liver microsomes (>98% parent remaining after 30 min incubation in rat, dog and human; 88.7% parent remaining in monkey). The corresponding microsomal intrinsic clearance values for ABT-267 were 1.9, 3.0, 3.4 and 18.4 µl/min/mg for rat, dog, human and monkey, respectively. Incubations with human recombinant CYPs suggested that CYP3A4/5 and CYP2C8 have the potential to metabolize [3H]A- ABT-267. Further characterization of enzyme kinetics in rhCYP3A4 and rhCYP2C8 suggested CYP3A4 was the primary enzyme responsible for metabolizing [3H]ABT-267. ABT-267 showed no inhibition of the major CYP enzymes including CYP1A2, 2B6, 2C9, 2C19, 2D6 or 3A4 (IC50 >30 µM), but inhibited CYP2C8 with IC50 of 7.4 µM. No time-dependent inhibition of CYP3A4 was observed in human liver microsome (HLM) at 10 and 50 µM of ABT-267. Likewise, time dependent inhibition of CYP1A2, 2B6, 2C8, 2C9, 2C19 and 2D6 by A-1233617 was not observed in HLM. ABT-267 did not cause CYP1A2, 2B6 and 3A4 mRNA induction in the test concentration range in vitro in three human donors.

The potential for ABT-267 to inhibit UGT1A1 was evaluated in human liver microsomes using probe substrates. Pooled human liver microsomes were incubated with UGT1A1 selective probe substrate and product formation was measured in the presence of increasing concentrations of ABT-267 (0-100 µM). A-1233617 showed inhibition of UGT1A1 with IC50 of 2.12 µM.

Similar to the observations with liver microsomes, [3H] ABT-267 was stable in rat, dog, monkey and human hepatocytes (~98.9%, 90.7%, 88.7% and 94.1% parent remaining at 0.5 µM for four hours, respectively). The in vitro metabolite profiles in liver microsomes across species showed seven metabolites formed in human liver microsomes, nine in monkey liver microsomes, four in rat liver microsomes and six in dog liver microsomes. Metabolic profiling was also performed using cross-species hepatocytes. In general, metabolite profiles in hepatocytes were similar to these in liver microsomes, suggesting CYP enzymes are responsible for the metabolism of [3H] ABT-267.

Metabolite identification showed that the metabolism was limited largely to pyrrolidine ring oxidation (M1 & M2) and t-butyl phenyl oxidation (M3, M4, M5). Minor metabolites (M6 & M7) involving hydrolysis of aniline amide linker were also observed, mostly in hepatocytes. Minor dehydrogenation and oxidation metabolites (M10 & M11) were observed.

Identification of ABT-267 metabolites in mouse, rat, rabbit and dog plasma was conducted at steady state, following oral dosing for eight days. At least eight metabolites were identified in mouse plasma, including the amide hydrolysis metabolites M6, M7 and M23, the oxidative metabolites M1, M2, M3, the

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hydration metabolite M9, and the tert-butyl hydroxyl metabolite, M26. In rat, M19 was the most significant metabolite in plasma, with at least eight additional metabolites identified. In rabbit, at least nine metabolites were identified, with M23 as the most abundant in plasma. In dog, six metabolites were identified in plasma, with the amide hydrolysis products M23 and M6, and the hydration product M9 as the most abundant metabolites. Metabolite identification and profiling in monkey plasma from preclinical studies were conducted by LC-MS/MS analysis. The preliminary metabolite profile of ABT-267 showed that unchanged parent drug was the major component in monkey plasma. A total of 9 metabolites were identified. The bis-amide cleavage product M23 and the corresponding oxidation product M26 were the major metabolites observed in plasma. Other metabolites included hydration metabolite M9, amide hydrolysis metabolites M6, M7, M23, the hydroxy tert-butyl M26 and the dihydroxy tert-butyl M25. In addition, trace levels of tert-butyl desmethyl oxidation products M29, M34 and M35 were also detected.

The preliminary metabolite identification of ABT-267 was conducted in pooled CByB6F1-Tg(HRAS)2Jic non-transgenic mice plasma samples (Study TD11-004). LC-MS profiling of ABT-267 metabolites showed that unchanged parent drug is the major component in mouse plasma. A total of nine minor metabolites were identified, including mono-oxidation metabolites M2, M3, M4, M5 and M9, dehydrogenation products M11, and amide hydrolysis related products M6, M23 and M19. All the metabolites were present in minor levels, ranging from 0.03% to ~4% relative to unchanged parent drug.

The metabolite profiles of [^{14}C] ABT-267 in milk and plasma were studied in female rats given a single 5 mg/kg oral dose of [^{14}C] ABT-267. [^{14}C] ABT-267 represented a majority of total radioactivity in both rat milk (~91.2%) and plasma (~72.4%). Metabolite M19 was detected in both milk (5.5%) and plasma (16.9%). In addition, low levels of M23, M24 and M26 are observed radiochemically in plasma and one minor uncharacterized radiochemical component U1 was noted in rat milk.

In four healthy males, unchanged ABT-267 recovered in feces and urine represented about 88% of an administered radiochemical dose (25 mg). In plasma, ABT-267 was the major component, accounted for ~36% of drug-related material, metabolite M36, M29, M37 accounted for approximately 27, 18 and 11% of total drug-related material in plasma, respectively. Other metabolites were relatively minor, each far less than 10% of the total drug-related material. The major human metabolism pathway involves hydrolysis of ABT-267 to dianiline M23 (A-1242846) which undergoes oxidation and C-demethylation of the tert-butyl group to form metabolite M29, M36 and M37.

Excretion

Mean total recovery of radioactivity from male rats was 95.0% ± 16.3% up to 72 hours post dose and 100.0% ± 9.8% for female rats. Drug-related radioactivity was mainly recovered in feces, 93.3% ± 15% for male rats, 99.8% ± 9.8% for female rats. Renal elimination was minimal.

Mean total recovery of excreted radioactivity from bile duct-cannulated rats was 102.8% after intravenous administration or 107.4% after oral administration. Drug-related radioactivity was mainly eliminated via the biliary route (91.1% of intravenous dose and 9.0% of oral dose). Renal elimination is relatively minimal (1.4% of intravenous dose and 0.22% of oral dose). For orally treated rats, the sum of urinary and biliary excretion indicated that about 9.2% of the dose was absorbed.

Mean total recovery of radioactivity from mice was 100.1% ± 8.5% up to 48 hours post dose. Drug-related radioactivity was mainly recovered in feces, 98.1% ± 8.6%. Renal elimination was minimal (0.8% ± 0.3% of dose).

In healthy male subjects, a mean of 90.2% of the dose was recovered in feces and 1.91% was recovered in urine through the last collection interval.

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Transporters

ABT-267 does not appear to significantly inhibit OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1 or MATE2K, P-gp and BCRP. Inhibition of MRP2 was <30% at 100 µM. A-1233617 inhibited the ATP-dependent transport of taurocholic acid (TCA) by Bile Salt Export Protein in a concentration-dependent manner, with an apparent IC50 value of 95 µM (n=2).

In genetically-altered mice, the absence of both P-gp and BCRP resulted in a significantly higher area under the concentration–time curve in plasma, brain and liver after an intravenous dose, indicating P-gp or BCRP or both played important roles in the disposition and elimination of A-1233617. The AUCs in plasma, brain and liver increased more significantly after oral administration, indicating that P-gp or BCRP or both have an impact on the absorption of ABT-267, in addition to disposition and elimination. No exposure changes in Mrp2 knockout mouse were observed after either intravenous or oral dosing, indicating Mrp2 transporter is not involved in the absorption, disposition and elimination of ABT-267.

General Toxicology

Single-Dose Toxicity

No single dose studies were conducted.

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Repeat-Dose Toxicity

Study title: Non-GLP Study Report Two-Week Oral Dosing Studies of A-1233617 in CD1 Mice Studies CMET09-147 and CMET09-148

Study no.:	R&D/10/493 (CMET09-147 and CMET09-148)
Study report location:	Electronic
Conducting laboratory and location:	Abbott Laboratories, Abbott Park, IL
Date of study initiation:	Not noted
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	A-1233617 Free Base; Lot No.: 1727006 89.7%

Key Study Findings

- Mice were administered 2.85, 28.5, or 95.1 mg/kg ABT-267 via oral gavage for 14 days.
- Moderate increases in liver function enzymes (ALT, AST, GLDH) correlated with histopathological findings (single cell hepatocellular necrosis) in males at middle and high doses.
- The NOAEL was defined as the low dose, 2.85 mg/kg. No exposure data is available from low dose animals.
- Systemic exposures were only assessed at the high dose. At that dose systemic exposures were 127.3 and 63.9 µg*hr/mL in male and female mice, respectively.

Methods

Doses:	2.85, 28.5, 95.1 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	PEG-400: Tween 20: Poloxamer 124: Vitamin E TPGS, 50: 20: 10: 20, w/w/w/w
Species/Strain:	Mouse, CD1
Number/Sex/Group:	5
Age:	46-49 days old
Weight:	male mice weighed 23.2 to 31.9 g and female mice weighed 21.3 to 24.4 g
Satellite groups:	Toxicokinetics: 3/timepoint at high dose
Unique study design:	None
Deviation from study protocol:	The test article purity was lower than anticipated such that the actual dose was adjusted. No other significant deviations occurred.

Results

Mortality: One low dose male was euthanized on Day 8 due to a dosing related injury. There were no other early deaths.

Clinical Signs: There were no remarkable clinical signs.

Body Weights: There were no significant dose-related changes in body weight.

Hematology: There were no significant dose-related effects on hematology parameters.

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Clinical Chemistry: In male mice, mild to moderate increases in alanine aminotransferase (ALT) were noted at the low dose (2.85 mg/kg; 1/2 mice), middle dose (28.5 mg/kg; 2/3 mice), and high dose (95.1 mg/kg; 3/3 mice). Also in males, mild to moderate increases in aspartate aminotransferase (AST) were seen (2/2 mice, 3/3 mice and 3/3 mice at low, middle and high doses, respectively) with mild to moderate increases in glutamate dehydrogenase (GLDH) (2/3 mice and 3/3 mice at the middle and high doses, respectively). In female mice, an increase in AST was observed in 1/3 female mice in the high dose group.

Gross Pathology: There were no remarkable findings.

Organ Weights: Liver, spleen, brain, heart, kidneys, testes, ovaries, and thymus were weighed. There were no remarkable findings.

Histopathology

Adequate Battery: No. A full histopathological battery was not collected/analyzed in this non-GLP study.

Peer Review: None noted.

Histological Findings

Minimal to mild single cell hepatocellular necrosis was observed in 4/5 low-dose male mice and 5/5 high-dose male mice.

Toxicokinetics

Toxicokinetic parameters were determined following a single high dose (95.1 mg/kg) of ABT-267. Exposures in males were approximately twice those in females (see table below, *excerpted from sponsor*).

PO (95.1 mg/kg)	Days	$AUC_{0\text{-}24hr}$	C_{max}	T_{max}
Female	1	63.9	4.61	5.3
Male	1	127.3	8.59	12.0

Stability and Homogeneity

Formulations were prepared daily so that stability was not an issue. Homogeneity was not assessed.

Reference ID: 3628623

Study title: Four-Week Oral Toxicity Study of A-1233617 Free Base in CD-1 Mice

Study no.:	TD09-269
Study report location:	Electronic
Conducting laboratory and location:	Abbott Laboratories 100 Abbott Park Road Abbott Park, IL 60064, USA
Date of study initiation:	05 Jan 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 free base (A-1233617, A-1233617.0, ABT-267), 83192PP01, 90.8%

Key Study Findings

- Mice were administered 1, 5, 20 or 60 mg/kg (males) or 2, 10, 40, or 120 mg/kg (females) ABT-267 via oral gavage for 28-days.
- There were no remarkable findings.
- The No-Observed-Adverse-Effect-Level (NOAEL) was the high dose (60 mg/kg/day for males and 120 mg/kg/day for females).
- These dosages corresponded with systemic exposures of 103 µg•hr/mL (AUC_{0-24}) in the males, and 67.3 µg•hr/mL (AUC_{0-24}) in females.

Reference ID: 3628623

Methods

Doses:	Males: 1, 5, 20 or 60 mg/kg Females: 2, 10, 40, or 120 mg/kg
Frequency of dosing:	Daily for 28 days
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	50% Polyethylene glycol (PEG 400):20% Tween 20:10% Poloxamer 124:20% Vitamin E TPGS
Species/Strain:	Mice/ CD-1 [Crl: CD® (ICR)]
Number/Sex/Group:	13
Age:	9 weeks
Weight:	The male mice weighed 23.2 to 35.7 grams and the female mice weighed 19.6 to 29.8 grams
Satellite groups:	8 (control) or 39 mice/sex/dose group were used to measure plasma concentrations
Unique study design:	None
Deviation from study protocol:	None

Results

Mortality: Mice 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 11 early deaths; all were considered to be related to the dosing procedure as evidenced at necropsy by lung discoloration, failure of lung to collapse, perforated esophagus and/or food-filled pocket.

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

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: Blood samples were collected at necropsy. Clinical chemistry was the priority, and remaining blood, if any, was used to assess hematologic parameters. There were no remarkable findings.

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Clinical Chemistry: There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Brain, heart, kidney, liver, spleen, testes, 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 at 1, 3, 6, 9, 12, and 24 hours after dosing on Day 1 and Day 25. Toxicokinetic parameters for ABT-267 in mice following 28-days of dosing via oral gavage are presented below (*excerpted from sponsor*).

Mean Toxicokinetic Parameters for A-1233617

		Day 1				Day 25			
Dosage (mg/kg/day)	Sex	C_{max} (µg/mL)	C_{max}/D (µg/mL/ mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	AUC/D (µg•hr/mL/ mg/kg/day)	C_{max} (µg/mL)	C_{max}/D (µg/mL/ mg/kg/day)	$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	AUC/D (µg•hr/mL/ mg/kg/day)
1	Male	0.075	0.0750	1.12	1.12	0.089	0.0890	1.15	1.15
2	Female	0.149	0.0745	1.74	0.870	0.0896	0.0448	1.48	0.741
5	Male	0.349	0.0698	5.93	1.19	0.476	0.0952	7.64	1.53
10	Female	0.809	0.0809	9.77	0.977	0.551	0.0551	9.02	0.902
20	Male	1.64	0.0820	26.2	1.31	2.16	0.108	33.8	1.69
40	Female	2.56	0.0640	40.1	1.00	2.43	0.0608	32.7	0.817
60	Male	5.48	0.0913	80.5	1.34	6.33	0.106	103	1.72
120	Female	5.42	0.0452	80.0	0.667	4.51	0.0376	67.3	0.561

Stability and Homogeneity: The homogeneity results for all dose formulations prepared on Day 1, as reflected by relative standard deviations of concentrations, were within ± 10%. Stability of dosing formulation (prepared weekly and stored [redacted] (b) (4)) was not assessed in the current study.

Reference ID: 3628623

Study title: Thirteen Week Oral Dose Toxicity Study of A-1233617 in Mice

Study no.:	126-518 (Sponsor Study no. TD10-071)
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	31 March 2010
GLP compliance:	Yes.
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 (ABT-267) 83192PP01, 91.1%

Key Study Findings

- Mice were administered 2.5, 20 or 400 mg/kg (males) or 5, 40, or 400 mg/kg (females) ABT-267 via oral gavage for 13 weeks.
- There were no remarkable findings.
- The No-Observed-Adverse-Effect-Level (NOAEL) was the high dose (400 mg/kg/day, corresponding with systemic exposures of 63.3 µg•hr/mL (AUC_{0-24}) in the males, and 37.0 µg•hr/mL (AUC_{0-24}) in females.

Reference ID: 3628623

Methods

Doses:	Males: 2.5, 20 or400 mg/kg Females: 5, 40, or 400 mg/kg
Frequency of dosing:	Daily for 91 days (13 weeks)
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	50% Polyethylene glycol (PEG 400):20% Tween 20:10% Poloxamer 124:20% Vitamin E TPGS
Species/Strain:	Mice/ CD-1 [Crl: CD® (ICR)]
Number/Sex/Group:	See sponsor table below
Age:	~ 8 weeks
Weight:	The male mice weighed 25.4 to 30.6 grams and the female mice weighed 20.2 to 25.7 grams
Satellite groups:	See sponsor table below
Unique study design:	None
Deviation from study protocol:	As a result of mis-dosing of groups 1 and 5, original animals were replaced on Day 5.

Group Assignments

Group Number	Dose Level (mg/kg/day) Males/Females	Number of Animals	
		Male	Female
1	0	16	16
2	2.5/5	16	16
3	20/40	16	16
4	400	16	16
5[a]	0	6+2[b]	6+2[b]
6[a]	2.5/5	36+3[b]	36+3[b]
7[a]	20/40	36+3[b]	36+3[b]
8[a]	400	36+3[b]	36+3[b]

[a] Designated as toxicokinetic animals
[b] Additional animals assigned as potential replacement animals

Observations and Results

Mortality: Mice were observed for survival and clinical signs twice a day during dosing. Detailed observations were made at six hours after dosing twice a week.

A summary of early deaths is provided in the table below (*excerpted from sponsor*). None of the deaths were considered to be related to test article, as the incidence and timing of deaths did not show dose-dependence.

Reference ID: 3628623

Animal Number	Sex	Group Number	Dose Level (mg/kg/day) Male/Female	Study Day	Clinical History	Gross Findings
R4013	Male	1	0	9	Limb function impaired; swelling	Left knee joint: fracture; severe. Spleen: enlarged; mild
4091	Male	6	2.5/5	12	No abnormalities detected	Thoracic cavity; fluid, clear, mild (approximately 1.0 mL)
4175	Male	8	400	17	Posture Hunched; Unkempt Appearance	Adhesion, moderate, pericardium adhered to multiple lobes of the lung and cavity wall. Mediastinal Lymph Node enlarged, mild Skin abscess, green , moderate, left axillary area Spleen, enlarged, mild Thymus, small, severe
4133	Male	7	20/40	25	Activity decreased, tremors, hair discolored (yellow, anogenital region), skin cold to touch and breathing difficult	All tissues were within normal limits.
4679	Female	8	400	74	Abdomen distended, scabbed area, cervical region, breathing audible	Skin abrasion/scab; dorsal cervical region; mild
4687	Female	8	400	75	Skin cold to touch, whole body, Unkempt appearance, Breathing audible, Breathing difficult	All tissues were within normal limits
R4568	Female	5	0	77	Carriage Low, Material around mouth, brown; Posture hunched, lip swelling, Skin cold to touch, whole body, activity decreased, breathing shallow	All tissues were within normal limits

Clinical Signs: A dose-dependent increase in unkempt appearance and yellow discolored hair was noted in middle dose and high dose males.

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

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

Reference ID: 3628623

Hematology: 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: Brain, heart, kidney, liver, spleen, testes, and thymus were weighed. There were no remarkable findings.

Histopathology

Adequate Battery: Yes.

Peer Review: Yes.

Histological Findings: Minimal (1/16) or mild (3/16) vaginal mucification was noted in high dose females. The finding was not considered to be toxicologically significant. There were no other remarkable findings.

Toxicokinetics

Blood samples were collected at 1, 3, 6, 9, 12, and 24 hours after dosing on Day 1 and 91. Toxicokinetic parameters for ABT-267 in mice following 91 days of dosing via oral gavage are presented below (*excerpted from sponsor*).

Mean (±SD) Toxicokinetic Parameters for A-1233617

Dose Level (mg/kg/day)	Day	Sex	C_{max} (µg/mL)	AUC (µg·hr/mL)
2.5	1	Male	0.0956	1.06
5	1	Female	0.155	2.18
2.5	91	Male	0.0861	1.36
5	91	Female	0.200	2.23
20	1	Male	0.774	11.6
40	1	Female	1.13	11.8
20	91	Male	1.05	17.4
40	91	Female	2.04	21.7
400	1	Male	2.04	28.4
400	1	Female	1.99	24.2
400	91	Male	4.10	63.3
400	91	Female	3.44	37.0

Stability and Homogeneity: The concentration results for all dose formulations were within 90 - 101% of nominal with the exception of the middle sample of the 200 mg/mL dose preparation on Day 1. The value was 87% of theoretical. Subsequent analysis of the backup homogeneity samples revealed a value of 94% of theoretical for all three samples. Stability of dosing formulation was not assessed in the current study. The Sponsor has provided the testing lab with stability data to cover the current study conditions.

Reference ID: 3628623

Study title: A 6-Month Toxicity Study of A-1233617 in Mice with a 1-Month Recovery Period

Study no.:	126-574 (Sponsor Study no. TD10-204)
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	29 November 2010
GLP compliance:	Yes. Note: Recovery data to be added by amendment.
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 (ABT-267) 85247PP00, 98.7%

Key Study Findings

- Mice were administered 5, 20 or 200 mg/kg (males) or 10, 40, or 200 mg/kg (females) ABT-267 via oral gavage for six months.
- There were no remarkable findings.
- The No-Observed-Adverse-Effect-Level (NOAEL) was the high dose (200 mg/kg/day, corresponding with systemic exposures of 40.8 µg•hr/mL (AUC_{0-24}) in the males, and 32.1 µg•hr/mL (AUC_{0-24}) in females.

Reference ID: 3628623

Methods

Doses:	Males: 5, 20 or 200 mg/kg Females: 10, 40, or 200 mg/kg
Frequency of dosing:	Daily for 182 days (6 months)
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	50% Polyethylene glycol (PEG 400):20% Tween 20:10% Poloxamer 124:20% Vitamin E TPGS
Species/Strain:	Mice/ CD-1 [Crl: CD® (ICR)]
Number/Sex/Group:	See sponsor table below
Age:	~ 8 weeks
Weight:	The male mice weighed 26.2 to 31.7 grams and the female mice weighed 22.2 to 26.2 grams
Satellite groups:	See sponsor table below
Unique study design:	None
Deviation from study protocol:	No reported deviations are expected to have affected study conclusions. Note: Recovery data to be added by amendment.

Group Assignments

Group Number	Dose Level (mg/kg/day) Male/Female	Number of Animals	
		Male	Female
	Main Study		
1	0 (vehicle)	30[b]	30[b]
2	5/10	20	20
3	20/40	20	20
4	200/200	30[c]	30[d]
	Toxicokinetic[a]		
5	0 (vehicle)	9+2	9+2
6	5/10	54+6	54+6
7	20/40	54+6	54+6
8	200/200	54+6	54+6

[a]Additional animals/sex/group included as possible replacement animals
[b]Ten animals/sex were assigned to a 1-month recovery period.
[c]Due to survival only eight males were assigned to a 1-month recovery period.
[d]Due to survival only nine females were assigned to a 1-month recovery period.

Results

Mortality: Mice were observed for survival and clinical signs twice a day during dosing. Detailed observations were made four to six hours after dosing at least once a week. There were eight early deaths from the main study groups:

Three mice (a male from the 5 mg/kg/day group, a male from the 200 mg/kg/day group, and a female from the 40 mg/kg/day group, respectively) were euthanized in poor condition with chronic active inflammation of the skin and/or ears. Microscopic observations occurring secondary to the skin/ear inflammation included increased extramedullary hematopoiesis in the spleen, increased

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lymphocyte/plasmacyte cellularity in lymph nodes, and/or increased cellularity of granulocytic series in femur bone marrow.

A male from the control group and a male from the 200 mg/kg/day group had evidence of probable dosing errors including pleural/subpleural inflammation of the lung, exudate in the thoracic cavity, and/or diffuse inflammation and necrosis of the epicardium of the heart.

The cause of death for one female from the 200 mg/kg/day group was moderate renal amyloidosis and chronic progressive nephropathy.

The cause of death for a male and female from the 200 mg/kg/day group was undetermined although both animals had microscopic observations of hemorrhage involving the lung, mediastinum, and/or thymus likely associated with dosing trauma.

There were 12 early deaths from the toxicokinetic groups. No cause of death was determined in those animals.

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

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

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

Hematology: 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: Brain, heart, kidney, liver, spleen, testes, 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 at 1, 3, 6, 9, 12, and 24 hours after dosing on Day 1 and during weeks 12 and 26. Toxicokinetic parameters for ABT-267 in mice following 182 days of dosing via oral gavage are presented below (*excerpted from sponsor*).

Reference ID: 3628623

Mean Toxicokinetic Parameters for A1233617

Treatment Group	Dose (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 C_{max}/D (µg/mL/mg/kg/day)	Day 1 T_{max} (hr)	Day 1 AUC (µg*hr/mL)	Day 1 AUC/D (µg*hr/mL/mg/kg/day)	Day 84 C_{max} (µg/mL)	Day 84 C_{max}/D (µg/mL/mg/kg/day)	Day 84 T_{max} (hr)	Day 84 AUC (µg*hr/mL)	Day 84 AUC/D (µg*hr/mL/mg/kg/day)
6M	5	Male	0.312	0.0624	6.0	4.53	0.906	0.272	0.0544	9.0	3.74	0.748
6F	10	Female	0.543	0.0543	6.0	5.92	0.592	0.408	0.0408	9.0	5.15	0.515
7M	20	Male	1.32	0.0660	6.0	17.9	0.896	1.50	0.0750	3.0	21.0	1.05
7F	40	Female	1.78	0.0445	6.0	24.8	0.620	1.60	0.0400	3.0	24.6	0.614
8F	200	Female	3.65	0.0183	6.0	51.9	0.260	3.15	0.0158	6.0	47.7	0.238
8M	200	Male	4.40	0.0220	12.0	69.7	0.348	5.05	0.0253	6.0	63.1	0.316

Mean Toxicokinetic Parameters for A1233617

Treatment Group	Dose (mg/kg/day)	Sex	Day 182 C_{max} (µg/mL)	Day 182 C_{max}/D (µg/mL/mg/kg/day)	Day 182 T_{max} (hr)	Day 182 AUC (µg*hr/mL)	Day 182 AUC/D (µg*hr/mL/mg/kg/day)
6M	5	Male	0.205	0.0410	3.0	3.57	0.715
6F	10	Female	0.320	0.0320	3.0	3.38	0.338
7M	20	Male	0.813	0.0407	9.0	10.5	0.526
7F	40	Female	1.60	0.0400	3.0	17.9	0.446
8F	200	Female	2.33	0.0117	6.0	32.1	0.160
8M	200	Male	2.98	0.0149	6.0	40.8	0.204

Stability and Homogeneity
The homogeneity results for all dose formulations, as reflected by relative standard deviations of concentrations, were within ± 5%. Stability of dosing formulation was not assessed in the current study. The Sponsor has provided the testing lab with stability data to cover the current study conditions.

Study title: Study RD11643. Five-Day Oral Toxicokinetic Study of A-1233617 in Male CByB6F1-Tg(HRAS)2Jic Non-Transgenic Mice TD11-143

Study no.:	TD11-143
Study report location:	Electronic
Conducting laboratory and location:	Abbott Laboratories 100 Abbott Park Road Abbott Park, IL 60064, USA
Date of study initiation:	21 January 2011 (Experimental start)
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 (ABT-267) 85247PP00, 88.9% potency

Key Study Findings

- Male CByB6F1-Tg(HRAS)2Jic non-transgenic mice (12/group) were administered A-1233617 for five days by oral gavage at dosages of 150, 200 and 300 mg/kg/day.
- Animals were observed twice daily to assess survival and body weights were measured twice during the five-day dosing period for the purpose of dose administration.
- All mice survived to scheduled, terminal blood collection and there were no effects on body weight.

Toxicokinetic Parameters for A-1233617, Mean Values

		Day 5		
Dosage (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0-24\ hr}$ (µg•hr/mL)
150	Male	3.13	6.0	53.6
200	Male	2.92	9.0	50.6
300	Male	3.14	6.0	43.5

Reference ID: 3628623

Study title: Four-Week Oral Toxicity Study of A-1233617 in CByB6F1-Tg(HRAS)2Jic non-Transgenic Mice

Study no.:	TD11-004
Study report location:	Electronic
Conducting laboratory and location:	Abbott Laboratories 100 Abbott Park Road Abbott Park, IL 60064, USA
Date of study initiation:	21 January 2011 (Experimental start)
GLP compliance:	Yes.
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 (ABT-267) 85247PP00, 88.9% potency

Key Study Findings

- Mice were administered 5, 25 or 150 mg/kg (males) or 10, 50, or 150 mg/kg (females) ABT-267 via oral gavage for 28 days.
- There were no remarkable findings.
- The No-Observed-Adverse-Effect-Level (NOAEL) was the high dose (150 mg/kg/day, corresponding with systemic exposures of 50.7 µg•hr/mL (AUC_{0-24}) in the males, and 38.9 µg•hr/mL (AUC_{0-24}) in females.

Reference ID: 3628623

Methods

Doses:	Males: 5, 25 or 150 mg/kg Females: 10, 50, or 150 mg/kg
Frequency of dosing:	Daily for 28 days
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	40% Phosal 53 MCT: 20% Polyethylene Glycol 400: 20% Poloxamer 124: 20% Cremophor RH40, by weight
Species/Strain:	Mice/ CByB6F1-Tg (HRAS)2 Jic (-/- homozygous c-Ha-ras),non-transgenic
Number/Sex/Group:	See sponsor table below
Age:	~ 9 weeks
Weight:	17.1 to 31.1 grams
Satellite groups:	See sponsor table below
Unique study design:	None
Deviation from study protocol:	No reported deviations are expected to have affected study conclusions.

Test Group	Dosage A-1233617 (mg/kg/day)[a]	Concentration of A-1233617 (mg/mL)[b]	Number of Animals[c]	
			Male	Female
Group 1 (vehicle control)	0[d]	0[e]	12	12
Group 2 (low)	5	2.5	12(2)	
	10	5		12(2)
Group 3 (mid)	25	12.5	12(2)	
	50	25		12(2)
Group 4 (high)	150	75	12(2)	12(2)

a. A-1233617.0 (Lot 85247P00). Assigned chemical potency = 889 mg/g of test item (88.9%).
b. Concentrations are expressed as mg active moiety/mL.
c. An additional two/sex/group (Groups 2-4) were dosed for the duration of the study and used as replacements in the event of mortality or in the event additional blood samples were required for toxicokinetic analysis. No mice were required for these purposes; therefore they were euthanized at the end of the dosing period and the carcasses were discarded.
d. Vehicle: 40% Phosal 53 MCT: 20% Polyethylene Glycol 400: 20% Poloxamer 124: 20% Cremophor RH40, by weight.
e. The dose volume was 2 mL/kg.

Results

Mortality: Mice were observed for survival and clinical signs twice a day during dosing. Detailed observations were made four to six hours after dosing at least two days a week. There were no early deaths among dosed animals.

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

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

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

Hematology: 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: Brain, heart, kidney, liver, spleen, testes, 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 at 1, 3, 6, 9, 12, and 24 hours after dosing on Day 1 and Day 24. Toxicokinetic parameters for ABT-267 in mice following 182 days of dosing via oral gavage are presented below (*excerpted from sponsor*).

Toxicokinetic Parameters for ABT-267, Mean Values (Male/Female)

ABT-267 Dose (M/F) (mg/kg/day)	Day	C_{max} (M/F) (µg/mL)	T_{max} (M/F) (hr)	AUC_{0-24} (M/F) (µg•hr/mL)
5/10	1	0.420/0.525	6/6	6.57/8.05
	24	0.411/0.597	6/6	7.46/6.86
25/50	1	1.97/2.50	9/6	32.3/35.2
	24	2.31/2.22	9/6	36.3/32.9
150/150	1	2.94/3.06	9/9	52.5/43.2
	24	3.00/2.29	6/9	50.7/38.9

Stability and Homogeneity

The homogeneity results for all dose formulations, as reflected by relative standard deviations of concentrations, were within ± 5%. Stability of dosing formulation was not assessed in the current study. The Sponsor has provided the testing lab with stability data to cover the current study conditions.

Reference ID: 3628623

Study title: 2-week Oral Range-finding Toxicity Study with A-1233617 in Rats (Non-GLP)

Study no.:	126-473 (sponsor no. TA09-188)
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 14, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	A-1233617.0, 1727006, 98.2%

Key Study Findings

- Rats were administered 2.5, 5, 15, or 30 mg/kg ABT-267 via oral gavage for 14 days.
- Based on histopathological findings in liver (single cell necrosis) from high dose animals, the no-observed-adverse-effect-level (NOAEL) was defined by this reviewer as the middle dose, 15 mg/kg/day, corresponding to Day 14 systemic exposures of 8.0 µg·hr/mL.
- The sponsor did not discuss the hepatic findings in high dose group animals and defined the NOAEL as the high dose, 30 mg/kg ($AUC_{Day\ 14}$=16 µg·hr/mL).

Reference ID: 3628623

Methods

Doses:	2.5, 5, 15, 30 mg/kg
Frequency of dosing:	Once daily (2.5, 5, 15 mg/kg) or twice daily (30 mg/kg) for 14 days
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	50% Polyethylene glycol (PEG 400), 20% Tween® 20, 10% Poloxamer 124, and 20% Vitamin E TGPS
Species/Strain:	Rat/ CD® [Crl:CD®(SD)]
Number/Sex/Group:	5
Age:	~ 6 weeks
Weight:	Male: 177 g to 210 g Female: 156 g to 179 g
Satellite groups:	Toxicokinetic: 6/Sex/Group
Unique study design:	none
Deviation from study protocol:	Reported deviations are not likely to have affected reported results.

Results

Mortality: Morbidity checks were performed twice daily. There were no deaths in treated groups. Death of one control animal was attributed to dosing error.

Clinical Signs: Detailed observations for clinical signs were performed twice weekly. There were no remarkable findings.

Body Weights: Body weights were recorded twice (week 1) or three times (week 2) a week. There were no remarkable findings.

Feed Consumption: Food consumption was recorded twice a week. There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted on surviving animals prior to terminal necropsy. There were no remarkable findings.

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

Clinical Chemistry: There were no toxicologically-significant findings.

Urinalysis: There were no toxicologically-significant findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Adrenals, brain, heart, kidney, liver, lung, 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: Minimal single cell hepatocellular necrosis was noted in three of five males from the high dose group. Minimal to mild hepatocellular vacuolation was noted in

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three of five high dose females. These findings were neither noted nor discussed by the sponsor.

Toxicokinetics

Samples for toxicokinetic assessment were collected prior to dosing and at 1, 3, 6, 9, 12, and 24 hours post-dose. Systemic exposures increased in proportion to dose from 2.5 to 30 mg/kg.

Summary of the Pharmacokinetic Parameters for A-1233617

Group	Dose	Day	C_{max}	C_{max}/D	T_{max}	$t_{1/2}$	$AUC_{0-\infty}$	$AUC_{0-\infty}/D$
6	2.5	1	90	36	3.0	5.3	829	331
		14	169	68	5.0	5.9	1416	567
7	5	1	173	35	4.0	5.7	1654	331
		14	280	56	5.0	5.7	2604	521
8	15	1	655	44	4.0	5.5	5622	375
		14	957	64	5.0	5.3	7986	532
9	30	1	886	30	12.0	5.2	9937	331
		14	1415	47	11.0	n.f.	16029	534

Units: Dose (mg acid/kg/day of A-1233617); C_{max} (ng/mL); C_{max}/D (ng/mL per mg/kg/day); T_{max} (hr); $t_{1/2}$ (hr); $AUC_{0-\infty}$ (ng•hr/mL); $AUC_{0-\infty}/D$ (ng•hr/mL per mg/kg/day); harmonic mean; n.f. = unable to estimate plasma elimination half-life

Stability and Homogeneity

Stability and homogeneity were not discussed in this non-GLP study report.

Study title: A Thirteen-Week Oral Maximum Tolerated Dose Toxicity Study of A-1233617 in Sprague-Dawley Rats

Study no.: TA10-249
Study report location: Electronic
Conducting laboratory and location: Abbott Laboratories
100 Abbott Park Road
Abbott Park, IL 60064, USA
Date of study initiation: 03 December 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-1233617 (A-1233617.0, ABT-267); Lot: 85247PP00; Potency: 88.9%

Key Study Findings

- Sprague-Dawley rats were administered ABT-267 (0, 10, 30 or 300 mg/kg/day) by oral gavage for 13 weeks.

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- Findings were limited to decreased food consumption in high dose females and minimal decreases in triglycerides (-10% to -31%) in all dose groups (males and females). The change in triglyceride levels was not considered to be toxicologically significant.
- Peak plasma drug level (Cmax) and systemic exposure (AUC0-24) increased with dose between 10 and 30 mg/kg/day in males, with little to no increase at 300 mg/kg/day.
- Based on a lack of adverse findings at any dose level, the no-observed-adverse-effect-level (NOAEL) was defined as the high dose, 300 mg/kg/day, corresponding to mean systemic exposures (AUC_{0-24}) of 22.4 µg●hr/mL in males and 35.3 µg●hr/mL in females.

Methods

Doses:	10, 30, and 300 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	40% Phosal 53 MCT: 20% Polyethylene Glycol 400: 20% Poloxamer 124: 20% Cremophor RH40, by weight
Species/Strain:	Rats/ Sprague-Dawley [Crl: CD® (SD)]
Number/Sex/Group:	12
Age:	Males: 8 weeks Females: 9 weeks
Weight:	213.1-308.1 grams
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	Reported deviations do not appear to have affected the validity of study conclusions.

Test Group	Dosage (A-1233617.0) (mg/kg/day)[a]	Concentration (mg/mL of A-1233617.0)	Number of Rats	
			Males	Females
Group 1 (control)	0[b]	0[c]	12	12
Group 2	10	5	12	12
Group 3	30	15	12	12
Group 4 (high)	300	150	12	12

a. A-1233617 (A-1233617.0, Lot 85347PP00); assigned chemical potency = 889 µg A-1233617.0/mg drug substance "as is."

b. Vehicle: Phosal 53 MCT: Polyethylene Glycol 400: Poloxamer 124: Cremophor RH40 (40:20:20:20), mixed by weight.

c. The dose volume: 2 mL/kg.

Results

Mortality: Rats were observed for morbidity/mortality twice daily during the dosing period. A high dose male was euthanized on Day 49 due to poor clinical condition including decreased activity, red muzzle staining, yellow ano-genital staining and favoring of the left forelimb. A food-filled pocket in the left axillary region, also containing fluid and white particulate, was observed grossly at necropsy. Therefore this animal's poor condition was considered to be the result of gavage-related trauma, and not test item-related.

Clinical Signs: Detailed clinical observations were recorded five to seven hours after dosing on two days per week during the dosing period. No test item-related changes in behavior or physical condition were reported.

Body Weights: Body weights were recorded twice weekly during dosing. No test item-related effects on bodyweight or bodyweight gain were observed.

Feed Consumption: Food consumption was recorded once weekly during dosing. No test item-related effects on food consumption were observed in males. In high dose females, mean food consumption values were decreased compared to controls (-10.5%) without corresponding effects on bodyweight. Therefore the effect on food consumption was not considered adverse.

Ophthalmoscopy: Ophthalmologic examinations were performed on Baseline Day 2 and dosing Day 91, prior to dose administration. All animals were within normal limits.

Clinical Pathology: Samples of blood were collected immediately prior to necropsy.

Hematology: No test item-related changes in hematology parameters were observed.

Clinical Chemistry: Test item-related, non dose-dependent, minimal decreases in mean triglyceride concentrations were seen in all dose groups (-10% to -31%: males, -13% to -22%: females) on Dosing Day 97.

Urinalysis: Low sample number in females (due to spillage) precludes definitive evaluation. No test item-related changes in urinalysis parameters were observed in males.

Gross Pathology: Abnormal content (yellow or white) was present in the stomach of four high dose animals (three males and one female) and large intestine of three high dose animals (one male and two females). There were no associated microscopic findings. The texture and color of the contents were consistent with the test item.

Organ Weights: The following organs were weighed: Adrenal glands, brain, epididymis, heart, kidney, liver, ovaries, pituitary gland, prostate gland, spleen, testes, thymus, thyroid and parathyroid. There were no test item-related changes in organ weight.

Histopathology

Adequate Battery: Yes. [Note: microscopic examination was limited to Groups 1 and 4.]

Peer Review: Yes

Histological Findings: There were no item item-related microscopic observations in this study.

Toxicokinetics

Samples of blood were collected at 1, 3, 6, 9, 12 and 24 hours after dosing from two rats/sex/group/time point on Days 1 and 96. Toxicokinetic parameters are presented in the table below (*excerpted from sponsor*).

Peak plasma drug level (C_{max}) and systemic exposure (AUC_{0-24}) increased with increasing dose between 10 and 30 mg/kg/day on both Days 1 and 96, and were similar in both sexes. Relative to the 30 mg/kg/day dose level, no additional increases in either C_{max} or AUC_{0-24} were observed in males administered 300 mg/kg/day, on either Day 1 or Day 96. In females administered 300 mg/kg/day, Day 1 C_{max} and AUC_{0-24} values were similar to those of animals administered 30 mg/kg/day. On Day 96, C_{max} and AUC_{0-24} values were approximately four-fold and three-fold higher than values measured on Day 1.

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Toxicokinetic Parameters for A-1233617 Mean ± Standard Deviation (SD)

Collection Interval	Sex	A-1233617 Dosage (mg/kg/day) 10	30	300
		Mean Plasma AUC_{0-24} (µg•hr/mL)		
Day 1	Males	3.93	11.7	10.4
	Females	3.83	9.65	10.8
Day 96	Males	10.7	20.4	22.4
	Females	9.23	25.4	35.3
		Mean Plasma C_{max} (µg/mL)		
Day 1	Males	0.267	0.773	0.785
	Females	0.333	0.703	0.822
Day 96	Males	0.954	1.50	1.74
	Females	0.546	1.51	3.25

Stability and Homogeneity

Assay results indicate that the formulations were prepared at the correct concentrations (± 3% of intended) and were homogeneous (relative standard deviations ranged from 0.6% to 0.8%).

Reference ID: 3628623

Study title: 2-week Oral Range-finding Toxicity Study with A-1233617 in Beagle Dogs (Non-GLP)

Study no.:	126-474
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 14, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	A-1233617 Free Base; Lot No.: 1727006 89.7%

Key Study Findings

- There were no adverse findings in this pilot study.
- The NOAEL was defined as the high dose (60 mg/kg). The corresponding systemic exposures (AUC) was 81.4 µg·hr/mL.

Methods

Doses:	3, 20 or 60 mg/kg
Frequency of dosing:	Daily for 14 days
Route of administration:	Oral (gelatin capsules)
Dose volume:	1 mL/kg
Formulation/Vehicle:	Gelatin capsule/50% Polyethylene glycol (PEG 400), 20% Tween® 20, 10% Poloxamer 124, and 20% Vitamin E TGPS
Species/Strain:	Dog/Beagle
Number/Sex/Group:	2
Age:	5 months
Weight:	Male: 6.16 to 7.42 kg Female: 5.65 to 6.17 kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Results

Mortality: Morbidity/mortality checks were performed twice daily. There were no early deaths.

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

Reference ID: 3628623

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

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

Ophthalmoscopy: Ophthalmoscopic examinations were performed pre-dosing and prior to terminal necropsy. There were no remarkable findings.

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

Clinical Chemistry: Alanine aminotransferase (ALT) was increased less than 2-fold over controls in one high dose male and one high dose female dog. This finding is not toxicologically significant.

Urinalysis: Urine was collected over 16 hours in under-cage pans prior to terminal necropsy. There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

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

Histopathology

Adequate Battery: Yes.

Peer Review: No.

