CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209884Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 209884
Submission date: nonclinical summaries and study reports submitted March 28, 2018
Drug: siponimod
Applicant: Novartis Pharmaceuticals
Indication: Adult patients with secondary progressive multiple sclerosis

Reviewing Division: Division of Neurology Products

Discussion:
The pharmacology/toxicology reviewer and supervisor conducted a thorough review of the nonclinical information submitted and concluded that siponimod could be approved from the pharmacology/toxicology perspective.

Siponimod is an agonist of the sphingosine-1-phosphate (SIP) receptor, selective for subtypes 1 and 5. Sphingosine 1-phosphate receptor modulator is an appropriate Established Pharmacologic Class for siponimod.

Developmental and reproductive toxicity studies revealed embryofetal and postnatal effects at clinically relevant exposures. Labeling will reflect these effects.

Two-year carcinogenicity studies were conducted in mice and rats. Hemangiomas and hemangiosarcomas were observed at all doses in mice. In rats, thyroid follicular cell adenoma and carcinomas were observed, however, these are not considered relevant to humans based on data showing induction of thyroid hormone metabolism.

Conclusions: I agree that this NDA can be approved from a pharm/tox perspective.

Comments on labeling were provided separately.

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/s/

PAUL C BROWN
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MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: March 25, 2019

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 209-884 (Mayzent, siponimod, BAF-312)

NDA 209-884 has been submitted by Novartis Pharmaceuticals to support marketing approval of Mayzent for treatment of adult patients with Secondary Progressive Multiple Sclerosis. The NDA was submitted for rolling review, with the nonclinical summaries and study reports included in Part 1 (March 28, 2018). Additional sections of the NDA were submitted on April 27, 2018, June 8, 2018, June 28, 2018, and July 26, 2018. The NDA was filed as a Priority application because the sponsor submitted a (b) (4) voucher (NDA Acknowledgement/Priority Review Designation letter, September 21, 2018). Clinical development was conducted under IND 76122.

A full battery of nonclinical studies was conducted to support clinical development and an NDA. The nonclinical data have been reviewed in detail by Dr. Siarey (Pharmacology/Toxicology NDA Review and Evaluation, NDA 208-884, Richard Siarey, Ph.D., February 17, 2019). Based on the review, Dr. Siarey has concluded the nonclinical data support approval.

Summary and Discussion of Selected Data

Pharmacology: Siponimod is an agonist of the sphingosine-1-phosphate (SIP) receptor, selective for subtypes 1 and 5. Binding of siponimod to the S1P1 receptor results in internalization and degradation of the receptor (functional antagonism), leading to sequestration of lymphocytes in lymph nodes and reduction of circulating lymphocytes. It is through this mechanism that siponimod is thought to be efficacious in MS patients. S1P5 receptors are located primarily on oligodendrocytes and natural killer cells, but the contribution of siponimod S1P5 binding to its therapeutics effects in patients with multiple sclerosis is unknown.

PK/TK/ADME:

Data from the in vitro and in vivo (oral) PK/ADME studies conducted by the sponsor indicate that siponimod is highly protein bound (>99%) in all species tested (including human) and that siponimod-related radioactivity is widely distributed to tissues (albino mouse and rat; pigmented

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rat), with levels in rat brain ~4 times that in plasma at 8 hrs post dose (3 mg/kg PO). An acute dose (^{14}C-sipinomod, 10 mg/kg PO) study in lactating rat demonstrated excretion of siponimod, metabolites, or both into milk.

In humans, the plasma $AUC_{tau,ss}$ for siponimod at the recommended human dose of 2.0 mg/day (b) (4) was 558 ng*hr/mL following 6-10 daily doses. A plasma $AUC_{(0\text{-inf})}$ for M17 at 2.0 mg was not provided; however, $AUC_{(0\text{-}24\ hr)}$ is estimated to be 896 ng*hr/mL, based on a value of 112 ng*hr/mL at 0.25 mg. The main circulating metabolites are M3 (a glucuronide of a hydroxylated metabolite, M5) and M17 (or LYS815, a cholesterol ester metabolite). In plasma, siponimod accounts for ~57% of circulating radioactivity after oral dosing; M3 and M17 account for ~18, and 80-97% of parent, respectively. M3 is a non-acyl glucuronide, inactive at S1P 1 and S1P receptors (EC_{50} values of >10 μM) and is, therefore, of limited toxicological concern. M17 is active at S1P1 and S1P5 receptors, although it is less potent than the parent compound (S1P1: EC_{50} of 341 ± 86 nM vs 4.3 ± 0.5 nM for PC; S1P5: EC_{50} of 341 ± 86 nM vs 4.3 ± 0.5 nM for PC).

Toxicokinetic data for siponimod were collected in mouse, rat, monkey, and rabbit; however, plasma exposure data are not available at all the doses tested in the pivotal nonclinical studies. For M3 and M17, toxicokinetic data were not collected in any of the pivotal studies. Plasma M3 exposures (AUC) at the high dose (HD) tested in the 52-week oral toxicity study in monkey were estimated to be ~50 times that in humans at the recommended daily dose of 2 mg, based on data from a non-GLP single-dose (15 mg/kg PO) study of ^{14}C-siponimod in monkey. Plasma M17 exposures were only assessed in investigative studies in male mouse (only at 5 hrs post dose) and rat; no TK data are available for M17 in female mouse or rat. The TK data are discussed (below) with the results of the relevant nonclinical studies.

General Toxicology

Pivotal oral (gavage) toxicity studies were conducted in Wistar rat (up to 26 weeks) and cynomolgus monkey (up to 52 weeks).

In the 26-week (+8-week recovery) study in rat (males: 0, 15, 50, and 150 mg/kg; females: 5, 15, and 50 mg/kg), the primary drug-related findings were consistent with the expected pharmacological effects of siponimod (reduction in circulating lymphocytes at all doses; microscopic changes in lymphoid tissue) and hepatic enzyme induction (thyroid follicular cell and hepatocellular hypertrophy). An NOAEL was not identified; however, plasma siponimod exposures (AUC) at the lowest doses tested (48200 and 77000 ng*hr/mL in males and females, respectively) provided substantial safety margins (>50-fold) compared to that at the recommended human daily dose.

In the 52-week (+ 8-week recovery) study in monkey (0, 10, 30, and 100 mg/kg), there were 3 deaths at the HD. Microscopic findings in these animals included lymphoid and bone marrow depletion, renal tubular epithelium vacuolar degeneration, hepatocellular necrosis, pancreatic acinar atrophy, and GI effects (necrotizing stomatitis and necrotizing lesions of the intestine). Convulsions and watery feces were observed at the HD, in animals that died (or were sacrificed) prematurely and in survivors. A reduction in circulating lymphocytes was observed at all doses (non-dose related), consistent with the pharmacological effects of siponimod. The primary drug-

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related microscopic findings in survivors were detected in lymphoid tissue (B- and T-cell depletion), bone marrow (including increased myeloid hypercellularity), skeletal muscle (myofiber degeneration), skin (follicular atrophy), and large intestine (mucosal hyperplasia, crypt necrosis). The low dose (LD) was identified as an NOAEL. Plasma siponimod exposures (AUC) at that dose were 95400 and 124000 ng*hr/mL in males and females, respectively, which provide substantial safety margins (>50-fold) compared to that at the recommended human daily dose.

Reproductive and Developmental Toxicology

A battery of oral (gavage) reproductive and development toxicology studies was conducted; separate male and female fertility and early embryonic development studies in Wistar rat, embryofetal development studies in Wistar rat and New Zealand White rabbit, and a pre- and postnatal development study in Wistar rat.

In the male fertility study, siponimod (0, 2, 20, or 200 mg/kg) was administered to males prior to and during the mating period (to untreated females). Body weight loss was observed at the HD during the first week of dosing and remain reduced, compared to controls, throughout the remainder of the dosing period. A dose-related increase in precoital interval was observed, with all doses affected. Findings at the HD consisted of decreases in corpora lutea and implantation sites, an increase in preimplantation loss, and a decrease in the number of viable fetuses. The effect on corpora lutea is not plausibly related to drug because females were not dosed; however, the other findings reflect a drug-related effect on male fertility, with the mid dose (MD) identified as an NOAEL. TK data were not collected during the study.

In the female fertility study, no drug-related effects on fertility parameters were observed when siponimod (0, 0.1, 0.3, or 1 mg/kg) was administered prior to and during the mating period (to untreated males) and continuing to gestation day (GD) 6. Plasma siponimod exposure ($AUC_{(0\text{-}24\ hr)}$ was 9030 ng*hr/mL on Day 10.

In the embryofetal development study in rat, siponimod was administered at doses of 0, 1, 5, and 40 mg/kg on GDs 6-17. Clinical signs (decreased stools, increased activity) were observed only at the HD. Post implantation loss was increased at all doses (70, 100, and 100%, respectively), resulting in only 66 viable fetuses (2.8/litter) at the LD and none at the higher doses. Fetal examinations at the LD revealed external (malrotated limbs, cleft palate), visceral (cardiomegaly), and skeletal (cleft palate, misshapen clavicles) malformations and external (generalized edema) and skeletal (incomplete ossification at multiple sites, misshapen sternebra, cervical ribs) variations. An NOAEL for adverse effects on embryofetal development was not identified. The plasma $AUC_{(0\text{-}24\ hr)}$ for siponimod at the LD was 10300 ng*hr/mL.

In the embryofetal development study in rabbit, siponimod was administered at doses of 0, 0.1, 1, and 5 mg/kg on GDs 7-20. There was no evidence of maternal toxicity. Developmental findings consisted of an increase in abortion at the HD, an increase in total resorptions at all but the LD, and a decrease in viable fetuses at the HD. Fetal examinations revealed an increase in skeletal variations (incomplete or lack of ossification at one or more sites, bent hyoid, fused or misshapen sternebra) at the MD and HD. No drug-related malformations were reported. The LD, the NOAEL for adverse effects on embryofetal development, was associated with a plasma

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siponimod C_{max} of 6.52 ng/mL (Day 20); an AUC could not be calculated because of insufficient data. At the MD (an effect-dose), the plasma siponimod $AUC_{(0\text{-}24\ hr)}$ was 958 ng*hr/mL. At the LD, siponimod was not detected in maternal or fetal plasma at 24 hrs post dose on Day 20; at the higher doses, concentrations were higher in fetal circulation (41 vs 16.5 ng/mL at the MD, 146 vs 89.6 ng/mL at the HD).

In the pre- and postnatal development study in rat, siponimod was administered at doses of 0, 0.05, 0.15, and 0.5 mg/kg from GD 6 to lactation day 20. Such low doses were required because of the embryolethality observed in the embryofetal development study at doses as low as 1 mg/kg. In dams, there were no drug-related clinical signs. Absolute body weight was not affected during gestation; however, a slight body weight loss was observed at the HD during GDs 18-20. Body weight (relative to control) in dams was slight reduced (≤10%) throughout the lactation period at the HD. An increase in gestation length was observed at the MD and HD (0.4 and 0.5 days, respectively, compared to control). The number of dead (stillborn) pups was increased at all doses, but to a lesser extent at the MD (pups/litters affected: 8/5, 3/2, and 18/14 at the LD, MD, and HD, respectively), resulting in a decrease in the live birth index at the HD.

In offspring, there were decreases in survival by postnatal (PND) 4 at the MD and throughout the lactation period at the HD, resulting in a substantial decrease in evaluable HD pups. Litter size was reduced at the MD and HD. Clinical signs observed in MD and HD pups included "dehydration, empty stomach, thinness, weakness, decreased activity, [and] coldness to touch." When pups found dead before PND 7 and those born with external abnormalities were histologically examined, an abdominal finding, uterine horn-like tissue, in male pups was observed at the MD (1 pup) and HD (10 pups); according to the study pathologist, this finding "did not appear to correlate with any distinct structure histologically and is of unknown etiology." Drug-related malocclusion of the incisors (without an effect on tooth quality) was observed at all doses in males and females; flat cranium was detected in HD pups. Additional findings in offspring included reduced body weight (compared to control) during the early postnatal period and delayed sexual maturation (preputial separation and vaginal opening) at the HD. A NOAEL for adverse effects on postnatal development was not identified. Toxicokinetic data were not collected during the study and plasma AUC data are not available for the LD from any study in Wistar rat.

Genetic Toxicology

Siponimod was negative in a standard battery of genetic toxicology studies: Ames assay, in vitro chromosomal aberration assay in human peripheral blood lymphocytes, oral in vivo mouse and rat micronucleus assays.

Carcinogenicity

The carcinogenic potential of siponimod was assessed in lifetime oral (gavage) carcinogenicity studies in CD-1 mouse (0, 2, 8, or 25 mg/kg/day) and Wistar rat (0, 10, 30, or 90 mg/kg/day in males, 0, 3, 10, or 30 mg/kg in females).

In mouse, a dose-related effect on survival resulted in dosing cessation for the MD and HD males (Week 91), LD females (Week 97), and MD and HD females (Week 92) and early termination of MD and HD males (Week 93 and Week 96, respectively) and at all doses in

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females (Week 101, 95, and 98 at C and LD, MD, and HD, respectively). Control and LD males were continued to Week 104. Tumor findings consisted of increases in malignant lymphoma in females at all doses and in hemangiosarcoma and combined hemangioma and hemangiosarcoma at all doses in males and females. Toxicokinetic data were collected only at 3 and 6 hrs post dose (Weeks 5 and 28); therefore, no AUC values were available. No plasma AUC data are available at the LD from any study in CD-1 mouse; at 5 mg/kg in the 13-week dose-ranging study, plasma $AUC_{(0\text{-}24\ hr)}$ was 44700 and 40100 ng*hr/mL in males and females, respectively.

In rat, there was no drug-related effect on survival; animals were dosed daily for up to 104 weeks. Tumor findings consisted of an increase in thyroid follicular cell adenoma and combined thyroid follicular cell adenoma and carcinoma in HD males. As in the mouse study, plasma samples were collected only at 3 and 6 hrs post dose (Weeks 5 and 26) for toxicokinetic analysis; therefore, AUC values are not available. However, in an investigative study (1370783), plasma $AUC_{(0\text{-}24hr)}$ for siponimod and M17 were 115000 and 4080 ng*hr/mL, respectively, on Day 28. (M17 exposure may be an underestimate; the highest plasma M17 concentration occurred at 24 hrs post dose.) In a 4-week oral toxicity study, plasma $AUC_{(0\text{-}24\ hr)}$ for siponimod at oral (gavage) doses of 10 and 50 mg/kg/day on Day 29 in males were 21700 and 83600 ng*hr/mL, respectively.

Investigative studies: The sponsor conducted a series of exploratory (non-GLP) studies to investigate the potential mechanism(s) underlying the tumor findings in mouse and rat. The results of these studies indicated:

- Oral administration of siponimod to mouse (0, 25, and 75 mg/kg PO) for up to 274 days resulted increased PLGF2 (soluble pro-angiogenic factor) in plasma from the first dose on and a decrease in endoglin (soluble anti-angiogenic factor) from 28 days of dosing on, at both doses but with no dose-response. Gene expression (endothelial cell activation-specific genes) and immunohistochemical (endothelial/angiogenic markers) analyses on skeletal muscle, liver, and skin did not result in consistent findings among tissues. However, in skeletal muscle there were persistent increases in markers of endothelial cell activation and mitosis. Toxicokinetic analyses were conducted; however, plasma siponimod and M17 exposures were assessed only at 5 hrs post dose; therefore, AUC data are not available.
- In an in vitro study, siponimod and M17 (LYS815) were incubated with isolated vascular endothelial cells from mouse (skeletal muscle), rat (pulmonary, aortic), and human (pulmonary, dermal) tissues and evaluated for changes in endothelial cell markers and soluble growth factors and incorporation of EdU into nuclear DNA. Markers of endothelial cell activation were increased only in mouse cells (10 nM to 1 μM; decreased above 1 μM).
- Oral (gavage) administration of siponimod (0 or 90 mg/kg/day) daily for 4 weeks to male Wistar rats resulted in a significant increase in T4-UDP-GT activity in the liver and increased TSH levels in plasma, accompanied by centrilobular hepatocellular and thyroid follicular cell hypertrophy. Decreases in plasma T4 levels would have been expected, but T3 and T4 data were not available because of "analytical issues." However, the available data suggest that the thyroid tumors in the rat carcinogenicity study were likely the result

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of increased circulating TSH levels due to induction of T4-UDP-GT, a mechanism not considered relevant to humans, particularly if TSH is not increased at the clinical dose.

Gene expression analysis of endothelial cell activation markers in skeletal muscle and skin indicated sustained up-regulation in both tissues but only a transient increase in markers of mitosis in skeletal muscle (mitosis-specific gene expression data not available for skin).

Overall, the data from these exploratory studies provide evidence suggestive of an increased sensitivity of mouse vascular endothelial cells to siponimod; however, they are not sufficient to warrant dismissing the clinical relevance of the tumor findings in mouse.

Conclusions and Recommendations

A standard battery of nonclinical studies was conducted on siponimod. While generally adequate, there was a notable lack of toxicokinetic data for siponimod, particularly at the lowest doses tested in some of the pivotal studies, and for M17, for which there are minimal data in male rodents and no data in female rodents or in monkey. Based on what data are available, it appears that M17 was not adequately assessed at the doses tested in the female fertility or pre- and postnatal development studies. However, considering the adverse developmental effects of siponimod itself on the developing organism, there is no need for additional studies. Overall, the nonclinical studies conducted on siponimod are adequate to support approval for the proposed indication.

A post-marketing requirement for a juvenile animal toxicology study is recommended to support clinical development of siponimod in the pediatric population under PREA.

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LOIS M FREED
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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:	209-884
Supporting document/s:	001 – 011
Serial number:	0001 – 0011
Applicant's letter date:	March 28, 2018 - July 26, 2018
CDER stamp date:	July 26, 2018
Product:	MAYZENT™ (Siponimod, BAF312)
Indication:	Secondary progressive multiple sclerosis
Applicant:	Novartis Pharmaceuticals Corp.
Review Division:	Division of Neurology Products
Reviewer:	Richard Siarey
Supervisor:	Lois Freed
Division Director:	Billy Dunn
Project Manager:	Rebecca N Lopez

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 209-884 are owned by Novartis Pharmaceuticals Corp. or are data for which Novartis Pharmaceuticals Corp. has obtained a written right of reference. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application are for descriptive purposes only and are not relied upon for approval of NDA 209-884.

Reference ID: 4392197

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1 Executive Summary

1.1 Introduction

Sphingosine-1-phosphate (S1P) is an endogenous signaling molecule, which has a role in the immune, cardiovascular, and central nervous systems (CNS) through action on five G-protein coupled receptors. Siponimod is an S1P agonist that is selective for $S1P_1$ and $S1P_5$ receptors. The sponsor is developing siponimod (0.25 and 2 mg tablets) for treatment of patients with secondary progressive multiple sclerosis.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies conducted during clinical development and submitted to support the marketing of siponimod include pharmacology, safety pharmacology, pharmacokinetic, chronic toxicology, genetic toxicology, carcinogenicity, and reproductive and developmental toxicology studies.

Siponimod acts at S1P receptors to promote internalization and degradation of $S1P_1$ receptors. This deprives lymphocytes of the obligatory signal to egress from lymphoid organs, preventing the recirculation of lymphocytes to other sites, and sequestering them in secondary lymphoid tissue. In animal models of experimental autoimmune encephalomyelitis (EAE), oral and ICV administration of siponimod resulted in reduce adverse clinical signs.

In cardiovascular safety pharmacology studies, oral siponimod acutely decreased heart rate and caused second-degree AV block in guinea pigs and monkeys, and decreased blood pressure in guinea pigs, with NOAELs of <0.03 mg/kg in guinea pigs and 60 mg/kg in monkeys. On a body surface area basis, NOAELs were <0.24 and 720 mg/m^2 for guinea pig and monkey, respectively. The human dose is 0.3 mg/m^2, resulting in no safety margin. In addition, decreased heart rate was observed in rat and rabbit after IV siponimod. Effects of siponimod on respiratory function included an increase in tidal volume and a decrease in respiration rate in rats.

In mouse, rat, and monkey repeat-dose toxicology studies of oral siponimod, deaths and early sacrifice were observed at 300, ≥50, and ≥100 mg/kg, respectively. The main clinical signs included breathing difficulties in mice and rats at 25 and 50 mg/kg, respectively and convulsions in mice and monkeys at 25 and 100 mg/kg, respectively. Decreased body weight was noted in rats at doses ≥ 50 mg/kg and in monkeys at doses ≥ 100 mg/kg. Target organs were the kidney (nephrotoxicity), lungs (fibrin/hyaline material, fibrosis, and smooth muscle hyperplasia), liver (hypertrophy), and thyroid gland (hypertrophy) in mice, lungs (alveolar macrophages and inflammation), liver (hypertrophy), and uterus in rats, and GI tract (inflammation, crypt hyperplasia, and mucosal erosion) and skeletal muscle (myofiber necrosis) in monkeys. Lymphoid tissues (depletion) were target organs in all species and was accompanied by decreased circulating levels of WBC and lymphocytes; this effect of siponimod was attributed to the pharmacological action of the drug. The NOAEL for lung toxicities were <5 mg/kg in mice, 50 and 10 mg/kg in M and F rats, and 100 mg/kg in monkeys. The safety margin

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for lung toxicity based on plasma exposure was < 73-fold in mice and > 100-fold in rats and monkey.

In genetic toxicology studies, siponimod was negative in the in vitro reverse mutation assay in bacterial cell (Ames) and mammalian cell assays and in in vivo clastogenicity assays (micronucleus) in mouse and rat.

The carcinogenicity study in mouse was adequate and positive for malignant lymphoma in F at all doses and for hemangiosarcoma and combined hemangioma and hemangiosarcoma in M and F at all doses. The carcinogenicity study in rat was adequate and positive for thyroid follicular cell adenoma and combined follicular cell adenoma and carcinoma in HDM.

In the reproductive and development studies, oral siponimod was teratogenic in rats and lethal to fetuses in both rats and rabbits, without a clear NOAEL for fetal toxicity in rats (<1 mg/kg/day) and a NOAEL of 0.1 mg/kg/day in rabbit. The safety margin for fetal toxicity based on plasma exposure is < 18-fold and 0.2-fold (extrapolated from the LOAEL) in rat and rabbit, respectively. The F_1 NOAEL (0.05 mg/kg/day) from the pre- and postnatal study was due to F_1 pup deaths in MD and HD litters, signs of abnormal flexure of the left and right hindlimbs, flat cranium, and malocclusion of the incisors in HD pups, and increased incidence of malformations that included absence of anal opening at the MD and cleft palate, split tongue, truncus arteriosus, and subcutaneous edema over the entire body at the HD. The F_1 NOAEL is 0.3 mg/m^2 on a body surface area basis and 0.9-fold (extrapolated from TK data from the rat EFD study) on a plasma exposure basis, resulting in no safety margin for lethality and teratogenicity. Siponimod crosses the placenta and has been found in milk of lactating animals.

Metabolites M3 and M17 were identified as the major circulating metabolites in humans, each accounting for ≥10% of total drug-related material in plasma. M3 is formed by hydroxylation and glucuronidation, and M17 is a cholesterol ester. M3 is a non-acyl glucuronide metabolite and has adequate systemic exposure in monkeys and adequate systemic exposure to M17 was measured in mouse and rat.

1.3 Recommendations

1.3.1 Approvability

Safety margins to toxicities observed with administration of siponimod are adequate (lung), can be monitored (cardiovascular), or are described in the labeling (reproductive and development, and carcinogenicity). Therefore, it is recommended, from a nonclinical perspective, that siponimod be approved.

1.3.3 Labeling

8.1 Pregnancy section

Animal Data

(b) (4)

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No NOAEL was established for rats in the EFD study, as mortality was observed at the LD; therefore, doses should be included in this paragraph.

(b) (4)

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(b) (4)

The following comment (b) (4) is not accurate, for example no TK data was conducted in safety pharmacology studies so there is no exposure data for the effect of siponimod on the cardiovascular system.

(b) (4)

Although dysregulated angiogenesis/erythropoiesis coupled with increase in endothelial cell proliferation has been described in mice, there appears to be insufficient data describing the mechanism of action, with a specific trigger, that siponimod may induced these changes and whether the key events in the mechanism of action are plausible and relevant in humans. (b) (4)

(b) (4)

After review of the 26-week rat (see Thompson review May 12, 2009), it was concluded that no NOAEL was established.

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2 Drug Information

2.1 Drug

CAS Registry Number: 1234627-85-0

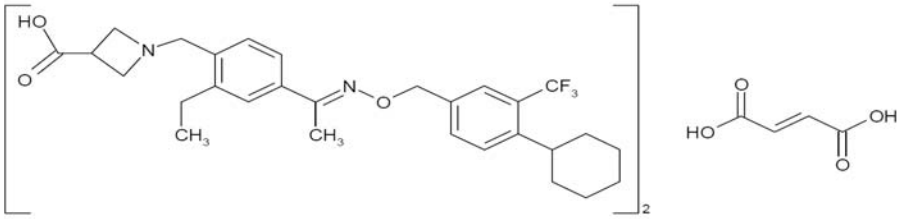
Generic Name: Siponimod

Code Name: BAF312

Chemical Name: (2E)-But-2-enedioic acid—1-({4-[(1E)-N-{[4-cyclohexyl-3-(trifluoromethyl)phenyl]methoxy}ethanimidoyl]-2-ethylphenyl}methyl)azetidine-3-carboxylic acid

Molecular Formula/Molecular Weight: $2(C_{29}H_{35}F_3N_2O_3) \cdot C_4H_4O_4$; (2*516.61) + 116.07 =1149.29

Structure or Biochemical Description: from the sponsor



Pharmacologic Class: $S1P_1$ and $S1P_5$ agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

The sponsor has submitted the following INDs for BAF312 (siponimod); IND 76,122 (DNP, treatment of multiple sclerosis), IND (b) (4) (b) (4) and IND (b) (4)

2.3 Drug Formulation

Film coated tablets (0.25 and 2 mg).

2.4 Comments on Novel Excipients

All excipients are standard pharmacopoeia excipients commonly used in pharmaceutical formulations. The concentration of each excipient is within the usual range of administration for a solid oral dosage form.

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2.5 Comments on Impurities/Degradants of Concern

The sponsor submitted information on 4 impurities, [redacted] (b) (4) and [redacted] (b) (4) The FDA's Computational Toxicology Consultation Service was consulted and concluded that the sponsor's information was sufficient to treat the 4 impurities as [redacted] (b) (4) impurities. Therefore, no further information is required (see Banks-Muckenfuss review, August 9, 2018, IND 76,122). The CMC reviewer noted that the proposed specification limit (NMT [redacted] (b) (4)%) for the drug substance degradant [redacted] (b) (4) exceeds the ICH qualification threshold (NMT [redacted] (b) (4)%). A 4-week toxicology study in rats used spiked-BAF312 (with [redacted] (b) (4) and pure BAF312 to assess impurity [redacted] (b) (4). The spiked-BAF312 batch had a similar severity in toxic effects to the pure BAF312 batch. The batch (with [redacted] (b) (4) of siponimod used for the carcinogenicity studies resulted in [redacted] (b) (4) doses similar or greater than the human dose. A limit of [redacted] (b) (4) [redacted] (b) (4) is set, by the sponsor, for the drug substance, resulting in a daily dose of 20 ng. The Federal regulations state, that [redacted] (b) (4) must be not more than [redacted] (b) (4) in [redacted] (b) (4) for drugs (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=73.1299); therefore, [redacted] (b) (4) is controlled appropriately.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population for siponimod are individuals with secondary progressive multiple sclerosis. The dosing regimen is 2 mg once daily administered orally after the following dose escalation; 0.25 mg/day on Days 1 and 2, 0.5 mg on Day 3, 0.75 mg on Day 4, and 1.25 mg on Day 5. The maintenance dose in patients with a CYP2C9 *1*3 or *2*3 genotype is 1 mg/day.

2.7 Regulatory Background

IND 76,122 was originally received on September 18, 2006, and allowed to proceed (Agency Letter, October 19, 2006). An EoP2 Meeting between the sponsor and FDA was held on September 28, 2011 (see Meeting Minutes, October 28, 2011). A pre-NDA meeting between the sponsor and FDA was held on October 10, 2017 (see Meeting Minutes, November 8, 2017).

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology, safety pharmacology, PK/ADME, chronic toxicology, carcinogenicity, reproductive and developmental toxicology, and genetic toxicology studies were submitted to the NDA.

Toxicology

- Study №: 0770339. A 52-week toxicity study of BAF312 administered orally via nasal gavage to Cynomolgus monkeys, with an 8-week recovery period.

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Genotoxicity

- Study №: 0612011. Mutagenicity test using Salmonella typhimurium.
- Study №: 0612109. Chromosome aberration test with cultured human peripheral blood lymphocytes.
- Study №: 0414047. Micronucleus test in vitro using TK6 cells.
- Study №: 0770326. Rat bone marrow micronucleus test after oral administration.
- Study №: 1470042. Induction of micronuclei in the bone marrow of treated mice.

Carcinogenicity

- Study №: 0870139. 104-week oral (gavage) carcinogenicity study in mice.
- Study №: 0870138. 104-week oral (gavage) carcinogenicity study in rats.

Reproductive and Developmental

- Study №: 0970446. An oral (gavage) male fertility study in rats.
- Study №: 1070054. BAF312: An oral gavage female fertility and early embryonic development study in rats.
- Study №: 0770653. An oral (gavage) embryofetal development study in rats.
- Study №: 0770652. An oral (gavage) embryofetal development study in rabbits.
- Study №: 0970449. An oral (gavage) pre- and postnatal study with immunological endpoints in the rat.

3.2 Studies Not Reviewed

Toxicology

- Single-dose toxicity studies.
- Studies by the IV route.

3.3 Previous Reviews Referenced

- Pharmacology/Toxicology Review, P. Roney, January 12, 2007
- Pharmacology/Toxicology Review, D.C. Thompson, June 12, 2008
- Pharmacology/Toxicology Review, D.C. Thompson, March 27, 2009
- Pharmacology/Toxicology Review, D.C. Thompson, May 12, 2009
- Exec CAC Minutes, A.S. Seifried, December 21, 2011
- Pharmacology/Toxicology Review, D.C. Thompson, February 3, 2012
- Pharmacology/Toxicology Review, M.K. Banks-Muckenfuss, August 9, 2018

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4 Pharmacology

4.1 Primary Pharmacology

Siponimod is an orally-administered selective $S1P_1$ and $S1P_5$ receptor agonist. In vitro studies to detect agonist-induced GTP[γ-^{35}S] binding demonstrated that siponimod (see table below) and several metabolites (M5, M16, and M17; see tables of metabolite data below) have high affinity for G-protein-coupled $S1P_1$ and $S1P_5$ receptors, with minimal activity at the other S1P receptors. Metabolite M3 had no activity at $S1P_1$ receptors.

Table: Affinity of siponimod for the S1P receptors (Sponsor's)

	EC_{50} [nM]	Agonism in [%] #)
hS1P1	0.39 ± 0.07	91± 6
hS1P2	>10000	3 ± 3
hS1P3	>1000	61 ± 11
hS1P4	750 ± 487	90 ± 9
hS1P5	0.98 ± 0.43	112 ± 20

#) maximum receptor stimulation compared to S1P

Table: Affinity of metabolites (M3 and M5) of siponimod for the S1P receptors (Sponsor's)

Receptor subtype	M5 EC_{50}±SD (nmol/L)a) % E_{max} b)	M3 EC_{50}±SD (nmol/L) a) % E_{max} b)	NVP-BAF312 EC_{50}±SD (nmol/L) a) % E_{max} b)	S1P EC_{50}±SD(nmol/L)a) % E_{max} b)
hS1P1	470±91	>10000	1.1±0.41	1.9±0.6
	78±8.4	36±13	100±0	100±1.7

a) Mean ± SD of three independent experiments

b) %E_{max} corresponds to the percent of maximal stimulation of the receptor by the compound compared to the reference NVP-BAF312.

Table: Affinity of metabolites (M16 [NVP-BAF312-NX3] and M17 [NVP-LYS815-NX-1]) of siponimod for S1P receptors (Sponsor's)

Receptor subtype	**NVP-BAF312-AE-1** EC50 ± SD (nmol/L) % Emax	**NVP-LYS815-NX-1** EC50 ± SD (nmol/L) % Emax	**NVP-LYG778-NX-3** EC50 ± SD (nmol/L) % Emax	**S1P** EC50 ± SD (nmol/L) % Emax
hS1P1	4.3 ± 0.5	341 ± 86	73 ± 25	0.92 ± 0.01
	100 ± 0	92 ± 4	84 ± 4	105 ± 10
hS1P5	2.2 ± 0.5	159 ± 9	16 ± 6	
	100 ± 0	80 ± 16	74 ± 14	

EC50 and % Emax data are means ± SD from four independent concentration-response curves for the metabolites NVP-LYS815 and NVP-LYG778 and from two curves for the reference compounds NVP-BAF312 and S1P (two replicates per concentration). Examples of concentration-response curves are shown in Figure 3-1 and Figure 3-2. % Emax levels were calculated based on the stimulation levels of the reference compound NVP-BAF312-AE-1.

Lymphocytes require $S1P_1$ receptor activation for migration from lymphoid organs; siponimod acts at S1P receptors to induce internalization and degradation of the receptor. The internalization and degradation of the receptor by siponimod causes these cells to become insensitive to S1P, depriving the cells of the obligatory signal to egress from peripheral lymphoid organs and, thus, prevents the recirculation of these cells to other sites. Decreased levels of circulating lymphocytes have been observed in all species administered siponimod. $S1P_5$ receptors are expressed on oligodendrocytes and oligodendrocyte precursor cells. Siponimod action at $S1P_5$ receptors may enhance remyelination, as it has been suggested that $S1P_5$ receptors could be part of the

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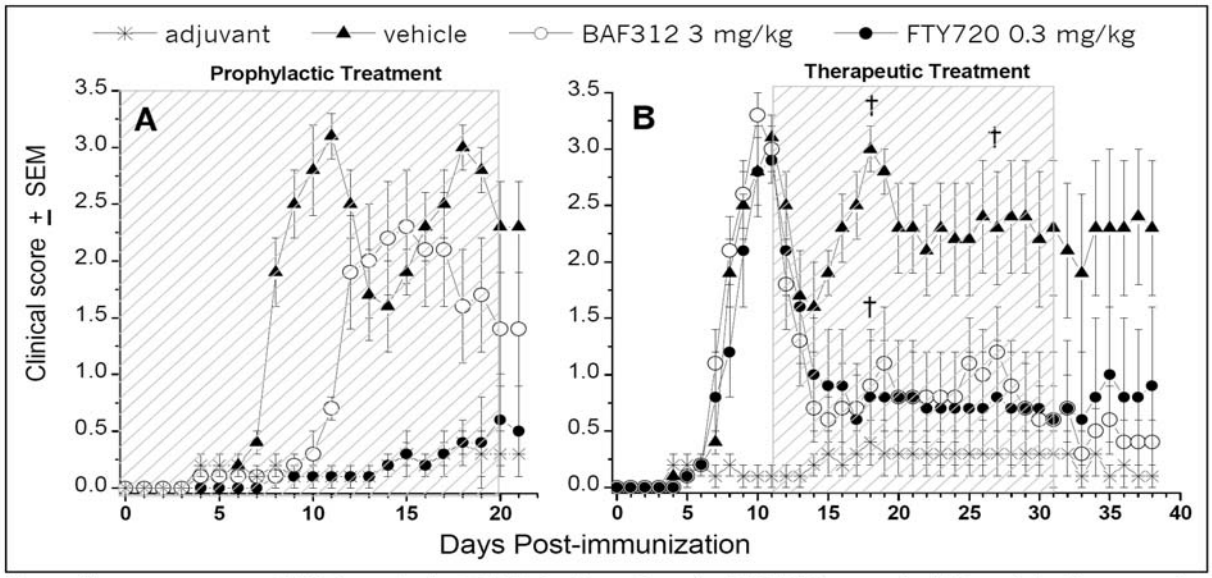
neuron-oligodendroglial communication network, regulating oligodendrocyte precursor cell migration that may provide directional guidance cues for migrating oligodendrocyte precursor cells in the developing brain (Novgorodov et al. FASEB J. 2007 May;21(7):1503-14).

Effects of siponimod in autoimmune encephalomyelitis (EAE) models

Siponimod has been investigated in rodent EAE models. Daily dosing of F C57/Bl6 mice with oral siponimod (3 mg/kg) for 25-27 days, after induction of EAE (SC myelin oligodendrocyte glycoprotein [MOG] emulsified in complete Freund's adjuvant followed by IP pertussis toxin), reduced adverse clinical signs and microscopic evidence of inflammation and demyelination in the spinal cord. In the Dark Agouti rat EAE model induced with intradermal encephalitogenic emulsion in complete Freund's adjuvant, oral siponimod was assessed either prophylactically (3 mg/kg from Day 0) or therapeutically (1-30 mg/kg on days 11/12 to 31). Prophylactic dosing with oral siponimod delayed the onset of adverse clinical signs by approximately 3 days. Dosing with oral siponimod after the induction of EAE reduced adverse clinical signs. Circulating lymphocytes counts were reduced by siponimod. Macrophage infiltration/microglial cell activation and demyelination were reduced after siponimod dosing in the rat EAE model.

Figure: Clinical scores after prophylactic and therapeutic dosing of siponimod (Sponsor's)



Mean disease scores ± SEM from study CrEAE-6 where 3 mg/kg BAF312 was administered for three weeks to compare prophylactic and therapeutic treatment. [A] Pairwise multiple comparison of AUC values from d 0-21 showed that BAF312 treatment was less effective than FTY720 ($p < 0.001$) yet still offered some protection compared to vehicle ($p = 0.02$). [B] Therapeutic dosing of BAF312 from d 11-31 tended to suppress the development of severe neurological symptoms in most animals relative to the vehicle group ($p = 0.05$). In comparison to EAE controls, FTY720 was more effective ($p = 0.006$). † = EAE-related mortality on the indicated post-immunization day.

In a published article (Gentile et al. Journal of Neuroinflammation, 2016; 13:207), data were presented that suggest siponimod may have actions in the CNS. ICV infusion of siponimod, for 4 weeks initiated 1 week before the induction of EAE (subcutaneous MOG_{35-55}), induced a decrease in adverse clinical scores, with only a small decrease in

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peripheral lymphocytes. However, ICV siponimod dosing was initiated prior to induction of EAE, the level of reduced adverse clinical score with 0.45 µg/day may relate to the approximate 25% decrease in peripheral lymphocytes, and infiltrating lymphocytes to the striatum were reduced. Therefore, the data does not appear to be conclusive evidence that the changes are due to a central action of siponimod and significant in multiple sclerosis.

Figure: Effects of siponimod on EAE motor deficits and peripheral lymphocytes (Gentile et al., 2016)

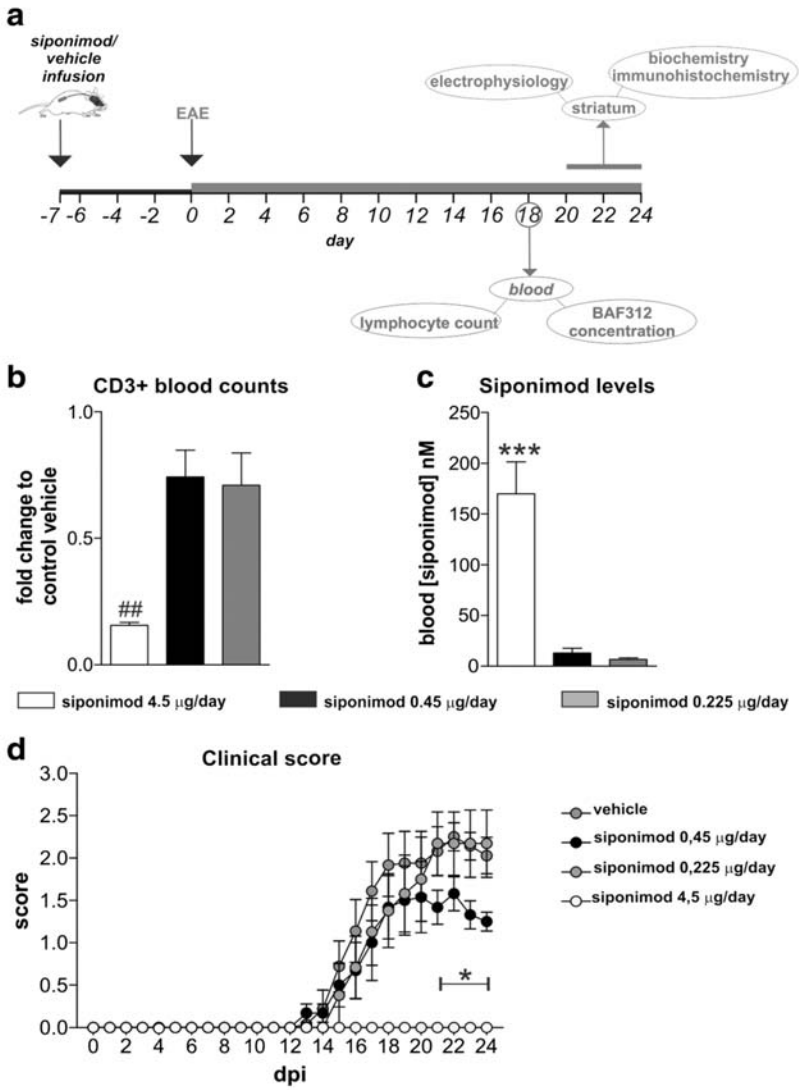

Fig. 1 Intracerebroventricular administration of 0.45 µg/day siponimod attenuates EAE motor deficits without inducing peripheral lymphocyte depletion. **a** Schematic representation of the experimental design used in the study. Minipump implantation and siponimod/vehicle release preceded the induction of EAE by 1 week. All examinations were performed on animals during the peak of the symptomatic phase of the disease 18–24 dpi, both in the peripheral blood samples and in the striatum of the animals. **b** $CD3^+$ lymphocytes were counted in the peripheral blood of EAE mice receiving vehicle or different siponimod dosages: significant reduction was observed for the 4.5 µg/day dosage. Siponimod 4.5 µg/day vs vehicle $\#\#p < 0.01$ unpaired *T* test; siponimod 0.45 µg/day vs vehicle $p > 0.05$ unpaired *T* test; siponimod 0.225 µg/day vs vehicle $p > 0.05$ unpaired *T* test. **c** Siponimod concentrations were determined in peripheral blood samples of EAE mice receiving intracerebroventricular release of different siponimod dosages: the highest concentration (4.5 µg/day) used was significantly higher compared to both 0.450 µg/day and 0.225 µg/day, while there were no statistically differences between the other two dosages. Tukey's post hoc $***p < 0.001$. **d** Representative clinical course of EAE mice treated with three different siponimod dosages: EAE disease progression is strongly affected by 4.5 µg/day dosage during the symptomatic phase of the disease and significantly attenuated by 0.45 µg/day concentration in the dpi range of 18–24. Daily statistical significance was evaluated by non-parametric Mann-Whitney test

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Other pharmacology studies
In mice, oral administration of siponimod dose-dependently inhibited T helper cell-dependent (KLH-induced) and -independent (LPS-induced) antigen-specific IgM and IgG. The delayed-type hypersensitivity response in mice, expressed after a second injection of sheep red blood cells, was not affected by oral siponimod (3-100 mg/kg), suggesting there is no direct effect of siponimod on the effector function of T memory cells.

In naïve monkeys, priming with tetanus toxoid induced a reservoir of memory T and B cells that are in lymphoid and peripheral tissues. These memory cells can effectively be re-stimulated by tetanus toxoid in naïve monkeys and monkeys dosed with oral siponimod during the recall. These data suggest that siponimod does not interfere with T and B cell memory functions. This suggests that the immune response to vaccination could remain partially intact during siponimod therapy.

In mice, oral siponimod (0.03 - 30 mg/kg) increased Evans Blue accumulation in lungs by 2- to 3-fold, likely mediated via the $S1P_1$ receptor. The increase in Evans Blue accumulation suggests an increase in capillary permeability. However, siponimod (30 mg/kg for 70 days), similar to fingolimod, did not cause blood-retina-barrier damage in rats.

4.2 Secondary Pharmacology

Siponimod was tested in a panel of in vitro radioligand binding assays of 98 targets consisting of GPCRs, transporters, ion channels, and enzymes. Siponimod had affinity for a number of human targets, with the following IC_{50}'s: histamine H_2 (0.12 μM), adrenergic α_{2A} (0.56 μM) and α_{2B} (0.21 μM), and the serotonin transporter (0.60 μM) (see Roney review, January 12, 2007; IND 76,122). Metabolite M3 (LNL925), assayed at 124 targets, had no affinity at any target up to 10 μM, and M16 (LYG778) and M17 (LYS815), assayed at 56 targets, had no affinity at any target (IC_{50} > 1 μM).

4.3 Safety Pharmacology

The effects of siponimod was investigated in a core battery of safety pharmacology studies. Neurological function was assessed in M Wistar rats; no changes in behavior or body temperature were observed after single oral doses up to 200 mg/kg siponimod.

Several in vitro assays were conducted to assess the effect of siponimod on cardiovascular function. The hERG tail current, expressed in HEK293 cells, was minimally inhibited by siponimod (91% of control at 25 μM). Siponimod activated the G-protein coupled inwardly rectifying potassium (GIRK) current in guinea pig myocytes (maximum activation of approximately 60% at 3 μM) and in isolated human atrial monocytes (EC_{50} of 16 nM). Another study demonstrated that incubation of guinea pig atrial myocytes with siponimod prevented GIRK channel activation by carbachol, suggesting siponimod desensitizes the activation of GIRK currents. In isolated rabbit

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tissues, siponimod (up to 10 μM) did not show a risk for QT interval prolongation and did not induce vasoconstriction of the thoracic aorta or coronary artery.

In in vivo cardiovascular studys, hemodynamic and electrocardiographic effects of siponimod were investigated in several species. In the telemeterized M Hartley guinea pig, oral administration of siponimod (0.03, 0.3, and 1 mg/kg) resulted in decreases in mean blood pressure for about 7-11 hours post-dose, heart rate for about 3-5 hours post-dose, QTc for about 4 hours post-dose, an increase in mean PR interval for about 5-8 hours post-dose, and second-degree AV block. In a second study in telemeterized M Hartley guinea pigs, administration of atropine (0.1 mg/kg) either 15 minutes before and 30 minutes after or 60 minutes after oral siponimod (0.3 mg/kg) did not change the effect of siponimod on blood pressure. Administration of atropine 15 minutes before and 30 minutes after siponimod reversed the siponimod decrease in heart rate and QTc and prevented or delayed the occurrence of AV block. In a non-GLP study in monkeys, one monkey administered 100 mg/kg orally had a reduced heart rate (47-68 bpm) between 1-2 hours post-dose, with a second-degree AV block of about 1-hour duration. In a later GLP study in monkeys, oral administration of siponimod at doses up to 150 mg/kg had no effect on blood pressure, heart rate, or ECG parameters.

