CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203858Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 203858
Agency receipt date: February 29, 2012
Drug: lomitapide
Sponsor: Aegerion Pharmaceuticals Inc.
Indication: homozygous familial hypercholesterolemia (HoFH) as an adjunct to diet and other lipid-lowering treatments

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharm/tox reviewer and supervisor concluded that the nonclinical data support approval of lomitapide for the indication listed above.

Lomitapide is a microsomal triglyceride transfer protein inhibitor. This is a new pharmacologic class.

Lomitapide produced embryofetal death and fetal malformations in rats. Some effects were observed even at clinically relevant doses. Malformations were also noted in ferrets in a dose range-finding study. Adverse fetal effects were not observed in rabbits although the highest dose tested was only 3 times the clinical exposure. The sponsor and the primary and secondary pharm/tox reviewers recommended that the drug be contraindicated in pregnancy.

Lomitapide was tested in a two-year oral gavage carcinogenicity study in rats and a two-year dietary study in mice. These studies were reviewed by the division and the Executive Carcinogenicity Assessment Committee. The Committee found the mouse study to be acceptable in spite of high mortality. A drug-related increase in animals with hepatocellular adenomas, carcinomas, and combined adenomas or carcinomas was observed in male mice at ≥1.5 mg/kg/day and in female mice at ≥7.5 mg/kg/day. In addition, the combined incidence of animals with adenomas or carcinomas in the small intestine was observed at ≥15 mg/kg/day in both male and female mice. The Committee also found the rat study to be acceptable and no drug-related neoplasms were observed at doses producing up to approximately 6-8 fold the human exposure.

Given the nature of the indication, lomitapide might be used in pediatric patients. The pharm/tox reviewer and supervisor believe that the possible impact of lomitapide on the neurological development of young children has not been adequately assessed in animals. This concern arises from the critical role played by lipids in neuron development. In addition, lomitapide alters fat-soluble vitamin absorption. The impact of lomitapide on vitamin malabsorption has also not been assessed in young animals. The reviewer and supervisor have recommended that a Postmarketing Requirement for a juvenile rat study with and without

vitamin and fatty acid supplementation be considered to help address the impact lomitapide may have on learning and memory in pediatric patients. The division is recommending that this be completed prior to trials in pediatric patients.

Conclusions:
I agree with the division pharm/tox conclusion that lomitapide can be approved from the pharm/tox perspective. I agree that lomitapide appears to carry risk for developing fetuses and, therefore, contraindication in pregnancy seems appropriate. I also agree that a Postmarketing Requirement for a juvenile animal study should be considered to help address the outstanding uncertainty around the impact of lomitapide on neurological development of young children.

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/s/

PAUL C BROWN
12/07/2012

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: November 13, 2012
From: Karen Davis-Bruno PhD; Pharmacology Supervisor; DMEP
Subject: Supervisory Pharmacology/Toxicology Memo
To: NDA 203-858

Reference is made to the Pharmacology/Toxicology Review of NDA 203-858 which recommends approval, which I am in agreement with.

Lomitapide inhibits microsomal triglyceride transfer protein (MTP) an intracellular protein that regulates lipid transfer between membranes in the lumen of the endoplasmic reticulum. MTP inhibition prevents ApoB-lipoprotein (LDL-C) assembly in various tissues including hepatocytes and enterocytes reducing the production of lipid containing particles into the systemic circulation. This mechanism differs from currently approved lipid lower drugs which act by increasing the clearance of LDL-C systemically.

Toxicology: Repeat dose toxicity studies were performed in rats, dogs and mice. The most significant effect was lipid accumulation in the liver, small intestine, and lungs. This was observed at comparable human therapeutic exposures and is consistent with the pharmacology of the drug. While reversibility of the lipid accumulation was demonstrated with discontinuation of drug treatment in rats, fat accumulation remains with long-term treatment and involves a potential risk of hepatotoxicity and perhaps liver carcinogenicity. In addition to lipid accumulation, hepatocellular lipid vacuolation is seen minimal-marked severity in the 6 month rat at 2 mg/kg/day (≥2X human AUC @ MRHD=60 mg) in addition to multifocal subacute inflammation in all females at this dose. Hepatic lipid accumulation is seen in dogs treated for 6 months at doses ≥1 mg/kg/day (17X human AUC @ 60 mg) and minimal vacuolization. Increases in liver enzymes (ALT, AST, ALP) were observed in drug treated rats (3-fold increase over controls) and dogs (7-fold mean increase and up to 25-fold individually). Patients with abetalipoproteinemia have elevated transaminases so this is not an unexpected finding.

Increased incidences of multifocal minimal-moderate liver fibrosis and cystic degeneration (males; minimal-moderate) were observed with treatment ≥ 93 weeks in rats. The fibrosis was considered random and did not involve the majority of the liver. The sponsor attributes the hepatic related histopathology in the rat to greater hepatic drug and metabolite levels than the dog despite higher plasma exposures. This may account for the larger spectrum of hepatic related histopathology.

Tissue levels of lomitapide and metabolites were assessed in the 6-month rat and dog toxicity studies. Although dog had higher plasma exposure at half the rat dose the rat liver and lung tissue had higher exposure to lomitapide. The elevated tissues levels may

explain the dose-related incidence and severity of findings in the rat compared to the dog.

6-Month Toxicity	Plasma AUC (ng*h/ml)		Liver (ng/g)		Lung (ng/g)	
Lomitapide Dose	M	F	M	F	M	F
Rat (2 mg/kg/d)	117	584	817	12579	782	7873
M1	13	53	BLQ	BLQ	BLQ	23
M3	331	1165	17	153	BLQ	40
Dog (1mg/kg/d)	430	685	286	686	741	206
M1	523		228	148	242	282
M3	289		BLQ	43	59	BLQ

BLQ=below limit of quantitation < 90 ng/g

Multifocal hemorrhages and increased APTT and PT were observed in rats at 20 mg/kg/day (>20X human AUC@ 60 mg). This was attributed to vitamin K deficiency due to inhibition of fat soluble vitamin absorption. The increased mortality associated with multifocal hemorrhages was reduced by weekly vitamin supplementation in conjunction with lompitapide dosing. Poikilocytosis and/or anisocytosis and decreased RBC parameters were observed in rats and dogs not associated with hemorrhage and in animals receiving vitamin supplementation. The initial 6-month rat toxicity study identified the vitamin K deficiency and therefore in subsequent 6-month rat and dog toxicity studies and rodent carcinogenicity studies, groups with and without vitamin supplementation were included. Fat soluble vitamin supplementation was not considered to impact the 6-month dog toxicity study and therefore the 12-month dog toxicity study did not have vitamin supplementation. None of the developmental/reproductive toxicity studies used vitamin supplementation.

Testicular tubular degeneration was observed in the 6-month dog toxicity study at 10 mg/kg/day (200X human AUC @ 60 mg) but not present at 5 mg/kg/day (>50X human AUC @ 60 mg) in a 12-month dog toxicity study. This observation was not observed in the rat. Based on the exposure at which this finding is observed it is unlikely to have clinical relevance in a therapeutic setting.

Lipid Accumulation in Tissues: Lipid accumulation was not restricted to the liver. The small intestine (absorptive epithelial cells) had lipid vacuolization (minimal to severe) in the rat and dog (minimal to moderate). Pulmonary histiocytosis was observed in mice (minimal), rats (minimal-marked; with foamy macrophages in the alveolar space), and dog (≥0.01 mg/kg/d at 6 months; at 12 months dogs given ≥0.05 mg/kg/d had pleural fibrosis at exposures less than human therapeutic exposure). Initial toxicology studies considered the pulmonary histiocytosis in rodents to be indicative of phospholipidosis. The basis of this diagnosis was ultrastructural transmission electron microscopy (TEM) evaluations of lungs from four lompitapide treated rats given 2 or 20 mg/kg/d (≥2X human AUC @ 60 mg human dose) which exhibited the characteristic concentric lamellar structures of phospholipidosis[1]. Aegerion performed further evaluations by

[1] Nonoyama 2008, J Toxicol Pathol ; Reasor 2006 Expert Opin Drug Safety

TEM in a 3 month study with rats given 4 mg/kg/d as well as during the carcinogenicity study where mice were given 45 mg/kg/d (79X human AUC @ 60 mg). The results of the later evaluations suggested that phospholipidosis was not present due to the absence of intra-lysosomal concentric lamellar inclusions. The histiocytosis noted under light microscopy was considered reflective of excessive accumulation of neutral lipids in the cytoplasm of alveolar macrophages.

Increased incidences of foamy alveolar macrophages (histiocytes) were observed in the lungs of lomitapide treated rodents. In mice, histiocytes were observed in alveolar spaces at ≥5X maximum clinical exposures after 3 months. Pulmonary histopathology performed during the mouse 2-year carcinogenicity study revealed increased incidences of alveolar spicules and lymphocyte infiltration at ≥22X maximum clinical exposures. While histiocytosis was not noted by light microscopy, a low incidence was observed when examined by electron microscopy. In rats multifocal histiocytes accumulation was observed in the alveoli and subpleural tissues at less than clinical therapeutic exposure after 3 months of treatment. Dose related histiocytosis characterized by multifocal aggregates of large foamy macrophages within alveoli with or without subacute inflammation, necrotic debris, cholesterol-like clefts and/or type II alveolar cell proliferation were observed in rats following 6 months of treatment at exposures less than the clinical therapeutic exposure. EM evaluations of this lung tissue characterized the histiocytosis as focal aggregates containing residual/lamellar inclusion bodies, lipid droplets and phagolysosomes varying in size, shape and electron density. Some occasional, adjacent type II cells were enlarged with lipid droplets and lamellar inclusion bodies. A second 6-month rat study confirmed the observation of pulmonary phospholipidosis. Special staining indicated the presence of phospholipids and neutral lipids in the macrophages and adjacent type II alveolar cells. Histiocytosis appeared to be recoverable following a 6 months drug free period. These studies were performed early in development and subsequent studies with different pathologists did not confirm the pulmonary phospholipidosis which is characterized by the excessive accumulation of concentric lamellar inclusions in the lysosomes of macrophages or other pulmonary cells.

In the rat 2-year carcinogenicity study increased incidence of alveolar spicules, lymphocyte infiltration with macrophage accumulation and thickened alveolar speta with macrophage infiltrates were observed at less than therapeutic exposures. An increase in septal cell mineralization was noted for male rats given lomitapide at 2X therapeutic exposure. Pleural/subpleural fibrosis was observed in female rats at this same exposure level. Although concentric lamellar inclusions were seen in rats they rarely observed in lysosomes and were present in cells from both control and lomitapide treated animals. No evidence of chronic interstitial inflammation in the alveolar wall adjacent to vacuolated macrophages was observed. Subsequent pathology examinations from longer duration studies (2-year carcinogenicity) showed some lipid accumulation but the findings were inconsistent with classical phospholipidosis. In dogs treated for one year, a low incidence of minimal to mild pleural fibrosis was observed at ≥3X therapeutic exposure. A slight increase in minimal focal mineraliaiton and mild chronic-active inflammation was observed in males and minimal to mild alveolar edema was seen in females in the absence of histiocytosis at >55X therapeutic exposures. Lung histiocytosis

was observed across dose groups but not in a dose-related incidence in the dogs treated for 6 months at high lomitapide exposures (>100X therapeutic exposure). Chronic inflammation and edema were observed across dose levels at an incidence and severity similar to controls. This suggests that the finding may have been an incidental background finding in dog.

EM evaluation of lung tissue from mice treated for 2 years indicated that excessive lipid accumulation in macrophages occurred in the absence of concurrent epithelial degeneration or inflammatory changes in the alveolar wall. The sponsor did evaluate the function of rat macrophages isolated by bronchioalveolar lavage after 3 months of treatment. The results showed that the isolated macrophages had similar phagocytic and respiratory burst activity as control macrophages, although it is felt that the design of the study could have been improved to obtain more definitive results.

Genotoxicity testing in a bacterial mutagenicity (Ames) assay, chromosomal aberration study in human lymphocytes and oral rat micronucleus assay were negative, demonstrating that lomitapide does not interact with DNA or pose a genotoxic risk.

Carcinogenicity was assessed in a 2-year mouse bioassay by dietary administration at 0.3, 1.5, 7.5, 15 or 45 mg/kg/day. Survival issues resulted in some mice being on study without being dosed from week 90 to study termination. Female dose groups were sacrified at week 99, mid dose males on week 101 and high dose males on week 101. Despite the high mortality ECAC considered the study to be acceptable. Drug related neoplasms included: hepatocellular adenomas, carcinomas and combined adenomas or carcinomas in males at ≥1.5 mg/kg/day (2X human AUC @ 60 mg) and in females at ≥7.5 mg/kg/day (9X human AUC@60 mg); combined incidence of mice with adenomas or carcinomas in the small intestine of males and females at ≥15 mg/kg/day (>20X human AUC @ 60 mg).

A 2-year bioassay was conducted in rats which were given 0.25, 1.7, 7.5 mg/kg/day in males or 0.03, 0.35, 2 mg/kg/day in females by oral gavage. The low dose females were sacrificed week 94 and remaining female dose groups by week 97. All the male dose groups were sacrificed during week 98. ECAC considered the study suboptimal and cautioned that the vehicle (75% PEG-400) may have affected the incidence of pancreatic acinar neoplasms. However drug-related neoplasms were not observed at 6-8X human AUC @ 60 mg.

Reproductive/Developmental Toxicity: Embryonic death and major fetal malformations were noted in rats at drug exposures that are approximately 10-fold higher than the anticipated clinical exposure. Some malformations and developmental delays were also noted in rats at clinically relevant exposures. Effects in rats appeared to occur in the absence of overt maternal toxicity as decrements in maternal body weight were only observed at the high dose (10-fold clinical exposure) and were likely due to embryonic death and decreased fetal weights that occurred at the high dose. Similar findings were not observed in rabbits at clinically relevant doses; however, higher doses (> 3-fold clinical exposure) were not tested. Treatment with AEGR-733 was associated with

malformations in ferrets at doses that are below the proposed clinical dose; however, these effects occurred in conjunction with signs of maternal toxicity (significant body weight loss and decreased food consumption). Based on the findings in rats and ferrets, the risk of embryonic death and/or teratogenicity at clinical exposures is possible unless additional information currently under review suggests a species specific explanation for the developmental findings observed. Therefore, pregnant women should not take lomitapide and women should take precautions so that they do not become pregnant while taking lomitapide. Women who become pregnant while taking lomitapide should consider discontinuation of lomitapide based on their risk:benefit with their physcician. Additional safety information will be needed prior to consideration of any pediatric dosing.

The indicated patient population having homozygous familial hypercholesterolemia (HoFH) will likely have concomitant lipid lowering medications such as statins administered as well as lomitapide based on their presumed high cardiovascular risk. Statin are contraindicated in pregnancy based on the clinical risk:benefit with similar reproductive toxicity profiles in animals to lomitapide. This contraindication is managed by product labeling for statins and can be similarly managed with lomitapide.

Lomitapide Reprotox Summary prepared by Tim Hummer PhD

Type of Study Study Number	Species/Strain	Dosing Duration	Dose Level (mg/kg/d)	Exposure Margin*	Key Findings
Fertility and early embryonic development #733PC0001	Rat/ Sprague-Dawley (25/group)	15 days before mating through GD7; necropsy on GD13	0.04	<1X	No treatment-related effects.
			0.2	~1X	No treatment-related effects.
			1 (NOAEL)	~3X	No treatment related effects on maternal body weight, food consumption, fertility indices, number of corpora lutea, implantation sites, or viable embryos. (maximum tolerated dose was not used as the high dose for this study. 4 mg/kg/d would have been better)
Embryo-fetal development #96039	Rat/ Sprague-Dawley (22/group)	GD6 through GD15; necropsy on GD20	0.04 (NOAEL)	<1X	No treatment-related effects.
			0.4	~2X	• Slight decrease in fetal body weights. • The following fetal malformations were observed: o Abdomen: umbilical hernia, gastroschisis o Tail: short, stubbed, bent, or absent o Heart: alterations in size and/or shape

					○ Limbs: malrotation ○ Anus: imperforate • The following fetal variations were observed: ○ Delays in ossification of the crainial, vertebral, and pelvic bones
			4	~10X	• Decreased maternal body weight gain, especially between GD16 and GD20, likely due to decreased litter size. • Increased maternal spleen weights. • Post-implantation loss of ~50% due primarily to early resorptions. • Decreased mean fetal body weights. • The following fetal malformations were observed: ○ Abdomen: umbilical hernia, gastroschisis ○ Tail: short, stubbed, bent, kinked, curled, filamentous ○ Heart: alterations in size and/or shape ○ Limbs/paws: malrotation, shortening ○ Anus: imperforate ○ Brain: exencephaly, hydrocephaly, cerebral hernia, misshaped cerebral hemispheres • The following fetal variations were observed: ○ Delays in ossification of the crainial, vertebral, pelvic, sternal, and metacarpal bones ○ Variations in development of brain: slight dilation of the lateral and/or third ventricles ○ Skin: friable
Embryo-fetal development	Rabbit/ New Zealand white	GD6 through	0.1	0.03X[†]	No treatment-related effects.
			1	0.3X[†]	• Maternal body weight

#96032	(20/group)	GD20; necropsy on GD29			gain was 65% less than controls during the treatment period. • No treatment-related effects on number of implantation sites, resorptions, number of viable fetuses, litter size, male/female ratio, fetal body weights, or fetal alterations.
			10 (NOAEL)	3X†	• Maternal body weight gain was 76% less than controls during the treatment period. • No apparent treatment-related effects on number of implantation sites, resorptions, number of viable fetuses, litter size, male/female ratio, fetal body weights, or fetal alterations.

Embryo-fetal development (range-finding study) #97008	Ferret (6/group)	GD12 through GD28; necropsy on GD35	1.6 (NOAEL not identified)	0.3X†	• Profound body weight loss between GD12 and GD29 - 15% loss from GD12 weight, which correlated with decreased food consumption. • Mean fetal body weights were slightly lower than controls. • The following fetal malformations were observed at a low incidence (1-4 fetuses and 1-2 litters per finding: o Limbs/paws: rotated medially, digits absent or fused o Head: cleft palate, red eyes, open eye lids, low set ears o Kinked tail o Body: umbilical hernia
			4	0.8X†	• Profound body weight loss between GD12 and GD29 - 18% loss from GD12 weight, which correlated with decreased food consumption. • Increased vomiting • Slight increase in

					resorptions, resulting in slight decrease in number of live fetuses/litter. • The following fetal malformations were observed at a low incidence, except for limbs rotated medially and umbilical hernia (5-9 fetuses and 3-4 litters): o Limbs/paws: rotated medially or short, digits absent or fused o Head: red eyes, open eye lids o Body: umbilical hernia
			10	2X†	• Profound body weight loss between GD12 and GD29 - 21% loss from GD12 weight, which correlated with decreased food consumption. • Increased vomiting • Increased resorptions, resulting in decreased number of live fetuses/litter. • Mean fetal body weights were lower than control. • The following fetal malformations were observed at a low incidence, except for limbs rotated medially and umbilical hernia (11-16 fetuses and 4-5 litters): o Limbs/paws: rotated medially or shortened, digits absent o Head: red eyes, open eye lids, low set ears o Kinked tail o Body: umbilical hernia
			25	5X†	• Profound body weight loss between GD12 and GD29 - 24% loss from GD12 weight, which correlated with decreased food consumption. • Increased vomiting

					• Increased resorptions, resulting in decreased number of live fetuses/litter. • Mean fetal body weights were lower than control. • The following fetal malformations were observed at a low incidence, except for limbs rotated medially and umbilical hernia (19 fetuses and 5 litters): o Limbs/paws: rotated medially, digits fused o Head: cleft palate, cleft facial, red eyes, open eye lids, low set ears o Short tail o Body: umbilical hernia o Skull: depressed
Peri- and post-natal development #AEGR-733PC0014	Rat/ Sprague-Dawley (25/group)	GD7 through LD20	0.1 (NOAEL for F1 development)	<1X	No treatment-related effects
			0.3 (NOAEL for F0 reproduction)	~1X	• Slightly lower maternal body weights, likely due to lower fetal body weights. • Fetal eye anomalies (missing eye, microphthalmia) at low incidence. 2 pups had dilation of the lateral ventricles of the brain
			1 (NOAEL for F0 maternal toxicity)	~3X	• Lower maternal body weights, likely due to decreased litter size and lower fetal body weights. • Slightly increased gestation period • Decreased indices of live liter size/litter, number of live pups, viability index, lactation index, and fetal body weight. • Increased number of stillborn pups and pups dying on LDs 1-7. • Fetal eye anomalies (missing eye,

					microphthalmia) at low incidence. Increase in tail anomalies (bent, short, tail or tip of the tail missing or absent, purple, red and/or black), pale body, or a head mass. • 1 pup had dilation of the lateral ventricles of the brain and 1 pup and malformed forelimb. • F1 generation rats were not able to overcome the deficits in postpartum body weights (remained lower after weaning). • No statistically significant or biologically important differences occurred in watermaze performance of the F1 generation male and female rats regarding learning, short-term retention, long-term retention, or response inhibition. • There were no statistically or biologically important effects on the mating and fertility parameters evaluated in the F1 generation male and female rats. • All fetal gross alterations of the F2 generation were considered unrelated to in utero maternal (F1) exposure.

*Estimated based on exposure data for non-pregnant animals.
†Based on body surface area extrapolation; exposure data not available for this species.
GD = gestation day; LD = lactation day; NOAEL = no observed adverse effect level.

Proposed Labeling:

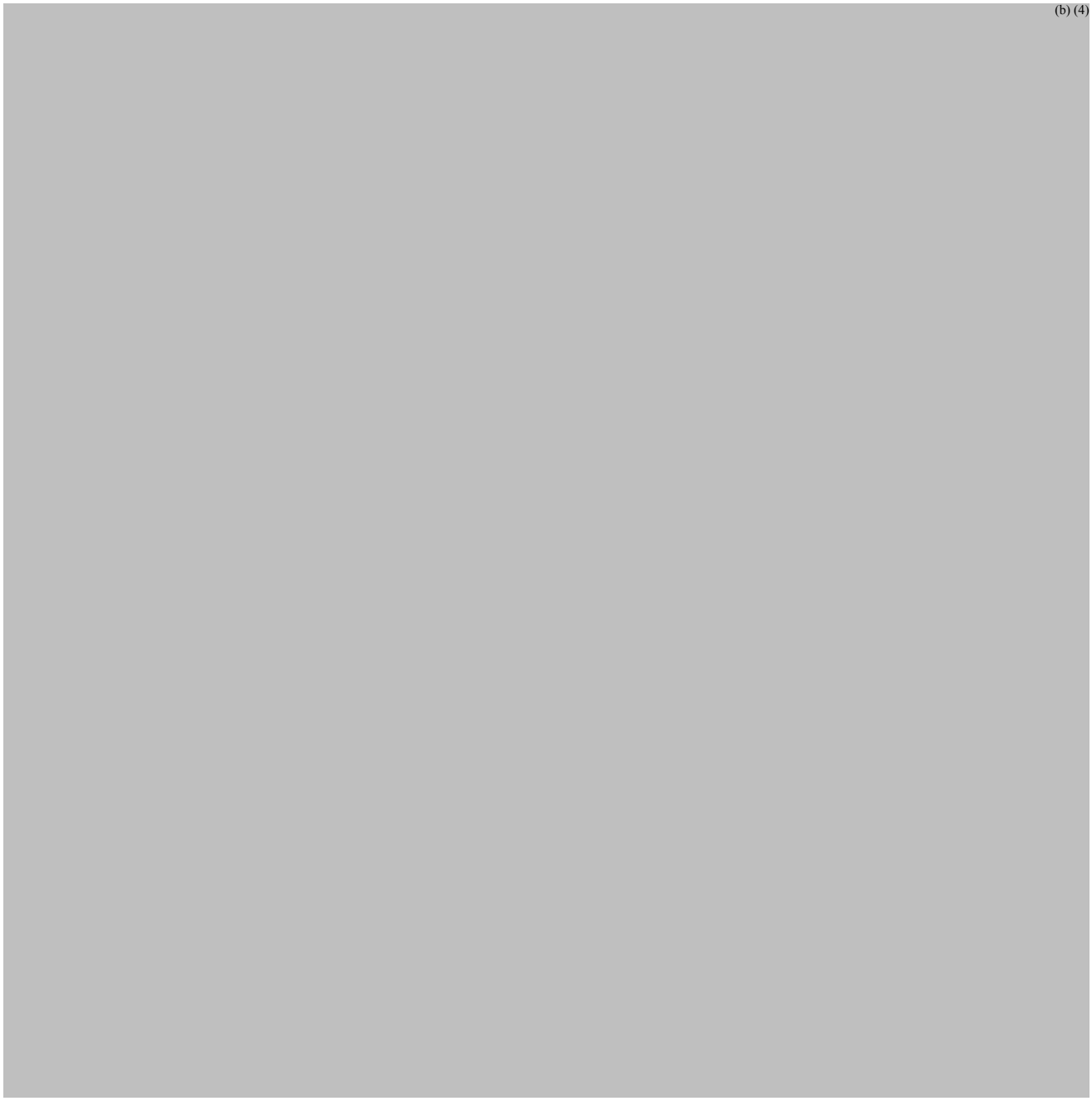
8.1 Pregnancy

Pregnancy Category X.

Risk Summary

TRADENAME is contraindicated during pregnancy because TRADENAME may cause fetal harm when administered to a pregnant woman. Lomitapide was teratogenic in rats and ferrets at exposures estimated to be less than human therapeutic exposure at 60 mg (AUC<67 ng h/ml) when administered during organogenesis. There was no evidence of teratogenicity in rabbits at 3 times the maximum recommended human dose (MRHD) of 60 mg based on body surface area. Embryo-fetal lethality was observed in rabbits at 6-times the MRHD. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to a fetus.

(b) (4)



decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

Safety and effectiveness have not been established in pediatric patients.

8.8 Females of Reproductive Potential

TRADENAME may cause fetal harm [see *Use in Specific Populations (8.1)*]. Females who become pregnant during TRADENAME therapy should stop TRADENAME immediately and notify their healthcare provider.

Pregnancy testing

Females of reproductive potential should have a negative pregnancy test before starting TRADENAME.

Contraception

Females of reproductive potential should use effective contraception during TRADENAME therapy.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Lomitapide did not exhibit genotoxic potential in a battery of studies, including the *in vitro* Bacterial Reverse Mutation (Ames) assay, an *in vitro* cytogenetics assay using primary human lymphocytes, and an oral micronucleus study in rats.

Lomitapide had no effect on fertility in rats at doses up to 5 mg/kg/day at systemic exposures estimated to be 4-times (females) and 5-times (males) higher than in humans at 60 mg based on AUC.

Nonclinical Support for Pediatric Use: Based on the severity of dyslipidemia in the HoFH population, it is likely that lomitapide will be used in the pediatric population. Based on the age of the dogs (6-months) at the initiation of the chronic dog toxicity study it is likely that the results of this study would be informative for an adolescent population. This is similar for the 7-8 week old rat at initiation of the chronic rodent toxicity studies. The pre- and post-natal developmental study assessed rats up to 20 days old which developmentally equivalent to a child (~3 yrs). In this study there were no biologically important differences in learning or memory (short or longterm). Only the 6-month toxicity and carcinogenicity studies utilized vitamin supplementation in conjunction with lomitapide. There is an informational gap for rats postnatal day 20-50 for assessment of learning and memory. Based on this consideration of a juvenile rat study ± fat soluble vitamin and essential fatty acid supplementation as a PMR can be considered to address concerns for malabsorption and any potential for learning and memory prior to lomitapide exposure in developing pediatrics • 5 years old.

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/s/

KAREN L DAVIS BRUNO
11/13/2012
Pharm/Tox Supervisory Memo

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:	203858
Review number	1
Supporting document/s:	1
Applicant's letter date:	28 February 2012
CDER stamp date:	29 February 2012
Product:	Lomitapide (AEGR-733)
Indication:	Hypercholesterolemia
Applicant:	Aegerion Pharmaceuticals, Inc.
Review Division:	Metabolism and Endocrinology Products
Reviewer:	B. Timothy Hummer, PhD, DABT
Supervisor/Team Leader:	Karen Davis-Bruno, PhD
Division Director:	Mary Parks, MD
Project Manager:	Kati Johnson

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203858 are owned by Aegerion Pharmaceuticals, Inc. or are data for which Aegerion Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 203858 that Aegerion Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203858.

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1 Executive Summary

1.1 Introduction

Lomitapide is a microsomal triglyceride transfer protein (MTP) inhibitor that has been developed to reduce cholesterol and triglycerides in patients with homozygous familial hypercholesterolemia (HoFH), an orphan indication. Lomitapide is the first MTP inhibitor being reviewed by the FDA for marketing approval in the United States. Lomitapide is intended to be used as an adjunct therapy to diet and other lipid-lowering medications. The recommended starting dose will be 5 mg taken orally once daily. After 2 weeks, the dose can be escalated to 10 mg followed by incremental increases to 20, 40, and 60 mg at a minimum of 4-week intervals.

1.2 Brief Discussion of Nonclinical Findings

Treatment with lomitapide resulted in the accumulation of neutral lipids in small intestinal epithelial cells and hepatocytes in mice, rats, and dogs. Lipid accumulation in alveolar macrophages was also observed in mice and rats. These findings in liver and intestine are considered to be related to the pharmacodynamic activity of lomitapide by inhibiting the incorporation of lipids with apolipoprotein B (apo B), thereby resulting in excess intracellular lipids. Neutral lipid accumulation in alveolar macrophages is also likely related to the pharmacodynamic activity of lomitapide.

Lipid accumulation in small intestine occurred at clinically relevant exposures in mice, rats, and dogs and was shown to be reversible in mice after a 2-year exposure. Lipid accumulation in the small intestine was not associated with signs of toxicity or inflammation. It is anticipated that a similar effect will occur in humans with uncertain safety implications.

Lipid accumulation in hepatocytes occurred at or near clinically relevant exposures in mice, rats, and dogs. In rats treated for 6 months, lipid vacuolation had reversed after a 6-month treatment-free period. Lipid accumulation was also generally associated with statistically significantly increased liver weights in all species tested. Slight increases in mean serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) were noted in rats and dogs. Slightly larger increases in mean serum alkaline phosphatase (ALP) were also observed in rats. In rats, minimal single cell necrosis and minimal subacute inflammation was seen in the liver after 3 and 6 months of treatment at clinically relevant exposures. After 2 years of dosing in rats, an increase in the incidence and severity (minimal to moderate) of focal/multifocal fibrosis and centrilobular hepatocyte vacuolation were observed at clinically relevant exposures. These changes were not associated with an increased incidence of hepatocellular tumors.

Because treatment-related fatty liver has been observed clinically, this effect has already been identified to occur in humans with uncertain safety implications. Humans with steatosis are at risk for developing nonalcoholic steato-hepatitis, especially when other risk factors are present, such as insulin resistance. Because other risk factors are

generally not present in laboratory animals, it is difficult to predict how the presence of hepatocellular lipid accumulation observed in nonclinical studies will translate to clinical safety in the general population.

In the 2-year rat carcinogenicity study, hepatic fibrosis was observed, which appeared to correlate with the presence of hepatocyte vacuolation. However, after 2 years of treatment in mice and 1 year of treatment in dogs, hepatocellular lipid vacuolation was not observed even though it was observed in shorter duration studies. In humans it has been noted that the degree of lipid accumulation in the liver appears to plateau and maybe even reverse in some patients with continued lomitapide treatment. The observation of apparent reversibility of fatty liver in mice, dogs, and humans in spite of continued lomitapide treatment could indicate that hepatocytes may be able to regulate intracellular lipid levels over time, thereby reducing the risk for long-term liver injury associated with fatty liver. However, this possibility remains uncertain and therefore, patients should be adequately monitored for the development of fatty liver and potential liver injury.

An increased incidence in the presence of foamy alveolar macrophages (histiocytes) was observed in rats at clinically relevant exposures and in mice at slightly greater than clinical exposure. The severity of histiocytosis was generally minimal in mice and minimal to mild in rats. Histiocytosis appeared to be reversible after a 6-month recovery period in rats. In dogs, histiocytosis was not observed at a higher incidence or severity compared with the control group. Based on the most recent electron microscopy (EM) evaluations, the excessive vacuolation was described as morphologically consistent with neutral lipid vacuoles. Earlier EM examinations characterized the histiocytosis as a mixture of both neutral lipid and phospholipid vacuoles. It is uncertain why the conclusions regarding potential phospholipidosis have changed. It is also uncertain whether there is a meaningful difference between the presence of neutral lipids versus phospholipids with regard to pulmonary function and overall safety.

EM evaluation of lung tissue from mice treated for 2 years indicated that excessive lipid accumulation in macrophages occurred in the absence of concurrent epithelial degeneration or inflammatory changes in the alveolar wall. The sponsor did evaluate the function of rat macrophages isolated by bronchioalveolar lavage after 3 months of treatment. The results showed that the isolated macrophages had similar phagocytic and respiratory burst activity as control macrophages, although it is felt that the design of the study could have been improved to obtain more definitive results.

After long-term treatment in dogs (1 year) and rats (2 years), there were some apparent treatment-related findings in lung. In the dog study, there was a low incidence of minimal to mild pleural fibrosis as well as minimal focal mineralization, mild chronic active inflammation, and minimal to mild alveolar edema. With the exception of pleural fibrosis, which was observed in both genders at the mid- and high-dose levels, these findings were only observed in a single gender (1 or 2 animals out of 4) at the high-dose level. Although these pulmonary findings were generally low with regard to both incidence and severity, the fact that they only occurred at the highest dose levels

suggests that they could be drug-related rather than incidental findings. In the 2-year rat study, there were increases in the incidence of alveolar spicules, lymphocyte infiltration with macrophage accumulation, thickened alveolar septa with macrophage infiltrates, septal cell mineralization, and pleural/subpleural fibrosis. Although these findings were also observed in the control animals at a low incidence, the incidence and severity generally increased in a dose-related manner suggesting that the findings were treatment related.

Treatment for two years in mice resulted in an increased incidence of hepatocellular tumors at clinically relevant exposures in males and at approximately 9-fold higher than clinical exposures in females. Evaluation of liver tissue did not reveal an increase in liver toxicity or chronic inflammation, which are potential mechanisms for tumor development. Some liver fibrosis was observed in the rat carcinogenicity study but an increase in hepatocellular tumors was not observed in rats. A small, but statistically significant increase in small intestinal tumors was also observed in mice but only at approximately 25-fold expected clinical exposures. The high clinical exposure margin in conjunction with the fact that this tumor type was only observed in a single species lowers the overall concern for human safety with regard to small intestinal tumors. Overall, it is felt that the possible tumor risk with lomitapide treatment in humans can be adequately addressed in the label.

Treatment with lomitapide during the period of organogenesis resulted in embryonic death and fetal malformations of the abdomen, limbs, tail, and head in rats and ferrets at clinically relevant exposures. Treatment with lomitapide in pregnant rabbits resulted in embryonic loss at doses that are approximately 6.5-fold higher than the clinical dose of 60 mg (based on body surface area extrapolation). Because of the observed teratogenic activity in rats and ferrets, it is recommended that lomitapide should not be administered to pregnant women or to women who may become pregnant.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data submitted in this application support the marketing approval of lomitapide for the treatment of HoFH.

1.3.2 Additional Nonclinical Recommendations

Because of a concern for off-label use of lomitapide in pediatric patients, the nonclinical review team is recommending that the sponsor conduct a juvenile toxicology study as a post-marketing requirement. This study should be designed to evaluate the effects of lomitapide on learning, memory, and behavior with and without vitamin supplementation to determine whether any observed effects are due directly to lomitapide or secondarily to the inhibition of absorption of fat soluble vitamins. The study should be completed before any formal pediatric studies are initiated.

1.3.3 Labeling

Section 8 Pregnancy Category X (see Section 4 Contraindications)

(b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Lomitapide did not exhibit genotoxic potential in a battery of studies, including the in vitro Bacterial Reverse Mutation (Ames) assay, an *in vitro* cytogenetics assay using primary human lymphocytes, and an (b) (4) oral micronucleus study in rats.

Lomitapide had no effect on fertility in rats at doses up to 5 mg/kg/day at systemic exposures estimated to be 4-times (females) and 5-times (males) higher than in humans at 60 mg based on AUC.

13.2 Animal Toxicology and/or Pharmacology

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 202914-84-9

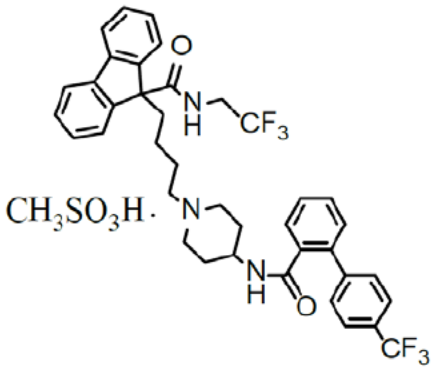
Generic Name: Lomitapide, lomitapide mesylate

Code Name: AEGR-733, BMS-201038, BMS-201038-04

Chemical Name: methanesulfonic acid; N-(2,2,2-trifluoroethyl)-9-[4-[4-[[2-[4-(trifluromethyl)phenyl]benzoyl]amino]piperidin-1-yl]fluorine-9-carboxamide

Molecular Formula/Molecular Weight: $C_{39}H_{37}N_3O_2F_6 \bullet CH_4SO_3$ / 693.7 (789.8 with salt)

Structure or Biochemical Description:



Pharmacologic Class: Microsomal triglyceride transfer protein (MTP) inhibitor - (first in class)

2.2 Relevant INDs

IND 50,820 - lomitapide - Aegerion

(b) (4)

2.3 Drug Formulation

Components and Composition of Lomitapide Capsules (modified from Sponsor's table)

Component	Function	Grade	Amount per Capsule		
			5 mg	10 mg	20 mg
(b) (4)					
Lomitapide mesylate[1]	Active ingredient	In house	5.69 mg (5.00 mg free base)	11.39 mg (10.00 mg free base)	22.77mg (20.00 mg free base)
Pregelatinized starch	(b) (4)	NF, Ph.Eur.			
Microcrystalline cellulose		NF, Ph.Eur.			
Lactose monohydrate[2]		NF, Ph.Eur.			
Sodium starch glycolate		NF, Ph.Eur.			
(b) (4)					
Colloidal silicon dioxide (USP/ (b) (4)	(b) (4)	USP, Ph.Eur			
Magnesium stearate		NF, Ph.Eur.			
Sodium starch glycolate		NF, Ph.Eur.			
Total amount			100.00 mg	200.00 mg	200.00 mg
Drug Product Capsules					
Capsule body: Size 1	Capsule shell	In house	1 (combined weight of body and cap = 76 mg) Swedish orange gelatin imprinted with "5 mg"	1 (combined weight of body and cap = 76 mg) Swedish orange gelatin imprinted with "10 mg"	1 (combined weight of body and cap = 76 mg) White gelatin imprinted with "20 mg"
Capsule cap: Size 1	Capsule shell	In house	1 Swedish orange gelatin imprinted with "A733"	1 White gelatin imprinted with "A733"	1 White gelatin imprinted with "A733"

NF = National Formulary; Ph.Eur. = European Pharmacopoeia; (b) (4); USP = United States Pharmacopeia

(b) (4)

Composition of Lomitapide Drug Product Capsule Shells (modified from Sponsor's table)

Component	Qualitative composition	Grade	Amount per Capsule (mg)		
			5 mg	10 mg	20 mg
Capsule body	Gelatin	USP/NF, Ph.Eur.	(b) (4)		
	Titanium dioxide	(b) (4) USP/NF, Ph. Eur.			
	(b) (4) red iron oxide	(b) (4) USP/NF			
Capsule cap	Gelatin	USP/NF, Ph.Eur.			
	Titanium dioxide	(b) (4) USP/NF, Ph. Eur.			
	(b) (4) red iron oxide	(b) (4) USP/NF			
Imprinting ink (b) (4)	Shellac (b) (4) Iron oxide black (b) (4) Propylene glycol (b) (4)	USP/NF, Ph.Eur. NF USP, Ph.Eur. USP, FCC, Ph.Eur., JSFA NF,Ph.Eur., FCC			

CFR = Code of Federal Regulations; FCC = Food Chemicals Codex; JSFA = Japanese Standards for Food Additives; NF = National Formulary; Ph.Eur. = European Pharmacopoeia; USP = United States Pharmacopeia

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients used in the drug product. All excipients are currently used in approved drug products at similar or higher amounts for the same route of administration.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities or degradants of concern that have been identified.

2.6 Proposed Clinical Population and Dosing Regimen

The clinical population will be patients with HoFH, an orphan population. The dosing regimen will be once daily oral dosing at doses up to 60 mg per day.

A summary of human PK data is shown in the sponsor-generated table below. Most studies were single-dose studies rather than repeat-dose studies. The AUC values for the single-dose studies were calculated as $AUC_{0\text{-}inf}$. In these studies, plasma concentrations were measured for up to 72 or 120 hours. Therefore, the $AUC_{0\text{-}inf}$ is felt to approximate the $AUC_{0\text{-}24h}$ (or AUC_{tau} for once daily dosing) after repeated dosing.

Based on the available information, the sponsor has estimated that the mean $AUC_{0\text{-}24h}$ after repeated dosing of 60 mg will be 69.5 ng·h/mL, although it is not clear which PK data the sponsor used for this calculation.

Table 2: Mean Pharmacokinetic Parameters for Lomitapide across Studies at Similar Doses

Study No.	Drug Substance Lot No.	Drug Product Lot No.	N	Pharmacokinetic Parameter				
				Dose (mg)	C_{MAX} (ng/mL)	T_{MAX}[A] (h)	AUC[B] (h·ng/mL)	$T_{1/2}$ (h)
CV145-001(SD)[c]	R005A	N96074, N96070	6	50	2.3	7.5	102.5	34.4
CV145-002 (SD) CV145-002 (MD)[d]	R005A	N96074, N96070	24	50	3.3 8.5	5 3.25	33.6 132.6	51.1 42.0
CV-145-003 (SD, Oral)[c] CV-145-003 (SD, IV)	R005A	N96074	6 24	50 60	2.2 350.7	ND ND	96.7 1776.5	43.6 24.8
CV145-005 (SD)[c,e]	R005A	N96074	24	50	2.51	6	94.0	38.2
AEGR733-017 (SD)[c,f]	1713-1713-07-001	L0302139	8 8	60 60	1.45 1.05	4 7	74.3 92.9	68 74.6
AEGR733-018 (SD)[c,g]	1713-1713-07-001	L0302139	30	60	1.21	6	62.39	39.7
AEGR733-021 (SD)[h]	1713-1713-07-001	L0302139	7	60	1.26	8	52.68	46.6

IV=intravenous, MD=multiple dose, SD=single dose; ND=not done
[a] Median
[b] $AUC_{0\text{-}inf}$ for single dose; $AUC_{0\text{-}tau}$ for multiple dose
[c] Used in C_{max} and AUC comparison across studies
[d] Value presented is for Day 14.
[e] Fasted
[f] Healthy controls, fasted
[g] Lomitapide only
[h] $AUC_{0\text{-}inf}$, N=4

The effects of renal impairment on exposure to lomitapide, M1 (BMS-203215) and M3 (BMS-203304) are shown in the sponsor-generated tables below. Because M1 is primarily eliminated through renal excretion, end-stage renal disease (ESRD) resulted in a 3-fold increase in M1 exposure, whereas only small increases in exposure ($AUC_{0\text{-}inf}$) were noted for lomitapide (↑39%) and M3 (↑11%). C_{max} values for lomitapide and M1 were approximately 50% and 100% higher in patients with ESRD. No effect on C_{max} was noted for M3.

Table 11-3 Summary of Plasma Pharmacokinetic Parameters of Lomitapide (Pharmacokinetic Population)

Parameter (Unit)	Normal Healthy Subjects Mean (CV) (N=7)	Subjects with ESRD[a] Mean (CV) (N=7)
AUC_{0-t} (ng•hr/mL)	51.94 (36)	69.76 (36)[c]
AUC_{0-inf} (ng•hr/mL)	52.68 (18)[d]	73.39 (41)[d]
AUC_{0-72} (ng•hr/mL)	41.56 (34)	58.01 (36)[c]
C_{max} (ng/mL)	1.26 (52)	1.89 (58)
T_{max} (hr)[b]	8.00 (1.00, 24.00)	4.00 (2.00, 6.00)
λ_z (1/hr)	0.0158 (26)	0.0171 (42)[c]
$t_{1/2}$ (hr)	46.58 (28)	47.77 (47)[c]

Abbreviations: CV, coefficient of variation; ESRD, end-stage renal disease; hr, hour.
Note: Geometric means are presented for AUCs and C_{max}. The AUC_{0-inf} of lomitapide was not reported for Subjects 104, 105, 203, 205 and 206 since the percent extrapolation was >20%. Subject 106 terminated early and a terminal phase was not identified.
[a] Subjects required hemodialysis.
[b] For T_{max}, the median (minimum, maximum) values are presented.
[c] n=6.
[d] n=4.

Table 11-4 Summary of Plasma Pharmacokinetic Parameters of M1 (Pharmacokinetic Population)

Parameter (Unit)	Normal Healthy Subjects Mean (CV) (N=7)	Subjects with ESRD[a] Mean (CV) (N=7)
AUC_{0-t} (ng•hr/mL)	69.93 (32)	210.97 (42)[c]
AUC_{0-inf} (ng•hr/mL)	73.90 (32)	221.11 (50)[d]
AUC_{0-72} (ng•hr/mL)	61.85 (33)	170.56 (48)[c]
C_{max} (ng/mL)	2.26 (36)	4.69 (70)
T_{max} (hr)[b]	6.00 (3.00, 6.00)	6.00 (4.00, 8.00)
$t_{1/2}$ (hr)	28.74 (12)	38.19 (41)[e]

Abbreviations: CV, coefficient of variation; ESRD, end-stage renal disease; hr, hour.
Note: Geometric means are presented for AUCs and C_{max}. The AUC_{0-inf} of M1 was not reported for Subject 105 since the percent extrapolation was >20%. Subject 106 terminated early and a terminal phase was not identified. A clear terminal elimination phase was not identified for Subject 104.
[a] Subjects required hemodialysis.
[b] For T_{max}, the median (minimum, maximum) values are presented.
[c] n=6.
[d] n=4.
[e] n=5.

Table 11-5 Summary of Plasma Pharmacokinetic Parameters of M3 (Pharmacokinetic Population)

Parameter (Unit)	Normal Healthy Subjects Mean (CV) (N=7)	Subjects with ESRD[a] Mean (CV) (N=7)
AUC_{0-t} (ng•hr/mL)	413.70 (21)	456.89 (62)[c]
AUC_{0-inf} (ng•hr/mL)	441.58 (21)[c]	490.49 (62)[c]
AUC_{0-72} (ng•hr/mL)	377.08 (22)	408.97 (61)[c]
C_{max} (ng/mL)	25.72 (25)	26.56 (41)
T_{max} (hr)[b]	3.00 (1.00, 4.00)	3.00 (2.00, 4.00)
$t_{1/2}$ (hr)	32.92 (9)[c]	32.29 (28)[c]

Abbreviations: CV, coefficient of variation; ESRD, end-stage renal disease; hr, hour.
Note: Geometric means are presented for AUCs and C_{max}. Subject 106 terminated early and a terminal phase was not identified. A clear terminal elimination phase was not identified for Subject 202.
[a] Subjects required hemodialysis.
[b] For T_{max}, the median (minimum, maximum) values are presented.
[c] n=6.

2.7 Regulatory Background

The development program for lomitapide (initially called BMS-201038) was initiated by Bristol-Myers Squibb (BMS) in the mid-1990s for the treatment of hypercholesterolemia. Due to safety and tolerability concerns, BMS discontinued the development program and donated lomitapide to the University of Pennsylvania under the direction of Dr. Daniel Rader in 2002. The applicant, Aegerion Pharmaceuticals, Inc., licensed lomitapide from the University of Pennsylvania in 2006. Lomitapide was granted orphan designation for the treatment of HoFH in 2007.

3 Studies Submitted

3.1 Studies Reviewed

Primary pharmacology studies
Secondary pharmacology studies
Safety pharmacology studies

Neurobehavior in mice (BMS study report - Nov 1998a)
Neurobehavior in rats - Irwin test (Study 733PC0017, BMS report - May 1996)
Body temperature in rats (BMS study report - Nov 1998a)
Hypnotic activity in mice (BMS study report - Nov 1998a)
Seizure response in mice (BMS study report - Nov 1998a)
Analgesic activity in mice (BMS study report - Nov 1998a)
hERG assay (Study 733PC0019)
Cardiovascular effects in rats (BMS report - May 1996)
Cardiovascular effects in dogs (BMS study report - Nov 1998a)
Respiratory effects in dogs (BMS study report - Nov 1998a)

Gastric motility in mice (BMS study report - Nov 1998a)
Gastric motility with rabbit ileum (BMS study report - Nov 1998a)
Gastric motility with guinea pig ileum (BMS study report - Nov 1998a)
Renal effects in rats (BMS study report - Nov 1998a)

PK/ADME studies

Bioanalytical method validation reports
Absorption in rats (Study 910056240)
PK in plasma and liver of rats (Study 910056291)
PK in plasma and liver of rats (Study 910061832)
Absorption in dogs (Study 910056234)
Absorption in monkeys (Study 910056239)
Plasma protein binding (Study 910060036)
Tissue distribution and mass balance in rats (Study 910059453)
Tissue distribution in rats (Study 910059992)
Metabolism in mouse, rat, dog, and human hepatocytes (Study 733PC0009)
Metabolite profile in rat plasma, bile, urine, and liver (Studies 910064099 and 910064100)
Metabolite profile in rat bile and urine (Study 910056489)
Metabolite profile in rat plasma, bile, and urine (Study 733PC0016)
Metabolite profile in dog plasma and urine (Study 910064102)
Metabolite profile in dog plasma, urine, and feces (Study 733PC0013)
Metabolite profile in monkey plasma and urine (Study 910064101)

General Toxicology

Single dose in mice (Study 96011; Study 96035)
Single intravenous dose in rats (Study 96036)
Single dose in mice (Study 96011; Study 96035)
Single intravenous dose in rats (Study 96036)
Single dose in dogs (Study 96041)
3-day and 21-day studies in hamsters (BMS Study - May 1996a)
2-week study in rats (Study 96043)
2-week study in rats (Study 95051)
2-week intravenous study in rats (Study 96209)
2-week intravenous study in dogs (Study 96331)
3-week study in mice (Study 96056)
2- and 4-week study in mice (Study 96042)
1-month study in rats (Study 96003)
1-month investigative study in rats with vitamin supplementation (Study 96016)
1-month study in dogs (Study 96004)
3-month range finding study in mice (Study 99008)
3-month dietary study in mice (Study 96057)
3-month dietary study in rats (Study 96058)
3-month investigative study in rats with electron microscopy (Study 733PC0008)
6-month study in rats (Study 97027)
6-month study in rats (Study 96024)
6-month study in dogs (Study 96025)
1-year study in dogs (Study 97057)

Genetic Toxicology

Exploratory in vitro reverse mutation assay (Study 95712)
In vitro reverse mutation assay (Study 96630)
In vitro reverse mutation assay with metabolite M1 (Study CHV-177787-0-401R)
In vitro chromosomal aberration study in human lymphocytes (Study 96686)
Micronucleus study in rats (Study 96629)

Carcinogenicity

2-year carcinogenicity study in mice (Studies 733PC0003 and 733PC0018)
2-year carcinogenicity study in rats (Studies 733PC0002 and 733PC0004)

Developmental and Reproductive Toxicology

Fertility study in rats (Study 733PC0001)
Embryo-fetal development range-finding study in rats (Study 96015)
Investigative embryo-fetal development study in rats (Study 96022)
Embryo-fetal development study in rats - pivotal (Study 96039)
Embryo-fetal development range-finding study in rabbits (Study 96031)
Exploratory embryo-fetal development study in rabbits (Study 96324)
Embryo-fetal development range-finding study in ferrets (Study 97008)
Embryo-fetal development study in rabbits - pivotal (Study 96032)
Pre- and post-natal development in rats (Study 733PC0014)

Special Toxicology Studies

Rat lung macrophage function (Study 733PC0011)
Antigenicity in guinea pigs (Study 0740580)
Streptococcal host resistance model (Study 733PC0012)
MTP expression in lungs (Study 910065346)
Bovine corneal opacity and permeability assay with M1 (Study A96BB27.350)

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Some studies were previously reviewed by Dr. Indra Antonipillai or Dr. Dylan Yao. Portions of these reviews have been included within this document with some modification, where indicated.

4 Pharmacology

MTP is a soluble protein found primarily in the endoplasmic reticulum of hepatocytes and intestinal epithelium. MTP forms a heterodimer with protein disulfide isomerase and together regulate the assembly of triglycerides with apolipoprotein B (apoB) resulting in the formation of very low-density lipoprotein cholesterol (VLDL), the precursor of low-density lipoprotein cholesterol (LDL). Inhibition of MTP activity prevents the transfer of lipid to apoB resulting in the proteolytic destruction of nascent apoB. MTP is also essential for the formation and secretion of chylomicrons in the intestine. Inhibition of MTP in enterocytes prevents the formation of chylomicrons and thereby inhibits the transfer of dietary-derived lipids to the liver.

Patients with HoFH have high levels of plasma LDL due to mutations in genes involved in the regulation of lipids. LDL receptor mutations are the predominant genetic defect identified in the HoFH population, although the involvement of mutations in other genes, such as apoB, have been reported at a lower incidence. In the absence of a functional LDL receptor or apoB molecule, LDL cannot be efficiently taken up by the liver resulting in a longer circulating half-life and higher plasma LDL levels. By inhibiting MTP, hepatic VLDL and intestinal chylomicron formation and secretion is inhibited, thereby lowering circulating plasma levels of VLDL and LDL.

BMS-201038 was shown to inhibit the triglyceride transfer activity of MTP in vitro by evaluating vesicle stability using bovine MTP. In this study, the IC_{50} for inhibition was 15.5 nM for BMS-201038; the IC_{50} for metabolite M1 was approximately 250-fold less potent at 6.3 µM and M3 did not have a significant effect on MTP activity. In the small unilamellar vesicle (SUV) assay, IC_{50} values for isolated rat, hamster, and human MTP were 5, 7, and 5 nM, respectively. In a separate SUV assay, it was shown that BMS-201038 inhibited the transfer of triglyceride, cholesteryl ester, and diglyceride with similar potency (0.5 to 1.2 nM) but the inhibition of phosphatidylcholine transfer was weak (IC_{50} could not be determined). Additionally, it was shown that BMS-201038 inhibits triglyceride transfer by directly binding to MTP. BMS-201038 inhibited apoB secretion for HepG2 cells with an IC_{50} of 0.8 nM.

In vivo, BMS-201038 decreased total cholesterol, VLDL/LDL, and HDL by up to 70% in Sprague-Dawley rats treated for 3 days at doses ≥0.3 mg/kg/day. Similar effects were observed in Golden Syrian hamsters receiving doses of ≥1 mg/kg for 3 days. After 21 days of treatment in hamsters fed a high-fat diet, significant decreases in total cholesterol, VLDL/LDL, HDL, and triglycerides were observed at ≥3 mg/kg. Triglyceride levels were increased in liver and small intestine within 7 days of treatment, but the accumulation appeared to stabilize after 14 days of treatment. The cholesterol lowering activity of BMS-201038 was also demonstrated in cynomolgus monkeys receiving ≥2.5 mg/kg/day for 7 days. In addition to effects on plasma lipid parameters, treatment in hamsters and monkeys resulted in slight increases (<2X) in plasma ALT, AST, and/or CPK.

4.1 Primary Pharmacology

Evaluation of lomitapide (AEGR-733; BMS-201038) and its primary metabolites, M1 (BMS-203215) and M3 (BMS-203304) for MTP inhibition using fluorescent-labeled triglyceride transfer assay kit (Study 733-PC0024)

MTP inhibition was measured in vitro by evaluating the stability of vesicles in the presence of different concentrations of test substance with and without the addition of bovine MTP. Vesicle stability was determined by measuring the background fluorescence units after a 30-minute incubation. The background and transferred fluorescence readings were used to calculate the percent triglyceride transfer activity of MTP at each concentration. From these calculations, an IC_{50} was determined. Based on this analysis, AEGR-733 and M1 had IC_{50} values of 15.5 nM and 6.3 µM, respectively. In contrast, M3 did not have a significant effect on MTP activity.

The inhibition of rat, hamster, and human MTP triglyceride transfer by BMS-201038 (BMS Study Report - March 1996a)

MTP activity was determined by measuring the transport of ^{14}C-triglyceride between donor and acceptor in an SUV assay. MTP was isolated from rat and hamster livers as well as from Sf9 cells infected with human MTP large subunit and PDI baculovirus. Concentrations of BMS-201038 ranged from 1 to 30 nM. The IC_{50} values for inhibition of triglyceride transfer were 5, 7, and 5 nM for rat, hamster, and human MTP, respectively. Thus, BMS-201038 showed equivalent potency against rat, hamster, and human MTP in the SUV assay.

Mechanism of inhibition of microsomal triglyceride transfer protein inhibitor BMS-201038 (BMS Study Report - April 1996a)

Lipid transfer from donor to acceptor membranes was measured in the small unilamellar (SUV) assay to evaluate the mechanism of MTP inhibition. The inhibition of lipid binding to MTP was also determined. The SUV assay showed that BMS-201038 inhibited the transfer of triglyceride, cholesteryl ester, and diglyceride with IC_{50} values of 0.5, 0.55, and 1.2 nM, respectively. BMS-201038 was a weak inhibitor of phosphatidylcholine transfer, with a maximum inhibition of only 18%. Therefore, BMS-201038 was found to be a potent inhibitor of neutral lipid transfer, but a very weak inhibitor of phospholipid transfer.

In vitro experiments showed that BMS-201038 inhibits triglyceride transfer by directly binding to MTP. In HepG2 cells, BMS-201038 was shown to bind to the 97 kD subunit of MTP within the ER lumen. Inhibition of apoB secretion from HepG2 cells by BMS-201038 had an IC_{50} value of 0.8nM, which is similar to the value obtained above for the inhibition of human MTP triglyceride transport in vitro (0.5 nM).

Effects of the microsomal triglyceride transfer protein inhibitor BMS-201038 on lipoprotein secretion from HepG2 cells (BMS Study Report - December 1995a)

The secretion of apoB and apoAI (negative control) from HepG2 cells was measured with and without BMS-201038. The amount of DNA replication was also measured using ^{3}H-thymidine incorporation. BMS-201038 was shown to inhibit apoB secretion with an IC_{50} of 0.8 nM. In contrast, the test article had an IC_{50} against apoAI secretion of 6.5 µM, over 8000 times higher than the IC_{50} against apoB secretion. The inhibition of apoB secretion was not due to cytotoxicity as the IC_{50} for ^{3}H-thymidine incorporation occurred at a high concentration of 12.4 µM, which was similar to the concentration required to inhibit apoAI secretion.

The acute efficacy of BMS-201038 in the rat triton model of VLDL secretion (BMS Study Report - January 1996a)

Intravenous administration of triton WR1339, a nonionic surfactant, blocks the clearance of plasma triglyceride rich lipoproteins and has been used to study lipoprotein production in animals. In this study, rats underwent fasting so that the intestinal lipoprotein secretion did not contribute to the lipoproteins being measured and therefore enabled a more accurate determination of the hepatic contribution of accumulated plasma triglycerides. Another group of rats were fed to determine the effect of MTP

inhibition on intestinal lipoprotein secretion. Rats were fasted for 18 hours and were orally administered 0.3, 1, or 3 mg/kg BMS-201038 1 hour prior to receiving an intravenous injection of triton. Separate groups of animals received an intravenous dose of 0.03, 0.1, or 0.3 mg/kg BMS-201038 simultaneously with the triton.

Administration of triton alone produced a time dependent accumulation of plasma triglycerides. Oral pretreatment of fasted rats with BMS-201038 inhibited the triton-induced accumulation of plasma triglycerides. The ED_{50} for this effect was 0.19 mg/kg. The ED_{50} for BMS-201038 treatment in fasted rats was 0.15 mg/kg. The efficacy of BMS-201038 in fed rats, ED_{50} of 0.15 mg/kg, demonstrated that both intestinal and hepatic lipoprotein secretion can be inhibited. A comparison of triglyceride secretion inhibition in fed and fasted rats is shown in sponsor-generated Figure 3.

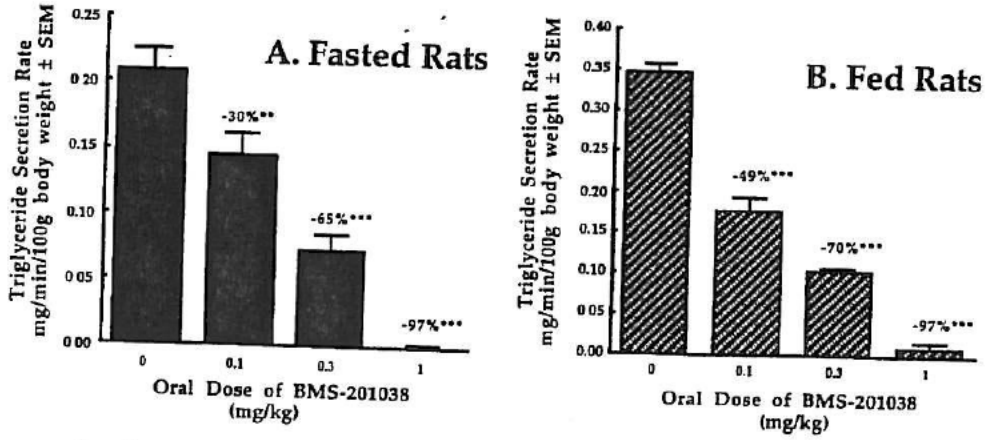


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Figure 3. Comparison of triglyceride secretion inhibition efficacy in fed and fasted rats by BMS-201038. Rats were dosed orally 1 hour before intravenous triton (250 mg/kg)injection with vehicle or BMS-201038. Plasma triglycerides were determined 2 1/2 hours later. *** $p < 0.001$; ** $p < 0.005$

The effect of three day oral treatment with BMS-201038, a potent inhibitor of MTP, on rat plasma cholesterol (BMS Study Report - April 1996b)

Sprague-Dawley rats (4/group) were orally administered BMS-201038 at 0.3, 1, 3, or 10 mg/kg or vehicle (10% M-pyrol:80% water:10% cremophore [50% cremophore:50% ethanol]) once daily for 3 days. Plasma lipid levels and other clinical chemistry parameters were measured. A dose-dependent, statistically significant decrease (10% to 72%) in cholesterol was noted at all dose levels. Statistically significant decreases in HDL cholesterol (up to ~65%) and VLDL/LDL cholesterol (up to ~70%) occurred at ≥0.3 and ≥1 mg/kg/day, respectively. Plasma triglycerides were increased at 0.3 (↑58%) and 1 mg/kg/day (↑88%), whereas they were decreased at 3 (↓38%) and 10 mg/kg/day (↓63%). Increases in plasma ALT or AST were not observed at any dose level. The EC_{50} in rats after 3 days of oral treatment was determined to be 2.5 mg/kg/day.

The effect of three day oral treatment with BMS-201038, a potent inhibitor of MTP, on hamster plasma cholesterol (BMS Study Report - January 1996b)

Golden Syrian hamsters (4-5/group) were orally administered 1, 3, or 10 mg/kg BMS-201038 or vehicle (10% M-pyrol:80% water:10% cremophore [50% cremophore:50% ethanol]) once daily for 3 days. At the end of treatment, plasma lipid levels and chemistries were measured.

As shown in the sponsor-generated figure below, after 3 days of treatment, total cholesterol decreased by approximately 35%, 49%, and 83% for the 1, 3, and 10 mg/kg/day groups, respectively. Similar effects on HDL and VLDL+LDL were observed. The EC_{50} for cholesterol lowering was calculated to be 2.0 mg/kg. Treatment at the high dose resulted in a decrease in plasma triglycerides (↓26%). Slight increases in mean plasma ALT (↑32%) and AST (↑69%) were also observed at the high dose.

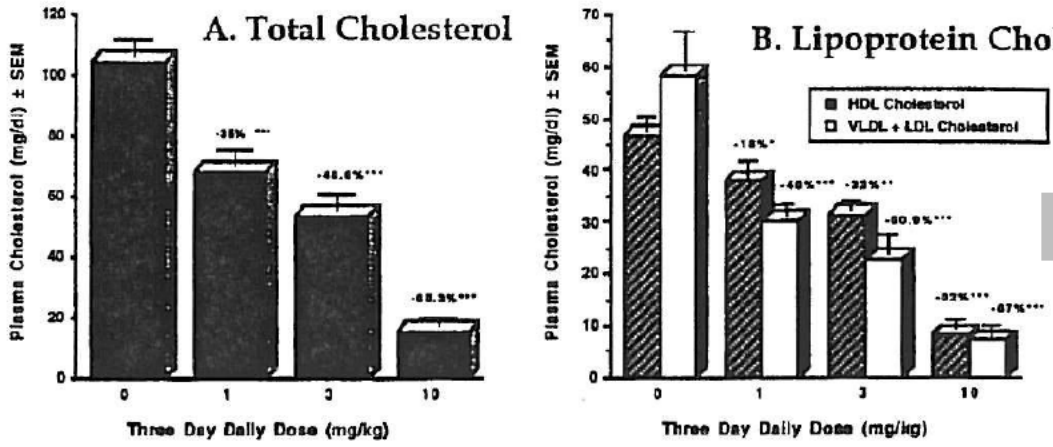

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Figure. The Cholesterol lowering efficacy of BMS-201038 in hamsters.
Treatment was orally for three days. Plasma was obtained 18 hours after last dose.
p values: * <0.5; ** <0.05; ***< 0.005.

Reversibility of hepatic and intestinal triglyceride accumulation in BMS-201038 treated hamsters (BMS Study Report - April 1996c)

Golden Syrian hamsters (4/group) were orally administered 10 mg/kg BMS-201038 or vehicle (10% M-pyrol:80% water:10% cremophore [50% cremophore:50% ethanol]) once daily for 7 days. A recovery period of up to 7 days was also implemented. Subgroups were sacrificed at various time points during the treatment and recovery periods. For each sacrifice time point, sections of liver and small intestine were collected and quick frozen for the determination of triglyceride levels. Additionally, plasma samples were collected for measurement of plasma lipid levels and other clinical chemistry parameters. The results are shown in the sponsor-generated figures below.

As shown in Figure 1, plasma total cholesterol and triglycerides were reduced by approximately 85% after 3 days of treatment, which was maintained throughout the remainder of the treatment period. Cholesterol levels rebounded to pre-treatment levels after 48 hours of recovery. Plasma triglyceride levels actually increased by 30% above pre-treatment levels but then decreased to pre-treatment 2 days later. Liver and small intestine triglyceride accumulation increased during the 7-day treatment period and then quickly decreased within approximately 48 hours after the start of recovery, reaching control values by the end of the 7-day recovery period (Figure 2).

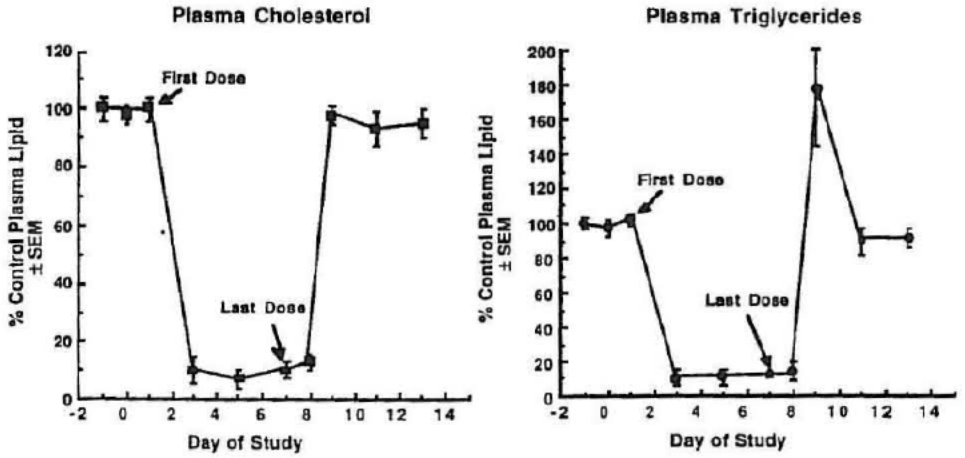

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Figure 1. Reversibility of Cholesterol Lowering Efficacy of BMS-201038. Hamsters were treated orally for 7 days with 10 mg/kg. Fasting plasma samples were obtained at the intervals post treatment as indicated.

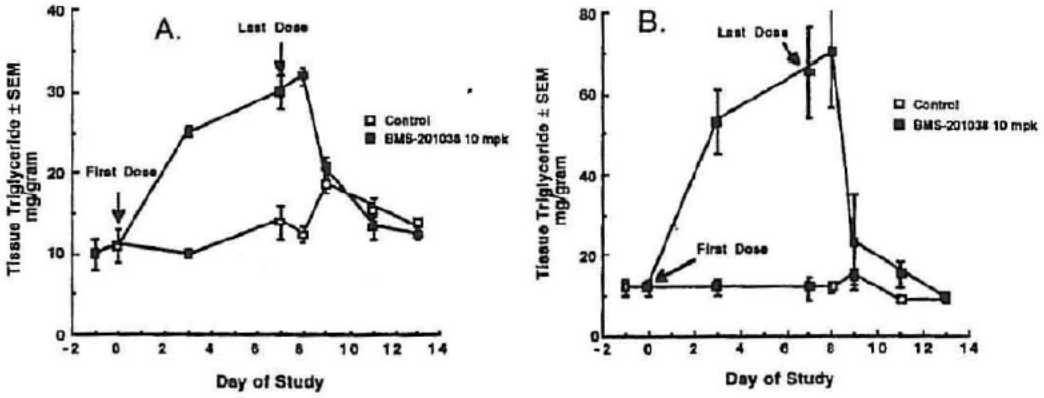

Figure 2. Recovery From MTP Inhibitor Induced Tissue Lipidosis. Hamsters were treated for various times up to 7 days. Hepatic (A.) and intestinal (B.) tissue samples from fasted animals were then examined at the times indicated for triglyceride levels.

Three week treatment of hamsters with BMS-201038: Effect on plasma and tissue lipids (BMS Study Report - February 1996a)

Golden Syrian hamsters were orally administered 1, 3, or 6 mg/kg BMS-201038 or vehicle (10% M-pyrol:80% water:10% cremophore [50% cremophore:50% ethanol]) once daily for 7, 14, or 21 days. Groups were fed either standard diet or a high-fat diet. At sacrifice, sections of liver and small intestine were fixed in formalin or quick frozen. Serum chemistry was also assessed.

Animals on high-fat diet had a 138% increase in total cholesterol, 219% increase in VLDL plus LDL, and 248% increase in triglycerides compared with animals on standard diet. The cholesterol lowering effect of BMS-201038 in hamsters fed standard diet or a high-fat diet is shown in sponsor-generated Figure 2 below.

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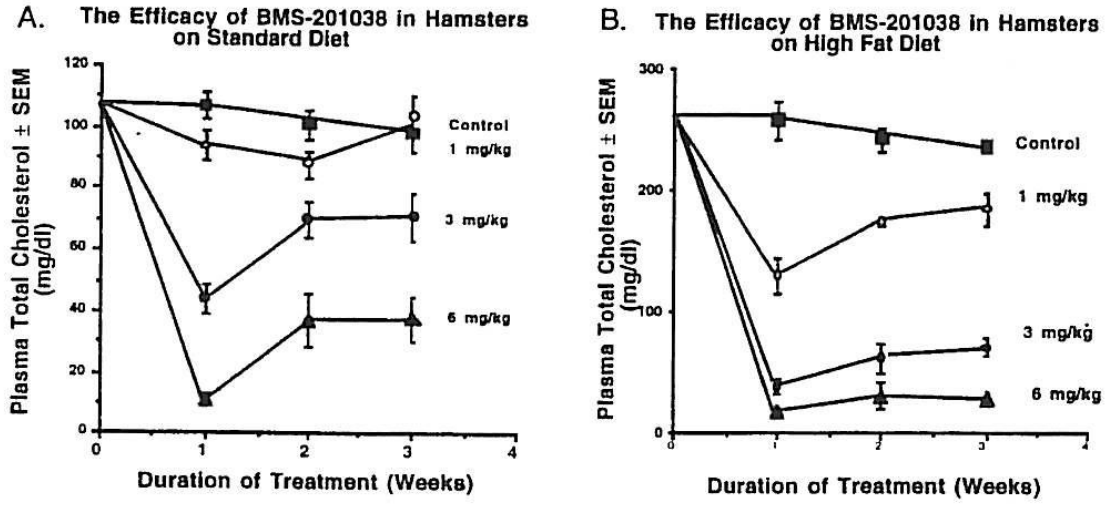


Figure 2. Cholesterol Lowering Efficacy of BMS-201038. Hamsters maintained on either a standard or high fat diet were orally dosed with BMS-201038 for the duration indicated. Fasting plasma samples were obtained 18 hours after the last dose of compound.

Lipid parameters after 21 days of treatment are shown in the sponsor-generated tables below.

Standard Diet

21 Day Treatment

Treatment	Total Cholesterol	HDL Cholesterol	VLDL + LDL Cholesterol	Triglycerides
Control mean ± sem	mg/dl 95.5 ± 6.3	mg/dl 50.8 ± 3.9	mg/dl 44.8 ± 2.7	mg/dl 41.0 ± 5.6
1 mg/kg mean ± sem % change p value	104.5 ± 1.4 9.4% 0.21	54.5 ± 2.4 7.3% 0.45	50.0 ± 1.4 11.6% 0.13	37.0 ± 5.2 -9.7% 0.57
3 mg/kg mean ± sem % change p value	71 ± 8 -25.7% 0.05	41.8 ± 4.1 -17.7% 0.17	29.3 ± 4.1 -34.6% 0.02	37 ± 5.2 -9.8% 0.62
6 mg/kg mean ± sem % change p value	37.3 ± 7.3 -60.9% <0.001	20 ± 8.5 -60.6% <0.001	17.3 ± 3.1 -61.4% <0.001	34.5 ± 4.4 -15.9% 0.40

High-Fat Diet

21 Day Treatment

Treatment	Total Cholesterol	HDL Cholesterol	VLDL + LDL Cholesterol	Triglycerides
Control mean ± sem	mg/dl 206.3 ± 2.8	mg/dl 86.3 ± 4.1	mg/dl 120.0 ± 5.6	mg/dl 136.3 ± 30.9
1 mg/kg mean ± sem % change p value	184.5 ± 13.9 -10.6% 0.18	83.3 ± 6 -3.5% 0.69	101.3 ± 10.7 -15.6% 0.17	98.3 ± 29.9 -27.9% 0.41
3 mg/kg mean ± sem % change p value	69.0 ± 7.6 -66.6% <0.001	35.7 ± 3.5 -58.6% <0.001	33.3 ± 4.1 -72.3% <0.001	63.3 ± 1.3 -53.6% 0.10
6 mg/kg mean ± sem % change p value	28 ± 1 -86.4% <0.001	10.5 ± 5 -87.8% <0.001	17.5 ± 6 -85.4% <0.001	43 ± 13 -68.5% 0.12

In animals fed a high-fat diet, CPK was elevated for the 3 and 6 mg/kg groups after 7 and 21 days (up to 238% for the high dose group). Similar effects were not seen for the animals fed a standard diet. No effects on body weight were noted. Triglyceride levels were increased in liver and small intestine within 7 days of treatment after which time the triglyceride levels appeared to stabilize, as triglyceride levels were similar at Day 14 and Day 21 (sponsor-generated Table 7). Tissue triglyceride levels were slightly higher in animals receiving a high-fat diet.

Table 7. Triglyceride levels of liver and small intestine of hamsters on a standard or high fat diet treated with BMS-201038 for three weeks.

Treatment	7 Days		14 Days		21 Days	
	liver	small intestine	liver	small intestine	liver	small intestine
Standard Diet						
0 mg/dl	9.1 ± 0.6	10.7 ± 1.0	8 ± 0.4	10 ± 0.6	9 ± 0.5	13 ± 0.7
1 mg/dl	11 ± 0.3	43 ± 1.8	11 ± 1.1	43 ± 4.5	11 ± 1.5	38 ± 2.2
3 mg/dl	18 ± 1.8	64 ± 4.0	12 ± 0.8	50 ± 4.3	23 ± 3.5	55 ± 7.6
6 mg/dl	24 ± 0.4	70 ± 11.3	19 ± 3.4	52 ± 7.7	19 ± 1.0	50 ± 3.9
High Fat Diet						
0 mg/dl	14 ± 1.5	21 ± 2	10 ± 0.4	13 ± 1.9	15 ± 0.6	18 ± 2.2
1 mg/dl	17 ± 2.0	35 ± 2.8.	20 ± 1.2	26 ± 1.9	17 ± 3.0	43 ± 2.9
3 mg/dl	22 ± 3.1	69 ± 10.9	27 ± 3.1	56 ± 1.8	25 ± 4.8	66 ± 12.5
6 mg/dl	32 ± 13	76 ± 16.2	31 ± 1.6	71 ± 8.7	36 ± 3.9	60 ± 6.2

Microscopic evaluation of the liver and small intestine showed there no apparent progression of the degree of lipid vacuolation observed on Day 7 compared with Day 21. Additionally, a more severe form of prefibrosis characteristic of pathologic lipidosis was not observed.

The effect of BMS-201038 on plasma triglyceride concentrations evaluated 10 and 24 hours after 7 days of treatment in the cynomolgus monkey (Macaca fascicularis) (BMS Study Report - November 1995a)

Cynomolgus monkeys were orally administered 2.5 or 5.0 mg/kg BMS-201038 or vehicle (50% ethanol in water) once daily for 7 days. Ten hours after the final dose (6-hour fast) and 24 hours after the final dose (18-hour fast), plasma lipid parameters were measured.

The effects of BMS-201038 on plasma triglycerides and total cholesterol are shown in sponsor-generated Figure 2 and 3, respectively. Control animals showed an increase in plasma triglyceride levels at the 10-hour time point, which was 8 hours after feeding. Animals receiving BMS-201038 showed a slight decrease in triglyceride levels compared with the time zero time point. By 24 hours, triglyceride levels for control animals were equivalent to the test article treated animals. Effects on cholesterol lowering were similar at the 10- and 24-hour time points. Treatment resulted in slight increases (<2X) in plasma ALT, AST, and CK (5 mg/kg only). The results of this study showed that the effect of BMS-201038 on triglycerides is more transient compared with the effect on plasma cholesterol levels, which is consistent with a more rapid turnover rate for triglycerides compared with cholesterol.