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 14. Toxicokinetic parameters in dogs following 14 days of oral administration of ABT-267 are presented below (*table excerpted from sponsor*).

Summary of the Pharmacokinetic Parameters for A-1233617

Group	Dose	Day	C_{max}	C_{max}/D	T_{max}	$t_{1/2}$	$AUC_{0-\infty}$	$AUC_{0-\infty}/D$
2	3	1	0.5	0.15	3.8	8.8	7.6	2.54
		14	0.5	0.17	4.5	11.1	6.8	2.26
3	20	1	2.5	0.13	3.0	8.6	35.5	1.78
		14	2.6	0.13	4.5	9.1	35.5	1.77
4	60	1	6.3	0.10	6.8	9.2	112.0	1.87
		14	6.1	0.10	4.5	10.9	81.4	1.36

Units: Dose (mg acid/kg/day of A-1233617); Cmax (µg/mL); Cmax/D (µg/mL per mg/kg/day); Tmax (hr); t1/2 (hr); AUC0-∞ (µg•hr/mL); AUC0-∞ /D (µg•hr/mL per mg/kg/day).

Reference ID: 3628623

Stability and Homogeneity

Dosing formulations were prepared daily: Stability testing was ongoing at the time of this study. Homogeneity testing was not discussed and presumably not done.

Study title: Four-Week Oral Capsule Toxicity Study of A-1233617 in Beagle Dogs

Study no.:	TB09-270
Study report location:	Electronic
Conducting laboratory and location:	Abbott Laboratories 100 Abbott Park Road Abbott Park, IL 60064, USA
Date of study initiation:	21 December 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 free base (A-1233617.0, ABT-267) Lot: 83192PP01, 90.8%

Key Study Findings

- Beagle dogs were dosed with 2, 10 or 60 mg/kg ABT-267 in liquid-filled capsules for 28 days.
- There were no toxicological findings.
- The no-observed-adverse-effect level (NOAEL) was defined as the high dose, 60 mg/kg/day, corresponding to systemic exposure values of 48.9 µg•hr/mL (AUC_{0-24}; males and females combined).

Methods

Doses:	2, 10 or 60 mg/kg
Frequency of dosing:	Once daily for 28 days
Route of administration:	Oral (gelatin capsules)
Dose volume:	1 mL/kg
Formulation/Vehicle:	Gelatin capsule/50% Polyethylene glycol (PEG 400), 20% Tween® 20, 10% Poloxamer 124, and 20% Vitamin E TGPS
Species/Strain:	Dog/Beagle
Number/Sex/Group:	3
Age:	11 - 14 months
Weight:	6.4 – 11.1 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	The stated deviation is not likely to have affected study results.

Results

Mortality: Morbidity/mortality checks were performed twice daily. There were no early deaths.

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

Reference ID: 3628623

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

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

Ophthalmoscopy: Ophthalmoscopic examinations were performed pre-dosing and prior to terminal necropsy. There were no remarkable findings.

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

Electrocardiogram: ECGs were recorded pre-dosing, at the end of one week of dosing, and near the end of the four week dosing period. There were no remarkable findings.

Clinical Chemistry: Alanine aminotransferase (ALT) was increased less than 2-fold over controls in one high dose male and one high dose female dog. This finding is not toxicologically significant.

Urinalysis: Urine was collected over 16 hours in under-cage pans prior to terminal necropsy. 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 28. Toxicokinetic parameters in dogs following 28 days of oral administration of ABT-267 are presented below (*table excerpted from sponsor*). Exposures were similar in males and females. Systemic exposures increased in proportion to dose from 2 to 10 mg/kg but increases were less than dose proportional between 10 and 60 mg/kg.

Reference ID: 3628623

Collection Interval	Sex	A-1233617 Dosage (mg/kg/day) 2	10	60
		Mean Plasma AUC_{0-24} (µg•hr/mL) ± SD		
Day 1	Males	2.75 ± 0.302	15.8 ± 6.26	32.0 ± 8.90
	Females	2.62 ± 1.24	12.3 ± 3.76	51.9 ± 19.0
	Combined	2.69 ± 0.811	14.1 ± 4.99	41.9 ± 17.2
Day 28	Males	3.47 ± 0.727	17.8 ± 9.24	39.5 ± 11.2
	Females	3.10 ± 1.66	13.7 ± 3.37	58.3 ± 20.2
	Combined	3.28 ± 1.17	15.8 ± 6.61	48.9 ± 17.9
		Mean Plasma C_{max} (µg/mL) ± SD		
Day 1	Males	0.246 ± 0.0213	1.11 ± 0.213	2.18 ± 0.488
	Females	0.204 ± 0.0619	0.981± 0.123	3.64 ± 1.52
	Combined	0.225 ± 0.0474	1.04 ± 0.171	2.91± 1.29
Day 28	Males	0.264 ± 0.0655	1.31± 0.572	2.86 ± 0.388
	Females	0.253 ± 0.107	1.23 ± 0.228	4.09 ± 1.40
	Combined	0.259 ± 0.0797	1.27 ± 0.392	3.47 ± 1.14

Stability and Homogeneity

Stability and homogeneity of the test article formulation was assessed in the current study. Both stability and homogeneity met acceptance criteria.

Reference ID: 3628623

Study title: 3-Month Oral Dose Toxicity Study With A-1233617 in Beagle Dogs

Study no.:	126-522
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 31, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ABT-267 (A-1233617 free base), Lot No.: 83192PP01, 91%

Key Study Findings

- Beagle dogs were dosed with 2, 10 or 100 mg/kg ABT-267 in liquid-filled capsules for 91 days.
- In high dose animals, minimal to mild histopathology findings in small intestine, bile duct and liver were not considered adverse based on a lack of clinical signs of body weight effects.
- The NOAEL is defined as the high dose, 100 mg/kg, corresponding to systemic exposure values of 61 and 106 µg•hr/mL (AUC_{0-24}) in males and females, respectively.

Methods

Doses:	2, 10 or 100 mg/kg
Frequency of dosing:	Daily for 3 months
Route of administration:	Oral (gelatin capsules)
Dose volume:	≤ 5 mL/capsule
Formulation/Vehicle:	Gelatin capsule/50% Polyethylene glycol (PEG 400), 20% Tween® 20, 10% Poloxamer 124, and 20% Vitamin E TGPS
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Age:	6-7 months
Weight:	Male: 8.33 to 10.13 kg Female: 5.96 to 6.85 kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

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 approximately 2 hours and 50 minutes to 3 hours and 10 minutes postdose. There were no remarkable findings.

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 pre-dosing period (Days -11 to -7 and Days -7 to -4), and twice weekly during the study. There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted pretest (Day -4) and prior to the terminal necropsy (Day 88). There were no remarkable findings.

Reference ID: 3628623

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

Electrocardiogram: Physical examinations were conducted pretest (Day -5), and electrocardiographic examinations were conducted prior to the initiation of dosing (Day -11) and at approximately 2 hours and 52 minutes to 3 hours and 2 minutes postdose on Days 1 and 87. There were no remarkable findings.

Clinical Chemistry: Blood samples for clinical pathology evaluations were collected from all animals twice pretest (Days -8 and -5), once during Week 4 (Day 25), and prior to the terminal necropsy (Day 88). Alanine aminotransferase (ALT) was increased less than 2-fold over controls in one high dose male and one high dose female dog. This finding is not toxicologically significant.

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, spleen, ovaries, testes, thyroid/parathyroid, and thymus were weighed. In one high dose female, increased liver weight correlated with increased hepatocellular vacuolation microscopically.

Histopathology

Adequate Battery: Yes.
Peer Review: Yes.
Histological Findings: In high dose groups, males (3/4) and females (3/4) had minimal to mild focal dilatation of the lymphatic capillaries (lacteals) within villi of the small intestine. In one high dose female, diffuse, mild hepatocellular vacuolation which correlated with increased liver weight. Finally, in one high dose female, extrahepatic bile duct was inadvertently collected (attached to the pancreas sample), and on examination histopathology was noted. Mild subacute/chronic inflammation involving the tunica mucosa and muscularis was seen microscopically, without correlative findings in the biliary tree. The study pathologist classified these findings as non-adverse given the lack of clinical signs or effects on body weight.

This reviewer raises the possible connection between findings in lacteals and bile ducts, but acknowledges that none of the noted findings are considered adverse.

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 ABT-267 are presented below (*table excerpted from sponsor*). Exposures were similar in males and females.

Reference ID: 3628623

A-1233617 Dosage (mg/kg/day)				
Collection Interval	Sex	2	10	100
		Mean Plasma AUC_{24} (µg•hr/ml) ± SD		
Day 1	Male	1.98 ± 0.664	11.9 ± 0.690	38.4 ± 13.9
	Female	2.21 ±0.371	9.81 ± 3.09	50.7 ± 13.5
Day 28	Male	1.87 ± 0.530	14.4 ± 3.99	48.4 ± 15.4
	Female	1.98 ± 0.225	14.0 ± 4.44	75.8 ± 23.4
Day 91	Male	2.15 ±0.922	19.3 ± 4.38	61.2 ± 16.8
	Female	4.07 ± 1.48	16.8 ± 10.7	106 ± 33.7
		Mean Plasma C_{max} (µg/ml) ± SD		
Day 1	Male	0.185 ± 0.0506	1.06 ± 0.114	2.47 ± 0.812
	Female	0.199 ± 0.0348	0.762 ± 0.281	3.04 ± 0.676
Day 28	Male	0.166 ± 0.0348	1.20 ± 0.385	3.06 ± 0.724
	Female	0.173 ± 0.0137	1.03 ± 0.300	4.93 ± 1.40
Day 91	Male	0.185 ± 0.0581	1.61 ± 0.337	3.82 ± 0.812
	Female	0.340 ± 0.138	1.31 ± 0.820	6.53 ± 2.37

Stability and Homogeneity

According to the test lab, the sponsor provided data indicating the stability of the test article in formulation for at least 7 days (b) (4) Homogeneity of the test article formulation was assessed in the current study. Both stability and homogeneity met acceptance criteria.

Study title: Twenty-Seven Week Oral Capsule Toxicity Study of A-1233617 Free Form in Beagle Dogs

Study no.: TB10-205
Study report location: Electronic
Conducting laboratory and location: Global Pharmaceutical Research and Development
Preclinical Safety Division
Toxicology
Abbott Laboratories
100 Abbott Park Road
Abbott Park, IL 60064, USA
Date of study initiation: November 1, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ABT-267 (A-1233617 free base), Lot No.: 85247PP00, 89%

Key Study Findings

- Administration of A-1233617 for 189 to 191 days at dose levels of 4, 20 and 100 mg/kg/day did not result in adverse findings.
- Test item related, non-adverse findings were limited to minimal to mild vacuolation of small intestinal villi (duodenum and/or jejunum) in animals administered 20 and 100 mg/kg/day.
- Based on the lack of adverse findings at any dose level, the no observed adverse effect level (NOAEL) in this study was 100 mg/kg/day, resulting in a combined AUC of 83.3 µg*hr/mL.

Methods

Doses:	4, 20 or 100 mg/kg
Frequency of dosing:	Daily for 27 weeks
Route of administration:	Oral (gelatin capsules)
Dose volume:	1 mL/kg
Formulation/Vehicle:	Gelatin capsule/50% Polyethylene glycol (PEG 400), 20% Tween® 20, 10% Poloxamer 124, and 20% Vitamin E TGPS
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Age:	8 months
Weight:	5.7 to 11.4 kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Results

Mortality: Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. One male from the middle dose group was found dead on day 59. Death was attributed to spontaneous intestinal torsion.

Clinical Signs: Cageside observations were conducted twice daily during dosing. Detailed clinical observations were conducted twice weekly at approximately 3 hours postdose. There were no remarkable findings.

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 pre-dosing period and twice weekly during the study. There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted pretest and prior to the terminal necropsy (Day 186). There were no remarkable findings.

Hematology: Blood samples for clinical pathology were collected once during the baseline period, at approximately one and 12 weeks after the initiation of dosing, and near the end of the dosing period. There were no remarkable findings.

Electrocardiogram: There were no remarkable findings.

Clinical Chemistry: Blood samples for clinical pathology were collected once during the baseline period, at approximately one and 12 weeks after the initiation of dosing, and near the end of the dosing period. There were toxicologically significant findings.

Urinalysis: There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Reference ID: 3628623

Organ Weights: Adrenals, brain, epididymis, heart, kidney, liver, pituitary, spleen, ovaries, testes, thyroid/parathyroid, and thymus were weighed. In one high dose female, increased liver weight correlated with increased hepatocellular vacuolation microscopically.

Histopathology

Adequate Battery: Yes.

Peer Review: Yes.

Histological Findings: Histopathology was noted in the duodenum and/or jejunum of middle and high dose males and females. Minimal to mild vacuolation within intestinal villi was present among most animals at greater than or equal to 20 mg/kg/day (middle dose), without dose-dependent effects on incidence or severity. The vacuoles were variably sized, smoothly rounded, clear spaces distinct from lacteals and not associated with a cellular reaction. Similar vacuoles were noted within the outer medulla and subcapsular sinuses of the mesenteric lymph node from a high-dose female. The intestinal villar vacuoles were considered not adverse, due to lack of an appreciable functional effect.

Toxicokinetics
Blood samples were collected predose and at 1, 3, 6, 9, 12, and 24 hours postdose on Days 1, 91 and 189.

Toxicokinetic parameters in dogs following 189 days of oral administration of ABT-267 are presented below (*table excerpted from sponsor*). Exposures were similar in males and females.

Reference ID: 3628623

Mean (±SD) Toxicokinetic Parameters for A-1233617

Test Group	Dose (mg/kg/day)	Sex	Day 189				
			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)
2	4	Male	0.660	0.165	3.0	8.47	2.12
			±0.0258	±0.00648	±0.0	±1.11	±0.280
		Female	0.533	0.133	3.0	5.99	1.50
			±0.147	±0.0366	±0.0	±1.26	±0.315
		Overall	0.596	0.149	3.0	7.23	1.81
			±0.119	±0.0296	±0.0	±1.72	±0.432
3	20	Male	3.51	0.176	3.0	53.8	2.69
			±0.266	±0.0133	±0.0	±6.77	±0.336
		Female	3.41	0.171	3.0	41.5	2.08
			±0.804	±0.0400	±0.0	±13.4	±0.670
		Overall	3.46	0.173	3.0	46.8	2.34
			±0.591	±0.0294	±0.0	±12.2	±0.608
4	100	Male	5.36	0.0536	4.5	89.8	0.898
			±0.815	±0.00815	±1.7	±17.3	±0.173
		Female	5.01	0.0501	4.5	76.9	0.769
			±2.08	±0.0208	±1.7	±25.5	±0.255
		Overall	5.18	0.0518	4.5	83.3	0.833
			±1.47	±0.0147	±1.6	±21.3	±0.213

Stability and Homogeneity
According to the test lab, the sponsor provided data indicating the stability of the test article in formulation for at least 7 days [redacted] (b) (4) Homogeneity of the test article formulation was assessed in the current study. Both stability and homogeneity met acceptance criteria.

Genetic Toxicology

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

Study title: Salmonella − Escherichia coli/Mammalian − Microsome Reverse Mutation Assay with a Confirmatory Assay with A − 1233617

Study no.:	8222070 (sponsor no. TX09-277)
Study report location:	Electronic
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	14 January 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617, 83192PP01, 91.1%

Key Study Findings

ABT-267 was negative for mutations under the conditions of this *Salmonella–E. coli*/Mammalian-Microsome Reverse Mutation Assay

Reference ID: 3628623

Methods

Strains:	TA98, TA100, TA1535, TA1537, WP2*uvr*A
Concentrations in definitive study:	1.00, 5.00, 10.0, 50.0, 100, 500, 1000, 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 (*excerpted from sponsor*)
Formulation/Vehicle:	dimethylsulfoxide (DMSO),
Incubation & sampling time:	52 ± 4 hour

Positive Control Articles

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)	CAS No.	Lot No.
TA98	–	2-nitrofluorene	1.0	607-57-8	01508BE
TA100, TA1535	–	sodium azide	2.0	26628-22-8	017K0136
TA1537	–	ICR-191	2.0	17070-45-0	116K1026
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0*	56-57-5	117K1485
TA98	+	benzo[a]pyrene	2.5	50-32-8	087K0733
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	613-13-8	12317CE
WP2*uvr*A	+	2-aminoanthracene	25.0	613-13-8	12317CE

* 0.4 µg/plate for the preincubation exposure method

Study Validity

Study validity criteria were met.

Results

ABT-267 was negative for mutations under the conditions of this *Salmonella–E. coli*/Mammalian-Microsome Reverse Mutation Assay

***In Vitro* Chromosomal Aberration Assays in Mammalian Cells**

Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with A-1233617.

Study no.:	8222071 (sponsor no. TX09-278)
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	14 January 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617, 83192PP01, 91.1%

Key Study Findings

No statistically significant increase in the number of cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed. ABT-267 was considered to be negative for causing chromosomal aberrations under the conditions of this assay.

Methods

Cell line:	Cultured human lymphocytes

Reference ID: 3628623

Concentrations in definitive study:	18.8, 37.5, 75.0, 113, 150, 225, 300, and 400 µg/mL
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 (*excerpted from sponsor*)

Summary of Treatment Schedule in Hours (approximate)

S9Activation Mix	Test Article Added	Exposure Completed	Colcemid® Added	Harvest Started
Without	0	3	20*	22
Without	0	22	20*	22
With	0	3	20*	22

Study Validity

All validity criteria were met.

Results

ABT-267 was considered to be negative for causing chromosomal aberrations under the conditions of this assay.

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

Study title: In Vivo Mouse Bone Marrow Micronucleus Assay with A-1233617

Study no:	TD09-279
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09 March 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-998821, Lot No. 83192PP01, 90.8%

Key Study Findings

Administration of A-1233617 did not induce an increase in micronucleated immature erythrocytes in male mice under the conditions of this assay. Therefore, A-1233617 was concluded to be negative in the *in vivo* micronucleus assay.

Reference ID: 3628623

Methods

Doses in definitive study:	500, 1000 or 2000 mg/kg
Frequency of dosing:	single administration
Route of administration:	oral
Dose volume:	20 mL/kg (10 mL/kg for cyclophosphamide).
Formulation/Vehicle:	80% (w/w) polyethylene glycol 400 (PEG-400)/20% (w/w) polysorbate 20 (Tween™ 20)
Species/Strain:	Hsd:ICR(CD-1) mice
Number/Sex/Group:	5
Satellite groups:	Toxicokinetics (18/group)
Basis of dose selection:	The dose levels were selected based on the absence of findings at the high dose in a preliminary toxicity assessment.
Negative control:	vehicle
Positive control:	cyclophosphamide

Study Validity

Results of the toxicokinetic analysis confirmed bone marrow exposure of the test article. All criteria for a valid assay were met.

Results

The group mean of micronucleus frequency in low dose (500 mg/kg) group (0.09±0.04%) was found statistically significantly higher than that in concurrent vehicle control group (0.01±0.02%), but was not considered biologically relevant. The test article, A-1233617, was determined to be negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

Carcinogenicity

Study title: Study RD12574. 26-Week Oral Gavage Carcinogenicity Study with A-1233617 Free Form in Model 001178-T (Hemizygous)CByB6F1-Tg(HRAS)2Jic Mice

Study no.:	TD12-080
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4 June 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 (A-1233617.0, A-1233617 free form, ABT-267; Batch Number 95438PP00), 92.4%
CAC concurrence:	Yes

Key Study Findings

- There were no treatment related neoplastic or non-neoplastic findings.
- At the high dose, the mean AUC value was 37 µg.hr/mL for both sexes.

Adequacy of Carcinogenicity Study

Reference ID: 3628623

Mice were exposed to adequate concentrations of test article, and assessments were sufficient to determine the carcinogenicity of test article.

Appropriateness of Test Models
The transgenic mouse species used here is an appropriate model in which to assess the carcinogenicity of chemical agents.

Evaluation of Tumor Findings
There were no tumor findings associated with test article administration.

Methods

Doses:	0 (water), 0 (vehicle), 2.5 (male), 5 (female), 10 (male), 20 (female), 150 mg/kg/day ABT-267, 75 mg/kg MNU (positive control group)
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	40% Phosal 53 MCT: 20% Polyethylene Glycol 400: 20% Poloxamer 124: 20% Cremophor RH40
Basis of dose selection:	1 month study, maximum feasible exposures, saturation of absorption, CAC concurrence.
Species/Strain:	CByB6F1-Tg(HRAS)2Jic mice
Number/Sex/Group:	25 (15 positive control)
Age:	6-7 weeks at receipt
Animal housing:	individually housed in polyboxes with nonaromatic bedding (SaniChips™) in an environmentally controlled room
Paradigm for dietary restriction:	Not applicable.
Dual control employed:	yes
Interim sacrifice:	no
Satellite groups:	Yes (TK)
Deviation from study protocol:	No significant deviations.

Observations: All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. A detailed clinical examination of each main study animal was performed prior to dosing and at 3 hours (±30 minutes) postdose on Day 1, and twice weekly thereafter during the study. Body weights and food consumption were measured weekly during the study. Blood samples for clinical pathology were collected at necropsy.

Results

Mortality: No dose relationship was noted in the incidence of mortality.

Reference ID: 3628623

Number of Animals Not Surviving to the Scheduled Terminal Necropsy (Week 27)*		
Dose Level (mg/kg/day)	Male	Female
0 (Water)	1/25	2/25
0 (Vehicle)	0/25	1/25
2.5	1/25	-
5	-	2/25
10	0/25	-
20	-	0/25
150	1/25	1/25
Positive Control	4/15	3/15

*Number of early deaths/Number per group
- Not applicable

The complete list of unscheduled deaths is given below (table excerpted from sponsor).

Unscheduled Deaths of Main Study Terminal Males and Females					
Animal Number	Sex	Dose Level (mg/kg/day)	Fate	Fate Day	Cause of Death
1012	M	0 mg/kg/day (Water Control)	FD	164	Urogenital inflammation/obstruction/calculi
1074	M	2.5 mg/kg/day	FD	67	Urogenital inflammation/obstruction/calculi
1133	M	150 mg/kg/day	EE	109	Undetermined
1141	M	Positive Control	FD	70	Gastrointestinal tumor
1144	M	Positive Control	EE	72	Gastrointestinal tumor
1147	M	Positive Control	EE	120	Lymphoid tumor
1154	M	Positive Control	EE	168	Gastrointestinal tumor
2012	F	0 mg/kg/day (Water Control)	EE	112	Undetermined
2020	F	0 mg/kg/day (Water Control)	FD	85	Hemangiosarcoma/Hemangioma
2041	F	0 mg/kg/day (Vehicle Control)	FD	62	Dosing injury
2069	F	5 mg/kg/day	FD	62	Dosing injury
2076	F	5 mg/kg/day	EE	163	Skin tumor
2134	F	150 mg/kg/day	EE	184	Accidental injury
2141	F	Positive Control	EE	158	Gastrointestinal tumor
2145	F	Positive Control	EE	163	Lymphoid tumor
2146	F	Positive Control	FD	28	Uterine tumor
M – Male F – Female			FD – Found Dead EE – Euthanized *in extremis*		

Clinical Signs: No test article-related clinical findings were observed.

Body Weights: No test article or vehicle-related effects on body weight were observed.

Feed Consumption: Sporadic differences between control and treated animals with respect to feed consumption were not dose related.

Gross Pathology: There were no treatment-related macroscopic findings present in either sex in this study.

Histopathology

Peer Review: Yes.

Neoplastic: There were no drug-related neoplastic findings. There was no statistically significant difference in tumor incidence between the control and treated groups.

Non Neoplastic: There were no treatment-related non-neoplastic findings.

Toxicokinetics

Reference ID: 3628623

Despite the difference between the low and middle dose levels, systemic exposures (AUC) were nearly identical in males and females at all doses (see sponsor's table below). At the high dose, the mean AUC value was 37 µg.hr/mL for both sexes.

Mean Toxicokinetic Parameters for A-1233617					
		Day 87			
Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	C_{max}/Dose (µg/mL/ mg/kg/day)	AUC (µg*hr/mL)	AUC/Dose (µg*hr/mL/ mg/kg/day)
2.5	male	0.159	0.0636	2.67	1.07
5	female	0.216	0.0432	2.98	0.597
10	male	0.798	0.0798	10.7	1.07
20	female	0.885	0.0443	12.4	0.618
150	male	1.98	0.0132	36.6	0.244
150	female	2.51	0.0167	36.9	0.246

Dosing Solution Analysis

The sponsor provided data indicating the test article was stable under the conditions of this study. Formulations were considered to be homogeneous (0.5 to 1.4% S.D.). Concentrations ranged from 95 to 104% of theory for all test article groups and were considered acceptable.

Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study title: An Oral (Gavage) Fertility and Early Embryonic Development Study of A-1233617 in Mice

Study no.: TD11-274
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 21 February 2012 (protocol signed)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-1233617, 95438PP11 (sublot of 95438PP00), 99.8%

Key Study Findings

- Mating and fertility were unaffected by doses of A-1233617.
- A-1233617-related changes in male reproductive organ weights included: increases weights of the prostate at 20 and 200 mg/kg/day; the seminal vesicles (without fluid) at 200 mg/kg/day; and decreases in the absolute and relative weights of the testes at 200 mg/kg.

Reference ID: 3628623

Methods

Doses:	See tables below
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	PEG 400 (50%), Poloxamer 124 (10%), Tween 20 (20%) and Vitamin E TPGS (20%), by weight
Species/Strain:	Crl:CD1(ICR) mice
Number/Sex/Group:	See table below
Satellite groups:	No
Study design:	See tables below
Deviation from study protocol:	None noted

Table Experimental Design - Male Mice

Group No.	Test Material	A-1233617 Dose Level (mg/kg)	A-1233617 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	No. of Male Mice
1	Control Article	0	0	2	30
2	A-1233617	5	2.5	2	30
3	A-1233617	20	10	2	30
4	A-1233617	200	100	2	30

a. The assigned potency is 924 mcg/mg bulk; a correction factor of 1.082 was used for the purpose of dose and preparation calculations.

Table Experimental Design - Female Mice

Group No.	Test Material	A-1233617 Dose Level (mg/kg)	A-1233617 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	No. of Female Mice
5	Control Article	0	0	2	30
6	A-1233617	10	5	2	30
7	A-1233617	40	20	2	30
8	A-1233617	200	100	2	30

a. The assigned potency is 924 mcg/mg bulk; a correction factor of 1.082 was used for the purpose of dose and preparation calculations.

Results

The following parameters and end points were evaluated in this study: viability, clinical signs, body weights, body weight changes, food consumption, estrous cycling, ovarian and uterine examinations (female mice), reproductive organ weights (male mice only) and gross necropsy findings.

Reference ID: 3628623

Mortality: One male at 20 mg/kg/day was found dead on DS 29, and the cause of death could not be established. Two males at 200 mg/kg/day were euthanized; one male on DS 3 was euthanized due to a limb injury and another one on DS 28 was euthanized due to suspected gavage error based on veterinary examination and signs of respiratory distress. All other male mice survived to scheduled euthanasia.

All female mice survived to scheduled euthanasia, and there were no A-1233617-related clinical signs during the premating or gestation periods.

Clinical Signs: Clinical signs that occurred in individual animals included mild or moderate dehydration (based on skin turgor), scant feces, ungroomed coat, ptosis, tachypnea and lacrimation. However, these were not attributed to A-1233617 because the observations were transient or were not dose-dependent.

Body Weight: Terminal body weights were comparable among the four dose groups and did not significantly differ.

Feed Consumption: Absolute (g/day) and relative (g/kg/day) food consumption values were unaffected by doses of A-1233617 as high as 200 mg/kg/day.

Toxicokinetics
Toxicokinetics were not assessed.

Stability and Homogeneity
The results for A-1233617.0 concentration ranged from 90% to 110% of the theory for all other treatment groups, except the Group 3 Top (start of study; 10 mg/mL), Group 6 (mid-point; 5 mg/mL) and Group 2 (end of study; 2.5 mg/mL) samples which were 122%, 125% and 89% of theory, respectively.

The relative standard deviation for homogeneity of A-1233617.0 concentrations ranged from 0.0% to 13.6% within each treatment group. Laboratory investigations conducted to verify the accuracy of these out of range results confirmed the results outside of the 90% to 110% range. It was discovered that the formulations were prepared on a mg/g basis, and the weight-based calculations did not take into account any adjustment for the density of the control article.

Necropsy [Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)]
Mating and fertility parameters [numbers of days in cohabitation, mice that mated, the fertility index (number of pregnancies per number of mice that mated), mice with confirmed mating dates during the first or second week of cohabitation and number of pregnancies per number of mice in cohabitation] were unaffected by doses of A-1233617.

A-1233617-related changes in male reproductive organ weights included: increases in the absolute and relative (% terminal body weight) weights of the prostate at 20 and 200 mg/kg/day; increases in the absolute and relative weights of the seminal vesicles (without fluid) at 200 mg/kg/day; and decreases in the absolute and relative weights of the testes at 200 mg/kg/day (7% to 11% lower than control).

The absolute and relative weights of the prostate were significantly increased ($p \leq 0.01$) in the 20 mg/kg/day dose group, as compared to controls (137% and 139% of controls). Similar increases in the weight of the prostate were observed at 200 mg/kg/day, with the absolute and relative weights reaching statistical significance ($p \leq 0.05$ or $p \leq 0.01$), as compared to controls.

The absolute and relative weights of the seminal vesicles (without fluid) at 200 mg/kg/day were also significantly increased ($p \leq 0.01$), as compared to controls. The increases in the weights (absolute and relative) of the seminal vesicles (without fluid) and prostate in the 200 mg/kg/day dose group ranged from 121% to 132% of controls.

Reference ID: 3628623

Embryonic Fetal Development

Study title: A Dose Range-finding Embryo-fetal Development Study of A-1233617 by Oral Gavage in Mice

Study no.: **Study RD101462/TD10-221**
Study report location: **EDR**
Conducting laboratory and location: (b) (4)
Date of study initiation: **29 November 2010 (protocol signed)**
GLP compliance: **Yes**
QA statement: **Yes**
Drug, lot #, and % purity: **A-1233617, 85247PP00, 98.7%**

Key Study Findings

- Administration of A-1233617 did not result in any test article-related mortality, or increases the incidence of clinical signs or gross lesions at any dosage level.
- There were no effects on maternal body weight, body weight gain, Caesarean-sectioning or litter parameters at any dosage level.
- As measured on GD15, maximum maternal exposures (AUC) of 59 µg.hr/mL were achieved at the middle dose (150 mg/kg, defined as the NOAEL). Fetal exposures were also maximized at the middle dose (0.844 µg.hr/mL).

Methods

Doses: 15, 50, 150, 500 mg/kg/day
Frequency of dosing: Once per day
Dose volume: 2 ml/kg
Route of administration: Oral gavage
Formulation/Vehicle: PEG 400, TWEEN® 20, Poloxamer 124 and Vitamin E TPGS [50%, 20%, 10% and 20% w/w, respectively]
Species/Strain: Crl:CD1(ICR) mice
Number/Sex/Group: 10
Satellite groups: 6 (control) or 18 (toxicokinetics
Study design:

Experimental Design Experimental Design

Group Number	Test Material	A-1233617 Dosage Level (mg/kg)	A-1233617 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	Number of Main Study Mice	Number of Toxicokinetic Mice[b]
1	Control Article	0	0	2	10	6
2	A-1233617	15	7.5	2	10	18
3	A-1233617	50	25	2	10	18
4	A-1233617	150	75[c]	2	10	18
5	A-1233617	500	250	2	10	18

a. The test article A-1233617 potency was 889 mg/gram bulk; formulation concentrations were adjusted for potency.
b. Toxicokinetic mice were used for blood collection purposes only.
c. The 75 mg/mL concentration was actually prepared as 77.9 mg/mL. This error occurred during dose formulation preparation.

Deviation from study protocol: Documented deviations are not expected to have adversely affected data validity.

Results

Reference ID: 3628623

Mortality: Mice were checked for morbidity/mortality twice daily. There was no test article-related mortality at any dosage level.

Clinical Signs: General appearance was assessed daily prior to dosing. Postdose observations were conducted 4-6 hours after dosing. No clinical signs occurred at any dosage level.

Body Weight: Body weights were recorded daily during the dosing and postdosing periods. Body weights and body weight gains were comparable among the dosage groups. The average maternal body weight on GD 18 was 103%, 104%, 104% and 101% in the 15, 50, 150 and 500 mg/kg/day dosage groups, respectively. The average maternal body weight gains in the 15, 50, 150 and 500 mg/kg/day dosage groups were 102%, 109%, 110% and 110% of the control article group value, respectively, for the cumulative dosage period (calculated as GDs 6 through 16). For the combined dosage and postdosage periods (GDs 6 to 18), the average maternal body weight gains in the 15, 50, 150 and 500 mg/kg/day dosage groups were 105%, 106%, 108% and 102% of the control article group value, respectively.

Toxicokinetics

Following maternal blood collection on GD 15 (approximately 1, 3, 6, 9 and 12 hours postdosage) or GD 16 (approximately 24 hours after the last maternal dosage on GD 15), fetal blood samples (0.10 mL to 0.25 mL, pooled by litter) were collected by decapitation.

Summary of Mean Toxicokinetic Parameters for A-1233617

Dosage Group	GD 15 Maternal			GD 15 Fetal			AUC Fetal to Dam Ratio
	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)	
2 15 mg/kg/day	0.807	3.0	10.5	0.00947	6.0	0.133	0.013
3 50 mg/kg/day	2.56	9.0	40.9	0.0492	9.0	0.679	0.017
4 150 mg/kg/day	3.56	9.0	59.1	0.0643	9.0	0.844	0.014
5 500 mg/kg/day	3.71	9.0	40.0	0.0587	9.0	0.583	0.015

Stability and Homogeneity

The Sponsor provided data that demonstrate that the test article is stable in the control article when prepared and stored (b) (4) at concentrations bracketing those used in the present study. The results for A-1233617.0 concentrations ranged from 97 to 109% of the theory for all other treatment groups.

Necropsy

A gross necropsy of the thoracic, abdominal and pelvic viscera was performed for each mouse assigned to the main study. Tissues were collected and preserved as described in the sponsor's table below (Tissue Collection and Preservation). Images were generated for illustration of or consultation on gross observations. Images and associated documentation were retained and archived.

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

Reference ID: 3628623

As a result of the early deliveries in the 150 and 500 mg/kg/day dosage groups, Caesarean-sectioning observations on GD 18 were based on 9, 10, 10, 9 and 9 mice in the 0 (Control Article), 15, 50, 150 and 500 mg/kg/day dosage groups, respectively.

Fetal body weights (combined, male and female) were slightly reduced (6% to 7%) in the 500 mg/kg/day dosage group, in comparison to the control article group values. This fetal body weight finding was not considered adverse, since it was small in magnitude and each of the average values was within the range observed historically at the Testing Facility. In addition, each of the average values was within the historical range of the Testing Facility (historical range of combined fetal weights: 1.24 g to 1.41 g; male fetal weights: 1.27 g to 1.43 g; female fetal weights: 1.20 g to 1.39 g).

No other Caesarean-sectioning or litter parameters were affected by dosages as high as 500 mg/kg/day. The litter averages for corpora lutea, implantations, the percentage of preimplantation loss, litter sizes, live fetuses, early and late resorptions, the percentage of postimplantation loss, the percentage of resorbed conceptuses per litter, and the percentage of live male fetuses were comparable among the five dosage groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

Offspring (Malformations, Variations, etc.)

None of the fetal gross external or soft tissue alterations were attributed to maternal treatment with A-1233617 because of the lack of dose response to exposure and the low incidence of the findings.

Summary of Historical Incidence of Fetal Alterations Observed on Study

Alteration		Dosage Group					HCD Range/Study[a]	
		0 mg/kg/day	15 mg/kg/day	50 mg/kg/day	150 mg/kg/day	500 mg/kg/day	N	%
Fetal Gross External Alterations								
Hindlimbs: Rotated	L	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0-2	0-40.0
	F	0 (0.0%)	0 (0.0%)	1 (0.8%)	0 (0.0%)	0 (0.0%)	0-3	0-3.8
Eye: Lid(s) Open	L	0 (0.0%)	0 (0.0%)	2 (20.0%)	2 (22.2%)	0 (0.0%)	0-1	0-16.7
	F	0 (0.0%)	0 (0.0%)	2 (1.6%)	2 (1.7%)	0 (0.0%)	0-1	0-1.3
Head: Exencephaly	L	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0-1	0-16.7
	F	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.9%)	0 (0.0%)	0-1	0-1.3
Fetal Soft Tissue Alterations								
Eye: Lid(s) Open	L	1 (11.1%)	0 (0.0%)	2 (20.0%)	1 (11.1%)	0 (0.0%)	0-1	0-4.3
	F	1 (0.9%)	0 (0.0%)	2 (1.6%)	1 (0.9%)	0 (0.0%)	0-1	0-0.8
Eye: Retina, Folded	L	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0-1	0-10.0
	F	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.9%)	0 (0.0%)	0-2	0-1.9
Brain: Misshapen	L	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	-	-
	F	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.9%)	0 (0.0%)	-	-

a. Testing Facility Historical Control Data, June 2006 to June 2010 (See Appendix 11).
"-": Denotes finding not included in the current historical control data.

Embryonic Fetal Development

Study title: An Embryo-Fetal Development Study of A-1233617 by Oral Gavage in Mice

Study no.:	Study RD11369/TD 11-036
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	05 April 2011 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 (ABT-267), 85247PP00, 98.7%

Key Study Findings

- Administration of ABT-267 did not result in any test article-related mortality, or increases the incidence of clinical signs or gross lesions at any dose level.
- There were no effects on maternal body weight, body weight gains, Caesarean-sectioning parameters, litter parameters or on embryo-fetal development at any dose level.
- Administration of ABT-267 by oral gavage was well tolerated in mice at doses achieving saturation of systemic exposure (40.4 µg*hr/mL achieved at 50 mg/kg/day) and higher (150 mg/kg/day).
- On the basis of these data, the maternal and developmental no-observed-adverse-effect level (NOAEL) for A-1233617 is defined as 50 mg/kg/day (Sponsor considered the NOAEL to be 150 mg/kg/day).

Methods

Doses:	15, 50, 150 mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	2 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Phosal 53 MCT: PEG 400: Poloxamer 124: Cremophor RH40 [40:20:20:20, by weight]
Species/Strain:	Crl:CD1(ICR) mice
Number/Sex/Group:	25
Satellite groups:	6 (control) or 18 (toxicokinetics
Study design:	

Experimental Design

Group No.	Test Material	A-1233617 Dose Level (mg/kg)	A-1233617 Concentration (mg/mL)[a]	Dose Volume (mL/kg)	Number of Main Study Mice	Number Toxicoki Mice[b]
1	Control Article	0	0	2	25	6
2	A-1233617	15	7.5	2	25	18
3	A-1233617	50	25	2	25	18
4	A-1233617	150	75	2	25	18

[a] The bulk test article A-1233617 potency was 889 mg/gram bulk; formulation concentrations were adjusted potency.
[b] Toxicokinetic mice were used for blood collection purposes only.

Deviation from study protocol:	Documented deviations are not expected to have adversely affected data validity.

Reference ID: 3628623

Results

Mortality: Mice were checked for morbidity/mortality twice daily. There was no test article-related mortality at any dosage level.
Clinical Signs: General appearance was assessed daily prior to dosing. Postdose observations were conducted 4-6 hours after dosing. No clinical signs occurred at any dosage level.
Body Weight: Body weights were recorded daily during the dosing and postdosing periods. There were no significant effects on body weight.

Toxicokinetics

Following maternal blood collection on GD 15 (approximately 1, 3, 6, 9 and 12 hours postdose) or GD 16 (approximately 24 hours after the last maternal dose on GD 15), fetal blood samples (0.10 mL to 0.4 mL, pooled by litter) were collected by decapitation.

Toxicokinetic parameters are summarized in the sponsor's table below.

Summary of Mean Toxicokinetic Parameters for A-1233617

Dose Group	GD 15 Maternal			GD 15 Fetal			AUC Fetal to Dam Ratio
	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24} (µg*hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC_{0-24} (µg*hr/mL)	
2 15 mg/kg/day	1.10	6.0	13.6	0.0140	12.0	0.197	0.014
3 50 mg/kg/day	2.49	6.0	40.4	0.0452	12.0	0.688	0.017
4 150 mg/kg/day	2.73	6.0	38.0	0.0442	9.0	0.670	0.018

Stability and Homogeneity
The Sponsor provided data that demonstrate that the test article is stable in the control article when prepared and stored (b) (4) at concentrations bracketing those used in the present study. The results for A-1233617.0 concentrations ranged from 97 to 103% of the theory for all other treatment groups. The homogeneity of the active dose formulations prepared on April 18, 2011 was determined by assaying samples from the top, middle, and bottom. The relative standard deviation of concentrations ranged from 0.8 to 1.8% within each treatment group.

Necropsy: There were no test article-related gross lesions.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):
No Caesarean-sectioning or litter parameters were affected at any dose level of A-1233617.

Offspring (Malformations, Variations, etc.): No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by doses of A-1233617 as high as 150 mg/kg/day. There were no dose-dependent or significant differences in the litter or fetal incidences of any gross external, soft tissue or skeletal alterations. Fetal ossification site averages were comparable among the 4 dose groups. The only statistically significant finding was a decrease (significant at $p \leq 0.05$) in the average number of ossified hindlimb tarsals in the 50 mg/kg/day dose group, in comparison to the control article group value. This reduction was considered unrelated to maternal administration of A-1233617 because the reduction was not dose-dependent, and the average value was within the historical range of the Testing Facility.

Embryonic Fetal Development

Study title: An Oral Developmental Toxicity Study with A-1233617 in Rabbits, Including a Toxicokinetic Evaluation

Study no.:	Study RD11406/TE11-037
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	21 April 2011 (protocol signed)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617, 85247PP00, 88.9%

Key Study Findings

- No external malformations or variations were observed in the 10 mg base/kg/day group.
- Two fetuses from two different litters in the 60 mg base/kg/day group were observed with external malformations and variations. One was observed with microphthalmia, absent incisors, fused nares, and absent papillae (variation). The second was observed with thoracogastroschisis, craniorachischisis, underdeveloped skin, ectrodactyly, abnormal flexure of the fore- and hind paw, microphthalmia, open eye, incisors absent, cleft palate, pinna smaller than normal, hind limbs malrotated, and protruding tongue (variation). The incidence of these external malformations and variations were within historical control ranges for this laboratory, or in the case of craniorachischisis, were within historical ranges in rabbits in general, and considered unrelated to treatment.
- Visceral malformations were observed in four fetuses from three different litters at 60 mg base/kg/day. These malformations consisted of malpositioned kidney and microphthalmia, diaphragmatic hernia, and persistent truncus arteriosus and interventricular septum absent.
- These few visceral malformations and the few visceral variations (small gallbladder, ureter dilated were seen at a low incidence and were either similar to controls or within historical control ranges for this laboratory and considered unrelated to treatment.
- The two fetuses in the 60 mg base/kg/day group that were observed with several external malformations also had several skeletal malformations which involved misshapen, fused, and absent skull and/or limb bones. As these findings were only seen in two fetuses they were considered spontaneous and unrelated to treatment. A few additional skeletal malformations (branched, fused, misshapen, and/or misaligned skull and vertebrae bones) and skeletal variations (sternebrae not ossified, additional ossification centers were observed in the 10 and 60 mg base/kg/day groups, but were seen at a similar incidence in the control group or were within historical control ranges for this laboratory and considered unrelated to treatment.
- Based on these results, the no observed adverse effect level (NOAEL) for maternal or embryo-fetal toxicity was 60 mg base/kg/day, resulting in a systemic exposure (AUC0-24) of 6.26 µg•hr/mL.

