

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

213498Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

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

Center for Drug Evaluation and Research

Date: March 18, 2021

From: Lois M. Freed, Ph.D.
Director, Division of Pharmacology/Toxicology-Office of Neuroscience

Subject: NDA 213498 (Ponvory, ponesimod, JNJ-678961453)

NDA 213498 was submitted by the sponsor to support marketing authorization of ponesimod for the “treatment of relapsing forms of multiple sclerosis..., to include clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease, in adults.”

To support clinical development and the NDA, the sponsor conducted a full battery of nonclinical studies. These studies were reviewed in detail by Dr. Richard Siarey (Pharmacology/Toxicology NDA Review and Evaluation, March 12, 2021), who has recommended that, from a nonclinical standpoint, the NDA be approved.

Ponesimod is a selective sphingosine-1-phosphate (S1P) 1 receptor agonist, with an EC₅₀ of 5.7 nM (maximum effect compared to 100% for the endogenous ligand, S1P); however, ponesimod may also have agonist effects at the S1P3 receptor and partial agonist effects at the S1P4 and S1P5 receptors, as noted in the table below (human S1P receptors).

	S1P1	S1P2	S1P3	S1P4	S1P5
EC ₅₀ (nM)	5.7 ±	>10000	108 ± 17	1119 ± 157	69 ± 17
maximum effect (%)	99 ± 1	--	101 ± 4	17.5 ± 3.5	42 ± 3

Following an initial activation of S1P1, ponesimod results in a functional antagonism through internalization and desensitization of the receptor and subsequent sequestration of lymphocytes in lymphoid tissue and decreases in circulating lymphocytes.

The pivotal nonclinical studies were conducted in CD-1 mouse, Wistar rat, beagle dog, and New Zealand White rabbit. Of these species, pharmacological activity at S1P1 and S1P3 receptors were tested in mouse, rat, and dog.

	MOUSE		RAT		DOG	
	S1P1	S1P3	S1P1	S1P3	S1P1	S1P3
EC ₅₀ (nM)	1.4 (0.7-2.7)	586 (484-709)	1.9 (0.2-16.1)	1496 (1236-1810)	3.5 (1.2-10.1)	841 (374-1892)
maximum effect (%)	97	88	99	45	97	74

The two most abundant circulating metabolites identified in humans were M12 and M13; however, only M13, formed by “truncation of the ethylene glycol side chain...and oxidation to the carboxylic acid,” was a major metabolite, accounting for ~18% of total drug-related material in humans at oral doses of 40-100 mg.

The in vitro pharmacological activity of M13 at human S1P receptors was characterized, as follows:

	S1P1	S1P2	S1P3	S1P4	S1P5
M13					
EC ₅₀ (nM)	1608	>10000	990	>10000	>10000
maximum effect (%)	67	--	57	--	--

Dr. Siarey states that both metabolites were adequately tested in the pivotal nonclinical studies.

The primary toxicities observed in the nonclinical studies consisted of cardiovascular/cardiac toxicity (dog), lung toxicity (mouse, rat, and dog), developmental toxicity (rat and rabbit), and carcinogenicity (mouse).

Cardiovascular/cardiac toxicity

In the pivotal toxicity studies in dog, coronary arterial lesions (thickening of the vessel wall, hyperplasia/hypertrophy of smooth muscles cells of the tunica media, subendocardial fibrosis) involving the papillary muscle of the left ventricle were observed in oral toxicity studies of 13 to 52 weeks in duration. There were no drug-related effects observed on ECG or echocardiography. At the NOAEL (2 mg/kg/day) for cardiac lesions, plasma exposures (AUC) were approximately 2 times that expected in humans at the RHD.

The sponsor conducted studies to investigate the potential mechanism(s) underlying the cardiac lesions observed in dog (but not rat). In vitro studies in rat, dog, and human coronary arteries identified interspecies differences in contractile properties of ponesimod: vasoconstriction was observed in dog and human, but not in rat, arteries. In dog large coronary arteries, ponesimod (1, 10, 100, and 300 nM and 1 µM) caused vasoconstriction at concentrations of 100 nM and above (0.8, 2.6, 12, 48, and 70.2%, respectively). In human coronary arteries, ponesimod (100 nM and 1 µM) caused vasoconstriction at 1 µM (approximately 46%) but not at 100 nM (0.6%). The sponsor noted that the potential for hemodynamic effects may be related to species differences in tissue S1P3:S1P1

receptor mRNA, with a higher ratio in dog (2:1) compared to rat (1:15), and intermediate in human (1:1); however, the potency of ponesimod at S1P3 receptors (demonstrated to mediate contraction) also differed substantially among species, as noted in the tables above. Specifically, in vitro potency at the dog S1P3 receptor was approximately 8-fold lower compared to that at the human S1P3 receptor. Therefore, how these data inform potential interspecies differences in sensitivity regarding the cardiac findings in dog is unclear. As noted, no drug-related effects on ECG or echocardiography were observed in the toxicity studies in dog; however, the sensitivity of these assessments to detect any drug-related hemodynamic effects is also unclear. Based on the available information, the cardiac lesions observed in dog are considered relevant to humans; potential differences in sensitivity between dog and human are not fully understood.

Lung toxicity

For ponesimod, increases in lung weight and histopathology (alveolar histiocytosis, edema) were observed in oral toxicity studies in mice, rats, and dogs. At the higher doses tested in short-term studies, alveolar histiocytosis was associated with lung edema, emphysema, or hyalinosis, and with alveolar hyperplasia after cessation of dosing in rats and alveolar histiocytosis and hyalinosis in dogs. Effects tended to be absent or less severe after chronic dosing. These findings are considered secondary to increased vascular permeability caused by S1P1 receptor modulation (Sanna MG et al. Nat Chem Biol 2:434-441, 2006). The NOAELs for lung findings in the 4-week oral toxicity studies in rats and dogs were associated with plasma exposures (AUC) similar or lower than that expected in humans at the recommended human dose (RHD) of 20 mg/day.

Lung was also identified as a target organ in juvenile rat (see Reproductive and Developmental Toxicology)

Reproductive and Developmental Toxicology

A standard battery of reproductive and developmental toxicology studies was conducted for ponesimod.

No effects on mating or fertility parameters were observed in separate studies in male and female Wistar rats orally administered ponesimod at doses of 0, 10, 30, and 100 mg/kg prior to and throughout the mating period and continuing in females to Day 6 of gestation. Plasma ponesimod exposures (AUC) at the highest dose tested were approximately 10 (males) and 30 (females) times that in humans at the recommended human dose (RHD) of 20 mg/day.

Embryofetal development (EFD) studies were conducted in Wistar rat and New Zealand White rabbit. Oral administration of ponesimod during the period of

organogenesis results in embryolethality and fetal malformations in both species, in the absence of maternal toxicity.

In pregnant rat, ponesimod (0, 1, 10, and 40 mg/kg/day) increased the incidences of fetal malformations primarily involving the limbs (syndactyly and ectrodactyly) and cardiovascular system (including ventricular septal defects) at all but the lowest dose tested. A high incidence of embryofetal death was observed at the highest dose tested. Maternal toxicity was not observed, indicating a selective effect on the fetus. Plasma exposure (AUC) at the no-effect dose (1 mg/kg/day) for adverse effects on embryofetal development in rats was lower than that in humans at the recommended human dose (RHD) of 20 mg/day.

In the pivotal EFD study in pregnant rabbit, ponesimod (0, 0.25, 1, and 4 mg/kg/day) increased post-implantation loss and fetal variations (visceral and skeletal) were noted at the highest dose tested. No maternal toxicity was observed. Plasma exposure at the no-effect dose (1 mg/kg/day) for adverse effects on embryofetal development in rabbits was lower than that in humans at the RHD. In a dose-range finding study in pregnant rabbits, oral administration of ponesimod (0, 6, 20, or 60 mg/kg/day) during organogenesis, an increase in embryofetal death and fetal limb malformation (brachydactyly) were observed at the lowest dose tested; at the higher doses, there were no live fetuses.

Effects on pre- and postnatal development were assessed in Wistar rat. Following oral administration of ponesimod (0, 5, 10, and 20 mg/kg/day) throughout pregnancy and lactation, the offspring exhibited decreased survival, reduced body weight gain, and reduced fertility and reproductive performance (increases in pre- and postimplantation loss) at the highest dose tested, neurobehavioral impairment (increased locomotor activity) at the mid and high doses, and delayed sexual maturation at all doses tested. A no-effect dose for adverse effects on pre- and postnatal development in rats was not identified. Plasma exposure (AUC) in dams at the lowest dose tested was less than that in humans at the RHD.

The serious developmental toxicity observed in rat and rabbit are consistent with the essential role of S1P receptors in embryogenesis (e.g., Mendelson K et al. J Biol Chem 288(4):2143-2156, 2013; Xiong Y et al. J Clin Invest 124(11):4823-4828, 2014).

Juvenile Animal Toxicology

Although ponesimod is under evaluation for the treatment of relapsing forms of multiple sclerosis only in adults, the sponsor conducted studies to assess potential adverse effects when ponesimod was administered during the postnatal period. In the pivotal juvenile animal study, oral administration of ponesimod (0, 1, 10, 30, and 100 mg/kg/day) to young Wistar rat from postnatal day 28 to 91 resulted in lung histopathology (alveolar histiocytosis/edema) and decreased

immune function (T-cell dependent antibody response) at the two highest doses tested. Decreased body weight gain and long bone growth were observed at all but the low dose, and neurobehavioral impairment (increased locomotor activity) was observed at the highest dose tested. The T-cell dependent antibody response was similar among groups by the end of the 4-week recovery period; however, decreased lymphocyte count and neurobehavioral impairment persisted.

Genetic Toxicology

A standard battery of genetic toxicology studies was conducted for ponesimod.

Ponesimod was negative in in vitro (Ames, chromosomal aberration in mammalian cells) and in vivo (micronucleus in rat) assays.

Carcinogenicity

The carcinogenic potential of ponesimod was tested in 2-year oral carcinogenicity studies in CD-1 mouse and Wistar rat.

In mouse, oral administration of ponesimod (0, 50, 150, or 400 mg/kg/day in males and 30, 100, or 300 mg/kg/day in females) resulted in incidences in hemangiosarcoma and combined hemangioma and hemangiosarcoma in males at all doses and at the highest dose tested in females. Plasma exposures (AUC) at the lowest dose tested in males (50 mg/kg/day) were approximately 5 times that in humans at the recommended human dose (RHD) of 20 mg.

In rat, oral administration of ponesimod (0, 3, 10, or 30 mg/kg/day in males and 0, 10, 30, or 100 mg/kg/day in females) did not result in an increase in tumors. Plasma exposures at the highest dose tested in males (30 mg/kg/day) were approximately 4 times that in humans at the recommended human dose (RHD) of 20 mg.

Conclusions and Recommendations

The pivotal nonclinical studies of ponesimod were adequately conducted and identified target organs for toxicity (including cardiovascular and lung) and serious developmental (teratogenic) effects.

With appropriate labeling, the nonclinical studies support approval of the IND for the proposed indication.

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/s/

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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: 213-498
Supporting document: 001
Serial number: 0001
Applicant's letter date: March 18, 2020
CDER stamp date: March 18, 2020
Product: VIVMORY (Ponesimod)
Indication: Relapsing multiple sclerosis to include clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease
Applicant: Janssen Research and Development, LLC
Clinical Review Division: Division of Neurology 2
Reviewer: Richard Siarey
Supervisor: Lois Freed
DN2 Division Director: Nick Kozauer
Project Manager: Kristen Haslam

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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 through action on five G-protein coupled receptors. Ponesimod is an S1P agonist that is selective for the S1P₁ receptor. The sponsor has developed ponesimod for treatment of patients with relapsing multiple sclerosis to include clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease with a recommended maintenance dose of 20 mg/day.

1.2 Brief Discussion of Nonclinical Findings

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

Ponesimod acts at S1P receptors to promote internalization and degradation of S1P₁ 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 ponesimod resulted in neurological impairments.

In cardiovascular safety pharmacology studies, a single oral dose of ponesimod decreased heart rate, blood pressure, and caused first-, second-, and third-degree AV block in guinea pigs. However, repeated doses resulted in a reduction of the cardiovascular effects observed after the initial dose. The reduced electrocardiographic effects were dependent on the timing of the second dose, with effects weaker when the second dose was administered 12-36 hours post-first dose but not when the delay was 48 hours. The NOAEL for the acute cardiovascular effect in guinea pigs was 0.1 mg/kg (0.8 mg/m²), resulting in a minimal safety margin with the clinical first dose (2 mg; 1.2 mg/m²) on a body surface basis. Effects of ponesimod on respiratory function included increases in enhanced pause (bronchoconstriction), tidal volume, and peak expiratory flow up to 15 days of oral ponesimod (≥ 10 mg/kg) in rats. Some of the changes in respiration maybe due to ponesimod actions at the S1P₃ receptor in the lower trachea, as ponesimod induced contraction of the lower trachea that has higher expression of S1P₃ receptor mRNA. Lung vascular permeability was induced by single and repeated doses of ponesimod and corresponded to the level of lymphopenia, but was maximal after the first dose, peaking at 3-7 hours post-dose. These mechanistic studies may suggest there was adaption in the lung, at least up to 14 days.

In rat and dog repeat-dose toxicology studies of oral ponesimod, deaths and early sacrifice were observed at 400 and ≥ 75 mg/kg, respectively. Clinical signs included breathing difficulties in rats at ≥ 10 mg/kg and tremor, collapse, uncoordination,

impaired motility, and recumbency in dogs at ≥ 40 mg/kg. Target organs were the liver (hypertrophy) and lungs (alveolar histiocytosis, hematoidin crystals, hyalinosi, interstitial fibrosis, bronchioloalveolar hyperplasia, osseous metaplasia, and increased weight) in mice, liver (hypertrophy) and lungs (alveolar histiocytosis, edema, emphysema, hyalinosi, osseous metaplasia, and increased weight) in rats, and heart (arteriopathy and subendocardial fibrosis) and lungs (alveolar histiocytosis, foamy macrophages, pleural fibrosis, osseous metaplasia, fibrosing alveolitis, and fibrous thickening) in dogs. Lymphoid tissues were target organs in all species, with microscopic findings (atrophy, hypocellularity, and medulla hypertrophy) accompanied by decreased circulating levels of WBC and lymphocytes; these findings were attributed to the pharmacological action of the drug. The NOAELs for lung toxicity were 90 and 30 mg/kg in M and F mice, respectively, 4 mg/kg in rats, and 3 mg/kg in dogs. The safety margins for lung toxicity based on plasma exposure were 12- and 2.4-fold in M and F mice, respectively, 0.7-fold in rats, and 4.4-fold in dogs. The NOAEL for cardiac toxicity was 3 mg/kg in dogs, with the safety margin of 4.4-fold based on plasma exposure.

In genetic toxicology studies, ponesimod was negative in the in vitro reverse mutation assay in bacterial cell (Ames) and mammalian cell clastogenicity assays, and in in vivo clastogenicity assays (micronucleus) in mouse and rat. In the 2-year rat carcinogenicity study, there were no drug-related increases in neoplasms in M or F. The mouse study was positive for hemangiomas and hemangiosarcomas, with an increase in incidences at all doses in M and in HDF.

In the reproductive and development studies with oral ponesimod, fertility was not affected in F or M (NOAEL was 100 mg/kg/day), but teratogenicity and fetal lethality was observed in both rats and rabbits. Malformations in rats included fused ascending aorta trunk, muscular ventricular septal defect, membranous ventricular septal defects, syndactyly, ectrodactyly, malrotated hindlimbs, and eye microphthalmia and rabbits had morphological changes in the cardiovascular and limb systems. The fetal NOAEL was 1 mg/kg/day in both rats and rabbits, with a safety margin for fetal toxicity of 0.2-fold in rat and rabbit based on plasma exposure. In the pre- and postnatal study, ponesimod was well tolerated in F₀ dams and the developmental NOAEL was determined at less than the LD (<1 mg/kg/day) due to delayed sexual maturation in pups from all ponesimod dose groups; decreased viability, decrease pre-weaning body weight gain, lower fertility of F₁ F, increased motor fine movements and ambulatory movements, and reduced reproductive performance were noted at the HD.

Metabolite M13 was identified as a major circulating metabolite in humans. Adequate systemic exposure to M13 was demonstrated in mouse and rat.

1.3 Recommendations

1.3.1 Approvability

Toxicities of ponesimod observed in nonclinical studies can be monitored (cardiovascular) or are described in the labeling (lung and reproductive and

development). Therefore, it is recommended, from a nonclinical perspective, that ponesimod be approved.

1.3.3 Labeling

The following are suggested edits (in red) to the sponsors proposed nonclinical sections of the label.

8.1 Pregnancy

Data

Animal Data

When ponesimod was orally administered (b) (4)

(b) (4)

When ponesimod was orally administered (b) (4) to female rats throughout pregnancy and lactation, (b) (4)

(b) (4)

Plasma exposure in dams at the lowest dose was less than (b) (4) that in humans at the RHD (b) (4)

(b) (4)

8.2 Lactation

Risk Summary

There are no data on the presence of TRADENAME in human milk, the effects on the breastfed infant, or the effects of the drug on milk production. (b) (4)

(b) (4)

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for TRADENAME and any potential adverse effects on the breastfed (b) (4) from TRADENAME or from the underlying maternal condition.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Ponesimod is a sphingosine 1-phosphate (S1P) receptor 1 modulator (b) (4)
binds with high affinity to S1P receptor 1 (b) (4).

Ponesimod blocks the capacity of lymphocytes to egress from lymph nodes reducing the number of lymphocytes in peripheral blood. The mechanism by which ponesimod exerts therapeutic effects in multiple sclerosis is **unknown but** may involve reduction of lymphocyte migration into the central nervous system.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

(b) (4)

(b) (4)

Mutagenesis

Ponesimod was negative in a battery of in vitro (Ames, chromosomal aberration in mammalian cells) and in vivo (micronucleus in rat) assays.

Fertility

(b) (4)

(b) (4)

13.2 Animal Toxicology and/or Pharmacology

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 854107-55-4

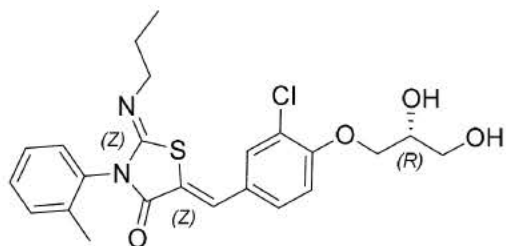
Generic Name: Ponesimod

Code Name: ACT-128800, JNJ-67896153

Chemical Name: (2Z,5Z)-5-[3-chloro-4-[(2R)-2,3-dihydroxypropoxy]benzylidene]-3-(2-methylphenyl)-2-(propylimino)-1,3-thiazolidin-4-one

Molecular Formula/Molecular Weight: C₂₃H₂₅ClN₂O₄S / 460.97 g/mol

Structure or Biochemical Description: from the sponsor



Pharmacologic Class: S1P₁ modulator

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 101,722 (DN2, treatment of multiple sclerosis),

(b) (4)

2.3 Drug Formulation

Film coated tablets (2, 3, 4, 5, 6, 7, 8, 9, 10, and 20 mg).

2.4 Comments on Novel Excipients

There are no issues with the excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no impurity issues.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population for ponesimod are individuals with relapsing forms of multiple sclerosis, to include clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease, in adults. Following an initial dose-escalation period (2 to 20 mg/day), the recommended maintenance dose is 20 mg/day.

2.7 Regulatory Background

A pre-IND meeting was held on April 24, 2008 (Minutes; May 23, 2008). IND 101,722 was originally received on December 5, 2008, and was allowed to proceed (Agency Letter; March 2, 2009). An EoP2 Meeting between the sponsor and FDA was held on December 6, 2011 (see Meeting Minutes; January 27, 2012). A pre-NDA meeting was held on September 4, 2019 (Meeting Minutes; September 26, 2019).

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 (see Appendix).

3.3 Previous Reviews Referenced

- Pharmacology/Toxicology Review, R. Houghtling, May 12, 2009
- Exec CAC Minutes, A.S. Seifried, April 5, 2013
- Pharmacology/Toxicology Review, R. Houghtling, July 25, 2013
- Pharmacology/Toxicology Review, R. Houghtling, May 1, 2015

4 Pharmacology

4.1 Primary Pharmacology

Ponesimod is an iminothiazolidinone derivative that is an orally administered selective S1P1 receptor modulator. In vitro binding studies to detect agonist-induced GTP[γ -³⁵S] incorporation activity demonstrated that ponesimod (see table below) has high affinity and selectivity for the G-protein-coupled S1P1 receptor. The main metabolites (M12 and M13 [major]; see tables of metabolite data below) had minimal activity at the S1P receptors and are, therefore, expected to be inactive.

Table: Affinity of ponesimod for human S1P receptors (Sponsor's)

Receptor	EC ₅₀ (nM) (mean ± SEM)		% Maximal Effect (mean ± SEM) ^a	
	Ponesimod	S1P	Ponesimod	S1P
S1P ₁	5.7 ± 0.4	25.5 ± 1.4	99 ± 1	100%
S1P ₂	>10,000	44.5 ± 5.3	NA	100%
S1P ₃	108 ± 17	0.7 ± 0.1	101 ± 4	100%
S1P ₄	1119 ± 157	166 ± 25	17.5 ± 3.5 ^b	100%
S1P ₅	69 ± 17	122 ± 9	42 ± 3 ^b	100%

EC₅₀ results are expressed as arithmetic mean ± SEM (n = 2–5), with the internal maximal effect defined as 100%.

^a The maximal activity of ponesimod at 10 μM is also indicated as % activity relative to the effect of 1 μM S1P (defined as 100%).

^b Ponesimod acts as partial agonist at S1P₄ and S1P₅.

EC₅₀: agonist concentration inducing 50% of its maximal response; NA: not applicable; S1P: sphingosine 1-phosphate; S1P_{1–5}: human sphingosine 1 phosphate receptor 1–5.

Table: Affinity of metabolites (M12 and M13) of ponesimod for human S1P receptors (Sponsor's)

Receptor	EC ₅₀ (nM) (mean, 95% CI)			% Maximal Effect ^a		
	M12	M13	S1P	M12	M13	S1P
S1P ₁	787 (498-1244)	1608 (1165-2221)	67 (27-165)	100%	67% ^b	100%
S1P ₂	>10,000	>10,000	41 (16-102)	NA	NA	100%
S1P ₃	854 (532-1373)	990 (511-1917)	0.5 (0.1-1.6)	117%	57% ^b	100%
S1P ₄	>10,000	>10,000	498 (298-831)	NA	NA	100%
S1P ₅	1372 (842-2235)	>10,000	30 (7.2-127)	40% ^b	NA	100%

EC₅₀ results are expressed as geometric mean and 95% confidence interval (CI) (n = 3–9), with the internal maximal effect defined as 100%.

^a The maximal activity of M12 and M13 at 10 μM is also indicated as % activity relative to the effect of 1 μM S1P (defined as 100%).

^b partial agonist

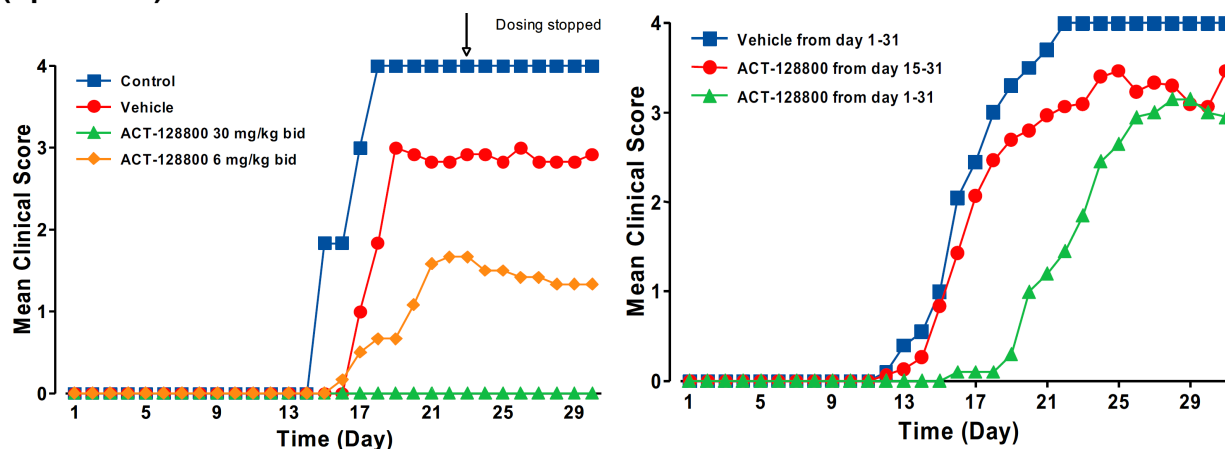
EC₅₀: agonist concentration inducing 50% of its maximal response; NA: not applicable; S1P: sphingosine 1-phosphate; S1P_{1–5}: human sphingosine 1 phosphate receptor 1–5.

Lymphocytes require S1P1 receptor activation for migration from lymphoid organs; ponesimod acts at S1P1 receptors to induce internalization and degradation of the receptor. The internalization and degradation of the receptor 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 were observed in all species administered ponesimod.

Effects of ponesimod in autoimmune encephalomyelitis (EAE) models

Ponesimod was investigated in the mouse EAE model. F C57BL/6 mice were dosed orally with ponesimod (30 mg/kg BID) either immediately after induction of EAE (on Days 1 to 23 or 29) or therapeutically (on Days 15 to 31), after induction of EAE (SC myelin oligodendrocyte glycoprotein [MOG] emulsified in complete Freund's adjuvant followed by IP pertussis toxin). Daily dosing with oral ponesimod immediately after induction of EAE almost completely prevented adverse clinical signs. Therapeutic daily dosing with oral ponesimod reduced adverse clinical signs and microscopic evidence of inflammation, demyelination, and axonal damage in the spinal cord.

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



4.2 Secondary Pharmacology

Ponesimod was tested in a panel of in vitro radioligand binding assay of 107 targets consisting of GPCRs, transporters, ion channels, and enzymes. At 10 μM , ponesimod demonstrated affinity for several human targets: endothelin ET_A receptor (74%), monoamine oxidase MAO-B (62%), and protein serine/threonine kinase Ca^{2+} /calmodulin-dependent II (63%). An in vivo study in mouse demonstrated no effect of ponesimod (100 mg/kg BID for 9 days) on the immune control of a *Listeria monocytogenes* infection compared to control animals.

4.3 Safety Pharmacology

Neurological function was assessed for 24 hours in M and F Wistar rats, with no changes in behavior observed after single oral doses up to 100 mg/kg ponesimod.

Several in vitro assays were conducted to assess the effect of ponesimod on cardiovascular function. The hERG tail current, expressed in CHO cells, was minimally reduced by ponesimod (22% at 3 μM). The major metabolite M13 (ACT-338375) dose-dependently decreased the hERG tail current by 25% and 46% at 10 and 100 μM , respectively. In M and F rat, M guinea pig, and F rabbit isolated atrial tissues, ponesimod (up to 10 μM) did not increase the rate of sinus node firing. In isolated F

Wistar rat arteries, ponesimod (1 μ M) induced a contractile response of the basilar artery (high expression of S1P₃ receptors) in the presence of endothelium and a greater contractile response in the absence of endothelium. In contrast, ponesimod (1 μ M) did not induce a response in the rat isolated aorta (low expression of S1P₃ receptors) with and without endothelium. Therefore, as S1P₁ receptors are expressed at similar levels in both arteries, S1P₃ receptors appear to mediate the contractile response of vascular smooth muscles to ponesimod. However, in the presence of phenylephrine-induced contraction of the aorta, ponesimod (1 μ M) evoked an endothelium-dependent relaxation that was reduced by an S1P₁ receptor antagonist (ACT-180806A). When effects of ponesimod on vascular reactivity were assessed in other species, it was found that the vasoconstriction action of ponesimod was greater in dog small mesenteric arteries, in contrast to rat mesenteric arteries that did not contract. Differences were also found using the coronary arteries from dog and rats, as dog coronary arteries were sensitive to the vasoconstrictor actions of ponesimod, in contrast to rat coronary arteries that did not contract.

In in vivo cardiovascular studies, hemodynamic and electrocardiographic effects of ponesimod were investigated in several species. In M normotensive rat, single oral doses of ponesimod (30, 100, 300, and 600 mg/kg) had no effect on mean arterial pressure or heart rate. With administration of ponesimod (30 mg/kg) in food for 4 weeks increase in mean arterial pressure was observed from Day 10 until the end of dosing. In M hypertensive rats, ponesimod (100 mg/kg) had no effect on mean arterial pressure or heart rate when given during the inactive phase but decreased the normal increase in heart rate during early active phase when administered during the active phase.

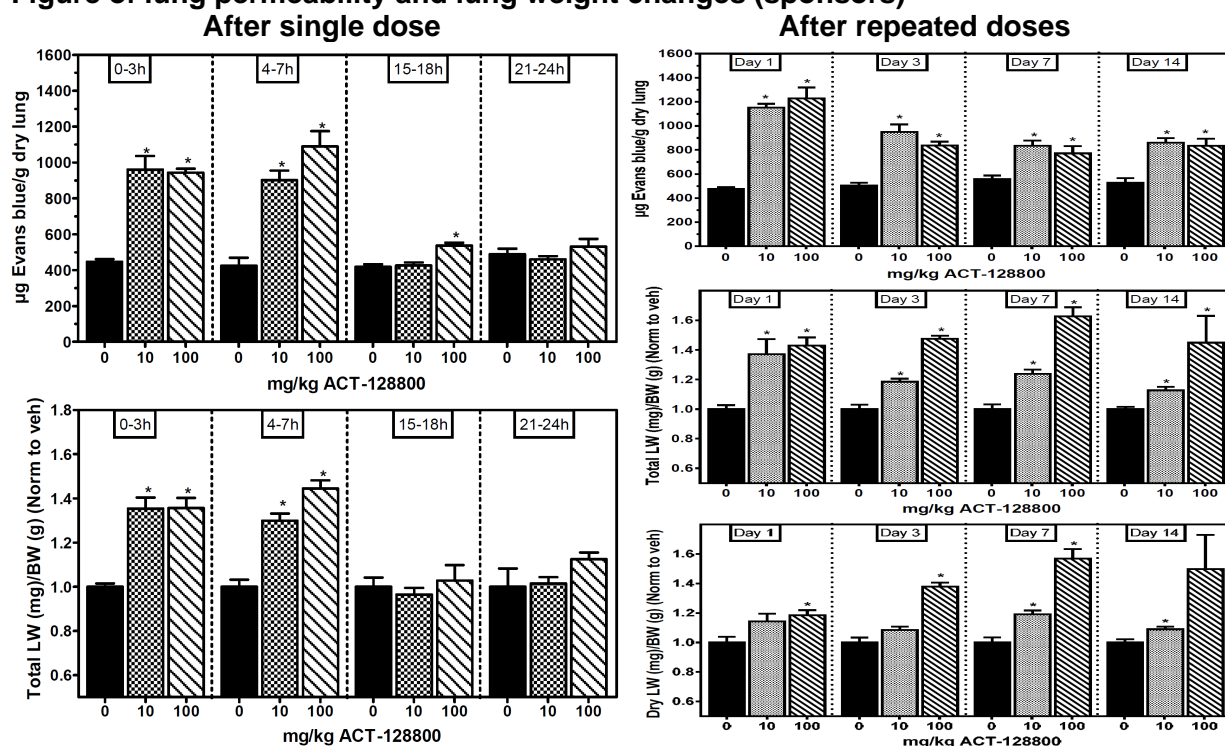
In telemeterized M Dunkin Hartley guinea pig, single oral administration of ponesimod (0.3, 1, 3, 10, and 30 mg/kg) resulted in dose-dependent decreases in mean blood pressure and heart rate, an increase in ventricular rate, and first-degree AV block followed by second-degree AV block and sometimes third-degree AV block that lasted no longer than 4 hours; no effect was observed at 0.1 mg/kg. Repeat administration of oral ponesimod (1 and 3 mg/kg) resulted in second-degree AV block after the initial dose, but the electrocardiographic effects were reduced after the second dose and were dependent on the timing of the second dose. Administration of the second dose, 12-36 hours post first dose, resulted in a much weaker effect and, therefore, suggests desensitization. However, when the second dose was administered at 48 hours post first dose, similar electrocardiographic effects were observed with greatly reduce plasma concentrations of ponesimod, suggesting resensitization is related to the plasma concentration of ponesimod. An up-titration regimen of ponesimod, with increasing doses from 0.1 (4 doses), 0.2 (3 doses), to 0.4 (1 dose) mg/kg every 12 hours, reduced the effects on heart rate and rhythm of conscious guinea pigs, whereas using an initial dose of 0.4 mg/kg induced second-degree AV block in 3 of 4 animals. In anesthetized M Dunkin Hartley guinea pigs, IV administration of ponesimod (0.03 mg/kg/20 min) induced decreases in mean blood pressure and first-degree AV block followed by second-degree AV block and occasionally third-degree AV block. These effects of ponesimod were rapidly reversed by a β -adrenoceptor agonist but not by a muscarinic receptor antagonist.