In IV studies, siponimod administration to rats decreased heart rate at 0.5 and 1 minute post-dose, with the heart rate returning to normal after 5 minutes. Slow IV bolus doses of siponimod (0.03, 0.3, and 1 mg/kg) administered to anesthetized M guinea pigs resulted in a decrease in mean blood pressure within 2 minutes post-dose, a decrease in heart rate 5 minutes post-dose, and development of AV conduction abnormalities. In rabbits, IV siponimod (1 mg/kg) decreased heart rate up to 2.5 hours post-dose and arrhythmias were observed in 4 of 6 rabbits.

The effect of oral siponimod on respiratory function was assessed in M Wistar rats. Siponimod (100 and 200 mg/kg) increased tidal volume and decreased respiration rate at 1 hour post-dose. No significant effects were observed on minute volume at any time point or on tidal volume or respiration rate at other time points up to 24 hours post-dose.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Absorption

After oral administration, the T_{max} for siponimod ranged from 2 to 8 hours and $t_{½}$ ranged from 16 to 62 hours in mice, rats, and monkeys. Data in humans were similar, with T_{max} between 3 and 8 hours and a median value of 4 hours. Absolute oral bioavailability of siponimod was 49-52% in M rats, 54-83% in monkeys, and 84% in humans. In rats, plasma exposure to siponimod was greater in F than M.

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Table: Pharmacokinetic parameters of siponimod in plasma of animals and human after single (SD) and multiple (MD) oral administrations (Sponsor's)

Species (Reference)	Gender, (n)	Dose [a] (mg/kg/day)	Tmax (h)	Cmax (ng/mL)	Cmax/dose (ng/mL)/(mg/kg) [b]	AUClast (ng·h/mL)	AUC interval (h)	AUClast/dose (ng·h/mL)/(mg/kg) [b]	T1/2 (h)	CL/F (L/h)	Vz/F (L)	F (%)
Mouse (DMPK R1300411-02)	m, (3/time point)	8 (SD)	8	2300	287	64060	0-168	7870	27.2	-	-	-
Mouse (DMPK R1500775)	m, (n=2/ timepoint)	25 (SD)	6	5950	241	112000	0-24	4530	-	-	-	-
		25 (MD)	6	7910	319	141000	0-24	5690				
Rat (DMPK R0500017-02)	m, (3)	3 (SD)	6.67	259	86.0	4050	0-96	1340	-	-	-	48.7
Rat (DMPK R0900164-01)	m, (3)	3 (SD)	8 [c]	235	73.1	3470	0-96	1080	-	-	-	52.1
Rat (DMPK R1300583)	f, (8)	10 (SD)	8	2580	266	94540	0-72	9740	28.1	-	-	-
Monkey (DMPK R1000166-01)	m, (3)	15 (SD)	7	3800	253	111000	0-336	7400	16.0 62 [g]	-	-	-
Monkey (RD-2005-00583) [d]	m,(2)+ f,(2)	1 (SD)	2.5 [e]	354	354	7550	0-168	7550	-	-	-	71 [e]
Human (Study A2104)	m, (4)	0.143 [f] (SD)	4 [c]	80.4	591	3200	0-144 to 0-216	23500	56.6	3.97	291	84

a) Referred to the free compound (MW: 516.61), nominal values of single dose (SD) or multiple doses (MD).
b) Calculated with exact dose if available.
c) median
d) All concentrations were determined in whole blood.
e) Tmax ranged between 2 and 4.8 hours. BAF312 was orally bioavailable (54-83% at doses between 0.03 and 3 mg/kg).
f) Nominal dose of 10 mg assuming 70 kg body weight
g) T1/2alpha and T1/2beta were calculated separately (AUCbeta: <6%)

Source: [Table 2.6.5.3A-DMPK R1300411-02], [Table 2.6.5.3B-DMPK R0500017-02], [Table 2.6.5.3C-DMPK R0900164-01], [Table 2.6.5.3E-Report RD-2005-00583], [Table 2.6.5.3F-DMPK R1000166-01], [Table 2.6.5.4A-DMPK R1500775], [Table 2.6.5.7A-DMPK R1300583], [Study A2104], [SCP]

In mice and rats, after oral administration of siponimod the T_{max} of metabolite M17 was approximately 24 hours and accumulation observed.

5.1.2 Distribution

Distribution of siponimod was evaluated in blood of several animal species. The fraction of siponimod in plasma was approximately 60% in mouse, 48% in rat, 59% in rabbit, 71% in dog, 56% in monkey, and 68% in human. Plasma protein binding was high for siponimod and metabolite M17 (>99%) in mouse, rat, rabbit, dog, monkey, and human.

Tissue distribution studies were conducted in several species, including mice and rats. Siponimod was widely distributed into tissues in both rodent species, consistent with the steady state volume of distribution (2.2-3.0 L/kg for rats). The highest exposures were found in bile, liver, penis, adrenal cortex, esophagus, and kidney cortex.

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Table: Siponimod distribution after oral dosing (Sponsor's)

Tissues	AUC (nmol*h/g)	AUC ratio *	Tissues	AUC (nmol*h/g)	AUC ratio *
Adrenal (cortex)	184	13.1	Liver	231	16.5
Adrenal (medulla)	63	4.5	Lung	40	2.9
Bile	408	29.1	Lymph nodes	34	2.4
Blood	14	1.0	Mouth (mucosa)	31	2.2
Blood vessel wall	3	0.2	Muscle	28	2.0
Bone marrow	34	2.4	Nasal turbinates	28	2.0
Bone mineral	6	0.4	Nerves	75	5.4
Brain (Cerebellar white matter)	64	4.6	Oesophageus	168	12.0
Brain (Cerebellum)	40	2.9	Pancreas	78	5.6
Brain (Cerebral cortex)	38	2.7	Penis (corpus cavernosum)	199	14.2
Brain (Dura mater)	52	3.7	Pineal gland	56	4.0
Brain (Inf. Colliculus)	50	3.6	Pituitary gland	57	4.1
Brain (Lateral septal nu)	43	3.1	Preputial gland	23	1.6
Brain (mean)	43	3.1	Salivary gland	59	4.2
Brain (Olfactory bulb)	31	2.2	Seminal vesicle	6	0.4
Brain (Reticular nu)	44	3.1	Seminal vesicle (contour)	30	2.1
Brain (Spinal chord gray)	50	3.6	Skin	19	1.4
Brain (Spinal chord white)	61	4.4	Skin (subcutis)	36	2.6
Brain (Spinal chord) mean	57	4.1	Spleen	47	3.4
Brain (Sup.colliculus)	46	3.3	Spleen (red pulp)	40	2.9
Brown fat	73	5.2	Spleen (white pulp)	20	1.4
Cartilage	24	1.7	Stomach (glandular)	90	6.4
Choroid plexus	33	2.4	Stomach (non glandular)	27	1.9
Epididymis	38	2.7	Testis	57	4.1
Eye (choroid)	48	3.4	Thymus	36	2.6
Eye (lens)	5	0.4	Thyroid gland	61	4.4
Eye (vitreous body)	5	0.4	Tongue	50	3.6
Hair (follicle)	43	3.1	Tooth (enamel)	21	1.5
Hair (tactile)	19	1.4	Tooth (pulp)	29	2.1
Harderian gland	86	6.1	Urinary bladder	53	3.8
Heart	64	4.6	White fat	8	0.6
Intest. wall (colon)	71	5.1			
Intest. wall (duodenum)	59	4.2			
Kidney (CM junction)	84	6.0			
Kidney (cortex)	114	8.1			
Kidney (medulla)	58	4.1			
Kidney (pelvis)	33	2.4			
Lachrymal gland	86	6.1			

* $AUC_{tissue} / AUC_{blood}$

In rats, siponimod was detected in brain and CSF after oral administration. Siponimod exposures in brain and spinal cord were between 0.8- and 4.4-fold that in blood. Highest tissue/blood ratios in the CNS were observed in white matter of the spinal cord, cerebellum, corpus callosum, and medulla oblongata.

A single oral dose of 10 mg/kg [^{14}C]siponimod was administered to lactating rats on Day 11 after parturition. Milk and blood samples were collected up to 72 hours post-dose and siponimod and metabolites measured. The T_{max} of siponimod in milk was 8 hours post-dose and $t_{½}$ was 27.8 hours; similar values were observed for siponimod in plasma (T_{max} of 8 hours and $t_{½}$ of 28.1 hours). The milk/plasma concentration ratios ranged from 0.09 (at 0.25 hours) to 0.61 (at 24 hours) for siponimod. Although metabolites M1, M2, M6, M8, and M16 were detected in milk, all were minor components, with siponimod accounting for 90% of radioactive dose.

Table: Siponimod and metabolites blood and milk concentrations

	Plasma		**Milk**	
	AUC_{0-72} (nmol/L*h)	**%**	**AUC_{0-72} (nmol/L*h)**	**%**
Siponimod	165000	95.29	90100	89.79
M1	8.13	0.00	186	0.19
M2	-	-	6.9	0.01
M6	595	0.34	305	0.30
M8	1.19	0.00	459	0.46
M16	1480	0.86	123	0.12

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Placental transfer of siponimod was investigated in pregnant rabbits after oral doses of 0.1, 1, and 5 mg/kg on Gestation Day (GD) 20. Plasma exposure to siponimod in dams was generally dose-proportional between the LD and MD and greater than dose-proportional between the MD (958 ng*h/mL) and HD (5510 ng*h/mL). Fetal (pooled fetuses) exposure to siponimod was observed at the MD (41 ng/g) and HD (146 ng/g). Blood levels of siponimod in fetuses were lower than in dams.

5.1.3. Metabolism

Two major human metabolites were identified; M3 formed by hydroxylation and glucuronidation and M17, a cholesterol ester. Systemic exposure of M3 in monkey was approximately 60-fold that observed in humans and is a non-acyl glucuronide metabolite. Adequate systemic exposure of M17 was obtained in mouse and rat (see table below).

Table: Pharmacokinetics of metabolite M3 (Sponsor's)

Animals							
Species	NOAEL [b)] (mg/kg/day)	Sex	AUC0-24h (ng/mL•h) [b)] BAF312	AUC0-24h (nmol/L•h) [b)] BAF312	AUC0-24h (ng/mL•h) [c)] M3	AUC0-24h (nmol/L•h) [c)] M3	Exposure multiple [a, b)] Based on a mean AUCtau, ss of M3 358 nmol/L•h [d, e)]
52-week monkey	10	M	95400	184665	12290	17359	48.5
	10	F	124000	240026	15975	22562	63.0

a) Animal exposure multiples of a 2 mg dose in humans for metabolite M3
b) NOAELs 52-week (monkey) repeat-dose toxicity studies [Table 2.6.7.7F-Study 0770339]
c) Calculated based on M3- AUCinf as a percentage of BAF312- AUCinf in monkey ADME study: 9.4% (based on molar values) [Table 2.6.5.9E-DMPK R1000166-01]
d) Data from (2 mg, qd, [Study A2118]), geo-mean: 254 h*ng/mL (MW: 708.7)
e) In a pharmacogenetic study [Study A2128] after single dose of 0.25 mg siponimod, a 0.34-ratio of exposure for M3 between CYP2C9*3/*3 and CYP2C9*1/*1 subjects was calculated. Consequently, the exposure ratio will be approx. 3-times higher in CYP2C9*3/*3 patients when compared to CYP2C9*1/*1 patients.

Reference ID: 4392197

Table: Pharmacokinetics of metabolite M17 (Sponsor's)

Species (Reference)	Gender	Dose (mg/kg/day)	Tmax (h)	Cmax (ng/mL)	(nmol/L) [a]	AUC0- (ng·h/mL)	(nmol*h/L) [a]	AUC interval (h)	T1/2 (h)	Cmax ratio M17/ BAF312 ratio [b] *(Exposure multiples animal/human)* [c]	AUC ratio M17/ BAF312 ratio [b] *(Exposure multiples animal/human)* [c]
Mouse (DMPK R1500775)	m	25 (SD)	24	9500	10700	111000	125000	0-24	na	0.932	0.578
	m	25 (MD) [d]	24	26800	30300	591000 [e]	668000 [e]	0-24	na	1.98 *(209)*	2.45 *(660 [m])*
Rat (Study 1370783)	m	90 (SD)	24	143	162	2230	2520	0-24	na	0.00726	0.00779
	m	90 (MD) [f]	3	206	233	4080 [e]	4600 [e]	0-24	na	0.0141 *(1.60)*	0.0207 *(4.55 [g])*
Monkey (DMPK R1000166-01)	m	15 (SD)	nd	nd	nd	nd	nd	nd	nd	nd	nd
Human (Study A2126)	m/f	0.00357 (SD) [h]	96 [i]	0.356 [j]	0.402 [j]	112 [j,k]	127 [j,k]	na	155 [j]	0.122 [j]	0.974 [j,k]
Human (Study CBAF312 A2118)	m/f	0.0286 (MD) [h]	na	129 [j,l]	145 [j,l]	896 [j,l]	1012 [j,l]	0-24	na	na	na

nd: not detectable; na: not applicable

a) MW of M17: 885.25

b) Calculated using molar BAF312 (Cmax or AUC) values of the same study: mouse ((Table 3-3), [Table 2.6.5.4A-DMPK R1500775]), rat [Table 2.6.7.17B-Study 1370783], monkey (Table 3-3), [Table 2.6.5.9E-DMPK R1000166-01]), human [Study A2126]

c) Exposure multiples were calculated using data in healthy human subjects 2 mg steady-state (predicted) [Study A2118]

d) 14 days

e) AUCtau, ss

f) 28 days

g) Exposure multiples calculated at the NOAEL of 50 mg/kg/day for male rats and 15 mg/kg/day for female rats using a 26-weeks toxicity study [Table 2.6.7.7C-Study 0770059] are 4.2 and 7.6, respectively.

h) Nominal dose assuming 70 kg body weight

i) Median

j) Geo-mean

k) AUCinf

l) Extrapolated from Study A2126 data assuming dose and time linearity PK for M17

m) Exposure multiple calculated at the LOAEL of 5 mg/kg/day using a 13-weeks toxicity study [Table 2.6.7.7A-Study 0770649] in male and female mice is 209 and 188, respectively.

Source: Table 2.6.5.4A-DMPK R1500775, Table 2.6.7.17B-Study 1370783, Table 2.6.5.9E-DMPK R1000166-01, Study A2126, Study A2118

5.1.3.1 In vitro

In vitro metabolism of siponimod by liver microsomes of mouse, rat, monkey, and human (dog microsomes did not metabolize siponimod) and rat and human hepatocytes had similar metabolic patterns. In hepatocytes, the primary isozymes involved in metabolism were CYPs 2C9 (79%) and 3A4 (19%). Siponimod was metabolized differently by the CYP2C9 genotypes, genotype 3 was 10-fold slower than genotype 1, and genotype 2 had intermediate activity.

Reference ID: 4392197

Table: Comparing siponimod metabolites in different species (Sponsor's)

Compo-nents	Metabolic reaction	Incubations with liver microsomes					Incubations with hepatocytes	
		Species					Species	
		Human	*Rat*	*Monkey*	*Dog*	*Mouse*	*Human*	*Rat*
M3	Hydroxylation and glucuronidation	X	XX	XX		X	XX	XX
M4	Hydroxylation and sulfate conjugation						X	X
M5	Hydroxylation	X	X	X			X	X
M6	Hydroxylation		XX	X		X	X	XX
M7	Hydroxylation	X	X	XX			XX	X
BAF312	Parent drug	XX	XX	XX	XX	XX	XX	XX

5.1.3.2. In vivo

In a CD-1 mouse ADME study, PK of metabolites M16 and M17 were assessed after single and repeated oral doses of siponimod (25 mg/kg/day). M16 was not detected in mouse plasma. M17 was detected, with similar C_{max} and $AUC_{0\text{-}24\ h}$ to siponimod on Day 1 and higher C_{max} and $AUC_{0\text{-}24}$ than siponimod on Day 14, T_{max} was 24 hours.

In rats, metabolites of siponimod were formed by hydroxylation (M5, M6, M7, and M9), hydroxylation and glucuronidation (M3 and M14), hydroxylation and sulfation (M4 and M10), and cleavage/hydrolysis of the oxime ether with oxidation (M11 and M13). After oral dosing in rats, the main plasma metabolites were M6 (12%), M11 (23%), and M13 (6%). Metabolites detected in urine included M10, M11, and M13, which ranged between 0.03% and 0.47% of the total radioactive dose. Metabolites detected in bile included M3, M4, and M14, which accounted for 11%, 26%, and 10% of the dose, respectively, and metabolites detected in feces included metabolites M4, M5, and M6, which accounted for 16%, 14%, and 27% of the radioactive dose, respectively. In a rat mechanistic study (№ 1370783; see section 10.4), PK of metabolites M16 and M17 were assessed after single and repeated doses of siponimod (90 mg/kg/day). M16 was not detected in rat plasma. M17 was detected with lower plasma exposure (C_{max} and $AUC_{0\text{-}24\ h}$) than siponimod and a T_{max} of 24 hours.

Reference ID: 4392197

Table: Siponimod and metabolites in rat plasma after oral dosing (Sponsor's)

Experiment	Rat 3 mg/kg p.o.							
Sample type	Plasma pools of n = 2 (Rat 11 excluded)							
Sample collection time (h)	0.25	2	4	8	24	48	AUC0-48h	
Component	Concentration (µM)						h*µM	(%)
P22.2	n.d.	n.d.	n.d.	n.d.	0.004	0.002	0.111	0.8
P24.4	n.d.	n.d.	n.d.	n.d.	0.005	0.001	0.114	0.8
M11	n.d.	0.009	0.018	0.045	0.116	0.022	3.104	22.7
P26.6	n.d.	n.d.	0.002	0.006	0.012	0.003	0.350	2.6
M13	n.d.	n.d.	0.001	0.006	0.027	0.021	0.862	6.3
M5	n.d.	0.004	0.008	0.007	0.001	n.d.	0.122	0.9
M6	n.d.	0.035	0.068	0.094	0.018	0.002	1.586	11.6
M7	n.d.	n.d.	n.d.	0.001	n.d.	n.d.	0.011	0.1
BAF312	0.077	0.292	0.383	0.350	0.035	0.006	6.047	44.3
Sum of additional components	0.004	0.004	0.010	0.003	0.005	0.002	0.198	1.5
Total components detected	0.081	0.345	0.489	0.513	0.224	0.058	12.505	91.6
Lost during sample processing	0.003	0.022	0.039	0.050	0.020	0.008	1.153	8.4
Lost during HPLC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
Total radiolabeled components in original sample [a]	0.084	0.367	0.528	0.563	0.244	0.066	13.658	100.0

n.d. not detectable
- not calculated/ not meaningful
a: Data from Table 6-6

Table: Siponimod and metabolites in rat feces and urine after oral dosing (Sponsor's)

Experiment	Intact rats; 3 mg/kg p.o. (pool of n=2)		
Sample type	**Urine**	**Feces**	**Total excretion**
Excretion period (h)	0 – 96 h	0 – 96 h	0 – 96 h
Component	Excretion (% of dose)		
P17.4	0.0923	0.304	0.396
P19.1 + P19.2	0.269	0.911	1.18
P20.0	0.105	n.d.	0.105
P20.6	0.0244	n.d.	0.0244
P22.2	0.105	2.19	2.29
M10	0.468	2.26	2.73
M11	0.160	0.883	1.04
M13	0.028	5.09	5.11
P30.2	n.d.	2.90	2.90
M3	n.d.	0.727	0.727
M14	n.d.	1.35	1.35
P34.8	n.d.	1.39	1.39
P35.0	n.d.	2.35	2.35
P35.8	n.d.	1.69	1.69
M9	n.d.	0.579	0.579
M4	n.d.	16.4	16.4
M5	n.d.	14.2	14.2
M6	n.d.	26.6	26.6
M7	n.d.	1.52	1.52
P45.0	n.d.	n.d.	n.d.
BAF312	n.d.	9.41	9.41
Sum of additional components	0.042	1.18	1.22
Total components detected	1.30	92.0	93.3
Lost during sample processing	0.016	0.462	0.478
Lost during HPLC	n.d.	n.d.	n.d.
Total radiolabeled components in original sample (pool 0-96h) [a]	1.32	92.4	93.8
Total excretion of radiolabeled components (0-168h) [a]	1.44	94.7	96.1

n.d. not detectable
a: Data from Table 6-23

Reference ID: 4392197

In monkeys, metabolites of siponimod were formed by hydroxylation (M5, M6, and M7), hydroxylation and glucuronidation (M3), hydroxylation and sulfation (M4a, M4b, and M4c), and cleavage/hydrolysis of the oxime ethers (M1 and M2) and further reduction (M8) or oxidation (M11, M13 and M15). The main plasma metabolite after oral dosing was M3 at 7% of total radioactivity. Metabolites detected in urine included M1, M2, M3, and M8 ranging between 0.17% and 0.49% of the total radioactive dose. Metabolites detected in feces included M4, M5, M6, and M7 that accounted for 28%, 28%, 8% and 8% of the radioactive dose, respectively.

Table: Siponimod and metabolites in monkey plasma after oral dosing (Sponsor's)

Experiment	Monkey (15 mg/kg) p.o.								
Sample type	Plasma pools of n = 3								
Sample collection time (h)	1	4	7	10	24	72	144	AUC0-144h	
Component	Concentration (µM)							h*µM	(%)
P7.0	n.d.	n.d.	n.d.	n.d.	0.0296	n.d.	n.d.	0.917	0.27
P26.6	n.d.	n.d.	n.d.	n.d.	0.0285	n.d.	n.d.	0.883	0.26
P29.2	n.d.	n.d.	n.d.	n.d.	0.0289	n.d.	n.d.	0.897	0.26
M3	0.171	0.528	0.559	0.498	0.291	0.0875	0.0469	23.8	6.95
P32.2	n.d.	n.d.	n.d.	0.0355	0.0278	n.d.	n.d.	1.16	0.34
M5	0.0185	0.0695	0.0889	0.0624	0.0285	0.00913	n.d.	2.47	0.72
M6	0.0230	0.124	0.0699	0.109	0.0703	0.0159	n.d.	4.69	1.37
M7	0.0274	0.123	0.144	0.114	0.0738	0.0139	n.d.	4.94	1.44
P57.2	n.d.	n.d.	0.0650	0.0771	0.0789	0.0289	n.d.	5.03	1.47
P72.6	n.d.	n.d.	n.d.	0.0400	n.d.	n.d.	n.d.	0.340	0.10
BAF312	1.67	8.04	7.62	6.89	3.66	0.638	0.309	272	79.4
Sum of additional components	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
Total components detected	1.91	8.88	8.55	7.83	4.32	0.794	0.356	317	92.6
Lost during sample processing	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
Lost during HPLC	0.152	0.710	0.683	0.626	0.345	0.0634	0.0284	25.3	7.40
Total radiolabeled components in original sample [a)]	2.06	9.59	9.23	8.46	4.66	0.857	0.384	342	100

n.d.: not detected

a): Data from Table 5-2 or Table 5-3

To obtain concentrations in ng-eq/mL (= ng/mL for parent compound), multiply the concentrations in µM by 516.61 g/mol.

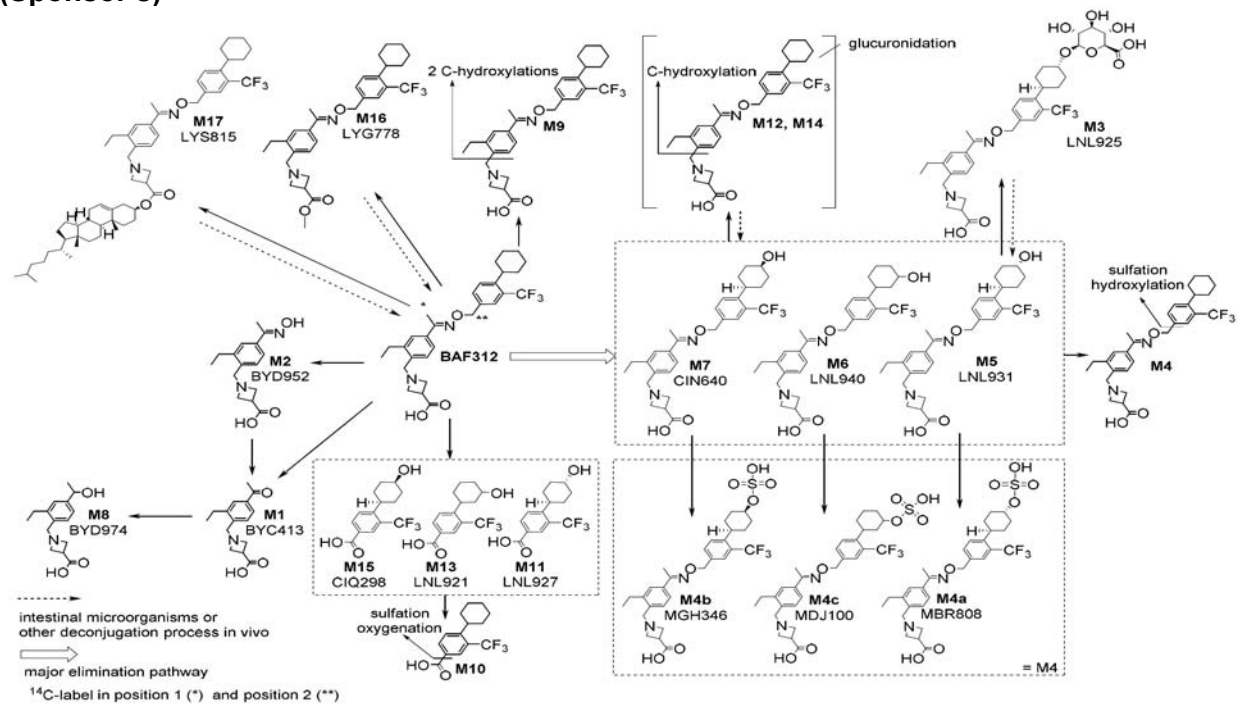
Reference ID: 4392197

Table: Siponimod and metabolites in monkey feces and urine after oral dosing (Sponsor's)

Experiment	Monkey; (15 mg/kg) p.o. (pool of n=3)		
Sample type	**Urine**	**Feces**	**Total excretion**
Excretion period (h)	0-144	0-144	0-144
Component	Excretion (% of dose)		
M8	0.49	1.49	1.99
P15.4	0.02	n.d.	0.02
M1	0.38	4.63	5.00
P17.4	0.01	n.d.	0.01
M2	0.17	n.d.	0.17
P19.2	0.01	n.d.	0.01
P21.8	n.d.	0.69	0.69
M3	0.32	n.d.	0.32
P35.8	n.d.	1.25	1.25
M4 [b)]	n.d.	27.90	27.90
M5	n.d.	28.31	28.31
M6	n.d.	8.25	8.25
M7	n.d.	7.63	7.63
P52.6	n.d.	1.05	1.05
BAF312	n.d.	9.36	9.36
Sum of additional components	0.05	3.59	3.64
Total components detected	1.45	94.17	95.62
Lost during sample processing	0.01	1.89	1.90
Lost during HPLC	0.18	-	0.18
Total radiolabeled components in original sample (0-144h)	1.65	96.06	97.71
Total excretion of radiolabeled components (0-336h) [b)]	1.71	98.2	99.9

n.d.: not detected
- : data not obtained
a): Metabolite M4 was formed by sulfation of M5 (M4a: 34.9%), M6 (M4c: 9.9%) and an other sulfate (M4b: 55.2%) as describe in Section 3.3
b): Data from Table 5-4

Figure: Proposed metabolism of siponimod in mouse, rat, monkey, and human (Sponsor's)



Reference ID: 4392197

5.1.4 Excretion

After oral dosing of siponimod in rats and monkeys, the major excretion pathway was in the feces (approximately 98% and 95%, respectively), with approximately 9% of siponimod dose excreted unchanged.

5.1.5 Drug interactions Studies

No significant inhibition of cytochrome P450 enzymes was observed at concentrations up to 100-200 µM siponimod. Solute-carrier mediated uptake of siponimod was not observed in hepatocytes (rat, monkey, and human) and no inhibition was observed in the presence of rifamycin (OATP family inhibitor), p-aminohippuric acid (OAT family substrate), or tetraethylammonium (an OCT family substrate).

6 General Toxicology

6.2 Repeat-Dose Toxicity

6.2.1 Previously reviewed studies

Mouse studies

- **2-Week oral dose-range finding in mice (Study No. 0770648; DARRTs, IND 76,122; Thompson review Feb 3, 2012); 0, 50, 150, and 300 mg/kg/day.**
  Key findings included mortality in HDM due to nephrotoxicity, clinical signs of reduced muscle tone, trembling, decreased motor activity, hunched posture, pale appearance, cold to touch, and recumbency was observed in MDM and HDM. Dose-dependent increase in liver weight, lymphopenia, lymphoid depletion in the lymph nodes, lymphoid atrophy in the spleen, centrilobular hypertrophy in the liver, and foci with fibrin/hyaline material with inflammatory reaction, fibrosis, smooth muscle hyperplasia, and increased alveolar macrophages in the lungs was observed at all doses. No NOAEL could be determined.

- **13-Week oral gavage dose range-finding toxicity study in the mouse (Study No. 0770649; DARRTs, IND 76,122; Thompson review Feb 3, 2012); 0, 5, 15, 35, and 80 mg/kg/day.**
  Key findings include 4 unscheduled deaths among TK animals (1 control M, 1 LMDF, and 2 HMDF; the control M and 1 HMDF were due to gavage errors), increases in liver and thymus weights, lymphopenia, histiocytosis in the lymph nodes, lymphoid hyperplasia and atrophy in the spleen, and foci with fibrin/hyaline material, inflammation/fibrosis, and alveolar macrophages with accumulation in the lungs at all doses; centrilobular hypertrophy was observed at ≥ LMD. A NOAEL could not be determined based on adverse findings in the lung, liver, and lymphoid organs.

Reference ID: 4392197

Rat studies

- **2-Week oral exploratory study in male rats (Study No. 0410113; DARRTs, IND 76,122; Roney review Jan 12, 2007); 0, 10, 30, and 100 mg/kg/day.**
  Key findings included lymphopenia, lymphoid depletion in the spleen and lymph nodes, and smooth muscle hypertrophy and increased alveolar macrophages in the lungs at all doses.

- **4-Week oral (gavage) toxicity study in rats with a 4-week recovery period (Study No. 0670037; DARRTs, IND 76,122; Roney review Jan 12, 2007); 0, 10, 50, and 200 mg/kg/day.**
  Key findings included decrease in the HD in F to 100 mg/kg/day on Day 13 due to lack of toleration in HDF. Other findings included lymphopenia, dose-dependent increase in liver and thymus weights, dose-dependent decrease in spleen and uterine weights, lymphoid depletion in the lymph nodes, lymphoid atrophy in the spleen, and medullary enlargement in the thymus at all doses, centrilobular hypertrophy in liver at the MD and HD, alveolitis and foam cell aggregates in the lung in HDM, MDF, and HDF, and uterine atrophy at the HD. Plasma exposure was 3-5-fold higher in F. There was no dose limiting toxicity in M; the NOEL for F was 10 mg/kg based on reduced feces and alveolitis at higher doses.

- **26-Week oral gavage toxicity study in rats with an 8-week recovery period (Study No. 0770059; DARRTs, IND 76,122; Thompson review May 12, 2009); 0, 15, 50, and 150 mg/kg/day in M and 0, 5, 15, and 50 mg/kg/day in F.**
  Key findings included 5 premature deaths (2 control F, 1 MDF, 1 HDM, and 1 HDF on Days 152, 104, 95, 187, and 144, respectively). The sponsor did not consider these deaths drug-related, due to the lack of dose responsiveness and the lack of clear toxicity at the end of the study in surviving animals; however, the HDM displayed weakness, suspected dehydration, severe uncoordination, and abnormal gait and the HDF displayed labored breathing and abnormal breathing sounds on the day of death and the lungs were spongy and uncollapsed at necropsy. Other observations include lymphopenia, dose-dependent increase in liver weight and decrease in spleen weight, lymphoid atrophy in the lymph nodes and spleen, lymphoid hyperplasia in the spleen, loss of medullary distinction in the thymus at all doses and follicular cell hypertrophy at all doses, and centrilobular hypertrophy at the MD and HD. In lungs, macrophage accumulation was observed in 1 HDF (minimal) and 1 recovery HDF (slight). Plasma exposure was 5-7-fold higher in F. No NOAEL could be determined, although most adverse findings appeared to be reversible.

Reference ID: 4392197

Table: Siponimod toxicokinetic data (Sponsor's)

		Males			Females		
Day	Parameter	15 mg/kg/d	50 mg/kg/d	150 mg/kg/d	5 mg/kg/d	15 mg/kg/d	50 mg/kg/d
26	C_{max} (ng/mL)	2250	6520	13,500	4140	7830	17,500
	T_{max} (hr)	1.00	3.00	6.00	6.00	6.00	6.00
	AUC (ng•hr/mL)	31,400	93,600	185,000	70,200	150,000	320,000
152	C_{max} (ng/mL)	3330	7060	13,300	3820	9680	19,400
	T_{max} (hr)	6.00	6.00	6.00	6.00	6.00	6.00
	AUC (ng•hr/mL)	48,200	106,000	198,000	77,000	191,000	372,000

Monkey studies

- **2-Week oral (gavage) dose range-finding study in monkeys (Study No. 0570040; DARRTs, IND 76,122; Roney review Jan 12, 2007); 0, 10, 30, and 100 mg/kg/day.**
  Key findings included increased soft feces at the HD, smooth muscle hyperplasia and inflammation in the lung in HDM. No dose-limiting toxicity was observed.

- **4-Week oral (gavage) toxicity study in monkeys with a 4-week recovery period (Study No. 0670007; DARRTs, IND 76,122; Roney review Jan 12, 2007); 0, 10, 50, and 200 mg/kg/day.**
  Key findings included clonic convulsions, ataxia, tremors, emesis, hypoactivity, and dilated pupils at the HD (200/150 mg/kg), early moribund sacrifices of 2 M after reduction of the HD to 150 mg/kg, soft feces and diarrhea at ≥ 50 mg/kg, and lymphopenia at all doses. The NOEL was 10 mg/kg.

- **26-Week oral gavage toxicity and immunotoxicity assessment study in monkeys with a 12-week recovery period (Study No. 0770061; DARRTs IND 76,122; Thompson review May 12, 2009); 0, 10, 50, and 100 mg/kg/day.**
  Key findings included premature deaths of 1 MDM (on Day 104) and 1 HDF (on Day 173) with no definitive cause. Clinical signs included liquid feces at all doses, distended abdomen and tremors at the MD and HD, and convulsions at the HD. Changes in organ weights included increase in liver weight at all doses and decrease in spleen weight at the HD. Lymphopenia was observed at all doses and macroscopic findings in lymphoid tissues include lymphoid atrophy in the spleen at all doses and thymus and lymph node at the MD and HD. Other microscopic findings noted were dose-related inflammation and vasculopathy at all doses and increased number of goblet cells at the MD and HD in the stomach and GI tract. No NOAEL could be determined based on findings in the lymphoid organs and intestinal tract, although most adverse findings appeared to be reversible.

  In the immunotoxicity assessment, a decrease in the number of peripheral blood lymphocytes was observed at all doses in M and F; the relative proportions of most T lymphocyte subsets were decreased. Following the recovery period, there was some evidence suggestive of recovery of the lymphocyte populations, although recovery was not reached after 12 weeks in most cases.

Reference ID: 4392197

6.2.2 Reviewed studies

Study title: A 52-week toxicity study of BAF312 administered orally via nasal gavage to Cynomolgus monkeys, with an 8-week recovery period

Study no.:	0770339
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 4, 2007
GLP compliance:	Yes
QA statement:	Yes, Nov 10, 2010
Drug, lot #, and % purity:	BAF312, Batch 0651002, 99.5%

Key Study Findings

- Three HDM died or were euthanized early due to drug-related effects.
- There was a dose-dependent increase in incidences of watery feces that was considered adverse at the HD. Tremors were noted in 1 HDM and 2 HDF. Convulsions occurred in 1 HDM.
- Decreased body weights occurred in HDM.
- Decreased WBCs and lymphocytes occurred at all doses in M and F.
- The main macroscopic findings included lymphoid follicles or aggregates and myeloid hypercellularity in bone marrow, reduced size of germinal centers and hypocellularity of the paracortex in lymphoid tissue at all doses, and degeneration/necrosis of myofibers skeletal muscle at MD and HD. A full recovery was not always observed.
- The sponsor considered the LD, 10 mg/kg/day, as the NOAEL due to increase in muscle necrosis at the higher doses; this appears appropriate. The NOAEL was associated with plasma exposures (AUC) to siponimod on Day 357 of 95400 and 124000 ng*h/mL in M and F, respectively.

Methods

Doses:	0, 10, 30, and 100 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral by nasal gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4)
Species/Strain:	Monkey / Cynomolgus
Number/Sex/Group	
Main:	4
Recovery:	2 for control and HD groups
Age:	Males 2.1-3.6 years; Females 2.5-3.7 years
Weight:	Males 2.2-2.9 kg; Females 2.3-2.7 kg

Observations and Results

Mortality

Observations for morbidity and mortality were conducted twice daily.

Reference ID: 4392197

There were 3 early deaths. One HDM died on Day 99 and 2 HDM were euthanized early on Days 208 and 396. The HDM that died had decreased skin turgor consistent with dehydration from Day 84; a veterinary evaluation was conducted, and it was decided to initiate diet enrichment. There was a reduction in body weight (approximately 7%) between Weeks 8 and 13, with watery feces observed from Day 90 and poor appetite and emesis from Day 94. Microscopic findings included erythroid hypocellularity of bone marrow, renal tubular epithelial degeneration/vacuolation, liver cell necrosis, and infections of the oral cavity (necrotizing stomatitis) and in the GI tract (necrotizing typhlitis). Premature sacrifice of 2 HDM was due to deteriorating physical condition that was first observed during dosing and included watery feces, distended abdomen, hunched posture, reduced body weight, reduced food consumption, and dehydration. Convulsions were also observed on Day 122 in the HDM sacrificed on Day 396. The expected pharmacological action of siponimod, decreases in WBC and lymphocytes, and microscopic findings of decreased lymphoid depletion, were observed. Other microscopic findings included renal tubular epithelial hyperplasia and hypertrophy in the HDM sacrificed on Day 208.

Clinical Signs

Observations of clinical signs were conducted once daily from Day -7, prior to initiation of dosing, and during the dosing and recovery periods.

Clinical signs included a dose-dependent increase in incidences of watery feces (see table below) that was considered adverse at the HD. Convulsions occurred on Day 122 in 1 HDM and the same HDM and 2 HDF displayed tremors on Days 364, 13-15, and 195, respectively.

Table: Incidence of watery feces (Sponsor's)

	Group 1 (0 mg/kg/day)	Group 2 (10 mg/kg/day)	Group 3 (30 mg/kg/day)	Group 4 (100 mg/kg/day)
Group size	12 (4)	8 (0)	8 (0)	12 (3)
Number of observations	15 (0)	194 (0)	166 (0)	924 (14)
Number of animals with watery feces	7 (0)	8 (0)	7 (0)	12 (1)*

* Animal no. 4006, which was euthanized prematurely on day 396

Body Weights

Body weights were recorded weekly prior to initiation and during the dosing and recovery periods.

At the end of 52 weeks of dosing, an 80% decrease in mean body weight gain was observed in HDM. The change in absolute body weight was -29%. In F, there was no decrease in mean body weight gain. There was no increase in body weight gain observed during the recovery period in M or F.

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Food Consumption
Food consumption was evaluated by observing the number of biscuits remaining from the previous day's food ration.

Reduced food consumption was observed in several HD animals.

Ophthalmoscopy
Examination of all animals was conducted once prior to initiation of dosing and on Days 270 and 358 during dosing.

There were no abnormalities detected.

ECG
Examination of all animals was conducted once prior to initiation of dosing and on Days 90, 180, 270, and 358 during dosing at approximately 4 hours post-dose.

There were no abnormalities detected; however, no individual animal line listings were provided.

Hematology
Blood samples were taken from all animals pre-dose on Days 1, 90, 270, and 365 during dosing and at the end of the recovery period (Day 420).

Marked decreases in WBC (approximately 70%), lymphocytes (approximately 90%), and basophils (approximately 80%) were observed from Day 90 in all siponimod dosed M and F groups. At the HD, these decreases had only partial recovered.

Table: Changes in selected hematology parameters compared to control animals

	RDW	Platelets	WBC	Lymphocytes	Basophils	Unclassified cell
MALE						
LD						
Day 1:	0%	-5%	+9%	-11%	+18%	-7%
Day 90:	0%	+27%	-65%*	-85%*	-70%*	-56%*
Day 135:	+2%	+26%	-67%*	-85%*	-76%*	-48%*
Day 270:	+3%	+36%	-60%*	-86%*	-76%*	-30%
Day 365:	+3%	+46%	-66%*	-88%*	-81%*	-29%
MD						
Day 1:	+1%	+9%	-3%	+2%	+21%	+48%
Day 90:	+3%	+18%	-77%*	-86%*	-84%*	-80%*
Day 135:	+6%	+13%	-64%*	-87%*	-67%*	-61%*
Day 270:	+6%	+25%	-74%*	-89%*	-87%*	-57%
Day 365:	+5%	+23%	-75%*	-90%*	-82%*	-73%
HD						
Day 1:	+3%	+27%	+20%	+3%	+40%	+8%
Day 90:	+7%	+63%*	-67%*	-91%*	-84%*	-76%*
Day 135:	+10%*	+71%*	-65%*	-87%*	-90%*	-77%*
Day 270:	+11%*	+56%*	-70%*	-91%*	-80%*	-35%
Day 365:	+10%*	+82%*	-64%*	-91%*	-73%*	-38%
Day 420:	-2%	+31%	-39%	-74%	-71%	-15%

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	RDW	Platelets	WBC	Lymphocytes	Basophils	Unclassified cell
FEMALE						
LD						
Day 1:	+2%	-18%	-11%	-22%	-34%	-22%
Day 90:	+4%	-9%	-72%*	-85%*	-87%*	-87%*
Day 135:	+7%	-7%	-60%*	-85%*	-76%*	-60%*
Day 270:	+8%	-5%	-59%*	-88%*	-83%*	-61%*
Day 365:	+6%	-2%	-58%	-85%*	-79%	-10%
MD						
Day 1:	+1%	-14%	-21%	-37%	-50%	-50%
Day 90:	+4%	-3%	-80%*	-91%*	-89%*	-80%*
Day 135:	+6%	-7%	-79%*	-91%*	-87%*	-77%*
Day 270:	+5%	-3%	-84%*	-92%*	-89%*	-86%*
Day 365:	+5%	+11%	-81%*	-92%*	-90%*	-73%*
HD						
Day 1:	+4%	+1%	+21%	-18%	-4%	-15%
Day 90:	+11%*	+18%	-66%*	-88%*	-80%*	-78%*
Day 135:	+11%*	+24%	-71%*	-89%*	-78%*	-75%*
Day 270:	+12%*	+22%	-70%*	-90%*	-84%*	-69%*
Day 365:	+10%*	+35%	-73%*	-88%*	-81%*	-70%*
Day 420:	+8%	+40%	-60%	-78%	-84%	-71%

*** statistically significant from control.**

Clinical Chemistry

Blood samples were taken from all animals pre-dose on Days 1, 90, 270, and 365 during dosing and at the end of the recovery period (Day 420).

There were decreases in albumin, globulin, and total protein in all siponimod dosed M and F groups. At the HD, recovery from these decreases was generally only partial. There was a dose-dependent decrease in ALP that was observed from Day 90 in M; recovery was observed.

Table: Changes in selected clinical chemistry parameters compared to control animals

	ALT	ALP	Protein	Albumin	Globulin	Triglycerides
MALE						
LD						
Day 1:	-30%	-26%	-2%	+2%	-6%	-6%
Day 90:	-13%	-37%*	-9%	-6%	-12%	+13%
Day 270:	+13%	-20%	-18%*	-17%	-19%*	+3%
Day 365:	-19%	-33%	-20%*	-20%*	-19%*	-31%
MD						
Day 1:	+28%	-19%	-2%	-3%	-1%	+30%
Day 90:	-38%	-43%*	-9%	-3%	-11%	+30%
Day 270:	-37%	-39%	-14%*	-10%	-17%*	+24%
Day 365:	-5%	-42%*	-10%	-9%	-11%	+32%
HD						
Day 1:	-12%	-8%	+2%	0%	+3%	+13%
Day 90:	+24%	-58%*	-4%	-9%	-1%	+48%*
Day 270:	+45%	-57%*	-19%*	-23%*	-16%*	+25%
Day 365:	+36%	-51%*	-23%*	-28%*	-18%*	+34%
Day 420:	+65%	-4%	-9%	0%	-18%	+2%
FEMALE						
LD						
Day 1:	-18%	-28%	-1%	0%	-1%	-13%
Day 90:	-12%	-22%	-13%*	-8%	-16%*	+7%
Day 270:	+15%	-27%	-21%*	-18%	-23%*	+21%
Day 365:	+41%	-28%	-14%	-18%*	-12%*	+11%

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	ALT	ALP	Protein	Albumin	Globulin	Triglycerides
MD						
Day 1:	-5%	-3%	-3%	+2%	-6%	+18%
Day 90:	+10%	-6%	-11%*	-2%	-18%*	+41%
Day 270:	+104%	+8%	-16%*	-8%	-23%*	+40%
Day 365:	+169%	+6%	-19%*	-12%	-23%*	+14%
HD						
Day 1:	-7%	-3%	-2%	+2%	-5%	+12%
Day 90:	+69%	-42%	-9%*	-3%	-14%*	+34%
Day 270:	+153%*	-14%	-14%*	-11%	-17%*	+31%
Day 365:	+137%	-14%	-16%*	-15%*	-17%*	+32%
Day 420:	+120%	+41%	-16%	-23%	-10%	-15%

*** statistically significant from control.**

Urinalysis

Urine samples were taken prior to initiation of dosing in Week -1, on Days 113 and 358 during dosing, and at the end of the recovery period.

There were no abnormal changes.

Gross Pathology

Gross pathology was assessed in all animals.

Few macroscopic findings were noted. There was an increased incidence in reduced thymus size noted in HDM (1) and HDF (4).

Organ Weights

At necropsy the organs, listed in the table below from the study report, were weighed.

Organs weighed	
Adrenals	Brain
Kidneys	Heart
Lungs	Liver
Pituitary	Ovaries
Testes	Uterus
Prostate	Spleen
Thyroid with parathyroids	Thymus

There was a decrease in absolute spleen and thymus weight at all doses in M and F. Spleen and thymus weights in control, HDM, and HDF were comparable in recovery animals. Other changes included an increase in the lung weight at all doses in M and F and a decrease in heart weight at the HD in M and F.