Methods

Doses:	0, 10, 60 mg/kg
Frequency of dosing:	Once per day
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Phosal 53 MCT (40% w/w, PEG400 (20% w/w), Poloxamer 124 (20% w/w), and

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	Cremophor RH40 (20% w/w)
Species/Strain:	New Zealand White Hra:(NZW)SPF rabbits
Number/Sex/Group:	23
Satellite groups:	Toxicokinetics (5/group)
Study design:	Dosing from GD 7 to 19
Deviation from study protocol:	Noted deviations are not expected to affect study results.

Observations of the main study animals included clinical signs, gestation body weights and body weight change, and food consumption. On GD 29, each main study animal was euthanized and subjected to a complete necropsy, including a uterine examination in which the total number of implantations, early and late resorptions, viable and nonviable fetuses, sex, and individual body weights of the fetuses were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded and adjusted GD 29 body weights and body weight changes (GD 0 to 29 and GD 7 to 29) were calculated. All fetuses were given an external and visceral examination and were processed for skeletal examination. Malformations and developmental variations were recorded.

Results

Mortality: All animals survived to scheduled necropsy.

Clinical Signs: There were no remarkable clinical signs.

Body Weight: Body weight and body weight gain were not affected by treatment.

Toxicokinetics

Pregnant rabbits were administered daily oral doses of 10 or 60 mg/kg/day of A-1233617 starting on GD 7 and continuing through GD 19. The estimated mean AUCs on GD 7 were 2.61 and 7.39 µg*hr/mL in the low and high dose groups, respectively. The estimated mean AUCs on GD 18 were 3.44 and 6.26 µg*hr/mL in the low and high dose groups, respectively. The mean dose-normalized exposure (AUC/Dose) appeared to be less than proportional to dose at the two dose levels on GD 7 and GD 18. The average Tmax occurred approximately 3.0 to 5.4 hours post dosing. The mean plasma A-1233617 concentrations in fetuses on GD 19 were consistently lower as compared to the dams across all dose groups.

Mean (±SD) Toxicokinetic Parameters for A-1233617

Dose (mg base/kg/day)	GD 7			GD 18		
	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)
10	0.282±0.0875	3.0±0.0	2.61±0.664	0.372±0.0582	4.2±1.6	3.44±0.905
60	0.695±0.239	5.4±1.3	7.39±2.66	0.641±0.247	3.0±0.0	6.26±2.62

Mean (±SD) Plasma Concentrations of A-1233617 in Maternal Animals and their Fetuses

Dose (mg base/kg/day)	GD 19 Plasma Concentration (µg/mL)		
	Maternal	Fetal	Mean Fetal/Maternal Ratio
10	0.338±0.0543	0.00380±0.00351	0.0105
60	0.606±0.157	0.00829±0.00197	0.0139

Stability and Homogeneity

The sponsor provided information regarding stability of test article under the conditions of this study, so that no additional assessments were conducted here. Homogeneity and concentrations of dose formulations were confirmed.

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

The pregnancy index was 100%, 95.7%, and 87% in the control, 10, and 60 mg base/kg/day groups, respectively. There were one and three animals not pregnant in the 10 and 60 mg base/kg/day groups, respectively. The pregnancy index in the treated groups was lower than controls and the historical control mean (96.44%) for this laboratory, however per the study design implantation occurs prior to the dosing period and the lower pregnancy index was not considered test article-related. Corpora lutea counts and uterine parameters (implantation sites, viable fetuses, resorptions, and pre- and post-implantation loss) in the treated groups were comparable to the control group and unaffected by treatment.

Mean gravid uterine weight, adjusted body weight, and adjusted weight change (GD 0-29 and GD 7-29) in the treated groups were comparable to the control group and unaffected by treatment.

Offspring (Malformations, Variations, etc.)

Fetal sex ratios were not affected by treatment.

Fetal body weights were comparable among control and treated groups.

No external malformations or variations were observed in the 10 mg base/kg/day group.
Two fetuses from two different litters in the 60 mg base/kg/day group were observed with external malformations and variations. One was observed with microphthalmia, absent incisors, fused nares, and absent papillae (variation). The second was observed with thoracogastroschisis, craniorachischisis, underdeveloped skin, ectrodactyly, abnormal flexure of the fore- and hind paw, microphthalmia, open eye, incisors absent, cleft palate, pinna smaller than normal, hind limbs malrotated, and protruding tongue (variation). The incidence of these external malformations and variations were within historical control ranges for this laboratory and considered unrelated to treatment.

Visceral malformations were observed in four fetuses from three different litters at 60 mg base/kg/day. These malformations consisted of malpositioned kidney and microphthalmia, diaphragmatic hernia, and persistent truncus arteriosus and interventricular septum absent.

These few visceral malformations and the few visceral variations (small gallbladder, ureter dilated were seen at a low incidence and were either similar to controls or within historical control ranges for this laboratory and considered unrelated to treatment.

The two fetuses in the 60 mg base/kg/day group that were observed with several external malformations also had several skeletal malformations which involved misshapen, fused, and absent skull and/or limb bones. As these findings were only seen in two fetuses they were considered spontaneous and unrelated to treatment. A few additional skeletal malformations (branched, fused, misshapen, and/or misaligned skull and vertebrae bones) and skeletal variations (sternebrae not ossified, additional ossification centers were observed in the 10 and 60 mg base/kg/day groups, but were seen at a similar incidence in the control group or were within historical control ranges for this laboratory and considered unrelated to treatment.

Prenatal and Postnatal Development

Study title: A Developmental and Perinatal/Postnatal Reproduction Study of A-1233617 by Oral

Reference ID: 3628623

(Gavage) in Mice, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	Study RD12711/TD12-079
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	27July2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1233617 (A-1233617.0, A-1233617 hydrate (b) (4) ABT-267), 95438PP11 (equivalent to lot 95438PP00), 99.8%

Key Study Findings

- There were no treatment related effects (i.e., maternal health) in pregnant mice treated with 10, 40 and 200 mg/kg ABT-267.
- There were no apparent effects on gestation, parturition, lactation or maternal behavior at any dose level tested.
- In addition, there were no effects on survival, growth, sexual maturation, motor activity, learning and memory, mating and fertility, male reproductive organ weights or ovarian and uterine parameters in the F1 generation mice.
- There were no treatment-related fetal external abnormalities in the F2 generation fetuses.
- The maternal and reproductive no-observable-adverse-effect-level (NOAEL) for ABT-267 is 200 mg/kg/day. The NOAEL for viability and growth in the offspring is also 200 mg/kg/day, the highest dose tested. Systemic exposures were not determined.

Methods

Doses:	0, 10, 40, 200 mg/kg
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Polyethylene Glycol 400 (PEG 400) (50%), Poloxamer 124 (10%), Tween® 20 (20%) and Vitamin E TPGS (20%), by weight
Species/Strain:	Crl:CD1(ICR) mice
Number/Sex/Group:	25
Satellite groups:	
Study design:	Maternal dosing: from GD 6 continuing through Day 20 postpartum (mice that delivered a litter) or GD 22 (mice that did not deliver a litter).
Deviation from study protocol:	The deviations are not likely to affect study validity.

Results

The following parameters and end points were evaluated for the F0 generation dams in this study: viability, clinical signs, body weights, body weight changes, maternal behavior, natural delivery observations, maternal toxicokinetics, and gross pathology findings.

The following parameters and end points were evaluated for the F1 generation litters through weaning: viability, clinical signs, body weights, body weight changes, toxicokinetics and gross pathology findings.

F1 generation mice randomly selected for continuation on study were evaluated for the following parameters and endpoints: viability, clinical signs, body weights, body weight changes, sexual maturation, behavioral testing (passive avoidance, motor acitvity and watermaze), reproductive capacity, gross pathology findings, organ weights (F1 generation male mice), ovarian and uterine examinations (F1 generation female mice), and fetal external examinations (F2 generation fetuses).

F_0 Dams

Survival: Two mice, one from the low dose and one from the high dose, were euthanized early due to gavage error.

Clinical signs: No clinical signs were related to dose administration.

Body weight: No effects of dose on body weight.

Feed consumption: No effects of dose on feed consumption.

Uterine content: Pregnancy was confirmed in 20, 21, 22 and 23 mice in the 0, 10, 40 and 200 mg/kg/day dose groups.

Necropsy observation: No dose-related findings.

Toxicokinetics:

Dose Group	Dose Level (mg/kg)	Average Dam Concentration (µg/mL)	Average Pup (Pooled by Litter) Concentration (µg/mL)	Average Ratio (Pup Concentration/ Dam Concentration)
2	10	0.00576 ± 0.00749	0.00 ± 0.00	0.00 ± 0.00
3	40	0.0807 ± 0.0838	0.00740 ± 0.0165	0.0326 ± 0.0729
4	200	0.132 ± 0.0692	0.0141 ± 0.0126	0.160 ± 0.141

Stability and homogeneity: The sponsor provided stability data. Homogeneity and concentration of dose preparations met acceptance criteria.

F_1 Generation

Survival: One male and one female from the high dose (200 mg/kg) group failed to thrive and were found dead on day 23 and 26.

Clinical signs: Clinical signs were not considered to be related to test article since incidences were not dose-related. These clinical signs included: reduced maternal care (not nursing, not nesting, no milk band present, ungroomed coat), bruising or injuries (discoloration on the tail or head, scabbing at one or more locations), cold to touch, varying degrees of dehydration (mild to severe), abdominal distention, bent tail, thin body condition, decreased motor activity, a malformed right hindlimb with limited use, a mass on the head and a missing tail.

Body weight: There were no dose-related effects on body weight.

Physical development: There were no effects on sexual maturation. There were no treatment related findings at necropsy.

Neurological assessment: There were no dose-related effects on learning or motor activity.

Reproduction: There were no dose-related effects on mating and/or fertility.

Reference ID: 3628623

F_2 Generation

The litter averages for corpora lutea, implantations, the percentage of preimplantation loss, litter sizes, live and dead fetuses, early and late resorptions, the percentage of postimplantation loss, fetal body weights, the percentage of dead or resorbed conceptuses, and the percentage of live male fetuses were comparable among the 4 dose groups and did not significantly differ from controls.

Other Toxicology Studies

Two metabolites of ombitasvir, M29 and M36 (A-1538855 and A-1548255, respectively), were identified as unique human metabolites (i.e., metabolites that were not present in significant amounts in nonclinical species). The toxicological profile of each metabolite was assessed in repeat dose studies, genetic toxicology assays, and reproductive toxicology studies. The studies are summarized and reviewed below.

Repeat-dose Studies

Study title: Four-Week Oral Toxicity Study of A-1548255 (b) (4) in CD-1 Mice (with a Four-Week Recovery Period)

Study no.:	TD13-184
Study report location:	EDR
Conducting laboratory and location:	AbbVie Inc. Research and Development Preclinical Safety Division Toxicology 1 North Waukegan Road North Chicago, IL 60064, USA
Date of study initiation:	19 August 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1548255 free form; (also known as A-1548255, A-1548255.0, ABT-267 metabolite M36), lot# 2132644, 2117513, 146.8 mg A-1548255.0/g (b) (4)

Key Study Findings

- Administration of A-1548255 at a dosage of 1.5, 3 or 6 mg/kg/day resulted in no adverse effects and did not induce micronuclei in bone marrow.
- The No-Observed-Adverse-Effect Level (NOAEL) in this study was 6 mg/kg/day corresponding with an AUC of 16.8 µg•hr/mL on Day 28.

Methods

Doses: 0, 1.5, 3, 6 mg/kg/day
Frequency of dosing: Once/day for 28 to 29 days
Route of administration: Oral gavage
Dose volume: 10 mL/kg
Formulation/Vehicle: 50 mM sodium phosphate buffer, pH 7.4 ± 0.05 with 0.1% Hydroxypropylmethyl Cellulose (HPMC) in purified water.
Species/Strain: CD-1[CD® (SD)]
Number/Sex/Group:

Test Group	Dosage A-1548255 (mg/kg/day)[b]	Concentration of A-1548255 (mg/mL)[c]	Main Study		Satellite Animals[a]	
			Male	Female	Male	Female
Group 1 (control)	0[d]	0[e]	10 (5)[f]	10 (5)	4	4
Group 2 (low)	1.5	0.15	10	10	15	15
Group 3 (mid)	3	0.3	10	10	15	15
Group 4 (high)	6	0.6	10 (5)	10 (5)	15	15

a. Satellite mice used for terminal toxicokinetic blood sampling. Cohorts of up to three animals per sex in Groups 2-4 were bled at each time point at the end of study.
b. A-1548255 free form (b) (4) (Lot 10024544-0869). Expressed as free active moiety. Assigned chemical potency = 146.8 mg A-1548255.0/g (b) (4)
c. Expressed as mg A-1548255/mL.
d. Control: (b) (4) control was given to provide the same mg/kg dose as the excipient level in the high dose group.
e. Test item and control item were formulated with vehicle, (50 mM sodium phosphate buffer, pH 7.4 ± 0.05 with 0.1% Hydroxypropylmethyl Cellulose (HPMC) in purified water). The dose volume was 10 mL/kg.
f. Five mice per sex within parentheses were designated recovery mice.

Age: 10 weeks
Weight: 23.5 to 42.9 g
Satellite groups: See table above
Deviation from study protocol: Deviations described in the final report are not likely to impact study validity.

Results

Mortality: All main and recovery group animals survived to scheduled necropsies.
Clinical Signs: During the dosing period detailed observations of physical condition and behavior were recorded twice weekly for main study and recovery animals. There were no treatment related clinical signs.

Body Weights: Body weight was measured twice weekly during the dosing and recovery periods for all mice on study. There were no treatment related effects on body weight.

Feed Consumption: Quantitative assessment of food consumption was recorded at least once weekly for all main study and recovery mice. There were no treatment related effects on food consumption.

Clinical Pathology: Blood samples and bone marrow smears were collected from mice during scheduled necropsies.

Micronuclei: For micronucleus induction evaluation, slides from the treated and control groups were analyzed (males only). At least 2000 polychromatic erythrocytes (PCEs)/animal were scored.
The test article did not induce micronuclei and was not cytotoxic to bone marrow, and was therefore found to be negative in the micronuclei assay under the conditions of this study.

Hematology: There were no treatment related effects on hematology parameters.

Reference ID: 3628623

Clinical Chemistry: There were no treatment related effects on clinical chemistry parameters.

Gross Pathology: There were no treatment related findings.

Organ Weights: Heart, kidneys, liver/gall bladder, spleen, testes, and thymus were weighed. There were no treatment related effects on body weights.

Histopathology

Adequate Battery: Yes. Control and high dose animals only.

Peer Review: Yes.

Histological Findings: There were no histological findings.

Toxicokinetics

Blood samples for determination of plasma test item concentration were collected from up to three satellite mice/sex/group/time point in Groups 2-4 at 0.5, 2, 6, 12 and 24 hours on Day 28. Toxicokinetic parameters are summarized in the table below (*excerpted from sponsor*):

Overall Mean Pharmacokinetic Parameters for A-1548255 on Dosing Day 28

A-1548255 Dosage (mg/kg/day)		
1.5	3	6
Mean Plasma AUC[a] (µg•hr/mL)		
3.07	6.55	16.8
Mean Plasma C_{max} (µg/mL)		
0.47	1.02	2.75

a. AUC calculated as AUC_{0-24}.

Dosing Solution Analysis

The assay results indicate that the formulations were prepared at the correct concentrations (94 to 100% of the theory) and were homogeneous (relative standard deviation ranged from 1.7% to 2.2%). Assay of the formulations with concentrations of 0.1 and 60 mg/mL confirmed stability within the preparation interval.

Reference ID: 3628623

Study title: Four-Week Oral Toxicity Study of A-1538855 (b) (4) in CD-1 Mice (with a Four-Week Recovery Period)

Study no.:	TD13-188
Study report location:	EDR
Conducting laboratory and location:	AbbVie Inc. Research and Development Preclinical Safety Division Toxicology 1 North Waukegan Road North Chicago, IL 60064, USA
Date of study initiation:	05 September 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1538855 free form (A-1538855.0 (b) (4) (b) (4) Lot 10024544-0866

Key Study Findings

- Administration of A-1538855 at a dosage of 1, 2, 3.5 mg base/kg/day in males and 1, 2, 3 mg/kg/day in females resulted in no adverse effects and did not induce micronuclei in bone marrow.
- Therefore, the No-Observed-Adverse-Effect Level (NOAEL) in this study was 3.5 mg base/kg/day in males and 3 mg/kg/day in females, respectively.
- Corresponding systemic exposures (AUC) were 15.8 µg•hr/mL in males, 18.1 µg•hr/mL in females, respectively.

Methods

Doses:	0, 1.0, 2, or 3.5 (male) 3.0 (female) mg/kg/day
Frequency of dosing:	Once/day for 28 to 29 days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	A-1538855:HPMC-AS:Copovidone (15:25:60 (w%)/ 50 mM sodium phosphate buffer, pH 7.4 ± 0.05 with 0.1% Hydroxypropylmethyl Cellulose (HPMC) in purified water.
Species/Strain:	CD-1[CD® (SD)]

Reference ID: 3628623

Number/Sex/Group:

Test Group	Dosage A-1538855 (mg/kg/day)[a]		Concentration of A-1538855 (mg/mL)[b]		Main Study		Satellite Animals[c]	
	Males	Females	Males	Females	Male	Female	Male	Female
Group 1 (vehicle control)	0[d]	0[d]	0[e]	0[e]	11 (5)[f]	11 (5)	3	3
Group 2 (low)	1	1	0.1	0.1	11	11	15	15
Group 3 (mid)	2	2	0.2	0.2	11	11	15	15
Group 4 (high)	3.5	3	0.35	0.3	11 (5)	11 (5)	15	15

a. A-1538855 free form (Lot 10024544-0866). Expressed as free active moiety. Assigned chemical potency = 150.7 mg/g (b) (4)
b. Expressed as mg free active moiety of A-1538855/mL.
c. Satellite mice used for terminal toxicokinetic blood sampling. Cohorts of up to three animals per sex in Groups 2-4 were bled at each time point and at the end of study.
d. Control: (b) (4) control was given to provide the same mg/kg dose as the excipient level in the high dose group.
e. The dose volume was 10 mL/kg. Test item and control item were formulated with vehicle (50 mM sodium phosphate buffer, pH 7.4± 0.05 with 0.1% Hydroxypropylmethyl Cellulose (HPMC) in purified water).
f. Five mice per sex within parentheses were designated recovery mice.

Age: 10 weeks
Weight: 22.5 to 39.7 g
Satellite groups: See table above
Deviation from study protocol: Deviations described in the final report are not likely to impact study validity.

Results

Mortality: Three mice died prior to scheduled necropsy. Two deaths of low dose animals were related to gavage error. The cause of the third death, a middle-dose male found dead on day 9 was not determined, but is not considered to be treatment related given the lack of dose relationship (i.e., no deaths in high dose group).

Clinical Signs: During the dosing period detailed observations of physical condition and behavior were recorded twice weekly for main study and recovery animals. There were no treatment related clinical signs.

Body Weights: Body weight was measured twice weekly during the dosing and recovery periods for all mice on study. There were no treatment related effects on body weight.

Feed Consumption: Quantitative assessment of food consumption was recorded at least once weekly for all main study and recovery mice. There were no treatment related effects on food consumption.

Clinical Pathology: Blood samples and bone marrow smears were collected from mice during scheduled necropsies.

Micronuclei: For micronucleus induction evaluation, slides from the treated and control groups were analyzed (males only). At least 2000 polychromatic erythrocytes (PCEs)/animal were scored.

The test article did not induce micronuclei and was not cytotoxic to bone marrow, and was therefore found to be negative in the micronuclei assay under the conditions of this study.

Hematology: There were no treatment related effects on hematology parameters.

Clinical Chemistry: There were no treatment related effects on clinical chemistry parameters.

Gross Pathology: There were no treatment related findings.

Organ Weights: Heart, kidneys, liver/gall bladder, spleen, testes, and thymus were weighed. There were no treatment related effects on body weights.

Histopathology

Adequate Battery: Yes. Control and high dose animals only.

Peer Review: Yes.

Histological Findings: There were no histological findings.

Toxicokinetics

Blood samples for determination of plasma test item concentration were collected from up to three satellite mice/sex/group/time point in Groups 2-4 at 0.5, 2, 6, 12 and 24 hours on Day 28. Toxicokinetic parameters are summarized in the table below (*excerpted from sponsor*):

Mean Pharmacokinetic Parameters for A-1538855 on Dosing Day 28

	A-1538855 Dosage (mg base/kg/day)		
Sex	1.0	2.0	3.5/3.0 (males/females)
		Mean Plasma AUC[a] (µg•hr/mL)	
Males	3.93	6.96	15.8
Females	3.05	7.11	18.1
Overall	3.49	7.03	NA
		Mean Plasma C_{max} (µg/mL)	
Males	0.48	1.02	2.29
Females	0.43	1.19	2.40
Overall	0.44	1.10	NA

a. AUC calculated as AUC_{0-24}.

NA: Not applicable

Dosing Solution Analysis

Formulations were prepared at the correct concentrations (91 to 104% of the theory) and were homogeneous (relative standard deviation ranged from 1.9% to 5.2%).

Study title: Four-Week Oral Safety Study of ABT-450/Ritonavir and ABT-267 Using Milled Fixed Dose Combination Tablets in CD-1 Mice TD13-238

Reference ID: 3628623

Study no.:	TD13-238
Study report location:	EDR
Conducting laboratory and location:	AbbVie Inc. Research and Development Preclinical Safety Division Toxicology AbbVie Inc. 1 North Waukegan Road North Chicago, IL 60064, USA
Date of study initiation:	03 September 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ABT-450 (A-1043422, A-1043422.0); Ritonavir (ABT-538; A-84538, A-84538.0); ABT-267 (A-1233617, A-1233617.0). Received as fixed dose combination tablets. There were two types of tablets, specified below: ● Combination tablets containing 75 mg ABT-450 (A-1043422.0), 50 mg Ritonavir (ABT-538; A-84538.0), and 12.5 mg ABT-267 (A-1233617.0). ● Combination tablets containing 75 mg ABT-450 (A-1043422) and 50 mg Ritonavir (ABT-538; A-84538). (b) (4) Lot: Lot 13-000510 (ABT-450/Ritonavir/ABT-267 combination tablets). Lot 12-007880 (ABT-450/Ritonavir combination tablets). Assigned Chemical Potency: ABT-450/Ritonavir/ABT-267 combination tablets: ● ABT-450: 99.8% ● Ritonavir: 99.0% ● ABT-267: 100.5% ABT-450/Ritonavir combination tablets: ● ABT-450: 101.2% ● Ritonavir: 99.6%

Key Study Findings

- Administration of a fixed dose combination of ABT-450/RTV/ABT-267 to male and female mice at 30/20/2 mg/kg/day, respectively, by oral gavage once daily for 28 consecutive days resulted in no adverse or toxicologically significant effects.
- Systemic exposures (AUC) after 28 days of dosing were 19, 23, and 4.7 µg•h/mL.
- Based on the lack of findings, the NOAEL in this single dose level study can be defined as 30/20/2 mg/kg/day ABT-450/RTV/ABT-267.

Reference ID: 3628623

Methods

Doses: 0, 30/20/2 mg/kg (ABT-450/RTV/ABT-267)
Frequency of dosing: Once daily
Route of administration: Oral gavage
Dose volume: 10 mL/kg
Formulation/Vehicle: Suspension/Aqueous 0.1% Medical Antifoam C.
Species/Strain: Mice/ CD-1[CD® (SD)]
Number/Sex/Group:

Test Group	Dosage (mg/kg/day)[b]	Concentration (mg/mL)[c]	Main Study		Satellite Animals[a]	
			Male	Female	Male	Female
Group 1 (placebo control)	0[d]	0[e]	10	10	4	4
Group 2	30/20/2	3/2/0.2	10	10	18(2)	18(2)

Age: 9 weeks
Weight: Males: 29.8 to 40.4 g
Females: 20.7 to 26.9 g
Satellite groups: See table above
Deviation from study protocol: There were no significant deviations.

Results

Mortality: Moribund/mortality checks were conducted at least twice daily. All animals survived to the scheduled necropsies.

Clinical Signs: Detailed clinical observations were conducted twice following dosing at least twice for week. Two minor clinical observations in the treated group, pilo-erection and rough hair coat, were not considered adverse. Both animals gained weight and there was no decrease in food consumption.

Body Weights: Body weights were collected twice weekly. No test item-related changes were observed in the body weight data.

Feed Consumption: Food consumption was quantified once weekly. No test item-related changes were observed in the food consumption data.

Clinical Pathology: Blood and bone marrow samples were collected from mice during scheduled necropsies.

Hematology: No test item-related changes in hematology parameters were observed.

Clinical Chemistry: No test item-related changes in clinical chemistry parameters were observed.

Gross Pathology: No test item-related gross observations were observed.

Organ Weights: Brain, heart, kidneys, liver/gall bladder, spleen, testes, thymus were weighed. No test item-related changes in organ weights were observed.

Reference ID: 3628623

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: none

Toxicokinetics

Blood samples for determination of plasma test item concentration were collected from up to three satellite mice/sex/time point in Group 2 at 1, 3, 6, 9, 12, and 24 hours after dosing near the end of the dosing period. Toxicokinetic parameters are presented below (*table excerpted from sponsor*):

Day 28 Mean Toxicokinetic Parameters for ABT-450, Ritonavir, and ABT-267

Test Item Component	Dosage (mg/kg/day)	Sex	C_{max} (µg/mL)	AUC (µg•h/mL)[a]
ABT-450	30	M	4.79	13.7
		F	8.35	24.4
		O	6.57	19
Ritonavir	20	M	7.49	21.8
		F	9.54	24.1
		O	8.51	23
ABT-267	2	M	0.367	6.24
		F	0.265	3.17
		O	0.308	4.69

a. Day 28 calculated as AUC_{0-24}.

Dosing Solution Analysis

The formulations were prepared at the correct concentrations (93% to 95% of the theoretical concentrations) and were homogeneous (relative standard deviation ranged from 1.1% to 1.4%). Assay of the formulations confirmed stability of the test item during the study.

Genetic Toxicology

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

Study title: *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with A-1538855 Free Form and A-1548255 Free Form

Study no.: TX13-149
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 12 June 2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-1538855 free form (lot number 2117515) and A-1548255 free form (lot number 2117513)

Key Study Findings

A-1538855 and A-1548255 were negative in the *Salmonella–E.coli*/Mammalian-Microsome Reverse Mutation Assay under the conditions, and according to the criteria, of the test protocol.

Methods

Strains:

Table II. Tester Strain Genotypes

Tester Strain	*his*/*trp* Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA1535	*his*G46	*uvr*B	*rfa*	–
TA1537	*his*C3076	*uvr*B	*rfa*	–
WP2*uvr*A	*trp*	*uvr*A	–	–

Concentrations in definitive study: 1.60, 5.00, 16.0, 50.0, 160, 500, 1600, and 5000 µg/plate with and without S9

Basis of concentration selection: Limit dose

Negative control: DMSO

Positive control:

Positive Control Articles

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)	CAS No.	Lot No.
TA98	–	2-nitrofluorene	1.0	607-57-8	S43858
TA100, TA1535	–	sodium azide	2.0	26628-22-8	MKBF6507V
TA1537	–	ICR-191	2.0	17070-45-0	031M1947V
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0	56-57-5	A0305157
TA98	+	benzo[a]pyrene	2.5	50-32-8	090M1400V
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5	613-13-8	STBB1901
WP2*uvr*A	+	2-aminoanthracene	25.0	613-13-8	STBB1901

Formulation/Vehicle: DMSO

Incubation & sampling time: 52 ± 4 hr

Study Validity: All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

Results**:** A-1538855 and A-1548255 were negative in the *Salmonella–E.coli*/Mammalian-Microsome Reverse Mutation Assay under the conditions, and according to the criteria, of the test protocol.

Reproductive Toxicology

In a preliminary study, pregnant CD-1 mice were administered dosages of 0.5, 1.5, 3, or 5 mg/kg/day A-1538855 or 0.5, 1.5, 3, or 6 mg/kg/day A-1548255 by oral gavage from Gestation Days (GD) 6 through 15. The high doses were selected in order to provide systemic exposures at least 25x the human exposure to these metabolites at the recommended dose of ombitasvir. Mortality, clinical signs, body weight, food consumption, pregnancy status and resorptions were assessed. No test item-related changes occurred with either test item for any of the parameters evaluated.

Study title: A-1538855 (M29): An Oral Developmental Toxicity Study in Mice, Including a Toxicokinetic Evaluation

Study no.:	TD13-180
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-1538855.0 (ABT-267 Metabolite) Lot Number: 10024544-0866, 100% by HPLC

Key Study Findings

- A-1538855 was not teratogenic at the dose levels evaluated.
- Based on the lack of adverse findings in any dose group, the No-Observed-Adverse-Effect Level (NOAEL) for maternal and developmental/fetal toxicity was 4.5 mg/kg/day (AUC 17.4 µg*hr/mL), the highest dose level evaluated.

Methods

Doses:	1, 2.5, or 4.5 mg/kg/day
Frequency of dosing:	Once daily from Gestation Day (GD) 6 to 15
Dose volume:	10 mL/kg/day
Route of administration:	oral gavage
Formulation/Vehicle:	50 mM Na phosphate buffer (pH 7.4±0.05) with 0.1% Hydroxypropylmethyl Cellulose (HPMC) in purified water/ HPMC-AS:Copovidone (29.4:70.6; wt:wt) Placebo (b) (4)
Species/Strain:	Crl:CD1® (ICR) mice
Number/Sex/Group:	30
Satellite groups:	four groups of 6 (control) and 15 animals/dose group served as toxicokinetic (TK) animals
Deviation from study protocol:	Reported deviations do not appear to have affected study validity.

Results

Mortality: All animals (main study and TK) survived to terminal euthanasia.

Clinical Signs: There were no treatment related clinical signs.

Body Weight: There were no treatment related body weight changes.

Feed Consumption: There were no treatment related changes in food consumption.

Toxicokinetics

The average AUCs on GD 15 were 2.24, 7.76, and 17.4 µg*hr/mL in the 1, 2.5, and 4.5 mg/kg/day groups, respectively.

Dosing Solution Analysis

Reference ID: 3628623

Homogeneity and concentration analyses confirmed that dose formulations were homogeneous (Relative Standard Deviation of 0.2 to 0.8%) and that animals received the targeted concentrations (95-103% of theory).

Necropsy

On GD 17, each main study animal was subjected to a complete necropsy, including a uterine examination in which the total number of implantations, early and late resorptions, viable and nonviable fetuses, sex, and individual body weights of the fetuses were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded and adjusted GD 17 body weights and adjusted body weight changes (GD 0 to 17 and 6 to 17) were calculated. All fetuses were given an external examination; approximately one-half of the fetuses in each litter were processed for visceral examination, and the remaining fetuses for skeletal examination. All malformations and variations were recorded.

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

Uterine and ovarian parameters were unaffected by treatment.

Offspring (Malformations, Variations, etc.)

Fetal parameters (body weights, sex ratios, external, visceral, and skeletal malformations/variations) were unaffected by the test article.

Study title: A-1548255: An Oral Developmental Toxicity Study in Mice, Including a Toxicokinetic Evaluation

Study no.:	TD13-181
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 26, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1548255 (ABT-267) Lot Number: 10024544-0864, 96.5%

Key Study Findings

- A-1548255 was not teratogenic at the dose levels evaluated.
- Based on the lack of adverse findings in any dose group, the No-Observed-Adverse-Effect Level (NOAEL) for maternal and developmental/fetal toxicity was 6 mg/kg/day (11.6 AUC µg*hr/mL), the highest dose level evaluated.

Methods

Doses:	1.5, 3, or 6 mg/kg/day
Frequency of dosing:	Once daily from Gestation Day (GD) 6 to 15
Dose volume:	10 mL/kg/day
Route of administration:	oral gavage
Formulation/Vehicle:	50 mM Na phosphate buffer (pH 7.4±0.05) with 0.1% Hydroxypropylmethyl Cellulose (HPMC) in purified water/ HPMC-AS:Copovidone (29.4:70.6; wt:wt) Placebo (b) (4)

Reference ID: 3628623

Species/Strain:	Crl:CD1® (ICR) mice
Number/Sex/Group:	30
Satellite groups:	four groups of 6 (control) and 16 animals/dose group served as toxicokinetic (TK) animals
Deviation from study protocol:	Reported deviations do not appear to have affected study validity.

Results

Mortality: All animals (main study and TK) survived to terminal euthanasia.

Clinical Signs: There were no treatment related clinical signs.

Body Weight: There were no treatment related body weight changes.

Feed Consumption: There were no treatment related changes in food consumption.

Toxicokinetics: The average mean AUCs on GD 15 were 1.68, 4.65, and 11.6 µg*hr/mL in the 1.5, 3, and 6 mg/kg/day groups, respectively.

Dosing Solution Analysis: Homogeneity and concentration analyses confirmed that dose formulations were homogeneous (Relative Standard Deviation of 0.0 to 1.3%) and that animals received the targeted concentrations (92-106% of theory).

Necropsy: On GD 17, each main study animal was subjected to a complete necropsy, including a uterine examination in which the total number of implantations, early and late resorptions, viable and nonviable fetuses, sex, and individual body weights of the fetuses were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded and adjusted GD 17 body weights and adjusted body weight changes (GD 0 to 17 and 6 to 17) were calculated. All fetuses were given an external examination; approximately one-half of the fetuses in each litter were processed for visceral examination, and the remaining fetuses for skeletal examination. All malformations and variations were recorded.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.): Uterine and ovarian parameters were unaffected by treatment.

Offspring (Malformations, Variations, etc.): Fetal parameters (body weights, sex ratios, external, visceral, and skeletal malformations/variations) were unaffected by the test article.

Special Toxicology Studies

Study title: Repeat Dosage Phototoxicity Study of A-1233617 by Oral Gavage in Hairless Mice

The phototoxic potential of ABT-267 was assessed in female Crl:SKH1-*hr* hairless mice (6/group) following three days of treatment (0 and 200 mg/kg/day).

Reference ID: 3628623

A-1233617: Toxicokinetic Parameters Assessed in the Tolerability Phase (Day 3)				
Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)
10	Female	0.150	3.0	0.988
40	Female	0.603	3.0	5.26
200	Female	1.13	6.0	11.3

Three daily doses of ABT-267 at a dose of 200 mg/kg/day to female Crl:SKH1-*hr* hairless mice followed by a single exposure to solar-simulated ultraviolet radiation did not result in skin reactions indicative of cutaneous phototoxicity.

Therefore, the NOAEL for this study was 200 mg/kg, a dose which results in a Cmax of 1.13 µg/mL and an AUC of 11.3 µg*hr/mL.

Appendix 3 Dasabuvir/ABT-333/A-99821

Studies Reviewed

Secondary Pharmacology
Study RD08215. In Vitro Pharmacology Study of A-998821.0

Study RD08216. In Vitro Pharmacology Study of A-998821.0

Study RD08217. In Vitro Pharmacology: Human V1a Receptor Functional Assay Study of A-998821.0

Study RD08218. In Vitro Pharmacology Study of A-998821.0

Safety Pharmacology
Study RD071328. A-998821.0: CNS/Neurobehavioral Safety Pharmacology Profile in the Rat (P.O. Administration)

Study RD071191. A Neurobehavioral Safety Evaluation of Orally Administered A-998821 (ABT-333) in Rats

Study RD08154. A-998821: In Vitro Effects on hERG Current

Study RD071194. Effects of A-998821 (ABT-333) on Cloned hERG Potassium Channels Expressed in Mammalian Cells

Study RD08155. A-998821: In Vitro Effects on Cardiac Purkinje Fiber Repolarization

Study RD08167. Effects of A-998821 on Cardiovascular and Hemodynamic Function in the Anesthetized Dog

Study RD071193. Cardiovascular Safety Evaluation of Orally Administered A-998821 (ABT-333) in Beagle Dogs

Study RD071192. A Respiratory Safety Evaluation of Orally Administered A-998821 (ABT-333) in Rats

Study RD08153. A-998821: Effects on Ferret Emetic Liability and Rat Gastrointestinal Transit

Pharmacokinetics/ADME
Study RD08394. Physicochemical Properties of A-998821 Free Acid and Its Mono Sodium Salt

Study A-998821 Drug Metabolism Memo No. 07. The In Vitro Permeability and Transport Characteristics of A-998821 Across Human Caco-2 Cells

Study RD071144. Preclinical Pharmacokinetic Summary of A-998821 in Mouse, Rat, Rabbit, Monkey and Dog

Study RD13785. Integration of Pharmaceutics, Formulations and Pharmacokinetics for the Definition of Maximum Feasible Exposures in Preclinical Studies with A-998821

Study A-998821 Drug Metabolism Memo No. 16. A-998821 Pharmacokinetics following Oral Dosing in Mouse

Study RD13223. Metabolism and Excretion of [^{14}C]A-998821 (ABT-333) following Oral Administration to Mice

Reference ID: 3628623

Study A-998821 Drug Metabolism Memo No. 03. A-998821 Pharmacokinetics following Intravenous or Oral Dosing in Rat

Study A-998821 Drug Metabolism Memo No. 06. Effect of Formulation on A-998821 Bioavailability following Oral Dosing in Rat

Study RD071142. Preliminary Metabolism and Disposition of [^{3}H]A 998821 in Male Rats

Study A-998821 Drug Metabolism Memo No. 24. Pharmacokinetics of A-45271 (Ribavirin) after Oral Dosing (± A-998821) in Rat and Monkey

Study A-998821 Drug Metabolism Memo No. 20. A-998821 Pharmacokinetics following Oral Dosing in Rabbit

Study A-998821 Drug Metabolism Memo No. 04. A-998821 Pharmacokinetics following Intravenous or Oral Dosing in Dog

Study A-998821 Drug Metabolism Memo No. 05. A-998821 Pharmacokinetics following Intravenous or Oral Dosing in Monkey

Study RD121006. Absorption, Distribution, Metabolism, and Excretion (ADME) Study of [^{14}C]ABT-333 in Healthy Male Subjects Following a Single Oral Dose Administration

Study RD13187. Determination of the Binding of A-998821 and the M1 Metabolite, A-1041392, to Human and Animal Plasma Proteins

Study A-998821 Drug Metabolism Memo No. 08. Determination of the Blood-to-Plasma Concentration Ratios Following Incubations of A-988821 in Human, Monkey, Dog and Rat Whole Blood

Study RD12152. Quantitative Whole-Body Autoradiography of Rats Following Oral Administration of ^{14}C-ABT-333

Study RD13519. Placental Transfer, Lacteal Excretion, and Tissue Distribution of Radioactivity in Pregnant Female Sprague Dawley Rats Following Oral Administration of ^{14}C ABT-333

Study RD071141. In Vitro Metabolism of [^{3}H]A−998821

Study A-998821 Drug Metabolism Memo No. 29. Uptake of A-998821 by Organic Anion Transporter Polypeptide (OATP) 1B1 and 1B3

Study A-998821 Drug Metabolism Memo No. 30. Uptake of A-1041392 (A-998821 M1 metabolite) by Organic Anion Transporting Polypeptide (OATP) 1B1 and 1B3

Study A-998821 Drug Metabolism Memo No. 22. Assessment of A-998821 Efflux Mediated by P-glycoprotein (P-gp/MDR1) and Breast Cancer Resistance Protein (BCRP)

Study A-998821 Drug Metabolism Memo No. 25. Assessment of A-1041392 (A-998821 M1 Metabolite) Efflux Mediated by P-glycoprotein (P-gp/MDR1) and Breast Cancer Resistance Protein (BCRP)

Study A-998821 Drug Metabolism Memo No. 19. Metabolism and Excretion of [^{14}C]A-998821 Following Oral Administration to CByB6F1 Tg(HRAS)2Jic Mice

Study A-998821 Drug Metabolism Memo No. 17. Preliminary Metabolite Identification of A-998821 in Mouse and Rat Plasma Samples (TD09-196, TD09-141, TA08-229)

Reference ID: 3628623

Study A-998821 Drug Metabolism Memo No. 35. Metabolite Profiles of [^{14}C]A-998821 in Rat Milk and Plasma after a 5 mg/kg Oral Dose in Female Sprague Dawley Rats

Study A-998821 Drug Metabolism Memo No. 36. Metabolism and Disposition of [^{14}C]A-998821 (ABT-333) after a Single 1 mg/kg Oral Dose in Beagle Dogs

Study A-998821 Drug Metabolism Memo No. 14. Preliminary Metabolite Identification of A-998821 in Phase I Human Plasma Samples (M10-687)

Study RD12843. Metabolism and Disposition of [^{14}C]ABT-333 (A-998821) in Male Subjects After a Single 400 mg Oral Dose

Study RD071140. Assessment of the effects of A−998821 on the activity of cytochrome P450 (CYP450) enzymes in human liver microsomes

Study RD13206. Assessment of the Effect of A-1041392 (M1 Metabolite of A-998821) on the Activity of Cytochrome P450 (CYP450) Isoforms in Human Liver Microsomes

Study RD13202. Assessment of CYP Time Dependent Inhibition Potential by A-998821 in Human Liver Microsomes

Study RD13203. Assessment of CYP Time Dependent Inhibition Potential by A-1041392 (M1 Metabolite of A-998821) in Human Liver Microsomes

Study RD121090. An In Vitro Investigation of Cytochrome P450 Induction by A-998821 (ABT-333) and A-1041932 (ABT-333 M1) in Cultured Human Hepatocytes

Study RD13524. Assessment of the Effect of A-998821 and the M1 Metabolite (A-1041392) on the Activity of UDP glucuronosyltransferases 1A1 (UGT1A1) Isoform in Human Liver Microsomes

Study A-998821 Drug Metabolism Memo No. 23. Inhibitory Interaction of A-998821 on P-glycoprotein (P-gp/MDR1) and Breast Cancer Resistance Protein (BCRP)

Study A-998821 Drug Metabolism Memo No. 26. Inhibitory Interaction of A-1041392 (A-998821 M1 Metabolite) across Canine MDCKII MDR1 and MDCKII-BCRP Cells

Study A-998821 Drug Metabolism Memo No. 31. Inhibitory Interaction of A-998821 on Multidrug Resistance Protein 2 (MRP2)

Study A-998821 Drug Metabolism Memo No. 32. Inhibitory Interaction of A-998821 on Bile Salt Export Pump (BSEP)

Study A-998821 Drug Metabolism Memo No. 33. Inhibitory Interaction of A-1041392 (A-998821 M1 Metabolite) on Multidrug Resistance Protein 2 (MRP2)

Study A-998821 Drug Metabolism Memo No. 34. Inhibitory Interaction of A-1041392 (A-998821 M1 Metabolite) on Bile Salt Export Pump (BSEP)

Study A-998821 Drug Metabolism Memo No. 27. Inhibitory Interaction of A-998821 on Organic Anion Transporting Polypeptides (OATP) 1B1, 1B3, Organic Anion Transporters (OAT) 1, 3, Organic Cation Transporters (OCT) 1, 2, and Multi-drug and Toxin Extrusion Proteins (MATE) 1 and 2K

Study A-998821 Drug Metabolism Memo No. 28. Inhibitory Interaction of A-1041392 (A-998821 M1 Metabolite) on Organic Anion Transporting Polypeptides (OATP) 1B1, 1B3, Organic Anion Transporters (OAT) 1, 3, Organic Cation Transporters (OCT) 1, 2, and Multidrug and Toxin Extrusion Proteins (MATE) 1 and 2K

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Repeat-Dose Toxicity
Study RD09915. 14-Day Dose-Range Finding Oral Gavage Toxicity and Toxicokinetic Study with A-998821 Sodium in Mice TD09-140

Study RD091187. 13-Week Oral Gavage Maximum Tolerated Dose Study with A-998821 Sodium in Mice TD09-141

Study RD10313. 4-Week Oral Gavage Maximum Tolerated Dose Study with A-998821 Sodium in Model 001178-W (Wild Type) CByB6F1 Tg(HRAS)2Jic Mice TD09-196

Study RD071174.Seven-Day Oral Dosing Study of A-998821 in Male Sprague-Dawley Rats CMET07-038

Study RD101465. A-998821: A Three-Week Oral Palatability (Dietary) Pharmacokinetic Study in Sprague-Dawley Rats TA10-272

Study RD08013.Four-Week Oral Toxicity Study of A-998821 in Sprague-Dawley Rats (with a Four-Week Recovery Period) TA07-343

Study RD081262.13 Week Oral Toxicity Study of A-998821 Sodium in Combination with Ribaviran in Sprague Dawley Rats with a 1-Month Recovery Period TA07-438

Study RD081422.6-Month Oral Dose Toxicity Study with A-998821 Sodium in Rats with a 1-Month Recovery Period TA08-229

Study RD07962. Five-Day Oral Capsule Dosage Range-Finding Toxicity Study of A-998821 in Beagle Dogs TB07-235

Study RD071081. Four-Week Oral (Capsules) Toxicity Study of A-998821 in Beagle Dogs Including a Four-Week Recovery Period TB07-342

Study RD09310. 9 Month Oral Capsule Toxicity Study with A-998821 Sodium in Beagle Dogs with a 4 Week Recovery Period TB07-439

Study RD08695. Two Week Oral dose Range-Finding Study with A-998821 Sodium in Cynomolgus Monkeys TC07-436

Study RD081138. Four Week Oral Dose Toxicity Study with A-998821 Sodium in combination with Ribavirin and Interferon in Cynomolgus Monkeys with a 4-Week Recovery Period TC07-437

Study RD081421. Amended Report for A 1-Month Oral Dose Toxicity Study with (b) (4) and A-998821 Sodium Salt in Combination with Subcutaneous-Dose PEG-interferon and Oral-Dose Ribavirin in Cynomolgus Monkeys, with a 1-Month Recovery Period TC08-029

Genotoxicity
Study RD071401. Bacterial Mutation Assay TX07-344

Study RD071402. Chromosome Aberration Test TX07-345

Study RD071403. A-998821 Rat Micronucleus Test TA07-346

26-Week Oral Gavage Carcinogenicity Study with A-998821 Sodium in Model 001178-T (Hemizygous) CBYB6F1-TG(HRAS)2JIC Mice TD12-027

Reproductive Toxicology

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Study RD091012. An Oral Fertility Study with A-998821 Sodium in Rats, including a Toxicokinetic Evaluation TA08-260

Study RD081858. A Range-finding Oral Developmental Toxicity study with A-998821 Sodium in the Rat, Including a Toxicokinetic Evaluation TA08-300

Study RD09105. An Oral Developmental Toxicity with A-99 8821 Sodium in Rats TA08-301

Study RD081855. A Range-finding Oral Developmental Toxicity study with A-998821 Sodium in the Rabbit, Including a Toxicokinetic Evaluation TE08-302

Study RD09104. An Oral Developmental Toxicity with A-99 8821 Sodium in Rabbits TE08-303

Study RD12272. Oral Developmental and Perinatal/Postnatal Reproduction Toxicity Study of A-998821 Sodium Salt in Rats, Including a Postnatal Behavioral/Functional Evaluation TA11-281

Other Toxicity Studies
Study RD12338. Four-Week Oral Gavage Toxicity Study of A-998821 Sodium (with Impurities) in Sprague-Dawley Rats TA12-049

Ames TX13-164 (b) (4)

Chrom Abs TX13-165 (b) (4)

Previous Reviews Referenced

Nonclinical studies, including safety pharmacology, ADME, repeat-dose toxicology, and genetic toxicology studies to support the Dasabuvir (ABT-333) portion of the NDA have been reviewed previously by Dr. Pritam (Pete) Verma. The reviews are included in Appendix 4 and are summarized in the appropriate sections of this review.