In conscious M Beagle dogs, oral ponesimod (10, 30, and 100 mg/kg) produced no effect on heart rate or ECG parameters at any dose. However, at 30 mg/kg, blood pressure was increased in 1 dog, lasting for 4 hours, and at 100 mg/kg blood pressure was increased in 2 dogs, lasting for 7 hours. Repeated doses of ponesimod (40 mg/kg/day) increased blood pressure at 0.5-2 hours post-dose. In anesthetized F Beagle dogs IV administration of ponesimod (1.4, 4, and 8 mg/kg/30 min) induced increases in mean blood pressure associated with an increase in total peripheral resistance, followed by a decrease in stroke volume.

Respiration was assessed for 24 hours in M Wistar rats after administration of oral ponesimod. An approximate 2-fold increase in enhanced pause (Penh; a marker of bronchoconstriction) was observed at 3 and 6 hours post-dose after 100 mg/kg oral ponesimod compared to 3-fold increase in rats dosed with oral fingolimod (10 mg/kg). In another study in M Wistar rats, a single oral dose of ponesimod (100 mg/kg) increased tidal volume and peak expiratory flow at 1.5-2.5 hours post-dose. In a repeat dose study in M Wistar rats, there were increases in enhanced pause, tidal volume, and peak expiratory flow up to Day 15 at all doses of ponesimod (10 and 100 mg/kg/day, PO), but no change in respiration parameters was observed after 4 weeks of repeat dosing of ponesimod up to 100 mg/kg/day. Microscopic findings in the lungs included an increase in pulmonary alveolar histiocytosis. There was no effect of ponesimod (≥ 10 mg/kg) on respiration in dogs.

Lung vascular permeability was assessed in M Wistar rats using IV injected Evans blue dye as a marker for plasma protein leakage. Single oral doses of ponesimod (0, 0.2, 0.5, 1, 2, 4, 6, 10, 30, and 100 mg/kg) induced a dose-dependent increase in lung vascular permeability that corresponded to the level of lymphopenia. This suggests that the S1P₁ receptor is responsible for both increased lung vascular permeability and reduced blood lymphocyte count. Lung permeability and lung weight were observed after repeated doses of ponesimod (0, 10, and 100 mg/kg/day for 14 days, n = 4 M/group). After the first dose, increase in permeability and lung weight appeared to peak at 4-7 hours post-dose. With repeated doses there was a slight decrease in the increased permeability after the peak at the first dose, which appeared to plateau by Day 14; lung weight was always statistically significantly increased at both doses.

Figure of lung permeability and lung weight changes (sponsors)



Top panel, lung EB leakage; bottom panel, total lung weight.

Top panel, lung Evans Blue leakage; middle panel, total lung weight; bottom panel, dry lung weight.

Results are expressed as mean $\pm$ SEM; *p<0.05 versus vehicle. LW, lung weight; BW, body weight; Norm, normalized; veh, vehicle

In M Wistar rats (n = 2 control and 3 poniesimod), lung vascular permeability and histopathology changes were assessed after single and 7-day repeat doses of oral poniesimod (0 and 100 mg/kg/day) using IV injected FITC-albumin as a marker for lung capillary leakage. Distended perivascular spaces containing fluid, with the presence of macrophages and eosinophils, were observed in lungs 3-24 hours post single dose. These changes corresponded with an increase in lung weight. After 7-days, the increased lung weight was slightly reduced, with microscopic findings of perivascular distension with edema fluid, cellular infiltration by eosinophils/macrophages, and alveolar histiocytosis slightly less severe compared to single dose observations.

In in vitro studies using isolated trachea from different anatomical sections, poniesimod (0.1-1 μ M) induced a contractile response that was greater in a lower tracheal segment compared to an upper tracheal segment. The contractile response was not affected by an S1P₁ receptor antagonist (ACT-868599) but was inhibited by an S1P₃ receptor antagonist (ACT-893599) and was consistent with a higher expression ratio (3-fold) of s1p3:s1p1 receptor mRNA in the lower tracheal segment.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Absorption

After oral administration of ponesimod to nonclinical species (mice, rats, dogs, and monkeys), T_{max} for ponesimod generally ranged from 0.5 to 8 hours and $t_{1/2}$ ranged from 1.4 to 25 hours. Data in humans were similar, with T_{max} between 1.5 and 6 hours. Absolute oral bioavailability of ponesimod was 35-61% in rats, 57-74% in dogs, and 84% in humans.

Table: Pharmacokinetic parameters of ponesimod in plasma of animals and human after single and multiple oral administrations

Parameter	Rat ^a				Dog ^b				Human ^c	
	Male		Female		Male		Female		Day 1	Day 7
	Day 1	Week 26	Day 1	Week 26	Day 1	Week 52	Day 1	Week 52		
Dose (mg/kg)	0.4	0.4	0.4	0.4	3	3	3	3	20 mg	20 mg
T_{max} (h)	2.0	1.0	1.0	0.5	1.5	1.5	2	2	4.0	2.5
C_{max} (ng/mL)	29.9	55.8	48.4	77.8	616	881	1230	1370	91.7	207
AUC_{0-24h} (ng*h/mL)	63.3	129	99.0	191	6740	8330	14300	16300	1318	3473
$t_{1/2}$ (h)	2.0	2.6	3.2	2.8	7.7	6.6	8.3	8.4	-	32.7
Accumulation Factor ^d	-	-	1.6	1.5	-	-	2.1	2.0	-	2.6

a - Rat 26 Week study (№: T-05.134); b - Dog 52 Week study (№: T-06.186); c - Human study (№: AC-058-102); d - Accumulation factor = AUC_{0-24h} multiple dose/ AUC_{0-24h} single dose (Day 1).

5.1.2 Distribution

Distribution of ponesimod in blood and plasma protein binding were evaluated in several animal species. In mouse, rat, guinea pig, rabbit, dog, monkey, and human, the blood-to-plasma ratios of ponesimod was 0.54-0.85; for the major metabolite, M13, it was 0.50-0.57 in mouse, rat, dog, and human. Plasma protein binding was high (>98%) for ponesimod and M13 in the same species.

Tissue distribution studies were conducted in albino and pigmented rats. In albino rats, radioactivity was widely distributed into most tissues and was maximal at 1-4 hours post-dose. The highest exposures were found in GI tract and liver but was also observed in the brain and spinal cord at higher levels than blood at 1-4 hours post-dose. The radioactivity in the brain was primarily unchanged ponesimod, with no detectable levels of metabolites M12 and M13. In pigmented rats, radioactivity levels were higher than in the albino rats, including the eye uveal tract and skin.

Table: Ponesimod tissue distribution after oral dosing (Sponsor's)

Sampling Time	Concentration (ng equivalents of ponesimod/g tissue)							
	Albino					Pigmented		
	1 hour	4 hours	1 day	3 days	7 days	1 day	7 days	21 days
Tissues/Organs								
Whole blood	405	234	27	25	BQL	30	19	BQL
Brain	936	453	15	BQL	BQL	BQL	BQL	BQL
Pineal body	1480	916	24	BQL	BQL	30	BQL	BQL
Pituitary gland	1260	809	37	BQL	BQL	58	BQL	BQL
Spinal cord	777	554	BQL	BQL	BQL	18	BQL	BQL
Nasal mucosa	341	-	43	17	BQL	25	30	BQL
Tongue	1810	878	32	18	BQL	26	21	BQL
Tooth pulp	869	357	44	BQL	BQL	30	BQL	BQL
Esophagus	2010	563	80	BQL	BQL	37	BQL	BQL
Heart	1670	837	19	BQL	BQL	22	BQL	BQL
Kidney - cortex	2500	1350	117	41	24	84	36	BQL
- medulla	1720	850	41	20	16	44	16	BQL
Kidney pyramid ^b	moderate	moderate	low	low	RND	low	RND	RND
Urinary bladder ^b	high	moderate	moderate	low	-	-	-	RND
Bulbo-urethral gland	934	945	44	BQL	BQL	28	BQL	BQL
Liver	6360	3830	246	63	27	201	57	BQL
Lungs	754	390	34	15	BQL	34	19	BQL
Pancreas	2300	1050	51	19	BQL	40	19	BQL
Spleen	1190	544	47	26	20	47	26	BQL
Adrenal - cortex	2270	988	61	34	BQL	74	33	BQL
- medulla	1300	629	BQL	BQL	BQL	35	BQL	BQL
Harderian glands	2080	2050	259	78	BQL	245	35	BQL
Lacrimal glands - intraorbital	1710	1810	66	54	BQL	52	22	BQL
- exorbital	1940	1650	59	29	BQL	63	20	BQL
Uveal tract	603	269	36	BLO	BLO	126	35	BLO
Lymph nodes - mandibular	919	490	43	BQL	BQL	41	BQL	BQL
Salivary gland	1970	1010	51	21	BQL	43	25	BQL
Thymus	994	452	46	28	BQL	37	BQL	BQL
Thyroid	1520	680	82	BQL	BQL	89	BQL	BQL
Seminal vesicles	216	194	39	28	BQL	45	34	BQL
Prostate	1270	993	32	18	BQL	31	BQL	BQL
Testes	426	406	25	15	BQL	15	17	BQL
Epididymis	295	626	39	24	BQL	30	17	BQL
Preputial gland	1140	2280	681	74	BQL	705	BQL	BQL
Bone	-	-	-	-	-	-	-	-
Bone marrow	577	381	58	BQL	BQL	77	BQL	BQL
Fat (brown)	1610	845	206	95	BQL	138	40	BQL
Fat (white)	276	276	39	BQL	BQL	BQL	BQL	BQL
Muscle (skeletal)	623	466	15	BQL	BQL	BQL	BQL	BQL
Skin	462	380	33	79	37	66	106	46
Stomach contents ^b	high	high	low	low	RND	low	low	low
Stomach mucosa - fundus	3770	1690	52	23	BQL	50	BQL	BQL
- nonfundic	3720	2480	72	19	BQL	105	BQL	BQL
Cecum contents ^b	low	high	high	moderate	RND	high	moderate	low
Cecum mucosa	905	7320	3040	62	BQL	641	415	BQL
Small intestine contents ^b	moderate	high	moderate	moderate	RND	moderate	RND	low
Small intestine mucosa	1350	10200	145	74	BQL	105	BQL	BQL
Large intestine contents ^b	low	low	high	moderate	RND	high	moderate	-
Large intestine mucosa	1180	2790	234	86	BQL	774	188	BQL
Bile ducts ^b	high	high	moderate	RND	RND	moderate	RND	RND
Rectum contents ^b	low	low	high	moderate	-	high	moderate	low
Rectum mucosa	984	436	484	62	BQL	1330	523	BQL

^a Ponesimod is known as ACT-128800 in studies conducted/sponsored by Actelion Pharmaceuticals, Ltd.

^b Relative levels of radioactivity were described as "high", "medium", "low", or not detected (RND) based on visual inspection of the autoradiogram printouts because tissues did not lend themselves to quantitative analysis.

- = not available, not assessed; BQL = below the limit of quantification; RND = radioactivity not detected

In the reproduction and developmental studies, ponesimod was detected in pups from dams dosed with ponesimod. Plasma exposures (AUC) in pups from dams dosed with 5, 10, and 20 mg/kg ponesimod (Study No: 13502) were 163, 265, and 740 ng*h/mL, respectively, on lactation Day 12.

5.1.3. Metabolism

Two main human metabolites were identified; M12 and M13, both carboxylic acids, with M12 determined as less than 10%, but M13 greater than 10% of total drug related exposure in healthy subjects. Both M12 and M13 are less potent than ponesimod at S1P receptors. Adequate systemic exposure of M13 was obtained in mouse and rat (see tables below).

Table: Pharmacokinetics of metabolites M12 and M13 in humans (Sponsor's)**Table 11: PK of Ponesimod, M12 and M13 in Plasma After Multiple-dose Oral Administration of Ponesimod in Healthy Subjects (AC-058-109)**

Parameter	Geometric Mean (95% CI); t _{max} : Median (Range)					
	Ponesimod		M12		M13	
	40 mg Day 9	100 mg Day 18 ^a	40 mg Day 9	100 mg Day 18 ^a	40 mg Day 9	100 mg Day 18 ^a
N	12	11	12	11	12	11
t _{max} , h	2.5 (2.5-6.0)	2.5 (2.5-10.0)	2.5 (2.5-10.0)	2.5 (2.5-10.0)	10.0 (2.5-16.0)	10.0 (2.5-10.0)
C _{max} , ng/mL	316 (270-371)	711 (595-850)	27.0 (23.8-30.7)	60.0 (50.6-71.1)	68.7 (60.2-78.3)	168 (137-207)
AUC _{0-24h} , ng.h/mL	5352 (4602-6224)	11,841 (9905-14,156)	415 (365-472)	977 (856-1114)	1301 (1123-1507)	3133 (2551-3848)
t _{1/2} , h	-	31.4 (28.2-34.9)	-	29.0 (23.2-36.3)	-	34.3 (30.4-38.7)

AUC_{0-24h}=area under the plasma concentration-time curve from time 0 to 24 hours; CI=confidence interval; C_{max}=maximum plasma concentration; M12=ACT-204426; M13=ACT-338375; N=maximum number of subjects with data; PK=pharmacokinetics; t_{1/2}=half-life; t_{max}=time to reach the maximum plasma concentration.

^a One subject had withdrawn prior to Day 18 and was only included in the Day 9 analysis.

Source: Mod5.3.4.1/AC-058-109/Tab6, Tab7, Tab8, Tab9, Tab10 and Tab11

Table: Pharmacokinetics of metabolite M13 from the carcinogenicity studies (Sponsor's) Mice

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
30	26	f	2	103	454 [#]	3.43	15.1
30	52	f	2	97.6	387 [#]	3.25	12.9
50	26	m	2	143	566 [#]	2.86	11.3
50	52	m	1	115	511 [#]	2.30	10.2
100	26	f	4	274	1530	2.74	15.3
100	52	f	2	256	2210	2.56	22.1
150	26	m	2	382	2070	2.55	13.8
150	52	m	2	394	2460	2.63	16.4
300	26	f	2	579	3660	1.93	12.2
300	52	f	2	509	3620	1.70	12.1
400	26	m	2	739	5450	1.85	13.6
400	52	m	2	558	4090	1.40	10.2

[#]AUC0-6h, all samples were BLQ at 24h

Rat

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
3	26	m	2	18.8	86.7 [#]	6.27	28.9 [#]
3	52	m	2	20.8	109 [#]	6.93	36.2 [#]
10	26	f	2	36.5	162 [#]	3.65	16.2 [#]
10	52	f	2	67.6	457	6.76	45.7
10	26	m	2	51.6	324	5.16	32.4
10	52	m	2	74.6	438	7.46	43.8
30	26	f	4	64.5	359 [#]	2.15	12.0 [#]
30	52	f	2	131	1110	4.37	37.0
30	26	m	2	104	715	3.47	23.8
30	52	m	2	152	1020	5.07	33.9
100	26	f	2	156	1250	1.56	12.5
100	52	f	2	332	3490	3.32	34.9

[#]AUC0-8h, more than 50% of samples were BLQ at 24h

5.1.3.1 In vitro

In vitro metabolism of ponesimod was assessed in hepatocytes; all human metabolites were formed in at least one nonclinical species

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

Species ^b	Time (h)	M4	M7	M9	M10	M11	M12	M13	Ponesimod	Σ all products ^a
Human ^c	24	-	-	-	4.9	12.0	9.3	32.4	31.4	92.3
Human	24	1.2	1.2	2.1	1.5	4.7	6.6	8.6	72.4	98.3
Mouse	24	11.2	1.7	3.7	7.7	-	10.9	2.7	35.6	99.2
Rat ^c	4	-	-	14.5	4.5	-	2.0	3.9	49.5	88.5
Rat ^c	24	-	7.3	3.0	4.5	-	<1	5.6	4.4	59.2
Dog	24	-	-	-	5.5	3.5	10.0	1.4	74.9	97.7
Monkey	24	-	-	6.9	1.4	2.1	10.2	3.5	67.2	95.7
Minipig	4	-	-	-	9.1	13.0	-	-	65.4	100.1
Minipig	24	-	-	-	26.2	37.9	-	1.6	5.9	93.3

Source: [Mod4.2.2.4/B-05.108](#)

Notes: Samples were analyzed using HPLC coupled to radiodetection. Values represent relative amounts of metabolites, as a percentage of the sum of all metabolites plus parent compound. Only metabolites generated by human hepatocytes with an abundance >1% are listed.

^a Values <100% indicate formation of species-specific metabolites not included in this table.

^b Cryopreserved hepatocytes unless noted.

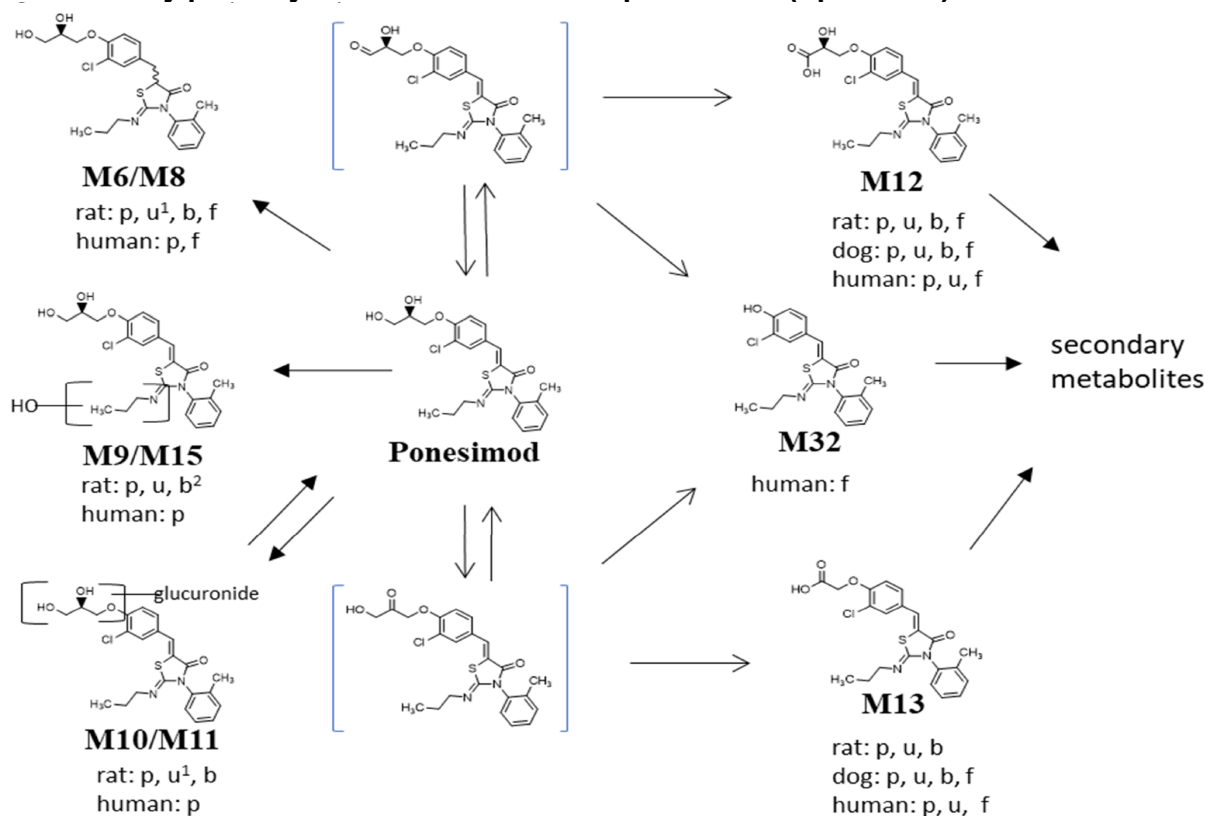
^c Fresh hepatocytes.

- = not detected; HPLC = high performance liquid chromatography

5.1.3.2. In vivo

After oral and/or IV dosing in rats, unchanged ponesimod was most abundant in plasma with metabolites M1, M7, M9, M10, M12-M16, and M24 detected. Metabolites detected in urine included M2, M5, M9, M10, M14, M20-23, and M25, with M2 being the main metabolite. Many metabolites were detected in bile with the main metabolites including M5-M14, M16, and M18; metabolites detected in feces included metabolites M2, M6, M8, M12, M16, M20, and M22-M24.

After oral dosing in dogs, unchanged ponesimod was most abundant in plasma with metabolites M12 and M13 detected. Metabolites detected in urine included M2, M4, M10, M12, M13, M16, M21, M22, M24, and M25, with M2, M10, M12, M14, and M16 the main metabolites. The metabolites detected in bile included M1, M10, M11, M12, and M13, and metabolites detected in feces included metabolites M1, M12, and M13, with M12 the main metabolite in bile and feces.

Table: Primary pathways in the metabolism of ponesimod (Sponsor's)

1 – M6 and M10 were detected in rat urine, but not M8 or M11.

2 – M9 was detected in rat bile, but not M15.

b = bile; f = feces; p = plasma; u = urine

5.1.4 Excretion

After oral dosing of ponesimod in rats and dogs, the major excretion pathway was in feces (approximately 93% and 42%, respectively), with unchanged ponesimod a minor component.

5.1.5 Drug interactions Studies

Ponesimod had an inhibitory effect on activity of CYPs 2C19, 2C9, 2C8, 2D6, 2J2, and 3A4, with IC_{50} values from 7.8-36 μ M; the metabolite M13 had an inhibitory effect on activity of CYPs 2C8, 2C9, and 3A4 (astemizole) with IC_{50} values of 9.7, 43, and 20 μ M, respectively. However, neither ponesimod nor M13 had a time-dependent inhibition on activity of CYPs (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4). Ponesimod did not significantly induce CYPs 1A2, 2B6, and 2C9 but did slightly induce CYP3A4 (activity and mRNA) and M13 did not induce CYPs 1A2, 2B6, and 3A4 significantly.

Table: Ponesimod inhibition of CYP and UGT enzyme activity (Sponsor's)

Enzyme	Test System	Ponesimod			M13			
		IC ₅₀ (μM)		K _i (μM)	IC ₅₀ (μM)		K _i (μM)	
		Bound	Unbound ^a		Bound	Unbound ^a		
CYP	1A2	HLM	>50	>9	-	>100	>72	-
	2A6	HLM	>100	>18	-	>100	>72	-
	2B6	recombinant ^b	>50	>9	-	>100	>72	-
	2C8	HLM	28	5.0	19	9.7	7.0	6.4
	2C9	HLM	14	2.5	6.5	43	31	-
	2C19	recombinant ^b	9.6	1.7	5.9	>100	>72	-
	2D6	HLM	36	6.5	10	>100	>72	-
	2E1	HLM	>50	>9	-	-	-	-
	2J2	HLM	22	4.0	-	>50	>36	-
	2J2	recombinant ^b	7.8	1.4	-	>50	>36	-
	3A4-midazolam	HLM	19	3.4	15	160	115	-
	3A4-testosterone	HLM	22	4.0	-	200	144	-
	3A4-astemizole	HLM	8.0	1.4	-	20	14	-
	3A4-astemizole	recombinant ^b	26	4.7	-	>50	>36	-
UGT	1A1	recombinant ^b	>50	>9	-	24	17	-
	2B7	recombinant ^b	17	3.1	-	>50	>36	-

Source: [Mod4.2.2.6/B-05.078](#), [B-13.080](#), and [FK13543](#).

Note: Ponesimod was tested at 8 to 9 concentrations up to 100 μM for CYPs 2A6, 2C8, 2C19, and 2D6, and at 7 to 8 concentrations up to 50 μM for the other CYPs (B-05.078 and B-13.080). M13 was tested at 11 concentrations up to 500 μM for CYPs 2C9 and 3A4, and at 8 to 9 concentration up to 100 μM for the other CYPs (B-05.078 and B-13.080). In addition, ponesimod and M13 were tested 7 concentration up to 50 μM for CYPs 2J2 and 3A4 (FK13543). Ponesimod and M13 were tested at 8 concentrations up to 50 μM for UGTs 1A1 and 2B7 (B-13.080).

^a Unbound IC₅₀ values for ponesimod and M13 were calculated using $f_{u,inc} = 0.18$ and 0.72 based on non-specific binding to liver microsomes of 82.1% and 28.1%, respectively. These values were determined under the conditions of the CYP2C8 assay (0.25 mg/mL microsomes) and applied to the other assays using HLM and also to assays using recombinant enzyme expressed in baculovirus-infected Sf9 insect cells, which used lower amounts of microsomal protein (0.1-0.15 mg/mL) as it provides a conservative approach.

^b Recombinant enzyme expressed in baculovirus-infected insect cells.

- = not applicable/not determined; CYP = cytochrome P450; $f_{u,inc}$ = unbound fraction in the incubation; HLM = human liver microsomes; IC₅₀ = concentration that elicits 50% inhibition; K_i = inhibition constant; UGT = uridine 5'-diphosphate-glucuronosyl transferase

6 General Toxicology

6.2 Repeat-Dose Toxicity

6.2.1 Previously reviewed studies

Mouse studies

- “The Sponsor conducted two 13-week oral toxicity studies in mouse to establish suitable doses for a 2-year carcinogenicity study. In the first GLP study (T-06.049), an MTD was not established as the NOAEL was at the high dose of 90 mg/kg/day; thus, a second GLP study was conducted and reviewed, below” **DARRTs, IND 101,722; Houghtling review Jul 25, 2013.)**
- **ACT-128800 – Follow-up 13-Week oral (gavage) toxicity study in the mouse (Study No. T-07.015; DARRTs, IND 101,722; Houghtling review Jul 25, 2013); 0, 150, 400, and 800 mg/kg/day.**

Key findings included findings that liver, lung, mesenteric lymph node, spleen, and thymus were the target organs. The MTD was established at 150 mg/kg/day

based upon liver toxicity. No NOAEL was established due to the findings in the lung (increased weight and alveolar hyalinoses and histiocytosis) and liver in LDM. The histopathology findings identified in the mesenteric lymph node, spleen, and thymus are likely mediated through the immunomodulatory activity of ACT-128800 at S1P receptors.

Tables: Lung histopathological data

Group	0		150		400		800	
	mg/kg/day		mg/kg/day		mg/kg/day		mg/kg/day	
Treatment	M	F	M	F	M	F	M	F
Sex	M	F	M	F	M	F	M	F
No. of animals examined	11	11	10	10	10	10	11	13
- alveolar hyalinoses	-	-	1	1	3	1	6	3
Minimal	-	-	1	1	3	1	3	3
Slight	-	-	-	-	-	-	2	-
Moderate	-	-	-	-	-	-	1	-
- alveolar histiocytosis	-	-	2	1	-	1	3	-
Minimal	-	-	2	1	-	1	2	-
Slight	-	-	-	-	-	-	1	-

Toxicokinetic data (Sponsor's)

Occasion	Dose (mg/kg/day)	Sex	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-8h} (ng.h/mL)
day 0	150	Male	7197	1	41193
		Female	9240	1	36843
	400	Male	11833	3	79730
		Female	11567	3	65560
	800	Male	16967	3	91633
		Female	16833	3	95833
week 13	150	Male	9607	1	50818
		Female	10967	3	59473
	400	Male	14267	3	77658
		Female	12000	3	81612
	800	Male	19050	3	130208
		Female	17200	8	118400

Rat studies

- **ACT-128800: 26 Week oral (gavage) administration toxicity study in the rat with a 13-week treatment free period (Study No. T-05.134; DARRTs, IND 101,722; Houghtling review Jul 25, 2013); 0, 0.4, 4, 30, and 100 mg/kg/day.**

Key findings included unscheduled death of 1 LDM on Day 6. The cause of death was undetermined, but findings included: gaseous distension in the stomach, duodenum, and jejunum, dark salivary glands and mandibular lymph nodes, red foci on the thymus, and reddened lung lobes and several dark areas on all lung lobes. The major target organs were, in M, liver, lung, and lymphoid tissues and in F, liver, lung, spleen, and heart (organ weight, only). The expected pharmacodynamic effects were observed and included dose-dependent increased incidence and severity of atrophy in spleen and decreased marginal zone in both sexes at the LMD, HMD, and HD, decreased numbers and size of white pulp follicles in the thymus and lymph nodes characterized by loss of definition between the cortex and medulla or at minimal grade, a loss of lymphocytes near the edge of the medulla (thymus), and an absence of germinal centers and reduction in paracortical lymphocytes (mesenteric LN) in HMMD and HMD and in LMDH, HMDF, and HDF, reduction in lymphocyte counts at the HMD and HD in M and F found at Weeks 13 and 26 with the reduction still apparent in HMD at Week 39 (treatment-free period). Sex-dependent increase in systemic ACT-128800 exposure was found in F, which was 2.8-fold higher than in M at all dose levels at the end of the 26-week dosing period. The MTD is considered 30 mg/kg/day (however, the Sponsor

considered the MTD to be 100 mg/kg/day). The NOAEL is 0.4 mg/kg/day based upon findings of lymphoid organ atrophy in M and F, reduction in body weight gain in M, increased lung weight, and microscopic findings of foamy alveolar macrophages in M and F. The HED at the NOAEL = 0.065 mg/kg/day.

**Table: Lung histopathological data (Sponsor's)
Main**

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

ORGAN AND FINDING DESCRIPTION	SEX: -----MALE-----					SEX: -----FEMALE-----				
	GROUP: -1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:	15	15	15	15	15	15	15	15	15	14
LUNG NUMBER EXAMINED:	15	15	15	15	15	15	15	15	15	14
--OSSEOUS METAPLASIA	0	1	2	0	0	0	0	0	0	3
--ALVEOLAR MACROPHAGES	6	6	1	0	0	7	3	1	0	5
--PIGMENT IN ALVEOLAR MACROPHAGES	0	1	0	0	0	0	0	0	0	5
--VASCULAR MINERALISATION	0	0	0	1	0	0	0	0	0	0
--AGONAL CONGESTION/HAEMORRHAGE	0	1	0	0	2	0	0	1	1	0
--INFLAMMATORY CELL FOCI	7	7	10	6	6	5	2	2	4	4

Recovery

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

ORGAN AND FINDING DESCRIPTION	SEX: -----MALE-----					SEX: -----FEMALE-----				
	GROUP: -1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NUMBER:	5	5	5	5	5	5	4	5	5	5
LUNG NUMBER EXAMINED:	5	5	5	5	5	5	4	5	5	5
--ALVEOLAR MACROPHAGES	4	0	2	1	4	3	3	0	3	3
--PIGMENT IN ALVEOLAR MACROPHAGES	0	0	0	0	1	0	0	0	0	1
--VASCULAR MINERALISATION	0	0	0	1	0	0	0	0	0	0
--AGONAL CONGESTION/HAEMORRHAGE	0	0	1	0	0	0	1	0	0	1
--INFLAMMATORY CELL FOCI	2	4	1	2	1	3	1	1	1	0

Table: Toxicokinetic (Sponsor's)

Occasion	Dose	Sex	AUC(0-τ) (ng.h/mL)	AUC(0-τ) (norm)	C _{max} (ng/mL)	C _{max} (norm)	t _{max} (h)	t _{1/2} (h)	RA _{AUC}	RA _{C_{max}}
Day 1	0.4	Male	63.3	158	12.0	29.9	2.00	2.02	-	-
		Female	129	323	22.3	55.8	1.00	2.61	-	-
	4	Male	1410	353	263	65.8	2.00	2.50	-	-
		Female	2280	570	341	85.3	2.00	3.06	-	-
	30	Male	12900	430	1730	57.6	2.00	2.62	-	-
		Female	24000	801	2840	94.8	2.00	3.38	-	-
100	Male	38900	389	4780	47.8	4.00	3.38	-	-	
	Female	77300	773	6100	61.0	2.00	5.94	-	-	
Week 26	0.4	Male	99.0	248	19.4	48.4	1.00	3.15	1.56	1.62
		Female	191	477	31.1	77.8	0.50	2.79	1.48	1.39
	4	Male	1690	421	290	72.4	2.00	3.41	1.19	1.10
		Female	2540	636	394	98.4	2.00	3.53	1.12	1.15
	30	Male	19800	661	2310	76.8	4.00	3.26	1.53	1.33
		Female	39600	1320	4080	136	2.00	5.24	1.65	1.43
	100	Male	36200	362	3760	37.6	2.00	5.06	0.931	0.787
		Female	103000	1030	8480	84.8	2.00	6.02	1.34	1.39

C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]
AUC(0-τ) (norm) = AUC [ng.h/mL] / dose [mg/kg/day]
τ = 24 hours

6.2.2 Reviewed studies

Study title: ACT-128800: Oral (gavage) MTD (dose evaluation) toxicity study in the Wistar rat.