Table: Organ changes in mean absolute weight compared to control organ weights

	Adrenal	Brain	Heart	Kidney	Liver	Lungs	Pituitary	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
MALES												
LD:	+4%	-3%	-2%	-8%	+7%	+13%	-17%	-17%	-53%*	-31%	-5%	+14%
MD:	+21%	+2%	-2%	+8%	+16%	+16%	-7%	-12%	-45%*	+72%	-29%	+10%
HD:	+8% (-21%)	-3% (-4%)	-18% (-28%)	+4% (-19%)	+11% (-26%)	+17% (-25%)	-23% (+3%)	-40% (-82%)	-50% (-37%)	-77% (-90%)	-47% (+224%)	+22% (0%)

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	Adrenal	Brain	Heart	Kidney	Liver	Lungs	Pituitary	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
FEMALES												
LD:	+7%	-2%	0%	-1%	+14%	+14%	-15%	+14%	-29%*	+10%	-32%	-15%
MD:	+2%	-3%	-11%	+4%	+3%	+21%	-4%	+42%	-48%*	+12%	-20%	+25%
HD:	-7% (-10%)	-2% (-2%)	-15% (+9%)	+8% (+13%)	+17% (+17%)	+13% (+7%)	-12% (-34%)	+12% (-19%)	-35%* (-18%)	-4% (+276%)	-63% (+3%)	0% (+90%)

*** Statistically significant from control; recovery values in parenthesis.**

Table: Organ changes in mean weight (relative to body weight) compared to control organ weights

	Adrenal	Brain	Heart	Kidney	Liver	Lungs	Pituitary	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
MALES												
LD:	+14%	+3%	+6%	-2%	+13%	+20%	-8%	-8%	-52%*	-24%	0%	+25%
MD:	+32%	+5%	-1%	+9%	+19%*	+20%	-3%	-13%	-46%*	+81%	-29%	+16%
HD:	+36% (-17%)	+18% (+43%)	-1% (+7%)	+27% (+22%)	+35% (+11%)	+45% (+12%)	-1% (+50%)	-19% (-73%)	-39% (-6%)	-69% (-85%)	-39% (+374%)	+54% (+51%)
FEMALES												
LD:	0%	-8%	-6%	-8%	+7%	+5%	-21%	+7%	-34%*	+3%	-36%	-20%
MD:	0%	-3%	-10%	+4%	+3%	+18%	-3%	+37%	-49%*	+12%	-21%	+27%
HD:	-6% (-14%)	0% (-6%)	-12% (+5%)	+11% (+8%)	+19%* (+12%)	+13% (+3%)	-12% (-37%)	+16% (-22%)	-35%* (-22%)	0% (+270%)	-61%* (-2%)	+2% (+78%)

*** Statistically significant from control; recovery values in parenthesis.**

Histopathology

At necropsy, the following organs, as listed in the study report, were collected and examined microscopically.

W	P	Adrenal	W	P	Ovary
	P	Aorta (thoracic)		P	Oviducts
	P	Bone (femoral head)		P	Pancreas
	P	Bone (seventh rib)	W	P	Parathyroid[a]
	P	Bone marrow (sternum)	W	P	Pituitary
W	P	Brain (forebrain, midbrain, cerebellum, and medulla oblongata)	W	P	Prostate
	P	Cecum		P	Rectum
	P	Cervix		P	Salivary gland (mandibular)
	P	Colon		P	Sciatic nerve
	P	Duodenum		P	Seminal vesicles
	P	Epididymis		P	Skeletal muscle (biceps femoris)
	P	Esophagus		P	Skin
	P	Eye with optic nerve		P	Spinal cord (cervical, thoracic, lumbar)
	P	Femur (distal w/ joint)	W	P	Spleen
	P	Gallbladder		P	Stomach
W	P	Heart (including section of aorta)	W	P	Testis
	P	Ileum	W	P	Thymus
	P	Jejunum	W	P	Thyroid
W	P	Kidney		P	Tongue

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	P	Lacrimal gland		P	Trachea
	P	Larynx (cross-section)		P	Ureter cross-section
W	P	Liver (2 lobes)		P	Urinary bladder
W	P	Lung (2 lobes)	W	P	Uterus
	P	Lymph node – mandibular		P	Vagina
	P	Lymph node – mesenteric		P	Macroscopic lesions
	P	Lymph node – tracheobronchia			Animal identification
	P	Mammary gland			

W = weighed; P = processed
[a] The occasional absence of the parathyroid gland from the routine tissue section required a recut of the section to attempt to collect it.

Adequate Battery: Yes.
Peer Review: Yes, by [redacted] (b) (4), DVM, PhD, DACVP.
Signed Pathology report: Yes, by [redacted] (b) (4), DVM, DACVP (page 2091 of the study report).

Histological Findings
Histological findings noted in the 3 HDM that died or were euthanized early included lymphoid depletion, sporadic hair follicle atrophy, and renal interstitial and tubular amphophilic deposits. Other findings likely associated with siponimod were observed in kidney (tubular epithelial hypertrophy/hyperplasia accompanied by deposition of amphophilic material and disrupted and vacuolated tubular epithelium), liver (individual cell hepatocyte necrosis and hepatocyte vacuolation), bone marrow (erythroid cell depletion), pancreas (acinar cell atrophy), oral cavity (mucosal and gingiva necrosis), and GI tract (crypt abscess and necrosis). Siponimod-associated kidney, liver, and oral cavity lesions were not observed in HD animals that survived to scheduled necropsy.

Histological findings in surviving animals were noted in bone marrow, large intestine, lymphoid tissues, and skeletal muscle. Siponimod-associated findings in the bone marrow included an increased incidence and severity of myeloid hypercellularity (likely related to the extramedullary hematopoiesis in lymph nodes) at all doses. Lymphoid tissues with siponimod-associated findings at all doses included gut-associated lymphoid tissue (GALT), lymph nodes, spleen, and thymus. An increased incidence and/or severity of reduced size of germinal centers was observed in GALT, lymph nodes, and spleen. In the lymph nodes, an increased incidence and severity of extramedullary hematopoiesis and hypocellularity of the paracortex were also observed. In the thymus, an increased incidence and severity of hypocellularity of the cortex and hypercellularity of the medulla was observed. In the skeletal bicep femoris muscle, increased incidence and severity of degeneration and degeneration/regeneration of myofibers was observed in siponimod-dosed animals.

In recovery animals, the germinal centers of GALT, lymph nodes, and spleen in HD animals were similar to control animals, suggesting recovery from B-cell lymphoid depletion in these organs. Lymphoid changes in the thymus were similar between control and HDM, but there were incidences of cortical hypocellularity in HDF and medulla hypercellularity in HDM, suggesting there was not full recovery. Similarly, there were incidences of lymph node lymphoid hypocellularity in HD animals, suggesting there was not full recovery after 8 weeks.

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Table: Summary of selected microscopic findings

		MALE						FEMALE					
		Main				Rec		Main				Rec	
	(mg/kg):	0	10	30	100	0	100	0	10	30	100	0	100
	Number of monkeys												
	Surviving animals:	4	4	4	2	2	1	4	4	4	4	2	2
	Premature deaths:	0	0	0	(2)	0	(1)	0	0	0	0	0	0
Tissue	**Finding**												
Bone marrow - sternum	Erythroid hypercellular												
	minimal:	2	1	0	1 (0)	1	0 (0)	3	1	3	1	1	1
	mild:	0	0	0	1 (0)	0	1 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Myeloid hypercellular												
	minimal:	0	1	2	1 (0)	0	0 (0)	1	1	1	2	0	2
	mild:	0	1	0	0 (0)	0	0 (1)	0	0	3	0	0	0
	Lymphoid aggregate												
	present:	0	2	4	1 (0)	1	1 (0)	1	2	2	1	0	1
Cavity - oral	Necrosis												
	present:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Cecum	Reduced germinal centers												
	minimal:	0	1	1	1 (0)	1	1 (0)	0	0	0	0	0	0
	mild:	0	1	0	0 (1)	0	0 (0)	1	0	1	1	0	0
	moderate:	0	0	1	0 (1)	0	0 (0)	0	0	2	1	0	0
	Lymphoplasmacytic infiltration												
	minimal:	0	1	1	0 (1)	0	0 (0)	0	2	0	2	0	0
	mild:	0	1	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (0)	0	0 (1)	0	0	0	0	0	0
	Crypt abscess												
	minimal:	0	1	0	0 (1)	0	0 (0)	0	1	0	0	0	0
	Crypt necrosis												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (1)	0	0	0	0	0	0
	Hyperplasia												
	minimal:	0	1	0	0 (1)	0	0 (1)	0	1	0	0	0	0
	Necrosis												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Colon	Lymphoplasmacytic infiltrate												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	1	0	1	0	0
	Crypt abscess												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	1	0	1	0	0
	Crypt necrosis												
	minimal:	0	0	0	0 (1)	0	0 (1)	0	0	0	0	0	0
	Hyperplasia												
	minimal:	0	1	0	0 (1)	0	0 (1)	0	1	0	0	0	0
	Hemorrhage												
	minimal:	0	0	0	1 (0)	0	0 (0)	0	0	0	1	0	0
Duodenum	Reduced germinal centers												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	1	0	0	0	0
	mild:	1	2	1	0 (1)	1	0 (0)	2	1	1	4	1	0
	moderate:	1	1	0	1 (1)	0	0 (1)	1	0	0	0	0	0
Ileum	Reduced germinal centers												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	1	1	2	0 (2)	2	1 (1)	0	2	1	2	1	0
	moderate:	0	1	0	0 (0)	0	0 (0)	0	0	0	1	0	0
	Crypt abscess												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0

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		MALE						FEMALE					
		Main				Rec		Main				Rec	
	(mg/kg):	0	10	30	100	0	100	0	10	30	100	0	100
	Number of monkeys												
	Surviving animals:	4	4	4	2	2	1	4	4	4	4	2	2
	Premature deaths:	0	0	0	(2)	0	(1)	0	0	0	0	0	0
Tissue	**Finding**												
Jejunum	Reduced germinal centers												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	2	1 (2)	0	0 (1)	1	0	0	1	0	0
Kidney	Tubule disruption/vacuolation												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Hypertrophy/hyperplasia												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Amphophilic deposition												
	minimal:	0	0	0	0 (1)	0	0 (1)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Congestion												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Atrophy												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Leukocyte cell infiltration												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Mineralization												
	minimal:	0	0	1	0 (1)	0	0 (0)	0	1	0	0	0	0
Liver	Vacuolation												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Hypertrophy/hyperplasia												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Necrosis												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Abscess												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Lung	Pleural fibrosis												
	minimal:	0	4	1	1 (0)	0	1 (1)	3	1	2	1	2	0
	mild:	1	0	1	0 (0)	1	0 (0)	0	1	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Increase alveolar macrophages												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Lymph node – Mandibular	Hypocellular lymphoid												
	minimal:	2	2	2	1 (0)	0	0 (0)	0	2	2	2	0	2
	mild:	0	1	1	1 (0)	0	0 (0)	0	1	1	1	0	0
	moderate:	0	0	0	0 (1)	0	1 (0)	0	0	0	0	0	0
	marked:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Reduced germinal centers												
	minimal:	0	3	1	2 (0)	0	0 (0)	1	2	1	2	0	0
	mild:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (1)	1	0	0	0	0	0

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		MALE						FEMALE					
		Main				Rec		Main				Rec	
	(mg/kg):	0	10	30	100	0	100	0	10	30	100	0	100
	Number of monkeys												
	Surviving animals:	4	4	4	2	2	1	4	4	4	4	2	2
	Premature deaths:	0	0	0	(2)	0	(1)	0	0	0	0	0	0
Tissue	**Finding**												
	marked:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Lymph node - Mandibular	Neutrophilic infiltration												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	1	0 (0)	0	0 (0)	0	0	0	1	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	marked:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Extramedullary hematopoiesis												
	minimal:	2	1	1	1 (0)	0	1 (0)	1	0	2	0	0	1
	mild:	0	1	1	0 (0)	0	0 (0)	0	2	1	2	0	1
Lymph node - Mesenteric	Hypocellular lymphoid												
	minimal:	2	1	2	0 (0)	1	0 (0)	1	0	1	1	0	2
	mild:	0	3	2	2 (0)	0	1 (1)	0	4	3	2	0	0
	moderate:	0	0	0	0 (0)	0	0 (0)	0	0	0	1	0	0
	marked:	0	0	0	0 (2)	0	0 (0)	0	0	0	0	0	0
	Reduced germinal centers												
	minimal:	1	3	1	1 (0)	1	1 (0)	4	3	1	0	0	1
	mild:	0	1	2	1 (0)	1	0 (0)	0	1	1	3	0	0
	moderate:	0	0	0	0 (2)	0	0 (1)	0	0	0	1	0	0
Lymph node – Tracheo bronchial	Hypocellular lymphoid												
	minimal:	1	1	2	1 (1)	0	1 (0)	0	1	2	3	0	1
	mild:	0	2	1	1 (0)	0	0 (0)	0	1	0	1	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	2	1	0	0	0
	Reduced germinal centers												
	minimal:	0	0	1	0 (0)	0	0 (0)	0	0	a	0	0	0
	mild:	3	0	1	1 (1)	0	1 (0)	2	2	2	2	0	1
	moderate:	0	3	1	1 (1)	0	0 (0)	2	2	0	2	2	0
	marked:	0	0	0	0 (0)	0	0 (1)	0	0	0	0	0	0
Pancreas	Acinar atrophy												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Rectum	Reduced germinal centers												
	minimal:	1	2	0	0 (1)	0	1 (0)	1	0	1	0	0	0
	mild:	2	1	3	2 (1)	2	0 (0)	0	2	1	2	2	0
	moderate:	0	0	0	0 (0)	0	0 (0)	0	0	1	0	0	0
	Crypt abscess												
	minimal:	0	0	0	0 (0)	0	0 (1)	0	0	0	0	0	0
Skeletal muscle – femoris	Myocyte Degen/regeneration												
	minimal:	0	0	1	1 (0)	0	0 (0)	2	2	1	2	0	0
	mild:	0	1	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	1	0 (1)	0	0 (0)	0	0	0	1	0	0
	Myofiber degeneration (necrosis)												
	minimal:	0	0	1	0 (1)	0	0 (0)	0	0	0	1	0	0
	mild:	0	0	0	1 (0)	0	0 (0)	0	0	0	0	0	0
	Edema												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (0)	0	0 (0)	0	0	1	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (1)	0	0	0	0	0	0
	Hemorrhage												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0

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		MALE						FEMALE					
		Main				Rec		Main				Rec	
	(mg/kg):	0	10	30	100	0	100	0	10	30	100	0	100
	Number of monkeys												
	Surviving animals:	4	4	4	2	2	1	4	4	4	4	2	2
	Premature deaths:	0	0	0	(2)	0	(1)	0	0	0	0	0	0
Tissue	**Finding**												
	mild:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Skin	Reduced hair follicle												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	1	0	0	0
	mild:	0	0	2	0 (0)	0	1 (0)	0	0	0	0	0	0
	moderate:	0	0	0	1 (1)	0	0 (0)	0	1	1	0	0	0
	Hyperplasia												
	minimal:	0	0	0	0 (0)	0	0 (1)	0	0	0	0	0	0
	mild:	0	0	1	1 (0)	0	0 (0)	0	0	0	0	0	0
	Dermatitis												
	minimal:	0	0	1	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	1	0 (0)	0	0 (0)	0	0	0	0	0	1
	moderate:	0	0	0	1 (0)	0	0 (0)	0	0	0	0	0	0
	Parakeratosis												
	present:	0	0	0	1 (0)	0	0 (0)	0	0	0	0	0	0
	Hemorrhage												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Edema												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	moderate:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Fibrosis												
	minimal:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
	Increased apoptosis												
	present:	0	0	0	0 (1)	0	0 (0)	0	0	0	0	0	0
Spleen	Reduced germinal centers												
	minimal:	1	0	1	0 (0)	0	1 (0)	1	0	1	1	1	0
	mild:	0	1	3	0 (0)	1	0 (0)	0	1	1	1	1	0
	moderate:	0	2	0	1 (0)	0	0 (0)	1	1	1	2	0	0
	marked:	0	0	0	1 (2)	0	0 (1)	0	1	0	0	0	0
Thymus	Cortical hypocellularity												
	minimal:	2	2	2	1 (0)	1	1 (0)	1	1	2	3	0	1
	mild:	0	2	1	1 (0)	0	0 (0)	0	3	2	1	0	0
	moderate:	0	0	1	0 (0)	1	0 (0)	0	0	0	0	0	0
	marked:	0	0	0	0 (2)	0	0 (1)	0	0	0	0	0	0
	Medulla hypercellularity												
	minimal:	0	0	0	1 (0)	0	1 (1)	0	1	1	1	0	0
	mild:	0	2	3	1 (0)	0	0 (0)	0	2	3	3	0	0
	moderate:	0	2	1	0 (0)	0	0 (0)	0	1	0	0	0	0
	Edema												
	minimal:	0	0	0	0 (0)	0	0 (0)	0	0	0	0	0	0
	mild:	0	0	0	1 (0)	0	0 (0)	0	0	0	0	0	0

Rec – Recovery; Data from animals that died prematurely (found dead and early euthanasia) are in parenthesis;

Toxicokinetics

Blood samples were taken on Days 1, 28, 154, and 357, pre-dose and at 0.5, 1, 3, 6, and 24 hours post-dose. At termination, brain and lung samples were collected from all animals in the terminal and recovery groups.

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Increase in plasma exposure (C_{max} and AUC) to siponimod was generally less than dose-proportional, with plasma exposure greater in M. T_{max} was at 4-6 hours in M and F. Accumulation occurred after 4 weeks at all doses (approximately 2.3-fold) in M and F.

Table: Toxicokinetic parameters for siponimod (Sponsor's)

Dose	Study day	Gender	Tmax	Cmax	SD	Cmax/ dose	AUC	SD	AUC/ dose	n
10	1	Male	4.50	4040	628	404	67400	9160	6740	4
		Female	4.50	3200	685	320	51900	20000	5190	4
	28	Male	4.50	5110	1090	511	92500	22600	9250	4
		Female	6.00	5800	2630	580	101000	54400	10100	4
	154	Male	4.50	5250	717	525	94000	8790	9400	4
		Female	4.50	5910	3190	591	106000	63100	10600	4
	357	Male	4.50	5440	785	544	95400	16600	9540	4
		Female	3.75	6620	3170	662	124000	80900	12400	4
30	1	Male	5.25	11000	772	365	198000	5450	6580	4
		Female	4.50	9380	1810	313	157000	32300	5220	4
	28	Male	4.50	19200	1490	639	351000	53200	11700	4
		Female	5.25	13900	2470	462	242000	53700	8040	4
	154	Male	5.25	23800	5700	792	427000	100000	14200	4
		Female	5.25	16100	2870	536	278000	88700	9260	4
	357	Male	5.25	18000	2960	600	331000	54000	11000	4
		Female	6.00	12100	3130	403	215000	54200	7160	4
100	1	Male	6.00	19100	5640	191	339000	89200	3390	6
		Female	6.00	16800	4760	168	293000	76800	2930	6
	28	Male	5.00	41900	16400	419	786000	323000	7860	6
		Female	5.50	40600	11700	406	761000	244000	7610	6
	154	Male	4.80	53300	32300	533	1020000	628000	10200	5
		Female	6.00	40200	10600	402	750000	212000	7500	6
	357	Male	6.00	46000	20300	460	884000	387000	8840	4
		Female	6.00	31000	8560	310	565000	170000	5650	6

Units: dose [mg/kg/day], Tmax [hours], Cmax [ng/mL], Cmax/dose [(ng/mL)/(mg/kg/day)], AUC [ng*Hours/mL], AUC interval [0-24 Hours], AUC/dose [(ng*Hours/mL)/(mg/kg/day)].

Exposure to siponimod in brain and lung was observed and was generally less than dose-proportional, with exposure similar between the sexes.

Table: Siponimod tissue exposure (ng/g) (Sponsor's)
Brain

Dose	Study day	Gender	Mean	% CV	n
10	366	Male	24200	8.3	4
		Female	29500	78	4
30	366	Male	84200	16.9	4
		Female	54000	44.1	4
100	208†	Male	200000	n.c.	1
	366	Male	237000	n.c.	2
		Female	170000	14.1	4
	396‡	Male	2380	n.c.	1
	421	Male	1900	n.c.	1
		Female	1520	n.c.	2
	Ratio day 421 / 366	Male	0.0080		
		Female	0.0089		

Unit: dose [mg/kg/day]. † The animal 4002M died on day 208. ‡ The animal 4006M died on day 396. n.c., not calculable.

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Lung

Dose	Study day	Gender	Mean	% CV	n
10	366	Male	6010	12.9	4
		Female	8170	118.1	4
30	366	Male	17300	85.5	4
		Female	10400	41.7	4
100	208†	Male	30200	n.c.	1
	366	Male	34800	n.c.	2
		Female	48200	18.7	4
	396‡	Male	1630	n.c.	1
	421	Male	424	n.c.	1
		Female	777	n.c.	2
	Ratio day 421 / 366	Male	0.012		
		Female	0.016		

Unit: dose [mg/kg/day]. † The animal 4002M died on day 208. ‡ The animal 4006M died on day 396. n.c., not calculable.

Flow cytometry

Blood samples were taken prior to initiation of dosing on Day -5, on Days 1 and 365 during dosing, and at the end of the recovery period (Day 420).

On Day 365, there were decreases in absolute counts of T-lymphocyte subsets ($CD3^+$, $CD3_+/CD4^+$, and $CD3^+/CD8^+$) and B lymphocytes ($CD20^+$) in all animals. Partial recovery was observed.

Table: Changes in lymphocyte counts compared to pre-dosing

	$CD20_+$	$CD3+/CD4^+$	$CD3^+/CD8^+$	$CD3^-/CD14^+$ (Monocytes)	$CD3^-/CD16^+$ (NK-cells)	$CD3^+$
MALE						
Control:	+27% (-1%)	+45% (-25%)	+48% (-18%)	+44% (+23%)	+23% (+28%)	+59% (-16%)
LD:	-86%*	-99%*	-83%*	+106%	-8%	-92%*
MD:	-91%*	-99%*	-90%*	+35%	-7%	-94%*
HD:	-93%* (-84%)	-95%* (-95%)	-76%* (-60%)	+41% (-18%)	-12% (-9%)	-88%* (-79%)
FEMALE						
Control:	-19% (-30%)	+17% (-4%)	+5% (+1%)	+5% (-21%)	+1% (-4%)	+17% (+4%)
LD:	-92%*	-98%*	-75%*	+68%	-18%	-87%*
MD:	-93%*	-100%*	-91%*	-7%	-5%	-95%*
HD:	-86%* (-62%)	-99%* (-93%)	-91%* (-84%)	-23% (-34%)	-19% (-30%)	-94%* (-87%)

*** statistically significant from control; recovery values in parenthesis.**

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7 Genetic Toxicology

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

Study title: Mutagenicity test using *Salmonella typhimurium*

Study no.:	0612011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAF312, Batch № 0651001, 98.7%
Previous review:	IND 76,122, DARRTs; Roney review Jan 12, 2007

Study Validity
Negative and positive controls were within the respective historical control range.

Results
No increases in revertants were observed up to 5000 µg/plate using the plate incorporation and preincubation methods.

7.2 In Vitro Assays in Mammalian Cells

Study title: Chromosome aberration test with cultured human peripheral blood lymphocytes

Study no.:	0612109
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAF312, Batch № 0651001, 98.7%
Previous review:	IND 76,122, DARRTs; Roney review Jan 12, 2007

Study Validity
Negative and positive controls were within the respective historical control range. Concentrations used were 0, 32.5, 54.2, and 90.4 µg/mL for 20 hour exposure without S9, 0, 49.3, 66.6, and 90 µg/mL for 3 hour exposure without S9, 0, 26.5, 43.1, and 70.1 µg/mL for 3 hour exposure with S9, and 0, 27, 49.3, and 66.6 µg/mL for a repeat 3 hour exposure with S9. The highest concentration (HC), in the absence of S9, reduced the mitotic index by more than 50%. In the first study in the presence of S9, the HC reduced the mitotic index by only 44.8%. The study was repeated, and the HC reduced the mitotic index by greater than 50% (52%).

Results
No significant effects were observed up to 90.4 µg/mL after 20-hour exposure in the absence of S9, 90 µg/mL after 3-hour exposure in the absence of S9, and 66.6 µg/mL after 3-hour exposure in the presence of S9.

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Micronucleus test in vitro using TK6 cells. (*Study №: 0414047*)
In a non-GLP micronucleus assay using TK6 cells, siponimod, up to 150 mg/mL, did not induce an increased in the number of cells containing micronuclei after 3-hour dosing in the presence of S9, nor after 20-hour dosing in the absence of S9.

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Induction of micronuclei in the bone marrow of treated mice

Study no:	1470042
Study report location:	EDR
Conducting laboratory and location:	Dept of Safety Profiling & Assessment, Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	February 20, 2014
GLP compliance:	Yes, UK and OECD
QA statement:	Yes
Drug, lot #, and % purity:	BAF312, Batch № 1113020, 100%

Key Study Findings: Siponimod did not induce micronuclei in M rats in vivo up to 500 mg/kg.

Methods

Doses in first experiment:	0, 250, 500, and 1000 mg/kg
Doses in second experiment:	0 and 125 mg/kg
Frequency of dosing:	Daily dosing for 2 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4)
Species/Strain:	Mouse/CD-1 (ICR)
Number/Sex/Group:	6 M 3 M for positive control group
Age:	6-7 Weeks
Weight:	29 – 38 g
Basis of dose selection:	Doses were based on study № 0770648 (2-week oral dose-ranging finding study in mice) and a dose finding experiment (500, 1000, and 2000 mg/kg/day in 3 M and 3 F/group and 1400 mg/kg in 3 M
Negative control:	Vehicle
Positive control:	Cyclophosphamide (40 mg/kg) used for both experiments

Study Validity
The assay was a valid study. The MTD was estimated at 1000 mg/kg/day from the dose finding study based on observations of piloerection, tremors, twitching, head tilting, and

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decreased activity, with early sacrifice in extremis on Day 2 due to declining condition at 1400 and 2000 mg/kg/day. The time between last dose administration and tissue (bone marrow) harvest was 24 hours; 2000 polychromatic erythrocytes per animal were analyzed for the frequency of micronuclei.

Results

Clinical signs in HD animals included piloerection, tremors, and head tilting, with more severe signs observed in 2 M resulting in early sacrifice. No increase in micronuclei was observed at doses of 125, 250, and 500 mg/kg siponimod. In contrast, cyclophosphamide induced a significant increase in micronuclei compared to vehicle control (see table below). Therefore, siponimod showed no clastogenic potential up to 500 mg/kg/day.

Table: Summary of micronucleus data (Sponsor's)

Group / Treatment (mg/kg/day)	PCE scored	Number of MN PCE	% PCE	MN / 2000 PCE	% MN PCE	SD	Heterogeneity		2 x 2 Contingency	
							X^2	S	X^2C	S
1M / Vehicle (0)	12000	11	45.40	1.83	0.09	0.07	5.91	NS		
2M / BAF312 (250)	12000	16	47.10	2.67	0.13	0.11	8.76	NS	0.59	NS
3M / BAF312 (500)	12000	8	40.07	1.33	0.07	0.06	5.50	NS	0.21	NS
5M / CPA (40)	6000	57	58.33	19.00	0.95	0.98			76.04	p≤0.001
6M / Vehicle (0)	12000	8	35.87	1.33	0.07	0.03	1.00	NS		
7M / BAF312 (125)	12000	7	39.50	1.17	0.06	0.06	5.86	NS	0.00	NS
8M / CPA (40)	6000	63	41.13	21.00	1.05	0.10			95.96	p≤0.001

M	Male	MN	Micronucleated
PCE	Polychromatic erythrocyte	NCE	Normochromatic erythrocyte
NS	Not significant	S	Significance
SD	Standard deviation		

Study title: Rat bone marrow micronucleus test after oral administration

Study no:	0770326
Study report location:	EDR
Conducting laboratory and location:	Dept of Safety Profiling & Assessment, Novartis Pharma AG, Basel, Switzerland
Date of study initiation:	April 23, 2007
GLP compliance:	Yes, Swiss GLP based on OECD
QA statement:	Yes
Drug, lot #, and % purity:	BAF312, Batch № 0651001, 99.3%

Key Study Findings: Siponimod did not induce micronuclei in M rats in vivo up to 1250 mg/kg.

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Methods

Doses in definitive study:	0, 125, 395, and 1250 mg/kg
Frequency of dosing:	Daily dosing for 2 days
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4)
Species/Strain:	Rat/Wistar (Han)
Number/Sex/Group:	7 M 3 M for positive control group
Age:	8 Weeks
Weight:	225.98 – 286.43 g
Basis of dose selection:	Doses were based on a dose-finding study
Negative control:	Vehicle
Positive control:	Cyclophosphamide (10 mg/kg)

Study Validity

The assay was valid. No clinical signs were reported; however, clinical signs observed in the dose-finding study and a second study (1 M and 1 F, 1250 mg/kg) were discussed. Clinical signs, i.e., reduced motor activity, piloerection, hunched posture, and tremor, were observed after the first and/or second dose. Animals were sacrificed 48 post-first dose; therefore, the time between last dose administration and tissue (bone marrow) harvest was 24 hours; 4000 polychromatic erythrocytes per animal were analyzed for the frequency of micronuclei.

Results

No increase in micronuclei was observed with siponimod. In contrast, cyclophosphamide produced a significant increase in micronuclei compared to vehicle control (see table below). Therefore, siponimod showed no clastogenic potential up to 1250 mg/kg/day.

Table: Summary of micronucleus data (Sponsor's)

Group	Dose (mg/kg)	Sex	No. of animals	Frequency (%)**	
				MPE	PCE
Vehicle	0	Male	7	0.11 +/- 0.08	31.2 +/- 5.3
Cyclophosphamide	10	Male	3	3.39 +/- 2.04	32.1 +/- 2.2
BAF312	125	Male	7	0.13 +/- 0.05	27.6 +/- 5.9
BAF312	395	Male	7	0.14 +/- 0.03	25.1 +/- 3.5
BAF312	1250	Male	7	0.11 +/- 0.06	27.0 +/- 5.3

4000 cells/animal were analyzed.

Note: Each value represents mean ± SD (standard deviation) of a treatment group.

**frequencies are calculated as follows:

Frequency MPE: number of MPE x 100 / number of PCE

Frequency PCE: number of PE x 100 / number of (PCE+NCE)

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8 Carcinogenicity

Study title: 104-week oral (gavage) carcinogenicity study in mice

Study no.:	0870139
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	May 2, 2012
GLP compliance:	Yes, OECD
QA statement:	Yes
Drug, lot #, and % purity:	BAF312, Lot №: 1113020, 100%
CAC concurrence:	Yes, Dated Dec 21, 2011

Protocol recommendations

The protocol was reviewed by the Exec CAC under IND 76,122 (Meeting Minutes, December 21, 2011). The Exec CAC recommended doses of 0, 2, 8, and 25 mg/kg/day based on MTD and on > 25-fold plasma AUC ratio but noted that the study may not be acceptable if the clinical dose increases such that the ratio is no longer at least 25-fold. At the time of the protocol review the sponsor asserted that the maximum anticipated clinical dose would be < 5 mg/day; the proposed dose is 2 mg/day.

Key Study Findings

- There was a statistically significant, dose-dependent decrease in the survival rate in MDM and HDM and at all doses in F.
- Decreases in body weight gain were observed at all doses.
- There was a statistically significant increase in the incidence of malignant lymphomas at all doses in F and in the incidence of vascular tumors at all doses in M and F.

Adequacy of Carcinogenicity Study

Early cessation of dosing (in Week 91 in MDM and HDM, Week 92 in MDF and HDF, and Week 97 in LDF) and termination (in Week 93 in MDM, Week 96 in HDM, Week 101 in control F and LDF, Week 98 in MDF, and Week 95 in HDF) of animals did not compromise the study since the duration of dosing was sufficient.

Appropriateness of Test Models

The CD-1 mouse is an appropriate model to test for carcinogenicity over a 2-year period. In mouse pharmacokinetic studies (PK), M17, a major circulating metabolite in humans, was observed at a greater plasma exposure compared to human plasma exposure (approximately 100-fold using data from PK study № DMPK R1500775 in M CD-1 mice [25 mg/kg/day] and clinical trial CBAF312 A2118). The major circulating human metabolite, M3, was not measured; however, M3 is formed by hydroxylation and glucuronidation of siponimod and, therefore, as a glucuronidation metabolite that is not an acyl glucuronide, the metabolite is of minimal toxicological concern.

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Evaluation of Tumor Findings
A statistically significant increase in the incidence of malignant lymphoma was observed at all doses in F. An increased incidence of hemangiomas and hemangiosarcomas was seen at all doses in M and F that reached statistical significance.

Methods

Doses:	0 (vehicle), 2, 8, and 25 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4)
Basis of dose selection:	Based on findings from 2-week and 13-week oral (gavage) repeat dose toxicity studies in CD-1 mice.
Species/Strain:	Mouse / Crl:CD1 (ICR) BR
Number/Sex/Group:	70
Age:	6 weeks at initiation of dosing
Weight:	Males – 27.8-38.2 g; Females – 21.2-28.1 g
Animal housing:	Group housed (3 of same sex and dosing group)
Paradigm for dietary restriction:	Ad libitum access
Satellite groups	
TK analysis:	8/sex/group
Deviation from study protocol:	The duration of dosing was 104 weeks for control and LDM, 91 weeks for MDM and HDM, 97 weeks for the control and LDF, and 92 weeks for MDF and HDF. Dosing was shortened at the MD and HD because of significantly decreased survival rates.

Observations and Results
Mortality
Observations for mortality were conducted twice daily.

There was a dose-related decrease in the survival rate (see table below) at all doses in M and F that reached statistical significance (as reported by the FDA statistical reviewer) for all groups except LDM. Dosing was discontinued for MDM and HDM on Day 633, for MDF and HDF on Day 643, and for control F and LDF on Day 678. The following groups; MDM, HDM, control F, LDF, MDF, and HDF were terminated early on Days 651, 667, 706, 706, 684, and 660, respectively, as recommended by the Exec CAC and Division (see advice to sponsor; DARRTS December 6, 2013).

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Table: Survival incidences (Sponsor's)

Study week	Males Dose (mg/kg/day) 0	2	8	25	Females Dose (mg/kg/day) 0	2	8	25
16	68 (97%)	69 (99%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)	70 (100%)
28	68 (97%)	69 (99%)	70 (100%)	68 (97%)	69 (99%)	70 (100%)	69 (99%)	70 (100%)
56	65 (93%)	64 (91%)	65 (93%)	63 (90%)	67 (96%)	64 (91%)	58 (83%)	62 (89%)
80	51 (73%)	46 (66%)	34 (49%)	33 (47%)	53 (76%)	42 (60%)	36 (51%)	35 (50%)
96	37 (53%)	29 (41%)	**15[a] (21%)**	**15[a] (21%)**	38 (54%)	21 (30%)	18 (26%)	**15[b] (21%)**
100	32 (46%)	22 (31%)	-	-	32 (46%)	17 (24%)	**16[b] (23%)**	-
104	26 (37%)	18 (26%)	-	-	30[c] (43%)	**16[c] (23%)**	-	-

Note: n = 70 for each dose group.

[a] Dosing was discontinued starting on week 91 (day 633). Group 3 terminated on week 93 (day 651) and group 4 on week 96 (day 667).

[b] Dosing was discontinued starting on week 92 (day 643). Group 4 terminated on week 95 (day 660) and group 3 on week 98 (day 684).

[c] Dosing was discontinued starting on week 97 (day 678) and terminated on week 101 (day 706).

For each premature death the most probable cause of death was determined (see table below). Hemangiosarcoma (M and F) and lymphoma (F) were considered a cause of death; other drug-related causes of death included lung lesions in M and F and ovarian cysts in F.

Table: Causes of mortality (Sponsor's)

Sex	Males				Females			
Dose (mg/kg/day)	0	2	8	25	0	2	8	25
No. of animals[a]	44	52	55	56	40	54	54	55
Undetermined	6	5	5	3	2	1	2	1
Accidental (including gavage accident)	2	0	1	1	2	0	2	0
Systemic:								
Obstructive uropathy	6	3	4	2	0	0	0	0
Urinary tract infection	0	2	1	2	0	0	0	0
Neoplastic:								
Hemangiosarcoma	0	20↑	28↑	26↑	2	13↑	16↑	18↑
Lymphoma	10	9	8	11	10	22↑	17↑	19↑
Lung bronchioloalveolar carcinoma	5	5	1	1	5	1	1	0
Non-Neoplastic:								
Lung lesions	0	1↑	5↑	4↑	0	4↑	3↑	5↑
Ovarian cyst	—	—	—	—	1	8↑	9↑	6↑
Nephropathy	2	0	1	0	9	1	0	2

[a] Total number of found dead plus preterminally euthanized animals.

↑ = Increased.

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Clinical Signs
Detailed exams were conducted weekly and animals were examined weekly for the presence of palpable masses.

Blue, black, and purple discoloration of the skin was observed at the LD, MD, and HD starting in Week 38 for M and Week 25 for F. Breathing was abnormal (deep, labored, and shallow) and respiration rate was increased/irregular in HDF. Convulsions were observed in animals from all groups with increased incidences in 4 HDM and 8 HDF. Other clinical signs included signs of deterioration such as prominent backbone, hunched posture, erected fur, uncoordination, decreased activity, coldness, thinness, and thin cover of fur in animals from all groups in M and F.

The number of palpable masses was comparable across dose groups for F and slightly increased for M.

Table: Palpable masses in males and females (Sponsor's)

Group no. identification	Dose level base/salt (mg/kg/day)	Masses			
		Males	%	Females	%
1/ Vehicle control	0	11/70	16	10/70	14
2/ BAF312	2	18/70	26	9/70	13
3/ BAF312	8	24/70	34	10/70	14
4/ BAF312	25	17/70	24	12/70	17

Body Weights
Body weights were recorded once prior to initiation of dosing, weekly through Week 14, every four weeks during Weeks 18-78, and every two weeks thereafter.

Body weight gain was not affected in M and F up to Week 26. However, at Week 78, significant decreases in body weight gain were observed in MDM (20%), HDM (18%), LDF (30%), MDF (29%), and HDF (19%).

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Tables: Summary of body weight gain (g) (Sponsor's)
MALE

Group	Summary Information	Abs Gain -1 to 78	% Gain -1 to 78	Week Abs Gain -1 to End of study	Week % Gain -1 to End of study
1	Mean	20.42	62.166	18.25	55.368
	SD	6.00	18.493	7.25	21.808
	N	53	53	26	26
2	Mean	17.92	54.242	12.56 e	38.524 b
	SD	6.41	19.163	5.37	16.166
	N	47	47	18	18
3	Mean	16.25 B	50.000 B		
	SD	5.50	16.350		
	N	36	36		
4	Mean	16.74 A	50.578 A		
	SD	5.65	17.133		
	N	35	35		

Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (*t*-test)
d - P ≤ 0.05 e - P ≤ 0.01 f - P ≤ 0.001 (Wilcoxon)

FEMALE

Group	Summary Information	Abs Gain -1 to 78	% Gain -1 to 78	Week Abs Gain -1 to End of study	Week % Gain -1 to End of study
1	Mean	23.70	97.127	20.66	85.962
	SD	6.51	26.461	7.10	30.176
	N	53	53	32	32
2	Mean	16.62 C	68.148 C	14.56 b	61.087 b
	SD	6.27	25.179	5.80	23.088
	N	44	44	17	17
3	Mean	16.71 C	69.514 C		
	SD	7.17	29.305		
	N	39	39		
4	Mean	19.27 B	80.970 A		
	SD	6.71	26.740		
	N	43	43		

Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (*t*-test)
d - P ≤ 0.05 e - P ≤ 0.01 f - P ≤ 0.001 (Wilcoxon)

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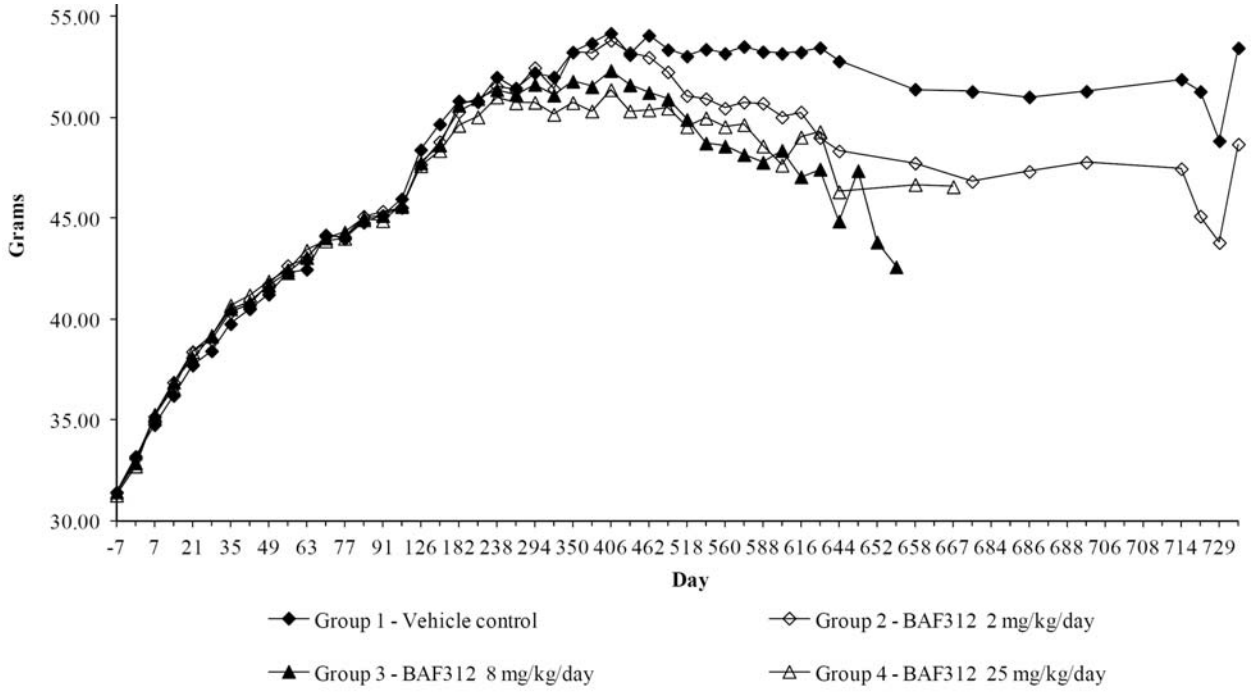
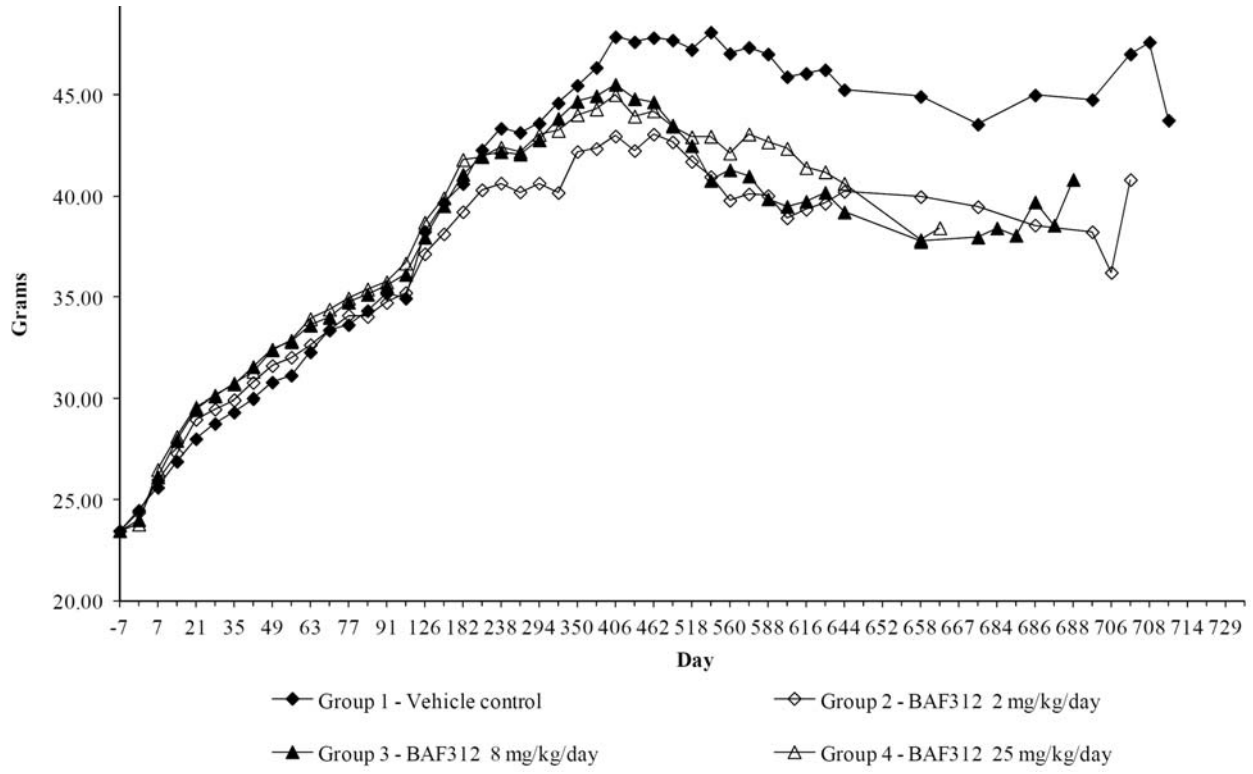
Figure: Body weight changes (Sponsor's)

MALE



FEMALE



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Food Consumption
Food consumption was recorded once prior to initiation of dosing, weekly through Week 14, every four weeks during Weeks 18-78, and every two weeks thereafter.

There were no drug-related changes in mean food consumption.

Hematology
Blood samples were taken prior to terminal sacrifice.

No data were provided.

Gross Pathology
Macroscopic examination occurred at necropsy at the end of the dosing period for surviving animals, at the time of euthanasia for humane reasons, and as soon as possible for animals found dead.

There was a non-dose-dependent increase in the incidence of pale foci in the lungs of all siponimod groups, except LDM. The pale foci correlated with an increase in alveolar macrophages and eosinophilic material. Subcutaneous tissue of LDM, MDM, HDM, and HDF had increased findings of thickness that often correlated with edema. Raised foci and increased incidences of masses were observed in the stomach of MDM, HDM, LDF, MDF, and HDF.

Table: Gross necropsy findings in mice dosed with siponimod (Sponsor's)

Tissue/Finding Sex	Males				Females			
Dose (mg/kg/day)	0	2	8	25	0	2	8	25
No. of animals examined	70	70	70	70	70	70	70	70
Lung								
Pale foci	3	2	10↑	9↑	4	16↑	12↑	10↑
Subcutaneous tissue								
Thick	4	18↑	21↑	19↑	9	10	11	14↑
Stomach								
Raised foci	0	2	7↑	12↑	3	10↑	8↑	10↑
Mass	3	6	3	9↑	0	10↑	4↑	9↑

↑ = Increased.

Histopathology
At necropsy, the following organs, as listed in the study report, were collected from control, LD, MD, and HD animals. Microscopic examination included all tissues.