Pharmacology

Primary Pharmacology

Refer to Microbiology review.

Secondary Pharmacology

Significant (>50%) interaction (i.e., inhibition of control specific binding) was noted at the following receptors: Adenosine (A3), peripheral benzodiazepine, cholecystokinin A (CCK1), serotonin (5-HT1B), glucocorticoid, vasopressin (V1a), and chloride.

Moderate inhibition of control specific binding was noted at the following receptors:
Cannabinoid (CB1), dopamine (D3), peroxisome proliferator-activated receptor (PPARγ), prostaglandin (EP4), serotonin (5-HT2A), and the norepinephrine (NE) transporter.

The lack of effects in safety pharmacology studies, lack of findings in repeat dose nonclinical toxicology studies, and safety data from clinical trials suggests that secondary binding to endogenous receptors is not significant *in vivo*.

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Safety Pharmacology

(*The following is taken from a review by Dr. Pritam (Pete) Verma*.)
A-998821 was tested in a battery of safety pharmacology assays. A-998821 produced no clinically significant neurobehavioral, respiratory or gastrointestinal effects. In *in vitro* electrophysiology assays, A-998821 produced an IC50 of 0.3 µg/mL in a hERG assay but did not affect canine Purkinje fiber repolarization up to and including 14.93 µg/mL. In addition, A-998821 had no effect on QTc in the conscious dog at plasma concentrations as high as 6190 ng/mL. In the anesthetized dog, A-998821 produced modest self- limiting increases in mean arterial pressure (7 mmHg), which were not produced at slower intravenous infusion rates, suggesting that the pressor effect was potentially mediated by the rate of increase in A-998821 concentration and was mitigated by tachyphylaxis/desensitization. Finally, A-998821 produced a slight decrease in mean arterial pressure in conscious dogs but only at plasma concentrations (6190 ng/mL) well above those predicted to be efficacious in humans.

Safety pharmacology studies
1. Effect of A-998821 on *In Vitro* hERG Current (R&D/08/154)
A-998821 was evaluated at measured mean bath concentrations of 0.4, 1.4, and 4.3 µg/mL (n = 4). These concentrations reduced hERG tail current by 19, 48, and 77%, respectively (after correction for vehicle/rundown). The reduction of hERG tail current was statistically different from vehicle. The IC50 value for hERG block was 1.5 µg/mL, a concentration above those predicted to be efficacious in humans (estimated efficacious human Cmax of 126 to 505 ng/mL).

Study RD071194. Effects of A-998821 (ABT-333) on Cloned hERG Potassium Channels Expressed in Mammalian Cells

In addition, a hERG study was performed in accordance with GLP regulations and ICH guidelines. A-998821 was tested at concentrations of 0.0336, 0.0944 and 0.571 µg/mL
(n = 5 to 6). A-998821 inhibited hERG potassium current by 4.7 ± 0.5%, 21.0 ± 1.2% and 67.1 ± 1.7% *versus* -0.2 ± 0.1% (n = 5, mean ± SEM) in control. The IC_{50} for the inhibitory effect of A-998821 on hERG potassium current was 0.3 µg/mL; a concentration similar to those predicted to be efficacious in humans.

2. Effect of A-998821 on Canine Purkinje Fiber Repolarization *In Vitro* (R&D/08/155)
A-998821 was examined at measured concentrations of 0.18 ± 0.02, 1.43 ± 0.07, and 14.93 ± 0.57 µg/mL (mean ± SEM, n = 4) in comparison to vehicle controls, n = 4/group). During slow stimulation (2 sec), chosen to emphasize repolarization delays, no effect of A-998821 on the APD was noted up to and including the highest concentration of 14.93 µg/mL; a plasma concentration well above those predicted to be efficacious in humans (estimated human Cmax of 126 to 505 ng/mL).

3. Effects of A-998821 on Cardiovascular Parameters in the Anesthetized Dog

Study RD08167. Effects of A-998821 on Cardiovascular and Hemodynamic Function in the Anesthetized Dog

A-998821 was tested in three separate cardiovascular studies using the anesthetized dog model. In each study, A-998821 was administered to anesthetized dogs as three escalating 30-minute intravenous infusions. In all three studies, the compound produced no physiologically relevant effects on HR (contractility=dP/dtmax, dP/dt50mmHg), central venous pressure, hematocrit or the PR interval. In the mid- and high-dose studies (infusion rates of 0.003 to 0.032 mg/kg/min), A-998821 produced modest self-limiting increases in MAP (7 mmHg *vs*. vehicle) and maximum shortening of the QT interval (14 msec *vs*. vehicle) at plasma concentrations of 0.23 and 1.84 µg/mL, respectively. When administered in a low-dose study over a slower range of escalating infusion rates (0.001 to 0.010 mg/kg/min), A-998821 did not produce an effect on MAP at concentrations as high as 0.7 µg/mL suggesting that the pressor effect

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observed at high infusion rates was mediated by rate of rise. Of note, the QT shortening observed in the anesthetized dog study at supratherapeutic plasma concentrations was in contrast to the hERG study findings suggesting a potential for QT prolongation related to inhibition of hERG.

4. Effects of A-998821 on Cardiovascular Parameters in Conscious Dogs
Study RD071193. Cardiovascular Safety Evaluation of Orally Administered A-998821 (ABT-333) in Beagle Dogs
The study was conducted using male beagle dogs orally administered 1, 3 or 10 mg/kg of A-998821. The animals were instrumented with radiotelemetry transmitters for measurement of blood pressure, heart rate, the electrocardiogram (ECG), and body temperature. Hemodynamics and the ECG (QRS duration and the RR, PR, and QT intervals) were monitored continuously for 22 hours. Blood samples for determination of plasma concentrations of A-998821 were collected from the dogs just prior to dosing and at eight hours after dosing. Oral administration of A-998821 at 1, 3, and 10 mg/kg produced plasma concentrations of 598 ± 15.8, 1860 ± 44.6 and 6190 ± 272 ng/mL, respectively. Oral administration of A-998821 at doses of 1 and 3 mg/kg did not produce any effects on blood pressure, heart rate or any of the ECG parameters. Compared with vehicle, 10 mg/kg (exposure = 6190 ± 272 ng/mL) produced a slight decrease in blood pressure (13 mmHg at two hrs after dosing). In summary, oral administration of A-998821 produced no cardiovascular effects in conscious dogs at doses up to and including 3 mg/kg, a dose associated with plasma concentrations of 1860 ± 44.6 ng/mL.

5. CNS Safety and Neurobehavioral Evaluation of A-998821 (R&D/07/1328) and Study RD071191. A Neurobehavioral Safety Evaluation of Orally Administered A-998821 (ABT-333) in Rats
The general behavioral effects of A-998821 were examined using the primary observation (Irwin) test in rats. No effects were observed with the oral dose of 1 mg/kg, but a mild, transient, dose-independent excitatory effect (increased sniffing behavior) was observed in at least half of the animals dosed with 3 to 100 mg/kg. No other consistent effects occurred in this assay.

In other CNS/neurobehavioral safety pharmacology assays in rats administration of A-998821 had no significant or consistent effects on spontaneous locomotor activity and on acute thermal nociception (hot plate test) up to the highest dose tested, 100 mg/kg, p.o.

The neurobehavioral responses to A-998821 were additionally evaluated in the rat modified Irwin assay (FOB) and were conducted in accordance with Good Laboratory Practices (GLP) regulations and the International Conference on Harmonization (ICH) guidelines. Female Sprague-Dawley rats were orally administered A-998821 at doses of 3, 10, and 30 mg/kg (n = 8/group). One additional group of animals received the vehicle (n = 8). Blood samples were collected from a satellite group of three animals/group at three and six hours after dosing for the determination of A-998821 plasma concentrations. Oral doses of 3, 10, and 30 mg/kg A-998821 produced maximal plasma concentrations of 201 ± 54.0, 1140 ± 106, and 5030 ± 337 ng/mL (mean ± SEM), respectively. No effects of A-998821 were observed through the highest dose of
30 mg/kg, a dose associated with maximal plasma concentrations of 5030 ± 337 ng/mL.
Taken together, these results suggest that A-998821 produces no CNS/neurobehavioral effects in rats at the dose of 1 mg/kg, p.o. At 3 to 30 mg/kg, no effects were observed in female Sprague-Dawley rats, but a mild, transient, dose-independent excitatory effect (increased sniffing behavior) was seen in male Wistar rats over the same dose range. At 100 mg/kg, no adverse effects were observed; however, a mild, transient increase was found in sniffing behavior. Since the excitatory effect was non-dose dependent and was not observed in the GLP CNS study at supratherapeutic plasma concentrations, the effect is not likely to occur in A-998821 dose-escalating studies in humans.

6. Effects of A − 998821 on Respiratory Function in Conscious Rats (R&D/07/1192)
Male, Sprague-Dawley rats were orally administered A-998821 at doses of 3, 10, and 30 mg/kg (n = 8/group). One additional group of animals served as the control and received the vehicle control (n = 8). Blood samples were collected from a satellite group of three animals/group at three hours after dosing for the determination of A-998821 plasma concentrations. Oral doses of 3, 10, and 30 mg/kg A-998821 produced plasma concentrations of 271 ± 40.4, 977 ± 237, and 4660 ± 517 ng/mL (mean ± SEM), respectively. Oral administration of A-998821 did not produce any physiologically relevant effects on

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respiratory rate, tidal volume or minute volume up to and including 30 mg/kg, a dose associated with plasma levels of 4660 ± 517 ng/mL.

7. Effects of A − 998821 in Gastrointestinal Tolerability Models
Ferret Emesis Study and Study RD08153. A-998821: Effects on Ferret Emetic Liability and Rat Gastrointestinal Transit
Overnight-fasted, male ferrets were used to assess the emetic liability of A-998821. A-998821 was administered by oral gavage at doses of 1.5, 5, and
15 mg/kg (n = 5-6/dose). After dosing, the number of emetic episodes and the presence of behaviors believed to correlate with nausea in ferrets were recorded for each animal over a period of 120 minutes. A blood sample for determination of A-998821 plasma concentrations was obtained from each ferret 120 minutes after oral dosing. Oral administration of A-998821 at doses of 1.5, 5, and 15 mg/kg produced maximum mean plasma concentrations of 550 ± 30, 1140 ± 130 and 880 ± 250 ng/mL (mean ± SEM), respectively, measured in samples obtained 120 minutes after dosing. No emesis or nausea were observed in ferrets up to and including the highest oral dose of 15 mg/kg (exposure of 1140 ± 130 ng/mL).

Gastrointestinal Transit: Overnight-fasted, male Sprague Dawley rats were used to determine the effects of A-998821 on gastrointestinal transit. A-998821 was administered orally at doses of 3, 10, and 30 mg/kg (n = 8/dose). Gastrointestinal transit rate was determined by measuring the position of the leading edge of the charcoal meal relative to the total length of the small intestinal segment. Oral administration of A-998821 at doses of 3, 10, and 30 mg/kg produced plasma concentrations of 450 ± 40, 1310 ± 210, and 3640 ± 460 ng/mL (mean ± SEM), respectively, 2.75 hours after dosing. A-998821 had no significant effects on gastrointestinal transit up to and including the highest oral dose of 30 mg/kg (plasma concentration of 3640 ± 460 ng/mL).

Pharmacokinetics/ADME/Toxicokinetics

PK/ADME

(*The following is taken from a review by Dr. Pritam (Pete) Verma*.)

The ABT-333 pharmacokinetic profile in mouse, rat, monkey and dog was characterized by a wide range of plasma clearance values (CLp =0.04 L/hr•kg (dog) to 1.2 L/hr•kg [monkey]), with high volumes of distribution in all species (Vss > 1.1 L/kg). The ABT-333 plasma elimination half-life (t 1/2) was short in monkey (2 hr), but averaged 3.6 hours in rat and 19.5 hours in dog. Bioavailability from an oral dose was low in both monkey (4.5%) and rat (21.3%), but high in dog (95.9%). Bioavailability from a suspension of the sodium salt was equivalent to that obtained from a solution formulation in dog. Bioavailability following an oral dose was lower when dogs were fed prior to dosing when compared to animals provided food 4 to 12 hours after drug administration.
ABT-333 is extensively metabolized in vivo in the rat and the drug-related material is primarily eliminated in the bile. ABT-333 is cleared primarily by cytochrome P450 (CYP)-mediated oxidative metabolism to A-1041392 (M1, *t*- butyl hydroxylation), with subsequent conjugation (M3, ether glucuronide or M2, sulfate) or further oxidation to its acid (A-1039710, M5) with subsequent glucuronidation. ABT-333 is not a potent inhibitor of CYP1A2, 3A4/5 or 2D6 (IC50 >40 μM) and is a weak-to-moderate competitive inhibitor of CYP2C8, 2C9 and 2C19 (IC50 ~17, ~9 and ~18 μM, respectively). CYP2C8, 3A4 and 2D6 contribute to its metabolism in human liver microsomes (~60, 30 and 10% of the control activity, respectively). ABT-333 is not an inducer of CYP3A4/5 mRNA. There was no evidence of active efflux. In combination, these data suggest that ABT-333 has low potential to elicit clinically significant drug-drug interactions.

Pharmacokinetics and ADME studies

Absorption
Rat (R&D/07/1144, 2008)

Reference ID: 3628623

Dose absorption following a single oral administration at 5 and 10 mg/kg of [^{3}H]ABT − 333 duct-cannulated (BDC) male SD rats was relatively low (~20 to 25%), with good overall recoveries of radioactivity observed 72 hours postdose (92 and 89.1% of the dose, respectively). The low absorption observed in the rat is attributed to poor dissolution due to the low solubility of ABT-333.

Distribution

Plasma Protein Binding (R&D/07/1142, 2008)
Preliminary data indicate that ABT-333 (5 µM) is highly bound (> 99%) to rat, dog, monkey and human plasma proteins. When incubated in whole blood, [^{3}H]ABT-333 distributed preferentially into the plasma compartment, with a blood-to-plasma concentration ratio ranging between 0.6 and 0.7 in rat, dog, monkey, and human independent of the 0.3 to 30 µM concentration evaluated.

Tissue Distribution Studies (R&D/07/1144, 2008)
In intact male SD rats (5 mg ABT-333/kg, PO), the dosed radioactivity distributed well to liver (44.1), adrenal glands (8.4), kidney (4.9), heart (3), lung (2.6), and lymph nodes (2.3), with tissue-to-plasma ratios at 3 hour postdose, had limited distribution to brain, eyes, and blood (T/P ~0.08, 0.3, 0.66) and there was no preferential distribution for all other tissues evaluated. The T/P ratios remained relatively constant or decreased through the duration of the study, indicating that the half-life of radioactivity in all tissues was similar to plasma.

Metabolism
In Vitro Metabolism (R&D/07/1141, 2008)
[^{3}H]ABT-333 had moderate stability in incubations with rat, monkey and human liver microsomes, with microsomal intrinsic clearances (~22.0, 12.4, and 22.8 µL/min/mg protein) that predicted plasma clearances of ~1.1, 0.44, and 0.50 L/h•kg, respectively.
Turnover was negligible in the dog (< 0.01% parent metabolized in 30 minutes). M1, the *t*-butyl hydroxyl metabolite (A-1041392) was detected in incubations with all species as the only product. This metabolite represented up to 30% of the drug-related material in rat, monkey, and human and was negligible in the dog. ^{3}H-ABT-333 also had moderate stability in rat, monkey, and human hepatocytes. The hepatocyte clearance (6.03, 7.35, and 7.97 µL/min/million cells, respectively) predicted a CLp of 0.89, 0.51, and 0.47 L/hr•kg, respectively, that were approximately within 2-fold of the observed plasma clearances. Turnover in the dog was negligible. M1 was the most abundant radiolabeled component, representing ~24, 6, and 31% of the drug related material in rat, monkey, and human, respectively. M3 (M1 glucuronide) represented up to ~8, 27, and 5% of the drug-related material. M2 and M4 (sulfate conjugate and *t*-butyl aldehyde, respectively) were minor products of metabolism. In vitro, M2 and M4 were only detected in human hepatocytes; however, both metabolites were detected in the rat *in vivo*.

It is predicted that in the human, ABT-333 will undergo a moderate rate of metabolism primarily to M1, with minor contributions from other metabolites.

In Vivo Metabolism
In bile duct cannulated rats (5 mg/kg, IV), [^{3}H]ABT-333 underwent extensive oxidative metabolism (< 2% of the dose was recovered as intact parent) in the rat. The most significant product of metabolism was the M3 (proposed as the glucuronide conjugate of M1) representing ~50% of the dose, followed by M1 (A-1041392, *t*-butyl hydroxy) and M5 (A-1039710, *t*-butyl acid), representing 8 and 5% of the total dose, respectively. Other products of metabolism included a M2, a sulfate conjugate, M6, a glucuronide conjugate of the *t*-butyl acid M5 and M4, a proposed *t*-butyl aldehyde. Similar metabolite profiles were observed following a single 5 or 10 mg/kg dose.

Parent drug was the most significant radiolabeled component in plasma after a 5 mg/kg dose (~80 to 90% of the total plasma radioactivity), followed by M1 (A-1041392, *t*-butyl hydroxyl; ~10 to 20% of the total plasma radioactivity). M3 was detected in trace amounts (< 3% of the total plasma radioactivity).

Reference ID: 3628623

In healthy male subjects, 94% of a radiolabelled dose of 400 mg ABT-333 was eliminated in feces. Only 2% was excreted in urine. Dasabuvir was the major component of drug related radiochemical material in plasma (60% of radiochemical). A tert-butyl hydroxylate metabolite, M1 (22%), was the major metabolite in plasma. The other metabolites, including a sulfate conjugate M2, a glucuronide conjugate M3, secondary oxidation products (M4 and M5), a glucuronide conjugate of M5 (M6) and a trace desmethyl metabolite M11, were considered minor metabolites as each accounted for less than 5% of radiochemical in plasma.

Inhibition and CYP Involvement in Metabolism (R&D/07/1140, 2008)
ABT-333 is not a potent inhibitor of CYP1A2, 3A4/5 or 2D6 (IC50 > 40 µM) and is a weak- competitive inhibitor of CYPs 2C8, 2C9 and 2C19 (IC50 ~17, ~9, and ~18 µM, respectively). An [I]/Ki ratio ≥ 0.1 indicates a remote potential for the drug under consideration to elicit a clinically significant drug-drug interaction. Assuming competitive inhibition, the Ki values are estimated to be 50% of the experimental IC50.
For ABT-333, data suggest a remote potential to cause clinically significant drug-drug interactions with clinical concentrations ≤ 0.43 µM (CYP2C9) or 0.8 µM (CYPs 2C19 and 2C8). Similarly, there will be a remote potential for interactions with CYPs 1A2, 2D6 and 3A4/5 with clinical concentrations ≤ 2 µM.

Following incubations of [^{3}H]ABT-333 (0.25 µM, 60 minutes) in human recombinant CYPs and flavin-containing monooxygenases (FMOs), metabolism was only observed with CYPs 2C8, 2B6, 3A4, and 2D6. [3H]ABT-333 incubations in human liver microsomes in the presence of known inhibitors of specific CYP isoforms indicated that CYP2C8 had the most significant contribution to metabolism followed by CYP3A4 and CYP2D6 (~60, 30, and 10% of the control activity was inhibited by quercetin, ketoconazole, and quinidine, respectively). The contributions of CYP2B6 were negligible (< 1% inhibition was observed with 2-phenyl-2-(1-piperidinyl)propane). The potential for a comedication to significantly inhibit the metabolism of ABT-333 is low, given that multiple isoforms are involved in the metabolism of ABT-333.

Induction
Preliminary data obtained from studies conducted in human hepatocytes indicate that ABT-333 is not an inducer of CYP3A4/5 mRNA (< 7% of rifampin, no dose response, 1 to 10 µM).

Excretion/Elimination (R&D/07/1142, 2008)
Following a single IV administration of [^{3}H]ABT-333 to bile duct cannulated male SD rats at 5 mg/kg, good recoveries of radioactivity were achieved at 72 hours postdose (89% of the dose). The dosed radioactivity was eliminated primarily into the bile (71.7%), with minimal elimination via the urine (2.8%). Dose absorption following a single oral administration at 5 and 10 mg/kg of [^{3}H]ABT-333 to bile duct cannulated male SD rats was relatively low (~20 to 25%), with good overall recoveries of radioactivity observed 72 hours postdose (92 and 89.1% of the dose, respectively).

General Toxicology

Single-Dose Toxicity

No single dose studies were conducted.

Repeat-Dose Toxicity

Several studies were conducted to determine appropriate dose ranges, or in a single case palatability of test compound, to be used for pivotal repeat dose toxicity studies. An additional study assessed the toxicity of co-administration of ABT-333 with PEG-interferon and Ribavirin in monkeys. As clinical development of ABT-333 did not include co-administration with PEG-interferon and Ribavirin, that study is not considered a pivotal study in the current application. The non-pivotal studies are summarized here. Pivotal studies have been reviewed in detail.

Reference ID: 3628623

CMET07-038: Seven-Day Oral Dosing Study of A-998821 in Male Sprague-Dawley Rats

Male rats (3/dose group) received ABT-333 via oral gavage at one of three doses: 10, 30, and 60 mg/kg (30 mg/kg BID) for 7 days. Possible test-article related findings in rats dosed with 60 mg/kg included decreased spleen weight correlating with mild splenic red pulp atrophy in 1 of 3 rats.

TA10-272: A-998821: A Three-Week Oral Palatability (Dietary) Pharmacokinetic Study in Sprague-Dawley Rats

Following 21 days of dietary administration to male and female rats at 20 and 50 mg A-998821/g bulk food (which represented approximately 2% and 5% concentrations of A-998821 in the diet), there were no test article-related clinical findings, effects on body weight, or gross findings at necropsy. Food consumption was transiently lower among females at both treated levels during the Day 1-3 interval; however, A-998821 mixed in the diet up to 50 mg A-998821/g bulk food was generally palatable to male and female rats. Average daily consumption was 4026 (Standard Deviation [SD] ± 332.4) and 3742 (SD ± 369.3) mg/kg/day and AUC values were 124 µg*hr/mL and 256 µg*hr/mL for males and females, respectively.

TB07-235: Five-Day Oral Capsule Dosage Range-Finding Toxicity Study of A-998821 in Beagle Dogs

ABT-333 (Lot 1463220) was formulated in Tween-20 (20%):PEG-400 (80%) and administered daily to beagle dogs (two/sex/group) for five to six consecutive days. Dogs received daily doses of 1, 10, 30, or 60 mg acid/kg/day. The formulations were prepared so that all dosages were administered in a 1 mL/kg volume as a liquid formulation in gelatin capsules. Dogs in the control group (two/sex) were administered vehicle (1 mL/kg) only. Evaluated parameters included body weights (prestudy only), food consumption, clinical observations, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), anatomic pathology (organ weights, gross and microscopic observations), and plasma/liver concentrations of ABT-333. The no observed adverse effect level (NOAEL) for ABT-333 free acid was 60 mg acid/kg/day (corresponding to a Cmax and AUC of 11.0 µg/mL and 204.2 µg•hr/mL, respectively) when formulated in Tween-20 (20%): PEG-400 (80%) and administered orally by capsule to two beagle dogs/sex/group once daily for five to six consecutive days.

TC07-436: Two Week Oral dose Range-Finding Study with A-998821 Sodium in Cynomolgus Monkeys

Three treatment groups of 2 cynomolgus monkeys (*Macaca fascicularis*)/sex were administered the test article at dose levels of 100, 150, or 200 mg/kg/day. The test article or vehicle was administered to all groups via oral gavage, once per day, for 14 consecutive days at a dose volume of 1.0 mL/kg. Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Clinical observations were conducted on Days 3, 7, 10, and 14. Body weights were measured and recorded on Days -1, 3, 7, 10, and 14. Blood and urine samples for clinical pathology evaluations were collected from all animals on Days -7, -1, 3, and 14. Blood samples for determination of the plasma concentrations of the test article were collected from all animals at designated time points on Days 1 and 14. At study termination, the animals were transferred to the stock colony. Within the study design limits of only two animals per gender per dose level, the mean AUC and Cmax values for A-998821 generally increased with increasing dose level. The mean AUC, dose normalized AUC, and Cmax values increased from Days 1 to 14. The mean (combined sex) toxicokinetic parameters are summarized in the following table.

Reference ID: 3628623

Dose Level (mg/kg/day)	Study Day	C_{max} (µg/mL)	C_{max}/Dose (µg/mL per mg/kg/day)	T_{max} (hour)	$T_{1/2}$ (hour)	AUC (µg·hr/ml)	AUC/Dose (µg·hr/mL per mg/kg/day)
100	1	2.5	0.03	3.0	3.0	14.1	0.14
	14	5.4	0.05	3.8	2.6	44.6	0.45
150	1	5.7	0.04	3.0	4.4	51.8	0.35
	14	11.3	0.08	4.5	4.1	115.1	0.77
200	1	6.9	0.03	3.0	3.5	57.6	0.29
	14	12.1	0.06	5.3	4.1	142.2	0.71

All animals survived to the study termination. There were no test article-related effects on body weights, hematology, coagulation, clinical chemistry, or urinalysis parameters. Based on these data, the no-observed-adverse-effect-level (NOAEL) for this study was 200 mg/kg/day, the highest dose tested, for males and females, corresponding with an AUC of 142 µg·hr/mL.

Study #RD081421. Amended Report for A 1-Month Oral Dose Toxicity Study with (b) (4) and A-998821 Sodium Salt in Combination with Subcutaneous-Dose PEG-interferon and Oral-Dose Ribavirin in Cynomolgus Monkeys, with a 1-Month Recovery Period TC08-029

The objectives of this study were to determine the potential toxicity of (b) (4) in combination with ribavirin and PEG-interferon, and A-998821 (A-998821.5 salt) in combination with ribavirin and PEG-interferon, when administered orally (via nasal gavage) to cynomolgus monkeys for 28 days, and to evaluate recovery from any effects of either combination treatment over a dose-free period of 29 days.

There were no test article-related clinical signs; effects on food consumption, ECGs, ophthalmic examinations, body weights, and clinical pathology parameters; or post-mortem findings. There were minimal and non-adverse changes in hematology parameters resulting from the administration of the reference articles (ribavirin and PEG-interferon) including decreased circulating levels of red cell mass (RBC, Hb, Hct, and MCHC) and increased RDW and MCV on Days 14 and 27. Neither (b) (4) nor A-998821.5 (ABT-333) had an effect on these hematological responses to ribavirin and PEG-interferon administration. All of these parameters recovered partially or fully by the end of the 29-day dose-free period.

Exposure levels of PEG-interferon decreased with increased days of dosing. On Study Day 25, most of the samples analyzed were below the limit of quantitation of the assay. This observation is consistent with previous data in which time-dependent induction of immunogenicity was observed. In contrast, Ribavirin exposure levels increased with increasing days of dosing. For both PEG-interferon and Ribavirin, exposure levels did not change with co-dosing of A-998821.5 or (b) (4) and there were no sex differences in exposures. Exposure data for (b) (4) and A-998821 are summarized in the table below (*excerpted from sponsor*).

Mean (±SD) Toxicokinetic Parameters for A-998821

			Day 1			Day 28		
Group	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg•hr/mL)
5	100	male	1.49 (±1.18)	2.6 (±0.9)	10.4 (±7.99)	1.24 (±0.709)	1.8 (±1.1)	8.86 (±6.35)
		female	0.286 (±0.200)	2.6 (±0.9)	1.82 (±0.925)	2.19 (±2.56)	2.2 (±1.1)	14.8 (±18.8)
		overall	0.886 (±1.02)	2.6 (±0.8)	6.10 (±7.01)	1.72 (±1.84)	2.0 (±1.1)	11.8 (±13.6)
6	150	male	1.26 (±1.02)	3.0 (±0.0)	10.8 (±11.5)	3.78 (±2.32)	3.0 (±0.0)	34.5 (±29.4)
		female	1.59 (±0.772)	3.2 (±1.8)	11.8 (±6.07)	2.15 (±1.59)	3.2 (±1.8)	19.2 (±20.6)
		overall	1.43 (±0.872)	3.1 (±1.2)	11.3 (±8.66)	2.96 (±2.06)	3.1 (±1.2)	26.9 (±25.3)

The no-observed-adverse-effect level (NOAEL), based on absence of effects, was considered to be [redacted] (b) (4) for [redacted] (b) (4) and 150 mg/kg for A-998821.5, in combination with 15 mcg/kg PEG-Interferon and 50 mg/kg ribavirin, under the conditions of this study. These dosages resulted in exposures of [redacted] (b) (4) for [redacted] (b) (4) and 26.9 µg•hr/mL for A-998821.5.

(*The following summaries are taken from reviews by Dr. Pritam (Pete) Verma. See Appendix 4 for the full reviews.*)

1. **Four-week Oral Toxicity Study in Sprague-Dawley Rats:** Groups of male and female animals (10/sex/group main study; 5/sex/group TK) received daily oral gavage (2 ml/kg) doses of ABT-333 at dose levels of 0 [vehicle control consisting of [vitamin E TPGS:VP-dimer (20%: 80%, w/w)], 0 (negative control consisting of normal saline), 10 (low), 30 (mid) or 60 mg/kg/day (high) for four weeks followed by a 4-week recovery period. Noteworthy clinical signs observed during the dosing period included moderate urine stained hair around the anogenital area in one female and two male rats dosed at 60 mg acid/kg/day, as well as slight red and severe urine stained hair around the anogenital area in one female rat treated with 10 mg acid/kg/day. Based on results of the study, a dose level of 60 mg/kg/day may be considered the NOAEL. At the NOAEL, based on the body surface area factor, an equivalent dose in humans would be 9.7 mg/kg/day or 585 mg/day for a 60 kg person.

2. **Four-week Oral Toxicity Study in Beagle Dogs with a Four-week Recovery Period:** Groups of male and female beagle dogs (5/sex/group) received daily ABT-333 at dose levels of 0 [control: capsules filled with gelatin and PEG 400:Tween 20, (80:20)], 1 (low), 3 (mid) or 10 mg/kg/day (high) for four weeks followed by a 4-week recovery period. Two dogs/sex/group were designated recovery animals. Clinical signs included occasional emesis and abnormal feces (with mucus or white particles) in control dogs as well as dogs dosed with A-998821 during the treatment period. Clinical chemistry revealed slight elevations (less than 2-fold) in alkaline phosphatase serum levels in mid and high dose males and females in Weeks 2 and 5 of treatment. Based on results of the study, the NOAEL of ABT-333 when administered to male and female dogs for at least 4 weeks was 10 mg/kg/day. At the NOAEL, based on the body surface area factor, an equivalent dose in humans would be 5.4 mg/kg/day or 324 mg/day for a 60 kg person.

3. **ABT-333: Four-Week Oral Toxicity Study of A-998821 in Combination with Ribavirin and Interferon in Cynomolgus Monkeys (with a Four-Week Recovery Period):** Groups of male and female animals were administered the test article, RBV, IFN (vehicle: 0.2% hydroxypropyl methylcellulose in water) for four weeks followed by a 4- week recovery period. No toxicologically remarkable findings were noted among animals given A-998821 alone or in combination with RBV and IFN. The NOAEL for A-998821 noted for this study was 150 mg/kg/day (corresponding to a Cmax and AUC of 9.77 µg/mL and 108 µg•hr/mL, respectively). Comparable A-998821 exposures were observed when coadministered RBV and IFN. No toxicologically remarkable findings were noted among animals given A-998821 alone or in combination with RBV and IFN.

4. **ABT-333: Thirteen-Week Oral Toxicity Study of A-998821 in Combination with Ribavirin in rats (with a Four-Week Recovery Period):** Groups of male and female rats were administered the test article in combination with RBV (vehicle: 0.2% hydroxypropyl methylcellulose in water) for thirteen weeks followed by a 4-week recovery period. No toxicologically remarkable findings were noted among animals given A-998821 alone or in combination with RBV. The NOAEL for A-998821 (alone) noted for this study was 200 mg/kg/day for males and female (corresponding to an AUC 0-24 of 34 and 94 µg•hr/mL and Cmax of 3.5 and 6.8 µg/ml for males and females, respectively). Co administration of A-998821 did not exacerbate any test article related observations nor induce any new combination toxicities.

5. **Study title: 6-Month oral dose toxicity study with A-998821 in rats with a 1-month recovery period**: Three groups of 25 male and 25 female Sprague-Dawley rats were administered A-998821 at dose levels of 50, 200, or 800 mg/kg/day (25, 100 or 400 mg/kg, BID., 6 hours apart). One additional group of 25 animals/sex served as the control and received the vehicle, 0.2% hydroxypropyl methylcellulose in distilled water, BID. Following the dosing period, five animals/sex/group were maintained for a 1-month recovery period. There were no treatment-related mortalities and no adverse test article-related effects on clinical observations, body weight, food consumption, ophthalmoscopic examinations, hematology, coagulation, clinical chemistry, urinalysis, or organ weights. Alveolar histiocytosis at all doses and granulomatous inflammation of the ileum at 800 mg/kg/day were test article-related changes but were not associated with adverse clinical effects. The NOAEL for this study was 800 mg/kg/day (400 mg/kg, BID) associated with AUC values of 119 and 319 µg*hr/mL in males and females, respectively, at Week 23. Microscopic change in target tissues, lung and ileum, were not considered to be adverse effects.

6. **9-Month Oral Capsule Toxicity Study with A-998821 in Beagle Dogs with a 4-Week Recovery Period:** Three treatment groups of five dogs/sex were administered the test article at dose levels of 10, 30, or 60 mg/kg/day. One additional group of five animals/sex served as the controls and received the vehicle, 0.2% hydroxypropyl methyl cellulose. Following 273 days of administration, two animals/sex/group were maintained for a 28 day recovery period. In males and females given 60 mg/kg/day, mild increases in total bilirubin, ALT, sorbitol dehydrogenase, and gamma glutamyltransferase were seen relative to controls at the termination interval suggesting a mild test article-related hepatic effect at this dose. In addition, beginning by Week 4 and generally persisting through termination there were mild, non-progressive, yet test article-related increases in ALP in males given 60 mg/kg/day and in females given 30 mg/kg/day, relative to controls. These changes correlate to the moderately increased mean absolute liver weight in males given 60 mg/kg/day and the increased hepatocellular vacuolation noted microscopically. In liver from male dogs administered A-998821, there was an increase in the amount of glycogen in centrilobular hepatocytes compared to concurrent vehicle controls. The areas of increased glycogen likely correspond to the vacuolation noted in hepatocytes by histopathology. The ultrastructure of hepatocytes was otherwise normal. Based upon an absence of any adverse findings during this study, the NOAEL for A- 998821 sodium during this study was 60 mg/kg/day, for males and females; corresponding with an overall Day 270 AUC of 839 µg*hr/mL, and a Cmax of 41.7 µg/mL.

Reference ID: 3628623

Study title: 14-Day Dose-Range Finding Oral Gavage Toxicity and Toxicokinetic Study with A-998821 Sodium in Mice

Study no.:	TD09-140
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	24July2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	A-998821, 62749PP00, 92.2% potency

Key Study Findings

- There were no treatment related findings.
- The NOAEL is 4000 mg/kg/day, which correlates to overall mean Cmax of 51.58 µg/mL and AUC of 388.4 µg•hr/mL, on Day 14.

Methods

Doses:	600, 2000, 4000 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.2 % (w/v) hydroxypropyl methylcellulose (4000 cps) in RO water
Species/Strain:	Hsd:ICR(CD-1®) mice
Number/Sex/Group:	5
Age:	6-8 weeks
Weight:	31.2 – 41.9 g
Satellite groups:	Toxicokinetics: 5 (control) or 20/group

Results

Mortality: Early deaths were attributed to gavage error (three females) or undetermined (one male).

Unscheduled Deaths

Animal No.	Sex	Group No.	Dose (mg/kg/day)	Study Day	Clinical History	Relevant Gross Findings	Cause of Death
A33432	F	4	4000	12	Swollen midline ventral abdomen, squinted eyes, hypoactive, cold to touch	Inflammation and foreign material in the lung	Accidental (gavage-related)
A33387	M	7	2000	10	Found dead	None	Unknown
A33487	F	8	4000	5	Retching and foaming at mouth	Discolored lung, white semi-solid material in trachea	Accidental (gavage-related)
A33493	F	8	4000	13	Hunched, few feces, squinted eyes, left eye cloudy discharge, rough haircoat, swollen left ventral thorax	White/granular foreign material in, and perforation of the esophagus	Accidental (gavage-related

Clinical Signs: There were no significant treatment related clinical signs.

Body Weights: Although there were no effects noted on body weight per se, mean body weight gain was higher in middle dose males and high dose females, compared to controls.

Reference ID: 3628623

Mean Body Weight Change Data

Test Article	Control	A-998821 sodium		
Group	1	2	3	4
Level (mg/kg/day)	0	600	2000	4000

Day		Mean body weight gain (g) for Group: 1M	2M	3M	4M	Statistics
DSNG 1- DSNG 5	Mean	0.0	1.0	2.2*	2.0*	P
	SD	1.22	0.72	0.61	0.97	
	N	5	5	5	5	
DSNG 5- DSNG 8	Mean	0.6	0.3	0.6	0.1	P
	SD	1.08	0.45	0.93	0.69	
	N	5	5	5	5	
DSNG 8- DSNG 13	Mean	0.8	0.9	-0.8	0.5	P
	SD	0.49	1.01	2.22	1.37	
	N	5	5	5	5	
DSNG 13- DSNG 16	Mean	0.2	0.8	-0.4	0.7	P
	SD	0.76	0.46	1.47	1.08	
	N	5	5	5	5	
DSNG 1- DSNG 16	Mean	1.6	3.0	1.7	3.3	P
	SD	1.03	0.90	3.88	1.15	
	N	5	5	5	5	

* P< or =0.05

P = ANOVA (and Dunnett's, if applicable)

These findings were transient and were not considered adverse.

Feed Consumption: Feed consumption was higher from day 1 to 4 in high dose males and females. Because the finding was transient and did not affect the well-being of the mice, it was not considered to be adverse.

Clinical Pathology: There were no treatment related findings on hematology and clinical chemistry parameters.

Gross Pathology: There were no remarkable findings.

Organ Weights: There were no remarkable findings.

Histopathology

Adequate Battery: No, in this non-GLP dose range finding study.

Peer Review: No.

Histological Findings: A minimal increase in extramedullary hematopoiesis in the liver of females given 4000 mg/kg/day had no clinical pathology correlate (hematology) and therefore was not considered test article-related. Other organs (bone marrow and spleen) that may also show potential effects on the hematopoietic system were not examined due to the experimental design for this study.