Study no.: T-05.018
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: February 21, 2005
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: ACT-0128800, Batch AA-016498-batch- (b) (4) 95.19%

Key Study Findings included clinical signs of sedation, ruffled fur, and tachypnea at the LD and HD, with incidences more severe at the HD but no premature deaths. Decrease in body weights occurred at the HD, which was greater in F. Hematology findings attributed to the pharmacological action of ponesimod included decreased WBCs and lymphocytes at all doses in M and F. The sponsor considered the LD as the maximum dose for a 14-Day study.

Methods

Doses: 400 and 600 mg/kg
 Frequency of dosing: Daily for 3 days
 Route of administration: Oral by gavage
 Dose volume: 10 mL/kg
 Formulation/Vehicle: 0.25% Methylcellulose and 0.05% Tween80
 Species/Strain: Rat / Wistar Han
 Number/Sex/Group: 2
 Age: 6 weeks
 Weight: Males 152.1-155.5 g; Females 115.2-132.4 g

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

	Rat nr	Dose PO mg/kg	Time PO h	Conc PO ng/mL
ACT-128800 (b) (4) study 859304	Gr1. 1M	600	3	18500
	Gr1. 2M	600	3	14800
	Gr1. 5F	600	3	11800
	Gr1. 6F	600	3	11900
	Gr2. 3M	400	3	15100
	Gr2. 4M	400	3	7080
	Gr2. 7F	400	3	10900
	Gr2. 8F	400	3	12600

Study title: ACT-128800: 14-Day oral (gavage) dose range finding toxicity study in the rat.

Study no.: T-05.019
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: March 14, 2005
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: ACT-0128800, Batch AA-016498-batch-
 (b) (4), 95.19%

Key Study Findings included 2 HDF found dead; therefore, the other HD animals were euthanized early. Decreased body weight gain was observed at the LD and MD, and body weight was decreased at the HD, which correlated with a decrease in food consumption. Hematology findings included decreased WBCs and lymphocytes at all doses in M and F. The main macroscopic finding included discolored lungs in HDM, MDF, and HDF and microscopic findings included fatty change in the adrenal cortex, cellular debris in the epididymides in HDM, alveolar histiocytosis, edema, emphysema, and hyalinosis in the lungs in MDM, HDM, MDF, and HDF, hypocellularity in lymph nodes at all doses in M and F, and white pulp atrophy in the spleen at all doses in M and F. No NOAEL could be established due to changes observed at the LD, 30 mg/kg/day.

Methods

Doses: 0, 30, 125, and 400 mg/kg
 Frequency of dosing: Daily
 Route of administration: Oral by gavage
 Dose volume: 10 mL/kg
 Formulation/Vehicle: 0.25% Methylcellulose and 0.05% Tween80
 Species/Strain: Rat / Wistar Han
 Number/Sex/Group
 Main: 5
 Toxicokinetic: 3
 Age: 6 weeks
 Weight: Males 137.6-162.3 g; Females 115.2-133.9 g

Histological Findings

Histological findings included an increase in severity of diffuse fatty change in the adrenal cortex, cellular debris in the epididymides in HDM, alveolar histiocytosis, edema, emphysema, and hyalinosis in the lungs in MDM, HDM, and HDF, hypocellularity in lymph nodes at all doses in M and F, and white pulp atrophy in the spleen at all doses in M and F.

Table: Summary of selected microscopic findings

Tissue	Finding	(mg/kg): Number of rats:	MALE				FEMALE				
			0	30	125	400	0	30	125	400	
			5	5	5	0 (5)	5	5	5	0 (5)	
Adrenal	Diffuse fatty change	Grade 1:	4	2	3	0 (1)	2	1	2	0 (5)	
		Grade 2:	0	1	1	0 (2)	0	0	0	0 (0)	
		Grade 3:	0	0	0	0 (1)	0	0	0	0 (0)	
Epididymides	Cellular debris	Grade 1:	0	0	0	0 (1)	-	-	-	-	
		Grade 2:	0	0	0	0 (0)	-	-	-	-	
		Grade 3:	0	0	0	0 (3)	-	-	-	-	
Liver	Glycogen deposits	Grade 1:	0	0	0	0 (1)	0	0	0	0 (3)	
		Grade 2:	0	0	0	0 (3)	0	0	0	0 (2)	
Lung	Alveolar histiocytosis	Grade 1:	0	1	0	0 (0)	0	0	0	0 (0)	
		Grade 2:	0	0	0	0 (0)	0	0	3	0 (0)	
		Grade 3:	0	0	1	0 (1)	0	0	2	0 (3)	
		Grade 4:	0	0	1	0 (4)	0	0	0	0 (2)	
	Alveolar edema	Grade 1:	0	0	0	0 (0)	0	0	0	0 (0)	
		Grade 2:	0	0	0	0 (1)	0	0	0	0 (2)	
		Grade 3:	0	0	0	0 (0)	0	0	0	0 (1)	
	Alveolar emphysema	Grade 1:	0	0	0	0 (1)	0	0	0	0 (1)	
		Grade 2:	0	0	1	0 (0)	0	0	0	0 (0)	
		Grade 3:	0	0	0	0 (1)	0	0	0	0 (0)	
	Alveolar hyalinosi	Grade 1:	0	0	2	0 (1)	0	0	0	0 (1)	
		Grade 2:	0	0	1	1 (0)	0	0	0	0 (1)	
		Grade 3:	0	0	0	0 (1)	0	0	0	0 (0)	
	Lymph node - Mesenteric	Hypocellularity	Grade 1:	0	0	0	0 (0)	0	1	3	0 (4)
			Grade 2:	0	3	3	0 (3)	0	0	2	0 (1)
			Grade 3:	0	1	2	0 (1)	0	0	0	0 (0)
Grade 4:			0	1	0	0 (0)	0	0	0	0 (0)	
Lymph node - Popliteal	Hypocellularity	Grade 1:	0	0	0	0 (0)	0	1	0	0 (1)	
		Grade 2:	0	1	0	0 (1)	0	0	2	0 (0)	
Spleen	Congestion	Grade 1:	0	0	0	0 (2)	0	0	0	0 (1)	
		Grade 2:	0	0	3	0 (2)	3	3	2	0 (3)	
		Grade 3:	1	0	0	0 (1)	1	2	2	0 (1)	
	White pulp atrophy	Grade 1:	0	1	3	0 (5)	0	1	1	0 (4)	
		Grade 2:	0	0	2	0 (0)	0	0	2	0 (1)	
		Grade 3:	0	0	0	0 (0)	0	1	2	0 (0)	
	Granulocytic infiltration	Grade 1:	0	0	0	0 (0)	0	0	0	0 (3)	
		Grade 2:	0	0	0	0 (0)	0	0	0	0 (1)	
Thyroid	Follicular cell hypertrophy	Grade 1:	4	2	1	0 (2)	0	0	2	0 (3)	
		Grade 2:	0	0	1	0 (0)	0	0	0	0 (0)	

Data from animals that died prematurely (found dead and early euthanasia) are in parenthesis.

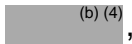
Table: Toxicokinetic parameters for ponesimod (Sponsor's)

TK parameters		ACT-128800 _ Day 1					
		Group 2 (30 mg/kg)		Group 3 (125 mg/kg)		Group 4 (400 mg/kg)	
		Male	Female	Male	Female	Male	Female
C_{max}	[ng/ml]	2810	4590	6620	7170	9610	16100
T_{max}	[h]	2	1	2	4	12	2
AUC_{0-t}	[ng·h/ml]	18911	33122	65393	97262	134064	184943
$t_{1/2}$	[h]	2.2	2.9	3.4	8.8	n.c.	n.c.

n.c.: not calculated (see Tables 1 and 2)

TK parameters		ACT-128800 _ 2 weeks			
		Group 2 (30 mg/kg)		Group 3 (125 mg/kg)	
		Male	Female	Male	Female
C_{max}	[ng/ml]	4830	5610	11200	12300
T_{max}	[h]	2	2	2	2
AUC_{0-t}	[ng·h/ml]	20949	32350	71666	112611
$t_{1/2}$	[h]	2.6	3.6	2.0	4.9

Study title: ACT-128800: 4-Week oral (gavage) toxicity study in the Wistar rat with a 4-week recovery period

Study no.: T-05.043
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: June 10, 2005
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch AA-016498-batch-
 (b) (4), 98.93%

Key Study Findings included 3 deaths; 1 control F, 1 LDF, and 1 HDF; the cause of death was undetermined. No adverse clinical signs or body weight changes were noted during dosing. Decreases in WBCs and lymphocytes occurred at the MD and HD in M and F. Mean IgM and IgG titers were decreased during dosing and dose-dependent decreases in T-cells (CD3⁺), T-helper (CD3⁺/CD4⁺), and cytotoxic T-cells (CD3⁺/CD8⁺), and increases in B-lymphocytes (CD45R-B220⁺) and monocytes/granulocytes (CD11b⁺) were observed, with the changes in lymphocytes reversed at the end of the recovery period. Microscopic findings included diffuse fatty change in adrenals, hyaline droplets in kidneys, hepatic centrilobular hypertrophy in M only, and alveolar histiocytosis and foamy appearance in lungs at all doses, which partially recovered. In lymphoid tissues, hypocellularity was observed in lymph nodes and spleen and medullary hypertrophy in

the thymus that generally recovered. The sponsor concluded that a NOAEL could not be established due to the lung findings; this appears appropriate.

Methods

Doses:	0, 10, 30, and 100 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral by nasal gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.25% (w/v) Methylcellulose and 0.05% (v/v) Tween 80
Species/Strain:	Rat / Wistar Han
Number/Sex/Group	
Main:	10
Recovery:	5
Toxicokinetic:	3 for control and 8 for LD, MD, and HD
Antibody response:	6
Age:	6 weeks
Weight:	Males 133.5-173.1 g; Females 110.2-140.8 g

Histological findings

Histological findings were noted in adrenal, kidney, liver, lung, lymph nodes, spleen, and thymus. Findings in M and F included an increase in incidence and severity of diffuse fatty change in adrenal, hyaline droplets in kidney, hepatic centrilobular hypertrophy in M only, and alveolar histiocytosis and foamy appearance in lung. In lymphoid tissues, hypocellularity was observed in lymph nodes and spleen and medullary hypertrophy in the thymus; this effect of ponosimod are considered related to the pharmacological action of the drug. Generally, there was partial reversal of the adrenal, kidneys, and liver findings and full recovery of lymphoid tissue findings in recovery animals.

Table: Summary of selected microscopic findings

Tissue	Finding	Number of rats	MALE								FEMALE							
			Main				Recovery				Main				Recovery			
			(mg/kg):	0	10	30	100	0	10	30	100	0	10	30	100	0	10	30
			10	10	10	10	5	5	5	5	10	10	10	9	5	5	5	5
Adrenal	Diffuse fatty change	Grade 1:	7	5	5	6	2	3	4	2	3	3	3	1	0	3	3	2
		Grade 2:	2	4	3	3	2	1	1	3	0	0	0	0	0	0	0	0
		Grade 3:	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Kidney	Hyaline droplets	Grade 1:	3	4	1	2	3	0	0	1	0	0	0	0	0	0	0	0
		Grade 2:	2	2	4	3	1	3	0	2	0	0	0	0	0	0	0	0
		Grade 3:	1	0	3	4	0	1	5	1	0	0	0	0	0	0	0	0
	Pelvic dilation	Grade 1:	0	4	2	3	1	1	0	1	5	3	2	1	0	1	1	1
		Grade 2:	0	1	0	0	0	2	2	0	0	0	0	1	0	0	0	0
	Tubular Atrophy	Grade 1:	0	1	2	2	0	0	0	0	2	1	1	1	0	0	0	0
Liver	Centrilobular hypertrophy	Grade 1:	0	2	1	5	0	0	2	0	0	0	0	0	0	0	0	0
		Grade 2:	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0

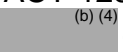
Tissue	Finding	Number of rats	MALE								FEMALE							
			Main				Recovery				Main				Recovery			
			0	10	30	100	0	10	30	100	0	10	30	100	0	10	30	100
			10	10	10	10	5	5	5	5	10	10	10	9	5	5	5	5
Lung	Alveolar histiocytosis	Grade 1:	0	0	3	6	0	1	0	3	0	6	6	4	0	0	0	1
		Grade 2:	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0
		Grade 3:	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	Foamy appearance	Grade 1:	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0
		Grade 2:	0	0	0	2	0	0	0	2	0	0	0	3	0	0	0	1
		Grade 3:	0	0	0	2	0	0	0	1	0	0	1	1	0	0	0	0
	Alveolar hyalinosi	Grade 1:	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
		Grade 2:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Grade 3:	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Osseous metaplasia	Grade 1:	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
		Grade 2:	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Bronchiolo-alveolar hyperplasia	Grade 1:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
		Grade 2:	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Lymph node - Mandibular	Hypocellularity	Grade 1:	0	0	0	0	0	0	0	0	0	0	0	4	0	0	1	0
Lymph node - Mesenteric	Hypocellularity	Grade 1:	0	4	4	2	0	0	0	0	0	1	3	4	0	0	0	0
		Grade 2:	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	Plasmacytic infiltration	Grade 1:	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0
		Grade 2:	0	0	2	1	0	0	0	0	0	0	4	6	0	0	1	0
Lymph node - Popliteal	Hypocellularity lymphoid	Grade 1:	1	4	1	4	0	0	0	0	3	1	1	0	0	0	0	0
		Grade 2:	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0
		Grade 3:	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Spleen	PALS hypercellularity	Grade 1:	0	3	3	0	0	0	0	0	0	2	0	0	0	0	0	0
		Grade 2:	0	1	2	4	0	0	0	0	0	1	4	1	0	0	0	0
		Grade 3:	0	0	2	3	0	0	0	0	0	2	5	5	0	0	0	0
		Grade 4:	0	0	0	1	0	0	0	0	0	0	0	3	0	0	0	0
	Extramedullary hematopoiesis	Grade 1:	0	0	0	0	1	1	4	2	0	0	1	0	1	0	2	0
		Grade 2:	6	5	7	3	4	4	1	3	8	8	4	0	4	5	3	5
		Grade 3:	4	5	3	7	0	0	0	0	2	2	4	4	0	0	0	0
		Grade 4:	0	0	0	0	0	0	0	0	0	0	1	5	0	0	0	0
	Hemosiderin deposits	Grade 1:	0	0	0	0	0	0	1	0	0	0	0	2	4	4	3	2
		Grade 2:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Thymus	Medulla hypertrophy	Grade 1:	0	4	2	3	0	0	0	0	0	4	4	1	0	0	0	1
		Grade 2:	0	0	2	4	0	0	0	0	0	0	4	6	0	0	0	0
		Grade 3:	0	0	0	3	0	0	0	0	0	0	0	2	0	0	0	0

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

TK parameters		ACT-128800 Day 2					
		Group 2 (10 mg/kg)		Group 3 (30 mg/kg)		Group 4 (100 mg/kg)	
		Males	Females	Males	Females	Males	Females
C _{max}	[ng/ml]	1500	2440	5080	5170	10800	13100
T _{max}	[h]	1	1	2	1	2	2
AUC _{0-24h}	[ng·h/ml]	6260	12100	28200	37100	96200	128000
t _{1/2}	[h]	2.24	2.66	2.53	2.57	2.24	2.78

TK parameters		ACT-128800 week 4					
		Group 2 (10 mg/kg)		Group 3 (30 mg/kg)		Group 4 (100 mg/kg)	
		Males	Females	Males	Females	Males	Females
C _{max}	[ng/ml]	1910	2590	4880	5900	10100	12300
T _{max}	[h]	1	1	2	2	2	2
AUC _{0-24h}	[ng·h/ml]	6410	11300	24500	35300	63600	111000
t _{1/2}	[h]	3.07	3.07	3.10	2.80	3.06	5.95

Study title: ACT-128800: Follow-up 4-Week oral (gavage) toxicity study in the Wistar rat with a 4-week recovery period

Study no.: T-05.129
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: November 11, 2005
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch AA-016498-batch-
 (b) (4), 99.06%

Key Study Findings included decreases in WBCs and lymphocytes in HDF. Decreases in T-cells (CD3⁺) and T-helper (CD3⁺/CD4⁺) and increases in B-lymphocytes (CD45RA⁺) and monocytes/granulocytes (CD11b⁺) were observed in HDF, with the changes reversed at the end of the recovery period. Microscopic findings included an increase in the incidence of alveolar histiocytosis in the lung (with an increase in weight in HD animals), lymphoid organ changes of hypercellularity in splenic white pulp, and medulla hypertrophy in the thymus of HD animals. Splenic and thymic findings were reversed in recovery animals. There were no premature deaths and no adverse clinical signs or changes in body weight, food consumption, ophthalmoscopy, urinalysis, or macroscopic findings. The sponsor considered the MD, 2 mg/kg/day, as the NOEL due to increase lung changes at the HD; this appears appropriate. The NOEL was associated with plasma exposures (AUC) to ponesimod on Day 28 of 764 and 1113 ng·h/mL in M and F, respectively.

Methods

Doses: 0, 0.4, 2, and 10 mg/kg
 Frequency of dosing: Daily
 Route of administration: Oral by gavage
 Dose volume: 10 mL/kg
 Formulation/Vehicle: 0.25% (w/v) Methylcellulose and 0.05% (v/v) Tween 80
 Species/Strain: Rat / Wistar Han
 Number/Sex/Group
 Main: 10
 Recovery: 5 Control and HD Groups
 Toxicokinetic: 3 for control and 9 for LD, MD, and HD
 Age: 6 weeks
 Weight: Males 131.1-151.1 g; Females 111.5-130.1 g

Histological findings

The main target organs included the lung, spleen, and thymus. An increase in incidence of alveolar histiocytosis in the lung, hypercellularity in the white pulp in the spleen, and medulla hypertrophy in the thymus was observed in HD animals. The splenic and thymic findings were reversed in recovery animals.

Table: Summary of selected microscopic findings

Tissue	Finding	Number of rats	MALE						FEMALE					
			Main				Rec		Main				Rec	
			0	0.4	2	10	0	10	0	0.4	2	10	0	10
			10	10	10	10	5	5	10	10	10	10	5	5
Brain	Hydrocephalus													
		Grade 1:	0	-	-	0	0	0	0	-	-	0	0	0
		Grade 2:	0	-	-	1	0	0	0	-	-	0	0	0
Lung	Alveolar histiocytosis													
		Grade 1:	2	2	2	5	1	3	2	0	2	6	2	0
	Foamy appearance													
		Grade 1:	1	0	0	0	0	3	0	0	0	0	2	0
Lymph node - Mandibular	Hypocellularity													
		Grade 1:	1	4	2	2	0	0	0	0	2	1	0	0
	Plasmacytic infiltration													
		Grade 1:	0	3	0	2	0	0	0	0	0	0	0	0
Lymph node - Mesenteric	Mast cell hyperplasia													
		Grade 1:	2	0	3	3	1	0	3	5	4	2	1	1
		Grade 2:	0	1	0	2	1	1	1	0	0	1	2	1
		Grade 3:	0	1	1	0	0	0	0	0	0	1	0	0
Lymph node - Popliteal	Mast cell hyperplasia													
		Grade 1:	0	2	0	1	0	1	0	0	2	1	0	0
		Grade 2:	0	0	0	0	0	0	0	0	0	0	2	0
Spleen	PALS hypercellularity													
		Grade 1:	0	2	1	6	0	0	1	1	1	6	0	0
Testes	Cellular debris													
		Grade 1:	0	-	-	1	0	0	-	-	-	-	-	-
		Grade 2:	0	-	-	1	0	0	-	-	-	-	-	-
Thymus	Medulla hypertrophy													
		Grade 1:	0	0	0	1	0	0	0	0	0	4	0	0

Rec – Recovery.

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

TK parameters ACT-128800	Group 2 (0.4 mg/kg)		Group 3 (2 mg/kg)		Group 4 (10 mg/kg)		
	Males	Females	Males	Females	Males	Females	
Day 1							
C _{max}	[ng/ml]	23.7	27.3	154	194	917	1312
t _{max}	[h]	0.5	2	0.5	0.5	0.5	2
AUC _{0-inf}	[ng·h/ml]	112	176	627	1124	4273	7357
t _{1/2}	[h]	2.96	3.00	2.66	2.40	2.44	2.79
Day 28							
C _{max}	[ng/ml]	38.3	50.9	252	312	1483	1630
t _{max}	[h]	0.5	0.5	0.5	0.5	0.5	0.5
AUC _{0-inf}	[ng·h/ml]	119	199	764	1113	5958	8697
t _{1/2}	[h]	2.64	2.48	1.86	2.19	2.57	3.22

Study title: ACT-128800: Dose range finding oral (gavage) toxicity study in the dog.

Study no.: T-05.020
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: March 22, 2005
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: ACT-0128800, Batch AA-016498-batch-
(b) (4), 96.8%

Key Study Findings included 2 deaths (1 M and 1 F) after 8 doses of 200 mg/kg/day, with clinical signs of tremor, uncoordinated movements, collapse, and decreased activity observed at doses $\geq$ 200 mg/kg/day and body weight decrease with repeat doses of 200 mg/kg/day. Blood pressure was decreased by a single dose of 300 mg/kg and with repeat doses of 100 and 200 mg/kg/day. Decreased lymphocytes occurred at all doses in M and F. Moderate bronchopneumonia, alveolar histiocytosis, and hyalinoses were noted in the lung of the F that was sacrificed early, and cortical atrophy was noted in the thymus in 2 M and 2 F. No adverse changes in ECGs, clinical chemistry, urinalysis, organ weights, and macroscopic findings were noted. The sponsor considered doses $\geq$ 200 mg/kg/day above the MTD.

Methods

Doses: 50, 100, 200, and 300 mg/kg (see tables below)
 Frequency of dosing: Daily
 Route of administration: Oral by gavage
 Dose volume: 2 and 4 mL (see below)
 Formulation/Vehicle: 0.25% Methylcellulose and 0.05% Tween80
 Species/Strain: Dog / Beagle
 Number/Sex/Group: 1 (see below)
 Age: 9-10 months
 Weight: 7.4-10.3 kg

	Group 1	Group 2	Group 3
Dose Level* (rising dose)	50 mg/kg/day*	100 mg/kg/day	300 mg/kg/day 200 mg/kg/day
Dose Level+ (repeat dose 1)	200 mg/kg/day	200 mg/kg/day	
Dose Level+ (repeat dose 2)		100 mg/kg/day	100 mg/kg/day
Male Numbers	1	2	3
Female Numbers	4	5	6

+ Dose levels are stated in terms of the material as supplied.

* Initial dose level

Dose levels, volumes and concentrations

	<u>Rising dose phase</u>
Group 1	Day 1 50 mg/kg; 2 mL/kg; 25 mg/mL
Group 2	Days 5,7,8 100 mg/kg; 2 mL/kg; 50 mg/mL
Group 3	Day 12, 13 300 mg/kg; 4 mL/kg; 75 mg/mL
Group 3	Day 15 200 mg/kg; 4 mL/kg; 50 mg/mL

Groups 1 and 2
 Groups 2 and 3

Repeat dose phases

1: Days 1-8 200 mg/kg; 4 mL/kg; 50 mg/mL
 2: Days 1-14 100 mg/kg; 4 mL/kg; 25 mg/mL

Histological findings

The main histological findings included moderate bronchopneumonia, alveolar histiocytosis, and hyalinosis in the lung of the F that was sacrificed early and cortical atrophy in the thymus in 2 M and 2 F.

Table: Summary of selected microscopic findings

Tissue	Finding	Group: Number of rats	MALE			FEMALE		
			1	2	3	1	2	3
Bone Marrow - Sternum	Fatty atrophy	minimal:	0	0	0	0	0	0
		slight:	0	0	0	1	0	0
		moderate:	1	0	1	0	0	0
		marked:	0	1	0	0	1	1
Liver	Glycogen deposits	minimal:	0	0	0	0	0	0
		slight:	0	0	0	0	0	0
		moderate:	1	1	1	0	1	1
Spleen	Congestion	minimal:	0	0	0	0	0	0
		slight:	0	0	0	0	0	1
		moderate:	0	0	1	0	1	0
		marked:	1	1	0	1	0	0

Tissue	Finding	Group: Number of rats	MALE			FEMALE		
			1	2	3	1	2	3
Thymus	Cortical atrophy		1	1	1	1	1	1
		minimal:	0	0	0	0	0	0
		slight:	1	1	0	0	0	0
		moderate:	0	0	0	0	1	0
		marked:	0	0	0	0	0	1

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

Dose (mg/kg/day)	Day	Gender	Dog	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-24h} (ng.h/mL)
100	1	F	F5	8420	2	107000
			F6	4570	2	77300
			Mean*	6500	2	92200
		M	M2	8090	2	89800
			M3	4220	2	56700
			Mean*	6160	2	73300
100	14	F	F5	8340	1	63600
			F6	6200	2	56900
			Mean*	7270	1.5	60300
		M	M2	7340	2	65700
			M3	5150	2	59500
			Mean*	6250	2	62600
200	1	F	F4	11600	2	138000
			F5	13700	4	175000
			Mean*	12700	3	157000
		M	M1	3710	2	50500
			M2	7570	2	75300
			Mean*	5640	2	62900

* Median for T_{max}**Study title: ACT-128800: 4-Week oral (gavage) toxicity study in the dog with a 4-week recovery period**

Study no.: T-05.042
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: May 30, 2005
GLP compliance: Yes, (b) (4)
QA statement: Yes
Drug, lot #, and % purity: ACT-128800, Batch AA-016498-batch-
(b) (4), 98.93%

Key Study Findings included early sacrifice of 2 HDM due to the severity of clinical signs that included vomiting, loose feces, recumbency, tremor, and decreased activity. Recumbency, tremor, and decreased activity also were observed at all doses in M. Decreased body weight gain was observed in HDF, and decreases in WBCs and lymphocytes were noted at all doses in M and F. Microscopic findings included an increase in incidence of alveolar histiocytosis in the lung and lymphoid organ changes of atrophy in splenic white pulp and medulla hypertrophy in the thymus at all doses. Splenic and thymic changes were reversed in recovery animals. No adverse changes were noted in food consumption, ophthalmoscopy, ECGs, or urinalysis. According to

the sponsor, an NOAEL could not be established due to the lung findings at the LD; this appears appropriate.

Methods

Doses: 0, 10, 30, and 100 reduced to 75 mg/kg
 Frequency of dosing: Daily
 Route of administration: Oral by gavage
 Dose volume: 2 mL/kg
 Formulation/Vehicle: 0.25% (w/v) Methylcellulose and 0.05% (v/v) Tween 80
 Species/Strain: Dog / Beagle
 Number/Sex/Group
 Main: 3
 Recovery: 2 for control and HD
 Age: 9-12 months
 Weight: 7-12 kg

Histological findings

The main target organs were the lung, spleen, and thymus; findings included an increase in incidence of alveolar histiocytosis (macrophages) in the lung with foamy appearance, atrophy of white pulp in the spleen, and medulla hypertrophy in thymus at all doses. These findings were reversed in recovery animals.

Table: Summary of selected microscopic findings

Tissue	Finding	Number of dogs:	MALE						FEMALE						
			Main				Rec		Main				Rec		
			(mg/kg):	0	10	30	75/100	0	75/100	0	10	30	75/100	0	75/100
			3	3	3	3 (2)	2	1	3	3	3	3	2	2	
Lung	Alveolar histiocytosis	Grade 1:	0	1	1	0 (0)	0	0	0	2	1	0	0	0	
		Grade 2:	0	0	1	3 (0)	0	0	0	0	0	2	0	0	
	Foamy appearance	Grade 1:	0	1	0	0 (0)	0	0	0	0	1	1	0	0	
		Grade 2:	0	0	0	1 (0)	0	0	0	0	0	0	0	0	
	Spleen	White pulp atrophy	Grade 1:	0	0	2	1 (2)	0	0	0	0	2	0	0	0
			Grade 2:	0	1	0	0 (0)	0	0	0	0	1	1	0	0
Grade 3:			0	1	0	0 (0)	0	0	0	0	0	0	0	0	
Grade 4:			0	0	0	0 (0)	0	0	0	0	0	0	0	0	
Extramedullary hematopoiesis		Grade 1:	2	1	1	1 (2)	1	0	2	1	1	0	2	0	
		Grade 2:	0	0	2	2 (0)	0	0	1	1	1	0	0	0	
		Grade 3:	0	2	0	0 (0)	0	0	0	1	1	2	0	1	
		Grade 4:	0	0	0	0 (0)	0	0	0	0	0	0	0	0	
Congestion		Grade 1:	0	0	0	0 (0)	0	0	0	0	0	0	0	0	
		Grade 2:	0	0	0	0 (0)	0	0	0	0	0	0	0	0	
		Grade 3:	0	0	0	0 (0)	0	0	1	0	0	0	0	0	
		Grade 4:	0	0	0	1 (1)	1	0	1	0	0	0	0	0	
Thymus	Medulla hypertrophy	Grade 1:	0	1	1	0 (0)	0	1	0	2	0	2	0	0	
		Grade 2:	0	0	0	0 (1)	0	0	0	1	1	1	0	0	

Rec - Recovery; Data from animals that died prematurely are in parenthesis.

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

TK parameters	ACT-128800 _ Day 1						Day 8	
	Group 2 (10 mg/kg)		Group 3 (30 mg/kg)		Group 4 (100 mg/kg)		Group 4 (75 mg/kg)	
	Males	Females	Males	Females	Males	Females	Males	Females
C _{max} [µg/ml]	4.82	3.90	5.32	7.1	9.61	10.3	12.5	20.8
T _{max} ^a [h]	2	2	2	24	8	8	12	24
AUC _{0-24h} [µg·h/ml]	56.8	50.1	77.5	123	166	181	191	356
t _{1/2} [h]	10.2	15.9	15.9	n.a.	17.5	18.0	11.2	n.a.

TK parameters	ACT-128800 _ week 4					
	Group 2 (10 mg/kg)		Group 3 (30 mg/kg)		Group 4 (75 mg/kg)	
	Males	Females	Males	Females	Males	Females
C _{max} [µg/ml]	4.06	4.63	6.93	7.46	9.89	13.9
T _{max} ^a [h]	2	2	2	2	2	4
AUC _{0-24h} [µg·h/ml]	60.1	57.0	109	116	148	229
t _{1/2} [h]	9.23	8.77	12.2	10.0	9.80	12.9

^a: For T_{max}, median is presented instead of mean

n.a: not applicable since only one individual value was available for mean calculations

Study title: ACT-128800: Follow-up 4-Week oral (gavage) toxicity study in the dog with a 4-week recovery period

Study no.: T-05.148
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: January 17, 2006
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch AA-016498-batch-
 (b) (4), 99.06%

Key Study Findings included sporadic tremors at all doses, with 1 HDF displaying tremors on 11 occasions. Decreased WBCs and lymphocytes occurred at all doses in M and F. There was an increase in lung weight in HDM, and the main microscopic finding was alveolar histiocytosis in the lungs of in MDM, HDM, and HDF. No premature deaths or adverse changes in body weight, food consumption, ECGs, ophthalmoscopy, urinalysis, or gross pathology were observed. The sponsor considered the LD, 0.4 mg/kg/day, the NOAEL due to alveolar histiocytosis at the MD and HD and lung weight increase at the HD; this appears appropriate. The NOAEL was associated with plasma exposures (AUC) to ponesimod on Day 24 of 1657 and 2184 ng·h/mL in M and F, respectively.