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	Animal identification
P	Artery, aorta
	Bone marrow smear
P	Bone marrow, femur
P	Bone marrow, sternum
P	Bone, femur
P	Bone, sternum
P	Brain
P	Cervix
P	Epididymis
P	Esophagus
P	Eye
P	Gallbladder
P	Gland, adrenal
P	Gland, Clitoral
P	Gland, harderian
P	Gland, lacrimal
P	Gland, mammary
P	Gland, parathyroid
P	Gland, pituitary
P	Gland, preputial
P	Gland, prostate
P	Gland, salivary
P	Gland, seminal vesicle
P	Gland, thyroid
P	Gross lesions/masses
P	Gut-associated lymphoid tissue
P	Heart
P	Kidney
P	Large intestine, cecum
P	Large intestine, colon
P	Large intestine, rectum
P	Larynx
P	Liver
P	Lung
P	Lymph node, mandibular
P	Lymph node, mesenteric
P	Lymph node, drainage of clinically observed mass
P	Muscle, skeletal
P	Nasal Cavities
P	Nerves, optic
P	Nerve, sciatic
P	Ovary
P	Oviduct
P	Pancreas
P	Skin
P	Small intestine, duodenum
P	Small intestine, jejunum
P	Small intestine, ileum
P	Spinal cord
P	Spleen
P	Stomach
P	Testis
P	Thymus/thymus area
P	Tongue
P	Trachea
P	Urinary bladder
P	Ureters
P	Uterus
P	Vagina

Peer Review: Yes
Signed Pathology report: Yes

Neoplastic
A statistically significant non-dose-dependent increase in malignant lymphoma was observed at all doses in F. There were slightly higher incidences of malignant lymphoma in siponimod-dosed M, but the increase was not statistically significant. There was a statistically significant non-dose-dependent increase in vascular tumors (hemangioma and/or hemangiosarcoma) in M and F at all doses; tissues most affected were adipose tissue, bone marrow, heart, jejunum, liver, lung, ovary, skeletal muscle, spleen, subcutaneous tissue, tail, and uterus. There was an increase in kidney tubular cell adenomas and lung bronchioloalveolar adenoma in HDM, but the increases were not statistically significant.

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Table: Number of animals with observed neoplasms after siponimod dosing for up to 104 weeks

	MALE				FEMALE			
(mg/kg):	**0**	**2**	**8**	**25**	**0**	**2**	**8**	**25**
Number mice examined								
Surviving animals:	**26**	**18**	**15**	**14**	**30**	**16**	**16**	**15**
Premature deaths:	**44**	**52**	**55**	**56**	**40**	**54**	**54**	**55**
Neoplasm								
Malignant lymphoma	6 (10)	11 (14)	7 (14)	5 (15)	15 (11)	12 (30)	11 (27)	11 (29)
Total:	**16**	**25**	**21**	**20**	**26**	**42***	**38***	**40***
Hemangioma	2 (2)	5 (5)	2 (15)	4 (9)	1 (3)	2 (9)	1 (8)	2 (5)
Total:	**4**	**10**	**17***	**13***	**4**	**11**	**9**	**7**
Hemangiosarcoma	3 (3)	12 (32)	6 (40)	9 (37)	1 (5)	6 (28)	7 (24)	11 (28)
Total:	**6#**	**44***	**46***	**46***	**6#**	**34***	**31***	**39***
Hemangioma/sarcoma	5 (5)	13 (34)	7 (42)	9 (39)	2 (7)	6 (31)	8 (26)	11 (28)
Total:	**10#**	**47***	**49***	**48***	**9#**	**37***	**34***	**39***

Data from animals that died prematurely are in parenthesis. The tumor data were analyzed by the FDA statistician for dose response relationships and pairwise comparisons of control group with each of the dosed groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method (see statistical review; NDA 209-884, January 14, 2019). * Total incidences are statistically significant; # indicate statistically significant dose response.

Non-Neoplastic

Target organs included the bone marrow, kidney, liver, lung, and subcutis. Findings included an increase in severity in hematopoietic hypercellularity in bone marrow, increase in incidence and severity of tubule karyomegaly in kidney, pigmentation and necrosis in the liver, alveolar macrophages and alveolar eosinophil material in the lung, and increase in incidence of chronic inflammation in lungs and edema and inflammation in the subcutis. Increased extramedullary hematopoiesis, which is likely a response to the vascular tumors, was noted with greater incidences and severity in several organs including adrenal gland, liver, lymph nodes, ovary, and spleen. Lymphoid hyperplasia/atrophy was observed in lymphoid tissues (thymus and lymph nodes), which is consistent with the pharmacological action of siponimod.

Table: Summary of selected microscopic findings

		MALE				FEMALE			
	(mg/kg):	**0**	**2**	**8**	**25**	**0**	**2**	**8**	**25**
	Number of mice examined								
	Surviving animals:	**26**	**18**	**15**	**14**	**30**	**16**	**16**	**15**
	Premature deaths:	**44**	**52**	**55**	**56**	**40**	**54**	**54**	**55**
Tissue	**Finding**								
Adrenal gland	Extramedullary hematopoiesis								
	minimal:	**0 (1)**	**0 (2)**	**0 (0)**	**0 (1)**	**0 (2)**	**0 (5)**	**1 (3)**	**1 (6)**
	mild:	**0 (0)**	**0 (0)**	**0 (1)**	**0 (1)**	**0 (0)**	**0 (3)**	**0 (1)**	**0 (1)**
Bone marrow – Femur	Hematopoietic hypercellularity	**57**	**55**	**61**	**58**	**53**	**60**	**54**	**53**
	minimal:	**2 (3)**	**3 (0)**	**1 (0)**	**3 (1)**	**8 (3)**	**0 (0)**	**0 (0)**	**1 (3)**
	mild:	**11 (6)**	**1 (6)**	**5 (8)**	**3 (4)**	**9 (5)**	**2 (9)**	**3 (9)**	**1 (6)**
	moderate:	**10 (22)**	**8 (20)**	**5 (27)**	**7 (26)**	**6 (15)**	**12 (24)**	**11 (26)**	**9 (18)**
	marked:	**0 (3)**	**1 (16)**	**0 (15)**	**0 (14)**	**0 (6)**	**1 (12)**	**0 (5)**	**3 (12)**
	severe:	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (1)**	**0 (0)**	**0 (0)**	**0 (0)**

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		MALE				FEMALE			
	(mg/kg):	0	2	8	25	0	2	8	25
	Number of mice examined								
	Surviving animals:	26	18	15	14	30	16	16	15
	Premature deaths:	44	52	55	56	40	54	54	55
Tissue	**Finding**								
Bone marrow – Femur	Lymphoid follicle								
	minimal:	0 (0)	2 (4)	4 (3)	2 (0)	6 (1)	3 (2)	2 (5)	7 (2)
	mild:	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (2)	0 (0)	0 (0)
	moderate:	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)
	marked:	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)
Kidney	Tubule karyomegaly								
	minimal:	1 (1)	3 (5)	5 (7)	2 (3)	0 (3)	0 (5)	3 (7)	2 (7)
	mild:	0 (0)	1 (7)	2 (2)	0 (5)	0 (0)	1 (6)	3 (8)	5 (4)
	moderate:	0 (0)	0 (6)	0 (1)	1 (4)	0 (0)	0 (7)	3 (7)	3 (5)
	marked:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
Liver	C. hepatocellular hypertrophy								
	minimal:	1 (5)	5 (4)	8 (7)	8 (7)	3 (3)	1 (3)	2 (1)	4 (2)
	mild:	0 (1)	1 (1)	0 (2)	0 (4)	1 (0)	1 (0)	0 (0)	0 (0)
	Hepatocellular hypertrophy								
	minimal:	1 (2)	0 (12)	0 (5)	0 (5)	1 (4)	2 (5)	2 (6)	3 (8)
	mild:	2 (1)	1 (4)	1 (2)	2 (5)	1 (2)	0 (4)	1 (1)	2 (2)
	moderate:	0 (0)	0 (0)	0 (1)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
	Pigmentation								
	minimal:	5 (2)	2 (10)	8 (10)	9 (16)	6 (14)	6 (21)	14 (23)	4 (15)
	mild:	0 (0)	0 (6)	0 (4)	4 (16)	3 (1)	0 (9)	1 (7)	11 (29)
	moderate:	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (1)
	Necrosis								
	minimal:	2 (3)	1 (6)	1 (3)	1 (3)	1 (1)	0 (4)	0 (4)	0 (3)
	mild:	0 (0)	0 (1)	0 (2)	0 (3)	0 (0)	0 (4)	0 (1)	1 (3)
	moderate:	0 (0)	0 (2)	0 (1)	1 (2)	0 (0)	0 (2)	0 (1)	0 (0)
	marked:	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	Single cell necrosis								
	minimal:	4 (5)	1 (14)	0 (15)	3 (8)	2 (8)	3 (5)	0 (6)	2 (10)
	mild:	0 (2)	1 (2)	0 (1)	2 (3)	0 (0)	0 (1)	0 (2)	0 (3)
	moderate:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
	Extramedullary hematopoiesis								
	minimal:	0 (5)	1 (6)	1 (15)	2 (9)	2 (9)	3 (9)	0 (15)	4 (14)
	mild:	0 (2)	1 (8)	1 (1)	1 (3)	0 (1)	0 (8)	0 (4)	0 (7)
	moderate:	0 (0)	0 (1)	0 (0)	0 (1)	0 (1)	1 (2)	0 (2)	0 (3)
	marked:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
Lung	Increased alveolar macrophages								
	minimal:	3 (6)	7 (15)	5 (17)	3 (18)	11 (14)	9 (11)	1 (6)	1 (7)
	mild:	1 (3)	8 (19)	4 (15)	4 (12)	0 (1)	5 (18)	7 (17)	2 (22)
	moderate:	0 (2)	3 (7)	5 (16)	5 (14)	1 (2)	1 (19)	8 (25)	11 (18)
	marked:	0 (0)	0 (2)	0 (5)	0 (3)	0 (0)	0 (2)	0 (3)	1 (6)
	severe:	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)	0 (1)	0 (0)	0 (0)
	Alveolar eosinophilic material								
	minimal:	0 (1)	12 (30)	10 (32)	9 (31)	0 (5)	4 (33)	13 (27)	12 (29)
	mild:	0 (1)	1 (6)	2 (8)	0 (10)	0 (0)	0 (9)	1 (18)	2 (17)
	moderate:	0 (0)	0 (1)	0 (2)	0 (2)	0 (1)	0 (0)	0 (3)	0 (3)
	marked:	0 (0)	0 (0)	0 (2)	0 (2)	0 (0)	0 (2)	0 (2)	0 (2)
	Chronic inflammation								
	minimal:	0 (2)	5 (15)	7 (11)	5 (19)	0 (0)	3 (15)	8 (19)	7 (17)
	mild:	0 (2)	2 (4)	3 (8)	0 (4)	0 (1)	2 (6)	3 (3)	4 (6)
	moderate:	0 (1)	0 (2)	0 (1)	1 (0)	0 (1)	1 (2)	0 (2)	0 (3)
	marked:	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

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		MALE				FEMALE			
	(mg/kg):	0	2	8	25	0	2	8	25
	Number of mice examined								
	Surviving animals:	26	18	15	14	30	16	16	15
	Premature deaths:	44	52	55	56	40	54	54	55
Tissue	**Finding**								
Lung	Mononuclear cell infiltration								
	minimal:	7 (3)	7 (17)	6 (19)	6 (14)	9 (4)	4 (16)	0 (20)	2 (9)
	mild:	1 (0)	3 (2)	3 (3)	4 (1)	3 (4)	3 (7)	7 (6)	2 (11)
	moderate:	1 (0)	1 (1)	2 (0)	1 (0)	2 (2)	0 (0)	0 (1)	1 (1)
	marked:	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lymph node	Erythrocytosis								
	minimal:	0 (2)	2 (9)	1 (7)	1 (7)	4 (2)	4 (13)	6 (11)	2 (14)
	mild:	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)	0 (0)	0 (2)	0 (0)
	Incr extramedullary hematopoiesis								
	minimal:	0 (1)	0 (5)	0 (1)	2 (0)	0 (1)	0 (5)	3 (3)	3 (4)
	mild:	0 (0)	0 (0)	0 (3)	0 (2)	0 (0)	0 (2)	0 (2)	0 (4)
	moderate:	0 (0)	0 (1)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)
	marked:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)
	Lymphoid hyperplasia								
	minimal:	1 (3)	1 (4)	0 (4)	5 (3)	4 (3)	2 (6)	1 (3)	0 (7)
	mild:	0 (0)	0 (1)	1 (1)	0 (1)	0 (3)	0 (3)	0 (3)	0 (1)
	moderate:	0 (0)	0 (0)	1 (1)	0 (0)	2 (1)	1 (1)	1 (2)	0 (2)
	marked:	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
Lymph node - Mandibular	Erythrocytosis								
	minimal:	0 (1)	1 (5)	0 (3)	0 (9)	5 (4)	2 (13)	1 (13)	2 (15)
	mild:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
	Incr extramedullary hematopoiesis								
	minimal:	1 (1)	1 (7)	2 (5)	4 (7)	0 (2)	0 (6)	3 (4)	2 (12)
	mild:	0 (0)	1 (2)	0 (1)	2 (4)	0 (0)	1 (2)	1 (1)	0 (4)
	moderate:	0 (0)	0 (1)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (2)
Lymph node - Mesenteric	Incr extramedullary hematopoiesis								
	minimal:	1 (0)	2 (1)	1 (0)	2 (2)	0 (1)	0 (4)	0 (3)	1 (3)
	mild:	0 (2)	0 (0)	0 (0)	0 (2)	1 (0)	0 (3)	0 (3)	0 (4)
	moderate:	0 (1)	0 (2)	0 (0)	0 (1)	0 (1)	0 (0)	0 (1)	0 (1)
Ovary	Extramedullary hematopoiesis								
	minimal:	-	-	-	-	0 (0)	1 (0)	0 (1)	1 (3)
	mild:	-	-	-	-	0 (0)	0 (1)	0 (0	0 (0
Spleen	Incr extramedullary hematopoiesis								
	minimal:	6 (7)	4 (3)	5 (2)	2 (3)	11 (6)	5 (2)	2 (4)	1 (2)
	mild:	4 (11)	7 (11)	5 (10)	3 (8)	8 (6)	2 (9)	6 (4)	6 (6)
	moderate:	2 (4)	1 (13)	1 (24)	6 (18)	2 (9)	3 (15)	3 (22)	3 (11)
	marked:	0 (3)	0 (12)	0 (9)	0 (11)	0 (5)	1 (13)	0 (13)	0 (19)
	severe:	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Subcutis	Edema								
	minimal:	1 (0)	1 (6)	0 (12)	2 (7)	0 (6)	0 (7)	0 (3)	1 (6)
	mild:	0 (1)	1 (8)	1 (7)	1 (7)	0 (1)	0 (0)	0 (3)	1 (4)
	moderate:	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
	Inflammation								
	minimal:	0 (2)	0 (2)	0 (2)	1 (4)	0 (0)	0 (1)	0 (2)	1 (2)
	mild:	0 (0)	0 (0)	0 (2)	0 (1)	0 (0)	0 (1)	0 (0)	0 (0)
Thymus	Lymphoid atrophy/necrosis								
	minimal:	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	mild:	0 (2)	0 (1)	0 (0)	0 (1)	1 (1)	1 (0)	0 (0)	0 (1)
	moderate:	6 (4)	2 (2)	7 (11)	2 (7)	8 (5)	1 (11)	0 (9)	2 (9)
	marked:	5 (15)	7 (33)	1 (23)	6 (26)	1 (13)	1 (13)	0 (13)	3 (18)
	severe:	2 (5)	3 (0)	2 (5)	1 (7)	0 (4)	0 (0)	0 (0)	0 (2)

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Data from animals that died prematurely are in parenthesis; C. – centrilobular; Incr – increased.

Toxicokinetics

Blood samples were taken from TK animals in Weeks 5 and 26 at 3 and 6 hours post-dose; C_{max} and AUC were not calculated. Levels of siponimod were measured, but no assessment of metabolites was conducted.

Siponimod was not found in control samples. Plasma exposure to siponimod appeared approximately dose-proportional, with exposure generally greater in M than F. Accumulation was not apparent over the course of the study.

Table: Siponimod plasma levels (Sponsor's)

Dose	Week	Time (h)	Plasma concentration (ng/mL)		Ratio Male/Female
			Male	Female	
2	5	3	1260	959	1.31
		6	1290	1170	1.10
	26	3	1500	1470	1.02
		6	1440	994	1.45
8	5	3	5620	4020	1.40
		6	5800	3130	1.85
	26	3	4370	4630	0.944
		6	6100	3900	1.56
25	5	3	17000	8850	1.92
		6	15700	9070	1.73
	26	3	12600	9040	1.39
		6	14800	8350	1.77

Dosing Solution Analysis

All siponimod samples analyzed for concentration from Weeks 1, 9, 24, 39, 54, 69, 84, and 104 were within the ± 15% accuracy criteria (actual range: 96% to 108% of the nominal concentrations).

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Study title: 104-Week oral (gavage) carcinogenicity study in rats

Study no.:	0870138
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 2, 2012
GLP compliance:	Yes; OECD
QA statement:	Yes
Drug, lot #, and % purity:	BAF312, Lot №: 1113020, 100%
CAC concurrence:	Yes; Dated Dec 21, 2011

Protocol recommendations

The protocol was reviewed by the Exec CAC under IND 76,122 (Meeting minutes December 21, 2011). The Exec CAC concurred with the sponsor's proposed doses based on the > 25-fold plasma AUC ratio but noted that the study may not be acceptable if the clinical dose increases such that the ratio is no longer at least 25-fold. At the time of the protocol review, the sponsor asserted that the maximum anticipated clinical dose would be < 5 mg/day; the proposed dose is 2 mg/day.

Key Study Findings

- There was a decrease in survival rate in F that reached statistical significance at the LD and HD.
- Decreases in body weight gain were observed at all doses in M and F.
- There was a dose-dependent increase in the incidence of follicular cell adenoma or combined with carcinoma in M that was statistically significant at the HD.

Adequacy of Carcinogenicity Study

There was no difference in survival rate in M. A statistically significance decrease in survival rate was observed in LDF and HDF. Survivors in all groups were dosed for the full duration of the 104-week study.

Appropriateness of Test Models

The Wistar Han rat is an appropriate model to test for carcinogenicity over a 2-year period. In rat pharmacokinetic studies (PK), M17, a major circulating metabolite in humans, was also observed at a greater plasma exposure compared to human plasma exposure (approximately 4.5-fold using data from PK study № 1370783 in M rats [90 mg/kg/day] and clinical trial CBAF312 A2118). The major circulating human metabolite, M3, was not measured; however, M3 is formed by hydroxylation and glucuronidation of siponimod and, therefore, as a glucuronidation metabolite and not an acyl glucuronide, the metabolite is of minimal toxicological concern.

Evaluation of Tumor Findings

A statistically significant increase in the incidence of follicular cell adenoma or combined with carcinoma was observed in HDM.

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Methods

Doses:	Males: 0, 10, 30, and 90 mg/kg Females: 0, 3, 10, and 30 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4)
Basis of dose selection:	Findings from a 26-week oral (gavage) repeat dose with 8-week recovery toxicity study in Wistar Han rats.
Species/Strain:	Rat / Wistar Han
Number/Sex/Group	
Main groups:	50
Toxicokinetic groups:	4
Age:	Approximately 7 weeks at initiation of dosing
Weight:	Males – 122-192 g; Females – 97-156 g
Animal housing:	Group housed (3 of same sex and dosing group)
Deviation from study protocol:	There were no major deviations.

Observations and Results

Mortality

Observations for moribundity and mortality were conducted twice daily.

There was no difference in survival rate in M, but there was a decrease in survival rate in F that reached statistical significance at the LD and HD.

Table: Mortality incidences (Sponsor's)

Study week	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	10	30	90	0	3	10	30
16	50 (100%)	50 (100%)	50 (100%)	50 (100%)	49 (98%)	50 (100%)	50 (100%)	50 100%)
28	50 (100%)	50 (100%)	49 (98%)	49 (98%)	49 (98%)	50 (100%)	50 (100%)	50 (100%)
56	48 (96%)	47 (94%)	49 (98%)	44 (88%)	49 (98%)	50 (100%)	49 (98%)	49 (98%)
80	42 (84%)	41 (82%)	48 (96%)	40 (80%)	45 (90%)	43 (86%)	44 (88%)	43 (86%)
96	34 (68%)	36 (72%)	33 (66%)	35 (70%)	38 (76%)	28 (56%)	30 (60%)	30 (60%)
100	32 (64%)	31 (62%)	30 (60%)	32 (64%)	36 (72%)	22 (44%)	29 (58%)	25 (50%)
104	30 (60%)	29 (58%)	26 (52%)	26 (52%)	35 (70%)	20* (40%)	27 (54%)	22 (44%)

Note: n = 50 for each dose group.
* P-value ≤ 0.01.

For each premature death the most probable cause of death was determined (see table below). Thyoma (M and F) and uterine hemangiosarcoma (F) were considered causes

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of death, however, incidences of these tumors were not statistically significant between the control and siponimod dosed groups at the end of the study. Other drug-related causes of death included polyarteritis in M and F and uterine changes, including dilatation, inflammation, hemorrhage, endometrial hyperplasia, vascular hypertrophy/hyperplasia, and thrombosis in F (see non-neoplastic microscopic findings in table below).

Table: Causes of mortality (Sponsor's)

Sex	Males				Females			
Dose (mg/kg/day)	0	10	30	90	0	3	10	30
No. of animals[a]	20	21	24	25	15	30	24	28
Undetermined	2	2	0	1	1	2	1	3
Accidental (including gavage accident)	0	0	0	1	0	0	0	0
Systemic:								
Polyarteritis	0	5↑	4↑	2↑	0	4↑	4↑	4↑
Uterine changes	-	-	-	-	0	7↑	4↑	5↑
Neoplastic:								
Pituitary tumors[b]	10	3	5	3	4	2	2	2
Thymoma	0	4	1	2	0	2	2	3
Lymphoma	3	1	5	2	0	0	0	1
Endometrial stromal sarcoma	-	-	-	-	2	0	0	1
Endometrial adenocarcinoma	-	-	-	-	1	0	2	1
Uterine hemangiosarcoma	-	-	-	-	0	2	1	2
Non-Neoplastic:								
Chronic progressive nephropathy	0	0	1	0	0	1	2	0
Cardiomyopathy	1	1	0	0	0	0	0	0

[a] Total number of found dead plus preterminally euthanized animals.
[b] Including adenoma/carcinoma of pars distalis, intermedia and nervosa.
↑ = Increased.

Clinical Signs

Detailed exams were conducted weekly. Animals were examined weekly for the presence of palpable masses.

Signs considered drug-related included non-dose-dependent signs related to the eye observed from Weeks 32 and 43 at all doses in M and HDF, respectively. Changes to the eye included protruding nictitating membrane and signs considered related to corneal inflammation; pale, damaged, enlarged, and/or opaque eyeballs, partly closed eyes, and eye discharge. Other clinical signs included labored breathing and abnormal breathing sounds, salivation, and abdominal distension.

The number of palpable masses was generally comparable across groups for M and slightly increased in control F.

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Table: Palpable masses in males and females (Sponsor's)

Group no. identification	Dose level base/salt (mg/kg/day)	Masses			
		Males	%	Females	%
1/ Vehicle control	0	13/50	26	26/50	52
2/ BAF312	10	13/50	26	-	-
3/ BAF312	30	8/50	16	-	-
4/ BAF312	90	11/50	22	-	-
5/ BAF312	3	-	-	15/50	30
6/ BAF312	10	-	-	10/50	20
7/ BAF312	30	-	-	8/50	16

Body Weights

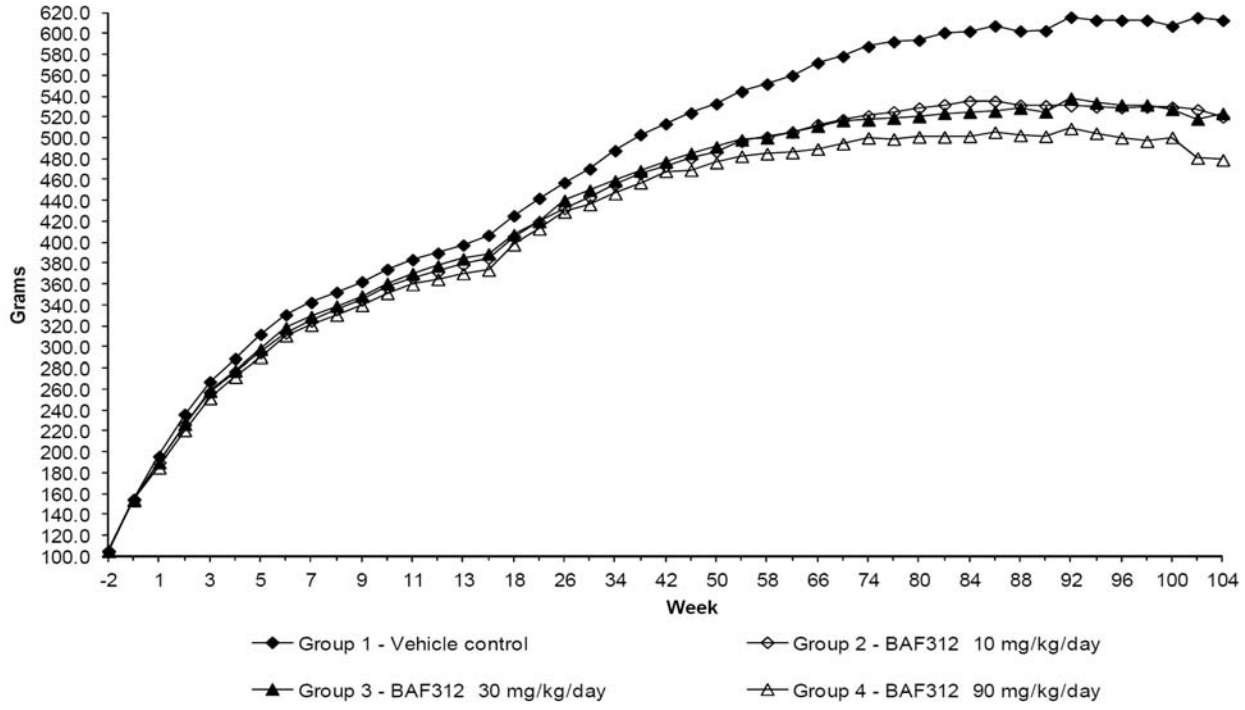
Body weights were recorded once prior to initiation of dosing, weekly through Week 14, every four weeks during Weeks 18-78, and every two weeks thereafter.

At Week 104, decreased body weight gain was observed in all siponimod dosed groups in M and F.

Table: Mean body weight comparisons to control (Sponsor's)

Sex	Males			Females		
Dose (mg/kg/day)	10	30	90	3	10	30
Group	2	3	4	5	6	7
Week 26	-5.4	-3.7	-6.1	-3.7	-3.2	-6.5
Week 54	-8.8	-8.5	-11	-7.0	-8.5	-12
Week 104	-15	-15	-22	-16	-21	-19

Figure: Body weight (Sponsor's)
MALE



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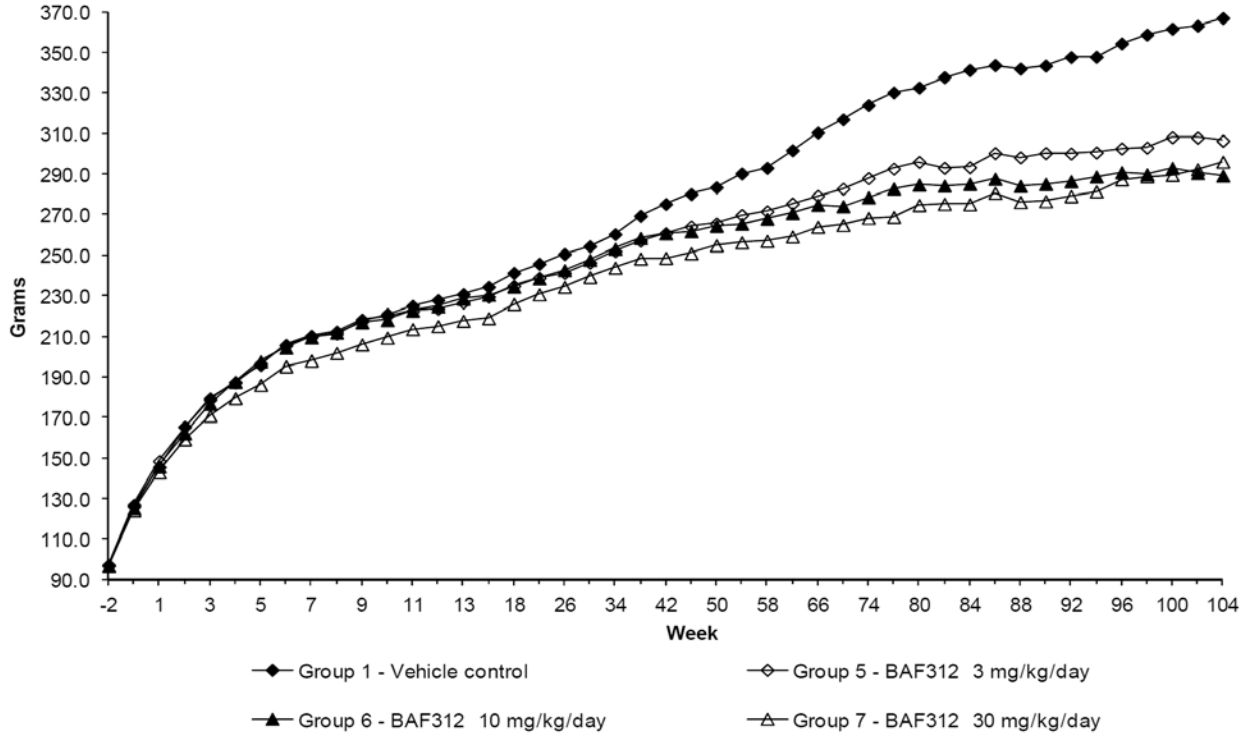
FEMALE



Food Consumption
Food consumption was recorded once prior to initiation of dosing, weekly through Week 14, every four weeks during Weeks 18-78, and every two weeks thereafter.

There was a slight decrease in food consumption in HDM and HDF.

Ophthalmology
Examinations of all animals were conducted once prior to initiation of dosing. No ocular assessments were conducted during the dosing period.

Blood smears
Samples were collected from all animals euthanized in extremis and all survivors for possible future evaluation but were not examined.

Gross Pathology
A complete necropsy was conducted on all animals.

Drug-related macroscopic changes included thyroid gland enlargement and increases in dark foci and masses in HDM that generally correlated with neoplastic findings. Other macroscopic findings observed at all doses included aorta dilatation, fluid accumulation in body cavities, dark foci in brain, irregular surface of the kidney, enlarged and irregular surface of the liver, cysts in lymph nodes, ovary, and oviduct, uterine fluid accumulation and dilatation. Soft consistency and pale foci of testis were observed at the HD.

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Table: Gross necropsy findings in rats dosed with siponimod (Sponsor's)

Tissue/Finding Sex	Males				Females			
Dose (mg/kg/day)	0	10	30	90	0	3	10	30
No. of animals examined	50	50	50	50	50	50	50	50
Artery, aorta								
Dilatation	0	12↑	9↑	5↑	0	7↑	8↑	10↑
Body cavity, thoracic								
Material/fluid accumulation[a]	1	4↑	5↑	8↑	0	4↑	3↑	6↑
Brain								
Focus; dark	0	4↑	1	0	1	1	5↑	4↑
Thyroid								
Enlargement	2	5	3	7↑	1	0	0	2
Mass	0	2	4	7↑	2	0	0	0
Focus; dark	1	3	2	6↑	0	0	1	1
Kidney								
Irregular surface	1	14↑	7↑	5↑	0	20↑	19↑	16↑
Liver								
Irregular surface	0	2↑	5↑	4↑	0	4↑	9↑	4↑
Enlargement	3	3	6	14↑	2	1	1	3
Focus; raised	1	2	3	8↑	1	1	3	2
L.N. Mandibular[b]								
Cyst; pale	3	16↑	19↑	14↑	1	8↑	7↑	12↑
L.N. Mesenteric[b]								
Cyst; pale	2	8↑	4↑	10↑	1	2	3	4
Ovary								
Cyst; pale	—	—	—	—	13	19↑	25↑	22↑
Oviduct								
Dilatation/Cyst; pale	—	—	—	—	0	2↑	2↑	2↑
Uterus								
Material/fluid accumulation[a]	—	—	—	—	3	13↑	17↑	20↑
Dilatation	—	—	—	—	0	8↑	11↑	10↑
Focus; dark/raised	—	—	—	—	0	7↑	5↑	0
Cyst; dark	—	—	—	—	0	5↑	7↑	1
Nodule	—	—	—	—	0	3	2	2
Vessel, blood								
Dilatation	0	4↑	4↑	1	0	1	7↑	3↑
Testis								
Focus; pale	3	7	8	11↑	—	—	—	—
Abnormal consistency; soft	6	6	7	12↑	—	—	—	—

[a] Including fluid or material accumulation; pale or dark.
[b] L.N. = lymph node.
↑ = Increased.

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Histopathology
At necropsy, the following organs, as listed in the study report, were collected from control, LD, MD, and HD animals. Microscopic examination included all tissues.

Tissue list for collection and/or processing (P)

	Animal identification	P	Large intestine, rectum
P	Artery, aorta	P	Larynx
	Bone marrow smear	P	Liver
P	Bone marrow, femur	P	Lung
P	Bone marrow, sternum	P	Lymph node, mandibular
P	Bone, femur	P	Lymph node, mesenteric
P	Bone, sternum	P	Lymph node, drainage of clinically observed mass
P	Brain	P	Muscle, skeletal
P	Cervix	P	Nasal Cavities
P	Epididymis	P	Nerves, optic
P	Esophagus	P	Nerve, sciatic
P	Eye	P	Ovary
P	Gland, adrenal	P	Oviduct
P	Gland, Clitoral	P	Pancreas
P	Gland, harderian	P	Skin
P	Gland, lacrimal	P	Small intestine, duodenum
P	Gland, mammary	P	Small intestine, jejunum
P	Gland, parathyroid	P	Small intestine, ileum
P	Gland, pituitary	P	Spinal cord
P	Gland, preputial	P	Spleen
P	Gland, prostate	P	Stomach
P	Gland, salivary	P	Testis
P	Gland, seminal vesicle	P	Thymus/thymus area
P	Gland, thyroid	P	Tongue
P	Gross lesions/masses	P	Trachea
P	Gut-associated lymphoid tissue	P	Urinary bladder
P	Heart	P	Ureters
P	Kidney	P	Uterus
P	Large intestine, cecum	P	Vagina
P	Large intestine, colon		

Peer Review: Yes, by (b) (4), BVSc & AH, MS, PhD, DACVP.
Signed Pathology report: Yes, by (b) (4), DVM, DES, MSc, DACVP, DABT.

Neoplastic
There was a statistically significant increase in thyroid gland follicular cell adenoma and combined with carcinoma in HDM. The sponsor considered the increased incidence of follicular cell carcinoma in M at ≥ MD were siponimod-related, due to the presence of significant differences for the follicular cell adenoma/carcinoma combination and the follicular cell carcinoma being considered as the last step in follicular cell carcinogenesis. There were increases in osteosarcoma in various tissues in HDM (head, subcutis, and tongue) and thymoma in M at all doses, but the increases were not statistically significant.

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Table: Number of animals with observed neoplasms after siponimod dosing for up to 104 Weeks

	MALE				FEMALE			
(mg/kg):	**0**	**10**	**30**	**90**	**0**	**3**	**10**	**30**
Number rats examined								
Surviving animals:	**30**	**29**	**26**	**25**	**35**	**20**	**26**	**22**
Premature deaths:	**20**	**21**	**24**	**25**	**15**	**30**	**24**	**28**
Neoplasm								
Primary Osteosarcoma								
Head:	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Subcutis:	0 (0)	0 (0)	0 (0)	0 (1)	1 (0)	0 (0)	0 (0)	0 (0)
Tongue:	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Total:	**0**	**0**	**0**	**3**	**1**	**0**	**0**	**0**
Meninges								
Secondary Osteosarcoma	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Total:	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
Thymus								
Benign thymoma	2 (0)	4 (5)	3 (2)	4 (2)	6 (0)	1 (4)	4 (2)	1 (6)
Total:	**2**	**9**	**5**	**6**	**6**	**5**	**6**	**7**
Malignant thymoma	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)
Total:	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**
Thyroid								
Follicular cell adenoma	2 (0)	5 (1)	3 (4)	11 (5)	2 (0)	0 (0)	1 (0)	1 (0)
Total:	**2#**	**6**	**7**	**16***	**2**	**0**	**1**	**1**
Follicular cell carcinoma	1 (0)	2 (0)	3 (2)	3 (2)	1 (0)	0 (0)	0 (0)	0 (0)
Total:	**1**	**2**	**5**	**5**	**1**	**0**	**0**	**0**
Follicular cell adenoma/carcinoma	3 (0)	7 (1)	6 (5)	11 (6)	3 (0)	0 (0)	1 (0)	1 (0)
Total:	**3#**	**8**	**11**	**17***	**3**	**0**	**1**	**1**

Data from animals that died prematurely are in parenthesis. The tumor data were analyzed by the FDA statistician for dose response relationships and pairwise comparisons of control group with each of the dosed groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method (see statistical review; NDA 209-884, January 14, 2019). * Total incidences are statistically significant; # indicate statistically significant dose response.

Non-Neoplastic

Target organs of siponimod included bone marrow, brain, eye, kidneys, liver, lungs, lymph nodes, spleen, testis, thymus, thyroid gland, and uterus. Findings included an increase in incidence in hypercellularity of the bone marrow, pleural fibrosis in the lung, and extramedullary hematopoiesis in the spleen at all doses. Increases in incidence and severity were observed in non-neuronal focal/multifocal necrosis (often accompanied by vascular inflammation) and mineralization in the brain, chronic inflammation of the cornea and lens degeneration in the eye, chronic progressive nephropathy in kidney, hepatocellular hypertrophy, bile duct hyperplasia, and eosinophilic cellular alteration in the liver, and hyperplasia/hypertrophy, hemorrhage, ulceration, inflammation, and dilatation in the uterus. Lymphoid hyperplasia/atrophy seen in lymphoid tissues (spleen and lymph nodes) and thymic medullary increased cellularity appeared to be consistent with the pharmacological action of siponimod. Changes in follicular cell hypertrophy were present in the thyroid gland at all doses in M and in HDF. Compared to occasional

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incidences of minimal to mild findings in 4 M and 1 F control animals there was an increase in vascular inflammation in several tissues that included brain, cecum, cervix, heart, kidney, larynx, ovary, pancreas, rectum, spleen, testes, tongue, and uterus; marked to severe incidences were observed in cervix and uterus at the HD, kidney, pancreas, and testes at all doses.

Table: Summary of selected dose response microscopic findings

		MALE				FEMALE			
	(mg/kg):	0	10	30	90	0	3	10	30
	Number rats examined								
	Surviving animals:	30	29	26	25	35	20	26	22
	Premature deaths:	20	21	24	25	15	30	24	28
Tissue	**Finding**								
Bone marrow - Femur	Hypercellularity								
	minimal:	0 (0)	0 (0)	1 (3)	0 (8)	2 (6)	2 (11)	6 (11)	2 (11)
	mild:	1 (2)	0 (2)	0 (2)	1 (3)	0 (2)	0 (6)	1 (3)	0 (2)
	moderate:	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bone marrow - Sternum	Hypercellularity								
	minimal:	3 (1)	4 (1)	2 (5)	4 (10)	3 (6)	2 (10)	7 (16)	1 (13)
	mild:	1 (2)	0 (2)	0 (3)	1 (5)	0 (3)	0 (6)	1 (4)	1 (3)
	moderate:	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Brain	Necrosis								
	minimal:	0 (0)	1 (2)	0 (1)	0 (0)	0 (0)	0 (0)	1 (0)	0 (1)
	mild:	0 (0)	0 (2)	0 (1)	0 (0)	0 (0)	0 (3)	1 (3)	1 (1)
	moderate:	0 (0)	0 (2)	0 (1)	0 (0)	0 (0)	0 (2)	0 (2)	0 (2)
	Mineralization								
	minimal:	1 (1)	15 (6)	16 (12)	13 (14)	0 (0)	12 (8)	16 (5)	18 (5)
	mild:	0 (0)	8 (3)	7 (3)	7 (4)	0 (0)	3 (1)	2 (2)	1 (2)
	moderate:	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Vascular inflammation								
	minimal:	0 (0)	0 (2)	0 (1)	0 (1)	0 (0)	0 (2)	0 (0)	0 (2)
	mild:	0 (0)	0 (2)	0 (0)	0 (0)	0 (0)	0 (3)	1 (2)	1 (2)
	moderate:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	1 (0)	0 (0)
Eye	Cornea chronic inflammation								
	minimal:	0 (0)	7 (5)	9 (4)	9 (4)	0 (0)	4 (1)	6 (2)	8 (4)
	mild:	0 (0)	3 (2)	0 (1)	2 (2)	0 (0)	2 (2)	1 (5)	1 (2)
	moderate:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	marked:	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Kidney	CPN								
	minimal:	12 (5)	12 (2)	8 (6)	8 (5)	5 (4)	12 (8)	16 (2)	11 (4)
	mild:	11 (0)	7 (5)	7 (5)	10 (9)	1 (0)	4 (8)	2 (6)	4 (6)
	moderate:	1 (1)	5 (7)	5 (2)	4 (3)	0 (0)	2 (6)	3 (9)	2 (6)
	marked:	0 (0)	2 (2)	1 (3)	0 (1)	0 (0)	0 (3)	3 (2)	0 (2)
Liver	Centrilobular hypertrophy								
	minimal:	0 (0)	19 (0)	20 (1)	21 (15)	0 (0)	1 (0)	12 (0)	13 (3)
	mild:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
	Bile duct hyperplasia								
	minimal:	2 (0)	11 (3)	4 (6)	12 (6)	7 (1)	8 (6)	5 (9)	7 (5)
	mild:	1 (0)	6 (4)	7 (1)	1 (1)	5 (3)	6 (6)	12 (3)	6 (6)
	moderate:	0 (0)	3 (1)	0 (1)	0 (1)	0 (0)	0 (4)	2 (3)	2 (5)
	marked:	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)
	Eosinophilic cellular alteration								
	minimal:	0 (1)	7 (6)	16 (10)	11 (7)	2 (0)	7 (1)	9 (2)	13 (5)
	mild:	0 (0)	2 (0)	3 (1)	5 (0)	0 (0)	0 (1)	1 (0)	0 (1)
	moderate:	0 (0)	0 (1)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Reference ID: 4392197

		MALE				FEMALE			
	(mg/kg):	0	10	30	90	0	3	10	30
	Number rats examined								
	Surviving animals:	30	29	26	25	35	20	26	22
	Premature deaths:	20	21	24	25	15	30	24	28
Tissue	**Finding**								
Lung	Macrophage aggregation								
	minimal:	3 (4)	2 (3)	3 (5)	7 (7)	10 (4)	2 (3)	4 (7)	4 (6)
	mild:	1 (1)	1 (0)	1 (4)	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	moderate:	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (1)	0 (0)	0 (0)
	Plural fibrosis								
	minimal:	0 (0)	13 (6)	13 (5)	12 (2)	0 (0)	0 (3)	5 (0)	6 (0)
	Bronchiolalveolar inflammation								
	minimal:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	mild:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	moderate:	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Lymph node - Mandibular	Lymphoid depletion								
	minimal:	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	mild:	0 (0)	0 (3)	0 (2)	0 (3)	0 (0)	0 (0)	0 (0)	0 (1)
Lymph node – Mesenteric	Lymphoid depletion								
	minimal:	0 (0)	0 (0)	1 (0)	0 (2)	0 (0)	0 (1)	0 (1)	0 (0)
	mild:	0 (0)	1 (4)	1 (1)	0 (2)	0 (0)	0 (0)	0 (0)	0 (1)
Spleen	Extramedullary hematopoiesis								
	minimal:	3 (2)	10 (0)	10 (2)	5 (2)	4 (2)	8 (2)	5 (0)	4 (4)
	mild:	1 (1)	6 (3)	4 (1)	3 (2)	1 (2)	4 (6)	4 (2)	0 (4)
	moderate:	1 (1)	0 (0)	1 (2)	2 (2)	1 (3)	1 (8)	3 (8)	4 (5)
	marked:	1 (0)	0 (2)	0 (2)	0 (2)	3 (4)	0 (5)	3 (7)	2 (4)
	severe:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
	Lymphoid depletion								
	minimal:	0 (2)	0 (2)	0 (1)	0 (2)	0 (0)	0 (1)	0 (1)	0 (1)
	mild:	0 (1)	0 (1)	0 (2)	1 (6)	0 (0)	0 (1)	0 (0)	0 (1)
	moderate:	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	marked:	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)	0 (0)	0 (1)
Thymus	Medullary increased cellularity								
	minimal:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	mild:	0 (0)	1 (0)	1 (0)	0 (0)	0 (0)	1 (0)	1 (0)	0 (0)
	moderate:	0 (0)	17 (2)	16 (1)	14 (1)	0 (5)	9 (5)	9 (1)	8 (1)
	marked:	0 (0)	6 (10)	6 (13)	8 (12)	0 (0)	9 (17)	12 (13)	14 (14)
	severe:	0 (0)	0 (1)	0 (0)	0 (6)	0 (0)	0 (0)	0 (3)	0 (0)
Thyroid	Follicular cell hypertrophy								
	minimal:	1 (0)	14 (0)	13 (2)	17 (3)	0 (0)	0 (0)	1 (1)	6 (4)
	Follicular cell hyperplasia								
	minimal:	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	1 (0)	0 (0)
	mild:	3 (2)	1 (0)	2 (1)	2 (3)	0 (0)	0 (0)	0 (0)	0 (0)
	moderate:	2 (1)	0 (0)	3 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
	marked:	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	severe:	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Uterus	Dilatation								
	minimal:	-	-	-	-	0 (0)	1 (1)	0 (0)	0 (0)
	mild:	-	-	-	-	0 (0)	1 (2)	3 (0)	1 (3)
	moderate:	-	-	-	-	0 (0)	0 (5)	2 (6)	2 (7)
	marked:	-	-	-	-	0 (0)	0 (1)	0 (3)	1 (1)
	severe:	-	-	-	-	0 (0)	0 (1)	0 (0)	0 (0)
	Vascular hyperplasia/trophy								
	minimal:	-	-	-	-	0 (0)	1 (0)	1 (0)	0 (1)
	mild:	-	-	-	-	0 (0)	0 (4)	3 (2)	2 (4)
	moderate:	-	-	-	-	0 (0)	1 (1)	2 (0)	0 (1)
	marked:	-	-	-	-	0 (0)	0 (0)	0 (2)	0 (1)
	severe:	-	-	-	-	0 (0)	0 (2)	0 (1)	1 (0)

Reference ID: 4392197

		MALE				FEMALE			
	(mg/kg):	0	10	30	90	0	3	10	30
	Number rats examined								
	Surviving animals:	30	29	26	25	35	20	26	22
	Premature deaths:	20	21	24	25	15	30	24	28
Tissue	**Finding**								
Uterus	Inflammation								
	minimal:	-	-	-	-	0 (0)	0 (0)	3 (2)	1 (0)
	mild:	-	-	-	-	0 (0)	1 (1)	1 (0)	1 (1)
	moderate:	-	-	-	-	0 (0)	0 (0)	1 (2)	2 (1)
	marked:	-	-	-	-	0 (0)	0 (4)	2 (1)	1 (3)
	Hemorrhage								
	minimal:	-	-	-	-	0 (0)	0 (0)	3 (3)	0 (3)
	mild:	-	-	-	-	0 (0)	0 (7)	1 (5)	2 (3)
	moderate:	-	-	-	-	0 (0)	0 (3)	0 (2)	0 (4)
	marked:	-	-	-	-	0 (0)	0 (1)	0 (0)	0 (0)
	Endometrial hyperplasia								
	minimal:	-	-	-	-	0 (0)	0 (1)	0 (0)	0 (0)
	mild:	-	-	-	-	0 (0)	0 (2)	0 (1)	2 (0)
	moderate:	-	-	-	-	0 (0)	0 (1)	0 (0)	1 (0)
	marked:	-	-	-	-	0 (0)	0 (0)	0 (0)	0 (0)
	severe:	-	-	-	-	0 (0)	0 (0)	1 (0)	0 (0)

Data from animals that died prematurely are in parenthesis; Semi. – seminiferous; CPN - Chronic progressive nephropathy.