Toxicokinetics

Reference ID: 3628623

Mean Toxicokinetic Parameters for A-998821

			Day 14				
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)
6	600	Male	20.8	0.03	2.3	107.8	0.18
		Female	25.1	0.04	1.0	140.3	0.23
		Overall	22.9	0.04	1.7	124.0	0.21
7	2000	Male	24.5	0.01	2.3	199.0	0.10
		Female	38.3	0.02	7.0	326.7	0.16
		Overall	31.4	0.02	4.7	262.8	0.13
8	4000	Male	48.8	0.01	4.0	286.9	0.07
		Female	54.4	0.01	6.0	489.9	0.12
		Overall	51.6	0.01	5.0	388.4	0.10

Study title: 13-week Oral Gavage Maximum Tolerated Dose Study with A-998821 Sodium in Mice

Study no.: TD09-141
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 14September2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-998821, 75991PP00, 91.9% potency

Group	Subgroup[b]	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration[c,d] (mg/mL)
1 (Control)[a]	1 (Toxicity)	10	10	0	0
	2 (Toxicokinetic)	8	8	0	0
2 (Low)	1 (Toxicity)	10	10	600	60
	2 (Toxicokinetic)	38	38	600	60
3 (Mid)	1 (Toxicity)	10	10	2000	200
	2 (Toxicokinetic)	38	38	2000	200
4 (High)	1 (Toxicity)	10	10	5000	500
	2 (Toxicokinetic)	38	38	5000	500

a Group 1 received vehicle control article (0.2% [w/v] hydroxypropyl methylcellulose [4000 cps] in RO water) only.
b Toxicokinetic animals were included solely for the purpose of blood sample collections. Two animals/sex/group were included as extras for possible mortality.
c A-998821 sodium (A-998821.5, ABT-333), Lot No. 75991PP00, assigned chemical potency of 919 µg A-998821.0/mg of drug substance (a correction factor of 1.09 was used) and Lot No. 82163PP00 assigned chemical potency of 922 µg A-998821.0/mg of drug substance (a correction factor of 1.08 was used).
d The dose volume was 10 mL/kg.

Key Study Findings

- There were no treatment related findings, so that a maximum tolerated dose was not determined.
- The NOAEL is 5000 mg/kg/day, which correlates to an overall mean AUC of 197 µg·hr/mL on Day 80.

Methods

Doses:	600, 2000, 5000 mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.2 % (w/v) hydroxypropyl methylcellulose (4000 cps) in RO water
Species/Strain:	Hsd:ICR(CD-1®) mice
Number/Sex/Group:	10
Age:	6-8 weeks
Weight:	20.9- 43.0 g
Satellite groups:	Toxicokinetics: 8 (control) or 38/group

Results

Mortality: Twenty early deaths, six from the toxicity dose groups and 14 from the toxicokinetic dose groups, were attributed to gavage error (eight) or undetermined. Of the 12 undetermined deaths, the clinical signs were suggestive of gavage error. Early deaths are not considered to be treatment related.

Reference ID: 3628623

Animal ID	Sex	Group/ Subgroup No.	Dose (mg/kg/day)	Death Study Day	Clinical History	Pertinent Pathology Findings	Cause of Death
Toxicity							
A34494	M	3/1	2000	63	Unremarkable	Inflammation of thoracic cavity, esophagus, thyroid, trachea, findings in various tissues	Accidental (gavage-related)
A34521	M	4/1	5000	67	Swollen mid-abdomen, hypoactive, cold to touch, discolored haircoat	Inflammation of aorta, esophagus (with erosion), heart, stomach, thyroid, trachea, findings in various tissues	Accidental (gavage-related)
A34567	M	4/1	5000	65	Hunched, audible and irregular respiration, pale skin	Findings in various tissues	Undetermined
A34583[a]	F	1/1	0	27	Limited hind limb use	Unremarkable	Undetermined
A34609	F	2/1	600	10	Hunched, lack of feces, rough haircoat	Tan semifluid in thoracic cavity, perforated esophagus, inflammation of thoracic cavity, esophagus, lung	Accidental (gavage-related)
A34703	F	4/1	5000	73	Tremors, hypoactive squinted eyes, audible, irregular, and labored respiration	Findings in various tissues	Undetermined
Toxicokinetic[b]							
A34544	M	4/2	5000	62	Audible respiration	Large jejunal lumen	Undetermined
A34633	F	2/2	600	11	Hunched, hypoactive, squinted eyes, labored respiration, cold to touch	Unremarkable	Undetermined
A34661	F	3/2	2000	65	Hunched, tremors, circling, irregular respiration	Tan semifluid in thoracic cavity	Accidental (gavage-related)
A34670	F	3/2	2000	24	Hunched, swollen ventral cervical area, few feces, audible respiration	Tan, semifirm lobulated contents surrounding thyroid in thoracic cavity	Accidental (gavage-related)
A34674	F	3/2	2000	22	Hunched, thin, few feces, irregular respiration	Adhesions of, and perforated esophagus, discoloration of heart and lungs, adhesions of lungs, white semisolid contents of thoracic cavity	Accidental (gavage-related)
A34675	F	3/2	2000	9	Hunched, tremors, irregular respiration, cold to touch	Perforated esophagus, tan semisolid contents of thoracic cavity	Accidental (gavage-related)
A34707	F	4/2	5000	41	Hypoactive, labored respiration, cold to touch	Unremarkable	Undetermined
A34708	F	4/2	5000	73	Swollen midline ventral abdomen, hypoactive, squinted eye, cold to touch	Unremarkable	Undetermined
A34710	F	4/2	5000	40	Hunched, swollen midline ventral abdomen, hypoactive, cold to touch	Enlarged small and large intestines	Undetermined
A34713	F	4/2	5000	22	Hunched, tremors, hypoactive, few feces, pale	Perforated esophagus, white semisolid contents of thoracic cavity	Accidental (gavage-related)
A34714	F	4/2	5000	40	Squinted eyes, labored respiration	Unremarkable	Undetermined
A34718	F	4/2	5000	22	Swollen abdomen, tremors, hypoactive, irregular respiration, cold to touch, red haircoat, pale	Unremarkable	Undetermined
A34720	F	4/2	5000	45	Hypoactive, few feces, audible and labored, respiration, cold to touch	Enlarged small and large intestines	Undetermined
A34727	F	4/2	5000	24	Hunched, few feces, labored respiration, red haircoat	Unremarkable	Undetermined

a Necropsy was limited to an examination of the oral, cervical, and thoracic cavities and regions. All tissues and carcasses were discarded and no histopathological examination performed (see Protocol Deviations).
b Observations were limited to observations for mortality, abnormalities, and signs of pain or distress and body weight determinations. Necropsy was limited to an examination of the oral, cervical, and thoracic cavities and regions. All tissues and carcasses were discarded and no histopathological examination performed.

Clinical Signs: There were no clinical signs that are considered to be treatment related. Noted clinical signs were intermittent, transient, and/or not dose-related..

Body Weights: There were no treatment related effects on body weights.

Feed Consumption: There were no treatment related effects on feed consumption.

Clinical Pathology

Hematology: Hematological findings included increased total leukocyte counts in males given >2000 mg/kg/day (1.79x and 1.74x increase from control values at 2000 and 5000 mg/kg/day, respectively). Although the increased leukocyte counts were due to increased lymphocyte counts (1.83x and 1.77x increase from control values at dose levels of 2000 and 5000 mg/kg/day, respectively), it is noted that the increases in lymphocyte counts were not significantly different from control values. Due to the minimal increases these changes were not considered to be toxicologically significant.

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Clinical Chemistry: Mild to marked increases were noted in mean aspartate aminotransferase (AST; 3.36x to 6.68 x increase from control values) and alanine aminotransferase (ALT; 4.52x to 6.52x increase from control values) activities in females given >600 mg/kg/day, although these increases in AST and ALT activity were not dose-related.

Males, but not females, given 2000 or 5000 mg/kg/day had a minimal (1.45x) or mild (2.16x) increase in alkaline phosphatase (ALP) activity compared with mean control values, respectively. The increases in AST and ALT activity were of uncertain relationship to test article administration, because similar marked but unexplained increases in AST and/or ALT activities were sporadically observed in males in the control and lower dose groups. Increased AST, ALT, and ALP activities were not associated with hepatic histopathology.

Gross Pathology: There were no remarkable findings.

Organ Weights: There were no remarkable findings.

Histopathology

Adequate Battery: Yes.

Peer Review: No peer review was necessary as there were no histopathological findings related to test article administration.

Histological Findings: There were no histopathological findings related to test article administration.

Toxicokinetics

Mean Toxicokinetic Parameters for A-998821

			Day 80				
Test 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)
2	600	Male	13.2	0.022	1	72.2	0.12
		Female	9.5	0.0158	3	53.7	0.0895
		Overall	10	0.0167	3	62.8	0.105
6	2000	Male	22.7	0.0114	3	163	0.0815
		Female	21.4	0.0107	3	148	0.074
		Overall	22.1	0.0111	3	156	0.0778
4	5000	Male	22.7	0.00454	1	167	0.0335
		Female	36.9	0.00738	6	242	0.0485
		Overall	27.3	0.00546	6	197	0.0395

Dosing Solution Analysis: Concentration and homogeneity parameters met the acceptance criteria.

Study title: 4-Week Oral Gavage Maximum-Tolerated Dose Study with A-998821 Sodium in Model 001178-W (Wild Type) CByB6F1-Tg(HRAS)2Jic Mice

Reference ID: 3628623

Study no.:	RD10313 ([redacted] (b) (4) study no. 8217409)
Study report location:	Electronic Document Room
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	18 February 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ABT-333, lot no 82163PP00, 92.2% purity/lot no. 85224PP00, 92.6% purity

Key Study Findings

- This study was conducted to identify the maximum tolerated dose of ABT-333 in CByB6F1-Tg(HRAS)2Jic mice. Mice were administered ABT-333 at doses of 600, 2000, 6000, and 12000 mg/kg/day.
- Daily oral administration of ABT-333 was not tolerated at 12,000 mg/kg/day and resulted in early termination due to poor survival.
- Adverse stomach findings, including glandular stomach hyperplasia of foveolar (surface) mucosal epithelium, mucus metaplasia of gastric glands, and increased inflammation and nonglandular stomach hyperkeratosis and squamous hyperplasia, that may have been due to a mild irritant effect of the semisolid test article material in the stomach lumen were observed in animals dosed with >6,000 mg/kg/day.
- Also in the middle-high and high dose animals, histopathology in adrenal cortex (increased cytoplasmic microvacuolation and diffuse hyperplasia), liver (slight to marked cytoplasmic vacuolation) and thymus (lymphoid depletion/necrosis) was noted.
- The maximum-tolerated dose was defined as 2,000 mg/kg/day, which resulted in a mean Cmax of 34.6 µg/mL and AUC0-24 of 396 µg·hr/mL on Day 27 of the dosing phase.

Reference ID: 3628623

Methods

Doses:	600, 2000, 6000, 12000 mg/kg/day
Frequency of dosing:	Once (600, 2000, 6000 mg/kg) or twice 6000 mg/kg BID
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.2% [w/v] hydroxypropyl methylcellulose
Species/Strain:	Model 001178-W (wild type), CByB6F1-Tg(HRAS)2Jic mice
Number/Sex/Group:	15
Age:	8-9 weeks
Weight:	Males: 21.4 to 32.2 g Females: 17.8 to 23.8 g
Satellite groups:	Toxicokinetics: 23/sex/group (low, middle mid-high), 26/sex/group (high)
Unique study design:	Middle-high and high doses prepared at maximum feasible dose due to viscosity limitations.
Deviation from study protocol:	Adrenal cortex, liver and stomach tissue were examined from only 10 animals in low and middle dose groups, rather than from all 15 animals in those dose groups as per protocol. Although unlikely, assessment of more animals may have identified histopathology at those doses where no effects were seen.

Results

Mortality: Animals were checked twice daily for mortality. One male administered 2000 mg/kg/day was sacrificed moribund on day 16 of dosing. The cause of death was not determined but was not considered to be test article related due to the lack of similar effects in other animals at this dose level. Three males administered 6000 mg/kg/day ABT-333 were sacrificed moribund, one each on days 13, 24 and 26. Findings among the three animals included swollen abdomen, squinted eyes, white feces, cold to touch, irregular and audible respiration, hypoactivity, rough haircoat, no feces, semisolid foreign material in the stomach and distended urinary bladder. Dosing of animals in the high dose (12,000 mg/kg/day) group was terminated early due to poor survival. Seventeen animals were sacrificed moribund between days 8 and 26. No specific histopathologic finding was associated with the early deaths. A few animals given 12,000 mg/kg/day were observed with swollen abdomens and the many of the animals were observed at necropsy with the presence of white, semisolid foreign material (likely a combination of test article and food).

Clinical Signs: Animals were checked twice daily for abnormalities and signs of distress. Clinical signs associated with moribund condition in middle-high and high dose groups are described above.

Body Weights: Body weights were recorded twice weekly during dosing. Animals in the middle (2000 mg/kg) and middle-high (6000 mg/kg) dose groups had increased body weight gain over the 28 day dosing period. The increased weight may be associated with an accumulation of viscous test article and food. Aside from the impact on histopathology in the stomach, this finding is not considered to be toxicologically significant.

Feed Consumption: Food consumption was recorded twice weekly during dosing. Increases in food consumption ranging from 10 to 40% were noted in males dosed with ≥2000 mg/kg and females dosed with ≥600 mg/kg. The increases were test article related but sporadic and were not considered adverse.

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Hematology: Samples for hematology and clinical chemistry were collected via cardiac puncture at necropsy. Test article-related effects on hematology parameters were limited to mildly higher variability in red cell size for males and females given >6,000 mg/kg/day [note that effects on red cell mass (i.e., red blood cell count, hemoglobin, and hematocrit) and absolute reticulocyte counts were not noted], and mildly lower absolute lymphocyte count for males and females given 12,000 mg/kg/day. There were no histopathological correlates with clinical pathology findings.

Clinical Chemistry: Clinical pathology effects were limited to minimally higher total protein for females given >6,000 mg/kg/day, and mildly higher alkaline phosphatase for males given >2,000 mg/kg/day and females given 2,000 or 6,000 mg/kg/day.

Gross Pathology: Dose related macroscopic findings were limited to foreign material present in stomach or distended stomachs and intestines infrequently in high dose animals.

Organ Weights: Increased liver weights in females at 2000 mg/kg and all animals at higher doses correlated with microscopic findings of cytoplasmic vacuolation (glycogen) at 6000 or 12000 mg/kg/day. Decreased thymus weights correlated with lymphoid depletion/necrosis at 6000 or 12000 mg/kg/day. Changes in spleen and brain did not have microscopic correlates and were not considered to be test article related.

Histopathology

Adequate Battery: Yes. All tissues from middle-high and high dose groups were examined microscopically. Only target organs identified in the high dose groups were examined microscopically in low and middle dose group animals. Adrenal cortex, liver and stomach tissue were examined from only 10 animals in low and middle dose groups, rather than from all 15 animals in those dose groups as per protocol. Although unlikely, assessment of more animals may have identified histopathology at those doses where no effects were seen.

Peer Review: Yes

Histological Findings: Stomach (glandular and non-glandular), adrenal cortex, liver and thymus were identified as target organs in high dose groups. Minimal to moderate test article-related changes were present in the glandular and nonglandular stomach of animals given 6,000 or 12,000 mg/kg/day, including the following: glandular stomach hyperplasia of foveolar (surface) mucosal epithelium, mucus metaplasia of gastric glands, and increased inflammation and nonglandular stomach hyperkeratosis and squamous hyperplasia. Effects were possibly due to irritation caused by the presence of test article in the lumen.

Incidence and Mean Severity Grade of Test Article-Related Changes in the Glandular and Nonglandular Stomach

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Number Examined	10	10	10	11	14	10	10	10	10	15
Glandular Stomach										
Gastric glands, mucus metaplasia	0	0	0	10 (1.5)	13 (1.9)	0	0	0	5 (0.8)	11 (1.5)
Hyperplasia, foveolar (surface) mucosal epithelium	0	0	0	4 (0.5)	10 (1.3)	0	0	0	6 (0.9)	7 (0.9)
Inflammation	0	0	0	11 (2.0)	10 (1.4)	1 (0.1)	1 (0.2)	0	6 (1.0)	7 (0.9)
Nonglandular Stomach										
Hyperplasia, squamous	0	0	0	2 (0.3)	11 (1.1)	0	0	0	6 (0.6)	5 (0.4)
Hyperkeratosis	0	0	0	5 (0.5)	12 (1.4)	0	0	1 (0.1)	5 (0.6)	10 (0.9)

Number in parentheses indicates severity grade.

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Test article-related changes were present in the zona fasciculata of the adrenal cortex of animals given 2000; 6000; or 12000 mg/kg/day. Microscopic changes in this region occurred in the outer zona fasciculata adjacent to the overlying zona glomerulosa. The test article-related changes consisted of increased cytoplasmic microvacuolation and diffuse hyperplasia in males and females, respectively.

Incidence and Mean Severity Grade of Test Article-Related Changes in the Adrenal Cortex

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Number Examined	10	10	10	11	14	10	10	10	10	15
Adrenal Cortex										
Zona fasciculata (outer), hyperplasia, diffuse	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (1.1)	10 (2.2)	14 (1.9)
Zona fasciculata (outer), increased microvacuolation	0 (0.0)	0 (0.0)	1 (0.2)	10 (1.4)	10 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Number in parentheses indicates severity grade.

Cytoplasmic vacuolation was notably increased in animals given 6,000 or 12,000 mg/kg/day, where it was graded slight to marked (present diffusely throughout the liver section) and correlated with increased liver weight parameters in these groups.

Incidence and Mean Severity Grade of Test Article-Related Changes in the Liver

Sex	Males					Females				
Group	1	2	3	4	5	1	2	3	4	5
Number Examined	10	10	10	11	14	10	10	10	10	15
Liver										
Increased vacuolation (glycogen) hepatocellular	1 (0.1)	1 (0.1)	2 (0.2)	9 (1.7)	11 (2.6)	2 (0.2)	1 (0.1)	1 (0.1)	7 (1.6)	8 (1.9)

Number in parentheses indicates severity grade.

Lymphoid depletion/necrosis was noted in the thymus of females given 6,000 or 12,000 mg/kg/day. This finding was graded minimal to moderate and correlated with decreased weight parameters in these groups. Thymic lymphoid depletion/necrosis was considered to be associated with stress in these dose group animals.

Toxicokinetics

Samples were collected from three animals/sex/timepoint. Timepoints were approximately 1, 3, 6, 9, 12, and 24 hours postdose. Plasma concentration on Day 27 of the dosing phase, as characterized by AUC0-24 and Cmax, was similar for males and females given 6,000 mg/kg/day and 12,000 mg/kg/day. For animals given 600 or 2,000 mg/kg/day, exposures for females were less than 2-fold higher than males. The mean exposure appeared less-than-proportional for both sexes at 600, 2,000, and 6,000 mg/kg/day. On Day 27 of the dosing phase, mean AUC was comparable for animals given 6,000 and 12,000 mg/kg/day.

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Mean Toxicokinetic Parameters for A-998821 for Day 27 of the Dosing Phase

Group	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)		AUC_{0-24} (µg•hr/mL)
7	600	male	15.4		87
		female	20.7		165
		overall	18.1		126
8	2,000	male	30.8		292
		female	38.3		499
		overall	34.6		396
9	6,000	male	66.9		755
		female	62.7		744
		overall	62.1		750
Group	**Dose (mg/kg/day)**	**Sex**	**C_{max}^1 (µg/mL)**	**C_{max}^2 (µg/mL)**	**AUC (µg•hr/mL)**
10	12,000	male	45.4	71.4	788
		female	50.6	75.1	836
		overall	48.0	73.3	812

C_{max} = Maximum plasma concentration; AUC_{0-t} = Area under the plasma concentration-time curves; C_{max}^1 = Period following the first of the two daily doses; C_{max}^2 = Period following the second of the two daily doses.

Genetic Toxicology

Refer to Appendix 4 for reviews of mutagenicity and clastogenicity with ABT-333. In summary, ABT-333 was negative in an in vitro mutagenicity assay and an in vitro chromosome aberration test.

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

Study title: A-998821 Rat Micronucleus Test

Study no: TA07-346
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 27 November 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-998821, Lot No. 57651PP00, 99.1%

Key Study Findings

Administration of A-998821 did not induce an increase in micronucleated immature erythrocytes in male rats under the conditions of this assay. Therefore, A-998821 was concluded to be negative in the *in vivo* micronucleus assay.

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Methods

Doses in definitive study:	500, 1000 or 2000 mg/kg
Frequency of dosing:	single administration
Route of administration:	oral
Dose volume:	20 mL/kg (10 mL/kg for cyclophosphamide).
Formulation/Vehicle:	80% (w/w) polyethylene glycol 400 (PEG-400)/20% (w/w) polysorbate 20 (Tween™ 20)
Species/Strain:	Sprague-Dawley SD (Hsd:SD) albino outbred rats (*Rattus norvegicus*)
Number/Sex/Group:	5
Satellite groups:	Toxicokinetics (6/group)
Basis of dose selection:	The dose levels were selected based on the absence of findings at the high dose in a preliminary toxicity assessment.
Negative control:	vehicle
Positive control:	cyclophosphamide

Study Validity

Results from negative and positive control samples were consistent with a valid assay.

Results

A marginal increase was noted in the incidence of micronucleated immature erythrocytes (MIE) in 48-hour post-treatment samples from treated rats. However, the incidence of MIE fell among historical controls for control animals. A re-read of the 48-hour samples did not confirm the increased incidence of MIE.

Carcinogenicity

Study title: 26 –Week Oral Gavage Oncogenicity Study with A-998821 Sodium in Model 001178-T (Hemizygous) CBYB6F1-Tg(HRAS)2Jic mice

Study no.:	(b) (4) Study Number 126-701 ABBVIE Study Number: 12-027
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	23 February 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-998821.5, A-998821, ABT-333; Batch Number 91377PP00, 99.5%
CAC concurrence:	Yes

Key Study Findings

- There were no dose-related neoplastic findings. The high dose (2000 mg/kg/day) resulted in systemic exposures (AUC) of 265 µg*hr/mL.
- Non-neoplastic microscopic findings were limited to neutrophil/macrophage infiltration and foreign material within the small intestine (jejunum and/or ileum) and increased multinucleated

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macrophages and foreign material within the mesenteric lymph node of males and females at 2000 mg/kg/day.

- Minimal neutrophil/macrophage infiltration with or without foreign material was also noted within the small intestine (ileum) of females at 600 mg/kg/day.

Adequacy of Carcinogenicity Study

Mice were exposed to adequate concentrations of test article, and assessments were sufficient to determine the carcinogenicity of test article.

Appropriateness of Test Models

The transgenic mouse species used here is an appropriate model in which to assess the carcinogenicity of chemical agents.

Evaluation of Tumor Findings

There were no dose-related neoplastic findings.

Methods

Doses:	200, 600, 2000 mg/kg
Frequency of dosing:	once a day
Dose volume:	2 mL
Route of administration:	oral gavage
Formulation/Vehicle:	0.2% hydroxypropyl methylcellulose (HPMC) prepared in distilled water
Basis of dose selection:	1-month study (max. feasible exposures at high dose) and ECAC agreement
Species/Strain:	Model 00178-T (Hemizygous) CByB6F1-Tg(HRAS)2Jic mice
Number/Sex/Group:	25
Age:	7-9 weeks
Animal housing:	individually in solid bottom cages
Paradigm for dietary restriction:	none
Dual control employed:	Yes (water)
Interim sacrifice:	None
Satellite groups:	Positive controls, N-Nitroso-N-methylurea (NMU (15/sex) Sentinel animals for serological health screen purposes (10/sex) Toxicokinetic (Taconic model 001178-W (nontransgenic), CByB6F1-Tg(HRAS)2Jic: 6/sex controls or 21/sex treated
Deviation from study protocol:	Deviations noted are not expected to have influenced the conclusions of this study.

Results

Mortality

No dose relationship was noted in the incidence of mortality. The complete list of unscheduled deaths is given below (table excerpted from sponsor).

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Unscheduled Vehicle Control and A-998821-treated Main Study Deaths

Animal Number	Sex	Dose Level	Fate	Fate Day	Cause of Death
1004	M	0 mg/kg/day	EE	180	Dosing Injury
1005	M	0 mg/kg/day	FD	180	Undetermined
1007	M	0 mg/kg/day	FD	113	Hemangiosarcoma/hemangioma
1025	M	0 mg/kg/day	DPE	133	Urogenital inflammation/obstruction/calculi
3020	M	600 mg/kg/day	FD	157	Dosing Injury
3022	M	600 mg/kg/day	FD	25	Accidental injury
4019	M	2000 mg/kg/day	EE	52	Dosing Injury
2519	F	200 mg/kg/day	EE	113	Hemangiosarcoma/hemangioma
2524	F	200 mg/kg/day	EE	120	Skin tumor
3501	F	600 mg/kg/day	EE	100	Undetermined
3506	F	600 mg/kg/day	EE	166	Dosing Injury
3514	F	600 mg/kg/day	EE	139	Hemangiosarcoma/hemangioma
3520	F	600 mg/kg/day	FD	169	Accidental injury

M – Male
F – Female

DPE – Died Prior to Euthanasia
FD – Found Dead
EE – Euthanized *in extremis*

Clinical Signs: Cageside observations were made twice daily. Detailed clinical examinations were conducted twice weekly during dosing. There were no treatment related clinical signs.

Body Weights: Mean body weights in high dose females tended to be greater than controls throughout the dosing period. The finding was considered to be incidental, not related to treatment.

Feed Consumption: Sporadic differences in feed consumption among control and treated mice were noted. Definitive attribution to treatment could not be made.

Gross Pathology: All macroscopic observations were considered incidental and/or of the type occasionally observed in mice of this strain and age based on presence in concurrent vehicle control, lack of microscopic correlates, lack of dose dependency, and/or low incidence rate.

Macroscopic findings in the positive control group were associated with induction of various neoplasms related to the administration of N-Nitroso-N-methylurea (MNU).

Histopathology

Peer Review: Yes.

Neoplastic: There was no evidence of treatment-induced carcinogenicity. None of the tumor statistical tests showed significant results (all unadjusted p-values>0.05). All neoplasms in treated males and females were considered incidental and/or of the type occasionally observed in mice of this age and strain based on presence of similar neoplasms at similar incidence rates in concurrent vehicle control animals, single or low occurrence, and/or lack of dose dependency.

Non Neoplastic: Non-neoplastic A-998821-related microscopic findings were limited to neutrophil/macrophage infiltration and foreign material within the small intestine (jejunum and/or ileum) and increased multinucleated macrophages and foreign material within the mesenteric lymph node of

Reference ID: 3628623

males and females at 2000 mg/kg/day. Minimal neutrophil/macrophage infiltration with or without foreign material was also noted within the small intestine (ileum) of females at 600 mg/kg/day.

Summary of Non-neoplastic A-998821-related Microscopic Findings								
Dose level: mg/kg/day	0		200		600		2000	
Sex	M	F	M	F	M	F	M	F
Number Examined	25	25	25	25	25	25	25	25
Small intestine, jejunum								
infiltration, neutrophils/macrophages								
- minimal	0	0	0	0	0	0	7	2
foreign material								
- minimal	0	0	0	0	0	0	6	2
Small intestine, ileum								
infiltration, neutrophils/macrophages	0	0	0	0	0	3	15	25
- minimal	0	0	0	0	0	3	12	16
- mild	0	0	0	0	0	0	3	9
foreign material	0	0	0	0	0	1	15	25
- minimal	0	0	0	0	0	1	14	25
- mild	0	0	0	0	0	0	1	0
Lymph node, mesenteric								
macrophages, multinucleated, increased	0	0	0	0	0	0	23	23
- minimal	0	0	0	0	0	0	22	19
- mild	0	0	0	0	0	0	1	4
infiltration, neutrophils/macrophages								
- mild	0	0	0	0	0	0	0	1
foreign material	0	0	0	0	0	0	23	22
- minimal	0	0	0	0	0	0	22	22
- mild	0	0	0	0	0	0	1	0

M - Male
F - Female

Toxicokinetics

Mean Toxicokinetic Parameters for A-998812				
		Day 91		
Dose Level (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)
200	Female	11.3	1.0	54.3
	Male	10.6	3.0	48.1
	Overall	9.93	3.0	51.2
600	Female	18.3	6.0	137
	Male	16.1	1.0	101
	Overall	16.3	3.0	119
2000	Female	31.1	6.0	321
	Male	22.8	6.0	208
	Overall	27.0	6.0	265

Dosing Solution Analysis: Homogeneity and concentrations were confirmed.

Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study title: An Oral Fertility Study with A-998821 Sodium in Rats, Including a Toxicokinetic Evaluation

Study no.: 126-386
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: February 6, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-998821/68859PP01/ 91.9 %

Key Study Findings

- There were no adverse effects on fertility parameters in treated animals. Estrous cycle length, copulatory interval, mating, fertility and fecundity indices were not affected at any dose levels. Likewise, no effect was observed on organ weights or uterine and sperm parameters.
- The no-observed-adverse-effect-level (NOAEL) was considered to be 800 mg acid/kg/day, corresponding to repeat-dose AUC's of 198 and 250 µg•hr/mL in males and females, respectively.

Methods

Doses:	60, 300, or 800 mg acid/kg/day (30, 150, and 400 mg acid/kg/dose [BID])
Frequency of dosing:	BID
Dose volume:	10 mL/kg/dose
Route of administration:	oral gavage
Formulation/Vehicle:	0.2% hydroxypropylmethylcellulose (HPMC) in water
Species/Strain:	CD®[Crl:CD®(SD)]
Number/Sex/Group:	25
Satellite groups:	none
Study design:	Males dosed beginning 28 days prior to mating, and to the females beginning 14 days prior to mating. Dosing of the males continued through the mating and postmating periods until euthanasia on Day 64 to 66 of treatment, while dosing of the females continued through the mating period and to Gestation Day (GD) 7. The females that mated were dosed for a total of 23 to 43 days.

Observations

Observations of the animals included clinical signs, body weights, and food consumption measurements. Females were examined daily for estrous cycle determination during the premating and mating periods until evidence of mating was observed. Mated females were euthanized on GD 13, uterine examinations were conducted, and the location of normally developing implantations, resorptions, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded. Complete necropsies were performed on all animals. Sperm analyses (motility, concentration, and morphology) were also conducted.

Results

Mortality: One male from the control group dies as the result of technician error (no further details given). A female rat in the middle dose (300 mg/kg/day) was found dead. Based on findings at necropsy, death was attributed to gavage error.

Clinical Signs: No clinical signs were considered to be related to test article administration.

Body Weight: There were no changes in body weight that were considered to be related to test article administration.

Feed Consumption: Due to sporadic nature of incidences of increased food consumption, changes were not considered to be test article related.

Toxicokinetics

Toxicokinetic parameters are summarized in the sponsor's table below.

The mean dose-normalized exposure (AUC/Dose) appeared to be less than proportional to dose for both sexes at all three dose levels. Repeat-dose exposures (AUC) in females on Day 14 were higher than repeat-dose AUC's in males on Days 28 and 66.

Reference ID: 3628623

Mean (±SD) Toxicokinetic Parameters for A-998821								
				C_{max} (µg/mL)		T_{max} (hr)		
Group	Day	Dose (mg acid/kg/day)	Sex	1	2	1	2	AUC (µg•hr/mL)
2	14	60	female	2.63	3.84	6	9	60.0
	28	60	male	2.98	2.73	3	7.5	34.7
	66	60	male	2.16	3.07	3	9	36.0
3	14	300	female	7.90	11.4	3	9	160
	28	300	male	3.55	7.00	3	7.5	83.2
	66	300	male	5.15	9.36	6	9	128
4	14	800	female	9.85	20.5	1.5	9	250
	28	800	male	8.28	11.5	6	7.5	162
	66	800	male	12.4	14.1	6	9	198

Dosing Solution Analysis: The Sponsor has provided documentation that the test article formulation is stable at these concentrations for at least 14 days; therefore, no stability analyses were performed.

Formulations were tested per the sponsor's table below. All analyses met study requirements.

Dosing Formulation Analysis Sample Collection							
			Number of Samples per Concentration				
Sample Type	Dose Level Sampled (mg acid/kg/day)	Stratum	Collected	Analyzed	Backup	Sample Volume (mL)	Intervals
Homogeneity Analyses[a]	60, 300, 800	Top	2	1	1	2.0	First preparation
		Middle	2	1	1	2.0[b]	
		Bottom	2	1	1	2.0	
Concentration Analyses[a]	0, 60, 300, 800	Middle	2	1	1	2.0[b]	Weeks 1[c], 4, and last preparation

[a]The samples were stored [redacted] (b) (4) pending analyses or final disposition.
[b]Sample volume at 0 mg acid/kg/day was 5.0 mL.
[c]Samples were only collected at 0 mg acid/kg/day during Week 1.

Necropsy

Fertility Parameters
There were no adverse effects on fertility parameters in treated animals. Estrous cycle length, copulatory interval, mating, fertility and fecundity indices were not affected at any dose levels. Likewise, no effect was observed on organ weights or uterine and sperm parameters.

Embryonic Fetal Development

Study title: An Oral Developmental Toxicity Study with A-998821 Sodium in Rats

Study no.:	TA08-301 (R&D/12/272):
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 31, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-998821/ 68859PP01/ 92%

Key Study Findings

- Pregnant rats were treated with 60, 300 or 800 mg acid/kg/day on GDs 6 to 17.
- No effects of treatment were seen on maternal clinical findings, gestation body weights or body weight change, food consumption, or macroscopic evaluations.
- Similarly, no effects of treatment were seen from uterine implantation data, fetal body weights and sex ratios, or fetal external, visceral, or skeletal evaluations.
- On the basis of these data, the No-Observed-Adverse-Effect-Level (NOAEL) for maternal and developmental toxicity with A-998821 Sodium in the rat was 800 mg acid/kg/day, which was the highest dose level evaluated and corresponded to a maternal AUC of 329 µg·hr/mL on GD 16.

Methods

Doses:	60, 300, 800 mg acid/kg/day
Frequency of dosing:	BID
Dose volume:	20 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Aq. 0.2% hydroxypropyl methylcellulose
Species/Strain:	CD® [Crl:CD®(SD)] rats
Number/Sex/Group:	25
Satellite groups:	Toxicokinetic (5/group)
Study design:	See table below
Deviation from study protocol:	Reported deviations do not raise concerns about data integrity.

Reference ID: 3628623

Group Assignments					
Group Number	Dose Level		Concentration[b]		Number of Time-mated Females
	mg acid/kg/day[a]	mg acid/kg/dose	mg acid/mL	mg bulk/mL	
Main Study					
1	0	0	0	0	25
2	60	30	3.0	3.26	25
3	300	150	15.0	16.32	25
4	800	400	40.0	43.52	25
Toxicokinetic					
5	0	0	0	0	5
6	60	30	3.0	3.26	5
7	300	150	15.0	16.32	5
8	800	400	40.0	43.52	5

[a]Administered twice daily in equally divided doses.
[b]The test article has an assigned chemical potency of 919µg A-998821/mg bulk test article

Observations

Observations of the main study animals included clinical signs, gestation body weights and body weight change, and food consumption. Blood samples for determination of the plasma concentrations of the test article were collected from TK animals at 1.5, 3, 6 (prior to second dose), 7.5 (1.5 hour after second dose), 9, 12, and 24 hours postdose on GD 6 and 16. On GD 17, approximately 3 hours postdose, the TK animals were euthanized, pregnancy status was determined, fetal blood was collected, and the carcasses were discarded. On GD 20, all main study animals were euthanized and subjected to a complete necropsy, including a uterine examination in which the total number of implantations, early and late resorptions, viable and nonviable fetuses, sex, and individual body weights of the fetuses were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded and adjusted body weight changes were calculated for the main study animals. All fetuses were given an external examination, approximately one-half of the fetuses in each litter were processed for visceral examination and the remaining fetuses in each litter were processed for skeletal examination. Malformations and developmental variations were recorded.

Results

Mortality: All animals survived to scheduled necropsy.

Clinical Signs: No remarkable findings.

Body Weight: No remarkable findings.

Feed Consumption: Slight but statistically significant increases in maternal food consumption were not considered to be toxicological significant.

Reference ID: 3628623

Toxicokinetics

Toxicokinetic parameters are presented in the sponsor's table below.

Mean (±SD) Toxicokinetic Parameters for A-998821

Group	GD	Dose (mg/kg/day)	C_{max} (µg/mL) 1	C_{max} (µg/mL) 2	T_{max} (hr) 1	T_{max} (hr) 2	AUC (µg•hr/mL)
6	6	60	1.76 (±0.279)	3.01 (±1.01)	4.2 (±1.6)	9.3 (±1.6)	41.0 (±13.8)
6	16	60	2.92 (±0.825)	4.01 (±0.693)	4.2 (±1.6)	9.6 (±1.3)	59.1 (±7.98)
7	6	300	5.85 (±1.09)	11.6 (±1.09)	5.4 (±1.3)	10.2 (±1.6)	162 (±14.9)
7	16	300	8.64 (±1.90)	13.9 (±2.13)	4.2 (±1.6)	9.0 (±0.0)	212 (±44.5)
8	6	800	8.90 (±0.975)	17.5 (±1.34)	6.0 (±0.0)	9.8 (±1.5)	252 (±14.1)
8	16	800	14.2 (±2.64)	20.3 (±2.98)	3.0 (±0.0)	9.4 (±1.9)	329 (±57.3)

Dosing Solution Analysis: Dose formulations were found to be homogenous, and concentrations were within acceptable parameters.

The Sponsor has provided documentation that the test article is stable for at least 14 days when formulated at concentrations bracketing those used in this study.

Necropsy

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

Pregnancy rates were 100% in each group. No effect of treatment was noted.

Offspring (Malformations, Variations, etc.)

No effect of treatment was noted with respect to fetal parameters.

Reference ID: 3628623

Study title: An Oral Developmental Toxicity Study with A-998821 Sodium in Rabbits

Study no.:	**TE08-303**
Study report location:	**EDR**
Conducting laboratory and location:	(b) (4)
Date of study initiation:	**04 Mar 2012**
GLP compliance:	**Yes**
QA statement:	**Yes**
Drug, lot #, and % purity:	**A-998821/ 91377PP00/ 92%**

Key Study Findings

- Pregnant rabbits were treated with 100, 200, or 400 mg/kg/day from GD 7 to GD 20.
- No effects of treatment at the dose levels evaluated were seen from maternal clinical examinations, gestation body weights or body weight change, food consumption, or macroscopic evaluations.
- Similarly, no effects of treatment were seen from uterine implantation data, fetal body weights and sex ratios, or fetal external, visceral, or skeletal evaluations.
- On the basis of these data, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity with A-998821 Sodium in the rabbit was 400 mg acid/kg/day, which was the highest dose level evaluated and corresponded to a maternal AUC of 83.5 µg·hr/mL on GD 19.

Methods

Doses:	100, 200, 400 mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	4 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.2% aq. Hydroxypropyl methylcellulose
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	20
Satellite groups:	Toxicokinetic (5/group)
Study design:	See table below
Deviation from study protocol:	No significant deviations

Reference ID: 3628623

Group Assignments				
Group Number	Dose Level (mg acid/kg/day)	Concentration		Number of Time-mated Females
		mg acid/mL[a]	mg bulk/mL[a]	
Main Study				
1	0	0	0	20
2	100	25	27.2	20
3	200	50	54.4	20
4	400	100	108.8	20
Toxicokinetic				
5	0	0	0	5
6	100	25	27.2	5
7	200	50	54.4	5
8	400	100	108.8	5

[a]A-998821 Sodium, Lot Number 68859PP01, has an assigned chemical potency of 919 µg A-998821 acid/mg bulk test article.

Observations

Observations of the main study animals included clinical signs, gestation body weights and body weight change, and food consumption. On GD 29, each surviving main study animal was euthanized and subjected to a complete necropsy, including a uterine examination in which the total number of implantations, early and late resorptions, viable and nonviable fetuses, sex, and individual body weights of the fetuses were recorded. The total number of corpora lutea on each ovary was also recorded. Gravid uterine weights were recorded and adjusted body weight and body weight changes were calculated. All fetuses were given an external and visceral examination and were processed for skeletal examination. Malformations and developmental variations were recorded.

Results

Mortality: One high dose animal aborted on GD 27. This animal had stopped eating on GD11, and the aborted pregnancy was attributed to inappetence. Because there was only one incidence, this finding was not considered to be test article related.

Clinical Signs: No treatment-related effects were noted.

Body Weight: No treatment-related effects were noted.

Feed Consumption: No treatment-related effects were noted.

Toxicokinetics
Blood samples for determination of the plasma concentrations of the test article were collected from TK animals at 0.5, 1, 3, 6, 9, 12, and 24 hours postdose on GD 7 and 19. On GD 20, at 2.5 hours postdose, the TK animals were euthanized, pregnancy status was determined, fetal blood was collected, and the carcasses were discarded.

Toxicokinetic parameters are presented in the sponsor's table below.

Reference ID: 3628623

Mean (±SD) Toxicokinetic Parameters for A-998821							
		GD 7			GD 19		
Group	Dose (mg acid/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg·hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg·hr/mL)
6	100	2.79 (±1.09)	1.4 (±0.9)	13.3 (±4.11)	3.86 (±1.85)	2.0 (±2.2)	23.8 (±14.8)
7	200	3.81 (±1.29)	1.0 (±0.0)	22.9 (±10.4)	5.70 (±2.24)	1.0 (±0.0)	37.1 (±19.2)
8	400	5.21 (±0.988)	1.8 (±1.1)	28.5 (±4.03)	10.6 (±1.18)	3.6 (±1.3)	83.5 (±34.7)

Dosing Solution Analysis: Dose formulations were found to be homogenous, and concentrations were within acceptable parameters. The Sponsor has provided documentation that the test article is stable for at least 14 days when formulated at concentrations bracketing those used in this study.

Necropsy

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

The pregnancy rate was 100% in the control, 100, and 400 mg acid/kg/day groups, and 95% in the 200 mg acid/kg/day group. Exclusive of the nonpregnant animal and the aborted pregnancy, there were 20, 20, 19, and 19 pregnancies with viable fetuses for evaluation on GD 29 in the control, 100, 200, and 400 mg acid/kg/day groups, respectively.

No treatment-related effects were noted.

Offspring (Malformations, Variations, etc.)

No treatment-related effects were noted.

Prenatal and Postnatal Development

Study title: Oral Developmental and Perinatal/Postnatal Reproduction Toxicity Study of A-998821 Sodium Salt in Rats, Including a Postnatal Behavioral/Functional Evaluation.

Study no.: TA11-281 (R&D/12/272):
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 04 Mar 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: A-998821/ 91377PP00/ 92%

Key Study Findings

- Pregnancy was confirmed in 18 (94.7%), 17 (89.5%), 20 (100.0%) and 18 (90.0%) F1 generation rats in the 0, 50, 200 and 800 mg/kg/day dose groups, respectively.

- No treatment-related effects on prenatal and postnatal development were noted.
- The NOAEL was defined as 800 mg/kg/day based on the lack of findings. Corresponding systemic exposures were 302 µg*hr/mL.