Figure 2

Effect of BMS-201038 Given by Gavage for 7 Consecutive Days and Evaluated 10 and 24 hrs After Last Dose on Plasma Triglyceride Levels

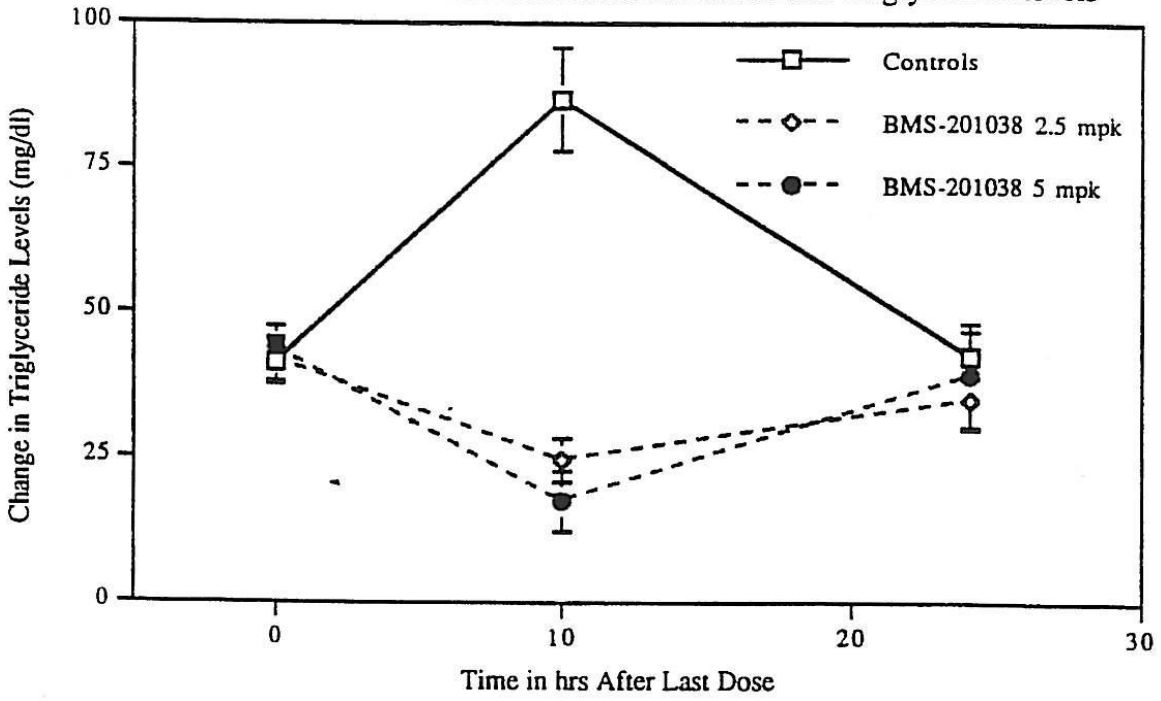
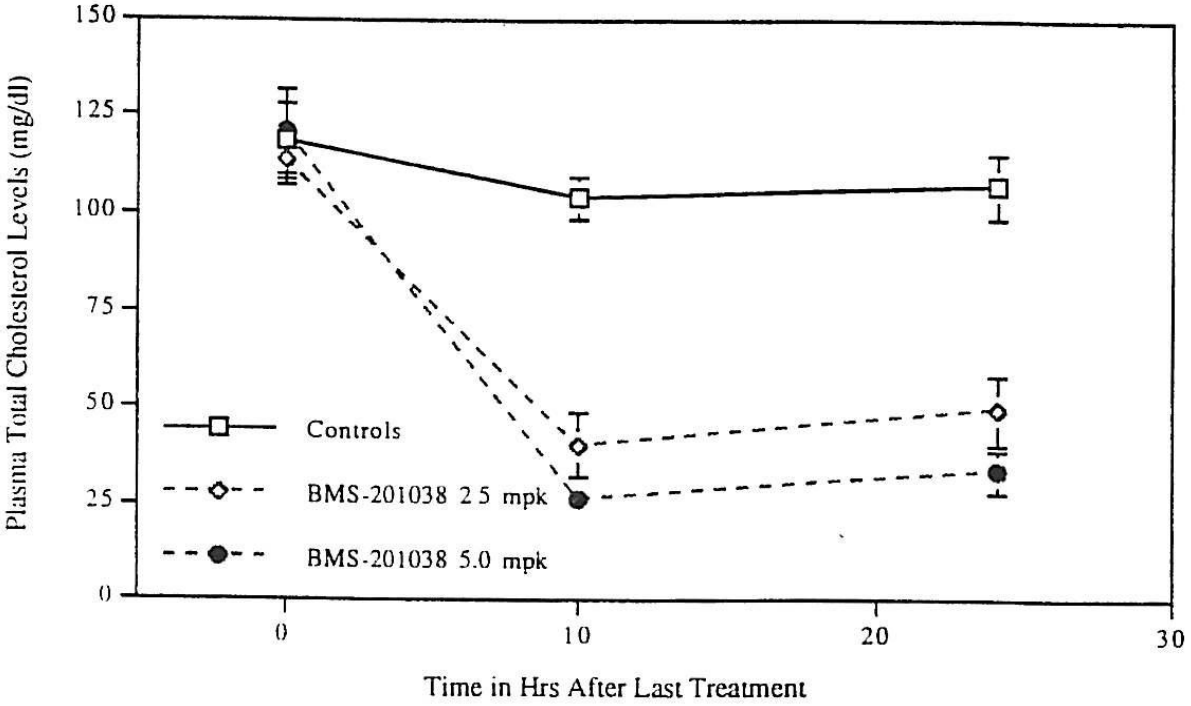


Figure 3

Effect of BMS-201038 Given by Gavage for 7 Days and Evaluated at 10 and 24 hrs After Last Dose on Plasma Total Cholesterol Levels



The effects of BMS-201038 on plasma cholesterol, lipoprotein cholesterol, plasma triglyceride concentrations, liver enzymes, creatinine kinase, and body weight in the Cynomolgus monkey (Macaca fascicularis) (BMS Study Report - October 1995a)
Cynomolgus monkeys were orally administered 1.25, 2.5, or 5.0 mg/kg BMS-201038 or vehicle (50% ethanol in water) once daily for 14 days. Clinical chemistry parameters were measured pre-treatment and on Days 7 and 14 of treatment; animals underwent a 16-hour fast prior to plasma sampling. Feces were collected on Days 12 through 14 to determine the percent of fatty acids excreted in feces.

Dose-dependent decreases in total cholesterol, VLDL/LDL, and HDL were observed at all dose levels after 7 and 14 days of dosing. On Day 14, lipid parameters for the high-dose group had decreased by 76% to 79% compared with Day 0 baseline values. Decreases in mean triglyceride concentrations were also observed after 7 days, but the decrease was not as noteworthy on Day 14 and was only observed at the high dose (↓39%), as vehicle-treated animals also showed a decrease. Treated animals showed an increase in ALT, AST, and CK at all dose levels on Days 7 and 14 compared with baseline values and untreated controls. Increases were generally less than 2 fold, although ALT increases for the 5 mg/kg/day group were 2 to 3-fold higher than baseline values. Effects on liver enzymes were similar on Day 7 and Day 14. Animals treated with 5 mg/kg/day also had a higher percentage of fecal fatty acids compared with vehicle control animals (sponsor-generated Table 14).

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Table 14
Quantative Fecal Fat Fractionation in
Normal Chow Fed Cynomolgus Monkeys

Group	24 hr Total Fecal Fatty Acids (gram)	24 hr Average Diet Consumed (gm)	24 hr Total Diet Fat (gram)	24 hr % Dietary Fecal Fatty Acids Excreted	72 hr Total Fecal Sample Wt (gms)
Control					
Average	0.6	100.0	5.0	12.0	103.8
SEM	0.1	0.0	0.0	1.7	8
BMS-201038 5.0 mg/kg of body wt/day					
Average	1.6	100.0	5.0	31.2	143.4
SEM	0.2	0.0	0.0	3.9	5.3
p value	*0.002*			*0.005*	

The effects of BMS-201038 on plasma cholesterol, lipoprotein cholesterol, plasma triglyceride concentrations, liver enzymes, creatine kinase, and body weight in the hyperlipidemic cynomolgus monkey (Macaca fascicularis) (BMS Study Report - October 1995b)

Male and female Cynomolgus monkeys were placed on a high-fat diet for 4 weeks, resulting in an average increase in total cholesterol of 91%. At this point, monkeys were orally administered 5.0 mg/kg BMS-201038 or vehicle (50% ethanol in water) once daily for 14 days while maintained on the high-fat diet. Clinical chemistry parameters and body weights were assessed at baseline and on Days 7 and 14 of treatment after fasting. Feces were collected on Days 12 through 14 to determine the percent of fatty acids excreted in feces.

After 7 days, animals receiving 5 mg/kg/day had mean decreases in total cholesterol, VLDL/LDL, and HDL levels of 73%, 78%, and 59%, respectively. After 14 days, mean decreases for these parameters were 84%, 89%, and 67%, respectively. As in the previous study, effects on triglycerides were difficult to determine because the control animals also showed decreases at Day 7 and Day 14. Plasma ALT, AST, and CK were increased in treated animals; mean increases ranged from approximately 2 to 2.5 fold on Day 14. Total and percent fecal fatty acids were increased by approximately 4 fold in animals receiving BMS-201038 (sponsor-generated table below).

The Effect of BMS-201038 on
Total Fecal Fatty Acids Determined From a 72 hr Quantitative Fecal Fat Fractionation
in Hyperlipidemic Cynomolgus Monkeys

	24 hr Total Fecal Fatty Acids (gram)	24 hr Total Dietary Fat (gram)	24 hr Percent Fecal Fatty Acids Excreted
Controls	0.2 ± 0.1	14.8 ± 1.6	1.5 ± 0.6
Treated	0.8 ± 0.3	14.5 ± 1.5	5.4 ± 1.8
p value	*0.108*	*0.894*	*0.071*

4.2 Secondary Pharmacology

Receptor antagonism assay (May 7, 1996 safety pharmacology report)

The potential for BMS-201038 to antagonize the binding of appropriate radioligands to 31 receptors and channels was evaluated in vitro. A concentration-dependent inhibition (0.01 µM to 10 µM) of ligand binding was observed for the serotonin 5-HT1 (70% inhibition), serotonin 5-HT2 (70% inhibition), and sigma (85% inhibition) receptors and sodium channel-2 (109% inhibition). It was concluded that displacement from sigma binding sites appeared to be related to functional receptor antagonism and that compound reduction of electrically stimulated guinea pig vas deferens contractile responses suggested that antagonism may in part be physiologic rather than pharmacologic. Displacement from the serotonin 5-HT2 and sodium channel-2 binding

sites appeared to be unrelated to functional receptor agonism or antagonism. Displacement from serotonin 5-HT binding sites was noted but not described further.

Using preclinical models to explore the mechanism of HDL lowering and the potential for amelioration of steatosis with MTP inhibition (Study 733PC0010)

Steatosis was not ameliorated in wild-type mice when concomitantly administered fenofibrate (1 mg/kg) or niacin (1% in diet). In the LDLR+/- apobec-/-hapobtsg (LAHB) mouse model, concomitant treatment with fenofibrate did reduce steatosis by 52% (duration of treatment not specified), but ezetamibe did not. Fish oil (Menhaden oil) in comparison to soybean oil resulted in significantly lower levels of hepatic triglycerides in both the vehicle and MTP inhibitor treated groups.

The sponsor hypothesized that agents that inhibit DGAT2-catalyzed triglyceride synthesis can ameliorate steatosis while agents that inhibit DGAT1, affect fatty acid flux to the liver, or inhibit cholesterol absorption do not appear to be effective. Thus a combination of fibrate and omega-3 fatty acids may decrease steatosis in humans receiving a MTP inhibitor. No conclusions were made regarding the mechanism for HDL lowering.

Treatment of rats with high doses of BMS-201038: Effect of vitamin K supplementation on altered clotting times (May 1996c study report)

Sprague-Dawley rats were treated with 125 or 250 mg/kg BMS-201038 or vehicle by oral gavage once daily for 14 days. For each treatment group, half of the animals received vitamin K supplementation and the other half did not. Vitamin K supplementation consisted of daily oral doses of 200 µg/kg starting two days before test article dosing as well as a subcutaneous administration of a 30% solution of vitamin K. Animals were assessed for blood clotting time as well as other measures of toxicity.

Treatment resulted in decreased body weight gain (125 mg/kg/d) or body weight loss (250 mg/kg/d). Gross pathology showed lipid accumulation in the liver and intestine in both test article groups. BMS-201038-reated animals also had residual food material in the GI tract even though they had undergone an 18 hour fast. Occasional fluid and gas retention resulting in protrusion of the stomach and intestine was also observed. The pleural cavity of treated rats not receiving vitamin K supplementation had a bright pink appearance and frequent light hemorrhage into the cavity wall. The vitamin K supplemented groups did not show signs of hemorrhage. Treated animals had decreased hematocrit, which was more noteworthy in animals not receiving vitamin K. Unsupplemented animals had increased prothrombin time by 10 seconds at 125 mg/kg/d and prothrombin time was tripled for the high-dose group. Activated partial thromboplastin time was doubled and tripled in the 125 and 250 mg/kg/d groups, respectively. Vitamin K supplementation prevented the blood clotting prolongations. Both dose groups showed elevations in ALT. Cholesterol was lowered by 50% to 72% and triglycerides were lowered by 80%.

4.3 Safety Pharmacology

4.3.1 Nervous System

General activity and behavior in mice (Nov 1998a study report; non-GLP)

Male CD-1 mice (10/group) were administered a single oral dose of vehicle (75% PEG 400 in water) or 3, 10, 30, or 100 mg/kg BMS-201038. Mice were observed for awareness, mood, motor activity, CNS excitation, posture, motor incoordination, muscle tone, reflexes, and autonomic syndromes before dosing and at 0.5, 1, 2, 3, 4, 5, 6, and 24 hours after dosing.

No effects on general behavior were observed.

Effects on spontaneous locomotor activity in mice (Nov 1998a study report; non-GLP)

Male CD-1 mice (10/group) were administered a single oral dose of 10, 30, or 100 mg/kg BMS-201038 or vehicle (75% PEG 400 in water). Locomotor activity was measured before treatment and from 15 to 30, 45 to 60, 105 to 120, 165 to 180, 225 to 240, 285 to 300, and 345 to 360 minutes after dosing.

Compared with the vehicle group, spontaneous locomotor activity was significantly decreased in the 10 and 100 mg/kg groups at 225 to 240 minutes after administration and in the 30 mg/kg group at 105 to 240 minutes after administration. Non-statistically significant decreases were also observed for the 45 to 60 minute and 285 to 300 minute time points for all BMS-201038 treatment groups, although the effects did not always occur in a dose-related manner.

Hypnotic activity in mice (Nov 1998a study report; non-GLP)

Male CD-1 mice (10/group) were administered a single oral dose of 10, 30, or 100 mg/kg BMS-201038 or vehicle (75% PEG 400 in water). The mice received an intraperitoneal injection of 70 mg/kg hexobarbital at 120 minutes after oral administration of the vehicle or BMS-201038. Sleeping time, the duration from the disappearance of the righting reflex until reappearance, was timed.

Sleeping times for BMS-201038-treated animals was similar to the vehicle group. Therefore, hypnotic activity was not observed.

Effects on seizure response induced by electroshock in mice (Nov 1998a study report; non-GLP)

Male CD-1 mice (10/group) were administered a single oral dose of 10, 30, or 100 mg/kg BMS-201038 or vehicle (75% PEG 400 in water). To evaluate the antagonistic effects to provoke convulsions, a silver bipolar electrode was firmly attached to each eye of the animal at 120 minutes after oral administration of the vehicle or BMS-201038, and then an electroshock of 165 volts for 0.7 seconds was applied to the eye. The

presence or absence of tonic flexor/extensor convulsions and death were monitored for 60 minutes after the electric stimulation.

No antagonistic activity to provoke convulsions was observed. Tonic flexor/extensor convulsions were observed in all animals. Following convulsions, 2, 4, 1, and 2 animals from the vehicle, 10, 30, and 100 mg/kg groups died, respectively.

To assess for pro-convulsive activity, another group of mice received an electroshock (electric current: 40 mA, pulse width: 1 msec, cycle: 50 Hz, duration: 0.2 sec.) 120 minutes after receiving vehicle or BMS-201038. The presence or absence of tonic convulsions and death were monitored for 60 minutes after the electric stimulation.

Pro-convulsive activity was not observed. No animals experienced convulsions or died.

Effects on seizure response induced by pentetrazol in mice (Nov 1998a study report; non-GLP)

Male CD-1 mice (10/group) were administered a single oral dose of 10, 30, or 100 mg/kg BMS-201038 or vehicle (75% PEG 400 in water). To evaluate the antagonistic effects to provoke convulsions, mice received a single intraperitoneal injection of 150 mg/kg pentetrazol 120 minutes after oral dosing of vehicle or BMS-201038. The presence or absence of clonic convulsions, tonic flexor/extensor convulsions, and death were monitored for 60 minutes after the electric stimulation.

No antagonistic activity to provoke convulsions was observed. Clonic convulsions and tonic flexor/extensor convulsions were observed in all animals. Following convulsions, all animals died.

To assess for pro-convulsive activity, mice received a single intraperitoneal injection of 41 mg/kg pentetrazol 120 minutes after oral dosing of vehicle or BMS-201038. The presence or absence of clonic convulsions, tonic convulsions, and death were monitored for 60 minutes after the electric stimulation.

Pro-convulsive activity was not observed. Clonic or tonic convulsions did not occur in any of the groups.

Analgesic activity in mice (Nov 1998a study report; non-GLP)

Male CD-1 mice (10/group) were administered a single oral dose of 10, 30, or 100 mg/kg BMS-201038 or vehicle (75% PEG 400 in water). Mice received an intraperitoneal injection of 0.6% acetic acid at 0.1 mL/g 120 minutes after oral dosing of vehicle or BMS-201038. Starting 5 minutes after acid injection, the number of writhings was counted for 10 minutes.

Some analgesic effects were noted at the 30 and 100 mg/kg dose levels, as the number of writhings (10 and 9, respectively) was decreased for those groups compared with the vehicle control group (23 writhings).

Effects on general behavior in rats (May 7, 1996 safety pharmacology report)
BMS-201038 was evaluated for effects on general behavior in male Sprague-Dawley rats (8/group) as a non-GLP exploratory study. One hour and approximately 3 hours after oral administration of 150 mg/kg or vehicle, general activity in an open field was measured for 10 minutes.

No effects on general activity were noted.

General activity and behavior in rats (Nov 1998a study report; non-GLP)
Male Sprague-Dawley rats (10/group) were administered a single oral dose of vehicle (75% PEG 400 in water) or 3, 10, 30, or 100 mg/kg BMS-201038. Mice were observed for awareness, mood, motor activity, CNS excitation, posture, motor incoordination, muscle tone, reflexes, and autonomic syndromes before dosing and at 0.5, 1, 2, 3, 4, 5, 6, and 24 hours after dosing.

No effects on general behavior were observed.

Lomitapide (also known as AEGR-733 and BMS-201038): Effects on Gross Behavioral and Physiological Status in Sprague Dawley Rats Using the Irwin Test (Study 733PC0017)
A GLP-compliant Irwin test was conducted to evaluate the potential effects of lomitapide on behavior and neurological function. Female Sprague-Dawley rats (6/group) were administered a single oral dose of vehicle (75% PEG 400 in water), lomitapide (1, 30, or 100 mg/kg), or 10 mg/kg chlorpromazine (positive control). Irwin test parameters were evaluated at 1, 4, 8, and 24 hours after dosing.

Loose stools, red staining around head and face, and lack of grooming were observed at 1, 4, and 8 hours after receiving either control or lomitapide. As the incidence and severity was similar across all groups, this appears to have been a vehicle-related effect. Rats receiving chlorpromazine exhibited behavioral and physiological effects consistent with its known pharmacological activity (e.g., decreased motor activity, catalepsy, and decreased alertness). In conclusion, lomitapide did not show adverse effects on behavior or neurological function under the conditions of this study.

Effects on body temperature in rats (Nov 1998a study report; non-GLP)
Male Sprague-Dawley rats (8/group) were administered a single oral dose of vehicle (75% PEG 400 in water) or 10, 30, or 100 mg/kg BMS-201038. Rectal temperatures

were measured before dosing and at 30, 60, 120, 180, 240, 300, and 360 minutes after dosing of vehicle or BMS-201038.

A slight decrease in mean body temperature (-0.3 to -0.5°C) was observed in rats receiving 100 mg/kg between 120 and 360 minutes compared with the control group (-0.1 to +0.4°C). This difference was statistically significant at 300 minutes, which was likely the result of a rise in temperature in the control group at that particular time point.

Cardiovascular

Lomitapide (also known as AEGR-733 and BMS-201038), BMS-203215, and BMS-203304: Effects on hERG Tail current Recorded from Stably Transfected HEK293 Cells (Study 733PC0019)

The effects of lomitapide and two major metabolites on tail currents of human ether-a-go-go-related gene (hERG)-encoded channels were evaluated in HEK293 cells stably transfected with hERG cDNA. A standard voltage profile was used, in which the cell membrane was depolarized from a holding voltage of -0 mV to a test voltage of +20 mV for 4.8 seconds, and then re-polarized to -50 mV for 5 seconds.

A dose response curve was generated from a minimum of four cells per concentration for all test articles assessed. Lomitapide concentrations included 0 (0.1% DMSO), 0.03, 0.1, 0.3, 1.0, and 3.0 µM. Concentrations for each of the metabolites were 9 (0.1% DMSO), 3, 10, 30, and 300 µM. Top concentrations were based on maximum solubility.

The IC50 values for IK_R channel inhibition was 1.72 µM for lomitapide, 135 µM for BMS-203215 (M1), and >300µM for BMS-203304 (M3).

Effects on blood pressure and heart rate in rats (May 7, 1996 safety pharmacology report; non-GLP)

BMS-201038 was evaluated for effects on blood pressure and heart rate in normotensive male Sprague-Dawley rats (5/group) as a non-GLP exploratory study. Blood pressure and heart rate were monitored at 1, 4, and 24 hours after administration of 150 mg/kg or vehicle. A statistically significant decrease in heart rate was observed at 24 hours after dosing compared with both the baseline value for that group and the 24-hour value for the vehicle control group (390 bpm vs. 450 bpm at baseline and vs. 474 bpm for 24-hour control). No effects on blood pressure were noted.

Effects on the cardiovascular system in dogs (Nov 1998a study report; non-GLP)

Male beagle dogs (3/group) received a single intravenous dose of vehicle or 0.8, 1.8, 4, or 20 mg/kg BMS-201038 at a rate of 1 mL/minute. Group 1 received vehicle and the 4 mg/kg dose level, Group 2 received the 0.8 and 1.8 mg/kg doses, and Group 3 received the 20 mg/kg dose. Parameters of heart rate, arterial pressure, femoral artery flow, and ECGs were evaluated by using non-invasive methods while under anesthesia

(pentobarbital and phenobarbital). Data were obtained before dosing and at 1, 5, 15, 30, 45, and 60 minutes after intravenous administration. Some respiratory parameters were also evaluated simultaneously as described in the next section below.

Noteworthy changes in cardiovascular parameters are discussed below. Maximal changes in parameter measurements are shown in parentheses; changes at other time points were often less severe.

At 0.8 mg/kg, one of three animals showed a decrease in mean blood pressure (↓9%), heart rate (↓27%), and femoral arterial blood flow (↓12%), and a rise in T wave height during the administration and for 60 minutes after administration.

At 1.8 mg/kg, one of three animals showed a decrease in mean blood pressure (↓8% to 14%) at 15 and 60 minutes after administration. No other effects on cardiovascular parameters were noted.

At 4 mg/kg, one of three animals showed a decrease in mean blood pressure (↓5% to 9%) and femoral arterial blood flow (↓11%) during administration and for 60 minutes after.

At 20 mg/kg, three of three animals showed a decrease in mean blood pressure (↓49% to 11%) and femoral arterial blood flow (↓18% to 67%). Effects had mostly reversed by 30 minutes. One animal had a decrease in heart rate (↓34%) and a rise in T wave height during administration and for 15 minute to 60 minutes after. A second animal had a decrease in heart rate (↓44%) along with extrasystole (disappearance of P wave, shortened or prolonged P-R interval, a rise in QRS duration, and a fall in the ST segment) during administration and for 15 minutes after.

Respiratory

Effects on the respiratory system in dogs (Nov 1998a study report; non-GLP)

Male beagle dogs (3/group) received a single intravenous dose of vehicle or 0.8, 1.8, 4, or 20 mg/kg BMS-201038 at a rate of 1 mL/minute. Group 1 received vehicle and the 4 mg/kg dose level, Group 2 received the 0.8 and 1.8 mg/kg doses, and Group 3 received the 20 mg/kg dose. Parameters of respiratory depth and rate were evaluated while under anesthesia (pentobarbital and phenobarbital). Data were obtained before dosing and at 1, 5, 15, 30, 45, and 60 minutes after intravenous administration. Cardiovascular parameters were also evaluated simultaneously as described above.

Noteworthy changes in respiratory changes are discussed below. Maximal changes in parameter measurements are shown in parentheses; changes at other time points were often less severe.

At 0.8 mg/kg, there were no effects on respiratory parameters.

At 1.8 mg/kg, two of three animals showed an increase in respiratory rate (↑71%) during administration and for 5 minutes after.

At 4 mg/kg, one of three animals showed an increase in respiratory rate (↑100%) during administration and for 1 minute after.

At 20 mg/kg, three of three animals showed an increase in respiratory rate (↑40% to 500%) during administration and for 60 minutes after. For one animal, the increase in respiratory movement occurred after the disappearance of respiratory movement and a second animal had a transient decrease in respiratory width.

For all dose levels, increases in respiratory rates were generally greatest at 1 minute after administration and then decreased over time.

Gastrointestinal

Effects on spontaneous motility of the isolated ileum in rabbits (Nov 1998a study report; non-GLP)

Ilea from five male JW rabbits were incubated with vehicle (DMSO) or BMS-201038 concentrations of 6×10^{-7}, 6×10^{-6}, or 6×10^{-5} mg/mL. Each ileum segment was suspended in Tyrode's solution at 37° C Magnus bath and incubated under each of the four treatment concentrations. Spontaneous motility was measured using a transducer before treatment and for 10 minutes after application of the vehicle or BMS-201038. Wave heights were measured about 1, 5 , and 10 minutes after the test substance application, and the ratio (%) of each wave height to that recorded 1 minute before the test substance application was calculated.

BMS-201038 did not affect the spontaneous motility of isolated rabbit ileum.

Antagonistic effects on ileum contractions induced by agonists in guinea pigs (Nov 1998a study report; non-GLP)

Ilea from five male Hartley guinea pigs (2 to 4 dose levels per specimen) were incubated with vehicle or BMS-201038 concentrations of 6×10^{-7}, 6×10^{-6}, or 6×10^{-5} mg/mL. Each ileum segment was suspended in Tyrode's solution at 37° C Magnus bath and incubated under each of the four treatment concentrations. Spontaneous motility was measured using a transducer. The vehicle (DMSO) or BMS-201038 was added into the Magnus bath when spontaneous motility of the isolated ileum disappeared. Each of the following agonists were added to the water bath 2 minutes after vehicle or BMS-201038: acetylcholine (6.8×10^{-7} M), histamine (9.0×10^{-7} M), serotonin (2.8×10^{-5} M), and barium chloride (4.8×10^{-3} M). The wave height showing the maximal agonist-induced contraction recorded was measured; the ratio (%) of the wave height showing the maximal agonist-induced contraction after application of the vehicle or BMS-201038 to that obtained before the application (the previous reading) was calculated.

BMS-201038 did not affect isolated ileum contractions induced by the agonists tested.

Effects on the propulsion ability in mice (Nov 1998a study report; non-GLP)
Male CD-1 mice (10/group) were administered a single oral dose of 10, 30, or 100 mg/kg BMS-201038 or vehicle (75% PEG 400 in water). Mice then received 0.2 mL/animal activated charcoal 120 minutes after test article administration. After 10 minutes, the full length of the small intestine was isolated and the distance that the charcoal traveled in the gastrointestinal tract was measured.

No effects on GI propulsion were noted for the 10 and 30 mg/kg groups; GI propulsion was significantly suppressed for the 100 mg/kg group. Intestinal passage rate was 2.6% for the high-dose group compared with 53% for the control group.

Renal
Effects on water and electrolyte metabolism in rats (Nov 1998a study report; non-GLP)
Male Sprague-Dawley rats (8/group) were administered a single oral dose of vehicle (75% PEG 400 in water) or 10, 30, or 100 mg/kg BMS-201038. Immediately after being treated, the animals received water for injection at 25 mL/kg by oral gavage and were placed in a metabolic cage individually. Urine was collected for 3 and 6 hours under deprivation of feed and water. The 3-hour urine was subjected to measurement of urinary volume and urine qualitative tests, and the 6-hour urine was subjected to measurement of urinary volume and concentrations of urinary electrolytes.

Urinary volume significantly decreased in the 6-hour urine sample for the 30 mg/kg group and at the 3- and 6-hour urine samples for the 100 mg/kg group. Urinary sodium and potassium were significantly decreased for all BMS-201038-treated groups. Urinary chloride was also decreased for the 100 mg/kg group. Urinary protein was higher than control for all eight animals receiving 100 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Method validation
An LC-MS/MS method was validated for the measurement of BMS-201038, BMS-203304 (M3), and BMS-203215 (M1) in dog plasma (Study 910062417). The method was then cross-validated for mouse and rat plasma as well as for metabolite BMS-224433 (M2) for all three matrices. The quantitation ranges for BMS-201038 were 0.2-100 ng/mL, 0.25-250 ng/mL, and 0.5-250 ng/mL for dog, rat, and mouse plasma, respectively. The quantitation ranges for both M3 and M1 were 0.4-100 ng/mL, 0.5-250 ng/mL, and 1-250 ng/mL for dog, rat, and mouse plasma, respectively. For M2, the quantitation ranges in dog, rat, and mouse plasma were 2-1000 ng/mL, 2.5-2500 ng/mL, and 5-2500 ng/mL, respectively. Ambient temperature stability of the four analytes in plasma was demonstratied over a 24-hour period in all matrices with the exception of BMS-201038 in mouse plasma, which was only stable for 6 hours, and M1 in dog plasma, for which the data were inconclusive. Long-term stability at -70°C was shown

for parent, M1, and M2 for 21 days. BMS-201038 and M3 were stable after three freeze/thaw cycles, whereas M1 showed high variability and M2 was not evaluated.

An LC-MS/MS method was validated for the measurement of BMS-201038 and BMS-203304 (M3) in rat and monkey plasma (Study BMS-910062026). The method was also applied to metabolite BMS-203215 (M1); however, the method did not meet the acceptance criteria for validation of rat plasma. Because of this, even though the acceptance criteria were met for M1 in monkey plasma, this method was not recommended for the determination of M1 in monkey plasma. For rat and monkey plasma, BMS-201038 was linear over a concentration range of 0.5 ng/mL to 500 ng/mL. M3 was linear between 2.5 ng/mL and 500 ng/mL. M1 was linear between 5 ng/mL and 500 ng/mL. Samples were found to be stable after three freeze/thaw cycles. The analytes were found to be stable in the extract after storage at room temperature for 24 hours or refrigerated for 4 days. When stored in rat plasma at room temperature for 6 hours, M1 and M3 appeared to be unstable; however these analytes were found to be stable in monkey plasma at room temperature for 6 hours.

An LC-MS/MS method was cross-validated for the measurement of BMS-201038, BMS203304 (M3), BMS-203215 (M1), and BMS-224433 (M2) in mouse plasma (Study 920000849). This method was found to be comparable in sensitivity, specificity, accuracy, and precision to the original API III^+ method. The lower limits of quantitation were 0.5 ng/mL (BMS-201038), 1.0 ng/mL (M1 and M3), and 10 ng/mL (M2).

An LC-MS/MS method was validated for the measurement of BMS-201038 and BMS203304 (M3) in rat liver (Study 910059768). The method was shown to be sensitive, accurate, and precise. The linear range was from 8.75 ng/g (BMS-201038) or 17.5 ng/g (BMS-203304) to 17,500 ng/g, using 0.1 mL samples of liver homogenate.

An LC-MS/MS method was validated for the measurement of BMS-201038, BMS-203215 (M1), BMS-203304 (M3), and BMS-224433 (M2) in dog lung (Study BMS-910065060). The method was found to be sensitive, accurate, and precise. The linear range was 31.5 ng/g to 180,000 ng/g using 1 mL samples of lung homogenate.

An LC-MS/MS method was validated for the measurement of AEGR-733 (BMS-201038), BMS-203304 (M3), BMS-203215 (M1), and BMS-224433 (M2) in plasma from mouse and rat carcinogenicity studies (Studies 7881-103, 7881-107, and 7881-108). Calibration curves for both matrices ranged from 1 to 1000 ng/mL for parent, 2 to 1000 ng/mL for M3 and M1, and 2.5 to 2500 ng/mL for M2. Samples stored at -60°C to -80°C were found to be stable for 252 days (mouse) and 251 and 560 days (rat). Short term stability was shown after three freeze/thaw cycles and after storage at room temperature for 24 hours.

Absorption

Rats:

Three Sprague-Dawley rats received a single intravenous injection of 4 µmol/kg (2.8 mg/kg of free amino) BMS-201038 (HCl salt) as a solution in 50% ethanol (BMS Study 910056240). Three additional rats received 20 µmol (13.9 mg/kg of free amine) BMS-201038 (HCl salt) by oral gavage. Serial blood sampling was conducted for 48 hours after dosing. Concentrations of BMS-201038 in plasma were measured by using an LC/MS/MS method. Results are summarized in the sponsor-generated table below.

Mean (SD) Pharmacokinetic Parameters and Bioavailability of BMS-201038 in Rats (N=3)

Parameter	Unit	Intravenous	Oral
Dose	mg/kg[a]	2.77	13.9
Cl	mL/min/kg	38.7 (5.8)	
V_{ss}	L/kg	14.4 (4.0)	
$t_{1/2\beta}$	hr	7.5 (0.2)	10.7 (4.8)
C_{max}	ng/mL		174 (100)
T_{max}	hr		0.83 (0.29)
MRT	hr	6.1 (1.0)	14.0 (7.2)
MAT	hr		7.9 (7.2)
$AUC_{0-\infty}$	ng/mL x hr	1210 (195)	1300 (445)
$NAUC_{0-\infty}$[b]	ng/mL x hr	1210 (195)	260 (89)
Bioavailability	%		21.4 (7.4)

[a] As the free amine of BMS-201038

[b] NAUC = AUC normalized to 2.77 mg/kg.

Limited evaluation - The concentrations of BMS-201038 in the liver and plasma of rats after single oral doses of BMS-201038 (BMS Study Report - 910056291)

Male Sprague-Dawley rats received a single oral dose of 14.6 mg/kg BMS-201038 (HCl salt) in 50% ethanol. Drug concentrations in plasma and liver were measured at 0.5, 1, 2, 4, 6, and 8 hours after dosing (2 rats/time point) by using an LC/MS/MS method.

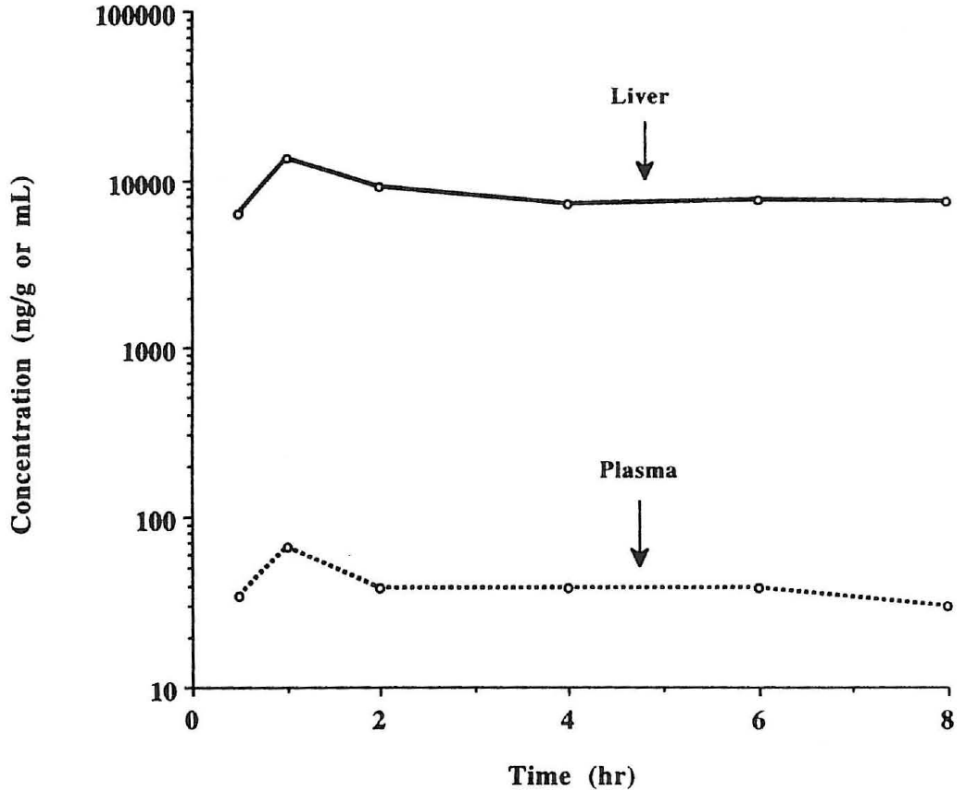
BMS-201038 concentrations in liver ranged between 6,350 and 13,900 ng/g for all time points. These concentrations were estimated to be 18,000- to 40,000-fold higher than the IC_{50} for MTP inhibition (0.5 nM; 0.35 ng/mL). Plasma concentrations ranged between 30.1 and 66.4 ng/mL. C_{max} occurred 1 hour after dosing for both liver and plasma. Concentrations in liver were about 200-fold higher than in plasma, indicating that liver is a highly exposed organ. The data are summarized in the sponsor-generated table and figure below.

The Average Concentrations of BMS-201038 in the Liver and Plasma (N=2) of Rats After Single 14.6-mg/kg Oral Doses

Time	Average Concentration (ng/g or mL)		Liver/Plasma	% Dose
(hr)	Liver	Plasma	Ratio	in Liver[a]
0.5	6350	34.3	185	1.4
1	13900	66.4	221	3.0
2	9210	39.0	227	2.1
4	7360	38.9	196	1.5
6	7690	38.3	202	1.7
8	7520	30.1	234	1.5

[a] % Dose in Liver = [Concentration in Liver (ng/g) x Liver Weight (g)] X 100 /Dose (µg) x 1000

Concentrations of BMS-201038 in the Liver and Plasma of Rats After Single 14.6-mg/kg Oral Doses



Evaluation of the relationship between the pharmacologic effects and concentrations of BMS-201038 in plasma and liver rats (BMS Study Report 910061832)

Male Sprague-Dawley rats (4/group) received daily oral doses of 0.625, 1.25, 2.5, 5, 10, or 20 mg/kg BMS-201038 in 25% PEG 400 for 4 consecutive days. Plasma was obtained pre-dose and at 1 hour post-dose each day. Blood was collected from a separate group of rats receiving 2.5 mg/kg/day for 4 days at 0.5, 2, 4, 8, and 24 hours or at 1, 3, 6, 12, and 48 hours post-dose on Day 4. Liver was collected from all rats at the time of last collection. Concentrations of 201038, BMS-203215 (M1), and BMS-203304 (M3) were measured by using an LC/MS/MS method. Plasma samples were also analyzed for total cholesterol, triglyceride, creatine kinase, ALT, and AST. Pharmacokinetic/pharmacodynamic analyses of the BMS-201038 plasma concentration versus time data and the cholesterol and triglyceride concentrations were also conducted for the 2.5 mg/kg/day dose level.

Maximum cholesterol lowering on Day 4 was observed at the 20 mg/kg/day dose level. Decreases in triglyceride levels were also seen at concentrations of 5 mg/kg/day and higher. There were no apparent treatment-related effects on ALT, AST, or creatine kinase levels. Concentrations of BMS-201038 and metabolites in plasma and liver increased in a dose-related manner. Concentrations of BMS-201038 in the liver were about 105 to 300 times the plasma concentration.

The pharmacokinetic/pharmacodynamic relationship showed that there was a delay in the development of the maximum pharmacological response. Modeling of this relationship predicted an IC_{50} of 6.59 ng/mL for cholesterol lowering and an IC_{50} of 5.53 ng/mL for the triglyceride lowering effect. The data are summarized in the sponsor-generated tables and figures below.

Table 4
Mean (SD) Concentrations of Cholesterol,Triglyceride, BMS-201038, BMS-203304, and BMS-203215 in Plasma of Rats (N=4) on Day 1
(Protocol No. 744/201038/002)

0 hr

Dose (mg/kg)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	BMS-201038 (ng/mL)	BMS-203304 (ng/mL)	BMS-203215 (ng/mL)
0	91.0 (7.0)	48.0 (10.5)	a	a	a
0.625	142 (16.8)	114 (13.2)	b	c	e
1.25	115 (11 4)	92.5 (15.5)	b	c	e
2.5	142 (1.1)	115 (11.2)	b	c	e
5	69.6 (3.9)	50.3 (11.5)	b	c	e
10	81.2 (5.1)	41.6 (10.6)	b	c	e
20	76.6 (9.6)	33.6 (7.7)	25.6 (51 2)	25.2 (50.5)	21.5 (42.9)

1 hr

Dose (mg/kg)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	BMS-201038 (ng/mL)	BMS-203304 (ng/mL)	BMS-203215 (ng/mL)
0	79.7 (3.0)	55.8 (6.0)	a	a	a
0.625	159 (7.6)	155 (22.5)	0.73 (0.9)	2.41 (1.6)[d]	e
1.25	117 (17.0)	95.1 (11.5)	12.8 (10.8)	16.0 (9.6)	e
2.5	99.7 (47.8)	75.0 (32.8)	22.9 (13.0)	24.5 (8.8)	7.15 (14.3)
5	65.1 (6.5)	23.6 (2.5)	28.5 (6.0)	29.2 (5.1)	6.59 (13.2)
10	71.9 (3.7)	19.9 (4.8)	89.0 (51.7)	51.2 (8.8)	23.5 (16.2)
20	66.6 (12.1)	21.0 (0.6)	177 (47.0)	77.3 (11.0)	39.2 (6.6)

a Sample was not analyzed.
b Plasma concentrations of all rats were below the lower limit of quantitation (LLQ) of 0.5 ng/mL.
c Plasma concentrations of all rats were below the LLQ of 2.5 ng/mL.
d Mean concentration is below the LLQ of 2.5 ng/mL because plasma concentrations of one or more rats were below LLQ.
e Plasma concentrationsof all rats were below the LLQ of 5 ng/mL.

Table 7
Mean (SD) Concentrations of Cholesterol,Triglyceride, BMS-201038, BMS-203304, and BMS-203215 in Plasma of Rats (N=4) on Day 4
(Protocol No. 744/201038/002)

0 hr

Dose (mg/kg)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	BMS-201038 (ng/mL)	BMS-203304 (ng/mL)	BMS-203215 (ng/mL)
0	67.3 (3.2)	36.7 (8.0)	a	a	a
0.625	125 (13.0)	89.5 (17.2)	0.25 (0.5)[b]	c	d
1.25	103 (4.7)	85.7 (13.7)	1.90 (0.5)	4.77 (3.4)	d
2.5	110 (17.7)	80.9 (23.4)	2.76 (0.7)	8.01 (10.8)	d
5	58.9 (7.5)	44.8 (20.3)	5.88 (1.9)	31.2 (19.8)	d
10	45.6 (5.2)	25.3 (5.2)	19 1 (10.8)	41.0 (9.7)	d
20	39.0 (7.0)	20.7 (5.9)	23.7 (13.7)	72.2 (15.8)	d

1 hr

Dose (mg/kg)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	BMS-201038 (ng/mL)	BMS-203304 (ng/mL)	BMS-203215 (ng/mL)
0	67.8 (3.9)	37.5 (5.2)	a	a	a
0.625	119 (7.3)	71.5 (5.1)	16.5 (3.0)	12.0 (8.6)	1.30 (2.6)[e]
1.25	84.1 (16.1)	55.6 (10.5)	17.5 (10.9)	22.1 (11.5)	d
2.5	88.3 (26.0)	43.7 (19.6)	28.7 (11.4)	44.1 (16.1)	6.78 (13.6)
5	54.3 (9.1)	27.8 (6.3)	37.9 (23.3)	73.2 (30.5)	22.9 (16.0)
10	44.8 (4.3)	14.4 (1.6)	81.8 (35.1)	149 (110)	28.9 (19.3)
20	38.5 (5.6)	16.4 (2.3)	83.0 (33.1)	175 (26.8)	31.4 (3.8)

a No sample was analyzed.
b Mean concentration is below the LLQ of 0.5 ng/mL because plasma concentrations of one or more rats were below LLQ and were treated as 0 in calculation of the mean and SD.
c Plasma concentrations in all rats were below the LLQ of 2.5 ng/mL.
d Plasma concentrations in all rats were below the LLQ of 5 ng/mL.
e Mean concentration is below the LLQ of 5 ng/mL because plasma concentrations of one or more rats were below LLQ and were treated as 0 in calculation of the mean and SD.

Table 8

Mean (SD) Concentrations of BMS-201038, BMS-203304, and BMS-203215 in Liver of Rats on Day 4 Following Daily Oral Doses BMS-201038
(Protocol No. 744/201038/002)

Leg	Dose (mg/kg)	Time After Dose (hr)	BMS-201038 (ng/mL)	BMS-203304 (ng/mL)	BMS-203215 (ng/mL)
A	0.625	1	1731 (278)	35.4 (9.3)	1884 (307)
	1.25	1	2498 (393)	73.4 (24.5)	2728 (434)
	2.5	1	3589 (1494)	193 (67.7)	3932 (1648)
	5	1	5285 (2317)	272 (61.6)	5802 (2555)
	10	1	8619 (3064)	559 (323)	9478 (3378)
	20	1	11796 (2847)	1213 (620)	12980 (3139)
B	2.5	24	847 (87.3)	55.2 (8.6)	909 (96.3)
	2.5	48	344 (130)	13.2 (16.0)[a]	354 (143)

a Mean concentration is below the LLQ of 17.5 ng/mL because plasma concentrations of one or more rats were below LLQ and were treated as 0 in calculation of the mean and SD.

Table 13
Mean (SD) Concentrations of BMS-201038, BMS-203304, and BMS-203215 in Rat Plasma after the Last of Daily Oral Doses of 2.5 mg/kg BMS-201038 for 4 Days (Leg B)
(Protocol No. 744/201038/002)

Time After Last Dose (hr)	BMS-201038 (ng/mL)	BMS-203304 (ng/mL)	BMS-203215 (ng/mL)
0	2.50 (0.3)	5.13 (4.6)	b
0.5	10.7 (2.1)	20.4 (6.2)	b
1	19.1 (3.5)	42.0 (11.4)	11.8 (1.2)
2	18.0 (2.6)	24.8 (4.5)	b
3	13.5 (4.9)	31.4 (10.1)	b
4	16.6 (2.9)	28.1 (7.1)	7.06 (6.1)
6	10.5 (5.5)	26.9 (10.3)	2.56 (5.1)[c]
8	13.3 (0.2)	19.2 (4.5	b
12	4 61 (2.8)	41.6 (3.9)	b
24	2.70 (0.8)	9.03 (2.4)	b
48	a	3.49 (4.1)	b

a Plasma concentrations in all rats were below the LLQ of 0.5 ng/mL.
b Plasma concentrations in all rats were below the LLQ of 5 ng/mL.
[c] Mean concentration is below the LLQ of 5 ng/mL because plasma concentration in one or more rats was below the LLQ and treated as 0 in calculation of the mean and SD.

Mean (SD) Concentrations of BMS-201038, Cholesterol, and Triglycerides in Plasma of Ra 1 hr on Days 1, 2, 3, and 4 After Daily Oral Administration of 0.625, 1.25, 2.5, 5, 10 and 20 mg/kg BMS-201038
(Protocol No. 744/201038/002)

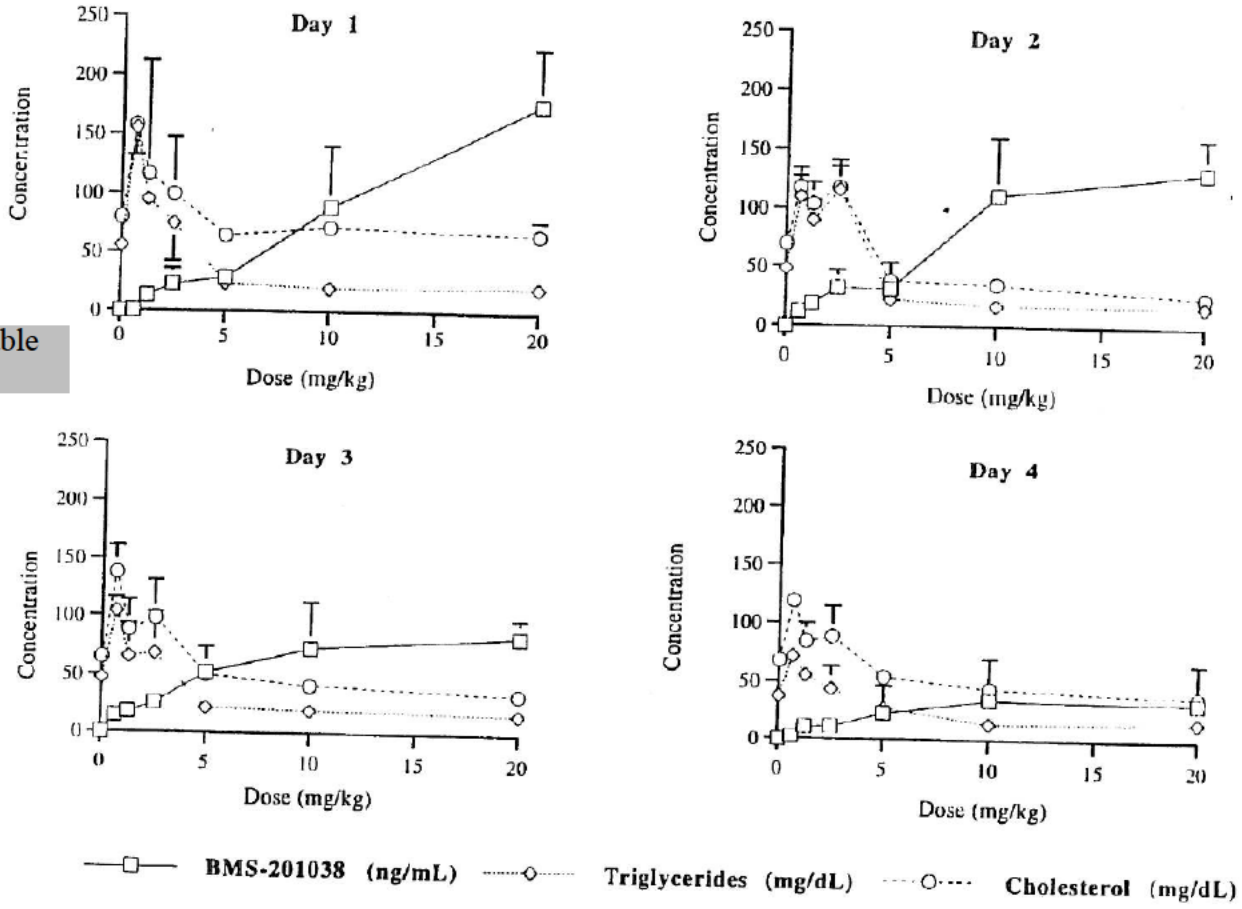


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Figure 2

Mean Observed Percent Difference (from Predose Levels) in Cholesterol/Triglyceride after Daily Oral Administration of 0.625, 1.25, 2.5, 5, 10, and 20 mg/kg Doses of BMS-201038 to Rats (Protocol No. 744/201038/002)

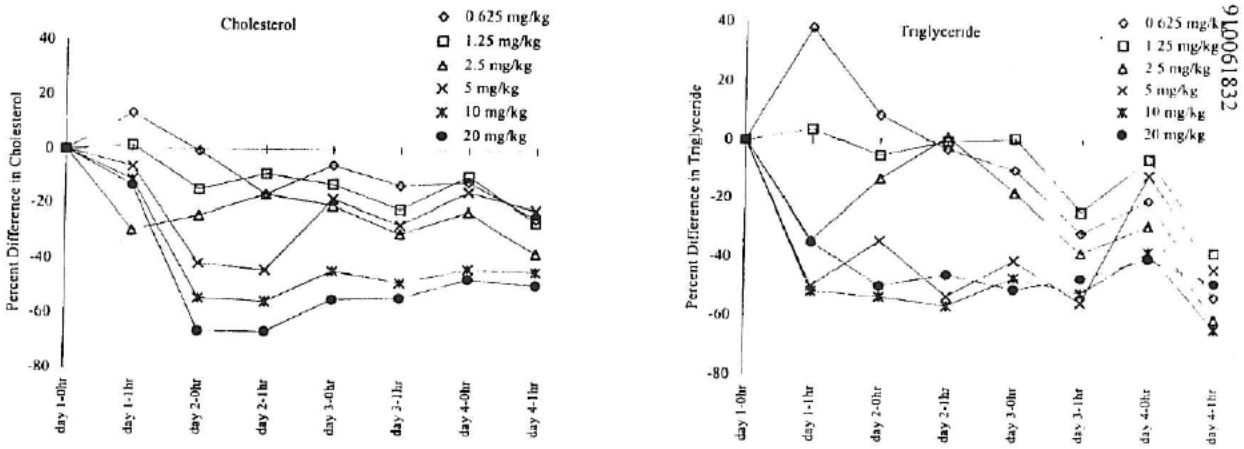
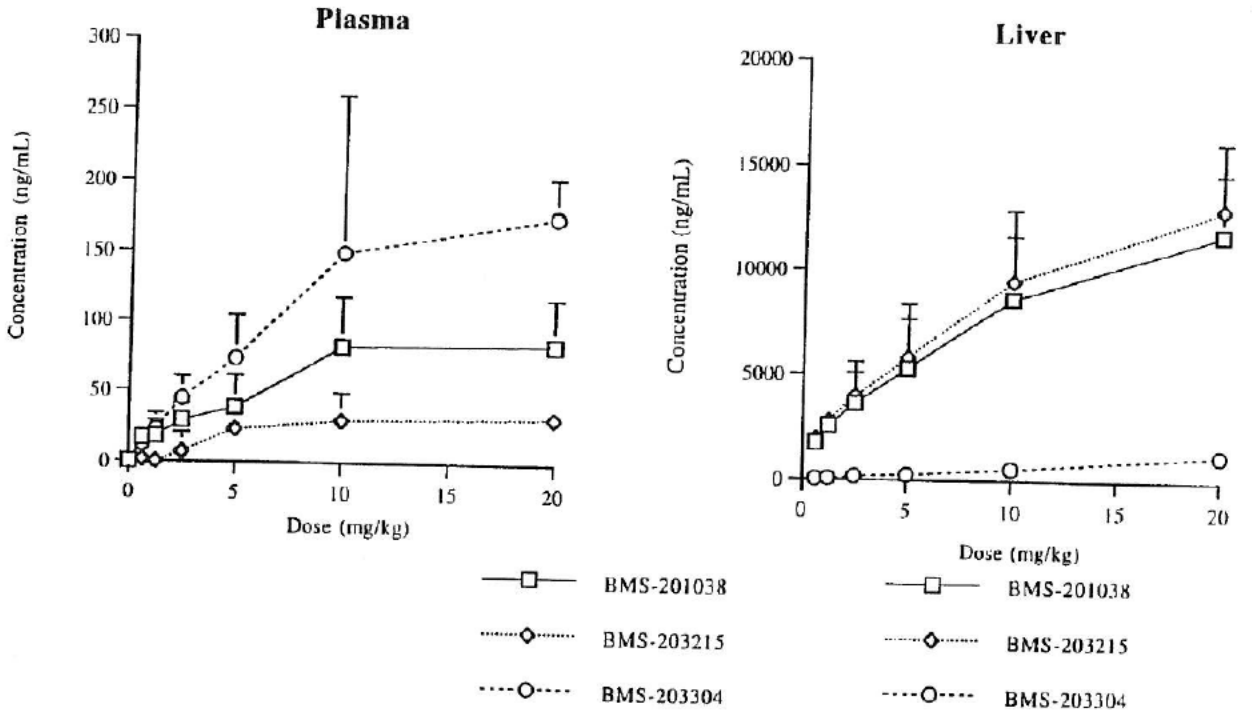


Figure 3

Mean (SD) Concentrations of BMS-201038, BMS-203304, and BMS-203215 in Plasma and Liver of Rats at 1 hr on Day 4 After Daily Oral Doses of BMS-201038
(Protocol No. 744/201038/002)



Dogs:
Four male beagle dogs were administered a single dose of BMS-201038 (methanesulfonate salt) by intravenous injection (0.8 mg/kg free base; n=4) or oral dosing (8 mg/kg free base; solution [25% PEG 400 in water]; n=4, and gelatin capsule; n=3) (BMS Study 910056234). There was at least 1 week between each dose administration. Serial blood sampling was conducted for 48 hours after each dose. BMS-201038 concentrations in plasma were measured by using an LC/MS/MS method. Results are summarized in the sponsor-generated table below.

TABLE 5

Mean (SD) Pharmacokinetic Parameters and Bioavailability of BMS-201038 Following Single Intravenous and Oral Administrations of BMS-201038 to Dogs (N=4) (Protocol No 744/201038/001)

Formulation	Nominal Dose (mg)	Cmax (ng/mL)	Tmax [a] (hr)	T-HALF (hr)	AUC(INF) (ng x hr/mL)	CLT (mL/min/kg)	VSS (L/kg)	MRT	MAT	Bioavailibility (%)
BMS-201038-04 (Methanesulfonate Salt in IV Solution)	10	[b]	[b]	9.10 (2.06)	2931 (453)	4.66 (0.78)	3.16 (0.34)	11.5 (1.48)	-	-
BMS-201038-04 (Methanesulfonate Salt in PO Solution)	100	663 (164)	4 (4.0, 8.0)	13.6 (2.70)	13451 (3471)	[b]	[b]	20.9 (4.64)	9.42 (4.21)	47.0 (9.93)
BMS-201038-04 (Methanesulfonate Salt in Capsules)	97 [e]	559 (225) [e]	24 (4, 28) [e]	11.0 [d]	16615 [e] (9185)	[b]	[b]	24.8 [e] (2.85)	13.5 [e] (1.97)	53.8 [e] (25.4)

[a] Median (Minimum, Maximum)
[b] Not applicable.
[c] N = 3. Dog No. 305 was not included in the calculations of the mean and SD. The capsule broke during dose administration and the animal did not receive the full dose. Therefore, plasma samples were not collected from Dog # 305.
[d] N = 2. T-Half for dog No. 303 not calculated.
[e] Dog No. 303 AUC(INF) was hand calculated based on Riegelman's equation.

Four male beagles received 20 mg/animal of different lot numbers of AEGR-733 (BMS-201038) by oral administration via gelatin capsules (Aegerion Study 733PC0020). There was an approximate 7-day wash-out period between the administration of each lot. Serial blood sampling was conducted for 96 hours after dosing. Concentrations of AEGR-733, BMS-203215 (M1), and BMS-203304 (M3) in plasma were measured by using an LC/MS/MS method. A summary of the results for each lot is presented in the sponsor-generated tables below.

Lot L0303315 (also called L0302139) was (b) (4) to yield pools of different sized test article particles, which were combined in different ratios to form two new lots. It is believed that the test article used in this study was the mesylate salt, but it is not stated in the study report. The different particle fractions for each lot are shown in the sponsor-generated tables below.

The data show that C_{max} and AUC values were approximately (b) (4) for the lot that contained particle sizes between (b) (4) compared with the original lot that contained particle sizes between (b) (4). The lot that contained particle sizes between (b) (4) microns also had an apparent improved bioavailability with C_{max} and AUC values being approximately (b) (4) than the original lot.

Pharmacokinetic parameters for Lomitapide, M1, and M3 in plasma collected from male dogs (Phase 1) following oral administration of Lomitapide (Lot No. L0303315/L0302139)

Animal Number	Phase	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng•h/mL)	AUC_{0-inf} (ng•h/mL)	$t_{1/2}$ (h)
			Lomitapide			
H02660	1	14.7	3.0	256	274	26.1
H02661	1	53.3	3.0	860	874	15.2
H02662	1	2.98	10	81.3	92.3	35.1
H02663	1	7.35	2.0	156	183	46.1
	Mean	19.6	—	338	356	30.6
	SD	23.0	—	355	353	13.1
	Median	11.0	3.0	206	229	30.6
	Range	—	(2-10)	—	—	—
			M1			
H02660	1	4.57	3.0	109	112	18.3
H02661	1	20.0	6.0	403	410	14.2
H02662	1	3.36	2.0	52.3	53.2	12.2
H02663	1	7.17	2.0	212	224	25.3
	Mean	8.78	—	194	200	17.5
	SD	7.65	—	154	157	5.79
	Median	5.87	2.5	161	168	16.3
	Range	—	(2-6)	—	—	—
			M3			
H02660	1	5.94	1.0	71.2	ND	ND
H02661	1	16.5	1.0	295	311	18.8
H02662	1	10.9	0.5	38.9	39.4	11.3
H02663	1	17.0	0.5	83.5	84.6	19.9
	Mean	12.6	—	122	145	16.7
	SD	5.22	—	117	146	4.68
	Median	13.7	0.8	77.4	84.6	18.8
	Range	—	(0.5-1)	—	—	—

Pharmacokinetic parameters for Lomitapide, M1, and M3 in plasma collected from male dogs (Phase 2) following oral administration of Lomitapide (Lot No. A13050-16)

Animal Number	Phase	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng•h/mL)	AUC_{0-inf} (ng•h/mL)	$t_{1/2}$ (h)
			Lomitapide			
H02660	2	42.2	4.0	610	635	24.3
H02661	2	54.8	24	1350	1370	14.5
H02662	2	12.5	4.0	223	235	29.9
H02663	2	89.1	4.0	1450	1490	21.1
	Mean	49.7	—	908	933	22.5
	SD	31.7	—	591	599	6.43
	Median	48.5	4.0	980	1000	22.7
	Range	—	(4-24)	—	—	—
			M1			
H02660	2	7.20	2.0	210	217	18.5
H02661	2	16.3	24	590	607	15.0
H02662	2	8.73	4.0	185	187	14.8
H02663	2	23.6	6.0	826	879	23.0
	Mean	14.0	—	453	473	17.8
	SD	7.56	—	310	332	3.85
	Median	12.5	5.0	400	412	16.8
	Range	—	(2-24)	—	—	—
			M3			
H02660	2	10.3	1.0	178	180	11.6
H02661	2	18.0	24	907	ND	ND
H02662	2	18.6	1.0	196	197	12.3
H02663	2	19.3	2.0	278	283	19.1
	Mean	16.6	—	390	220	14.3
	SD	4.20	—	348	55	4.14
	Median	18.3	1.5	237	197	12.3
	Range	—	(1-24)	—	—	—

Pharmacokinetic parameters for Lomitapide, M1, and M3 in plasma collected from male dogs (Phase 3) following oral administration of Lomitapide (Lot No. A13050-17)

Animal Number	Phase	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng•h/mL)	AUC_{0-inf} (ng•h/mL)	$t_{1/2}$ (h)
			Lomitapide			
H02660	3	54.3	4.0	977	1010	21.5
H02661	3	73.7	6.0	1300	1320	20.2
H02662	3	6.87	3.0	177	187	27.9
H02663	3	47.8	2.0	578	613	29.5
	Mean	45.7	—	758	783	24.8
	SD	28.1	—	487	491	4.61
	Median	51.1	3.5	778	812	24.7
	Range	—	(2-6)	—	—	—
			M1			
H02660	3	9.22	12	307	320	19.9
H02661	3	19.1	2.0	476	482	13.6
H02662	3	6.30	6.0	134	136	14.9
H02663	3	14.0	2.0	459	487	24.7
	Mean	12.2	—	344	356	18.3
	SD	5.61	—	159	166	5.07
	Median	11.6	4.0	383	401	17.4
	Range	—	(2-12)	—	—	—
			M3			
H02660	3	10.7	1.0	268	ND	ND
H02661	3	25.8	1.0	503	506	7.34
H02662	3	19.1	1.0	128	129	18.1
H02663	3	33.5	1.0	151	155	22.6
	Mean	22.3	—	263	263	16.0
	SD	9.70	—	172	211	7.84
	Median	22.5	1.0	210	155	18.1
	Range	—	N/A	—	—	—

Particle Size Distribution (%) of Drug Substance Lot L0109571 used to Prepare Drug Product Lot L0303315/L0302139 Based on (b) (4) Analysis*

STUDY PHASE	DRUG PRODUCT LOT NO.	(b) (4)
1	L0303315/L0302139	

*Mean of 5 measurements

(b) (4) of Drug Substance Lot L0109571 to Create Lot A13050-16 and Lot A13050-17 (% of Batch)

STUDY PHASE	LOT NO.	(b) (4)
2	A13050-16	
3	A13050-17	

Monkeys:
Three cynomolgus monkeys received 3.8 µmol/kg (2.63 mg/kg of free amine) BMS-201038 (HCl salt) in 50% ethanol by intravenous injection (BMS Study 910056239). One week later, the same monkeys received 23.8 µmol/kg (16.5 mg/kg of free amine) BMS-201038 (HCl salt) by oral gavage. Serial blood sampling was conducted for 72 hours after dosing. Concentrations of BMS-201038 in plasma were measured by using an LC/MS/MS method. A summary of the results are shown in the sponsor-generated table below.

Mean (SD) Pharmacokinetic Parameters and Bioavailability of BMS-201038 in Cynomolgus Monkeys (N=3)

Parameter	Unit	Intravenous	Oral
Dose	mg/kg	2.63	16.5
Cl	mL/min/kg	44.7 (4.1)	
V_{ss}	L/kg	37.7 (2.3)	
$t_{1/2\beta}$	hr	12.3 (2.8)	a
C_{max}	ng/mL		30.0 (15.6)
T_{max}	hr		8.0 (4.0)
MRT	hr	14.2 (2.1)	17.4 (1.5)
AUC_∞	ng/mL x hr	990 (95)	497 (208)
$NAUC_\infty$	ng/mL x hr	990 (95)	79 (33)b
Bioavailability	%		7.9 (2.1)

a Could not be determined due to insufficient data.
b Normalized to 2.63 mg/kg.

Distribution

In vitro:

In vitro binding of [^{14}C]-BMS-201038 to plasma proteins (BMS Study Report 910060036)

In vitro protein binding of [^{14}C]-BMS-201038 was assessed in freshly pooled plasma samples from rats, dogs, monkeys, and humans at nominal concentrations of 250, 500, 1000, 2500, and 5000 ng/mL.

The percent protein binding was found to be extensive and independent of drug concentration for all species over the concentration range tested. Percent protein binding averaged 99.5% in rat plasma, 99.8% in dog plasma, 99.6% in monkey plasma, and 99.8% in human plasma.

Rats:

Limited evaluation - The absorption and disposition of [^{3}H]-BMS-201038 in bile-duct cannulated rats (BMS Study Report 910056235)

Two bile duct cannulated (BDC) rats received an oral dose of 20 µmol/kg (13.9 mg/kg) ^{3}H-BMS-201038 (HCl salt) in 50% ethanol. Animals were sacrificed 24 hours after dosing and radioactivity was measured in various tissues. Results are summarized in the sponsor-generated tables below.

The Percent of Radioactive Dose Recovered After Single 20-µmol/kg Oral Doses of [^{3}H]-BMS-201038 to Two Bile Duct-Cannulated Rats

Sample	R493	R494	Average
Bile	63.54	66.24	64.89
Urine	4.96	1.04	3.00
Feces	11.01	14.79	12.90
Liver	3.76	2.47	3.12
Kidney	0.39	0.22	0.31
Lungs	0.56	0.32	0.44
Heart	0.06	0.05	0.06
Brain	0.00	0.00	0.00
G.-I. Tract	4.55	4.91	4.73
Carcass	10.94	8.00	9.47
Total	99.77	98.04	98.91
Absorption (%)[a]	84.21	78.34	81.28

[a] Estimated by subtracting the radioactivity in feces and the G.-I. tract from the total radioactivity recovered in all tissues.

The Concentration of Radioactivity in Selected Tissues at 24 hours
After Single 20-µmol/kg Oral Doses of [^{3}H]-BMS-201038 to Two Bile Duct-Cannulated Rats

	µg-Equivalent/g or mL			Ratio
Sample	R493	R494	Average	(Tissue/Plasma)
Blood	0.41	0.26	0.34	0.4
Plasma	0.92	0.92	0.92	1.0
Heart	3.88	2.78	3.33	3.6
Brain	0.13	0.09	0.11	0.1
Liver	19.96	14.17	17.07	18.6
Kidneys	11.61	6.21	8.91	9.7
Lungs	23.50	15.46	19.48	21.2
Skin	2.58	2.04	2.31	2.5
Adipose	4.55	3.61	4.08	4.4
Carcass	2.92	2.14	2.53	2.8

Limited tissue distribution and mass balance of total radioactivity following intravenous and oral administration of [^{14}C]-BMS-201038 to male Long-Evans rats (BMS Study Report 910059453)

Male Long-Evans rats (pigmented) received a single intravenous dose of 2.5 mg/kg or an oral dose of 10 mg/kg [^{14}C]-BMS-201038 (methanesulfonate salt). For each route of administration, rats (3/time point) were sacrificed at 1, 3, 6, 12, 24, or 48 hours after dosing. Tissue samples were assayed for total radioactivity from several organs.

Tissue concentrations were generally greatest at 1 hour post-dosing. The tissues having the greatest levels of radioactivity after intravenous administration were: lungs > thyroid > adrenals > liver > kidney > spleen > small intestine > bone marrow > heart. After oral dosing, tissue concentrations were generally greatest at 6 hours dosing, not including the gastrointestinal tract, which had the greatest levels of radioactivity at 1 hour or 3 hours post-dose (large intestine). Tissues have the greatest amount of radioactivity were: small intestine > stomach > liver > lungs > large intestine contents > adrenals > small intestine contents > large intestine > thyroid > spleen > kidney > bone marrow > heart. For both routes of administration, levels were low in the blood, brain, plasma, and testes. A summary of the results is presented in the sponsor-generated tables below.

Mean (SD) Concentrations of Total Radioactivity in Tissues, Organs and Specimens of Long-Evans Rats (N=3) Following Single 2.5 mg/kg Intravenous Doses of [^{14}C] BMS-201038

	Concentration of Total Radioactivity (μg equivalent BMS-201038/g)											
Tissue/Organ/Specimen	1 hr		3 hr		6 hr		12 hr		24 hr		48 hr	
Adrenals	21.7	(3.97)	12.4	(4.35)	7.74	(2.09)	3.15	(0.22)	1.45	(0.41)	0.49	(0.16)
Bladder	1.36	(0.36)	1.65	(0.57)	0.90	(0.12)	0.48	(0.00)	0.17	(0.03)	0.04	(0.01)
Blood	0.22	(0.02)	0.19	(0.02)	0.16	(0.02)	0.11	(0.01)	0.06	(0.01)	0.03	(0.01)
Bone Marrow	5.48	(0.33)	5.14	(0.76)	4.00	(0.47)	1.83	(0.37)	0.60	(0.16)	0.15	(0.04)
Brain	0.13	(0.00)	0.10	(0.01)	0.09	(0.01)	0.07	(0.00)	0.04	(0.01)	0.02	(0.01)
Carcass	1.97	(0.10)	1.43	(0.27)	1.13	(0.15)	0.56	(0.08)	0.21	(0.05)	0.06	(0.01)
Eyes	1.85	(0.14)	1.79	(0.06)	1.99	(0.19)	2.11	(0.17)	1.61	(0.20)	1.31	(0.19)
Heart	4.22	(0.28)	2.76	(0.46)	1.73	(0.24)	0.77	(0.07)	0.28	(0.07)	0.09	(0.02)
Kidney	9.32	(0.55)	5.80	(0.61)	3.38	(0.50)	1.68	(0.12)	0.82	(0.17)	0.28	(0.05)
Large Intestine	2.14	(0.18)	1.76	(0.10)	3.82	(0.62)	1.71	(0.51)	0.41	(0.10)	0.10	(0.02)
L.I. Contents	0.02	(0.00)	0.14	(0.09)	4.41	(0.29)	2.07	(0.63)	0.74	(0.28)	0.18	(0.06)
Liver	13.3	(0.92)	9.38	(1.17)	6.93	(0.48)	4.92	(0.32)	3.05	(0.25)	1.48	(0.13)
Lungs	38.8	(3.95)	29.8	(5.14)	18.6	(1.70)	8.30	(0.43)	1.66	(0.47)	0.37	(0.13)
Plasma	0.18	(0.01)	0.17	(0.01)	0.18	(0.02)	0.14	(0.01)	0.07	(0.01)	0.03	(0.01)
Skeletal Muscle	1.91	(0.27)	1.65	(0.27)	1.18	(0.26)	0.46	(0.04)	0.16	(0.04)	0.04	(0.01)
Skin (nonpigmented)	1.17	(0.06)	1.29	(0.08)	1.14	(0.18)	0.64	(0.09)	0.29	(0.09)	0.09	(0.03)
Skin (pigmented)	1.60	(0.13)	1.46	(0.13)	1.23	(0.13)	0.74	(0.09)	0.44	(0.16)	0.21	(0.02)
Small Intestine	6.10	(0.24)	8.21	(1.07)	4.08	(0.19)	2.52	(0.15)	1.62	(0.20)	0.77	(0.23)
S.I. Contents	0.78	(0.10)	2.38	(0.29)	1.06	(0.14)	0.51	(0.05)	0.21	(0.03)	0.09	(0.02)
Spleen	7.80	(0.34)	7.17	(0.94)	5.78	(0.97)	2.65	(0.28)	0.90	(0.11)	0.22	(0.09)
Stomach	3.15	(0.29)	1.67	(0.33)	0.78	(0.08)	0.37	(0.03)	0.15	(0.04)	0.05	(0.01)
Stomach Contents	0.07	(0.02)	0.06	(0.06)	0.03	(0.01)	0.01	(0.00)	0.01	(0.01)	0.00	(0.01)
Testes	0.18	(0.03)	0.20	(0.06)	0.23	(0.02)	0.23	(0.04)	0.15	(0.03)	0.08	(0.02)
Thyroid	28.2	(14.8)	15.3	(7.83)	4.33	(0.49)	3.79	(0.43)	1.11	(0.48)	0.37	(0.15)

Mean (SD) Concentrations of Total Radioactivity in Tissues, Organs and Specimens of Long-Evans Rats (N=3) Following Single 10 mg/kg Oral Doses of [^{14}C] BMS-201038

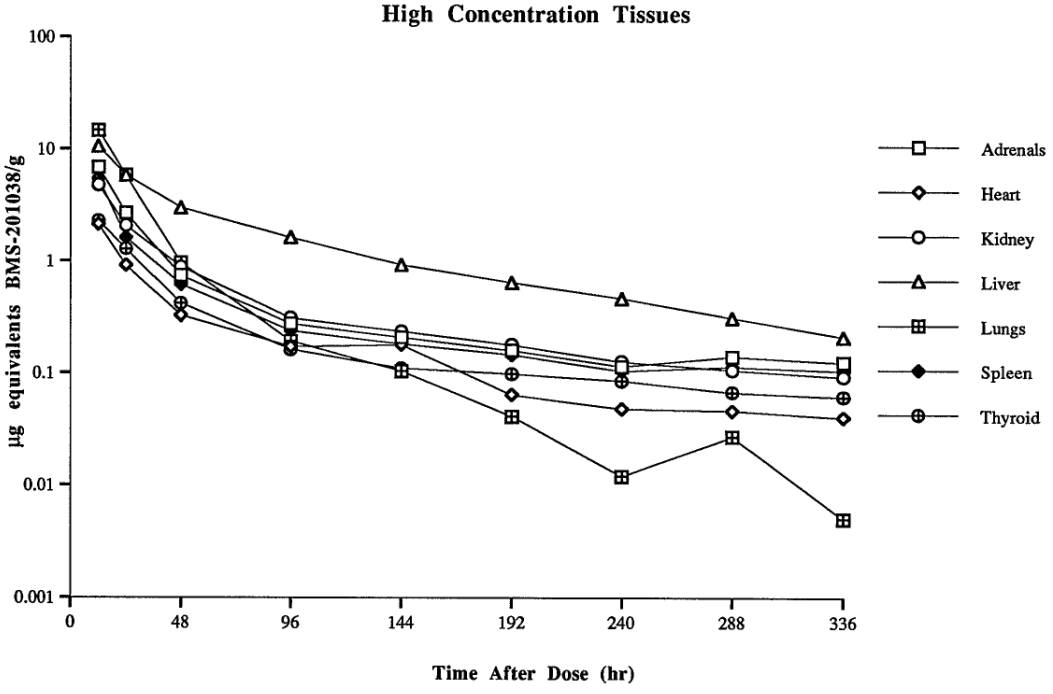
	Concentration of Total Radioactivity (μg equivalent BMS-201038/g)											
Tissue/Organ/Specimen	1 hr		3 hr		6 hr		12 hr		24 hr		48 hr	
Adrenals	10.3	(0.32)	11.4	(6.94)	14.1	(0.38)	6.69	(0.55)	2.32	(0.15)	0.84	(0.35)
Bladder	0.76	(0.04)	1.21	(0.85)	1.58	(0.09)	1.11	(0.07)	0.41	(0.08)	0.10	(0.04)
Blood	0.49	(0.08)	0.58	(0.23)	0.73	(0.05)	0.46	(0.05)	0.20	(0.03)	0.08	(0.03)
Bone Marrow	2.67	(0.08)	3.41	(2.32)	5.53	(0.65)	3.29	(0.42)	1.10	(0.06)	0.30	(0.16)
Brain	0.14	(0.03)	0.22	(0.08)	0.44	(0.05)	0.31	(0.05)	0.14	(0.01)	0.04	(0.02)
Carcass	0.84	(0.04)	1.32	(0.90)	1.93	(0.07)	1.25	(0.16)	0.43	(0.03)	0.14	(0.06)
Eyes	0.49	(0.02)	0.86	(0.49)	1.90	(0.08)	2.17	(0.35)	1.73	(0.12)	1.46	(0.72)
Heart	3.67	(0.30)	3.47	(1.93)	3.73	(0.28)	1.73	(0.24)	0.61	(0.04)	0.22	(0.09)
Kidney	6.50	(0.10)	7.22	(3.89)	8.19	(0.17)	3.71	(0.28)	1.71	(0.14)	0.71	(0.18)
Large Intestine	2.35	(0.86)	4.23	(3.87)	11.8	(3.58)	6.03	(1.53)	1.23	(0.42)	0.29	(0.08)
L.I. Contents	1.49	(1.25)	4.76	(3.93)	20.4	(2.61)	6.83	(2.26)	1.86	(0.21)	0.52	(0.19)
Liver	42.4	(2.27)	25.5	(8.87)	18.0	(1.79)	9.48	(0.81)	5.01	(0.44)	2.76	(0.14)
Lungs	14.3	(0.69)	17.4	(11.5)	25.3	(2.62)	10.4	(1.95)	2.45	(0.33)	0.60	(0.41)
Plasma	0.62	(0.11)	0.74	(0.28)	0.92	(0.01)	0.60	(0.09)	0.24	(0.03)	0.08	(0.02)
Skeletal Muscle	0.75	(0.07)	1.02	(0.61)	1.82	(0.04)	1.03	(0.10)	0.31	(0.03)	0.09	(0.03)
Skin (nonpigmented)	0.70	(0.10)	0.98	(0.50)	1.77	(0.09)	1.24	(0.24)	0.59	(0.09)	0.20	(0.07)
Skin (pigmented)	0.83	(0.04)	1.14	(0.64)	1.84	(0.07)	1.42	(0.09)	0.76	(0.10)	0.52	(0.22)
Small Intestine	102	(18.4)	77.5	(4.69)	18.1	(1.77)	4.22	(0.08)	2.21	(0.34)	1.17	(0.20)
S.I. Contents	8.02	(1.53)	12.9	(4.58)	6.61	(2.05)	1.30	(0.03)	0.43	(0.15)	0.15	(0.02)
Spleen	5.91	(0.72)	6.22	(3.39)	8.94	(0.66)	4.43	(0.61)	1.53	(0.06)	0.49	(0.21)
Stomach	64.0	(15.9)	27.9	(13.2)	3.08	(0.93)	0.83	(0.10)	0.48	(0.18)	0.12	(0.04)
Stomach Contents	8.73	(2.94)	6.99	(5.63)	0.86	(0.40)	0.06	(0.03)	0.35	(0.31)	0.01	(0.02)
Testes	0.14	(0.03)	0.26	(0.12)	0.50	(0.06)	0.46	(0.06)	0.28	(0.00)	0.15	(0.06)
Thyroid	7.73	(2.36)	10.5	(5.93)	10.2	(0.37)	6.37	(6.65)	1.08	(0.10)	0.52	(0.07)

A limited study of tissue distribution of total radioactivity following oral administration of [^{14}C]-BMS-201038 to Long Evans and Sprague Dawley rats (BMS Study Report 910059992)

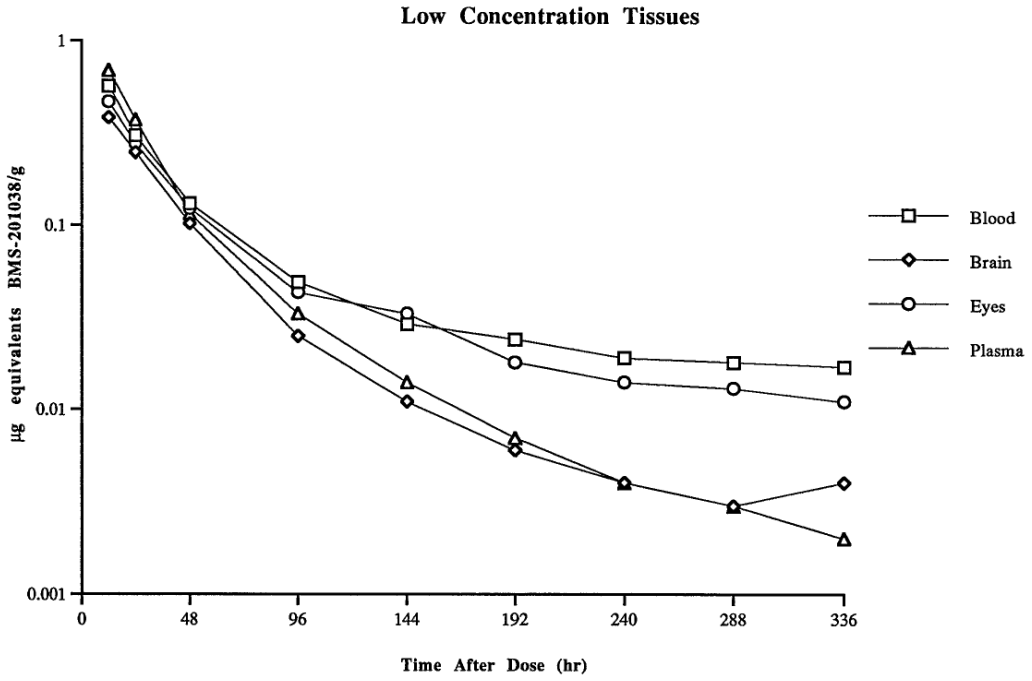
Male Sprague Dawley (albino) and Long Evans (pigmented) rats were administered a single oral dose of 10 mg/kg [^{14}C]-BMS-201038. Three rats from each strain were sacrificed at the following time points after dosing: 12, 24, 48, 96, 144, 192, 240, 288, and 336 hours. For Long Evans rats, eyes were collected up to the 336 hour time point and blood samples were collected up to 48 hours. For Sprague Dawley rats, a limited list of organs and tissues were collected for all time points.