Toxicokinetics

Blood samples were taken from TK animals in Weeks 5 and 26 at 3 and 6 hours post-dose; C_{max} and AUC were not calculated. Levels of siponimod were measured, but no assessment of metabolites was conducted.

Siponimod was not detected in control samples. Plasma exposure to siponimod generally appeared less than dose-proportional, with exposure greater in F than M. Accumulation was apparent over the course of the study.

Table: Siponimod plasma levels (ng/mL) (Sponsor's)
MALE

Dose (mg/kg/day)	Day	Time (h)	Mean	SD	CV%
10	34	3	1550	332	21.5
		6	1060	279	26.2
	181	3	2220	346	15.6
		6	1230*	NC*	NC*
30	34	3	3240	240	7.4
		6	2750	509	18.5
	181	3	4140	622	15
		6	3970	233	5.9
90	34	3	5400	530	9.8
		6	9100	1170	12.8
	181	3	5770	495	8.6
		6	11600	1270	11

NC, not calculated
* n=1

Reference ID: 4392197

FEMALE

Dose (mg/kg/day)	Day	Time (h)	Mean	SD	CV%
3	34	3	1880	559	29.8
		6	1340	91.9	6.9
	181	3	2590	742	28.7
		6	1610	106	6.6
10	34	3	5480	721	13.2
		6	5320	566	10.6
	181	3	7030	919	13.1
		6	7630	2770	36.3
30	34	3	9510	658	6.9
		6	12000	2050	17.2
	181	3	13600	141	1
		6	16100*	NC*	NC*

NC, not calculated
* n=1

Dosing Solution Analysis
All siponimod samples analyzed for concentration from Day 1 and Weeks 9, 24, 39, 54, 69, 84, and 104 were within the ± 15% accuracy criteria (actual range: 86% to 107% of the nominal concentrations).

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: An oral (gavage) male fertility study in rats

Study no.: 0970446
Study report location: EDR
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover Facility, New Jersey, 07936 USA
Date of study initiation: February 1, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BAF312, Batch № 0822008, 100.0%

Key Study Findings

- There were no deaths or adverse clinical signs, but significant decreases in body weight gain and food consumption were observed at the HD.
- Decrease in epididymis weight was observed in HDM, and a dose-dependent increase in the precoital interval and a dose-dependent decrease in mean corpora lutea, implantations, and viable fetuses was observed in the undosed F.
- The NOAEL for M fertility was 20 mg/kg/day.

Reference ID: 4392197

Methods

Doses:	0, 2, 20, and 200 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	[redacted] (b) (4)
Species/Strain:	Rat/Wistar Han
Number/Sex/Group:	25
Age:	8-10 Weeks
Weight:	Males - 279-358 g; Females - 138-237 g
Study design:	M were dosed for 28 days prior to mating, during the 2-week mating period, and then until the termination. F were not dosed

Observations and results

Mortality

Observations for mortality were conducted twice daily on weekdays and at least once daily on weekend days.

No deaths occurred during the study.

Clinical Signs

Prior to dosing, animals were observed on days that body weights were taken. During dosing M were observed twice daily, pre-dose and approximately 3 hours post-dose, and F were observed once daily.

No adverse clinical signs were noted. Salivation and decreased stools were observed in HDM.

Body Weight

Animals were weighed once prior to dosing. During dosing, M body weights were recorded twice weekly until termination. Body weights were recorded in F twice weekly premating and on GDs 0, 3, 6, 9, and 13.

In M, body weight gain was significantly decreased at the HD (53% on Day 29).

Food Consumption

Food consumption was recorded weekly on dosing Days 1, 8, 15, 22, and 29 for M and on premating Days 1, 8, and 15 and GDs 0, 3, 6, 9, and 13 for F.

Food consumption was deceased on Days 1-8 (45%), 8-15 (17%), and 15-22 (9%) at the HD. Food consumption was decreased 19% at the HD on Days 1-29.

Vaginal cytology

Vaginal cytology was determined daily, and estrous cycles assessed.

Reference ID: 4392197

Vaginal cytology and mating parameters were similar across the groups. Most mating indices were unaffected in M at any dose level, although there was a dose-dependent increase of the precoital interval from 2.3 days in the control group to 3.9 days in the HD group.

Gross findings
M were terminated on completion of mating and F were terminated on GD 13. Visceral organs were examined macroscopically in F.

Increased incidences of small renal papilla were observed in M: 1 control, 1 LD, 2 MD, and 3 HD.

Male Reproductive Parameters
Testis and epididymis were weighed. The left testis was taken and used to determine testicular sperm counts. Sperm samples were collected from the vas deferens and motility determined manually from videotaped images. The number of corpora lutea from the ovaries were recorded and implantation sites were identified as either a live fetus or early resorption.

There was a decrease in total and relative to body epididymis weight at the HD (20% and 14%, respectively). Otherwise, there were no changes in M reproductive parameters. Numbers of corpora lutea, implantations, and viable fetuses were dose-dependently reduced. However, the sponsor stated that the values were within historical control values for the facility.

Reference ID: 4392197

Table: Summary of cesarean data (Sponsor's)

	DOSAGE				
Females Mated	N	25	25	25	25
Pregnant	N	23	20	19	21
	%	92.0	80.0	76.0	84.0
Aborted	N	0	0	0	0
Premature Births	N	0	0	0	0
Female Mortality	N	0	0	0	0
Pregnant at C-section	N	23	20	19	21
Dams with Viable Fetuses	N	23	20	19	20
Dams with all Resorptions	N	0	0	0	1
Corpora Lutea	N	324	273	260	251
	MEAN	14.1	13.6	13.7	12.0*
	S.D.	2.2	2.9	2.9	2.6
Implantation Sites	N	277	252	222	182
	MEAN	12.0	12.6	11.7	8.7*
	S.D.	4.1	4.2	4.3	5.0
Preimplantation Loss	%	14.5	7.7	14.6	27.5
Postimplantation Loss	%	6.5	3.6	5.4	6.6
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	18	9	12	12
	%	6.5	3.6	5.4	6.6
	MEAN	0.8	0.4	0.6	0.6
	S.D.	1.1	0.8	1.0	0.7
Early Resorptions	N	18	9	12	12
	%	6.5	3.6	5.4	6.6
	MEAN	0.8	0.4	0.6	0.6
	S.D.	1.1	0.8	1.0	0.7
Late Resorptions	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.0	0.0	0.0
Viable Fetuses	N	259	243	210	170
	%	93.5	96.4	94.6	93.4
	MEAN	11.3	12.1	11.1	8.1
	S.D.	4.3	4.2	4.2	5.0

Statistical key: * = p<0.05

Study title: BAF312: An oral gavage female fertility and early embryonic development study in rats

Study no.: 1070054
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: October 18, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BAF312, Batch № 1113015, 100%

Reference ID: 4392197

Key Study Findings

- There were no deaths or adverse clinical signs, but a decrease in body weight gain and food consumption was observed during the premating period at all doses.
- No drug-related changes were observed in F fertility or early embryonic development at any dose.
- The NOAEL was 1 mg/kg/day associated with an $AUC_{0\text{-}24h}$ of 9030 ng*h/mL.

Methods

Doses:	0, 0.1, 0.3, and 1 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4)
Species/Strain:	Rat/Wistar Han
Number/Sex/Group:	24
Age:	9 Weeks
Weight:	Females - 191-226 g
Study design:	F were dosed for 14 days prior to mating, during the mating period, and then until Day 6 post coitum. One F was paired with 1 M, with the cohabitation lasting a maximum of 14 days.

Observations and results

Mortality

Observations for mortality and moribundity were conducted twice daily.

No deaths occurred during the study.

Clinical Signs

Cage-side observations were conducted once daily on non-dosing days. On dosing days, observations were conducted pre-dose and within 3 hours post-dose. Detailed examinations were conducted on days the body weight was recorded.

No drug-related clinical signs were noted at any dose.

Body Weight

Animals were weighed twice prior to dosing. During dosing body weights were recorded twice weekly until the end of dosing, including the first day of dosing and the day animals were placed for mating. In F, body weights were recorded on Days 0, 3, 6, 9, and 13 post coitum.

Body weight gain was decreased during the premating period (Days 0-13) at the LD (55%), MD (34%), and HD (15%). No change in body weight gain was observed during gestation.

Reference ID: 4392197

Food Consumption
Food consumption was recorded twice weekly during dosing until the initiation of mating. Food consumption of mated F was recorded on Days 0-3, 3-6, 6-9, and 9-13 post coitum.

Food consumption was dose-dependently decreased during the premating period (Days 0-13) at the LD (4%), MD (7%), and HD (8%), but was similar during gestation.

Vaginal cytology
Vaginal cytology was determined, and estrous cycles assessed. Samples were collected for 14 days beginning with the first day of dosing.

There was no drug-related change in the estrus cycle.

Gross findings
F were terminated on post coitum Day 13. A complete necropsy examination was performed that included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. Organs examined included kidney, lung, lymph node, thymus, and trachea.

There were no drug-related macroscopic findings.

Ovarian and uterine examinations
Uterus and ovary were removed at necropsy and the number of corpora lutea, implantation sites, resorptions, live and dead embryos, and placentae recorded.

There were no drug-related changes in the mean day to mating, fertility, conception rate, and mating indices, and number of corpora lutea, implantations, live embryos, dead embryos, resorptions, and pre- and post-implantation losses.

Toxicokinetics
Blood samples were taken on Days 1 and 11 at 0.5, 1, 3, 7, and 24 hours post-dose.

Increase in plasma exposure (C_{max} and AUC) to siponimod was generally dose-proportional between the LD and MD and greater than dose-proportional between the MD and HD; T_{max} was at 3-7 hours. Accumulation occurred at all doses (approximately 2.3-fold).

Reference ID: 4392197

Table: Toxicokinetic data of siponimod (Sponsor's)

Study days	Parameter	Dose (mg/kg/day) 0.1	0.3	1.0
0/1	Tmax	7.00	7.00	7.00
	Cmax	17.4	46.1	236
	Cmax/Dose	174	154	236
	AUC	317	915	4410
	AUC/Dose	3170	3050	4410
10/11	Tmax	3.00	3.00	7.00
	Cmax	46.4	103	468
	Cmax/Dose	464	344	468
	AUC	841	2100	9030
	AUC/Dose	8410	7010	9030

Units: Tmax (Hours), Cmax (ng/mL), Cmax/Dose [(ng/mL)/(mg/kg/day)], AUC (ng*h/mL), AUC/Dose [(ng*h/mL)/(mg/kg/day)], SE of Composite AUC (NJH) (ng*h/mL), Co (ng/mL), C24h/Cmax (%).

9.2 Embryonic Fetal Development

Study title: An oral (gavage) embryo-fetal development study in rats

Study no: 0770653
Study report location: EDR
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936 USA
Date of study initiation: October 22, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BAF312, Batch № 0651001, 99.2%

Key Study Findings

- Increases in resorptions resulted in no viable fetuses at the MD and HD and a decrease in viable fetuses at the LD.
- Increases in malformations (external, visceral, and skeletal) were noted at the LD.
- The fetal NOAEL was < LD due to increased embryofetal deaths (early resorptions) and malformations. Plasma exposure (AUC) at the LD was 10300 ng*h/mL.

Reference ID: 4392197

Methods

Doses:	0, 1, 5, and 40 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral, gavage
Formulation/Vehicle:	(b) (4)
Species/Strain:	Rat/Wistar Han
Number/Sex/Group	
Main:	25 Pregnant F
Toxicokinetics:	2 Control and 5 LD, MD, and HD groups
Age:	Approximately 10 weeks
Weight:	Females - 176-250 g at GD 0
Study design:	Siponimod was administered daily on GDs 6-17

Observations and results

Mortality

Observations for mortality and moribundity were conducted twice daily on weekdays and once daily on weekends.

No unscheduled deaths occurred in dams.

Clinical Signs

Observations were conducted at least once daily prior to initiation of dosing, twice daily during dosing (pre-dose and within approximately 3 hours post-dose), and after the termination of dosing.

No clinical signs were noted in dams at the LD and MD. Decreased stools and increased activity were observed in HD dams.

Body Weight

Body weight recordings were taken on GDs 0, 3, 6, 9, 12, 15, 18, and 21.

During gestation (Days 0-21) decrease in body weight gain was observed in dams at the LD (23%), MD (36%), and HD (39%).

Food Consumption

Food consumption recordings were taken on GDs 3, 6, 9, 12, 15, 18, and 21.

Decrease in food consumption occurred in HD dams during Days 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21; the decreases ranged between 8-30%.

Necropsy

On GD 21, F were sacrificed, and visceral organs and placentas examined macroscopically. The uterus was removed for assessment of fertility parameters. The uterus (with contents), ovaries, and oviducts were weighed.

Reference ID: 4392197

Uterine weight was decreased, compared to control values, by 51%, 86%, and 84% at the LD, MD, and HD, respectively. Macroscopic findings noted in dams included small kidney (1 LD), small renal papilla (1 LD), and urinary bladder calculi (1 LD and 1 HD).

Cesarean Section Data

Implantation sites were identified as either live fetus, dead fetus, early resorptions, or late resorptions. Numbers of corpora lutea were recorded.

There were no differences in numbers of corpora lutea, implantations, and pre-implantation losses. However, post-implantation loss was increased in LD dams, and there was total post-implantation loss in all MD and HD dams that were mainly early resorptions. MD and HD dams had no viable fetuses and number of fetuses were reduced in litters from LD dams. Weights of viable fetuses from the LD group were similar to control fetal weight.

Table: Cesarean section data (Sponsor's)

	DOSAGE	0 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY	40 MG/ KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	22	24	23	23
	%	88.0	96.0	92.0	92.0
Aborted	N	0	0	0	0
Premature Births	N	0	0	0	0
Female Mortality	N	0	0	0	0
Pregnant at C-section	N	22	24	23	23
Dams with Viable Fetuses	N	21	18	0	0
Dams with all Resorptions	N	1	6	23	23
Corpora Lutea	N	248	279	261	268
	MEAN	11.3	11.6	11.3	11.7
	S.D.	1.8	1.7	1.6	1.2
Implantation Sites	N	190	226	193	213
	MEAN	8.6	9.4	8.4	9.3
	S.D.	3.5	3.1	3.2	2.9
Preimplantation Loss	%	23.4	19.0	26.1	20.5
Postimplantation Loss	%	5.8	70.8	100.0	100.0
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	11	160**	193**	213**
	%	5.8	70.8	100.0	100.0
	MEAN	0.5	6.7**	8.4**	9.3**
	S.D.	0.7	3.0	3.2	2.9
Early Resorptions	N	10	114**	183**	195**
	%	5.3	50.4	94.8	91.5
	MEAN	0.5	4.8**	8.0**	8.5**
	S.D.	0.7	2.9	3.2	3.0
Late Resorptions	N	1	46**	10*	18**
	%	0.5	20.4	5.2	8.5
	MEAN	0.0	1.9**	0.4	0.8
	S.D.	0.2	1.8	0.7	1.2

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Viable Fetuses	N	179	66**	0**	0**
	%	94.2	29.2	0.0	0.0
	MEAN	8.1	2.8**	0.0**	0.0**
	S.D.	3.6	2.5	0.0	0.0
Viable Male Fetuses	N	88	40		
	%	49.2	60.6		
Live Fetal Body Weight (g)	MEAN	5.3	5.1		
	S.D.	0.4	0.4		
Male Fetuses	MEAN	5.4	5.2		
	S.D.	0.4	0.5		
Female Fetuses	MEAN	5.1	4.9		
	S.D.	0.4	0.5		

Statistical key: ** = p<0.01

Offspring (Malformations, Variations, etc.)
Viable fetuses were sexed, weighed, and examined for external findings. A visceral examination was conducted on approximately one-half of each litter. A skeletal examination was conducted on the other half of fetuses.

External observations: There were no viable MD or HD fetuses. Malrotated limbs (malformation), cleft palate (malformation), and edema (variation) were noted at the LD in 12, 1, and 10 of 66 fetuses, respectively. Other variations included subcutaneous hemorrhage and discoloration in the dorsal abdominal areas in 1 LD fetus.

Table: External malformations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY	40 MG/ KG/DAY
Litters Evaluated	N	21	18	0	0
Fetuses Evaluated	N	179	66	0	0
Live	N	179	66	0	0
Dead	N	0	0	0	0
CLEFT PALATE					
Fetal Incidence	N	0	1		
	%	0.0	1.5		
Litter Incidence	N	0	1		
	%	0.0	5.6		
LIMB(S)-MALROTATED					
Fetal Incidence	N	1	12**		
	%	0.6	18.2		
Litter Incidence	N	1	9**		
	%	4.8	50.0		
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	1	12**		
	%	0.6	18.2		
Litter Incidence	N	1	9**		
	%	4.8	50.0		

Statistical key: ** = p<0.01

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Table: External variations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY	40 MG/ KG/DAY
Litters Evaluated	N	21	18	0	0
Fetuses Evaluated	N	179	66	0	0
Live	N	179	66	0	0
Dead	N	0	0	0	0
GENERALIZED EDEMA					
Fetal Incidence	N	0	10**		
	%	0.0	15.2		
Litter Incidence	N	0	7**		
	%	0.0	38.9		
DISCOLORATION-BILATERAL DORSAL ABDOMINAL AREA					
Fetal Incidence	N	0	1		
	%	0.0	1.5		
Litter Incidence	N	0	1		
	%	0.0	5.6		
SUBCUTANEOUS HEMORRHAGE					
Fetal Incidence	N	0	1		
	%	0.0	1.5		
Litter Incidence	N	0	1		
	%	0.0	5.6		
TOTAL FETAL EXTERNAL VARIATIONS					
Fetal Incidence	N	0	10**		
	%	0.0	15.2		
Litter Incidence	N	0	7**		
	%	0.0	38.9		

Statistical key: ** = p<0.01

Visceral findings: Cardiomegaly (malformation) was noted in 1 LD fetus. Variations were observed in a single LD fetus and included discolored liver, small renal papilla, and dilated ureter.

Table: Visceral malformations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY	40 MG/ KG/DAY
Litters Evaluated	N	20	16	0	0
Fetuses Evaluated	N	84	30	0	0
Live	N	84	30	0	0
Dead	N	0	0	0	0
CARDIOMEGALY					
Fetal Incidence	N	0	1		
	%	0.0	3.3		
Litter Incidence	N	0	1		
	%	0.0	6.3		
TOTAL FETAL VISCERAL MALFORMATIONS					
Fetal Incidence	N	0	1		
	%	0.0	3.3		
Litter Incidence	N	0	1		
	%	0.0	6.3		

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Table: Visceral variations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY	40 MG/ KG/DAY
Litters Evaluated	N	20	16	0	0
Fetuses Evaluated	N	84	30	0	0
Live	N	84	30	0	0
Dead	N	0	0	0	0
LIVER-DISCOLORED					
Fetal Incidence	N	0	1		
	%	0.0	3.3		
Litter Incidence	N	0	1		
	%	0.0	6.3		
RENAL PAPILLA-SMALL					
Fetal Incidence	N	0	1		
	%	0.0	3.3		
Litter Incidence	N	0	1		
	%	0.0	6.3		
URETER-DILATED					
Fetal Incidence	N	0	1		
	%	0.0	3.3		
Litter Incidence	N	0	1		
	%	0.0	6.3		
TOTAL FETAL VISCERAL VARIATIONS					
Fetal Incidence	N	0	2		
	%	0.0	6.7		
Litter Incidence	N	0	2		
	%	0.0	12.5		

Skeletal findings: Skeletal malformations of cleft palate and misshapen clavicles were noted in 2 and 6 LD fetuses, respectively. Skeletal variations included statistically significant increases in incomplete ossification of the interparietal and sternebra, unossified forepaw and hindpaw phalanx, misshapen sternebra, and cervical rib in LD fetuses.

Table: Skeletal malformations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY	40 MG/ KG/DAY
Litters Evaluated	N	21	18	0	0
Fetuses Evaluated	N	95	36	0	0
Live	N	95	36	0	0
Dead	N	0	0	0	0
PALANTINE-SPLIT(CLEFT)					
Fetal Incidence	N	0	2		
	%	0.0	5.6		
Litter Incidence	N	0	2		
	%	0.0	11.1		
CLAVICLE-MISSHAPEN					
Fetal Incidence	N	0	6**		
	%	0.0	16.7		
Litter Incidence	N	0	6**		
	%	0.0	33.3		
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	0	6**		
	%	0.0	16.7		
Litter Incidence	N	0	6**		
	%	0.0	33.3		

Statistical key: ** = p<0.01

Toxicokinetics

Maternal blood samples were taken from TK animals on GD 17 at 0.5, 3, 7, and 24 hours post-dose.

Increase in plasma exposure (C_{max} and AUC) to siponimod was generally dose-proportional between the LD and MD and less than dose-proportional between the MD and HD; T_{max} was 3-7 hours.

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Table: Toxicokinetic data of siponimod (Sponsor's)

Treatment	Parameter	Units	BAF312	S.D.	%CV	n
Low	Dose	mg/kg/day	1	0.00	0.00	4
	Tmax	Hours	[3-7]			4
	Cmax	ng/mL	498	35.5	7.13	4
	Cmax/Dose	ng/mL/mg/kg/day	498	35.5	7.13	4
	AUC	ng*Hours/mL	10300	660	6.41	4
	AUC/Dose	ng*Hours/mL/mg/kg/day	10300	660	6.41	4
	AUC Interval (0-24 Hours)			-		4
	Co	ng/mL	344	22.8	6.63	4
Mid	Dose	mg/kg/day	5	0.00	0.00	5
	Tmax	Hours	[3-7]			5
	Cmax	ng/mL	2870	252	8.78	5
	Cmax/Dose	ng/mL/mg/kg/day	575	50.4	8.77	5
	AUC	ng*Hours/mL	57900	6360	11.0	5
	AUC/Dose	ng*Hours/mL/mg/kg/day	11600	1260	10.9	5
	AUC Interval (0-24 Hours)			-		5
	Co	ng/mL	1830	303	16.6	5
High	Dose	mg/kg/day	40	0.00	0.00	5
	Tmax	Hours	[3-7]			5
	Cmax	ng/mL	14800	1850	12.5	5
	Cmax/Dose	ng/mL/mg/kg/day	370	46.3	12.5	5
	AUC	ng*Hours/mL	316000	47900	15.2	5
	AUC/Dose	ng*Hours/mL/mg/kg/day	7900	1200	15.2	5
	AUC Interval (0-24 Hours)			-		5
	Co	ng/mL	11700	2670	22.8	5

Since one female from the low dose group was not pregnant its samples were not sent for analysis and therefore the mean PK parameters were calculated on 4 animals.

[...] T_{max} given as a range.

An oral (gavage) embryo-fetal development study in rabbits

Study no: 0770652
Study report location: EDR
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936 USA
Date of study initiation: November 7, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BAF312, Batch № 0651001, 99.2%

Key Study Findings

- Four HD dams aborted their litters.
- Mean body weight gain and uterine weights were decreased at the HD.
- Increased resorption of fetuses occurred at the MD and HD resulting in decreases in viable fetuses.
- Malformations were noted in fetuses at the HD and included a single incidence of fused kidney (visceral) and of fused frontal and misaligned sternebra (skeletal).
- The maternal NOAEL was 1 mg/kg/day. The fetal NOAEL was 0.1 mg/kg/day due to abortions at the HD, increased embryofetal deaths (resorptions) at the MD and HD, and increased variations, visceral at the HD and skeletal at the MD and HD. Plasma exposure (AUC) at 0.1 mg/kg/day was not calculated, but C_{max} was 6.5 ng/mL. Plasma exposure (AUC) at 1 mg/kg/day was 958 ng*h/mL.

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Methods

Doses:	0, 0.1, 1, and 5 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	(b) (4)
Species/Strain:	Rabbit/New Zealand White, Hra:(NZW)SPF
Number/Sex/Group	
Main:	20 Pregnant females
Toxicokinetic:	3 Control and 5 LD, MD, and HD groups
Age:	Approximately 23 weeks at dose initiation
Weight:	2.81-3.79 kg at dose initiation
Study design:	Siponimod was administered daily on GDs 7-20

Observations and Results for Dams

Mortality

Observations were conducted twice daily on weekdays and once daily on weekends and holidays.

There was no drug-related dam mortality. One HD dam was sacrificed on GD 12 due to an injured hindlimb and 4 HD dams aborted their litters and were sacrificed between GDs 24-27.

Clinical Signs

Observations were conducted at least once daily prior to initiation of dosing, twice daily during dosing (pre-dose and within approximately 3 hours post-dose), and after the termination of dosing.

No drug-related clinical signs were observed.

Body Weight

Body weight recordings were taken on GDs 0, 5, 7, 10, 14, 17, 21, 24, and 29.

During gestation (Days 0-29), decrease in body weight gain was observed in HD dams (43%).

Food Consumption

Food consumption recordings were taken daily during GDs 5-29.

There were no food consumption changes observed.

Necropsy

On GD 29, dams were sacrificed and major visceral organs, including placentas examined macroscopically. The ovary, oviducts, and uterus and contents were removed and weighted.

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Compared to control dam values, there was a dose-dependent decrease in uterine weight by 3%, 6%, and 57% at the LD, MD, and HD, respectively. Ovarian cysts were observed in 1 HD dam and 1 HD dam had a broken hindlimb (see Mortality section).

Cesarean Section Data

Reproductive parameters were evaluated: corpora lutea, implantation sites, resorptions, dead fetuses and viable fetuses.

One control dam delivered prematurely on GD 29, 4 HD dams aborted between GDs 24-27, and 1 HD dam had no viable fetuses. There were no differences in numbers of corpora lutea, implantations, and pre-implantation losses. However, post-implantation loss was increased in dams at the MD and HD, mainly due to early resorptions. This resulted in a decrease in mean viable fetuses (67%) at the HD. There were no changes in fetal weights of viable fetuses at all doses.

Table: Cesarean section data (Sponsor's)

	DOSAGE	0 MG/KG/DAY	0.1 MG/KG/DAY	1 MG/KG/DAY	5 MG/KG/DAY
Females Mated	N	20	20	20	20
Pregnant	N	17	19	20	16
	%	85.0	95.0	100.0	80.0
Aborted	N	0	0	0	4
Premature Births	N	1	0	0	0
Female Mortality	N	0	0	0	1
Pregnant at C-section	N	16	19	20	11
Dams with Viable Fetuses	N	16	19	20	10
Dams with all Resorptions	N	0	0	0	1
Corpora Lutea	N	159	194	200	113
	MEAN	9.9	10.2	10.0	10.3
	S.D.	1.7	1.5	1.7	1.8
Implantation Sites	N	142	173	186	101
	MEAN	8.9	9.1	9.3	9.2
	S.D.	1.9	1.8	1.5	2.3
Preimplantation Loss	%	10.7	10.8	7.0	10.6
Postimplantation Loss	%	6.3	8.7	13.4	70.3
Dead Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Resorptions, total	N	9	15	25*	71**
	%	6.3	8.7	13.4	70.3
	MEAN	0.6	0.8	1.3	6.5**
	S.D.	1.0	0.6	1.4	1.8
Early Resorptions	N	6	13	19	60**
	%	4.2	7.5	10.2	59.4
	MEAN	0.4	0.7	0.9	5.5**
	S.D.	0.5	0.7	1.2	2.8
Late Resorptions	N	3	2	6	11**
	%	2.1	1.2	3.2	10.9
	MEAN	0.2	0.1	0.3	1.0*
	S.D.	0.8	0.3	0.5	1.7
Viable Fetuses	N	133	158	161*	30**
	%	93.7	91.3	86.6	29.7
	MEAN	8.3	8.3	8.1	2.7**
	S.D.	1.9	1.7	1.7	1.6
Viable Male Fetuses	N	69	90	80	18
	%	51.9	57.0	50.6	60.0
Live Fetal Body Weight (g)	MEAN	41.9	41.5	40.3	41.1
	S.D.	4.4	4.2	4.2	5.0
Male Fetuses	MEAN	42.3	42.5	41.0	42.8
	S.D.	4.3	4.1	5.0	6.7
Female Fetuses	MEAN	42.2	40.3	39.6	42.4
	S.D.	5.0	4.6	4.7	4.2

Statistical key: * = p<0.05 ** = p<0.01

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Offspring (Malformations, Variations, etc.)
Fetuses were weighed and eviscerated fetuses stained with alizarin red.

External observations: There were no malformations and only edema (variation) was noted in 1 MD fetus.

Visceral findings: Malformations noted in HD fetuses included absent gallbladder (also in LD) and malpositioned kidney; however, these malformations were noted in control fetuses, with higher numbers of incidences. Fused kidney was noted in 1 HD fetus. Variations observed included dose-dependent increase in small gallbladder and coagulated blood in the abdomen in a single HD fetus.

Table: Visceral malformations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	0.1 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY
Litters Evaluated	N	16	19	20	10
Fetuses Evaluated	N	133	158	161	30
Live	N	133	158	161	30
Dead	N	0	0	0	0
HYDROCEPHALY					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.3	0.0	0.0
BRAIN-REDUCED IN SIZE					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.3	0.0	0.0
LUNG-SMALL					
Fetal Incidence	N	0	1	1	0
	%	0.0	0.6	0.6	0.0
Litter Incidence	N	0	1	1	0
	%	0.0	5.3	5.0	0.0
GALLBLADDER-ABSENT					
Fetal Incidence	N	2	1	0	1
	%	1.5	0.6	0.0	3.3
Litter Incidence	N	2	1	0	1
	%	12.5	5.3	0.0	10.0
KIDNEY-MISSHAPEN					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	6.3	0.0	0.0	0.0
KIDNEY-MALPOSITIONED					
Fetal Incidence	N	2	0	0	1
	%	1.5	0.0	0.0	3.3
Litter Incidence	N	2	0	0	1
	%	12.5	0.0	0.0	10.0
Litters Evaluated	N	16	19	20	10
Fetuses Evaluated	N	133	158	161	30
Live	N	133	158	161	30
Dead	N	0	0	0	0
KIDNEY-FUSED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	3.3
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	10.0
TOTAL FETAL VISCERAL MALFORMATIONS					
Fetal Incidence	N	4	3	1	2
	%	3.0	1.9	0.6	6.7
Litter Incidence	N	4	3	1	2
	%	25.0	15.8	5.0	20.0

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Table: Visceral variations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	0.1 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY
Litters Evaluated	N	16	19	20	10
Fetuses Evaluated	N	133	158	161	30
Live	N	133	158	161	30
Dead	N	0	0	0	0
ABDOMEN-COAGULATED BLOOD					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.6	0.0	3.3
Litter Incidence	N	0	1	0	1
	%	0.0	5.3	0.0	10.0
GALLBLADDER-SMALL					
Fetal Incidence	N	2	4	4	6**
	%	1.5	2.5	2.5	20.0
Litter Incidence	N	2	3	4	4
	%	12.5	15.8	20.0	40.0
GALLBLADDER-ENLARGED					
Fetal Incidence	N	0	6*	0	0
	%	0.0	3.8	0.0	0.0
Litter Incidence	N	0	3	0	0
	%	0.0	15.8	0.0	0.0
RENAL PAPILLA-SMALL					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.3	0.0	0.0
TOTAL FETAL VISCERAL VARIATIONS					
Fetal Incidence	N	2	11*	4	7**
	%	1.5	7.0	2.5	23.3
Litter Incidence	N	2	6	4	4
	%	12.5	31.6	20.0	40.0

Statistical key: * = p<0.05 ** = p<0.01

Skeletal findings: Malformations of fused frontal and misaligned sternebra were noted in single fetuses at the HD; however, there were a greater number of malformations, of a similar type, noted in control fetuses. Skeletal variations included statistically significant increases in percent incidences of unossified forepaw phalanx at the HD, incomplete ossification of the hyloid at the MD and HD, forepaw and hindpaw phalanx at the HD, and talus at the HD, bent hyoid at the HD, and fused and misshapen sternebra at the HD.

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Table: Skeletal malformations (Sponsor's)

DOSAGE		0 MG/ KG/DAY	0.1 MG/ KG/DAY	1 MG/ KG/DAY	5 MG/ KG/DAY
Litters Evaluated	N	16	19	20	10
Fetuses Evaluated	N	133	158	161	30
Live	N	133	158	161	30
Dead	N	0	0	0	0
FRONTAL-FUSED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	3.3
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	10.0
HYOID-MISSHAPEN					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	6.3	0.0	0.0	0.0
METACARPAL-ABSENT					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	6.3	0.0	0.0	0.0
FOREPAW PHALANX-ABSENT					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	6.3	0.0	0.0	0.0
STERNEBRA-MISALIGNED					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	3.3
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	10.0
RIB-BRANCHED					
Fetal Incidence	N	2	1	0	0
	%	1.5	0.6	0.0	0.0
Litter Incidence	N	2	1	0	0
	%	12.5	5.3	0.0	0.0
Litters Evaluated	N	16	19	20	10
Fetuses Evaluated	N	133	158	161	30
Live	N	133	158	161	30
Dead	N	0	0	0	0
CERVICAL CENTRUM-MISALIGNED					
Fetal Incidence	N	3	0	0	0
	%	2.3	0.0	0.0	0.0
Litter Incidence	N	2	0	0	0
	%	12.5	0.0	0.0	0.0
CERVICAL CENTRUM-FUSED					
Fetal Incidence	N	3	0	1	0
	%	2.3	0.0	0.6	0.0
Litter Incidence	N	2	0	1	0
	%	12.5	0.0	5.0	0.0
CERVICAL VERTEBRA-FUSED					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	6.3	0.0	0.0	0.0
THORACIC CENTRUM-FUSED					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.6	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	5.3	0.0	0.0
THORACIC CENTRUM-HEMICENTRIC					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	6.3	0.0	0.0	0.0
THORACIC CENTRUM-MISALIGNED					
Fetal Incidence	N	1	0	1	0
	%	0.8	0.0	0.6	0.0
Litter Incidence	N	1	0	1	0
	%	6.3	0.0	5.0	0.0

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Litters Evaluated	N	16	19	20	10
Fetuses Evaluated	N	133	158	161	30
Live	N	133	158	161	30
Dead	N	0	0	0	0
THORACIC CENTRUM-MISSHAPEN					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.6	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.0	0.0
THORACIC VERTEBRA-FUSED					
Fetal Incidence	N	1	0	1	0
	%	0.8	0.0	0.6	0.0
Litter Incidence	N	1	0	1	0
	%	6.3	0.0	5.0	0.0
THORACIC VERTEBRA-MALPOSITIONED					
Fetal Incidence	N	1	0	0	0
	%	0.8	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	6.3	0.0	0.0	0.0
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	6	1	2	2
	%	4.5	0.6	1.2	6.7
Litter Incidence	N	4	1	2	2
	%	25.0	5.3	10.0	20.0

Toxicokinetics

Maternal blood samples were taken from satellite animals on GD 20 at 0.5, 3, 7, and 24 hours post-dose. Fetuses from each litter were pooled and analyzed for siponimod.

Increase in plasma exposure (C_{max} and AUC) to siponimod was generally dose-proportional between the LD and MD and greater than dose-proportional between the MD and HD; T_{max} was 3-7 hours. The levels of siponimod could only be measured in MD and HD fetal tissue, with increases in siponimod levels less than dose-proportional.

Table: Maternal toxicokinetic data for siponimod (Sponsor's)

Study Day	Parameter	Units	Mean	S.D.	%CV	n
20	Dose	mg/kg/day	0.1	0.00	0.00	4
	T_{max}	Hours	[3-7]			
	C_{max}	ng/mL	6.52	0.755	11.6	4
	C_{max}/Dose	ng/mL/mg/kg/day	65.2	7.55	11.6	4
	AUC	ng*Hours/mL	NR			
	AUC/Dose	ng*Hours/mL/mg/kg/day	NR			
	AUC Interval		(0-24 Hours)			
	Co	ng/mL	NR			
	Dose	mg/kg/day	1	0.00	0.00	5
	T_{max}	Hours	[3-7]			
	Cmax	ng/mL	66.4	10.3	15.5	5
	Cmax/Dose	ng/mL/mg/kg/day	66.4	10.3	15.5	5
	AUC	ng*Hours/mL	958	258	26.9	5
	AUC/Dose	ng*Hours/mL/mg/kg/day	958	258	26.9	5
	AUC Interval		(0-24 Hours)	-		5
	Co	ng/mL	16.5	6.30	38.2	5
	Dose	mg/kg/day	5	0.00	0.00	5
	T_{max}	Hours	3	0	0	5
	C_{max}	ng/mL	426	48.8	11.5	5
	C_{max}/Dose	ng/mL/mg/kg/day	85.2	9.76	11.5	5
	AUC	ng*Hours/mL	5510	646	11.7	5
	AUC/Dose	ng*Hours/mL/mg/kg/day	1100	130	11.8	5
	AUC Interval		(0-24 Hours)	-		5
	Co	ng/mL	89.6	17.1	19.1	5

NR: value not reported due to insufficient data point in the TK profiles.
[ ] Tmax given as a range
No samples for rabbit 619 from low dose group (0.1 mg/kg/day) since this female was not pregnant

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Table: Maternal plasma and fetal tissue concentration of siponimod (Sponsor's)

Study Day	Treatment (mg/kg/day)	Time (h)	Plasma (ng/mL)				Fetus (ng/g)			
20			Mean	S.D.	%CV	n	Mean	S.D.	%CV	n
	Control (0)	0.5	0.00			3				
		3	0.00			3				
		7	0.00			3				
		24	0.00			3	0.00			3
	Low (0.1)	0.5	0.00			4				
		3	6.50	0.779	12.0	4				
		7	2.97	3.44	115.8	4				
		24	0.00			4	0.00			4
	Mid (1)	0.5	20.7	2.30	11.1	5				
		3	66.0	9.87	15.0	5				
		7	54.1	17.6	32.5	5				
		24	16.5	6.30	38.2	5	41.0	13.3	32.4	5
	C24/Cmax (%)		25							
	High (5)	0.5	119	31.0	26.1	5				
		3	426	48.8	11.5	5				
		7	301	42.0	14.0	5				
		24	89.6	17.1	19.1	5	146	26.8	18.4	5
	C24/Cmax (%)		21							

No samples available from rabbit 619 from low dose group since this female was not pregnant

9.3 Prenatal and Postnatal Development

Study title: An oral (gavage) pre and postnatal study with immunological endpoints in the rat

Study no: 0970449
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: January 28, 2014
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: BAF312, Batch № 1113020, 100.8%

Key Study Findings

- Several F_0 HD dams were euthanized early including 1 with dystocia and 6 due to loss of live fetuses.
- Body weight gain was decreased at all doses during gestation and in MD and HD dams during lactation.
- Reproductive changes for F_0 dams included a slight decrease in gestation index at the HD and an increase in gestation length at the MD and HD. The number of live fetuses was reduced at the HD.
- F_1 pup deaths were observed at all doses between PNDs 0 and 4 that increased dose-dependently. The increase in F_1 pup death occurred in 5, 15, and 19 dams at the LD, MD, and HD, respectively. Further deaths of F_1 pups, in MD and HD litters, occurred during lactation.

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- Signs observed in HD F_1 pups included abnormal flexure of the left and right hindlimbs, flat cranium, and malocclusion of the incisors.
- Malformations noted in necropsied pups included absence of anal opening in a MDF and cleft palate, split tongue, truncus arteriosus, and subcutaneous edema over the entire body in HD pups.
- Decreases in body weight gain, food consumption, and delays in sexual maturation were noted at the HD, but with no effect on reproductive function.
- The NOAEL for toxicity and reproductive performance in F_0 dams and F_1 generation development was 0.05 mg/kg/day.

Methods

Doses:	0, 0.05, 0.15 and 0.5 mg/kg
Frequency of dosing:	Daily
Dose volume:	2 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	(b) (4)
Species/Strain:	Rat/Wistar Han
Number/Sex/Group:	24 Pregnant females/group
Age:	81-85 Days at initiation of dosing
Weight:	196-245 g
Deviation from study protocol:	siponimod was administered daily from GDs 6 to Lactation Day 20, 21, or 22

Observations and Results for F_0 Dams

Mortality

Observations were conducted twice daily.

One HD dam was euthanized early on GD 22 due to birthing difficulties (dystocia); clinical signs included red liquid vaginal discharge, areas of red staining of the fur in the urogenital region, firm abdominal interstructure, hunched posture, decreased activity, partly closed eyes, decreased respiratory rate, and bent tail. Six dams were euthanized between PNDs 1 and 15 because no live fetuses remained. No litters were born to 1 control, 1 MD, and 1 HD dams; however, at necropsy the HD dam was found to be pregnant.

Clinical Signs

Observations were conducted once daily on non-dosing days and twice daily during dosing, i.e., pre-dose and within 3 hours post-dose. Detailed examinations were conducted on days body weight was measured.

No adverse clinical signs were observed during the study.

Body Weight

Body weight recordings for F_0 dams were taken on GDs 0, 3, 6, 9, 12, 15, 18, and 20 and on Lactation Days 0, 4, 7, 10, 14, 17, and 21.

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During gestation (Days 6-20), decrease in body weight gain was observed in dams at the LD (8%), MD (9%), and HD (36%). Body weight gain was also reduced during lactation (Days 0-21) in dams at the MD (16%) and HD (25%).

Food Consumption
Food consumption for F_0 dams were recorded on GDs 3-6, 6-9, 9-12, 12-15, and 15-18 and on Lactation Days 0-4, 4-7, 7-10, and 10-14.

Food consumption in MD and HD dams was significantly decreased during lactation. In MD dams, decreases were observed between Lactation Days 4 and 14, and in HD dams, decreases were observed between Lactation Days 0 and 14.

Reproduction parameters
The time of onset, number of pups (dead and alive), implantation scars, and completion of parturition were recorded, with any sign of dystocia noted.

There was a slight decrease in gestation index at the HD due to 1 HD dam euthanized early with dystocia and 1 HD dam with 11 implantation sites, but no delivered pups. An increase in number of dead pups was observed in HD dams that resulted in a statistically significant decrease in the live birth index. Gestation length was increased at the MD and HD.

Table: Summary of maternal reproduction parameters (Sponsor's)

Group	No. of Mated Females	No. of Pregnant Females	Pregnancy Rate (%)	Gestation Index (%)	Dead Pups Litters Affected	Dead Pups Pups Affected	Malformed Pups Litters Affected	Malformed Pups Pups Affected
1	24	23	95.8	100.0	0	0	0	0
2	24	24	100.0	100.0	5 *	8 **	0	0
3	24	23	95.8	100.0	2	3	0	0
4	24	24	100.0	91.7	14 ***	18 ***	0	0

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Group	Summary Information	Length of Gestation (Days)	Sex Ratio (% Males)	Number of Pups at Birth/Litters Live	Dead	Malformed	No. of Implant Scars	Live Birth Index (%)
1	Mean	21.5	48.92	11.3	0.0	0.0	12.0	94.29
	SD	0.5	14.28	2.0	0.0	0.0	1.9	7.70
	N	23	23	23	23	23	23	23
2	Mean	21.4	51.48	10.6	0.3	0.0	11.6	91.36
	SD	0.5	12.97	2.2	0.9	0.0	2.3	10.31
	N	24	24	24	24	24	23	23
3	Mean	21.9 D	47.40	10.2	0.1	0.0	11.3	94.09
	SD	0.3	16.41	2.5	0.5	0.0	2.3	5.69
	N	23	23	23	23	23	22	22
4	Mean	22.0 F	42.92	4.7 F	0.8	0.0	11.1	47.66 F
	SD	0.2	23.17	2.7	0.7	0.0	1.7	24.62
	N	23	21	22	22	22	19	19

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

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Necropsy
Necropsy of F_0 dams occurred at scheduled termination after weaning (Lactation Days 21-23).

There were no remarkable macroscopic findings in F_0 dams.

Observations and Results for F_1 Generation (pre-weaning)
Mortality and Clinical signs
Observations for general condition were conducted on F_1 pups daily during the lactation period.

There were 8, 25, and 56 pups that died between PNDs 0 and 4 from 5, 15, and 19 F_0 dams at the LD, MD, and HD, respectively; there were no F_1 pup deaths of control pups. There was further death of F_1 pups during PNDs 4 and 21 of 2 and 7 pups from 2 and 6 litters at the MD and HD, respectively. This resulted in decreased F_1 pup survival rates to PND 21 of 3%, 11%, and 61% at the LD, MD, and HD, respectively. In F_1 pups, clinical signs included dehydration, emptiness of the stomach, thinness, weakness, decreased activity, and coldness to touch that were generally observed between PNDs 0 and 4. Other signs observed in HD F_1 pups included abnormal flexure of the left (18 M and 11 F) and right (17 M and 9 F) hindlimbs between PNDs 0 and 4. Flat cranium was noted in 10 HDM and 7 HDF pups from PND 14 and malocclusion of the incisors was observed in 1 MDM pup and 1 HDF pup between PNDs 18 and 23. Abnormal flexure of the left and right forelimbs was observed in 1 MDM that also displayed flat cranium and abnormal gait. Anal atresia was observed in 1 MDF pup.

Body Weight
Body weight recordings for F_1 pups were taken on PNDs 4, 7, 14, 17, and 21.

Litter mean F_1 pup body weight was increased on PND 0 in M pups by 4% and 12% and in F pups by 5% and 8% at the MD and HD, respectively. The increase in body weight may be due to increase in gestation length noted at these doses. A 16-21% decrease in body weight in HDM and HDF pups was noted on PNDs 4, 7, and 14; however, body weights were comparable with controls at weaning.

Physical development
Observations and tests were conducted on all pups for righting reflex from PND 2, auricular startle response from PND 12, pinna unfolding from PND 2, and eye opening from PND 12.

Eye opening was statistically significantly earlier in MDF, HDM, and HDF by 0.5, 1.2, and 0.8 days, respectively. Auricular startle response was approximately 1 day later in HDM and HDF pups. No difference was observed in righting reflex or pinna detached development.