Methods

Doses: 50, 200, 800 mg/kg /day

Frequency of dosing: BID
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: 0.2% (w/v) Hydroxypropyl Methylcellulose (E4M Premium CR) (HPMC) in Reverse Osmosis Deionized Water
Species/Strain: Crl:CD(SD) Sprague Dawley rats
Number/Sex/Group: 20
Satellite groups: Toxicokinetic study groups (5 per dose group)
Study design:

Experimental Design - F0 Generation Rats

Group No.	Test Material	Total Daily Dose Level (mg/kg/day)	Dose Level (mg/kg/dose)[a]	Concentration (A-998821.0 mg/mL)[b]	Dose Volume (mL/kg)	No. of Main Study Rats (Rat Numbers)	No. of Toxicokinetic Rats (Rat Numbers)
1	Control Article	0	0	0	10	20 (2901-2920)	5 (2981-2985)
2	A-998821	50	25	2.5	10	20 (2921-2940)	5 (2986-2990)
3	A-998821	200	100	10	10	20 (2941-2960)	5 (2991-2995)
4	A-998821	800	400	40	10	20 (2961-2980)	5 (2996-3000)

a. Doses were administered twice daily approximately 6 hours apart.
b. A-998821.5, Lot No. 91377PP00 has an assigned chemical potency of 920 µg A-998821.0 /mg bulk test article, respectively. A correction factor of 1.08 was used for the purpose of dose and preparation calculations.

Experimental Design - F1 Generation Rats

Group No.	Test Material	Maternal Total Daily Dose Level (mg/kg/day)	Maternal Concentration (A-998821.0 mg/mL)	Maternal Dose Volume (mL/kg)	No. of Rats/Sex	Assigned F1 Generation Rat Numbers	
						Male	Female
1	Control Article	0	0	10	20	401 - 420	501 - 520
2	A-998821	50	2.5	10	20	421 - 440	521 - 540
3	A-998821	200	10	10	20	441 - 460	541 - 560
4	A-998821	800	40	10	20	461 - 480	561 - 580

Deviation from study protocol: No significant deviations.

Observations

F0 generation dams in this study: viability, clinical signs, maternal body weights, maternal body weight changes, maternal food consumption, maternal behavior, natural delivery observations, maternal plasma sample collection and analysis, and gross pathology.

F1 generation litters through weaning: viability, clinical signs, body weights, body weight changes, and gross pathology (F1 generation pups that were euthanized or were not selected for continued evaluation).

F1 generation rats selected for continuation on study were evaluated for the following parameters and endpoints: viability, clinical signs, body weights, body weight changes, food consumption, sexual maturation, behavioral testing (acoustic startle, motor activity and watermaze), reproductive capacity, gross pathology, reproductive organ weights (F1 generation male rats), ovarian and uterine examinations (F1 generation female rats), and fetal gross external examinations (F2 generation fetuses).

Results

F_0 Dams

Survival: All dams survived to scheduled necropsy.

Clinical signs: No treatment related effects.

Body weight: No treatment related effects.

Feed consumption: No treatment related effects.

Uterine content: Pregnancy was confirmed in 20 dams from each group, except 19 in the low dose group. All dams delivered except one control dam. There were no treatment effects on delivery or litter parameters. A statistically significant decrease in percent liveborn pups in the high dose group was not considered to be a treatment effect, as the percentage of stillborn pups was within the labs historical range.

Necropsy observation: No gross pathology findings.

Toxicokinetics:

Dose Level (mg/kg/day)	Cmax[1] (µg/mL)	Cmax[2] (µg/mL)	Cmax[1]/D (µg/mL/ mg/kg/day)	Cmax[2]/D (µg/mL/ mg/kg/day)	Tmax[1] (hr)	Tmax[2] (hr)	AUC (µg*hr/mL)	AUC/D (µg*hr/mL/ mg/kg/day)
50	3.50 ± 0.578	5.75 ± 1.15	0.0700 ± 0.0116	0.115 ± 0.0230	3.6 ± 1.3	9.6 ± 1.3	76.3 ± 15.6	1.52 ± 0.308
200	9.84 ± 1.82	15.9 ± 2.60	0.0492 ± 0.00911	0.0797 ± 0.0130	3.0 ± 0.0	9.3 ± 1.6	223 ± 26.4	1.11 ± 0.131
800	12.3 ± 2.77	19.7 ± 7.60	0.0154 ± 0.00346	0.0246 ± 0.00952	3.3 ± 1.6	9.9 ± 2.0	302 ± 110	0.377 ± 0.137

Cmax[1] and Tmax[1] refer to the period following the first of the two daily doses.
Cmax[2] and Tmax[2] refer to the period following the second of the two daily doses.

Dosing Solution Analysis: Concentrations and homogeneity was confirmed in dose preparations.

F_1 Generation

Survival: One F1 control female and one F1 middle dose male were euthanized due to injuries not related to test article administration. All other animals survived to scheduled necropsy.

Clinical signs: No treatment related effects.

Body weight: No treatment related effects.

Feed consumption: No treatment related effects.

Physical development: No treatment related effects.

Neurological assessment: No treatment related effects.

Reproduction: No treatment related effects. Pregnancy was confirmed in 18 (94.7%), 17 (89.5%), 20 (100.0%) and 18 (90.0%) F1 generation rats in the 0, 50, 200 and 800 mg/kg/day dose groups, respectively.

Reference ID: 3628623

Mean (SD) Plasma Concentrations (µg/mL) and Ratios of A-998821 in Maternal Rats and Their Pups on Day 14 Postpartum

Dose Level (mg/kg/day)	Pup	Maternal	Pup/Maternal
50	0.0637 ± 0.0478	0.617 ± 0.292	0.0954 ± 0.0368
200	0.243 ± 0.0499	1.70 ± 0.593	0.157 ± 0.0552
800	0.490 ± 0.164	4.64 ± 3.54	0.140 ± 0.0671

F_2 Generation

Survival: No treatment related effects.
Body weight: No treatment related effects.
External evaluation: No treatment related effects.
Male/Female ratio: No treatment related effects.

Reference ID: 3628623

Appendix 4. Reviews by Dr. Pete Verma

APPEARS THIS WAY ON ORIGINAL

Reference ID: 3628623

Appendix 5: Review of Degradants/Impurities of Concern by Dr. Mark Powley

APPEARS THIS WAY ON ORIGINAL

Reference ID: 3628623

PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

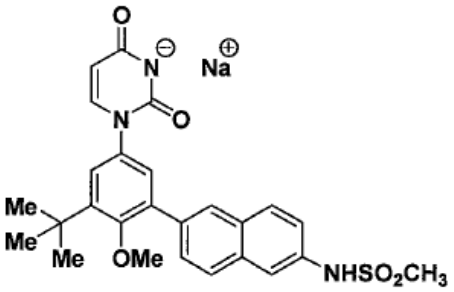
IND number: 101,636.000
Review number: 000
Sequence number/date/type of submission: May 2, 2008
Information to sponsor: Yes
Sponsor and/or agent: Abbott Laboratories
Global Pharmaceutical Research and Development
Abbott Park, Illnois 60064 USA

Manufacturer for drug substance: Abbott Laboratories
Global Pharmaceutical Research and Development
Abbott Park, Illnois 60064

Reviewer name: Pritam S. Verma, Ph.D.
Division name: DAVP
HFD #: 530
Review completion date: February 20, 2009

Drug:

Trade name: not available
Generic name: not available
Code name: ABT-333 (A-998821)
Chemical name: Methanesulfonamide, N- (6- (5 -(3 ,4-dihydro-2,4-dioxo-1 (2H)-pyrimidiny 1)- 3-(1,1-dimethy lethy 1)- 2- methoxypheny 1) - 2-naphthalenyl)-, monosodium salt
CAS registry number: not available
Molecular weight: 515.56 (salt); 493.57 (acid)
Molecular formula/molecular weight: $C_{26}H_{26}N_{30}O_5SNa$ (salt); (acid)
Structure:



Relevant INDs/NDAs/DMFs: None

Drug class: ABT-333 is a nonnucleoside inhibitor of the hepatitis C virus (HCV) RNA-dependent RNA polymerase

Intended clinical population: male and female patients

Clinical formulation: Dosage Form: Capsules; Strength: 5 and 50 mg

Excipients: Microcrystalline cellulose, lactose, sodium croscarmellose, magnesium stearate, (b) (4) titanium dioxide, and iron oxide black

Route of administration: oral

Previous clinical experience: None
Proposed clinical protocol:

Single Dose Escalating, Food Effect, and Multiple Dose (2 Day) Escalating Study (M10-351)

M10-351 is a Phase 1, a double-blind, randomized, placebo-controlled study in healthy and HCV Genotype 1-infected adults to evaluate the safety, tolerability, antiviral activity, and pharmacokinetic profiles of single and multiple doses [2 days] of ABT-333.

The clinical single doses are: 10, 25, 50, 100, 200, 400 or 600 mg

Introduction and History: ABT -333, a non-nucleoside inhibitor of NS5B polymerase, is being developed for the treatment of HCV infection. The NS5B gene product of HCV possesses RNA-dependent RNA polymerase activity, an activity not present in mammalian cells. The RNA polymerase plays an essential role in viral replication by directing the synthesis of both the replicative intermediate minus-strand RNA as well as the progeny plus-strand RNA and is therefore a potential target for therapeutic inhibition. ABT-333 has not previously been administered to humans.

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

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Mechanism of action: ABT-333 is a nonnucleoside inhibitor of the hepatitis C virus (HCV) RNA-dependent RNA polymerase encoded by the nonstructural protein 5B (NS5B) gene, with inhibitory concentrations in the nanomolar range against genotype 1a and 1b NS5B enzymes in biochemical assays and subgenomic replicon systems.

2.6.2 PHARMACOLOGY

2.6.2.4 Safety pharmacology

Brief summary

A-998821 was tested in a battery of safety pharmacology assays. A-998821 produced no clinically significant neurobehavioral, respiratory or gastrointestinal effects. In *in vitro* electrophysiology assays, A-998821 produced an IC_{50} of 0.3 µg/mL in a hERG assay but did not affect canine Purkinje fiber repolarization up to and including 14.93 µg/mL. In addition, A-998821 had no effect on QTc in the conscious dog at plasma concentrations as high as 6190 ng/mL. In the anesthetized dog, A-998821 produced modest self-limiting increases in mean arterial pressure (7 mmHg), which were not produced at slower intravenous infusion rates, suggesting that the pressor effect was potentially mediated by the rate of increase in A-998821 concentration and was mitigated by tachyphylaxis/desensitization. Finally, A-998821 produced a slight decrease in mean arterial pressure in conscious dogs but only at plasma concentrations (6190 ng/mL) well above those predicted to be efficacious in humans.

Safety pharmacology studies

1. Effect of A-998821 on *In Vitro* hERG Current (R&D/08/154)

A-998821 was evaluated at measured mean bath concentrations of 0.4, 1.4, and 4.3 µg/mL (n = 4). These concentrations reduced hERG tail current by 19, 48, and 77%, respectively (after correction for vehicle/rundown). The reduction of hERG tail current was statistically different from vehicle. The IC_{50} value for hERG block was 1.5 µg/mL, a concentration above those predicted to be efficacious in humans (estimated efficacious human C_{max} of 126 to 505 ng/mL).

In addition, a hERG study was performed in accordance with GLP regulations and ICH guidelines. A-998821 was tested at concentrations of 0.0336, 0.0944 and 0.571 µg/mL (n = 5 to 6). A-998821 inhibited hERG potassium current by 4.7 ± 0.5%, 21.0 ± 1.2% and 67.1 ± 1.7% *versus* -0.2 ± 0.1% (n = 5, mean ± SEM) in control. The IC_{50} for the inhibitory effect of A-998821 on hERG potassium current was 0.3 µg/mL; a concentration similar to those predicted to be efficacious in humans.

2. Effect of A-998821 on Canine Purkinje Fiber Repolarization *In Vitro* (R&D/08/155)

A-998821 was examined at measured concentrations of 0.18 ± 0.02, 1.43 ± 0.07, and 14.93 ± 0.57 µg/mL (mean ± SEM, n = 4) in comparison to vehicle controls,

n = 4/group). During slow stimulation (2 sec), chosen to emphasize repolarization delays, no effect of A-998821 on the APD was noted up to and including the highest concentration of 14.93 µg/mL; a plasma concentration well above those predicted to be efficacious in humans (estimated human C_{max} of 126 to 505 ng/mL).

3. Effects of A-998821 on Cardiovascular Parameters in the Anesthetized Dog (R&D/08/07)

A-998821 was tested in three separate cardiovascular studies using the anesthetized dog model. In each study, A-998821 was administered to anesthetized dogs as three escalating 30-minute intravenous infusions. In all three studies, the compound produced no physiologically relevant effects on HR (contractility=dP/dt_{max}, dP/dt_{50mmHg}), central venous pressure, hematocrit or the PR interval. In the mid- and high-dose studies (infusion rates of 0.003 to 0.032 mg/kg/min), A-998821 produced modest self-limiting increases in MAP (7 mmHg *vs*. vehicle) and maximum shortening of the QT interval (14 msec *vs*. vehicle) at plasma concentrations of 0.23 and 1.84 µg/mL, respectively. When administered in a low-dose study over a slower range of escalating infusion rates (0.001 to 0.010 mg/kg/min), A-998821 did not produce an effect on MAP at concentrations as high as 0.7 µg/mL suggesting that the pressor effect observed at high infusion rates was mediated by rate of rise. Of note, the QT shortening observed in the anesthetized dog study at supratherapeutic plasma concentrations was in contrast to the hERG study findings suggesting a potential for QT prolongation related to inhibition of hERG.

4. Effects of A-998821 on Cardiovascular Parameters in Conscious Dogs (R&D/07/1193)

The study was conducted using male beagle dogs orally administered 1, 3 or 10 mg/kg of A-998821. The animals were instrumented with radiotelemetry transmitters for measurement of blood pressure, heart rate, the electrocardiogram (ECG), and body temperature. Hemodynamics and the ECG (QRS duration and the RR, PR, and QT intervals) were monitored continuously for 22 hours. Blood samples for determination of plasma concentrations of A-998821 were collected from the dogs just prior to dosing and at eight hours after dosing. Oral administration of A-998821 at 1, 3, and 10 mg/kg produced plasma concentrations of 598 ± 15.8, 1860 ± 44.6 and 6190 ± 272 ng/mL, respectively. Oral administration of A-998821 at doses of 1 and 3 mg/kg did not produce any effects on blood pressure, heart rate or any of the ECG parameters. Compared with vehicle, 10 mg/kg (exposure = 6190 ± 272 ng/mL) produced a slight decrease in blood pressure (13 mmHg at two hrs after dosing). In summary, oral administration of A-998821 produced no cardiovascular effects in conscious dogs at doses up to and including 3 mg/kg, a dose associated with plasma concentrations of 1860 ± 44.6 ng/mL.

5. CNS Safety and Neurobehavioral Evaluation of A-998821 (R&D/07/1328)

The general behavioral effects of A-998821 were examined using the primary observation (Irwin) test in rats. No effects were observed with the oral dose of 1 mg/kg,

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but a mild, transient, dose-independent excitatory effect (increased sniffing behavior) was observed in at least half of the animals dosed with 3 to 100 mg/kg. No other consistent effects occurred in this assay.

In other CNS/neurobehavioral safety pharmacology assays in rats, administration of A-998821 had no significant or consistent effects on spontaneous locomotor activity and on acute thermal nociception (hot plate test) up to the highest dose tested, 100 mg/kg, p.o.

The neurobehavioral responses to A-998821 were additionally evaluated in the rat modified Irwin assay (FOB) and were conducted in accordance with Good Laboratory Practices (GLP) regulations and the International Conference on Harmonization (ICH) guidelines. Female Sprague-Dawley rats were orally administered A-998821 at doses of 3, 10, and 30 mg/kg (n = 8/group). One additional group of animals received the vehicle (n = 8). Blood samples were collected from a satellite group of three animals/group at three and six hours after dosing for the determination of A-998821 plasma concentrations. Oral doses of 3, 10, and 30 mg/kg A-998821 produced maximal plasma concentrations of 201 ± 54.0, 1140 ± 106, and 5030 ± 337 ng/mL (mean ± SEM), respectively. No effects of A-998821 were observed through the highest dose of 30 mg/kg, a dose associated with maximal plasma concentrations of 5030 ± 337 ng/mL.

Taken together, these results suggest that A-998821 produces no CNS/neurobehavioral effects in rats at the dose of 1 mg/kg, p.o. At 3 to 30 mg/kg, no effects were observed in female Sprague-Dawley rats, but a mild, transient, dose-independent excitatory effect (increased sniffing behavior) was seen in male Wistar rats over the same dose range. At 100 mg/kg, no adverse effects were observed; however, a mild, transient increase was found in sniffing behavior. Since the excitatory effect was non-dose dependent and was not observed in the GLP CNS study at supratherapeutic plasma concentrations, the effect is not likely to occur in A-998821 dose-escalating studies in humans.

6. Effects of A − 998821 on Respiratory Function in Conscious Rats (R&D/07/1192)

Male, Sprague-Dawley rats were orally administered A-998821 at doses of 3, 10, and 30 mg/kg (n = 8/group). One additional group of animals served as the control and received the vehicle control (n = 8). Blood samples were collected from a satellite group of three animals/group at three hours after dosing for the determination of A-998821 plasma concentrations. Oral doses of 3, 10, and 30 mg/kg A-998821 produced plasma concentrations of 271 ± 40.4, 977 ± 237, and 4660 ± 517 ng/mL (mean ± SEM), respectively. Oral administration of A-998821 did not produce any physiologically relevant effects on respiratory rate, tidal volume or minute volume up to and including 30 mg/kg, a dose associated with plasma levels of 4660 ± 517 ng/mL.

7. Effects of A − 998821 in Gastrointestinal Tolerability Models (R&D/07/1328)

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Ferret Emesis Study: Overnight-fasted, male ferrets were used to assess the emetic liability of A-998821. A-998821 was administered by oral gavage at doses of 1.5, 5, and 15 mg/kg (n = 5-6/dose). After dosing, the number of emetic episodes and the presence of behaviors believed to correlate with nausea in ferrets were recorded for each animal over a period of 120 minutes. A blood sample for determination of A-998821 plasma concentrations was obtained from each ferret 120 minutes after oral dosing. Oral administration of A-998821 at doses of 1.5, 5, and 15 mg/kg produced maximum mean plasma concentrations of 550 ± 30, 1140 ± 130 and 880 ± 250 ng/mL (mean ± SEM), respectively, measured in samples obtained 120 minutes after dosing. No emesis or nausea were observed in ferrets up to and including the highest oral dose of 15 mg/kg (exposure of 1140 ± 130 ng/mL).

Gastrointestinal Transit: Overnight-fasted, male Sprague Dawley rats were used to determine the effects of A-998821 on gastrointestinal transit. A-998821 was administered orally at doses of 3, 10, and 30 mg/kg (n = 8/dose). Gastrointestinal transit rate was determined by measuring the position of the leading edge of the charcoal meal relative to the total length of the small intestinal segment. Oral administration of A-998821 at doses of 3, 10, and 30 mg/kg produced plasma concentrations of 450 ± 40, 1310 ± 210, and 3640 ± 460 ng/mL (mean ± SEM), respectively, 2.75 hours after dosing. A-998821 had no significant effects on gastrointestinal transit up to and including the highest oral dose of 30 mg/kg (plasma concentration of 3640 ± 460 ng/mL).

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The ABT-333 pharmacokinetic profile in mouse, rat, monkey and dog was characterized by a wide range of plasma clearance values (CLp =0.04 L/hr•kg (dog) to 1.2 L/hr•kg [monkey]), with high volumes of distribution in all species (Vss > 1.1 L/kg). The ABT-333 plasma elimination half-life ($t_{1/2}$) was short in monkey (2 hr), but averaged 3.6 hours in rat and 19.5 hours in dog. Bioavailability from an oral dose was low in both monkey (4.5%) and rat (21.3%), but high in dog (95.9%). Bioavailability from a suspension of the sodium salt was equivalent to that obtained from a solution formulation in dog. Bioavailability following an oral dose was lower when dogs were fed prior to dosing when compared to animals provided food 4 to 12 hours after drug administration. ABT-333 is extensively metabolized in vivo in the rat and the drug-related material is primarily eliminated in the bile. ABT-333 is cleared primarily by cytochrome P450 (CYP)-mediated oxidative metabolism to A-1041392 (M1, *t*- butyl hydroxylation), with subsequent conjugation (M3, ether glucuronide or M2, sulfate) or further oxidation to its acid (A-1039710, M5) with subsequent glucuronidation.

ABT-333 is not a potent inhibitor of CYP1A2, 3A4/5 or 2D6 (IC_{50} >40 µM) and is a weak-to-moderate competitive inhibitor of CYP2C8, 2C9 and 2C19 (IC50 ~17, ~9 and ~18 µM, respectively). CYP2C8, 3A4 and 2D6 contribute to its metabolism in human liver microsomes (~60, 30 and 10% of the control activity, respectively). ABT-333 is not

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an inducer of CYP3A4/5 mRNA. There was no evidence of active efflux. In combination, these data suggest that ABT-333 has low potential to elicit clinically significant drug-drug interactions.

Pharmacokinetics and ADME studies

Absorption

Rat (R&D/07/1144, 2008)

Dose absorption following a single oral administration at 5 and 10 mg/kg of [^{3}H]ABT − 333 duct-cannulated (BDC) male SD rats was relatively low (~20 to 25%), with good overall recoveries of radioactivity observed 72 hours postdose (92 and 89.1% of the dose, respectively). The low absorption observed in the rat is attributed to poor dissolution due to the low solubility of ABT-333.

2. Single oral dose pharmacokinetics in rat, dog and monkeys (U04-3273; U04-3276; U04-3275)

Distribution

Plasma Protein Binding (R&D/07/1142, 2008)

Preliminary data indicate that ABT-333 (5 µM) is highly bound (> 99%) to rat, dog, monkey and human plasma proteins. When incubated in whole blood, [^{3}H]ABT-333 distributed preferentially into the plasma compartment, with a blood-to-plasma concentration ratio ranging between 0.6 and 0.7 in rat, dog, monkey, and human independent of the 0.3 to 30 µM concentration evaluated.

Tissue Distribution Studies (R&D/07/1144, 2008)

In intact male SD rats (5 mg ABT-333/kg, PO), the dosed radioactivity distributed well to Liver (44.1), adrenal glands (8.4), kidney (4.9), heart (3), lung (2.6), and lymph nodes (2.3), with tissue-to-plasma ratios at 3 hour postdose, had limited distribution to brain, eyes, and blood (T/P ~0.08, 0.3, 0.66) and there was no preferential distribution for all other tissues evaluated. The T/P ratios remained relatively constant or decreased through the duration of the study, indicating that the half-life of radioactivity in all tissues was similar to plasma.

Metabolism

In Vitro Metabolism (R&D/07/1141, 2008)

[^{3}H]ABT-333 had moderate stability in incubations with rat, monkey and human liver microsomes, with microsomal intrinsic clearances (~22.0, 12.4, and 22.8 µL/min/mg protein) that predicted plasma clearances of ~1.1, 0.44, and 0.50 L/h•kg, respectively. Turnover was negligible in the dog (< 0.01% parent metabolized in 30 minutes). M1, the

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t-butyl hydroxyl metabolite (A-1041392) was detected in incubations with all species as the only product. This metabolite represented up to 30% of the drug-related material in rat, monkey, and human and was negligible in the dog.

^{3}H-ABT-333 also had moderate stability in rat, monkey, and human hepatocytes. The hepatocyte clearance (6.03, 7.35, and 7.97 µL/min/million cells, respectively) predicted a CL_p of 0.89, 0.51, and 0.47 L/hr•kg, respectively, that were approximately within 2-fold of the observed plasma clearances. Turnover in the dog was negligible. M1 was the most abundant radiolabeled component, representing ~24, 6, and 31% of the drug related material in rat, monkey, and human, respectively. M3 (M1 glucuronide) represented up to ~8, 27, and 5% of the drug-related material. M2 and M4 (sulfate conjugate and *t*-butyl aldehyde, respectively) were minor products of metabolism. In vitro, M2 and M4 were only detected in human hepatocytes; however, both metabolites were detected in the rat in vivo.

It is predicted that in the human, ABT-333 will undergo a moderate rate of metabolism primarily to M1, with minor contributions from other metabolites.

In Vivo Metabolism

In BDC rats (5 mg/kg, IV), [^{3}H]ABT-333 underwent extensive oxidative metabolism (< 2% of the dose was recovered as intact parent) in the rat. The most significant product of metabolism was the M3 (proposed as the glucuronide conjugate of M1) representing ~50% of the dose, followed by M1 (A-1041392, *t*-butyl hydroxy) and M5 (A-1039710, *t*-butyl acid), representing 8 and 5% of the total dose, respectively. Other products of metabolism included a M2, a sulfate conjugate, M6, a glucuronide conjugate of the *t*-butyl acid M5 and M4, a proposed *t*-butyl aldehyde. Similar metabolite profiles were observed following a single 5 or 10 mg/kg dose.

Parent drug was the most significant radiolabeled component in plasma after a 5-mg/kg dose (~80 to 90% of the total plasma radioactivity), followed by M1 (A-1041392, *t*-butyl hydroxyl; ~10 to 20% of the total plasma radioactivity). M3 was detected in trace amounts (< 3% of the total plasma radioactivity).

Inhibition and CYP Involvement in Metabolism (R&D/07/1140, 2008)

ABT-333 is not a potent inhibitor of CYP1A2, 3A4/5 or 2D6 (IC_{50} > 40 µM) and is a weak- competitive inhibitor of CYPs2C8, 2C9 and 2C19 (IC_{50} ~17, ~9, and ~18 µM, respectively). An [I]/Ki ratio ≥ 0.1 indicates a remote potential for the drug under consideration to elicit a clinically significant drug-drug interaction. Assuming competitive inhibition, the Ki values are estimated to be 50% of the experimental IC_{50}. For ABT-333, data suggest a remote potential to cause clinically significant drug-drug interactions with clinical concentrations ≤ 0.43 µM (CYP2C9) or 0.8 µM (CYPs2C19 and 2C8). Similarly, there will be a remote potential for interactions with CYPs 1A2, 2D6 and 3A4/5 with clinical concentrations ≤ 2 µM.

Following incubations of [^{3}H]ABT-333 (0.25 µM, 60 minutes) in human recombinant

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CYPs and flavin-containing monooxygenases (FMOs), metabolism was only observed with CYPs 2C8, 2B6, 3A4, and 2D6.

[^{3}H]ABT-333 incubations in human liver microsomes in the presence of known inhibitors of specific CYP isoforms indicated that CYP2C8 had the most significant contribution to metabolism followed by CYP3A4 and CYP2D6 (~60, 30, and 10% of the control activity was inhibited by quercetin, ketoconazole, and quinidine, respectively). The contributions of CYP2B6 were negligible (< 1% inhibition was observed with 2-phenyl-2-(1-piperidinyl)propane). The potential for a comedication to significantly inhibit the metabolism of ABT-333 is low, given that multiple isoforms are involved in the metabolism of ABT-333.

Induction

Preliminary data obtained from studies conducted in human hepatocytes indicate that ABT-333 is not an inducer of CYP3A4/5 mRNA (< 7% of rifampin, no dose response, 1 to 10 µM).

Excretion/Elimination (R&D/07/1142, 2008)

Following a single IV administration of [^{3}H]ABT-333 to BDC male SD rats at 5 mg/kg, good recoveries of radioactivity were achieved at 72 hours postdose (89% of the dose). The dosed radioactivity was eliminated primarily into the bile (71.7%), with minimal elimination via the urine (2.8%). Dose absorption following a single oral administration at 5 and 10 mg/kg of [^{3}H]ABT-333 to BDC male SD rats was relatively low (~20 to 25%), with good overall recoveries of radioactivity observed 72 hours postdose (92 and 89.1% of the dose, respectively).

2.6.6.1 Overall toxicology summary

General toxicology:

1. Four-week Oral Toxicity Study in Sprague-Dawley Rats: Groups of male and female animals (10/sex/group main study; 5/sex/group TK) received daily oral gavage (2 ml/kg) doses of ABT-333 at dose levels of 0 [vehicle control consisting of [vitamin E TPGS:VP-dimer (20%: 80%, w/w)], 0 (negative control consisting of normal saline), 10 (low), 30 (mid) or 60 mg/kg/day (high) for four weeks followed by a 4-week recovery period. Noteworthy clinical signs observed during the dosing period included moderate urinestained hair around the anogenital area in one female and two male rats dosed at 60 mg acid/kg/day, as well as slight red and severe urine stained hair around the anogenital area in one female rat treated with 10 mg acid/kg/day. Based on results of the study, a dose level of 60 mg/kg/day may be considered the NOAEL. At the NOAEL, based on the body surface area factor, an equivalent dose in humans would be 9.7 mg/kg/day or 585 mg/day for a 60 kg person.

2. Four-week Oral Toxicity Study in Beagle Dogs with a Four-week Recovery Period: Groups of male and female beagle dogs (5/sex/group) received daily ABT-333

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at dose levels of 0 [control: capsules filled with gelatin and PEG 400:Tween 20, (80:20)], 1 (low), 3 (mid) or 10 mg/kg/day (high) for four weeks followed by a 4-week recovery period. Two dogs/sex/group were designated recovery animals. Clinical signs included occasional emesis and abnormal feces (with mucus or white particles) in control dogs as well as dogs dosed with A-998821 during the treatment period. Clinical chemistry revealed slight elevations (less than 2-fold) in alkaline phosphatase serum levels in mid and high dose males and females in Weeks 2 and 5 of treatment. Based on results of the study, the NOAEL of ABT-333 when administered to male and female dogs for at least 4 weeks was 10 mg/kg/day. At the NOAEL, based on the body surface area factor, an equivalent dose in humans would be 5.4 mg/kg/day or 324 mg/day for a 60 kg person.

Genetic toxicology:

ABT-333 was negative in 2 in vitro assays evaluating the potential to induce mutation or chromosomal aberrations. Both assays were conducted in the presence and absence of metabolic activation (rat S9). In the in vitro *Salmonella-Escherichia*/mammalian microsome reverse mutation (Ames) assay, ABT-333 was not mutagenic up to the limit dose of 5000 µg/plate[5] using both the plate incorporation and preincubation versions of the bacterial mutation test. In the in vitro chromosome aberration assay, ABT-333 was not clastogenic when evaluated in cultured human peripheral blood lymphocytes up to concentrations of 40 µg/mL without metabolic activation or 80 µg/mL with metabolic activation.

Reproductive toxicology: not reported.

2.6.6.2 Single-dose toxicity

Not reported.

2.6.6.3 Repeat-dose toxicity

Study title: 1. ABT-333: Draft Report Four-Week Oral Toxicity Study of A-998821 in Sprague-Dawley Rats (with a Four-Week Recovery Period)

Key study findings: Groups of male and female animals (10/sex/group main study; 5/sex/group TK) received daily oral gavage (2 ml/kg) doses of ABT-333 at dose levels of 0 [vehicle control consisting of (Vitamin E TPGS:VP-dimer (20%: 80%, w/w)], 0 (negative control consisting of normal saline, 5 animals/sex/group), 10 (low), 30 (mid) or 60 mg/kg/day (high) for four weeks. Reversibility of observed toxicities was assessed in a set of recovery rats (five/sex/group) maintained without dosing for an additional 30 or 31 days after 28 consecutive doses.

Noteworthy clinical signs observed during the dosing period included moderate urinestained hair around the anogenital area in one female and two male rats dosed at 60 mg acid/kg/day,

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as well as slight red and severe urine stained hair around the anogenital area in one female rat treated with 10 mg acid/kg/day.

Based on results of the study, a dose level of 60 mg/kg/day may be considered the NOAEL. At the NOAEL, based on the body surface area factor, an equivalent dose in humans would be 9.7 mg/kg/day or 585 mg/day for a 60 kg person.

Study no.: Study TA07-343

Conducting laboratory and location: Global Pharmaceutical Research and Development
Development Sciences, Toxicology and Pathology
Abbott Laboratories, Abbott Park, IL 60064, USA

Date of study completion: December 28, 2007

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: 57651PP00; **purity 99.8%**

Methods

Doses: Groups of male and female animals (10/sex/group main study; 5/sex/group TK) received daily oral gavage (2 ml/kg) doses of ABT-333 at dose levels of 0 [vehicle control consisting of (Vitamin E TPGS:VP-dimer (20%: 80%, w/w)], 0 (negative control consisting of normal saline), 10 (low), 30 (mid) or 60 mg/kg/day (high) for four weeks followed by a 4-week recovery period.

Species/strain: male and female rats; Sprague-Dawley [Crl: CD® (SD)]

Number/sex/group or time point (main study): 10 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg/day

Satellite groups used for toxicokinetics: 5 animals/sex/dose, plasma concentrations of ABT-333.

Weights: The rats weighed 196.0-337.1 grams at the start of the dosing period.

Sampling times: blood samples for clinical pathology immediately prior to necropsy.

Gross and Histopathology: adequate.

Results

Mortality: there were no unscheduled mortalities during this study.

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Clinical signs: Noteworthy clinical signs observed during the dosing period included moderate urinestained hair around the anogenital area in one female and two male rats dosed at 60 mg acid/kg/day, as well as slight red and severe urine stained hair around the anogenital area in one female rat treated with 10 mg acid/kg/day. Also apparent was slight urine-stained hair on the abdomen in one male rat at 60 mg acid/kg/day and one female rat at 10 mg acid/kg/day. These clinical signs were considered possibly treatment related, but not adverse or toxicologically relevant due to their transient nature and low overall incidence. Slight urine-stained hair near the anogenital area was also noted in both male and female rats in the vehicle control and A-998821-treated groups. The incidence of this finding was slightly higher in male and female rats (all dosage groups) treated with A-998821 compared to the vehicle control group. Urine staining was not apparent in rats that were administered 0.9% sodium chloride for injection (negative control) only. Slight urine staining near the anogenital area was considered possibly test-item and/or vehicle (Vitamin E TPGS:VP-dimer (20%: 80%, w/w) related, but not adverse or toxicologically relevant due to the sporadic nature, low overall incidence, and generally mild severity. No test item-related clinical signs were apparent during the recovery period.

Body weights: There were no statistically significant or toxicologically relevant differences in body weight data throughout the dosing and recovery periods.

Food consumption: there were no remarkable changes.

Ophthalmology: there were no drug related ocular changes.

Hematology & Coagulation: no changes were seen.
Serum Chemistry: no changes were seen.

The following findings were attributed to administration of the vehicle, Vitamin E TPGS: VP-dimer (20%: 80%, w/w):

Urinalysis: An increased incidence of proteinuria detected by routine urinalysis in both males and females at the end of the dosing period .

Organ weights: Increases in both absolute and relative liver and kidney weights for males at the end of the dosing period.

Histopathology: Adequate Battery: yes; Peer review: yes

Gross pathology: there were no macroscopic findings interpreted to be related to the administration of drug.

Histopathology: microscopic observations in liver (centrilobular hypertrophy in males and females and periportal hepatocellular vacuolation only in females), kidneys (hyaline droplets in tubular epithelial cells in males and tubular basophilia in males), and pituitary gland in males (vacuolation of the pars distalis).

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Ultrastructural Pathology: Liver and kidney cortex specimens were examined from male and female rats administered vehicle only (Vitamin E TPGS: VP-dimer (20%:80%, w/w), 60 mg acid/kg/day of A-998821 in vehicle or negative control (0.9% sodium chloride). In liver from male and female rats administered A-998821, and from male and female rats administered vehicle only there was an increase in size of centrilobular hepatocytes. The increase in size was concurrent with an increase in the amount of smooth endoplasmic reticulum and rough endoplasmic reticulum. In periportal hepatocytes from one of two female rats administered A-998821 and one of two female rats administered only the vehicle there were large amounts of intracellular lipid. In kidney cortex of one male rat administered A-998821 and one male rat administered only the vehicle, proximal tubule epithelial cells contained large, electron-dense, polyangular inclusions characteristic of those seen in lysosomal accumulation of $\alpha_{2\mu}$-globulin in male rats. Liver and kidney in the lower dosage main study groups and recovery groups were not examined. No changes in kidney cortex or liver were apparent in the negative control group, and all of the above-mentioned changes in the liver and kidney were attributed to the test-item vehicle (Vitamin E TPGS: VP-dimer (20%: 80%, w/w)).

Toxicokinetics: A summary of ABT-333 plasma concentrations is provided in Table 9. Male and female rats were administered daily doses of A-998821 at either 10, 30 or 60 mg/kg/day for 28 days and serial plasma samples were collected on Day 0 and Day 27. Though mean female toxicokinetic values appear to be greater than those for male rats, the range of values for individual male rats and individual female rats overlap, indicating no real differences in male and female Cmax and AUC values. The time to maximum plasma concentration (Tmax) occurred generally between three and six hours. Therefore, for many individual rats there was not enough time-points post Cmax to calculate half-life. Therefore half-lives were not calculated and AUC values on Day 0 were not extrapolated.

Table 9

Mean plasma concentrations in male and female Sprague Dawley rats during the 4-week toxicity study

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Treatment Group	Dose (mg/kg/day)	Sex	Day 0 C_{max} (µg/mL)	Day 0 T_{max} (hr)	Day 0 AUC* (µg•hr/mL)	Day 27 C_{max} (µg/mL)	Day 27 T_{max} (hr)	Day 27 AUC* (µg•hr/mL)
T_1	10	male	0.546	4.2	5.72	0.390	3.6	3.39
		female	0.766	4.2	8.91	0.704	3.1	5.45
		overall	0.656	4.2	7.31	0.547	3.4	4.42
T_2	30	male	1.94	4.8	19.7	1.22	2.5	9.51
		female	3.87	5.4	47.1	2.32	4.4	25.1
		overall	2.91	5.1	33.4	1.77	3.5	17.3
T_3	60	male	3.82	3.6	34.6	1.91	2.6	13.4
		female	4.70	4.2	61.1	3.24	2.2	30.9
		overall	4.26	3.9	47.8	2.57	2.4	22.1

* AUC calculated as AUC_{0-24} on both Day 0 and Day 27

Conclusion: The NOAEL noted for this study was 60 mg acid/kg/day of A-998821 (corresponding to a C_{max} and AUC of 2.6 µg/mL and 22.1 µg•hr/mL, respectively) when formulated in 20% Vitamin E TPGS and 80% VP-dimer and administered by oral gavage daily for 28 to 30 consecutive days to Sprague-Dawley rats.

Study title: **2. ABT-333: Four Week Oral (Capsules) Toxicity Study of A−998821 in Beagle Dogs Including a Four Week Recovery Period**

Key study findings: Groups of male and female beagle dogs (5/sex/group) received daily ABT-333 at dose levels of 0 [control: capsules filled with gelatin and PEG 400:Tween 20, (80:20)], 1 (low), 3 (mid) or 10 mg/kg/day (high) for four weeks followed by a 4-week recovery period. Two dogs/sex/group were designated recovery animals. Clinical signs included occasional emesis and abnormal feces (with mucus or white particles) in control dogs as well as dogs dosed with A-998821 during the treatment period. Clinical chemistry revealed slight elevations (less than 2-fold) in alkaline phosphatase serum levels in mid and high dose males and females in Weeks 2 and 5 of treatment.

Based on results of the study, the NOAEL of ABT-333 when administered to male and female dogs for at least 4 weeks was 10 mg/kg/day. At the NOAEL, based on the body surface area factor, an equivalent dose in humans would be 5.4 mg/kg/day or 324 mg/day for a 60 kg person.

Study no.: TB07 − 342

Volume # and page #: electronic

Conducting laboratory and location: Abbott GmbH & Co. KG
Research and Development
67061 Ludwigshafen, Germany

Reference ID: 3628623

Date of study completion: January 9, 2008

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: 57651PP00; **purity 99.8%**

Methods

Doses: Groups of male and female beagle dogs (5/sex/group) received daily ABT-333 at dose levels of 0 [control: capsules filled with gelatin and PEG 400:Tween 20, (80:20)], 1 (low), 3 (mid) or 10 mg/kg/day (high) for four weeks followed by a 4-week recovery period. Two dogs/sex/group were designated recovery animals.

Species/strain: Beagle dogs
Number/sex/group or time point (main study): 5 animals/sex/group

Route, formulation, volume, and infusion rate: oral, capsules

Age &Weight: The dogs were 6.5 to 7 months old at arrival and approximately 7.5 to 8 months old at dosing. The weight was 8.3-11.0 kg (males) and 5.4-7.6 (females) at the initiation of dosing (weights Day -1).

Sampling times: Blood samples for toxicokinetic investigations were collected.

Gross and Histopathology: Adequate Battery: yes

Results

Clinical signs: All animals survived until scheduled termination. Emesis, abnormal feces (with mucus or white particles) and diarrhea were occasionally observed in control dogs as well as dogs dosed with A-998821 during the treatment period. Since these are common observations in dogs and incidence was not dose dependant, these findings were not regarded as test item-related. One female dog at 3 mg/kg showed bloody diarrhea and one female at 1 mg/kg bw had diarrhea with blood traces, which could be attributed to *giardia canis* infection.

Body weights and food consumption: no change

Ophthalmology: there were no drug related changes.

Cardiology: there were no EKG findings and the QT intervals were normal.

Urinalysis: no change.

Reference ID: 3628623

Clinical chemistry: Serum evaluation revealed test item-induced increases of alkaline phosphatase in males and females at 3 and 10 mg/kg in Weeks 2 and 5. However, these elevations which were always slight in severity (less than 2-fold) returned to predose values at the end of the four-week recovery period.

Hematology, coagulation : Mean platelet volume was slightly and statistically significantly increased in males at 10 mg/kg in Week 2 but was within normal limits. Therefore, this change lacking deviations in platelet counts was not considered toxicologically meaningful.

Organ weights: no change.

Gross pathology: no findings

Histopathology: Adequate Battery: yes; peer review: yes

Histopathology: no findings

Toxicokinetics: data are shown in Table 11. In the low dose group, plasma concentrations and kinetic values in males tend to be higher than in females but in the higher groups, this difference disappeared, therefore combined males and female values were considered only. Due to the estimated apparent length of the half-lives, half-lives could not be accurately estimated and are therefore not reported. In all Groups, both C_{max} and AUC increased with multiple dosing; mean overall values on Day 28 were more than double those on Day 1. This increase may be at least partly attributable to the estimated length of the half-lives (greater than 12 hours). Both C_{max} and AUC were dose proportional on both Days 1 and 28.

Table 11

Mean Pharmacokinetic Parameters of ABT-333 in male and female beagle dogs

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Collection Interval	Sex	A-998821 (mg/kg/day) 1	3	10
		Mean Plasma AUC* (µg•hr/mL) ± SD/Mean Plasma AUC per Dose		
Day 1	Males	9.64 (±0.959)/9.64	25.4 (±4.56)/8.46	92.0 (±8.27)/9.20
	Females	7.43 (±1.08)/7.43	24.4 (±2.95)/8.14	71.2 (±38.7)/7.12
Day 28	Males	22.1 (±1.23)/22.1	58.8 (±7.34)/19.6	242 (±44.4)/24.2
	Females	16.4 (±2.50)/16.4	50.5 (±8.43)/16.8	227 (±29.3)/22.7
		Mean Plasma C_{max} (µg/mL) ± SD/Mean Plasma C_{max} per Dose		
Day 1	Males	0.55 (±0.0477)/0.553	1.47 (±0.252)/0.491	5.76 (±0.546)/0.576
	Females	0.46 (±0.0550)/0.462	1.48 (±0.177)/0.493	4.22 (±2.29)**/0.422
Day 28	Males	1.23 (±0.0336)/1.236	3.51 (±0.381)/1.17	14.1 (±2.45)/1.41
	Females	0.97 (±0.136)/0.974	3.01 (±0.581)/1.00	13.1 (±1.44)/1.31

* AUC calculated as AUC_{0-24} on both Day 1 and Day 28.