The highest concentrations of radioactivity in Sprague Dawley rats were in liver > lungs > adrenals > kidneys > spleen > thyroid > heart. The blood, brain, plasma, and eyes showed low concentrations. Total radioactivity in the gastrointestinal tract was not assessed in this study. With the exception of the eyes, concentrations at 12, 24, and 48 hours were similar to those seen in the tissues from Long Evans rats in the tissue distribution study described above (Study 910059453). The concentration of total radioactivity in the eyes of Long Evans rats decreased with an apparent elimination half-life of about 235 hours. The results of ocular autoradiography of eyes collected at 1 and 24 hours after dosing indicated that the radioactivity was associated with the Hardarian glands, iris, and the retinal pigmented epithelium of Long Evans rats, whereas the radioactivity was associated with only the Hardarian glands of Sprague Dawley rats. The results of this analysis suggested that [^{14}C]-BMS-201038 associates with both melanin and porphyrin pigment-laden tissue. A summary of the results is presented in the sponsor-generated tables and figures below.

Mean Concentrations of Total Radioactivity in Tissues of Male Sprague Dawley Rats Following a Single 10 mg/kg Oral Dose of [14C]BMS-201038



Mean Concentrations of Total Radioactivity in Tissues of Male sprague Dawley Rats Following a Single 10 -mg/kg Oral Dose of [14C]BMS-201038



Mean (SD) Concentrations of Total Radioactivity (µg equivalents of BMS-201038/g or mL) in Tissues, Organs, and Specimens of Sprague Dawley Rats (N=3) Following Single 10-mg/kg Oral Doses of [14C]BMS-201038

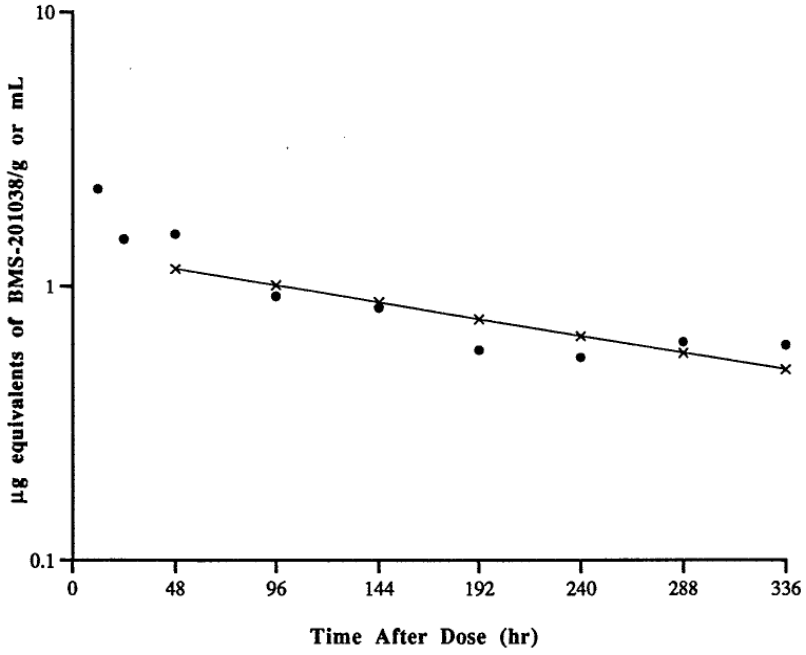
	Concentration of Total Radioactivity (µg equivalents of BMS-201038/g or mL)								
	12 hr	24 hr	48 hr	96 hr	144 hr	192 hr	240 hr	288 hr	336 hr
Adrenals	6.866	2.674	0.737	0.275	0.208	0.159	0.114	0.140	0.124
	(1.540)	(0.181)	(0.151)	(0.070)	(0.027)	(0.026)	(0.012)	(0.008)	(0.012)
Blood	0.569	0.306	0.131	0.049	0.029	0.024	0.019	0.018	0.017
	(0.043)	(0.026)	(0.008)	(0.001)	(0.001)	(0.001)	(0.002)	(0.001)	(0.002)
Brain	0.383	0.248	0.102	0.025	0.011	0.006	0.004	0.003	0.004
	(0.013)	(0.021)	(0.006)	(0.005)	(0.001)	(0.002)	(0.00)	(0.00)	(0.001)
Eyes	0.466	0.276	0.123	0.043	0.033	0.018	0.014	0.013	0.011
	(0.052)	(0.013)	(0.011)	(0.003)	(0.001)	(0.003)	(0.002)	(0.001)	(0.001)
Heart	2.116	0.913	0.325	0.171	0.178	0.064	0.048	0.046	0.040
	(0.421)	(0.046)	(0.045)	(0.065)	(0.143)	(0.003)	(0.005)	(0.001)	(0.004)
Kidney	4.759	2.057	0.883	0.308	0.235	0.178	0.126	0.106	0.092
	(0.806)	(0.169)	(0.099)	(0.059)	(0.003)	(0.010)	(0.007)	(0.012)	(0.005)
Liver	10.528	5.834	2.987	1.617	0.924	0.642	0.463	0.310	0.209
	(0.202)	(0.602)	(0.316)	(0.152)	(0.109)	(0.078)	(0.055)	(0.020)	(0.076)
Lungs	14.664	5.814	0.954	0.192	0.104	0.041	0.012	0.027	0.005
	(2.125)	(0.851)	(0.28)	(0.081)	(0.051)	(0.014)	(0.012)	(0.013)	(0.005)
Plasma	0.690	0.373	0.115	0.033	0.014	0.007	0.004	0.003	0.002
	(0.030)	(0.028)	(0.006)	(0.003)	(0.002)	(0.000)	(0.001)	(0.00)	(0.00)
Spleen	5.439	1.612	0.612	0.238	0.181	0.145	0.104	0.113	0.104
	(0.776)	(0.796)	(0.108)	(0.038)	(0.014)	(0.009)	(0.024)	(0.005)	(0.003)
Thyroid	2.276	1.278	0.417	0.161	0.110	0.098	0.085	0.067	0.061
	(1.028)	(0.477)	(0.158)	(0.050)	(0.024)	(0.008)	(0.037)	(0.012)	(0.007)

Mean (SD) Concentration of Total Radioactivity (µg equivalents of BMS-201038/g) in Eyes of Long Evans Rats Following Single 10-mg/kg Oral Doses of [^{14}C]BMS-201038

Time After Dose (hr)	Mean[a]	SD[a]
12	2.264	0.304
24	1.480	0.128
48	1.542	0.206
96	0.918	0.039
144	0.833	0.102
192	0.586	0.037
240	0.550	0.043
288	0.625	0.098
336	0.611	0.116

a Calculations of mean and SD were based on untruncated numbers.

Mean Concentration of Total Radioactivity in Eyes of Male Long Evans Rats Following a Single 10-mg/kg Oral Dose of [14C]BMS-201038[a]



Metabolism

In Vitro:

Metabolism of [^{14}C]-AEGR-733 in cryopreserved hepatocytes from mice, rats, dogs, and humans (Aegerion Study Report 733PC0009)

[F-^{14}C]-AEGR-733 (Fluorine carboxamine carbonyl) and [B-^{14}C]-AEGR-733 (Biphenyl carboxamine carbonyl) were each incubated with cryopreserved human hepatocytes for 4 hours at concentrations of 1 and 10 µM. A 1:1 mixture of these two radiolabeled compounds were also incubated with cryopreserved mouse, rat, and dog hepatocytes at 1 and 10 µM for 4 hours. Metabolite profiling was conducted by using RP-HPLC radiochromatography and specific metabolites were identified by LC/MS analysis.

Under the conditions of this study, AEGR-733 was metabolized by human hepatocytes moderately at 10 µM and extensively at 1 µM. Seven major metabolites (M1, M2, M3, M5, M9, M10, and M11) and three minor metabolites (M6, M7, and M8) were identified. The metabolism of AEGR-733 in mouse, rat, and dog hepatocyte incubations was less extensive compared with human hepatocytes; however, all metabolites produced in human hepatocyte incubations were also found in at least two animal hepatocyte incubations. The major metabolic routes are mono-oxidation (M7, M8, M9, and M11) and N-dealkylation (M1, M2, M6), followed by further oxidation (M3) and/or glucuronidation (M10). Summaries of the relative percent distribution of metabolites for human, mouse, rat, and dog hepatocytes are shown in the sponsor-generated tables below. The proposed metabolic pathways for humans, mice, rats, and dogs are shown in the sponsor-generated figure below.

Relative Percent Distribution of [F-^{14}C]AEGR-733 and [B-^{14}C]AEGR-733 and Individual Metabolites in 4-Hour Human Hepatocyte Incubations

Radioactivity Peak/Region (Met. Code)	R_t (min)	[F-^{14}C]AEGR-733		[B-^{14}C]AEGR-733	
		1 μM	10 μM	1 μM	10 μM
1 (M1)	26.63-27.63	ND	ND	17.80	13.77
2 (M5)	27.63-27.63	10.49	3.99	ND	ND
3 (M10)	30.38-30.38	8.39	5.37	ND	ND
4	32.13-32.38	ND	ND	6.80	2.73
5	33.63-33.63	ND	1.34	ND	1.11
6	35.88-36.13	ND	0.75	ND	0.83
7 (M6)	36.13-36.88	ND	0.15	ND	0.74
8 (M2)	38.13-38.38	ND	ND	15.53	5.50
9 (M7)	41.00-41.38	ND	1.79	ND	1.66
10 (M3)	41.88-41.88	18.65	8.98	ND	ND
11 (M8)	42.13-43.13	ND	0.75	ND	1.02
12 (M11)	43.38-43.38	15.62	13.01	19.74	13.45
13 (AEGR-733)	45.13-45.13	27.97	48.23	28.16	42.33
14 (M9)	45.88-46.63	5.13	5.52	2.91	6.01
Others[a]		13.75	10.12	9.06	10.85

ND: Non-detectable.

[a] Including other multiple minor radioactivity peaks.

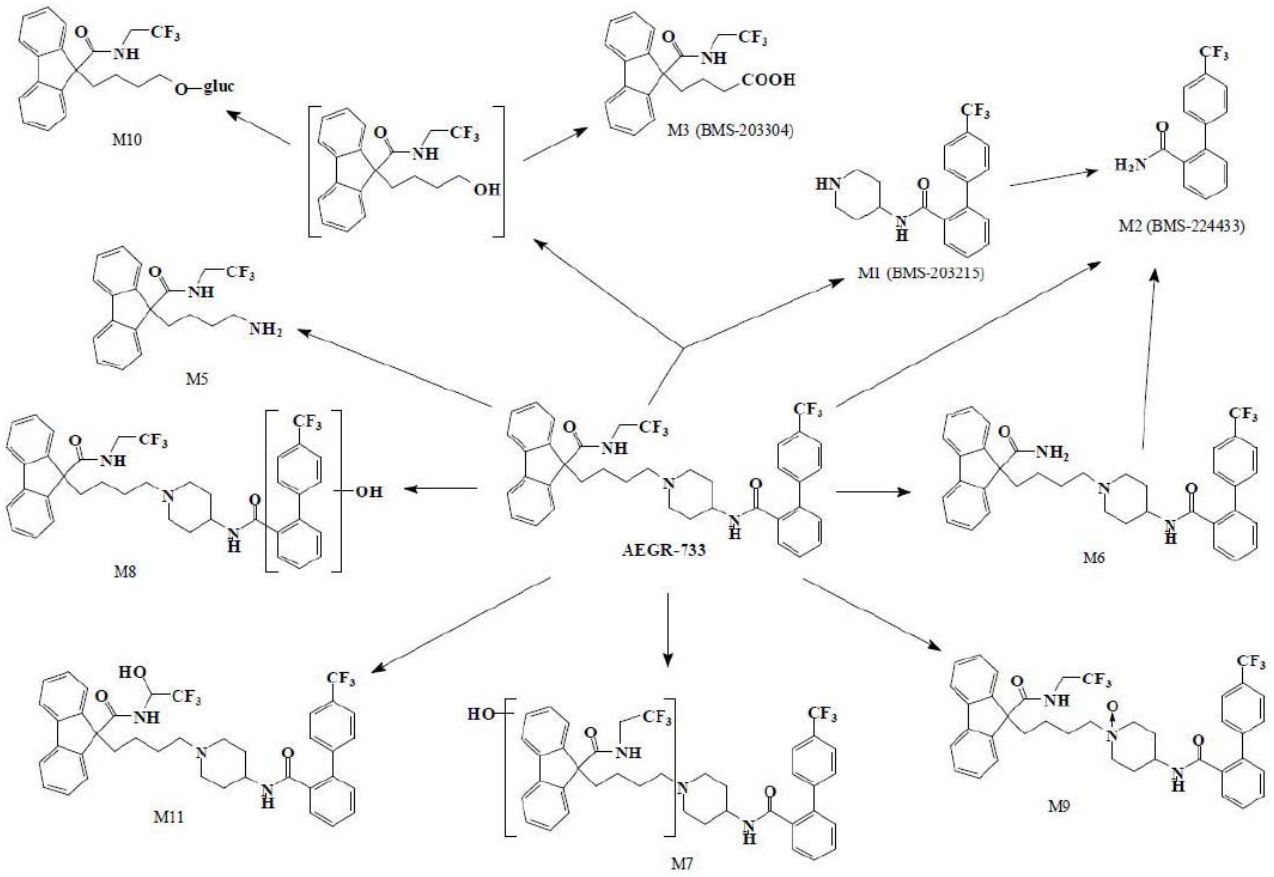
Relative Percent Distribution of [^{14}C] AEGR-733 and Individual Metabolites in 4-Hour Mouse, Rat, and Dog Hepatocyte Incubations

Radioactivity Peak/Region (Met. Code)	R_t (min)	MH		RH		DH	
		1 μM	10 μM	1 μM	10 μM	1 μM	10 μM
1 (M1)	26.63-27.63	4.55	10.19	1.56	2.12	17.70	10.67
2 (M5)	27.50-27.63	ND	0.97	ND	0.36	1.77	0.74
3 (M10)	30.38-30.50	ND	ND	ND	0.64	5.31	3.56
4	32.13-32.38	3.94	3.68	6.56	2.24	ND	0.55
5	33.63-34.00	ND	0.68	ND	0.97	1.77	0.58
6	35.63-35.88	ND	0.21	3.75	1.06	ND	0.26
7 (M6)	36.13-37.13	ND	0.14	ND	ND	ND	0.19
8 (M2)	38.13-38.38	1.52	1.47	2.81	1.27	4.42	2.04
9 (M7)	41.00-41.63	1.82	4.36	2.19	0.85	1.33	5.85
10 (M3)	41.88-41.88	9.70	9.72	ND	3.27	13.72	9.15
11 (M8)	42.13-42.63	1.82	0.46	2.19	0.30	1.77	0.13
12 (M11)	43.38-43.88	1.82	1.14	8.13	11.37	4.42	2.26
13 (AEGR-733)	45.13-45.63	66.06	58.78	65.00	67.63	45.13	54.14
14 (M9)	45.63-47.00	1.21	1.50	2.50	1.57	2.65	1.58
Others[a]		7.56	6.70	5.31	6.35	0.01	8.30

ND: Non-detectable.

[a] Including other multiple minor radioactivity peaks.

Proposed metabolic pathways for AEGR-733 in human and animal hepatocytes



Rats:

Analysis of [^{14}C]-BMS-201038 and metabolites in rat bile and liver and Analysis of [^{14}C]-BMS-201038 and metabolites in rat plasma and urine (BMS Study Reports 910064099 and 910064100, respectively)

Rats (strain not specified) were administered a single oral dose of 10 mg/kg [^{14}C]-BMS-201038. Bile samples were collected in 2-hour intervals through 12 hours and an additional 12-24 hour collection from each rat (5 rats/ time point). Liver samples were collected at 12 and 24 hours after dosing (3 animals/time point). Plasma was collected at 1, 6, and 24 hours post-dose (3 rats/time point). Urine was collected from time 0 to 24 hours post-dose (5 rats/time point).

The prominent radioactive peak isolated from bile was metabolite M304-AG (M18) and a minor radioactive peak was observed for M304 (M3). Little or no radioactivity was

present in samples at retention times corresponding to M215 (M1), M433 (M2), or parent compound.

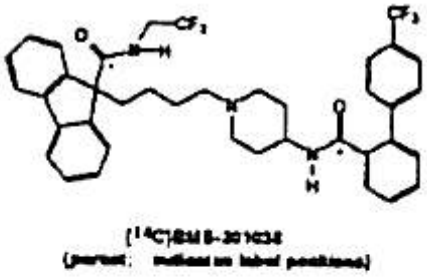
Liver samples showed that the major retention time peaks were metabolite M2 and parent compound. A minor radioactive peak represented M3 with little or no radioactivity in retention time peaks corresponding to M18 or M1.

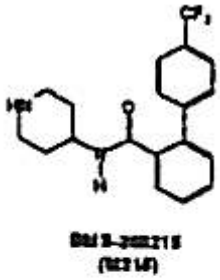
Total radioactivity in the soluble acetonitrile from plasma was 77%, 66%, and 64% at the 1, 6, and 24 hour time points. The majority of the radioactive retention time peaks represented metabolites M18 or M1, or the parent compound. Additional smaller, unidentified peaks were also detected.

Approximately 3% of the dose was isolated in urine. The prominent radioactive peak corresponded with M1 and a minor peak was M18. Little or no radioactivity was present in the retention time peaks corresponding to M2, M3, or parent compound. Additional smaller, unidentified peaks were also noted.

Structures of the metabolites (and parent) isolated in bile, liver, plasma, and/or urine are shown in the sponsor-generated figure below.

BMS-201038 M215 (M1)





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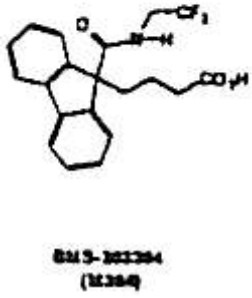
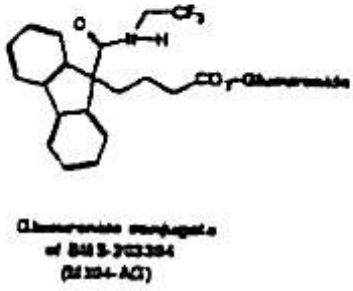
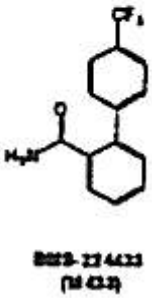






M304 (M3) M304-AG (M18) M433 (M2)

Limited evaluation - excretion of unchanged BMS-201038 in bile and urine of rats (BMS Study Report 910056489)

Groups of three male BDC rats (strain not specified) were each administered an intravenous dose of 3 mg/kg or an oral dose of 6.5 mg/kg BMS-201038 (free base; HCl salt) in 50% ethanol. Bile and urine were collected for 24 hours. The concentration of BMS-201038 in bile and urine was determined by using an LC/MS/MS method.

After 24 hours, the percentage of administered dose of parent compound isolated in bile was 0.72% and 0.79% for the intravenous and oral routes, respectively. The percentage of administered dose of parent compound isolated in urine was 0.07% and 0.06% for the intravenous and oral routes, respectively. Therefore, the amount of unchanged parent compound in bile or urine was minimal.

Biotransformation of AEGR-733 (lomitapide) in male Sprague-Dawley rats after a single oral dose of [^{14}C]-AEGR-733 (Aegerion Study Report AEGR733-PC0016)

Male Sprague-Dawley rats were orally administered a single dose of 10 mg/kg [^{14}C]-AEGR-733. One group of rats had blood samples taken at 1, 2, 4, 6, 24, 36, and 48 hours post-dose (3 rats / time point). Another group of 3 rats and a group of 3 BDC rats had urine collected for the following time periods: 0 to 6, 6 to 24, and 24 to 48 hours; feces was collected at 0 to 24 and 24 to 48 hours post-dose. The BDC rats also had bile collected at pre-dose and for the following time periods: 0 to 6, 6 to 24, and 24 to 48 hours. A discussion regarding elimination routes for this study is presented in the elimination section below.

Unchanged AEGR-733 represented 5.6% of the administered dose in rat plasma. Nine metabolites (M1, M2, M3, M11, M14, M18, M20, M24, and M28) were detected in plasma, with M2 (33.2%), M11+M24 (coeluting; 14.1%), M14 (20.3%), and M28 (4.57%) being the major metabolites. Plasma total radioactivity reached a C_{max} of 1105 ng Eq/g at 4 hours with a mean half-life of 9.6 hours.

Urine was a minor elimination route; more than 10 minor metabolites were identified in urine: M1, M2, M3, M5, M10, M13+M27 (coeluting), M14, M15, M16, M17, M18, M20, M21, and M22. None of these metabolites represented more than 1% of the administered dose. Metabolites M8, M21, and M22 were the prominent fecal metabolites, with M1, M3, M11+M24 (coeluting), M23, M26, and M27 being minor metabolites, with each making up less than 3% of the administered dose. The major metabolites in bile were M18 and M22. Minor bile metabolites included M1, M3, M11+M24 (coeluting), M10, M17, and M21, with each accounting for less than 2% of the administered dose.

Exposure profiles for parent and major metabolites in plasma are shown below in sponsor-generated Table 3. The major metabolic pathways were oxidation (M8, M11, M23, M24, M25, M28), oxidative *N*-dealkylation (M1, M2, M3, M5), followed by oxidation (M12, M13, M20, M21, M22, M26, M27), glucuronide conjugation (M10, M14, M15,

M17, M18), and piperidine ring opening (M16, M19). Among them, M12, M19, and M25 were detected only by LC/MS. The metabolic pathways proposed for rats are shown in sponsor-generated Figure 9 below.

Table 3 Concentration (AEGR-733 ng Eq./mL) of AEGR-733 and Its Metabolites in 1-, 2-, 6-, 24-, 36- and 48-Hour Plasma Extracts and Estimated $AUC_{0\text{-}48hr}$ Values Following Oral Administration of AEGR-733 to Rats

Radioactivity Peak/Region	Concentration (AEGR-733- ng Eq./mL)[a]						$AUC_{0\text{-}t}$	
	1 hr	2 hr	6 hr	24 hr	36 hr	48 hr	hr*ng Eq/mL[c]	%[d]
TRA[b]	547	765	906	542	206	96.1	23600	100
1 (M14)	47.9	109	201	148	ND	6.60	4790	20.3
2 (M1)	28.4	28.6	13.0	4.34	ND	ND	282	1.19
3	ND	6.96	8.24	ND	ND	ND	NA	NA
4 (M18)	6.24	11.2	ND	8.02	ND	ND	107	0.45
5 (M20)	20.1	55.5	31.7	9.27	ND	ND	591	2.50
6	ND	ND	5.89	ND	7.09	3.31	170	0.72
7	7.60	11.2	ND	13.0	7.79	3.63	345	1.46
8 (M2)	56.9	118	224	190	146	68.0	7830	33.2
9 (M3)	70.6	65.9	32.9	17.2	ND	ND	752	3.19
10 (M28)	8.31	29.5	63.4	33.3	ND	ND	1079	4.57
11 (M11+M24)	107	163	176	49.3	9.21	4.30	3330	14.1
12 (AEGR-733)	130	97.2	64.5	12.3	3.54	1.65	1320	5.59

ND: not detected; NA: Not applicable due to lack of of enough data points for calculation of AUC values.

[a] Concentrations (AEGR-733 ng Eq./mL) = Relative Percent Distribution (Appendix L) × Concentration of TRA

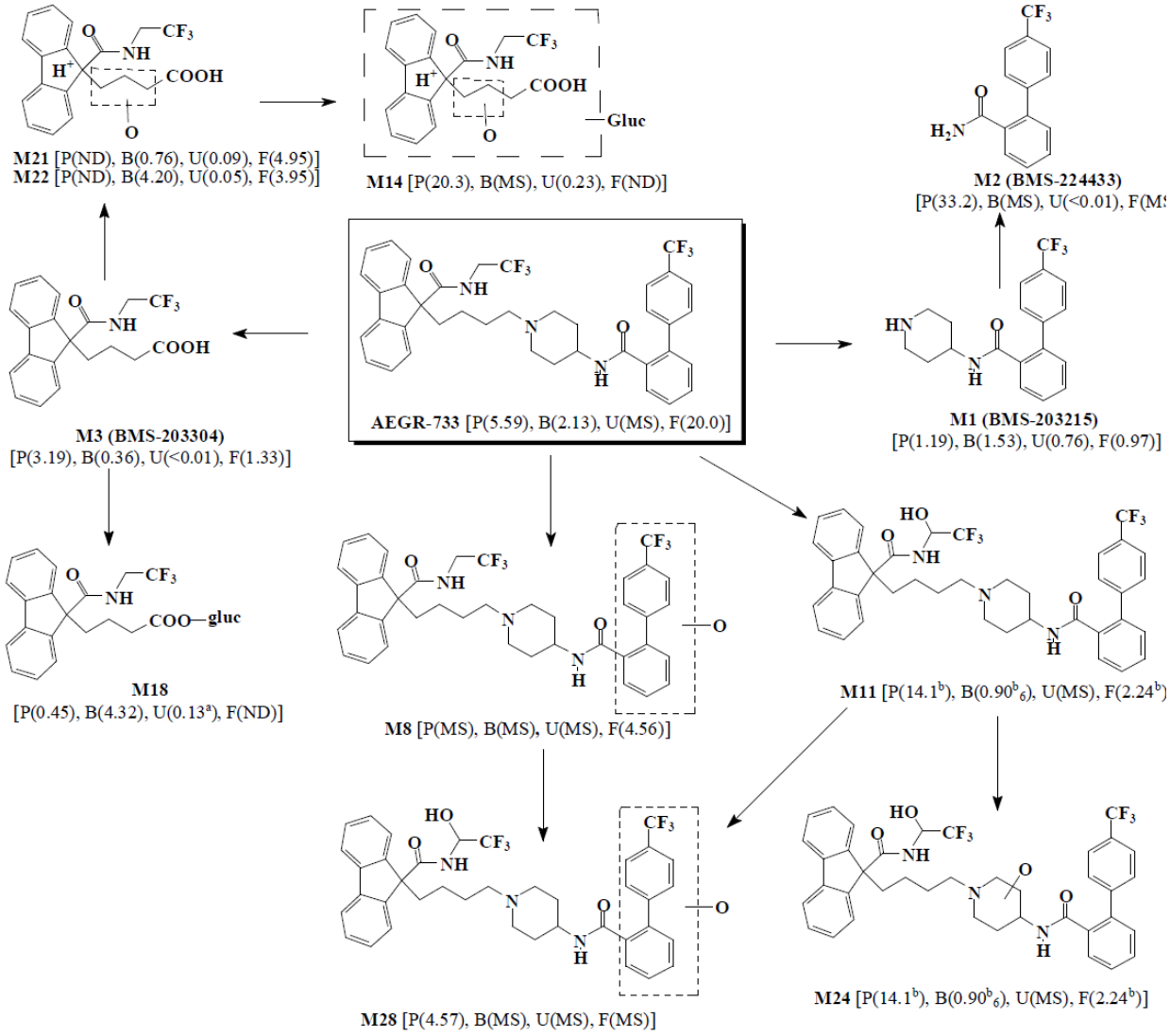
[b] Concentration of total radioactivity in plasma samples from rats (Table C-1)

[c] The AUC values were obtained using WinNonlin for AEGR-733, M1, M2, M3, M11 +M24 (coeluting), M14, and M20. (Table C-2). The AUC values of the other metabolites were obtained by trapezoidal calculation using Excel.

[d] $\%AUC_{0\text{-}t}$ = $AUC_{0\text{-}t}$ (individual metabolite region)/$AUC_{0\text{-}t}$ (plasma TRA)[c] x 100%.

Note that the plasma samples were taken in volumes rather than in weights; therefore, the concentration is expressed as ng Eq./mL instead of ng Eq./g.

Figure 9 Proposed Major Metabolic Pathways of AEGR-733 in Rats



Gluc = glucuronic acid, ND = Not detectable by MS and radioprofiling, MS = Detectable by LC/MS only.

Note: those metabolites accounting for >3% of AUC0-48 hr and/or >3% of dose were presented except for M1.

Values in parenthesis are the percent of AUC of the total plasma radioactivity for rat plasma (P) and percent of dose for bile (B), urine (U), and feces (F).

[a] M18 coeluted with M10 in rat urine. The value was combined with M10.

[b] M11 coeluted with M24 in rat plasma, bile, and urine. The value was combined with M24.

The metabolic pathways of all metabolites identified in rats are depicted in Figure P-55.

Dogs:

Analysis of [^{14}C]-BMS-201038 and metabolites in dog plasma and urine (BMS Study Report 910064102)

Six male beagle dogs received a single oral dose of 2 mg/kg [^{14}C]-BMS-201038. The metabolite profile was examined in plasma and urine by using reverse phase HPLC.

The amount of total radioactivity in the soluble acetonitrile fraction was 93%, 84%, and 75% for the 1, 8, and 24 hour time points. The major retention time peak corresponded to the parent compound and minor peaks corresponded to metabolites M215 (M1), M304 (M3), M304-AG (M18), and M433 (M2). The amount of radioactivity recovered from urine after 24 hours was not reported. The major retention time peak in urine corresponded to M1. Another prominent radioactive peak corresponded to an unidentified metabolite. A minor peak was observed for M18 and M3. Little or no radioactivity was associated with peaks for M2 or parent compound. Other minor, unidentified peaks were observed in plasma and urine.

Biotransformation of AEGR-733 (lomitapide) in male Beagle dogs after a single oral dose of [^{14}C]-AEGR-733 (Aegerion Study Report AEGR733-PC0013)

Three male beagle dogs were orally administered a single dose of 2 mg/kg [^{14}C]-BMS-201038. Blood, urine, and feces were collected up to 24 hours post-dose for the determination of lomitapide metabolite profiles.

AEGR-733 was the major circulating drug-related component in dog plasma. Radioprofiling and LC/MS/MS methods detected 14 metabolites. The major urinary metabolites were M1 and M13, representing 3.65% and 3.53% of the dose. In feces, unchanged AEGR-733, M3, and M11+M24 (co-eluting) represented 2.46%, 4.30%, and 1.23% of the administered radioactivity, respectively. Metabolic pathways of AEGR-733 were oxidation (M8, M11, M23, M24, M25, M28), oxidative N-dealkylation (M1, M2, M3, M5), followed by oxidation (M13, M22, M26, M27), glucuronide conjugation (M10, M15, M17, M18), and piperidine ring opening (M19). Pharmacokinetic results of the parent and major metabolites in plasma over a 24-hour period are shown in sponsor-generated Table 5. The major metabolic pathways for dogs are shown in sponsor-generated Figure S-1.

Table 5 Pharmacokinetic Parameters for Total Radioactivity, AEGR-733, and Its Metabolites after a Single Oral (2 mg/kg) Dose of [^{14}C]AEGR-733 to Male Beagle Dogs (Group 1)

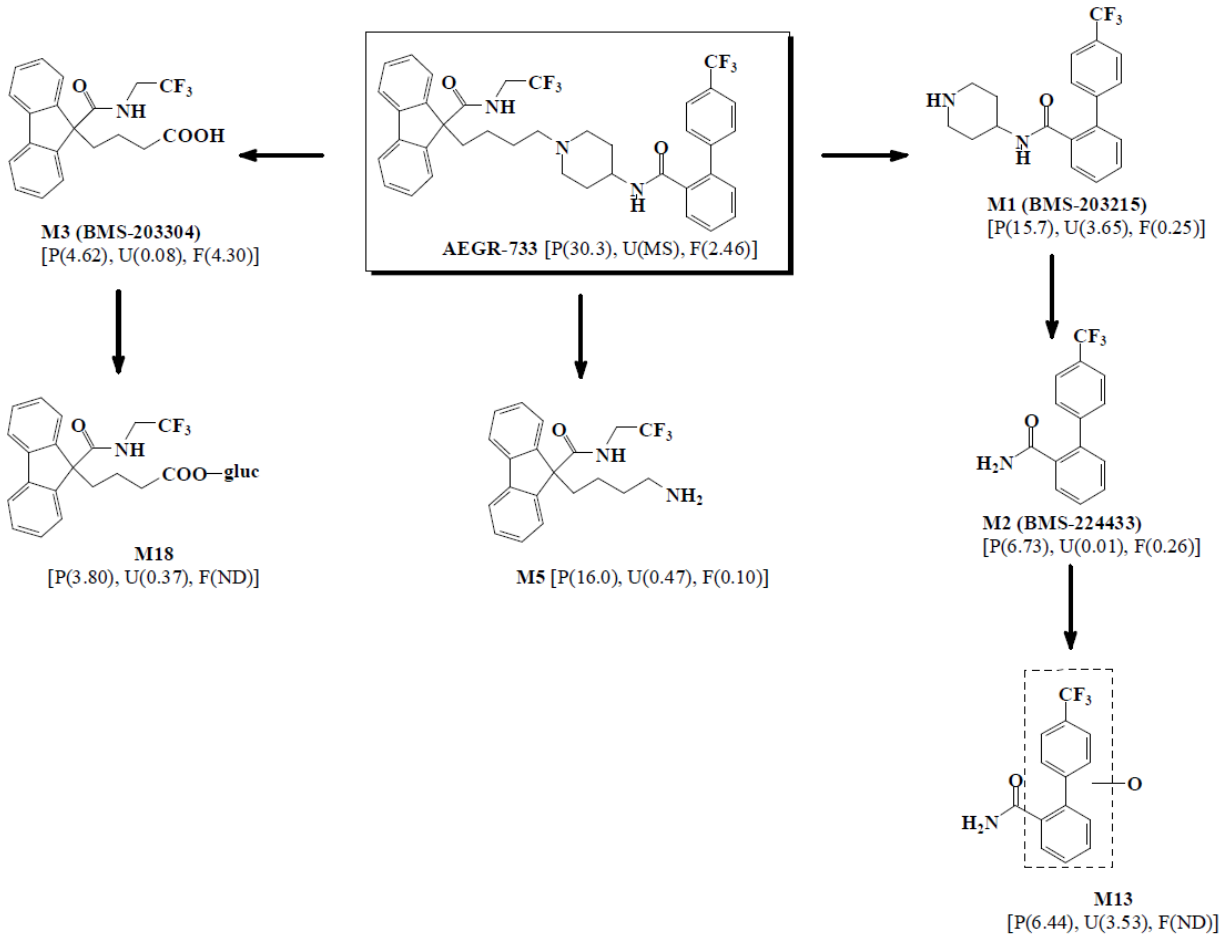
Radioactivity Region	C_{max} (ng.Eq./mL)	T_{max} (hr)	$T_{1/2}$ (hr)	AUC_{0-t} (hr*ng.Eq./mL)
TRA[b]	402	2.00	17.3	6210
1 (M13)	29.6	1.00	9.19	400
2 (M26)	6.95	2.00	10.7	125
3 (M1)[a]	54.8	8.00	30.2	978
4 (M5)[a]	57.4	8.00	19.2	993
6 (M18)	21.5	1.00	27.2	236
7 (M10)[a]	4.56	8.00	19.5	72.0
8 (M2)	38.3	2.00	10.8	418
9 (M3)	29.0	1.00	17.5	287
10 (M25)	8.60	2.00	14.9	114
11 (M11 + M24)	9.25	2.00	ND	167
12 (AEGR-733)	144	2.00	14.4	1880

[a] C_{max} value included for $T_{1/2}$ determination.

ND: Not determined. Terminal elimination phase not defined.

[b] Note that TRA in plasma and concentration of AEGR-733 and its metabolites in 1-, 2-, 8-, 12-, and 24-hr plasma samples were used for the calculation of the above PK parameters. This yielded slightly different PK parameters for TRA from those presented in Table E-3.

Figure S-1 Proposed Major Metabolic Pathways of AEGR-733 in Dogs



gluc = glucuronic acid, MS = Detectable by LC/MS only, ND = Not detected by radioprofiling and MS method.

Values in parenthesis are the percent of AUC of the total plasma radioactivity for dog plasma (P) and percent of dose for urine (U) and feces (F).

Note: those metabolites accounting for >3% of $AUC_{0\text{-}24\ hr}$ and/or >3% of dose were presented.

Monkeys:

Analysis of [^{14}C]-BMS-201038 and metabolites in monkey plasma and urine (BMS Study Report 910064101)

Six Cynomolgus monkeys received a single oral dose of 20 mg/kg [^{14}C]-BMS-201038. The metabolite profile was examined in plasma and urine by using reverse phase HPLC.

The amount of total radioactivity in the soluble acetonitrile fraction was 88%, 75%, and 61% for the 1, 8, and 24 hour time points. The major retention time peak corresponded to metabolite M433 and minor peaks corresponded to M215 (M1), M304 (M3),

M304-AG (M18), and parent compound. The amount of radioactivity recovered from urine after 24 hours was 20% of the administered dose. The major retention time peak in urine corresponded to an unknown metabolite. A minor peak was observed for M18. Little or no radioactivity was associated with peaks for M1, M2, M3, or parent compound. Other minor, unidentified peaks were observed in plasma and urine.

Best Available Copy

Comparative Metabolite Profile Across Species
Sponsor-generated table from BMS Study Report 910064103

Species (dose)	Time of Plasma Collection (hr)	Quantitative Plasma Biotransformation Profiles [b] (% of plasma radioactivity)					
		Acetonitrile Insoluble Fraction [a]	BMS-201038	BMS-203215	BMS-203304	BMS-224433	Remaining Unidentified Fraction [c]
Rat (10 mg/kg)	1	23	9	4	27	9	28
	6	34	3	5	14	19	25
	24	36	1	9	7	22	25
Dog (2 mg/kg)	1	7	34	9	12	9	29
	8	16	34	16	3	5	26
	24	25	26	18	5	2	24
Monkey (20 mg/kg)	1	12	7	12	12	12	45
	8	25	3	14	5	21	32
	24	39	3	11	3	23	21

[a] radioactivity remaining associated with the protein precipitate following acidified acetonitrile extraction
[b] parent drug and metabolites were determined by comparison to retention time of authentic standard
[c] unidentified radioactivity in the acetonitrile-soluble fraction

Excretion

Rat:

Limited tissue distribution and mass balance of total radioactivity following intravenous and oral administration of [^{14}C]-BMS-201038 to male Long-Evans rats (BMS Study Report 910059453)

Male Long-Evans rats (pigmented) received a single intravenous dose of 2.5 mg/kg or an oral dose of 10 mg/kg [^{14}C]-BMS-201038 (methanesulfonate salt). Recovery of total radioactivity in cage wash, blood, liver, and carcass was determined at 96 hours after dosing. Urine and feces were collected continuously for 96 hours at 24-hour intervals.

Total recovery of radioactivity after 96 hours for the intravenous and oral routes was 101.4% and 96.3%, respectively. Recovery in feces was 94.3% and 89.2% for the intravenous and oral routes, respectively. Recovery in urine was 3.5% and 4.7% for the intravenous and oral routes, respectively. Based on the high degree of fecal excretion after intravenous dosing, it appears that biliary excretion is a primary route of drug elimination. A summary of the data is presented in the sponsor-generated table below.

Mean (SD) Recovery of Total Radioactivity (% of Dose) in Urine, Feces, Cage Wash, Blood, Liver and Carcasses of Male Long-Evans Rats (N=5) Per Route of Administration Following Single 2.5 mg/kg Intravenous and 10 mg/kg Oral Doses of [^{14}C]-BMS-201038

Specimen	Time (hr)	Dose Route[a] PO		IV	
Urine	0-24	3.86	(0.49)	2.65	(0.36)
	24-48	0.65	(0.06)	0.63	(0.18)
	48-72	0.15	(0.02)	0.18	(0.05)
	72-96	0.06	(0.01)	0.07	(0.01)
Subtotal		4.73	(0.49)	3.53	(0.59)
Feces	0-24	75.67	(4.85)	66.60	(9.88)
	24-48	11.14	(4.13)	21.84	(8.16)
	48-72	1.70	(0.27)	4.43	(1.23)
	72-96	0.66	(0.04)	1.46	(0.13)
Subtotal		89.18	(1.44)	94.34	(0.74)
Cage W.-Wat	0-24	0.65	(0.38)	0.28	(0.20)
	24-48	0.09	(0.06)	0.04	(0.03)
	48-72	0.03	(0.02)	0.00	(0.00)
	72-96	0.01	(0.01)	0.00	(0.00)
Subtotal		0.79	(0.46)	0.32	(0.19)
Cage W.-Met	96	0.08	(0.06)	0.01	(0.02)
Subtotal		0.08	(0.06)	0.01	(0.02)
Blood	96	0.01	(0.00)	0.01	(0.00)
Subtotal		0.01	(0.00)	0.01	(0.00)
Liver	96	0.74	(0.05)	1.55	(0.12)
Subtotal		0.74	(0.05)	1.55	(0.12)
Carcass	96	0.80	(0.10)	1.64	(0.20)
Subtotal		0.80	(0.10)	1.64	(0.20)
Total[b]		96.32	(1.19)	101.39	(0.48)

[a] All values rounded

[b] Includes urine, feces, cage washes, blood, liver and carcass

Biotransformation of AEGR-733 (lomitapide) in male Sprague-Dawley rats after a single oral dose of [^{14}C]-AEGR-733 (Aegerion Study Report AEGR733-PC0016)
Male Sprague-Dawley rats were orally administered a single dose of 10 mg/kg [^{14}C]-AEGR-733. A group of 3 rats and a group of 3 BDC rats had urine collected for the following time periods: 0 to 6, 6 to 24, and 24 to 48 hours; feces was collected at 0 to 24 and 24 to 48 hours post-dose. The BDC rats also had bile collected at pre-dose and for the following time periods: 0 to 6, 6 to 24, and 24 to 48 hours. Additionally, another group of rats had blood samples taken at 1, 2, 4, 6, 24, 36, and 48 hours post-dose to determine the metabolite profile in plasma, as discussed above in the metabolism section.

Urinary excretion was a minor elimination pathway, with 3.15% of the radioactive dose recovered in urine. Parent compound was only detected in urine from rats by LC/MS, but not by radioprofiling. Approximately 88.1% of the total administered dose was recovered in rat feces after the 48 hour collection period. In BDC rats, 36.7% of the radioactivity was recovered in bile and 49.9% was recovered in feces. This indicates that at least 40% of the administered dose was absorbed from the gastrointestinal tract. Unchanged parent compound represented 20% of the administered dose in feces. A summary of the excretion data are shown in sponsor-generated Figures 1 and 2 below.

Figure 1 Mean Cumulative Recovery of Radioactivity in Excreta of Male Intact Sprague-Dawley Rats (Group 2, n=3) after a Single Oral Administration of [^{14}C]AEGR-733 (10 mg/kg)

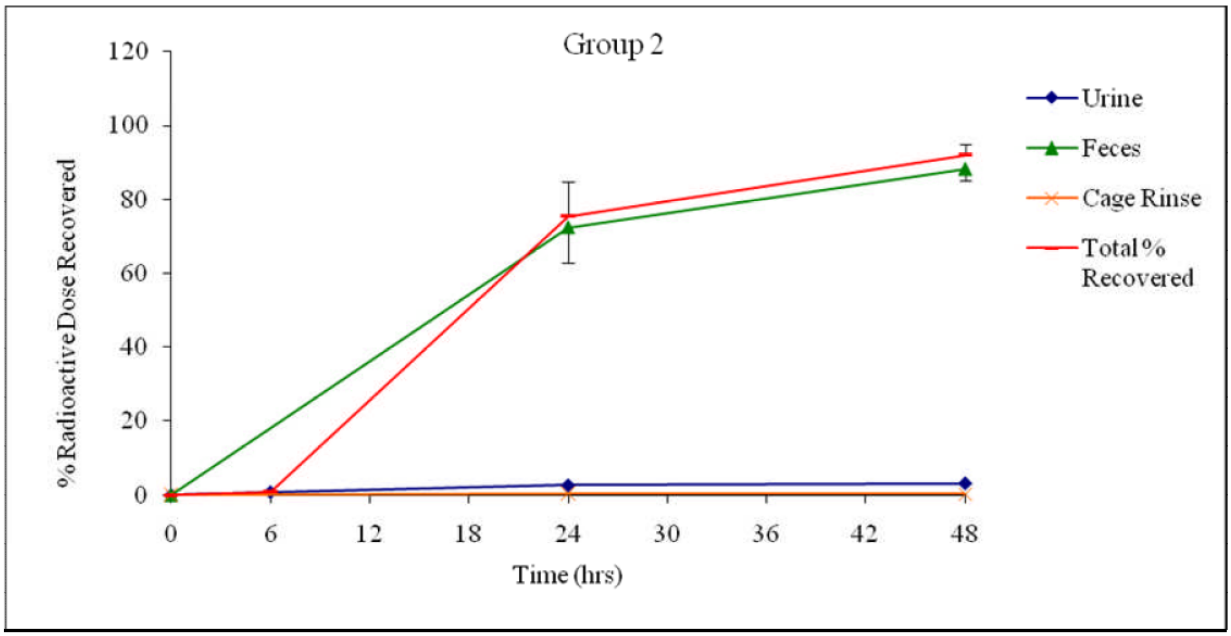
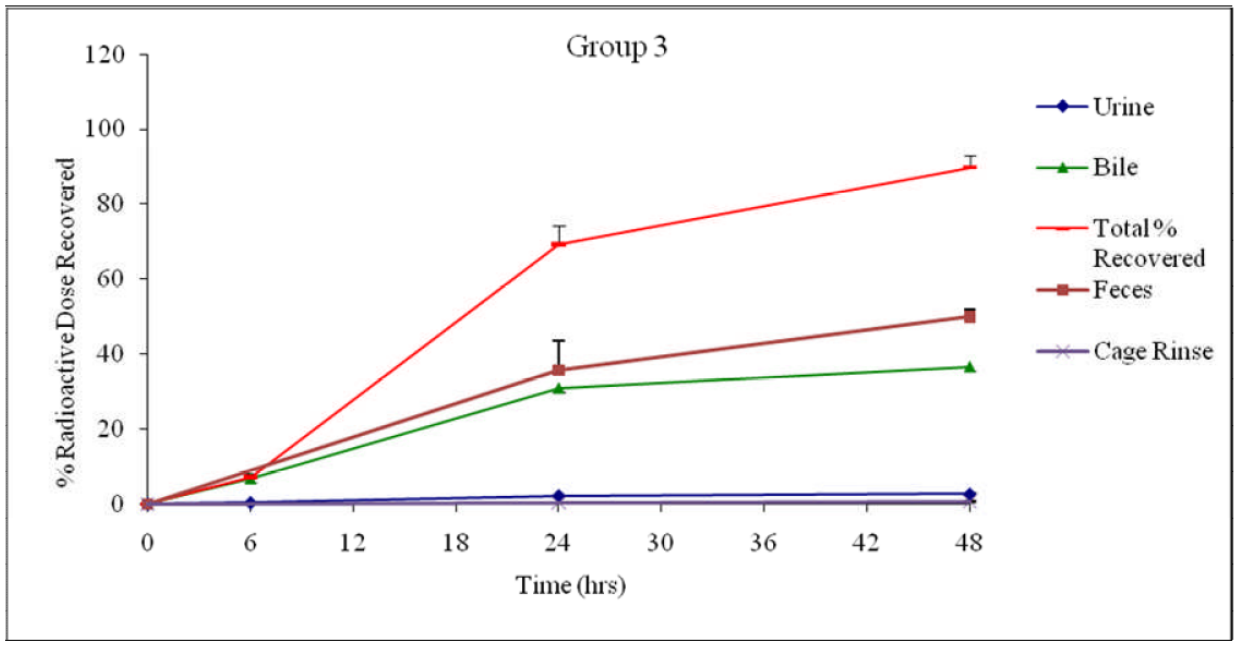


Figure 2 Mean Cumulative Recovery of Radioactivity in Excreta of Male BDC Sprague-Dawley Rats (Group 3, n=3) after a Single Oral Administration of [^{14}C]AEGR-733 (10 mg/kg)



Dogs:

Biotransformation of AEGR-733 (lomitapide) in male Beagle dogs after a single oral dose of [^{14}C]-AEGR-733 (Aegerion Study Report AEGR733-PC0013)

Three male beagle dogs were orally administered a single dose of 2 mg/kg [^{14}C]-BMS-201038. Blood, urine, and feces were collected up to 24 hours post-dose for the determination of AEGR-733 metabolite profile.

The mean total recovery of excreted radioactivity was 31% after 24 hours, with 10.5% recovered in urine and 20.5% recovered in feces. Unchanged AEGR-733 was only detected at a trace level in urine. Other endpoints of this study are discussed above in the metabolism section.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-Dose Toxicity

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (mg/kg)	Endpoints	Findings
Single dose - oral 96011 GLP	Mouse/CD-1 5/sex/group	0, 300, 600, 1200, and 2400	Clinical signs; BW; standard gross pathology and histopathology on stomach only for early decedents and after 2-week observation period	• Dose-related, transient, minimal to marked BW losses noted for all groups. • At 2400 mg/kg, decreased activity (2-24 hr), fecal staining and deaths of 1 M and 1 F within 24 hours. • Early decedents had moderate distention of the stomach with fluid, but no microscopic correlate. • No macroscopic or microscopic findings after 2 week recovery.
Single dose - IV bolus 96035 GLP	Mouse/CD-1 5/sex/group	0, 12.5, 25, and 50	Clinical signs; BW; gross pathology on early decedents and after 2-week observation period for 25 and 50 mg/kg groups only	• At 50 mg/kg, all mice had convulsions, collapsed, and died within 2 minutes of dosing. • Any effects on BW could not be distinguished from similar effects noted for control animals. • No macroscopic findings were observed.
Single dose - oral 96010 GLP	Rat/Sprague-Dawley 7/sex/group	0, 0.1, 1, 10, 100, 300, 600, 1200, and 2400	Clinical signs, BW; standard gross pathology and histopathology on liver and small intestine only for early decedents and after on Day 3 (2/sex/group) and	• At 1200 mg/kg, 1 F died on D8. At 2400 mg/kg, 1 M and 1 F died on D10 and 1 M died on D12. • At 2400 mg/kg, clinical signs included decreased activity, chromorhinorrhea, rales, discolored haircoat, and inactivity (1 F). • At 300 mg/kg, minimal to mild BW loss on D3. Dose-related minimal to marked BW loss at ≥600 mg/kg. • Tan discoloration of mucosal surface of small

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (mg/kg)	Endpoints	Findings
			after 2-week observation period (5/sex/group)	intestine (mostly distal duodenum and proximal jejunum) and pale discoloration of liver at ≥10 mg/kg (F) and ≥100 mg/kg (M). • Microscopic lipid vacuolation of the absorptive epithelium in small intestine (moderate) and liver hepatocytes (predominantly periportal; minimal to mild) on D3 (≥10 mg/kg) and on D15 (≥100 mg/kg).
Single dose - IV bolus 96036 GLP	Rat/ Sprague-Dawley 5/sex/group	0, 12.5, 25, 35, and 50	Clinical signs, BW; standard gross pathology for early decedents and after 2-week observation period, and histopathology on liver, small intestine, and injection site (tail) at end of 2-week observation period	• At 50 mg/kg, all M and 1 F had ataxia, tremors, convulsions, and/or collapse and died within 30 minutes of dosing; no macroscopic findings noted for early decedents. • All M at 25 mg/kg and all animals at 35 mg/kg showed ataxia and/or convulsions and collapse on D1; all animals recovered by 24h postdose. • Transient minimal BW loss at ≥25 mg/kg on D3. • Tan discoloration in liver and small intestine at 50 mg/kg (F). • Minimal to moderate microscopic lipid vacuolation in hepatocytes at ≥25 mg/kg. Mild to moderate lipid vacuolation in absorptive epithelium of jejunum at 50 mg/kg (F). • At injection site, mild focal scab formation with mild fibrosis of underlying dermis at ≥25 mg/kg and moderate to marked multifocal ulceration with minimal to moderate hemorrhage and/or fibrosis in underlying dermis at ≥35 mg/kg.

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (mg/kg)	Endpoints	Findings
Single dose - IV bolus 96041 GLP	Dog/Beagle 2/sex/group	0, 0.05, 0.5, and 5	Clinical signs, BW, FC, ophthalmic exams, ECG, BP, and gross pathology (control and HD) after 2-week observation period	• At 0.5 mg/kg, head shaking in all dogs and emesis or decreased activity in some dogs immediately after dosing. At 5 mg/kg, mild to moderate ataxia and decreased activity in all dogs, head shaking and/or emesis in some dogs immediately after dosing. • No effects on ophthalmic exams, ECG intervals, BW, or FC. • Decreased BP at 0.05 (minimal), 0.5 (moderate), and 5 mg/kg (mild to marked). • No macroscopic findings were noted after 2 week observation period.

BP = blood pressure; BW = body weight; D = day; ECG = electrocardiogram; F = female; FC = food consumption; GLP = Good Laboratory Practice; HD = high dose; M = male.

6.2 Repeat-Dose Toxicity

Repeat-Dose Range-Finding Studies

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
Repeat-dose exploratory toxicity study BMS-May 1996a Non-GLP	Hamster 4/group 0, 1, 3, and 10 mg/kg/d for 3 days (in standard diet or high-fat diet)	Histopathology on liver and small intestine	• Intracytoplasmic microvesiculation, compatible with lipid, was observed in periportal hepatocytes of MD and HD group animals fed either diet. • Lipid accumulation in the small intestinal enterocytes was observed in MD and HD group animals on standard diet and in all groups receiving high-fat diet. • The nature and severity of lipid accumulation in liver and small intestine were similar for both the standard diet and high-fat diet.
	Hamster 4/group/time point 0, 1, 3, and 6 mg/kg/d for 7, 14, or 21 days (in standard diet or high-fat diet)	Histopathology on liver and small intestine	• Dose-related lipid accumulation was observed in the liver and small intestine of test article-treated animals and was similar in nature for both standard and high-fat diet, but was slightly more severe in the high-fat fed group. • The degree of lipid accumulation in the liver and small intestine tended to decrease as the duration of treatment increased.
3 week oral RF study 96056 Non-GLP	Mouse/CD-1 5/sex/group 0, 4, 25, 100, and 400 mg/kg/d (gavage)	Clinical signs, BW, FC	• 1 M and 1 F control animals died on D3 and D5; at 100 mg/kg/d, 3 M and 1 F died starting on D3; all 400 mg/kg/d animals died starting on D4. • At 100 mg/kg/d, 1 M was inactive and had hard feces on D8-D10. At 400 mg/kg/d, 2 M and 1 F were inactive and/or collapsed on D3. • Mild to marked decreases in BW at ≥100 mg/kg/d, which correlated with mild to marked decreases in FC.
2 or 4 week palatability study	Mouse/CD-1 2-weeks with vitamins	Clinical signs, BW, FC	• At 200 mg/kg/d, 3 M and 1 F died between D6 and D13. All animals at 250 mg/kg/d died or were sacrificed moribund from D7 to D10. • No overt clinical signs of toxicity. • For M, transient BW loss at ≥4 mg/kg/d with slightly decreased BW

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
96042 Non-GLP	6/sex/group 0, 50, 100, 200, and 250 mg/kg/d (in diet) 4 weeks without vitamins 5/sex/group 0, 4, 10, and 25 mg/kg/d (in diet)		gain at 10 and 25 mg/kg/d by Week 4; moderate to marked BW loss at 50 to 200 mg/kg/day and severe BW loss at 250 mg/kg/d. • For F, transient BW loss at 10 and 25 mg/kg/d; moderate to marked BW loss at 50 to 200 mg/kg/d; severe BW loss at 250 mg/kg/d. • Effects on BW generally correlated with decreases in FC.
3 month oral RF study 99008 GLP	Mouse/CD-1 10/sex/group + TK group animals 0, 1.25, 5, 20, and 80 mg/kg/d (gavage)	Clinical signs, BW, FC, clinical pathology, organ weights, gross pathology, histopathology, TK	• There were no treatment-related mortalities or clinical signs. • Slight decrease in BW gain for M at 80 mg/kg/d and slight increase in BW gain for F at ≥20 mg/kg/d. Slight increase in FC for HD F. • Increased neutrophils at ≥5 mg/kg/d. • Decreased cholesterol at all doses and decreased triglycerides at 5 and 20 mg/kg/d only. Mild decrease in serum globulins (≥20 mg/kg/d), total protein and albumin (80 mg/kg/d; M only), and mild increases in urea nitrogen (80 mg/kg/d; F only). • Increased liver weights (15% to 34%) at ≥20 mg/kg/d and decreased prostate/seminal vesicle and thymus weights at 80 mg/kg/d (M only). • Minimal to moderate pale discoloration of small intestinal mucosa at ≥5 mg/kg/d and minimal to mild pale discoloration of the liver at ≥20 mg/kg/d. • Minimal to mild hepatocellular lipid vacuolation at all doses. Minimal to marked, dose-related increase in intracytoplasmic lipid vacuolation of absorptive epithelial cells lining the small intestinal villi, which was most prominent in the jejunum. • TK data from D29 are shown in the sponsor-generated table below:

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/Strain
Number/Group
Dose Levels</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><th rowspan="2">Dose (mg/kg/day)</th><th rowspan="2">Sex</th><th colspan="4">AUC (0-24 hr) (ng x hr/mL)</th></tr>
<tr><th>BMS-201038</th><th>BMS-203215</th><th>BMS-203304</th><th>BMS-224433</th></tr>
<tr><td rowspan="2">1.25</td><td>M</td><td>291</td><td>255</td><td>2091</td><td>a</td></tr>
<tr><td>F</td><td>377</td><td>509</td><td>1540</td><td>a</td></tr>
<tr><td rowspan="2">5</td><td>M</td><td>1690</td><td>1219</td><td>7840</td><td>520</td></tr>
<tr><td>F</td><td>1309</td><td>1320</td><td>4655</td><td>583</td></tr>
<tr><td rowspan="2">20</td><td>M</td><td>6685</td><td>5445</td><td>44265</td><td>3641</td></tr>
<tr><td>F</td><td>9138</td><td>7429</td><td>35046</td><td>3525</td></tr>
<tr><td rowspan="2">80</td><td>M</td><td>14099</td><td>14149</td><td>105407</td><td>13758</td></tr>
<tr><td>F</td><td>24301</td><td>19042</td><td>83362</td><td>20409</td></tr>
</table>
a - Most plasma concentrations were below the lower limit of quantification (LLQ) of 10 ng/ml.
</td></tr>
<tr><td>2 week palatability study

96043

Non-GLP</td><td>Rat/Sprague-Dawley

6/sex/group

0, 25, 50, 100, and 200 mg/kg/d (in diet)</td><td>Clinical signs, BW, FC</td><td>• No mortalities or overt clinical signs.
• Initial BW loss at D3 in M at 200 mg/kg/d and F at ≥100 mg/k/g/d. By end of dosing, BWs were 13% to 31% less than control for all M groups and 12% less than controls for HD F.
• Dose-related decreased FC for all groups on D1-D4. At the end of dosing, FC was ~15% less for M at ≤100 mg/kg/d and 35% less for M at 200 mg/kg/d compared with control. For F at 200 mg/kg/d, FC was ~15% less than control.</td></tr>
<tr><td>2 week oral RF study

95051

Non-GLP</td><td>Rat/Sprague-Dawley

5/sex/group

0, 4, 20, and 100 mg/kg/d (gavage)</td><td>Clinical signs, BW, clinical pathology, organ weights, gross pathology, histopathology, TK</td><td>• 3 M and all F at 100 mg/kg/d died during the second week.
• At 100 mg/kg/d, decreased activity ~2 hr after dosing each day with recovery by 24 hr. Two M had gasping with distinct expiratory “noise” at the end of the respiratory cycle; may indicate gavage error.
• BW loss at 100 mg/kg/d.
• Increased poikilocytosis at 4 mg/kg/d (F) and at ≥20 mg/kg/d for both sexes. Decreased erythrocyte parameters, with slightly increased neutrophils and reticulocytes at 100 mg/kg/d.
• Dose-related decreases in cholesterol and triglycerides at ≥4 mg/kg/d; minimal increases in ALT (F) and ALP at ≥4 mg/kg/d. At 100 mg/kg/d, mild decreases in serum calcium (M); and increases in urea nitrogen (M), sodium, chloride (M), and mild decreases in potassium (F) and phosphorus.</td></tr>
</table>

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/Strain
Number/Group
Dose Levels</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td></td><td></td><td></td><td>
• Increased liver weight at 4 and 20 mg/kg/d and increased spleen weight at 20 mg/kg/d. Decreased thymus and prostate/ seminal vesicle weights at 100 mg/kg/d.
• Pale discoloration in liver at ≥4 mg/kg/d and small intestine for 1 LD F
• Lipid vacuolation (Oil-Red-O stain positive) in hepatocytes and small intestine enterocytes (most prominent in jejunum followed by duodenum) at ≥4 mg/kg/d. Mild to moderate thymic atrophy at 100 mg/kg/d. Drug-related hemorrhage in several tissues at ≥20 mg/kg/d.
• TK data are shown below in the sponsor-generated table:
<table>
<tr><th rowspan="2">BMS-201038
Daily Dose
(mg/kg)</th><th rowspan="2">Sex</th><th colspan="2">$AUC_{24\ hr}$ (ngxhr/ml)</th></tr>
<tr><th>Day 1</th><th>Day 14</th></tr>
<tr><td>4</td><td>M</td><td>98</td><td>162</td></tr>
<tr><td></td><td>F</td><td>614</td><td>718</td></tr>
<tr><td>20</td><td>M</td><td>862</td><td>552</td></tr>
<tr><td></td><td>F</td><td>2260</td><td>1770</td></tr>
<tr><td>100</td><td>M</td><td>4570</td><td>a</td></tr>
<tr><td></td><td>F</td><td>6460</td><td>a</td></tr>
</table>
a = no sample
</td></tr>
<tr><td>2 week IV toxicity study

96209

GLP</td><td>Rat/ Sprague-Dawley

10/sex/group

0, 0.1, 1, and 10 mg/kg/d

IV dosing was changed to IP dosing on D5 for</td><td>Clinical signs, BW, ophthalmology, clinical pathology, organ weights, gross pathology, histopathology</td><td>
• There were no mortalities.
• At 10 mg/kg/d, vocalization, bluish discoloration at injection site, discoloration of tail, prolonged bleeding from tail injection sites, swelling of tail, and tail skin lesions. Tails developed necrosis and injections were changed to IP, resulting in thickening of abdominal skin.
• BW gain was 10% less than controls for HD M; no effect on F.
• Slight decreases in RBC parameters at 1 mg/kg/d. At 10 mg/kg/d, increased leukocytes, lymphocytes, neutrophils, and monocytes; decreased RBC parameters and increased reticulocytes; and increased APTT and fibrinogen.
</td></tr>
</table>

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
	HD group and D10 for all other animals.		• Decreased serum cholesterol and triglycerides and increased AST (F) and ALP at ≥1 mg/kg/d. Mild to moderate increase in serum bilirubin and globulins and mild decrease in serum albumin at 10 mg/kg/d. • Increased liver weights at ≥1 mg/kg/d (M) and ≥0.1 mg/kg/d (F); increased spleen weights at 10 mg/kg/d; decreased kidney, prostate/ seminal vesicle weights at 10 mg/kg/d (M). • Pale discoloration of liver at ≥0.1 mg/kg/d and small intestine (primarily jejunum) at ≥1 mg/kg/d. • Lipid vacuolation of hepatocytes and absorptive epithelial cells of small intestinal villi (most severe in jejunum) at all doses, and was more severe in females than in males. Increased hematopoiesis in spleen at 10 mg/kg/d. Injection site inflammation at ≥0.1 mg/kg/d, thrombosis at ≥1 mg/kg/d, and necrosis at 10 mg/kg/d.
1 month oral toxicity study 96003 GLP	Rat/ Sprague-Dawley 10/sex/group and 5/sex/group (recovery) 0, 0.5, 5, and 50 mg/kg/d (gavage)	Clinical signs, BW, FC, clinical pathology, organ weights, gross pathology, histopathology, TK	• At 50 mg/kg/d, all but 2 F died or were sacrificed moribund between Weeks 2 and 4. • Increased chromorhinorrhea and chromodacryorrhea at 50 mg/kg/d • Slight decrease in BW gain for HD F; decreased BW gain at Week 2 followed by BW loss at Week 3 for HD M. Minimal decreases in FC in Week 1 (M) or Week 3 (F). • Poikilocytosis at ≥0.5 mg/kg/d (F); decreased RBC parameters, decreased bone marrow myeloid/erythroid ratio, increased nucleated RBCs. Changes reversed after 1 month recovery. • Decreased cholesterol and triglycerides and increased ALP at ≥0.5 mg/kg/d; increased ALT and AST at ≥5 mg/kg/d; increased urea nitrogen and creatine kinase at 50 mg/kg/d. • Increased urine volume with decreased specific gravity in HD F. • Increased liver weight at ≥0.5 mg/kg/d; increased spleen weight and decreased uterine weight at ≥5 mg/kg/d. • Pale discoloration of liver at ≥0.5 mg/kg/d; mild red discoloration of the ventral wall of the cranial cavity in one MD M. In animals that died or sacrificed, accumulation of red fluid (blood) in body cavities

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/Strain
Number/Group
Dose Levels</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td></td><td></td><td></td><td>and discoloration and/or size changes in several organs.
• Early decedents showed hemorrhage in many organs, centrilobular hepatocellular necrosis, lipid vacuolation in liver and small intestines, and extramedullary hematopoiesis in spleen.
• Lipid vacuolation (Oil-Red-O stain positive) in hepatocytes and absorptive epithelial cells of small intestine at ≥0.5 mg/kg/d. Tissue hemorrhage in 1 MD M and the 2 surviving HD F. At recovery, minimal to mild lipid vacuolation in hepatocytes in LD and MD F.
• TK data are shown below in the sponsor-generated table:
BMS-201038 Plasma $AUC_{0\text{-}24\ hr}$ (ngxhr/ml)
Daily Dose (mg/kg) | Day 1 Males | Day 1 Females | Day 28 Males | Day 28 Females
0.5 | a | a | 48.2 | 163
5 | 274 | 620 | 366 | 849
50 | 3915 | 5840 | b | b
a: Concentrations in most of the plasma samples were below the lower limit of quantitation of 0.5 ng/ml. Due to a drug preparation error, these animals received a dose of 0.14 mg/kg on day 1 only.
b: Most of the animals died before day 28 of the study.</td></tr>
<tr><td>1-month oral investigative study

96016

GLP</td><td>Rat

5/sex/group - 2 week sacrifice

10/sex/group - 1 month sacrifice

0 or 50 mg/kg/d with or without vitamin K (0.2</td><td>Purpose: to determine whether the cause of deaths in earlier studies was due to vitamin K deficiency.

Mortality, clinical signs, BW, FC, opthalmoscopy, clinical pathology,</td><td>• 8 males and 10 females from the HD group without vitamin supplementation died before the end of the study; no test article-related deaths occurred in the HD group receiving vitamin K.
• Mild (females) to moderate (males) decreases in BW gain and FC were noted for HD group animals not receiving vitamin K; HD males given vitamin K had a minimal decrease in BW gain.
• Without vitamin K supplementation, there were mild decreases in RBC parameters (females), mild to moderate increases in platelet counts (males), marked increases in PT and APTT, mild increases in bleeding time and fibrinogen, and increased incidence of poikilocytosis. Animals receiving vitamin K had only minimal to mild</td></tr>
</table>

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
	mg/kg by SC 3x weekly)	organ weights, gross pathology	increases in total leukocyte, neutrophils, and lymphocyte counts and increased poikilocytosis. • All treated groups had decreased cholesterol, triglycerides, protein, and globulins and mild increases in ALT, AST, and ALP). Animals not receiving vitamin K also had minimal to mild increases in urea nitrogen, glucose (males), and creatine kinase; in females, moderate to marked decreased sodium, potassium, and chloride, along with a mild increase in serum phosphorus. • In early decedents, minimal to marked red discoloration/hemorrhage was noted in one or more tissues; 2 M and5 F had markedly increased thymus size. Animals receiving vitamin K did not have signs of hemorrhage. • All treated animals had mild to moderate tan discoloration of the small intestine. • The results of this study support the hypothesis that BMS-201038 interferes with the absorption of fat-soluble vitamin K, leading to vitamin K deficiency and subsequent clotting abnormalities.
2 week IV toxicity study 96331 GLP	Dog/ Beagle 2/sex/group 0, 0.05, 0.5, and 5 mg/kg/d (IV bolus)	Clinical signs, BW, FC, WC, ECG, BP, ophthalmology, clinical pathology, organ weights, gross pathology, histopathology	• There were no unscheduled deaths. • Clinical signs were mostly attributed to the vehicle, possibly the Tween 80 component; dose-related increases in emesis may have been drug related. Thin body condition in one or more animals at ≥0.05 mg/kg/d. • BW loss (3% to 6%) at ≥0.5 mg/kg/d that correlated with decreased FC; decreased WC at 5 mg/kg/d. • Decreased BP at all doses, which was attributed to Tween 80. QT interval prolongation within normal limits; slightly decreased HR; QTc was not calculated. • Poikilocytosis at ≥0.5 mg/kg/d. Increased WBCs and neutrophils at 5 mg/kg/d (1 M). • Decreased cholesterol and triglycerides at all doses. Decreased serum protein at ≥0.05 mg/kg/d and decreased albumin and globulin at ≥0.5 mg/kg/d. Minimally increased urea nitrogen at all doses.

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
			• White-yellowish discoloration of small intestine at ≥0.05 mg/kg/d. Mild to moderate small intestinal dilatation at ≥0.5 mg/kg/d. • Intracytoplasmic lipid vacuolation (Oil-Red-O stain positive for neutral lipids) of absorptive epithelial cells lining the upper areas of the small intestinal villi (most prominently in jejunum) at all doses. Hepatocellular lipid deposition (Oil-Red-O stain positive), primarily periportal, at all doses. Microscopic injection site lesions were noted, but are not considered to be relevant for the oral dosing route.
1 month toxicity study 96004 GLP	Dog/Beagle 3/sex/group 0, 0.02, 0.2, 2, and 20 mg/kg/d (via gelatin capsules)	Clinical signs, BW, FC, ECG, BP, ophthalmology, clinical pathology, fecal lipids, organ weights, gross pathology, histopathology, electron microscopy (liver and small intestine for control and HD only), TK	• There were no mortalities. • No effects on ECG intervals. Minimal to moderate decreases in BP and heart rate at 2 and 20 mg/kg/d. • BW loss at ≥2 mg/kg/d (7% to 11% [M] and 12% to 26% [F] less than starting weight). • Moderate to marked poikilocytosis at ≥2 mg/kg/d; mild decrease in APTT and minimal increase in monocytes at 20 mg/kg/d. • Mild to marked decrease in cholesterol and triglycerides at ≥0.2 mg/kg/d; mild decrease in serum globulins, mild to moderate increase in AST and moderate to marked increase in ALT at ≥2mg/kg/d; mildly increased urea nitrogen for 1 HD M. • No changes to fecal lipid content at any dose level. • No treatment-related effects on organ weights. • Yellow-white discoloration of mucosal surface of small intestine at ≥0.2 mg/kg/d. • Intracytoplasmic lipid vacuolation (Oil-Red-O stain positive) of absorptive epithelial cells lining the intestinal villi at ≥0.2 mg/kg/d, which was most prominent in the jejunum; minimal hepatocellular lipid deposition (F only) and single cell necrosis at ≥2 mg/kg/d. • EM for HD group: hepatocellular lipid deposition was characterized by one or more variably-sized homogenous pale-gray lipid droplets in the cytoplasm and in the small intestine lipid deposition was characterized by multiple, variably-sized, homogenous pale-gray lipid droplets that were partially enveloped by endoplasmic reticulum.

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/Strain
Number/Group
Dose Levels</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td></td><td></td><td></td><td>• TK data are shown below in the sponsor-generated table:
<table>
<tr><th rowspan="3">BMS-201038
Daily Dose
(mg/kg)</th><th colspan="4">Plasma $AUC_{24\ hr}$ Values (ng x hr/ml)</th></tr>
<tr><th colspan="2">Day 1</th><th colspan="2">Day 28</th></tr>
<tr><th>Males</th><th>Females</th><th>Males</th><th>Females</th></tr>
<tr><td>0.02</td><td>a</td><td>a</td><td>a</td><td>a</td></tr>
<tr><td>0.2</td><td>18.6</td><td>21.9</td><td>78.2</td><td>78.1</td></tr>
<tr><td>2</td><td>1297</td><td>1338</td><td>3239</td><td>1947</td></tr>
<tr><td>20</td><td>10520</td><td>10339</td><td>20415</td><td>11460</td></tr>
<tr><td colspan="5">a - Below the lower limit of quantitation.</td></tr>
</table></td></tr>
</table>

APTT = activated partial thromboplastin time; BP = blood pressure; BW = body weight; D = day; ECG = electrocardiogram; EM = electron microscopy; F = female; FC = food consumption; GLP = Good Laboratory Practice; HD = high dose; IP = intraperitoneal; IV = intravenous; LD = low dose; M = male; MD = mid dose; PT = prothrombin time; RBC = red blood cells; RF = range finding; TK = toxicokinetic; WC = water consumption.
Vehicle for oral studies was 75% PEG-400, 25% water.
Vehicle for IV studies was 10% PEG-400, 10% ethanol, and 1% Tween 80 in water or 0.9% saline for control.

Pivotal Studies

Study title: **BMS-201038: Three-Month Dietary Range-Finding Study in Mice**
Note: this study was submitted to IND 50,820 and reviewed by Dr. Da-Lin Yao (review submitted to DARRTS on 10 April 2007). The results of this study were used to determine the dose levels for the 2-year carcinogenicity study. Only minor edits to Dr. Yao's review have been made for clarity and formatting. Study drug BMS-201038 = AEGR-733

Study no.: 96057
Study report location: Module 4.2.3.2
Conducting laboratory and location: (b) (4)

Date of study initiation: 1/3/1997 (animals dosed from 1/24/1997 through 4/25/1997)
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: BMS-201038, Lot # R007A, and purity 87.2%

Key study findings:
CD-1 mice were dietary dosed with BMS-201038 at 0, 1.5, 5, 15, or 45 mg/kg/d for 3 months without vitamin supplementation:

- No drug-related mortalities up to 45 mg/kg/d (sporadic deaths in all groups including control resulted from blood sampling for hematology without findings of other causes; more deaths were seen at ≥5 mg/kg/d, indicating poor handling procedure).
- Increased body weight gain in treated animals at ≥5 mg/kg/d, remarkably at HD of 45 mg/kg/d (↑66% M and ↑36% F). Decreased FC for the first week in treated animals and comparable to or higher than the control thereafter, indicating possible transient palatability problem.
- Statistically significant (ss) increases in RBC and ss decreases in MCV, MCH and absolute neutrophils were noted at 15 and 45 mg/kg/d (M and F).
- Notable serum chemistry changes seem to be pharmacology-related, including decreased cholesterol and triglycerides at 15 and 45 mg/kg/d (M and F). Slightly decreased total protein, albumin, and globulin were also noted at the two higher doses (M and F).
- Dose-dependant, ss increase in liver wt (abs. and rel.) in treated animals (↑ 11% to 28% at all dose levels [M] and ↑ 8% to 26% at ≥ 5 mg/kg/d [F]), which may be associated with the lipid accumulation in hepatocytes.
- Dose-related increases in intracellular lipid accumulation (vacuolation) in hepatocytes and epithelial cells of the small intestine in all treated groups (≥1.5 mg/kg/d, M and F); histiocytosis with foamy alveolar macrophages in the alveolar spaces at ≥5 mg/kg/d (M and F), which was described as phospholipidosis.
- Systemic exposure to BMS-201038 and its three major metabolites were generally dose proportional. The $AUC_{0\text{-}24h}$ values of major metabolite BMS-203304 were 4X to 6X the parent drug.