Methods

Doses: 0, 0.4, 2, and 10 mg/kg
 Frequency of dosing: Daily
 Route of administration: Oral by gavage
 Dose volume: 2 mL/kg
 Formulation/Vehicle: 0.25% (w/v) Methylcellulose and 0.05% (v/v)
 Tween 80
 Species/Strain: Dog / Beagle
 Number/Sex/Group
 Main: 3
 Recovery: 2 for control and HD
 Age: 9-10.5 months
 Weight: 7.1-10.8 kg

Histological findings

The main histological finding was noted in lungs and was alveolar histiocytosis in MDM, HDM, and HDF. There were incidences of alveolar histiocytosis in HD recovery animals, suggesting there was not full recovery after 4 weeks.

Table: Summary of selected microscopic findings

Tissue	Finding	Number of dogs	MALE						FEMALE						
			Main				Rec		Main				Rec		
			(mg/kg):	0	0.4	2	10	0	10	0	0.4	2	10	0	10
			3	3	3	3	2	2	3	3	3	3	2	2	
Heart	Myocardial fibrosis	minimal:	0	0	0	0	0	0	0	0	0	0	0	0	0
		slight:	0	0	0	1	0	0	0	0	0	0	0	0	0
Lung	Alveolar histiocytosis	minimal:	1	0	2	1	0	1	0	0	0	1	0	2	
		slight:	0	0	0	1	0	0	0	0	0	0	0	0	
	Foamy appearance	minimal:	1	0	1	1	0	1	0	0	0	0	0	0	
		slight:	0	0	0	1	0	0	0	0	0	0	0	0	
Mammary glands	Glandular development	minimal:	0	0	0	0	0	0	0	1	0	1	0	0	
		slight:	0	0	0	0	0	0	0	1	1	1	0	0	
		moderate:	0	0	0	0	0	0	0	0	1	0	1	1	
		marked:	0	0	0	0	0	0	0	0	1	0	0	0	
	Secretory activity	minimal:	0	0	0	0	0	0	0	0	0	0	0	0	
		slight:	0	0	0	0	0	0	0	0	0	0	1	1	
		moderate:	0	0	0	0	0	0	0	0	1	0	0	0	
		marked:	0	0	0	0	0	0	0	0	0	1	0	0	
Lymph node - Popliteal	Hypocellularity	minimal:	0	0	0	0	0	0	0	0	0	0	0	0	
		slight:	0	0	0	1	0	0	0	0	0	0	0	0	
Spleen	Extramedullary hematopoiesis	minimal:	3	3	1	0	2	1	3	2	2	1	2	1	
		slight:	0	0	0	3	0	0	0	0	1	0	4	0	
		moderate:	0	0	0	0	0	0	0	0	0	1	0	0	
Thymus	Medulla hypertrophy	minimal:	0	0	2	1	0	1	0	1	1	1	0	1	
		slight:	0	0	0	0	0	0	0	0	1	1	0	0	

Rec – Recovery.

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

TK parameters ACT-128800		Group 2 (0.4 mg/kg)		Group 3 (2 mg/kg)		Group 4 (10 mg/kg)	
		Males	Females	Males	Females	Males	Females
Day 1							
C _{max}	[ng/ml]	199	216	982	840	3310	3450
t _{max} ^a	[h]	1.00	1.00	1.00	1.00	2.00	2.00
AUC _{0-24h}	[ng·h/ml]	1713	2204	10643	9952	36260	42228
Terminal t _{1/2}	[h]	7.55	6.62	7.34	7.55	9.17	10.3
Week 4							
C _{max}	[ng/ml]	192	225	1540	954	3608	3308
t _{max} ^a	[h]	2.00	1.00	1.00	2.00	4.00	2.00
AUC _{0-24h}	[ng·h/ml]	1657	2184	12563	9972	51019	45064
Terminal t _{1/2}	[h]	6.07	8.79	5.87	7.91	10.8	16.0

^a: For t_{max}, median is presented instead of mean

Study title: ACT-128800: 13 Week and supplemental 26 Week oral (gavage) administration toxicity study in the dog

Study no.: T-07.186
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: June 7, 2008
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch AA-016498-batch-
 (b) (4), 99.4%

Key Study Findings

- There were no deaths or adverse clinical signs.
- There were decreases in body weight gain at QD dosing of 5, 10, and 40 mg/kg/day, but not 2.5 mg/kg BID.
- Decreased WBC and lymphocytes occurred at all doses in M and F.
- There were increases in liver and lung weights; the main microscopic findings were arteriopathy in local vessels, typically located in papillary muscles, of the heart at 5, 10, and 40 mg/kg/day and pneumonitis in lungs at 5, 10 and 40 mg/kg/day. Lymphoid tissue findings included lymphoid atrophy in lymph nodes and spleen at 5, 10 and 40 mg/kg/day, consistent with the pharmacological effect.
- There was no NOAEL established for either 13- or 26-week dosing durations.

Methods

Doses
 13-Week: 0, 10 and 40 mg/kg
 26-Week: 0 and 5 mg/kg
 Frequency of dosing: Daily and BID (see table below)
 Route of administration: Oral by gavage
 Dose volume: 2 mL/kg
 Formulation/Vehicle: 0.25% (w/v) Methylcellulose and 0.05% (v/v) Tween 80
 Species/Strain: Dog / Beagle
 Number/Sex/Group: See table below
 Age: 49-59 weeks
 Weight: 8.75-14.6 kg
 Animal 36 (Group 5F) was found dead during pre-dosing (Day -2) following sham intubation. Animal 21 (Group 1F, from the 26-Week study) was re-allocated to the Low II 13-Week study to replace Animal 36. Animal 21 was re-identified as animal 36. Pre-dosing data for Animal 21 were transferred and reported as Animal 36.

Group Number	Description	Dose level (mg/kg/day)	Number of animals in group			
			Main Study (26 Weeks treatment)		Interim study (13 Weeks treatment)	
			Male	Female	Male	Female
1	Control I	0	3	3	-	-
2	Control II	0	-	-	3	3
3	Low I	2 x 2.5#	4	4	-	-
4	High I	5	4	4	-	-
5	Low II	10	-	-	3	3
6	High II	40	-	-	3	3

twice daily dosing approximately 8 hours apart

Observations and Results

Mortality

There were no early deaths after Week 13 or 26 of dosing.

Clinical Signs

Observations of clinical signs were conducted immediately after dosing and approximately 2- and 6-hours post-dose. Group 3 animals were observed immediately and 2 hours post-second dose for the first 12 days, and on Days 15 and 17. On all other days, observations were conducted immediately and 15 minutes post-second dose. Detailed examinations were conducted daily during dosing and weekly during recovery.

There were no adverse clinical signs observed.

Body Weights

Body weights were recorded once weekly from Week -1.

13-Week data

In M, there was a decrease in body weight gain over 13 weeks at 10 mg/kg and a decrease in body weight at 40 mg/kg and in F there was a decrease in body weight at 40 mg/kg/day.

26-Week data

There was a decrease in body weight gain over 26 weeks in M and F with QD dosing, but not BID dosing.

Table: Body weights changes (kg)

mg/kg	MALE						FEMALE					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	0	0	2.5 BID	5	10	40	0	0	2.5 BID	5	10	40
Week 13	0.46	0.82	0.17	0.47	0.16	-0.08	0.55	0.10	0.10	0.49	0.59	-0.35
Week 26	0.68	-	0.66	0.13	-	-	0.96	-	0.92	0.29	-	-

Food Consumption

Food consumption was recorded weekly from Week -1.

There were no adverse changes in food consumption.

Ophthalmoscopy

Examination of all animals was conducted once prior to initiation of dosing and in Week 13 for groups 2, 5, and 6 and Week 25 for groups 1, 3, and 4.

There were no adverse changes.

ECG

Examination of all animals was conducted once prior to initiation of dosing and in Week 13 for groups 2, 5, and 6 and Week 25 for groups 1, 3, and 4 pre-dosing and approximately 2 hours post-dose. QT interval was corrected using the Fridericia and Van de Water methods.

There were no adverse changes.

Hematology

Blood samples were taken from all animals prior to initiation of dosing, in Week 13 from groups 2, 5, and 6, and Week 26 from groups 1, 3, and 4.

Decreases in WBC and lymphocytes were observed at all doses from Weeks 6 to 26. Platelets and fibrinogen were increased at 10 and 40 mg/kg/day at Week 13. The decrease in WBC and lymphocytes were similar with 5 mg/kg/day QD and BID for 26 weeks.

Table: 13 Week changes in selected hematology parameters compared to pretest values

Group	Dose	Sample	WBC (%)	Lymphocytes (%)	Neutrophils (%)	Platelets (%)	Fibrinogen (%)
MALE							
2	0	Week 6:	-6%	+3%	-9%	-11%	+12%
		Week 13:	+7%	-3%	+13%	-2%	+18%
4	5	Week 13:	-33%	-64%	-18%	-7%	-17%
		Week 6:	-30%	-75%	-10%	+9%	+8%
5	10	Week 13:	-25%	-78%	-3%	+26%	+22%
		Week 6:	-24%	-77%	-1%	+20%	+62%
6	40	Week 13:	-3%	-47%	+22%	+47%	+33%
		FEMALE					
2	0	Week 6:	+19%	-8%	+31%	+6%	+20%
		Week 13:	-4%	-4%	+2%	0%	+11%
4	5	Week 13:	-29%	-59%	-14%	+9%	+1%
		Week 6:	-31%	-61%	-18%	-5%	+9%
5	10	Week 13:	-21%	-63%	-4%	+5%	+10%
		Week 6:	-47%	-82%	-32%	+14%	+39%
6	40	Week 13:	-43%	-82%	-26%	+31%	+46%

Table: 26 Week changes in selected hematology parameters compared to pretest values

Group	Dose	Sample	WBC (%)	Lymphocytes (%)	Neutrophils (%)	Platelets (%)	Fibrinogen (%)
MALE							
1	0	Week 13:	-11%	+7%	-15%	-1%	+2%
		Week 26:	-11%	-7%	-10%	-9%	+2%
3	2.5 BID	Week 13:	-32%	-73%	-16%	+23%	-4%
		Week 26:	-20%	-69%	-3%	-1%	+3%
4	5	Week 13:	-33%	-64%	-18%	-7%	-17%
		Week 26:	-33%	-67%	-18%	-4%	-12%
FEMALE							
1	0	Week 13:	-2%	-2%	-6%	+13%	-2%
		Week 26:	0%	0%	0%	+28%	+24%
3	2.5 BID	Week 13:	-39%	-39%	-29%	+7%	-5%
		Week 26:	-33%	-33%	-20%	+14%	+4%
4	5	Week 13:	-29%	-29%	-14%	+9%	+1%
		Week 26:	-31%	-31%	-15%	+17%	+21%

Clinical Chemistry

Blood samples were taken from all animals prior to initiation of dosing, in Week 13 from groups 2, 5, and 6, and Week 26 from groups 1, 3, and 4.

Increase in triglycerides was observed at all doses in animals from Weeks 6 to 26 and in phosphorus at 10 and 40 mg/kg/day in F. Cholesterol, albumin, protein, and calcium were decreased at 5, 10, and 40 mg/kg/day at all doses from Weeks 6 to 26.

Table: 13 Week changes in selected clinical chemistry parameters compared to pretest values

Group	Dose	Sample	Trigs (%)	Cholesterol (%)	Albumin (%)	Protein (%)	Calcium (%)	Phosphorus (%)
MALE								
2	0	Week 6:	0%	-3%	0%	+2%	0%	-14%
		Week 13:	-8%	-9%	+4%	+2%	+1%	-14%
4	5	Week 13:	+10%	-26%	0%	-5%	-3%	-20%
		Week 6:	+19%	-27%	-11%	-6%	-5%	-13%
5	10	Week 13:	+13%	-27%	-11%	-9%	-6%	-13%
		Week 6:	+100%	-21%	-24%	-12%	-5%	0%
6	40	Week 13:	+41%	-16%	-21%	-14%	-6%	-7%

Group	Dose	Sample	Trigs (%)	Cholesterol (%)	Albumin (%)	Protein (%)	Calcium (%)	Phosphorus (%)
FEMALE								
2	0	Week 6:	0%	+3%	-3%	+2%	0%	-14%
		Week 13:	+6%	+16%	0%	+3%	+1%	+7%
4	5	Week 13:	-2%	-21%	-9%	-5%	-2%	-7%
		Week 6:	+7%	-31%	-4%	-7%	-3%	+21%
5	10	Week 13:	+21%	-17%	-4%	-5%	-2%	+7%
		Week 6:	+95%	-2%	-20%	-15%	0%	+29%
6	40	Week 13:	+19%	-17%	-20%	-18%	-3%	+21%

Table: 26 Week changes in selected clinical chemistry parameters compared to pretest values

Group	Dose	Sample	Trigs (%)	Cholesterol (%)	Albumin (%)	Protein (%)	Calcium (%)	Phosphorus (%)
MALE								
1	0	Week 13:	+67%	+3%	+7%	+4%	0%	-20%
		Week 26:	+57%	+6%	+7%	+11%	+2%	-7%
3	2.5 BID	Week 13:	+6%	-35%	-7%	-9%	-3%	-13%
		Week 26:	+32%	-30%	-4%	-3%	-2%	-7%
4	5	Week 13:	+10%	-26%	0%	-5%	-3%	-20%
		Week 26:	+21%	-31%	+4%	0%	-4%	-13%
FEMALE								
1	0	Week 13:	0%	+2%	+3%	+3%	+3%	0%
		Week 26:	+21%	0%	+3%	+9%	+3%	0%
3	2.5 BID	Week 13:	-19%	-18%	-11%	-2%	-1%	+7%
		Week 26:	+38%	-13%	+4%	+7%	+2%	+14%
4	5	Week 13:	-2%	-21%	-9%	-5%	-2%	-7%
		Week 26:	+24%	-12%	-6%	+2%	-2%	0%

Urinalysis

Urine samples were taken from all animals prior to initiation of dosing, in Weeks 6 and 13 from groups 2, 5, and 6, and Week 25 from groups 1, 3, and 4.

There were no adverse changes in urinalysis.

Gross Pathology

Gross pathology was assessed in all animals.

Week 13

There were lung observations at 10 and 40 mg/kg/day that included discolorations such as dark, dark focus, red focus, and pale.

Week 26

There were no adverse macroscopic findings.

Organ Weights

At necropsy, the organs, listed in the table below (labeled with †) from the study report, were weighed.

Week 13

There were increases in liver and lung weights at 10 and 40 mg/kg/day and thymus at 40 mg/kg/day in M and F and decrease in lymph node weights at 10 and 40 mg/kg/day in F.

Table: Week 13 organ changes in mean absolute weight compared to control

Grp	Adrenal	Brain	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate /Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
							Ma	Me	P						
MALE															
5:	+6%	-3%	0%	+4%	+11%*	+43%	+5%	-39%	-15%	+6%	-13%	+15%	+13%	-8%	-15%
6:	+1%	-4%	+7%	-1%	+21%*	+33%	+11%	-6%	+3%	+9%	-47%*	+45%	+10%	+25%	-12%
FEMALE															
5:	-2%	-7%	+10%	+7%	+19%	+21%	-12%	-17%	-6%	-9%	+77%	-25%	+4%	+5%	+8%
6:	+13%	+5%	+5%	+5%	+18%	+47%	-17%	-50%*	-26%	0%	+16%	-5%	-29%	+46%	-15%

* Statistically significant from control; recovery values in parenthesis; Epid - epididymides; Ma - mandibular; Me - mesenteric; P - popliteal; Pit - pituitary.

Table: Week 13 organ changes in mean weight (relative to body weight) compared to control

Grp	Adrenal	Brain	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate /Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
							Ma	Me	P						
MALE															
5:	+8%	0%	+13%	+16%	+23%	+56%	+20%	-32%	-7%	-	-1%	-4%	+27%	+3%	0%
6:	+17%	0%	+13%	+13%	+37%*	+56%	+24%	+5%	+7%	-	-37%	-63%	+3%	+36%	0%
FEMALE															
5:	-6%	0%	+13%	+7%	+20%	+22%	-12%	-19%	-10%	0%	+69%*	-27%	+6%	+5%	0%
6:	+19%	+14%	+13%	+9%	+23%*	+44%	-15%	-48%*	-25%	0%	+15%	-5%	-28%	+47%	-12%

* Statistically significant from control; recovery values in parenthesis; Epid - epididymides; Ma - mandibular; Me - mesenteric; P - popliteal; Pit - pituitary.

Week 26

There was a statistically significant increase in adrenal gland weight at 5 mg/kg/day after BID dosing in M, in liver weight after QD and BID dosing in M, in lung weight after BID dosing in M, in mandibular lymph node after QD dosing in M, and in pituitary gland after QD and BID dosing in M.

Table: Week 26 organ changes in mean absolute weight compared to control

Grp	Adrenal	Brain	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate /Ovary	Spleen	Testes/ Uterus	Thymus	Thyroid
							Ma	Me	P						
MALE															
3:	+32%*	-3%	+7%	+12%	+27%*	+37%*	+19%	-25%	+9%	+37%*	-12%	+6%	+15%	+4%	-6%
4:	+18%	+3%	+4%	+13%	+23%*	+25%	+77%*	-15%	+10%	+48%*	+6%	+41%	+4%	+35%	-2%
FEMALE															
3:	+11%	-5%	-2%	+26%	+23%	+12%	-1%	-8%	+99%	-15%	+14%	0%	-33%	+20%	-29%
4:	-5%	-10%	0%	+15%	+10%	+30%	-15%	-15%	+38%	-20%	-47%	-8%	-73%*	+41%	-2%

* Statistically significant from control; recovery values in parenthesis; Epid - epididymides; Ma - mandibular; Me - mesenteric; P - popliteal; Pit - pituitary.

Table: Week 26 organ changes in mean weight (relative to body weight) compared to control

Grp	Adrenal	Brain	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate /Ovary	Spleen	Testes	Thymus	Thyroid
							Ma	Me	P						
MALE															
3:	+17%	0%	0%	+3%	+18%	+22%	+8%	-29%	0%	-	-16%	-1%	+7%	-1%	-11%
4:	+8%	0%	+11%	+7%	+18%	+22%	+69%	-20%	0%	-*	+1%	+34%	0%	+29%	-11%
FEMALE															
3:	+13%	0%	0%	+33%	+25%*	+25%	-3%	-6%	+113%	0%	+19%	+3%	-32%	+23%	-22%
4:	-7%	0%	0%	+19%	+13%	+38%*	-18%	-10%	+50%	0%	+43%*	-6%	-70%*	+36%	0%

* Statistically significant from control; recovery values in parenthesis; Epid - epididymides; Ma - mandibular; Me - mesenteric; P - popliteal; Pit - pituitary.

Histopathology

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

adrenals (†) (§)	oesophagus (§)
animal identification	ovaries (†) (§)
aorta (LS + TS) (§)	oviducts (§)
bone marrow smear (sternum) (a) (c)	pancreas (§)
brain inc pons (†) (§)	pituitary (†) (§)
caecum (§)	plantar nerve (TS + LS, unilateral) (§)
colon (§)	popliteal lymph nodes (†) (§)
dorsal root ganglia (lumbar x2, including spinal roots) (§)	prostate (†) (§)
duodenum (§)	rectum (§)
eyes and optic nerves (b) (§)	salivary gland mandibular, sublingual & parotid (§)
femur with bone marrow and articular surface (§)	sciatic nerves (§)
gall bladder (§)	skin and subcutaneous tissue (§)
gross lesions (§)	spinal cord cervical (TS + LS) (§)
heart (†) (§) (e)	spinal cord lumbar (TS + LS) (§)
ileum including Peyer's patch (§)	spinal cord thoracic (TS + LS) (§)
jejunum (§)	spleen (†) (§)
kidney (†) (§)	sternum with bone marrow (§)
lacrimal glands	stomach (§)
larynx (§)	testes + epididymides (d) (†) (§)
liver (†) (§)	thymus (†) (§)
lungs with mainstem bronchi and bronchioles (†) (§)	thyroids + parathyroids (†) (§)
mammary (§)	tongue (§)
mandibular lymph nodes (†) (§)	trachea (§)
mesenteric lymph nodes (†) (§)	ureters (§)
muscle (quadriceps) (§)	urinary bladder (§)
muscle (semimembranosus, TS, unilateral) (§)	uterus including cervix (†) (§)
muscle (medial, gastrocnemius, TS unilateral) (§)	vagina (§)

fixative = 10% neutral buffered formalin except where indicated by: a = methanol, b = Davidson's fluid
 c = see clinical pathology section, d = Bouin's fixative
 e = heart includes 2 x left ventricular papillary mm. (m. papillaris subauricularis & m. papillaris subatrialis) and 3 x right ventricular papillary mm. (m. papillaris magnus, m. papillaris subarteriosus & m. papillaris parvus)
 Bone designated for histopathological examination was decalcified using Kristenson's fluid
 LS = longitudinal section
 TS = transverse section

Adequate Battery: Yes.

Peer Review: Yes, but non-GLP so no details were given.

Signed Pathology report: Yes, by (b) (6).

Histological Findings Week 13

The main histological findings at Week 13 were noted in heart, liver, lungs, lymph nodes, and spleen. In the heart, arteriopathy was observed in local vessels, typically located in papillary muscles, at 10 and 40 mg/kg/day and in the ventricular wall in a M dosed at 40 mg/kg/day. Pneumonitis and foamy and/or partial foamy macrophages were observed in lungs at 10 and 40 mg/kg/day. Other lung findings included bronchitis/bronchiolitis and pleural fibrosis at 40 mg/kg/day. Lymphoid tissue findings included lymphoid atrophy in lymph nodes and spleen at 10 and 40 mg/kg/day.

Table: Summary of selected microscopic findings at Week 13

Tissue	Finding	Group (mg/kg) Number of dogs	MALE			FEMALE		
			2	5	6	2	5	6
			0	10	40	0	10	40
			3	3	3	3	3	3
Heart	Cardiomyopathy/fibrosis	minimal:	0	0	0	0	0	0
		slight:	0	0	2	0	0	1
	Arteriopathy left ventricular wall	minimal:	0	0	0	0	0	0
		slight:	0	0	0	0	0	0
		moderate:	0	0	1	0	0	0
	Arteriopathy left posterior papillary muscle	minimal:	0	0	1	0	1	0
		slight:	0	2	0	0	0	2
		moderate:	0	0	2	0	0	1
	Arteriopathy anterior papillary muscle	minimal:	0	1	0	0	0	0
		slight:	0	1	1	0	1	1
		moderate:	0	0	1	0	0	1
	Arteriopathy interventricular septum	minimal:	0	1	0	0	0	1
		slight:	0	0	0	0	0	0
		moderate:	0	0	1	0	0	0
	Vascular hemorrhage	minimal:	0	0	1	0	0	2
Pigment	minimal:	0	0	1	0	0	0	
Kidney	Tubular vacuolation	minimal:	0	0	0	1	1	0
		slight:	0	0	0	0	0	3
Lung	Hemorrhage	present:	0	1	1	0	0	0
	Foamy macrophages	minimal:	2	3	2	2	1	3
		slight:	0	0	1	0	0	0
	Partly foamy macrophages	minimal:	0	0	1	0	0	1
		slight:	0	1	1	0	0	1
	Pleural fibrosis	minimal:	0	0	0	0	0	1
		slight:	0	0	1	0	0	0
	Bronchitis/bronchiolitis	minimal:	0	0	2	0	0	0
	Pneumonitis	minimal:	0	0	1	0	1	2
		slight:	0	1	1	0	0	0
Alveolitis	minimal:	0	0	0	0	0	0	
	slight:	0	0	0	0	0	0	
	moderate:	0	1	0	0	0	0	
Lymph node - mandibular	Atrophy	minimal:	0	0	1	0	1	0
	Sinus histiocytosis	minimal:	1	0	0	0	0	1
Lymph node - mesenteric	Atrophy	minimal:	0	0	3	0	0	1
		slight:	0	1	0	0	0	1
	Sinus histiocytosis	minimal:	0	1	1	0	0	0
		slight:	0	1	1	0	0	0

Tissue	Finding	Group (mg/kg) Number of dogs	MALE			FEMALE		
			2	5	6	2	5	6
			0	10	40	0	10	40
			3	3	3	3	3	3
Lymph node - popliteal	Sinus histiocytosis	minimal:	0	0	0	0	0	1
		slight:	0	1	0	0	0	0
Skin	Dermatitis	minimal:	0	1	0	0	1	0
		slight:	0	0	2	0	0	1
	Granuloma	present:	0	0	1	0	0	1
Spleen	Lymphoid atrophy	minimal:	0	2	2	0	1	2
		slight:	0	1	1	0	0	1
	Capsular fibrosis	minimal:	0	0	0	0	0	0
		slight:	0	0	0	0	0	1
	Necrosis	minimal:	0	0	1	0	0	0
Thymus	Edema	minimal:	0	0	0	0	0	1

Histological Findings Week 26

The main histological findings at Week 26 were noted in heart, liver, lungs, lymph nodes, and spleen. In the heart, arteriopathy was observed in local vessels, typically located in papillary muscles, at 5 mg/kg/day with both QD and BID dosing and minimal subendocardial fibrosis was observed in the valve of the left ventricle of 1 M dosed BID. Pneumonitis was observed in lungs after both QD and BID dosing. Lymphoid tissue findings included lymphoid atrophy in lymph nodes and spleen after both QD and BID dosing.

Table: Summary of selected male microscopic findings at Week 26

Tissue	Finding	Group (mg/kg) Number of dogs	MALE			FEMALE		
			1	3	4	1	3	4
			0	2.5 BID	5	0	2.5 BID	5
			3	4	4	3	4	4
Heart	Arteriopathy left posterior papillary muscle	minimal:	0	0	1	0	0	0
		slight:	0	0	0	0	2	0
	Arteriopathy left anterior papillary muscle	minimal:	0	1	2	0	1	0
		slight:	0	1	1	0	0	0
	Vascular hemorrhage	minimal:	0	1	0	0	0	0
	Subendocardial fibrosis	minimal:	0	1	0	0	0	0
Liver	Glycogen vacuolation	minimal:	1	4	4	1	2	0
		slight:	0	0	0	1	0	0
Lung	Hemorrhage	present:	0	1	0	0	0	0
	Foamy macrophages	minimal:	3	1	1	0	1	1
		slight:	0	1	0	0	0	0

Tissue	Finding	Group (mg/kg)	Number of dogs	MALE			FEMALE		
				1	3	4	1	3	4
				0	2.5 BID	5	0	2.5 BID	5
				3	4	4	3	4	4
Lung	Macrophages aggregates	minimal:	1	0	1	1	2	1	
	Bronchitis/bronchiolitis	minimal:	1	1	0	0	1	0	
		slight:	0	1	0	0	0	0	
	Pneumonitis	minimal:	0	0	0	0	2	0	
		slight:	0	1	1	0	0	0	
	Alveolitis	minimal:	0	1	0	0	0	1	
	Osseous metaplasia	minimal:	0	0	0	0	1	0	
Lymph node	Hemorrhage	present:	0	1	2	0	2	0	
	Atrophy	minimal:	0	0	0	0	0	0	
		slight:	0	0	0	0	1	0	
Lymph node - mesenteric	Atrophy	minimal:	0	1	1	0	0	1	
		slight:	0	2	0	0	2	0	
Pancreas	Atrophy	minimal:	0	0	1	0	0	0	
Spleen	Lymphoid atrophy	minimal:	0	2	1	0	3	0	
		slight:	0	2	1	0	1	0	
	Capsular fibrosis	minimal:	0	0	1	0	0	0	

Toxicokinetics

Blood samples were taken on Day 1 and in Week 13 for groups 1-6 pre-dose and at 0.5, 1, 2, 4, 8, and 24 hours post-dose, on Day 1 and in Week 13 for groups 1, 3, and 4 at 9, 10, and 12 hours post-dose, and in Week 26 for groups 1, 3, and 4 pre-dose and at 0.5, 1, 2, 4, 8, 9, 10, 12, and 24 hours post-dose.

On Day 1, increase in ponesimod plasma exposure (C_{max} and AUC) was less than dose-proportional between the LD and MD and approximately dose-proportional between the MD and HD. In Week 13, increase in ponesimod plasma exposure (C_{max} and AUC) was less than dose-proportional between the LD and MD and greater than dose-proportional between the MD and HD. Generally, plasma exposure was greater in M. T_{max} ranged between 1 and 24 hours in M and F, and $t_{1/2}$ was 3.7-25 hours. Accumulation occurred after 13 weeks. Plasma exposure after BID and QD dosing at the LD was generally similar in M, but AUC levels were less with BID dosing in F.

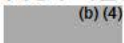
Table: Toxicokinetic parameters for ponesimod (Sponsor's)

ACT-128800 Dose Level (mg/kg/day)	Sex	Day 1				Week 13				Week 26				
		AUC _{0-24h} (ng.h/mL)	C _{max} (ng/mL)	t _{max} # (h)	t _{1/2} (h)	AUC _{0-24h} (ng.h/mL)	C _{max} (ng/mL)	t _{max} # (h)	t _{1/2} (h)	AUC _{0-24h} (ng.h/mL)	C _{max} (ng/mL)	t _{max} # (h)	t _{1/2} (h)	
2 x 2.5	Male	N	4	4	4	0	4	4	4	0	4	4	4	0
		Mean	23000	1460	12.0	NC	29700	1890	10.0	NC	35500	2260	10.0	NC
		SD	7900	433	(10.0-12.0)	NC	8860	590	(2.00-12.0)	NC	14900	859	(1.00-10.0)	NC
	CV%	34.4	29.6		NC	29.8	31.3		NC	42.0	38.1		NC	
	Female	N	4	4	4	0	4	4	4	0	4	4	4	0
		Mean	11000	847	10.0	NC	15000	999	6.00	NC	14300	1050	10.0	NC
		SD	6790	407	(10.0-10.0)	NC	17300	908	(2.00-12.0)	NC	12800	724	(10.0-12.0)	NC
	CV%	62.0	48.1		NC	115.7	90.9		NC	89.3	69.2		NC	
	5	Male	N	4	4	4	4	4	4	4	4	4	4	4
Mean			22700	1860	2.00	10.2	22400	1990	2.00	7.36	21300	1800	2.00	6.58
SD			11700	1110	(2.00-2.00)	3.41	10900	914	(2.00-2.00)	2.31	11900	596	(1.00-4.00)	2.14
CV%		51.7	59.5		33.6	48.7	46.0		31.4	55.9	33.1		32.5	
Female		N	4	4	4	3	4	4	4	4	4	4	4	4
		Mean	15300	1380	1.50	5.43	23200	2000	1.00	8.15	15600	1570	1.50	6.54
		SD	13200	770	(1.00-4.00)	3.04	18100	1130	(1.00-2.00)	5.60	12300	633	(0.500-2.00)	4.05
CV%		86.3	55.7		55.9	77.8	56.7		68.8	78.5	40.3		61.9	
10		Male	N	3	3	3	2	3	3	3	3			
	Mean		26400	2140	2.00	8.49	27100	2230	1.00	6.19				
	SD		3710	425	(1.00-2.00)	NC	3670	223	(1.00-2.00)	1.16				
	CV%	14.1	19.9		NC	13.6	10.0		18.7					
	Female	N	3	3	3	3	3	3	3	2				
		Mean	19600	1910	1.00	7.27	17400	1980	1.00	3.66				
SD		14300	540	(1.00-2.00)	3.87	13800	940	(1.00-2.00)	NC					
CV%	72.8	28.3		53.2	79.6	47.6		NC						
40	Male	N	3	3	3	0	3	3	3	0				
		Mean	94200	4860	4.00	NC	157000	10200	4.00	NC				
		SD	61000	2690	(1.00-24.0)	NC	122000	8130	(4.00-8.00)	NC				
	CV%	64.7	55.4		NC	77.5	80.1		NC					
	Female	N	3	3	3	1	3	3	3	1				
		Mean	86000	4190	24.0	25.0	174000	9640	8.00	15.4				
SD		27500	987	(2.00-24.0)	NC	26000	955	(2.00-8.00)	NC					
CV%	31.9	23.5		NC	15.0	9.9		NC						

AUC (norm) = AUC [ng.h/mL] / dose [mg/kg/day]
 C_{max} (norm) = C_{max} [ng/mL] / dose [mg/kg/day]
 NC = Not Calculable
 # = Median (min-max)

Study title: ACT-128800: 26 Week oral (gavage) administration toxicity study in the dog with a 13 Weeks treatment free

Study no.: T-05.139
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)

Date of study initiation: November 16, 2005
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch AA-016498-batch-
 (b) (4), 99.4%

Key Study Findings

- There were no deaths.
- Clinical signs included muscle twitching, tremors, and impaired motility at the HD and thin appearance at all doses.
- Decreased lymphocytes occurred from 1-2 hours at all doses in M and F and showed partial recovery.
- There was an increase in lung weight in HDM; the main microscopic findings were foamy and partially foamy alveolar macrophages in the lungs and arteriopathy in vessels, typically in papillary muscles, in the heart at the HMD and HD. Incidences of arteriopathy were still observed at the HD in recovery animals.
- The sponsor considered the LMD (2 mg/kg/day) the NOAEL due to arteriopathy in the heart at the HMD and HD; this appears appropriate. The NOAEL was associated with plasma exposures (AUC) to ponesimod in Week 26 of 4720 and 11400 ng*h/mL in M and F, respectively.