**Vomiting of test article was observed in one of five high dose females on Day 1 correlating with distinctly low plasma exposure in this animal

Conclusions: Oral administration of A-998821 (ABT-333) *via* capsules to male and female Beagle dogs at daily dosages of 1, 3 and 10 mg acid/kg bw (formulated in PEG 400:Tween 20, 80:20) over a period of 28-35 days induced no relevant test item-related changes regarding clinical signs, hematology, urinalysis, blood chemistry, organ weights, gross pathology and histopathology. Thus, the no-observed-adverse-effect-level (NOAEL) was 10 mg/kg bw, corresponding to C_{max} levels of 14.1 and 13.1 µg/mL and AUC levels of 242 and 227 µg•h/mL for male and female dogs, respectively.

2.6.6.4 Genetic toxicology

Study title: **1. ABT-333: Ames reverse mutation study in Salmonella and Escherichia coli**

Key findings: A-998821 was found to be soluble in dimethyl sulfoxide (DMSO). *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* were treated with the test article at a range of concentrations up to 5000 µg/plate (the standard limit dose for this assay) in the presence and absence of a supplemented liver fraction (S9 mix) using the plate incorporation and pre-incubation versions of the bacterial mutation test. No meaningful increases in colony counts were obtained with any strain following exposure to the test article in either the plate incorporation or pre-incubation assay in the presence or absence of S9 mix.
It is concluded that A-998821 did not show any evidence of genotoxic activity in this in vitro mutagenicity assay.

Study no.: **TX07-344**

Volume # and page #: electronic

Conducting laboratory and location: (b) (4) (b) (4)

Date of study completion: 12 March 2008

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: 57651PP00; purity-not known

Methods: A-998821 was found to be soluble in dimethyl sulfoxide (DMSO). *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* were treated with the test article at a range of concentrations up to 5000 µg/plate (the standard limit dose for this assay) in the presence and absence of a supplemented liver fraction (S9 mix) using the plate incorporation and pre-incubation versions of the bacterial mutation test. Bacteria were incubated with standard positive control agents, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix. Precipitation of the test article on plates was observed at higher dose levels; these were most marked in pre-incubation assay in absence of S9 mix.

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

Mixed function oxidase: crude rat liver extract (S-9) provided the mixed function oxidase metabolic activation system. The extract was obtained from male Sprague-Dawley rats which were stressed with a single intraperitoneal injection of Aroclor 1250 (500 mg/kg) 5 days prior to sacrifice.

Doses used in definitive study: 0 to 5000 µg/plate

Basis of dose selection: a non-GLP exploratory study.

Negative controls: DMSO

Positive controls: Sodium azide (NaAz), 9-Aminoacridine (9AC), 2-Nitrofluorene (2NF), 4-Nitroquinoline N-oxide (NQO) and Benzo[a]pyrene (BaP)

Incubation and sampling times: after solidification of the agar overlay, all plates were incubated aerobically at 37 degree C in darkness for 46-48 hr.

Results
No meaningful increases in colony counts were obtained with any strain following

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exposure to the test article in either the plate incorporation or pre-incubation assay in the presence or absence of S9 mix. All criteria for a valid study were met. It is concluded that A-998821 did not show any evidence of genotoxic activity in this in vitro mutagenicity assay.

Study validity: valid

Study outcome: ABT-333 was not mutagenic in the Ames assay.

Study title: 2. A-998821 Chromosome Aberration Test

Key findings: Cultures treated with A-998821 did not show any statistically significant increases in the incidence of cells with aberrant metaphases when tested up to the limits of toxicity and/or solubility. The positive control agents caused highly significant increases in the proportion of cells with chromosome damage, confirming the sensitivity of the system and the effectiveness of the S9 mix. All criteria for a valid assay were met.

Study no.: **TX07-345**

Volume # and page #: electronic

Conducting laboratory and location: (b) (4) (b) (4)

Date of study Completion: 12 March 2008

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: 57651PP00; purity-not known

Methods

A-998821 was found to be soluble in dimethyl sulfoxide (DMSO) up to approximately 360 mg/mL and was found to precipitate in medium at a final concentration of approximately 20 µg/mL. Human peripheral blood lymphocytes were stimulated into division in culture then treated with the test article from 0.160 µg/mL to 80.0 µg/mL. This range assured the test article would be evaluated up to a dose level showing visible precipitation in the culture medium at the end of treatment. Cultures were treated for 4 hours in the absence and presence of rat S9 mix and for 21 hours in the absence of rat S9 mix; appropriate concurrent vehicle and positive controls were included for each treatment regime.

Metaphases from cultures treated with the three highest dose levels of test article not producing excessive toxicity (together with appropriate vehicle and selected positive control cultures) were subjected to detailed examination for the presence of chromosomal aberrations using light microscopy.

Strains/species/cell line: Human peripheral blood lymphocytes

Doses used in definitive study: The concentrations examined were 10.0, 20.0 and 40.0 µg/mL (4-hour without S9); 20.0, 40.0 and 80.0 µg/mL (4-hour with S9); and 10.0, 20.0 and 40.0 µg/mL (21-hour without S9).

Basis of dose selection: dose range finding study.

Negative controls: DMSO

Positive controls: Mitomycin C (MMC) and Cyclophosphamide monohydrate (cyclophosphamide, CP)

Incubation and sampling times: Cultures tested in the absence of S9 mix were treated as indicated in the study design then returned to the incubator for either 4 or 21 hours as appropriate.

Results:

Cultures treated with A-998821 did not show any statistically significant increases in the incidence of cells with aberrant metaphases when tested up to the limits of toxicity and/or solubility. The positive control agents caused highly significant increases in the proportion of cells with chromosome damage, confirming the sensitivity of the system and the effectiveness of the S9 mix. All criteria for a valid assay were met.

It is concluded that A-998821 did not show any evidence of genotoxic activity in this *in vitro* test for induction of chromosome damage.

Study validity: valid

Study outcome: A-998821 did not show any evidence of genotoxic activity in this *in vitro* test for induction of chromosome damage.

2.6.6.6 Reproductive and developmental toxicology

None reported.

2.6.6.9 Discussion and Conclusions

General toxicology

The oral toxicity of ABT -333 was evaluated in rats and dogs studies in definitive GLP studies of 28-day duration. In the rat, ABT-333 was well tolerated when administered orally at plasma levels conservatively estimated to be 4 to 12 times greater than the predicted therapeutic AUC exposure in humans. In the 28-day toxicology study in beagle dogs, ABT-333 was also well tolerated when dosed orally at AUC exposures approximately 41- to 124-fold greater than the predicted therapeutic AUC exposure in humans. Changes associated with ABT-333 included slight elevations (less than 2-fold) in alkaline phosphatase serum levels in mid and high dose males and females in Weeks 2 and 5 of treatment. However, these effects were reversible and were not considered toxicologically meaningful. There were no relevant changes in clinical signs, hematology, urinalysis, blood chemistry, organ weights, gross pathology, and histopathology. Overall, no dose limiting toxicity was observed in either species.

While ABT-333 toxicity studies support a starting dose in human trials of 25 mg, the initial human dose to be studied is 10 mg as a single dose. This lower dose takes into consideration results from the GLP hERG assay, where ABT-333 inhibited hERG tail current with an IC_{50} of 300 ng/mL, which is 2.4 times the predicted Cmax achieved by a 25 mg dose. In order to provide a larger safety margin, an initial human dose of 10 mg was selected, which is predicted to produce a Cmax of approximately 41 ng/mL. This concentration is 7-fold below the IC_{50} in the hERG assay, which increases the safety margin and is also below the predicted efficacious drug exposure.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

		NOAEL mg/kg	HED (mg/kg)	Safety factor ‡ 10 mg (0.17 mg/kg)
1	*7-day rat tox*	10	1.62	9.7
2	*4-week rat tox*	60	9.7	58.4
3	*5-day dog tox*	60	32.4	195
4	*4-wk dog tox*	10	5.4	32.4
5	*Cardio dog*	3	1.62	9.7
Dose escalation		10 mg, 25 mg, 50 mg, 100 mg, 200 mg, 400 mg or 600 mg		
Final Conclusion: Reasonably safe to proceed				

Summary: ABT-333: This phase 1 study is reasonably safe to initiate at the starting dose of 20 mg.

External comments (to sponsor):

1. Please escalate dosing of ABT-333 in subsequent long term rat and dog studies in an effort to show frank toxicities at the high dose.

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2. Please initiate Segment I and II of ABT-333 reproductive toxicity studies in animals after completion of the single dose clinical studies.

3. Please conduct micronucleus assay in rodents to evaluate genotoxic potential of ABT-333.

4. Please provide a Gantt chart of proposed Preclinical studies to support the clinical development of ABT-333.

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

Reference ID: 3628623

Linked Applications	Sponsor Name	Drug Name / Subject
IND 101636	ABBOTT LABORATORIES	ABT-333

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

/s/

PRITAM S VERMA
04/09/2009

HANAN N GHANTOUS
04/09/2009

PHARMACOLOGY/TOXICOLOGY REVIEW

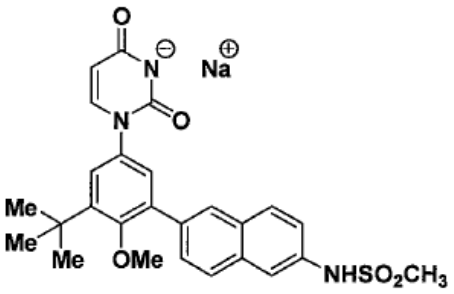
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 101,636.018
Review number: 000
Sequence number/date/type of submission: January 6, 2009
Information to sponsor: No
Sponsor and/or agent: Abbott Laboratories
Global Pharmaceutical Research and Development
Abbott Park, Illnois 60064 USA

Reviewer name: Pritam S. Verma, Ph.D.
Division name: DAVP
HFD #: 530
Review completion date: August 28, 2009

Drug:

Trade name: not available
Generic name: not available
Code name: ABT-333 (A-998821)
Chemical name: Methanesulfonamide, N- (6- (5 -(3 ,4-dihydro-2,4-dioxo-1 (2H)-pyrimidiny 1)- 3-(1,1-dimethy lethy 1)- 2- methoxypheny 1) - 2-naphthalenyl)-, monosodium salt
CAS registry number: not available
Molecular weight: 515.56 (salt); 493.57 (acid)
Molecular formula/molecular weight: $C_{26}H_{26}N_{30}O_5SNa$ (salt); (acid)
Structure:



Relevant INDs/NDAs/DMFs: None

Drug class: ABT-333 is a nonnucleoside inhibitor of the hepatitis C virus (HCV) RNA-dependent RNA polymerase

Introduction and History: ABT -333, a non-nucleoside inhibitor of NS5B polymerase, is being developed for the treatment of HCV infection. The NS5B gene product of HCV possesses RNA-dependent RNA polymerase activity, an activity not present in mammalian cells. The RNA polymerase plays an essential role in viral replication by directing the synthesis of both the replicative intermediate minus-strand RNA as well as the progeny plus-strand RNA and is therefore a potential target for therapeutic inhibition.

Toxicology:

Study title: 1. ABT-333: Four-Week Oral Toxicity Study of A-998821 in Combination with Ribavirin and Interferon in Cynomolgus Monkeys (with a Four-Week Recovery Period)

Key study findings: No toxicologically remarkable findings were noted among animals given A-998821 alone or in combination with RBV and IFN.

Study no.: 126-277

Conducting laboratory and location: (b) (4)

Date of study completion: December 8, 2008

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: 62749PP00; **purity 99.8%**

Methods

Doses: Groups of male and female animals were administered the test article, RBV, IFN (vehicle: 0.2% hydroxypropyl methylcellulose in water) for four weeks followed by a 4-week recovery period as shown in Table 1.

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Table 1

Group Assignments and Dose Administration					
Group Number	A-998821 Dose Level (mg acid/kg/day)	Ribavirin[a] Dose Level (mg/kg/day)	Interferon[b] Dose Level (µg/kg/dose)	Number of Animals	
				Male	Female
1	0	0	0	5[c]	5[c]
2	150	0	0	5[c]	5[c]
3	0	50	15	5[c]	5[c]
4	100	50	15	5[c]	5[c]
5	150	50	15	5[c]	5[c]

[a] RBV administered once daily via oral gavage approximately 3 hours after A-998821 administration.
[b] IFN administered twice weekly via subcutaneous injection approximately 3 hours after A-998821 administration.
[c] Two animals designated for recovery.

Species/strain: male and female cynomolgus monkeys

Number/sex/group or time point (main study): Table 1

Route, formulation, volume, and infusion rate: Table 1

Satellite groups used for toxicokinetics: Table 1

Age: 2-3 yrs

Sampling times: blood samples for clinical pathology immediately prior to necropsy.

Gross and Histopathology: adequate.

Results

Mortality: there were no unscheduled mortalities during this study.

Clinical signs: are shown in Table 2.

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Table 2

Remarkable Clinical Observations										
Dose [a]	0/0/0		150/0/0		0/50/15		100/50/15		150/50/15	
Clinical Observations	Total Number of Findings (Number of Monkeys Affected)									
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Feces Soft	4(1)	24(3)	24(4)	20(5)	10(4)	12(4)	22(5)	16(5)	27(4)	22(5)
Feces Watery	4(2)	1(1)	10(3)	10(3)	2(1)	1(1)	2(2)	3(2)	8(1)	8(2)
Skin Discolored	-	-	1(1)	-	-	15(1)	-	7(2)	2(1)	13(4)
Emesis after dosing	-	-	4(2)	3(2)	9(1)	18(4)	-	4(2)	6(3)	12(4)
Emesis	-	-	-	-	-	-	-	-	-	1(1)

[a] A-998821/RBV/IFN. A-998821 and RBV in mg/kg/day, IFN in µg/kg/dose.
- Not observed

Body weights: There were no statistically significant or toxicologically relevant differences in body weight data throughout the dosing and recovery periods.

Food consumption: there were no remarkable changes.

Electrocardiographic examinations: were within normal limits. No arrhythmias were found.

Hematology & Coagulation: on day 3, there were moderate decreased in erythrocyte mass (erythrocytes, hemoglobin and hematocrit) similar in magnitude in all groups and both sexes that were interpreted as procedure-related blood losses, In response to this blood loss, all groups had evidence of regeneration; however, all RBV/INF treated groups in both sexes had a relatively diminished/delayed regeneration response when compare to groups not receiving RBV/INF. This effect was interpreted as solely due to RBV/IFN treatment. None of these changes were present during recovery period.
No effects were seen among coagulation parameters.

Serum Chemistry: no changes were seen.

Urinalysis: no changes were seen.

Histopathology: Adequate Battery: yes; Peer review: yes

Gross pathology: treatment related macroscopic findings were limited to the injection site. One male at 100 mg A-998821/RBV/IFN had mild red discoloration on the injection site that correlated microscopically with hemorrhage associated with the trauma of injection procedures.

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Organ weights: no treatment related organ weight trends were present.

Statistically significant organ weight changes were limited to males at 100 and 150 mg A-998821/kg/day/RBV/IFN, and included: decreased brain/body weight ratios and increased mean absolute kidneys weights of males at these dose levels compared to males at 0 mg A-998821/kg/day and males at 0 mg A-998821/kg/day/RBV/IFN. These organ weight changes were primarily associated with the increased body weights of males at 100 and 150 mg A-998821/kg/day/RBV/IFN relative to males at 0 mg A-998821/kg/day and 0 mg A-998821kg/day/RBV/IFN, and were considered incidental to test article administration.

At recovery, there were not treatment related trends were present.

Histopathology: microscopic findings were present in the injection sites of animals treated with IFN in groups 3, 4 and 5.

The injection sites of animals treated with IFN in combination with 0, 100, or 150 mg A-998821/kg/day sodium frequently exhibited minimal to mild, treatment-related subacute inflammation. The inflammation was characterized by small, multifocal, predominantly perivascular accumulations of small lymphocytes and rare macrophages in the dermis and subcutis. All males at 100 mg A-998821/kg/day/RBV/IFN also had minimal hemorrhage that was attributed to trauma associated with injection procedures.

At recovery, no treatment related findings were identified.

Toxicokinetics: data are presented for A-998821, RBV and IFN in Tables 3, 4 and 5.

Toxicokinetics of A-998821 (Table 3): coadministration RBV and IFN did not remarkably alter the toxicokinetic parameters of A-998821. No remarkable gender differences for individual A-998821 plasma concentration were observed within a group. For the two groups coadministered RBV and IFN, both Cmax and AUC were dose proportional from 100 to 150 mg A-998821 on day 1 and day 28.

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Table 3
Mean (±SD) Toxicokinetic Parameters for A-998821

	A-998821.0		Day 1				Day 28			
Group	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{0-\infty}$ (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-24} (µg•hr/mL)
2	150	male	3.31	4.2	4.1	31.9	9.55	4.2	3.8	113
			±1.84	±1.6		±21.0	±2.82	±1.6		±46.5
		female	4.96	3.0	3.5	37.2	9.98	3.0	3.5	104
			±1.27	±0.0		±14.8	±1.51	±0.0		±23.6
		overall	4.14	3.6	3.8	34.6	9.77	3.6	3.7	108
			±1.72	±1.3		±17.3	±2.14	±1.3		±35.1
4	100[a]	male	2.32	3.0	4.3	12.6	3.91	3.0	3.1	30.7
			±1.78	±0.0		±9.07	±1.33	±0.0		±13.0
		female	1.41	3.0	5.2	13.9	3.33	3.6	3.1	24.8
			±1.68	±0.0		±18.1	±1.85	±1.3		±11.0
		overall	1.87	3.0	4.7	13.2	3.62	3.3	3.1	27.8
			±1.70	±0.0		±12.8	±1.55	±0.9		±11.7
5	150[a]	male	2.17	2.6	4.0	13.3	6.50	3.2	3.3	62.6
			±1.70	±0.9		±9.19	±2.83	±1.8		±39.8
		female	3.49	2.6	4.7	23.6	9.51	3.6	4.3	110
			±1.59	±0.9		±12.0	±2.82	±1.3		±47.3
		overall	2.83	2.6	4.3	18.5	8.01	3.4	3.7	86.3
			±1.70	±0.8		±11.5	±3.10	±1.5		±48.2

[a] Animals were coadministered RBV and IFN.

Toxicokinetics of Ribavirin (Table 4): coadministration of RBV with A-998821 did not alter RBV toxicokinetic parameters.

Reference ID: 3628623

Table 4
Mean (±SD) Toxicokinetic Parameters for Ribavirin

Group	RBV Dose (mg/kg/day)	Sex	Day 28 C_{max} (µg/mL)	Day 28 T_{max} (hr)	Day 28 AUC_{0-24} (µg•hr/mL)
3	50[a]	male	1.34 ±0.297	4.0 ±0.0	24.4 ±5.81
		female	1.06 ±0.0802	4.0 ±0.0	19.5 ±2.55
		overall	1.20 ±0.254	4.0 ±0.0	21.9 ±4.97
4	50[a,b]	male	1.35 ±0.271	4.0 ±0.0	25.1 ±5.64
		female	1.09 ±0.239	4.0 ±0.0	19.8 ±4.77
		overall	1.22 ±0.278	4.0 ±0.0	22.5 ±5.66
5	50[a,c]	male	1.24 ±0.199	4.0 ±0.0	23.4 ±3.92
		female	1.07 ±0.313	4.0 ±0.0	20.4 ±6.89
		overall	1.15 ±0.264	4.0 ±0.0	21.9 ±5.52

[a] Animals were coadministered IFN at 15 ug/kg/dose twice weekly.
[b] Animals were coadministered A-998821 at 100 mg/kg/day.
[c] Animals were coadministered A-998821 at 150 mg/kg/day.

Toxicokinetics of IFN (Table 5): coadministration of A-998821 with IFN did not alter measurable Day 1 Cmax values for RBV.

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Table 5

Mean (±SD) Toxicokinetic Parameters for IFN

Group	IFN Dose (ug/kg/dose)	Sex	Day 1 C_{max} (ng/mL)	Day 1 T_{max} (hr)
3	15[a]	male	40.1 ±10.6	16.0 ±9.2
		female	42.6 ±16.8	12.8 ±6.6
		overall	41.5 ±13.6	14.2 ±7.5
4	15[a,b]	male	27.8 ±4.42	25.6 ±14.3
		female	33.2 ±7.22	13.6 ±6.1
		overall	30.5 ±6.33	19.6 ±12.1
5	15[a,c]	male	46.6 ±5.86	9.0 ±2.0
		female	37.9 ±8.83	26.4 ±13.1
		overall	41.7 ±8.52	18.7 ±13.1

[a] Animals were coadministered RBV at 50 ug/kg/day.
[b] Animals were coadministered A-998821 at 100 mg/kg/day.
[c] Animals were coadministered A-998821 at 150 mg/kg/day.

Conclusion: The NOAEL for A-998821 noted for this study was 150 mg/kg/day (corresponding to a C_{max} and AUC of 9.77 µg/mL and 108 µg•hr/mL, respectively). Comparable A-998821 exposures ere observed when coadministered RBV and IFN. No toxicologically remarkable findings were noted among animals given A-998821 alone or in combination with RBV and IFN.

Study title: 1. ABT-333: Thirteen-Week Oral Toxicity Study of A-998821 in Combination with Ribavirin in rats (with a Four-Week Recovery Period)

Key study findings: No toxicologically remarkable findings were noted among animals given A-998821 alone or in combination with RBV.

Study no.: 126-275

Conducting laboratory and location: (b) (4)

Reference ID: 3628623

Date of study completion: December 19, 2008

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: 62749PP00; **purity 99.8%**

Methods

Doses: Groups of male and female rats were administered the test article in combination with RBV (vehicle: 0.2% hydroxypropyl methylcellulose in water) for thirteen weeks followed by a 4-week recovery period as shown in Table 1.

Table 1

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0 (Vehicle Control)	15[c, f]	15[c, f]
2	20 A-998821 + 0 RBV	15[c, f]	15[c, f]
3	60 A-998821 + 0 RBV	15[c, f]	15[c, f]
4	200 A-998821 + 0 RBV	15[c, f]	15[c, f]
5[b]	0 + 40 RBV	15[c, f]	15[c, f]
6[b]	20 A-998821 + 40 RBV	15[c, f]	15[c, f]
7[b]	200 A-998821 + 40 RBV	15[c, f]	15[c, f]
8[a]	0 (Vehicle Control)	6 + 2[d]	6 + 2[d]
9[a]	20 A-998821 + 0 RBV	6 + 2[d]	6 + 2[d]
10[a]	60 A-998821 + 0 RBV	6 + 2[d]	6 + 2[d]
11[a]	200 A-998821 + 0 RBV	6 + 2[d]	6 + 2[d]
12[a,b]	0 +40 RBV	12 + 3[e]	12 + 3[e]
13[a,b]	20 A-998821 + 40 RBV	12 + 3[e]	12 + 3[e]
14[a,b]	200 A-998821 + 40 RBV	12 + 3[e]	12 + 3[e]

[a] Designated for toxicokinetic analysis.
[b] RBV was administered approximately 3 hours after vehicle or A-998821 administration.
[c] The last four or five animals per sex were designated for evaluation during the 1 month recovery period.
[d] Two cohorts of three animals per sex were bled for A-998821 toxicokinetic samples. The remaining two animals per sex were retained as possible replacements per dose group.
[e] The first two cohorts of three animals per sex were bled for A-998821 toxicokinetic samples at alternating timepoints. The second two cohorts of three animals per sex were bled for RBV toxicokinetic samples at alternating timepoints. The remaining three animals per sex were retained as possible replacements per dose group.
[f] The first 10 animals were necropsied following completion of dose administration. Remaining animals were evaluated during the 1 month recovery period.

Species/strain: male and female rats/ CD[Crl:CD(SD)]

Number/sex/group or time point (main study): Table 1

Route, formulation, volume, and infusion rate: Table 1

Reference ID: 3628623

Satellite groups used for toxicokinetics: Table 1

Weights: 242-276 g and 170-208 g male and female, respectively.

Sampling times: blood samples for clinical pathology immediately prior to necropsy.

Gross and Histopathology: adequate.

Results

Mortality:

One male rat (Animal 4063) given 0 mg A-998821/kg/day + 40 mg RBV/kg/day was found dead on Day 34, one male rat (Animal 4105) given 200 mg A-998821/kg/day + 40 mg RBV/kg/day was found dead on Day 51, and one female rat (Animal 4292) given 0 mg A-998821/kg/day + 0 mg RBV/kg/day was found dead on Day 72.

The cause of death for Animal 4063 was interpreted to be accidental based on the presence of moderate red discoloration in multiple lung lobes consistent with a dosing error.

The cause of death for Animal 4105 was undetermined, but not considered test article-related given a lack of remarkable toxicologic findings for any animal at this dose level including those which survived past Day 72.

The cause of death for Animal 4292 was not considered test article-related given a lack of test article administration.

Clinical signs: there were no remarkable test article related signs observed at any dose level.

Body weights:

Mean body weights were increased for all groups during the course of the study. There were no A-998821-related effects on body weights noted at any dose level.

Mean body weights for animals given RBV were approximately 90% of vehicle control groups at Day 91. Mean body weight were comparable among animals given RBV with or without A-998821 at Day 91.

Food consumption:

There were no A-998821-related effects on food consumption noted at any dose level.

Mean food consumption values were consistently lower in animals receiving RBV relative to the animals in the absence of RBV, however, the decreases were negligible (<10%). Mean food consumption values were comparable for animals given RBV with or without A-998821.

Ophthalmoscopic examination: There were no A-998821 or RBV related effects at any dose level.

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Hematology: there were no effects.

Coagulation: there were no effects.

Serum Chemistry: no changes were seen.

Urinalysis: no changes were seen.

Toxicokinetics: data are presented for A-998821, RBV and IFN in Tables 3, 4 and 5.

Toxicokinetics of A-998821 (Table 2): The Cmax and AUC values were larger for females as compared to males at all dose levels on days 1 and 91. The Cmax and AUC increased less than dose proportionally for animals given A-998821 alone on days 1 and 91. Coadministration of RBV with A-998821 did not alter toxicokinetic parameters.

Table 2

Mean Toxicokinetic Parameters for A-998821

A-998821 Dose Level (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 $AUC_{0-\infty}$ (µg·hr/mL)	Day 91 C_{max} (µg/mL)	Day 91 T_{max} (hr)	Day 91 AUC_{0-24} (µg·hr/mL)
20	male	0.719	3.0	5.95	1.18	1.0	10.1
	female	1.57	3.0	12.4	3.04	3.0	26.8
	overall	1.14	3.0	9.08	1.97	3.0	18.5
20[a]	male	0.869	3.0	8.00	1.22	3.0	8.69
	female	2.07	3.0	20.3	2.45	3.0	26.3
	overall	1.47	3.0	14.1	1.84	3.0	17.5
60	male	2.29	3.0	13.7	1.86	3.0	13.0
	female	3.08	3.0	27.2	4.09	3.0	44.1
	overall	2.69	3.0	20.4	2.98	3.0	28.5
200	male	3.84	3.0	31.7	3.53	6.0	33.8
	female	4.95	3.0	54.0	6.80	3.0	93.5
	overall	4.40	3.0	42.9	5.10	6.0	63.6
200[a]	male	5.06	3.0	29.7	4.83	3.0	31.2
	female	7.50	3.0	88.3	9.22	3.0	102
	overall	6.28	3.0	58.9	7.03	3.0	66.6

[a] Animals were co-administered RBV at dose levels of 40 mg/kg/day.

Toxicokinetics of Ribavirin (Table 3): coadministration of RBV with A-998821 did not alter RBV toxicokinetic parameters.

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Table 3

Mean Toxicokinetic Parameters for RBV

RBV Dose Level (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 T_{max} (hr)	Day 1 $AUC_{0-\infty}$ (µg·hr/mL)	Day 91 C_{max} (µg/mL)	Day 91 T_{max} (hr)	Day 91 AUC_{0-24} (µg·hr/mL)
40	male	0.624	3.0	7.06	0.652	6.0	9.39
	female	0.387	6.0	5.96	0.826	6.0	10.3
	overall	0.495	3.0	6.04	0.739	6.0	9.84
40[a]	male	0.798	3.0	6.93	0.735	6.0	8.70
	female	0.434	6.0	6.48	0.959	6.0	10.4
	overall	0.582	3.0	6.04	0.847	6.0	9.55
40[b]	male	0.671	3.0	6.37	0.563	6.0	8.02
	female	0.293	6.0	5.88	0.699	3.0	9.25
	overall	0.447	3.0	5.43	0.615	3.0	8.64

[a] Animals were co-administered A-998821 at 20 mg/kg/day.
[b] Animals were co-administered A-998821 at 200 mg/kg/day.

Histopathology: Adequate Battery: yes; Peer review: yes

Gross pathology: there were no definitive microscopic findings in any of the terminal groups.

Organ weights: there were no definitive changes.

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Histopathology:

Test article-related microscopic findings in the terminal and/or recovery groups in this study include minimally increased adrenal cortical vacuolation and minimal alveolar histiocytosis. Both of these findings are not uncommon incidental lesions and thus the toxicological significance of these findings is unclear. Neither is considered to be an adverse finding, although there is persistence of the alveolar histiocytosis in the female recovery groups.

Minimally increased adrenal cortical vacuolation was seen at a slightly increased incidence over controls in terminal males in the 200 mg/kg/day A-998821 group and seen at a more prominently increased incidence in terminal males in the 0 mg/kg/day, 20 mg/kg/day and 200 mg/kg/day A-998821 + 40 mg/kg/day Ribavirin groups. Within the A-998821 + Ribavirin groups, there was only a slightly increased incidence over the control group (0 mg/kg/day A-998821 + 40 mg/kg/day Ribavirin) at the 200 mg/kg/day A-998821 + 40 mg/kg/day Ribavirin dose level. This parallels the modest increase in incidence over controls seen in the A-998821 alone groups. Although minimally increased adrenal cortical vacuolation was present in the 60 mg/kg/day A-998821, 20 mg/kg/day A-998821 + 40 mg/kg/day Ribavirin, and 200 mg/kg/day A-998821 + 40 mg/kg/day Ribavirin recovery groups as well, it was at an incidence so low as to be within range of normal incidental findings in rats at this facility. The increased adrenal cortical vacuolation manifested as adrenal cortical cells that were expanded by large, clear, well-demarcated vacuoles within the cytoplasm. Detailed incidence information is shown in the text tables below. The dose-dependent increased incidence suggests that this is a test article-related change, but it is apparently one that resolves with time, as seen in the recovery groups, and is not considered to be adverse.

Minimal alveolar histiocytosis was seen at an increased incidence in the lungs of both terminal males and terminal females in most of the A-998821 and A-998821 + 40 mg/kg/day Ribavirin groups. Recovery females in all of the A-998821 and A-998821 + 40 mg/kg/day Ribavirin groups showed persistence of alveolar histiocytosis, although this was not seen in the recovery males. The finding of alveolar histiocytosis consisted of small aggregates of foamy macrophages within the alveoli. Detailed incidence information is shown in the text tables that follow. This is not considered to be an adverse finding.

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Test Article-Related Microscopic Changes Male Terminal							
Dose Level (mg/kg/day)	0 (Vehicle Control)	20 A-998821	60 A-998821	200 A-998821	0 + 40 RBV	20 A-998821 + 40 RBV	200 A-998821 + 40 RBV
Sex	M	M	M	M	M	M	M
Number Examined	10	10	10	10	11	10	11
Adrenal glands Vacuolation, cortical, increased -minimal	0	1	1	3	4	5	7
Lung Histiocytosis, alveolar -minimal	1	3	5	5	2	6	4

Test Article-Related Microscopic Changes Female Terminal							
Dose Level (mg/kg/day)	0 (Vehicle Control)	20 A-998821	60 A-998821	200 A-998821	0 + 40 RBV	20 A-998821 + 40 RBV	200 A-998821 + 40 RBV
Sex	F	F	F	F	F	F	F
Number Examined	10	10	9	10	10	10	10
Lung Histiocytosis, alveolar -minimal	2	7	8	4	1	1	7

Test Article-Related Microscopic Changes Male Recovery							
Dose Level (mg/kg/day)	0 (Vehicle Control)	20 A-998821	60 A-998821	200 A-998821	0 + 40 RBV	20 A-998821 + 40 RBV	200 A-998821 + 40 RBV
Sex	M	M	M	M	M	M	M
Number Examined	5	5	5	5	4	5	4
Adrenal glands							
Vacuolation, cortical, increased							
-minimal	0	0	1	0	0	1	1
Lung							
Histiocytosis, alveolar							
-minimal	1	1	2	1	1	1	0

Test Article-Related Microscopic Changes Female Recovery							
Dose Level (mg/kg/day)	0 (Vehicle Control)	20 A-998821	60 A-998821	200 A-998821	0 + 40 RBV	20 A-998821 + 40 RBV	200 A-998821 + 40 RBV
Sex	F	F	F	F	F	F	F
Number Examined	5	5	5	5	5	5	5
Lung							
Histiocytosis, alveolar							
-minimal	0	3	3	3	1	4	2

Conclusion: The NOAEL for A-998821 (alone) noted for this study was 200 mg/kg/day for males and female (corresponding to an AUC $_{0\text{-}24}$ of 34 and 94 µg•hr/mL and Cmax of 3.5 and 6.8 µg/ml for males and females, respectively). Co-administration of A-998821 did not exacerbate any test article related observations nor induce any new combination toxicities.

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-101636	ORIG-1	ABBOTT LABORATORIES	ABT-333
IND-101636	ORIG-1	ABBOTT LABORATORIES	ABT-333

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/s/

PRITAM S VERMA
09/09/2009

HANAN N GHANTOUS
09/09/2009

PHARMACOLOGY/TOXICOLOGY REVIEW

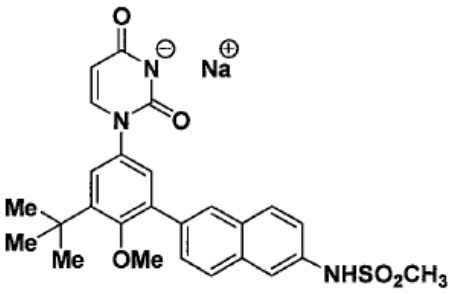
2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 101,636
Review number: 05
Sequence number/date/type of submission: SN042/October 21, 2009
Information to sponsor: No
Sponsor and/or agent: Abbott Laboratories
Global Pharmaceutical Research and Development
Abbott Park, Illinois 60064 USA

Reviewer name: Pritam S. Verma, Ph.D.
Division name: DAVP
HFD #: 530
Review completion date: January 6, 2010

Drug:

Trade name: not available
Generic name: not available
Code name: ABT-333 (A-998821)
Chemical name: Methanesulfonamide, N- (6- (5 -(3 ,4-dihydro-2,4-dioxo-1 (2H)-pyrimidiny 1)- 3-(1,1-dimethy lethy 1)- 2- methoxypheny 1) - 2-naphthalenyl)-, monosodium salt
CAS registry number: not available
Molecular weight: 515.56 (salt); 493.57 (acid)
Molecular formula/molecular weight: $C_{26}H_{26}N_{30}O_5SNa$ (salt); (acid)
Structure:



Relevant INDs/NDAs/DMFs: None

Drug class: ABT-333 is a nonnucleoside inhibitor of the hepatitis C virus (HCV) RNA-dependent RNA polymerase

Introduction and History: ABT -333, a non-nucleoside inhibitor of NS5B polymerase, is being developed for the treatment of HCV infection. The NS5B gene product of HCV possesses RNA-dependent RNA polymerase activity, an activity not present in mammalian cells. The RNA polymerase plays an essential role in viral replication by directing the synthesis of both the replicative intermediate minus-strand RNA as well as the progeny plus-strand RNA and is therefore a potential target for therapeutic inhibition.

Toxicology:

Study title: 6-Month oral dose toxicity study with A-998821 in rats with a 1-month recovery period

Key study findings: Three groups of 25 male and 25 female Sprague-Dawley rats were administered A-998821 at dose levels of 50, 200, or 800 mg/kg/day (25, 100 or 400 mg/kg, BID., 6 hours apart). One additional group of 25 animals/sex served as the control and received the vehicle, 0.2% hydroxypropyl methylcellulose in distilled water, BID. Following the dosing period, five animals/sex/group were maintained for a I-month recovery period. Results: There were no treatment-related mortalities and no adverse test article-related effects on clinical observations, body weight, food consumption, ophthalmoscopic examinations, hematology, coagulation, clinical chemistry, urinalysis, or organ weights. Alveolar histiocytosis at all doses and granulomatous inflammation of the ileum at 800 mg/kg/day were test article-related changes but were not associated with adverse clinical effects.

The NOAEL for this study was 800 mg/kg/day (400 mg/kg, BID) associated with AUC values of 119 and 319 µg*hr/mL in males and females, respectively, at Week 23. Microscopic change in target tissues, lung and ileum, were not considered to be adverse effects.

Study no.: 126-359

Conducting laboratory and location: (b) (4)

Date of study completion: September 25,2009

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: 62749PP00; **purity 99.8%**

Methods

Doses: 50, 200, or 800 mg/kg/day (25, 100 or 400 mg/kg, BID., 6 hours apart).

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Experimental design: is shown in Table 1. The vehicle and test article were administered twice a day via oral gavage with approximately 6 hours between doses (15 minutes) for 182 days. The dose levels were 0, 50, 200, and 800 mg/kg/day administered at a dose volume of 10 mL/kg/dose.

Table 1

Group Assignments			
Group Number	Dose Level (mg/kg/day) [c]	Number of Animals [a]	
		Male	Female
1	0	25	25
2	50	25	25
3	200	25	25
4	800	25	25
89 [b]	N/A [d]	10	10

[a] Five animals/sex/group (control and treated groups) were retained for the recovery period.
[b] 10 animals/sex were assigned as sentinel animals.
[c] Dose levels expressed are the total daily dose levels administered to the groups. Doses were administered twice daily (BID).
[d] N/A - Not applicable.

Species/strain: male and female rats/CD(Crl:CD(SD) rats

Number/sex/group or time point (main study): Table 1

Route, formulation, volume, and infusion rate: Table 1

Satellite groups used for toxicokinetics: Blood samples (approximately 0.5 mL) were collected from warmed and restrained (unanesthetized) animals via the lateral tail vein for determination of the plasma concentrations of the test article. Samples were collected at 1.5, 3, 6 (prior to the second dose), 7.5, 9, 12, and 24 hours after the first dose on Day 1 and once during Week 23.

Age & weight: 110 male and 110 female animals (weighing 192 to 221 g and 161 to 190 g, respectively)

Sampling times: blood samples for clinical pathology immediately prior to necropsy.

Gross and Histopathology: adequate.

Results

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Mortality: No test article-related mortality was noted at any dose level

One control male (animal number 1005) was found dead on Day 63. One male (1043) and one female (animal number 2027) at 50 mg/kg/day, and one male at 200 mg/kg/day (animal number 1072), were found dead on Days 155, 107, and 121, respectively. Although there were no findings to suggest a cause of death in the control male, deaths of the latter three animals were considered to be gavage-related based on the general lack of toxicity, the proximity of death following dose administration, microscopic observation of minimal hemorrhage in the lungs, and/or the presence of white frothy fluid in the trachea.

Clinical signs: There were no test article-related clinical observations

Body weights: There were no test article-related body weight effects.

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

Ophthalmology: There were no test article-related ophthalmoscopic findings.

Hematology & Coagulation: There were no adverse test article-related effects on hematology parameters. Neutrophils and monocytes in females at 800 mg/kg/day were greater than controls at termination. Although these higher values were relatively consistent within the group and deemed test article related, most values remained within expected ranges and were not considered adverse at these magnitudes. One male at 800 mg/kg/day (animal number 1095) exhibited moderately to markedly increase total leukocytes and neutrophils. At recovery, there were no statistically significant differences. Few sporadic, individual increases of neutrophils were noted and considered incidental. There were no test article-related effects on coagulation parameters.

Serum Chemistry: There were no toxicologically significant effects on clinical chemistry parameters. The means for AST and ALT were increased in males at 800 mg/kg/day. These increases were attributable to primarily one male (animal number 1078). Other statistically significant differences between treated and control groups occurred in several parameters, but these were not considered to be biologically relevant effects because of the low magnitude of the difference, direction of change, and/or the lack of dose-dependency. At recovery, AST, ALT, and sorbitol dehydrogenase (SDH) were moderately to markedly increased in one male at 200 mg/kg/day (animal number 1075). Since these changes in AST, ALT, and SDH were isolated findings and not dose-related, they were considered unrelated to treatment.

Urinalysis: no changes were seen.

Toxicokinetics: data are presented in Table 2. Plasma A-998821 exposures at Week 23 were greater in females than in males at all doses. Plasma Cmax values at Week 23 were 4.75, 5.03, and 12.5 µg/mL in males and 7.33, 16.7, and 29.2 µg/mL in females at 50, 200, and 800 mg/kg/day, respectively. Following the second daily dose, plasma AUC

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values at Week 23 were 31.5, 51.8, and 119 µg*hr/mL in males and 74.3, 170, and 319 µg*hr/mL in females at 50, 200, and 800 mg/kg/day, respectively.

Table 2
Mean toxicokinetic parameters

Mean Toxicokinetic Parameters for A-998821

Group	Day	Dose (mg/kg/day)	Sex	Cmax (µg/mL) 1	Cmax (µg/mL) 2	Tmax * (hr) 1	Tmax * (hr) 2	AUC (µg•hr/mL)
2	1	50	male	0.701	3.93	6	9	17.2
			female	1.45	4.88	6	9	26.4
	160	50	male	2.60	4.75	3	7.5	31.5
			female	5.04	7.33	3	9	74.3
3	1	200	male	5.13	8.17	3	7.5	46.0
			female	7.54	10.5	3	7.5	104
	160	200	male	3.73	5.03	3	12	51.8
			female	11.8	16.7	1.5	9	170
4	1	800	male	8.75	25.1	3	7.5	134
			female	13.4	24.8	3	9	257
	160	800	male	6.67	12.5	3	7.5	119
			female	23.0	29.2	3	7.5	319

* Both T_{max} 1 and T_{max} 2 are hours relative to first daily dose.

Histopathology: Adequate Battery: yes; Peer review: yes

Gross pathology: Test article-related macroscopic findings were observed in the lungs of males and females. At terminal necropsy, discoloration white, tan or white focus foci were observed in a dose-related fashion in males and females at 200 and 800 mg/kg/day, with a higher incidence and severity in females relative to males. At recovery necropsy, tan focus foci were observed in two females at 50 and 200 mg/kg/day. Discoloration white and tan or white focus foci corresponded microscopically to alveolar histiocytosis. Other macroscopic observations were considered incidental and unrelated to treatment.

Organ weights: There were no test article-related changes in organ weights.

Histopathology: Test article-related microscopic findings were observed in the lungs and ileum of males and females (Table 4).