Methods

Doses: 0, 1.5, 5, 15, or 45 mg/kg/d
Species/strain: Mice/CD-1
Number/sex/group or time point (main study): 28/sex/group for dosing groups and 10/sex for control
Route, formulation, volume: Oral via dietary admixture (continuous dosing), each animal received test material in the diet, *ad libitum*, 7 days per week. Fresh diets were provided weekly.
Satellite groups used for toxicokinetics or recovery: 18/sex/group (out of 28/sex/group) in the 4 dosing groups were used for TK (4-week); no recovery group was remained; 10/sex/group in C and HD groups were examined for histopathology, and only a few mice in three lower dose groups examined for histopathology as illustrated in the sponsor-generated table below:
Age: 45 days at initiation of dosing
Weight: 29 to 33 g for males and 24 to 27 g for females
Sampling times: Main study animals were necropsied after 3-months of dosing

Group	Dose Level[a]	Total		Toxicokinetic Studies[c] 1 Month		Hematology 3 Month		Clinical Chemistry and Necropsy[d] 3 Month		Microscopic Pathology[e]	
	mg/kg/day	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
I	0[b]	10	10	0	0	9	9	6	9[f]	10	10
II	1.5	28	28	18	18	10	10	9	9	1	1
III	5	28	28	18	18	10	10	5	8	5	2
IV	15	28	28	18	18	10	10	6	5	4	5
V	45	28	28	18	18	10	10	1	7	10	10

[a]Doses given were expressed in terms of BMS-201038 free base.
[b]Control animals received untreated Purina Certified Rodent Chow (5002).
[c]Animals were bled for the toxicokinetic studies on Test Days 33 and 34. Blood was collected from three males and three females (from the animals assigned for toxicokinetic evaluation) from each dose group at approximately 4, 8, 12, 16, 20, and 24 hours after the start of the room dark cycle. After the bleeding procedure, the mice were sacrificed via CO_2 inhalation and/or cervical dislocation and the carcasses were discarded.
[d]All survivors (up to 10/sex/group) were examined macroscopically after 3 months of treatment.
[e]Tissues listed in Table I (pages 31 and 32) were examined for all animals in Groups I and V and for those animals which died unscheduled deaths during the study; target organs identified in Group V (liver, lung, and small intestine) were examined at the lower doses.
[f]Clinical chemistry values were only obtained on 8 females.

Observation and Times:

Clinical signs:	Twice pretest and weekly thereafter
Body weights:	Twice pretest, weekly during treatment and terminally (after fasting)
Food consumption:	Weekly beginning one week prior to dosing; test material intake was calculated as: food consumed (g/kg/d) x dietary concentration (mg drug/g of diet)
Ophthalmoscopy:	Not conducted
Hematology:	Collected on test day 85
Clinical chemistry:	Collected on test day 92
Urinalysis:	Not conducted
Gross pathology:	At necropsy
Organ weights:	See histopathology inventory table
Histopathology:	Control and HD only, except lung, liver, and small intestine for all groups - See histopathology inventory table

Results:

Mortality: 35 deaths occurred in all groups (M and F) including the control shortly following blood collection (for hematology) at week 12 or were found dead on Days 86 to 87 after being bled; no other causes could be determined, but more deaths were seen at ≥ 5 mg/kg/d, possibly indicating poor handling procedure.

Dosing group (m/k/d)	Total dead		Total survivors	
	Male	Female	Male	Female
C	4	1	6	9
1.5	1	1	9	9
5	5	2	5	8
15	4	5	6	5
45	9	3	1	7

Clinical signs: Unremarkable

Body weights: Body weight gain increased in almost all tread groups (M and F) in a dose-dependent manner.

Body weight gains at the end of dosing (week 13) vs. week 0: g (change in %)

	Male					Female				
Doses, m/k/d	C	1.5	5	15	45	C	1.5	5	15	45
Weight gain	6.8 -	8.4 ↑23%	8.3 ↑22%	8.5 ↑25%	11.3 ↑66%	6.1 -	6 ↓2%	7.1 ↑16%	7.3 ↑20%	8.3 ↑36%

Food consumption: Mean food consumption in the 15 and 45 mg/kg/d groups (M and F) was lower than controls in the first week of treatment, suggesting a possible transient palatability problem. Subsequent food consumption values for treated groups were comparable or higher than concurrent control. Test material intake was roughly close to desired levels.

Hematology: Statistically significant increase in mean RBC and decreased mean MCV, MCH, and absolute neutrophils were noted at 15 and 45 mg/kg/d in M and F.

	Male					Female				
Doses, m/k/d	**C**	**1.5**	**5**	**15**	**45**	**C**	**1.5**	**5**	**15**	**45**
RBC, 10^6/µl	9.53	9.48	9.56	10.10	10.66*	9.19	9.09	9.62	9.95*	10.07*
MCV, fL	48.0	48.4	47.8	46.2	44.9**	49.4	48.7	47.8	47.3*	45.8**
MCH, pg	16.2	16.3	16.3	15.6*	15.1**	16.4	16.4	16.0	15.8	15.1**
Abs Neut, 1000/µl	1.24	0.80	0.63	0.54*	0.16**	0.45	0.43	0.20	0.11**	0.11*

*,** Significantly different from control mean; p≤0.05, p≤0.01.

Clinical chemistry: Notable changes seem to have been related to pharmacology, including decreased cholesterol and triglycerides at 15 and 45 mg/kg/d in M and F. Slightly decreased total protein, albumin, and globulin were also noted at the two higher doses in M and F.

Gross pathology: Gross pathology assessments were unremarkable in the necropsied animals. For those that died prior to termination, the deaths seemed to result from blood collection without other apparent gross findings.

Organ weights: Statistically significant increase in liver weight (abs. and rel.) in treated animals at ≥ 5 mg/kg/d in M and F. Females at 45 mg/kg/d had statistically significantly decreased spleen and kidney weights (abs. and rel.). The increased liver weight appears to be related to lipid accumulation in hepatocytes.

Liver wt (abs. wt g) and changes vs. Control (%):

	Male					Female				
Doses, m/k/d	**C**	**1.5**	**5**	**15**	**45**	**C**	**1.5**	**5**	**15**	**45**
Liver	2.296	2.559 ↑11%	2.680* ↑17%	2.717* ↑18%	2.935** 28%	1.852 -	1.860 -	2.008 ↑8%	2.278** ↑23%	2.336** ↑26%

Organ wt (relative to body wt [%])

	Male					Female				
Doses, m/k/d	**C**	**1.5**	**5**	**15**	**45**	**C**	**1.5**	**5**	**15**	**45**
Liver	6.00	6.41	6.75**	6.76**	n/d	5.84	6.00	6.36	6.99**	7.09**
Kidney	1.95	1.99	1.99	1.77	n/d	1.63	1.5	1.52	1.49	1.32**
Spleen	2.82	2.85	2.84	2.60	n/d	4.65	3.88	3.77	3.67	3.16*

Histopathology:

Unscheduled-necropsied animals*:* Multifocal or diffuse hepatocellular vacuolation was observed in several mice at all dose levels of the early decedent mice; the vacuolation was confirmed to be lipid droplets by ORO staining (0/4, 1/1, 5/5, 3/4, and 6/9 in males and 0/1, 0/1, 0/2, 5/5, and 2/3 in females for control, 1.5, 5, 15, and 45 mg/kg/d groups, respectively).

Similar vacuolation was also present in the epithelial cells of the duodenum, jejunum and ileum. The incidence in males was 0/4, 3/4, 4/4, and 9/9 for control, 5, 15, and 45 mg/kg/d groups and the incidence in females was 0/1, 0/1, 2/2, 3/3, and 3/3 for the control, 1.5, 5, 15, and 45 mg/kg/d groups, respectively.

Histiocytosis with foamy alveolar macrophages in the alveolar spaces of the lungs was noted at doses ≥5 mg/kg/d. The incidence in males was 0/4, 0/1, 1/5, 0/4, and 4/9 and the incidence in females was 0/1, 0/1, 0/2, 1/5, and 3/3 for the control, 1.5, 5, 15, and 45 mg/kg/d groups, respectively.

A female control mouse had malignant lymphoma involving multiple lymph organs, which was consistent with the common background finding in the young CD-1 mice ((b) (4) no historical data provided).

Histopathology findings in premature deaths: (sponsor-generated table)

ORGAN/TISSUE EXAMINED		--- NUMBER-OF-ANIMALS-AFFECTED ---									
TABLE INCLUDES: SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL; DEATH=UNSCHED;FIND=ALL;SUBSET=T	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
LIVER	NUMBER:	4	1	5	4	9	1	1	2	5	3
--VACUOLATION HEPATOCELLULAR	NUMBER EXAMINED:	4	1	5	4	9	1	1	2	5	3
	1>	0	1	5	3	6	0	0	0	4	2
	2>	0	0	0	0	0	0	0	0	1	0
	TL>	0	1	5	3	6	0	0	0	5	2
--CONGESTION	2>	1	0	1	0	7	0	1	2	0	3
	3>	3	0	0	0	2	0	0	0	0	0
	TL>	4	0	1	0	9	0	1	2	0	3
--S-METASTATIC/INVASIVE NEOPLASM	+>	0	0	0	0	0	1	0	0	0	0
	TL>	0	0	0	0	0	1	0	0	0	0
--INFILTRATES,MONONUCLEAR	1>	0	0	0	0	1	0	0	0	0	0
	TL>	0	0	0	0	1	0	0	0	0	0
--BASOPHILIC FOCUS	1>	0	0	0	0	1	0	0	0	0	0
	TL>	0	0	0	0	1	0	0	0	0	0
--INFLAMMATION,SUBACUTE	TL>	0	0	0	0	0	0	0	0	0	0
--EXTRAMEDULLARY HEMATOPOIESIS	TL>	0	0	0	0	0	0	0	0	0	0
DUODENUM	NUMBER EXAMINED:	4	0	4	4	9	1	1	2	3	3
--VACUOLATION	1>	0	0	0	0	1	0	0	0	0	0
	2>	0	0	3	3	5	0	0	2	2	2
	3>	0	0	0	1	2	0	0	0	1	1
	4>	0	0	0	0	1	0	0	0	0	0
	TL>	0	0	3	4	9	0	0	2	3	3
JEJUNUM	NUMBER EXAMINED:	2	0	3	3	9	1	1	2	3	3
--VACUOLATION	1>	0	0	1	0	1	0	0	0	0	0
	2>	0	0	2	1	5	0	0	2	2	2
	3>	0	0	0	2	2	0	0	0	1	1
	4>	0	0	0	0	1	0	0	0	0	0
	TL>	0	0	3	3	9	0	0	2	3	3
ILEUM	NUMBER EXAMINED:	4	0	3	1	9	1	1	2	3	3
--VACUOLATION	1>	0	0	2	0	2	0	0	0	0	0
	2>	0	0	0	1	1	0	0	0	2	1
	3>	0	0	0	0	0	0	0	0	1	0
	4>	0	0	0	0	1	0	0	0	0	0
	TL>	0	0	2	1	4	0	0	0	3	1
LUNGS	NUMBER EXAMINED:	4	1	5	4	9	1	1	2	5	3
--HISTIOCYTOSIS	1>	0	0	1	0	4	0	0	0	1	3
	TL>	0	0	1	0	4	0	0	0	1	3
--CONGESTION	2>	1	1	3	4	7	0	1	2	1	0
	3>	3	0	2	0	2	0	0	0	4	3
	TL>	4	1	5	4	9	0	1	2	5	3
--INFILTRATES,MONONUCLEAR	1>	2	0	0	0	0	0	0	0	0	0
	TL>	2	0	0	0	0	0	0	0	0	0
--S-METASTATIC/INVASIVE NEOPLASM	+>	0	0	0	0	0	1	0	0	0	0
	TL>	0	0	0	0	0	1	0	0	0	0
--HEMORRHAGE ALVEOLAR	TL>	0	0	0	0	0	0	0	0	0	0
--PERIVASCULAR: INFILTRATES,SUBACUTE	2>	0	0	0	0	1	0	0	0	0	0
	TL>	0	0	0	0	1	0	0	0	0	0

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1> = Minimal
2> = Slight
3> = Moderate
4> = Moderately Severe (Marked)
5> = Severe
P> = Present
TL = Total

Terminal-sacrifice animals (end of dosing): Findings were seen in the liver, lungs, and small intestines, similar to those seen in the unscheduled dead animals.

Hepatocellular vacuolation: 0/6, 1/9, 3/5, 1/6, and 0/1 for males and 0/9, 0/9, 3/8, 4/5, and 3/7 for females for the control, 1.5, 5, 15, and 45 mg/kg/d groups, respectively.

Lipid vacuolation in the small intestines: 0/6, 4/9, 5/5, 6/6, and 1/1 for males and 0/9, 7/9, 8/8, 5/5, and 7/7 for females for the control, 1.5, 5, 15, and 45 mg/kg/d groups, respectively.

Lung histiocytosis: 0/6, 0/9, 1/5, 5/6, and 1/1 for males and 0/9, 0/9, 2/8, 3/5, and 4/7 for females for the control, 1.5, 5, 15, and 45 mg/kg/d groups, respectively.

Histopathology findings in terminal autopsy: (sponsor-generated table)

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TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=T

--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---

ORGAN/TISSUE EXAMINED		SEX:	MALE					FEMALE				
		GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
		NUMBER:	6	9	5	6	1	9	9	8	5	7
LIVER	NUMBER EXAMINED:		6	9	5	6	1	9	9	8	5	7
--VACUOLATION HEPATOCELLULAR	1>		0	1	3	1	0	0	0	3	4	3
	TL>		0	1	3	1	0	0	0	3	4	3
--CONGESTION	TL>		0	0	0	0	0	0	0	0	0	0
--S-METASTATIC/INVASIVE NEOPLASM	TL>		0	0	0	0	0	0	0	0	0	0
--INFILTRATES,MONONUCLEAR	1>		1	2	1	1	0	3	2	2	1	0
	TL>		1	2	1	1	0	3	2	2	1	0
--BASOPHILIC FOCUS	TL>		0	0	0	0	0	0	0	0	0	0
--INFLAMMATION,SUBACUTE	1>		0	0	0	0	0	0	0	1	0	0
	TL>		0	0	0	0	0	0	0	1	0	0
--EXTRAMEDULLARY HEMATOPOIESIS	1>		0	0	0	0	0	0	2	0	0	0
	TL>		0	0	0	0	0	0	2	0	0	0
DUODENUM	NUMBER EXAMINED:		6	8	5	6	1	9	9	8	5	7
--VACUOLATION	1>		0	2	0	0	0	0	5	2	0	0
	2>		0	1	3	1	1	0	2	5	0	0
	3>		0	0	2	5	0	0	0	1	5	2
	4>		0	0	0	0	0	0	0	0	0	5
	TL>		0	3	5	6	1	0	7	8	5	7
JEJUNUM	NUMBER EXAMINED:		6	9	5	6	1	9	9	8	5	7
--VACUOLATION	1>		0	3	0	0	0	0	1	0	0	0
	2>		0	1	3	3	1	0	1	4	0	0
	3>		0	0	2	3	0	0	2	4	5	2
	4>		0	0	0	0	0	0	0	0	0	5
	TL>		0	4	5	6	1	0	4	8	5	7
ILEUM	NUMBER EXAMINED:		6	9	5	6	1	9	9	8	5	7
--VACUOLATION	1>		0	0	1	0	0	0	0	5	0	0
	2>		0	0	1	5	1	0	0	2	1	7
	3>		0	0	0	0	0	0	0	0	4	0
	TL>		0	0	2	5	1	0	0	7	5	7
LUNGS	NUMBER EXAMINED:		6	9	5	6	1	9	9	8	5	7
--HISTIOCYTOSIS	1>		0	0	1	5	1	0	0	2	3	3
	2>		0	0	0	0	0	0	0	0	0	1
	TL>		0	0	1	5	1	0	0	2	3	4
--CONGESTION	1>		0	0	1	0	0	0	0	0	0	0
	2>		0	0	0	0	0	0	1	0	0	0
	TL>		0	0	1	0	0	0	1	0	0	0
--INFILTRATES,MONONUCLEAR	1>		1	0	0	0	0	0	0	0	0	0
	TL>		1	0	0	0	0	0	0	0	0	0
--S-METASTATIC/INVASIVE NEOPLASM	TL>		0	0	0	0	0	0	0	0	0	0
--HEMORRHAGE ALVEOLAR	1>		0	1	0	2	0	0	0	1	0	0
	2>		0	1	0	0	0	0	0	0	0	0
	TL>		0	2	0	2	0	0	0	1	0	0
--PERIVASCULAR: INFILTRATES,SUBACUTE	TL>		0	0	0	0	0	0	0	0	0	0

1> = Minimal
2> = Slight
3> = Moderate
4> = Moderately Severe (Marked)
5> = Severe
P> = Present
TL = Total

Toxicokinetics: Systemic exposures to BMS-201038 and metabolites were generally dose proportional in this dietary study. Nominal BMS-201038 doses increased in a 1:3:10:30 proportion. The $AUC_{0\text{-}24h}$ values of BMS-201038 increased in a 1:3:10:36 ratio in male mice and in a 1:2:6:23 ratio in female mice, respectively. The $AUC_{0\text{-}24h}$ values of major metabolite BMS-203304 (M3) were 4 to 6X the parent drug while $AUC_{0\text{-}24h}$ values of another metabolite BMS-203215 (M1) were slightly higher than the parent. The overall exposure of the mice to the 4 compounds was: BMS-203304 (M3) > BMS-203215 (M1) > BMS-201238 (parent) > BMS-224433 (M2). The $AUC_{0\text{-}24h}$ values for the parent and three metabolites are presented in the sponsor-generated table below.

AUC0-24h (ng.h/mL)

Daily dose	1.5 mg/kg		5 mg/kg		15 mg/kg		45 mg/kg	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
BMS-201038	116	144	371	291	1192	878	4201	3334
BMS-203304	567	351	1749	1201	5080	4485	26021	15811
BMS-203215	150	150	370	379	1052	1105	5861	6574
BMS-224433	n/c	n/c	209	177	731	819	3242	2680

n/c: not calculated (below LLQ of 5 ng/mL)

Study title: **BMS-201038: Three-Month Dietary Range-Finding Study in Rats**

Note: this study was previously reviewed by Dr. Da-Lin Yao (review in DARRTS on 10 April 2007); only minor edits to Dr. Yao's review have been made for clarity and formatting. Study drug BMS-201038 = AEGR-733

Study no.: 96058
Study report location: Module 4.2.3.2
Conducting laboratory and location: Bristol-Myers Squibb, Pharmaceutical Research Institute, Dept. of Toxicology and Pathology, New Brunswick, NJ, USA
Date of study initiation: 12/9/1996
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BMS-201038 as mesylate salt, lot #R007A, and 87.2% pure

Key Findings

- Dose-dependent mortalities occurred at ≥5 mg/kg/d, with the effect being greater in males. Mortalities may have been due to treatment-related hemorrhage as moribund animals showed a red exudate.
- Decreased body weight gain (up to 35% less than control) was noted in males at ≥5 mg/kg/d. No effects on body weight gain were noted in females. Food consumption was lower for animals receiving ≥5 mg/kg/d during the first week, after which time food consumption was similar to or greater than controls.
- Decreased mean erythrocyte parameters were noted in males receiving ≥10 mg/kg/d. Increased mean white blood cell counts were observed at all dose levels and increased neutrophils and lymphocytes were seen at ≥5 mg/kg/d. Clotting parameter

times were prolonged at ≥5 mg/kg/d, which is consistent with the observation of hemorrhage.

- Increased mean ALT, AST, and ALP were observed at all dose levels but the increases were not always dose dependent. Decreased mean cholesterol and triglycerides were observed at all dose levels.
- Mean liver weights were increased at all dose levels and increased mean spleen and ovary weights were observed at ≥5 mg/kg/d.
- Discoloration of the liver (pale) and small intestine (tan) was observed for most females and many males treated with the test article. Most animals that died early showed macroscopic and microscopic evidence of extensive hemorrhage in numerous organs.
- In liver, lipid vacuolation and subacute inflammation were observed at ≥1 mg/kg/d and single-cell necrosis was seen at 10 mg/kg/d. In small intestine, lipid vacuolation was observed in all treated females and most treated males in the absence of accompanying necrosis or inflammation. In lung, small multifocal collections of foamy macrophages (histiocytes) were observed in the alveoli of animals treated at 10 mg/kg/d. Histiocytosis was called phospholipidosis in the study report, although this conclusion was not confirmed by electron microscopy.

Methods

Doses:	0, 1, 5, 10, and 15 mg/kg/day
Species/strain:	Rat/Sprague-Dawley
Number/sex/group (main study):	10
Route and formulation:	Orally dosed via dietary admixture
Satellite groups used for toxicokinetics:	10/sex/group
Age:	39 days at initiation of dosing
Weight:	182 to 206 g (males) and 129 to 150 g (females)
Sampling times:	Necropsied after 3-months of dosing

Observation and Times:

Clinical signs:	Twice daily
Body weights:	Twice pretest, weekly during dosing and terminally (after fasting)
Food consumption:	Weekly, beginning one week prior to dosing
EKG:	Not applicable
Hematology:	Collected at termination Days 92 and 93
Clinical chemistry:	Collected at termination Days 92 and 93
Urinalysis:	Not done
Gross pathology:	At necropsy
Organ weights:	See histopathology inventory table
Histopathology:	Control and HD only, except lung, liver, and small intestine for all groups - See histopathology inventory table

Results:

Mortality: Dose-related mortalities occurred at ≥5 mg/kg/d in males and at ≥10 mg/kg/d in females, but the cause of deaths was not described (probably due to drug-induced hemorrhage when considering the signs and histopathology results):

Mortality table (n=10/sex/group):

	Male					female				
Dose, m/k/d	**0**	**1**	**5**	**10**	**15**	**0**	**1**	**5**	**10**	**15**
Total dead	0	0	1	4	7	0	0	0	1	2

Clinical signs: Signs noted in animals that died prematurely included a pale appearance and red exudate, consistent with internal bleeding and attributed to BMS-201038.

Body weights: Dose-related decreases in body weight gain were noted at ≥5 mg/kg/d in males with weight gain being 13%, 23%, and 35% less than control at 5, 10, and 15 mg/kg/d, respectively. In females, weight gain changes were comparable to control.

Food consumption: A transient decrease in food consumption was observed during the first week of dosing for males and females at ≥5 mg/kg/d, suggesting a possible transient palatability problem. Subsequent food consumption was comparable or greater in these dose groups compared with control.

Hematology: Statistically significant decreases in mean hemoglobin, hematocrit, and red blood cells were observed in treated males at 10 and 15 mg/kg/d but not in females; statistically significant increases in mean white blood cells, absolute neutrophils, and absolute lymphocytes occurred at ≥5 mg/kg/d in both males and females. Coagulation parameter changes were seen at ≥5 mg/kg/d in both genders, including increased platelet counts, prothrombin time, activated partial thromboplastin time, and fibrinogen (males only). Refer to the table below:

Drug-related hematology parameter changes:

	Male					female				
Dose, m/k/d	**0**	**1**	**5**	**10**	**15**	**0**	**1**	**5**	**10**	**15**
HGB, g/dL	15	15.3	13.9	12.7	12.4*	14.3	14.6	14.2	14.0	14.0
HCT, %	44.4	45.2	42.1	40.4*	39.5*	42.0	42.6	41.8	41.2	41.5
RBC, mil/µl	8.25	8.49	7.6	6.75**	6.66**	7.62	7.77	7.71	7.65	7.76
PLT, thous/µl	672	784	1039**	1034**	1167**	643	703	712	659	684
WBC, thous/µl	10.36	13.49*	16.54**	17.53**	17.60**	7.65	10.52*	14.39**	14.39**	13.86**
Abs Neut, thous/µl	1.15	2.30	3.43**	5.31**	2.93	0.79	1.33	2.07**	2.29**	1.90**
Abs Lymph, hroud/µl	8.58	10.08	12.00**	11.27*	14.02*	6.20	8.42	11.41**	11.11*	11.04**
PT, sec	9.4	11.7	32.5**	37.5**	40.0**	9.4	9.4	15.2*	19.1**	20.9**
APTT, sec	22.1	35.3	106.8	116.3**	119.7**	18.7	20.2	52.6**	61.8**	69.2**
Fibrin, mg/dL	241	255	283	326**	322**	199	191	214	223	199

Abs Neut = absolute neutrophils; APTT = activated partial thromboplastin time; HCT = hematocrit; HGB = hemoglobin; PLT = platelet; PT = prothromibin time; RBC = red blood cells; WBC = white blood cells.

Clinical chemistry: Drug-related, statistically significant increases in mean AST, ALT, and ALP at ≥1 mg/kg/d were observed in both males and females and statistically significant decreases in cholesterol and triglycerides at ≥5 mg/kg/d were observed in both males and females (see the summary table below).

	Male					female				
Dose, m/k/d	**0**	**1**	**5**	**10**	**15**	**0**	**1**	**5**	**10**	**15**
AST, IU/L	79	97*	113**	120**	112**	89	172**	167**	164**	151**
ALT, IU/L	54	82**	72**	71*	71	66	100**	78	81*	72
ALKP, IU/L	189	649**	489**	375**	378**	187	584**	472**	414**	404**
CHOL, Mg/dL	82	50	28**	29**	32	89	30**	31**	35**	36**
TRIG, Mg/dL	86	22	3**	2**	3**	60	10	5**	4**	3**

Gross pathology: 15 animals died between Weeks 5 and 13 at ≥10 mg/kg/d, including 4 M and1 F at 10 mg/kg/d and 7 M and 2 F at 15 mg/kg/d. All animals had gross and/or microscopic evidence of extensive hemorrhage in numerous organs and tissues, which was considered treatment-related. One male died at 5 mg/kg/d but the cause of death was not determined.

For the terminal sacrificed animals, drug-related findings were noted in the liver and small intestines. In the liver, pale discoloration was observed in most of the test article-treated females and many of the treated males. In the small intestines, the duodenum and jejunum were discolored (tan) in almost all animals receiving BMS-201038.

Organ weights: A dose-related increase in mean liver weights associated with microscopic lipid deposition was observed for all treated groups. Increased mean spleen and ovary weights were observed at ≥5 mg/kg/d. No gross or microscopic findings were associated with the increased spleen and ovary weights.

Histopathology:
Unscheduled deaths: No histopathology was conducted for the 15 mg/kg/d group. Drug-related changes were seen in the liver and small intestine. Trace to moderate diffuse hepatocellular vacuolation was noted in 4/4 M and 1/1 F at 10 mg/kg/d; ORO stain confirmed that the vacuoles in the liver cells were lipid droplets. Single hepatocyte necrosis and trace foci of subacute inflammation were seen in the dead female rat in this group.

Terminal sacrifice: Findings were predominantly seen in the liver, lungs, small intestine (15 mg/kg/d group was not processed for microscopic evaluation). Drug-related hemorrhage was found in many tissues at 10 mg/kg/d, including spinal cord, pancreas heart, urinary bladder, testes, epididymides, prostate, seminal vesicles, and adrenals.

Liver: trace to moderate diffuse hepatocellular vacuolation was observed in most rats at 1, 5, or 10 mg/kg/d. The vacuoles were confirmed to be lipid droplets by ORO staining. Single cell necrosis was noted in 1/6 M and 4/9 F at 10 mg/kg/d. Multiple foci of subacute inflammation were seen in all treated females and a few males. A summary of liver findings is presented in the sponsor-generated table below.

Group	I		IV	
Dose (mg/kg/day)	0		10	
Sex	M	F	M	F
Number of Animals	10	10	6	9
LIVER				
HEPATOCELLULAR LIPID VACUOLATION				
Minimal	0	0	1	0
Slight	0	0	1	0
Moderate	0	0	1	2
Marked	0	0	0	7
SINGLE CELL NECROSIS				
Minimal	0	0	1	4
INFLAMMATION, SUBACUTE				
Minimal	0	0	0	9

Small intestine: epithelial lipid vacuoles were seen in the absorptive epithelial cells in the duodenum and jejunum in all treated females and most males. There were no findings of inflammation or necrosis.

Lung: multifocal small collections of foamy macrophages (histocytosis) were noted in alveoli, frequently in subpleural locations in 4/6 males and 8/9 females at 10 mg/kg/d, and approximately 50% of treated animals at 1 and 5 mg/kg/d. Focal, subacute inflammation at 5 and 10 mg/kg/d and proliferation of alveolar type II cells at 10 mg/kg/d was stated by the study director in the results section; however, these specific findings could not be found in the histopathology summary tables in the study report.

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MICROSCOPIC FINDINGS --- EXPANDED INCIDENCE SUMMARY

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=T

ORGAN/TISSUE EXAMINED		--- NUMBER-OF-ANIMALS-AFFECTED ---									
	SEX:	MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	10	10	9	6	3	10	10	10	9	8
LIVER	NUMBER EXAMINED:	10	10	9	6	0	10	10	10	9	0
--VACUOLATION HEPATOCELLULAR	1>	0	1	3	1	0	0	0	0	0	0
	2>	0	0	2	1	0	0	1	0	0	0
	3>	0	0	4	1	0	0	4	3	2	0
	4>	0	0	0	0	0	0	5	7	7	0
	TL>	0	1	9	3	0	0	10	10	9	0
--CONGESTION	TL>	0	0	0	0	0	0	0	0	0	0
--LYMPHOID CELL INFILTRATE(S)/AGGREGATE(S)	1>	3	0	0	3	0	3	0	0	5	0
	TL>	3	0	0	3	0	3	0	0	5	0
--SINGLE CELL NECROSIS	1>	0	0	0	1	0	0	0	0	4	0
	TL>	0	0	0	1	0	0	0	0	4	0
--INFLAMMATION,SUBACUTE	1>	0	1	3	0	0	0	6	0	9	0
	2>	0	0	0	0	0	0	4	4	0	0
	3>	0	0	0	0	0	0	0	6	0	0
	TL>	0	1	3	0	0	0	10	10	9	0
--PPL: INFILTRATES SUBA	1>	0	0	1	0	0	0	0	0	0	0
	TL>	0	0	1	0	0	0	0	0	0	0
--NECROSIS,COAGULATION	TL>	0	0	0	0	0	0	0	0	0	0
DUODENUM	NUMBER EXAMINED:	10	10	9	6	0	10	10	10	9	0
--VACUOLATION	1>	0	5	0	2	0	0	1	0	5	0
	2>	0	2	0	3	0	0	9	0	4	0
	3>	0	0	9	1	0	0	0	10	0	0
	TL>	0	7	9	6	0	0	10	10	9	0
JEJUNUM	NUMBER EXAMINED.	10	10	9	6	0	10	10	10	9	0
--VACUOLATION	1>	0	1	0	0	0	0	0	0	0	0
	2>	0	9	0	4	0	0	0	0	1	0
	3>	0	0	9	2	0	0	9	10	8	0
	TL>	0	10	9	6	0	0	9	10	9	0
LUNGS	NUMBER EXAMINED:	10	10	9	6	0	9	10	10	9	0
--HISTIOCYTOSIS	1>	1	5	5	4	0	1	4	9	8	0
	TL>	1	5	5	4	0	1	4	9	8	0
--CONGESTION	TL>	0	0	0	0	0	0	0	0	0	0
--PVS: EDEMA	TL>	0	0	0	0	0	0	0	0	0	0
--PVS: INFILTRATES,SUBA	1>	1	0	1	2	0	0	0	0	1	0
	2>	1	0	0	0	0	0	0	0	0	0
	TL>	2	0	1	2	0	0	0	0	1	0
--PULMONARY VESSELS: MINERAL DEPOSIT(S)	1>	0	2	1	0	0	0	0	0	0	0
	TL>	0	2	1	0	0	0	0	0	0	0
--HEMORRHAGE	1>	0	1	0	0	0	0	0	0	0	0
	2>	0	3	0	0	0	0	0	0	0	0
	TL>	0	4	0	0	0	0	0	0	0	0

1 = Minimal; 2 = Slight; 3 = Moderate; 4 = Moderately Severe (Marked); 5 = Severe; p = Present; TL = Total

Toxicokinetics: Dose-related exposures to BMS-201038 and its three metabolites were observed in this dietary study. Exposures in females were much higher than in males at the same dose levels (3 to 5X for the parent AUC). $AUC_{0\text{-}24h}$ values of Metabolite BMS-203304 and BMS-224433 were about 2- to 3X and 2 to 6X higher than the parent. Overall, systemic exposure of the rats to the four compounds was as follows: BMS-224433 (M2) > BMS-203304 (M3) > BMS-201038 (parent) > BMS-203215 (M1). A summary of the TK data is presented in the sponsor-generated table below.

AUC(24 hr) (ng.hr/mL)								
Intended Daily Dose	BMS-201038		BMS-203304		BMS-203215		BMS-224433	
	M	F	M	F	M	F	M	F
1 mg/kg	60.2	192	70.5	239	a	b	230	380
5 mg/kg	233	1243	438	2637	12.9	61.4	1348	2603
10 mg/kg	491	2379	805	6422	41.5	167	2732	6443
15 mg/kg	791	3489	1921	10226	94.4	340	4886	9248

a Not calculated. Plasma concentrations in all animals were below the LLQ of 0.5 ng/mL
b Not reported. Plasma concentrations in most animals were below the LLQ of 0.5 ng/mL

Study title: **3-month oral gavage investigative toxicity study with AEGR-733 (formerly BMS-201038) in rats**

Study no.: AEGR-733PC0008
Study report location: Module 4.2.3.7.7
Conducting laboratory: (b) (4)
Date of study initiation: 21 January 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: AEGR-733 (BMS-201038; mesylate salt),
Batch #1713-1713-07-001, 86.6% pure "as is", free base

Key Findings

- There were no apparent treatment-related mortalities, adverse clinical signs, or effects on body weight.
- A slight increase in the incidence of minimal to slight intra-alveolar histiocytosis in the lung was observed in the 0.01, 0.04, and 0.15 mg/kg/d dose groups. A more noteworthy increase in both incidence and severity was observed at the high dose of 4 mg/kg/d. A slight increase in incidence of histiocytosis was observed in the high-dose group at both the 2- and 4-month recovery time points. An increase in histiocytosis was not observed at the 6-, 8-, or 12-month time points.

- When evaluated by TEM, the excessive vacuolation in alveolar macrophages was considered to be morphologically consistent with neutral lipid vacuoles. Concentric lamellar inclusions, characteristic of phospholipidosis, were rarely seen in lysosomes and were observed in both control and treated animals. The lipid vacuolation in the cytoplasm of lung macrophages was not associated with chronic interstitial lung injury.
- The results of a respiratory burst assay conducted with lung lavaged macrophages were inconclusive because of a poor yield of viable cells; this assay was repeated in Study 733PC0011, reviewed in the Special Toxicology Studies section.

Methods

Species/strain: Rat/Sprague-Dawley (females only)

Study design: See sponsor-generated table below

Group[a]	No. of Animals[b,c,d]	AEGR-733 Dose Level (mg/kg/day)	AEGR-733 Dose Concentration (mg/mL)
1 (Control)	65	0	0
2 (Low)	25	0.01	0.01
3 (Mid)	25	0.04	0.04
4 (Mid-High)	25	0.15	0.15
5 (High)	65	4.0	4.0

a Group 1 received control article only.

b At the dosing phase sacrifice (after 3 months of dosing), the first 15 animals in each group (dependent on survival) were designated for pathologic evaluation of the lungs; the lungs from 5 animals in each group were also designated for transmission electron microscopy. The next 10 animals in each group (dependent on survival) were designated for collection of bronchoalveolar (BAL) lavage samples for macrophage function assessments.

c After 2, 4, and/or 6 months of recovery, 10 rats each from the control and 4.0 mg/kg/day groups (dependent on survival) were designated to assess the reversibility of pathologic changes in the lungs by light microscopy and/or effects on macrophage function. After 2, 4, 6 and 8 months of recovery, 5 rats each from the control and 4.0 mg/kg/day groups were also designated for transmission electron microscopy of the lung.

d After 12 months and 2 weeks of recovery, any remaining animals were sacrificed, and lung tissue was held for possible future examination.

Route and volume: Oral gavage at 1 mL/kg

Formulation: 75% PEG-400 in reverse osmosis water

Satellite groups for recovery: See sponsor-generated table above. Additional animals were included in control and HD groups for 2, 4, 6, 8, and 12 months of recovery. Tissue samples from the 8 and 12 month recovery groups were archived but not analyzed.

Age: 6 to 7 weeks at initiation of treatment

Weight: 145 to 217 g

Toxicokinetics: Blood samples collected at 1, 2, 4, 8, 12, and 24 hours postdose during Week 14 from 15 main study animals per group designated for pathological evaluation.

Special evaluations: Transmission electron microscopy (TEM) on lung tissue and evaluation of alveolar macrophages isolated by bronchoalveolar lavage (BAL) for assessment of activity in a respiratory burst assay.

Observation and Times:

Clinical signs: Twice daily
Body weights: Twice pretest, weekly during dosing and terminally
Food consumption: Not conducted
Clinical pathology: Not conducted
Gross pathology: At necropsy
Organ weights: Lungs only
Histopathology: Lungs only; lungs for TEM (5/group/time point) were infused with and collected in McDowell-Trump fixative and imbedded in epoxy resin. Lungs for standard light microscopy (10 to 15 animals per group) were preserved in 10% neutral-buffered formalin.
Peer Review: A TEM peer review was conducted by (b) (4) however, the results of the peer review are maintained in the study files and no peer review statement or signature of the peer reviewer is included in the study report.

Results:

Mortality:
There were no deaths attributed to the test article. Two control animals died on Day 92 following blood collection. One animal receiving 0.15 mg/kg/d was found dead on Day 26, with no clinical signs of toxicity prior to death. One 4 mg/kg/d group animal was sacrificed moribund on Day 199 of the recovery phase. Clinical signs prior to death included few feces and red haircoat.

Clinical signs:
No test article-related clinical signs occurred during the dosing or recovery phases.

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

Gross pathology:
The presence of few or multiple discolored tan areas involving all lung lobes in one main group animal given 0.01 mg/kg/d and 2 main group animals receiving 4 mg/kg/d, one 4-month recovery animal receiving 4 mg/kg/d, and one 6-month recovery animal from the control group. These observations were not considered to be related to the test article.

Organ weights:
No treatment-related effects on lung weights were noted.

Histopathology:
An increase in the incidence of histiocytosis was observed for treated groups at end of treatment. A slight increase in the incidence of histiocytosis was observed in the high-dose group after 2 and 4 months of recovery. No microscopic lesions (e.g., histiocytosis) were observed at the 6-, 8-, or 12-month recovery time points. The incidence and severity of intra-alveolar histiocytosis for the main study group and recovery groups 1 (2 months) and 2 (4 months) are shown in the sponsor-generated tables below. No other treatment-related microscopic effects were noted in the lungs.

Incidence and Mean Severity of Test Article-Related Microscopic Findings in the Lung at the Dosing Phase Sacrifice

	AEGR-733 (formerly BMS-201038) mg/kg/day				
Sex	Females				
Dose Level	0	.01	.04	.15	4.0
Number Examined	13	15	15	15	15
Lung					
Histiocytosis, Intraalveolar					
Minimal	3	6	6	6	8
Slight	0	0	1	1	5
Average severity	0.2	0.4	0.5	0.5	1.2

Incidence and Mean Severity of Test Article-Related Microscopic Findings in the Lung at Recovery Sacrifice 1

	AEGR-733 (formerly BMS-201038) mg/kg/day				
Sex	Females				
Dose Level	0	.01	.04	.15	4.0
Number Examined	10	0	0	0	10
Lung					
Histiocytosis, Intraalveolar					
Minimal	2	-	-	-	2
Slight	0	-	-	-	2
Average severity	0.2	-	-	-	0.6

Incidence and Mean Severity of Test Article-Related Microscopic Findings in the Lung at Recovery Sacrifice 2

	AEGR-733 (formerly BMS-201038) mg/kg/day				
Sex	Females				
Dose Level	0	.01	.04	.15	4.0
Number Examined	10	0	0	0	10
Lung					
Histiocytosis, Intraalveolar					
Minimal	1	-	-	-	2
Slight	0	-	-	-	0
Average severity	0.1	-	-	-	0.2

Transmission Electron Microscopy:
Lung tissue from a subset of animals at each sacrifice time point was evaluated by TEM. This analysis revealed evidence of excessive cytoplasmic vacuolation in alveolar macrophages of some test article-treated animals at the end of the dosing period and at the end of the 2- and 4-month recovery periods, which was consistent with the vacuolation noted by light microscopy. The TEM pathologist reported that "the excessive vacuolation was considered to be morphologically consistent with neutral lipid vacuoles because of the presence of smooth contoured vacuoles with homogeneous translucent content, characteristic of the ultrastructural appearance of neutral lipid". The evaluation did not reveal evidence of excessive accumulation of concentric lamellar inclusions in the lysosomes of macrophages or other lung cells that would be characteristic of phospholipidosis. Although vacuoles often contained heterogenous deeply electron-dense structures and irregular single and multilamellar membranous profiles, it was the pathologist's opinion that these findings were effects of fixation and processing. Concentric lamellar inclusions, characteristic of phospholipidosis, were rarely seen in lysosomes of cells within the lung samples and were observed in cells from both control and treated animals.

In the lung tissues examined, there was no evidence of chronic interstitial reaction in the alveolar wall adjacent to vacuolated macrophages. The absence of chronic reactions suggests that the observed macrophage alterations were not associated consequential injury to pulmonary tissue. At the end of the 6- and 8-month recovery periods, lipid vacuoles were observed in alveolar macrophages of both control and treated animals. Because the lipid vacuolation seen in control animals was similar to that observed in the treated animals, these effects were not considered treatment-related. Additionally, very few macrophages were in alveoli of animals from the 6- and 8-month recovery groups.

The pathologist's overall conclusions were that the administration of AEGR-733 led to excessive accumulation of lipid vacuoles in the cytoplasm of lung macrophages but this accumulation did not result in chronic interstitial lung injury. Excessive lipid accumulation resolved within 6 months after treatment. The vacuoles were felt to contain neutral lipids and phospholipidosis was discounted because there was no evidence of increased concentric lamellar inclusions within lysosomes of lung macrophages. A summary of the incidence of lipid vacuolation is shown in the sponsor-generated table below.

Summary of TEM Findings of Excessive Lipid Vacuoles in Lung Macrophages

Time Point	Group	Dose (mg/kg/day)	Number Examined	Number of Animals with Excessive Lipid Vacuoles in Macrophages	Animals with Excessive Lipid Vacuoles in Macrophages
Terminal	1	0	5	0	
Terminal	2	0.01	5	1	B69812
Terminal	3	0.04	5	2	B69838, B69846
Terminal	4	0.15	5	3	B69861, B69862, B69867
Terminal	5	4.0	5	2	B69890, B69896
Recovery 1 - 2 mo.	1	0	5	0	
Recovery 1 - 2 mo.	5	4.0	5	2	B69915, B69917
Recovery 2 - 4 mo.	1	0	5	1	B69785
Recovery 2 - 4 mo.	5	4.0	5	3	B69921, B69923, B69924
Recovery 3 - 6 mo.	1	0	5	2	B69796, B69797
Recovery 3 - 6 mo.	5	4.0	5	1	B69931
Recovery 4 - 8 mo.	1	0	5	2	B69801, B69802
Recovery 4 - 8 mo.	5	4.0	5	1	B69941

Macrophage function:
Individual BAL samples had poor cell yields and low viability. BAL samples from each treatment group were combined for the respiratory burst assay. The results showed a non-dose-related decrease in respiratory burst (H_2O_2 production), with 18%, 88%, 88%, and 76% decreases compared with the control group for the 0.01, 0.04, 0.15, and 4 mg/kg/d groups, respectively. Cell sample yield and viability from the first recovery group were also poor. Overall, no definitive conclusions could be made and this assay was repeated in Study 733PC0011 (reviewed in Special Toxicology Section).

Toxicokinetics: (sponsor-generated tables)

Toxicokinetic Parameters for AEGR-733 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg/day)]
3	0.04	0.718	18.0	24.0	8.50	212
4	0.15	3.51	23.4	2.00	71.5	477
5	4.0	75.8	19.0	2.00	1507	377

Notes: No toxicokinetic analysis was performed on Group 2 due to the lack of quantifiable results.
AUC_{0-24} is equivalent to AUC_{0-t}

Toxicokinetic Parameters for BMS-203215 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/day)]	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/ (mg/kg/day)]	M:P Ratio AUC_{0-24}
5	4.0	13.4	3.35	2.00	186	46.5	0.123

Notes: No toxicokinetic analysis was performed on Groups 2, 3, and 4 due to the lack of quantifiable results.
AUC_{0-24} is equivalent to AUC_{0-t}

Toxicokinetic Parameters for BMS-224433 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/day)]	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/ (mg/kg/day)]	M:P Ratio AUC_{0-24}
4	0.15	2.92	19.5	12.0	51.4	343	0.719
5	4.0	127	31.8	8.00	2405	601	1.60

Notes: No toxicokinetic analysis was performed on Groups 2 and 3 due to the lack of quantifiable results.
AUC_{0-24} is equivalent to AUC_{0-t}

Toxicokinetic Parameters for BMS-203304 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/day)]	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/ (mg/kg/day)]	M:P Ratio AUC_{0-24}
4	0.15	4.77	31.8	24.0	96.5	643	1.35
5	4.0	194	48.5	24.0	3530	883	2.34

Notes: No toxicokinetic analysis was performed on Groups 2 and 3 due to the lack of quantifiable results.
AUC_{0-24} is equivalent to AUC_{0-t}

Dosing formulation analysis:
Mean concentrations of prepared dose formulations of the 0.01, 0.4, .015, and 4.0 mg/mL preparations were 93.9%, 94.2%, 95.9%, and 96.0%, respectively, for Week 1; 94.6%, 97.1%, 94.3%, and 96.5%, respectively, for Week 6, and 91.9%, 95.2%, 108%, and 98.3%, respectively, for Week 13.

Study title: BMS-201038 - Six Month Oral Toxicity Study in Rats

Study no.:	96024
Study report location:	Module 4.2.3.2
Conducting laboratory and location:	Bristol-Myers Squibb, Pharmaceutical Research Institute, Dept. of Toxicology and Pathology, New Brunswick, NJ, USA
Date of study initiation:	23 July 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038 (as the mesylate salt), Batch #40983-012-32 and R006A, 82.5% pure (free base)
Note:	Review of this study was initially conducted by Dr. Dylan Yao and submitted to DARRTS on 10 April 2007. Edits to Dr. Yao's review have been made for clarity and formatting.

Key Study Findings

- Drug-related deaths occurred in 20 mg/kg/d treated animals (without vitamin supplementation) during the treatment (mortality rate 90%). Cause of death was extensive internal hemorrhage in multiple organs/tissues resulting from drug-induced malabsorption of fat-soluble vitamins. No drug-related deaths occurred at 20 mg/kg/d with vitamin supplementation.
- No effects on body weights or food consumption were observed.
- No remarkable findings in hematology parameters were noted. Slightly decreased APTT was noted for all groups with vitamin supplementation compared with controls receiving vitamins. All vitamin supplemented groups including control had increased fibrinogen levels compared with unsupplemented controls.
- Drug-related decreases in mean serum cholesterol and triglycerides were observed at ≥2 mg/kg/d with and without supplementation. Increased mean ALT and AST (1.5X the control values) was observed only in females receiving 2 mg/kg/d without vitamin supplementation. Increased mean ALP (2 to 3X the control values) was observed at ≥ 2 mg/kg/d with and without vitamin supplementation.
- Drug-related microscopic findings included lipid accumulation in the hepatocytes and intestinal epithelial cells, and multifocal histiocytosis (foamy macrophage accumulation in alveolar space) in the lungs at 0.2, 2, and 20 mg/kg/d, which was considered to be consistent with phospholipidosis. Minimal (males) to moderate (females) changes were noted for these lesions at 2 and 20 mg/kg/d.
- Exposures to BMS-201038 and its metabolites increased in a dose proportional manner, and were similar in the presence or absence of vitamin supplements. Exposures to the parent and metabolites were higher in females than in males. Overall, systemic exposures to the four compounds in rats were as follows: BMS-224433 > BMS-203304 > BMS-201038 > BMS-203215.

- Decreased vitamin A and E levels in the liver occurred at 2 mg/kg without vitamin supplementation (↓17% M and ↓69% F); however no decrease in liver concentration of vitamin A and E was noted at 2 and 20 mg/kg/d with vitamin supplementation.

Methods

Species/Strain: Rat/Sprague-Dawley

Dose groups: See sponsor-generated table below:

Group Number	Daily Dose		BMS-201038 Concentration (mg/ml)	Vitamin Supplement[a]	Number of Rats
	BMS-201038 (mg/kg)	Dose Volume (ml/kg)			
1 (Control)	0 (75% PEG-400)	1	0	No	10M, 10F
2 (Control)	0 (75% PEG-400)	1	0	Yes	10M, 10F
3	0.02	1	0.02	No	10M, 10F
4	0.02	1	0.02	Yes	10M, 10F
5	0.2	1	0.2	No	10M, 10F
6	0.2	1	0.2	Yes	10M, 10F
7	2	1	2	No	10M, 10F
8	2	1	2	Yes	10M, 10F
9	20	1	20	No	10M, 10F
10	20	1	20	Yes	10M, 10F

a - 10,000 IU/kg of vitamin A, 1,500 IU/kg of vitamin D, 50 IU/kg of vitamin E, and 0.3 mg/kg of vitamin K were administered subcutaneously.

Frequency of dosing: Once daily

Route of administration: Oral gavage

Formulation/Vehicle: BMS-201038 solution in 75% PEG-400/25% water

Age: ~5 weeks

Weight: 86 to 116 g (males) and 81 to 103 g (females)

Satellite groups: No, TK samples were taken from treated animals at 1, 2, 4, 8, 12, and 24 hours (3 animals/group/time point) after dosing on Day 1 and during the third and sixth months.

Unique study design: Subcutaneous multiple vitamin supplements (A, D, E, K) were given weekly to half of the animals at each dose level to protect against drug-induced fat-soluble vitamin deficiency. Electron microscopy was conducted on lung, liver, and small intestine.

Deviation from study protocol: There were no protocol deviations that affected the integrity or interpretability of the study results.

Observations and Results

Mortality

Drug-related deaths occurred in the 20 mg/kg/d group not receiving vitamin supplementation (Group 9). The mortality rate for this group was 90%, with 18 out of 20 rats (9 M and 9 F) dying or being sacrificed moribund during Weeks 4 through 11. All of these deaths resulted from extensive hemorrhage, which was attributed to drug-induced malabsorption of fat-soluble vitamin K.

Other sporadic deaths occurred in most groups, including 1 M and 1 F from Group 9, with gross and/or microscopic lesions that were consistent with gavage errors. One male in Group 10 died with a large incidental mammary adenoma. A summary of deaths is shown in Dr. Yao's table below. Only the 18 deaths in Group 9 were considered to be drug related.

Group No.	Doses, mg/kg/d	Sex	No. of rats dosed	Died	Moribund-sacrifice
1	0	M	10	0	0
		F	10	0	0
2	0 + v [a]	M	10	2	0
		F	10	1	0
3	0.02	M	10	0	0
		F	10	0	0
4	0.02 + v	M	10	2	0
		F	10	1	0
5	0.2	M	10	1	0
		F	10	0	0
6	0.2 + v	M	10	1	0
		F	10	0	0
7	2	M	10	1	0
		F	10	1	0
8	2 + v	M	10	1	0
		F	10	0	0
9	20	M	10	6	4
		F	10	5	5
10	20 + v	M	10	2	0
		F	10	2	0

[a] v+ = with vitamin supplementation.

Clinical Signs

Major signs relevant to treatment were noted in animals receiving 20 mg/kg/d without vitamin supplementation, including rhinorrhea and chromorhinorrhea, blood on nares, urine stain, rough coat, decreased activity, limb paralysis, and few or no feces. Alopecia was mainly seen in animals receiving 2 or 20 mg/kg/d with vitamin supplementation. A summary of noteworthy clinical observations is shown in the sponsor-generated table below.

Observations	NUMBER OF RATS											
	Group 1 Control 0 mg/kg		Group 2 BMS-201038 0 mg/kg+VS		Group 3 BMS-201038 0.02 mg/kG		Group 4 BMS-201038 0.02 mg/kg+VS		Group 5 BMS-201038 0.2 mg/kg		Group 6 BMS-201038 0.2 mg/kg+VS	
	M	F	M	F	M	F	M	F	M	F	M	F
Chromodacryorrhea	0	0	0	0	0	0	0	0	0	0	0	0
Chromorhinorrhea	2	0	0	1	0	0	1	0	0	0	0	1
Rhinorrhea	0	0	1	0	0	0	0	0	0	0	0	1
Frank blood on nares	0	0	0	0	0	0	0	0	0	0	0	0
Dyspnea, labored breathing, rales	0	0	0	0	0	0	1	1	1	0	0	1
Alopecia	0	0	0	0	0	0	0	1	0	0	0	0
Subcutaneous mass at injection site	0	0	0	0	0	0	0	0	0	0	0	0
Urine stain	0	0	0	0	0	0	0	0	0	0	0	0
Rough coat	0	0	1	0	0	0	1	0	0	0	0	0
Inactivity	0	0	0	1	0	0	0	1	1	0	0	1
Limb paralysis	0	0	0	0	0	0	0	0	0	0	0	0
Few or no feces	1	0	1	0	0	0	0	0	0	0	0	0

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Observations	NUMBER OF RATS							
	Group 7 BMS-201038 2 mg/kg		Group 8 BMS-201038 2mg/kg+VS		Group 9 BMS-201038 20 mg/kg		Group 10 BMS-201038 20 mg/kg+VS	
	M	F	M	F	M	F	M	F
Chromodacryorrhea	0	0	0	0	1	1	0	0
Chromorhinorrhea	1	0	0	0	1	5	0	0
Rhinorrhea	0	1	0	0	0	3	0	0
Frank blood on nares	0	0	0	0	3	1	0	0
Dyspnea, labored breathing, rales	0	1	1	0	0	0	0	0
Alopecia	0	0	1	4	0	0	2	5
Subcutaneous mass at injection site	0	0	0	0	0	0	5	0
Urine stain	0	1	0	0	2	3	0	0
Rough coat	0	0	0	0	0	2	0	0
Inactivity	0	0	0	0	3	1	0	0
Limb paralysis	0	0	0	0	1	1	0	0
Few or no feces	1	1	0	0	1	2	0	0

Body Weights

A slight decrease in mean body weight gain (6%) was seen for males receiving 20 mg/kg/day. There were no drug-related effects on body weight for the other dose groups.

Feed Consumption:
No treatment-related effects on food consumption were noted at any dose level.

Ophthalmoscopy
No treatment-related ophthalmic changes were noted at any dose level.

Hematology
There were no toxicologically meaningful changes in hematology parameters. Mean total leukocyte counts were statistically significantly increased at 2 and 20 mg/kg/day with vitamin supplementation compared with controls without vitamin supplementation, but only slightly higher than controls with vitamin supplementation. Neutrophil counts were slightly higher for groups receiving vitamins compared with controls not receiving vitamins.

Mean prothrombin time was slightly longer for females receiving 2 mg/kg/day plus vitamin supplementation, but not at the high dose. Mean activated partial thromboplastin time (APTT) was slightly shorter for high-dose males and females with vitamin supplementation. Males receiving vitamin supplementation, including controls, also had slight decreases in APTT compared with controls not receiving vitamins. All groups receiving vitamins had higher levels of fibrinogen compared with controls not receiving vitamins.

Clinical Chemistry:
Treatment-related serum chemistry changes were noted for cholesterol (CHOL), triglycerides (TRIG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) at ≥2 mg/kg/day.

Doses, mg/kg/d		0	0 +v	0.02	0.02+v	0.2	0.2 +v	2	2 +v	20	20 +v
CHOL, mg/dL	M	103	100	123	105	106	112	68**	94	-	52**
	F	113	124	111	121	108	107	48**	53**	-	39**
TRIG, mg/dL	M	108	100	115	108	91	105	89	90	-	12**
	F	82	82	68	65	55**	62	26**	25**	-	6**
ALT, U/L	M	79	57	64	61	61	60	80	60	-	66
	F	67	64	62	62	86	58	102**	85	-	68
AST, U/L	M	112	90	93	86	82	87	113	91	-	101
	F	103	101	93	99	107	92	167**	139	-	132
ALP, U/L	M	187	173	175	180	215	214	378**	294	-	539**
	F	163	179	161	181	193	173	562**	505**	-	489**

- : animals died before the end of doing.

Urinalysis:
There were no drug-related changes in urinalysis parameters. At 2 and 20 mg/kg/d with vitamin supplementation, mild decreases in urine output with corresponding increases in urine specific gravity were noted in females.

Vitamin concentrations in liver:
Vitamin A and E levels in liver were decreased at 2 mg/kg without vitamin supplementation (↓17% M and ↓69% F); however there was no decrease of liver concentration of liver vitamin A and E with vitamin supplementation. Note that vitamin levels for the 20 mg/kg/day group without vitamin supplementation were not assessed because this group did not survive until the end of the study.

Gross Pathology
***Premature deaths**:*
In the 9 males and females given BMS-201038 at 20 mg/kg/d without vitamin supplementation that died or sacrificed moribund, mild to marked hemorrhage/red discoloration was noted in one or more of the following organs or tissues: thymus, salivary gland, urinary bladder, epididymides, testes, prostate, skeletal muscle, seminal vesicles, vagina, lung, skin (head), esophagus, plural cavity (along vertebral column), and ocular accessory glands. Additional findings in some of these animals included blood within body cavities (thoracic, cranial, and/or abdominal) and the pericardial sac. Blood in the subcutaneous tissue of the cervical and axillary areas, thorax, and/or thoracic inlet as well as dried blood or red discharge around the mouth, nose, eyes, or on other body surfaces was also noted.

Other drug-related gross lesions in the 20 mg/kg/d group without vitamin supplementation were seen in the liver and small intestine:

> Liver - moderate pale discoloration in 6 of the 20 rats (1 M, 5 F);
> Small intestine - mild tan discoloration in the jejunum and duodenum in 7 of the 20 rats (5 M, 2 F).

Besides these dead or moribund sacrificed animals, gross findings in other dead animals were consistent with dosing accidents, including moderate to marked red discoloration of the lungs. Drug-related gross lesions were also noted in the liver and small intestine of a few rats at 20 mg/kg/d with vitamin supplementation that died due to dosing accidents, consisting of mild, pale discoloration in the liver and mild, tan discoloration of small intestine. A summary of macroscopic findings for unscheduled deaths is presented in the sponsor-generated table below.

Summary of Gross Pathology Findings (unscheduled deaths)

Group	2		4		5	6	7		8
Dose(mg/kg)	0/Vit		0.02/Vit		0.2	0.2/Vit	2		2/Vit
Sex	M	F	M	F	M	M	M	F	M
Animals On Study	10	10	10	10	10	10	10	10	10
Animals Logged	2	1	2	1	1	1	1	1	1
Liver	2	1	2	1	1	1	1	1	1
Not Remarkable	2	0	2	1	1	1	1	1	1
Remarkable Observations	0	1	0	0	0	0	0	0	0
Discoloration, Dark	0	1	0	0	0	0	0	0	0
Lung	2	1	2	1	1	1	1	1	1
Remarkable Observations	2	1	2	1	1	1	1	1	1
Discoloration, Red	2	1	2	1	1	1	1	1	1

Group	9		10	
Dose(mg/kg)	20		20/Vit	
Sex	M	F	M	F
Animals On Study	10	10	10	10
Animals Logged	10	10	2	2
Liver	10	10	2	2
Not Remarkable	9	5	1	1
Remarkable Observations	1	5	1	1
Discoloration, Pale	1	5	0	1
Discoloration, Red	0	0	1	0
Lung	10	10	2	2
Not Remarkable	8	8	1	0
Remarkable Observations	2	2	1	2
Discoloration, Red	2	1	1	2
Discoloration, Red; Right Caudal Lobe	0	1	0	0
Small Intestine	10	10	2	2
Not Remarkable	5	8	1	1
Remarkable Observations	5	2	1	1
Discoloration, Tan	5	2	1	1

Terminal sacrifice (end of dosing):
At the end of dosing, drug-related gross lesions were observed in the liver, small intestine, and lungs at 0.2, 2, and 20 mg/kg/d, and there were no substantial differences for these lesions between vitamin-treated and non-vitamin-treated animals given equivalent doses of BMS-201038:

- Liver: mild, pale discoloration in 5 F at 0.2 mg/kg/d, 18 F at 2 mg/kg/d, 7 M and 8 F at 20 mg/kg//d with vitamin supplementation that survived to scheduled necropsy.
- Small intestine: mild, tan discoloration in duodenum and jejunum in 2 M at 0.2 mg/kg/d; 3 M and 16 F at 2 mg/kg/d; and 8 M and 8 F at 20 mg/kg/d with vitamin supplementation that survived to scheduled necropsy.

- Lung: minimal, multifocal tan discoloration in 1 M and 1 F at 0.2 mg/kg/d; 1 M and 9 F at 2 mg/kg/d; and 8 M and 8 F at 20 mg/kg/d with vitamin supplementation that survived to scheduled necropsy.

A summary of macroscopic findings for scheduled necropsies are presented in the sponsor-generated table below.

Summary of Gross Pathology Findings (scheduled necropsy)

Group	1		2		3		4		5		6	
Dose(mg/kg)	0		0/Vit		0.02		0.02/Vit		0.2		0.2/Vit	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Animals On Study	10	10	10	10	10	10	10	10	10	10	10	10
Animals Logged	10	10	8	9	10	10	8	9	9	10	9	10
Liver	10	10	8	9	10	10	8	9	9	10	9	10
Not Remarkable	10	10	8	9	9	9	8	9	9	8	9	6
Remarkable Observations	0	0	0	0	1	1	0	0	0	2	0	4
Discoloration, Pale	0	0	0	0	0	0	0	0	0	1	0	4
Hepatodiaphragmatic Nodule; Median Lobe	0	0	0	0	1	1	0	0	0	1	0	0
Lung	10	10	8	9	10	10	8	9	9	10	9	10
Not Remarkable	10	10	8	9	10	10	8	9	9	10	8	9
Remarkable Observations	0	0	0	0	0	0	0	0	0	0	1	1
Discoloration, Tan	0	0	0	0	0	0	0	0	0	0	1	1
Skin	10	10	8	9	10	10	8	9	9	10	9	10
Not Remarkable	10	10	6	6	10	10	6	8	9	10	8	9
Remarkable Observations	0	0	2	3	0	0	2	1	0	0	1	1
Alopecia	0	0	0	0	0	0	0	1	0	0	0	0
Thickness Increased	0	0	1	3	0	0	2	0	0	0	1	1
Scab	0	0	1	0	0	0	0	0	0	0	0	0
Small Intestine	10	10	8	9	10	10	8	9	9	10	9	10
Not Remarkable	10	10	8	9	10	10	8	9	7	10	9	10
Remarkable Observations	0	0	0	0	0	0	0	0	2	0	0	0
Discoloration, Tan	0	0	0	0	0	0	0	0	2	0	0	0
Testis	10	0 *	8	0 *	10	0 *	8	0 *	9	0 *	9	0 *
Not Remarkable	10	0	8	0	10	0	7	0	9	0	9	0
Remarkable Observations	0	0	0	0	0	0	1	0	0	0	0	0
Absent	0	0	0	0	0	0	1	0	0	0	0	0
Uterus	0 *	10	0 *	9	0 *	10	0 *	9	0 *	10	0 *	10
Not Remarkable	0	9	0	7	0	8	0	9	0	9	0	8
Remarkable Observations	0	1	0	2	0	2	0	0	0	1	0	2
Dilatation	0	1	0	2	0	2	0	0	0	1	0	2

* - Fishger's not calculated (less than 4 tissues)

Group	1		7		8		10	
Dose(mg/kg)	0		2		2/Vit		20/Vit	
Sex	M	F	M	F	M	F	M	F
Animals On Study	10	10	10	10	10	10	10	10
Animals Logged	10	10	9	9	9	10	8	8
Liver	10	10	9	9	9	10	8	8
Not Remarkable	10	10	9	1	9	0	1	0
Remarkable Observations	0	0	0	8	0	10	7	8
Discoloration, Pale	0	0	0	8 b	0	10 b	7 b	8 b
Lung	10	10	9	9	9	10	8	8
Not Remarkable	10	10	9	6	8	4	0	0
Remarkable Observations	0	0	0	3	1	6	8	8
Discoloration, Tan	0	0	0	3	1	6 a	8 b	8 b
Skin	10	10	9	9	9	10	8	8
Not Remarkable	10	10	9	9	5	5	1	0
Remarkable Observations	0	0	0	0	4	5	7	8
Alopecia	0	0	0	0	0	0	1	2
Thickness Increased	0	0	0	0	4 a	5 a	6 b	6 b
Small Intestine	10	10	9	9	9	10	8	8
Not Remarkable	10	10	7	1	8	2	0	0
Remarkable Observations	0	0	2	8	1	8	8	8
Discoloration, Tan	0	0	2	8 b	1	8 b	8 b	8 b
Uterus	0 *	10	0 *	9	0 *	10	0 *	8
Not Remarkable	0	9	0	8	0	7	0	8
Remarkable Observations	0	1	0	1	0	3	0	0
Dilatation	0	1	0	1	0	3	0	0

* - Fishger's not calculated (less than 4 tissues)

Organ Weights

At the end of dosing, there were no substantial differences in organ weights between vitamin-treated and non-vitamin-treated animals given equivalent doses of BMS-201038. Drug-related organ weight changes were seen in liver and spleen when compared with controls:

- Mean liver weight was increased at 0.2 mg/kg/d (↑23%) and 2 mg/kg/d (↑83%) in females and at 20 mg/kg/d (↑16% and ↑81%) in males and females, respectively.
- Mean spleen weight was increased for females receiving 20 mg/kg/d (↑ 44%).

Histopathology:

Adequate Battery: Yes

Peer Review: Yes, conducted by a second BMS pathologist

Histological Findings:
Major microscopic lesions were observed in the liver, small intestine, and lungs (see sponsor-generated tables below for both unscheduled and terminal deaths):

Liver: An increase in incidence and severity of hepatocellular lipid vacuolation occurred in a dose-dependent manner at all dose levels (≥0.02 mg/kg/d), which correlated with increased liver weights and pale discoloration of the liver seen at necropsy. The incidence at the two lowest dose levels was higher in females than in males. A severity summary of the hepatocellular lipid vacuolation is shown below:

0.02 mg/kg/d – minimal;
0.2 mg/kg/d – mild to moderate;
2 and 20 mg/kg/d – minimal (males) and marked (females).

Small intestine: lipid vacuolation of absorptive epithelial cells in the small intestine was observed at 0.2, 2, and 20 mg/kg/d, predominantly in the jejunum and duodenum, which correlated with tan discoloration of the small intestine. The incidence and severity were also dose-dependent. The incidence was higher in females at 0.2 and 2 mg/kg/d. A severity summary of the epithelial cell lipid vacuolation is shown below:

0.2 mg/kg/d – minimal;
2 and 20 mg/kg/d – minimal to moderate.

Lung: multifocal histiocytosis was observed with increased incidence and /or severity at 0.2 (with vitamins only), 2, and 20 mg/kg/d, which was characterized by multifocal aggregates of large foamy macrophages within alveoli, either alone or associated with a dose-related increase in incidence and/or severity of subacute inflammation, necrotic cellular debris, cholesterol-like clefts, and/or Type II alveolar cell proliferation. Special stain with Luxol fast blue and Baker's acid hematin stain for phospholipids and Oil-Red-O for neutral lipid demonstrated that both types of lipid were contained in the macrophages and adjacent Type II alveolar lining cells. The severity of the lung phospholipidosis was as follows:

0.2 mg/kg/d – minimal;
2 mg/kg/d – minimal to mild;
20 mg/kg/d – minimal to mild (males) and minimal to marked (females)

Major Histopathology Findings (premature deaths; sponsor-generated table)

Group	2		4		5	6	7		8
Dose(mg/kg)	0/Vit		0.02/Vit		0.2	0.2/Vit	2		2/Vit
Sex	M	F	M	F	M	M	M	F	M
Animals On Study	10	10	10	10	10	10	10	10	10
Animals Logged	2	1	2	1	1	1	1	1	1
Liver	2	1	2	1	1	1	1	1	1
Remarkable Observations	2	1	2	1	1	1	1	1	1
Congestion	2	1	2	1	1	1	1	1	1
Fibroplasia/Fibrosis; Subcapsular	0	0	1	0	0	0	0	0	0
Infiltration, Mononuclear Cell	0	0	0	0	0	0	1	0	0
Inflammation, Subacute	0	0	0	0	0	0	0	1	0
Necrosis, Single Cell	0	0	0	0	1	0	0	0	0
Vacuolation	0	0	0	0	1	0	0	1	1
Lung	2	1	2	1	1	1	1	1	1
Remarkable Observations	2	1	2	1	1	1	1	1	1
Congestion	2	0	2	0	1	1	1	1	1
Hemorrhage	2	1	2	1	1	1	1	1	1
Histiocytosis	1	0	0	0	0	0	0	0	0
Inflammation, Subacute	0	1	2	1	0	1	1	1	0

Group	9		10	
Dose(mg/kg)	20		20/Vit	
Sex	M	F	M	F
Animals On Study	10	10	10	10
Animals Logged	10	10	2	2
Liver	10	10	2	2
Not Remarkable	1	0	0	0
Remarkable Observations	9	10	2	2
Congestion	2	1	2	0
Extramedullary Hematopoiesis	1	1	0	0
Hemorrhage	2	0	0	0
Infiltration, Mononuclear Cell	1	3	0	1
Inflammation, Subacute	2	4	0	1
Necrosis; Centrilobular	0	3	0	0
Necrosis, Coagulative	1	2	0	0
Necrosis, Single Cell	1	1	0	1
Vacuolation	7	10	1	2
Lung	10	10	2	2
Not Remarkable	1	0	0	0
Remarkable Observations	9	10	2	2
Congestion	2	2	2	1
Hemorrhage	3	5	2	2
Histiocytosis	5	6	1	1
Infiltration, Mononuclear Cell	6	2	1	1
Inflammation, Subacute	2	3	0	0
Small Intestine	10	10	2	2
Not Remarkable	0	1	0	0
Remarkable Observations	10	9	2	2
Vacuolation	10	9	2	2

Major Histopathology Findings (scheduled necropsy; sponsor-generated table)

Group	1		2		3		4		5		6	
Dose(mg/kg)	0		0/Vit		0.02		0.02/Vit		0.2		0.2/Vit	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Animals On Study	10	10	10	10	10	10	10	10	10	10	10	10
Animals Logged	10	10	8	9	10	10	8	9	9	10	9	10
Liver	10	10	8	9	10	10	8	9	9	10	9	10
Not Remarkable	7	6	5	6	5	2	5	3	0	0	4	0
Remarkable Observations	3	4	3	3	5	8	3	6	9	10	5	10
Fibroplasia/Fibrosis; Subcapsular	0	0	0	0	1	1	0	0	0	1	0	0
Infiltration, Mononuclear Cell	3	4	3	3	5	2	2	4	4	9	3	7
Inflammation, Acute	0	0	0	0	1	1	0	0	0	0	0	0
Inflammation, Subacute	0	0	0	0	0	0	0	0	2	5 a	0	4
Necrosis, Coagulative	0	0	1	0	0	0	0	0	0	0	0	0
Necrosis, Single Cell	0	0	0	0	0	0	0	0	2	6 a	0	5 a
Vacuolation	0	0	0	0	0	6 a	1	6 b	6 b	10 b	4 a	10 b
Lung	10	10	8	9	10	10	8	9	9	10	9	10
Not Remarkable	8	9	7	7	8	9	6	8	8	9	5	6
Remarkable Observations	2	1	1	2	2	1	2	1	1	1	4	4
Granuloma	1	0	0	0	0	0	0	0	0	0	0	0
Histiocytosis	1	1	1	2	2	1	2	1	1	1	4	4
Small Intestine	10	10	8	9	10	10	8	9	9	10	9	10
Not Remarkable	10	10	8	9	10	10	8	9	7	9	9	10
Remarkable Observations	0	0	0	0	0	0	0	0	2	1	0	0
Vacuolation	0	0	0	0	0	0	0	0	2	1	0	0

Group	1		7		8		10	
Dose(mg/kg)	0		2		2/Vit		20/Vit	
Sex	M	F	M	F	M	F	M	F
Animals On Study	10	10	10	10	10	10	10	10
Animals Logged	10	10	9	9	9	10	8	8
Liver	10	10	9	9	9	10	8	8
Not Remarkable	7	6	0	0	0	0	0	0
Remarkable Observations	3	4	9	9	9	10	8	8
Congestion	0	0	0	0	0	1	0	0
Cyst	0	0	1	0	0	0	0	0
Infiltration, Mononuclear Cell	3	4	6	7	5	7	5	7
Inflammation, Subacute	0	0	1	9 b	0	10 b	2	8 b
Necrosis, Single Cell	0	0	3	9 b	2	10 b	1	7 b
Vacuolation	0	0	9 b	9 b	9 b	10 b	8 b	8 b
Lung	10	10	9	9	9	10	8	8
Not Remarkable	8	9	7	2	4	2	0	0
Remarkable Observations	2	1	2	7	5	8	8	8
Granuloma	1	0	0	0	0	0	0	0
Histiocytosis	1	1	2	7 b	5	8 b	8 b	8 b
Small Intestine	10	10	9	9	9	10	8	8
Not Remarkable	10	10	5	0	7	0	0	0
Remarkable Observations	0	0	4	9	2	10	8	8
Vacuolation	0	0	4 a	9 b	2	10 b	8 b	8 b

a = $p < 0.05$, b = $p < 0.01$

Special Evaluation:
Liver and small intestine from the control, 2, and 20 mg/kg/d groups with and without vitamin supplementation (5/sex/group) were evaluated by electron microscopy (the 20 mg/kg/d group not receiving vitamin supplementation were not evaluated because of early mortality). EM examination results indicated that deposited lipid in the hepatocytes and epithelial cells of small intestine were multiple, variable-sized, homogenous pale-gray lipid droplets in the cytoplasm.

Lung tissue from 1 male and 1 female from the 20 mg/kg/d group with vitamin supplementation was evaluated by electron microscopy (control animals were not evaluated). Pulmonary histiocytosis was initially observed by light microscopy and was characterized by dense aggregates of large, foamy alveolar macrophages filling alveolar air spaces that were occasionally associated with an increase in size (hypertrophy) and number (hyperplasia) of adjacent Type II cells. Ultrastructurally, the alveolar macrophages were enlarged with abundant cytoplasm packed with neutral lipid droplets, electron-dense multi-laminated osmiophilic structures, electron-lucent vacuoles (attributed to the extraction of the lipid during processing), and cleft-like electron-lucent structures (cholesterol clefts). Free cholesterol cleft-like structures were also observed in the alveolar air spaces, which are likely the same finding as the "alveolar spicules" noted in the rodent carcinogenicity studies. The pathologist concluded that the EM results of the lungs were consistent with pulmonary phospholipidosis.

Toxicokinetics:
On Day 1 and during Weeks 10 and 24 of dosing, C_{max} and $AUC_{0\text{-}24h}$ values of BMS-201038 and metabolites BMS-203215 (M1), BMS-224433 (M2), and BMS-203304 (M3) increased in a dose-proportional manner. Systemic exposures to the parent and metabolites were similar in the presence or absence of vitamin supplements. Exposures to the parent and metabolites were higher in females than in males. A summary of exposure data is shown in the sponsor-generated tables below. Overall, systemic exposures to the four compounds were as follows:
BMS-224433 (M2) > BMS-203304 (M3) > BMS-201038 (parent) > BMS-203215 (M1)

AUC values of the parent drug at scheduled time points:

Dose Group (mg/kg)	Period of Sample Collection	AUC(0-t) Values of BMS-201038 (ng hr/ml)			
		Males		Females	
		Without Vitamins	With Vitamins	Without Vitamins	With Vitamins
0.02	Day 1	a	a	a	a
	Week 10	a	a	25.4	19 8
	Week 24	53.2	6.70	28.1	27.7
0.2	Day 1	24.2	19.2	38.9	20.1
	Week 10	18.2	26.0	96.2	88.1
	Week 24	21.0	22.0	70.9	77.2
2	Day 1	151	104	300	358
	Week 10	147	153	526	783
	Week 24	117	151	584	644
20	Day 1	1388	1233	3524	3322
	Week 10	909	2121	b	5332
	Week 24	b	1200	b	3778

a AUC value not calculated because plasma concentrations of BMS-201038 were below LLQ of 0 25 ng/mL
b All rats in the high dose group that did not receive vitamin supplements died.

A summary of $AUC_{0\text{-}24h}$ values of the parent drug and its metabolites at Week 24 are shown in the sponsor-generated table below. The data are an average of the mean AUC values for both the vitamin-supplemented or unsupplemented rats for each dose level.

	$AUC_{0\text{-}24\ hr}$ (ng x hr/ml)							
	Males				Females			
	0.02 mg/kg	0.2 mg/kg	2 mg/kg	20 mg/kg	0.02 mg/kg	0.2 mg/kg	2 mg/kg	20 mg/kg
BMS-201038	30	22	134	1200	28	74	614	3778
BMS-203215	a	a	13	396	a	a	53	1508
BMS-203304	68	34	331	5680	46	129	1165	29673
BMS-224433	a	63	1186	11603	43	167	2642	26336

a - AUC values could not be calculated as most of the plasma concentrations were below the lower limit of quantitation.

Dosing Solution Analysis:

Dosing formulations were assessed for concentration at Day 1, Week 15, Week 16, and Week 28. The 0.02, 0.2, and 2 mg/kg/d formulations were approximately only 80% of nominal at Week 15. Concentrations were ±10% of nominal for all other time points. The dosing solution was shown to be stable for 7 days when stored under refrigeration.