Methods

Doses:	0, 0.4, 2, 10, and 40 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral by gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	0.25% (w/v) Methylcellulose and 0.05% (v/v) Tween 80
Species/Strain:	Dog / Beagle
Number/Sex/Group	
Main:	4
Recovery:	2 for control, LD, and HD
Age:	4-9.5 months
Weight:	M – 6.75-14.1 kg; F – 5.55-11.0 kg

Observations and Results

Mortality

There were no early deaths.

Clinical Signs

Observations of clinical signs were conducted once daily. Detailed examinations were conducted weekly prior to dosing and during recovery and daily during dosing.

Clinical signs observed mainly in the first week of dosing at the HD included salivation, liquid feces, muscle twitching, tremors, and impaired motility. Thin appearance was first observed in Week 4-5 in 1 LDM, 1 HMDM, 2 HDM, and 1 HMDF, by Week 26 it was noted in 1 LMDM, 1 HMDM, and 4 HDM and 1 LMDF, 1 HMDF, and 2 HDF.

Body Weights

Body weights were recorded once weekly from Week -1.

There were no adverse changes in body weight.

Food Consumption

Food consumption was recorded weekly from Week -1.

There were no adverse changes in food consumption.

Ophthalmoscopy

Examination of all animals was conducted once prior to initiation of dosing, in Week 25 of dosing, and Week 39 of recovery.

There were no adverse changes.

ECG

Examination of all animals was conducted once prior to initiation of dosing, in Week 25 during dosing pre-dosing and approximately 2 hours post-dose, and Week 39 of recovery.

There were no adverse changes.

Hematology

Blood samples were taken from all animals prior to initiation of dosing, in Weeks 13 and 26 of dosing, and toward the end of recovery. Blood samples were taken to analyze WBC parameters on Day 1 and in Week 26 pre-dose and at 0.5, 1, 2, 3, 4, 8, and 24 hours post-dose. To assess WBC parameters recovery, blood samples were taken in Week 27 at 24, 36, 48, 72, and 96 hours and 7 days post last dose.

The main changes were decreases in WBC and lymphocytes, with the decrease in lymphocytes observed from 1-2 hours post-dose on Day 1. The decrease in lymphocytes lasted the duration of dosing, with partial recovery in Week 39.

Figure: Lymphocyte counts with inset graphs of data from Days 1 (dosing) and 179 (recovery)

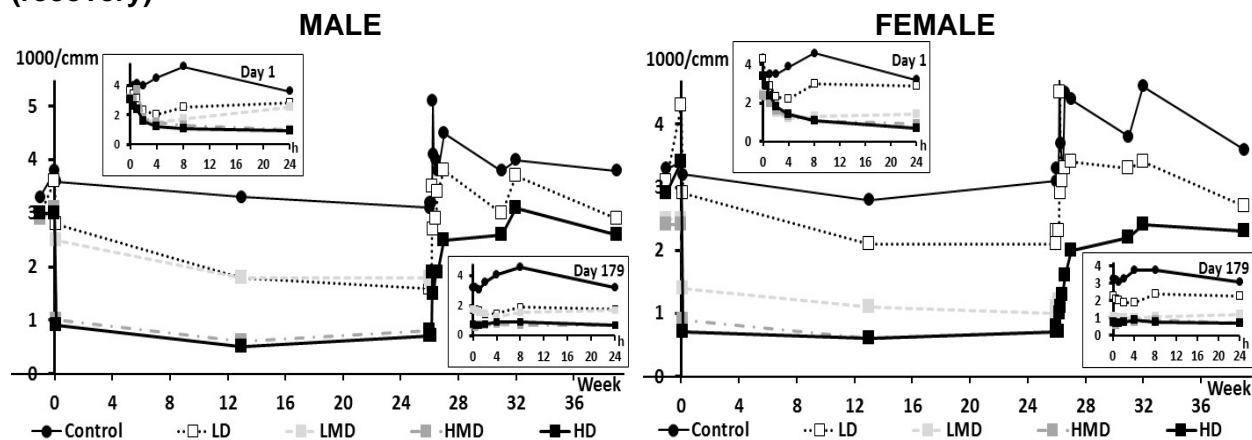


Table of hematology parameter changes compared to pretest values

Dose	Sample	WBC	Lymph	Eosin	Basophils	Reticulo	Platelets	Fibrinogen	APPT (s)
MALE									
0	Week 13:	+2%	0%	0%	0%	+25%	+7%	-17%	+0.8
	Week 26:	+3%	-6%	0%	0%	-25%	+2%	-9%	0
	Week 39:	+35%	+15%	+35%	0%	-25%	+15%	-17%	+2.0
LD	Week 13:	-10%	-40%	+33%	0%	+13%	+2%	-25%	+0.1
	Week 26:	-3%	-47%	+33%	0%	0%	+2%	-14%	-0.5
	Week 39:	+8%	-3%	0%	0%	-25%	+22%	-25%	+0.4
LMD	Week 13:	-21%	-40%	-40%	0%	+11%	+11%	-4%	-0.1
	Week 26:	-22%	-40%	-40%	0%	-11%	+3%	-8%	-0.4
HMD	Week 13:	-35%	-79%	-33%	-100%	+11%	+10%	-4%	+1.2
	Week 26:	-29%	-72%	-33%	-100%	-22%	+9%	-8%	+0.7
HD	Week 13:	-36%	-83%	-67%	-100%	+50%	+36%	-26%	+1.2
	Week 26:	-20%	-77%	-33%	-100%	+17%	+27%	+11%	+1.2
	Week 39:	+12%	-13%	-33%	-100%	+17%	-12%	-26%	+0.8
FEMALE									
0	Week 13:	-2%	-15%	-25%	0%	-17%	+4%	+26%	+0.9
	Week 26:	+3%	-6%	0%	0%	-33%	0%	+11%	+0.9
	Week 39:	+3%	+9%	0%	+100%	-33%	+3%	-11%	+0.2
LD	Week 13:	+1%	-32%	0%	0%	+13%	+14%	+10%	+0.1
	Week 26:	-10%	-32%	0%	0%	-25%	+6%	0%	-0.6
	Week 39:	+4%	-13%	-57%	0%	-25%	-11%	+24%	+3.2
LMD	Week 13:	-26%	-56%	0%	0%	+9%	+10%	-11%	+0.2
	Week 26:	-30%	-60%	0%	0%	-9%	-1%	-18%	+0.4
HMD	Week 13:	-19%	-75%	-33%	-100%	+33%	+23%	-12%	+0.6
	Week 26:	-24%	-71%	-33%	-100%	+50%	+18%	+4%	+0.6
HD	Week 13:	-30%	-79%	-60%	-100%	+67%	+29%	+5%	+1.1
	Week 26:	-18%	-76%	-60%	-100%	0%	+39%	+33%	+1.7
	Week 39:	-6%	-21%	-60%	-100%	+33%	+10%	+10%	+5.1

Lymph – lymphocytes; Eosin – eosinophils; Reticulo – reticulocytes.

Clinical Chemistry

Blood samples were taken from all animals prior to initiation of dosing, in Weeks 13 and 26 of dosing, and toward the end of recovery.

There were increases in urea and ALP in HDM and HDF and decreases in ALT, cholesterol, protein, calcium, magnesium, and albumin at the HMD and HD. Generally, recovery from these changes were observed.

Table: Changes in selected clinical chemistry parameters compared to pretest values

Dose	Sample	ALT	ALP	Cholesterol	Urea	Protein	Albumin	Magnesium	Calcium
MALE									
0	Week 13:	-8%	-7%	-11%	0%	-3%	+7%	+5%	-11%
	Week 26:	0%	-6%	-14%	+2%	0%	+4%	+5%	-6%
	Week 39:	+2%	-32%	-17%	-6%	+2%	0%	+5%	-5%
LD	Week 13:	-13%	-11%	-24%	-2%	-7%	+4%	+1%	-11%
	Week 26:	-9%	-19%	-21%	-2%	-2%	0%	+3%	-5%
	Week 39:	+16%	-24%	-24%	+6%	+7%	+4%	+6%	-4%
LMD	Week 13:	-16%	+14%	-24%	+2%	-5%	-11%	-3%	-9%
	Week 26:	-10%	+3%	-26%	-6%	-2%	0%	-3%	-6%
HMD	Week 13:	-34%	-13%	-23%	-3%	-12%	-14%	-1%	-11%
	Week 26:	-25%	-11%	-36%	-5%	-12%	-14%	-3%	-8%
HD	Week 13:	-42%	+26%	-5%	+26%	-17%	-15%	-3%	-15%
	Week 26:	-40%	+9%	-3%	+9%	-12%	-10%	-3%	-10%
	Week 39:	+4%	-2%	-5%	-2%	+3%	-4%	-3%	-4%

Dose	Sample	ALT	ALP	Cholesterol	Urea	Protein	Albumin	Magnesium	Calcium
FEMALE									
0	Week 13:	-7%	-23%	+3%	+2%	-5%	0%	-5%	-9%
	Week 26:	-5%	-18%	+8%	0%	+2%	0%	+4%	-5%
	Week 39:	0%	-43%	-11%	-6%	+2%	0%	-2%	-4%
LD	Week 13:	-17%	-11%	-17%	-2%	-5%	0%	-2%	-11%
	Week 26:	+26%	-9%	-10%	+7%	-3%	-7%	-6%	-8%
	Week 39:	-20%	-35%	+23%	+35%	-7%	-17%	+1%	-7%
LMD	Week 13:	-22%	+3%	-12%	+20%	-8%	0%	-1%	-10%
	Week 26:	-15%	+3%	-21%	+8%	-10%	-10%	-1%	-8%
HMD	Week 13:	-15%	-4%	-32%	-2%	-15%	-10%	-9%	-12%
	Week 26:	-6%	+11%	-40%	-4%	-11%	-13%	-6%	-9%
HD	Week 13:	-32%	0%	-21%	+13%	-17%	-17%	-10%	-13%
	Week 26:	-37%	+44%	-23%	+20%	-14%	-20%	-5%	-12%
	Week 39:	-13%	-32%	-10%	+2%	0%	-7%	-6%	-7%

Urinalysis

Urine samples were taken from all animals prior to initiation of dosing, in Weeks 12 and 25 of dosing, and Week 39 of recovery.

There were no adverse changes in urinalysis.

Gross Pathology

Gross pathology was assessed in all animals.

Macroscopic findings included clear fluid, observed in the abdominal cavity in 3 HDM, 1 HMDF, and 2 HDF, thick and firm heart left ventricle in 1 HDM and 1 HDF, mottled liver in 1 HDM, and gelatinous thymus in 2 HDM and 1 HDF. Few macroscopic findings were noted in recovery animals.

Organ Weights

At necropsy, the organs listed in the table below (labeled with †) from the study report were weighed.

Statistically significant increases in absolute lung weight in HDM (35%), absolute liver weight in HDM (33%) and HDF (32%), and absolute popliteal lymph node weight in HDF (87%) and decrease in absolute prostate weight in HDM (51%) were noted. Partial reversal of the organ weight increases and reversal in the decrease in prostate weight was observed by the end of the recovery period.

Histopathology

At necropsy, the following organs, as listed in the study report, were collected and tissues labeled with (Š) examined microscopically apart from brain sections that were examined in control and HD animals.

- adrenals (†) (§)
- animal identification
- aorta (TS and LS) (§)
- bone marrow smear(sternum) (a) (c)
- brain including Pons (†) (§)
- caecum (§)
- colon (§)
- dorsal root ganglia (lumbar, including spinal roots) (§)
- duodenum (§)
- eyes + optic nerves (b) (§)
- femur with bone marrow and articular surface (§)
- gall bladder (§)
- gross lesions (§)
- heart (†) (§)
- ileum including Peyer’s patch (§)
- jejunum (§)
- kidneys (†) (§)
- lacrimal glands
- larynx (§)
- liver (†) (§)
- lungs with mainstem bronchi and bronchioles (†) (§)
- mammary (§)
- mandibular lymph nodes (†) (§)
- mesenteric lymph nodes (†) (§)
- muscle (medial gastrocnemius TS unilateral) (§)
- muscle (quadriceps) (§)
- muscle (semimembranosus TS, unilateral) (§)
- oesophagus (§)
- ovaries (†) (§)
- oviducts (§)
- pancreas (§)
- pituitary (†) (§)
- plantar nerve (TS and LS, unilateral) (§)
- popliteal lymph nodes (†) (§)
- prostate (†) (§)
- rectum (§)
- salivary glands, parotid, sublingual, mandibular (§)
- spinal cord thoracic (TS and LS) (§)
- sciatic nerves (§)
- skin and subcutaneous tissue (§)
- spinal cord cervical (TS and LS) (§)
- spinal cord lumbar (TS and LS) (§)
- spleen (†) (§)
- sternum with bone marrow (§)
- stomach (§)
- testes + epididymides (d) (†) (§)
- thymus (†) (§)
- thyroids + parathyroids (†) (§)
- tongue (§)
- trachea (§)
- ureters (§)
- urinary bladder (§)
- uterus including cervix (†) (§)
- vagina (§)

fixative = 10% neutral buffered formalin except where indicated by: a - methanol, b - Davidson’s fluid, c – clinical pathology section, d - Bouin’s fixative

LS = Longitudinal section; TS = Transverse section (†) Weighed organs

Bone tissue designated for histopathological examination was decalcified using Kristensons fluid. (§) tissues embedded in paraffin wax BP and stained with haematoxylin and eosin.

Adequate Battery: Yes.

Peer Review: No.

Signed Pathology report: Yes, by (b) (6)

Histological Findings

The main histological findings were noted in heart, liver, lungs, and thymus. In the heart, arteriopathy was observed in vessels, typically located in papillary muscles, at the HMD and HD. At the HMD and HD, increased glycogen was noted in the liver. Foamy and partially foamy alveolar macrophages were observed in all groups. Incidences of edema were observed at the HD in M and F in the thymus. Incidences of arteriopathy in HD recovery animals were noted, suggesting no recovery after 4 weeks.

Table: Summary of selected male microscopic findings

Tissue	Finding	(mg/kg): Number of dogs	Main					Recovery		
			0	0.4	2	10	40	0	0.4	40
Heart	Arteriopathy posterior papillary muscle		4	4	4	4	4	2	3	3
	Grade 1:		0	0	0	1	1	0	0	0
	Grade 2:		0	0	0	1	0	0	0	1
	Grade 3:		0	0	0	1	0	0	0	1
	Arteriopathy anterior papillary muscle									
	Grade 1:		0	0	0	0	0	0	0	1
	Grade 2:		0	0	0	0	0	0	0	1
	Arteriopathy septum									
	Grade 1:		0	0	0	0	0	0	0	2

Tissue	Finding	(mg/kg): Number of dogs	Main					Recovery		
			0	0.4	2	10	40	0	0.4	40
			4	4	4	4	4	2	3	3
Liver	Increased Glycogen	Grade 1:	0	0	1	2	1	0	0	0
		Grade 2:	0	0	0	0	3	0	0	0
Lung	Foamy/partially foamy macrophages	Grade 1:	1	0	1	1	0	1	1	1
		Grade 2:	0	0	0	0	1	0	0	0
Thymus	Edema	Grade 1:	0	0	0	0	0	0	0	0
		Grade 2:	0	0	0	0	0	0	0	0
		Grade 3:	0	0	0	0	2	0	0	0

Table: Summary of selected female microscopic findings

Tissue	Finding	(mg/kg): Number of dogs	Main					Recovery		
			0	0.4	2	10	40	0	0.4	40
			4	4	4	4	4	2	3	3
Heart	Arteriopathy posterior papillary muscle	Grade 1:	0	0	0	2	1	0	0	0
		Grade 2:	0	0	0	0	2	0	0	0
		Grade 3:	0	0	0	0	0	0	0	1
		Grade 4:	0	0	0	0	1	0	0	1
	Arteriopathy anterior papillary muscle	Grade 1:	0	0	0	0	0	0	0	0
		Grade 2:	0	0	0	0	1	0	0	2
		Grade 3:	0	0	0	0	0	0	0	0
		Grade 4:	0	0	0	0	1	0	0	0
	Arteriopathy septum	Grade 1:	0	0	0	0	1	0	0	1
		Grade 2:	0	0	0	0	0	0	0	0
Grade 3:		0	0	0	0	1	0	0	0	
Liver	Increased Glycogen	Grade 1:	0	0	0	2	1	0	0	0
		Grade 2:	0	0	0	0	2	0	0	0
Lung	Foamy/partially foamy macrophages	Grade 1:	2	1	1	2	2	2	0	1
		Grade 2:	0	0	0	2	0	0	0	0
Skin	Folliculitis	Grade 1:	1	1	2	1	2	0	0	1
		Grade 2:	0	0	0	2	1	0	0	0
		Grade 3:	0	0	0	1	0	0	0	0
Thymus	Edema	Grade 1:	0	0	0	0	0	0	0	0
		Grade 2:	0	0	0	0	0	0	0	0
		Grade 3:	0	0	0	0	1	0	0	0

Toxicokinetics

Blood samples were taken on Day 1 and during Week 26 pre-dose and at 0.5, 1, 2, 4, 8, and 24 hours post-dose.

Increase in C_{max} of ponesimod was generally less than dose-proportional and increase in AUC was dose-proportional, with plasma exposure similar between the sexes. T_{max}

was at 1-8 hours in M and F and $t_{1/2}$ was 4-11 hours. Minimal accumulation occurred after 26 weeks.

Table: Toxicokinetic parameters for ponosimod (Sponsor's)

Day 1						Week 26					
ACT-128800 Dose Level (mg/kg/day)	Sex	AUC _{0-τ} (ng.h/mL)	C _{max} (ng/mL)	t _{max} (h) median	t _{1/2} (h)	ACT-128800 Dose Level mg/kg/day	Sex	AUC _{0-τ} (ng.h/mL)	C _{max} (ng/mL)	t _{max} (h) median	t _{1/2} (h)
0.4	Male	1400	145	2.00	6.85	0.4	Male	1960	199	1.00	6.98
	Female	1500	163	2.00	5.34		Female	1850	174	2.00	7.58
2	Male	4630	645	2.00	NC	2	Male	4720	660	1.00	3.95
	Female	8000	775	2.00	7.75		Female	11400	988	1.00	7.48
10	Male	38000	2650	3.00	9.12	10	Male	36400	2840	1.50	10.6
	Female	19700	1780	2.00	11.0		Female	25600	2210	1.00	10.0
40	Male	95300	5910	2.00	NC	40	Male	100000	6730	2.00	7.71
	Female	102000	5250	8.00	NC		Female	144000	9170	4.00	7.93

τ = 24 hours

NC = Not calculable

τ = 24 hours

Neurological examination

Examination of all animals was conducted once prior to initiation of dosing, in Weeks 1, 5, and 25 during dosing, and Week 38 during recovery at approximately 2 hours post-dose.

There were no adverse changes in observed.

Study title: ACT-128800: 52 Week Oral (Gavage) Administration Toxicity Study in the Dog Followed by a 13 Week Treatment-Free Period

Study no.: T-06.186
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: September 14, 2006
 GLP compliance: Yes, (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch AA-016498-batch-(b) (4), 99.4%

Key Study Findings

- There were no deaths.
- One HDF had dosing stopped between Days 228-231 due to swollen/red ears, sores and hair loss to muzzle, sores on paws and sagging skin on face.
- Decreased lymphocytes occurred at all doses in M and F.
- The main microscopic findings were in the heart and included arteriopathy in the posterior and anterior papillary muscle and subendocardial fibrosis in the left ventricle value at the HD in M and F.

- The sponsor considered the MD, 3 mg/kg/day, the NOAEL due to microscopic findings in the heart at the HD; this appears appropriate. The NOAEL was associated with plasma exposures (AUC) to ponesimod in Week 52 of 14300 and 16300 ng*h/mL in M and F, respectively.

Methods

Doses:	0, 1, 3, and 10 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral by gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	0.25% (w/w) Methylcellulose and 0.05% (v/v) Tween 80
Species/Strain:	Dog / Beagle
Number/Sex/Group	
Main:	4
Recovery:	4 for control and HD
Age:	58-63 weeks
Weight:	11.25-17.75 kg

Observations and Results

Mortality

There were no early deaths.

Clinical Signs

Observations of clinical signs were conducted once daily. Detailed examinations were conducted weekly prior to dosing and during recovery and daily during dosing.

No adverse clinical signs were observed. Signs were mainly observed at the HD and included soft/liquid feces and incidences of sores and lesions particularly on paws and legs. Due to swollen/red ears, sores and hair loss to muzzle, sores on paws and sagging skin on face 1 HDF had dosing stopped between Days 228-231.

Body Weights

Body weights were recorded once weekly from Week -5.

There were no adverse changes in body weight.

Food Consumption

Food consumption was recorded daily from Week -5.

There was a statistically significant decrease in food consumption in HDF (10%) over the dosing period. Otherwise, no adverse changes in food consumption were noted.

Ophthalmoscopy

Examination of all animals was conducted once prior to initiation of dosing, in Week 51 of dosing, and Week 65 of recovery.

One HDF developed lens opacity by Week 51. Otherwise, no adverse changes were noted.

ECG

Examination of all animals was conducted once prior to initiation of dosing, in Week 51 during dosing pre-dosing and approximately 2 hours post-dose, and Week 65 of recovery. The QT_c was calculated by the Fredericia and van de Water methods. Echocardiography examinations were conducted in Week 49.

In Week 51, there was a statistically significant increase in QT_c of 14 and 7 ms in HDM 2 hours post-dose using the Fredericia and van de Water methods, respectively compared to control animals; increases in QT_c compared to pre-dose levels were 29 and 22 ms.

Hematology

Blood samples were taken from all animals twice prior to initiation of dosing, in Weeks 26 and 52 of dosing, and Week 65 during recovery.

Generally, dose-dependent decreases in WBC (approximately 8-47%) and lymphocytes (approximately 37-77%) were observed at all doses in M and F in Weeks 26 and 52. At the HD, these decreases partially recovered.

Clinical Chemistry

Blood samples were taken from all animals twice prior to initiation of dosing, in Weeks 26 and 52 of dosing, and Week 65 during recovery.

By Week 52, compared to pre-dosing levels, there were decreases in cholesterol at all doses in M, high density lipoprotein cholesterol (HHDL) at all doses in M, protein at all doses in M and F. Recovery from these decreases were observed.

Table: Changes in selected clinical chemistry parameters compared to pretest values

Dose	Sample	Cholesterol		HHDL		Protein	
		Mean	%	Mean	%	Mean	%
MALE							
0	Pretest:	3.9		3.59		63	
	Week 26:	4.3	+10%	4.25	+18%	64	+2%
	Week 52:	4.0	+3%	3.51	-2%	64	+2%
	Recovery:	3.8	-3%	3.40	-5%	64	+2%
LD	Pretest:	3.8		3.54		65	
	Week 26:	3.5	-8%	3.38	-5%	61	-6%
	Week 52:	3.1	-18%	2.60	-27%	60	-8%
MD	Pretest:	4.0		3.71		64	
	Week 26:	3.5	-12%	3.34	-10%	59	-8%
	Week 52:	3.2	-20%	3.39	-9%	58	-9%
HD	Pretest:	3.6		3.35		62	
	Week 26:	2.9	-19%	2.84	-15%	56	-10%
	Week 52:	2.8	-22%	2.39	-29%	57	-8%
	Recovery:	3.3	-8%	2.98	-11%	61	-2%

Dose	Sample	Cholesterol		HDL		Protein	
		Mean	%	Mean	%	Mean	%
FEMALE							
0	Pretest:	4.2		3.81		64	
	Week 26:	4.0	-5%	3.84	+1%	63	-2%
	Week 52:	3.8	-10%	3.24	-15%	64	0%
LD	Recovery:	4.7	+12%	4.26	+12%	67	+5%
	Pretest:	4.4		3.95		66	
	Week 26:	3.8	-14%	3.61	-9%	62	-6%
MD	Week 52:	3.9	-11%	3.39	-14%	60	-9%
	Pretest:	3.7		3.52		62	
	Week 26:	3.3	-11%	3.13	-11%	57	-8%
HD	Week 52:	2.8	-24%	2.48	-30%	57	-8%
	Pretest:	3.6		3.24		60	
	Week 26:	3.5	-3%	3.46	+7%	56	-7%
HD	Week 52:	3.4	-6%	3.02	-7%	56	-7%
	Recovery:	4.4	+22%	3.89	+20%	62	+3%

Urinalysis

Urine samples were taken from all animals once prior to initiation of dosing, in Weeks 25 and 51 of dosing, and Week 65 during recovery.

There were no adverse changes in urinalysis.

Gross Pathology

Gross pathology was assessed in all animals.

No adverse macroscopic findings were noted.

Organ Weights

At necropsy, the organs listed in the table below (labeled with †) from the study report were weighed.

Statistically significant increases in absolute adrenal weight at all doses in M, brain weight in HDM and HDF, ovaries in HDF, uterus in HDF were noted. There was reversal in the organ weight increases at the HD, except for adrenal glands in HDM.

Table: Organ changes in mean absolute weight compared to control

	Adrenal	Brain	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate /Ovary	Spleen	Testes /Uterus	Thymus	Thyroid
							Ma	Me	P						
MALE															
LD:	+32%*	-1%	+6%	+6%	-2%	+1%	+106%	-19%	-14%	-5%	-28%	+8%	-9%	+47%	+1%
MD:	+43%*	+5%	+5%	+21%	+6%	+17%	+24%	-15%	+7%	-7%	-12%	+25%	-4%	-8%	+23%
HD:	+22%*	+8%*	+1%	-4%	-4%	+14%	+30%	-5%	+42%	-18%	+16%	+2%	-14%	+17%	-5%
	(+22%)	(-1%)	(-3%)	(0%)	(-2%)	(-1%)	(-17%)	(+6%)	(+2%)	(+41%*)	(+15%)	(+38%)	(+17%)	(+15%)	(-14%)
FEMALE															
LD:	+29%*	+1%	-9%	+7%	+10%	+6%	+45%	-26%	+162%	+26%	+29%	+13%	-9%	-4%	+19%
MD:	+13%	-9%	0%	-1%	+2%	+5%	-3%	-1%	0%	+31%	+56%	+13%	+52%	+14%	+22%
HD:	+4%	-8%*	+2%	-1%	+26%	+3%	+22%	-5%	-12%	+23%	+68%*	+48%	+141%*	-8%	+16%
	(-15%*)	(-1%)	(+5%)	(+1%)	(-6%)	(+1%)	(-29%)	(-18%)	(-9%)	(-7%)	(-9%)	(*17%)	(-4%)	(+33%)	(0%)

* Statistically significant from control; recovery values in parenthesis; Ma – mandibular; Me – mesenteric; P – popliteal; Pit - pituitary.

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

	Adrenal	Brain	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate	Spleen	Testes	Thymus	Thyroid
							Ma	Me	P	/Ovary					
MALE															
LD:	+22%*	0%	-11%	-2%	-9%	0%	+94%	-23%	-25%	0%	-34%	+1%	-16%	+38%	0%
MD:	+33%*	0%	0%	+14%	0%	+11%	+13%	-20%	0%	0%	-16%	+19%	-10%	-13%	+14%
HD:	+22%*	0%	0%	-6%	-3%	+11%*	+31%	-7%	+38%	0%	+13%	+2%	-14%	+16%	0%
	(+9%)	(0%)	(-11%)	(-5%)	(-7%)	(0%)	(-20%)	(+1%)	(-11%)	(0%)	(+11%)	(+31%)	(+11%)	(+7%)	(-22%)
FEMALE															
LD:	+33%*	0%	0%	+9%	+11%	+13%	+43%	-27%	+163%	0%	+33%	+13%	-6%	-3%	+17%
MD:	+25%*	0%	+13%	+10%	+11%	+25%*	+7%	+8%	+13%	0%	+67%	+24%	+68%	+20%	+33%
HD:	+8%	0%	+13%	+6%	+32%*	+13%	+29%	0%	0%	0%	+78%*	+60%*	+151%*	-6%	+17%
	(-14%)	(0%)	(0%)	(+3%)	(-7%)	(0%)	(-28%)	(-16%)	(0%)	(0%)	(-7%)	(+20%)	(-3%)	(+35%)	(0%)

* Statistically significant from control; recovery values in parenthesis; Ma – mandibular; Me – mesenteric; P – popliteal; Pit - pituitary.

Histopathology

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

adrenals (†) (§)	oesophagus (§)
animal identification	ovaries (†) (§)
aorta (LS + TS) (§)	oviducts (§)
bone marrow smear (sternum)(a) (c)	pancreas (§)
brain including Pons (†) (§)	pituitary (†) (§)
caecum (§)	plantar nerve (TS + LS, unilateral) (§)
colon (§)	popliteal lymph nodes (†) (§)
dorsal root ganglia (§)	prostate (†) (§)
duodenum (§)	rectum (§)
eyes and optic nerves (b) (§)	salivary gland mandibular, sublingual, parotid (§)
femur with bone marrow and articular surface (§)	sciatic nerves (§)
gall bladder (§)	skin and subcutaneous tissue (§)
gross lesions (§)	spinal cord cervical (TS + LS) (§)
heart (†) (§) (e)	spinal cord lumbar (TS + LS) (§)
ileum including Peyer's patch (§)	spinal cord thoracic (TS + LS) (§)
jejunum (§)	spleen (†) (§)
kidney (†) (§)	sternum with bone marrow (§)
lacrimal glands	stomach (§)
larynx (§)	testes + epididymides (d) (†) (§)
liver (†) (§)	thymus (†) (§)
lungs (†) (§)	thyroids + parathyroids (†) (§)
mammary (§)	tongue (§)
mandibular lymph nodes (†) (§)	trachea (§)
mesenteric lymph nodes (†) (§)	ureters (§)
muscle (quadriceps) (§)	urinary bladder (§)
muscle (semimembranosus, TS unilateral) (§)	uterus including cervix (†) (§)
muscle (medial gastrocnemius, TS) unilateral) (§)	vagina (§)

fixative = 10% neutral buffered formalin except where indicated by: a = methanol, b = Davidson's fluid,

d = Bouin's fixative

c = see [clinical pathology section](#)

e = included slide production and examination of left papillary muscles and main right papillary muscle

Bone designated for histopathological examination was decalcified using Kristenson's fluid.

LS = Longitudinal section

TS = Transverse section

Adequate Battery: Yes.

Peer Review: Heart sections were peer reviewed.