Lung findings consisted of increased incidence and magnitude of alveolar histiocytosis. Alveolar histiocytosis is a common background finding in rats characterized by clusters of enlarged macrophages with abundant, foamy cytoplasm within alveoli. At terminal

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necropsy, males and females at all dose levels were affected in a dose-related fashion, with a higher severity observed in females. At recovery necropsy, alveolar histiocytosis was observed in only one male at 800 mg/kg/day, and at all dose levels in females, albeit with a lower incidence and severity at 50 and 200 mg/kg/day relative to terminal necropsy. These findings suggest a trend toward recovery, but also that the rats did not completely recover from alveolar histiocytosis during the one-month recovery period. The alveolar histiocytosis was not interpreted to be adverse in this study.

Small intestine findings consisted of granulomatous inflammation in the lamina propria of the ileum (one animal also had in the jejunum) in males and females at 800 mg/kg/day. The granulomatous inflammation expanded the lamina propria and was characterized primarily by epithelia macrophages and fewer foreign body-type multinucleated giant cells. Within the area of granulomatous inflammation, thin (1-2 micrometers in diameter), needle-shaped foreign material could be observed under polarized light (birefringence). The finding was present at terminal and recovery necropsy, with higher incidence and magnitude in females. This finding is not interpreted to be adverse in this study.

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Table 3

Test Article-Related Microscopic Findings Male and Female Terminal								
Dose level: mg/kg/day	**0**		**50**		**200**		**800**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number examined	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
Lung								
Histiocytosis, alveolar	1	0	6	9	10	13	8	19
-minimal	1	0	6	7	9	7	5	4
-mild	0	0	0	2	1	6	3	13
-moderate	0	0	0	0	0	0	0	2
Small intestine, ileum								
Inflammation, granulomatous	0	0	0	0	0	0	9	12
-minimal	0	0	0	0	0	0	6	7
-mild	0	0	0	0	0	0	3	2
-moderate	0	0	0	0	0	0	0	3

Test Article-Related Microscopic Findings Male and Female Recovery								
Dose level: mg/kg/day	**0**		**50**		**200**		**800**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number examined	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Lung								
Histiocytosis, alveolar	0	1	0	2	0	2	1	5
-minimal	0	1	0	2	0	1	1	0
-mild	0	0	0	0	0	1	0	5
Small intestine, ileum								
Inflammation, granulomatous	0	0	0	0	0	0	2	3
-minimal	0	0	0	0	0	0	1	1
-mild	0	0	0	0	0	0	1	1
-moderate	0	0	0	0	0	0	0	1

One male at 800 mg/kg/day from the recovery group had a benign nephroblastoma in one kidney. Nephroblastoma is a common spontaneously occurring tumor in rats.9, 10. This tumor and all other microscopic observations were either common background findings in rats or were considered incidental and unrelated to treatment.

Conclusion: The NOAEL for this study was 800 mg/kg/day (400 mg/kg BID) associated with AUC values of 119 and 319 µg*hr/mL in males and females, respectively, at Week 23. Microscopic change in target tissues, lung and ileum, were not considered to be adverse effects.

Reference ID: 3628623

9-Month Oral Capsule Toxicity Study with A-998821 in Beagle Dogs with a 4-Week Recovery Period

Key study findings: Three treatment groups of five dogs/sex were administered the test article (Lot Nos. 62757PPOO, 62749PPOO, 59685PPOO) at respective dose levels of 10, 30, or 60 mg/kg/day. One additional group of five animals/sex served as the control and received the vehicle, 0.2% hydroxypropyl methyl cellulose. Following 273 days of administration, two animals/sex/group were maintained for a 28 day recovery period.

In males and females given 60 mg/kg/day, there were mild increases in total bilirubin, ALT, sorbitol dehydrogenase, and gama-glutamyltransferase were seen relative to controls at the termination interval suggesting a mild test article-related hepatic effect at this dose. In addition, beginning by Week 4 and generally persisting through termination; there were mild, non-progressive, yet test article-related increases in ALP in males given 60 mg/kg/day and in females given 30 mg/kg/day, relative to controls. These changes correlate to the moderately increased mean absolute liver weight in males given 60 mg/kg/day and the increased hepatocellular vacuolation noted microscopically. In liver from male dogs administered A-998821, there was an increase in the amount of glycogen in centrilobular hepatocytes compared to concurrent vehicle controls. The areas of increased glycogen likely correspond to the vacuolation noted in hepatocytes by histopathology. The ultrastructure of hepatocytes was otherwise normal.

Based upon an absence of any adverse findings during this study, the NOAEL for A-998821 sodium during this study was 60 mg/kg/day, for males and females; corresponding with an overall Day 270 AUC of 839 µg*hr/mL, and a Cmax of 41.7 µg/mL.

Study no.: 126-278

Conducting laboratory and location: (b) (4)

Date of study completion: September 2, 2009

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: The following lot numbers of A-998821 sodium (A-998821.5, A-998821, ABT-333) were used during this study.

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Lot Number	Assigned Chemical Potency (mcg A-998821.0/mg A-998821.5	Assigned Retest Date
62757PP00	915	01 September 2008
62749PP00	922	01 February 2009
59685PP00	920	01 May 2009

Methods: The vehicle or test article was administered orally via capsule, once per day, for 9 consecutive months during the study. The dose levels were 10, 30, and 60 mg/kg/day. The control group received gelatin capsules filled with vehicle at the same dose volume as the high dose animals.

Experimental design: is shown in Table 4.

Table 4

Group Assignments			
Group Number	Dose Level (mg A-998821.0/kg/day)	Number of Animals[a]	
		Male	Female
1	0	5	5
2	10	5	5
3	30	5	5
4	60	5	5

[a]Two animals/sex/group were maintained for a 28 day recovery period following 273 days of dosing.

Species/strain: male and female beagle dogs

Number/sex/group or time point (main study): Table 4

Route, formulation, volume, and infusion rate: oral capsule

Satellite groups used for toxicokinetics: Blood samples (approximately 0.5 mL) were collected from all animals via the jugular vein for determination of the plasma concentrations of the test article. Samples were collected predose and at 1, 2, 4, 8, 12, and 24 hours postdose on Days 1 ,91, 179, and 270.

Age & weight: 6 to 7 months of age; 20 male and 20 female animals (weighing 7.20 to 9.25 kg and 5.31 to 6.61 kg, respectively

Sampling times: blood samples for clinical pathology immediately prior to necropsy.

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Gross and Histopathology: adequate.

Results

Mortality: No test article-related mortality was noted at any dose level

Clinical signs: There were no test article-related clinical observations

Body weights: There were no test article-related body weight effects were noted at any dose level

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

Ophthalmology: There were no test article-related ophthalmoscopic findings.

Electrocardiographic Examinations: All the electrocardiograms evaluated were qualitatively and quantitatively normal. No abnormalities in rhythm were found. There were no test article-related or otherwise abnormal electrocardiographic findings.

Hematology & Coagulation: There were no test article-related effects on hematology parameters. Occasional statistically significant changes in platelets and mean corpuscular hemoglobin concentration (MCHC) were observed and not considered toxicologically relevant due to the small magnitude of the changes. There were no test article-related effects on coagulation parameters.

Serum Chemistry: In both sexes at 60 mg/kg/day at the termination interval, there were mild increases in total bilirubin (range 1.33 to 1.50-fold), ALT (range 1.82 to 2.l5-fold), sorbitol dehydrogenase (range 1.72 to 2.02-fold), and gama-glutamyltransferase (GGT) (range 2.00 to 2.85-fold) relative to controls that indicate a mild test article-related hepatic effect at this dose. These changes correlate to the microscopic findings of hepatocellular vacuolation described elsewhere and are not considered adverse; they had generally resolved by the recovery interval.

Beginning by Week 4, there were mild test article-related increases in ALP (range 1.02 to 2.86-fold) in both sexes at all dose levels relative to controls; changes generally persisted though termination. These increases by and large reached statistical significance in males at 60 mg/kg/day and at >30 mg/kg/day in females and were not progressive over time but did follow a dose response in males (not females) at all three dose levels. The alterations in ALP may be due to enzyme induction although an association with the microscopic liver findings discussed previously is also a consideration.

They had resolved by and large by the recovery interval and were not considered adverse.

In both sexes at >30 mg/kg/day, there were test article-related increases in cholesterol (range 1.01 to 1.43-fold) relative to controls beginning by Week 4 and persisting through

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termination. The increases generally followed a dose response and progressively increased over time. The changes were minimal in males at the 30 mg/kg/day dose but there was a clear trend affecting both sexes at these dose levels. At recovery, these patterns of increase were not clearly evident hence they are considered resolved. These changes are not considered adverse.

Urinalysis: no changes were seen.

Toxicokinetics: data are presented in Table 5. No gender differences were observed in the toxicokinetic dataset. Exposures (AUC) were proportional to dose throughout the study.

Dose		Day 1			Day 270		
		C_{max}	T_{max}	AUC	C_{max}	T_{max}	AUC
(mg/kg/day)	Sex	(µg/mL)	(hr)	(µg•hr/mL)	(µg/mL)	(hr)	(µg•hr/mL)
10	male	2.70 (±0.316)	9.6 (±8.3)	53.8 (±6.90)	9.17 (±2.54)	4.8 (±3.0)	184 (±54.6)
	female	2.58 (±1.04)	3.2 (±1.1)	46.4 (±19.4)	8.29 (±4.21)	2.2 (±1.1)	154 (±78.5)
	overall	2.64 (±0.728)	6.4 (±6.5)	50.1 (±14.3)	8.73 (±3.31)	3.5 (±2.5)	169 (±65.7)
30	male	5.60 (±3.09)	13.6 (±10.0)	90.5 (±40.1)	21.7 (±8.81)	2.4 (±0.9)	424 (±160)
	female	5.14 (±1.48)	4.0 (±2.4)	95.9 (±28.6)	31.2 (±6.73)	3.6 (±0.9)	615 (±131)
	overall	5.37 (±2.30)	8.8 (±8.5)	93.2 (±33.0)	26.5 (±8.95)	3.0 (±1.1)	519 (±170)
60	male	8.01 (±1.30)	14.0 (±9.8)	138 (±21.6)	41.6 (±14.8)	4.8 (±1.8)	829 (±325)
	female	6.89 (±3.23)	7.2 (±9.4)	116 (±34.9)	41.7 (±21.6)	2.8 (±1.6)	848 (±452)
	overall	7.45 (±2.40)	10.6 (±9.8)	127 (±29.9)	41.7 (±17.5)	3.8 (±1.9)	839 (±371)

Histopathology: Adequate Battery: yes; Peer review: yes

Gross pathology: Treatment-related macroscopic findings were limited to mild enlargement of the adrenal glands of one male at 60 mg/kg/day at the terminal necropsy. Adrenal gland enlargement in this male correlated with increased adrenal gland weights and cortical hypertrophy /hyperplasia noted microscopically. The adrenal gland enlargement was likely stress related given the concomitant multicentric lymphoid depletion noted in all lymphoid organs (thymus, spleen, lymph nodes and Peyer's patches). Mild adrenal gland enlargement was also seen in one male at 30 mg/kg/day at the end of the dosing phase, and this was likely an incidental finding given that the

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adrenal gland weights of this animal were within the range noted in control males. Additionally, lymphoid depletion was limited to the thymus of this animal, likely reflecting normal biologic involution, and was not observed in the other lymphoid organs.

No test article-related or otherwise remarkable macroscopic findings were otherwise observed at terminal and recovery phase necropsies.

Organ weights: The liver weight and the liver/body weight ratio of 2/3 males at 60 mg/kg/day were moderately increased compared to controls at the dosing phase sacrifice. The liver weights of these animals were 588g and 428g, exceeding the range of 242 g to 286 g noted in concurrent control males. The increased liver weights likely reflected treatment-related hepatocellular vacuolation noted microscopically. The liver weights of all other animals at the terminal necropsy were similar to that noted in concurrent control animals, and no respective organ weight changes were observed at the recovery phase necropsy.

The adrenal gland weights of the same 2/3 males at 60 mg/kg/day at the terminal necropsy were also moderately increased relative to controls. The adrenal gland weights of these animals were 2.2 g and 2.3 g, compared to the range of 1.2 g to 1.4 g noted in control males. Increased adrenal gland weights likely reflected adrenal cortical hypertrophy/hyperplasia observed microscopically, and this finding was likely associated with stress given the concomitant multicentric lymphoid depletion noted in these animals. The adrenal gland weights of the 2/3 females at 60 mg/kg/day that exhibited diffuse cortical hypertrophy/hyperplasia were also slightly increased compared to controls, although slight increases in adrenal gland weight were also noted in several females at lower dose levels with no microscopic correlate. No organ weight changes were noted in the adrenal glands of animals at the recovery necropsy.

No other test article-related or otherwise noteworthy organ weight findings were observed at dosing or recovery phase necropsies. Any other statistically significant organ weight changes were considered incidental findings.

Histopathology: The liver in all males at 60 mg/kg/day at the terminal necropsy exhibited minimal to mild diffuse hepatocellular vacuolation, and this finding correlated with the increased liver enzyme values (ALT, SDH, GGT, and total bilirubin) and liver weights noted in males at this dose level. Hepatocellular vacuolation was not observed in other treated or control animals at this interval. This finding was characterized by diffuse enlargement of hepatocytes with abundant cytoplasm with poorly defined, irregular clear vacuoles. Hepatocellular vacuolation in the liver in males at 60 mg/kg/day was not associated with cellular degeneration or necrosis, and was not considered to be adverse. At the recovery necropsy, hepatocellular vacuolation was occasionally seen in control and treated animals with no apparent dose response. Given the absence of clinical chemistry effects and liver weight increases in affected animals at this interval, the vacuolation could not be attributed to treatment. It is unclear why there was a clear dose-

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related morphologic effect in hepatocytes at the terminal necropsy, while the vacuolation appeared to have more of a random distribution at the recovery necropsy.

The adrenal glands of two males and two females at 60 mg/kg/day at the terminal necropsy exhibited minimal to mild diffuse adrenal cortical hypertrophy/hyperplasia. Adrenal cortical hypertrophy/hyperplasia was likely stress induced in the males, particularly given that moderately increased adrenal gland weights and concurrent multicentric lymphoid depletion were noted in these animals. In the affected females, the adrenal gland weights were only slightly increased relative to controls, and gross glandular enlargement and multicentric lymphoid depletion were not observed. Cortical hypertrophy/hyperplasia can be a difficult change to assess in dogs due to variation in sectioning of the adrenal glands, and the apparent changes in these females may have been incidental. Similarly, at the recovery necropsy, diffuse adrenal cortical hypertrophy/hyperplasia was occasionally identified in control and treated animals with no clear dose response. Affected animals did not have a concomitant increase in adrenal gland weights or multicentric lymphoid depletion, and the apparent adrenal cortical hypertrophy/hyperplasia was likely an incidental finding.

Two males at 60 mg/kg/day at the terminal necropsy had severe lymphoid depletion in the thymus, as well as generalized lymphoid depletion in the spleen (mild), Peyer's patches (minimal to moderate), and mandibular, mesenteric, popliteal, and tracheobronchial lymph nodes (minimal to mild). The thymus weights of males at this dose level were considerably lower than controls (24% decrease in mean thymus weight), although this finding was not statistically significant. The affected males also had increased adrenal gland weights and mild diffuse adrenal cortical hypertrophy/hyperplasia, and all of these findings suggest a response to stress. In males at lower dose levels, there was a slight increase in the incidence and severity of generalized lymphoid depletion in the thymus relative to controls, although this finding may have reflected normal biologic variation in thymic involution and could not be ascribed to the test article, particularly given the absence of a clear effect on organ weights. In treated females, the incidence and severity of thymic lymphoid depletion did not differ significantly from controls. For other examined lymphoid organs in treated females, lymphoid depletion was limited to the Peyer's patches of one female at 10 mg/kg/day and was considered incidental, and this finding was not observed in the spleen or lymph nodes of any female.

At the recovery necropsy, the increased lymphoid depletion in the lymphoid organs noted at the terminal necropsy in males at 60 mg/kg/day was no longer present, suggesting resolution of this finding over the recovery period. Lymphoid depletion was variably noted in the thymus of control and treated animals with no relationship to dose, and was only identified in a single lymph node of one male at 60 mg/kg/day. Lymphoid depletion was not identified in the spleen or Peyer's patches of any animal at this interval.

Ultrastructural Pathology: Given the various changes to liver noted above, liver specimens from males given vehicle or 60 mg/kg/day test article were examined ultrastructurally. An increased amount of glycogen in centrilobular hepatocytes was

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observed for 60 mg/kg/day animals as compared to control animals. The areas of increased glycogen likely correspond to the hepatocellular vacuolization observed microscopically. The amount of glycogen in hepatocytes from dogs administered A-998821, though greater than in the concurrent controls, was considered to be within normal limits.

Conclusion: Based upon an absence of any adverse findings during this study, the NOAEL for A-998821 sodium was 60 mg/kg/day, for males and females; corresponding with an overall Day 270 AUC of 839 µg*hr/mL, and a Cmax of 41.7 µg/mL.

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

Reference ID: 3628623

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-101636	ORIG-1	ABBOTT LABORATORIES	ABT-333

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/s/

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01/20/2010

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01/22/2010

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 206-619

Supporting document/s:

Supporting Document	Sponsor Submission Date	CDER Received Date
1	1/14/14	1/15/14
2	2/28/14	2/28/14

Product: Paritaprevir/Ritonavir/Ombitasvir, Dasabuvir (ABT-450/r/ABT-267, ABT-333)

Indication: treatment of genotype 1 chronic hepatitis C infection, including in patients with cirrhosis

Applicant: AbbVie Inc.

Review Division: Division of Antiviral Products

Reviewer: Mark W. Powley, Ph.D.

Supervisor/Team Leader: Hanan Ghantous, Ph.D., DABT

Division Director: Debra B. Birnkrant, M.D.

Project Manager: Katherine Schumann, M.S.

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1 Executive Summary

1.1 Introduction

AbbVie Inc. has submitted an NDA to support the combination therapy of paritaprevir (ABT-450; HCV NS3/4A protease inhibitor), ritonavir (CYP3A4 inhibitor), ombitasvir (ABT-267; HCV NS5A inhibitor), and dasabuvir (ABT-333; HCV NS5B inhibitor) with or without ribavirin for treating chronic hepatitis C genotype 1 infection (GT1) in adult patients, including in patients with cirrhosis. The proposed clinical dose regimen includes 150 mg/day paritaprevir + 100 mg/day ritonavir + 25 mg/day ombitasvir + 500 mg/day dasabuvir for up to 24 weeks.

This review focuses on qualification of impurities and residual solvents. Regulatory decision making utilizes information presented in ICH guidelines M7 "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" Q3A(R2) "Impurities in New Drug Substances", Q3B(R2) "Impurities in New Drug Products", and Q3C(R5) "Impurities: Guideline for Residual Solvents".

All proposed specifications are considered acceptable from a pharmacology/toxicology perspective based on the results of general toxicology studies, empirical Ames assays, in vitro chromosomal aberration assays, and/or (quantitative) structure-activity relationship [(Q)SAR] predictions of mutagenicity.

Note that (Q)SAR evaluations were used in evaluating both specified and unspecified impurities. Unspecified impurities include unspecified identified impurities, unspecified unidentified impurities, and potential impurities from the manufacturing process that are eliminated or controlled upstream. The review of (Q)SAR data is limited to specified and unspecified identified impurities. Other impurities may have possessed structural alerts but are 1) effectively controlled (see detailed CMC reviews by Dr. Maotang Zhou or Dr. Milton Sloan) and do not pose substantial clinical risk or 2) qualified based on similarity with Ames negative API, or 3) shown to be negative in genotoxicity testing. A summary of relevant (Q)SAR data is provided in the Appendix.

2 Qualification of Paritaprevir Drug Substance

2.1 Impurities

2.1.1 Specified Impurities

Because specified impurities exceed the ICH Q3A(R2) qualification threshold, data was submitted from general toxicology studies and evaluations of mutagenic potential.

<u>General Toxicology</u> – Specified impurities are qualified by a 4-week study in Sprague Dawley rats (Study no. R&D/10/106). As results for 300 mg/kg/day of paritaprevir spiked with impurities are similar to 300 mg/kg/day paritaprevir alone, this dose is used to calculate qualified levels.

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Overall, the qualified levels of impurities summarized below are deemed adequate to support the proposed specifications.

Table 1. Paritaprevir drug substance specified impurity general toxicology qualification

Impurity	Toxicology Study Content	Non-Clinical Dose[a]	Qualified Level[b]	Proposed Specification
[redacted]	(b) (4)	300 mg/kg/day	[redacted]	(b) (4)
		300 mg/kg/day		
		300 mg/kg/day		
		300 mg/kg/day		
		300 mg/kg/day		
		300 mg/kg/day		
		300 mg/kg/day		
		300 mg/kg/day		
		300 mg/kg/day		

[a] only dose tested; no differences in toxicity profile for paritaprevir/ritonavir spiked with impurity vs. paritaprevir/ritonavir alone

[b] qualified level = [% impurity x non-clinical dose (mg/kg/day)] / [body surface area conversion factor x maximum clinical dose (i.e., 3 mg/kg/day)]

<u>Mutagenicity</u> – The Sponsor assessed potential mutagenicity using (Q)SAR predictions from an expert rule-based system (i.e., DEREK Nexus; DX) and a statistically-based system (i.e., CaseUltra; CU). Both (Q)SAR systems identified [redacted] (b) (4) *as potentially mutagenic based on the presence of a* (b) (4) *moiety. As a result, the combination of these impurities is controlled to ≤* (b) (4) *ppm (i.e.,* (b) (4) *µg/day total exposure). The exposure is well below the ICH M7 recommended limit of 20 µg/day for drugs administered between 1 to 12 months.*

CU predicted all remaining specified impurities to be mutagenic based on an alert (b) (4) [redacted] *structure. While these types of structures have* (b) (4) [redacted] *the alerting moiety is also present in the Ames negative and non-carcinogenic API. Therefore, impurities containing only this alert are considered non-mutagenic per ICH M7.*

2.1.2 Unspecified Impurities

Qualification of unspecified identified impurities is limited to (Q)SAR evaluations of potential mutagenicity. (b) (4) *is predicted positive by CU due to an alert* (b) (4) [redacted] *structure. The impurity is considered non-mutagenic because the alert is also present in the Ames negative and non-carcinogenic API.*

In addition, several unspecified impurities were shown to be negative in empirical Ames testing. These studies are listed below.

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Table 2. Paritaprevir drug substance unspecified impurity Ames assays

Impurity	Conclusion
(b) (4)	non-mutagenic impurity (Study no. R&D/13/392)
	non-mutagenic impurity (Study no. R&D/13/484)
	non-mutagenic impurity (Study no. R&D/13/684)

Other potentially mutagenic and/or carcinogenic impurities mentioned by the Sponsor include (b) (4)*. Each of these is effectively controlled to limits described in ICH M7 (see detailed CMC review by Dr. Milton Sloan).*

2.2 Residual Solvents

Proposed specifications for (b) (4) *as well as* (b) (4) *are consistent with recommendations provided in ICH Q3C(R5) and are, therefore, acceptable. Proposed specifications are summarized below.*

Table 3. Paritaprevir drug substance residual solvent proposed specifications

Solvent	Specification
(b) (4)	

3 Qualification of Ombitasvir Drug Substance

3.1 Impurities

3.1.1 Specified Impurities

Because specified impurities exceed the ICH Q3A(R2) qualification threshold, data was submitted from general toxicology studies and evaluations of mutagenic potential.

<u>General Toxicology</u> – Specified impurities are qualified by 4-week and 26-week studies in CD-1 mice (Study no. R&D/12/570 and Study no. R&D/11/561). Results for 200 mg/kg/day ombitasvir spiked with impurities (b) (4) *are similar to 200 mg/kg/day ombitasvir alone following 4-week of dosing. Therefore, this dose is used to calculate qualified levels. The NOAEL for the 26-week study in mice was used to qualify impurity* (b) (4) *Overall, the qualified levels of impurities summarized below are deemed adequate to support the proposed specifications.*

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Table 4. Ombitasvir drug substance specified impurity general toxicology qualification

Impurity	Toxicology Study Content	Non-Clinical Dose	Qualified Level[a]	Proposed Specification
[redacted] (b) (4)		200 mg/kg/day[b] 200 mg/kg/day[c] 200 mg/kg/day[b] 200 mg/kg/day[b] 200 mg/kg/day[b]	[redacted]	[redacted] (b) (4)

qualified level = [% impurity x non-clinical dose (mg/kg/day)] / [body surface area conversion factor x maximum clinical dose (i.e., 0.5 mg/kg/day)]

[b] 4-week study in mice (R&D/12/570); only dose tested; no differences in toxicity profile for ombitasvir spiked with impurity vs. ombitasvir alone

[c] 26-week study in mice (R&D/11/561); NOAEL

<u>Mutagenicity</u> – Two known mutagenic molecules, [redacted] (b) (4) [redacted] (b) (4) are both present in the drug substance. The combination of these impurities is controlled to ≤ (b) (4) ppm (i.e., (b) (4) µg/day total exposure), well below the ICH M7 recommended limit of 20 µg/day for drugs administered between 1 to 12 months.

A specification of ≤ (b) (4) % (i.e., (b) (4) µg/day) is proposed [redacted] (b) (4) This impurity was identified as a structural analog of [redacted] (b) (4) a known mutagenic carcinogen. The Sponsor indicates that the compound specific threshold of toxicological concern [redacted] (b) (4) is also appropriate [redacted] (b) (4) While structural analogs are sometimes useful for identifying potentially reactive impurities, quantitative assessments based on analogs are not routinely performed. In spite of this, the proposed specification is only slightly above the ICH M7 recommended limit of 20 µg/day for drugs administered between 1 to 12 months and is considered acceptable.

All other specified impurities in the drug substance are considered non-mutagenic based on (Q)SAR evaluation.

3.1.2 Unspecified Impurities

Qualification of unspecified identified impurities is limited to (Q)SAR evaluations of potential mutagenicity. Predictions from these evaluations indicate that all unspecified identified impurities are expected to be non-mutagenic.

Other potentially mutagenic and/or carcinogenic impurities mentioned by the Sponsor include [redacted] (b) (4) Each of these is effectively controlled to limits described in ICH M7 (see detailed CMC review by Dr. Milton Sloan).

3.2 Residual Solvents

The proposed specification for (b) (4) (i.e., ≤ (b) (4) ppm) is consistent with recommendations provided in ICH Q3C(R5) and, therefore, acceptable.

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4 Qualification of Dasabuvir Drug Substance

4.1 Impurities

4.1.1 Specified Impurities

Because specified impurities exceed the ICH Q3A(R2) qualification threshold and may be present at exposures ≤ (b) (4) mg/day, data was submitted from general toxicology studies and evaluations of genotoxic potential.

General Toxicology – Specified impurities are qualified by a 4-week study in Sprague Dawley rats (Study no. R&D/12/338). As results for 800 mg/kg/day dasabuvir spiked with impurities (b) (4) are similar to 800 mg/kg/day dasabuvir alone following 4-week of dosing, this dose is used to calculate qualified levels. Overall, the qualified levels of impurities summarized below are deemed adequate to support the proposed specifications.

Table 5. Dasabuvir drug substance specified impurity general toxicology qualification

Impurity	Toxicology Study Content	Non-Clinical Dose[a]	Qualified Level[b]	Proposed Specification
(b) (4)		800 mg/kg/day 800 mg/kg/day	(b) (4)	

[a] 4-week study in rats (R&D/12/338); only dose tested; no differences in toxicity profile for dasabuvir spiked with impurity vs. dasabuvir alone

[b] qualified level = [% impurity x non-clinical dose (mg/kg/day)] / [body surface area conversion factor x maximum clinical dose (i.e., 10 mg/kg/day)]

Genotoxicity – (b) (4) were shown to be non-genotoxic based on empirical Ames and in vitro chromosomal aberration assays (Study no. R&D/13/579 and Study no. R&D/13/580 – reviewed in Appendix).

4.1.2 Unspecified Impurities

Qualification of unspecified identified impurities is limited to (Q)SAR evaluations of potential mutagenicity. CU predicted (b) (4) to be mutagenic due to an alert (b) (4) structure. However, the Sponsor indicates the impurity is more similar to Ames negative training set molecules with the alert compared to those that are Ames positive. Because the alerts appear to be irrelevant for (b) (4) the impurity can be considered non-mutagenic.

(b) (4) were shown to be negative in empirical Ames testing (Study no. R&D/13/1119 - review in Appendix).

Other potentially mutagenic and/or carcinogenic impurities mentioned by the Sponsor include (b) (4)

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[redacted] (b) (4). *Each of these is effectively controlled to limits described in ICH M7 (see detailed CMC review by Dr. Maotang Zhou).*

4.2 Residual Solvents

Proposed specifications [redacted] (b) (4) *are consistent with recommendations provided in ICH Q3C(R5) and are, therefore, acceptable. Specifications are summarized below.*

Table 6. Dasabuvir drug substance residual solvent proposed specifications

Solvent	Specification
[redacted]	(b) (4)

5 Qualification of Ombitasvir/Paritaprevir/Ritonavir and Dasabuvir Drug Products

5.1 Specified Degradants

Proposed specifications for ombitasvir degradant [redacted] (b) (4) *and paritaprevir degradant* [redacted] (b) (4) *are considered acceptable based on the qualification data previously described. All proposed specifications for ritonavir are consistent with the commercial ritonavir table (NDA#22,417). There are no specified degradants for dasabuvir. Drug product specifications are described below.*

Table 7. Drug product impurity proposed specifications

	Specification	
Impurity	Release	Shelf Life
[redacted]		(b) (4)

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Appendix

(Q)SAR Evaluation

Evaluation of mutagenic potential was performed by AbbVie using DEREK Nexus (DX; v3.0.1) and CaseUltra (CU; v1.4.7). CU predictions made using the A7B (Salmonella 5 strains) and AT (E.coli/Salmonella TA102) training sets. Starting materials, raw materials, intermediates, and potential manufacturing impurities were evaluated. In total this amounted to ~250 structures evaluated by (Q)SAR. The following sections focus on evaluations of specified impurities and unspecified identified impurities.

Paritaprevir – Structural alerts were identified in numerous impurities. Many contained CU alert (b) (4)*. Although the alert is associated with potential* (b) (4) *the alert is also present in the Ames negative and non-carcinogenic API. Therefore, impurities containing only this alert are considered non-mutagenic per ICH M7.* (b) (4) *are both deemed potentially mutagenic* (b) (4)*.*

Impurity	Structural Alert(s)	Notes
(b) (4)		

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<u>Ombitasvir</u> – All specified and unspecified identified impurities are predicted to be non-mutagenic.

Impurity	Structural Alert(s)	Notes
(b) (4)		

<u>Dasabuvir</u> – The unspecified identified impurity (b) (4) *is predicted to be mutagenic by CU due to alerts* (b) (4) *structure. However, the Sponsor indicates the impurity is more similar to Ames negative training set molecules used to derive the alerts compared to those that are Ames positive. Because the alerts appear to be irrelevant* (b) (4) *the impurity can be considered non-mutagenic.*

Impurity	Structural Alert(s)	Notes
Unspecified Impurity		
(b) (4)		non-mutagenic (structural alerts deemed irrelevant)

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Genetic Toxicology Studies

Title: Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with (b) (4) Free Form and (b) (4) Free Form (Study no. R&D/13/579)

Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 7/12/13
GLP compliance: yes, with exceptions (e.g., incomplete test article characterization)
QA statement: yes
Test article, lot #, and % purity: (b) (4) lot#10047583-0103B, purity = 98.2%
(b) (4) lot#10047583-0104B, purity = 98.8%

Key Findings

- (b) (4) *was negative in the bacterial reverse mutation assay.*
- (b) (4) *was negative in the bacterial reverse mutation assay.*

Methods

Strains: TA98, TA100, TA1535, TA1537, and WP2*uvrA*
Concentrations in definitive assay: 1.6, 5.0, 16, 50, 160, 500, 1600, and 5000 µg/plate
Basis of concentration selection: limit dose of 5000 µg/plate
Negative control: DMSO
Positive control:

Strain	S9	Control	Dose
TA98	-	2-nitrofluorene	1 µg/plate
	+	benzo[a]pyrene	2.5 µg/plate
TA100	-	sodium azide	2 µg/plate
	+	2-aminoanthracene	2.5 µg/plate
TA1535	-	sodium azide	2 µg/plate
	+	2-aminoanthracene	2.5 µg/plate
TA1537	-	ICR-191	2 µg/plate
	+	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	-	4-nitroquinoline-N-oxide	1 µg/plate
	+	2-aminoanthracene	25 µg/plate

Formulation/Vehicle: DMSO
Incubation & sampling time: Plates were incubated for ~52 hr at 37 °C with or without metabolic activation (plate incorporation method). The metabolic activation system included 10% S9 fraction from Arochlor 1254 induced Sprague-Dawley rat liver.

Study Validity and Positive Response Criteria

- Tester Strain Integrity – *His*$^-$, *trp*$^-$, *rfa*, *uvrA*, and *uvrB* mutations as well as *pKM101* plasmid expression must be demonstrated in the appropriate tester strains.
- Tester Strain Density – Each culture must reach optical density demonstrated to be representative of $\geq 10^9$ cells/mL.

Reference ID: 3628623

- Vehicle Controls - Mean revertants/plate must fall within ranges determined by historical control data and published reports.
- Positive Control Values - Positive control articles must produce ≥3x revertants/plate compared to concurrent vehicle controls.
- Number of Dose Levels – Must have a minimum of 3 non-toxic dose levels.
- Maximum Dose Level – Must be the lowest of 5000 µg/plate, produce significant cytotoxicity, or exceed the limit of solubility.
- Positive Response – The test article must produce a ≥2x increase in the mean revertants/plate vs. concurrent vehicle control for TA98, TA100, and WP2*uvr*A or ≥3x increase for TA1535 and TA1537.

Results

(b) (4) *- There were no drug-related increases in mean revertants/plate. Precipitate was observed at doses ≥ 500 µg/plate in TA100, TA1535, and TA1537 with or without metabolic activation. Precipitate occurred in TA98 and WP2uvrA at doses ≥ 1600 µg/plate with and without metabolic activation. Toxicity (e.g., decreased colony counts) was observed in experiments without metabolic activation including TA1535 at doses ≥ 50 µg/plate, TA1537 at doses ≥ 160 µg/plate, and WP2uvrA at 5000 µg/plate. Toxicity in experiments with metabolic activation was limited to 5000 µg/plate for TA1535, TA1537, and WP2uvrA. The laboratory's criteria for a valid study were met.*

(b) (4) *- There were no drug-related increases in mean revertants/plate. Precipitate was observed at doses ≥ 500 µg/plate in all tester strains with or without metabolic activation. Toxicity (e.g., decreased colony counts) was observed in TA98 and WP2uvrA at 5000 µg/plate without metabolic activation. The laboratory's criteria for a valid study were met.*

Title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with (b) (4) Free Form and (b) (4) Free Form (Study no. R&D/13/580)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/12/13
GLP compliance:	yes, with exceptions (e.g., incomplete test article characterization)
QA statement:	yes
Test article, lot #, and % purity:	(b) (4) lot#10047583-0103B, purity = 98.2% (b) (4) lot#10047583-0104B, purity = 98.8%

Key Findings

- (b) (4) *was negative in the in vitro chromosomal aberration assay.*
- (b) (4) *was negative in the in vitro chromosomal aberration assay.*

Reference ID: 3628623

Methods

Cell line: human peripheral blood lymphocytes

Concentrations in definitive study:

(b) (4)

S9	time	Doses[a]
-	3 hr	3.05, 4.36, 6.23, 8.90, 12.7, 18.2, 25.9, 37.1, 52.9, 75.6, 108, 154, 220, 315, and 450 µg/mL
	24 hr	3.05, 4.36, 6.23, 8.90, 12.7, 18.2, 25.9, 37.1, 52.9, 75.6, 108, 154, 220, 315, and 450 µg/mL
+	3 hr	3.05, 4.36, 6.23, 8.90, 12.7, 18.2, 25.9, 37.1, 52.9, 75.6, 108, 154, 220, 315, and 450 µg/mL

[a] underlined doses were scored for aberrations

(b) (4)

S9	time	Doses[a]
-	3 hr	3.39, 4.84, 6.92, 9.89, 14.1, 20.2, 28.8, 41.2, 58.8, 84.0, 120, 172, 245, 350, and 500 µg/mL
	24 hr	3.39, 4.84, 6.92, 9.89, 14.1, 20.2, 28.8, 41.2, 58.8, 84.0, 120, 172, 245, 350, and 500 µg/mL
+	3 hr	3.39, 4.84, 6.92, 9.89, 14.1, 20.2, 28.8, 41.2, 58.8, 84.0, 120, 172, 245, 350, and 500 µg/mL

[a] underlined doses were scored for aberrations

Basis of concentration selection: Top doses evaluated for chromosomal aberrations resulted in ~50% cytotoxicity or were the lowest concentration where minimal precipitate was observed.

Negative control: DMSO

Positive control:

S9	Control	Dose
-	MMC	3 hr = 1.0 µg/mL 230 hr = 0.3 µg/mL
+	cyclophosphamide	25 µg/mL

Formulation/Vehicle: DMSO

Incubation & sampling time: 3 hr with and without metabolic activation and 24 hr without metabolic activation. All incubations were at 37 °C. Colcemid was present for the last 2 hr before harvest of mitotic cells. The metabolic activation system contained S9 fraction (1.5%) from Arochlor 1254-induced Sprague-Dawley rat liver.

Study Validity and Positive Response Criteria

- Vehicle Controls – Must contain < ~5% cells with aberrations.

Reference ID: 3628623

- Positive Controls – The % of cells with aberrations must be significantly higher ($p \leq 0.01$) than the vehicle controls.
- High Dose – If the aberration results are negative and there is no significant reduction (~50%) in mitotic index, the assay must include the highest applicable dose (target dose of 1 mM or 0.5 mg/mL, whichever is lower) or a dose exceeding the solubility limit in culture medium.
- Number of Doses – The assay must include ≥ 3 analyzable concentrations.
- Positive Response – Must have a significant increase ($p \leq 0.01$) in the number of cells with chromosomal aberrations at ≥ 1 concentration. A dose-response should be observed if a significant increase was seen at ≥ 1 concentration.

Results

(b) (4) – *There were no drug-related increases in the number of cells with chromosomal aberrations, polyploidy, or endoreduplication. Cytotoxicity (i.e., reduction in mitotic index) at the top doses were 0% for 3 hr incubations with metabolic activation, 54% for 24 hr incubations without metabolic activation, and 48% for 3 hr incubations with metabolic activation. Precipitate was observed at the highest dose evaluated for the 3 hr incubations with or without metabolic activation. The laboratory's criteria for a valid study was met.*

(b) (4) – *There were no drug-related increases in the number of cells with chromosomal aberrations, polyploidy, or endoreduplication. Cytotoxicity (i.e., reduction in mitotic index) at the top doses were 0% for incubations with metabolic activation and 42% for 3 hr incubations with metabolic activation. Precipitate was observed at the highest dose evaluated for all experimental conditions. The laboratory's criteria for a valid study was met.*

Title: Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with (b) (4) Free Form and (b) (4) Free Form (Study no. R&D/13/1119)

Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12/7/13
GLP compliance:	yes, with exceptions (e.g., incomplete test article characterization)
QA statement:	yes
Test article, lot #, and % purity:	(b) (4) lot#10032614-0530-1, purity = 99.9% (b) (4), lot#10032614-0529-1, purity = 99.6%

Key Findings

- (b) (4) *was negative in the bacterial reverse mutation assay.*
- (b) (4) *was negative in the bacterial reverse mutation assay.*

Reference ID: 3628623

Methods

Strains: TA98, TA100, TA1535, TA1537, and WP2*uvrA*
Concentrations in definitive assay: 1.6, 5.0, 16, 50, 160, 500, 1600, and 5000 µg/plate
Basis of concentration selection: limit dose of 5000 µg/plate
Negative control: DMSO
Positive control:

Strain	S9	Control	Dose
TA98	-	2-nitrofluorene	1 µg/plate
	+	benzo[a]pyrene	2.5 µg/plate
TA100	-	sodium azide	2 µg/plate
	+	2-aminoanthracene	2.5 µg/plate
TA1535	-	sodium azide	2 µg/plate
	+	2-aminoanthracene	2.5 µg/plate
TA1537	-	ICR-191	2 µg/plate
	+	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	-	4-nitroquinoline-N-oxide	1 µg/plate
	+	2-aminoanthracene	25 µg/plate

Formulation/Vehicle: DMSO
Incubation & sampling time: Plates were incubated for ~52 hr at 37 °C with or without metabolic activation (plate incorporation method). The metabolic activation system included 10% S9 fraction from Arochlor 1254 induced Sprague-Dawley rat liver.

Study Validity and Positive Response Criteria

- Tester Strain Integrity – *His⁻, trp⁻, rfa*, *uvrA*, and *uvrB* mutations as well as *pKM101* plasmid expression must be demonstrated in the appropriate tester strains.
- Tester Strain Density – Each culture must reach optical density demonstrated to be representative of $\geq 10^9$ cells/mL.
- Vehicle Controls - Mean revertants/plate must fall within ranges determined by historical control data and published reports.
- Positive Control Values - Positive control articles must produce ≥3x revertants/plate compared to concurrent vehicle controls.
- Number of Dose Levels – Must have a minimum of 3 non-toxic dose levels.
- Maximum Dose Level – Must be the lowest of 5000 µg/plate, produce significant cytotoxicity, or exceed the limit of solubility.
- Positive Response – The test article must produce a ≥2x increase in the mean revertants/plate vs. concurrent vehicle control for TA98, TA100, and WP2*uvr*A or ≥3x increase for TA1535 and TA1537.

Results

(b) (4) *- There were no drug-related increases in mean revertants/plate. Precipitate was observed at doses ≥ 160 in all tester strains with or without metabolic activation. Toxicity (e.g., decreased colony counts) was observed in TA1537 at doses 5000 µg/plate without metabolic activation and doses ≥ 500 µg/plate with metabolic activation. The laboratory's criteria for a valid study were met.*

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(b) (4) *- There were no drug-related increases in mean revertants/plate. Precipitate was observed at doses ≥ 500 µg/plate in all tester strains without metabolic activation. Precipitate was also noted at doses ≥ 1600 µg/plate with metabolic activation for all tester strains except WP2uvrA were it was observed at doses ≥ 500 µg/plate. Test article analysis indicated that the high-dose formulation was ~50% of the target concentration. The laboratory's criteria for a valid study were met.*

Reference ID: 3628623

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/s/

MARK J SEATON
09/16/2014

HANAN N GHANTOUS
09/16/2014
I concur with the recommendation of Dr. Mark Seaton that Viekira Pak be approved.

Comments on N206619a VIEKIRA

From. A. Jacobs, AD

Date: Sept 10, 2014

1. I concur that there are no pharm-tox approval issues.

Some other comments were conveyed to the reviewer and they will be addressed as appropriate.

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/s/

ABIGAIL C JACOBS
09/11/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 206-619 **Applicant:** AbbVie, Inc. **Stamp Date:** April 21, 2014

Drug Name: ABT-267/ABT-450/ritonavir + ABT-333 **NDA/BLA Type:** 3-NME

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

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

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

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

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

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

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

/s/

MARK J SEATON
05/22/2014

HANAN N GHANTOUS
05/23/2014