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Necropsy
Necropsy was conducted on F_1 pups that were found dead or euthanized between PNDs 0 and 7 and malformations and variations noted and on PNDs 21 to 23 on F_1 pups not selected for mating.

The malformation an absence of anal opening was noted in 1 MDF. Malformations in HD (2 M and 5 F) pups from 7 different litters included cleft palate (1 F), split tongue (1 F), truncus arteriosus (1 M), and subcutaneous edema over the entire body (1 M and 3 F). Variations noted in HD pups included vestigial liver lobe (1 M and 1 F), large spleen and kidney (1 F), edema/hematoma (4 M and 4 F), swollen soft cranium (4 F), and decreased anogenital distance (5 M). Abnormal flexure of hindlimbs was noted in 5 HDM and 3 HDF pups. The presence of testis and apparent uterine horn-like structure with no ovaries was noted in 1 MDM and 10 HDM pups. There were no remarkable findings in LD pups.

Macroscopic changes noted in 3 of 123 MD F_1 pups and in 2 out of 18 HD F_1 pups included an abnormal appearance of bone in 1 MDM and 1 HDM, a bent tail in 1 MDM, dilated ventricles in the heart in 1 MDF, and absence of digestive content in 1 HDF.

Observations and Results for F_1 Generation (post-weaning)
Placement of F_1 adults (post-weaning animals)

Group number	Number of Animals					
	Behavioral/ Reproductive subset		TDAR subset		Phenotyping subset	
	Males	Females	Males	Females	Males	Females
1/ Vehicle Control	23	23	10	10	10	10
2/ BAF312	24	24	10	10	10	10
3/ BAF312	22	23	10	10	10	10
4/ BAF312	11	12	-	-	-	-

TDAR = T-Cell Dependent Antibody Response

Mortality
Observations for mortality were conducted twice daily.

Deaths were observed in F_1 animals post-weaning; 1 control F (phenotyping subset) was euthanized due to poor condition on PND 24, 1 LDM and 1 MDF (phenotyping subset) were found dead on PND 24, and 1 MDM (TDAR subset) was found dead on Day 56, after blood collection. The cause of death of the LDM and MDF was undetermined. Due to low incidence and lack of dose-dependency the deaths were not considered drug-related.

Clinical signs
Observations were conducted once daily on the behavioral/reproductive subset. Detailed examinations were conducted on the days body weight was measured.

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Malocclusion of the incisors was noted from PND 21 in 2, 2, and 3 M and 2, 1, and 6 F at the LD, MD, and HD, respectively, and broken teeth were observed in 1 and 3 MDM and HDM, respectively. Flat cranium was noted in 1 HDM and 3 HDF from PND 21.

Body Weight
Body weight recordings were taken twice weekly after weaning. In mated F_1 F, body weight recordings were taken on GDs 0, 3, 7, 10, and 13.

There were slight decreases in body weight gain in M at PND 119 of 4%, 6%, and 7% at the LD, MD, and HD, respectively and in F at PND 84 of 4% at the HD. Body weight gain was comparable between the groups during gestation.

Food Consumption
Food consumption was recorded once weekly for the behavioral/reproductive subset until the start of cohabitation for mating and on GDs 0-3, 3-7, 7-10, and 10-13.

Food consumption was reduced during the first week post-weaning in HDM (89%) and HDF (84%) but was comparable between the groups for the remainder of the study, including gestation.

Hematology
Blood samples were taken from the phenotyping subset on PND 70 from control, LD, and MD groups; insufficient animals from HD F_0 dams were available for a HD group in the phenotyping subset (see placement of F_1 adult table above).

Dose-dependent decreases in WBC (15% and 18%), neutrophils (44% and 50%), lymphocytes (10% and 12%), monocytes (37% and 44%), and basophils (15% and 15%) were observed on PND 70 in LDM and MDM, respectively.

Physical development
Pupillary closure and visual placing responses of the behavioral/reproductive subset animals were tested on PND 21. Vaginal opening was assessed from PND 26; preputial separation was assessed from PND 35.

The pupillary closure response was positive in one eye in 1 MDM, negative in 2 HDM, positive in 1 MDF, and negative in 4 HDF; a positive response in both eyes was noted in all other pups. Preputial separation was approximately 2 days later in HDM and vaginal opening was statistically significantly later in HDF by 1.5 days.

Behavioral assessment
Tests conducted on the behavioral/reproductive subset animals included motor activity on PND 60 ± 2 for 1 hour, auditory startle on PND 55, and Cincinnati water maze (learning and memory) between PNDs 63 and 79. In the Cincinnati water maze, performance (ability to swim) was assessed and learning and memory was assessed using 2 paths.

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Motor activity: Ambulation was statistically significantly decreased in LDM and MDM and fine movement was decreased in MDM, but these changes were not observed at the HD.

Startle habituation: Startle habituation was comparable between siponimod-dosed and control groups in M and F.

Cincinnati water maze: Swimming ability, completion times, and number of errors in the Cincinnati water maze were comparable between siponimod-dosed and control groups in M and F.

Estrous cycle
The estrous cycle in behavioral/reproductive subset F were determined by examination of the vaginal lavage for at least 10 days prior to placement for mating and during mating until the day of positive identification of mating.

Estrous cycle length was comparable between siponimod-dosed and control F groups.

Reproduction parameters
On PNDs 86-89, mating pairs (1 M and 1 F) were set up from the behavioral/reproductive subset for up to 14 days. F were examined for evidence of mating. On GD 13, animals were euthanized, and the reproductive tract examined for number of corpora lutea, implantation sites, number of pups (dead and alive), and resorptions.

Mating and fertility indices, conception rate, mean number of days mating, number of corpora lutea, implantation sites, live and dead embryos, early resorptions, and pre- and post-implantation losses were comparable between siponimod-dosed and control groups.

Necropsy
Gross examination was conducted on behavioral/reproductive subset animals. Spleen and thymus from phenotyping subset animals were examined.

Macroscopic abnormalities of the teeth (malocclusion of the incisors) were noted in 1 and 3 in LDM and HDM groups, respectively and 2, 2, and 5 in LDF, MDF, and HDF groups, respectively.

Organ weights
At necropsy on PND 70, the spleen and thymus were collected from the phenotyping control, LD, and MD group animals and weighed; insufficient animals from HD F_0 dams were available for a HD group in the phenotyping subset (see placement of F_1 adult table above).

Dose-dependent increases in spleen and thymic weight were observed in LDF (8% and 3%, respectively) and MDF (16% and 10%, respectively).

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Histopathology
At necropsy, the following tissues were collected and examined microscopically; spleen and thymus from the phenotyping animals on PND 70 and bone (maxilla and mandible with teeth) from the behavioral/reproductive animals, after 2-3 weeks post-cohabitation period for M and at GD 13 for F.

No drug-related changes were observed in spleen or thymus of LD or MD animals. In mandibular incisors, abnormalities were observed in 2 HDM and 2 HDF that included a more tightly curved tooth, change in the position of the base of the tooth relative to the molar teeth, mild hemorrhage, and necrosis of the pulp cavity. These changes accompanied the malocclusion observed in 1 M and 2 F.

T-cell dependent antibody response (TDAR)
The T-cell dependent IgM and IgG antibody response was monitored in the TDAR subset using keyhole limpet hemocyanin (KLH). KLH was injected on PNDs 56 and 63. The anti-KLH IgM and IgG responses (antibody levels) were measured, by ELISA, 7 days following the KLH injections (i.e. PNDs 63 and 70). Blood samples were collected from control, LD, and MD animals (insufficient animals from HD F_0 dams were available for a HD group in the TDAR subset; see placement of F_1 adult table above), after each KLH injection and on PND 63 before KLH administration.

No anti-KLH IgM or IgG antibodies were detected in animals on PND 56 prior to KLH administration. Anti-KLH IgM antibodies were detected on PNDs 63 and 70 in control animals: 5 of 10 M and 9 of 10 F on PND 63 and all M and F on PND 70. Anti-KLH IgG antibodies were detected on PNDs 63 and 70 in control animals; 8 of 10 M and all F on PND 63 and all M and F on PND 70.

On PND 70, after 2 KLH injections, anti-KLH IgM and IgG antibody responses were observed in all animals from all groups. The mean levels of anti-KLH IgM and IgG antibodies were increased on PND 70 compared to PND 63 in all groups. The mean anti-KLH IgM and IgG antibody responses were comparable, with no statistically significant differences, in the LD and MD animals compared to control animals.

Table: Anti-KLH IgM responses (Sponsor's)

		MALES			FEMALES		
	Summary		Mean (µg/mL)			Mean (µg/mL)	
Group	Information	Day 56 post partum	Day 63 post partum	Day 70 post partum	Day 56 post partum	Day 63 post partum	Day 70 post partum
1	Mean	0.391 ø	4.825	15.425	0.391 ø	6.144	18.148
	Geometric Mean	0.391 ø	1.783	9.089	0.391 ø	4.768	14.689
	SD	0.000	6.008	15.632	0.000	3.091	11.104
	N	10	10	10	10	10	10
2	Mean	0.391 ø	7.433	24.224	0.391 ø	10.296	25.701
	Geometric Mean	0.391 ø	5.651	13.011	0.391 ø	5.567	13.320
	SD	0.000	3.654	25.952	0.000	12.668	30.033
	N	10	10	10	10	10	10
3	Mean	0.391 ø	6.553	12.951	0.391 ø	9.183	49.524
	Geometric Mean	0.391 ø	1.998	6.370	0.391 ø	5.402	27.912
	SD	0.000	9.552	15.694	0.000	9.634	58.889
	N	10	9	9	10	10	10

Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

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Table: Anti-KLH IgG responses (Sponsor's)

		MALES			FEMALES		
	Summary		Mean (µg/mL)			Mean (µg/mL)	
Group	Information	Day 56 post partum	Day 63 post partum	Day 70 post partum	Day 56 post partum	Day 63 post partum	Day 70 post partum
1	Mean	0.196 ø	1.105	43.773	0.196 ø	2.109	43.882
	Geometric Mean	0.196 ø	0.800	6.024	0.196 ø	1.974	28.084
	SD	0.000	0.898	123.314	0.000	0.776	36.094
	N	10	10	10	10	10	10
2	Mean	0.196 ø	1.296	26.285	0.196 ø	2.009	42.984
	Geometric Mean	0.196 ø	1.041	10.104	0.196 ø	1.716	22.024
	SD	0.000	0.922	33.795	0.000	1.201	48.343
	N	10	10	10	10	10	10
3	Mean	0.196 ø	1.032	5.709	0.196 ø	1.343	44.501
	Geometric Mean	0.196 ø	0.839	3.609	0.196 ø	1.201	20.190
	SD	0.000	0.586	7.015	0.000	0.649	54.036
	N	10	9	9	10	10	10

Significantly different from control group (group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Immunophenotyping

Blood (PND 70), spleen, and thymus samples were collected from the phenotyping subset control, LD, and MD groups (insufficient animals from HD F_0 dams were available for a HD group in the phenotyping subset; see placement of F_1 adult table above). The cellular antigens and cell populations identified in the table below were quantified, using specific antibodies against the marker antigens with the following exceptions: $CD3^-/CD45RA^+$ (B lymphocytes) and $CD3^-/CD161a^+$ (Natural-killer cells) were only assessed in blood and splenic samples and $CD4^+/CD8a^+$ and $CD4^-/CD8a^-$ (thymocyte cells) were only assessed in thymic samples.

Antigen marker(s)	Cell population identified
CD3-/CD45RA+	B lymphocytes
CD3+	Total T lymphocytes
CD3+/CD4+	Helper T lymphocytes
CD3+/CD8a+	Cytotoxic T lymphocytes
CD3-/CD161a+	Natural-killer cells
CD4+/CD8a+, and CD4-/CD8a-	Thymocyte subsets at different maturation stages

In blood, statistically significant increases were observed in absolute counts of T lymphocytes ($CD3^+$), helper T lymphocytes ($CD3^+/CD4^+$), and cytotoxic T lymphocytes ($CD3^+/CD8a^+$) on PND 70 in MDF. In spleen, statistically significant increases were observed in cells/spleen and/or cells/g counts of T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes, and total lymphocytes in MDF and in cytotoxic T lymphocytes in MDM. In thymus, no statistically significant changes were observed.

Table: Effect of siponimod on lymphocyte counts in blood compared to control animals

	Total Lymphocytes	B $CD3^-/CD45RA^+$	T $CD3^+$	Helper T $CD3^+/CD4^+$	Cytotoxic T $CD3^+/CD8a^+$	Natural-killer $CD3^-/CD161a^+$
MALE						
LD:	-10%	-13%	-10%	-10%	-6%	+2%
MD:	-12%	-20%	-3%	-9%	+10%	-46%
FEMALE						
LD:	+8%	-1%	+11%	+9%	+15%	-17%
MD:	+20%	-1%	+35%*	+33%*	+37%*	-31%

*** Statistically significant from control**

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Table: Effect of siponimod on lymphocyte counts in spleen compared to control animals

	Total Lymph ocytes	B CD3⁻/CD45RA⁺ cells/organ	B CD3⁻/CD45RA⁺ cells/g	T CD3⁺ cells/organ	T CD3⁺ cells/g	Helper T CD3⁺/CD4⁺ cells/organ	Helper T CD3⁺/CD4⁺ cells/g	Cytotoxic T CD3⁺/CD8a⁺ cells/organ	Cytotoxic T CD3⁺/CD8a⁺ cells/g	Natural-killer CD3⁻/CD161a⁺ cells/organ	Natural-killer CD3⁻/CD161a⁺ cells/g
MALE											
LD:	-1%	-10%	-5%	+4%	+10%	-5%	0	+21%	+31%	+39%	+44%
MD:	+5%	-5%	-1%	+17%	+22%	+8%	+10%	+38%	+46%*	+9%	+7%
FEMALE											
LD:	+27%	+30%	+22%	+25%	+18%	+20%	+13%	+31%	+23%	+11%	+6%
MD:	+60%*	+59%	+37%	+77%*	+54%*	+65%*	+43%*	+91%*	+67%*	+28%	+10%

*** Statistically significant from control**

Table: Effect of siponimod on lymphocyte counts in thymus compared to control animals

	Total Lymph ocytes	T CD3⁺ cells/organ	T CD3⁺ cells/g	Helper T CD3⁺/CD4⁺/CD8a⁻ cells/organ	Helper T CD3⁺/CD4⁺/CD8a⁻ cells/g	Cytotoxic T CD3⁺/CD4⁻/CD8a⁺ cells/organ	Cytotoxic T CD3⁺/CD4⁻/CD8a⁺ cells/g	Thymocyte CD4⁺/CD8a⁺ cells/organ	Thymocyte CD4⁺/CD8a⁺ cells/g	Thymocyte CD4⁻/CD8a⁻ cells/organ	Thymocyte CD4⁻/CD8a⁻ cells/g
MALE											
LD:	+4%	+3%	+9%	+6%	+11%	+5%	+7%	+3%	+9%	+2%	+7%
MD:	-20%	-18%	-17%	-14%	-13%	-15%	-16%	-21%	-20%	-21%	-20%
FEMALE											
LD:	+2%	+12%	+9%	+16%	+13%	+4%	+4%	+1%	-2%	-11%	-13%
MD:	+22%	+18%	+6%	+13%	+2%	+35%	+22%	+24%	+12%	-6%	-14%

*** Statistically significant from control**

10 Special Toxicology Studies

10.1 Local Tolerance

Primary skin irritation/corrosion study In the rabbit (4-hour semi-occlusive application). (*Study №: 0970033*)
The possible irritation or corrosion potential of siponimod (0.5 g moistened with 50% watery ethanol; 4-hour application) on skin was evaluated in New Zealand White rabbits (n = 3). Skin reactions were assessed at 1, 24, 48, and 72 hours post exposure.

No skin reactions to siponimod were observed.

Acute eye irritation/corrosion study In the rabbit. (*Study №: 0970034*)
The possible irritation or corrosion potential of siponimod (24 mg in 0.1 mL) on the eye was evaluated in New Zealand White rabbits (n = 3). Eye reactions were assessed at 1, 24, 48, and 72 hours and 7 days post-exposure.

Irritation, redness, chemosis, and discharge of the conjunctiva and iris were observed. Conjunctiva irritation was resolved in 7 days and iris irritation resolved in 24 hours.

Assessment of contact sensitizing potential with the murine local lymph node assay (LLNA TIER I). (*Study №: 0970036*)
The possible contact hypersensitivity potential of siponimod (0.5%, 5%, or 50% of 25 µL for 3 days) on dorsum ear was evaluated in Balb/c F mice (6/group). Ear reactions were assessed by visual examination of ears, sizes of ear-draining (auricular) lymph nodes, ear weight, ear-draining lymph node weights, and cell counts (lymph node hyperplasia)

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at 24 hours post-exposure. 1-Chloro-2,4-dinitrobenzene (0.5%) was used as the positive control.

Irritation of the ear was observed after visual examination in animals dosed at 0.5%, 5%, and 50% siponimod. An increase in lymph node weight was observed at 50% siponimod, but no increase in lymph node weight or cell counts was observed. The positive control (1-chloro-2,4-dinitrobenzene) animals had irritation of the ear and increased lymph node hyperplasia.

An acute intravenous, intra-arterial and perivenous administration tolerability study of BAF312 in the rabbit. (*Study №: 1270404*)
The tolerability of different routes of siponimod administration (0.025 mg/kg IV infusion over 5 hours, 0.00125 mg/kg IA over 15 minutes, and 0.0005 mg/kg perivenous over 20 minutes) was evaluated in New Zealand White M rabbits (3/group) for 5 days.

No drug-related macroscopic or microscopic findings were observed for any of the administration routes.

***In vitro* Balb/c 3T3 neutral red uptake phototoxicity assay.** (*Study №: 1770654*)
The possible phototoxicity potential of siponimod (0.316-1000 µg/mL incubated for 1 hour) was evaluated in Balb/c mouse 3T3 fibroblast cells using the neutral red assay. Chlorpromazine (0.25-100 µg/mL) was used as the positive control.

Cell survival, at the highest non-precipitating concentration of siponimod analyzed (100 µg/mL), was > 50% in the absence and presence of UV-A irradiation. IC_{50} values of neutral red uptake and PIF values could not be calculated. In contrast, cell survival in chlorpromazine had IC_{50} values equal to 54 and 4 µg/mL in the absence and presence of UV-A irradiation, respectively and a PIF value (14.9) > 5. Therefore, siponimod is non-phototoxic.

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10.2 Impurities

Study title: BAF312 (pure and spiked batches): 28 Day oral (gavage) administration impurity toxicity study in the rat with micronucleus assessment

Study no.:	1670150
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 22, 2016
GLP compliance:	Yes
QA statement:	Yes (Dated: December 11, 2017)
Drug, lot #, and % purity:	*TOX1/BAF312, Batch № 16/1, 96.3% (Nov 2017 assay) with (b) (4), and #BAF312, Batch № 1010010893, 99.7 (July 2017 assay) with (b) (4).

Key Study Findings

- Lymphocytes were reduced in all dose groups.
- Microscopic findings included increases in hyaline droplets in kidneys, hepatocyte and follicular cell hypertrophy, and vaginal mucification. Lymphoid findings were consistent with the pharmacological action of siponimod that included lymph node lymphoid depletion, splenic reduction in cellularity of periarteriolar lymphoid sheath, and thymic medullary enlargement and cortical thinning.
- TOX1/BAF312 did not induce micronuclei in the reticulocytes of peripheral blood.
- The groups dosed with TOX1/BAF312 appeared to have a similar severity of toxic effects to the BAF312 pure batch, with plasma exposure similar between spiked and pure BAF312 HD groups.
- These data suggest that degradation products/impurities (b) (4) at the levels used did not exacerbate the toxicities seen with siponimod.

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Methods

Doses:	0, 15*, 50*, and 50# mg/kg/day see above (Drug) for * and #; see table below for equivalent doses for impurities
Frequency of dosing:	Daily
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4)
Positive control for micronucleus assay:	Cyclophosphamide (10 mg/kg)
Species/Strain:	Rat/Wistar Han
Number/Sex/Group	
Main study:	10
Positive control group:	3
Age:	Approximately 10-11 weeks at start of dosing
Weight:	Males: 253.2-371.3 g; Females: 162.1-232.9 g

Group number	Group description	Dose level* (mg/kg/day)	Animal numbers Main Study (Subgroup 1) Male, n=10	Female, n=10
1	Control	0	R0001 - R0010	R0501 - R0510
2	Low (spiked)	15	R0101 - R0110	R0601 - R0610
3	High (spiked)	50	R0201 - R0210	R0701 - R0710
4	High (pure)	50	R0301 - R0310	R0801 - R0810

Group number	Group description	Dose level (mg/kg/day)	Animal numbers Positive control (Subgroup 2) Male n = 3
5	Positive control	10	R0401 - R0403

* Doses expressed in terms of the base. Salt/base ratio for BAF312 is 1.112

Table: Equivalent doses for components of formulations in Groups 2-4

Group	Dose mg/kg/day	BAF312 mg/kg/day (% of total)	Component (b) (4) mg/kg/day (% of total)	(b) (4) µg/m²	(b) (4) mg/kg/day (% of total)	(b) (4) µg/m²	(b) (4) mg/kg/day (% of total)	(b) (4) µg/m²
2	15	14.4 (96.3%¥)	0.210 (1.4%¥)	1260	0.165 (1.1%¥)	990	0.150 (1.0%¥)	900
3	50	48.2 (96.3%)	0.700 (1.4%)	4200	0.550 (1.1%)	3300	0.500 (1.0%)	3000
4	50	49.9 (99.7%⁺)	0.035 (0.07%⁺)	210	<0.025 (<0.05%⁺)	<150	<0.025 (<0.05%⁺)	<150
Human	0.0333*		0.0001	3.7	0.00005	1.8	0.00005	1.8

*** Human dose is 2 mg/day; ¥ Taken from Nov 2016 CoA; ⁺ Taken from July 2017 CoA. (b) (4) has a limit of (b) (4) has a limit of (b) (4) has a limit of (b) (4)**

Observations and Results

Mortality

Observations for ill health were conducted twice daily.

There were no deaths during the study.

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Clinical Signs
Observations were conducted daily at 3 hours post-dose. Detailed observations were conducted weekly.

There were no drug-related changes, with only occasional fur thinning/loss.

Body Weights
Body weights were recorded once prior to initiation of dosing, on Days 1, 4, 8, 11, 15, 18, 22, 25, and 28 during dosing, and at necropsy.

There was a decrease in body weight gain in pure BAF312 dosed animals during dosing that at Day 28 was 12% in M and 57% in F, compared to control animals. HDM TOX1/BAF312 decrease (16%) in body weight gain was similar to pure BAF312 HDM, but HDF TOX1/BAF312 decrease (6%) was less than the change in pure BAF312 HDF.

Table: Body weight gains

		Day 1-8			Day 1-28			
	Dose	Male		Female	Male		Female	
Group	(mg/kg/day)	Absolute	%	Absolute	Absolute	%	Absolute	%
Control	0	17.6 g	0%	3.8 g	53.8 g	0%	19.1 g	0%
TOX1/BAF312 spiked	15	11.3 g	-36%	-2.1 g	42.4 g	-21%	16.9 g	-12%
TOX1/BAF312 spiked	50	13.9 g	-21%	-4.5 g	45.1 g	-16%	18.0 g	-6%
BAF312 pure	50	15.5 g	-12%	-6.1 g	47.6 g	-12%	8.3 g*	-57%

Food Consumption
Food consumption was recorded weekly during dosing.

There was a decrease in food consumption during dosing with TOX1/BAF312 and BAF312 compared to control animals, especially during the first week (see table below).

Table: Mean food consumption (g/animal/day)

	Dose	Day 1-8				Day 1-29			
	(mg/kg/day)	Male		Female		Male		Female	
Group		Absolute	%	Absolute	%	Absolute	%	Absolute	%
Control	0	20.7 g	0%	13.7 g	0%	20.6 g	0%	13.9 g	0%
TOX1/BAF312 spiked	15	17.0 g	-18%	10.8 g	-21%	17.6 g	-15%	13.1 g	-6%
TOX1/BAF312 spiked	50	17.3 g	-16%	8.4 g	-39%	18.5 g	-10%	12.0 g	-14%
BAF312 pure	50	18.0 g	-13%	8.2 g	-40%	19.3 g	-6%	11.4 g	-18%

Ophthalmoscopy
Examinations were conducted prior to initiation of dosing on all animals and in Week 4 for animals in groups 1, 3, and 5.

No drug-related changes were observed.

Hematology
Blood samples were taken in Week 4.

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In M and F, all dosed groups had similar decreases in WBCs (65-73%), lymphocytes (87-90%), monocytes (18-62%), basophils (84-100%), and LUCs (91-100%).

Table: Hematology changes (Sponsor's)

	% Difference from Control					
	Male			Female		
	BAF312 (Spiked)		BAF312 (Pure)	BAF312 (Spiked)		BAF312 (Pure)
Dose (mg/kg/week)	15	50	50	15	50	50
Hemoglobin concentration	-5**	-6***	-5**	--	--	--
RBC	-8***	-4*	-7***	--	--	--
PCV	-6***	-5**	-4*	--	--	--
Absolute reticulocyte count	--	+17**	+19***	+31*	+25	+27
Percent reticulocyte	+18**	+22***	+28***	+33	+26	+31
White blood cell count	-68***	-69***	-72***	-73***	-65***	-72***
Lymphocyte absolute count	-89***	-89***	-90***	-90***	-87***	-89***
Monocyte absolute count	-50***	-54***	-62***	-38	-18	-30
Basophil absolute count	-100	-100	-93	-84	-100	-95
Large unstained cell absolute count	-91***	-91***	-94***	-92***	-100***	-96***
Fibrinogen concentration	--	--	--	+11**	+19***	+13***

-- = No test article-related effect; + = Increased; - = Decreased; Mean value for parameter in treated group differed from control mean at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Clinical Chemistry

Blood samples were taken in Week 4.

Statistically significant changes in clinical chemistry parameters (protein, albumin, globulin, calcium, and TCO2) in animals administered spiked TOX1/BAF312 or pure BAF312 were generally comparable between the sexes and between the doses at 50 mg/kg/day.

Table: Clinical chemistry changes (Sponsor's)

	% Difference from Control					
	Male			Female		
	BAF312 (Spiked)		BAF312 (Pure)	BAF312 (Spiked)		BAF312 (Pure)
Dose (mg/kg/week)	15	50	50	15	50	50
Total protein concentration	-9***	-9***	-8***	-10***	-12***	-10***
Albumin concentration	--	-5*	-5*	-13***	-16***	-14***
Globulin concentration	-19***	-17***	-15**	--	--	--
A: G ratio	+20**	+16*	+12	-13	-15*	-17*
Calcium concentration	--	--	--	-6***	-5***	-4*
TCO2 concentration	+6*	+8***	+10***	--	+8*	+10**

-- = No test article-related effect; + = Increased; - = Decreased; Mean value for parameter in treated group differed from control mean at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Urinalysis

Urine samples were taken in Week 4.

There were increases in mean specific gravity in M, pH in F, protein in M, and ketones in M administered spiked TOX1/BAF312 or pure BAF312. The increases were generally comparable between the HD of BAF312 pure and spiked batches.

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Table: Urinalysis changes (Sponsor's)

	% Difference from Control					
	Male			Female		
	BAF312 (Spiked)		BAF312 (Pure)	BAF312 (Spiked)		BAF312 (Pure)
Dose (mg/kg/week)	15	50	50	15	50	50
Urine specific gravity	--	+1**	+2***	--	--	--
Urine pH	--	--	--	+13**	+14**	+10*

-- = No test article-related effect; + = Increased; - = Decreased; Mean value for parameter in treated group differed from control mean at * p≤ 0.05; ** p≤ 0.01; *** p≤ 0.001

Gross Pathology

Gross pathology was assessed in all animals.

The macroscopic findings were generally unremarkable. Observed changes included dark (0, 0, and 2), large (0, 2, and 1), and mottled (1, 2, and 0) livers in spiked TOX1/BAF312 LD, spiked TOX1/BAF312 HD, and pure BAF312 HDM, respectively, and pale lungs at the spiked TOX1/BAF312 HD in 1 M and 1 F.

Organ Weights

At necropsy, the organs listed in the table below, in the histopathology section from the study report, were weighed.

In HDM groups, statistically significant changes were observed in the mean weight of liver and spleen; these changes were similar between the spiked TOX1/BAF312 and pure BAF312 groups. In HDF groups, statistically significant changes were observed in the mean weight of heart, liver, and spleen; there were no major differences between spiked TOX1/BAF312 and pure BAF312 groups, apart from spleen, which was not as reduced in spiked TOX1/BAF312 HDF. It is noted that lungs were not weighed.

Table: Organ changes in mean absolute weight compared to control organ weights

	Adrenal	Brain	Epidid	Heart	Kidney	Liver	Pituitary	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
MALES												
LD#:	-9%	0%	-7%	-0%	-10%	-3%	-11%	-8%	-20%*	+2%	-15%	-8%
HD#:	+8%	+3%	-10%	+1%	-6%	+14%*	-1%	-11%	-17%*	0%	+2%	+8%
HD:	+1%	+2%	-13%	+3%	-1%	+14%*	+3%	-14%	-19%*	-5%	-11%	+4%
FEMALES												
LD#:	+10%	+2%	-	+15%*	+5%	+21%*	-7%	-11%	-15%*	-11%	+28%	+3%
HD#:	+14%	+1%	-	+15%*	+4%	+32%*	-11%	-8%	-9%	-2%	+20%	+7%
HD:	+7%	+1%	-	+15%*	+4%	+24%*	-14%	-10%	-25%*	-22%	+16%	+15%

 # spiked siponimod groups; * Statistically significant from control; Epidid - epididymis.

Table: Organ changes in mean weight (relative to body weight) compared to control organ weights

	Adrenal	Brain	Epidid	Heart	Kidney	Liver	Pituitary	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
MALES												
LD#:	+1%	+12%*	+5%	+12%*	+1%	+9%*	+1%	+2%	-11%	+14%	-6%	+4%
HD#:	+14%*	+8%*	-5%	+7%	-1%	+21%*	+7%	-7%	-12%*	+6%	+7%	+15%
HD:	+8%	+9%*	-7%	+11%*	-7%	+24%*	+5%	-8%	-12%*	+2%	-5%	+13%

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	Adrenal	Brain	Epidid	Heart	Kidney	Liver	Pituitary	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
FEMALES												
LD#:	**+4%**	**-4%**	**-**	**+8%**	**-1%**	**+14%***	**-9%**	**-16%**	**-19%***	**-16%**	**+21%**	**-3%**
HD#:	**+10%**	**-2%**	**-**	**+13%***	**+2%**	**+29%***	**-11%**	**-11%**	**-12%**	**-6%**	**+17%**	**+4%**
HD:	**+6%**	**0%**	**-**	**+15%***	**+3%**	**+23%***	**-14%**	**-11%**	**-26%***	**-23%**	**+16%**	**+13%**

 # spiked siponimod groups; * Statistically significant from control; Epidid - epididymis.

Histopathology

At necropsy the following organs, listed in the study report, were prepared for histopathology examination. Tissues were fixed and all tissues were examined from control, spiked TOX1/BAF312 HD (Group 3), and pure BAF312 HD groups (Group 4), and lung and gross lesions were examined from spiked TOX1/BAF312 LD (Group 2).

Organ/Tissue			Organ/Tissue		
adrenal	W	E	nasopharynx		
animal identification			nerve, optic		E
aorta		E	nerve, sciatic		E
bone marrow smear (femur)[a,b]			ovary	W	E
brain	W	E	oviduct		E
caecum		E	pancreas		E
colon		E	parotid salivary gland		E
duodenum		E	pituitary	W	E
epididymis[d]	W	E			
esophagus		E	prostate	W	E
eye[c]		E	rectum		E
femur with bone marrow and femorotibial joint		E	seminal vesicle		E
GALT/Peyers patch		E	skin and subcutis		E
gross lesions		E	spinal cord, cervical		E
Harderian gland		E	spinal cord, lumbar		E
head			spinal cord, thoracic		E

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Organ/Tissue			Organ/Tissue		
heart	W	E	spleen	W	E
ileum		E	sternum with bone marrow		E
jejunum		E	stomach		E
kidney	W	E	sublingual salivary gland		E
lacrimal gland[e]		E	testis[d]	W	E
larynx		E	thymus	W	E
liver	W	E	thyroid with parathyroid	W[f]	E
lungs with main stem bronchi and bronchioles		E	tongue		E
lymph node, mandibular		E	trachea		E
lymph node, mesenteric		E	ureter		E
mammary gland		E	urinary bladder		E
mandibular salivary gland		E	uterus with cervix	W	E
muscle, quadriceps		E	vagina		E
nasal cavity					

W = weighed; E = processed and examined microscopically
Bone designated for histopathological examination was decalcified using Kristenson's fluid.
a methanol fixative
b see Clinical Pathology Section 3.4.2
c Davidson's fluid fixative
d Modified Davidson's fixative
e Preserved with head (*in situ*)
f Tissues weighed together

Adequate Battery: Yes
Peer Review: Yes, by (b) (4), Dr. Med. Vet., DECVP.
Signed Pathology report: Yes, by (b) (4), BVSc, MRCVS (page 295 of the study report).

Histological findings
The main histological findings were observed in the kidney, liver, thyroid gland, vagina, and lymphoid organs. Findings included increased severity of hyaline droplets in kidneys, hepatocyte hypertrophy, follicular cell hypertrophy, and vaginal mucification. Lymphoid findings were consistent with the pharmacological action of siponimod and included lymphoid depletion in lymph nodes, reduced cellularity of the periarteriolar lymphoid sheath (PALS) in spleen, and medullary enlargement and cortical thinning in thymus. The findings in the HD animal groups were generally similar, with no substantial difference between the spiked TOX1/BAF312 and pure BAF312 HD groups.

Table: Summary of microscopic findings

		MALE				FEMALE			
Tissue	**Finding**	**1 0**	**2 15#**	**3 50#**	**4 50**	**1 0**	**2 15#**	**3 50#**	**4 50**
Heart	Myocardium inflammation								
	minimal:	**0/10**	**0/0**	**0/10**	**0/10**	**0/10**	**0/0**	**0/10**	**0/10**
	slight:	**0/10**	**0/0**	**1/10**	**0/10**	**0/10**	**0/0**	**0/10**	**0/10**
Kidney	Hyaline droplets								
	minimal:	**3/10**	**0/0**	**0/10**	**0/10**	**0/10**	**0/0**	**0/10**	**0/10**
	slight:	**6/10**	**0/0**	**8/10**	**8/10**	**0/10**	**0/0**	**0/10**	**0/10**
	moderate:	**1/10**	**0/0**	**2/10**	**2/10**	**0/10**	**0/0**	**0/10**	**0/10**

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Tissue	Finding	MALE 1 0	MALE 2 15#	MALE 3 50#	MALE 4 50	FEMALE 1 0	FEMALE 2 15#	FEMALE 3 50#	FEMALE 4 50
Liver	Centrilobular Hepatocyte hypertrophy								
	minimal:	0/10	1/1	7/10	8/10	0/10	0/0	9/10	8/10
	slight:	0/10	0/1	3/10	2/10	0/10	0/0	1/10	2/10
	Agonal congest/hemorrhage								
	present:	0/10	1/1	2/10	2/10	0/10	0/0	0/10	0/10
Lungs	Increased alveolar macrophages								
	minimal:	0/10	0/10	1/10	0/10	0/10	1/10	0/10	0/10
	slight:	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
Lymph Node - Mandibular	Lymphoid depletion								
	minimal:	0/10	0/0	6/10	6/10	0/10	2/2	5/10	5/10
	slight:	0/10	0/0	2/10	1/10	0/10	0/2	3/10	1/10
	Sinus histiocytosis								
	minimal:	1/10	0/0	4/10	6/10	0/10	1/2	4/10	5/10
	slight:	0/10	0/0	2/10	4/10	0/10	1/2	4/10	2/10
	Agonal congestion/hemorrhage								
	present:	0/10	0/0	1/10	0/10	0/10	2/2	2/10	0/10
Lymph Node - Mesenteric	Lymphoid depletion								
	minimal:	0/10	0/0	3/10	4/10	0/10	1/1	4/10	2/10
	slight:	0/10	0/0	6/10	6/10	0/10	0/1	6/10	8/10
	Sinus histiocytosis								
	minimal:	0/10	0/0	5/10	4/10	0/10	0/1	5/10	7/10
	slight:	0/10	0/0	4/10	2/10	0/10	1/1	5/10	3/10
Ovary	Reduced corpora lutea								
	present:	-	-	-	-	0/10	0/0	4/10	3/10
Spleen	PALS reduced cellularity								
	minimal:	0/10	0/0	1/10	1/10	0/10	0/0	5/10	4/10
	slight:	0/10	0/0	7/10	6/10	0/10	0/0	4/10	5/10
	moderate:	0/10	0/0	1/10	3/10	0/10	0/0	1/10	1/10
	Hematopoiesis								
	minimal:	8/10	0/0	3/10	1/10	6/10	0/0	1/10	3/10
	slight:	1/10	0/0	7/10	8/10	2/10	0/0	8/10	7/10
	moderate:	1/10	0/0	0/10	1/10	0/10	0/0	1/10	0/10
Thymus	Medullary enlargement/cortical thinning								
	minimal:	0/10	0/0	4/10	1/10	0/10	0/0	5/10	6/10
	slight:	0/10	0/0	6/10	9/10	0/10	0/0	5/10	4/10
Thyroid	Follicular cell hypertrophy								
	minimal:	0/10	0/0	4/10	4/10	0/10	0/0	0/10	0/10
	slight:	0/10	0/0	1/10	0/10	0/10	0/0	0/10	0/10
Vagina	Mucification								
	minimal:	-	-	-	-	0/10	0/0	5/10	5/10
	slight:	-	-	-	-	0/10	0/0	0/10	0/10
	moderate:	-	-	-	-	0/10	0/0	1/10	0/10

Groups: 1 - Control, 2 - 15 mg/kg spiked TOX1/BAF312, 3 - 50 mg/kg spiked TOX1/BAF312, 4 -50 mg/kg pure BAF312; # spiked with impurities.

Toxicokinetics

Blood samples were taken during Week 4 at 0.5, 1, 3, 6, and 24 hours post-dose. Two animals/sex/group were sampled at each time point.

No siponimod was detected in control samples (LLOQ = 9.45 ng/ml). T_{max} was 3 hours in M and 3-6 hours in F and plasma exposure (C_{max} and AUC) increased approximate

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dose-proportionally in M and F, with plasma exposure similar between spiked TOX1/BAF312 and pure BAF312 HD groups. Plasma exposure was greater in F compared to M.

Table: Toxicokinetic parameters (Sponsor's)

Dose Group	Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	DN C_{max} [(ng/mL) / (mg/kg/day)]	t_{max} (h)	$AUC_{(0\text{-}24\ h)}$ (h*ng/mL)	DN $AUC_{(0\text{-}24\ h)}$ [(h*ng/mL) / (mg/kg/day)]
2	15 (spiked)	M	1420	94.7	3	14900	995
		F	5190	346	6	106000	7060
3	50 (spiked)	M	4750	95.0	3	48700	975
		F	12800	256	6	260000	5200
4	50 (pure)	M	5170	103	3	55700	1110
		F	12900	258	3	218000	4360

DN = dose normalized.

Micronucleus assay

Blood samples were taken on Day 3 approximately 24 hours post second-dose, in Week 4, and at necropsy no later than 36-48 hours post final dose. Only data from control and HD TOX1/BAF312 in Week 4 and positive control (10 mg/kg cyclophosphamide) collected on Day 29 were reported. Approximately 20000 reticulocytes were analyzed and the number of mature erythrocytes (ME), reticulocytes (RET), micronucleated reticulocytes (MN RET), and total RBC were recorded.

Mean frequencies of MN RET in HDM and HDF spiked TOX1/BAF312 group were similar to the mean frequencies observed in control M. Spiked TOX1/BAF312 did not induce MN RET in peripheral blood.

Table: Micronucleus data for spiked BAF312 (Sponsor's)

Group / Treatment (mg/kg/day)	%RET (Mean±SD)	%MNRET (Mean±SD)	Significance	%MNME (Mean±SD)
1 / Vehicle (0), males	1.37+0.20	0.14+0.04	-	0.011+0.006
3 / BAF312 (50), males	1.73+0.15	0.13+0.02	NS	0.008+0.004
5 / CPA (10), males	0.33+0.20	1.37+0.27	$p \leq 0.05$	0.010+0.001
1 / Vehicle (0), females	1.24+0.31	0.13+0.04	-	0.006+0.003
3 / BAF312 (50), females	1.46+0.16	0.09+0.03	NS	0.003+0.000

NS = Not Significant, RET = reticulocytes, MNRET = micronucleated reticulocytes, MNME = micronucleated mature erythrocytes, CPA = cyclophosphamide.

Reference ID: 4392197

Study title: (b) (4) **: Ames test**

Study no.:	0912575
Study report location:	EDR
Conducting laboratory and location:	No information was provided.
Date of study initiation:	No information was provided.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(b) (4) Batch No. 700-020-12, >97%

Key Study Findings:

- An increase in the number of revertants in TA100 in the presence of S9 was observed.

Methods

Strains:	*S. typhimurium* TA97a, TA98, TA100, TA102, and TA1535.
Concentrations in definitive study	
Experiment 1 with TA98, TA100, and TA1535 +/- S9:	12.71, 42.37, 127.11, 423.73, 1271.19, and 4237.29 µg per plate.
Experiment 2 with TA98 +S9, TA100 +S9, and TA1535 - S9:	211.86, 423.73, 635.59, and 847.46 µg per plate.
Experiment 2 with TA97a and TA102 +/- S9:	12.71, 42.37, 127.11, 423.73, 847.46, 1694.92 µg per plate.
Basis of concentration selection:	Not provided.
Negative control:	No information was provided.
Positive controls:	
Without S-9 mix:	3 µg Sodium azide for TA1535 and TA100, 100 µg 9-aminoacridine for TA97a, 2 µg 2-nitrofluorene for TA98, and 0.5 µg mitomycin C for TA102.
With S-9 mix:	3 µg 2-Aminoanthracene (2AA) on TA1535, TA98, and TA100, 10 µg 2AA for TA97a and TA102, 3 µg benzo(a)pyrene for TA98.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	No information was provided.

Study Validity

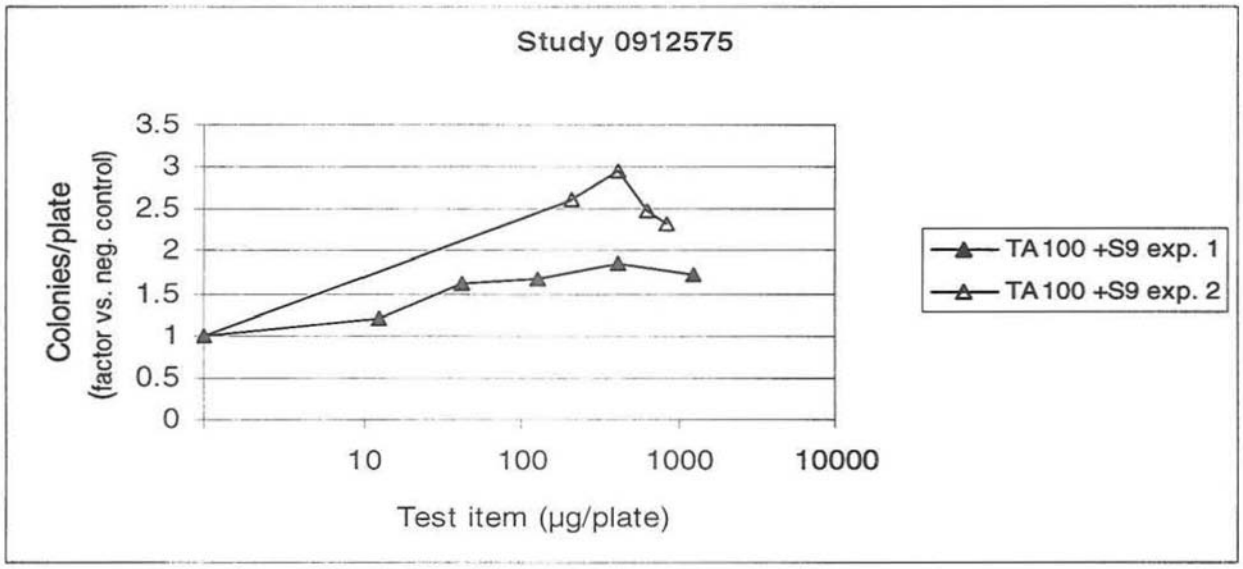
The plate incorporation test was conducted. The S-9 mix was prepared from M rats dosed with Aroclor 1254. No further information was provided; therefore, it is not possible to confirm if the study is valid.

Reference ID: 4392197

Results

There was no precipitation, but signs of cytotoxicity were observed at 1271.19 µg/plate in experiment 1, and 1694.92 µg/plate in strain TA97a in the presence and absence of S9 and ≥847.46 µg/plate in strain TA102 in the presence and absence of S9 in experiment 2. No individual data was provided, but the sponsor reported that "*the mean mutant numbers of negative control plates lay within the range of acceptable negative control values*" and "*treatment with the test item increased the number of revertants in strain TA100 +S9.*"

Figure: Increased number of revertants in strain TA100 (Sponsor's)



The CMC reviewer noted that [redacted] (b) (4) is covered by [redacted] (b) (4) test in DS specification. The TTC-based acceptable intake is set for NMT 1.5 ug/day (or 750 ppm for 2 mg/day MDD).

Study title: [redacted] (b) (4) **Ames test**

Study no.:	1112610
Study report location:	EDR
Conducting laboratory and location:	No information was provided.
Date of study initiation:	No information was provided.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[redacted] (b) (4) Batch No. BAF312-ehrsaan1-026, >97%

Key Study Findings:

- **No increase in number of revertants was observed in any strain at any dose of** [redacted] (b) (4)

Reference ID: 4392197

Methods

Strains:	*S. typhimurium* TA97a, TA98, TA100, TA102, and TA1535.
Concentrations in definitive study	
Expt 1: TA98 and TA100:	1.6, 8, 40, 200, 1000, and 5000 µg per plate.
Expt 2: TA97a, TA102, and TA1535:	1.6, 8, 40, 200, 1000, and 5000 µg per plate.
Basis of concentration selection:	The HD recommended by OECD guidelines
Negative control:	DMSO
Positive controls	
Without S-9 mix:	3 µg Sodium azide for TA1535 and TA100, 100 µg 9-aminoacridine for TA97a, 2 µg 2-nitrofluorene for TA98, and 0.5 µg mitomycin C for TA102.
With S-9 mix:	3 µg 2-Aminoanthracene (2AA) on TA1535, TA98, and TA100, 10 µg 2AA for TA97a and TA102, 3 µg benzo(a)pyrene for TA98.
Formulation/Vehicle:	DMSO.
Incubation & sampling time:	No information was provided.

Study Validity

The plate incorporation test was conducted. The S-9 mix was prepared from M rats dosed with Aroclor 1254. Triplicates were conducted in the study. No further information was provided; therefore, it is not possible to confirm if the study is valid.