Signed Pathology report: Yes, by (b) (6)

Histological Findings

The main histological finding was noted in heart, lungs, and lymphoid tissues (lymph nodes and spleen). In the heart, arteriopathy, characterized by vascular wall thickening,

was observed in the posterior and anterior papillary muscle and subendocardial fibrosis in the left ventricle value in HDM and HDF. Fibrosing alveolitis was observed in the lung in LDM and HDM and macrophage aggregates in MDF and HDM. Lymphoid tissue findings included lymphoid atrophy in at all doses. There was no reversal of heart arteriopathy at the HD. Otherwise, these findings were generally not observed in recovery animals.

Table: Summary of selected microscopic findings

Tissue	Finding	Number of dogs	MALE						FEMALE											
			Main				Rec.		Main				Rec.							
			(mg/kg):						0	1	3	10	0	10	0	1	3	10	0	10
			4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4		
Heart	Arteriopathy posterior papillary muscle		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
	Grade 1:		0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Grade 2:		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
	Grade 3:		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
	Arteriopathy anterior papillary muscle		0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	
	Grade 1:		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
	Grade 2:		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
	Grade 3:		0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	
	Subendocardial fibrosis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Grade 1:		0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	1	0	
	Grade 2:		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Coronary intimal proliferation		0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	
Grade 1:		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Grade 2:		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Grade 3:		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0		
Myocardial degeneration		0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0		
Grade 1:																				
Kidney	Nephropathy		0	1	0	2	3	0	0	1	1	1	0	1	0	1	1	1	0	1
Grade 1:																				
Liver	Glycogen vacuolation		0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	1	0	1
Grade 1:			0	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Grade 2:																				
Lung	Fibrosing alveolitis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grade 1:			0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
Grade 2:			0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grade 3:																				
Macrophage aggregate			0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Grade 1:																				
Lymph node - mesenteric	Atrophy		0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0
Grade 1:			0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grade 1:																				
Lymph node - popliteal	Atrophy		0	0	0	2	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Grade 1:			0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0
Grade 1:			0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Grade 2:																				
Spleen	Agonal congestion/hemorrhage present:		0	0	0	0	0	0	0	1	0	2	0	0	0	1	0	2	0	0
Lymphoid atrophy			0	1	2	4	0	0	0	1	1	1	0	0	0	1	1	1	0	0
Grade 1:			0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0
Grade 2:																				

Rec. - Recovery

Toxicokinetics

Blood samples were taken on Day 1 and during Week 52 pre-dose and at 0.5, 1, 2, 4, 8, and 24 hours post-dose.

On Day 1, increase in ponesimod plasma exposure (C_{max} and AUC_{0-24h}) was generally less than dose-proportional between the LD and MD and greater than dose-proportional between the MD and HD. In Week 52, increase in ponesimod plasma exposure (C_{max} and AUC_{0-24h}) was generally dose-proportional, with plasma exposure generally greater in F. T_{max} was at 4-8 hours in M and F and $t_{1/2}$ was 1-6 hours. Accumulation of ponesimod occurred after 52 weeks of dosing.

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

Day 1						Week 52							
ACT-128800 Dose Level (mg/kg/day)	Sex	$AUC_{0-\tau}$ (ng.h/mL)	C_{max} (ng/mL)	t_{max} # (h)	$t_{1/2}$ (h)	ACT-128800 Dose Level (mg/kg/day)	Sex	$AUC_{0-\tau}$ (ng.h/mL)	C_{max} (ng/mL)	t_{max} # (h)	$t_{1/2}$ (h)		
1	N	4	4	4	4	1	N	4	4	4	4		
	Mean	Male	3390	312	2.00		6.42	Mean	Male	5950	487	2.00	7.64
	SD		2300	142	(2.00-2.00)		2.32	SD		3960	199	(2.00-2.00)	3.30
	CV%		67.8	45.6			36.2	CV%		66.5	40.9		43.2
	N		4	4	4		3	N		4	4	4	4
	Mean	Female	5530	484	2.00		7.58	Mean	Female	7430	684	2.00	7.73
SD		3230	265	(2.00-4.00)	4.22	SD		4980	393	(1.00-2.00)	3.43		
CV%		58.5	54.7		55.7	CV%		67.0	57.4		44.3		
3	N	4	4	4	4	3	N	4	4	4	4		
	Mean	Male	6740	616	1.50		7.65	Mean	Male	14300	1230	2.00	8.32
	SD		3910	226	(1.00-2.00)		5.05	SD		9690	440	(1.00-4.00)	3.44
	CV%		58.0	36.7			66.0	CV%		67.8	35.9		41.4
	N		4	4	4		4	N		4	4	4	4
	Mean	Female	8330	881	1.50		6.60	Mean	Female	16300	1370	2.00	8.38
SD		4030	343	(1.00-2.00)	2.46	SD		11100	663	(1.00-2.00)	4.29		
CV%		48.4	38.9		37.3	CV%		67.9	48.3		51.2		
10	N	8	8	8	6	10	N	8	8	8	1		
	Mean	Male	32800	2610	2.00		7.87	Mean	Male	39900	2870	4.00	3.85
	SD		11100	946	(1.00-4.00)		2.91	SD		15200	597	(1.00-8.00)	NC
	CV%		33.8	36.2			36.9	CV%		38.2	20.8		NC
	N		8	8	8		2	N		8	8	8	5
	Mean	Female	32300	2900	2.00		3.69	Mean	Female	45200	3150	2.00	7.08
SD		21400	1830	(2.00-24.00)	NC	SD		36600	1620	(1.00-4.00)	5.70		
CV%		66.3	63.1		NC	CV%		80.8	51.6		80.6		

NC = Not calculable
 τ = 24 hours
 # = Median value (min-max)

NC = Not calculable
 τ = 24 hours
 # = Median value (min-max)

Neurological examination

Examination of all animals was conducted twice prior to initiation of dosing, in Weeks 25 and 51 during dosing, and Week 65 during recovery at approximately 2 hours post-dose.

There were no adverse changes in observed.

Study title: ACT-128800: 52-Week Follow-up Oral (Gavage) Toxicity Study in the Beagle Dog

Study no.: T-08.357
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: July 23, 2008
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch 4, 99.4 and 99.8%

Key Study Findings

- There were no deaths and few clinical signs apart from skin and dental changes
- Decreased WBC, lymphocytes, and basophils occurred at all doses in M and F.
- The main microscopic findings were in the heart, lungs, skin, spleen, and thymus and included arterial changes in the heart, alveolar histiocytosis in the lungs, lesions of the skin, and pharmacodynamic effects in the spleen, atrophy of PALS, and thymus, medullary hypertrophy.
- There was no NOAEL established due to microscopic findings of arterial lesions in the papillary muscle of the left ventricle, alveolar histiocytosis at all doses and skin lesions in the MD and HD groups.

Methods

Doses: 0, 10>20*, 40>80*, and 70>140*>280** mg/kg
 * Doses increased in Week 8
 ** Dose increased in Week 27
 Frequency of dosing: Daily
 Route of administration: Oral by gavage
 Dose volume: 2 mL/kg
 Formulation/Vehicle: 0.25% (w/w) Methylcellulose and 0.05% (v/v) Tween 80
 Species/Strain: Dog / Beagle
 Number/Sex/Group: 4
 Age: 9.5-11 months
 Weight: 10.1-15.3 kg

Observations and Results

Mortality

Observations for mortality were conducted at least twice daily.

There were no early deaths.

Clinical Signs

Observations of clinical signs were conducted at least twice daily. During dosing observations were conducted at 2- and 4-hour post dose, as well.

The main clinical signs observed included an increase in the severity in skin changes, such as erythema, crusts, nodules, wounds, and hair loss and dental calculus and erythema of the gingiva were observed from Week 39 at all doses.

Body Weights

Body weights were recorded twice weekly.

Mean body weight gains were decreased at all doses in M and in MDF and HDF.

Table: Body weight gains (kg; Sponsor's)

	Group 1 males	Group 2 males	Group 3 males	Group 4 males	Group 1 females	Group 2 females	Group 3 females	Group 4 females
Week 1 - 52	+ 2.4	+ 1.5	+ 1.5	+ 1.0	+ 1.3	+ 2.7	+ 0.7	+ 0.0

Food Consumption

Food consumption was recorded daily.

There were no adverse changes in food consumption.

Ophthalmoscopy

Examination of all animals was conducted once prior to initiation of dosing, in Weeks 26 and 52 of dosing.

There were no adverse changes.

ECG

Examination of all animals was conducted once prior to initiation of dosing, in Weeks 1, 13, 26, 27, and 52 during dosing at pre-dosing and 2 hours post-dose. The QT_c was calculated by the van de Water method. ECG examinations were conducted in Week 48.

There were no adverse changes.

Hematology

Blood samples were taken from all animals prior to initiation of dosing and in Weeks 13, 26, 31, 40, and 52 of dosing.

Decreases in WBC (approximately 9-39%), lymphocytes (approximately 66-83%), and basophils (approximately 33-86%) were observed at all doses in M and F, and platelets were increased in HDM, MDF, and HDF.

Clinical Chemistry

Blood samples were taken from all animals prior to initiation of dosing and in Weeks 13, 26, 31, 41, and 52.

There were decreases in ALT, cholesterol, bilirubin, protein, and calcium and increases in ALP in M and F.

Table: Changes in selected clinical chemistry parameters compared to pretest values

Dose	Sample	ALT		ALP		Cholesterol		Bilirubin		Protein		Albumin		Calcium	
		Mean	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
MALE															
0	Pretest:	34.5		79.1		3.74		2.58		60.69		34.00		2.81	
	Week 13:	36.2	+5%	64.1	-19%	3.36	-10%	3.03	+17%	59.30	-2%	34.27	+1%	2.71	-4%
	Week 26:	45.9	+33%	58.4	-26%	2.99	-20%	2.53	-2%	62.43	+3%	36.53	+7%	2.63	-6%
	Week 31:	36.4	+6%	60.0	-24%	2.69	-28%	2.50	-3%	60.45	0%	34.22	+1%	2.64	-6%
	Week 41:	33.4	-3%	58.1	-27%	3.03	-19%	2.83	+10%	60.55	0%	33.29	-2%	2.61	-7%
LD	Week 52:	37.9	+10%	59.9	-24%	2.96	-21%	2.20	-15%	60.44	0%	33.01	-3%	2.57	-9%
	Pretest:	36.7		107.3		4.13		2.78		59.58		31.55		2.67	
	Week 13:	33.3	-9%	104.4	-3%	3.29	-20%	2.05	-26%	51.34	-14%	26.47	-16%	2.49	-7%
	Week 26:	26.4	-28%	89.5	-17%	2.51	-39%	2.61	-6%	54.70	-8%	27.97	-11%	2.48	-7%
	Week 31:	24.0	-35%	83.7	-22%	2.29	-45%	2.35	-15%	51.44	-14%	25.42	-19%	2.43	-9%
MD	Week 41:	23.6	-36%	81.5	-24%	2.56	-38%	2.90	+4%	56.08	-6%	26.49	-16%	2.47	-7%
	Week 52:	28.0	-24%	85.1	-21%	2.47	-40%	2.08	-25%	55.62	-7%	27.08	-14%	2.35	-12%
	Pretest:	41.0		94.5		4.41		2.45		58.54		31.44		2.72	
	Week 13:	25.9	-37%	91.3	-3%	4.33	-2%	2.45	0%	49.98	-15%	25.15	-20%	2.49	-8%
	Week 26:	27.2	-34%	84.4	-11%	3.25	-26%	2.00	-18%	51.42	-12%	25.19	-20%	2.50	-8%
HD	Week 31:	27.1	-34%	91.2	-3%	3.07	-30%	2.38	-3%	51.63	-12%	25.91	-18%	2.55	-6%
	Week 41:	26.1	-36%	82.7	-12%	3.19	-28%	2.85	+16%	52.52	-10%	25.80	-18%	2.47	-9%
	Week 52:	28.4	-31%	93.6	-1%	2.86	-35%	1.85	-24%	52.84	-10%	24.49	-22%	2.35	-14%
	Pretest:	51.2		85.7		3.83		2.45		61.81		23.52		2.74	
	Week 13:	36.0	-30%	91.6	+7%	2.99	-22%	1.98	-19%	53.96	-13%	28.56	+21%	2.59	-5%
0	Week 26:	38.7	-24%	88.8	+4%	2.67	-30%	1.83	-25%	56.71	-8%	30.48	+30%	2.55	-7%
	Week 31:	35.5	-31%	97.5	+14%	2.53	-34%	1.95	-20%	55.22	-11%	28.24	+20%	2.52	-6%
	Week 41:	31.4	-39%	95.7	+12%	2.73	-29%	2.08	-15%	54.38	-12%	26.98	+15%	2.47	-10%
	Week 52:	36.5	-29%	95.0	+11%	2.60	-32%	1.58	-36%	54.72	-11%	26.44	+12%	2.36	-14%
	Pretest:	41.0		94.5		4.41		2.45		58.54		31.44		2.72	
FEMALE															
0	Pretest:	39.0		94.8		4.02		2.48		60.00		33.76		2.75	
	Week 13:	46.2	+18%	80.3	-15%	3.95	-2%	2.53	+2%	57.15	-5%	32.47	-4%	2.65	-4%
	Week 26:	39.5	+1%	75.5	-20%	3.64	-9%	2.75	+11%	61.31	+2%	34.40	+2%	2.62	-5%
	Week 31:	34.2	-12%	82.4	-13%	3.72	-7%	2.48	0%	59.20	-1%	30.79	-9%	2.62	-5%
	Week 41:	36.1	-7%	68.3	-28%	3.61	-10%	2.88	+16%	59.65	-1%	30.37	-10%	2.58	-6%
LD	Week 52:	33.6	-14%	85.5	-10%	4.53	+13%	2.30	-7%	62.60	+4%	30.12	-11%	2.53	-8%
	Pretest:	43.1		89.2		3.82		3.03		60.79		33.10		2.80	
	Week 13:	31.6	-27%	60.2	-33%	2.53	-34%	2.18	-28%	52.09	-14%	27.96	-16%	2.57	-8%
	Week 26:	30.5	-29%	71.3	-20%	2.98	-22%	2.45	-19%	57.90	-5%	29.41	-11%	2.57	-8%
	Week 31:	29.7	-31%	68.3	-23%	2.74	-28%	2.03	-33%	55.14	-9%	27.14	-18%	2.60	-7%
MD	Week 41:	32.4	-25%	63.0	-29%	2.43	-36%	3.00	-1%	55.93	-8%	27.73	-16%	2.54	-9%
	Week 52:	36.3	-16%	76.8	-14%	3.04	-20%	2.13	-30%	58.99	-3%	28.52	-14%	2.46	-12%
	Pretest:	38.1		86.9		3.52		2.58		60.72		33.46		2.72	
	Week 13:	26.9	-29%	63.4	-27%	2.42	-31%	1.85	-28%	49.83	-18%	25.50	-24%	2.43	-11%
	Week 26:	27.2	-29%	60.9	-30%	2.41	-32%	1.75	-32%	52.87	-23%	26.62	-20%	2.46	-10%
HD	Week 31:	24.7	-35%	61.8	-29%	2.13	-39%	1.23	-52%	51.04	-16%	23.89	-29%	2.45	-10%
	Week 41:	26.0	-32%	59.5	-32%	2.34	-34%	2.15	-17%	52.73	-13%	24.04	-28%	2.41	-11%
	Week 52:	24.7	-35%	68.8	-21%	2.82	-20%	1.38	-47%	54.03	-11%	22.75	-32%	2.37	-13%
	Pretest:	42.9		122.9		4.50		2.85		62.64		34.19		2.79	
	Week 13:	30.5	-29%	133.1	+8%	3.92	-13%	1.98	-31%	51.71	-17%	26.28	-23%	2.51	-10%
0	Week 26:	26.9	-37%	145.9	+19%	3.70	-18%	2.30	-19%	57.20	-9%	26.16	-23%	2.55	-9%
	Week 31:	28.8	-33%	165.8	+35%	3.43	-24%	1.90	-33%	55.18	-12%	26.04	-24%	2.57	-8%
	Week 41:	29.2	-32%	150.2	+22%	2.86	-36%	1.80	-37%	54.74	-13%	25.51	-25%	2.48	-11%
	Week 52:	31.6	-26%	186.5	+52%	3.09	-31%	1.48	-48%	55.99	-11%	26.09	-24%	2.37	-15%
	Pretest:	41.0		94.5		4.41		2.45		58.54		31.44		2.72	

Urinalysis

Urine samples were taken from all animals prior to initiation of dosing and in Weeks 13, 26, 31, 40, and 52 of dosing.

There were no adverse changes in urinalysis.

Gross Pathology

Gross pathology was assessed in all animals.

Macroscopic findings included yellowish areas in papillary muscle in 1 HDF, and fluid in the thoracic cavity, intestine, and yellowish area, and gelatinous thymus in 1 LDM, 2 HDM, and 1 HDF.

Organ Weights

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

There were increases in absolute mandibular lymph node, thymus, liver, and lung weights and a decrease in mesenteric lymph node weight.

Table: Organ changes in mean absolute weight compared to control

	Adrenal	Brain	Epi	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate /Ovary	Spleen	Testes /Uterus	Thymus	Thyroid
								Ma	Me	P						
MALE																
LD:	+4%	+7%	-3%	-1%	+7%	+9%	+3%	+21%	-34%*	-9%	-17%	-16%	+13%	+7%	-20%	+11%
MD:	+29%*	+2%	-8%	+4%	+10%	+19%	+29%*	+55%	-35%*	-23%	-13%	-15%	-1%	+12%	-10%	+15%
HD:	+14%	+5%	-21%*	-11%	+2%	+9%	+3%	+93%*	-40%*	-22%	-15%	-22%	+6%	-12%	+25%	+2%
FEMALE																
LD:	+18%	+7%	-	+10%	+20%*	+29%	+28%	+16%	-36%	+60%	+16%	-3%	-16%	-16%	+117%	+35%*
MD:	0%	-1%	-	+5%	+8%	+22%	+17%	-2%	-42%	-7%	+9%	+17%	-44%	+42%	+45%	+17%
HD:	+11%	+3%	-	-2%	+9%	+20%	+56%	+22%	-55%*	+35%	-9%	-42%	+5%	-48%	+79%	+2%

* Statistically significant from control; recovery values in parenthesis; Epi – epididymides; Ma – mandibular; Me – mesenteric; P – popliteal; Pit - pituitary.

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

	Adrenal	Brain	Epi	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prostate /Ovary	Spleen	Testes /Uterus	Thymus	Thyroid
								Ma	Me	P						
MALE																
LD:	+17%	+20%	+11%	0%	+18%	+21%	+14%	0%	-28%*	0%	-20%	-12%	+23%	+14%	-17%	+20%
MD:	+42%*	+20%	+3%	+13%	+18%	+29%*	+43%*	0%	-28%*	-19%	0%	-12%	+12%	+21%	-17%	+22%
HD:	+35%	+20%	-6%*	0%	+15%	+29%*	+29%	>0%*	-28%*	-9%	0%	0%	+26%	0%	+33%	+19%
FEMALE																
LD:	+6%	0%		0%	+7%	+16%	+14%	0%	-45%*	+36%	+20%	0%	-23%	-25%	+75%	+20%
MD:	+9%	0%		+13%	+14%	+31%	+29%	0%	-36%	0%	+40%	+26%	-40%	+50%	+50%	+28%
HD:	+29%	+17%	(-1%)	+13%	+26%*	+41%	+100%	>0%*	-45%*	+45%	+20%	-33%	+18%	-37%	+100%	+22%

* Statistically significant from control; recovery values in parenthesis; Epi – epididymides; Ma – mandibular; Me – mesenteric; P – popliteal; Pit - pituitary.

Histopathology

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

Tissue/Organ	Weighing	Fixation	Histological Examination
Adrenals	x	x	x
Aorta		x	x
Bone marrow (sternum)		x	x
Brain	x	x	
- cerebrum		x	x
- cerebellum		x	x
- medulla oblongata, pons		x	x
Caecum		x	x
Colon		x	x
Duodenum		x	x

Tissue/Organ	Weighing	Fixation	Histological Examination
Epididymides	x	modified Davidson's	x
Eyes (with optic nerve)		Davidson's	x
Femurhead		x	x
Gall bladder		x	x
Heart (entire organ)*	x	x	x
Ileum		x	x
Jejunum		x	x
Kidneys	x	x	x
Lacrimal glands		x	
Larynx		x	x
Liver	x	x	x
Lungs (with bronchi)	x	Instillation	x
Lymph nodes			
- popliteal	x	x	x
- mandibular	x	x	x
- mesenteric	x	x	x
Mammary Gland (with skin)		x	x
Nasal cavity (1-4 Levels)		x	
Nasopharynx / nares		x	
Oesophagus		x	x
Ovaries	x	x	x
Oviducts		x	x
Pancreas		x	x
Peripheral nerves			
- sciatic (bilateral)		x	x
- plantar (bilateral)		x	x
Peyer's patches (with ileum and jejunum)		x	x
Pharynx		x	
Pituitary	x	x	x
Prostate	x	x	x

Tissue/Organ	Weighing	Fixation	Histological Examination
Rectum		x	x
Salivary glands			
- mandibular		x	x
- sublingual		x	x
- parotid		x	x
Skeletal muscle			
- sartorius (bilateral)		x	x
- medial gastrocnemius (bilateral)		x	x
Skin and subcutaneous tissue (location different from mammary gland)			
- hindleg		x	x
- back		x	x
- abdomen		x	x
- head		x	x
Spinal cord			
- cervical		x	x
- thoracic		x	x
- lumbar		x	x
- dorsal root ganglia (lumbar) with spinal roots		x	x
Spleen	x	x	x
Stomach (2 zones)		x	x
Testes	x	modified Davidson's	x
Thymus	x	x	x
Thyroid with Parathyroid Glands	x	x	x
Tongue		x	x
Trachea		x	x
Uterus	x		
- horn		x	x
- corpus		x	x
- cervix		x	x
Ureters		x	x

Tissue/Organ	Weighing	Fixation	Histological Examination
Urinary bladder		x	x
Vagina		x	x
Animal identification		x	
Gross lesions		x	x
Bone marrow smears (sternum)		Methanol/ as appropriate	
Lower jaw		x	x

X = tissues / organs which were weighed, collected or examined

Adequate Battery: Yes.

Peer Review: No.

Signed Pathology report: Yes, by [redacted] (b) (6)

Histological Findings

The main microscopic findings were noted in heart, lungs, skin, spleen, and thymus. Heart changes included arterial changes in the papillary muscle of the left ventricle at all doses in M and F. Alveolar histiocytosis was observed in the lungs at all doses in M and F. Findings in different areas of the skin included acanthosis, granuloma, folliculitis/perifolliculitis, hyperkeratosis, and parakeratosis. Pharmacodynamic effects in the lymphoid tissues included atrophy of PALS in the spleen and medullary hypertrophy in the thymus.

Table: Summary of selected microscopic findings

Tissue	Finding	Number of dogs	MALE				FEMALE			
			0	LD	MD	HD	0	LD	MD	HD
			4	4	4	4	4	4	4	4
Heart	Arterial change	Grade 1:	0	2	1	1	0	2	2	1
		Grade 2:	0	1	1	1	0	1	1	0
		Grade 3:	0	0	1	1	0	1	1	3
Lung	Alveolar histiocytosis	Grade 1:	0	2	2	3	0	4	4	3
		Grade 2:	0	0	0	0	0	0	0	0
		Grade 3:	0	0	0	0	0	0	0	0
		Grade 4:	0	0	0	0	0	0	0	1
Lymph node - Mesenteric	Hemorrhage	Grade 1:	0	2	3	2	0	1	1	2
		Grade 2:	0	0	0	1	1	1	0	1
		Grade 3:	0	0	0	0	0	1	1	0
Lymph node - Popliteal	Plasmacytosis	Grade 1:	2	3	3	3	1	2	4	2
		Grade 2:	0	0	1	0	0	0	0	2
Prostate	Inflammation	Grade 1:	1	0	0	2	-	-	-	-
		Grade 2:	1	0	1	1	-	-	-	-
		Grade 3:	0	2	3	1	-	-	-	-
Skin - abdomen	Acanthosis	Grade 1:	0	1	2	0	0	1	2	0
		Grade 2:	0	0	0	1	0	0	0	1
	Granuloma	Grade 1:	0	1	1	0	0	1	1	2
		Grade 2:	0	1	0	0	0	0	0	0
Skin - back	Acanthosis	Grade 1:	0	0	0	1	0	0	0	1
		Grade 2:	0	0	0	0	0	0	0	0
		Grade 3:	0	0	0	0	0	0	1	1
	Parakeratosis	Grade 1:	0	0	0	0	0	0	1	1
Skin - head	Parakeratosis	Grade 1:	0	0	0	0	0	0	0	1
Skin - hindleg	Acanthosis	Grade 1:	1	0	0	0	0	0	2	1
		Grade 2:	0	0	0	0	0	0	1	1
	Granuloma	Grade 1:	1	1	1	1	0	0	1	3
		Grade 2:	0	0	0	0	0	1	0	0

Tissue	Finding	Number of dogs	MALE				FEMALE				
			0	LD	MD	HD	0	LD	MD	HD	
			4	4	4	4	4	4	4	4	
	Hyperkeratosis										
		Grade 1:	2	4	0	2	0	3	1	2	
	Parakeratosis										
		Grade 1:	0	0	0	0	0	0	0	2	
Skin/ Subcutis	Acanthosis										
		Grade 1:	0	0	0	0	0	1	0	0	
		Grade 2:	0	0	0	0	0	0	2	0	
		Grade 3:	0	0	1	0	0	1	1	1	
	Folliculitis/Perifolliculitis										
		Grade 1:	0	2	3	1	1	1	2	3	
	Papillary hyperplasia										
		Grade 1:	0	0	0	0	0	0	0	0	
		Grade 2:	0	0	0	0	0	0	0	0	
		Grade 3:	0	0	0	0	0	0	1	0	
		Grade 4:	0	0	0	1	0	0	0	1	
		Ulceration									
			Grade 1:	0	0	0	0	0	0	1	0
		Grade 2:	0	0	0	0	0	0	0	0	
		Grade 3:	0	0	1	0	0	0	1	0	
		Grade 4:	0	0	0	1	0	0	0	0	
Spleen	PALS atrophy										
		Grade 1:	0	0	3	4	0	0	1	4	
	Congestion										
		Grade 1:	0	0	0	0	1	0	0	0	
		Grade 2:	2	0	0	1	1	0	0	0	
		Grade 3:	0	0	2	0	0	2	0	0	
		Grade 4:	0	2	0	3	2	0	2	4	
Thymus	Medulla hypertrophy										
		Grade 1:	0	3	1	2	0	4	2	2	
		Grade 2:	0	0	2	1	0	0	2	1	
		Grade 3:	0	0	1	1	0	0	0	0	

Toxicokinetics

Blood samples were taken on Days 1 and Weeks 5, 8, 26, 27, 31, and 52 pre-dose and at 1, 2, 4, 8, and 24 hours post-dose.

Increase in ponesimod plasma exposure (C_{max} and AUC_{0-24h}) was generally less than dose-proportional with similar plasma exposure between 140 and 280 mg/kg/day suggesting saturation. T_{max} ranged between 1-13 hours in M and F.

Table: Toxicokinetic parameters for ponesimod (Sponsor's)

Group	Dose (mg/kg)	Study Week	T _{max} *		C _{max}		AUC _{0-24h}	
			(h)		(ng/mL)		(h*ng/mL)	
			Males	Females	Males	Females	Males	Females
1	0	1	24	24	2.12	1.77	NA	NA
1	0	5	24	2	3.38	2.08	60.7	34.4
1	0	8	24	24	7.78	2.87	119	64.5
1	0	26	24	24	3.48	2.36	63.6	47.9
1	0	27	24	24	5.99	4.73	117	81.2
1	0	31	1	1	6.35	2.78	99.4	65.3
1	0	52	24	12	3.90	2.87	62.9	43.8
2	10	1	3	1.5	1220	2260	12100	22700
2	10	5	2	2	1290	1680	14400	18700
2	20	8	2	2	2200	3000	27100	36200
2	20	26	2	13	2790	3730	28500	56600
2	20	27	3	2	1850	2320	20600	27000
2	20	31	2	3	2440	3440	26800	52500
2	20	52	2	2	1120	3040	8970	34100
3	40	1	8	2	2910	2960	52100	35200
3	40	5	1	2	3990	3380	54500	35600
3	80	8	4	4	9690	5380	153000	77100
3	80	26	2	3	4520	3740	62800	46300
3	80	27	2	3	4040	3620	42700	46900
3	80	31	2	3	4990	3950	62500	51300
3	80	52	4	4	4220	4540	60800	53700
4	70	1	8	8	3560	6660	55600	116000
4	70	5	2	1	3890	5940	42600	80500
4	140	8	3	3	7350	7730	96800	126000
4	140	26	3	3	5780	8860	60700	130000
4	280	27	4	4	6940	9220	100000	133000
4	280	31	4	4	7750	14300	89300	233000
4	280	52	2	4	5640	9130	60800	132000

Flow cytometry

Blood samples were collected prior to initiation of dosing and in Weeks 1, 26, 29, and 51, and the percentages of T-lymphocytes CD3/CD4 (T-helper cells) and CD3/CD8 (cytotoxic T-cells) were determined.

Dosing with ponesimod decreased relative levels of T-helper cells (CD3⁺/CD4⁺) and increased levels of cytotoxic T-cells (CD3⁺/CD8⁺) at all time points at all doses.

Table: Lymphocyte populations

	CD4+ of CD3+				CD8+ of CD3+				CD4+/CD8+ ratio			
	Pre	Wk 26	Wk 29	Wk 51	Pre	Wk 26	Wk 29	Wk 51	Pre	Wk 26	Wk 29	Wk 51
MALE												
Control:	63%	63%	59%	58%	21%	26%	28%	29%	3.06	2.53	2.17	2.27
LD:	58%	16%*	14%*	16%*	25%	73%*	77%*	76%*	2.70	0.25	0.21	0.23
MD:	65%	10%*	11%*	8%*	20%	80%*	81%*	86%*	3.36	0.13	0.15	0.09
HD:	60%	10%*	7%*	5%*	25%	78%*	84%*	90%*	2.85	0.14	0.09	0.06
FEMALE												
Control:	61%	56%	57%	52%	24%	28%	28%	32%	2.60	2.07	2.06	1.68
LD:	58%	11%*	8%*	9%*	25%	83%*	87%*	86%*	2.42	0.14	0.10	0.11
MD:	66%	7%*	8%*	6%*	23%	87%*	86%*	91%*	2.99	0.08	0.09	0.06
HD:	61%	7%*	7%*	7%*	25%	86%*	85%*	89%*	2.70	0.08	0.08	0.09

* statistically significant from control.

7 Genetic Toxicology

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

Study title: ACT-128800: Reverse mutation in five histidine-requiring strains of Salmonella typhimurium

Study no.: T-05.058
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 GLP compliance: Yes, (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch № AA-016498-
 batch-(b) (4) 98.93%

Key Study Findings: Ponesimod was negative in the study.

Methods

Strains: TA98, TA100, TA102, TA1535, and TA1537
 Concentrations in definitive study
 Experiment 1: 1.6, 8, 40, 200, 1000, and 5000 µg/plate
 Experiment 2: 20.48, 51.2, 128, 320, 800, and 2000 µg/plate
 Basis of concentration selection: Doses were selected from a preliminary study at a dose range of 1.6-5,000 µg per plate
 Negative control: See table below.
 Positive control: See table below.
 Formulation/Vehicle: DMSO
 Incubation & sampling time: 37°C for 72 hours
 Comment on Study Validity: The assay was a valid study. Six concentrations of ponesimod were assayed in triplicate.

	TA98	TA100	TA102	TA1535	TA1537
+ve control					
Without S9 mix:	2-NF 5 µg	NaAzide 2 µg	MMC 0.2 µg	NaAzide 2 µg	9-AA 50 µg
With S9 mix*:	B[a]P 10 µg	2-AA 5 µg	2-AA 20 µg	2-AA 5 µg	2-AA 5 µg
-ve control	DMSO	DMSO	DMSO	DMSO	DMSO

2-NF is 2-nitrofluorene, NaAzide is sodium azide, 9-AA is 9-aminoacridine, MMC is mitomycin C, B[a]P is benzo[a]pyrene, 2-AA is 2-aminoanthracene. The liver S9 mix was prepared from M Sprague-Dawley rats treated with Aroclor 1254.