Six-month oral Investigative Toxicity Study in Rats (Study No. 97027):

Note: this study was submitted to IND 50,820 and reviewed by Dr. Indra Antonipillai (review completed in July 1999). Edits to Dr. Antonipillai's review have been made for clarity and formatting. Study drug BMS-201038 = AEGR-733.

Study number:	97027
Study report location:	Module 4.2.3.7
Conducting laboratory:	Bristol-Myers Squibb, New Brunswick, NJ
Date of study initiation and final report:	May 21, 1997 and Feb 22, 1999
GLP compliance:	Yes
QA Statement:	Yes
Methods:	This study examined the reversibility effects of BMS-201038 (at 0 and 2 mg/kg/day) in a 6-month toxicity study in rats, followed by 3- and 6-month drug-free recovery periods.

Key Study Findings:

- One control and 5 treated animals died during the dosing period due to apparent gavage error. There were no apparent drug-related adverse clinical signs.
- Treatment-related hematology changes included increased neutrophils, lymphocytes, and platelets in females and poikilocytosis in both sexes, which had reversed after 3 months of recovery.
- Clinical chemistry changes included increased serum ALT, ALP, AST (females), cholesterol, and triglycerides (females). Slight increases in mean serum potassium and phosphorus were also noted in females. Clinical chemistry parameters were similar to control values after 3 months of recovery (except for cholesterol).
- At the end of dosing, mean percent liver lipid levels were increased by 71% in males and 353% females compared with controls. Mean total liver lipid levels (g/liver) were increased by 90% in males and 741% in females. Liver lipids were similar to control values after 3 months of recovery, although total liver lipids were still slightly higher in females (↑15%).
- Mild to moderate (males) and marked (females) decreases in liver concentrations of vitamins A and E were noted after 6 months of dosing. Vitamin A levels remained decreased in females by 30% and 18% at the end of 3- and 6-month recovery periods, respectively, while vitamin E levels recovered in both sexes by 3-months.
- After 6 months of dosing, mean liver weights were increased by 11% (males) and 73% (females) compared with controls. Liver weights were similar to controls after 6 months of recovery.
- Liver and small intestinal discoloration was observed in treated females and lung discoloration was observed in treated males and females.
- In liver, hepatocellular lipid vacuolation was observed in nearly all treated animals. Multifocal subacute inflammation was also seen in all treated females; the incidence in males was similar to controls. One treated female also had single cell hepatocyte necrosis. After 3 months of recovery, lipid vacuolation and subacute inflammation was similar to control levels; however, all treated females showed macrophage infiltration after 3 and 6 months of recovery. Using PAS and Oil-Red-O stains,

macrophages stained positive for lipofuscin and/or ceroid pigments, which are derived from oxidized lipids and lipoproteins.

- In lungs, a slight increase in congestion/edema/inflammation was observed for males only. After 6 months of treatment, mild multifocal histiocytosis was observed in 1/11 males and 2/8 females compared with zero control animals. The incidence of histiocytosis in treated animals was also similar after 3 and 6 months of recovery (1 to 2/sex), although the incidence of histiocytosis for the 6-month control recovery group increased (1/sex) compared with earlier time points (0/sex). By transmission electron microscopy, histiocytosis was observed in 1/5 males and 3/5 females compared with 1/4 male and 0/5 female controls. Lung tissue from the 3- and 6-month recovery periods were not assessed by TEM.
- In small intestine, lipid vacuolation was seen in all treated females and in 3/11 treated males. Vacuolation was not observed after the 3- and 6-month drug free periods.
- Females had significantly higher exposures to parent (4X) and metabolites (2X to 5X) compared with males. Liver concentrations of parent were approximately 15-fold higher in females. After the 6-month recovery period, females still had low, but detectable levels of parent compound in the liver. The plasma concentration of metabolite BMS-203304 [M3] was present in 4- to 8-fold higher amounts than the parent drug itself. Metabolite BMS-224433 [M2] was present in plasma at a slightly higher concentration than parent.

Dosing information:

Species/strain:	Rat/Harlem Sprague Dawley (outbred albino)
Dose levels:	0 (vehicle) or 2 mg/kg/d
Number/sex/group (main):	6-8/sex/group
Number/sex/group (recovery):	6-8/sex/group
Duration:	Once daily for 6 months with 3- and 6-month recovery
Route and dosing volume:	Oral (gavage), 1 mL/kg
Age:	Approximately 6 weeks old
Weight:	Males 164-184 g, females 122-145 g
Diet:	Harlan Teklad Certified LM-485 rat/mouse diet #7012 Vitamin supplementation was not employed
Satellite groups for toxicokinetics:	None
Dose selection:	Dose selection was based on a previous 6-month study (#96024) in rats (with and without vitamin supplementation), in which drug-related findings were observed in liver (lipid accumulation, inflammation and single cell necrosis of hepatocytes), small intestine (lipid accumulation) and lungs (histiocytosis). 2 mg/kg/day was chosen to induce similar liver, lung, and small intestine changes as seen in a previous study so that the reversibility of these findings could be evaluated.
Drug, lot #:	BMS-201038 as mesylate salt, Batch # N009A.
Formulation/vehicle:	75% polyethylene glycol (PEG-400) in 25% water

Times at which observations were made:

Clinical signs:	Daily
Body weights:	At the time of allocation to groups, on the day of treatment, and once weekly thereafter.
Hematology:	Prior to the daily dose during Week 26, and during recovery Weeks 13/14 and 25.
Clinical chemistry:	Prior to the daily dose during Week 26 and during recovery Weeks 13/14 and 25.
Ophthalmoscopy:	Prior to the first dose, during Week 26, and during recovery Weeks 13 and 25.
Gross pathology:	At 6 months and Week 13 and 25 of recovery
Organs weighed:	Only liver weights were obtained.
Histopathology:	Premature decedents and at sacrifice, sections of liver, lung, small intestine and gross lesions were examined. Liver sections from selected control, drug treated, and recovery animals were stained with Oil-Red-O and Periodic Acid Schiff (PAS) reagent and hematoxylin to detect lipofuscin and ceroid pigments.
Electron Microscopy:	Lung, 4M and 5F in control group and 5/sex in treated group - 6 month time point only. Leukocytes, 3/sex/group at 2 weeks and 1, 2, 3, 4, 5, and 6 months and at 1, 2, and 3 months of recovery
Toxicokinetics:	Plasma concentrations of the drug and metabolites (M1, M2, and M3) were measured at the end of the 6-month treatment period. Lung and liver samples were also collected after 6 months and at 3 and 6 months recovery, which were sent to Department of Metabolism and PK (Princeton, NJ) for tissue levels of the drug and metabolites.

Results:

Mortality:

In the control group, 3 rats died and 1 was sacrificed in poor condition on Day 25, Day 86, Day 155, and Day 158. In the drug-treated group, 5 males died during the 6-month dosing period, apparently due to dosing accidents.

Clinical Signs:

No drug-related effects were observed.

Body weight:

No drug-related effects were observed.

Ophthalmic Examination:

No drug-related effects were observed.

Hematology:
At the end of the 6-month treatment period, females had minimal to mild increases in total leukocytes (neutrophils 1.95* vs. 1.10 in controls, and lymphocytes 10.4* vs. 7.8) and platelet counts (1006* vs. 927 in controls). An increased incidence of poikilocytosis was observed in both sexes. These parameters were all comparable to controls by the end of 3-month recovery period.

Biochemistry:
At the end of the 6-month treatment period, mean serum cholesterol and triglycerides (females only) decreased. Mean serum ALT and ALP increased in both genders and AST, potassium, and phosphorus values were increased for females. At the end of the 3-month recovery period, the values for these parameters were comparable to controls, except cholesterol, which remained lower in females (Table 1).

Table 1. Blood Chemistry Parameters

	6-Months Dosing				3-Month Recovery				6-Month Recovery			
	Males		Females		Males		Females		Males		Females	
Doses	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**
Chol	136	98*	120	44*	158	177	127	152*	179	210	129	145*
TG	114	132	68	21*	127	162	58	65	209	209	97	99
ALT	67	78*	74	98*	103	87	82	69*	109	112	97	98
ALP	171	339*	163	560*	166	164	158	137*	152	171	151	158
AST	83	81	87	155*	132	105	111	91	115	113	123	110
Bilirubin	0.17	0.16	0.16	0.2*	0.19	0.17	0.18	0.19	0.19	0.18	0.19	0.16
Albumin	3.4	3.5	3.8	3.7*	3.4	3.4	3.9	3.7*	3.4	3.3	3.9	3.7*

*P= 0.05-0.01; Doses = mg/kg/day. ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Chol = cholesterol; TG = triglyceride.

Tissue Biochemistry:
Liver lipids: At the end of the 6-month treatment period, increases in liver lipids were noted in males (1.12* vs. 0.6 g/liver in controls) and females (2.69* vs. 0.32 g/liver in controls). Liver lipid levels reversed to control levels in males after 3 months of recovery, but were still slightly higher in females (0.38* vs 0.33 g/liver in controls). Liver lipid levels were not assessed after 6 months of recovery. (*p=0.05-0.01)

Liver Vitamin A and E levels: Liver vitamin A and E levels were decreased after the 6-month treatment period. Vitamin A levels were still decreased in females by 30% and 18% after the 3- and 6-month recovery periods, respectively, while vitamin E levels were similar to controls in both sexes after 3-months of recovery (Table 2).

Table 2. Liver Vitamin E and A Levels

	6-Months Dosing				3-Month Recovery				6-Month Recovery			
	Males		Females		Males		Females		Males		Females	
Doses	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**	**0**	**2mg**
Vitamin A	1413	1079*	2260	587*	1710	1763	2780	1940*	2010	2383	4050	3310*
Vitamin E	29	14*	54	6*	33	34	70	64	30	29	84	80

*p= 0.05-0.01; Doses = mg/kg/day.

Organ Weights:
Mean liver weights were increased in males (11%) and females (73%) compared with control values. Male liver weights were similar to control values after 3 months of recovery, but female liver weights were still slightly increased (7.07* vs. 6.12 in controls). Liver weights were similar to control values in both sexes after 6 months of recovery.

Gross pathology:
Liver discoloration (7/8 F); lung discoloration (minimal multifocal white in 1/11 M, 3/8 F); mottled lungs (2/11 M); and pale discoloration in the small intestine (3/8 F) were observed after 6-months of drug treatment. The lung findings were still observed after 3 months of recovery (1/7 M, 2/8 F) and after 6-months of recovery (1/6 M and 1/8 F).

Histopathology:
A summary of microscopic findings observed after 6 months of treatment and after 3 and 6 months of recovery is shown below. In liver, nearly all treated animals had hepatocellular lipid vacuolation. All females also had multifocal subacute inflammation and one female had single cell necrosis. After 3 months of recovery, lipid vacuolation had nearly reversed, while the incidence of inflammation was slightly higher than controls. Mild multifocal aggregates of macrophages distended with yellowish-brown foamy material were distributed predominantly in or around portal areas in all treated females after both the 3- and 6-month recovery periods. By PAS and Oil-Red-O stain the foamy material was shown to be lipofuscin and/or ceroid pigments, which are derived from oxidized lipids and lipoproteins. It was hypothesized that the lipofuscin/ ceroid pigments within the macrophages derived from the phagocytosis of the byproducts from metabolism of excess lipid in the liver.

In lungs, mild, multifocal histiocytosis was observed in 1/11 males and 2/8 females compared with 0/17 control animals. Histiocytosis was defined as focal aggregates of large foamy alveolar macrophages that contained residual/lamellar inclusion bodies, lipid droplets and phagolysosomes that varied in size, shape, and electron density. Occasionally adjacent type II cells were slightly enlarged and contained lipid droplets as well as a qualitative increase in the size or amount of lamellar inclusion bodies. Treated males also had a slightly higher incidence of congestion, edema, and/or inflammation compared with controls. After the 3-month recovery period, histiocytosis was observed in 2/15 treated animals compared with 0/16 controls, and was still slightly higher after 6 months of recovery (3/14 vs. 2/15 in controls).

Minimal to mild (males) and mild to moderate (females) lipid vacuolation was observed in small intestine after 6 months of treatment. This effect was not seen after the 3- and 6-month recovery periods.

Liver

After 6-months of drug treatment:	**Doses (0 and 2 mg/kg/day)**	
	Males	**Females**
Hepatocellular Lipid vacuolation[a]	0/9, 10/11	0/8, 8/ 8
Multifocal subacute inflammation[b]	2/9, 0/11	1/8, 8/8*
Single cell necrosis of hepatocytes	0/9, 0/11	0/8, 1/8
After 3-months of drug free recovery period		
Hepatocellular Lipid vacuolation[a]	0/7, 0/7	0/9, 1/8
Multifocal subacute inflammation[b]	4/7, 6/7	4/9, 6/8
Infiltration of macrophage	0/7, 0/7	0/0, 8/8*
After 6-months of drug free recovery period		
Multifocal subacute inflammation[b]	2/8, 2/6	1/7, 2/8
Infiltration of macrophage	0/8, 0/6	0/7, 8/8*

Lung

After 6-months of drug treatment:	**Doses (0 and 2 mg/kg/day)**	
	Males	**Females**
Congestion/edema/inflammation	1-2/9, 4-5/11	0/8, 0/ 8
Histiocytosis[c]	0/9, 1/11	0/8, 2/8
Histiocytosis by electron microscopy	1/4, 1/5	0/5, 3/5
After 3-months of drug free recovery period		
Inflammation (subacute)	2/7, 1/7	0/9, 0/8
Histiocytosis	0/7, 1/7	0/9, 1/8
After 6-months of drug free recovery period		
Inflammation (subacute)	0/8, 1/6	4/7, 1/8
Histiocytosis	1/8, 1/6	1/7, 2/8

Small intestine

After 6-months of drug treatment:	**Doses (0 and 2 mg/kg/day)**	
	Males	**Females**
Vacuolation[d]	0/9, 3/11	0/8, 8/8*

[a]Minimal in males, moderate to marked in females.
[b]Multifocal subacute inflammation with mononuclear cells.
[c]Mild multifocal histiocytosis.
[d]Lipid vacuolation of absorptive epithelial cells in the jejunum and duodenum, minimal to mild in males, mild to moderate in females.
*p<0.05-0.01

No ultrastructural differences in characteristics of peripheral lymphocytes and neutrophils were observed between control and drug-treated animals. In lungs, pulmonary histiocytosis was observed in 1/4 control male and 0/5 control female rats. By light microscopy the histiocytosis in the control animal was characterized by a focal aggregation of foamy alveolar macrophages that contained toluidine blue-stained cytoplasmic inclusions. Ultrastructurally, the cytoplasmic inclusions consisted of residual bodies and phagolysosomes that varied in size, shape, and electron density. Alveolar macrophages in nonremarkable control animals contained an irregularly-shaped nucleus, several phagolysosomes, lipofuscin, residual bodies, mitochondria, lipid droplets, and a few lamellar inclusion bodies.

In BMS-201038-treated animals, 1/5 males and 3/5 females had histiocytosis with focal aggregates of large foamy alveolar macrophages. Ultrastructurally, enlarged alveolar macrophages contained residual bodies, lipid droplets, numerous lamellar inclusion bodies, and many phagolysosomes that varied in size, shape and electron density. Occasionally adjacent type II cells were slightly enlarged and contained lipid droplets and a qualitative increase in the size and/or amount of lamellar inclusion bodies. Electron microscopy of the lung was not conducted for the 3 and 6-month recovery rats.

Toxicokinetics: As shown in the Table 3 below, females achieved a higher exposure of parent and metabolites than males. The two metabolites in plasma (BMS-203304, BMS-224433) were present in higher amounts than the drug itself.

Table 3. Plasma and tissue (liver and lung) concentration of BMS-201038 and its three metabolites (BMS-203215 [M1], BMS-224433 [M2], and BMS-203304 [M3])

Concentration	plasma (ng/mL)	Liver (ng/g)			Lung (ng/g)		
Duration	6-month	6-month	3-month recovery	6-month recovery	6-month	3-month recovery	6-month recovery
1. BMS-201038							
Males	5	817	BLQ	BLQ	782	BLQ	BLQ
Females	22	12579	197	101	7873	BLQ	BLQ
2. BMS-203215							
Males	0.2	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Females	0.8	BLQ	BLQ	BLQ	23	BLQ	BLQ
3. BMS-203304							
Males	7	17	BLQ	BLQ	BLQ	BLQ	BLQ
Females	39	153	BLQ	BLQ	40	BLQ	BLQ
4. BMS-224433							
Males	43	153	BLQ	BLQ	77	BLQ	BLQ
Females	83	147	BLQ	BLQ	118	BLQ	BLQ

BLQ = below limit of quantitation or not determined.

Study title: Six-month oral toxicity study in dogs

Study no.:	96025
Study report location:	Module 4.2.3.2
Conducting laboratory:	Bristol-Myers Squibb Pharmaceutical Research Institute Departments of Toxicology and Pathology New Brunswick, New Jersey, USA
Date of study initiation:	24 July 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038 (mesylate salt) Batch #R006A - 85.3% pure on an "as is" basis expressed as the free base Batch #N009A - 85.0% pure on an "as is" basis expressed as the free base Batch #40983-012-32 - 82.5% pure on an "as is" basis expressed as the free base

Key Study Findings

- There were no mortalities or adverse clinical signs.
- Final body weights were 15% less than controls for animals receiving 1 mg/kg/d plus vitamins (genders combined) and 14% to 19% less for the high-dose group without and with vitamins, respectively. This was consistent with half of the high-dose animals appearing thin and decreases in food consumption between Weeks 2 to 6 without vitamins and Weeks 2 to 18 with vitamins.
- Mean heart rates were approximately 20% to 30% lower for the high-dose animals compared with baseline and control values. Mean blood pressure values for high-dose animals were decreased by up to 40% compared with baseline values and up to 50% compared with control values, with the effects being more noteworthy for animals receiving vitamin supplementation.
- Mean erythrocyte parameters were decreased and mean leukocyte and neutrophils counts were increased for animals receiving ≥1 mg/kg/d with vitamins and at 10 mg/kg/d without vitamins. Platelets and fibrinogen were increased at ≥1 mg/kg/d.
- Mean cholesterol levels were decreased at ≥1 mg/kg/d and mean triglyceride levels were decreased at 10 mg/kg/d. Mean ALT levels were increased (~3-4X) at 10 mg/kg/d and mean AST levels were increased (~2X or less) at ≥1 mg/kg/d.
- Tan discoloration of the small intestine was noted at ≥0.1 mg/kg/d.
- Increased liver weights were observed at ≥1 mg/kg/d.
- Microscopic effects were noted in the liver (minimal deposition) at ≥1 mg/kg/d; small intestine (minimal to moderate vacuolation) at ≥0.01 mg/kg/d; lymph node (minimal to mild vacuolation) at ≥1 mg/kg/d; and testes (mild to moderate degeneration) at 10 mg/kg/d. In lung, minimal to mild histiocytosis, inflammation, and/or edema were noted for a few animals across all groups, including male controls. Because the incidence and severity did not increase with dose level and similar findings were observed in a few control animals, the findings do not appear to be drug related.

- Ultrastructurally, hepatocellular lipid deposition was characterized by multiple, variabley sized, homogeneous pale-gray lipid droplets in the cytoplasm. Small intestinal lipid distribution in the absorptive epithelial cells was characterized as multiple, variabley sized, homogeneous pale-gray lipid droplets that were partially enveloped by endoplasmic reticulum.
- The amount of total lipids was slightly increased at all dose levels was increased at all dose levels, ranging from 7% to 31% compared with controls.
- Exposures between vitamin- and non-vitamin-treated dogs receiving equivalent doses of BMS-201038 were similar. Exposure to parent and metabolites increased in a dose-related manner. Systemic exposures to each of the 4 analytes were as follows: BMS-201038 > BMS-203215 (M1) > BMS-203304 (M3) > BMS-224433 (M2). No gender-related differences in exposure were noted. The concentration of BMS-201038 in liver and lung were several hundred-fold higher than the concentrations in plasma.
- There did not appear to be noteworthy differences in the toxicity profile between groups that received vitamin supplementation and those that did not.

Methods

Species/Strain: Dog/ Beagle

Dose groups: See sponsor-generated table below:

Group Number	Daily Dose		BMS-201038 Concentration (mg/ml)	Vitamin Supplement[b]	Number of Dogs
	BMS-201038 (mg/kg)	Dose Volume[a] (ml/kg)			
1 (Control)	0 (Vehicle)	0.5	0	No	2M, 2F
2 (Control)	0 (Vehicle)	0.5	0	Yes	2M, 2F
3	0.01	0.5	0.02	No	2M, 2F
4	0.01	0.5	0.02	Yes	2M, 2F
5	0.1	0.5	0.2	No	2M, 2F
6	0.1	0.5	0.2	Yes	2M, 2F
7	1	0.5	2	No	2M, 2F
8	1	0.5	2	Yes	2M, 2F
9	10	0.5	20	No	2M, 2F
10	10	0.5	20	Yes	2M, 2F

a - Control dogs were given the same dose volume of the vehicle (75% PEG-400/25% water) in the same number and size gelatin capsules as dogs receiving BMS-201038.

b - 70,000 IU of vitamin A, 10,500 IU of vitamin D, 86 IU of vitamin E, and 0.6 mg of vitamin K were administered subcutaneously.

Frequency of dosing:	Once daily
Route of administration:	Orally in gelatin capsules
Formulation/Vehicle:	75% PEG-400, 25% water
Age:	7 to 12 months
Weight:	7.2 to 13.6 kg
Satellite groups:	None
Unique study design:	Half of animals/group received vitamin supplementation Liver lipids were measured Electron microscopy on liver and small intestine
Protocol deviations:	There were no protocol deviations that impacted the integrity of the study results.

Observations

Mortality and clinical signs:	Two to three times daily.
Body weights:	Once weekly.
Food consumption:	Estimated daily.
Ophthalmoscopy:	Once pretest and during Week 13 and 24 on all animals.
EKG:	Once pretest and once during Weeks 1, 4, 12, and 24 at 1 to 2 hours postdose on all animals.
Arterial blood pressure:	Once pretest and once during Weeks 13 and 24 at ~1 hour postdose on all animals.
Hematology:	Collected once pretest and once during Weeks 13 and 26.
Coagulation:	Collected once pretest and once during Weeks 8, 12, 17, 21 and 26.
Clinical chemistry:	Collected once pretest and once during Weeks 13 and 26.
Urinalysis:	24-hour urine collection once pretest and once during Weeks 12 and 25.
Liver lipids:	Total lipids were extracted and weighed from liver samples from 2/sex/group.
Gross pathology:	All animals at necropsy.
Organ weights:	All animals, refer to histopathology inventory table.
Histopathology:	Refer to histopathology inventory table (Liver sections from all animals stained with Oil-Red-O stain [neutral lipid] and from a subset of animals with Gomori's stain [iron]; mesenteric lymph nodes from selected animals stained with Oil-Red-O and Luxol fast blue [phospholipid]).
Adequate Battery:	Yes
Peer review:	Yes, all tissues from 2 dogs/sex for control (Groups 1 and 2) and all high-dose animals (Groups 9 and 10) were peer reviewed by the sponsor's pathologist. Additionally, sections of liver, lung, small intestine, testes, and mesenteric lymph node from all animals were reviewed.
Electron microscopy:	Control and high-dose groups only, with and without vitamins
Toxicokinetics:	Samples collected at 0.5, 1, 3, 6, and 24 hours post-dose on Day 1 and during Week 14 and 27 for all groups.

Results

Mortality:

There were no unscheduled deaths.

Clinical Signs:

One male and one female dog receiving 10 mg/kg/d and one male and female receiving 10 mg/kg/d plus vitamins were noted as having a thin body condition and decreased muscle mass (3 of the 4 animals) at the Week 13 and 24 physical exams.

Body Weights:

Mean Gender-Combined Body Weights

Dose (mg/kg/d)	0		0.01		0.1		1		10	
	-V	+V	-V	+V	-V	+V	-V	+V	-V	+V
Final Weight (kg)	10.7	11.0	10.7	10.3	10.7	10.8	10.1	9.3	9.2	8.9
Difference from control (kg)			0	-0.7	0	-0.2	-0.6	-1.7	-1.5	-2.1
% difference from control			-	↓6%	-	↓2%	↓6%	↓15%	↓14%	↓19%

V = vitamins.

Feed Consumption:

Compared with each respective control group, mean gender-combined food consumption was lower between Weeks 2 and 6 for the 10 mg/kg/d groups without vitamins and between Weeks 2 and 18 for the 10 mg/kg/d group with vitamins.

Ophthalmoscopy:

No drug-related ophthalmoscopic findings were noted.

ECG, heart rate, and blood pressure:

Heart rates were decreased for combined HD males and females at Weeks 12 and 24 compared with baseline (↓21 to 30%) and control values (↓21% to 34% [Week 24]). Systolic arterial blood pressure was decreased for combined HD males and females at Weeks 12 and 24 compared with baseline (↓20 to 43%) and control values (↓14% to 49%). Diastolic arterial blood pressure was decreased for combined HD males and females at Weeks 12 and 24 compared with baseline (↓18 to 38%) and control values (↓8% to 51%). Decreases for mean arterial pressures were similar. Effects on blood pressure were slightly more noteworthy for the group that received supplemental vitamins. There were no effects on body temperature.

Hematology:

Mean Gender-Combined Hematology Findings

Dose (mg/kg/d)	0		0.01		0.1		1		10	
	-V	+V	-V	+V	-V	+V	-V	+V	-V	+V
Poikilocytosis -Week 26	neg	neg	neg	slight	neg	mod	slight-mrkd	slight-mrkd	mod-mrkd	mod-mrkd
Anisocytosis -Week 26	neg	neg	neg	neg	neg	neg	neg	neg	slight	slight
Erythrocytes (10^6/mm^3) -Pretest	6.9	7.3	7.4	7.8	7.4	7.7	7.3	7.3	7.3	7.3
-Week 26	6.7	7.3	7.4	7.5	7.1	7.5	6.4	**5.6†**	**5.0†**	**5.4†**
Hemoglobin (g/dL) -Pretest	16.0	16.7	16.6	17.4	16.8	17.2	16.6	16.6	16.4	16.4
-Week 26	15.7	16.7	16.6	16.9	16.3	17.1	15.6	**13.9**	**12.5†**	**12.8†**
Hematocrit (%) -Pretest	46	48	48	51	50	51	48	48	48	48
-Week 26	47	50	50	51	48	51	45	**40†**	**37†**	**38†**
Leukocytes (10^3/mm^3) -Pretest	12.9	11.4	10.8	11.9	11.5	10.1	10.7	10.5	10.5	11.1
-Week 26	12.9	10.4	11.5	11.6	9.3	10.3	13.5	**15.8**	**12.6**	**16.6**
Neutrophils (10^3/mm^3) -Pretest	7.51	6.30	-	6.17	-	4.41†	-	5.70	-	6.14
-Week 26	7.71	6.46	-	7.04	-	6.48	-	**11.39**	-	**11.78**
Platelets (10^3/mm^3) -Pretest	296	245	264	266	299	280	262	291	281	250
-Week 26	329	238	297	263	333	246	**415**	**471**	**466**	**508†**
Fibrinogen (mg/dL) -Pretest	141	147	127	118	130	116	122	145	118	127
-Week 26	141	152	132	156	138	175	161	**196**	**188**	**247†**

*p<0.05; †p<0.01; V = vitamins. "-" = no meaningful difference from control or pretest value; mod = moderate; mrkd = marked; neg = negative.

Clinical Chemistry:

In addition to the parameters listed below, slight, but statistically significant decreases in mean serum total protein, albumin, and calcium were noted at 1 mg/kg/d with vitamins and 10 mg/kg/d with or without vitamins.

Mean Gender-Combined Clinical Chemistry Findings

Dose (mg/kg/d)	0		0.01		0.1		1		10	
	-V	+V	-V	+V	-V	+V	-V	+V	-V	+V
Cholesterol (mg/dL) -pretest	145	182	133	170	139	153	133	176	163	176
-Week 26	148	182	132	169	118	124	**44†**	**54†**	**24†**	**34†**
Triglycerides (mg/dL) -pretest	39	50	32	45	58	43	41	51	40	47
-Week 26	57	71	39	46	41	40	58	70	**9†**	**6†**
ALT (U/L) -pretest	32	31	33	40	39	38	36	42	50	46
-Week 26	40	30	33	43	41	38	41	35	**117**	**133**
AST (U/L) -pretest	34	26	31	30	34	30	34	30	32	30
-Week 26	35	26	30	25	40	34	**53**	**51**	**47**	**57**

ALT = alanine aminotransferase; AST = aspartate aminotransferase; V = vitamins.

Urinalysis:
The 1 and 10 mg/kg/d groups consumed slightly more water than at pretest or compared with controls at Week 25, which resulted in slightly higher urine output. Small, but statistically significant decreases in urine pH were noted at ≥1 mg/kg/d.

Gross Pathology:
Tan discoloration of the small intestine was noted at ≥0.1 mg/kg/d. At these dose levels, two animals per sex per group were noted with this finding, except for the 0.1 mg/kg/d male group not receiving vitamins (1 animal) and female group receiving vitamins (0 animals).

Organ Weights:
Mean Organ Weight Changes for Males

Dose (mg/kg/d)	0		0.01		0.1		1		10	
	-V	+V	-V	+V	-V	+V	-V	+V	-V	+V
Liver (g)	377.1	357.4	353.6	347.8	331.0	365.8	401.6	375.0	**433.7 ↑15%**	**446.3 ↑25%**
Relative to BW	3.718	3.206	3.023	3.133	3.090	3.249	3.855	**3.912 ↑22%**	**4.587 ↑23%**	**4.738 ↑48%**

BW = body weight; V = vitamins.

Mean Organ Weight Changes for Females

Dose (mg/kg/d)	0		0.01		0.1		1		10	
	-V	+V	-V	+V	-V	+V	-V	+V	-V	+V
Liver (g)	327.7	361.5	347.4	279.0	340.4	360.3	**361.8 ↑10%**	344.7	**413.4 ↑26%**	**415.7 ↑15%**
Relative to BW	3.065	3.391	3.440	2.908	3.101	3.393	**3.549 ↑16%**	**3.771 ↑11%**	**4.588 ↑50%**	**4.757 ↑40%**

BW = body weight; V = vitamins.

Histopathology:

Histopathology Findings for Males

Dose (mg/kg/d)	0		0.01		0.1		1		10	
	-V	+V	-V	+V	-V	+V	-V	+V	-V	+V
N	2	2	2	2	2	2	2	2	2	2
Liver - deposition (minimal)	-	-	-	-	-	-	1	1	2	2
Small intestine - vacuolation (minimal)	-	-	-	1	1	1	-	-	-	-
(mild)	-	-	-	-	1	1	-	1	-	1
(moderate)	-	-	-	-	-	-	2	1	2	1
Lung										
Histiocytosis (minimal)	1	1	1	-	-	1	-	-	2	-
Inflammation, chronic (minimal)	1	-	-	-	-	1	-	-	-	1
(mild)	-	-	-	1	-	-	-	1	-	-
Edema (minimal)	-	1	-	-	-	-	-	-	-	-
Lymph node - vacuolation (minimal)	-	-	-	-	-	-	-	1	-	-
(mild)	-	-	-	-	-	-	-	-	1	2
Tongue - inflammation, granulomatous (minimal)	-	-	1	-	-	-	-	-	-	-
Testes - degeneration (mild)	-	-	-	-	-	-	-	-	-	1
(moderate)	-	-	-	-	-	-	-	-	1	-
Spleen - fibroplasia/ fibrosis (mild)	-	-	-	-	-	-	-	-	1	-

V = vitamins.

Histopathology Findings for Females

Dose (mg/kg/d)	0		0.01		0.1		1		10	
	-V	+V	-V	+V	-V	+V	-V	+V	-V	+V
N	2	2	2	2	2	2	2	2	2	2
Liver - deposition (minimal)	-	-	-	-	-	-	2	2	2	2
Liver - coagulative necrosis (minimal)	-	-	-	-	-	-	-	-	1	-
Small intestine - vacuolation (minimal)	-	-	-	1	1	1	-	-	-	-
(mild)	-	-	-	-	1	-	-	-	1	-
(moderate)	-	-	-	-	-	-	2	2	1	2
Lung										
Histiocytosis (minimal)	-	-	1	1	1	1	-	-	1	1
Inflammation, chronic (minimal)	1	1	1	1	-	1	-	1	2	1
(mild)	-	-	-	-	-	-	1	1	-	-
Edema (minimal)	-	-	-	-	1	1	-	-	1	-
Lymph node - vacuolation (mild)	-	-	-	-	-	-	-	-	1	-
Tongue - inflammation, granulomatous (minimal)	-	-	-	-	-	-	1	1	1	-

V = vitamins.

Electron microscopy:
Liver - All high-dose animals showed lipid deposition in hepatocytes, while none of the control animals had lipid accumulation. Lipid disposition was often observed in centrilobular hepatocytes and was characterized ultrastructurally as multiple, variably sized, homogeneous pale-gray lipid droplets in the cytoplasm. Some droplets were partially to completely electron-lucent, which was attributed to the extraction of the lipid during processing. Hepatocellular mitochondria were nonremarkable and similar in appearance to controls.

Small intestine - All high-dose animals showed lipid deposition in the absorptive epithelial cells (enterocytes) of the small intestinal villi, while none of the control animals had lipid accumulation. Ultrastructurally, the lipid deposition was characterized as multiple, variably sized, homogeneous pale-gray lipid droplets that were partially enveloped by endoplasmic reticulum. The other organelles of the enterocytes, and the goblet cells and lamina propria were nonremarkable, with morphological characteristics similar to controls.

Liver Total Lipids

	Males and Females				
Dose (mg/kg/d)	**0**	**0.01**	**0.1**	**1**	**10**
Concentration (%)	3.6	4.1	4.1	4.1	3.6
Content (g)	11.7	12.5	13.6	15.3*	14.1

*p = 0.05 (Dunnett's test)

Toxicokinetics: (sponsor-generated tables)

Exposure Data for BMS-201038 at Day 1, Week 14, and Week 27

Daily Dose (mg/kg)	Period of Sample Collection	AUC (0-t) Values of BMS-201038 (ng.hr/mL)			
		Males		Females	
		Without Vitamins	With Vitamins	Without Vitamins	With Vitamins
0.01	Day 1	a	a	a	a
	Week 14	11.4	15.8	8.10	2.57
	Week 27	5.15	20.0	68.8	74.8
0.1	Day 1	12.3	5.84	41.1	8.94
	Week 14	41.8	28.3	38.7	15.3
	Week 27	60.7	70.6	153	184
1	Day 1	617	246	551	310
	Week 14	630	409	601	555
	Week 27	430	1721	685	1600
10	Day 1	6940	10032	6946	8139
	Week 14	11129	19742	10819	12777
	Week 27	12235	16195	11125	13805

a AUC value not calculated because plasma concentrations of BMS-201038 were below LLQ of 0.2 ng/mL.

Exposure Data for BMS-201038 and Metabolites at Week 27 for Males and Females, With and Without Vitamins, Combined

BMS-201038 Daily Dose (mg/kg)	Week 27 Plasma AUC_{24hr} Values (ngxhr/ml)[a]			
	BMS-201038	BMS-203215	BMS-203304	BMS-224433
0.01	42	b	b	b
0.1	117	32	36	b
1	1109	523	289	93
10	13340	4284	1683	564

a - AUCs for males and females combined.
b - Below the lower limit of quantitation.

Concentrations of BMS-201038, BMS-203215, BMS-203304, and BMS-224433 in Homogenates of Liver and Lungs of Dogs after 6 Month of Treatment

Concentration (ng/g)										
Group No. (Daily Dose)	Sex	Dog No.	BMS-201038		BMS-203215		BMS-203304		BMS-224433	
			Liver	Lung	Liver	Lung	Liver	Lung	Liver	Lung
4 0.01 mg/kg[b]	M	514	335	d	e	e	d	d	d	d
	F	515	274	d	e	e	d	d	d	d
5 0.1 mg/kg[c]	M	518	685	31.7	e	e	d	d	d	d
	F	520	693	55.9	e	e	d	d	d	d
7 1 mg/kg[c]	M	525	286	741	228	242	d	59.2	d	d
	F	528	686	206	148	282	42.8	d	d	d
9 10 mg/kg[c]	M	534	4051	16885	1780	8561	531	73.3	85.1	58.8
	F	535	2055	6563	1767	6534	279	66.5	62.9	34.7
10 10 mg/kg[b]	M	538	4459	9233	1648	2898	383	62.3	71.3	37.9
	F	540	3609	14647	2305	7151	848	81.7	79.2	38.9

a Animals were necropsied approximately 24 hr after the last dose.
b With vitamin supplement.
c No vitamin supplement.
d Below the lower limit of quantitation of 31.5 ng/g.
e Below the lower limit of quantitation of 90.0 ng/g.

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Dosing Solution Analysis:
Dosing formulations were measured for test article concentration on Days 1, 3, 8, 15, and during the third month. Dosing formulations were occasionally below or greater than 10% of the nominal concentration, especially during the first 8 days. These deviations were not judged to have a significant effect on the interpretability of the study results.

Study title: One-year oral toxicity study in dogs

Study no.:	97057
Study report location:	Module 4.2.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	27 November 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038, Batch #N009A, 86.2% pure (as is)
Note:	Review of this study was initially conducted by Dr. Dylan Yao and submitted to DARRTS on 18 January 2007. Edits to Dr. Yao's review have been made for clarity and formatting.

Key Study Findings

- There were no mortalities. Clinical signs including clear ocular discharge, diarrhea, and hypoactivity and/or decreased activity were noted at all three dose levels.
- Animals receiving 5 mg/kg/d initially showed body weight loss for up the first 7 weeks followed by decreased body weight gain. Mean final body weights for the 5 mg/kg/d group were lower after 1 year of dosing (↓14% in males and 13% in females).
- The 5 mg/kg/d group males showed decreased food consumption for the first 2-3 months, after which time food consumption was similar to or greater than controls.
- Decreased erythrocyte parameters were observed at Weeks 25 and 52 for males receiving ≥0.5 mg/kg/d and females receiving 5 mg/kg/d, with a correlating increase in reticulocytes for the 5 mg/kg/d animals. An increase in mean platelets was observed for all male dose levels and the 5 mg/kg/d females, with statistically significant differences occurring only for the 5 mg/kg/d males. Mean APTT was slightly increased at Week 52 for animals receiving 5 mg/kg/d.
- Decreases in mean total protein and globulin were observed at ≥0.5 mg/kg/d and decreased mean albumin was noted at 5 mg/kg/d. Mean cholesterol was decreased for all male dose levels and at ≥0.5 mg/kg/d for females. Mean triglyceride was decreased for all male dose levels and at 5 mg/kg/d for females. Females receiving ≥0.5 mg/kg/d had a slight increase (<2X) in mean AST. Animals receiving 5 mg/kg/d had a slight but statistically significant decrease in calcium at Week 52.
- Urine pH was decreased for animals receiving ≥0.5 mg/kg/d.
- No drug-related changes in EKG or blood pressure parameters were noted at Week 25 or Week 51.
- Increased mean liver weights were observed at all female doses and for males receiving ≥0.5 mg/kg/d, with statistical significance occurring at 5 mg/kg/d. Decreases in mean thyroid/parathyroid weight were seen for 5 mg/kg/d males.
- Liver lipid levels were similar between treated and control groups.
- Tan discoloration of the small intestine was observed at all dose levels. Tan discoloration was also noted in the lungs of two HD males that showed microscopic findings in the lungs and one HD female had lung adhesions.
- Microscopic findings included minimal to moderate epithelial vacuolation of the small intestine (duodenum, ileum and jejunum) at ≥0.5 mg/kg/d, minimal mesenteric lymph

node macrophage vacuolation at 5 mg/kg/d, and occasional minimal to mild focal mineralization, chronic active inflammation, and/or alveolar edema in the lung at 5 mg/kg/d. A low incidence of minimal to mild pleural fibrosis in the lung and minimal peracute hemorrhage in the mesenteric lymph node was also observed at ≥0.5 mg/kg/d. There were no microscopic findings in the liver.

- TK results showed a dose-related increase in exposure with parent and 3 metabolites.

Methods

Species/Strain: Dog/Beagle

Design:

Group Number	Daily Dose BMS-201038 (mg/kg)	Volume (ml/kg)	Concentration BMS-201038 (mg/ml)	Number Of Animals Males	Females
1	0	0.5	0	4	4
2	0.05	0.5	0.1	4	4
3	0.5	0.5	1	4	4
4	5	0.5	10	4	4

Frequency of dosing: Once daily
Route of administration: Orally fed via capsules (size 11 and 12)
Dose volume: Volume of drug plus vehicle in capsule or amount of water used to gavage the capsule was not specified in the report
Formulation/Vehicle: 75% polyethylene glycol (PEG)-400, 25% water
Age: 5.5 months
Weight: 6.2 to 7.7 kg (males) and 5.4 to 7.1 kg (females)
Unique study design: Liver lipids were measured
Protocol deviations: There were no protocol deviations that impacted the integrity of the study.

Observations

Mortality and clinical signs: Twice daily.
Body weights: Weighed once pretest, then once weekly until the end of dosing.
Food consumption: Recorded once pretest, once daily during entire study; weekly average weight was reported.
Ophthalmoscopy: Once pretest and during week 25 on all animals.

EKG: Once pretest, once during Weeks 25 and 51 at 1 to 2 hours post dosing on all animals. Blood pressure was also measured at the same time points.
Hematology: Collected once pretest, once during Week 25 and 51.
Clinical chemistry: Collected once pretest, once during Week 25 and 51.
Urinalysis: Overnight urine collected with metabolic cage once pretest, and once during Week 25 and 51, within 48 hours of the period during which water consumption was determined.
Gross pathology: All animals at necropsy.
Organ weights: Refer to histopathology inventory table.
Histopathology: All animals; refer to histopathology inventory table (EM samples from the liver, lungs, and small intestine were prepared but EM procedure was not performed as no findings on light microscopic examination were discovered).
Adequate Battery: Yes
Peer review: Yes, all tissues from 2 dogs/sex for control and 4/sex for high dose were peer reviewed by the sponsor's pathologist. Additionally, sections of liver, small intestine, and mesenteric lymph node from all animals were reviewed.

Results

Mortality:
There were no unscheduled deaths.

Clinical Signs:
- Diarrhea and/or occasionally colored feces at all three dose levels.
- Clear ocular discharge at all three dose levels.
- Decreased activity and/or hypoactivity at 5 mg/kg/d.

	Male				Female			
Doses (m/k/d)	**0**	**0.05**	**0.5**	**5**	**0**	**0.05**	**0.5**	**5**
Soft, mucoid feces and/or diarrhea	12/6	52/8	30/7	24/10	18/6	5/3	20/7	31/9
Eye discharge, clear (left and right)	0/0	48/3	9/2	156/7	1/1	2/2	60/6	123/5
Hypoactivity	0/0	0/0	0/0	14/2	0/0	0/0	0/0	1/1
↓ activity	0/0	3/2	0/0	37/4	0/0	1/1	0/0	8/3

(total number of occurrences of the observation/number of animals with at least one observation for that specific clinical sign)

Body Weights:
Treatment resulted in slight body weight loss for males and females receiving 5 mg/kg/d for the first 2 to 3 weeks of treatment. This resulted in statistically significantly lower body weights for males compared with controls at several time points during the study. By the end of the study, high-dose groups had lower mean body weights, but the difference from controls was not great enough to be statistically significant.

Final Body Weights at Week 52

Dose (mg/kg/d)	0		0.05		0.5		5	
Sex	M	F	M	F	M	F	M	F
Final weight (kg)	10.6	9.4	10.3	9.2	10.1	9.4	9.1	8.2
Diff from control (kg)			-0.3	-0.2	-0.5	0	-1.5	-1.2
% diff from control			↓3%	↓2%	↓5%	-	↓14%	↓13%

F = female; M = male.

Food Consumption:
Test article-related decreases, sometimes statistically significant, in food consumption occurred in the 5 mg/kg/d male group for the first 2 to 3 months of dosing, which correlated with effects on body weight during this time period. After this period, mean food consumption values for this group increased and generally remained greater than the control group values throughout the remainder of the study.

Ophthalmoscopy:
There were no treatment-related ocular changes.

ECG:
There were no apparent treatment-related changes in ECG intervals or blood pressure at Week 25 or 51.

Hematology:
In addition to the changes in hematology parameters shown below, a dose-related increase in the incidence of abnormal red blood cells (anisocytes, basophilic stippling, crenated cells, poikilocytosis, polychromasia, etc) was observed in all test article-treated groups at Week 25 and 52.

Doses (m/k/d)	Week	Male				Female			
		0	0.05	0.5	5	0	0.05	0.5	5
RBC	25	6.96	6.87	6.20	5.18**	6.27	6.76	6.68	5.44
(mil/µL)	52	7.15	6.98	6.56	5.15**	6.25	6.47	6.62	5.29
HGB	25	16.3	16.3	14.7*	12.5**	15.3	16.6	15.6	13.2
(g/dL)	52	16.5	16.2	15.0	12.2**	14.6	15.5	15.3	12.7*
HCT	25	46.8	46.6	41.1*	36.2**	42.4	46.4	44.3	38.2
(%)	52	48.7	47.7	44.3	36.9**	42.6	44.3	44.7	38.0
MCV	25	67.2	67.8	66.4	70.0	67.7	68.6	66.3	70.5
(femtoliters)	52	68.1	68.3	67.7	71.7*	68.2	68.7	67.7	71.8
MCH	25	23.4	23.7	23.7	24.1	24.4	24.6	23.3	24.3
(picograms)	52	23.1	23.2	22.9	23.8	23.4	24.0	23.1	24.0
PLT	25	284	335	334	498**	366	331	319	472
(thous/µL)	52	282	314	357	449**	362	350	349	435
APTT	25	13.0	13.1	12.7	12.3	14.0	12.6	13.6	12.6
(sec.)	52	11.8	11.7	11.9	12.4*	11.9	11.6	12.2	12.5
Reticulocyte	25	0.7	1.1	0.7	1.1	0.5	0.7	0.7	1.7**
(%)	52	0.9	0.7	0.9	1.3	0.3	0.3	0.3	1.7**

*p<0.05; **p<0.01; APPT = activated partial thromboplastin time; HCT = hematocrit; HGB = hemoglobin; MCH = mean cell hemoglobin; MCV = mean cell volume; PLT = platelet; RBC = red blood cell.

Clinical Chemistry:
Dose-related, statistically significant decreases in total protein, albumin and globulin, cholesterol, triglyceride, and calcium were noted at the mid dose and/or high dose; Minimal increases in AST were noted for the mid- and high-dose group females.

Doses (m/k/d)	Week	Male				Female			
		0	0.05	0.5	5	0	0.05	0.5	5
Albumin, g/dL	25	3.6	3.5	3.2	3.1	3.6	3.5	3.4	3.1*
	52	3.6	3.4	3.2	2.9*	3.4	3.4	3.3	2.8**
Total protein, g/dL	25	6.0	5.8	5.2*	4.9*	5.7	5.7	5.2	4.8**
	52	6.3	5.7	5.3*	4.7*	5.7	5.8	5.2*	4.5**
Globulin, g/dL	25	2.4	2.3	2.0	1.8*	2.1	2.1	1.8*	1.7**
	52	2.7	2.3	2.1**	1.9*	2.3	2.4	1.9	1.7**
AST, U/L	25	28	34	34	38	30	30	48*	42
	52	36	36	44	45	28	31	51**	44*
CHOL, Mg/dL	25	157	123*	90**	26**	164	188	67**	32**
	52	166	114**	70**	25**	176	228	75*	30**
TRIG, Mg/dL	25	45	30	65	10*	45	48	71	12*
	52	50	31*	32*	6**	53	47	46	7*
Calcium, Mg/dL	25	10.4	10.1	10.1	9.7*	10.2	10.2	10.3	9.8
	52	10.1	9.7	9.7	9.2	9.9	10.0	9.8	9.3*

*p<0.05; **p<0.01; AST = aspartate aminotransferase; CHOL = cholesterol; TRIG = triglyceride.

Urinalysis:
Mean pH was minimally but statistically significantly decreased in mid- and high-dose males (at week 25) and at MD females (at week 52).

Doses (m/k/d)	Week	Male				Female			
		0	0.05	0.5	5	0	0.05	0.5	5
Mean urine pH	25	7.1	7.3	6.1*	5.9*	7.1	6.6	6.5	6.1
	52	7.0	8.4	6.4	6.0	7.3	6.8	6.3	5.9*

Gross Pathology:
Tan discoloration of the small intestine was noted in 1/4 low-dose males and all mid- and high-dose groups. Tan discoloration was noted in the lungs for the two high-dose males that showed microscopic findings in the lungs. One high-dose female had lung adhesions.

Organ Weights:
Dose-related increases in liver weight (absolute in g and relative to body weight in %) in high-dose males and females; the mean absolute thyroid/parathyroid weight in high-dose males was lower, but not for relative weight.

Doses (m/k/d)	Abs. or rel. wt	Male				Female			
		0	0.05	0.5	5	0	0.05	0.5	5
Liver	Abs.	306.94	271.33	330.31	402.08**	286.25	308.82	308.02	322.79
	Rel. %	2.962	2.652	3.257	4.443**	3.053	3.313	3.281	3.870**
Thyroid/parathyroid	Abs.	1.0829	0.8763	0.9463	0.7307**	0.8986	1.0358	1.0307	0.8491
	Rel. %	0.011	0.009	0.009	0.008	0.010	0.012	0.011	0.010

**p<0.01.

Histopathology:
Cytoplasmic vacuolation in the epical epithelium of the villi of the small intestine (duodenum, jejunum, and/or ileum) was the major microscopic finding, which was dose-related in incidence and severity (see table below), and occasionally correlated with the gross discoloration of the intestine at necropsy. In the lung, minimal to mild pleural fibrosis was observed in 1 or 2 animals per group at ≥0.5 mg/kg/d. Chronic-active inflammation, mineralization, and alveolar edema were also noted at low incidence and severity at 5 mg/kg/d. Histiocytosis was not observed at any dose level.

Summary of Microscopic Findings

	Males				Females			
Dose (mg/kg/d)	**0**	**0.05**	**0.5**	**5**	**0**	**0.05**	**0.5**	**5**
N	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Duodenum								
Vacuoles, epithelium, Total	0	0	1	3	0	0	1	3
-Minimal	-	-	1	-	-	-	1	1
-Mild	-	-	-	3	-	-	-	1
-Moderate	-	-	-	-	-	-	-	1
Ileum								
Vacuoles, epithelium, Total	0	0	1	3	0	0	0	2
-Minimal	-	-	-	1	-	-	-	-
-Mild	-	-	1	2	-	-	-	2
Jejunum								
Vacuoles, epithelium, Total	0	0	3	4	0	0	2	4
-Minimal	-	-	1	-	-	-	-	-
-Mild	-	-	1	-	-	-	1	1
-Moderate	-	-	1	4	-	-	1	3
Lung								
Mineralization, focal, Total	0	0	0	1	0	0	0	0
-Minimal	-	-	-	1	-	-	-	-
Fibrosis, pleural, Total	0	0	2	1	0	0	1	1
-Minimal	-	-	2	-	-	-	1	-
-Mild	-	-	-	1	-	-	-	1
Inflammation, chronic-active, Total	0	0	0	1	0	0	0	0
-Mild	-	-	-	1	-	-	-	-
Edema, alveolar, Total	0	0	0	0	0	0	0	2
-Minimal	-	-	-	-	-	-	-	1
-Mild	-	-	-	-	-	-	-	1
Lymph node (mesenteric)								
Hemorrhage, peracute, Total	0	0	0	1	0	1	1	0
-Minimal	-	-	-	1	-	1	1	-
Vacuolation, macrophages, Total	0	0	0	1	0	0	0	2
-Minimal	-	-	-	1	-	-	-	-
-Mild	-	-	-	-	-	-	-	2

Special Evaluation:
At necropsy, a fresh liver sample from all dogs was collected for determination of liver lipid levels. The result showed that lipid levels between the control and BMS treated groups were comparable.

Toxicokinetics:
TK measured on Day 344 revealed that exposure to BMS-201038 and its metabolites, BMS-203215 (M1), BMS-203304 (M3), and BMS-224433 (M2), increased in a dose-related manner. TK data for Day 344 are summarized in the sponsor-generated table below.

Dose (mg/kg/day)	Sex	AUC(0-T) [ng.h/mL] (N=4)			
		BMS-201038	BMS-203215	BMS-203304	BMS-224433
0.05	M	14.6	3.72	a	b
	F	7.8	8.02	a	b
0.5	M	246	225	181	22.4[c]
	F	230	170	97.9	a
5	M	4423	2110	740	253
	F	3869	2423	875	349

a The mean AUC value was not reported. All or most plasma concentrations from individual dogs were below LLQ.
b The mean AUC value was not calculated. All plasma concentrations from individual dogs were below LLQ.
c N=1. Most plasma concentrations from the remaining three dogs were below LLQ and their AUC values were not reported.

Mean and individual T_{max} values for BMS-201038, BMS203215, and BMS-224433 ranged between 1 and 6 hours. Individual T_{max} values for BMS-20304 ranged between 0.5 and 24 hours. Mean values ranged between 1 and 3 hours with the exception of low-dose males, which had a mean value of 12.5 hours.

Dosing Solution Analysis

The concentration of BMS-201038 in the dosing formulation was determined on Day 1 and during Months 6, 9, and 12. Concentrations were within 10% of the nominal concentration at all time points.

Histopathology Inventory for NDA #203858

Species	MOUSE	RAT		DOG	
Study Number	96057	96058	96024	96025	97057
Study Duration	3 Months	3 Months	6 Months	6 Months	12 Months
Adrenals	X	X*	X*	X*	X*
Aorta	X	X	X	X	X
Bone Marrow	X	X	X	X	X
Bone (femur - mouse, rat) (rib, femur – dog)	X		X	X†	X
Brain	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymides	X	X	X	X	X
Esophagus	X	X	X	X	X
Eyes	X	X	X	X	X
Gall bladder	X			X	X
Gross lesions	X	X	X	X	X
Harderian gland			X		
Heart	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*
Lachrymal gland	X	X			
Larynx					
Liver	X*	X*	X*	X*	X*
Lungs	X	X*	X	X	X
Lymph nodes, suprapharyngeal					X
Lymph nodes, cervical			X	X	
Lymph nodes, mesenteric	X	X	X	X	X
Mammary Gland	X	X	X	X	
Nasal cavity					
Optic nerves	X	X			X
Ovaries	X*	X*	X*	X*	X*
Pancreas	X	X	X	X	X
Pharynx					
Pituitary	X	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*
Rectum	X	X	X†	X†	X
Salivary gland (submandibular -mouse, rat; submaxillary – dog)	X	X	X	X	X
Sciatic/peripheral nerve	X	X	X	X	X
Seminal vesicles -weighed with prostate	X*	X*	X*		
Skeletal muscle (biceps femoris, - mouse, rat; vastus medialis - dog)	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord (cervical, thoracic, lumbar)	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*
Sternum (with bone marrow)	X	X	X	X	smear
Stomach	X	X	X	X	X
Testes	X*	X*	X*	X*	X*
Thymus	X*	X	X*	X	X
Thyroid + parathyroid	X	X*	X*	X*	X*
Tongue	X	X	X	X	
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus + cervix	X*	X*	X*	X	X
Vagina	X	X	X	X	X

X, histopathology performed; *, organ weight obtained; †tissues collected but not evaluated microscopically

7 Genetic Toxicology

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

Exploratory

Study title: Exploratory Ames reverse-mutation study in Salmonella

Study no.:	95712
Study report location:	Module 4.2.3.3.1
Conducting laboratory:	Bristol-Myers Squibb, Syracuse, NY
Date of study initiation:	03 October 1995
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BMS-201038-01 (AEGR-733), Batch and purity not specified

Methods

Strains:	Salmonella: TA98 and TA100 only
Concentrations:	1, 10, 100, 500, 1000, 2500, and 5000 µg/plate
Basis of concentration selection:	This was a range-finding study
Negative control:	DMSO
Positive controls:	2.5 µg/plate 2-aminoanthracene (+S9); 2 µg/plate 2-nitrofluorene (-S9; TA98); 1 µg/plate sodium azide (-S9; TA100);
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Not indicated

Results

Under the conditions of this study, AEGR-733 (BMS-201038) was not mutagenic in the Salmonella tester strains TA98 and TA100 when tested to cytotoxic levels, with or without metabolic activation.

SUMMARY DATA

Table 1
Summary
Mean Histidine+ Revertant Counts from the Exploratory Study on BMS-201038

In the Presence[1] and Absence[2] of Metabolic Activation

Test Article	Dose Level (µg/plate)	TA 98[1] Mean ± SD	TA 100[1] Mean ± SD	TA 98[2] Mean ± SD	TA 100[2] Mean ± SD
DMSO	100 µl	17 ± 4	82 ± 17	13 ± 2	55 ± 4
BMS-201038	1	21	66	15	52
	10	24	66	14	52
	100	12	63	12	43
	500	4	*	2	*
	1000	*	*	*	*
	2500	*	*	*	*
	5000	*	0	*	0
Sodium azide	1.0				468
2-Nitrofluorene	2.0			686	
2-Aminoanthracene	2.5	717	504		

*Impossible to score plates due to the overgrowth of bacterial background lawn

Table 2

Bacterial Background Lawn Evaluation from the Exploratory Study on BMS-201038

In the Presence[1] and Absence[2] of Metabolic Activation				
BMS-201038[a]	TA98[1]	TA100[1]	TA98[2]	TA100[2]
1	0[b]	0	0	0
10	0	0	0	0
100	0	1	1	2
500	2	3	2	3
1000	3	3	3	3
2500[c]	3	3	3	3
5000[c]	3	4	3	4

[a] Concentrations in terms of μg of bulk BMS-201038/0.1 ml/plate
[b] BMS-201038-treated plates graded on a scale of 0—4 (compared to the negative controls)
[c] Visible test article precipitate was present on the bacterial culture plates for these doses

Grading system:

0 = No reduction of the bacterial background lawn
1 = Slight reduction of the bacterial background lawn
2 = Moderate reduction of the bacterial background lawn
3 = Marked reduction of the bacterial background lawn
4 = Complete annihilation of the bacterial background lawn

Pivotal

Study title: Ames reverse-mutation study in Salmonella and Escherichia coli

Study no.:	96630
Study report location:	Module 4.2.3.3.1
Conducting laboratory:	Bristol-Myers Squibb, Syracuse, NY
Date of study initiation:	11 March 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038-01 (AEGR-733), Batch GK1528, 96.8% purity (free base)

Key Study Findings

- Under the conditions of this assay, AEGR-733 (BMS-201038) was not mutagenic at concentrations up to and including cytotoxic levels with or without metabolic activation.

Methods

Strains:	Salmonella: TA98, TA100, TA1535, TA1537 E. coli: WP2 uvrA
Concentrations in definitive study:	Salmonella: 15.63, 31.25, 62.5, 125, 250 µg/plate E. coli: 31.25, 62.5, 125, 250, 500 µg/plate
Basis of concentration selection:	Observed cytotoxicity in a dose range-finding study in TA100 and E. coli WP2 uvrA.
Negative control:	DMSO
Positive controls:	0.025 mg/mL 2-aminoanthracene (+S9; salmonella strains); 0.1 mg/mL 2-amino-anthracene (+S9; E. coli); 0.02 mg/mL 2-nitrofluorene (-S9; TA98); 0.01 mg/mL sodium azide (-S9; TA100, TA1535); 1.0 mg/mL 9-aminoacridine (-S9; TA1537); 25 µL/plate methyl methanesulfonate (-S9; E. coli)
Metabolic activation:	10% (v/v) liver S9 fraction from male Sprague-Dawley rats treated with 500 mg/kg (ip) Aroclor 1254 five days prior to isolation
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Incubated for 46-50 hours

Study Validity

Criteria for a valid assay included:

- All Salmonella strains must exhibit sensitivity to crystal violet to demonstrate the presence of the rfa rough wall mutation. The E. coli strain must exhibit resistance to crystal violet to demonstrate the absence of the rfa mutation.
- Test strains TA98 and TA100 must exhibit resistance to ampicillin to demonstrate the presence of the R-factor plasmid pKM101.

- The mean number of revertants per plate for the negative control must fall with in the reference ranges.
- Positive control values must exhibit at least a 3-fold increase in mean number of revertants per plate above the mean value for the concurrent negative control.

Criteria for a positive response:
- A 2-fold increase in the mean number of revertants per plate above the negative control in strains TA98, TA100, and WP2 uvrA.
- A 3-fold increase in mean number of revertants per plate above the negative control in strains TA1535 and TA1537.
- Increases in revertant counts for all strains must be related to increases in test article concentration to warrant the designation of positive.
- A positive response in one tester strain either with or without exogenous metabolic activation is sufficient to designate the test article as a bacterial mutagen.

Results

The assays satisfied all criteria outlined above and are therefore considered valid.

The number of revertants for the negative control WP2 uvrA strain without metabolic activation (8 revertants) did not meet the acceptability criteria (must fall between 10 and 60 revertants), so this part of the assay was repeated.

Under the conditions of this assay, AEGR-733 (BMS-201038) was not mutagenic at concentrations up to and including cytotoxic levels with or without metabolic activation. A summary of the results is shown in the sponsor-generated tables below.

Table 3
Summary
Mean Histidine+ and Tryptophan+ Revertant Counts from
the Full Assay on BMS-201038

In the Presence of Metabolic Activation

Test Article	Dose Level (µg/plate)	TA 98 Mean ± SD	TA 100 Mean ± SD	TA 1535 Mean ± SD	TA 1537 Mean ± SD	WP2 uvrA Mean ± SD
DMSO	0	54 ± 7	143 ± 32	10 ± 3	9 ± 2	18 ± 2
BMS-201038	15.63	50 ± 4	123 ± 15	9 ± 3	6 ± 1	NT
	31.25	53 ± 5	127 ± 9	9 ± 4	7 ± 2	16 ± 6
	62.5	57 ± 3	141 ± 24	8 ± 5	5 ± 2	19 ± 4
	125	44 ± 10	114 ± 27	7 ± 2	5 ± 1	19 ± 8
	250ppt	26 ± 11	39 ± 10	3 ± 1	2 ± 2	18 ± 2
	500ppt	NT	NT	NT	NT	10 ± 4
2-Aminoanthracene	2.5	998	781	169	99	
2-Aminoanthracene	10					506

In the Absence of Metabolic Activation

Test Article	Dose Level (µg/plate)	TA 98 Mean ± SD	TA 100 Mean ± SD	TA 1535 Mean ± SD	TA 1537 Mean ± SD	WP2 uvrA Mean ± SD
DMSO	0	14 ± 5	127 ± 22	9 ± 5	4 ± 2	15 ± 3
BMS-201038	15.63	11 ± 1	95 ± 15	7 ± 5	4 ± 4	NT
	31.25	8 ± 9	68 ± 6	6 ± 5	3 ± 2	18 ± 3
	62.5	6 ± 1	52 ± 11	7 ± 5	2 ± 1	19 ± 1
	125	10 ± 4	38 ± 7	2 ± 1	1 ± 1	16 ± 3
	250ppt	5 ± 1	43 ± 5	2 ± 1	1 ± 2	8 ± 2
	500ppt	NT	NT	NT	NT	10 ± 5
2-Nitrofluorene	2	777				
Sodium azide	1		477	381		
9-Aminoacridine	100				508	
MMS	2.5 µl/plate					366

ppt Chemical precipitate present.

NT Strain not evaluated at this concentration.

Table 4
Summary
Mean Histidine$^+$ and Tryptophan$^+$ Revertant Counts from
the Confirmatory and Repeat Assays on BMS-201038

In the Presence of Metabolic Activation

Test Article	Dose Level (µg/plate)	TA 98 Mean ± SD	TA 100 Mean ± SD	TA 1535 Mean ± SD	TA 1537 Mean ± SD	WP2 uvrA Mean ± SD
DMSO	0	60 ± 13	124 ± 11	11 ± 4	8 ± 3	12 ± 2
BMS-201038	15.63	59 ± 5	107 ± 11	13 ± 1	7 ± 3	NT
	31.25	55 ± 10	90 ± 10	11 ± 3	7 ± 3	8 ± 2
	62.5	66 ± 9	111 ± 8	10 ± 4	10 ± 3	11 ± 5
	125	45 ± 4	66 ± 19	9 ± 2	6 ± 5	11 ± 6
	250[ppt]	43 ± 9	42 ± 5	0 ± 1	0 ± 1	8 ± 4
	500[ppt]	NT	NT	NT	NT	6 ± 0
2-Aminoanthracene	2.5	1044	1003	154	48	
2-Aminoanthracene	10					563

In the Absence of Metabolic Activation

Test Article	Dose Level (µg/plate)	TA 98 Mean ± SD	TA 100 Mean ± SD	TA 1535 Mean ± SD	TA 1537 Mean ± SD	WP2 uvrA Mean ± SD Confirmatory	WP2 uvrA Mean ± SD Repeat
DMSO	0	22 ± 7	81 ± 8	8 ± 3	5 ± 3	8 ± 3	34 ± 3
BMS-201038	15.63	21 ± 5	74 ± 1	8 ± 5	3 ± 1	NT	NT
	31.25	6 ± 2	56 ± 7	7 ± 3	5 ± 2	9 ± 4	34 ± 9
	62.5	10 ± 4	38 ± 8	7 ± 3	3 ± 1	6 ± 2	34 ± 6
	125	7 ± 2	35 ± 2	2 ± 1	0 ± 0	9 ± 3	27 ± 6
	250[ppt]	3 ± 1	40 ± 9	0 ± 0	0 ± 0	14 ± 2	29 ± 8
	500[ppt]	NT	NT	NT	NT	10 ± 2	19 ± 4
2-Nitrofluorene	2	979					
Sodium azide	.1		579	388			
9-Aminoacridine	100				1115		
MMS	2.5 µl/plate					714	798

[ppt] Chemical precipitate present.

[NT] Strain not evaluated at this concentration.

Table 5

Bacterial Background Lawn Evaluation[a] from the Full[1], Confirmatory[2] and Repeat[3] Assays

In the Presence of Metabolic Activation											
BMS-201038[b]	TA98		TA100		TA1535		TA1537		WP2 uvrA		
15.63	0[1]	0[2]	0[1]	0[2]	0[1]	0[2]	0[1]	0[2]	NT	NT	NT
31.25	0	0	0	0	0	0	0	0	0[1]	0[2]	NT
62.5	0	0	0	0	0	0	1	0	0	0	NT
125	1	1	1	1	1	1	2	2	0	0	NT
250	2[ppt]	2[ppt]	3[ppt]	3[ppt]	3[ppt]	3[ppt]	3[ppt]	3[ppt]	0[ppt]	0[ppt]	NT
500	NT	NT	NT	NT	NT	NT	NT	NT	1[ppt]	1[ppt]	NT
In the Absence of Metabolic Activation											
15.63	0[1]	0[2]	0[1]	0[2]	0[1]	0[2]	0[1]	0[2]	NT	NT	NT
31.25	0	1	0	1	1	1	0	1	0[1]	0[2]	0[3]
62.5	1	2	1	2	2	2	2	2	0	0	0
125	1	2	3	3	3	3	3	3	0	0	0
250	2[ppt]	3[ppt]	3[ppt]	3[ppt]	3[ppt]	3[ppt]	3[ppt]	4[ppt]	0[ppt]	0[ppt]	0[ppt]
500	NT	NT	NT	NT	NT	NT	NT	NT	1[ppt]	0[ppt]	0[ppt]

[a] The BMS-201038-treated plates were graded on a scale of 0 to 4 compared to the vehicle controls

[b] Concentration in terms of μg of BMS-201038 free-base/0.1 ml/plate

Grading system:
0 = No reduction of the bacterial background lawn
1 = Slight reduction of the bacterial background lawn
2 = Moderate reduction of the bacterial background lawn
3 = Marked reduction of the bacterial background lawn
4 = Complete annihilation of the bacterial background lawn

NT = Not tested
[ppt]Chemical precipitate present

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Cytogenetics study in primary human lymphocytes

Study no.:	96686
Study report location:	Module 4.2.3.3.1
Conducting laboratory:	Bristol-Myers Squibb, Syracuse, NY
Date of study initiation:	09 September 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038-01 (AEGR-733) mesylate salt, Batch R006A, 85.3% purity (free base)

Key Study Findings

- AEGR-733 (BMS-201038) was not shown to be clastogenic under the conditions of this study.

Methods

Cell line:	Human peripheral blood lymphocytes; isolated from two donors on day of study initiation
Concentrations in definitive study:	-S9: 0.5, 1, 2, 4 µg/mL +S9: 1, 2, 4, 8 µg/mL
Basis of concentration selection:	Range-finding study; mitotic indices were completely eliminated at ≥18.7 µg/mL
Negative control:	DMSO
Positive control:	0.1 µg/mL mitomycin C (-S9) 4 µg/mL cyclophosphamide (+S9)
Metabolic activation:	Liver S9 fraction from male Sprague-Dawley rats treated with 500 mg/kg (ip) Aroclor 1254 five days prior to isolation
Formulation/Vehicle:	DMSO
Incubation & sampling time:	5 hr incubation with S9, sampling at 24 hr 24 hr incubation without S9, sampling at 24 hr
Analysis:	Manual microscopic evaluation in a blinded manner.

Study Validity

Criteria for a valid study

- The mitotic index for the negative control cultures must exceed 2%.
- Positive control cultures must exhibit an increase in chromosome aberration frequency that is statistically significant (Students t test) at the 5% level.
- The average percentage of damaged metaphases in the negative control cultures must not exceed 6%.
- The test article should exhibit some cytotoxicity (i.e., reduced mitotic index)

Criteria for a positive response

- A significant concentration-dependent increase in the chromosome aberration frequency is shown. This is demonstrated by a statistically significant ($\alpha \leq 0.05$) positive β term in the regression analysis.
- A statistically significant ($\alpha \leq 0.05$) F value for treatment in the analysis of variance test is obtained and a statistically significant ($\alpha \leq 0.05$) effect in the positive direction is indicated by a multiple comparison test.
- If the two statistical criteria are not in agreement, the study director will determine whether the observed response is toxicologically significant. If the data are deemed equivocal, additional data would be collected.

Results

For cells treated without metabolic activation, the mitotic indices were approximately 90%, 65%, 52%, and 42% of the control cells at the 0.5, 1, 2, and 4 µg/mL concentrations. Mitotic indices for cells treated in the presence of metabolic activation were 86%, 71%, 65%, and 41% of controls. The negative and positive control data met the criteria for a valid study.

There was not a statistically significant increase in the number of damaged metaphase spreads for any of the treated groups when compared with the negative control values. A summary of the data is shown in the sponsor-generated table below. Based on these data, AEGR-733 (BMS-201038) was not shown to be clastogenic under the conditions of this study.

TABLE 2

BMS-201038

Cytogenetic Data Summarized by Dose Group

TREATMENT			ABERRATIONS							
			CELLS WITH ABERRATIONS		CHROMATID		CHROMOSOME			
	MITOTIC INDEX MEAN ± S.E.M	CELLS SCORED	MEAN% ± S.E.M.	ABS/CELL[1] ± S.E.M.	BKS [2]	EXC [3]	BKS [2]	EXC [3]	>10 [4]	TOTAL ABS [5]
DMSO	10.5% ± 0.1%	200	3.0 ± 0.6	0.03 ± 0.01	5	0	1	0	0	6
BMS-201038 (μg/ml) 24 HOUR EXPOSURE										
0.5	9.5% ± 0.8%	200	1.0 ± 1.0	0.01 ± 0.01	2	0	0	0	0	2
1.0	6.8% ± 0.6%*	200	1.5 ± 0.5	0.02 ± 0.01	3	0	0	0	0	3
2.0	5.5% ± 0.5%**	200	1.5 ± 0.5	0.02 ± 0.01	0	0	3	0	0	3
4.0	4.4% ± 0.4%**	200	1.5 ± 0.5	0.02 ± 0.01	2	0	1	0	0	3
MITOMYCIN C (μg/ml)										
0.10	5.1% ± 0.6%**	200	41.0 ± 1.7**	0.66 ± 0.05	84	23	24	0	0	131
DMSO + S-9	11.1% ± 0.5%	200	2.0 ± 0.8	0.02 ± 0.01	3	0	1	0	0	4
BMS-201038 (μg/ml) 5 HOUR EXPOSURE WITH S-9										
1.0	9.5% ± 0.4%	200	3.5 ± 0.5	0.04 ± 0.01	6	0	1	0	0	7
2.0	7.9% ± 0.8%**	200	3.0 ± 0.6	0.03 ± 0.01	2	1	3	0	0	6
4.0	7.2% ± 0.7%**	200	2.5 ± 0.5	0.03 ± 0.01	5	0	0	0	0	5
8.0	4.5% ± 0.4%**	200	1.5 ± 1.0	0.02 ± 0.01	2	0	1	0	0	3
CYCLOPHOSPHAMIDE (μg/ml) + S-9										
4	4.9% ± 0.6%**	200	38.0 ± 2.8**	0.69 ± 0.05	89	19	20	0	1	138

* Denotes significantly different from appropriate control at $P < 0.05$ by students "t" test.

** Denotes significantly different from appropriate control at $P < 0.01$ by students "t" test.

1 Total aberrations divided by the total number of metaphases evaluated.
2 Total of all breaks including chromatid and isochromatid type.
3 Total of all exchanges including interchanges and intrachanges.
4 Total cells observed with more than 10 separate aberrations.
5 Total aberrations observed for the treatment (sum of the individual totals plus 10 times the >10 frequency) .

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

Study title: Oral micronucleus study in rats

Study no:	96629
Study report location:	Module 4.2.3.3.2
Conducting laboratory:	Bristol-Myers Squibb, Syracuse, NY
Date of study initiation:	19 March 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038-01 (AEGR-733), Batch GK1528, 96.8% purity (free base)

Key Study Findings

- Under the conditions of this study, the results indicate that AEGR-733 (BMS-201038) did not exhibit genotoxic activity in rat bone marrow up to a lethal dose of 1000 mg/kg following three consecutive daily doses.

Methods

Doses in definitive study:	10, 100, and 1000 mg/kg
Frequency of dosing:	Once daily for 3 consecutive days
Route of administration:	Oral
Dose volume:	20 mL/kg
Formulation/Vehicle:	75% PEG 400
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	5
Satellite groups:	No
Basis of dose selection:	Range-finding study: 2 male and female Sprague-Dawley rats administered 125, 250, 500, or 1000 mg/kg by oral gavage on 3 consecutive days. (data not shown in this review)
Negative control:	75% PEG 400
Positive control:	7 mg/kg cyclophosphamide (3 daily doses by ip)

Study Validity

Criteria for a valid study

- A minimum of 2 test article doses must be available for microscopic evaluation.
- The mean percentage of micronucleated (MN) polychromatic erythrocytes (PCEs) in the negative control group must not exceed 0.5%.
- In bone marrow smears, 2000 PCEs per animal must be available for scoring.
- The incidence of MN-PCEs for the positive control group must be statistically significantly elevated and be at least 3-fold greater than the concurrent negative control group. This value must also exceed the laboratory's historical negative control data range.

- A minimum of 4 males and 4 females per dose level must survive test article treatment for bone-marrow sampling and subsequent microscopic analyses.

Criteria for a positive response

- A statistically significant, dose-related increase in mean MN-PCE value is observed.
- The mean MN-PCE value at the highest scorable dose level is statistically significant and at least 3-fold greater than the concurrent negative control mean value.
- The mean MN-PCE value of the highest scorable dose level exceeds the highest value for the laboratory's historical negative control data range of group means.

Results

In the definitive study, one apparent test article related death occurred in the 1000 mg/kg/d male group. At this dose level, males and females exhibited signs of abdominal bloating, decreased activity, hunched posture, labored breathing, and rough hair coat. Females treated with 100 mg/kg/d also showed rough hair coat. Other clinical signs were also observed in the vehicle control group and were not considered test article related (see sponsor-generated Table 2 below). Minimal, dose-related bone marrow toxicity, as determined by a decrease in bone marrow PCEs, occurred in treated females.

Mean frequencies of MN-PCEs for treated animals were not statistically significantly greater than for vehicle control animals. The mean frequency of MN-PCEs for the positive control group was statistically significantly greater than the concurrent controls. The data are summarized in sponsor-generated Table 4 below. The results of the study met the criteria for a valid study.

Under the conditions of this study, the results indicate that AEGR-733 (BMS-201038) did not exhibit genotoxic activity in rat bone marrow up to a lethal dose of 1000 mg/kg following three consecutive daily doses.

Table 3

BMS-201038: Oral Micronucleus Study in Rats

Summary of Clinical Observations

Sex	Dose BMS-201038 (mg/kg/day)	Incidence of Death	Toxic Signs
M	vehicle (75% PEG 400)	0/5	minimal dehydration, rasping, salivation, sneezing, soft feces, soiled genitals and muzzle
M	10	0/5	minimal dehydration, rasping, salivation, soft feces, soiled genitals and muzzle
M	100	0/5	diarrhea, minimal to moderate dehydration, rasping, salivation, soft feces, soiled forepaws, genitals and muzzle
M	1000	1/5[a]	abdominal bloating, decreased activity, diarrhea, hunched posture, labored breathing, minimal to moderate dehydration, rasping, rough hair coat, salivation, sneezing, soft feces, soiled abdomen, face, forepaws, genitals, and muzzle
M	cyclophosphamide (7 mg/kg/day)	0/5	No overt signs
F	vehicle (75% PEG 400)	0/5	diarrhea, minimal dehydration, soft feces, soiled genitals and muzzle
F	10	0/5	minimal dehydration, soft feces, soiled face, genitals and muzzle
F	100	0/5	diarrhea, minimal dehydration, rasping, rough hair coat, soft feces, soiled genitals and muzzle
F	1000	0/5	abdominal bloating, diarrhea, minimal dehydration, rasping, rough hair coat, soft feces, soiled abdomen, face, forepaws, genitals, and muzzle
F	cyclophosphamide (7 mg/kg/day)	0/5	No overt signs

a One animal died prior to scheduled necropsy

Table 4

BMS-201038: Oral Micronucleus Study in Rats

Summary of Bone-Marrow Analysis

Article	Dose (mg/kg/day)	Sex	No. Rats Evaluated	Mean % PCEs (±SD)	Mean % MN-PCEs (±SD)
vehicle (75% PEG 400)	0	M	5	50 ± 6.0	0.16 ± 0.15
		F	5	53 ± 2.5	0.08 ± 0.04
BMS-201038	10	M	5	47 ± 5.6	0.16 ± 0.12
		F	5	47 ± 4.1	0.10 ± 0.06
BMS-201038	100	M	5	45 ± 3.9	0.17 ± 0.12
		F	5	42 ± 2.7	0.14 ± 0.04
BMS-201038	1000	M	4[a]	47 ± 6.2	0.12 ± 0.09
		F	5	41 ± 1.9	0.13 ± 0.11
cyclophosphamide	7	M	5	47 ± 4.8	2.83 ± 0.50 **
		F	5	44 ± 1.3	1.55 ± 0.35 **

a One animal died prior to scheduled necropsy

** Statistically significant at $P<0.01$.

7.4 Other Genetic Toxicity Studies (Metabolites)

Study title: BMS-203215 [M1] in the *Salmonella*/Mammalian-microsome reverse mutation assay (Ames test) with a confirmatory assay

Study no.:	CHV-17787-0-401R
Study report location:	Module 4.2.3.7
Conducting laboratory:	(b) (4)
Date of study initiation:	24 June 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-203215-02 (Metabolite M1), Batch 38325-173-19, purity not specified

Key Study Findings

- Under the conditions of this assay, metabolite 203215 (M1) was not mutagenic at concentrations up to and including cytotoxic levels with or without metabolic activation.