Results

There was no precipitation, but signs of cytotoxicity were observed at 5000 µg/plate in all strains in the presence and absence of S9. No increase in revertant colonies was observed in any strain at any dose of [redacted] (b) (4)

The CMC reviewer noted that if [redacted] (b) (4) is controlled at NMT [redacted] (b) (4) % in D3, the remaining step and crystallization will likely purge to a high extent – also low MDD for this drug product (so high TTC ppm limit).

Reference ID: 4392197

10.3 Immunotoxicity

Study title: 4-Week oral (gavage) immunotoxicity study in rats with an 8-week recovery period

Study no:	0770500
Study report location:	EDR
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation, East Hanover Facility, New Jersey, 07936 USA
Date of study initiation:	August 29, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	BAF312, Batch № 0822007, 100%

Key Study Findings

- No deaths or adverse clinical signs were observed, but body weight gain was decreased at all doses.
- There were decreases in WBC and lymphocytes at all doses in M and F.
- Decreases in peripheral blood and splenic total T cell, T helper, T cytotoxic, and B cell counts were noted at all doses in M and F. Increases in $CD3^+CD4^+CD8a^-$, $CD3^+CD4^-CD8a^+$, and $CD3^+$ counts in the thymus were noted.
- A decrease in the primary and secondary response to the T cell dependent antigen, KLH, was noted at all doses in M and F.
- Generally, all these changes were reversible.

Reference ID: 4392197

Methods

Doses	
Males:	0, 0, 1.5, 10, 50, and 50 mg/kg
Females:	0, 0, 0.3, 10, 50, and 50 mg/kg (see table below)
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	(b) (4)
Species/Strain:	Rat/Wistar Han
Number/Sex/Group	
Main:	10
Recovery:	6 for control and HD groups
Age:	Approximately 8 weeks at initiation of dosing
Weight:	Males 296.8-235.0 g; Females 150.4-183.8 g

Group*	Number/sex	Animal numbers males	Dose (mg/kg/day) Base/Salt**	Concentration (mg/mL) Salt**
1 Control	10 +6 recovery	1001-10 1011-16	0	0
2 Control + KLH	10	2001-10	0	0
3 Low + KLH	10	3001-10	1.5/1.67	0.33
4 Mid + KLH	10	4001-10	10/11.1	2.2
5 High + KLH	10	5001-10	50/55.6	11.1
6 High	10 +6 recovery	6001-10 6011-16	50/55.6	11.1

* On days 11 and 19, Groups 2, 3, 4, and 5 received 300 µg KLH antigen formulation by subcutaneous injection and non-recovery animals from Groups 1 and 6 received antigen control (Sterile Water for Inj., USP) by subcutaneous injection. On recovery days 41 and 50, Groups 1 and 6 received 300 µg KLH antigen formulation by subcutaneous injection.
** Salt/base ratio for BAF312 is 1.112.

Observations and Results
Mortality
Observations were conducted twice daily on weekdays and at least daily on weekends.

No deaths occurred during the study.

Clinical Signs
Observations were conducted once daily on non-dosing days and twice daily during dosing (pre-dose and within 3 hours post-dose).

No adverse clinical signs were observed during the study.

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Body Weight
Body weight recordings were taken once prior to initiation of dosing and weekly thereafter.

In M, there was a decrease in mean body weight gain of 20%, 17%, and 10-13% at the LD, MD, and HD, respectively, during the 4 weeks of dosing. In F, there was a decrease in mean body weight gain of 11%, 1%, and 12-16% at the LD, MD, and HD, respectively, during the 4 weeks of dosing. Body weight gain was comparable in recovery control and HDM but was further decreased in HDF.

Food Consumption
Food consumption was recorded once weekly.

In M, food consumption at the LD, MD, and HD was significantly decreased during the first week of dosing by 9%, 9%, and 10-16%, respectively. In F, food consumption at the HD was significantly decreased during the first week of dosing by 23-24%.

Hematology
Blood samples were taken on Day 29 during dosing and during recovery on Day 41 (prior to KLH immunization), Day 50 (prior to KLH booster), and Day 60 (used for immunophenotyping).

The main changes in hematology parameters were marked decreases (approximately 80%) in WBC and lymphocytes at all doses in M and F. Other changes noted were decreases in monocytes, eosinophils, basophils, and large unstained cell counts at all doses in M and F. These changes were reversed during the recovery period with levels in HD animals comparable to control animals.

Table: Changes in WBC and lymphocytes compared to control animals (Sponsor's)

	MALE				FEMALE			
Parameter	1.5 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)	0.3 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)
White blood cell count	-76	-79	-81	-81	-82	-80	-78	-78
Total lymphocyte count	-84	-88	-90	-90	-90	-90	-88	-89

Mean % difference = (mean dose group value - mean control (Group 1) value)/ mean control (Group 1) value)x100%

Necropsy
Gross examination was conducted.

No major macroscopic findings were observed.

Organ weights
At necropsy, brain, spleen, and thymus were collected.

In HDM and HDF, statistically significant decreases in mean spleen weight (15% and 21%, respectively) and increases in mean thymic weight (26% and 19%, respectively)

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were observed. Mean spleen and thymus weights were comparable at the end of the recovery period in control and HD M and F.

Histopathology
At necropsy, the following tissues were collected and examined microscopically.

	P	bone marrow (in bone)	W	P	spleen
	P	bone marrow smear		P	sternum
W		brain	W	P	thymus
	P	injection sites		P	macroscopic lesions
	P	lymph node - mandibular			animal identification
	P	lymph node - mesenteric			

In thymus, medullary enlargement containing an increased number of small lymphocytes was observed at all doses in M and F. Lymphoid depletion in the periarteriolar lymphoid sheath areas at all doses in M and F and decreased erythropoiesis in HDM and HDF were observed in spleen. In lymph nodes, depletion of lymphocytes in the cortical/paracortical (mainly T-cell dependent) areas was observed in HDM and HDF. None of these changes were observed at the end of the recovery period.

T-cell dependent antibody response (TDAR)
The T-cell dependent IgM and IgG antibody response was monitored using KLH. KLH was injected on dosing Days 11 and 19 to animals in Groups 2, 3, 4, and 5 and on recovery Days 41 and 50 to animals in Groups 1 and 6. The anti-KLH IgM and IgG responses (antibody levels) were measured, by ELISA, on Days 11 (prior to KLH immunization), 19 (prior to KLH booster), and 29 during dosing and Days 41 (prior to KLH immunization), 50 (prior to KLH booster), and 60 during recovery.

Anti-KLH IgM and IgG antibodies were detected in the majority of control animals during dosing and all animals during recovery. On Days 19 and 29, after 2 KLH injections, anti-KLH IgM and IgG antibody responses were decreased in all M and F dosed groups compared to control, although the changes were not statistically significant. The decreases in the IgM and IgG antibody responses were reversible, with antibody levels comparable on Days 50 and 60 in control and HD animals.

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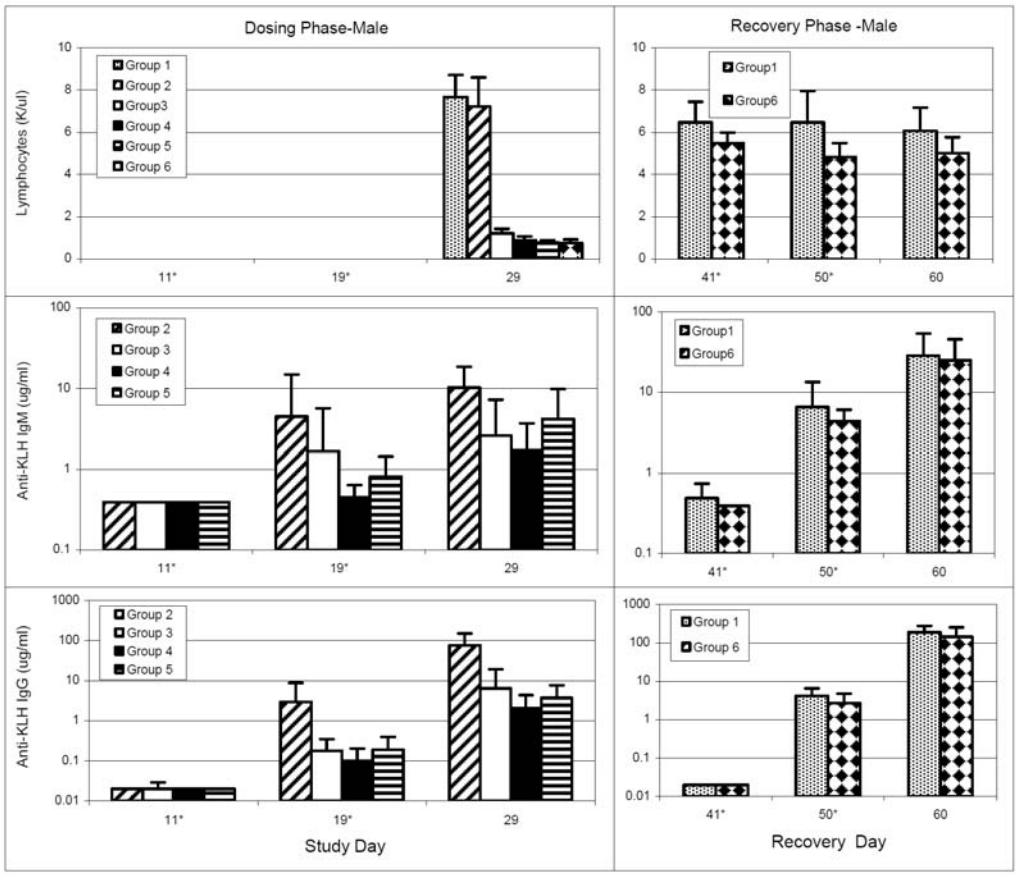
Figure: Changes in total lymphocyte counts correlated with anti-KLH IgM and IgG antibody concentrations following administration of siponimod (Sponsor's)
MALE



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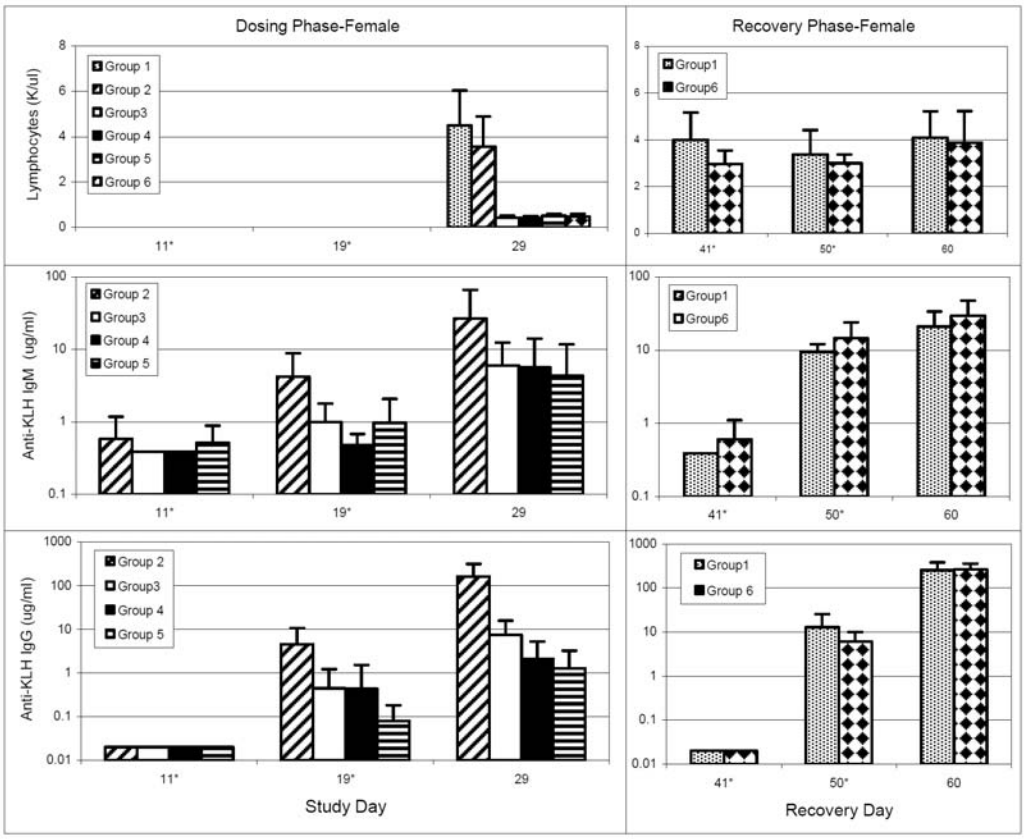
FEMALE



* indicates KLH injection time points

Note: samples for total lymphocyte counts were not collected during study days 11 and 19 of the dosing phase

Note: values for anti-KLH IgM antibodies are on log-scale (0.1 to 100) and values for anti-KLH IgG antibodies are on a log scale (0.01 to 1000)

Immunophenotyping

Blood samples from dosing Day 29 and during recovery on Day 60 were collected. Spleen and thymus samples were collected at necropsy. The cellular antigens and cell populations identified in the table below were quantified, using specific antibodies against the marker antigens: $CD3^+$ (total T lymphocytes), $CD3^+CD4^+$ (T helper lymphocytes), $CD3^+CD8a^+$ (T cytotoxic lymphocytes), $CD3^-CD161a^+$ (Natural killer (NK) lymphocytes), and $CD45RA^+$ (B lymphocytes) for blood and splenic samples, and $CD3^+$, $CD3^+CD4^+CD8a^+$, $CD3^-CD4^-CD8a^-$, $CD3^+CD4^+CD8a^-$, and $CD3^+CD4^-CD8a^+$ for thymic samples.

In blood, statistically significant marked decreases in absolute counts of T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes, and B lymphocytes were observed at all doses in M and F. In spleen, statistically significant decreases in cells/spleen and/or cells/g counts of T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes, B lymphocytes, and absolute lymphocytes were observed at all doses in M and F. No changes were noted in Natural Killer cells in blood and spleen. In thymus, statistically significant increases in absolute counts of $CD3^+/CD4^+/CD8a^-$, $CD3^+/CD4^-/CD8a^+$, and

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$CD3^+$ were observed at all doses in M and F. All the changes were reversible, with comparable counts observed in control and HD animals in Week 9.

Table: Mean percent differences in peripheral blood lymphocyte subsets following administration of siponimod (Sponsor's)

	MALE				FEMALE			
Parameter	1.5 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)	0.3 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)
Total T (CD3+)	-98	-99	-99	-99	-99	-99	-98	-98
T helper (CD3+CD4+)	-98	-99	-99	-100	-100	-100	-100	-100
T cytotoxic (CD3+CD8a+)	-95	-96	-97	-97	-98	-97	-95	-95
B (CD45RA+)	-66	-78	-80	-80	-83	-81	-75	-78

Mean % difference = (mean dose group value - mean control (Group 1) value)/ mean control (Group 1) value) x 100%

Table: Mean percent differences in spleen immunophenotyping parameters following administration of siponimod (Sponsor's)

MALE

Parameter	1.5 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)
Abs Lymph*/organ	-40	-41	-39	-41
Abs Lymph/gram	-30	-34	-33	-31
Total T (CD3+)/organ	-83	-82	-80	-84
T helper (CD3+CD4+)/organ	-85	-84	-81	-85
T cytotoxic (CD3+CD8a+)/organ	-80	-80	-78	-81
B (CD45RA+)/organ	-47	-47	-46	-40
Total T (CD3+)/gram	-81	-80	-78	-81
T helper (CD3+CD4+)/gram	-83	-82	-79	-83
T cytotoxic (CD3+CD8a+)/gram	-77	-78	-76	-78
B (CD45RA+)/gram	-38	-41	-40	-30

Mean % difference = (mean dose group value - mean control (Group 1) value)/ mean control (Group 1) value)x100%; * Abs Lymph= absolute lymphocyte count

FEMALE

Parameter	0.3 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)
Abs Lymph*/organ	-21	-25	-33	-39
Abs Lymph/gram	-19	-19	-20	-22
Total T (CD3+)/organ	-82	-82	-87	-85
T helper (CD3+CD4+)/organ	-83	-83	-88	-87
T cytotoxic (CD3+CD8a+)/organ	-82	-81	-85	-83
B (CD45RA+)/organ	-24	-44	-37	-50
Total T (CD3+)/gram	-81	-81	-84	-81
T helper (CD3+CD4+)/gram	-82	-81	-85	-83
T cytotoxic (CD3+CD8a+)/gram	-81	-80	-82	-79
B (CD45RA+)/gram	-22	-39	-26	-37

Mean % difference = (mean dose group value - mean control (Group 1) value)/ mean control (Group 1) value)x100%; * Abs Lymph= absolute lymphocyte count

Reference ID: 4392197

Table: Mean percent differences in thymic immunophenotyping parameters following administration of siponimod (Sponsor's)

	MALE				FEMALE			
Parameter	1.5 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)	0.3 mg/kg/day (Group 3)	10 mg/kg/day (Group 4)	50 mg/kg/day (Group 5)	50 mg/kg/day (Group 6)
Abs Lymph*/organ	16	24	25	40	25	37	36	40
Abs Lymph/gram	19	9	12	10	23	16	19	18
CD3+/organ	25	33	32	48	33	44	41	47
CD3+CD4+CD8a-/organ	320	305	262	258	322	300	308	316
CD3+CD4-CD8a+/organ	489	507	466	508	349	323	327	324
CD3+/gram	28	17	18	17	31	23	24	24
CD3+CD4+CD8a-/gram	330	259	227	185	319	242	256	253
CD3+CD4-CD8a+/gram	531	442	415	394	338	262	275	259

Mean % difference = (mean dose group value - mean control (Group 1) value)/ mean control (Group 1) value)x100%; * Abs Lymph = absolute lymphocyte count

10.4 Mechanistic studies

An investigative study of cellular proliferation markers in primary microvascular endothelial cells *in vitro*. (*Study №: 1420148*)
Vascular endothelial cells (VECs) from mice (skeletal muscle), rats (dermal, lung, aorta, and skeletal muscle), and humans (lung and dermal) were characterized by qPCR for expression of CD31, ZO-1, vWF, VECadherin, $S1P_1$, and α-Actin. Cells were cultured *in vitro* to confluence and then dosed with siponimod (0.001 nM – 30 μM) or metabolite M17 (0.001 nM - 5 μM) for 24 hours. Quantification of cell numbers, incorporation of 5-ethynyl-2'-deoxyuridine (EdU) into *de novo* DNA to quantify cells undergoing proliferation, and secretion of PLGF-2 was conducted.

In mouse VECs (skeletal muscle), siponimod (0.01-1.25 μM) induced an increase incorporation of EdU into VECs and PLGF-2 release from VECs; however, no changes were observed with M17. In rat (lung) and human (dermal) VECs, siponimod and M17 did not induce EdU incorporation or PLGF-2 release. Therefore, siponimod caused mouse VECs proliferation paralleled by PLGF2 release. In contrast, in rat and human, siponimod had no clear effect on EdU incorporation or PLGF-2 release.

Study title: 1-, 3-, 7-, 14-, 28-, 91- and 274-day exploratory study by the oral route (gavage) in male mice

Study no: 1370782
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: February 5, 2014
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: BAF312, Batch № 1113020, 100%

Reference ID: 4392197

Key Study Findings

- Hemangiosarcomas were observed in liver (1 HDM in Group 21) and testis (1 HDM in Group 21), but histopathological vascular changes consistent with preneoplastic stages of hemangiosarcoma not detected at any time point.
- PLGF-2 plasma levels were increased from the first-time point, with the highest levels observed in the 2 HDM with hemangiosarcomas.
- Gene expression analysis of skeletal muscle revealed sustained up-regulation of endothelial cell activation-specific and mitotic-specific signals during siponimod dosing.

Methods

Doses: 0, 25, and 75 mg/kg
Frequency of dosing: Daily for 1, 3, 7, 14, 28, 91, or 274 days
Dose volume: 10 mL/kg
Route of administration: Oral (gavage)
Formulation/Vehicle: [redacted] (b) (4)
Species/Strain: Mouse/CD-1
Number/Sex/Group: 30 M for 1, 3, 7, 14, 28, and 91 days (Groups 1-18)
50 M for 274 days (Groups 19-21)
Age: 7-8 Weeks at initiation of dosing
Weight: Males 29.7-47.6 g

Group	Number and identity of animals	Dose-level[a] (mg/kg/day)	Concentration[a] (mg/mL)	Days of treatment	Day of necropsy
1	30 males: C11371 to C11400	0	0	1	Day 1
2	30 males: C11401 to C11430	25	2.5		
3	30 males: C11431 to C11460	75	7.5		
4	30 males: C11191 to C11220	0	0	1 to 3	Day 3
5	30 males: C11221 to C 11250	25	2.5		
6	30 males: C11251 to C11280	75	7.5		
7	30 males: C11551 to C11580	0	0	1 to 7	Day 7
8	30 males: C11581 to C11610	25	2.5		
9	30 males: C11611 to C11640	75	7.5		
10	30 males: C11281 to C11310	0	0	1 to 14	Day 14
11	30 males: C11311 to C11340	25	2.5		
12	30 males: C11341 to C11370	75	7.5		

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13	30 males: C11461 to C11490	0	0	1 to 28	Day 28
14	30 males: C11491 to C11520	25	2.5		
15	30 males: C11521 to C11550	75	7.5		
16	30 males: C11801 to C11830	0	0	1 to 91	Day 91
17	30 males: C11831 to C11860	25	2.5		
18	30 males: C11861 to C11890	75	7.5		
19	50 males: C11641 to C11690	0	0	1 to 274	Day 274
20	50 males: C11691 to C11740	25	2.5		
21	50 males: C11741 to C11790	75	7.5		

[a]: doses and concentration refer to base, correction factor of 1.112 applied for the salt.

Observations and Results
Mortality
Observations were conducted twice daily.

Deaths occurred at all dose levels: 4/230 control M (1 and 3 from Groups 16 and 19, respectively), 14/230 LDM (1, 3, 2, and 8 from Groups 11, 14, 17, and 20, respectively), and 9/230 HDM (2 and 7 from Groups 18 and 21, respectively) were sacrificed prematurely or found dead. The cause of death for most animals found dead or sacrificed for humane reasons was gavage-related lesions or large subcutaneous abscesses with or without ulceration, which might be caused by cutaneous injuries.

Clinical Signs
Observations were conducted once daily. Detailed observations were conducted weekly. From Week 31, all surviving animals in Groups 19-21 were palpated every 2 weeks to record the time of onset, location, size, appearance, and progression of palpable masses.

Clinical signs included incidences of abdominal breathing in LDM from Groups 11, 14, and 20, and HDM from Groups 9, 12, 15, and 21, hunched posture in control M from Group 19, LDM from Group 14, and HDM from Groups 9, 12, and 18, dyspnea in control M from Group 19, LDM from Groups 17 and 20, and HDM from Groups 15 and 21. Palpable masses were recorded in 2 HDM.

Body Weight
Body weight recordings were taken once prior to initiation of dosing and then:

- Groups 1-3: Day 1 before dosing and fasting
- Groups 4-6: Day 1 and Day 3 before dosing and fasting
- Groups 7-9: Day 1 and Day 7 before dosing and fasting

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- Groups 10-21: Day 1 and then every 6 or 7 days, including the day of sacrifice, before dosing and fasting

There was an increase in mean body weight gain at the end of dosing (Day 91) in Groups 16-18 of 34% at the LD and 30% at the HD, compared to control. However, in Groups 19-21, comparable body weights were observed by the end of dosing (Day 274).

Food Consumption
Food consumption was recorded at the same intervals as body weights were taken.

Minimal changes in food consumption were observed.

Necropsy
Gross examination was conducted on all animals.

No major macroscopic findings were noted.

Histopathology
At necropsy, the following tissues were collected and examined microscopically:

- Liver, skeletal muscle, and subcutis from all animals in Groups 10-21
- Bone marrow from all animals in Groups 13-21
- Heart, lungs, kidney, macroscopic masses, and macroscopic findings from all animals in Groups 19-21
- Spleen and mesenteric lymph nodes of all animals in Groups 19-21

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Organs	Microscopy	IHC	Gene expression analysis	Determination of test items levels analysis[1]	Additional tissues for microscopy for cause of death/ moribundity[2]
Macroscopic lesions	X				
Macroscopic masses[3]	X	X	X		
Bone marrow (femur)	X		X		
Whole brain (including medulla/pons, cerebellar and cerebral cortex)				X	X
Cecum					X
Colon					X
Duodenum					X
Extraorbital lacrimal glands				X	
Heart	X (rest)	X (rest)	X (left ventricle)		
Ileum					X
Jejunum					X
Kidneys	X	X	X	X[4]	
Liver	X	X	X	X[5]	
Lungs	X (with bronchi)	X (with bronchi)	X[6]		
Lymph nodes (mesenteric)	X		X	X[4]	
Lymph nodes (mandibular)				X	
Pancreas					X
Rectum					X
Skeletal muscle (gastrocnemius)	X	X	X		
Skeletal muscle (quadriceps)				X	
Subcutis (sampled with skin, close to the inguinal mammary gland)	X		X	X[5]	
Spleen	X	X	X	X[4]	
Stomach with forestomach					X

For bilateral organs, the left side was taken for microscopy and immunohistochemistry, the right side was taken for gene expression analysis (except for groups 19 to 21),

[1]: from the first five surviving animals per group without macroscopic findings indicative of neoplastic lesions of group 19 to 21 animals,

[2]: tissues additionally preserved for any found dead or prematurely sacrificed animal,

[3]: macroscopic masses sampled (half for microscopy and immunohistochemistry, half for gene expression analysis) from groups 19 to 21 at scheduled sacrifice and from any animal prematurely sacrificed after signature of amendment No. 5,

[4]: for animals sampled for the determination of test item level analysis:

- the right kidney was sampled for the determination of test items level and the left kidney was halved transversally for microscopy/IHC and GEP,

- no spleen and mesenteric lymph nodes sampling for microscopy/immunohistochemistry and gene expression analysis for these animals,

[5]: for animals sampled for the determination of test item level analysis: tissues left over after sampling for genomics and histology,

[6]: the whole right side was sampled, lobes were separated and snap frozen altogether. All the lobes were later homogenized for gene expression analysis.

Histological Findings

Siponimod-related findings in M dosed for 14, 28, 91, and 274 days were noted in heart, liver, lungs, mesenteric lymph node, and spleen. In heart, myocardium and epicardium fibrosis, myocardium amyloid, and inflammatory cell infiltration were observed in LDM and HDM after 274 days of dosing. In liver, centrilobular hypertrophy was noted in LDM and HDM at all time points and an increase in incidence of inflammatory cell infiltration was noted at ≥ 91 days HDM. In lungs, eosinophilic crystals were noted in the alveoli or bronchiole at 274 days in LDM and HDM and dose-dependent increases in incidences and severity of fibrin/hyaline foci, alveolar macrophages, inflammation, and osseous metaplasia were noted after 274 days of dosing. In lymph nodes, increased incidence

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and severity in histiocytosis in the medullary sinus was noted in LDM and HDM after 274 days of dosing. In spleen, increased incidence in extramedullary hematopoiesis was noted in LDM and HDM after 274 days of dosing. Neoplastic findings were observed in M dosed for 274 days and included hemangiosarcomas in 2 HDM, in liver and testis, malignant lymphoma in 1 M control (in heart, lungs, and lymph node) and 1 HDM (in bone marrow, heart, lungs, and spleen), bronchiolo-alveolar adenoma in 1 control M and 1 LDM, and urinary bladder papilloma in 1 control M.

Table: Summary of selected microscopic findings

		Control				25 mg/kg/day				75 mg/kg/day			
	Day:	14	28	91	274	14	28	91	274	14	28	91	274
	Number mice examined												
	Surviving animals:	30	30	29	47	29	27	28	42	30	30	28	43
	Premature deaths:	0	0	1	3	1	3	2	8	0	0	2	7
Tissue	**Finding** Group:	10	13	16	19	11	14	17	20	12	15	18	21
Heart	Myocardium amyloid												
	minimal:	-	-	-	1 (0)	-	-	-	0 (0)	-	-	-	4 (0)
	mild:	-	-	-	0 (0)	-	-	-	1 (0)	-	-	-	1 (0)
	moderate:	-	-	-	0 (0)	-	-	-	0 (0)	-	-	-	0 (0)
	marked:	-	-	-	0 (0)	-	-	-	1 (0)	-	-	-	0 (0)
	Myocardium fibrosis												
	minimal:	-	-	-	2 (1)	-	-	-	3 (0)	-	-	-	4 (0)
	mild:	-	-	-	0 (0)	-	-	-	2 (0)	-	-	-	0 (0)
	Epicardium fibrosis												
	minimal:	-	-	-	0 (0)	-	-	-	1 (1)	-	-	-	0 (1)
	mild:	-	-	-	0 (0)	-	-	-	0 (0)	-	-	-	2 (0)
	moderate:	-	-	-	0 (0)	-	-	-	1 (0)	-	-	-	0 (0)
	marked:	-	-	-	0 (0)	-	-	-	0 (0)	-	-	-	1 (0)
	Inflammatory infiltration												
	minimal:	-	-	-	4 (1)	-	-	-	12 (2)	-	-	-	10 (4)
	mild:	-	-	-	0 (0)	-	-	-	0 (0)	-	-	-	0 (1)
Liver	Centrilobular hypertrophy												
	minimal:	0	0	0 (0)	0 (0)	18 (0)	15 (1)	16 (0)	0 (2)	21	19	11 (0)	0 (2)
	mild:	0	0	0 (0)	0 (0)	2 (0)	5 (0)	2 (0)	9 (0)	3	5	14 (0)	10 (0)
	Inflammatory infiltration												
	minimal:	1	7	6 (0)	11 (3)	8 (1)	7 (0)	5 (0)	16 (1)	7	8	15 (0)	32 (1)
	mild:	0	1	0 (0)	1 (0)	1 (0)	0 (0)	1 (0)	1 (0)	0	0	0 (1)	5 (5)
	moderate:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0	0	0 (0)	0 (0)
	Increase mitotic figures												
	minimal:	0	0	0 (0)	1 (0)	12 (0)	1 (0)	4 (0)	5 (0)	7	3	2 (0)	1 (0)
	Degeneration/Necrosis												
	minimal:	0	0	0 (0)	0 (0)	1 (0)	0 (0)	1 (0)	0 (0)	0	0	1 (1)	0 (0)
	mild:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0	0 (0)	0 (0)
	moderate:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0	0	0 (0)	0 (0)
	marked:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0	0	0 (0)	0 (0)
	Perivascular amyloid												
	minimal:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0	0 (0)	0 (0)
	mild:	0	0	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0	0 (0)	1 (0)
	moderate:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0	0	0 (0)	1 (0)
	Histiocytosis												
	minimal:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0	0 (0)	0 (0)
	mild:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0	0 (0)	10 (0)
	moderate:	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0	0 (0)	2 (0)
	Hemangiosarcoma	0	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	0	0 (0)	1 (0)

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		Control				25 mg/kg/day				75 mg/kg/day			
	Day:	14	28	91	274	14	28	91	274	14	28	91	274
	Number mice examined												
	Surviving animals:	30	30	29	47	29	27	28	42	30	30	28	43
	Premature deaths:	0	0	1	3	1	3	2	8	0	0	2	7
Tissue	**Finding** Group:	10	13	16	19	11	14	17	20	12	15	18	21
Lungs	Eosinophilic crystals												
	minimal:	-	-	-	0 (0)	-	-	-	18 (1)	-	-	-	12 (1)
	Fibrin/hyaline foci												
	minimal:	-	-	-	0 (0)	-	-	-	20 (1)	-	-	-	21 (1)
	mild:	-	-	-	0 (0)	-	-	-	9 (1)	-	-	-	11 (1)
	moderate:	-	-	-	0 (0)	-	-	-	1 (0)	-	-	-	1 (0)
	Incr alveolar macrophages												
	minimal:	-	-	-	1 (0)	-	-	-	16 (1)	-	-	-	21 (1)
	mild:	-	-	-	1 (0)	-	-	-	15 (1)	-	-	-	11 (1)
	moderate:	-	-	-	0 (0)	-	-	-	0 (1)	-	-	-	4 (1)
	Inflammation												
	minimal:	-	-	-	0 (0)	-	-	-	15 (0)	-	-	-	12 (1)
	mild:	-	-	-	0 (0)	-	-	-	4 (0)	-	-	-	6 (1)
	moderate:	-	-	-	0 (0)	-	-	-	0 (0)	-	-	-	1 (1)
	Osseous metaplasia												
	minimal:	-	-	-	0 (0)	-	-	-	7 (0)	-	-	-	15 (0)
Lymph Node – Mesenteric	Amyloid												
	minimal:	-	-	-	0 (0)	-	-	-	3 (0)	-	-	-	2 (0)
	mild:	-	-	-	2 (0)	-	-	-	2 (0)	-	-	-	3 (0)
	moderate:	-	-	-	1 (0)	-	-	-	0 (0)	-	-	-	1 (0)
	Histiocytosis												
	minimal:	-	-	-	9 (1)	-	-	-	16 (1)	-	-	-	14 (1)
	mild:	-	-	-	0 (0)	-	-	-	3 (2)	-	-	-	8 (1)
	Hyperplasia												
	minimal:	-	-	-	0 (0)	-	-	-	0 (0)	-	-	-	0 (0)
	mild:	-	-	-	0 (1)	-	-	-	0 (0)	-	-	-	2 (1)
	moderate:	-	-	-	0 (0)	-	-	-	1 (0)	-	-	-	0 (0)
	Sinus infiltration												
	minimal:	-	-	-	2 (1)	-	-	-	4 (1)	-	-	-	4 (0)
	mild:	-	-	-	1 (2)	-	-	-	3 (2)	-	-	-	1 (1)
	moderate:	-	-	-	0 (0)	-	-	-	1 (0)	-	-	-	0 (3)
	marked:	-	-	-	0 (0)	-	-	-	0 (1)	-	-	-	0 (2)
Spleen	Hematopoiesis												
	minimal:	-	-	-	23 (0)	-	-	-	17 (2)	-	-	-	16 (0)
	mild:	-	-	-	4 (1)	-	-	-	18 (5)	-	-	-	22 (2)
	moderate:	-	-	-	0 (1)	-	-	-	1 (0)	-	-	-	0 (4)
	marked:	-	-	-	0 (1)	-	-	-	0 (1)	-	-	-	0 (1)
Testes													
	Hemangiosarcoma	-	-	-	0 (0)	-	-	-	0 (0)	-	-	-	1 (0)

Data from animals that died prematurely are in parenthesis; Incr - increased.

Toxicokinetics

Blood samples were taken from 5 animals/group, before sacrifice, in Groups 1-3 and 16-21. Skeletal muscle samples were taken from 5 animals/group in Groups 16-21, liver tissue samples were taken from all animals in Group 16-21, and brain, kidney, spleen, mesenteric and mandibular lymph nodes, extraorbital lacrimal gland, and subcutis/skin samples were taken from 5 animals/group in Groups 19-21. Determination of tissue levels for siponimod and metabolites (when possible) were conducted.

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Plasma exposure to siponimod increased approximately dose-proportionally, with no accumulation noted. Plasma exposure to metabolite M17 increased less than dose-proportionally, with accumulation noted. Exposure to siponimod and M17 was higher in tissues than that reported in plasma, especially in all tissues for siponimod and in liver, kidney, and lymph nodes for M17.

Table: Siponimod exposure levels (Sponsor's)
Plasma

Dose-level (mg/kg/day)	Analyte	Mean plasma concentrations (ng/mL) and (% CV)		
		Day 1 (5 hours)	Day 91 (5 hours)	Day 274 (5 hours)*
25	BAF312	9380 (10.2)	13700 (17.2)	6970 (14.1)
	LYS815	455 (49.5)	5300 (39.6)	40600 (21.7)
	LYG778	n.r. (n.r.)	n.r. (n.r.)	8.04 (17.7)
75	BAF312	26000 (10.8)	27700 (7.4)	18400 (18.6)
	LYS815	627 (48.6)	7870 (28.3)	74400 (55.5)
	LYG778	n.r. (n.r.)	n.r. (n.r.)	18.4 (8.9)

n = 5.
n.r.: not reportable (due to back-conversion to BAF312 without stabilization using Venosafe tubes).
*: blood collection in Venosafe tubes.

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Tissues

Dose-level (mg/kg/day)	Analyte	Mean tissue concentrations (ng/g) and (% CV)		
		Tissue	Day 91 (5 hours)	Day 274 (5 hours)
25	BAF312	Liver	131000 (7.9)	117000 (8.4)
	LYS815		5710000 (53.2)	8640000 (32.3)
	LYG778		149 (34.4)	630 (92.7)
75	BAF312		503000 (11.9)	496000 (34.1)
	LYS815		20600000 (46.3)	31400000 (74.2)
	LYG778		1290 (31.8)	471 (50.7)
25	BAF312	Muscle	69000 (17.5)	59000 (16.2)
	LYS815		5670 (84.1)	8860 (41.4)
	LYG778		25.3 (33.2)	15.3 (69.3)
75	BAF312		128000 (19.0)	108000 (24.4)
	LYS815		42000 (37.9)	23200 (35.0)
	LYG778		32.4 (35.2)	25.6 (143.8)
25	BAF312	Kidney	n.a.	93400 (11.6)
	LYS815		n.a.	1640000 (35.4)
	LYG778		n.a.	1010 (27.8)
75	BAF312		n.a.	268000 (8.5)
	LYS815		n.a.	2150000 (17.2)
	LYG778		n.a.	376 (32.2)
25	BAF312	Brain	n.a.	57800 (13.0)
	LYS815		n.a.	9530 (33.2)
	LYG778		n.a.	2.92 (223.6)
75	BAF312		n.a.	215000 (18.0)
	LYS815		n.a.	33900 (54.0)
	LYG778		n.a.	0 (n.c.)
25	BAF312	Skin	n.a.	24600 (40.2)
	LYS815		n.a.	4890 (30.1)
	LYG778		n.a.	44.7 (66.2)
75	BAF312		n.a.	58600 (32.3)
	LYS815		n.a.	21600 (68.5)
	LYG778		n.a.	65.3 (53.4)
25	BAF312	Spleen	n.a.	65300 (25.3)
	LYS815		n.a.	n.c. (n.c.)
	LYG778		n.a.	439 (172.2)
75	BAF312		n.a.	188000 (23.6)
	LYS815		n.a.	n.c. (n.c.)
	LYG778		n.a.	155 (97.4)
25	BAF312	LN mandibular	n.a.	85100 (25.7)
	LYS815		n.a.	144000 (31.0)*
	LYG778		n.a.	225 (64.9)
75	BAF312		n.a.	236000 (45.3)
	LYS815		n.a.	159000 (n.c.)**
	LYG778		n.a.	177 (66.1)

n = 5, *: n = 3, **: n = 2.
n.a.: not applicable, n.c.: not calculable, LN: lymph nodes.

Soluble Angiogenic Growth Factors

At necropsy, blood samples were taken from all animals. Plasma levels of a panel of angiogenic growth factors (sALK-1, amphiregulin, EGF, endoglin, endothelin-1, sFASL, FGF-2, follistatin, G-CSF, HGF, IL-1β, IL-6, IL-17A, KC/CXCL1, leptin, MCP-1, MIP-1α, PLGF-2, prolactin, SDF-1, TNFα, VEGF-A, VEGF-C, and VEGF-D) were determined by the Luminex® xMAP® technology.

Changes in plasma levels of angiogenesis growth factors included an increase in levels of PLGF-2 (40-135%) and TNFα (79-291%) from Day 1 and a decrease in levels of endoglin (24-36%) after 28 days.

Gene Expression

At necropsy, the tissues identified in the table in the Histopathology section (above) were collected and profiled for gene expression.

<u>Endothelial cell gene signature:</u> In skeletal muscle, siponimod induced up-regulation of genes specific for mature endothelial cells (like multimerin 1 [*Mmrn*], collagen type IVα2

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[*Col4a2*], and von Willebrand factor homolog [*Vwf*]) from Day 3, endothelial cell activation-specific genes from Day 1, CD93 antigen from Day 1, prion protein gene complex (*Prn*) from Day 3, intercellular adhesion molecule 1 (*Icam*) on Day 1, and prominin (*CD133*) from Day 3. In skin, siponimod induced up-regulation of mature endothelial cells from Day 3, endothelial cell activation-specific genes from Day 1, and intercellular adhesion molecule 1 (*Icam*) from Day 1.

Figure: Mature endothelial cells gene signature data (Sponsor's)
Muscle

Skin

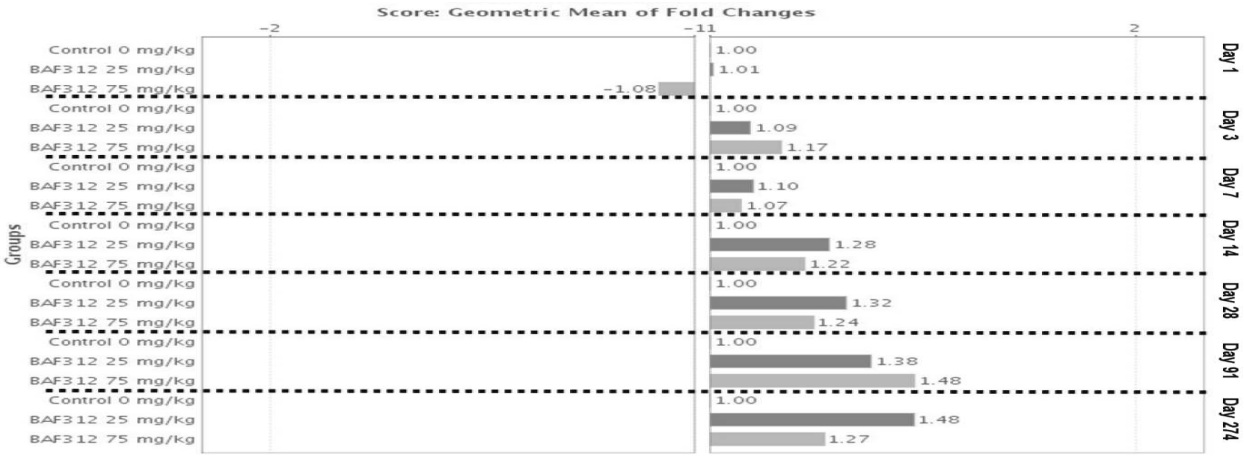

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Figure: Endothelial cell activation gene signature data (Sponsor's)
Muscle

Skin

Muscle CD93 (Sponsor's):

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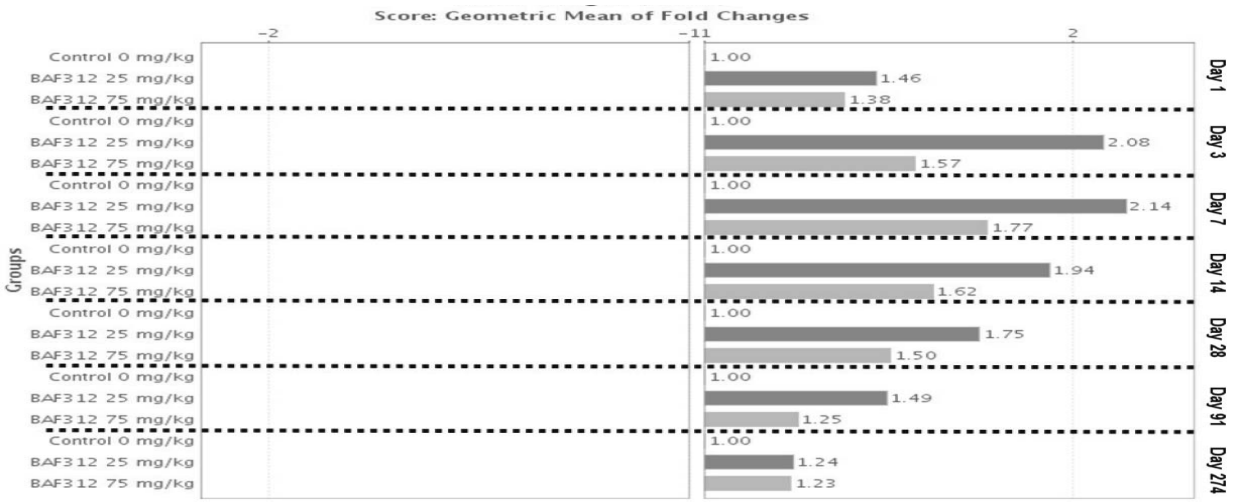
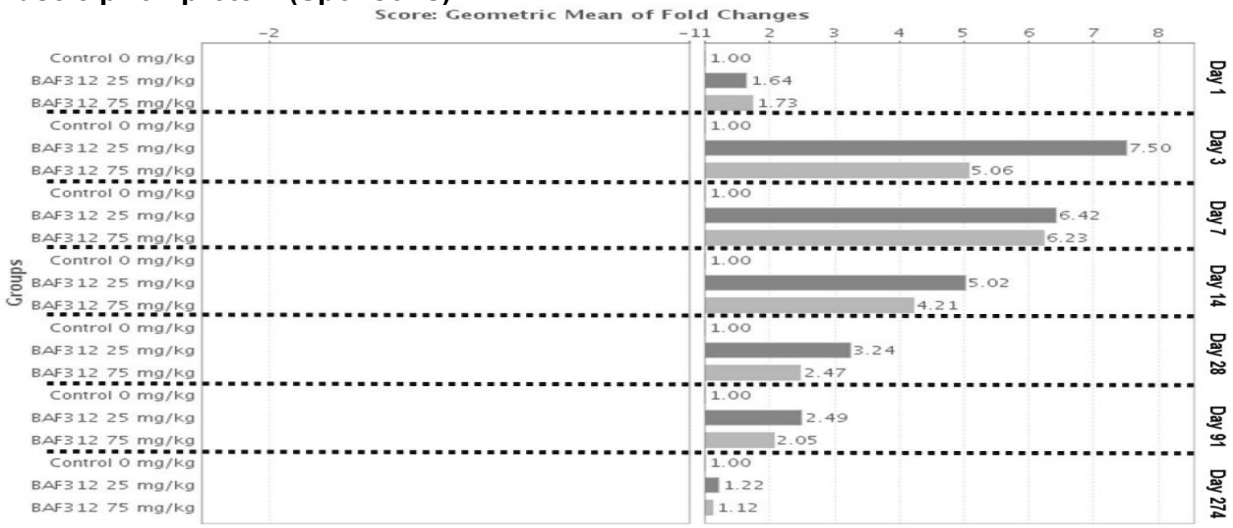
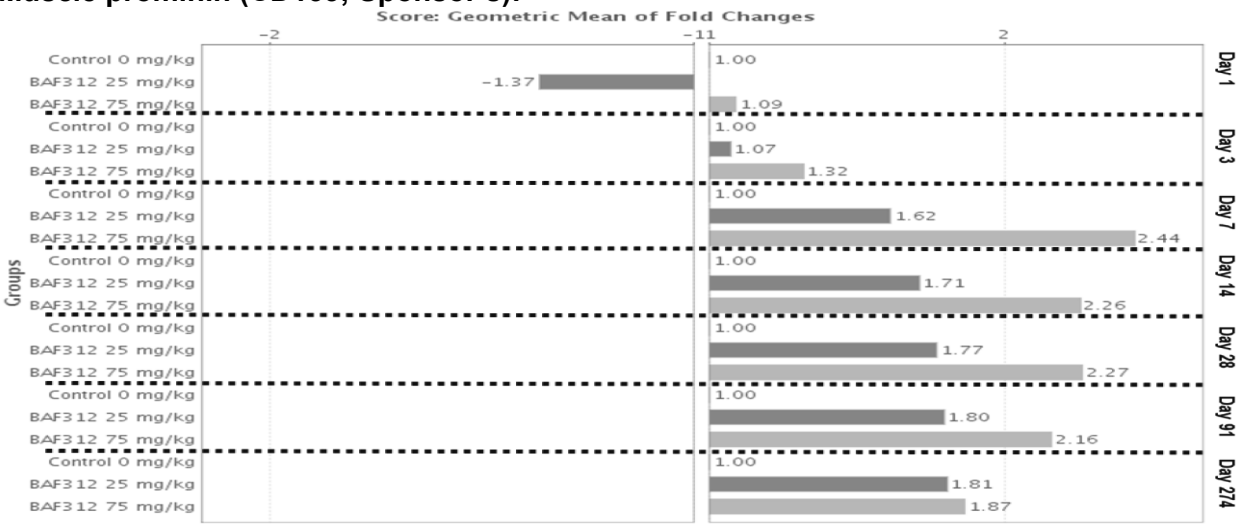


Muscle prion protein (Sponsor's):



Muscle prominin (CD133; Sponsor's):



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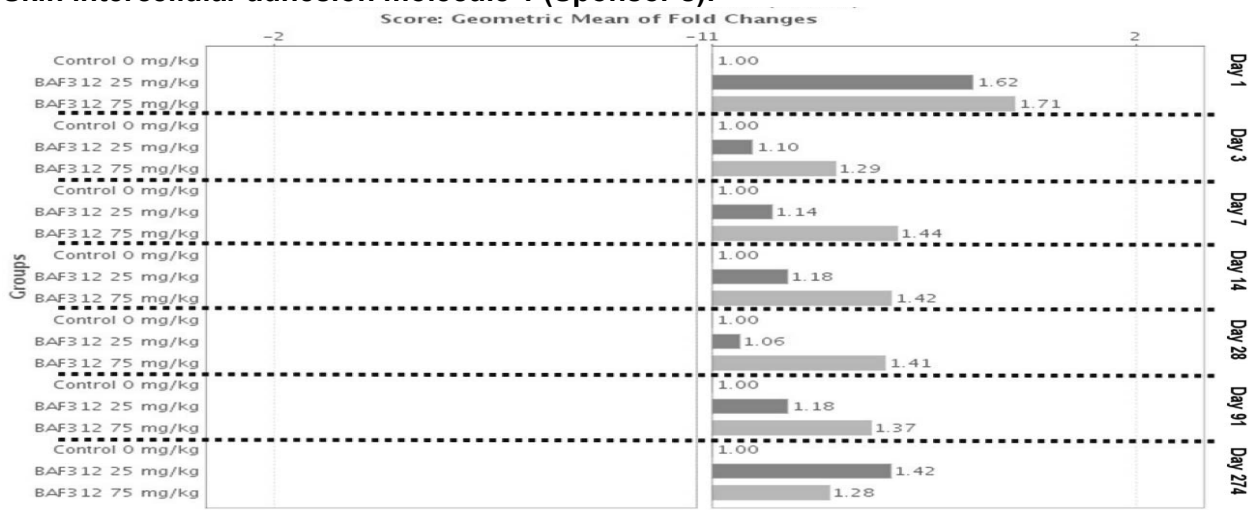
Muscle intercellular adhesion molecule 1 (Sponsor's):

Skin intercellular adhesion molecule 1 (Sponsor's):



<u>Mitosis gene signature:</u> In skeletal muscle, siponimod induced up-regulation of mitosis-specific genes like Ki67 (*Mki67*), cell division cycle 20 (*cdc20*), and histone cluster 1, H2ab (*histh2ab*) from Day 3. Analysis of mitosis-specific genes was not possible in skin due to proliferating hair follicles. In liver, siponimod induced up-regulation of mitosis-specific genes on Days 3 and 7.