Results

Precipitation of ponesimod was observed at 1000 and 5000 µg/plate in the absence and presence of S9 mix in experiment 1 and 800 and 2000 µg/plate in the absence and presence of S9 mix in experiment 2. Toxicity was observed at 5000 µg/plate in strain TA1537 in the presence of S9 mix in experiment 1 and at 2000 µg/plate in strains TA98 and TA102 in the absence of S9 mix and strain TA1537 in the presence of S9 mix. No increases in revertants were observed up to 5000 µg/plate using the plate incorporation and preincubation methods.

Study title: **Salmonella typhimurium reverse mutation assay with ACT-338375B**

Study no.: T-09.519
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-338375B, Batch № ELN023-0823.2, 98.8%

Key Study Findings: ACT-338375B was negative in the study.

Methods

Strains: TA98, TA100, TA102, TA1535, and TA1537
 Concentrations in definitive study: 33, 100, 333, 1000, 2500, and 5000 µg/plate
 Basis of concentration selection: Doses were selected from a preliminary study at a dose range of 3-5,000 µg/plate
 Negative control: See table below.
 Positive control: See table below.
 Formulation/Vehicle: DMSO
 Incubation & sampling time: 37°C for 72 hours
 Comment on Study Validity: The assay was a valid study. Six concentrations of ACT-338375B were assayed in triplicate and each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

	TA98	TA100	TA102	TA1535	TA1537
+ve control					
Without S9 mix:	4-NOPD 10 µg	NaAzide 10 µg	MMS 3.9 µg	NaAzide 10 µg	4-NOPD 50 µg
With S9 mix [*] :	2-AA 2.5 µg	2-AA 2.5 µg	2-AA 10 µg	2-AA 2.5 µg	2-AA 2.5 µg
-ve control	DMSO	DMSO	DMSO	DMSO	DMSO

4-NOPD is 4-nitro-o-phenylene-diamine, NaAzide is sodium azide, MMS is methyl methane sulfonate, 2-AA is 2-aminoanthracene. The liver S9 mix was prepared from M Wistar Han rats treated with phenobarbital/β-Naphthoflavone.

Results

Precipitation of ACT-338375B was observed at 2500 and 5000 µg/plate in the absence and presence of S9 mix. Reduced background growth was observed at 2500 and 5000 µg/plate in all strains in the absence and presence of S9 mix and at 1000 µg/plate in strain TA100 in the absence of S9 mix. No increases in revertants were observed up to 5000 µg/plate using the plate incorporation method.

Study title: *Salmonella typhimurium* reverse mutation assay with ACT-204426

Study no.: T-09.520
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 GLP compliance: Yes, (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-204426, Batch № ELN064-1725.1, 95.8%

Key Study Findings: ACT-204426 was negative in the study.

Methods

Strains: TA98, TA100, TA102, TA1535, and TA1537
 Concentrations in definitive study: 33, 100, 333, 1000, 2500, and 5000 µg/plate
 Basis of concentration selection: Doses were selected from a preliminary study at a dose range of 3-5,000 µg/plate
 Negative control: See table below.
 Positive control: See table below.
 Formulation/Vehicle: DMSO
 Incubation & sampling time: 37°C for 72 hours
 Comment on Study Validity: The assay was a valid study. Six concentrations of ACT-204426 were assayed in triplicate and each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

	TA98	TA100	TA102	TA1535	TA1537
+ve control					
Without S9 mix:	4-NOPD 10 µg	NaAzide 10 µg	MMS 3.9 µg	NaAzide 10 µg	4-NOPD 50 µg
With S9 mix [*] :	2-AA 2.5 µg	2-AA 2.5 µg	2-AA 10 µg	2-AA 2.5 µg	2-AA 2.5 µg
-ve control	DMSO	DMSO	DMSO	DMSO	DMSO

4-NOPD is 4-nitro-o-phenylene-diamine, NaAzide is sodium azide, MMS is methyl methane sulfonate, 2-AA is 2-aminoanthracene. The liver S9 mix was prepared from M Wistar Hanlbm rats treated with phenobarbital/β-Naphthoflavone.

Results

Precipitation of ACT-204426 was observed at 1000, 2500, and 5000 µg/plate in the presence of S9 mix. Reduced background growth was observed at 5000 µg/plate in TA1537 in the absence of S9 mix. No increases in revertants were observed up to 5000 µg/plate in 2 experiments using both the plate incorporation and preincubation methods.

7.2 In Vitro Assays in Mammalian Cells

Study title: ACT-128800: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes

Study no.: T-05.064
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 GLP compliance: Yes, (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch № AA-016498-batch-(b) (4), 98.93%

Key Study Findings: Ponesimod, up to the limit of cytotoxicity, did not induce chromosome aberrations in human lymphocytes in either the absence or presence of S9.

Methods

Cell line: Human blood lymphocytes
 Concentrations in definitive studies

Experiment 1

3 hours absence & presence S9: 20, 40, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150 µg/mL

Experiment 2

20 hours in absence of S9: 5, 7.5, 10, 12, 14, 16, 18, 20, 22.5, 25, 27.5, 30, 35, 40, and 50 µg/mL

3 hours in presence of S9: 38.1, 57.14, 76.19, 85.71, 95.23, 104.8, 114.3, 123.8, 133.3, 142.9, 152.4, and 171.4 µg/mL

Basis of concentration selection: Doses were selected from a preliminary study at a dose range of 2.251-4610 µg/plate (10 mM)

Negative control: DMSO

Positive control: 5 µg/mL 4-Nitroquinoline 1-oxide
6.25, and 11.9 µg/mL Cyclophosphamide

Formulation/Vehicle: DMSO

Incubation & sampling time: 3 and 20 hours in the absence of S9 and 3 hours in the presence of S9*; 37°C
* Aroclor 1254-induced Sprague-Dawley M rat liver S9

Comment on Study Validity: The assay was a valid study. Three or four doses were analyzed in at least duplicate in each treatment; 3- and 20-hours in the absence of S9 and 3-hours in the presence of S9 in 100 cells/duplicate.

Results

In the cytotoxicity dose finding study, concentrations greater than 144.1 µg/mL in the absence of S9 with 3- and 20-hour incubations and 288.1 µg/mL in the presence of S9 with 3-hour incubation were cytotoxic. In experiment 1, the mitotic index was 57 % in 100 µg/mL and 54% in 140 µg/mL with 3-hour incubation in the absence and presence of S9, respectively; the selected concentrations for analysis were 40, 70, 90, and 100 µg/mL and 80, 110, and 140 µg/mL in the absence and presence of S9, respectively. In experiment 2, the mitotic index was 55 % in 104.8 µg/mL with 3-hour incubation in the presence of S9 and 52% in 25 µg/mL with 20-hour incubation in the absence of S9; the selected concentrations for analysis were 76.19, 85.71, 95.23, and 104.8 µg/mL and 7.5, 20, and 25 µg/mL in the absence (20-hours) and presence (3-hours) of S9, respectively. 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.

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: ACT-128800: Induction of micronuclei in the bone marrow treated rats

Study no: T-07.143

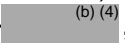
Study report location: EDR

Conducting laboratory and location:  (b) (4)

Date of study initiation: May 9, 2007

GLP compliance: Yes,  (b) (4)

QA statement: Yes

Drug, lot #, and % purity: ACT-128800, Batch № AA-016498-batch- (b) (4), 99.4%

Key Study Findings: Ponesimod did not induce micronuclei in M rats in vivo up to 400 mg/kg.

Methods

Doses in first experiment:	0, 50, 150, and 400 mg/kg
Frequency of dosing:	Daily dosing for 2 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.25% (w/v) Aqueous methylcellulose with 0.05% (v/v) Tween 80
Species/Strain:	Rat/Wistar Han
Number/Sex/Group	
Main:	6
Toxicokinetic:	3
Age:	7-8 weeks
Weight:	123 – 169 g
Basis of dose selection:	Doses were based on a dose finding experiment (400 and 600 mg/kg/day for 3 days in 2/sex/group).
Negative control:	Vehicle
Positive control:	Cyclophosphamide (20 mg/kg)
Sampling time	
Bone marrow:	24 Hours after the final administration
Blood:	1 and 2 Hours post-final dose
Comment on Study Validity:	The assay was a valid study. In the dose finding study, the following clinical signs were observed sedation, tachypnea, hunched posture, and emaciation at both dose levels. 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

There were no clinical signs of toxicity in HD animals. No increase in micronuclei was observed at doses of 50, 150, and 400 mg/kg ponesimod. In contrast, cyclophosphamide induced a significant increase in micronuclei compared to vehicle control. Therefore, ponesimod showed no clastogenic potential up to 400 mg/kg/day.

8 Carcinogenicity

Study title: ACT-128800: 104 Week Oral (Gavage) Administration Carcinogenicity Study in the Mouse

Study no.:	T13.023
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	May 28, 2013
GLP compliance:	Yes,  (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	ACT128800, Batch №: 605930003, 100.5%
CAC concurrence:	Yes, dated April 5, 2013

Protocol Recommendations

The protocol was reviewed by the Exec CAC under IND 101,722 (Meeting minutes, April 5, 2013). The Exec CAC recommended doses of 0, 0 (vehicle), 50, 150, and 400 mg/kg/day in M based on decreased body weight gain and 0, 0 (vehicle), 50, 150, and 300 mg/kg/day in F based on death noted in a 13-week study.

Key Study Findings

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

Adequacy of Carcinogenicity Study

Early termination (in Week 102) of all F groups 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 this study, plasma exposures for M12 and M13, major circulating metabolites in humans, were greater than those in humans (approximately 7- and 3-fold for M12 and M13 at the HD, respectively using data from clinical trial AC-058-109).

Evaluation of Tumor Findings

A dose-dependent increase in the incidence of hemangiomas and hemangiosarcomas was seen in M and F that reached statistical significance in all doses in M and in HDF.

Methods

Doses: Males: 0, 0, 50, 150, and 400 mg/kg
 Females: 0, 0, 30, 100, and 300 mg/kg
 Frequency of dosing: Daily
 Dose volume: 10 mL/kg
 Route of administration: Oral gavage
 Formulation/Vehicle: 0.25% (w/w) Methylcellulose and 0.05% Tween 80 (v/v)
 Basis of dose selection: Based on findings from two 13-week oral (gavage) toxicity studies in CD-1 mice
 Species/Strain: Mouse / Crl:CD1 (ICR)
 Number/Sex/Group: 60
 Age: 7-8 weeks at initiation of dosing
 Weight: Males: 28.6-44.1 g; Females: 21.1-32.3 g
 Animal housing: F were housed in groups of 3 and M were housed singly
 Satellite groups TK analysis: 24/sex/group
 Deviation from study protocol: The duration of dosing was 104 weeks for M. F groups were sacrificed in Week 102 because of decreased survival rates.

Observations and Results**Mortality**

Observations for clinical signs and overt toxicity were conducted twice daily.

There was a decrease in the survival rate in MDM and HDM, that reached statistical significance (as reported by the FDA statistical reviewer). In F, a decrease in survival rate in controls resulted in early termination of the F portion of the study early in Week 102 (see advice to sponsor. March 25, 2015). The main causes of death were lung carcinoma and lymphoma across all groups.

Table: Survival Incidences (Sponsor's)**MALES**

Group	Dose level (mg/kg/day)	Spontaneous Death		Killed in Extremis		Planned Necropsy	
		Abs.	%	Abs.	%	Abs.	%
1	0	7	11.7	27	45.0	26	43.3
2	0	5	8.3	23	38.3	32	53.3
4	50	12	20.0	18	30.0	30	50.0
6	150	9	15.0	34	56.7	17	28.3
8	400	13	21.7	30	50.0	17	28.3

FEMALES

Group	Dose level (mg/kg/day)	Spontaneous Death		Killed in Extremis		Planned Necropsy	
		Abs.	%	Abs.	%	Abs.	%
1	0	7	11.7	42	70.0	11	18.3
2	0	4	6.7	39	65.0	17	28.3
3	30	5	8.3	28	46.7	27	45.0
5	100	7	11.7	32	53.3	21	35.0
7	300	9	15.0	36	60.0	15	25.0

Number at start = 60 animals/group/sex

Females: Necropsy phase started on Day 713 (Week 102)

Males: Necropsy phase started on Day 730 (Week 105)

Table: Causes of Mortality (Sponsor's)**MALE**

Category	Group/Subgroup/Sex:	1/1/M	2/1/M	4/1/M	6/1/M	8/1/M
Observation	Number in Group:	60	60	60	60	60
Reason for Removal of Animal from Study						
Died after bleed		1	1	0	0	0
Died after dosing		1	1	1	0	1
Found Dead		5	3	10	9	12
Killed in Extremis		4	0	0	4	0
Musculoskeletal signs (impaired mobility)		0	0	0	1	0
Neurological Signs/Convulsions		2	0	0	0	0
Other reason (specify)		0	0	2	2	1
Poor condition		1	4	1	2	6
Respiratory signs		0	1	1	1	1
Severity of Clinical Observations		18	17	13	23	19
Size/position/condition of mass(es)		0	1	1	2	4
Skin lesions		1	0	2	0	0
Trauma (Specify)		0	0	1	0	0
Weight Loss		1	1	0	1	0

FEMALE

Category	Group/Subgroup/Sex:	1/1/F	2/1/F	3/1/F	5/1/F	7/1/F			
Observation	Number in Group:	60	60	60	60	60			
Reason for Removal of Animal from Study									
Died after bleed		0	0	0	0	0			
Died after dosing		0	0	0	0	0			
Found Dead		7	4	6	7	10			
Killed in Extremis		1	1	1	0	2			
Musculoskeletal signs (impaired mobility)		1	0	0	1	1			
Neurological Signs/Convulsions		0	0	1	1	1			
Other reason (specify)		1	0	1	1	2			
Poor condition		3	3	1	4	0			
Respiratory signs		1	1	2	1	0			
Severity of Clinical Observations		29	30	17	18	24			
Size/position/condition of mass(es)		5	3	3	3	5			
Skin lesions		1	1	1	1	2			
Trauma (Specify)		0	0	0	0	0			
Weight Loss		1	0	1	1	0			
Group									
Dose level (mg/kg/day)		1	2	3	4	5	6	7	8
		0	0	30	50	100	150	300	400

Clinical Signs

Detailed exams were conducted weekly including examination for the presence of palpable masses.

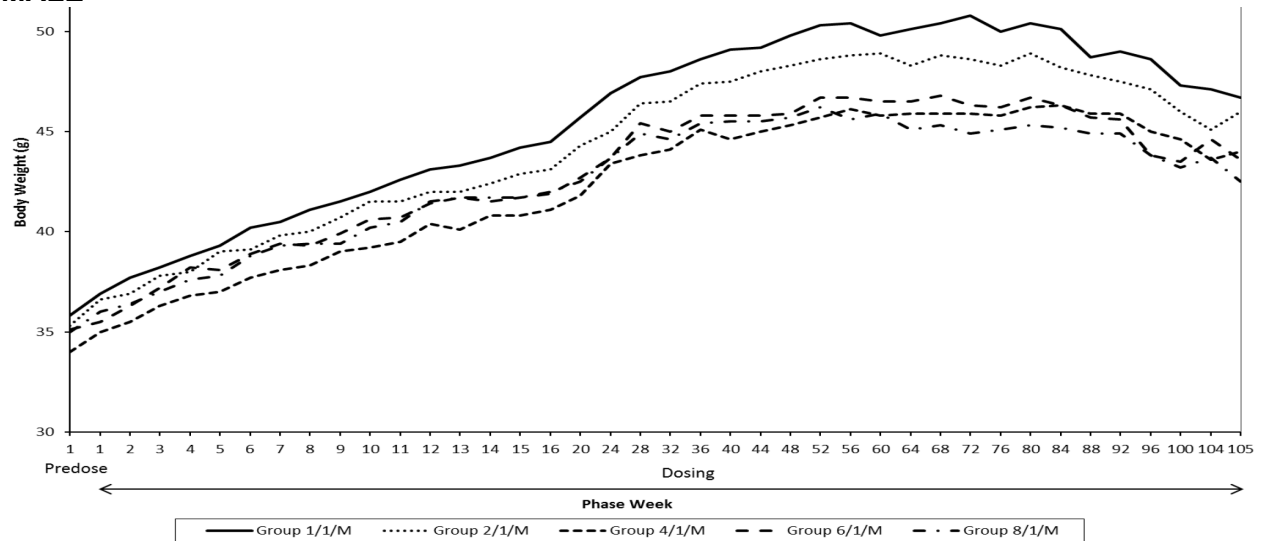
There were no drug-related adverse clinical signs noted. The number of palpable masses were greater in control animals.

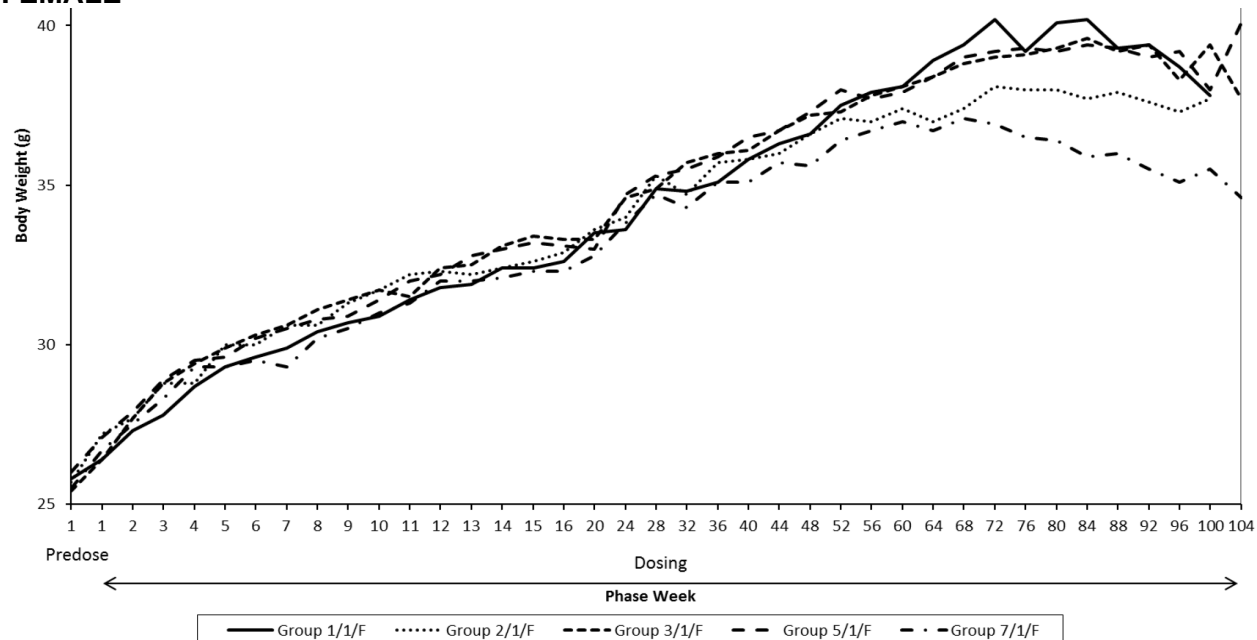
Body Weights

Body weights were recorded once prior to initiation of dosing (Day -7), weekly through Week 16, and every four weeks thereafter.

In M, there was a decreased in body weight in Week 105 by 5%, 6%, and 8% at the LD, MD, and HD, respectively. In F, body weight was decreased in Week 100 by 6% at the HD. Differences were relative to control groups combined.

Figure: Body Weight Changes (Sponsor's)
MALE



FEMALE**Food Consumption**

Food consumption was recorded weekly from Week -1 through Week 16, then every four weeks thereafter.

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

Ophthalmoscopy

Examination of 25/sex/group was conducted once prior to initiation of dosing and on 25/sex in control and HD groups during Weeks 26, 52, 78, and 104.

There were no drug-related changes.

Hematology

Blood samples were taken from all surviving M prior to terminal sacrifice (Week 104).

The pharmacodynamic effect of ponesimod, i.e., slight decreases in WBC and lymphocytes, were observed in HDM.

Clinical Chemistry

Blood samples were taken from all surviving M prior to terminal sacrifice (Week 104).

No adverse changes were observed.

Gross Pathology

Macroscopic examination occurred in all animals.

No adverse macroscopic findings were noted.

Organ Weights

At necropsy, the organs identified in the table in the histopathology section were weighed.

There were increases in mean liver, lung, and spleen weights and decreases in mean kidney and uterus weights in M and F, mainly at the HD.

Table: Organ Changes in Mean Absolute Weight Compared to Control Organ Weights

	Adrenal	Brain	Heart	Kidney	Liver	Lungs	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus
MALE										
LD:	-11%	+1%	+1%	-4%	-1%	+11%	+11%	+21%	+3%	+12%
MD:	-4%	+2%	+1%	-5%	+12%	+7%	-5%	+15%	-5%	-14%
HD:	0%	+1%	-6%	-12%	+14%	+29%	-5%	+41%	-3%	-8%
FEMALE										
LD:	-14%	+1%	0%	-1%	+7%	+5%	+507%	+74%	-44%	+60%
MD:	-22%*	-2%	-5%	-15%*	-5%	+4%	+233%	-29%	-25%	-9%
HD:	-22%*	-3%	-1%	-17%*	+10%	+76%*	+293%	+12%	-45%	-19%

* Statistically significant from control.

Table: Organ Changes in Mean Weight (Relative to Body Weight) Compared to Control Organ Weights

	Adrenal	Brain	Heart	Kidney	Liver	Lungs	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus
MALE										
LD:	-6%	+5%	+5%	0%	+3%	+15%	+15%	+25%	+7%	+18%
MD:	+6%	+10%	+8%	+2%	+23%*	+15%	+2%	+24%	+1%	-6%
HD:	+12%	+11%	+16%	-3%	+23%*	+42%*	+4%	+57%	+6%	+1%
FEMALE										
LD:	-16%	-2%	-4%	-4%	+3%	0%	+460%	+58%	-45%	+46%
MD:	-19%	+2%	-3%	-13%*	-3%	+7%	+251%	-26%	-22%	-17%
HD:	-15%	+4%	+6%	-10%	+17%*	+95%*	+262%	-21%	-41%	-14%

* Statistically significant from control.

Histopathology

At necropsy, the following organs, as listed in the study report, were collected from all animals. Microscopic examination included all tissues.

Organ/Tissue			Organ/Tissue		
adrenal	W	E	nerve, optic		E
animal identification			nerve, sciatic		E
aorta		E	oesophagus		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		E
Eye ^c		E	prostate	W	E
femur with bone marrow and femero-tibial		E	rectum		E
gall bladder		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
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	testis and epididymis ^d	W	E
larynx		E	thymus	W	E
liver	W	E	thyroid with parathyroid		E
lungs with main stem bronchi and bronchioles	W	E	tissue masses		E
lymph node, mandibular		E	tongue		E
lymph node, mesenteric		E	trachea		E
mammary gland		E	ureter		E
mandibular salivary gland		E	urinary bladder		E
muscle, quadriceps		E	uterus with cervix	W	E
nasal cavity		E	vagina		E
nasopharynx			Zymbal gland		E
nose/nares					

a = methanol fixative, c = Davidson's fluid fixative, d = Bouin's fixative;

b = see clinical pathology [section 3.5.2](#)

Bone designated for histopathological examination was decalcified using Kristenson's fluid.

Peer Review: Yes

Signed Pathology Report: Yes

Neoplastic

There was a statistically significant dose-dependent increase in vascular tumors (hemangioma and/or hemangiosarcoma) in M at all doses and in HDF; tissues most affected were liver and uterus. There was an increase in lung bronchioloalveolar adenocarcinomas in HDF, but the increase was not statistically significant.

Table: Observed Neoplasms After Ponesimod Treatment for up to 104 Weeks in Mice

Neoplasms	MALE					FEMALE				
	0	0	50	150	400	0	0	30	100	300
Malignant Lymphoma	0 (6)	3 (11)	3 (12)	3 (7)	4 (7)	7 (32)	7 (27)	13 (19)	10 (23)	10 (22)
Hemangioma	3 (1)	2 (0)	6 (0)	2 (4)	2 (1)	0 (0)	0 (1)	2 (3)	2 (5)*	1 (1)
Hemangiosarcoma	2 (0)#	1 (0)	2 (6)*	1 (7)*	4 (9)*	0 (1)#	1 (2)	1 (0)	0 (0)	1 (12)*
Hemangioma/sarcoma	5 (1)#	3 (0)	8 (6)*	3 (11)*	5 (10)*	0 (1)#	1 (2)	3 (3)	2 (5)	2 (12)*
Hemangioma; tissues	4	2	6	6	3	0	1	5	7	2
Body cavity	-	-	-	0 (1)	-	-	-	-	-	-
Bone marrow, femur	-	-	1 (0)	-	-	-	-	-	-	-
Bone marrow, sternum	-	-	-	-	1 (0)	-	-	-	-	-
Liver	-	1 (0)	2 (0)	1 (3)	1 (1)	-	-	-	2 (0)	1 (0)
Lymph node, mesenteric	1 (0)	-	1 (0)	1 (0)	-	-	-	-	1 (0)	-
Ovary	-	-	-	-	-	-	-	1 (1)	0 (2)	-
Skin	-	1 (0)	-	-	-	-	-	-	-	-
Spleen	0 (1)	-	-	-	-	-	0 (1)	-	-	0 (1)
Testes	2 (0)	-	-	-	-	-	-	-	-	-
Thymus	-	-	1 (0)	-	-	-	-	-	-	-
Tongue	-	-	1 (0)	-	-	-	-	-	-	-
Uterus	-	-	-	-	-	-	-	1 (2)	0 (3)*	-
Hemangiosarcoma; tissues	2	1	8	8	13	1	3	1	0	13
Body cavity	-	-	0 (1)	-	-	-	-	-	-	-
Cavity, nasal	-	-	-	0 (1)	-	-	0 (1)	-	-	-
Cavity, oral	-	-	-	-	0 (1)	-	-	-	-	-
Epididymis	-	-	-	-	1 (1)	-	-	-	-	-
Heart	-	-	-	-	0 (1)	-	-	-	-	-
Ileum	-	-	-	0 (1)	-	-	-	-	-	-
Liver	-#	-	2 (2)*	1 (4)*	1 (3)*	-#	-	-	-	0 (3)*
Lymph node, mesenteric	1 (0)	-	-	-	-	-	1 (0)	-	-	-
Muscle, skeletal	-	-	-	-	0 (1)	-	-	-	-	-
Ovary	-	-	-	-	-	0 (1)	-	-	-	-
Pancreas	-	-	-	-	0 (1)	-	-	-	-	-
Skin	-	-	-	-	-	-	-	-	-	0 (1)
Spleen	1 (0)	1 (0)	0 (3)	-	1 (1)	-	-	-	-	1 (1)
Stomach	-	-	-	0 (1)	-	-	-	-	-	-
Testes	-	-	-	-	0 (1)	-	-	-	-	-
Uterus	-	-	-	-	-	-#	1 (0)	1 (0)	-	0 (7)*

Data from animals that died/sacrificed prematurely are in parenthesis. The tumor data were analyzed by the FDA statistician for dose response (control groups were summed) 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-3 method (see statistical review; NDA 213-498, November 18, 2020). * Total incidences are statistically significant; # indicate statistically significant dose response.

Non-Neoplastic

The main findings were observed in liver and lung. An increase in incidence of hepatocellular hypertrophy was observed at all doses in M; increases in incidences and

severity of alveolar macrophages, hematoidin crystals, interstitial fibrosis, and osseous metaplasia were observed in the lung.

Table: Summary of Selected Microscopic Findings

Tissue	Finding	(mg/kg): Number:	MALE					FEMALE				
			0	0	50	150	400	0	0	30	100	300
			60	60	60	60	60	60	60	60	60	60
Adrenal	Subcapsular A hyperplasia											
	minimal:	11	9	7	14	4	21	16	26	24	19	
	slight:	8	10	12	8	6	22	16	25	17	20	
	moderate:	0	2	0	0	0	1	4	2	4	4	
	marked:	0	0	0	0	0	1	0	0	0	0	
	massive:	1	0	0	0	0	0	0	0	0	0	
	Subcapsular B hyperplasia											
	minimal:	1	1	0	1	0	1	0	0	0	1	
	slight:	3	5	4	2	0	1	1	0	0	0	
moderate:	2	2	1	1	0	0	0	0	0	0		
marked:	0	0	1	0	0	0	0	0	0	0		
Eyes	Osseous metaplasia											
	present:	0	1	4	6	4	1	0	3	1	3	
Joints	Arthropathy											
	minimal:	13	13	8	11	19	7	12	13	14	15	
	slight:	5	4	12	12	13	4	9	7	7	13	
	moderate:	0	6	6	3	6	0	1	1	6	3	
	marked:	0	0	2	0	0	0	2	0	0	1	
	massive:	0	0	0	0	0	0	1	0	0	0	
Kidney	Chronic progr nephrop											
	minimal:	31	33	24	36	22	11	15	23	23	27	
	mild:	7	5	3	2	5	4	0	3	3	3	
	moderate:	0	1	1	0	0	4	1	0	0	0	
	marked:	0	0	0	2	0	1	3	3	1	0	
	massive:	0	0	0	0	0	1	0	0	0	0	
Liver	Hepatocellular hypertrophy											
	minimal:	2	11	10	8	9	5	3	2	1	4	
	slight:	7	4	13	9	16	3	6	1	1	2	
	moderate:	3	4	4	6	3	0	1	0	2	0	
Lung	Alveolar macrophages											
	minimal:	3	7	15	13	3	1	4	7	14	9	
	slight:	3	8	8	13	15	2	2	6	9	19	
	moderate:	3	1	3	6	8	2	0	6	4	10	
	marked:	0	4	0	2	4	0	0	0	0	1	
	Alveolar hematoidin crystals											
	minimal:	0	0	2	6	8	0	0	1	4	4	
	slight:	0	0	1	3	2	0	0	0	0	1	
	moderate:	0	0	0	0	1	0	0	0	0	0	
	Interstitial fibrosis											
	minimal:	0	0	0	0	1	0	1	0	1	1	
	slight:	0	0	0	0	0	0	0	1	1	3	
	Interstitial inflammation											
	minimal:	2	2	1	4	3	1	0	2	1	2	
	mild:	1	0	3	0	4	2	1	0	0	2	
	moderate:	1	0	2	0	0	0	0	0	0	0	
	Osseous metaplasia											
minimal:	0	0	2	1	1	0	0	0	3	0		
slight:	0	0	0	1	2	0	0	0	0	1		

Tissue	Finding	(mg/kg): Number:	MALE					FEMALE				
			0	0	50	150	400	0	0	30	100	300
Lymph node - Mandibular	Sinus Erythrocytosis		60	60	60	60	60	60	60	60	60	
	present:		0	0	1	0	1	0	1	4	4	6
	Increased plasma cells											
	minimal:		0	0	0	0	0	0	0	0	0	0
	slight:		0	2	1	3	5	4	1	1	0	3
	moderate:		1	1	0	0	1	0	0	1	0	1
Lymph node - Mesenteric	Tingible body macrophages											
	minimal:		0	0	1	0	0	0	0	0	0	0
	slight:		0	0	0	1	2	0	0	0	0	0
	moderate:		0	0	0	0	1	0	0	0	0	0
	Decreased cellularity											
	minimal:		0	0	0	0	0	0	0	0	0	0
	slight:		1	0	4	2	5	1	0	0	2	2
	moderate:		2	0	3	5	0	0	0	2	0	1
	marked:		0	0	1	0	0	1	0	0	0	0
Sciatic nerve	Demyelination											
	minimal:		21	27	26	20	26	14	19	15	23	25
	slight:		3	9	4	3	4	7	6	16	11	8
	moderate:		3	2	3	2	2	1	3	5	2	2
	marked:		0	0	0	0	0	0	0	1	0	0
Spleen	Erythropoiesis											
	minimal:		18	18	10	10	9	8	23	10	13	1
	slight:		17	14	7	8	11	11	11	13	13	18
	moderate:		3	9	14	12	19	12	1	14	11	12
	marked:		4	2	5	6	6	6	3	6	6	8
	massive:		0	0	1	1	2	2	1	0	3	1
	Lymphoid depletion											
	minimal:		0	0	0	0	0	0	0	0	0	0
	slight:		1	1	2	5	7	0	2	0	4	1
	moderate:		1	0	6	7	2	1	1	1	1	5
	marked:		1	0	0	0	0	2	0	0	0	0
Thymus	Lymphoid hyperplasia											
	minimal:		0	0	0	0	0	0	0	0	0	0
	slight:		0	0	4	1	1	0	2	4	4	5
	moderate:		2	0	0	1	0	3	1	4	1	4
	marked:		0	0	1	0	0	0	1	2	1	1
	massive:		0	0	0	0	0	1	1	0	1	1

Toxicokinetics

Blood samples were taken from TK animals in Weeks 26 and 52 at 0.5, 1, 2, 4, 6, and 24 hours post-dose. Levels of ponesimod and metabolites ACT-204426 (M12) and ACT-338375 (M13) were quantitated.