Methods	
Strains:	Salmonella: TA98, TA100, TA1535, TA1537
Concentrations in definitive study:	-S9: 6.67 , 5000 µg/plate +S9: 66.7, 5000 µg/plate
Basis of concentration selection:	Observed cytotoxicity in a dose range-finding study in TA100 at ≥3,330 µg/plate.
Negative control:	DMSO
Positive controls:	2.5 µg/mL 2-aminoanthracene (+S9); 1.0 µg/mL 2-nitrofluorene (-S9; TA98); 2.0 µg/mL sodium azide (-S9; TA100, TA1535); 2.0 µg/mL ICR-191 (-S9; TA1537);
Metabolic activation:	Liver S9 fraction from male Sprague-Dawley rats treated with 500 mg/kg (ip) Aroclor 1254
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation: 48 ± 8 hours

Study Validity

Criteria for a valid assay included:

- All Salmonella strains must exhibit sensitivity to crystal violet to demonstrate the presence of the rfa rough wall mutation.
- Test strains TA98 and TA100 must exhibit resistance to ampicillin to demonstrate the presence of the R-factor plasmid pKM101.
- The mean number of revertants per plate for the negative control must fall within the reference ranges.
- Tester strain cultures should reach a density greater or equal to 0.5×10^9 bacteria/mL.
- Positive control values must exhibit at least a 3-fold increase in mean number of revertants per plate above the mean value for the concurrent negative control.

Criteria for a positive response:

- A 2-fold increase in the mean number of revertants per plate above the negative control in strains TA98 and TA100.
- A 3-fold increase in mean number of revertants per plate above the negative control in strains TA1535 and TA1537.

Results

The assays satisfied all criteria outline above and were considered valid.

In experiment 1, TA100 could not be scored because of confluent bacterial growth. Also in this assay, the revertant values for mean vehicle control for tester strain 1535 were higher than routinely observed for this strain. Both strains were retested.

Under the conditions of this assay, metabolite BMS-203215 (M1) was not mutagenic at concentrations up to and including cytotoxic levels with or without metabolic activation.

A summary of the results is shown in the sponsor-generated tables below.

Experiment 1

		MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION								BACKGROUND LAWN*
	DOSE/PLATE	TA98		TA100		TA1535		TA1537		
		MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	
MICROSOMES: Rat Liver										
VEHICLE CONTROL		25	5	NC	-	40	7	13	2	1
TEST ARTICLE	100 µg	22	6	NC	-	28	6	14	2	1
	333 µg	25	6	NC	-	44	6	14	2	1
	667 µg	25	7	NC	-	41	6	12	6	1
	1000 µg	29	10	NC	-	39	7	11	3	1
	3330 µg	17	7	NC	-	19	2	12	9	1
	5000 µg	3	1	NC	-	10	3	5	4	1/3[A]
POSITIVE CONTROL **		769	110	NC	-	123	14	147	3	1
MICROSOMES: None										
VEHICLE CONTROL		12	3	NC	-	29	9	8	1	1
TEST ARTICLE	100 µg	12	5	NC	-	19	2	7	3	1
	333 µg	18	7	NC	-	30	3	10	5	1
	667 µg	14	2	NC	-	19	2	9	3	1
	1000 µg	13	5	NC	-	18	6	4	1	1/2[B]
	3330 µg	1	2	NC	-	8	1	0	0	3
	5000 µg	0	0	NC	-	0	0	0	0	5
POSITIVE CONTROL ***		132	11	NC	-	521	28	242	44	1

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal 2 = slightly reduced 3 = moderately reduced
4 = extremely reduced 5 = absent 6 = obscured by precipitate
sp = slight precipitate mp = moderate precipitate (requires hand count) hp = heavy precipitate (requires hand count)

[A] The bacterial background lawn for tester strain TA98 was evaluated as moderately reduced (3) at this dose.
[B] The bacterial background lawn for tester strain TA1537 was evaluated as slightly reduced (2) at this dose.

NC = No count due to confluent bacterial growth (contamination) on these plates.
(See retest table below)

Experiment 1 - Retest of TA100 and TA1535

DOSE/PLATE		MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATION TA100 MEAN	TA100 S.D.	TA1535 MEAN	TA1535 S.D.	BACKGROUND LAWN*
MICROSOMES: Rat Liver						
VEHICLE CONTROL		86	17	8	1	1
TEST ARTICLE	100 µg	99	10	10	2	1
	333 µg	92	5	10	3	1
	667 µg	85	9	12	8	1
	1000 µg	91	16	11	4	1
	3330 µg	32	4	9	1	1
	5000 µg	9	5	10	5	3/1^
POSITIVE CONTROL **		957	115	166	10	1
MICROSOMES: None						
VEHICLE CONTROL		64	10	10	3	1
TEST ARTICLE	66.7 µg	80	4	9	2	1
	100 µg	74	4	12	3	1
	333 µg	69	14	12	2	1
	667 µg	70	15	13	4	1
	1000 µg	59	8	12	2	1
	3330 µg	12	5	5	5	4
POSITIVE CONTROL ***		652	17	583	58	1

** TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate

*** TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

^ The bacterial background lawn for tester strain TA1535 was evaluated as normal (1) at this dose.

Experiment 2 (confirmatory assay)

DOSE/PLATE	TA98 MEAN	TA98 S.D.	TA100 MEAN	TA100 S.D.	TA1535 MEAN	TA1535 S.D.	TA1537 MEAN	TA1537 S.D.	BACKGROUND LAWN*
MICROSOMES: Rat Liver									
VEHICLE CONTROL	16	3	92	15	9	2	4	2	1
TEST ARTICLE 100 µg	19	2	92	10	10	2	7	2	1
333 µg	14	4	95	5	14	5	5	3	1
667 µg	21	4	100	9	11	1	5	3	1
1000 µg	24	3	100	4	12	4	7	3	1
3330 µg	10	3	37	13	9	6	5	2	1
5000 µg	3	1	8	3	6	3	4	1	3/1[A]
POSITIVE CONTROL **	1226	116	1279	62	177	8	97	3	1
MICROSOMES: None									
VEHICLE CONTROL	12	4	80	7	10	5	5	2	1
TEST ARTICLE 66.7 µg	13	2	86	2	12	1	3	2	1
100 µg	13	3	91	4	12	5	3	1	1
333 µg	10	2	79	9	13	4	5	2	1
667 µg	10	4	79	11	10	2	4	3	1/2[B]
1000 µg	10	2	68	7	9	4	5	1	1/2[C]
3330 µg	1	1	1	1	5	2	1	1	3/4[D]
POSITIVE CONTROL ***	107	21	552	15	542	32	700	76	1

Best Available Copy

** TA98 2-aminoanthracene 2.5 µg/plate
TA100 2-aminoanthracene 2.5 µg/plate
TA1535 2-aminoanthracene 2.5 µg/plate
TA1537 2-aminoanthracene 2.5 µg/plate

*** TA98 2-nitrofluorene 1.0 µg/plate
TA100 sodium azide 2.0 µg/plate
TA1535 sodium azide 2.0 µg/plate
TA1537 ICR-191 2.0 µg/plate

* Background Lawn Evaluation Codes:
1 = normal
2 = slightly reduced
3 = moderately reduced
4 = extremely reduced
5 = absent
6 = obscured by precipitate
sp = slight precipitate
mp = moderate precipitate (requires hand count)
hp = heavy precipitate (requires hand count)

[A] The bacterial background lawn for tester strains TA1535 and TA1537 were evaluated as normal (1) at this dose.
[B] The bacterial background lawn for tester strain TA98 was evaluated as slightly reduced (2) at this dose.
[C] The bacterial background lawn for tester strains TA98 and TA100 were evaluated as slightly reduced (2) at this dose.
[D] The bacterial background lawn for tester strain TA100 was evaluated as extremely reduced (4) at this dose.

8 Carcinogenicity

Study title: 104-week dietary carcinogenicity study with AEGR-733 (formerly BMS-201038) in mice

Study no.:	7881-103 (AEGR 733-PC-0003) and 8245273 (AEGR 733-PC0018)
Study report location:	Module 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Diet:	AEGR-733 inhibits absorption of fat soluble vitamins from the intestine leading to toxicity from vitamin deficiency. All animals were fed Harlan Teklad rodent diet #2018S, which contains more vitamin A and K than standard rodent diet. Additional vitamin supplementation was not employed.
Date of study initiation:	14 May 2007
GLP compliance:	Yes, except vitamin A and E liver analysis
QA statement:	Yes
Drug, lot #, and % purity:	AEGR 733 (mesylate salt), Lot #3136 (85.5% pure) and L0109571 (84.7% pure)
CAC concurrence:	Yes, provided the 25X exposure margin is achieved. The sponsor has lowered the maximum recommended clinical dose to 60 mg. The AUC_{0-24} exposure margin at the highest dose tested in this study is 77X for the parent compound, >1000X for metabolite M1, and approximately 18X to 30X for metabolite M3.

Key Study Findings

- A dose-related trend in increased mortality was observed for males. Because of mortality issues, dosing was terminated for the male treatment groups from 2 to 14 weeks before necropsy and these groups were fed basal diet for the remainder of the study. Because of decreased survival, the 7.5 and 45 mg/kg/day male groups were sacrificed at Week 99 and Week 102, respectively; the remainder of the male groups was sacrificed at Week 105. No definitive treatment-related effect on mortality was observed for females. For some female groups, dosing was terminated from 2 to 8 weeks before necropsy because of survival issues; however a similar rate of early mortality was also observed for the female control group, which resulted in an unscheduled sacrifice for all female groups at Week 100. Deaths due to hepatic neoplasia were slightly increased for males and females receiving ≥1.5 and ≥7.5 mg/kg/day, respectively. A slight increase in death caused by gastrointestinal tumors was observed for both genders at 15 mg/kg/day (high mid-dose).
- A dose-related trend for decreased final mean body weights was observed for males receiving 7.5 mg/kg/day, with a statistically significant difference at ≥15 mg/kg/day

(~9% less then control) and for females receiving ≥1.5 mg/kg/day, with statistical significance at 45 mg/kg/day (16% less than control).

- Food consumption was generally increased in males and females receiving ≥7.5 mg/kg/day, which was considered to be related to the test article-related impairment of dietary fat absorption.
- Vitamin A and E levels in the liver decreased in a dose-related manner, with noteworthy decreases occurring at ≥7.5 mg/kg/day.
- Treatment-related macroscopic findings were noted in liver (mass present, discolored, cyst; ≥1.5 mg/kg/day for males and ≥7.5 mg/kg/day for females); lung (discolored; 45 mg/kg/day); small intestine (discolored, distended, thickened; ≥7.5 mg/kg/day); spleen (large; ≥1.5 mg/kg/day for males); and ovary (cyst; ≥1.5 mg/kg/day for females).
- A statistically significant increase in hepatocellular adenomas, carcinomas, and/or adenomas plus carcinomas was observed for males at ≥1.5 mg/kg/day (2X MHRD) and females at 7.5 mg/kg/day (9X MHRD). A statistically significant increase in small intestinal tumors (adenomas plus carcinomas) was observed for males and females at ≥15 mg/kg/day (~24X MHRD). Because small intestinal tumors are rare in this strain of mice (no tumors were observed in control animals), the low number of tumors (1 or 2 per group) observed in male mice at 0.3, 1.5, and 7.5 mg/kg/day may have also been treatment related. A dose-related decrease in bronchiolar-alveolar tumors was observed for males and females; however, the control tumor incidence was higher than historical control data, so the relevance of this finding is uncertain.
- Non-neoplastic microscopic findings included increased epithelial vacuaolation of the small intestine at ≥0.3 mg/kg/day for males and ≥1.5 mg/kg/day for females (note that dosing was discontinued for the high-dose group and for those animals that were sacrificed at study end, vacuolation was not observed, suggesting reversibility); increased incidence of alveolar spicules, lymphocyte infiltrate, and macrophage infiltrate in lung at 45 mg/kg/day for males and ≥7.5 mg/kg/day for females; increased incidence of inflammation/epidermal hyperplasia and ulcer/erosion of the skin/subcutis for females at 45 mg/kg/day; a dose-related increase in parovarian cysts at ≥1.5 mg/kg/day and decrease in ovarian amyloidosis at ≥7.5 mg/kg/day; increased extramedullary hematopoiesis in spleen at ≥7.5 mg/kg/day and myeloid hyperplasia of sternum marrow at 45 mg/kg/day. Dose-related decreases in lymphocyte/ macrophage inflammation/infiltrate and diffuse hepatocyte vacuolation in liver was observed for treated animals.
- Evaluation of lung tissue from 10 animals per group by transmission electron microscopy showed excessive cytoplasmic lipid accumulation, primarily in alveolar macrophages, in 1 to 3 males in the 0.3, 1.5, and 45 mg/kg/day group and in 1 to 2 females in the 15 and 45 mg/kg/day groups. This analysis did not show evidence that the lipid accumulation was phospholipidosis.
- The NOEL for tumorigenesis was considered to be 0.3 mg/kg/day for males and 1.5 mg/kg/day for females based on statistically significant increases in hepatocellular tumors at ≥1.5 mg/kg/day in males and ≥7.5 mg/kg/day in females.

Adequacy of Carcinogenicity Study

The carcinogenicity study was adequately designed and conducted.

Appropriateness of Test Models

The test model was appropriate to assess the carcinogenic potential of AEGR-733.

Evaluation of Tumor Findings

A statistically significant increase in the incidence of hepatocellular tumors (adenomas plus carcinomas) was observed in males given ≥1.5 mg/kg/day and females given ≥7.5 or 15 mg/kg/day. The tumor response was not completely dose-dependent as there was not a meaningful difference in tumor incidence between the LOEL and the higher doses. In fact, the tumor incidence at the highest dose level was less than at the lower doses, with the incidence for high-dose males not being statistically significant. Overall, there appears to be a threshold at which higher doses do not induce a greater amount of tumors. The pathologist characterized the hepatocellular carcinomas as invasive, whereas hepatocellular adenomas were generally well-demarcated and compressive to surrounding liver parenchyma. Both hepatocellular adenomas and carcinomas occurred singly or in multiples. Several animals had both adenomas and carcinomas.

Numerical increases in adenomas and/or carcinomas of the small intestine (duodenum, ileum, and/or jejunum) were observed for all treated male groups, with statistically significant increases occurring at ≥15 mg/kg/day. Numerical increases in adenomas and/or carcinomas of the small intestine in females was only observed at the two highest dose levels, with statistical significance only occurring at 15 mg/kg/day. The jejunum was the most common site for carcinomas. Intestinal adenomas were characterized as generally extending into the lumen, being noninvasive, and being composed of papillary formations of well-differentiated epithelial cells. Carcinomas were described as being variably differentiated, forming solid or acinar structures, and invading beyond the submucosa.

Although not statistically significant, it should be noted that small intestinal tumors were observed in one or two males given 0.3, 1.5, or 7.5 mg/kg/day and in three females receiving 45 mg/kg/day and no small intestinal tumors were observed in the control groups; it should also be noted that intestinal tumors are uncommon in this strain of mouse based on historical control data. Therefore, the absence of small intestinal tumors in control animals in conjunction with a low incidence of a rare tumor at low dose levels and statistical significance at higher doses suggest that the few tumors observed at the lower dose levels may have also been test-article related. As with the hepatocellular tumors, the occurrence of small intestinal tumors was not completely dose dependent, with the highest incidence occurring at the high-mid dose of 15 mg/kg/day for both sexes.

Interestingly, AEGR-733 was associated with statistically significant decreases in the incidence of combined bronchiolar-alveolar carcinomas and adenomas in treated males and females at all dose levels (except 7.5 mg/kg/day in females). Although the relevance of this apparent decrease in tumor development is uncertain and may have been artificially influenced by a higher incidence of these tumors in the control groups

compared with the laboratory's historical control data from similar studies, it does indicate that the microscopic changes observed in the lung (excessive cytoplasmic lipid accumulation primarily in alveolar macrophages, alveolar spicules, lymphocyte infiltration, and macrophage infiltration) were not pro-carcinogenic effects. This is noted because the other two tissues that had lipid accumulation, either in this study or in previously conducted mouse studies (small intestine and liver), also showed an increase in tumor incidence.

The NOEL for tumorigenesis was 0.3 mg/kg/day for males and 1.5 mg/kg/day for females based on the statistical significance of hepatocellular tumors at ≥1.5 mg/kg/day for males and ≥7.5 mg/kg/day for females. At the male NOEL, respective exposures for the parent, M1 metabolite, and M3 metabolite are approximately 0.4, uncertain (concentrations were under the LLOQ), and 0.2 times the anticipated clinical exposures at 60 mg/day. At the female NOEL, respective exposures for the parent, M1 metabolite, and M3 metabolite are approximately 2, 14, and 0.4 times the anticipated clinical exposures at 60 mg/day. At the observed LOELs, respective exposure margins are 2, 14, and 0.7 times for males and 9, 86, and 2 times for females.

Methods

Species/Strain: Mouse/CD1

Study design

Group	No. of Animals Male	No. of Animals Female	AEGR-733 Dose Level (mg/kg/day)
Carcinogenicity Animals			
1 (Control)[a]	60	60	0
2 (Low)	60	60	0.3
3 (Mid-Low)	60	60	1.5
4 (Mid)	60	60	7.5
5 (Mid-High)	60	60	15
6 (High)	60	60	45
Toxicokinetic Animals			
7 (Control)[a]	24	24	0
8 (Low)	24	24	0.3
9 (Mid-Low)	24	24	1.5
10 (Mid)	24	24	7.5
11 (Mid-High)	24	24	15
12 (High)	24	24	45
13 Sentinel Animals[b]	36	36	NA

NA = Not applicable.

a Groups 1, 7, and 13 received basal diet only.

b Nine sentinel animals/sex served as replacement animals to compensate for possible mortality (of sentinel animals).

Dose Group	Dosing Duration	Sacrifice Week	Dosing Holiday
Males			
0.3	694 days (Week 100)	Week 105	5 weeks
1.5	718 days (Week 103)	Week 105	2 weeks
7.5	688 days (Week 99)	Week 102	3 weeks
15	668 days (Week 96)	Week 105	9 weeks
45	596 days (Week 85)	Day 691 (Week 99)	14 weeks
Females			
0.3	680 days (Week 98)	Day 698 (Week 100)	2 weeks
1.5	680 days (Week 98)	Day 698 (Week 100)	2 weeks
7.5	698 days (Week 100)	Day 698 (Week 100)	None
15	698 days (Week 100)	Day 698 (Week 100)	None
45	641 days (Week 92)	Day 698 (Week 100)	8 weeks

Frequency of dosing: Once daily via diet
Dose volume: Not applicable
Route of administration: Oral
Formulation/Vehicle: Powdered test article admixed with food (Harlan Teklad Diet #2018S). Note that this diet contains higher concentrations of vitamins A and K than found in standard rodent chow. Dietary concentrations for Weeks 1 and 2 of the dosing phase were calculated based on projected estimates of body weight and food consumption values. Dietary concentrations for subsequent weeks were calculated using actual group/sex body weight data and food consumption data from previous carcinogenicity animal data collections. Control animals received only mouse food.
Basis of dose selection: ≥25X AUC to maximal clinical dose
Age: 6 to 6.5 weeks at initiation of treatment
Weight: 26.5 to 41.7 g (males) and 19.6 to 32.0 g (females)
Animal housing: Housed individually in stainless steel cages
Paradigm for dietary restriction: None; treatment-related effects on food consumption were not noted in range-finding studies.
Dual control employed: No
Interim sacrifice: No
Satellite groups: Yes, see above
Unique study design: 5 control males and 5 high-dose males sacrificed moribund with fatal liver carcinomas were evaluated by electron microscopy under a separate study number (733PC0018)
Deviation from study protocol: There were no deviations from the study protocol that affected the integrity or interpretability of the study.

Observations and Results

Mortality (twice daily)

Survival was slightly lower for the three highest dose levels for males. For females, survival was similar to controls or greater than controls (at 7.5 and 15 mg/kg/day). There was not a definitive increase in a particular cause of death for treated groups. A

slightly higher number of treated animals died as the result of a hepatic neoplasia (≥1.5 mg/kg/day for males and ≥7.5 mg/kg/day for females). There was also a slight increase in death from a GI neoplasia and inflammation for males and females treated at the higher dose levels, but the finding was not dose dependent for neoplasia. An increased number of females showed inflammation of the skin, whereas an apparent dose-dependent decrease in death due to amyloidois was noted for females.

Percentage Survival - Final Dosing Phase Necropsy

Sex	Males						Females					
AEGR-733 mg/kg/day	0	0.3	1.5	7.5	15	45	0	0.3	1.5	7.5	15	45
Total No. of Animals	60	60	60	60	60	60	60	60	60	60	60	60
Week of Necropsy	105	105	105	102	105	99	100	100	100	100	100	100
No. of Surviving Animals	19	19	17	15	14	14	15	18	18	25	27	17
Percentage	32	32	28	25	23	23	25	30	30	42	45	28

Summary of Mortality Findings

Gender	Males						Females					
Dose (mg/kg/day)	**0**	**0.3**	**1.5**	**7.5**	**15**	**45**	**0**	**0.3**	**1.5**	**7.5**	**15**	**45**
Scheduled sacrifices	19	19	17	15	14	14	15	18	18	25	27	17
Early deaths	41	41	43	45	46	46	45	42	42	35	33	43
Cause of Death												
Neoplasia, hepatic	11	13	21	20	20	17	1	0	0	3	4	4
Neoplasia, GI	0	0	0	1	5	1	0	0	0	1	4	1
Lymphosarcoma	3	4	3	1	0	0	9	9	7	1	4	3
Histiocytic sarcoma	0	1	2	0	1	0	3	3	6	5	2	2
Hemangiosarcoma/ hemangioma	0	1	3	1	1	1	1	2	0	3	0	0
Inflammation, skin	6	7	6	10	5	9	1	7	2	2	2	9
Inflammation, GI	1	0	0	2	3	4	0	0	0	1	1	2
Inflammation, lung	3	0	0	1	0	0	0	0	0	0	0	0
Inflammation, urinary/reproductive	3	2	1	0	0	0	0	0	1	0	0	0
Necrosis/malacia, CNS	1	0	1	0	0	0	0	0	1	2	0	3
Amyloidosis	0	1	0	0	0	0	12	5	8	7	1	0
Undetermined	7	8	2	4	7	13	8	7	5	7	9	11
Other*	6	4	4	5	4	1	10	9	12	3	6	8

*Only findings with an incidence of ≥3 animals (≥5%) in at least one group were listed as a separate finding; all other findings were included in the "other" category. CNS = central nervous system; GI = gastrointestinal.

As shown in the sponsor-generated figures below, the high-dose animals had a slightly higher mortality rate than the other treated groups. However, for females, the mortality rate for the high-dose group was similar to the control group.

Figure 1
Adjusted Survival Data (%) - Males

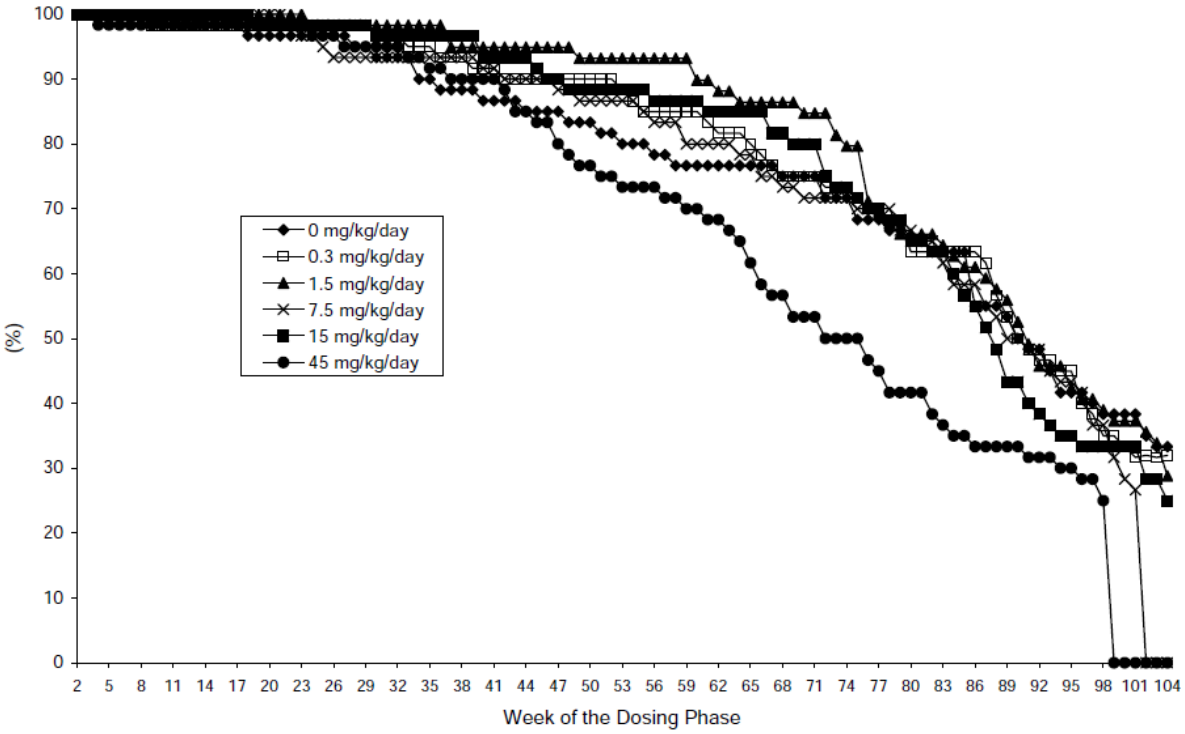
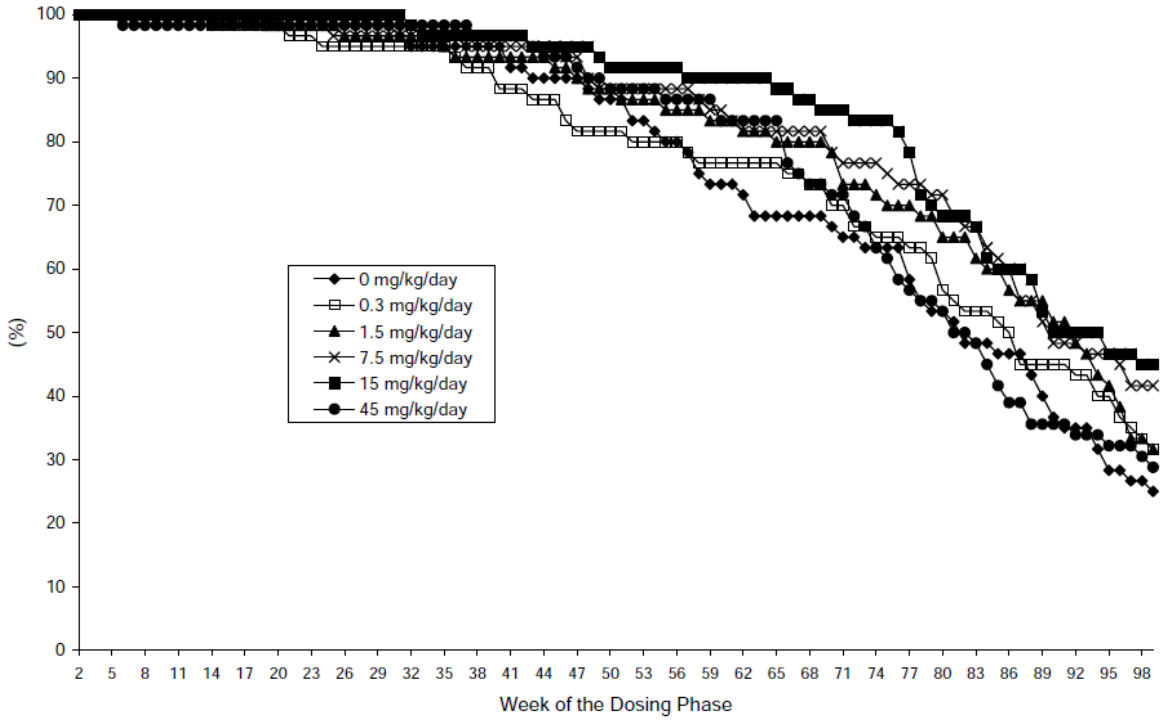


Figure 2
Adjusted Survival Data (%) - Females



Clinical Signs (twice daily and unscheduled observations; detailed exams once weekly)
Animals receiving 15 or 45 mg/kg/day had an increased incidence of swollen abdomen, which typically occurred in a transient fashion. This observation was considered to be test-article related because the intestine was a target organ.

Sores/scabs were observed on the ears, neck, and other areas of both control and treated animals, with a higher incidence (mostly on ears) seen in females receiving 3 or 45 mg/kg/day. Some lesions were diagnosed by a veterinarian as ulcerative dermatitis. In the most severe cases, animals were sacrificed as a result of the skin lesions. Because of a lack of dose response, the lesions were considered to have an uncertain relationship to the test article.

A slight increase in other clinical signs, such as hunched posture, hypoactivity, and pale appearance, were observed prior to death or moribund sacrifice and were consistent with agonal changes.

Body Weights (weekly through Week 38 and every other week thereafter)
As shown in the table and sponsor-generated figures below, mean body weights were less than controls for males receiving ≥7.5 mg/kg/day and females receiving ≥1.5 mg/kg/day.

Summary of Mean Final Body Weights

	Males						Females					
Dose (mg/kg/d)	**0**	**0.3**	**1.5**	**7.5**	**15**	**45**	**0**	**0.3**	**1.5**	**7.5**	**15**	**45**
Final Body Wt. (g) - Day 680 (M) or Day 698 (F)	50.0	49.8	50.1	46.5	45.2*	45.4*	45.3	45.7	42.7	41.0	41.6	38.0*
Difference from control (g)		-0.2	0.1	-3.5	-4.8	-4.6		0.4	-2.6	-4.3	-3.7	-7.3
% difference from control		-	-	↓7%	↓10%	↓9%		↑1%	↓6%	↓9%	↓8%	↓16%

*p≤0.05; g = grams; F = female; M = male.

Feed Consumption (weekly through Week 37 and every other week thereafter)
Food consumption was generally increased in male and females receiving ≥7.5 mg/kg/day compared with their respective controls. Increased food consumption was considered to be secondary to the test article-related impairment of dietary fat absorption. Mean weekly food consumption over the course of the study is shown in the sponsor-generated figures below.

Figure 3
Mean Body Weights Data - Males

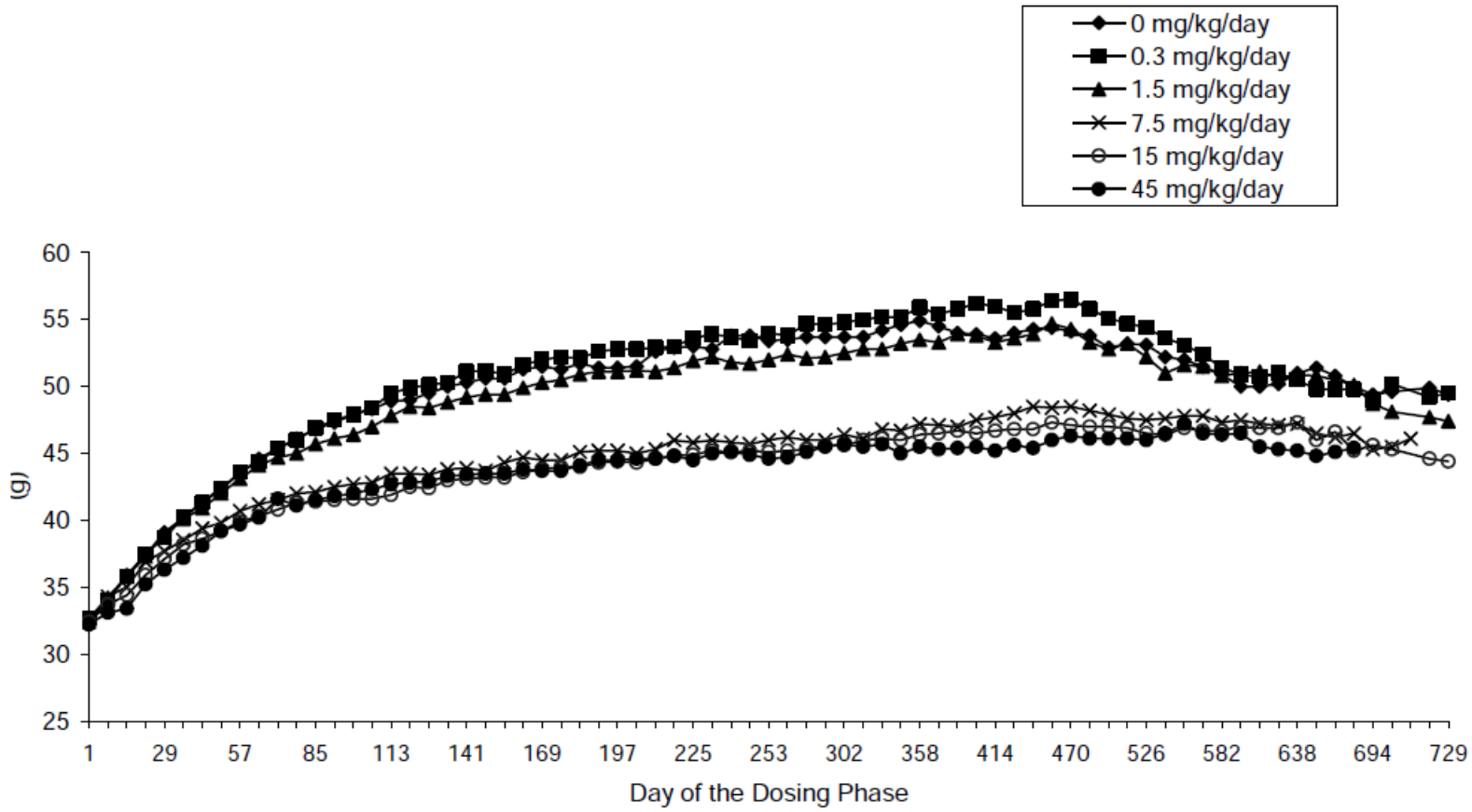


Figure 4
Mean Body Weights Data - Females

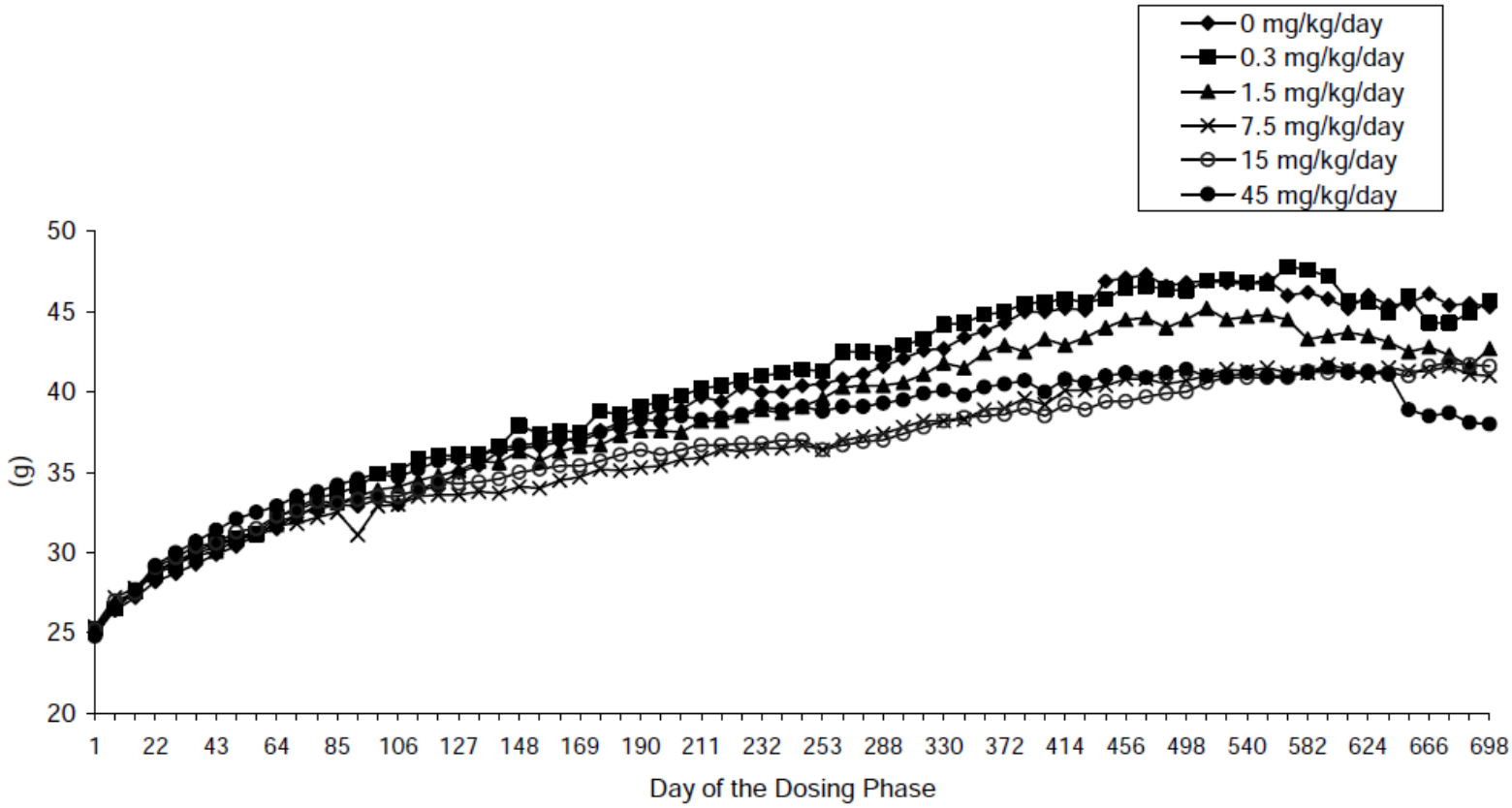


Figure 5
Mean Food Consumption Data - Males

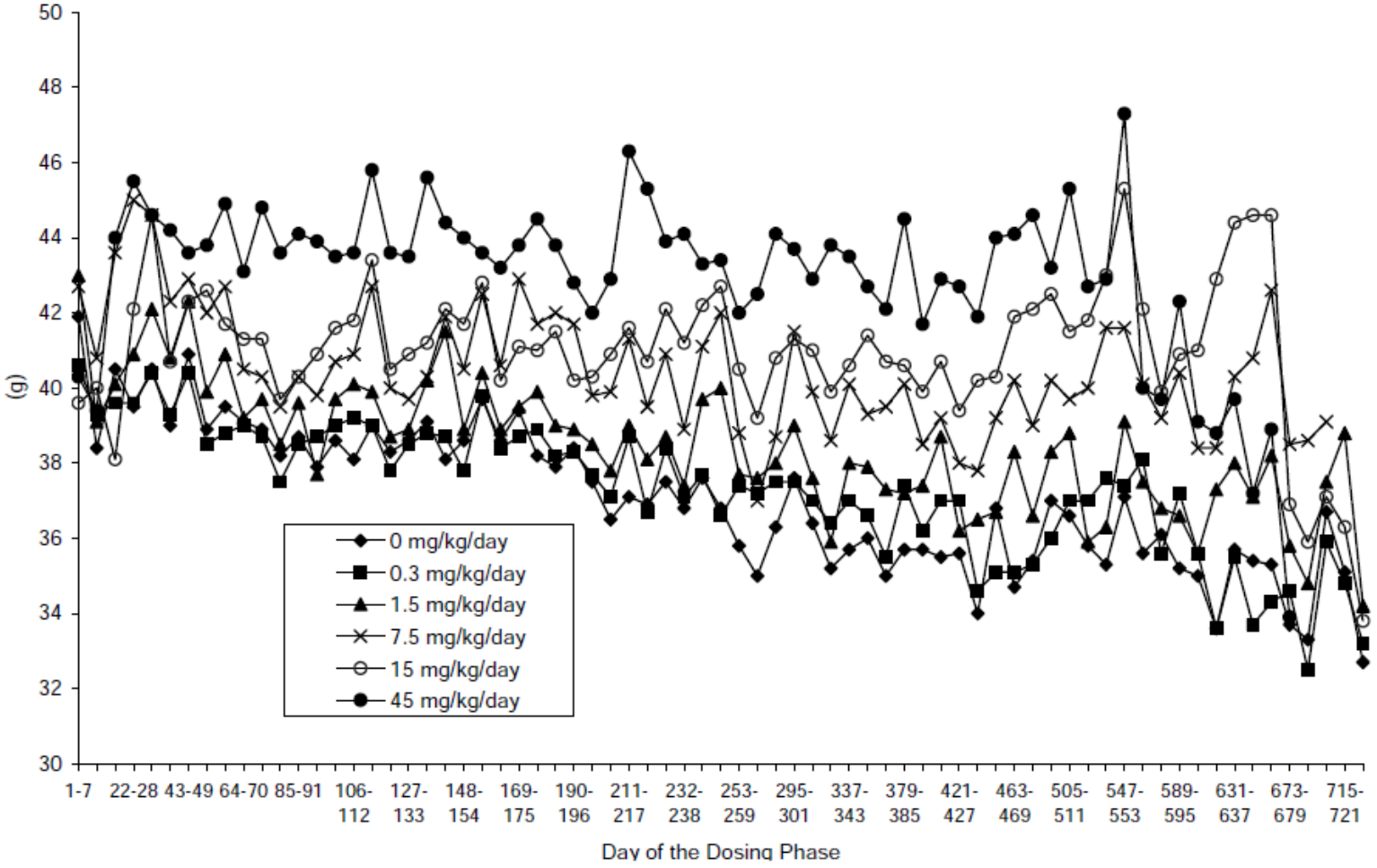
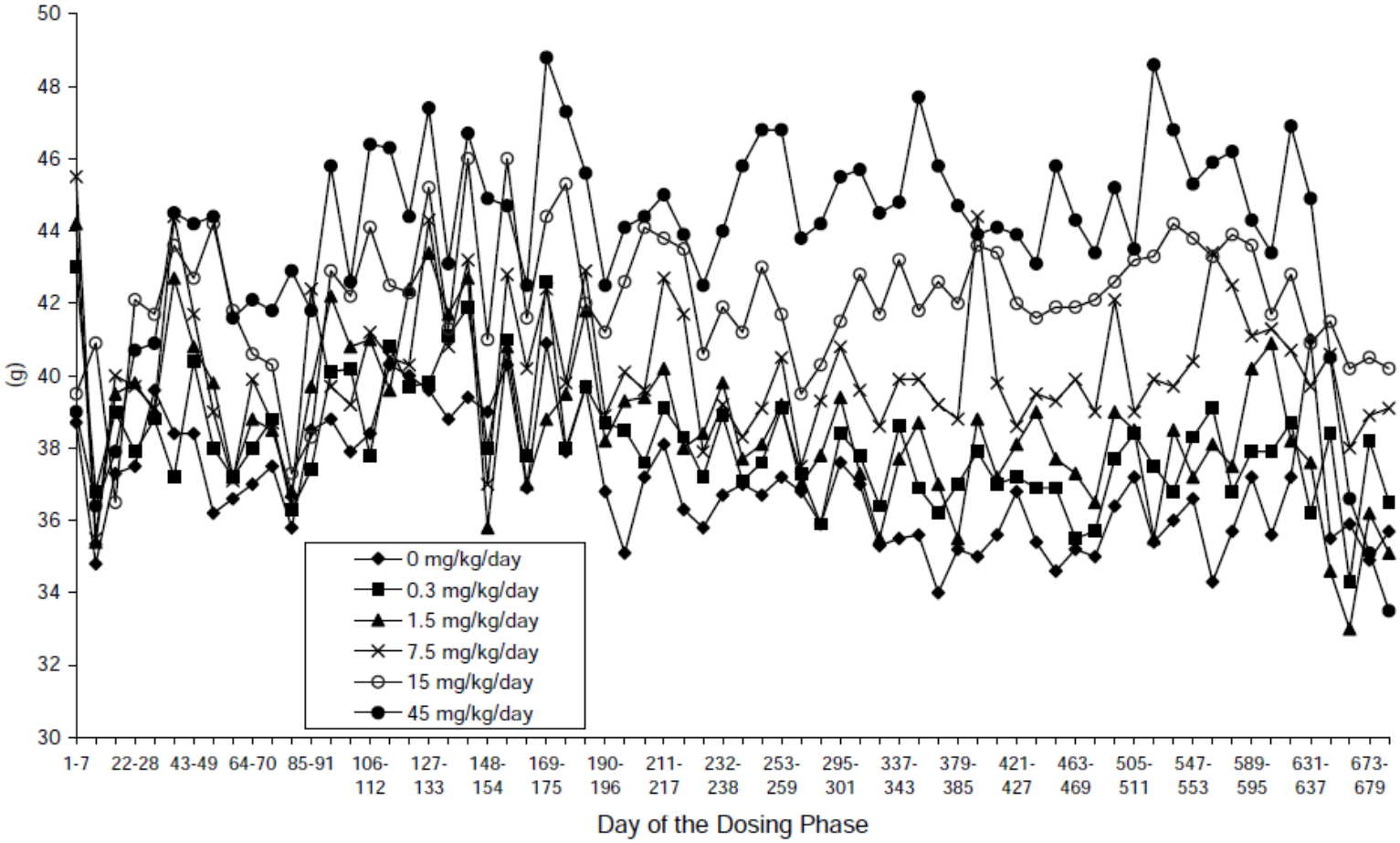


Figure 6
Mean Food Consumption Data - Females



Gross Pathology
Week 104 - Groups 1, 2, 3, and 5 males
Week 102 - Group 4 males
Week 99 - Group 6 males
Week 100 - All female groups

Gross Pathology Findings

	Males						Females					
Dose (mg/kg/d)	0	0.3	1.5	7.5	15	45	0	0.3	1.5	7.5	15	45
Liver												
Mass	19	22	33	31	22	25	6	1	1	11	13	9
Discolored	7	5	6	12	11	13	2	1	3	12	9	6
Cyst	2	5	3	1	9	8	1	3	1	4	5	2
Raised area	1	2	4	5	3	4	-	-	-	-	-	-
Spleen												
Large	2	3	7	12	13	10	-	-	-	-	-	-
Lung												
Discolored	7	3	3	1	3	11	6	8	5	8	6	12
Duodenum												
Discolored	0	0	1	0	2	3	0	0	0	2	3	4
Distended	4	0	2	4	5	8	2	1	1	0	7	7
Thickened	0	0	0	5	4	5	0	0	0	3	7	10
Ileum												
Discolored	0	0	0	0	2	0	0	0	0	0	1	2
Distended	0	0	0	2	1	2	1	0	0	0	0	2
Thickened	0	0	0	0	2	1	0	0	0	1	2	4
Jejunum												
Discolored	0	0	0	1	5	3	0	0	0	3	6	4
Distended	0	2	0	4	2	3	1	0	0	1	5	5
Thickened	0	0	1	3	6	9	0	0	0	11	14	10
Mass	0	0	1	1	3	0	0	0	1	1	3	2
Stomach - glandular												
Discolored	1	2	2	0	3	2	0	1	1	1	1	3
Ovary												
Cyst	NA	NA	NA	NA	NA	NA	34	34	44	49	50	44

"-" = no difference from control; NA = not applicable.

Histopathology

Peer Review: The pathology evaluation was reviewed by a second (b) (4) board-certified pathologist.

Summaries of noteworthy neoplastic and non-neoplastic microscopic findings are presented in the tables below.

Neoplastic Findings

	Males						Females					
Dose (mg/kg/d)	0	0.3	1.5	7.5	15	45	0	0.3	1.5	7.5	15	45
No. Examined	60	60	60	60	60	60	60	60	60	60	60	60
Liver												
Hepatocellular adenoma	**4*** (0.0011)	5	13	11	**16*** (0.0034)	**15*** (0.0013)	**1*** (<0.001)	0	1	**12*** (0.0035)	**12*** (0.0051)	**10*** (0.0065)
Hepatocellular carcinoma	23	22	**40*** (0.0088)	**38*** (0.0076)	36	30	**4*** (0.0016)	0	1	**17*** (0.0049)	16 (0.0119)	11
Both adenoma and carcinoma	2	2	9	7	9	8	0	0	0	6	3	2
Total with hepatocellular tumors	25 (0.0131)	25	**44*** (0.0061)	**42*** (0.0024)	**43*** (0.0071)	37 (0.0103)	**5*** (<0.001)	0	2	**23*** (<0.001)	**25*** (<0.001)	**19*** (0.0025)
Duodenum												
Adenoma	0	2	0	0	0	0	0	0	0	0	2	0
Carcinoma	**0*** (0.0107)	0	0	0	2 (0.2534)	2 (0.1875)	0	0	0	0	0	1
Total with duodenal tumor	0	2	0	0	2	2	0	0	0	0	2	1
Jejunum												
Adenoma	0	0	0	1	0	0	0	0	0	0	2	0
Carcinoma	**0*** (0.0110)	0	1	1	**6*** (0.0149)	3 (0.0786)	0	0	0	0	5 (0.0542)	1
Total with jejunal tumor	0	0	1	2	**6*** (0.0149)	3 (0.0786)	0	0	0	0	7	1
Ileum												
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0
Carcinoma	0	0	0	0	1	0	0	0	0	0	0	1
Total with ileal tumor	0	0	0	0	1	0	0	0	0	0	0	1

	Males						Females					
Dose (mg/kg/d)	0	0.3	1.5	7.5	15	45	0	0.3	1.5	7.5	15	45
No. Examined	60	60	60	60	60	60	60	60	60	60	60	60
Small Intestine Combined (duodenum + jejunum + ileum)												
Adenoma	0	2	0	1	0	0	0	0	0	0	3	0
Carcinoma	**0*** (<0.001)	0	1	1	**9*** (0.0019)	**5*** (0.0144)	**0*** (0.0086)	0	0	0	5 (0.0542)	3 (0.1304)
Total with small intestinal tumor	**0*** (0.0039)	2	1	2	**9*** (0.0019)	**5*** (0.0144)	**0*** (0.0111)	0	0	0	**8*** (0.0083)	3 (0.1304)
Colon												
Carcinoma	0	0	0	0	1	0	0	0	0	0	0	0
Cecum												
Adenoma	0	0	0	0	1	0	0	0	0	0	0	0
Large Intestine Combined (colon + cecum)												
Total with large intestinal tumor	0	0	0	0	2	0	0	0	0	0	0	0
Skin/Subcutis												
Fibrosarcoma	0	0	0	0	0	0	**0*** (0.0228)	0	0	0	0	2 (0.2536)
Lung												
Bronchiolar-alveolar adenoma	10	5	8	7	6	2	5	1	4	9	3	2
Bronchiolar-alveolar carcinoma	13	11	7	5	5	2	9	4	3	3	1	3
Total with bronchiolar-alveolar tumors	23	16	14	11	10	3	14	5	6	11	4	5

For Trend analysis: *p≤0.005 for common tumors and p≤0.025 for rare tumors.
For Pair-wise comparisons: *p≤0.01 for common tumors and p≤0.05 for rare tumors.

Non-neoplastic Findings

		Males						Females					
Dose (mg/kg/d)		0	0.3	1.5	7.5	15	45	0	0.3	1.5	7.5	15	45
Observation	**Grade**												
Duodenum - increased vacuolation, epithelium	-	57	57	45	32	27	25	59	56	49	12	9	27
	1	1	1	12	14	8	11	0	2	8	18	14	7
	2	0	1	2	11	14	16	0	0	1	29	32	22
	3	0	0	0	3	7	8	0	0	0	1	5	3
	Total	1	2	**14**	**28**	**29**	**35**	0	2	**9**	**48**	**51**	**32**
Jejunum - increased vacuolation, epithelium	-	57	49	35	36	27	26	60	55	43	14	7	27
	1	0	8	12	12	9	5	0	3	11	13	19	12
	2	0	2	10	9	15	16	0	0	5	15	24	14
	3	0	0	0	2	7	13	0	0	0	18	10	7
	Total	0	**10**	**22**	**23**	**31**	**34**	0	3	**16**	**46**	**53**	**33**
Ileum - increased vacuolation, epithelium	-	58	55	51	35	28	30	57	57	51	20	11	31
	1	0	1	9	20	14	15	0	1	3	19	18	17
	2	0	0	0	5	13	14	0	0	0	17	28	9
	3	0	0	0	0	1	0	0	0	0	1	2	0
	Total	0	1	**9**	**25**	**28**	**29**	0	1	3	**37**	**48**	**26**
Liver - lymphocyte/ macrophage inflammation/ infiltrate	-	35	37	43	53	51	51	25	30	29	34	32	40
	1	16	16	11	7	7	6	22	22	28	23	26	17
	2	6	4	3	0	2	2	12	6	1	2	2	0
	3	2	2	3	0	0	0	1	2	2	1	0	3
	4	1	1	0	0	0	1	0	0	0	0	0	0
	Total	25	23	17	7	9	9	35	30	31	26	28	20
Liver - increased hepatocyte vacuolation, diffuse	-	51	58	57	60	59	60	38	48	58	58	57	59
	1	5	1	1	0	0	0	15	11	2	1	1	0
	2	4	0	0	0	1	0	7	1	0	1	1	1
	3	0	1	1	0	0	0	0	0	0	0	1	0
	4	0	0	1	0	0	0	0	0	0	0	0	0
	Total	9	2	3	0	1	0	22	12	2	2	3	1
Lung - alveolar spicules	-	58	58	59	60	55	37	59	60	58	57	47	27
	1	2	2	1	0	5	21	1	0	2	3	12	28
	2	0	0	0	0	0	2	0	0	0	0	1	5
	Total	2	2	1	0	5	**23**	1	0	2	3	**13**	**33**
Lung - lymphocyte infiltrate	-	33	39	31	33	30	12	29	34	39	20	14	7
	1	17	10	20	17	25	33	21	16	11	26	29	24
	2	9	10	5	10	5	15	7	8	7	13	14	28
	3	1	1	3	0	0	0	3	2	3	1	3	1
	5	0	0	1	0	0	0	0	0	0	0	0	0
	Total	27	21	29	27	30	**48**	31	26	21	40	**46**	**53**
Lung - alveolar macrophage infiltrate	-	33	43	33	33	26	7	37	35	37	22	18	3
	1	13	9	18	18	27	36	11	14	17	27	26	24
	2	7	4	2	4	2	14	6	6	4	8	13	30
	3	2	3	6	5	3	3	2	4	1	3	3	3
	4	5	1	0	0	2	0	4	1	1	0	0	0

		Males						Females					
Dose (mg/kg/d)		0	0.3	1.5	7.5	15	45	0	0.3	1.5	7.5	15	45
Observation	**Grade**												
	5	0	0	1	0	0	0	0	0	0	0	0	0
	Total	27	17	27	27	34	**53**	23	25	23	**38**	**42**	**57**
Ovary - amyloidosis	-	NA	NA	NA	NA	NA	NA	46	51	54	55	58	60
	P	NA	NA	NA	NA	NA	NA	11	7	**5**	**5**	**2**	**0**
Ovary - parovarian cyst	-	NA	NA	NA	NA	NA	NA	17	18	12	6	7	6
	P	NA	NA	NA	NA	NA	NA	40	40	**47**	**54**	**53**	**54**
Skin/subcutis - inflammation/ epidermal hyperplasia	-	47	29	33	27	50	43	56	48	54	51	52	46
	1	1	1	1	0	0	0	0	1	0	2	2	0
	2	1	3	1	3	2	1	1	2	1	2	1	2
	3	3	9	5	5	4	4	3	3	4	2	3	3
	4	8	3	5	12	4	12	0	6	1	3	2	9
	Total	13	16	12	20	10	17	4	12	6	9	8	**14**
Skin/subcutis - ulcer/erosion	-	49	32	33	33	51	44	57	51	55	55	56	45
	1	0	0	1	0	0	0	0	0	0	0	0	0
	2	0	4	1	0	1	0	0	0	0	1	0	3
	3	7	5	4	2	3	3	3	3	3	1	2	5
	4	4	4	6	12	5	12	0	6	2	3	2	6
	5	0	0	0	0	0	1	0	0	0	0	0	1
	Total	11	13	12	14	9	16	3	9	5	5	4	**15**
Spleen - increased extramedullary hematopoiesis	-	31	26	30	12	17	12	32	22	32	15	17	21
	1	10	7	8	4	2	3	2	8	7	8	1	1
	2	10	12	13	10	5	8	10	17	10	14	12	8
	3	6	10	6	10	10	15	7	7	6	10	12	14
	4	3	4	2	23	25	22	9	6	5	12	17	15
	5	0	0	1	1	1	0	0	0	0	1	1	1
	Total	29	33	30	**48**	**43**	**48**	28	38	28	**45**	**43**	**39**
Sternum marrow - myeloid hyperplasia	-	42	28	32	25	42	35	43	27	30	25	47	33
	1	12	4	5	12	10	8	8	3	8	4	3	14
	2	5	8	6	8	5	16	6	13	4	5	8	12
	3	1	1	0	0	1	1	2	0	0	0	1	0
	Total	18	13	11	20	16	**25**	16	16	12	9	12	**26**

NA = not applicable.

In the sponsor-generated table below, a summary is presented for small intestinal epithelial vacuolation observed in animals that were evaluated at the scheduled necropsy (early decedents not included). With the exception of the 7.5 and 15 mg/kg/day female groups, dosing was discontinued for male and female animals between 2 and 14 weeks before sacrifice. Because mortality was not an issue for the 7.5 and 15 mg/kg/day female groups, dosing continued throughout the entire study for these two groups. It is noteworthy that of the animals that were necropsied at the scheduled sacrifice day, only the dose groups that were continually dosed had epithelial vacuolation, indicating that this was a reversible effect. This also indicates that the

incidences of epithelial vacuolation for the groups that received a dosing holiday are lower than if dosing had continued until the end of the study.

It is uncertain whether the dosing holiday had a similar effect on reversibility for hepatocyte vacuolation or alveolar foamy macrophage accumulation because these findings were not noted in this study, even at the two female dose levels that did not receive a dosing holiday. However, these findings were observed in the 3-month range-finding study in CD-1 mice. It is uncertain why these microscopic findings were not noted in this study but it is possible that the dosing holiday could have influenced the amount of lipid in hepatocytes and alveolar macrophages. It is also possible that the degree of lipid vacuolation decreases over time, as a similar result was observed in dogs with regard to hepatocellular lipid vacuolation.

Text Table 12
Incidences of Selected Nonneoplastic Microscopic Findings - Scheduled Dosing Phase Necropsies

Sex	Males						Females					
AEGR-733 mg/kg/day	0	0.3	1.5	7.5	15	45	0	0.3	1.5	7.5	15	45
Duodenum												
No. Examined	19	19	17	15	14	14	15	18	18	25	27	17
Vacuolation, Epithelium, Increased	0	0	0	0	0	0	0	0	0	25	27	0
Ileum												
No. Examined	19	17	17	15	14	14	14	18	17	24	27	17
Vacuolation, Epithelium, Increased	0	0	0	0	0	0	0	0	0	23	27	0
Jejunum												
No. Examined	19	19	17	15	14	14	15	18	18	25	27	17
Vacuolation, Epithelium, Increased	0	0	0	0	0	0	0	0	0	25	27	0

Transmission Electron Microscopy

Lung

Based on electron microscopy data from past studies, there has been some concern about possible drug-induced phospholipidosis in the lungs of treated rodents. Therefore, the sponsor conducted electron microscopy on the lungs from a subset of animals from this study (10/sex/group) to further evaluate the presence of lipid vacuolation. A summary of microscopic findings is shown in the sponsor-generated table below. Excessive cytoplasmic lipid vacuole accumulation in alveolar macrophages was observed in several drug-treated animals, particularly at the high dose. Two males receiving 0.3 mg/kg/day showed excessive cytoplasmic lipid vacuolation in type 2 pneumocytes; lipid vacuolation in this cell type was not observed at any of the higher dose levels. The TEM pathologist noted that the excessive lipid accumulation occurred in the absence of concurrent epithelial degeneration or inflammatory changes in the alveolar wall.

One female mouse at 15 mg/kg/day (not shown in summary table) had concentric lamellar inclusions with four lysosomes in one alveolar macrophage. Similar findings were not observed in other images for this same animal and therefore, the TEM pathologist did not consider the occurrence of lamellar structures in a single macrophage "to be indicative of excessive accumulation of concentric lamellar inclusions in lysosomes such that it would be definitive for phospholipidosis (Meador, 2005). Additionally, similar findings were not observed in the lung samples evaluated via TEM for other animals at any of the other doses of the test article evaluated in this study."

The TEM pathologist also made the following comment regarding phospholipidosis: "Relating to the differentiation of lipid accumulation from the occurrence of phospholipidosis, the description of the individual ultrastructural findings in lipid vacuoles in alveolar macrophages and pneumocytes frequently included description of membranous profiles within the lipid content of lipid vacuoles in control and drug-treated mice. Due to aldehyde fixation, tissue storage in aldehyde fixative, and tissue processing for TEM these membranous profiles may have been induced and lipids may have been partially or completely extracted from lipid vesicles (Ghadially, 1975; Hayat, 1989). While these effects may mimic phospholipidosis they showed no tendency to occur in lysosomes; therefore, they were not considered related to the phenomenon of phospholipidosis."

One additional finding that the TEM pathologist noted "was the frequent occurrence of crystalline inclusions in the cytoplasm of alveolar macrophages in mice of both sexes in the control and all AEGR-733 dosed groups. These crystals were long slender crystals that occurred singly or as aggregated multi-layered inclusions in the cytoplasm of macrophages. These crystals were usually free in the cytoplasm, but were occasionally closely associated with secondary lysosomes. In a very few animals, the crystals were also free in the alveolar space. The crystalline inclusions are known to occur as an idiopathic disease in mice and known to be predominantly composed of Ym1 protein that is secreted by alveolar macrophages and by neutrophils (Hoenerhoff et al., 2006)." The TEM pathologist considered these crystals to not be related to treatment. However, it is assumed that this observation correlates with the general pathologist's observation of "alveolar spicules", which after evaluating all 60 animals per group clearly shows a treatment-related effect at ≥7.5 mg/kg/day (see the table for non-neoplastic histopathology findings above). It should be noted that a similar dose-related finding was observed in the rat carcinogenicity study, so this effect was not limited to mice, as suggested by the reference that the TEM pathologist cited. The toxicological significance of the presence of alveolar spicules or crystalline inclusions is uncertain.

Overall, the TEM analysis of 10 animals/sex/group did not show evidence of classical drug-induced phospholipidosis at any dose level. The findings at ≥15 mg/kg/day were considered to be test-article related by the TEM pathologist; however, this reviewer feels that the lipid accumulation seen in a single male at 1.5 mg/kg/day cannot be ruled out as being treatment related. The TEM pathologist felt that the findings in two low-

dose males were of uncertain relevance because similar findings in type 2 pneumocytes were not observed at higher dose levels.

It should be noted that histiocytosis or the presence of foamy alveolar macrophages was not noted by light microscopy. However this finding was noted in a 3-month study that utilized the same strain of mouse and the same dose levels. It is uncertain why lipid accumulation in alveolar macrophages was not observed in the 2-year study. Although the dosing holiday at the end of the study could have affected the amount of lipid that remained in macrophages, as noted for small intestinal epithelium, the female 15 mg/kg/day group did not receive a dosing holiday and foamy macrophages were observed at 15 mg/kg/day in the 3-month mouse study at a low incidence.

Text Table 13
Summary of Transmission Electron Microscopy Findings of Excessive Lipid Vacuoles in Lung Macrophages or Pneumocytes

Time Point	Group/ Sex	Dose Level (mg/kg/day)	Number Examined	Number of Animals with Excessive Lipid Vacuoles in Macrophages or Pneumocytes
Dosing Phase - Final Phase	1M	0	10	0
Dosing Phase - Final Phase	2M	0.3	10	2
Dosing Phase - Final Phase	3M	1.5	10	1
Dosing Phase - Final Phase	4M	7.5	10	0
Dosing Phase - Final Phase	5M	15	10	0
Dosing Phase - Final Phase	6M	45	10	3
Dosing Phase - Final Phase	1F	0	10	0
Dosing Phase - Final Phase	2F	0.3	10	0
Dosing Phase - Final Phase	3F	1.5	10	0
Dosing Phase - Final Phase	4F	7.5	10	0
Dosing Phase - Final Phase	5F	15	10	1
Dosing Phase - Final Phase	6F	45	10	2

F = Female.
M = Male.

Liver carcinomas (Study Report #733-PC0018)
The purpose of this evaluation was to examine the livers by TEM from select mice from this study that were sacrificed moribund because of fatal liver carcinomas. The mice that were selected are shown in the sponsor-generated table below.

Group[a]	Animal Number	Day of Sacrifice	Dose Level (mg/kg/day)
1 (Control)	A28282	597	0
	A28298	624	
	A28331	624	
	A28336	636	
	A28338	596	
6 (High)	A28590	568	45
	A28600	568	
	A28609	540	
	A28634	526	
	A28637	583	

a Group 1 received basal diet only.

Ultrastructural Findings in the Liver Assessed by TEM

Finding	Control	45 mg/kg/d
N	**5**	**5**
Lipid globules, hepatocyte		
-minimally increased	1	0
-moderately increased	0	2
Lysosomes, hepatocyte		
-rare	1	2
-minimally increased	0	3
Peroxisomes, hepatocyte		
-minimally increased	0	1
-slightly increased	0	1
Vesiculation of rough ER, hepatocyte		
-slight	0	1

ER = endoplasmic reticulum; TEM = transmission electron microscopy

Vitamin Analysis

As shown in the sponsor-generated table below, vitamin A and E levels in the liver decreased in a dose-related manner. The decrease in the level of these fat soluble vitamins is an expected effect based on the mechanism of action of the test article as well as data from previously conducted toxicology studies.

Text Table 6
Liver Vitamin Analysis - Toxicokinetic Animals - 20 Hour Time Point During Week 27

Group		Vitamin A (µg/g)	Vitamin E (µg/g)
Control	M	2403 +/- 209	17.3 +/- 4.4
	F	2997 +/- 624	27.2 +/- 1.9
0.3 mg/kg/day	M	2255 +/- 193	29.5 +/- 5
	F	3218 +/- 412	33.8 +/- 6.1
1.5 mg/kg/day	M	2308 +/- 122	21.5 +/- 5.8
	F	2775 +/- 261	31.4 +/- 7.6
7.5 mg/kg/day	M	952 +/- 371	0
	F	937 +/- 569	19.6
15 mg/kg/day	M	521 +/- 233	0
	F	541 +/- 174	0
45 mg/kg/day	M	74 +/- 16	0
	F	136 +/- 36	0

F = Female.
M = Male.

Text Table 7
Liver Vitamin Analysis - Toxicokinetic Animals - 24 Hour Time Point During Week 27

Group		Vitamin A (µg/g)	Vitamin E (µg/g)
Control	M	2448 +/- 460	20.6 +/- 6.6
	F	3078 +/- 395	27.5 +/- 1.6
0.3 mg/kg/day	M	2303 +/- 331	33.4 +/- 9.7
	F	3143 +/- 332	37.0 +/- 9.2
1.5 mg/kg/day	M	1948 +/- 375	17.7 +/- 5.9
	F	2715 +/- 415	41.3 +/- 6.6
7.5 mg/kg/day	M	955 +/- 235	0
	F	1151 +/- 565	12.3 +/- 2.8
15 mg/kg/day	M	367 +/- 145	0
	F	710 +/- 360	0
45 mg/kg/day	M	99 +/- 26	0
	F	132 +/- 37	0

F = Female.
M = Male.

Text Table 8
Liver Vitamin Analysis - Carcinogenicity Animals - Dosing Phase Necropsy

Group		Vitamin A (µg/g)	Vitamin E (µg/g)
Control	M	4313 +/- 458	20.3 +/- 2.1
	F	6120 +/- 996	41.0 +/- 5.8
0.3 mg/kg/day	M	3390 +/- 1095	18.1 +/- 6.9
	F	4918 +/- 799	31.7 +/-6.6
1.5 mg/kg/day	M	3050 +/- 326	18.3 +/- 8.8
	F	4330 +/- 1651	32.2 +/- 9.7
7.5 mg/kg/day	M	1481 +/- 957	12.4 +/- 5.6
	F	1202 +/- 499	10.9 +/- 3.0
15 mg/kg/day	M	1067 +/- 218	10.8 +/-2.7
	F	324 +/- 53	0
45 mg/kg/day	M	793 +/- 215	6.78 +/- 2.1
	F	925 +/- 228	16.2 +/- 5.0

F = Female.
M = Male.

Toxicokinetics: (samples collected from up to 4 animals/sex/group during Week 27 at approximately 4, 8, 12, 16, 20, and 24 hours after start of dark room cycle, as rats are nocturnal feeders; it was not stated in the report when new food was placed in cages, but it is assumed it was at the beginning of the dark cycle)

A summary of TK results is shown in the sponsor-generated tables and figures below.

Table 1. Toxicokinetic Parameters for AEGR-733 in Mouse Plasma

Dose Group	AEGR-733 Salt Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg)]	Male/Female Ratio C_{max}	Male/Female Ratio AUC_{0-24}
8	0.3	M	1.59	5.28	16.0	28.0	28.0	93.2	0.988	1.04
		F	1.61	5.35	8.00	26.9	26.9	89.8		
9	1.5	M	6.68	4.45	12.0	139	139	92.7	0.990	1.18
		F	6.74	4.50	8.00	118	118	78.5		
10	7.5	M	43.6	5.81	16.0	772	772	103	0.904	1.18
		F	48.3	6.43	8.00	657	657	87.5		
11	15	M	95.0	6.34	12.0	1827	1827	122	0.876	1.18
		F	109	7.24	8.00	1546	1546	103		
12	45	M	277	6.16	8.00	5329	5329	118	1.01	0.994
		F	273	6.07	20.0	5364	5364	119		

Table 3. Toxicokinetic Parameters for BMS-203304 in Mouse Plasma

Dose Group	AEGR-733 Salt Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg)]	$t_{1/2}$ (hr)	M/P Ratio AUC_{0-24}	Male/Female Ratio C_{max}	Male/Female Ratio AUC_{0-24}
8	0.3	M	5.26	17.5	4.00	102	102	340	5.67	3.65	1.70	2.15
		F	3.10	10.3	8.00	42.2	47.5	158	NC	1.76		
9	1.5	M	28.4	18.9	16.0	436	436	290	NC	3.13	2.14	2.08
		F	13.3	8.86	8.00	210	210	140	NC	1.78		
10	7.5	M	133	17.7	8.00	2444	2444	326	NC	3.17	1.56	1.82
		F	84.8	11.3	4.00	1341	1341	179	NC	2.04		
11	15	M	369	24.6	12.0	6034	6034	402	NC	3.30	1.77	1.43
		F	208	13.8	4.00	4209	4209	281	NC	2.72		
12	45	M	1076	23.9	4.00	18285	18285	406	NC	3.43	1.64	1.73
		F	654	14.5	4.00	10552	10552	234	NC	1.97		

Table 5. Toxicokinetic Parameters for BMS-203215 in Mouse Plasma

Dose Group	AEGR-733 Salt Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg)]	M/P Ratio AUC_{0-24}	Male/Female Ratio C_{max}	Male/Female Ratio AUC_{0-24}
9	1.5	M	5.34	3.56	16.0	93.3	93.3	62.2	0.671	0.889	1.03
		F	6.01	4.00	16.0	90.2	90.2	60.2	0.766		
10	7.5	M	42.1	5.61	8.00	746	746	99.4	0.966	1.38	1.34
		F	30.4	4.05	8.00	557	557	74.2	0.848		
11	15	M	93.4	6.23	12.0	1970	1970	131	1.08	0.849	1.04
		F	110	7.34	16.0	1890	1890	126	1.22		
12	45	M	832	18.5	8.00	10397	10397	231	1.95	2.52	1.56
		F	330	7.33	4.00	6662	6662	148	1.24		

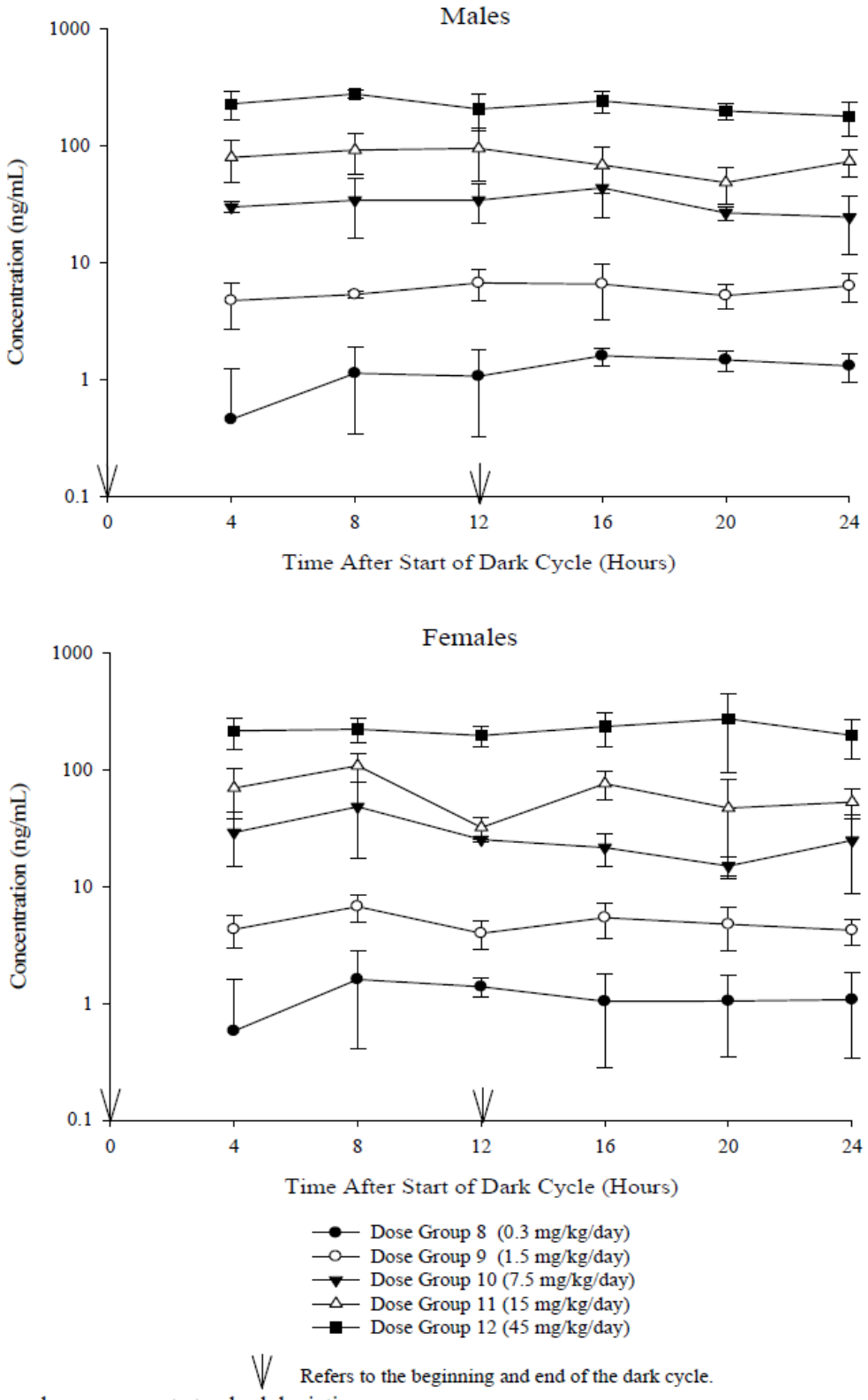
Note: All Dose Group 8 concentration values were below the lower limit of quantitation; therefore, no toxicokinetic analysis was performed.

Table 7. Toxicokinetic Parameters for BMS-224433 in Mouse Plasma

Dose Group	AEGR-733 Salt Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/(mg/kg)]	M/P Ratio AUC_{0-24}	Male/Female Ratio C_{max}	Male/Female Ratio AUC_{0-24}
9	1.5	M	0.695	0.463	16.0	4.16	5.55	3.70	0.0399	0.440	0.455
		F	1.58	1.05	16.0	10.8	12.2	8.14	0.104		
10	7.5	M	13.9	1.85	8.00	269	269	35.9	0.349	1.41	1.35
		F	9.84	1.31	12.0	199	199	26.5	0.303		
11	15	M	28.4	1.90	16.0	609	609	40.6	0.334	0.784	0.866
		F	36.3	2.42	16.0	704	704	46.9	0.455		
12	45	M	104	2.31	4.00	2261	2261	50.2	0.424	0.948	1.15
		F	109	2.43	4.00	1965	1965	43.7	0.366		

Note: All Dose Group 8 concentration values were below the lower limit of quantitation; therefore, no toxicokinetic analysis was performed.

Mean concentrations (ng/mL) of AEGR-733 in male and female mouse plasma as a function of dose



Note: Error bars represent standard deviations.

The mean concentration over time for the metabolites had a similar profile as the parent, and therefore, the figures are not included. As noted in the tables above, metabolites BMS-203215 (M1) and BMS-224433 (M2) were below the lower limit of quatitation (LLOQ) at all time points at the 0.3 mg/kg/day dose level. Additionally, BMS-224433 was below the LLOQ at the 8 and 16 hour time points for males and at the 8, 16, and 20 hour time points for females at 1.5 mg/kg/day. Metabolite GMS-203304 (M3) was below the LLOQ at the 24 hour time point for females receiving 0.3 mg/kg/day.

Dosing Solution Analysis

Dose concentration was evaluated during Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 104. Some drug-diet admixtures were occasionally found to be slightly outside of the allowable ±15% of target concentration during Weeks 1, 13, and 52. In these cases, the difference between the actual dose and nominal dose was generally small. Therefore, treated animals were considered to have received the intended doses and it was not felt that these deviations negatively impacted the interpretability of the study results.