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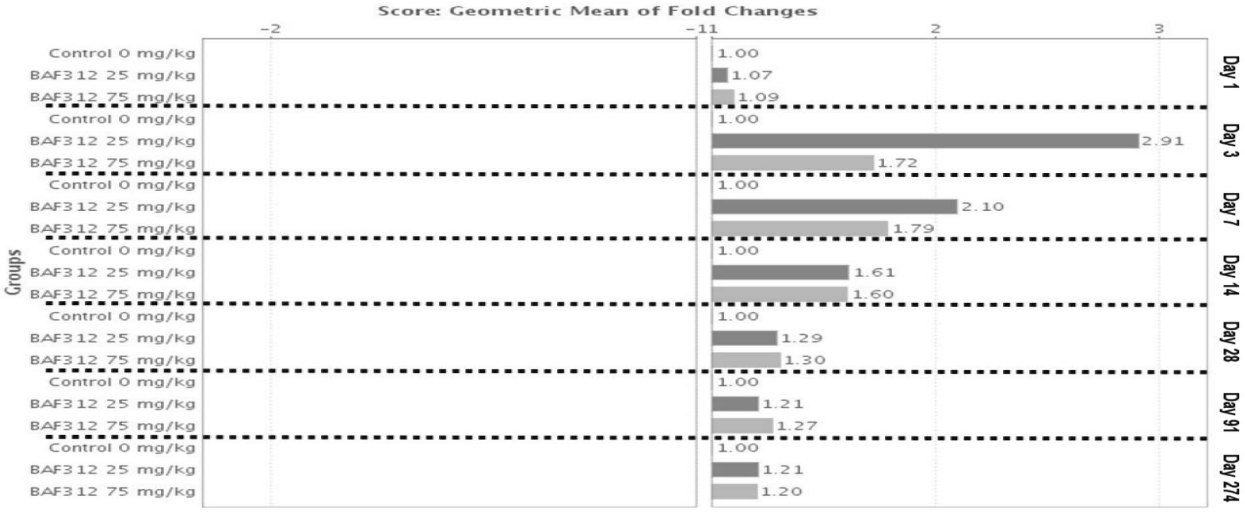
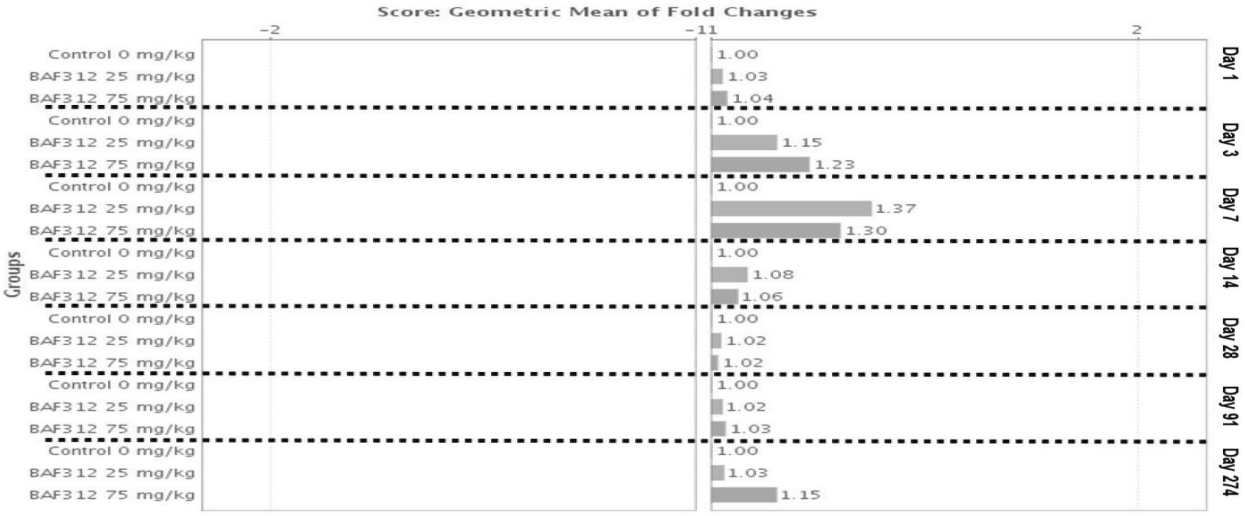
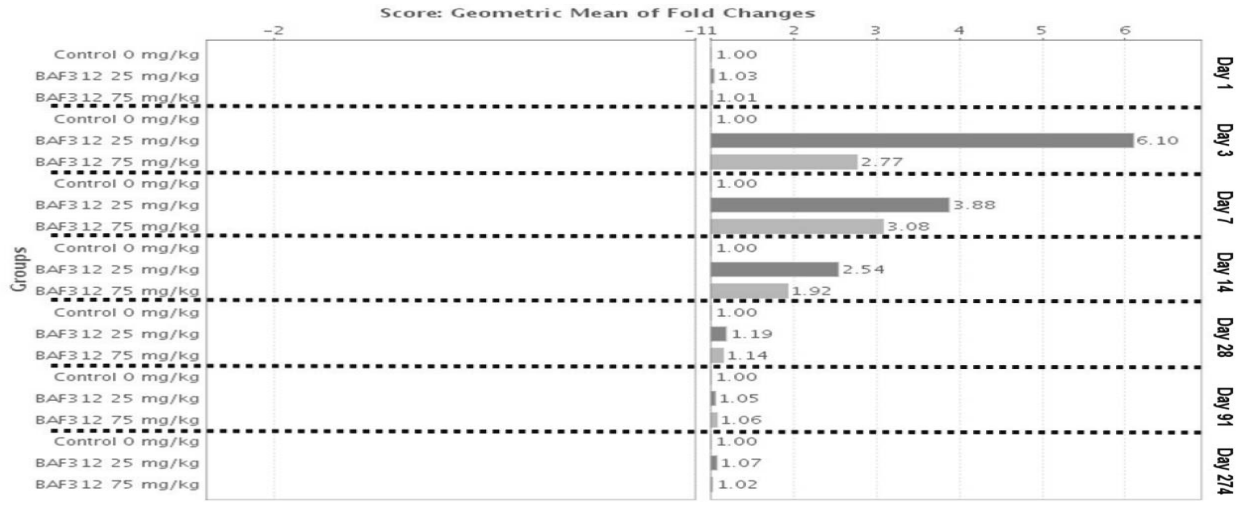
Figure: Mitosis gene signature data (Sponsor's)
Muscle



Liver



Mki67



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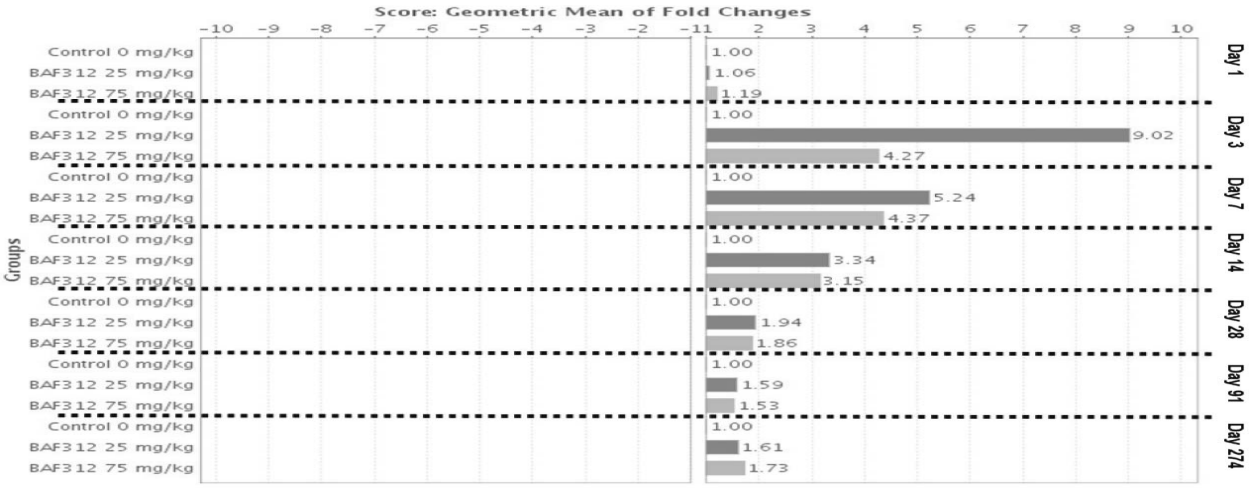
histh2ab



<u>Xenobiotic metabolism gene signature:</u> In liver, siponimod induced up-regulation of xenobiotic metabolism-specific genes (like cytochrome P450, family 2, subfamily b, polypeptide 10 [*Cyp2b10*], sulfotransferase family 1D, member 1 [*Sult1d1*], and UDP glucuronosyltransferase 1 family, polypeptide A1 [*Ugt1a1*]) from Day 1.

Figure: Liver xenobiotic metabolism gene signature data (Sponsor's)

Immunohistochemistry

At necropsy, the tissues identified in the table in the histopathology section were collected and the following tissues examined for Ki67, CD31, CD105, and CD93 staining:

Liver and muscle

- 5 animals in Groups 10, 13, and 16
- All animals in Groups 11, 12, 14, 15, 19, 20, and 21

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- Animals C11861, C11862, C11864, C11865, C11867, C11831, C11832, C11833, C11834, and C118315 from Groups 17 and 18

Subcutis, heart, lungs, spleen, kidneys, and masses
- All animals in Group 19-21

In liver and skeletal muscle, an increase in Ki67-positive proliferating cells was observed from Day 3, with the highest expression on Day 7 at the LD and HD; there was still a slight increase on Day 274. In skeletal muscle, proliferating Ki67-positive cells were regionally distributed and localized between muscle fibers. Ki67 staining was detected in some hepatocytes, especially with short dosing duration. With longer dosing duration, Ki67 staining in hepatocytes was confined to inflammatory foci. In the liver hemangiosarcoma in the HDM, there were numerous Ki67-positive cells throughout the tumor and occasionally lining vascular structures. In M dosed with vehicle and siponimod, positive staining for CD31 and CD105 was observed in the liver and skeletal muscle, with the staining confined to vascular structures. CD93 staining was detected in skeletal muscle of siponimod dosed M from Day 3, with the highest expression at Day 7; the staining was observed between the muscle fiber regions that also displayed Ki67 staining.

Study title: 1-, 3-, 7-, 14-, 28-, and 92-day exploratory study by the oral route (gavage) in male rats

Study no:	1370783
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 27, 2015
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	BAF312, Batch № 1113020, 100%

Key Study Findings

- Plasma PLGF-2 levels were increased but were not sustained beyond 14 days.
- Up-regulation of endothelial cell activation-specific genes and CD133, an early hematopoietic stem-cell marker, was observed in skeletal muscle with short siponimod administration (Days 1-3) and was sustained with longer dosing duration (Day 92).
- Up-regulation of mitosis-specific genes was observed on Day 3 but did not last longer than 14-days of dosing.

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Methods

Doses: 0 and 90 mg/kg
Frequency of dosing: Daily for 1, 3, 7, 14, 28, or 92 days
Dose volume: 5 mL/kg
Route of administration: Oral (gavage)
Formulation/Vehicle: [redacted] (b) (4)

Species/Strain: Rat/Wistar Han
Number/Sex/Group
Main: 30 M
Toxicokinetic 12 M (Group 11)
Age: 8-10 weeks at initiation of dosing
Weight: Males 209-375 g

Group	Number and identity of animals	Dose-level[a] (mg/kg/day)	Concentration[a] (mg/mL)	Days of treatment	Day of necropsy
1	30 males: E21741 to E21770	0	0	1	Day 1
2	30 males: E21771 to E21800	90	18		
3	30 males: E21801 to E21830	0	0	1 to 3	Day 3
4	30 males: E21831 to E21860	90	18		
5	30 males: E21861 to E21890	0	0	1 to 7	Day 7
6	30 males: E21891 to E21920	90	18		
7	30 males: E21933 to E21962	0	0	1 to 14	Day 14
8	30 males: E21963 to E21992	90	18		
9	30 males: E21993 to E22022	0	0	1 to 28	Day 28
10	30 males: E22023 to E22052	90	18		
11	12 males (TK): E21921 to E21932	90	18		
12	30 males: E22053 to E22082	0	0	1 to 92	Day 92
13	30 males: E22083 to E22112	90	18		

a: as active material; a correction factor of 1.112 was applied for the salt. TK = animals used for toxicokinetics.

Observations and Results

Mortality

Observations were conducted twice daily.

Two M in siponimod Group 10 were found dead on Day 4; one M displayed loud breathing, dyspnea, and enlarged lungs filled with foamy content, the other M displayed no clinical signs, but had red discolored lungs, along with clear fluid content in thoracic cavity.

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Clinical Signs
Observations were conducted once daily. Detailed observations were conducted weekly.

Dyspnea and loud breathing was observed in 1 siponimod dosed M from each of Groups 10 and 13, and hypersalivation was observed in 2 and 11 M from Groups 10 and 13, respectively. Otherwise, no adverse clinical signs were observed.

Body Weight
Body weight recordings were taken once prior to imitation of dosing and then:

- Groups 1-2: Day 1 before dosing and fasting
- Groups 3-4: Day 1 and Day 2 before dosing and fasting
- Groups 5-6: Day 1 and Day 5 before dosing and fasting
- Groups 7-10, 12-13: Day 1, then at least once a week, and before sacrifice
- Group 11: Day 1, then every 7 days

There was a decrease in mean body weight gain in siponimod groups during the first week of dosing by 44%, 26%, 60%, and 45% in Groups 6, 8, 10, and 13, respectively. The decrease in body weight gain continued to the end of dosing in Groups 8 (18%), 10 (26%), and 13 (17%).

Food Consumption
Food consumption was recorded at the same intervals as body weights were taken.

Food consumption was decreased in siponimod groups, especially during the first week of dosing, by 19%, 13%, 27%, and 19% in Groups 6, 8, 10, and 13, respectively.

Necropsy
Gross examination was conducted on all animals.

Macroscopic findings were observed in siponimod dosed M. Red discolored lungs were observed in the 2 Group 10 M that prematurely died on Day 4. Enlarged lungs were observed in 5 Group 6 M and 2 Group 8 M.

Organ weights
At necropsy, brain, liver, and thyroid were collected from Groups 9 and 10 M.

Statistically significant increases in mean liver (20%) and thyroid weight (11%) were observed in siponimod dosed M for ≥ 28 days.

Histopathology
At necropsy, the following tissues from Groups 1-10 were collected and examined microscopically.

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Organs	Preservation of tissues for potential microscopy	Preservation of tissues for gene expression analysis	Preservation of tissues in found dead animals[1]
Macroscopic lesions	X		X
Bone marrow (femur)	X	X	X
Brain (including medulla/pons cerebellar and cerebral cortex)			X
Cecum			X
Colon			X
Duodenum			X
Heart	X	X	X
Ileum			X
Jejunum			X
Kidneys	X	X	X
Liver	X	X	X
Lungs	X (with bronchi)	X	X
Lymph nodes (mesenteric)	X		X
Pancreas			X
Rectum			X
Skeletal muscle (gastrocnemius)	X	X	X
Subcutis (sampled with skin, close to the inguinal mammary gland)	X	X	X
Spleen	X	X	X
Stomach with forestomach			X

For bilateral organs, the left side was taken for microscopy, the right side was taken for gene expression analysis.
[1]: include additional tissues to be preserved in order to establish the cause of death.

Histological Findings

Siponimod-related findings in M dosed for 28 days included hepatocellular hypertrophy, lymphoid depletion of sinusoids and cortex/paracortex in mesenteric lymph node, and follicular cell hypertrophy in thyroid.

Table: Summary of microscopic findings

Tissue	Finding		Control 9 (28 Days)	90 mg/kg/day 10 (28 Days)
Liver	Hepatocellular hypertrophy			
		minimal:	**0/15**	**12/15**
		slight:	**0/15**	**3/15**
Lymph Node - Mesenteric	Germinal center development			
		minimal:	**11/15**	**6/14**
		slight:	**4/15**	**7/14**
	Sinusoidal erythrocytes			
		minimal:	**1/15**	**1/14**
		slight:	**0/15**	**1/14**
	Sinusoid Lymphoid depletion			
		present:	**0/15**	**14/14**
	Cortex/Paracortex lymphoid depletion			
		minimal:	**0/15**	**1/14**
		slight:	**0/15**	**4/14**
Thyroid gland	Follicular cell hypertrophy			
		minimal:	**3/15**	**7/15**
		slight:	**0/15**	**6/15**

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Toxicokinetics
Blood samples were taken on Days 1 and 28 from Group 11 M at 0.5, 1, 3, 6, 8, and 24 hours post-dose and from Groups 1-10 and 12-13 M before sacrifice. Skeletal muscle, liver, and skin samples were taken from Group 11 M. Determination of tissue levels for siponimod and metabolites (when possible) were conducted.

There was no accumulation of plasma exposure to siponimod; however, there was accumulation of M17. Exposure to siponimod and M17 was higher in liver than in plasma. M16 was not detected in plasma or tissue exposure.

Table: Siponimod and M17 exposure levels (Sponsor's)
Plasma: Siponimod

Day	Dose	Tmax	Cmax	Cmax/Dose	AUC	AUC/Dose	SE of Composite AUC	C0
1	90	3	11500	128	167000	1850	15170	0
28	90	6	8550	95.0	115000	1270	11690	1000

Units: Dose (mg/kg/day); Tmax (Hours); Cmax (ng/mL); Cmax/Dose ((ng/mL) / (mg/kg/day)); AUC (ng*Hours/mL); AUC/Dose ((ng*Hours/mL) / (mg/kg/day)); AUC interval (0-24h); SE of Composite AUC (ng*hours/mL); C0 (ng/mL).

Plasma: M17

Day	Dose	Tmax	Cmax	Cmax/Dose	AUC	AUC/Dose	SE of Composite AUC	C0
1	90	24	143	1.59	2230	24.8	538	0
28	90	3	206	2.29	4080	45.3	584	147

Units: Dose (mg/kg/day); Tmax (Hours); Cmax (ng/mL); Cmax/Dose ((ng/mL) / (mg/kg/day)); AUC (ng*Hours/mL); AUC/Dose ((ng*Hours/mL) / (mg/kg/day)); AUC interval (0-24h); SE of Composite AUC (ng*hours/mL); C0 (ng/mL).

Tissues:	**Siponimod**				**M17**			
Tissue	Mean	S.D.	%CV	n	Mean	S.D.	%CV	n
Liver	57500	27100	47.1	12	274	102	37.2	12
Skeletal muscle	2330	1900	81.5	12	0	n.c.	n.c.	12
Skin subcutis	1200	857	71.4	12	20.5	31.6	154.1	12

n.c.: not calculable

Units: Concentrations (ng/g)

Soluble Angiogenic Growth Factors
At necropsy, blood samples were taken from Groups 1-10 and plasma levels of a panel of angiogenic growth factors (KC/CXCL1, leptin, PLGF-2, TNFα, and VEGF) were determined using Luminex® xMAP® technology. Blood samples were taken from Groups 12-13 on Days 3, 25, 81, and 92 and PLGF-2 plasma level was determined using an ELISA method.

An increase in levels of PLGF-2 (159-349%) was observed, but not with dosing duration longer than 14 days.

TSH, T_3, and T_4
At necropsy, blood samples were taken from Groups 9 and 10 and evaluated for TSH, T_3, and T_4.

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There was an increase in TSH (159%) after 28 days of siponimod dosing. Neither T_3 nor T_4 could be determined accurately.

Microsomal Hepatic T_4-UDP-GT Activity
At necropsy, liver samples were taken from Groups 9 and 10 animals and evaluated for T_4-thyroxine-UDP-glucuronosyl transferase activity.

After 28 days of siponimod dosing, there was an increase in total liver T_4-thyroxine-UDP-glucuronosyl transferase activity (104%) normalized to body weight.

Gene Expression
At necropsy, the tissues identified in the table in the Histopathology section (above) were collected from Groups 1-10 and 12-13 and profiled for gene expression.

Endothelial cell gene signature: In skeletal muscle, siponimod induced up-regulation of genes specific for mature endothelial cells (like multimerin 1 [*Mmrn*], collagen type IVα2 [*Col4a2*], and von Willebrand factor homolog [*Vwf*]) from Day 3, endothelial cell activation-specific genes from Day 1, CD93 antigen from Day 1, prion protein gene complex (*Prn*) from Day 3, intercellular adhesion molecule 1 (*Icam*) from Day 1, and prominin (*CD133*) from Day 7. In skin, genes for mature endothelial cells were unaffected by siponimod dosing. Siponimod induced up-regulation endothelial cell activation-specific genes from Day 1.

Figure: Muscle Mature endothelial cells gene signature data (Sponsor's)

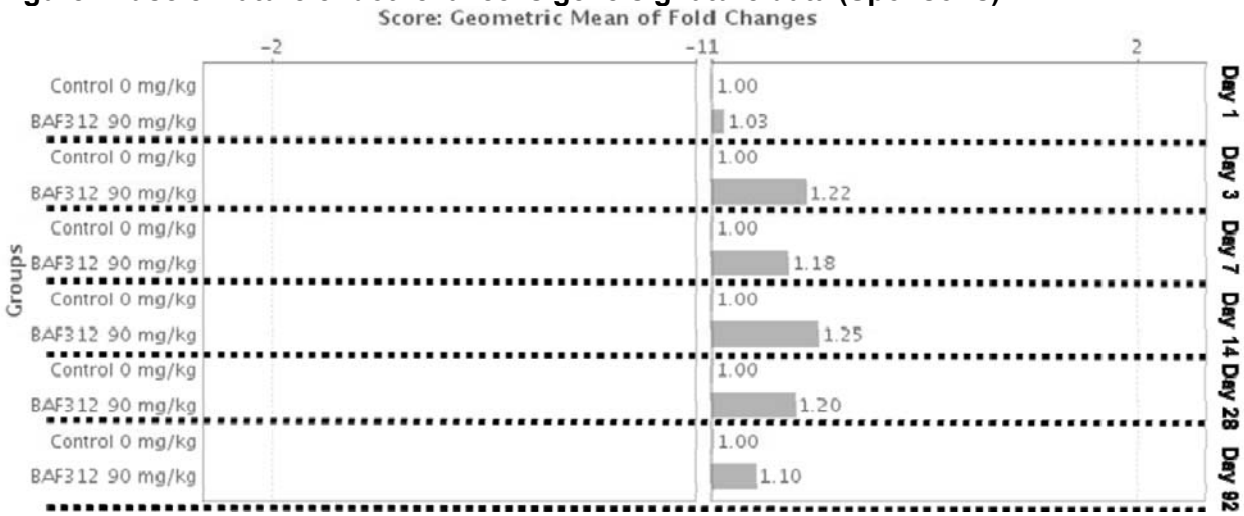

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Figure: Endothelial cell activation gene signature data (Sponsor's)
Muscle

Skin

Muscle CD93 (Sponsor's)

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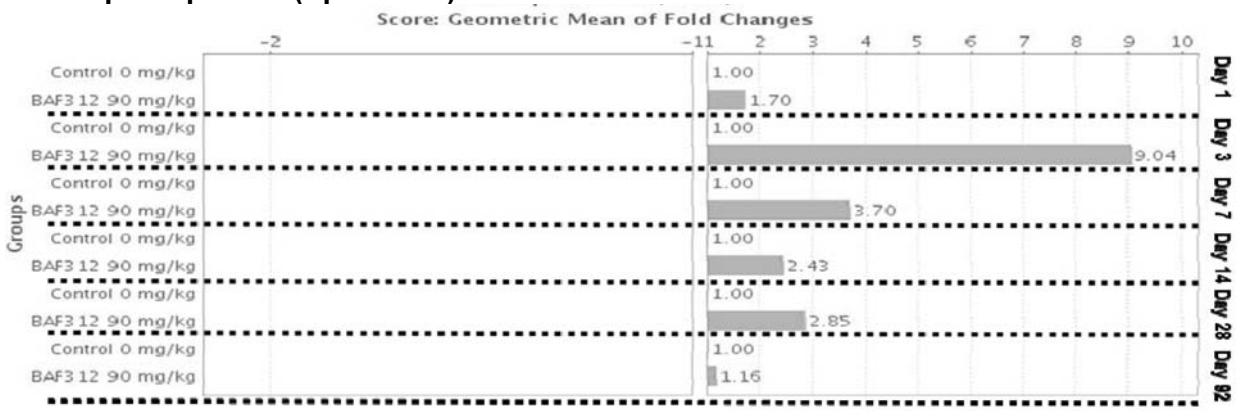
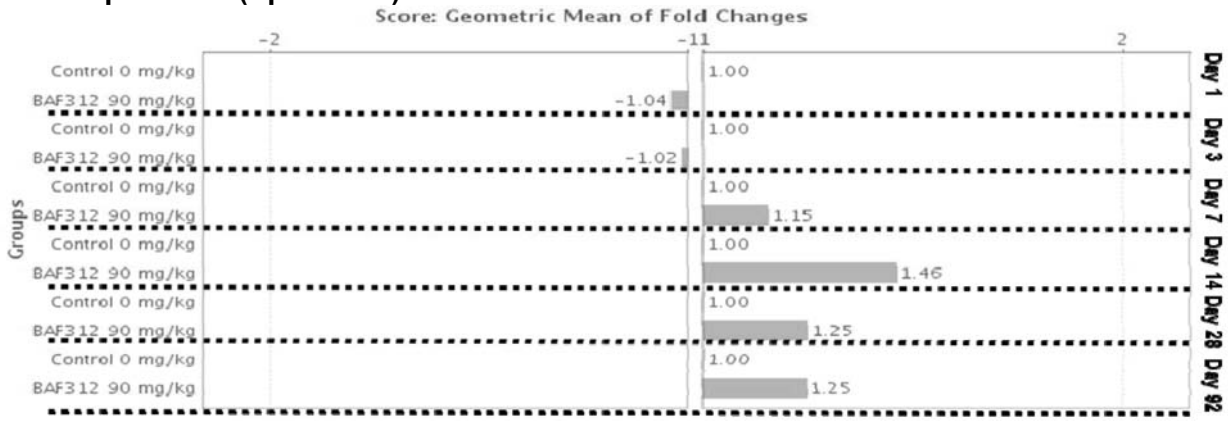
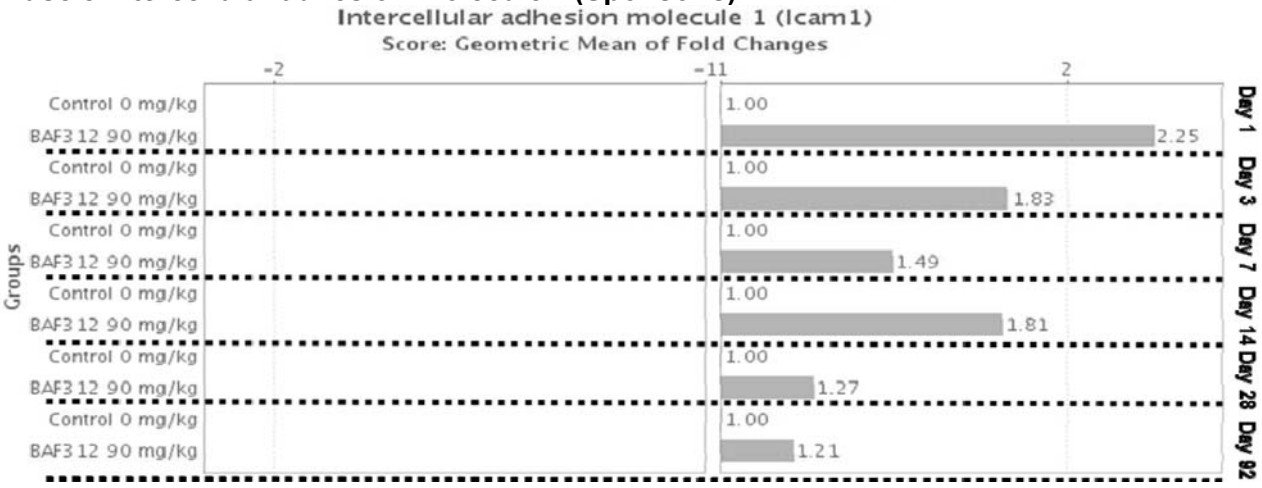
Muscle prion protein (Sponsor's)



Muscle prominin (Sponsor's)



Muscle intercellular adhesion molecule 1 (Sponsor's)



<u>Mitosis gene signature:</u> In skeletal muscle, siponimod induced up-regulation of mitosis-specific genes (like Ki67 [*Mki67*], cyclin A2 [*ccna2*], and cyclin-dependent kinase 1 [Cdk1]) on Day 3, but up-regulation did not last for the longer dosing durations.

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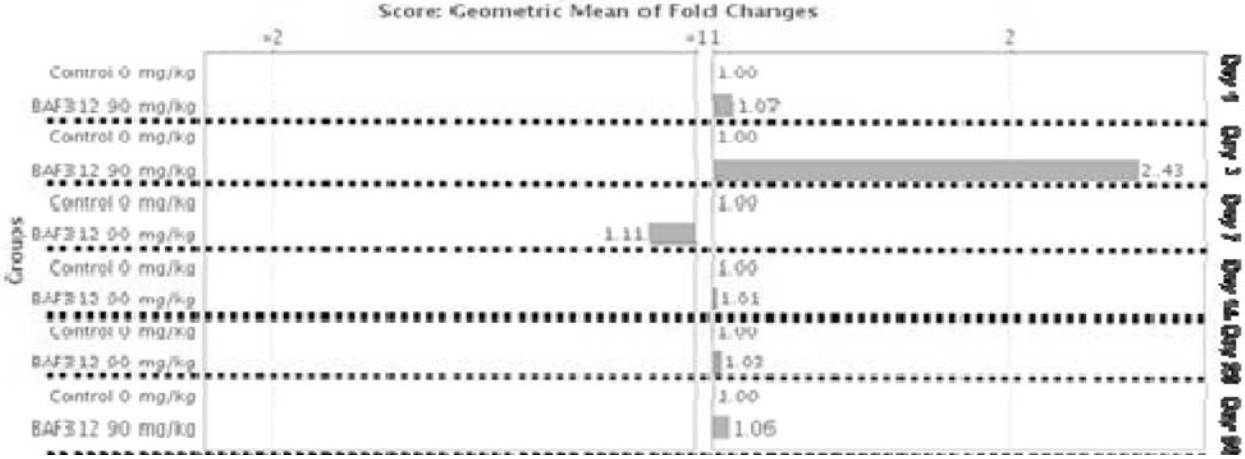
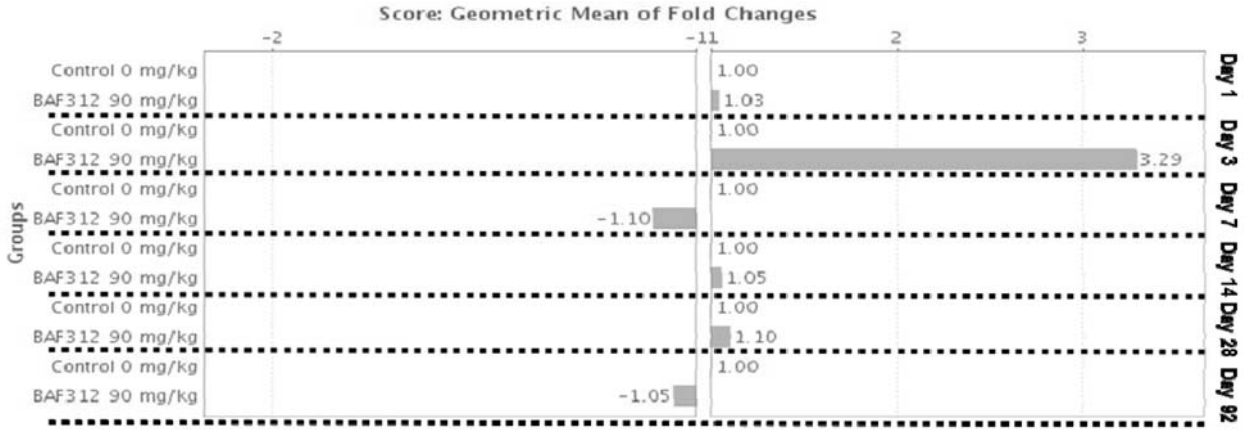
Analysis of mitosis-specific genes was not possible in skin due to proliferating hair follicles.

Figure: Mitosis gene signature data (Sponsor's)



Muscle *Mki67*



Immunohistochemistry

At necropsy, skeletal muscle was collected from Groups 1-6, 8, and 10 and examined for Ki67, cKit, CD31, and RnCd93 staining.

In skeletal muscle, an increase in Ki67-positive proliferating cells was observed after 3 days of siponimod dosing only. Positive staining, confined to endothelium vascular structures, was observed for CD31. CD93 staining was detected in skeletal muscle of vehicle and siponimod from Day 3; the staining was observed between the muscle fiber regions that also displayed Ki67 staining.

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10.5 Other

Lung Function

Effect of 4-week administration of BAF312 on rat lungs: Lung function test, S1P-R expression and histopathology. (*Study №: RD-2005-01357;* see Roney review, January 12, 2007, IND 76,122)

In a lung function study in M rat that displayed the pharmacological effect of lymphopenia with administration of oral siponimod (30 mg/kg), an increase in baseline airway resistance was observed in siponimod dosed rats (145 $cmH_2O/(L\ s^{-1})$ compared to control rats (127 $cmH_2O/(L\ s^{-1})$. Histological changes observed at 30 mg/kg siponimod included increases in alveolar macrophages, bronchial edema, and hyperplasia.

Airways hyperreactivity induced by BAF312 in the Brown Norway rat. (*Study №: RD-2006-01156*)

The effect of siponimod on lung function, following administration of oral doses of 0 (vehicle) and 30 mg/kg, was assessed in M Brown Norway rats using spasmogens, serotonin (0.3-30 µg/kg), methacholine (3-30 µg/kg), and adenosine (3 mg/kg) administered IV. Siponimod did not induce airway hyperresponsiveness.

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11 Integrated Summary and Safety Evaluation

Siponimod, an S1P agonist (selective for SIP_1 and SIP_5 receptors), is intended for the treatment of patients with secondary progressive multiple sclerosis. Siponimod acts at S1P receptors to promote internalization and degradation of $S1P_1$ receptors. This effect of siponimod deprives lymphocytes of the obligatory signal to egress from lymphoid organs, preventing the recirculation of lymphocytes to other sites, and sequestering them in secondary lymphoid tissue. Siponimod action at $S1P_5$ receptors that are expressed on oligodendrocytes and oligodendrocyte precursor cells may enhance remyelination. Siponimod also had affinity at a number of off target human receptors, although at much higher IC_{50}'s. Functionally, oral siponimod administered daily has been demonstrated to reduce adverse clinical signs and demyelination and macrophage infiltration/microglial cell activation in EAE rodent models. In a published article using the mouse EAE model, ICV infusion of siponimod, that produced a small decrease in peripheral lymphocytes, had a positive effect on EAE-induced adverse clinical scores. This may suggest that siponimod could have actions in the CNS. However, the data may not conclusively demonstrate a central action of siponimod, as siponimod dosing was initiated before induction of EAE, the reduction in EAE-induced adverse clinical score may still relate to the decrease in peripheral lymphocytes (~25%), and lymphocyte infiltration in the striatum was reduced.

In safety pharmacology studies, siponimod (PO and IV) acutely decreased heart rate in rats, guinea pigs, rabbits, and monkeys. The decrease in heart rate induced by siponimod in guinea pigs was partial reversed by atropine pre-treatment. Other cardiovascular observations included decreased blood pressure in guinea pigs and second-degree block in guinea pigs and monkeys. Siponimod activated the GIRK current in both guinea pig and human myocytes. The down-modulation of $S1P_1$ receptors induced by siponimod may prevent further activation of GIRK channels and could explain transient siponimod-induced bradycardia. NOAELs for heart findings in oral safety studies were <0.24, 3.6, and 720 mg/m^2 in guinea pigs, rabbits, and monkeys, respectively. (b) (4) there is no safety margin to heart findings observed in guinea pigs.

Oral bioavailability of siponimod was high in all species. Absorption of siponimod in animals after oral administration was slow, with T_{max} of 2-8 hours. Siponimod distributed to most tissues in rodents and was detected in the brain and CSF, with exposures 0.8- and 4.4-fold those in blood, respectively; highest exposure was observed in white matter.

In human plasma, the major circulating human metabolites are M3, formed by C-hydroxylation and then glucuronidation, and M17, a cholesterol ester of siponimod. After oral siponimod, the main components in plasma were siponimod and metabolites M11, M6, and M13 in rat plasma and siponimod and metabolite M3 in monkey plasma. M3 is a non-acyl glucuronide metabolite and has adequate systemic exposure in monkeys. Adequate systemic exposure to M17 was demonstrated in mouse and rat. Therefore, human plasma exposure to M3 and M17 are covered adequately in nonclinical species. Siponimod and metabolites were measured in rat milk up to

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72 hours post-dose, suggesting that nursing pups would be exposed to siponimod. Placental transfer of siponimod and metabolites occurred in rabbits.

In toxicology studies, deaths and early sacrificed were observed in mice (300 mg/kg; due to nephrotoxicity), rats (150 and 50 mg/kg in M and F, respectively; animals that died displayed adverse clinical signs), and monkeys (≥ 100 mg/kg; due to deteriorating physical condition). Few clinical signs were observed; however, breathing difficulties were observed in F mice at 25 mg/kg (carcinogenicity study) and F rats at 50 mg/kg and convulsions were observed in mice at 25 mg/kg (carcinogenicity study) and monkeys at ≥ 100 mg/kg. WBCs and lymphocytes were greatly reduced in all species at all doses, with accompanying histopathological changes in lymphoid tissues that included lymph node lymphoid depletion, splenic reduction in cellularity of periarteriolar lymphoid sheath, and thymic medullary enlargement and cortical thinning. These changes in lymphocytes and lymphoid lesions, can be attributed to the pharmacological action of siponimod. Findings of lung toxicity were observed in mice, rats, and monkeys. The most consistent microscopic finding was alveolar macrophage infiltration/accumulation. Other microscopic findings in the lungs included foci with fibrin/hyaline material, inflammation/fibrosis, and smooth muscle hypertrophy/hyperplasia. Effects on respiratory function in a safety pharmacology study in rat included increased tidal volume and decreased respiratory rate. NOAELs for lung toxicity were <5 mg/kg for mice, 50 and 10 mg/kg for M and F rats, respectively, and 100 mg/kg for monkeys. Safety margins to human plasma exposure from NOAELs are estimated at <73-, 343-, and 184-, and 1256-fold, respectively. The mouse is most sensitive species for lung toxicity. Findings in other organs have greater safety margins; nephrotoxicity in mice (safety margin 851-fold), increased number of goblet cells in the stomach and GI tract in monkeys (safety margin 373 to 574-fold), and degeneration of skeletal muscle myofibers in monkeys (safety margin 165- to 184-fold).

In genetic toxicology studies, siponimod did not demonstrate mutagenic potential in the Ames assay, human peripheral lymphocytes, or TK6 assay or clastogenic potential in the *in vivo* mouse and rat micronucleus assay.

The rat carcinogenicity study was positive for follicular cell adenoma or combined with carcinoma in HDM. The mouse study was positive for malignant lymphoma in F, with an increased incidence of malignant lymphoma observed at all doses, and for hemangiomas and hemangiosarcomas, with an increase in incidences at all doses in M and F.

Hemangiomas and hemangiosarcomas are common spontaneous tumors in mice.
In vitro mechanistic studies using VECs from mouse, rat, and human demonstrated that siponimod treated mouse cell cultures, in contrast to rat and human cells, increased proliferation and PLGF-2 release. An in vivo mechanistic study in mice suggests that there is an early and sustained local and systemic activation of VECs and release of PLGF-2 with persistent mitotic stimulation. In contrast, there was a transient induction of mitosis gene expression and PLGF-2 release, not lasting beyond 14 days of siponimod dosing, in a rat study, although there was a sustained activation of endothelial cells.

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These data may suggest that hemangiosarcomas induced by siponimod are mouse specific. However, although dysregulated angiogenesis/erythropoiesis (increased erythropoiesis was observed in several tissues in the mouse carcinogenicity study) coupled with increase in endothelial cell proliferation has been described in mice, there appears to be insufficient data describing the mechanism of action, with a specific trigger, by which siponimod may induced these changes and whether the key events in the mechanism of action are plausible and relevant in humans. Further, it has been noted that hemangiosarcomas in mice are generally observed after 18 months, whether spontaneously or chemically induced (see Cohen et al. Toxicological Sciences*, 2*009; 111:4-18). However, in the mouse 24-month study 27-43% of hemangiosarcomas in siponimod dosed groups were observed before 18 months, compared to 8% in control group. Therefore, the data do not appear to be sufficient to definitively state that the hemangiosarcomas in mice are irrelevant to humans.

Fertility was not affected in F (NOAEL was the HD, 1 mg/kg/day), but was minimally affected in M rats. At the HD (200 mg/kg/day) in M rats, an increase in precoital interval by 1.6 days and a decrease in epididymis weight was noted and decreased mean corpora lutea, implantations, and viable fetuses were noted in the undosed F mated with the M. In a rat embryofetal development study, siponimod was fetal lethal, with no viable fetuses born of dams administered 5 and 40 mg/kg/day and decreased live fetuses at 1 mg/kg/day. Teratogenicity was noted, with observed malformations including cardiomegaly, malrotated limbs, cleft palate, and misshapen clavicles. The fetal NOAEL was not established and is, therefore, < 1 mg/kg/day. In rabbits, the embryofetal development study (0, 0.1, 1, and 5 mg/kg) established the fetal NOAEL at 0.1 mg/kg/day, due to abortions in HD dams, increased embryofetal deaths at the MD and HD, and increased variations. No plasma exposures (AUC) were calculated. The NOAELs for rat and rabbit convert to < 3 and 1.2 mg/m^2, respectively, resulting in safety margins of approximately 4-fold. In the pre- and postnatal study (0, 0.05, 0.15, and 0.5 mg/kg/day), the NOAEL for maternal toxicity and reproductive performance was the 0.05 mg/kg due to early euthanasia of HD dams, decrease of body weight gains and food consumption in MD and HD dams during lactation, reproductive changes that included a slight decrease in gestation index at the HD, increase in gestation length at the MD and HD, and deceased number of live fetuses at the HD. The NOAEL for F_1 generation development in the pre- and postnatal study was 0.05 mg/kg due to decrease in F_1 survival between PNDs 0 and 4, further death of pups that occurred in MD and HD litters during lactation, signs of abnormal flexure of the left and right hindlimbs, flat cranium, malocclusion of the incisors, decreases in body weight and food consumption, delays in sexual maturation in HD pups, and increased incidence of malformations that included absence of anal opening at the MD and cleft palate, split tongue, truncus arteriosus, and subcutaneous edema over the entire body at the HD. The F_1 NOAEL is 0.3 mg/m^2 on a body surface area basis and 0.9-fold (extrapolated from TK data from the rat EFD study) on a plasma exposure basis, resulting in no safety margin for lethality and teratogenicity.

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