Study title: 104-Week Oral Gavage Carcinogenicity Study with AEGR-733 (formerly BMS-201038) in Rats - Amendment 1

Study no.:	733-PC0002 (7881-101) and 733-PC0004 (satellite groups to determine vitamin concentrations in blood and liver)
Study report location:	Module 4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	29 April 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AEGR-733, Lot #L0109571, 84.7%
CAC concurrence:	Yes

Key Study Findings

- Increased survival was noted for the mid- and high-dose groups, reaching statistical significance for females. Low-dose females were sacrificed during Week 95 due to decreased survival. All remaining female groups were terminated during Week 97 because the number of control animals reached 20. All male groups were terminated during Week 99 because the number of control animals reached 20.
- Mean final body weights were statistically significantly decreased compared with control for mid-dose females and high-dose males and females. Effects on body weight did not appear to be due to consistent differences in food consumption.
- Group 3 and 4 diets were fortified with additional fat-soluble vitamins starting at around Day 406 because of vitamin deficiencies noted in satellite group animals.
- Macroscopic findings included an increased incidence of discolored and large livers and discolored lungs for all treated male groups and mid- and high-dose females. Increased incidence of discolored, distended, and/or thickened duodenum was observed for HD animals, discolored and/or thickened ileum for HD females, and discolored and/or thickened jejunum for MD males and HD males and females.
- There was a statistically significant trend for increased pancreatic acinar cell adenomas in males, but the pair-wise comparison for the HD group fell short of the 0.01 p-value needed for statistical significance (p=0.077). There was an increase in tumor multiplicity for the HD group, with 8 of 15 (53%) animals having multiple acinar cell tumors compared with 1 of 7 (14%) animals in the control group. The acinar cell tumors found in HD animals did not appear to develop sooner than in the control animals. Small, non-statistical numerical increases were observed for combined hepatocellular adenomas plus carcinomas at the HD level for males and females and for thyroid follicular cell adenomas in HD males. One HD male had a carcinoma in the jejunum.
- Non-neoplastic findings were observed in the liver (cystic degeneration [MD and HD males]; focal/multifocal fibrosis [all male groups and MD and HD females]; centrilobular hepatocyte vacuolation [all treated groups]); lung (thicked alveolar septa associated with alveolar macrophage infiltrates [all treated groups]; alveolar spicules [all treated groups]; lymphocyte infiltration [all treated males and MD and HD

females]; alveolar macrophage infiltrate [MD and HD groups]; pleural/subpleural fibrosis [MD and HD females]; septal cell mineralization [MD and HD males]); pancreas (acinar cell hyperplasia [MD males and HD groups]; acinar epithelium vacuolation [all treated males and MD and HD females]; decreased zymogen [MD males and HD groups]); small intestine (increased epithelial vacuolation in jejunum and duodenum [all treated groups]; increased epithelial vacuolation in ileum [MD males and HD groups]; ovary (parovarian cyst [MD and HD females]); mesenteric lymph node (sinus erythrocytes [MD males and HD groups]); and adrenal gland (medulla hyperplasia [HD groups]).

- Although the sponsor concluded that the pancreatic acinar cell adenomas were likely treatment related because of statistical significance for trend analysis and increased tumor multiplicity, the Executive Carcinogenicity Assessment Committee did not feel that the increase in this benign tumor was treatment related. Therefore, there were no treatment-related increases in neoplasms at male doses up to 7.5 mg/kg/day and at female doses up to 2 mg/kg/day, which resulted in exposures that were approximately 6-fold and 8-fold higher than the clinical exposure, respectively.

Adequacy of Carcinogenicity Study

The carcinogenicity study was adequately designed and conducted, although it would have been preferable to have a water control group in addition to a vehicle control group. Also, the ECAC had some concern about dose groups receiving different amounts of supplemental vitamins; at Week 104, some of the vitamin-supplemented groups did have slightly higher mean vitamin A and/or E compared with controls.

It should be noted that the Division previously determined that for supporting a marketing application for the homozygous familial hypercholesterolemia indication, only a single carcinogenicity study would be required. An application that included patient populations not qualifying for an orphan designation would require data from a second carcinogenicity study. Because the NDA under review is only for the homozygous recessive population, the sponsor intends for the mouse carcinogenicity study to be the primary study to support marketing approval. The rat study is included as supportive information, and therefore, the fact that the high dose only resulted in an approximate 7-fold exposure margin to the clinical dose of 60 mg/day (rather than ≥25X) is not considered to be an issue for the evaluation of this marketing application.

Appropriateness of Test Model

The test model was appropriate to assess the carcinogenic potential of AEGR-733.

Evaluation of Tumor Findings

Small, non-statistically significant numerical increases were observed for combined hepatocellular adenomas plus carcinomas at the HD level for males and females, for thyroid follicular cell adenomas and pancreatic acinar cell adenomas in HD males. One HD male had a carcinoma in the jejunum. Although trend analysis showed statistical

significance and there was an increase in the percentage of males with multiple pancreatic acinar cell adenomas at the high dose, pair-wise analysis with the control group did not show statistical significance. Therefore, the numerical increase in pancreatic acinar cell adenomas was not considered to be treatment related. Because a statistically significant increase in hepatocellular and small intestinal tumors were observed in mice and there was a small numerical increase in these tumors in rats, it is possible that higher doses (i.e., up to 25X clinical exposure) in rats may have also resulted in statistically significant increases in these tumor types; however, under the conditions of this study, these findings are not considered to be treatment related.

Therefore, the NOEL for tumorigenesis is considered to be 7.5 mg/kg/day for males and 2 mg/kg/day for females (the highest doses tested). At the male NOEL, exposures for the parent, M1 metabolite, and M3 metabolite are approximately 6, 13, and 1 times the anticipated clinical exposures at 60 mg/day. At the female NOEL, respective exposures for the parent, M1 metabolite, and M3 metabolite are approximately 8, 8, and 3 times the anticipated clinical exposures at 60 mg/day.

Methods

Species/Strain: Rat/Sprague-Dawley
Study design: See sponsor-generated table below

Group	No. of Animals		Dose Level (mg/kg/day)		Dose Concentration (mg/mL)	
Carcinogenicity Animals	Male	Female	Male	Female	Male	Female
1 (Control)[a]	60	60	0	0	0	0
2 (Low)	60	60	0.25	0.03	0.25	0.03
3 (Mid)	60	60	1.7	0.35	1.7	0.35
4 (High)	60	60	7.5	2.0	7.5	2.0
Toxicokinetic Animals						
5 (Control)[a]	12	12	0	0	0	0
6 (Low)	12	12	0.25	0.03	0.25	0.03
7 (Mid)	12	12	1.7	0.35	1.7	0.35
8 (High)	12	12	7.5	2.0	7.5	2.0
9 Sentinel Animals[b]	10	10			NA	

NA = Not applicable.
a Groups 1 and 5 received control article only. The control article was 75% polyethylene glycol-400 solution (v/v) in reverse osmosis water.
b Sentinel animals were not dosed.

Frequency of dosing: Once daily
Dose volume: 1 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: 75% PEG-400 in reverse osmosis water
Basis of dose selection: Data from a 6-month repeat-dose toxicity study with and without subcutaneous vitamin supplementation.
Age: 5 to 6 weeks at initiation of dosing
Animal housing: Individually in stainless steel cages, or polycarbonate cages with bedding when necessary for health reasons.

Paradigm for vitamin replacement:	AEGR-733 inhibits absorption of fat soluble vitamins from the intestine leading to toxicity from vitamin deficiency. All animals were fed Harlan Teklad rodent diet #2018S, which contains more vitamin A and K than standard rodent diet. Beginning on Day 407, all animals in Group 3 received a vitamin fortified diet containing 5 times the concentrations of vitamins A, D, and E contained in the standard diet (#2018SC diet 5X A, D, E) and all animals in Group 4 received a vitamin fortified diet containing 10 times the concentrations of vitamins A, D, and E (#2018SC (10X A, D, E). The decision regarding when to change diet and how many extra fat soluble vitamins to add was dependent upon blood and liver sampling from another group of animals that were being treated concurrently with the same dose levels, but conducted and reported under a different study number (733PC0004).
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Yes, TK group for measuring drug plasma concentrations and separate satellite groups conducted under study 733PC0004 to assess for drug-induced vitamin deficiency.
Deviation from study protocol:	There were no deviations from the study protocol that affected the integrity or interpretability of the study.

Observations and Results

Mortality (checked twice daily)

As shown in the sponsor-generated table and figures below, groups receiving the mid- and high-dose levels had higher survival than controls, which was statistically significant for the mid-and high-dose females.

Sex	Males				Females			
AEGR-733 mg/kg/day	0	0.25	1.7	7.5	0	0.03	0.35	2.0
Total No. of Animals	60	60	60	60	60	60	60	60
Number of Animals at Dosing Phase Necropsy	20	17	28	30	20	15	32	41
Percentage Survival	33	28	47	50	33	25	53**	68*

* = Statistically significant at 1% level with Cox-Tarone Test and Gehan-Breslow Test.
** = Statistically significant at 5% level with Cox-Tarone Test at 1 % level with Gehan-Breslow Test.

Figure 1
Adjusted Survival Data (%) - Males

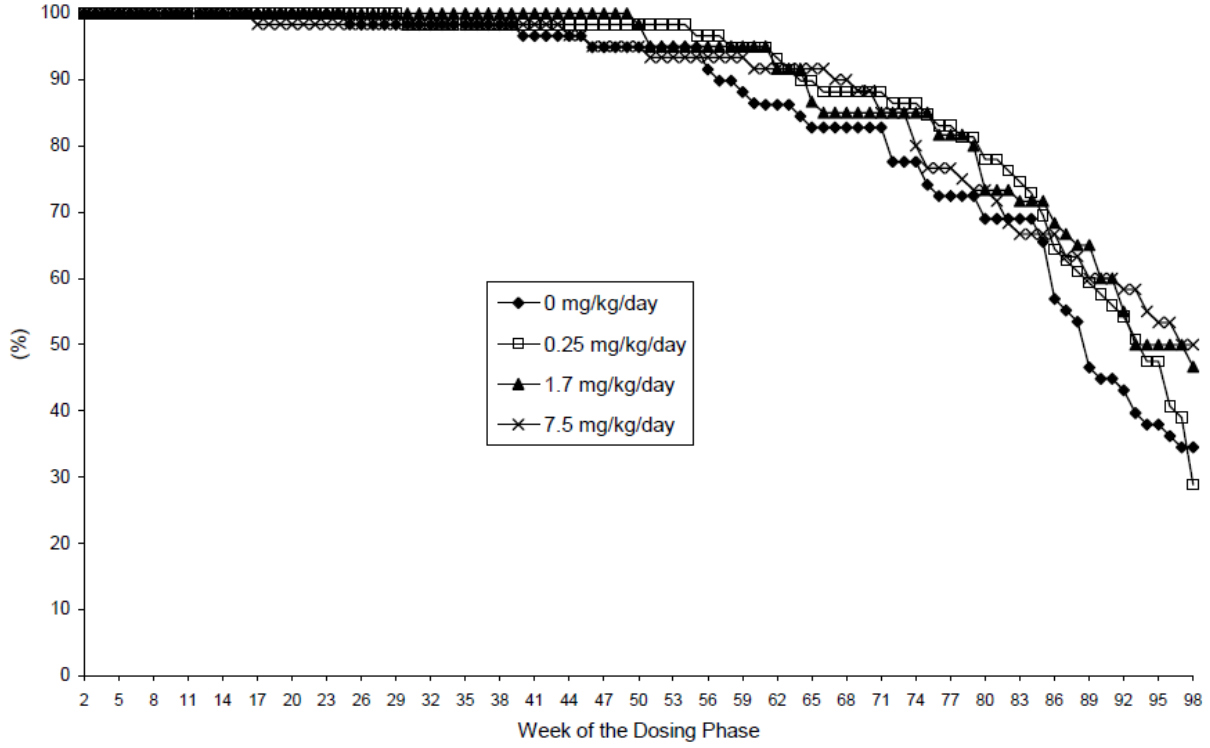
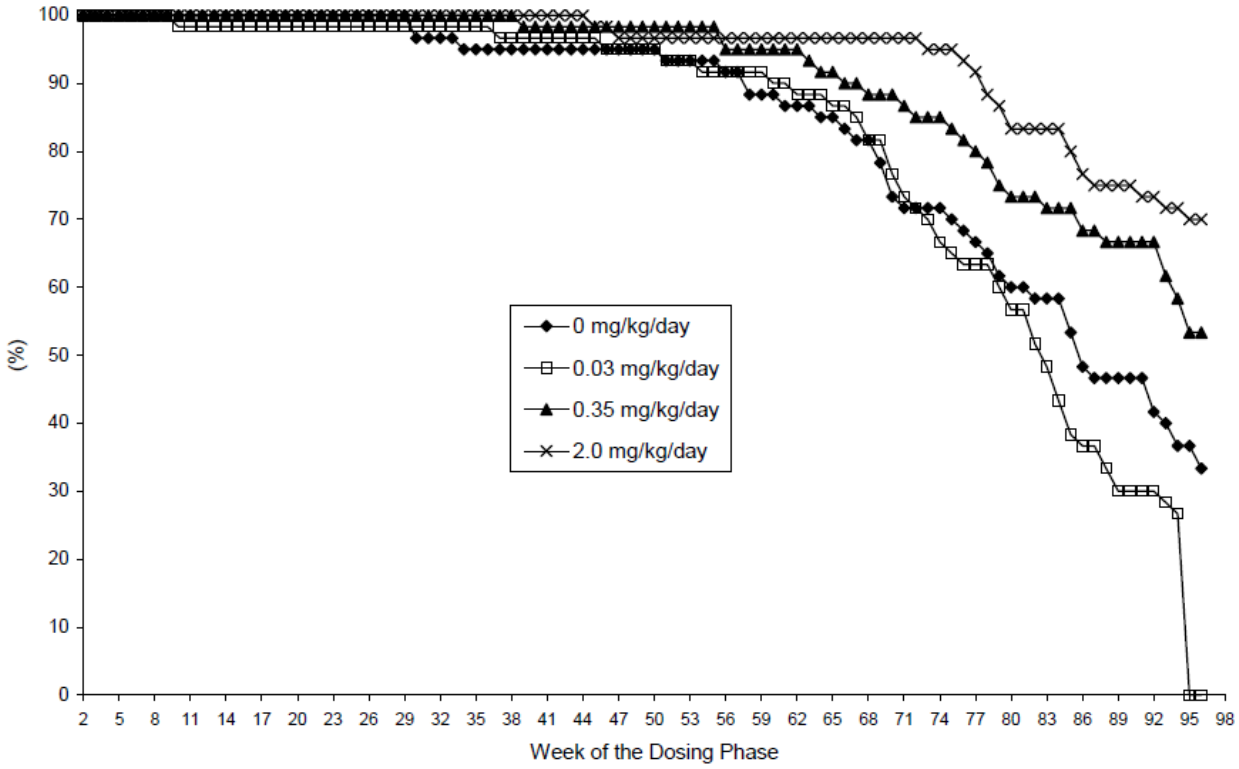


Figure 2
Adjusted Survival Data (%) - Females



Clinical Signs (checked twice daily with detailed examinations weekly)

There were no treatment-related, adverse clinical signs noted during the study.

Body Weights (measured weekly through Week 14 and every 4 weeks thereafter)

	Males				Females			
Dose (mg/kg/d)	**0**	**0.25**	**1.7**	**7.5**	**0**	**0.03**	**0.35**	**2.0**
Terminal Weight (g)	933	881	869	766*	597	575	533*	482*
Diff from control (g)		-52	-64	167		-22	-64	-115
% diff from control		↓6%	↓7%	↓18%		↓4%	↓11%	↓19%

*p<0.05.

Noteworthy decreases in final body weights were noted for males receiving 7.5 mg/kg/day and females receiving ≥0.35 mg/kg/day. This effect may have been due to the pharmacological activity of the test article, which limits fat absorption. There were no consistent differences in food consumption between treated and control animals. A summary of body weight and food consumption is presented in the sponsor-generated figures below.

Figure 3
Mean Body Weights - Males

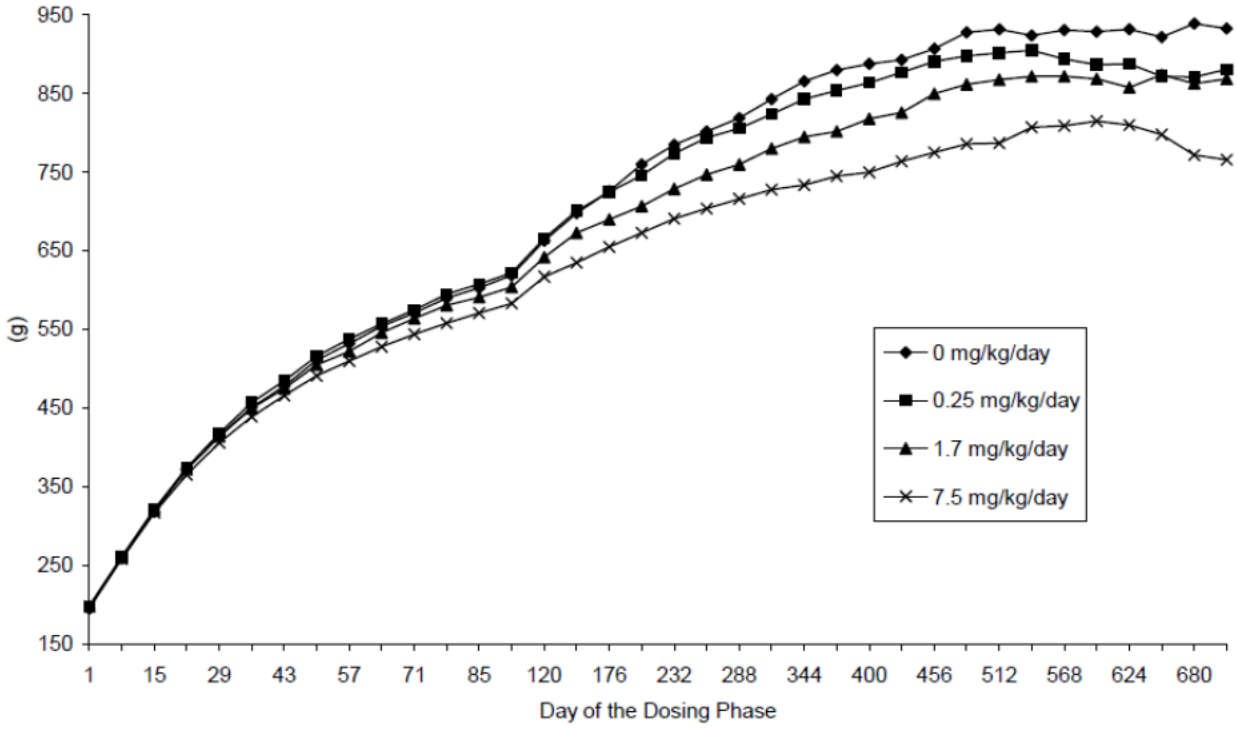
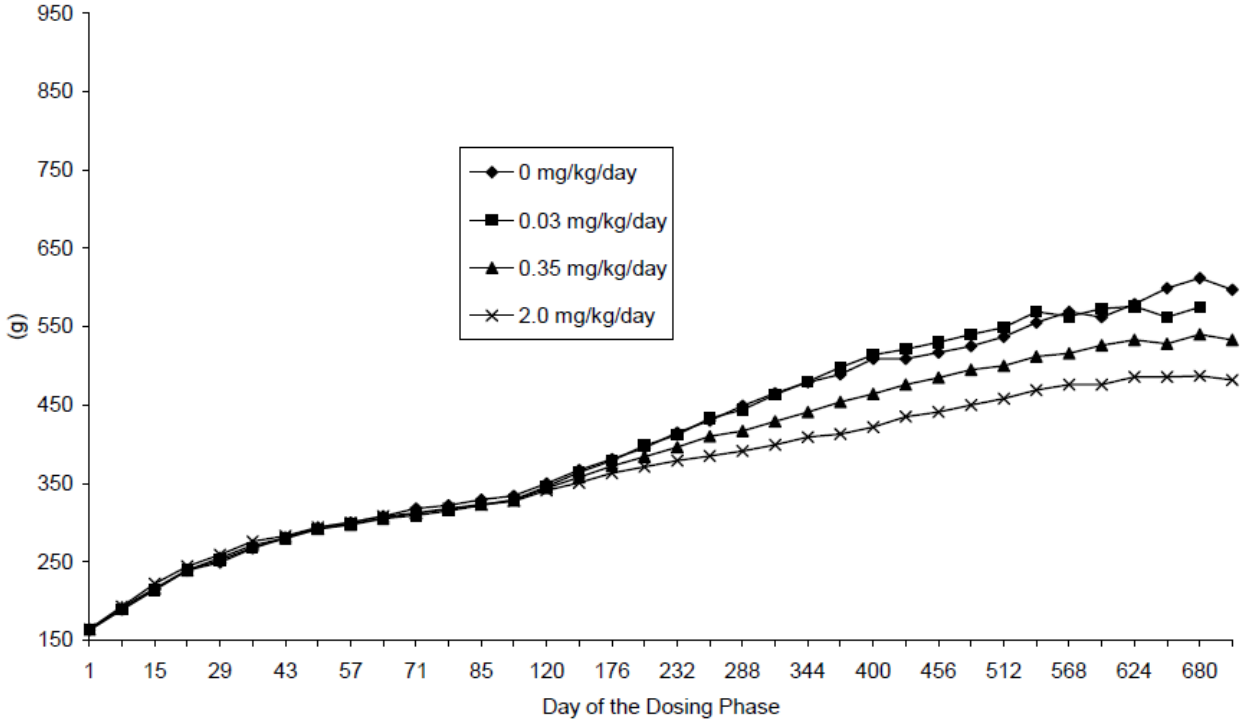
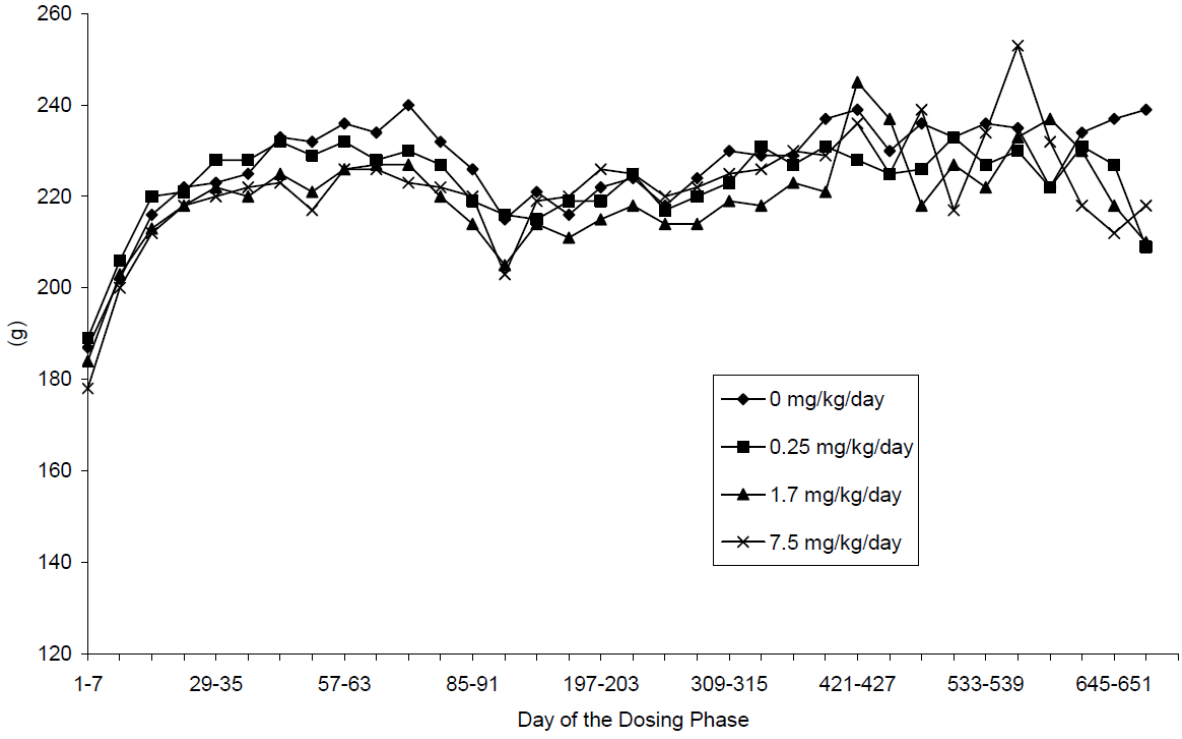


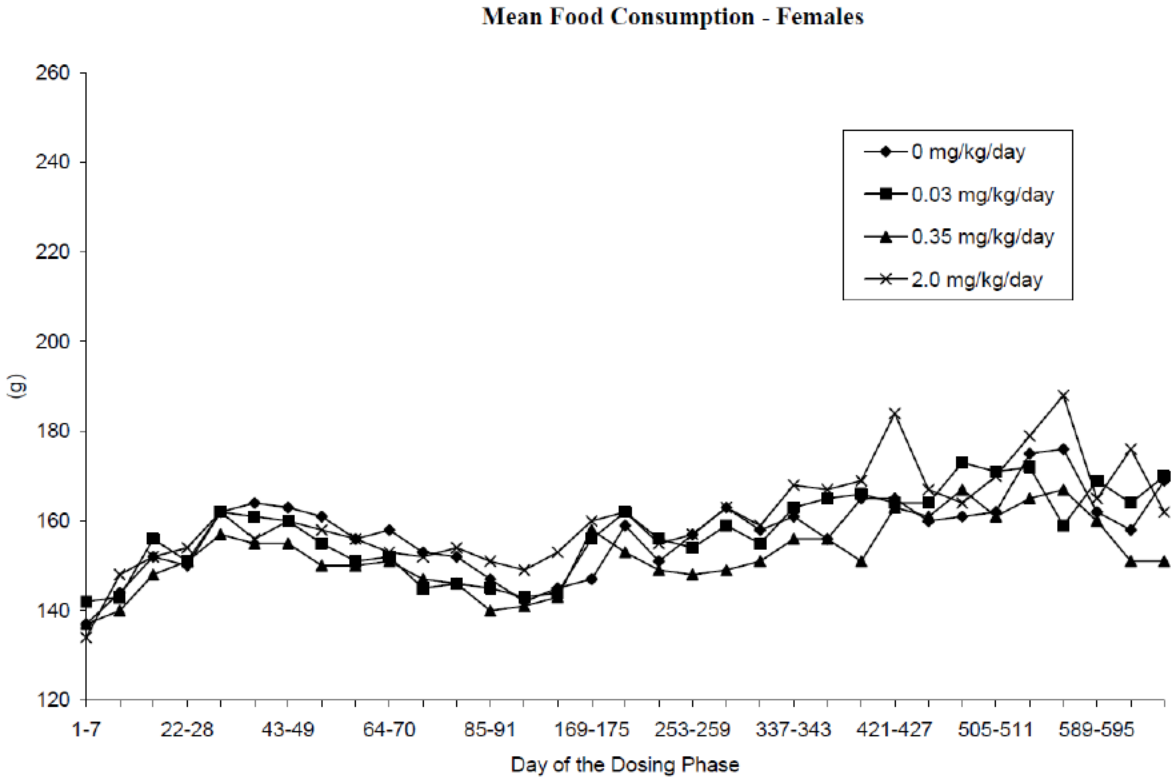
Figure 4
Mean Body Weights - Females



Feed Consumption

Mean Food Consumption - Males





Gross Pathology

Necropsies were conducted on all animals that died or were sacrificed at an unscheduled interval. Group 2 females were euthanized on Day 664 and all remaining female groups on Day 679. All male groups were euthanized on Day 688.

Macroscopic Findings

	Males				Females			
Dose (mg/kg/d)	**0**	**0.25**	**1.7**	**7.5**	**0**	**0.03**	**0.35**	**2.0**
Finding N	60	60	60	60	60	60	60	60
Liver								
-Discolored	11	**20**	**36**	**37**	14	18	**41**	**41**
-Large	2	**11**	**13**	**14**	0	2	**21**	**21**
Lung								
-Discolored	3	8	**21**	**20**	2	5	**7**	**34**
Duodenum								
-Discolored	1	0	1	**5**	0	0	1	**8**
-Distended	0	0	0	**7**	0	0	0	**2**
-Thickened	1	1	4	**8**	0	0	2	**14**
Ileum								
-Discolored	1	0	1	2	0	0	0	**4**
-Thickened	1	0	0	2	0	0	0	**2**
Jejunum								
-Discolored	1	2	**6**	**9**	0	0	1	**12**
-Thickened	1	0	**5**	**8**	0	0	2	**29**

Histopathology

Peer Review: Yes, by a second (b) (4) board-certified pathologist

Neoplastic Findings

		Males				Females			
Dose (mg/kg/d)		**0**	**0.25**	**1.7**	**7.5**	**0**	**0.03**	**0.35**	**2.0**
Finding									
Liver	N	60	59	60	60	60	60	60	60
Hepatocellular adenoma	P	1	0	2	2	4 (0.0404)	0	2	7 (0.4283)
Hepatocellular carcinoma	P	1	2	0	3	0	1	2	2
Adenoma plus carcinoma	Total	2 (0.0889)	2	2	5 (0.2787)	4	1	4	9
Pancreas	N	60	59	60	60	60	60	60	60
Acinar cell adenoma	P	7* (0.0036)	5	5	15 (0.0772)	0 (0.1316)	2	1	3 (0.1703)
Thyroid	N	60	59	60	60	60	48	29	60
Follicular cell adenoma	P	4 (0.0172)	5	9	12 (0.0443)	1	0	1	2
Follicular cell carcinoma	P	1	2	0	3	0	0	0	0
Adenoma plus carcinoma	Total	5 (0.0192)	6	9	13 (0.0556)	1	0	1	2
Jejunum	N	54	52	55	57	58	60	58	59
Carcinoma	P	0	0	0	1	0	0	0	0
Vascular	N	60	42	34	60	60	45	28	60
Hemangioma	P	0	0	1	1	0	0	1	0
Hemangio-sarcoma	P	0	3	1	2	0	0	0	0

N = number of animals examined; P = present.
For Trend analysis: *p≤0.005 for common tumors and p≤0.025 for rare tumors.
For Pairwise comparisons: *p≤0.01 for common tumors and p≤0.05 for rare tumors.

(sponsor-generated table)

Incidences of Multiplicity of Pancreatic Adenomas - Unscheduled and Scheduled Necropsies

Sex	Males				Females			
AEGR-733 mg/kg/day	0	0.25	1.7	7.5	0	0.03	0.35	2.0
Pancreas								
Number Examined	60	59	60	60	60	60	60	60
Acinar Cell Adenoma	7	5	5	15	0	2	1	3
Number of animals with Multiplicity[a]	1	0	2	8	0	0	0	0

a More than one tumor per animal.

(sponsor-generated table)

Incidences of Pancreatic Acinar Cell Adenoma at Selected Time points (Week of Sacrifice)

Sex	Males				Females			
AEGR-733 mg/kg/day	0	0.25	1.7	7.5	0	0.03	0.35	2.0
Acinar Cell Adenoma								
Time of Unscheduled or Scheduled Necropsy								
Weeks 0 through 52	0/4	0/1	0/3	0/4	0/4	0/4	0/1	0/2
Weeks 53 through 78	1/14	1/10	0/8	1/11	0/17	1/18	0/12	0/5
Weeks 79 through 92	3/17	1/16	0/16	1/10	0/14	1/20	0/7	0/9
Weeks 93 through Terminal Sacrifice	0/5	1/15	0/5	2/5	0/5	0/3	1/8	0/3
Terminal Sacrifice	3/20	2/17	5/28	11/30	0/20	0/15	0/32	3/41
Total	7/60	5/59	5/60	15/60	0/60	2/60	1/60	3/60

Non Neoplastic Findings

		Males				Females			
Dose (mg/kg/d)		**0**	**0.25**	**1.7**	**7.5**	**0**	**0.03**	**0.35**	**2.0**
Observation	**Grade**								
Liver - cystic degeneration	-	48	49	33	37	57	57	54	55
	1	10	9	22	20	2	3	4	2
	2	1	1	4	3	1	0	2	3
	3	1	0	1	0	0	0	0	0
	Total	12	10	**27**	**23**	3	3	6	5
Liver - fibrosis, focal/multifocal	-	56	51	43	32	56	57	43	42
	1	4	4	10	19	4	1	7	10
	2	0	4	4	8	0	2	8	7
	3	0	0	3	1	0	0	2	1
	Total	4	**8**	**17**	**28**	4	3	**17**	**18**
Liver - hepatocyte vacuolation, centrilobular	-	50	5	1	0	42	12	0	1
	1	3	0	2	0	9	10	0	0
	2	6	10	4	2	9	14	1	0
	3	1	20	10	12	0	19	10	15
	4	0	23	42	46	0	4	42	36
	5	0	1	1	0	0	1	7	8
	Total	10	**54**	**59**	**60**	18	**48**	**60**	**59**
Lung - pleural / subpleural fibrosis	-	45	48	41	50	56	56	48	39
	1	14	11	18	8	3	4	12	17
	2	0	0	1	2	1	0	0	4
	4	1	0	0	0	0	0	0	0
	Total	15	11	19	10	4	4	**12**	**21**

		Males				Females			
Dose (mg/kg/d)		0	0.25	1.7	7.5	0	0.03	0.35	2.0
Observation	Grade								
Lung - thickened, alveolar septa associated with alveolar macrophage infiltrates	-	57	51	25	18	59	56	39	33
	1	3	7	25	36	1	4	19	19
	2	0	1	10	6	0	0	2	7
	3	0	0	0	0	0	0	0	1
	Total	3	**8**	**35**	**42**	1	**4**	**21**	**27**
Lung - alveolar spicules	-	51	40	29	25	58	55	39	16
	1	9	18	25	33	2	5	19	24
	2	0	1	6	2	0	0	2	16
	3	0	0	0	0	0	0	0	4
	Total	9	**19**	**31**	**35**	2	5	**21**	**44**
Lung - lymphocyte infiltrate assoc. with alveolar macrophage accumulations	-	47	37	30	15	50	48	32	11
	1	12	18	20	35	10	12	24	24
	2	1	4	10	10	0	0	4	23
	3	0	0	0	0	0	0	0	2
	Total	13	**22**	**30**	**45**	10	12	**28**	**49**
Lung - macrophage infiltrate, alveolus	-	26	21	8	6	26	22	19	1
	1	28	28	26	32	33	36	30	24
	2	5	9	21	18	1	2	10	19
	3	1	1	5	4	0	0	1	16
	Total	34	38	**52**	**54**	34	38	**41**	**59**
Lung - septal cell mineralization	-	58	55	50	53	60	58	60	59
	1	2	4	10	5	0	2	0	1
	2	0	0	0	2	0	0	0	0
	Total	2	4	**10**	**7**	0	2	0	1
Pancreas - acinar cell hyperplasia	-	57	55	49	52	59	60	60	54
	1	1	3	3	1	0	0	0	0
	2	2	1	1	2	0	0	0	3
	3	0	0	2	3	1	0	0	2
	4	0	0	5	1	0	0	0	1
	5	0	0	0	1	0	0	0	0
	Total	3	4	**11**	**8**	1	0	0	**6**
Pancreas - vacuolation, acinar epithelium	-	60	53	52	55	60	60	57	23
	1	0	3	4	5	0	0	3	26
	2	0	3	4	0	0	0	0	11
	Total	0	**6**	**8**	**5**	0	0	**3**	**37**
Pancreas - decreased zymogen	-	49	45	38	27	16	46	50	20
	1	6	11	8	11	12	13	7	26
	2	5	3	14	22	2	1	3	14
	Total	11	14	**22**	**33**	14	14	10	**40**

		Males				Females			
Dose (mg/kg/d)		0	0.25	1.7	7.5	0	0.03	0.35	2.0
Observation	Grade								
Pancreas - focal atrophy/ loss, acinar epithelium	-	51	46	47	44	53	54	54	52
	1	4	10	10	11	6	5	5	5
	2	4	2	3	4	1	0	1	3
	3	1	1	0	0	0	0	0	0
	4	0	0	0	1	0	1	0	0
	Total	9	13	13	16	7	6	6	8
Jejunum - increased epithelial vacuolation	-	54	41	13	4	58	58	46	8
	1	0	6	8	5	0	1	4	3
	2	0	4	14	18	0	1	5	13
	3	0	1	20	30	0	0	3	35
	Total	0	**11**	**42**	**53**	0	**2**	**12**	**51**
Duodenum - increased epithelial vacuolation	-	55	51	56	57	59	57	50	2
	1	0	7	17	14	0	2	6	12
	2	0	6	13	32	0	1	3	37
	3	0	0	0	7	0	0	0	8
	Total	0	**13**	**30**	**53**	0	**3**	**9**	**57**
Ileum - increased epithelial vacuolation	-	52	50	45	41	59	60	57	52
	1	1	0	8	13	0	0	0	6
	2	0	0	0	1	0	0	0	0
	Total	1	0	**8**	**14**	0	0	0	**6**
Ovary - parovarian cyst	-	NA	NA	NA	NA	45	44	33	32
	P	NA	NA	NA	NA	15	16	**27**	**28**
Lymph node, mesenteric - sinus erythrocytes	-	56	53	42	50	59	58	58	49
	1	1	0	0	0	0	0	0	0
	2	1	5	18	7	1		1	10
	3	0	1	0	3	0	1	1	1
	5	0	0	0	0	0	1	0	0
	Total	2	6	**18**	**10**	1	2	2	**11**
Adrenal, medulla - hyperplasia	-	50	52	50	34	58	55	56	53
	1	3	3	4	3	1	1	1	2
	2	3	3	1	8	1	0	0	4
	3	4	0	3	5	0	2	3	1
	4	0	1	1	10	0	2	0	0
	5	0	0	1	0	0	0	0	0
	Total	10	7	10	**26**	2	5	4	**7**

Grading scores: "-" = not present; P = present; 1 = minimal; 2 = slight; 3= moderate; 4 = marked; 5 = severe.

Toxicokinetics

Samples were collected at 1, 2, 4, 8, 12, and 24 hours after dosing during Week 27. The first four animals/sex/group (dependent on survival) were bled approximately 1 and 8 hours postdose. The second four animals/sex/group (dependent on survival) were bled approximately 2 and 12 hours postdose. The last four animals/sex/group (dependent on survival) were bled approximately 4 and 24 hours postdose. A summary of TK data is shown in the sponsor-generated tables below.

Toxicokinetic Parameters for AEGR-733 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/ (mg/kg/day)]	$t_{1/2}$ (hr)
6	0.25	M	1.74	6.96	4.00	13.7	17.1	68.4	NC
	0.03	F	0.353	11.8	2.00	3.49	5.35	178	NC
7	1.7	M	11.6	6.79	4.00	155	155	91.4	7.53
	0.35	F	7.61	21.7	8.00	140	140	400	NC
8	7.5	M	30.4	4.06	4.00	435	435	58.0	9.14
	2.0	F	32.3	16.1	8.00	565	565	283	NC

Toxicokinetic Parameters for BMS-203304 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/ (mg/kg/day)]	$t_{1/2}$ (hr)	M/P Ratio AUC_{0-24}
6	0.25	M	2.24	8.97	4.00	10.8	11.8	47.3	NC	0.692
7	1.7	M	18.9	11.1	4.00	203	203	119	4.72	1.31
	0.35	F	16.2	46.4	2.00	235	235	671	NC	1.68
8	7.5	M	59.5	7.93	1.00	816	816	109	7.90	1.88
	2.0	F	129	64.7	2.00	1786	1786	893	NC	3.16

Note: No toxicokinetic analysis was performed on the Group 6 females, due to the lack of quantifiable results.

Toxicokinetic Parameters for BMS-203215 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/ (mg/kg/day)]	$t_{1/2}$ (hr)	M/P Ratio AUC_{0-24}
7	1.7	M	2.19	1.29	4.00	7.82	9.09	5.34	NC	0.0584
8	7.5	M	7.94	1.06	4.00	73.0	83.9	11.2	NC	0.193
	2.0	F	5.19	2.59	8.00	36.9	52.4	26.2	NC	0.0927

Note: No toxicokinetic analysis was performed on the Group 6 males and females and the Group 7 females, due to the lack of quantifiable results.

Toxicokinetic Parameters for BMS-224433 in Rat Plasma

Dose Group	AEGR-733 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/ (mg/kg/day)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL)/ (mg/kg/day)]	$t_{1/2}$ (hr)	M/P Ratio AUC_{0-24}
6	0.25	M	4.24	17.0	8.00	78.6	78.6	314	NC	4.60
7	1.7	M	33.0	19.4	8.00	678	678	399	NC	4.36
	0.35	F	10.3	29.3	12.0	208	208	595	NC	1.49
8	7.5	M	130	17.3	8.00	2625	2625	350	NC	6.03
	2.0	F	63.6	31.8	12.0	1320	1320	660	NC	2.34

Note: No toxicokinetic analysis was performed on the Group 6 females, due to the lack of quantifiable results.

Vitamin levels from satellite groups

These satellite groups were treated under a separate study number (733PC0004) to determine when treated animals in the main study should be switched to a vitamin fortified diet. The group assignments for the satellite study are shown in the sponsor-generated table below and satellite animal liver and plasma vitamin levels at various points during the study are shown in the sponsor-generated tables below. Based on the vitamin levels measured in the satellite animals, beginning on Day 407 of the dosing phase of the main study, all animals in Group 3 received a vitamin fortified diet containing 5 times the concentrations of vitamins A, D, and E contained in the standard diet, and all animals in Group 4 received a vitamin fortified diet containing 10 times the concentrations of vitamins A, D, and E contained in the standard diet.

	No. of Animals		AEGR-733 Dose Level (mg/kg/day)		AEGR-733 Dose Concentration (mg/mL)	
Group[a]	Male	Female	Male	Female	Male	Female
1 (Control)	30	30	0	0	0	0
2 (Low)	30	30	1.7	0.35	1.7	0.35
3 (High)	30	30	7.5	2	7.5	2.0

a Group 1 received control article only.

Liver Vitamin Analysis - Week 26

Group		Vitamin A (µg/g)	Vitamin E (µg/g)
Control	M	1660 +/- 45.8	48.0 +/- 8.5
1.7 mg/kg/day	M	928 +/- 101	30.3 +/- 1.8
7.5 mg/kg/day	M	316 +/- 251	5.6 +/- 1.2
Control	F	2283 +/-401	101 +/- 15.5
0.35 mg/kg/day	F	812 +/- 130	161 +/- 36.9
2 mg/kg/day	F	387 +/- 314	12.4 +/- 3.8

F = Female.
M = Male.

Liver Vitamin Analysis - Week 52

Group		Vitamin A (µg/g)	Vitamin E (µg/g)
Control	M	2110 +/- 52.9	49.7 +/- 2.8
1.7 mg/kg/day	M	1034 +/- 106	23.1 +/- 4.5
7.5 mg/kg/day	M	493 +/- 132	0
Control	F	2937 +/- 656	108 +/- 10.2
0.35 mg/kg/day	F	688 +/- 126	61.7 +/- 30.1
2 mg/kg/day	F	496 +/- 315	0

F = Female.
M = Male.

Liver Vitamin Analysis - Week 104

Group		Vitamin A (µg/g)	Vitamin E (µg/g)
Control	M	2848 +/- 601	74.7 +/- 10.2
1.7 mg/kg/day	M	1328 +/- 184	21.8 +/- 7.8
	M 5X	3914 +/- 995	81.6 +/- 58.4
7.5 mg/kg/day	M	660	4.6
	M 10X	4273 +/- 1010	69.9 +/- 47.8
Control	F	3610 +/- 1084	125 +/- 25.3
0.35 mg/kg/day	F	-	-
	F 5X	3272 +/- 782	555 +/- 172
2 mg/kg/day	F	578 +/- 432	13.4 +/- 10.8
	F 10X	4740 +/- 2874	115 +/- 55.5

F = Female.
F 5X = Rats received 5x the amounts of vitamins A, D, and E contained in the standard diet.
F 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.
M = Male.
M 5X = Rats received 5x the amounts of vitamins A, D, and E contained in the standard diet.
M 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.

Plasma Vitamin Analysis - Week 47

Group		Vitamin A (µg/mL)	Vitamin E (µg/mL)
Control	M	0.445 +/- 0.04	15.87 +/- 5.0
1.7 mg/kg/day	M	0.415 +/- 0.07	8.51 +/- 1.2
7.5 mg/kg/day	M	0.541 +/- 0.06	0
Control	F	0*	20.87 +/- 4.4
0.35 mg/kg/day	F	0.325 +/- 0.06	11.57 +/- 5.4
2 mg/kg/day	F	0.352 +/- 0.05	0

F = Female.
M = Male.
* = Data were below limit of detection.

Plasma Vitamin Analysis - Week 61

Group		Vitamin A (µg/mL)	Vitamin E (µg/mL)
Control	M	0.244 +/- 0.02	10.12 +/- 3.0
	M Aqu	0.261 +/- 0.05	18.1 +/- 3.0
1.7 mg/kg/day	M	0.325 +/- 0.06	5.37 +/- 1.8
7.5 mg/kg/day	M Aqu	0.363 +/- 0.02	3.64
	M 10X	0.289 +/- 0.06	4.29 +/- 2.1
Control	F	0.151 +/- 0.02	18.5 +/- 0.8
	F Aqu	0.205 +/- 0.09	27.6 +/- 3.8
0.35 mg/kg/day	F	0.218 +/- 0.04	21.1 +/- 8.3
2 mg/kg/day	F Aqu	0.243 +/- 0.02	1.6 +/- 0.14
	F 10X	0.168 +/- 0.04	9.52 +/- 4.8

F = Female.
F Aqu = Rats received 0.1 mL/day AquADEKs (liquid vitamin supplement).
F 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.
M = Male.
M Aqu = Rats received 0.1 mL/day AquADEKs (liquid vitamin supplement).
M 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.

Plasma Vitamin Analysis - Week 78

Group		Vitamin A (µg/mL)	Vitamin E (µg/mL)
Control	M	0.229 +/- 0.03	19.47 +/- 2.2
1.7 mg/kg/day	M	0.412 +/- 0.00	6.95 +/- 4.5
	M 5X	0.378 +/- 0.07	13.0 +/- 8.4
7.5 mg/kg/day	M	0.378 +/- 0.10	2.05 +/- 1.8
	M 10X	0.317 +/- 0.11	4.82 +/- 4.0
Control	F	0.207 +/- 0.02	27.37 +/-7.5
0.35 mg/kg/day	F	0.309 +/- 0.04	11.48 +/- 6.3
	F 5X	0.225 +/- 0.08	26.27 +/- 10.5
2 mg/kg/day	F	0.251 +/- 0.05	1.20 +/- 0.62
	F 10X	0.236 +/- 0.05	7.61 +/- 1.7

F = Female.
F 5X = Rats received 5x the amounts of vitamins A, D, and E contained in the standard diet.
F 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.
M = Male.
M 5X = Rats received 5x the amounts of vitamins A, D, and E contained in the standard diet.
M 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.

Plasma Vitamin Analysis - Week 104

Group		Vitamin A (µg/mL)	Vitamin E (µg/mL)
Control	M	0.225 +/- 0.02	19.90 +/- 3.66
1.7 mg/kg/day	M	0.296 +/- 0.06	5.09 +/- 4.3
	M 5X	0.235 +/- 0.04	8.26 +/- 7..8
7.5 mg/kg/day	M	0.404	0
	M 10X	0.282 +/- 0.12	8.09 +/- 7.0
Control	F	0.214 +/- 0.03	26.86 +/-5.8
0.35 mg/kg/day	F	-	-
	F 5X	0.257 +/- 0.05	38.58 +/- 11.0
2 mg/kg/day	F	0.318 +/- 0.11	1.53 +/- 0.54
	F 10X	0.238 +/- 0.04	6.63 +/- 2.8

F = Female.
F 5X = Rats received 5x the amounts of vitamins A, D, and E contained in the standard diet.
F 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.
M = Male.
M 5X = Rats received 5x the amounts of vitamins A, D, and E contained in the standard diet.
M 10X = Rats received 10x the amounts of vitamins A, D, and E contained in the standard diet.

Dosing Solution Analysis

With the exception of the low-dose concentrations (0.25 mg/mL for males and 0.03 mg/mL for females) at Week 39, all formulations were within ±10% of the target concentrations. The low-dose concentration for males at Week 39 was slightly higher (116%) than target, whereas the low-dose concentration for females at Week 39 was much greater (~300%) than target, but still approximately 4-fold less than the mid-dose concentration. Because these findings were only noted on a single occasion and the administered dose was still less than the mid-dose group, this error in dose formulation did not affect the integrity of the study. The test article was not detected in any of the control article formulations.

9 Reproductive and Developmental Toxicology

Developmental and Reproductive Toxicology Range-Finding and Investigative Studies

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
10-day RF study 96015 Non-GLP	Rat/Sprague-Dawley 8/group 0, 0.05, 0.5, 25, or 50 mg/kg/d from GD6 through GD15	Clinical observations, BW, FC, maternal gross pathology and organ weights, cesarean sectioning, and fetal evaluations	• There were no mortalities or treatment-related clinical signs. • Reduced maternal BW gain (58% & 53%) at 25 and 50 mg/kg/d. • Pale or mottled maternal livers at ≥0.5 mg/kg/d. • Increased maternal spleen weight (~40%) at ≥25 mg/kg/d. • ~80% post-implantation loss at ≥25 mg/kg/d. Decreased fetal body weights at ≥25 mg/kg/d. • At 0.5 mg/kg/d, one finding of forepaw digits absent or fused, anal atresia, stubbed tail, and kinked tail. Three fetuses had abdominal gastroschisis and 3 had umbilical hernia. • At 25 and/or 50 mg/kg/d, the following findings were observed in 1 or 2 fetuses: exencephaly, short snout, webbed, edematous neck, and anal atresia. A greater incidence was observed for abdominal gastroschisis, umbilical hernia, and effects on the tail (short, stubbed, kinked, absent, or curled).
Investigative embryo-fetal developmental study 96022 Non-GLP	Rat/Sprague-Dawley 8/group 0 and 5 mg/kg/d from GD 6 through GD15 with or without vitamin supplementation (A, D, E, and K)[†]	Clinical observations, BW, FC, maternal gross pathology and organ weights, cesarean sectioning, and fetal evaluations	• There were no mortalities or treatment-related clinical signs. • Decreased BW gain (15% to 17% at GD 20) and FC with or without vitamin supplements. • Both treated groups had increased spleen weights, pale and/or mottled livers, and perivaginal substance associated with drug-related resorption of conceptuses. • Both treated groups had marked increases in resorptions with associated decreased litter sizes and decreased fetal BW. • Drug-related malformations occurred at comparable incidences in both groups. BMS-201038 alone had malformations with hind limbs (inward rotation), abdomen (umbilical hernia, gastroschisis), and tail (short, stubbed, kinked, curled, or absent). BMS-201038 with vitamins had malformations with abdomen (umbilical hernia), tail (short, stubbed, kinked, curled, or absent), anus (inperforate), and short snout in one animal. • Vitamin supplementation did not provide protection against female toxicity or fetal malformations.

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
13-day RF study 96031 GLP	Rabbit/ New Zealand white 5/group 0, 5, 10, 20 or 40 mg/kg/d from GD6 through GD18	Clinical observations, BW, FC, maternal gross pathology, cesarean sectioning, and fetal evaluations	• There were no mortalities or treatment-related clinical signs. • There were no apparent effects on BW or FC. • There was an increase in pre-implantation loss at 40 mg/kg/d (26%) compared with control (14%). • Increased early resorptions of 26% and 35% at 20 and 40 mg/kg/d compared with 0% for controls. • Total loss based on the number of live fetuses versus number of corpora lutea was 38% and 54% for the 20 and 40 mg/kg/d groups compared with 14% for controls. • An increase in the number of fetuses with umbilical hernia occurred at the two lowest dose levels only (12% and 5%) compared with 0% for the other groups; therefore, this was likely not a treatment-related effect.
13-day exploratory study 96324 Non-GLP	Rabbit/New Zealand white 5/group 0, 0.5, or 25 mg/kg/d from GD6 through GD18	Clinical observations, BW, FC, maternal gross pathology and uterine weights, cesarean sectioning, and fetal evaluations	• Mortality occurred in all groups: 3 in control, 5 in low dose, and 4 in high dose. Clinical signs in moribund animals included stool abnormalities, hypoactivity, prostration, coolness to touch, loss of righting reflex, apparent abortion, increased respiration rate, miosis, and/or soiled haircoat. • Dose volumes were decreased from 5mL/kg to 1 mL/kg to reduce the amount of PEG 400 administered due to its apparent toxic affect in all groups. • All groups showed BW losses or reduced BW gains and markedly reduced FC, which were attributed to PEG 400. • No adverse effects were observed in fetuses examined grossly at scheduled cesarean sections. No adverse effects were observed in fetuses or embryos examined from does that died or were euthanized in moribund condition, except for hemorrhagic areas and/or growth retardation in embryos from a single doe dosed with 25 mg/kg/d. • No other parameters were assessed because of the high maternal mortality.

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
17-day embryo-fetal developmental RF study 97008 GLP (mostly)	Ferret 6/group 0, 1.6, 4, 10, and 25 mg/kg/d from GD12 through GD28; necropsy on GD35	Viability, clinical observations, BW, FC, gross pathology, caesarean sectioning, fetal weight, fetal gross examinations	• BW loss between GD12 and GD29 at all dose levels; mean final BWs were 15% to 24% less than GD12 weights. Effects on BW correlated with decreased FC. • Increased vomiting at ≥4 mg/kg/d. • Increased resorptions, resulting in decreased number of live fetuses/litter at ≥10 mg/kg/d. Slight effects on resorptions and number of live fetuses at 4 mg/kg/d. • Decreased mean fetal BW at ≥1.6 mg/kg/d. • A dose-related increased incidence in fetal malformations were noted at ≥1.6 mg/kg/d, including. o Limbs/paws: rotated medially, digits absent or fused o Head: cleft palate, red eyes, open eye lids, low set ears o Kinked tail o Body: umbilical hernia • Additionally, shortened limbs were observed at 4 mg/kg/d and cleft facial, depressed skull, and shortened tail were observed at 10 mg/kg/d. • Dose levels are 0.3-, 0.8-, 2-, and 5-fold of the maximum clinical dose based on body surface area extrapolation. • A NOAEL for maternal toxicity or fetal malformations was not identified.

[†]7000 IU/kg vitamin A, 1050 IU/kg vitamin D, 25 IU/kg vitamin E, and 0.16 mg/kg vitamin K_1.
BW = body weight; FC = food consumption; GD = gestation day; RF = range finding.

9.1 Fertility and Early Embryonic Development

Study title: Oral (Gavage) Fertility and General Reproduction Toxicity Study of AEGR-733 (formerly known as BMS-201038) in Rats

Study no.: AEGR-733PC0001 (b) (4)
Study report location: Module 4.2.3.5
Conducting laboratory: (b) (4)
Date of study initiation: 30 March 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: AEGR-733, Lot #R2873A, 97.5% pure

Key Study Findings

Male fertility

- There were no treatment-related effects on mortality, clinical signs, body weights, or male fertility indices. Mating of treated males with untreated females did not result in decreased fertility indices for the untreated females.

Female fertility and early embryonic development

- There were no treatment related effects on maternal body weight, food consumption, fertility indices, estrous cycling, number of corpora lutea, implantation sites, or viable embryos. It was not felt that the maximum tolerated dose was used as the high dose for this study. A high dose of 4 mg/kg/d seems to have been more appropriate.

Methods

Species/Strain: Rat/Sprague-Dawley
Study design: Males - See sponsor-generated table below

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned Numbers
I	0 (Vehicle)	0	1	25	27101 - 27125
II	0.2	0.2	1	25	27126 - 27150
III	1	1	1	25	27151 - 27175
IV	5	5	1	25	27176 - 27196, 4966[b], 27198 - 27200

a. The test article was expressed in terms of AEGR-733 free base.
b. Prior to the first day of dosage administration on 8 May 2007(DS 23), a rat that died (27197) was replaced with another rat (4966).

Study design: Females- See sponsor-generated table below

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned Numbers
I	0 (Vehicle)	0	1	25	26801 - 26825
II	0.04	0.04	1	25	26826 - 26850
III	0.2	0.2	1	25	26851 - 26875
IV	1	1	1	25	26876 - 26900

a. The test article was expressed in terms of AEGR-733 free base.

Frequency of dosing: Females: once daily beginning 15 days before cohabitation with untreated males through GD7
Males: once daily beginning 28 days before cohabitation with untreated females until the day before sacrifice
Route of administration: Oral gavage
Formulation/Vehicle: 75% PEG-400 in water
Satellite groups: None
Protocol deviations: None

Observations and Results

Mortality

One male from the 1 mg/kg/d group was found dead on Day 70 and was not considered to be related to treatment.

There were no treatment-related mortalities for the female-treated groups.

Clinical Signs

There were no clinical signs attributed to the test article for males or females.

Body Weight

There were no treatment-related effects on mean body weight for males or females.

Food Consumption

There were no treatment-related effects on mean food consumption for males or females.

Toxicokinetics

Not conducted

Fertility Parameters for Treated Males

There were no treatment-related effects on male fertility indices, including days in cohabitation, mating index, or fertility index. There were no treatment-related effects on male reproductive organ weights. A statistically significant increase in mean caudal epididymal sperm counts was observed for high-dose males (↑28%) compared with controls. Mean vas deferens sperm counts were slightly decreased for high-dose males (↓10%), but this did not occur in a dose-related manner. Overall, there does not appear

to be a biologically meaningful, treatment-related change in sperm counts. There were no treatment-related effects on implantations or embryonic viability for untreated females after mating with treated males. Summaries of male fertility findings are shown in the sponsor-generated tables below.

Mating and Fertility - Treated Males

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.2	1	5
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION	MEAN±S.D.	2.2 ± 1.0	2.8 ± 0.9	2.4 ± 1.4	3.4 ± 3.2
RATS THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX b	N/N	25/ 25	24/ 25	23/ 25	25/ 25
	(%)	(100.0)	(96.0)	(92.0)	(100.0)
RATS WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED WITH FEMALE					
DAYS 1-7	N(%)	25(100.0)	25(100.0)	25(100.0)	23(92.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	2(8.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	25/ 25	24/ 25	23/ 25	25/ 25
	(%)	(100.0)	(96.0)	(92.0)	(100.0)

a. Dosage occurred on day 23 of study through the day before sacrifice.
b. Number of pregnancies/number of rats that mated.

Sperm motility, count, and density

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.2	1	5
RATS TESTED	N	25	25	24b	25
VAS DEFERENS SPERM MOTILITY					
NUMBER MOTILE	MEAN±S.D.	461.2 ± 146.4	420.8 ± 155.5	459.8 ± 179.6	430.7 ± 135.3
MOTILE PERCENT	MEAN±S.D.	92.3 ± 7.3	93.2 ± 9.1	94.0 ± 7.1	94.9 ± 4.2
STATIC COUNT (NONMOTILE)	MEAN±S.D.	40.2 ± 52.6	31.1 ± 54.8	27.4 ± 25.2	23.0 ± 19.1
TOTAL COUNT c	MEAN±S.D.	501.4 ± 163.4	452.0 ± 158.7	487.2 ± 182.4	453.7 ± 139.2
CAUDA EPIDIDYMAL SPERM COUNT					
SPERM COUNT d	MEAN±S.D.	177.0 ± 33.7	187.0 ± 39.1	196.9 ± 48.9	226.8 ± 57.8**
SPERM DENSITY e	MEAN±S.D.	1438.32 ± 237.40	1545.45 ± 334.80	1665.54 ± 463.99	1866.27 ± 486.09

a. Dosage occurred on day 23 of study through the day before sacrifice.
b. Excludes values for rat 27165, which was found dead on day 70 of study.
c. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
d. Sperm count used in the calculation of sperm density. Ten fields were evaluated.
e. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3 x 10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table A20 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.
** Significantly different from the vehicle control group ($p \leq 0.01$).

C-sectioning and litter observations - untreated females mated with treated males

MALE DOSAGE GROUP MALE DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 0.2	III 1	IV 5
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	25(100.0)	24(96.0)	23(92.0)	25(100.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	25	24	23	25
CORPORA LUTEA	MEAN±S.D.	16.0 ± 1.9	15.7 ± 2.9	16.6 ± 1.7	16.3 ± 1.5
IMPLANTATIONS	MEAN±S.D.	15.4 ± 2.0	14.5 ± 4.5	16.1 ± 1.6	15.8 ± 1.6
VIABLE EMBRYOS	N	349	323	338	365
	MEAN±S.D.	14.0 ± 1.9	13.4 ± 4.7	14.7 ± 2.3	14.6 ± 2.0
NONVIABLE EMBRYOS	N	37	26	33	31
	MEAN±S.D.	1.5 ± 2.6	1.1 ± 1.0	1.4 ± 1.7	1.2 ± 1.4
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	13(52.0)	17(70.8)	17(73.9)	14(56.0)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	2(8.3)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	25(100.0)	22(91.7)	23(100.0)	25(100.0)
% NONVIABLE EMBRYOS/LITTER	MEAN±S.D.	8.5 ± 13.0	14.4 ± 27.0	8.9 ± 11.1	7.8 ± 8.8

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Fertility Parameters for Treated Females

There were no effects on the estrous cycle, mating index, or fertility index. There were no treatment-related effects on the number of corpora lutea, number of implantations, or implantation loss. Summaries of female fertility parameters are shown in the sponsor-generated tables below.

Mating and Fertility - Treated Females Mate with Untreated Males

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.04	III 0.2	IV 1
MATING OBSERVATIONS					
RATS IN COHABITATION	N	24b	25	25	25
DAYS IN COHABITATION	MEAN±S.D.	2.1 ± 1.1	1.8 ± 0.9	2.1 ± 1.2	1.8 ± 0.8
RATS THAT MATED	N(%)	24(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N (%)	23/ 24 (95.8)	23/ 25 (92.0)	25/ 25 (100.0)	23/ 25 (92.0)
RATS WITH CONFIRMED MATING DATES	N	24	25	25	25
MATED BY FIRST MALE					
DAYS 1-7	N(%)	24(100.0)	25(100.0)	25(100.0)	25(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	23/ 24 (95.8)	23/ 25 (92.0)	25/ 25 (100.0)	23/ 25 (92.0)

a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Excludes values for rat 26820, which was found dead on day 2 of study.
c. Number of pregnancies/number of rats that mated.

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C-sectioning and litter observations - treated females mated with untreated males

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.04	0.2	1
RATS TESTED	N	24b	25	25	25
PREGNANT	N(%)	23(95.8)	23(92.0)	25(100.0)	23(92.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	23	23	25	23
CORPORA LUTEA	MEAN±S.D.	15.6 ± 1.9	15.1 ± 2.9	15.5 ± 3.0	16.8 ± 2.4
IMPLANTATIONS	MEAN±S.D.	15.0 ± 2.4	14.4 ± 3.9	14.8 ± 3.2	15.9 ± 1.9
VIABLE EMBRYOS	N	318	302	345	334
	MEAN±S.D.	13.8 ± 3.3	13.1 ± 3.6	13.8 ± 3.8	14.5 ± 2.7
NONVIABLE EMBRYOS	N	27	29	24	31
	MEAN±S.D.	1.2 ± 1.4	1.3 ± 1.8	1.0 ± 1.8	1.3 ± 2.0
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	14(60.9)	12(52.2)	10(40.0)	10(43.5)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	23(100.0)	23(100.0)	25(100.0)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	23(100.0)	25(100.0)	23(100.0)
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	9.1 ± 13.3	7.8 ± 11.4	7.0 ± 14.4	8.5 ± 12.5

a. Dosage occurred on day 1 of study through day 7 of gestation.
b. Excludes values for rat 26820, which found dead on day 2 of study.

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9.2 Embryonic Fetal Development

Study title: Oral study of embryo-fetal development in rats

Study no.:	96039
Study report location:	Module 4.2.3.5.2
Conducting laboratory:	Bristol-Myers Squibb Pharmaceutical Research Institute Department of Pathology, New Brunswick, New Jersey
Date of study initiation:	02 October 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038-04 (AEGR-733), Batch #R007A, 87.2% pure "as is" as the free base

Key Study Findings

- There were no mortalities or clinical signs indicative of maternal toxicity.
- Mean maternal weight gain at 4 mg/kg/d was statistically significantly decreased between GD6 and GD9, which correlated with decreased food consumption. Between GD9 and GD16, no meaningful differences in body weight gain were noted. Between GD16 and GD20, a statistically significant decrease (↓47% compared with controls) in mean body weight was noted for HD females. The difference in mean body weight gain was likely the result of significant postimplantation loss in the HD group, as body weights excluding gravid uterus weights were similar across all groups.
- Mean postimplantation loss (primarily due to early resorptions) was statistically significantly increased for the HD group, with 52% of conceptuses being lost. A slight increase (6.2%) in postimplantation loss was observed for the MD group compared with controls (3.9%) but the difference was not statistically significant.
- In the HD group the number of live fetuses per litter ranged from 15 to 0, with a mean of 7.0 compared with 13.4 for the control group. All but 2 HD dams (90.5%) had at least one viable fetus on GD20; one dam had 100% resorbtions and another dam had no viable fetuses (13 resorptions plus 2 dead fetuses). Only one HD dam had no postimplantation loss.
- Statistically significant decreases in mean fetal body weights were noted for the MD (↓6%) and HD (↓25%) groups compared with control.
- 85% of viable fetuses in the HD group had at least one developmental alteration compared with 17.9% in the control group. 68.0% of all live fetuses in the HD group (73.2% fetuses/litter) had at least one malformation and 100% of litters had a fetus with at least one malformation compared with zero fetuses in the control group with a malformation. 55.1% of fetuses in the HD group had at least one variation compared with a control value of 17.9%. The number of litters with a fetus having a developmental variation was similar between control and HD groups. Malformations at the HD were observed for the head/brain (dilated ventricles, hydrocephaly, exencephaly, misshapen cerebral hemispheres), abdomen (umbilical hernia, gastroschisis), heart (small or misshapen ventricles), anus (imperforate), limbs (rotated, short), tail (short, stubbed, bent, absent, kinked, curled, filamentous), and

skeleton (incomplete ossification, hypoplastic). A statistically significant increase in the number of fetuses (5.5%) and litters (38.1%) with any malformation was also observed for the MD group.

- The NOAEL for maternal toxicity was 0.4 mg/kg/d based on decreased body weight gain at 4 mg/kg/d during the first 2 days of dosing. The NOAEL for embryonic toxicity and teratogenicity was 0.04 mg/kg/d based on effects on embryonic weight, embryonic death and/or malformations at 0.4 and 4 mg/kg/d.

Methods

Doses:	0, 0.04, 0.4 and 4 mg/kg/day
Frequency of dosing:	Once daily from GD6 through GD15
Dose volume:	1 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	75% PEG-400 in water
Species/Strain:	Rat / Sprague-Dawley
Number/Group:	22 presumed-pregnant rats per group
Satellite groups:	None
Study design:	Test article was administered from GD6 through GD15. Cesarean sectioning was conducted on GD20. Intact gravid uterus (including ovaries) and spleen were weighed. Examinations included number of corpora lutea, number and location of implantation sites, early and late resorptions, live and dead fetuses, and placenta. Fetuses were evaluated for weight, gender, and gross external alterations. Approximately half of the fetuses in each litter were examined for soft tissue alterations and the other half were evaluated for skeletal alterations.
Study protocol deviations:	There were no deviations that impacted the interpretability of the study data.

Observations and Results

Mortality

All rats survived to scheduled sacrifice day on GD20.

Clinical Signs

Perivaginal substance was noted for 9 HD dams, an observation that was believed to be related to the resorption of conceptuses.

Body Weight

HD dams gained 23% less weight (statistically significant) than controls between GD6 and GD9. No meaningful difference in body weight gain was noted between GD9 and GD16 and although there was a slight difference in weight gain between GD6 and GD16, the effect was not statistically significant. The most meaningful effect on body weight gain occurred between GD16 and GD20, during which time HD females gained 47% less weight than controls (statistically significant). The mean difference in body

weight gain between HD and control dams for the period between GD6 and GD20 was also statistically significant. On average, approximately 50% of implantations in the HD group underwent early resorption resulting in significantly decreased gravid uterine weights by GD20. As noted in the table below, the mean gravid uterine weight for HD dams were 44% less than for controls while the mean carcass weights were similar between HD and control dams indicating that the primary difference in final body weights was due to embryonic loss rather than from a direct loss of maternal weight due to maternal toxicity. However, the initial decrease in body weight gain, primarily between GD6 and GD8, was likely due to maternal toxicity and may have had a direct impact on embryonic viability.

Summary of Body Weight Gain

Dose (mg/kg/d)	0	0.04	0.4	4
Weight (g) - GD 6	246.5	250.1	248.4	247.8
Weight (g) - GD 16	333.5	339.0	335.1	327.0
Weight (g) - GD 20	403.4	405.8	399.9	364.0**
Weight gain (g) - GD 6-16	87.0	88.9	86.7	79.2
Diff from control (g)		1.9	-0.3	-7.8
% diff from control		↑2%	-	↓9%
Weight gain (g) - GD 6-20	156.9	155.7	151.5	116.2
Diff from control (g)		-1.2	-5.4	-40.7
% diff from control		↓1%	↓3%	↓26%
Gravid uterus weight	81.1	79.7	78.1	45.7**
Carcass weight	322.3	326.1	321.8	318.3 (↓1%)

**p≤0.01; GD = gestation day; carcass weight = (body weight on GD20) - (gravid-uterine weight).

Food Consumption

A statistically significant decrease in food consumption (↓13%) was observed for HD dams compared with controls during the first two days of dosing. After GD8, food consumption was similar among all groups.

Toxicokinetics

Not conducted; based on TK data from non-pregnant female Sprague-Dawley rats after receiving 4 mg/kg/day for 14 days, mean $AUC_{0\text{-}24}$ was 718 ng·h/mL (Study #95051). A dose of 0.5 mg/kg/day resulted in an $AUC_{0\text{-}24}$ value of 163 ng·h/mL after 28 days (Study #96003). TK assessments for doses less than 0.5 mg/kg/day have not been conducted.

Dosing Solution Analysis

Dosing solutions were prepared weekly. Day 1 dosing solutions for the LD, MD, and HD groups were shown to be 88.4%, 94.7%, and 93.1% of nominal concentrations, respectively. The sponsor states that stability of the test article in the dosing solution at concentrations bracketing those used in this study were determined previously (data not provided).

Necropsy

Macroscopic examination revealed 2 fluid-filled uteri and 1 friable uterus in the HD group. Pale and mottled liver was noted for 1 dam each in the MD and HD groups and liver with white streaks on the median lobe was noted for 1 MD dam. Mean absolute and relative spleen weights were statistically significantly increased for the HD group. A summary of macroscopic findings is shown in the sponsor-generated table below.

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SUMMARY OF SPLEEN WEIGHTS AND OBSERVATIONS AT NECROPSY

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED[a]:	N	22	22	22	22
UTERUS					
Fluid-filled	N	0	0	0	2
Friable	N	0	0	0	1
LIVER					
Pale and Mottled	N	0	0	1	1
Median lobe, white streaks	N	0	0	1	0
SPLEEN					
Misshapen	N	0	1	0	0
PREGNANT RATS SURVIVING TO GESTATION DAY 20[b]	N	20	22	21	21
CARCASS WEIGHT	MEAN	322.3	326.1	321.8	318.3
	SD	16.7	13.9	16.4	14.6
SPLEEN					
ABSOLUTE WEIGHT (g)	MEAN	0.73	0.78	0.73	1.03**
	SD	0.10	0.11	0.09	0.34
RELATIVE WEIGHT (% Carcass Weight)	MEAN	0.23	0.24	0.23	0.32**
	SD	0.03	0.03	0.03	0.10

CARCASS WEIGHT = (Body weight on day 20 of gestation) - (gravid-uterine weight)

Statistical Analysis: Analysis of Variance with Dunnett's procedure.
** Significantly different from control at P≤ 0.01.

a - Incidences include all animals necropsied at completion of the study regardless of pregnancy status.
b - Analysis of spleen-weights restricted to pregnant rats surviving to scheduled cesarean-sectioning on day 20 of gestation.

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

(sponsor-generated tables)

SUMMARY OF CESAREAN-SECTIONING OBSERVATIONS

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.9)	22(100.0)	21(95.5)	21(95.5)
PREGNANT RATS SURVIVING TO DAY 20 CESAREAN-SECTIONING	N	20	22	21	21
CORPORA LUTEA	MEAN	14.9	14.6	15.8	15.5
	SD	2.3	2.3	2.1	1.7
IMPLANTATIONS	MEAN	14.0	13.4	14.7	14.6
	SD	1.5	2.5	1.8	1.8
PREIMPLANTATION LOSS[a]	MEAN%	5.6	8.5	6.6	5.7
	SD	7.3	10.5	6.1	7.2
POSTIMPLANTATION LOSS[b]	MEAN%	3.9	4.6	6.2	52.0**
	SD	5.7	5.2	7.5	32.1
LITTER SIZE (LIVE + DEAD)	MEAN	13.4	12.8	13.8	7.1**
	SD	1.6	2.7	1.9	4.5
LIVE FETUSES	MEAN	13.4	12.8	13.8	7.0**
	SD	1.6	2.7	1.9	4.7
DEAD FETUSES	N	0	0	0	3
RESORPTIONS (EARLY + LATE)	N	11	12	19	156**
	MEAN	0.6	0.5	0.9	7.4
	SD	0.8	0.6	1.0	4.6
EARLY RESORPTIONS	N	11	12	19	154**
	MEAN	0.6	0.5	0.9	7.3
	SD	0.8	0.6	1.0	4.5
LATE RESORPTIONS	N	0	0	0	2
DAMS WITH ANY RESORPTIONS	N(%)	7(35.0)	11(50.0)	12(57.1)	20(95.2)**
DAMS WITH 100% RESORPTIONS	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
DAMS WITH VIABLE FETUSES	N(%)	20(100.0)	22(100.0)	21(100.0)	19(90.5)

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Kruskal-Wallis with Dunn's procedure used for enumeration data.
Fisher's Exact test was used for proportion data.
** Significantly different from control at P≤ 0.01.

a - Computed for each dam as [(corpora lutea-implantations/corpora lutea) x 100].
b - Computed for each dam as [(dead + resorbed conceptuses)/implantations) x 100].

SUMMARY OF FETAL OBSERVATIONS IN CESAREAN-DELIVERED LITTERS

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
LITTERS WITH ONE OR MORE LIVE FETUSES ON DAY 20 OF GESTATION	N	20	22	21	19
LIVE FETUSES	N	268	282	289	147
	MEAN	13.4	12.8	13.8	7.7**
	SD	1.6	2.7	1.9	4.3
LIVE MALE FETUSES	N	127	139	133	69
% LIVE MALE FETUSES PER LITTER	MEAN%	47.1	48.2	45.3	42.0
	SD	13.0	16.7	13.2	26.9
MEAN FETAL BODY WEIGHT (Grams) PER LITTER	MEAN	3.80	4.02*	3.57*	2.85**
	SD	0.25	0.25	0.29	0.38
MALE FETUSES	MEAN	3.93	4.15*	3.70*	3.01[a]**
	SD	0.23	0.24	0.30	0.36
FEMALE FETUSES	MEAN	3.68	3.90	3.48	2.76**
	SD	0.27	0.25	0.32	0.39
% DEAD OR RESORBED CONCEPTUSES PER LITTER	MEAN%	3.9	4.6	6.2	46.9**
	SD	5.7	5.2	7.5	29.4

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Kruskal-Wallis with Dunn's procedure used for enumeration data.
* Significantly different from control at P≤ 0.05.
** Significantly different from control at P≤ 0.01.

a - N=16; excludes litters 4F0072, 4F0079, and 4F0083 which had no male fetuses.

Offspring (Malformations, Variations, etc.) - sponsor-generated tables

SUMMARY OF FETAL ALTERATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.9)	22(100.0)	21(95.5)	21(95.5)
FETUSES EVALUATED	N	268	282	289	148
Live Fetuses	N	268	282	289	147
Dead Fetuses	N	0	0	0	1[d]
LITTERS EVALUATED	N	20	22	21	19[e]
ALTERATIONS (Malformations + Variations)					
Fetuses with Any Alterations	N(%)	48(17.9)	30(10.6)*	60(20.8)	125(85.0)**
Litters with Fetuses with Any Alterations	N(%)	18(90.0)	13(59.1)*	20(95.2)	19(100.0)
Percent Fetuses per Litter with Alterations	MEAN	17.7	10.6	21.4	87.1**
	SD	10.9	12.6	13.1	14.3
VARIATIONS[b]					
Fetuses with Any Variations	N(%)	48(17.9)	26(9.2)**	46(15.9)	81(55.1)**
Litters with Fetuses with Any Variations	N(%)	18(90.0)	12(54.5)*	19(90.5)	18(94.7)
Percent Fetuses per Litter with Variations	MEAN	17.7	8.8	16.2	56.1**
	SD	10.9	11.9	9.9	22.6
MALFORMATIONS[c]					
Fetuses with Any Malformations	N(%)	0	5(1.8)	16(5.5)**	100(68.0)**
Litters with Fetuses with Any Malformations	N(%)	0	3(13.6)	8(38.1)**	19(100.0)**
Percent Fetuses per Litter with Malformations	MEAN	0.0	2.1	5.8	73.2**
	SD	0.0	6.7	9.5	25.4

Statistical Analysis: Analysis of Variance with Dunnett's procedure was used for continuous data.
Fisher's Exact test was used for proportion data.
* Significantly different from control at P≤ 0.05.
** Significantly different from control at P≤ 0.01.

a - All percentages were calculated on the basis of the number of live fetuses in each group.
b - Common observations in this species and strain and reversible delays or accelerations in development.
c - Irreversible changes occurring at low incidences in this species and strain.
d - Excludes two dead fetuses from litter 4F0082 which had no viable conceptuses (13 early resorptions and two dead fetuses).
e - N=19; excludes litter 4F0073 that resorbed all (100%) conceptuses, and litter 4F0082 with 13 early resorptions and two dead fetuses.

SUMMARY OF FETAL GROSS EXTERNAL AND PLACENTAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
FETUSES EVALUATED	N	268	282	289	148
Live Fetuses	N	268	282	289	147
Dead Fetuses	N	0	0	0	1[b,c]
LITTERS EVALUATED	N	20	22	21	19[d]
HEAD: Exencephaly (M)					
Fetal Incidence	N(%)	0	0	0	17(11.6)**
Litter Incidence	N(%)	0	0	0	8(42.1)**
HEAD: Dome-shaped (M)					
Fetal Incidence	N(%)	0	0	1(0.3)	0
Litter Incidence	N(%)	0	0	1(4.8)	0
SKIN: Friable (V)					
Fetal Incidence	N(%)	0	0	0	3(2.0)*
Litter Incidence	N(%)	0	0	0	1(5.3)
ABDOMEN: Umbilical hernia (M)					
Fetal Incidence	N(%)	0	0	3(1.0)	28(19.0)**
Litter Incidence	N(%)	0	0	2(9.5)	12(63.2)**
ABDOMEN: Gastroschisis (M)					
Fetal Incidence	N(%)	0	0	4(1.4)	8(5.4)**
Litter Incidence	N(%)	0	0	3(14.3)	7(36.8)**
ANUS: Imperforate (M)					
Fetal Incidence	N(%)	0	0	1(0.3)	3(2.0)*
Litter Incidence	N(%)	0	0	1(4.8)	2(10.5)

(M) = Malformation (V) = Variation

Statistical Analysis: Fisher's Exact test.

* Significantly different from control at P≤ 0.05.
** Significantly different from control at P≤ 0.01.

a - All percentages were calculated on the basis of live fetuses in each group. There were no remarkable placental observations.
b - Excludes litter 4F0082 which had 13 early resorptions and two dead fetuses; both dead fetuses appeared unremarkable at gross external examination.
c - Fetus 4F0087-R6 had a stubbed tail.
d - N=19; excludes litter 4F0073 in which all (100%) conceptuses were resorbed and litter 4F0082 that had 13 early resorptions and two dead fetuses.

SUMMARY OF FETAL GROSS EXTERNAL AND PLACENTAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
FETUSES EVALUATED	N	268	282	289	148
Live Fetuses	N	268	282	289	147
Dead Fetuses	N	0	0	0	1[b,c]
LITTERS EVALUATED	N	20	22	21	19[d]
FORELIMBS AND HINDLIMBS: Total (Summarization of all Limb Alterations)					
Fetal Incidence	N(%)	0	0	3(1.0)	3(2.0)*
Litter Incidence	N(%)	0	0	3(14.3)	2(10.5)
FORELIMBS: Inward rotation[e] (M)					
Fetal Incidence	N(%)	0	0	0	1(0.7)
Litter Incidence	N(%)	0	0	0	1(5.3)
HINDLIMBS: Short[e] (M)					
Fetal Incidence	N(%)	0	0	0	1(0.7)
Litter Incidence	N(%)	0	0	0	1(5.3)
HINDPAWS: Inward rotation[e] (M)					
Fetal Incidence	N(%)	0	0	2(0.7)	1(0.7)
Litter Incidence	N(%)	0	0	2(9.5)	1(5.3)
HINDLIMBS: Inward rotation[e] (M)					
Fetal Incidence	N(%)	0	0	1(0.3)	0
Litter Incidence	N(%)	0	0	1(4.8)	0
TAIL: Short (M)					
Fetal Incidence	N(%)	0	0	2(0.7)	20(13.6)**
Litter Incidence	N(%)	0	0	1(4.8)	12(63.2)**

(M) = Malformation (V) = Variation

Statistical Analysis: Fisher's Exact test.

* Significantly different from control at P≤ 0.05.

a - All percentages were calculated on the basis of live fetuses in each group. There were no remarkable placental observations.
b - Excludes litter 4F0082 which had 13 early resorptions and two dead fetuses; both dead fetuses appeared unremarkable at gross external examination.
c - Fetus 4F0087-R6 had a stubbed tail.
d - N=19; excludes litter 4F0073 in which all (100%) conceptuses were resorbed and litter 4F0082 that had 13 early resorptions and two dead fetuses.
e - Right and/or left.

SUMMARY OF FETAL GROSS EXTERNAL AND PLACENTAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
FETUSES EVALUATED	N	268	282	289	148
Live Fetuses	N	268	282	289	147
Dead Fetuses	N	0	0	0	1[b,c]
LITTERS EVALUATED	N	20	22	21	19[d]
TAIL: Stubbed (M)					
Fetal Incidence	N(%)	0	0	1(0.3)	18(12.2)**
Litter Incidence	N(%)	0	0	1(4.8)	9(47.4)**
TAIL: Bent (M)					
Fetal Incidence	N(%)	0	1(0.4)	2(0.7)	12(8.2)**
Litter Incidence	N(%)	0	1(4.5)	2(9.5)	5(26.3)**
TAIL: Absent (M)					
Fetal Incidence	N(%)	0	0	1(0.3)	8(5.4)**
Litter Incidence	N(%)	0	0	1(4.8)	6(31.6)**
TAIL: Kinked (M)					
Fetal Incidence	N(%)	0	0	0	8(5.4)**
Litter Incidence	N(%)	0	0	0	7(36.8)**
TAIL: Curled (M)					
Fetal Incidence	N(%)	0	0	0	8(5.4)**
Litter Incidence	N(%)	0	0	0	5(26.3)*
TAIL: Filamentous (M)					
Fetal Incidence	N(%)	0	0	0	2(1.4)
Litter Incidence	N(%)	0	0	0	2(10.5)

(M) = Malformation (V) = Variation

Statistical Analysis: Fisher's Exact test.

* Significantly different from control at P≤ 0.05.
** Significantly different from control at P≤ 0.01.

a - All percentages were calculated on the basis of live fetuses in each group. There were no remarkable placental observations.
b - Excludes litter 4F0082 which had 13 early resorptions and two dead fetuses; both dead fetuses appeared unremarkable at gross external examination.
c - Fetus 4F0087-R6 had a stubbed tail.
d - N=19; excludes litter 4F0073 in which all (100%) conceptuses were resorbed and litter 4F0082 that had 13 early resorptions and two dead fetuses.

SUMMARY OF FETAL VISCERAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.9)	22(100.0)	21(95.5)	21(95.5)
FETUSES EVALUATED	N	134	140	145	74
Live Fetuses	N	134	140	145	74
Dead Fetuses	N	0	0	0	0[b]
LITTERS EVALUATED	N	20	22	21	17[c]
BRAIN: Total (Summarization of all Brain Alterations)					
Fetal Incidence	N(%)	0	0	1(0.7)	17(23.0)**
Litter Incidence	N(%)	0	0	1(4.8)	10(58.8)**
BRAIN: Lateral and/or third ventricles, dilated, slight (V)					
Fetal Incidence	N(%)	0	0	1(0.7)	12(16.2)**
Litter Incidence	N(%)	0	0	1(4.8)	7(41.2)**
BRAIN: Cerebral hemisphere(s), misshapen[d] (M)					
Fetal Incidence	N(%)	0	0	0	4(5.4)*
Litter Incidence	N(%)	0	0	0	4(23.5)*
BRAIN: Lateral and/or third ventricles, dilated, moderate or severe [Hydrocephaly] (M)					
Fetal Incidence	N(%)	0	0	0	2(2.7)
Litter Incidence	N(%)	0	0	0	2(11.8)
BRAIN: Lateral and/or third ventricles, small, and misshapen (interrelated with exencephaly) (M)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.9)
BRAIN: Cerebral hernia with absence of the left lateral and third ventricles (M)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.9)
EYES: Small[d] (M)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.9)
PALATE: Primary cleft, bilateral (M)					
Fetal Incidence	N(%)	0	0	1(0.7)	0
Litter Incidence	N(%)	0	0	1(4.8)	0

(M) = Malformation (V) = Variation
NOTE: Footnotes are located on the last page of this table.

Statistical Analysis: Fisher's Exact test.
* Significantly different from control at P≤ 0.05.
** Significantly different from control at P≤ 0.01.

SUMMARY OF FETAL VISCERAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.9)	22(100.0)	21(95.5)	21(95.5)
FETUSES EVALUATED	N	134	140	145	74
Live Fetuses	N	134	140	145	74
Dead Fetuses	N	0	0	0	0[b]
LITTERS EVALUATED	N	20	22	21	17[c]
HEART: Total (Summarization of all Heart Alterations)					
Fetal Incidence	N(%)	0	0	2(1.4)	3(4.1)*
Litter Incidence	N(%)	0	0	1(4.8)	3(17.6)
HEART: Ventricles, misshapen[d] (M)					
Fetal Incidence	N(%)	0	0	2(1.4)	0
Litter Incidence	N(%)	0	0	1(4.8)	0
HEART: Ventricles, small[d] (M)					
Fetal Incidence	N(%)	0	0	1(0.7)	1(1.4)
Litter Incidence	N(%)	0	0	1(4.8)	1(5.9)
HEART: Engored with red substance and necrotic; atria, enlarged (M)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.9)
HEART: Atria, small[d] (M)					
Fetal Incidence	N(%)	0	0	1(0.7)	2(2.7)
Litter Incidence	N(%)	0	0	1(4.8)	2(11.8)
THORACIC CAVITY: Necrotic organs (lungs, thymus, thyroid, and/or heart), some discolored and/or small (M)					
Fetal Incidence	N(%)	0	3(2.1)	2(1.4)	1(1.4)
Litter Incidence	N(%)	0	1(4.5)	1(4.8)	1(5.9)
THYROID: Discolored (V)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.9)
LIVER: Nodule, pale (interrelated with umbilical hernia or gastroschisis) (M)					
Fetal Incidence	N(%)	0	0	1(0.7)	2(2.7)
Litter Incidence	N(%)	0	0	1(4.8)	2(11.8)

(M) = Malformation (V) = Variation
NOTE: Footnotes are located on the last page of this table.

Statistical Analysis: Fisher's Exact test.
* Significantly different from control at P≤ 0.05.

SUMMARY OF FETAL VISCERAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.9)	22(100.0)	21(95.5)	21(95.5)
FETUSES EVALUATED	N	134	140	145	74
Live Fetuses	N	134	140	145	74
Dead Fetuses	N	0	0	0	0[b]
LITTERS EVALUATED	N	20	22	21	17[c]
LIVER: Misshapen (interrelated with gastroschisis) (M)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.9)
LIVER: Mottled, discolored, enlarged, firm, and/or necrotic (M)					
Fetal Incidence	N(%)	0	0	1(1.4)	1(2.7)
Litter Incidence	N(%)	0	0	1(9.5)	1(5.9)
ABDOMINAL CAVITY: Necrotic organs (spleen, liver, adrenals, kidneys, ureters, intestines, stomach, pancreas, and/or reproductive organs), and/or discolored (M)					
Fetal Incidence	N(%)	0	1(0.7)	1(0.7)	1(1.4)
Litter Incidence	N(%)	0	1(4.5)	1(4.8)	1(5.9)
KIDNEYS: Hemorrhagic[d] (V)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.9)

(M) = Malformation (V) = Variation

Statistical Analysis: Fisher's Exact test.

a - All percentages were calculated on the basis of the number of live fetuses in each group.
b - Excludes litter 4F0082 with 13 early resorptions and two dead fetuses, one of which (4F0082-R1) was assigned to visceral evaluation and appeared unremarkable.
c - N=17; excludes litter 4F0073 in which all (100%) conceptuses were resorbed, litter 4F0082 with 13 early resorptions and two dead fetuses, and litters 4F0072 and 4F0079 with only one viable fetus in each litter that was assigned to skeletal examination.
d - Right and/or left.

SUMMARY OF FETAL SKELETAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.0)	22(100.0)	21(95.5)	21(95.5)
FETUSES EVALUATED	N	134	142	144	74
Live Fetuses	N	134	142	144	73
Dead Fetuses	N	0	0	0	1[b,c]
LITTERS EVALUATED	N	20	22	21	18[d]
INTERPARIETAL: Incomplete ossification (V)					
Fetal Incidence	N(%)	0	0	2(1.4)	12(16.4)**
Litter Incidence	N(%)	0	0	2(9.5)	8(44.4)**
INTERPARIETAL: Hypoplastic (V)					
Fetal Incidence	N(%)	6(4.5)	2(1.4)	2(1.4)	10(13.7)*
Litter Incidence	N(%)	4(20.0)	2(9.1)	2(9.5)	4(22.2)
PARIETALS: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	2(1.5)	1(0.7)	0	9(12.3)**
Litter Incidence	N(%)	2(10.0)	1(4.5)	0	3(16.7)
PARIETALS: Hypoplastic[e] (V)					
Fetal Incidence	N(%)	2(1.5)	0	3(2.1)	7(9.6)**
Litter Incidence	N(%)	2(10.0)	0	3(14.3)	2(11.1)
FRONTALS: Hypoplastic[e] (V)					
Fetal Incidence	N(%)	0	0	1(0.7)	6(8.2)**
Litter Incidence	N(%)	0	0	1(4.8)	3(16.7)
FRONTALS: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	0	0	0	2(2.7)
Litter Incidence	N(%)	0	0	0	1(5.6)
SUPRAOCCIPITAL: Incomplete ossification (V)					
Fetal Incidence	N(%)	1(0.7)	1(0.7)	3(2.1)	2(2.7)
Litter Incidence	N(%)	1(5.0)	1(4.5)	3(14.3)	2(11.1)
SUPRAOCCIPITAL: Hypoplastic (V)					
Fetal Incidence	N(%)	6(4.5)	4(2.8)	2(1.4)	7(9.6)
Litter Incidence	N(%)	5(25.0)	3(13.6)	2(9.5)	3(16.7)

(M) = Malformation (V) = Variation
NOTE: Footnotes are located on the last page of this table.

Statistical Analysis: Fisher's Exact test.
* Significantly different from control at P≤ 0.05.
** Significantly different from control at P≤ 0.01.

SUMMARY OF FETAL SKELETAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.0)	22(100.0)	21(95.5)	21(95.5)
FETUSES EVALUATED	N	134	142	144	74
Live Fetuses	N	134	142	144	73
Dead Fetuses	N	0	0	0	1[b,c]
LITTERS EVALUATED	N	20	22	21	18[d]
HYOID: Incomplete ossification (V)					
Fetal Incidence	N(%)	20(14.9)	13(9.2)	13(9.0)	2(2.7)**
Litter Incidence	N(%)	10(50.0)	7(31.8)	10(47.6)	2(11.1)*
HYOID: Not ossified (V)					
Fetal Incidence	N(%)	15(11.2)	11(7.7)	10(6.9)	0**
Litter Incidence	N(%)	10(50.0)	6(27.3)	7(33.3)	0**
PREMAXILLAE: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.6)
MAXILLAE: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.6)
EXOCCIPITALS: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	0	0	0	1(1.4)
Litter Incidence	N(%)	0	0	0	1(5.6)
SQUAMOSALS: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	0	1(0.7)	0	0
Litter Incidence	N(%)	0	1(4.5)	0	0
VERTEBRAE: Centra, dumbbell-shaped (V)					
Fetal Incidence	N(%)	5(3.7)	1(0.7)	9(6.3)	52(71.2)**
Litter Incidence	N(%)	4(20.0)	1(4.5)	6(28.6)	17(94.4)**
VERTEBRAE: Centra, bifid (V)					
Fetal Incidence	N(%)	2(1.5)	1(0.7)	5(3.5)	43(58.9)**
Litter Incidence	N(%)	1(5.0)	1(4.5)	5(23.8)	17(94.4)**

(M) = Malformation (V) = Variation
NOTE: Footnotes are located on the last page of this table.

Statistical Analysis: Fisher's Exact test.
* Significantly different from control at P≤ 0.05.
** Significantly different from control at P≤ 0.01.

SUMMARY OF FETAL SKELETAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED RATS PREGNANT	N N(%)	22 20(90.0)	22 22(100.0)	22 21(95.5)	22 21(95.5)
FETUSES EVALUATED	N	134	142	144	74
Live Fetuses Dead Fetuses	N N	134 0	142 0	144 0	73 1[b,c]
LITTERS EVALUATED	N	20	22	21	18[d]
VERTEBRAE: Arches, bifid[e] (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	0 0	0 0	4(2.8) 2(9.5)	21(28.8)** 11(61.1)**
VERTEBRAE: Centra, not ossified (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	1(0.7) 1(5.0)	0 0	0 0	26(35.6)** 10 (55.6)**
VERTEBRAE: Centra, unilateral ossification, left or right (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	0 0	0 0	1(0.7) 1(4.8)	23(31.5)** 11(61.1)**
VERTEBRAE: Arches, incomplete ossification[e] (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	0 0	0 0	1(0.7) 1(4.8)	1(1.4) 1(5.6)
VERTEBRAE: Arches, fused[e] (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	0 0	0 0	0 0	1(1.4) 1(5.6)
VERTEBRAE: Centra: incomplete ossification (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	0 0	0 0	0 0	2(2.7) 1(5.6)
STERNEBRAE: Cleaved (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	0 0	0 0	0 0	2(2.7) 2(11.1)
STERNEBRAE: Fused (V)					
Fetal Incidence Litter Incidence	N(%) N(%)	0 0	0 0	1(0.7) 1(4.8)	0 0

(M) = Malformation (V) = Variation
NOTE: Footnotes are located on the last page of this table.

Statistical Analysis: Fisher's Exact test.
** Significantly different from control at P≤ 0.01.

SUMMARY OF FETAL SKELETAL OBSERVATIONS[a]

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
RATS EVALUATED	N	22	22	22	22
RATS PREGNANT	N(%)	20(90.0)	22(100.0)	21(95.5)	21(95.5)
FETUSES EVALUATED	N	134	142	144	74
Live Fetuses	N	134	142	144	73
Dead Fetuses	N	0	0	0	1[b,c]
LITTERS EVALUATED	N	20	22	21	18[d]
STERNEBRAE: Duplicated (V)					
Fetal Incidence	N(%)	0	0	1(0.7)	0
Litter Incidence	N(%)	0	0	1(4.8)	0
RIBS: Wavy[e] (V)					
Fetal Incidence	N(%)	3(2.2)	0	2(1.4)	2(2.7)
Litter Incidence	N(%)	3(15.0)	0	2(9.5)	2(11.1)
RIBS: Nodulated[e] (V)					
Fetal Incidence	N(%)	1(0.7)	0	1(0.7)	1(1.4)
Litter Incidence	N(%)	1(5.0)	0	1(4.8)	1(5.6)
PUBES: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	0	0	4(2.8)	4(5.5)*
Litter Incidence	N(%)	0	0	2(9.5)	2(11.1)
ISCHIA: Incomplete ossification[e] (V)					
Fetal Incidence	N(%)	0	0	1(0.7)	0
Litter Incidence	N(%)	0	0	1(4.8)	0

(M) = Malformation (V) = Variation
NOTE: Footnotes are located on the last page of this table.

Statistical Analysis: Fisher's Exact test.
* Significantly different from control at P≤ 0.05.

a - All percentages were calculated on the basis of the number of live fetuses in each group.
b - Excludes litter 4F0082 which had 13 early resorptions and two dead fetuses, one of which (4F0082-R2) was assigned to skeletal examination and had the following observations: Hyoid: Incomplete ossification; Vertebrae: Arches, C5 through C7, bilateral, bifid; Vertebrae: Centra, T1 through T4 and T6, not ossified; T5, dumbbell-shaped; T9 and T13, bifid; T10 through T12, unilateral ossification, left.
c - Fetus 4F0087-R6 had Vertebrae: Arches, C6 left, bifid.
d - N=18; excludes litter 4F0073 in which all (100%) conceptuses were resorbed, litter 4F0082 with 13 early resorptions and two dead fetuses, and litter 4F0083 with only one live fetus that was assigned to visceral examination.
e - Right and/or left.

SUMMARY OF FETAL OSSIFICATION AVERAGES

GROUP TREATMENT DAILY DOSE (mg/kg/day)		1 Control 0	2 BMS-201038 0.04	3 BMS-201038 0.4	4 BMS-201038 4
FETUSES EVALUATED	N	229	328	301	241
LITTERS EVALUATED	N	20	22	21	18[b]
OSSIFICATION SITES PER FETUS PER LITTER					
RIBS(Total)	MEAN	13.14	13.09	13.05	13.11
	SD	0.19	0.18	0.10	0.18
Right Rib	MEAN	13.11	13.06	13.03	13.08
	SD	0.19	0.13	0.08	0.16
Left Rib	MEAN	13.16	13.11	13.07	13.13
	SD	0.21	0.24	0.13	0.27
STERNEBRAE	MEAN	5.57	5.82	5.39	4.58**
	SD	0.48	0.24	0.42	0.80
FOREPAWS[a]					
Carpals	MEAN	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00
Metacarpals	MEAN	3.84	3.97	3.75	3.39**
	SD	0.23	0.07	0.29	0.41
Phalanges	MEAN	6.13	6.76	6.10	5.56
	SD	0.90	0.78	0.77	1.12
HINDPAWS[a]					
Tarsals	MEAN	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00
Metatarsals	MEAN	3.99	4.00	3.97	3.97
	SD	0.05	0.00	0.15	0.08
Phalanges	MEAN	5.11	5.36	5.11	4.80
	SD	0.80	0.69	0.50	1.10

Statistical Analyses: Analysis of Variance with Dunnett's procedure.
** Significantly different from control at P≤ 0.01.

a - Calculated as average per limb.
b - N=18; excludes litter 4F0082 which had 13 early resorptions and two dead fetuses, one of which (4F0082-R2) was assigned to skeletal examination (See Table 11 for skeletal alterations).

Study title: Oral study of embryo-fetal development in rabbits

Study no.:	96032 (b) (4)
Study report location:	Module 4.2.3.5.2
Conducting laboratory:	(b) (4)
Date of study initiation:	27 October 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BMS-201038-04 (AEGR-733), Batch #R007A, 87.2% pure "as is" as the free base

Key Study Findings

- There were no apparent test article-related mortalities. Five unscheduled deaths occurred that were likely the result of gavage errors (1, 1, and 3 deaths in the LD, MD, and HD groups, respectively).
- The HD group had fewer litters for assessment (14 compared with 19 for control) because 1 doe was found not to be pregnant, 2 does aborted, and 3 died prematurely because of gavage error.
- There were no apparent treatment-related clinical signs or necropsy findings.
- Maternal body weights were reduced for the MD and HD groups, which correlated with decreased food consumption. Body weight slightly rebounded after the dosing period ended.
- There were no apparent treatment-related effects on number of implantation sites, resorptions, number of viable fetuses, litter size, male/female ratio, or fetal body weights.
- The percentage of fetuses with any kind of alteration was statistically significantly higher for the HD group compared with controls (12.9% vs. 7.5%); however the mean historical control value for this parameter was 12.5%. The increase in this parameter was primarily due to a small but statistically significant increase in the percent fetal incidence of irregular ossification of the nasal bones (midline suture displacement). This finding was not considered to be toxicologically meaningful because it is a developmental variation rather than a malformation, was not accompanied by other alterations in ossification, and was within the laboratory's historical control range (values not provided).
- The NOAEL for embryonic development in rabbits is considered to be 10 mg/kg/day. Signs of maternal toxicity (decreased food consumption and body weight gain) were noted at the MD and HD levels.
- The NOAEL is approximately 3 times higher than the maximum clinical dose of 60 mg based on body surface area extrapolation. Because there is no available exposure data for rabbits, the clinical exposure margin cannot be determined.

Methods

Doses:	0, 0.1, 1, and 10 mg/kg/day
Frequency of dosing:	Once daily for 13 days (GD6 to GD18)
Dose volume:	1 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	75% (v/v) PEG 400
Species/Strain:	Rabbit/New Zealand white
Number/Sex/Group:	20 timed-pregnant females/group
Satellite groups:	None
Study design:	All rabbits were observed at least once daily for abnormal clinical signs, body weights, and food consumption. All surviving animals were sacrificed on GD29; the thoracic, abdominal, and pelvic viscera were examined for gross lesions. The C-section parameters included: number of corpora lutea, number and site of implantation, early and late resorptions, and live and dead fetuses. All fetuses were examined internally and externally for variation and malformation of viscera and skeleton.
Study protocol deviations:	There were no protocol deviations that impacted the interpretability of the study data.

Observations and Results

Mortality

There were 5 unscheduled deaths during the treatment period. The deaths were considered to be the result of dosing errors and/or aspiration of the test compound based on the necropsy findings in the trachea and lungs and the close proximity in time between dosing and death for 4 of 5 animals.

Summary of Mortality Incidence

Dose group (mg/kg/day)	Day of Death	Findings
0.1	Died 5 minutes after dosing on GD 16	Perinasal and perioral substance; white foam in the trachea and firm lung lobes.
1	Died 6 minutes after dosing on GD 17	White foam present in the trachea; firm and mottled lung lobes; tear in right diaphragmatic lung lobe.
10	Died 5 minutes after dosing on GD15	Pink perinasal and perioral substances; white foam in the trachea; mottled (pink to dark red) lung lobes.
	Died 5 minutes after dosing on GD17	White foam present in the trachea and firm, mottled lung lobes.
	Found dead on GD20	Tan to red mottling in lung lobes.

Clinical Signs and Necropsy Findings

Clinical signs noted during the study included soft or liquid feces, areas of localized alopecia, red substance in cage pan, dry feces, few or no feces, perioral and perinasal substance, and missing/broken incisors. In addition to the lung findings noted in the table above, tan and/or accentuated lobular pattern in the liver, pale or mottled liver lobes, red areas on the fundic mucosa, red fluid in the uterus and cervix, and parovarian cysts were observed. These observations were not considered to be related to the test article because the incidences were not dose dependent and/or the observation was noted in only one HD doe.

Abortions

One control group doe and two 10 mg/kg/day group does aborted. These events were considered to be unrelated to test article because the incidence was within the historical control range for the lab (historical mean = 2.5% vs. 10% for HD group; range not provided) and the difference from control was not statistically significant. Additionally, the sponsor notes that no abortions were noted in the range-finding study in which even higher doses were administered (40 mg/kg/day). A summary of findings are shown in the table below.

Summary of Abortion Incidence

Dose group (mg/kg/day)	Day of Abortion	Findings
0	GD28	Litter consisted of 10 apparent normal fetuses. Clinical observations included no feces in cage pan from GD22 to 25 and red substance in cage pan on GD27. Doe had intermittent weight losses between GD9 and GD15, followed by persistent weight loss associated with decreased food consumption.
10	GD25	Litter consisted of 8 fetuses that appeared normal for their development age. Clinical observations included weight loss after GD9 that was associated with decreased food consumption. Necropsy showed tan liver with an accentuated lobular pattern in all lobes.
	GD28	Litter consisted of 7 conceptuses; 2 were late resorptions, 2 were apparently normal, and 3 were presumed cannibalized. Clinical observations included soft or liquid feces on GD14 and 15, few feces on GD18, and a red substance in cage pan on GD27 and 28. Doe had intermittent weight losses after GD9 that were associated with decreased food consumption.

Body Weight

Effects on body weight were observed at the MD and HD levels. Decreased body weight gain was statistically significant between GD6 and GD9 for the MD group and between GD9 and GD12 for the MD and HD groups, with slight body weight loss occurring for the HD group during this period. Decreases in body weight gain were still noted at the end of dosing (GD18) but were not statistically significant. This effect was partially reversible during the non-dosing period (GD19 to GD29) as the body weight decrement was less at GD29 than at GD18. Body weights are summarized in the table below.

Summary of Body Weight Gain

Dose (mg/kg/d)	0	0.1	1	10
Weight (kg) - GD 6	3.90	3.91	3.90	3.89
Weight (kg) - GD 18	4.07	4.10	3.96	3.93
Weight (g) - GD 29	4.26	4.27	4.19	4.14
Weight gain (g) - GD 6-18	0.17	0.19	0.06	0.04
Diff from control (g)		0.02	-0.11	-0.13
% diff from control		↑12%	↓65%	↓76%
Weight gain (g) - GD 6-29	0.36	0.36	0.29	0.25
Diff from control (g)		0	-0.07	-0.11
% diff from control		-	↓19%	↓31%

GD = gestation day.

Food Consumption

Effects on body weight were consistent with decreased food consumption, which was statistically significantly lower for MD and HD groups between GD6 and GD19. Food consumption was similar to controls during the non-dosing period (GD19 to GD29).

Toxicokinetics

Not conducted in this study. Exposure of AEGR-733 or its metabolites was not measured in any rabbit study that was conducted by the sponsor. Therefore, clinical exposure margins can only be estimated based on body surface area extrapolation.

Dosing Solution Analysis

The concentrations of test article formulations for the first week of the study were found to be acceptable (between 97.9% and 99.4% of the intended concentration).

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

Litters from does that died prematurely or aborted were not included in this assessment. Therefore, C-section observations were based on 19, 19, 19, and 14 litters with live fetuses on GD29 for the control, LD, MD, and HD groups. All does evaluated on GD29 had viable fetuses; there were no does with all conceptuses resorbed.

There were no apparent treatment-related effects on number of implantation sites, resorptions, number of viable fetuses, litter size, male/female ratio, or fetal body weights. The litter size for the HD group (8.3 ± 2.5) was smaller than for the concurrent control group (9.2 ± 1.3); however, the number of corpora lutea were also less for the HD group (9.4 ± 2.2) compared with control (10.0 ± 1.3). Because the number of corpora lutea would have been established before the start of treatment, the apparent difference in litter size is not felt to be due to a treatment-related effect. The data are summarized in the sponsor-generated table below.

Caesarean-Sectioning Observations (sponsor-generated table)

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0.1	III 1	IV 10
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	20(100.0)	20(100.0)	20(100.0)	19(95.0)
FOUND DEAD	N(%)	0(0.0)	1(5.0)	1(5.0)	3(15.8)
ABORTED	N(%)	1(5.0)	0(0.0)	0(0.0)	2(10.5)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	19	19	19	14
CORPORA LUTEA	MEAN±S.D.	10.0 ± 1.3	9.7 ± 2.4	10.0 ± 2.2	9.4 ± 2.2
IMPLANTATIONS	MEAN±S.D.	9.7 ± 1.3	9.4 ± 2.6	9.3 ± 2.8	9.1 ± 2.5
LITTER SIZES	MEAN±S.D.	9.2 ± 1.4	9.0 ± 2.2	8.9 ± 2.7	8.3 ± 2.5
LIVE FETUSES	N	174	172	166	116
	MEAN±S.D.	9.2 ± 1.3	9.0 ± 2.2	8.7 ± 3.0	8.3 ± 2.5
DEAD FETUSES	N	1	0	4	0
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.2 ± 0.9	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.5 ± 0.8	0.3 ± 0.6	0.4 ± 0.6	0.8 ± 1.0
EARLY RESORPTIONS	N	4	2	4	7
	MEAN±S.D.	0.2 ± 0.6	0.1 ± 0.3	0.2 ± 0.5	0.5 ± 0.9
LATE RESORPTIONS	N	6	4	3	4
	MEAN±S.D.	0.3 ± 0.7	0.2 ± 0.5	0.2 ± 0.4	0.3 ± 0.6
DOES WITH ANY RESORPTIONS	N(%)	6(31.6)	5(26.3)	6(31.6)	7(50.0)
DOES WITH ALL CONCEPTUSES RESORBED	N	0	0	0	0
DOES WITH VIABLE FETUSES	N(%)	19(100.0)	19(100.0)	19(100.0)	14(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	19(100.0)	19(100.0)	14(100.0)

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LITTERS WITH ONE OR MORE LIVE FETUSES	N	19	19	19	14
IMPLANTATIONS	MEAN±S.D.	9.7 ± 1.3	9.4 ± 2.6	9.3 ± 2.8	9.1 ± 2.5
LIVE FETUSES	N	174	172	166	116
	MEAN±S.D.	9.2 ± 1.3	9.0 ± 2.2	8.7 ± 3.0	8.3 ± 2.5
LIVE MALE FETUSES	N	87	99	93	59
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	50.8 ± 23.2	57.1 ± 13.6	57.8 ± 18.2	50.8 ± 17.6
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.38 ± 4.80	42.90 ± 4.76	42.30 ± 4.49	42.26 ± 3.51 [13]b
MALE FETUSES	MEAN±S.D.	43.09 ± 4.17 [18]c	43.51 ± 4.71	42.50 ± 5.07	42.34 ± 3.62 [13]b
FEMALE FETUSES	MEAN±S.D.	42.55 ± 5.18 [18]d	41.90 ± 5.61	41.72 ± 4.00 [18]e	42.44 ± 3.46 [13]b
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.7 ± 8.4	2.6 ± 4.7	6.5 ± 13.6	8.7 ± 11.3

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 18 of gestation.
b. Excludes values that were not recorded.
c. Litter 3498 had no male fetuses.
d. Litter 3505 had no female fetuses.
e. Litter 3545 had no female fetuses.

Offspring (Malformations, Variations, etc.)

The percent of fetuses with any kind of alteration was statistically significantly higher for the HD group compared with controls (12.9% vs. 7.5%). This increase primarily is due to a small but statistically significant increase in the percent incidence of irregular ossification of the nasal bones, including fused internasal bones and midline suture displacement. These are considered to be skeletal variations rather than malformations. The percent incidence of litters with these findings was not statistically significantly increased.

The study director felt that the observed alterations were not related to AEGR-733 because 1) the litter incidence was not increased compared with concurrent controls and; 2) the values were within the lab's historical control ranges. The percentage of control group fetuses with any alteration observed for the ten most recent rabbit studies conducted at the laboratory ranged from 10.0% to 14.9% with a mean of 12.4% ± 1.6. Based on this information, it appears that the concurrent control value (7.5%) is below the lab's historical range and the HD group value (12.9%) is at the historical mean value. The study director also states that the specific finding of midline suture displacement of the nasal bones was also within the historical control range but the values were not provided. The increased incidence of angulated hyoid was also not felt to be treatment related because this finding did not occur in a dose-related manner and was not observed at the high-dose. A summary of noteworthy fetal alterations is shown in the sponsor-generated tables below.

Summary of Total Alterations (sponsor-generated table)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1	10
LITTERS EVALUATED	N	19	19	19	14
FETUSES EVALUATED	N	175	172	170	116
LIVE	N	174	172	166	116
DEAD	N	1b	0	4b	0
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	10(52.6)	11(57.9)	6(31.6)	10(71.4)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	13(7.5)	18(10.5)	7(4.2)	15(12.9)**
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	8.2 ± 10.5	9.9 ± 10.4	5.4 ± 9.5	13.0 ± 10.0

a. Dosage occurred on days 6 through 18 of gestation.
b. Dead fetuses were excluded from group averages and statistical analyses. Observations for these conceptuses are cited on Table 22.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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Fetal Skeletal Alterations (sponsor-generated table)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	1	10
LITTERS EVALUATED	N	19	19	19	14
FETUSES EVALUATED	N	175	172	170	116
LIVE	N	174	172	166	116
DEAD	N	1b	0	4b	0
SKULL - IRREGULAR OSSIFICATION:c (SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL d; INDIVIDUAL SUBCATEGORIES CITED BELOW)					
LITTER INCIDENCE	N(%)	6(31.6)	3(15.8)	2(10.5)	6(42.8)
FETAL INCIDENCE	N(%)	8(4.6)	3(1.7)	2(1.2)*	10(8.6)*
SKULL: NASAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTERNASAL; FUSED; INTRANASAL; MIDLINE SUTURE DISPLACED)					
LITTER INCIDENCE	N(%)	5(26.3)	3(15.8)	1(5.3)	6(42.8)
FETAL INCIDENCE	N(%)	7(4.0)	3(1.7)	1(0.6)*	9(7.8)*
SKULL: NASALS, CONTAINED AN INTERNASAL					
LITTER INCIDENCE	N(%)	3(15.8)	2(10.5)	0(0.0)	1(7.1)
FETAL INCIDENCE	N(%)	3(1.7)	2(1.2)j	0(0.0)	2(1.7)
SKULL: NASALS, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	2(14.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)i	0(0.0)	2(1.7)
SKULL: NASAL, CONTAINED AN INTRANASAL					
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
SKULL: NASALS, MIDLINE SUTURE DISPLACED					
LITTER INCIDENCE	N(%)	3(15.8)	0(0.0)	1(5.3)	3(21.4)
FETAL INCIDENCE	N(%)	3(1.7)e,h	0(0.0)	1(0.6)	5(4.3)**1
SKULL: FRONTALS, CONTAINED AN INTERFRONTAL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	1(7.1)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	1(0.9)
SKULL: PARIETAL, CONTAINED A HOLE					
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)g	0(0.0)	0(0.0)	0(0.0)
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	5(2.9)**j	1(0.6)	0(0.0)

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9.3 Prenatal and Postnatal Development

Study title: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of Lomitapide (also known as AEGR-733 and BMS-201038) in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	AEGR-733PC0014
Study report location:	Module 4.2.3.5
Conducting laboratory:	(b) (4)
Date of study initiation:	08 December 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AEGR-733, #L0109571, 84.3% pure "as is" free base

Key Study Findings

- There were no meaningful treatment-related effects at the low dose.
- Lower F_0 maternal body weights were observed at all dose levels, with statistical significance at the high dose. The effects on maternal body weight were likely due to decreased litter size and lower fetal body weights.
- A slightly increased gestation period was observed for the high-dose dams.
- Decreased indices of live liter size per litter, number of live pups, viability index, lactation index, and fetal body weight were observed for the high-dose litters.
- An increased number of stillborn pups and pups dying on LDs 1-7 occurred at the HD.
- Fetal eye anomalies (missing eye, microphthalmia) occurred at a low incidence in MD and HD groups. Increases in tail anomalies (bent, short, tail or tip of the tail missing or absent, purple, red and/or black), pale body, or a head mass was noted at the HD. One HD pup had dilation of the lateral ventricles of the brain and one HD pup had a malformed forelimb.
- F_1 generation rats from HD dams were not able to overcome the deficits in postpartum body weights (remained lower after weaning).
- No statistically significant or biologically important differences occurred in the passive avoidance or water maze performance of the F_1 generation male and female rats regarding learning, short-term retention, long-term retention, or response inhibition.
- There were no statistically or biologically important effects on the mating and fertility parameters evaluated in the F_1 generation male and female rats.
- All fetal gross alterations of the F_2 generation were considered unrelated to in utero maternal (F_1) exposure.
- The NOAEL for F1 development was 0.1 mg/kg/d (<1X MRHD); the NOAEL for F_0 reproduction was 0.3 mg/kg/d (~1X MRHD) due to delayed parturition at the HD; and the NOAEL for maternal toxicity was 0.3 mg/kg/d (~1X MRHD) due to the effects on maternal body weight at the HD, especially during Lactation Days 1 to 4.

Methods

Species/Strain: Rat/Sprague-Dawley

Frequency of dosing: Once daily from Gestation Day 7 through Lactation Day 20

Route of administration: Oral

Formulation/Vehicle: 75% (v/v) polyethylene glycol 400 in water

Study design: F_0 Generation - see sponsor-generated table below

Dosage Group	Number of Rats	Dosage (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)
I	25	0 (Vehicle)	0	1
II	25	0.1	0.1	1
III	25	0.3	0.3	1
IV	25	1	1	1

: F_1 Generation - see sponsor-generated table below

Dosage Group	Maternal Dosage (mg/kg/day)	Number of Rats per Sex	Assigned Rat Numbers	
			Male Rats	Female Rats
I	0 (Vehicle)	25	3501 - 3525	3601 - 3625
II	0.1	25	3526 - 3550	3636 - 3650
III	0.3	25	3551 - 3575	3651 - 3675
IV	1	25	3576 - 3588, 4001, 3590-3600	3676 - 3700

Toxicokinetics: Not conducted

Dosing Solution Analysis: Mean measured lomitapide concentrations for all dose formulations were within the acceptable limit of ±≤10% from nominal.

Protocol deviations: There were no protocol or GLP deviations that affected the interpretation of the study results.

Observations and Results

F_0 Dams

Survival: (Twice daily)
There were no unscheduled deaths.

Clinical signs: (Daily, before and after dosing)
There were no treatment-related effects on behavior.

Body weight and Food consumption: (BW daily during dosing period; FC approximately every 3 days)
Maternal body weight gain between GD7 and GD20 was 4%, 7%, and 16% less than controls for the 0.1, 0.3, and 1 mg/kg/d groups. The effect at the high dose was statistically significant, primarily due to decreased weight gain between GD15 and GD20. A decrease in mean food consumption for HD dams occurred between GD7 and

GD12, but was similar to control values thereafter. High-dose maternal weights decreased by 1.1 g between Lactation Days 1 and 4 compared with a 6.4 g weight gain for the control group. No effects on body weight were noted thereafter. High-dose females had decreased food consumption from Lactation Days 1 through 14.

Uterine content: (Day 21 of postpartum period)
There were no treatment-related differences in the number of implantation sites.

Necropsy observation: (Day 21 of postpartum period)
There were no noteworthy findings at necropsy.

Parturition:
The mean duration of gestation was slightly, but statistically significantly, longer for the high-dose dams (22.8 days) compared with the controls (22.3 days). There was a slight increase in the number of dams that had stillborn pups at the high dose (5/25) compared with control (2/24).

F_1 Generation

Survival: (Twice daily)
A statistically significant decrease in live births and increase in stillborns were observed at the high dose. There was also an increase in fetal death in the high-dose group during LD1 through LD7. Data are summarized in the sponsor-generated table below.

DOSAGE GROUP			I	II	III	IV
DOSAGE (MG/KG/DAY)a			0 (VEHICLE)	0.1	0.3	1
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS		N	24	25	25	25
PUPS DELIVERED (TOTAL)		N	324	346	326	289
		MEAN±S.D.	13.5 ± 2.1	13.8 ± 1.7	13.0 ± 1.9	11.6 ± 3.0
	LIVEBORN	MEAN±S.D.	13.4 ± 2.1	13.8 ± 1.7	13.0 ± 2.0	11.0 ± 3.2*
		N(%)	322(99.4)	344(99.4)	324(99.4)	274(94.8)**
	STILLBORN	MEAN±S.D.	0.1 ± 0.3	0.1 ± 0.3	0.0 ± 0.2	0.3 ± 0.6
		N(%)	2(0.6)	2(0.6)	1(0.3)	7(2.4)**
	UNKNOWN VITAL STATUS	N	0	0	1	8
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED						
DAY 1		N/N(%)	1/322(0.3)	3/344(0.9)	0/324(0.0)	23/274(8.4)**
DAYS 2- 4		N/N(%)	3/321(0.9)	1/341(0.3)	2/324(0.6)	34/251(13.5)**
DAYS 5- 7		N/N(%)	1/318(0.3)	0/340(0.0)	0/322(0.0)	4/217(1.8)**
DAYS 8-14		N/N(%)	0/317(0.0)	0/340(0.0)	0/322(0.0)	0/213(0.0)
DAYS 15-18		N/N(%)	0/317(0.0)	1/340(0.3)	1/322(0.3)	1/213(0.5)
DAYS 19-21		N/N(%)	0/317(0.0)	0/339(0.0)	1/321(0.3)	0/212(0.0)
VIABILITY INDEX b		%	98.8	98.8	99.4	79.2
		N/N	318/322	340/344	322/324	217/274**
LACTATION INDEX c		%	99.7	99.7	99.4	97.7
		N/N	317/318	339/340	320/322	212/217**

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
b. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
c. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 4 postpartum.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE A14 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	0.3	1
LITTERS EVALUATED	N	24	25	25	25
TOTAL PUPS STILLBORN OR FOUND DEAD a,b	N	5	7	3	26
STILLBORN	N	2	2	1	7
FOUND DEAD	N	3	5	2	19
NO MILK IN STOMACH c	N(%)	1(33.3)	2(40.0)	2(100.0)	13(68.4)
APPEARED NORMAL	N(%)	4(80.0)	4(57.1)	1(33.3)	12(46.2)

Clinical signs: (Daily during pre-weaning and weekly thereafter)
An increase in the number of pale pups was noted at the high-dose. Other clinical signs did not appear to be treatment related because of a low incidence or lack of dose relationship. Potential developmental defects noted in the sponsor-generated table below are discussed further in the physical development section.

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	0.1	0.3	1
LITTERS EXAMINED (N)		24	25	25	25
TRANSIENT CLINICAL OBSERVATIONS: a		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS			
TAIL, BENT	N/N	0/0	0/0	0/0	497/17**
TAIL, SHORT	N/N	0/0	0/0	0/0	455/15**
WHOLE BODY, PALE	N/N	0/0	0/0	0/0	7/5**
HEAD, MASS	N/N	0/0	0/0	0/0	38/4**
DEHYDRATION, TOTAL	N/N	4/1	12/2	68/3	18/3
MILD	N/N	4/1	11/2	63/3	18/3
MODERATE	N/N	0/0	0/0	3/2	0/0
SEVERE	N/N	0/0	1/1	2/1	0/0
HEAD, LOWER MIDLINE OR TIP OF TAIL, PURPLE, RED AND/OR BLACK	N/N	0/0	0/0	0/0	7/3**
NO MILK BAND PRESENT	N/N	0/0	0/0	2/1	5/2
NOT NURSING	N/N	0/0	0/0	1/1	3/1
LEFT EYE, UNABLE TO OPEN	N/N	0/0	0/0	1/1	1/1
HEAD OR TAIL, SCAB	N/N	8/2	3/1	0/0	4/1
LEFT FORELIMB, MALFORMED	N/N	0/0	0/0	0/0	8/1
RIGHT EYE, MICROPHTHALMIA	N/N	0/0	0/0	0/0	3/1
NOT NESTING	N/N	0/0	0/0	0/0	3/1
HEAD, SPARSE HAIR COAT	N/N	0/0	0/0	0/0	2/1

TRANSIENT CLINICAL OBSERVATIONS: a		TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS			
DECREASED MOTOR ACTIVITY	N/N	0/0	1/1	2/1	0/0
COLD TO TOUCH	N/N	0/0	0/0	6/3**	0/0
UNGROOMED COAT	N/N	0/0	0/0	16/2	0/0
HEAD, DOMED	N/N	0/0	0/0	1/1	0/0
LEFT EYE, OUT OF SOCKET	N/N	0/0	3/1	0/0	0/0
HEAD AND LEFT EYE, ULCERATION	N/N	0/0	1/1	0/0	0/0
PERSISTENT CLINICAL OBSERVATIONS: a		**TOTAL FREQUENCY (DAYS X PUPS)/LITTERS WITH OBSERVATIONS**			
TAIL OR TIP OF TAIL, MISSING OR ABSENT	N/N	18/1	0/0	0/0	162/10**
LEFT OR RIGHT EYE, MISSING	N/N	0/0	0/0	2/1	6/2

a. Tabulation restricted to adverse observations; all other pups appeared normal.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Body weight: (Lactation Days 1, 4, 7, 14, 18, and 21; weekly during post-weaning period; Gestation Days 0, 7, 10, 14, 17, and 21 [females only])

F_1 Pup Body Weight

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	0.1	0.3	1
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	25	25	25
PUP WEIGHT/LITTER (GRAMS)					
DAY 1	MEAN±S.D.	6.7 ± 0.3	6.7 ± 0.4	6.5 ± 0.5	5.7 ± 0.6**
DAY 4	MEAN±S.D.	9.8 ± 0.8	9.4 ± 0.7	9.2 ± 1.0*	8.3 ± 1.0**
DAY 7	MEAN±S.D.	14.5 ± 1.4	13.5 ± 1.4*	13.3 ± 1.6**	11.9 ± 1.4**
DAY 14	MEAN±S.D.	27.3 ± 3.0	25.4 ± 2.9*	25.4 ± 3.4*	23.5 ± 2.8**
DAY 18	MEAN±S.D.	34.5 ± 3.9	32.4 ± 3.8	32.5 ± 4.3	31.0 ± 3.6**
DAY 21	MEAN±S.D.	41.7 ± 5.1	39.1 ± 4.8	39.7 ± 5.9	37.2 ± 4.7**

DAY = DAY POSTPARTUM
a. Dosage occurred on day 7 of gestation through day 20 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Feed consumption: (Once weekly during post-weaning period; gestation Days 0, 7, 10, 14, 17, and 21)
There were no apparent treatment-related effects on food consumption. Absolute feed consumption was slightly lower for HD females post-weaning; however, when considering the lower body weights for this group, the relative feed consumption was similar to controls.

Physical development: (also see clinical signs table above)
Evaluation for vaginal patency started at Day 28 and evaluation for preputial separation started at Day 39. All pups not selected for continued evaluation were euthanized on Lactation Day 21 and examined for gross lesions.

There were no treatment-related differences in the time to preputial separation of vaginal patency compared with control animals. Mean body weights were statistically significantly lower for the high-dose group at these time points (45.9 days for males and 32.8 days for females).

For pups sacrificed and necropsied on Day 21 postpartum, tail effects (bent, short, tip missing, discolored) were noted at the HD level. Head mass was noted for 4 HD pups. One HD pup had a malformed left forelimb (flipper-like, split into two appendages). Missing eye was noted for 1 MD pup and 2 HD pups and microphthalmia of the right eye was noted for 1 HD pup. Two MD pups from the same litter and one HD pup had dilation of the lateral bilateral brain ventricles and one HD pup from a different litter had a head meningocele.

Neurological assessment:
Starting at Day 24 postpartum, one male and one female rat from each litter were evaluated in a passive avoidance test for learning, short-term memory, and long-term memory. Starting at Day 70 postpartum, one male and one female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning, and memory.

No treatment-related effects on performance were observed in the passive avoidance or water maze tests.

Reproduction:
At approximately 90 days of age, F1 generation rats within each dose group were assigned to cohabitation, one male and one female rat, excluding sibling matings. The cohabitation period lasted a maximum of 21 days. If mating had not occurred within 14 days, the female was reassigned another male from the same dose group that had mated. After completion of cohabitation, all males were euthanized and examined for gross lesions. Testes and epididymis weights were recorded and fixed for possible microscopic examination. All female rats were sacrificed on Gestation Day 21. C-section and gross necropsy was performed and fertility parameters were evaluated. Half of fetuses were stored in Bouin's solution and half were stored in alcohol for possible histopathological evaluation.

There was a statistically significant increase in the number of rats in the high-dose group (3 pairings compared with 0 pairings in the control group) that mated between Days 8 and 14 rather than between Days 1 and 7. However, the mean number of days in cohabitation before mating for the high-dose group was identical to the control group (3.5 days).

All of the high-dose pairs mated compared with 22 out of 24 control pairs and all high-dose F1 females became pregnant compared with 20 out of 22 control females that mated.

There was a slight, non-statistically significant increase in pre-implantation loss for the high-dose group (11.4%) compared with control females (4.4%) and the other two lower dose levels (3.6% and 3.1%). There were no apparent effects on other female fertility parameters including number of corpora lutea, resorptions, post-implantation loss, or dams with any resorptions.

Two high-dose females delivered early and were sacrificed without further evaluation. One dam delivered on Day 21 of gestation. The sponsor states that this is within their background control range for normal gestation; however, the historical control data range provided in the study report is 22.5 to 23.0 days with a mean of 22.7 days. The other dam delivered and was sacrificed on postpartum Day 119. Animals were paired on approximately Day 90 postpartum, but the mating date was not confirmed for this animal. This means that delivery occurred between GD15 and GD29. Since natural delivery normally occurs around GD22, it is likely that the duration of gestation for this dam was within the normal range considering the date of mating could not be confirmed.

F_2 Generation

Survival:
There were no effects on fetal survival after implantation. A slight increase in pre-implantation loss was observed, as noted above; the sponsor did not feel that this was treatment related because it did not reach statistical significance.

Body weight:
There was no apparent treatment-related effect on fetal body weight. Mean body weight was lower for the low- and mid-dose females, but the body weight for high-dose females were identical to controls.

External evaluation:
There were no apparent treatment-related effects on fetal development based on external evaluation.

Male/Female ratio:
There were no effects on male to female ratio.

10 Special Toxicology Studies

Other Toxicology Studies

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
Evaluation of rat lung macrophage function 733PC0011 GLP	Rat/Sprague-Dawley 10 females/group 0, 0.01, 0.04, 0.15, and 4.0 mg/kg/d (dosed once daily by oral gavage for 3 months)	Body weights and phagocytosis and respiratory burst of bronchoalveolar lavage (BAL) cells (macrophages)	• Phagocytic activity was determined by measuring the uptake of fluoroscein-labeled bioparticles. • Respiratory burst was evaluated by measuring the production of H_2O_2 using a fluorescent probe and cytofluorometric analysis. • Unscheduled deaths occurred for control (3), 0.01 mg/kg/d (2), 0.15 mg/kg/d (5), and 4 mg/kg/d (1) groups. Gavage trauma was suspected for 7 of the 11 deaths. Deaths were not determined for 2 animals each from the control and 0.15 mg/kg/d groups. • Mean final BWs were slightly higher (6%) for HD animals compared with the control value. • Mean phagocytosis was slightly lower for all treated groups compared with controls, although not statistically significant or dose related. • Mean respiratory burst was slightly higher than controls for the high-dose group, but not statistically significant. • A positive control article that is known to interfere with phagocytosis and/or respiratory burst was not used. A positive control would have been helpful to demonstrate the sensitivity of the assays. • The BAL cells were not characterized to determine what fraction of cells were macrophages versus other cell types. It is uncertain whether this impacted the study results. • The BAL cells were not characterized to determine the extent of lipid accumulation in macrophages. • Overall, it was concluded that there was no treatment-related effect on the phagocytic or respiratory burst activities of BAL cells; however, it seems additional controls/characterization could have been implemented to demonstrate the robustness of the assay and the relevance of the results.

Study Type Study No. GLP Status	Species/Strain Number/Group Dose Levels	Endpoints	Findings
Antigenicity study 0740580 GLP	Guinea pig 5 males/group/assay 1 mg/dose; orally 5x weekly for 3 weeks 0.5 mg/dose (SC) Positive control: 0.5 mg ovalbumin (SC) For SC injections, 3 injections given at 7-day intervals as emulsions with Freund's complete adjuvant or incomplete adjuvant	Passive cutaneous anaphylaxis (PCA) and Active systemic anaphylaxis ASA) tests	• For the orally administered group, a positive ASA reaction was not induced by intravenous injection of BMS-201038 at a dose of 1 mg/animal given 2 weeks after the last oral dose. • For the SC group, ASA nor PCA reactions were induced by the aforementioned challenge with BMS-201038. • Positive control animals exhibited typical anaphylactic signs upon challenge with ovalbumin.
Streptococcal host resistance model 733PC0012 GLP	Rat/Sprague-Dawley 20 females/group 0, 0.01, 0.04, 0.15, and 4.0 mg/kg/d (dosed once daily by oral gavage for 3 months)	None, study was terminated	• Based on the results of the BAL cell phagocytosis and respiratory burst assays, this study was terminated on Day 88 (prior to inoculation with *Streptoccus pneumoniae*) and no endpoints were assessed.
MTP expression in lungs 910065346	Multiple species (mouse, rat, dog, Cynomolgus monkey, and human)	RT-PCR using lung and liver (mouse, rat, dog, monkey, and human) and small intestine (monkey and	• Accumulation of lipids in lung has been observed in rodents treated with BMS-201038 for 3 months or longer. BMS identified the lipid accumulation as phospholipidosis (Aegerion's analysis of lung tissue from later studies contradicted the earlier finding of phospholipidosis,

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/Strain
Number/Group
Dose Levels</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td>Non-GLP</td><td></td><td>human only), in situ hybridization in lung tissue (mouse, rat, dog, monkey, and human), and ribonuclease protection assay (lung and liver from rat and dog)</td><td>characterizing the accumulation as neutral lipidosis).
• The purpose of this study was to evaluate a potential relationship between phospholipidosis in lungs and MTP gene expression in lung compared with expression in liver and small intestine (monkey and human only).
• The sponsor's conclusions were that the presence of MTP mRNA in the lung did not correlate with the development of phospholipidosis and that the pathogenesis of pulmonary phospholipidosis in rodents is not related to inhibition of MTP in the lung, but may be related to the structure of MTP inhibitors (cationic amphiphilic).
• A summary of MTP gene expression is shown in the sponsor-generated table below.
<table>
<tr><th rowspan="2">species</th><th rowspan="2">organ</th><th colspan="3">Result (+ or -)</th></tr>
<tr><th>RT-PCR</th><th>in situ</th><th>RPA</th></tr>
<tr><td>rat</td><td>lung</td><td>+</td><td>+</td><td>+</td></tr>
<tr><td></td><td>liver</td><td>+</td><td>+</td><td>+</td></tr>
<tr><td>mouse</td><td>lung</td><td>+</td><td>-</td><td>NE</td></tr>
<tr><td></td><td>liver</td><td>+</td><td>NE</td><td>NE</td></tr>
<tr><td>dog</td><td>lung</td><td>+</td><td>-</td><td>+</td></tr>
<tr><td></td><td>liver</td><td>+</td><td>-</td><td>+</td></tr>
<tr><td>monkey</td><td>lung</td><td>-</td><td>-</td><td>NE</td></tr>
<tr><td></td><td>liver</td><td>+</td><td>NE</td><td>NE</td></tr>
<tr><td>human (tissue)</td><td>lung</td><td>-</td><td>-</td><td>+</td></tr>
<tr><td></td><td>liver</td><td>NE</td><td>+</td><td>+</td></tr>
<tr><td>human (purchased RNA)</td><td>lung</td><td>+</td><td>NA</td><td>+</td></tr>
<tr><td></td><td>liver</td><td>+</td><td>NA</td><td>NE</td></tr>
</table>
NE= not evaluated, NA= not applicable</td></tr>
</table>

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/Strain
Number/Group
Dose Levels</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td>Bovine corneal opacity and permeability assay with metabolite M1

A96BB27.350

GLP</td><td>Bovine cornea (in vitro)

5 corneas

20% solution/ suspension of BMS-203215 in Eagle minimum essential medium with 1% fetal bovine serum (complete MEM)

Positive control: Imidazole</td><td>Freshly isolated bovine corneas were mounted in holders and treated with test article in complete MEM media for 4 hours; after the opacity evaluation, medium was replaced with a fluoroscein solution for the permeability assay.</td><td>• Based on the data in the sponsor-generated table below, BMS-203215 (metabolite M1) induced a similar degree of opacity and permeability as the positive control, imidazole. The resulting in vitro score placed the test article in the severe irritant range.

<table>
<tr><th>Test Material Identification</th><th>Opacity Measurement</th><th>Permeability Measurement</th><th>In Vitro Score</th><th>pH of dosing mixture</th></tr>
<tr><td>BMS 203215-02-BCOP1</td><td>89.9</td><td>1.186</td><td>107.7</td><td>11.0</td></tr>
<tr><td>Imidazole</td><td>65.5</td><td>1.671</td><td>90.6</td><td>NA</td></tr>
</table>
NA - Not Applicable
in vitro score:
from 0 to 25 = mild irritant
from 25.1 to 55 = moderate irritant
from 55.1 and above = severe irritant</td></tr>
</table>

GLP = good laboratory practice; MTP = microsomal triglyceride transfer protein.

11 Integrated Summary and Safety Evaluation

Safety pharmacology studies in dogs showed that single intravenous doses of lomitapide caused decreased blood pressure and heart rate and increased respiratory rate. These effects appeared to be related to C_{max} as the cardiovascular and respiratory changes were most noteworthy shortly after administration, after which time slowly returned to baseline values. Because TK evaluations were not conducted in this study, the exposures achieved through intravenous dosing are uncertain. In a 1-month repeat-dose toxicology study in dogs, minimal to moderate decreases in blood pressure and heart rate were observed at 2 and 20 mg/kg/d, which resulted in exposures that were approximately 37- and 230-times higher than at the maximum recommended human dose (MRHD) of 60 mg; in that study cardiovascular effects were not observed in dogs at clinically relevant doses.

In animal toxicology studies, the primary treatment-related finding was lipid accumulation in the liver, small intestine, and lung. Because lomitapide interferes with fat absorption from the small intestine, deficiencies in fat soluble vitamins (e.g., vitamins A, D, E, K) were observed in animal studies, which led to systemic hemorrhage in rats at exposures that were approximately 17-times higher than at MRHD, and resulted in death at higher exposures (~70X MRHD). When supplemental vitamin K was provided or lower doses were administered, systemic bleeding was not observed. In 6-month studies conducted in rats and dogs, one half of the animals in each group received vitamin supplementation. Longer duration studies were designed accordingly based on these results. For example, vitamin supplementation was employed for the mouse and rat carcinogenicity studies. However, vitamin supplementation was not utilized for the 1-year toxicology study in dogs or for any of the pivotal developmental and reproductive toxicity studies. The toxicity profile of lomitapide is discussed below by target organ.

Liver:
Treatment with lomitapide induces lipid accumulation in hepatocytes of mice, rats, hamsters, and dogs after a single dose (rats) after repeated daily dosing. Lipid vacuolation is primarily periportal and is positive for Oil-Red-O stain, indicating an accumulation of neutral lipids. For exposures between 3 and 6 months, vacuolation was generally scored as minimal to mild in mice, minimal to moderate in male rats, moderate to marked in female rats, and minimal in dogs. Lipid vacuolation was associated with increased mean absolute liver weight for mice, rats, and dogs by up to 28%, 83%, and 31%, respectively. The effect on liver weights was considerably more noteworthy for female rats compared with male rats. In an EM evaluation of liver sections from dogs treated for 1 month, hepatocellular lipid deposition was characterized as one or more variably-sized homogenous pale-gray lipid droplets in the cytoplasm. When reversibility was assessed in a 6-month rat study, lipid vacuolation was greatly diminished after a 3-month treatment-free period and not detected after 6 months; liver weights were similar to control values after a 6-month treatment-free period. After long-term exposure in mice (2 years) and dogs (1 year), hepatocyte vacuolation was not observed, although liver weights were slightly increased in dogs.

After 2 years of treatment in rats, hepatocyte vacuolation was observed at all dose levels tested.

In addition to lipid accumulation, slight increases in mean serum ALT and/or AST were noted in rats (≤2X increase) and dogs (≤3.5X increase) after 1 month of treatment or longer. In rats, mean serum ALP was increased up to 4 times compared with concurrent controls after 3 or 6 months of treatment. Minimal single cell necrosis was seen in rats at 10 mg/kg/d (7X [males] and 34X [females] MRHD) after 3 months and at ≥0.2 (females) or ≥2 mg/kg/d (males) after 6 months (≥2X [males] and ≥1X [females] MRHD). Minimal to moderate subacute inflammation was seen in rats at ≥1 mg/kg/d (≥1X [males] and ≥3X [females] MRHD) after 3 months and at ≥0.2 (females) or 20 mg/kg/d (males) after 6 months (17X [males] and ≥1X [females] MRHD), when vitamin supplementation was provided. After 2 years of dosing in rats, an increase in the incidence and severity (minimal to moderate) of focal/multifocal fibrosis was observed at ≥0.25 mg/kg/d for males (0.2X MRHD) and at ≥0.35 mg/kg/d for females (2X MRHD). An increased incidence of minimal to mild cystic degeneration was observed for males treated at ≥1.7 mg/kg/d (2X MRHD).

Small Intestine:
Treatment with lomitapide induced lipid accumulation in small intestinal absorptive epithelium of mice, rats, hamsters, and dogs at clinically relevant exposures. Moderate microscopic lipid vacuolation was seen as early as after a single oral dose of ≥10 mg/kg in rats (≥1.5X MRHD based on body surface area extrapolation). Within the small intestinal tissue, lipid vacuoles were most prominent in the jejunum followed by the duodenum. Lipid vacuoles were positive for Oil-Red-O staining, indicating an accumulation of neutral lipids. Vacuolation was not accompanied by necrosis or signs of chronic inflammation. EM evaluation of small intestine from dogs treated for 1 month showed lipid deposition as multiple, variably-sized, homogenous pale-gray lipid droplets that were partially enveloped by endoplasmic reticulum. Longer duration dog studies showed the presence of lipid vacuoles but the finding did not exhibit progression after exposures up to 12 months. Reversibility of lipid vacuolation in the small intestine was demonstrated in mice after treatment up to 2 years.

Lung:
In the lungs of treated animals, an increased incidence in the presence of foamy alveolar macrophages (histiocytes) was observed in mice and rats. In mice, foamy alveolar macrophages were observed in alveolar spaces at ≥5 mg/kg/d (≥5X MRHD) after treatment for 3 months. In a 2 year carcinogenicity study in mice, increases in the incidence of alveolar spicules and lymphocyte infiltration were observed at ≥15 mg/kg/d (≥22X MRHD); histiocytosis was not noted by standard microscopy, but was observed at a low incidence when lung tissue was examined by EM.

In rats, multifocal small collections of foamy macrophages were noted in alveoli, frequently in subpleural locations; an increased incidence of minimal histiocytosis was observed at doses as low as 0.01 mg/kg/d (<0.1X MRHD) after 3 months of treatment. After 6 months of treatment in rats at ≥0.2 mg/kg/d (≥0.3X MRHD), histiocytosis was

characterized by multifocal aggregates of large foamy macrophages within alveoli, either alone or associated with dose-related increasing incidence/severity of subacute inflammation, necrotic cellular debris, cholesterol-like clefts, and/or Type II alveolar cell proliferation. Special staining with Luxol fast blue and Baker's acid hematin stain for phospholipids and Oil-Red-O for neutral lipids demonstrated that both types of lipids were contained in the macrophages and adjacent Type II alveolar cells. Histiocytosis did appear to be reversible after a 6-month recovery period. In a 2-year carcinogenicity study in rats, increases in the incidence of alveolar spicules, lymphocyte infiltration with macrophage accumulation, and thickened alveolar septa with macrophage infiltrates were observed at ≥0.25 mg/kg/d (≥0.2X MRHD). An increase in septal cell mineralization was noted for males receiving ≥1.7 mg/kg/d (2X MRHD). An increased incidence of pleural/subpleural fibrosis was observed for females receiving ≥0.35 mg/kg/d (≥2X MRHD), although the incidence was similar to the male control group.

The results a 1-year study in dogs showed a low incidence of minimal to mild pleural fibrosis in males and females at ≥0.5 mg/kg/d (≥3X MRHD). Additionally, a slight increase in the incidence of minimal focal mineralization and mild chronic-active inflammation was observed at 5 mg/kg/d (64X MRHD) in males and minimal to mild alveolar edema was noted in females at 5 mg/kg/d (56X MRHD); these findings occurred in the absence of histiocytosis. Histiocytosis was also not observed in a 1-month toxicity study at doses up to 20 mg/kg/d. In dogs treated with lomitapide at doses up to 10 mg/kg/d for 6 months, minimal lung histiocytosis was observed for some animals across most groups including the male control group. The incidence or severity of histiocytosis did not increase with increasing dose level, and thus may have been an incidental background finding. In the 6-month study, chronic inflammation and edema were also observed across most dose levels at an incidence and severity similar to control groups.

To further characterize the observation of foamy alveolar macrophages, lung tissue from some rat and mouse studies was evaluated by EM. EM evaluation of lung tissue from a 6-month rat study characterized the histiocytosis as focal aggregates of large foamy alveolar macrophages containing residual/lamellar inclusion bodies, lipid droplets, and phagolysosomes varying in size, shape, and electron density. Occasionally adjacent type II cells were slightly enlarged and contained lipid droplets, as well as a qualitative increase in the size or amount of lamellar inclusion bodies. EM examination of lung tissue from another 6-month rat study showed that alveolar macrophages were enlarged with abundant cytoplasm packed with neutral lipid droplets, electron-dense multi-laminated osmiophilic structures, and cleft-like electron-lucent structures (cholesterol clefts). The pathologist concluded that the EM results of the lungs were consistent with pulmonary phospholipidosis. The EM evaluations for the 6-month studies were conducted during the early phase of drug development and EM evaluations from later studies, evaluated by a different pathologist, resulted in a slightly different interpretation, as described below.

After EM examination of rat lung tissue from a 3-month study, the pathologist described the excessive vacuolation as morphologically consistent with neutral lipid vacuoles

because the vacuoles were smoothly contoured with homogeneous translucent content. The pathologist concluded that the evaluation did not reveal the presence of phospholipidosis, which is characterized by the excessive accumulation of concentric lamellar inclusions in the lysosomes of macrophages or other lung cells. Although concentric lamellar inclusions were seen, they were rarely observed in lysosomes and were present in cells from both control and treated animals. The evaluation also showed no evidence of chronic interstitial reaction in the alveolar wall adjacent to vacuolated macrophages. After a 6-month recovery period, the number of macrophages with lipid vacuoles was low and similar to the control group, indicating that this effect is reversible once the drug is discontinued.

The pathologist also concluded that the lipid accumulation noted for alveolar macrophages in lung tissue from a 2-year mouse carcinogenicity study was not consistent with classical phospholipidosis. Observed alveolar spicules were characterized as long slender crystals that occurred singly or as aggregated multi-layered inclusions in the cytoplasm of alveolar macrophages. Excessive lipid accumulation occurred in the absence of concurrent epithelial degeneration or inflammatory changes in the alveolar wall.

The toxicity profile of lomitapide along with clinical safety margins for each finding is summarized in the table below.

Summary of Noteworthy Treatment-Related Effects by Target Organ/Finding

Observed Effect	Species	Study Duration	NOEL (mg/kg/d)	Safety Margin*
Liver - lipid vacuolation	Mouse	3 months	<1.5	<2X
		2 years	45†	~75X
	Rat	3 months	<1	<2X
		6 months	<0.02	<0.4X
		2 years	M: <0.25 F: <0.03	M: <0.25X F: <0.1X
	Dog	6 months	0.1	M: 1X F: 2X
		12 months	5††	60X
Liver - fibrosis, focal/multifocal	Rat	2 years	M: <0.25 F: 0.03	M: <0.25X F: 0.1X
Small intestine - lipid vacuolation	Mouse	3 months	<1.5	<2X
		2 years	<0.3	<0.4X
	Rat	3 months	<1	<2X
		6 months	0.02	0.4X
		2 years	M: <0.25 F: <0.03	M: <0.25X F: <0.1X
	Dog	6 months	<0.01	M: <0.2X F: <1X
		12 months	0.05	0.2X
Lung - histiocytosis/ foamy macrophages	Mouse	3 months	1.5	2X
		2 years	45†††	~75X
	Rat	3 months	<1	<2X
		3 months	<0.01	<0.1X
		6 months	0.02	0.4X
		2 years	M: 7.5† F: 2†	M: 6X F: 8X
	Dog	6 months	10†	200X
		12 months	5†	60X
Lung - pleural fibrosis	Rat	2 years	M: 7.5† F: 0.03	M: 6X F: 0.1X
	Dog	12 months	0.05	0.2X
Lung - thickened alveolar septa with macrophage infiltrates	Rat	2 years	M: 0.25 F: 0.03	M: 0.25X F: 0.1X
Lung - septal cell mineralization	Rat	2 years	M: 0.25 F: 2.0†	M: 0.25X F: 8X
Lung - alveolar spicules	Mouse	2 years	7.5	10X
	Rat	2 years	M: 0.25 F: 0.03	M: 0.25X F: 0.1X

NOEL = no observed effect level

*Safety margins were calculated using a mean $AUC_{0\text{-}24h}$ value of 69.5 ng·h/mL for humans at 60 mg.

†Finding not observed.

††Lipid vacuolation was not observed in hepatocytes from the 12-month dog study; however, mean liver weights were statistically significantly increased at 5 mg/kg/d compared with control values.

†††Lipid vacuoles were not observed in lung macrophages or type 2 pneumocytes by light microscopy but were noted in some animals by electron microscopy at ≥0.3 mg/kg/d for males and ≥15 mg/kg/d for females.

Carcinogenicity
Two 2-year carcinogenicity studies were conducted in mice and rats to evaluate the carcinogenic potential of lomitapide (summarized below). Data from genetic toxicology studies indicate that lomitapide is not a direct acting mutagen.

Mice
A 2-year bioassay was conducted in CD-1 mice. Mice (60/sex/group) were administered AEGR-733 by oral administration (mixed in diet) at dose levels of 0 (diet control), 0.3, 1.5, 7.5, 15 or 45 mg/kg/day. Study groups were assessed for neoplasms between Weeks 99 and 104, depending on animal survival for each group. Because AEGR-733 inhibits absorption of fat soluble vitamins from the intestine that can lead to toxicity due to vitamin deficiency, all animals were fed a rodent diet that contained more vitamin A and K than standard rodent diet.

A statistically significant increase in the incidence of hepatocellular neoplasms (adenomas or carcinomas, combined) was observed in males given ≥1.5 mg/kg/day (≥2X MRHD) and females given ≥7.5 mg/kg/d (≥9X MRHD). Both hepatocellular adenomas and carcinomas occurred singly or in multiples and several animals had both adenomas and carcinomas. Statistically significant increases in adenomas or carcinomas, combined, of the small intestine (duodenum, ileum, and jejunum) were observed in males and females at ≥15 mg/kg/day (24X MRHD). The jejunum was the most common site for carcinomas. The incidences of hepatocellular and small intestinal neoplasms was not completely dose dependent, as there were often fewer neoplasms at the high dose compared with lower dose levels; this effect was likely due to the higher mortality rate in the high-dose groups.

On the basis of a statistically significant increase in hepatocellular neoplasms, the NOEL for drug-related neoplasms in mice was 0.3 mg/kg/day for males and 1.5 mg/kg/day for females, which represent clinical exposure margins for the parent drug of 0.4X and 2X, respectively.

Rat
A 2-year bioassay was conducted in Sprague-Dawley rats. Rats (60/sex/group) were administered AEGR-733 once daily by oral gavage at dose levels of 0 (vehicle [75% PEG-400]), 0.25, 1.7, or 7.5 mg/kg/day in males or 0 (vehicle), 0.03, 0.35, or 2.0 mg/kg/day in females. Females received lower dose levels due to a greater drug exposure than males at equivalent doses. Treatment groups were assessed for neoplasms between Weeks 94 and 98, depending on animal survival for each group. Because AEGR-733 inhibits absorption of fat soluble vitamins from the intestine that can result in toxicity due to vitamin deficiency, throughout the study, all animals were fed a rodent diet that contains more vitamin A and K than standard rodent diet. Additionally, beginning on Day 407, mid-dose group animals received a vitamin-fortified diet containing 5 times the concentrations of vitamins A, D, and E contained in the standard diet and high-dose group animals received a vitamin-fortified diet containing 10 times the concentrations of vitamins A, D, and E.

There were no increases in neoplasms that were considered to be related to treatment at any dose tested. Therefore, the NOEL for drug-related neoplasms in rats was considered to be the highest dose tested: 7.5 mg/kg/day for males and 2 mg/kg/day for females, which represent 6X MRHD and 8X MRHD, respectively.

Developmental and Reproductive Effects:
The sponsor conducted a standard battery of toxicology studies to evaluate the potential effects of lomitapide on reproduction and embryo-fetal development. When dosed prior to and during mating, there were no treatment-related effects on reproductive endpoints for male or female Sprague-Dawley rats at dose levels up to approximately 3-fold higher than the MRHD.

In an embryo-fetal developmental toxicity study in Sprague-Dawley rats, fetal death and malformations were observed when lomitapide was administered during organogenesis from gestational days (GD) 6 through 15. Decreased fetal body weight and developmental defects were observed at ≥0.4 mg/kg/d (2X MRHD). Fetal malformations included defects to the abdomen (umbilical hernia, gastroschisis); tail (short, stubbed, bent, or absent); heart (alterations in size or shape); limbs (malrotation); and anus (imperforate). At 4 mg/kg/d (10X MRHD), shortened limbs, brain defects (exencephaly, hydrocephaly, cerebral hernia, misshaped cerebral hemispheres), and embryonic mortality were also noted. The no observed adverse effect level (NOAEL) for embryo-fetal development was 0.04 mg/kg/d (less than 1X MRHD). In an investigational developmental toxicology study, it was shown that supplementation with fat soluble vitamins did not provide protection against fetal malformations.

In an embryo-fetal developmental toxicity study in ferrets, treatment with lomitapide during organogenesis from GD12 through GD28 resulted in maternal body weight loss (associated with decreased food consumption), decreased fetal body weight, and fetal malformations at all doses tested. Malformations at ≥1.6 mg/kg/d included those involving the limbs/paws (rotated medially, digits absent or fused); head (cleft palate, open eye lids, low set ears); tail (kinked); and abdomen (umbilical hernia). The incidence of these findings tended to increase in a dose-related manner. At ≥4 mg/kg/d, increased embryonic resorptions and short limbs were also observed. Because effects on embryo-fetal development were observed at all dose levels, a NOAEL was not identified (<1.6 mg/kg/d; <0.3X MRHD based on body surface area extrapolation).

In an embryo-fetal developmental toxicity study in New Zealand white rabbits, doses of ≥1 mg/kg/d lomitapide resulted in biologically meaningful decreases in maternal body weight gain (65% to 76% less then controls) when administered during organogenesis from GD6 through GD20. Treatment did not result in adverse effects on embryonic survival or development at doses up to 10 mg/kg/d. Accordingly, the NOAEL for effects on embryonic development was 10 mg/kg/d (3X MRHD based on body surface area extrapolation). In a developmental toxicity range-finding study that tested higher doses than those used in the pivotal study, embryonic loss was observed at ≥20 mg/kg/d (6X MRHD based on body surface area extrapolation).

In a peri- and post-natal developmental toxicity study in Sprague-Dawley rats, treatment with 0.3 mg/kg/d lomitapide (1X MRHD) from GD7 through lactation day (LD) 20 resulted in decreased fetal body weights and a low incidence of fetal eye anomalies (missing eye, microphthalmia) and dilatation of the lateral ventricles of the brain. At 1 mg/kg/d (3X MRHD), litter sizes were smaller and there was an increase in still-born pups and pups dying between LD1 and LD7. In addition to the malformations noted for the 0.3 mg/kg/d group, an increase in tail anomalies (bent, short, missing or absent, discolored) were observed and a malformed limb was noted for one pup. Body weights for pups whose mothers were treated with 1 mg/kg/d remained lower than controls after weaning. There were no biologically meaningful effects on learning, short-term memory, long-term memory, response inhibition, or mating and fertility parameters for the F_1 generation at any dose. There were no treatment-related effects on fetal development for the F_2 generation. The NOAEL for the F1 generation was 0.1 mg/kg/d (<1X MRHD) based on the observed fetal malformations and effects on fetal body weight. The NOAEL for maternal reproduction was 0.3 mg/kg/d (1X MRHD) based on a slight increase in the length of gestation observed at 1 mg/kg/d.

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/s/

BRIAN T HUMMER
11/05/2012

KAREN L DAVIS BRUNO
11/05/2012
concur with recommendation

NDA 203858

45 Day Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

ITEM	YES	NO	COMMENT
1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		
2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review?	X		
3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		
4) Are all necessary and appropriate studies for this compound, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA. (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	X		All necessary and appropriate nonclinical studies appear to have been submitted. In Module 2.4, the sponsor has discussed the potential safety concerns of lipidosis and increased liver and small intestine tumors in mice. Pivotal toxicology studies include chronic studies in rats (6 months) and dogs (1 year); the standard battery of genotoxicity studies; the standard battery of developmental and reproductive studies; and 2-year carcinogenicity studies in mice and rats. No studies were identified as being omitted from this submission.
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	X		Based on past reviews of many of the studies resubmitted in this NDA, the pivotal studies appear to have been adequately designed and conducted. However, a more thorough evaluation of these aspects will be conducted during the review of the individual study reports.
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		Sponsor states in Module 2.6.6 (page 7) that: "All impurities in lomitapide drug substance and drug product were either lower than qualification thresholds stated in ICH guidelines (*Impurities in New Drug Substances* Q3A(R2), ICH Guideline October 2006; *Impurities in New Drug Products* Q3B(R2), ICH Guideline June 2006) and/or were represented in batches of lomitapide used in toxicology studies at higher levels than in clinical batches; therefore, no special qualifying studies were required."

ITEM	YES	NO	COMMENT
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		The route of administration used in the toxicology studies is the same as the route used in the clinic.
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	X		Draft labeling has been submitted. The drug is proposed to have Pregnancy Category X labeling; however, there is no descriptive text in Section 8.1 about findings in humans or animals that resulted in the proposed category. Also, there is no text in Section 13.3 describing the results from teratology studies conducted in rats and rabbits.
9) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file: None, this submission is acceptable for filing from a nonclinical perspective. Potential safety issues that will be focused upon in my review will be the finding of lipidosis in the lung, small intestine, and liver, and an increased tumor incidence in small intestine and liver in mice. A concern that some human metabolites were not adequately tested in toxicology studies was brought up during the pre-NDA meeting based on data that the sponsor provided in their meeting package. The sponsor later stated that the data presented in the package was not accurate and all human metabolites were adequately qualified in toxicology studies. Therefore, the human metabolite profile will also be further evaluated to ensure that all human metabolites were also identified in animals and sufficiently evaluated in toxicology studies.			

Reviewing Toxicologist: Tim Hummer, PhD
Supervisory Pharmacologist: Karen Davis-Bruno, PhD

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

/s/

BRIAN T HUMMER
05/04/2012

KAREN L DAVIS BRUNO
05/07/2012