Increase in plasma exposure (C_{max} and AUC) to ponesimod was generally less than dose-proportional. T_{max} was at 1-4 hours. No accumulation was observed between Weeks 26 and 52.

Table: Ponesimod Plasma Levels (Sponsor's)

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
30	26	f	1	1830	9400	61.0	313
30	52	f	1	1960	8490	65.3	283
50	26	m	1	3030	14300	60.6	286
50	52	m	1	3440	17300	68.8	346
100	26	f	1	4280	19300	42.8	193
100	52	f	1	4140	25800	41.4	258
150	26	m	2	5530	34500	36.9	230
150	52	m	2	5750	41400	38.3	276
300	26	f	2	8230	49500	27.4	165
300	52	f	1	6770	47600	22.6	159
400	26	m	2	9200	63200	23.0	158
400	52	m	4	7780	54700	19.5	137

Increase in plasma exposure (C_{max} and AUC) to metabolite ACT-204426 was generally less than dose-proportional, with plasma exposure greater in F. T_{max} was at 1-2 hours in M and F. No accumulation was observed between Weeks 26 and 52.

Table: ACT-204426 (M12) Plasma Levels (Sponsor's)

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
30	26	f	1	212	744*	7.07	24.8
30	52	f	1	249	817#	8.30	27.2
50	26	m	1	191	679#	3.82	13.6
50	52	m	1	215	708#	4.30	14.2
100	26	f	1	396	1630	3.96	16.3
100	52	f	1	388	2220	3.88	22.2
150	26	m	1	295	1450	1.97	9.64
150	52	m	2	344	1960	2.29	13.1
300	26	f	2	894	4610	2.98	15.4
300	52	f	1	687	3390	2.29	11.3
400	26	m	2	551	4120	1.38	10.3
400	52	m	2	347	2350	0.868	5.86

*AUC0-6h, more than 50% of samples were BLQ

#AUC0-6h, all samples were BLQ at 24h

Increase in plasma exposure (C_{max} and AUC) to metabolite ACT-338375 was generally less than dose-proportional, with plasma exposure greater in M. T_{max} was at 2-4 hours. No accumulation was observed between Weeks 26 and 52.

Table: ACT-338375 (M13) Plasma Levels (Sponsor's)

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
30	26	f	2	103	454 [#]	3.43	15.1
30	52	f	2	97.6	387 [#]	3.25	12.9
50	26	m	2	143	566 [#]	2.86	11.3
50	52	m	1	115	511 [#]	2.30	10.2
100	26	f	4	274	1530	2.74	15.3
100	52	f	2	256	2210	2.56	22.1
150	26	m	2	382	2070	2.55	13.8
150	52	m	2	394	2460	2.63	16.4
300	26	f	2	579	3660	1.93	12.2
300	52	f	2	509	3620	1.70	12.1
400	26	m	2	739	5450	1.85	13.6
400	52	m	2	558	4090	1.40	10.2

[#]AUC0-6h, all samples were BLQ at 24h

Dosing Solution Analysis

All ponosimod samples analyzed during Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 104 were within $\pm 10\%$ (range: 94-107%) of nominal concentrations.

Study title: ACT-128800: 104-Week Oral (Gavage) Administration Carcinogenicity Study in the Rat

Study no.: 8278919
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)

Date of study initiation: May 13, 2013
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT128800, Batch No: 605930003, 100.5%
 CAC concurrence: Yes, dated April 5, 2013

Protocol Recommendations

The protocol was reviewed by the Exec CAC under IND 101,722 (Meeting minutes April 5, 2013). The Exec CAC recommended doses of 0, 0 (vehicle), 3, 10, and 30 mg/kg/day in M based on decreased body weight noted in a 26-week study and 0, 0 (vehicle), 10, 30, and 100 mg/kg/day in F based on death noted in a 2-week study.

Key Study Findings

- There was a decrease in survival rate in F that reached statistical significance at the MD and HD.
- Decreases in body weight gain were observed at all doses in M and F.

Adequacy of Carcinogenicity Study

There was no difference in survival rate in M. A statistically significance decrease in survival rate was observed in MDF and HDF. Survivors in all M groups were dosed for the full duration of the 104-week study. Dosing was stopped at Week 96 in HDF.

Appropriateness of Test Models

The Wistar Han rat is an appropriate model to test for carcinogenicity over a 2-year period. In this study, plasma M12 and M13 (major circulating metabolites in humans) exposures were similar to expected human plasma exposures (using data from clinical trial AC-058-109).

Evaluation of Tumor Findings

No statistically significant increase in the incidence tumors was observed in M or F.

Methods

Doses:	Males: 0, 0, 3, 10, and 30 mg/kg Females: 0, 0, 10, 30, and 100 mg/kg
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.25% (w/w) methylcellulose and Tween 80 (0.05% v/v)
Basis of dose selection:	Findings from a 26-week repeat dose toxicity study in Wistar rats
Species/Strain:	Rat / Wistar Han
Number/Sex/Group	
Main groups:	51
Toxicokinetic groups:	9
Age:	Approximately 7 weeks at initiation of dosing
Weight:	Males: 149-220 g; Females: 115-177 g
Animal housing:	Group housed (up to 3 of same sex)
Deviation from study protocol:	The duration of dosing was 104 weeks for M but was stopped during Week 96 in HDF.

Observations and Results

Mortality

Observations for ill health and overt toxicity were conducted twice daily.

There was no difference in survival rate in M, but a decrease in survival rate was observed in F that reached statistical significance in MDF and HDF (as reported by the

FDA statistical reviewer), with dosing stopped in Week 104 in MDF and Week 96 in HDF when survivors fell to less than 20 (see advice to sponsor, January 4, 2015).

Table: Mortality Incidences (Sponsor's)

MALES

Group Number	1	2	3	5	7
Dose levels mg/kg/day	0	0	3	10	30
Incidence absolute	16/51	17/51	9/51	12/51	17/51
Incidence relative	31%	33%	18%	24%	33%

FEMALES

Group Number	1	2	4	6	8
Dose levels mg/kg/day	0	0	10	30 ^a	100 ^b
Incidence absolute	25/51	19/51	20/51	33/51	34/51
Incidence relative	49%	37%	39%	65%	67%

Number at start = 51 animals/group/sex

Necropsy phase started on Day 730 (Week 105)

^a Last dose administered Day 723 (Wk 104), ^b Last dose administered Day 672 (Wk 96).

Table: Causes of Mortality (Sponsor's)

MALE

Category	Group/Subgroup/Sex:	1/1/M	2/1/M	3/1/M	5/1/M	7/1/M
Observation	Number in Group:	51	51	51	51	51
Reason for Removal of Animal from Study						
Found Dead		3	3	1	2	0
Killed in Extremis		0	0	0	0	1
Musculoskeletal signs (impaired mobility)		0	0	0	0	1
Other reason (specify)		1	0	0	0	0
Poor condition		1	1	1	0	1
Respiratory signs		0	0	1	1	1
Severity of Clinical Observations		8	9	6	6	8
Size/position/condition of mass(es)		1	2	0	4	4
Weight Loss		2	2	0	0	1

FEMALE

Category	Group/Subgroup/Sex:	1/1/F	2/1/F	4/1/F	6/1/F	8/1/F
Observation	Number in Group:	51	51	51	51	51
Reason for Removal of Animal from Study						
Found Dead		4	0	2	1	2
Killed in Extremis		0	0	0	4	3
Musculoskeletal signs (impaired mobility)		0	0	0	0	2
Other reason (specify)		0	0	1	0	0
Poor condition		1	3	1	3	5
Respiratory signs		0	0	0	0	1
Severity of Clinical Observations		14	11	11	20	17
Size/position/condition of mass(es)		7	4	4	4	1
Weight Loss		1	2	1	2	5

Most animals were sacrificed due to moribund condition. Many of the deaths were associated with findings in the pituitary gland, especially pars distalis adenoma in M and

F, and an increase in kidney disease, especially chronic progressive nephropathy in F, which was dose-dependent.

Table: Incidences of Pituitary Gland and Kidney Related Mortality

	0		0		LD		MD		HD	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Pituitary	10/16	19/25	8/17	11/19	4/9	9/20	5/12	11/33	4/17	6/34
Kidneys	1/16	0/25	1/17	1/19	0/9	4/20	0/12	15/34	2/17	25/34

Clinical Signs

Detailed exams were conducted weekly on all animals including examination for the presence of palpable masses. Twelve/sex/group were observed twice daily immediately and 4 hours post-dose during Weeks 1 and 2 and once weekly at 4 hours post-dose from Week 3.

Signs considered drug-related included an increase in red tears in HDM, MDF, and HDF, an increase in irregular, noisy, and labored breathing in MDM, HDM, and HDF, hunched posture and thin in MDF and HDF. The number of palpable masses was generally comparable across groups for M and slightly increased in control F.

Body Weights

Body weights were recorded once prior to initiation of dosing (Day -7), weekly through Week 16, and every four weeks thereafter.

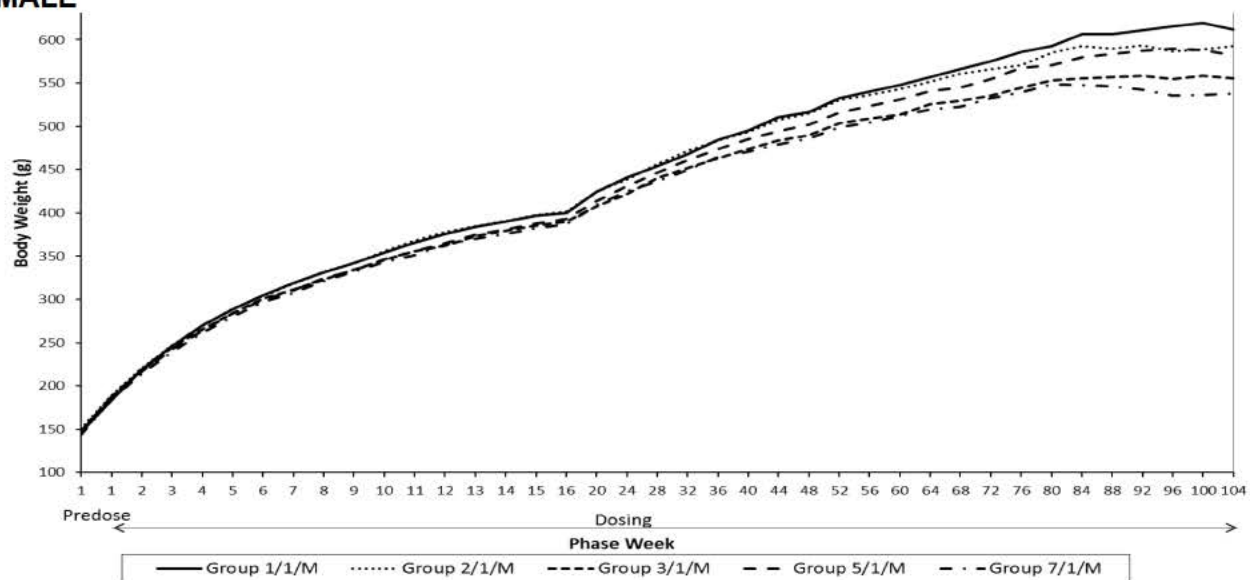
In M, there was a decreased in body weight in Week 104 by 8%, 3%, and 11% at the LD, MD, and HD, respectively. In F, body weight was decreased in Week 105 by 12%, 21%, and 17% at the HD. Differences were relative to control groups combined. By the end of the study there was a decrease in body weight gain observed in all ponesimod dosed groups in M and F.

Table: Mean Body Weight Change Comparisons to Combined Control Groups (Sponsor's)

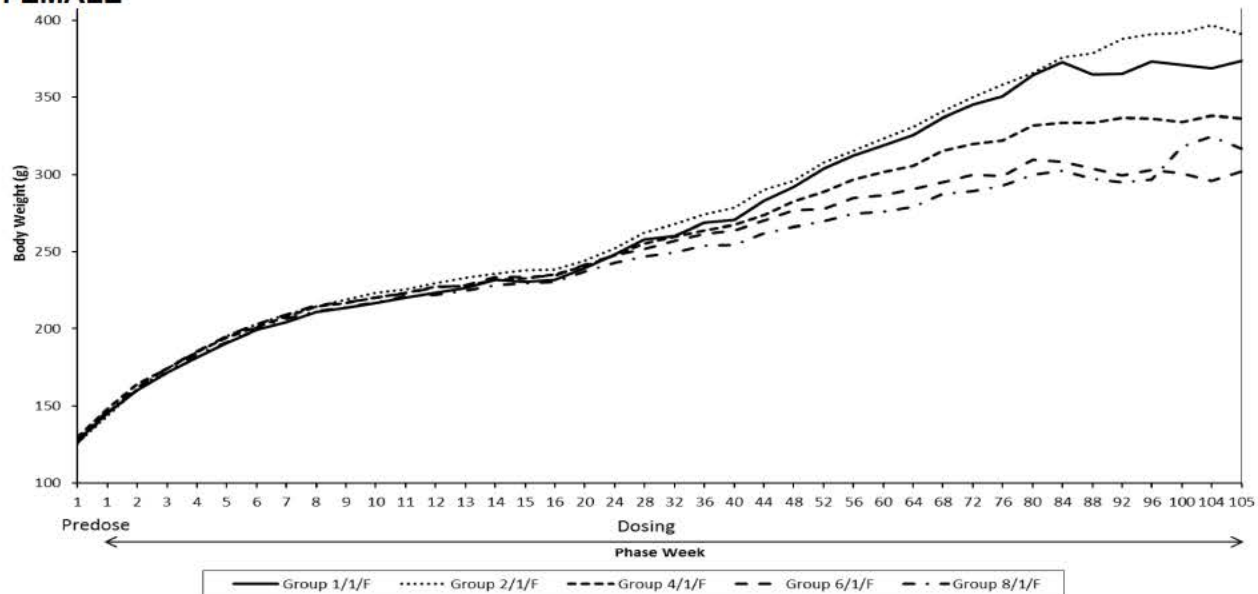
	Week 1-5	Week 5-14	Week 14-28	Week 28-52	Week 52-105	Week 1-105
MALE						
LD	-6%	-6%	-6%	-18%*	-26%*	-12%*
MD	-6%	-4%	0%	-10%*	-7%	-5%*
HD	-7%*	-5%	-6%	-19%*	-34%*	-15%*
FEMALE						
LD	-4%	-8%*	11%*	-27%*	-35%*	-21%*
MD	+3%	-6%	-31%*	-44%*	-68%*	-35%*
HD	-7%*	+10%*	-29%*	-50%*	-47%*	-28%*

* statistically significant from control.

**Figure: Body Weight (Sponsor's)
MALE**



FEMALE



Food Consumption

Food consumption was recorded weekly from Week -1 through Week 16, then every four weeks thereafter.

There was no drug-related effect on food consumption.

Ophthalmology

Examination of 25/sex/group was conducted once prior to initiation of dosing and on 25/sex in control and HD groups during Weeks 26, 52, 78, and 104 during dosing.

There were no drug-related changes.

Hematology

Blood samples were taken from TK animals in Week 52 and from surviving animals in Week 104.

The pharmacodynamic effect of ponesimod, decreases in WBC and lymphocytes, were observed at the MD and HD in M and F in Week 52, these decreases were also observed in Week 104 in M, but not in F.

Clinical Chemistry

Blood samples were taken from TK animals in Week 52 and from surviving animals in Week 104.

There were increases in cholesterol at the HD and creatinine in all dose groups in F.

Blood smears

Samples were collected from all animals at necropsy but were not examined.

Gross Pathology

A complete necropsy was conducted on animals.

Drug-related macroscopic changes included increase in incidences of thin appearance in MDF and HDF, aorta distension in MDM and HDF, kidneys exhibiting irregular surface in MDF and HDF, and mandibular lymph node cysts in all M and F dose groups. Decreases in incidences of pale lungs in all M and F ponesimod dose groups and pituitary and skin masses.

Table: Gross Necropsy Findings in Rats Dosed with Ponesimod (Sponsor's)

Finding	MALE					FEMALE				
	0	0	3	10	30	0	0	10	30	100
General appearance										
Thin	6/51	10/51	6/51	3/51	7/51	13/51	11/51	11/51	25/51	31/51
Aorta										
Distension	0/51	0/51	0/51	0/51	1/51	0/51	0/51	1/51	6/51	3/51
Kidney										
Irregular surface	0/51	1/51	0/51	1/51	3/51	1/51	0/51	1/51	14/51	19/51
Lung										
Pale area	8/51	6/51	4/51	1/51	0/51	8/51	3/51	2/51	1/51	0/51
Pale focus	23/51	17/51	1/51	1/51	3/51	20/51	17/51	2/51	2/51	1/51
Mandibular Lymph node										
Cyst	3/51	1/51	7/51	8/51	12/51	1/51	1/51	5/51	5/51	4/51
Pituitary gland										
Mass	10/51	10/51	7/51	5/51	4/51	23/51	21/51	11/51	13/51	8/51
Skin/Subcutis										
Mass	5/51	4/51	3/51	12/51	10/51	23/51	26/51	20/51	9/51	6/51

Organ Weights

At necropsy, the organs identified in the table in the histopathology section were weighed.

There were statistically significant increases in mean absolute lung weight in M and F and heart weight in F.

Table: Organ Changes in Mean Absolute Weight Compared to Control Organ Weights

	Adrenal	Brain	Heart	Kidney	Liver	Lungs	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus
MALE										
LD:	-1%	+1%	-1%	-2%	-8%*	-1%	+11%	-3%	-5%*	-5%
MD:	+13%	+1%	+5%	0%	+1%	+5%	+8%	-1%	-3%	+92%
HD:	+30%	+2%	-3%	-1%	+1%	+7%*	+7%	-3%	-3%	128%
FEMALE										
LD:	-5%	+1%	+8%	-2%	-1%	+8%	+10%	-20%*	+175%	+37%
MD:	-2%	+2%	+20%*	-1%	+3%	+14%*	+11%	+3%	+434%	+160%
HD:	+6%	0%	+18%*	-1%	+3%	+20%*	+17%	+11%	+902%	+66%

* Statistically significant from control.

Table: Organ Changes in Mean Weight (Relative to Body Weight) Compared to Control Organ Weights

	Adrenal	Brain	Heart	Kidney	Liver	Lungs	Prostate/ Ovary	Spleen	Testes/ Uterus	Thymus
MALE										
LD:	+12%	+14%*	+11%*	+11%*	+3%	+12%*	+28%	+9%	+7%	+5%
MD:	+21%	+8%	+13%*	+7%	+3%	+12%*	+15%	+6%	+4%	+111%
HD:	+53%*	+18%*	+18%*	-15%*	+16%*	+23%*	+24%	+12%	+13%*	+149%
FEMALE										
LD:	+7%	+12%*	+20%*	+10%*	+10%	+20%*	+23%	-8%	+189%	+60%*
MD:	+21%*	+25%*	+49%*	+22%*	+28%*	+41%*	+39%	+28%*	+570%*	+214%*
HD:	+27%*	+17%*	+40%*	+18%*	+23%*	+44%*	+35%	+35%	+1021%*	+106%*

* Statistically significant from control.

Histopathology

At necropsy, the following organs, as listed in the study report, were collected from all animals. Microscopic examination included all tissues.

W = weighed; E = processed and examined microscopically

Organ/Tissue			Organ/Tissue	
adrenal	W	E	nerve, optic	E
animal identification			nerve, sciatic	E
aorta		E	oesophagus	E
bone marrow smear (femur)			ovary	W E
brain	W	E	oviduct	E
caecum		E	pancreas	E
colon		E	parotid salivary gland	E
duodenum		E	pituitary	E
eye ¹		E	preputial/clitoral gland	E
femur with bone marrow ²		E	prostate	W E
GALT/Peyers patch		E	rectum	E
gross lesions		E	seminal vesicle	E
Harderian gland		E	skin and subcutis	E
head			spinal cord, cervical	E
heart	W	E	spinal cord, lumbar	E
ileum		E	spinal cord, thoracic	E
jejunum		E	spleen	W E
kidney	W	E	sternum with bone marrow	E
lacrimal gland		E	stomach	E
larynx		E	sublingual salivary gland	E
liver	W	E	testis and epididymis ³	W E
lungs with main stem bronchi and bronchioles	W	E	thymus	W E
lymph node, mandibular		E	thyroid and parathyroid	E
lymph node, mesenteric		E	tissue masses	E
lymph node, popliteal		E	tongue	E
mammary gland		E	trachea	E
mandibular salivary gland		E	ureter	E
muscle, quadriceps		E	urinary bladder	E
nasal cavity		E	uterus with cervix	W E
nasopharynx			vagina	E
nose/nares			Zymbal gland	E

1. Tissue taken into Davidson's fixative

2. Tissue includes femoro-tibial joint.

3. Tissue taken into Bouin's fixative

Bone designated for histopathological examination was decalcified using Kristenson's fluid.

Peer Review: Yes

Signed Pathology report: Yes

Neoplastic

Several rare tumors were detected with statistical significance. However, the sponsor noted that the rare tumors had low (1-2) rates of incidence and therefore, considered them not to provide evidence of a carcinogenic effect. There was a significant increase in Leydig cell adenoma in the testes at the LD, but not at the MD or HD. Based on the FDA statistical reviewer's tumor analysis, there were no other statistically significant increases in neoplastic findings.

Table: Observed Neoplasms After Ponesimod Treatment for up to 104 Weeks in Rats

Neoplasms	MALE					FEMALE				
	0	0	3	10	30	0	0	10	30	100
Malignant Lymphoma	0 (1)	0 (3)	0 (0)	1 (0)	0 (2)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
Hemangioma	1 (0)	2 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)
Hemangiosarcoma	0 (0)	0 (0)	2 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hemangioma/sarcoma	1 (0)	2 (0)	2 (0)	1 (0)	1 (1)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)
Adrenal										
Pheochromocytoma [‡]	0 (0)	0 (0)	0 (0)	2 (0)	2 (0)	0 (0)	0 (0)	0 (0)	0 (1)	1 (0)
Bone										
Osteosarcoma	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)
Heart										
Mesothelioma [‡]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
Liver										
Cholangioma [‡]	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Skin										
Osteosarcoma	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Rabdomyoma [‡]	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Testes										
Leydig cell adenoma	0 (0)	0 (0)	3 (1)	0 (1)	0 (0)	-	-	-	-	-
Thymus										
Thymoma	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)
Uterus										
Adenocarcinoma	-	-	-	-	-	1 (0)	1 (0)	3 (2)	2 (2)	2 (2)
Benign Polyp	-	-	-	-	-	3 (1)	2 (1)	6 (3)	2 (3)	4 (3)

Data from animals that died/sacrificed prematurely are in parenthesis. [‡] Rare tumors. The tumor data were analyzed by the FDA statistician for dose response (control groups were summed) 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-3 method (see statistical review; NDA 213-498, November 18, 2020). * Total incidences are statistically significant; # indicate statistically significant dose response.

Non-Neoplastic

Target organs of ponesimod included adrenal glands (F), brain, kidneys, liver, lymph nodes, spleen, thymus, and thyroid gland. Findings included an increase in incidence and severity in zona glomerulosa hypertrophy in F, brain mineralization and vacuolation in M and F and necrosis in F, chronic progressive nephropathy in F, bile duct hyperplasia in M and F, lymphangiectasis in lymph nodes in M and F, and follicular cell hypertrophy in M and F. Increases in incidences were observed in thymic tinctorial change in M and F. Increase in severity was observed in mastocytosis in mesenteric lymph node in M and F and splenic extramedullary hematopoiesis in M and F.

Table: Summary of Selected Microscopic Findings

Tissue	Finding	(mg/kg): Number	MALE					FEMALE					
			0	0	3	10	30	0	0	10	30	100	
			51	51	51	51	51	51	51	51	51	51	
Adrenal gland	Diffuse Z. glomerulosa hypertrophy	minimal:	3	1	2	2	3	1	0	3	20	19	
		mild:	0	0	0	0	1	0	0	0	3	5	
		moderate:	0	0	0	0	0	0	0	0	0	0	
Brain	Mineralization	minimal:	1	0	6	8	14	0	0	10	10	11	
		mild:	0	0	2	4	7	0	0	8	2	10	
		moderate:	0	0	0	1	5	0	0	3	8	9	
	Necrosis	minimal:	0	0	0	0	0	0	0	0	0	2	
		mild:	0	0	0	0	0	0	0	0	0	3	
		moderate:	0	0	0	0	0	0	0	0	0	1	
		marked:	0	0	0	0	0	0	0	0	0	1	
		marked:	0	0	0	0	0	0	0	0	0	1	
	Vacuolation	minimal:	5	11	8	17	13	15	10	18	23	19	
		mild:	1	1	1	4	5	1	2	1	6	4	
		moderate:	0	0	0	0	0	0	0	0	0	2	
	Kidney	Tubule atrophy	minimal:	14	13	13	12	12	9	7	25	14	10
			mild:	3	1	3	4	4	0	1	0	8	3
			moderate:	0	0	0	0	1	0	0	0	0	1
		Tubular Basophilia	minimal:	12	14	13	15	9	7	4	15	8	13
mild:			5	1	1	0	4	0	0	0	1	2	
moderate:			1	0	0	0	0	0	0	0	0	0	
Chronic progressive neuropathy		minimal:	0	0	0	0	0	0	0	0	0	0	
		mild:	0	0	0	0	0	0	0	0	0	0	
		moderate:	2	5	2	1	9	2	0	5	12	9	
		marked:	1	0	1	0	0	0	0	1	10	17	
		massive:	0	1	0	0	0	0	0	0	0	2	
Liver		Bile duct hyperplasia	minimal:	5	9	11	19	22	15	20	13	18	19
			mild:	1	2	2	3	9	9	4	21	13	16
			moderate:	0	1	1	0	2	3	0	2	3	1
Lung		Congestion	minimal:	2	3	3	2	4	0	0	0	5	2
	mild:		2	3	0	2	3	1	2	3	2	3	
	moderate:		1	2	1	0	1	0	2	1	1	2	
	marked:		2	0	1	2	0	1	0	0	0	0	
	Macrophage aggregation	minimal:	21	14	5	8	4	20	18	7	9	5	
		mild:	9	7	0	0	0	3	2	0	0	0	
		moderate:	1	1	0	0	0	0	0	0	0	1	
	Lymphocytic infiltration	minimal:	19	22	26	24	8	13	21	13	17	18	
		mild:	4	2	1	0	1	2	0	0	0	0	
	Perivascular infiltrate	minimal:	0	0	0	0	0	0	0	0	0	1	
		minimal:	0	0	0	0	0	0	0	0	0	1	
	Osseous metaplasia	minimal:	2	2	4	5	3	0	1	1	0	1	
		mild:	0	0	0	0	2	0	0	0	0	1	

Tissue	Finding	(mg/kg): Number	MALE					FEMALE				
			0	0	3	10	30	0	0	10	30	100
			51	51	51	51	51	51	51	51	51	
Lung	Hemorrhage	minimal:	0	0	0	0	2	0	2	0	0	2
		mild:	0	0	0	0	0	0	1	0	0	0
		moderate:	0	0	0	0	0	0	0	0	0	0
		marked:	0	0	0	1	0	0	0	0	0	0
		Br/alveol hyperplasia	minimal:	0	0	0	0	0	0	0	1	0
	mild:	0	0	0	0	0	0	0	0	0	0	
	moderate:	0	0	0	1	0	0	0	0	0	0	
Lymph node - Mandibular	Lymphangiectasis	minimal:	5	1	2	2	5	0	2	3	5	2
		mild:	1	4	6	10	5	2	1	1	2	6
		moderate:	4	1	2	9	9	1	0	10	6	3
		marked:	2	0	10	12	13	0	0	2	7	4
		massive:	0	0	1	0	2	0	0	1	0	0
Lymph node - Mesenteric	Lymphangiectasis	minimal:	3	3	3	1	1	1	1	1	2	0
		mild:	0	0	1	1	6	0	0	1	0	3
		moderate:	0	0	1	0	1	0	0	0	1	0
		marked:	0	0	1	0	0	0	1	1	0	0
		massive:	0	0	1	1	0	0	0	0	0	0
	Mastocytosis	minimal:	35	24	27	26	20	30	26	22	30	17
		mild:	8	14	10	13	19	3	7	16	13	14
		moderate:	1	0	1	2	2	0	0	2	2	6
	Decreased cellularity	minimal:	14	7	8	8	11	7	6	22	18	18
		mild:	0	0	2	1	1	1	2	4	9	8
moderate:		0	0	0	0	0	1	0	0	1	8	
marked:		0	0	0	0	0	0	0	1	0	1	
Spleen	Extramedullary hematopoiesis	minimal:	9	7	5	4	4	9	8	2	4	3
		mild:	8	6	3	7	3	10	10	9	7	2
		moderate:	23	31	35	35	27	23	20	33	18	18
		marked:	8	5	7	3	14	7	12	6	20	24
		massive:	0	0	0	0	0	0	0	0	2	0
Testes	Arteritis	minimal:	0	0	0	0	0	-	-	-	-	-
		mild:	0	0	0	0	5	-	-	-	-	-
Thymus	Lymphoid hyperplasia	minimal:	0	0	0	0	1	0	0	0	3	3
		mild:	2	0	0	0	3	1	2	1	3	4
		moderate:	1	0	1	1	3	1	2	0	6	2
		marked:	0	3	2	1	0	1	2	1	0	5
		massive:	0	0	1	0	2	1	1	4	5	5
	Tinctorial change	present:	1	0	1	13	26	0	0	21	18	21
Thyroid	Follicular cell hypertrophy	minimal:	22	13	14	16	19	2	3	6	10	16
		mild:	1	10	4	10	10	1	0	1	3	3

Toxicokinetics

Blood samples were taken from TK animals in Weeks 26 and 52 at 0.5, 1, 2, 4, 8, and 24 hours post-dose. Levels of ponesimod and metabolites ACT-204426 (M12) and ACT-338375 (M13) were quantitated.

Increase in plasma exposure (C_{max} and AUC) to ponesimod was generally less than dose-proportional. T_{max} was at 1-2 hours. Accumulation was observed between Weeks 26 and 52.

Table: Ponesimod Plasma Levels (Sponsor's)

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
3	26	m	2	266	1450	88.7	482
3	52	m	1	290	1660	96.7	553
10	26	f	1	1200	7500	120	750
10	52	f	2	1650	10300	165	1030
10	26	m	2	803	4200	80.3	420
10	52	m	2	979	5480	97.9	548
30	26	f	1	2000	14500	66.7	483
30	52	f	2	2910	20300	97.0	676
30	26	m	2	1620	10100	54.0	336
30	52	m	2	1800	12900	60.0	431
100	26	f	2	4670	35500	46.7	355
100	52	f	2	7390	66400	73.9	664

Increase in plasma exposure (C_{max} and AUC) to metabolite ACT-204426 was generally less than dose-proportional, with plasma exposure greater in F. T_{max} was at 1-2 hours in M and F. Accumulation was observed between Weeks 26 and 52.

Table: ACT-204426 (M12) Plasma Levels (Sponsor's)

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
3	26	m	1	17.9	84.6 [#]	5.97	28.2 [#]
3	52	m	1	28.6	113 [#]	9.53	37.7 [#]
10	26	f	1	35.2	155 [#]	3.52	15.5 [#]
10	52	f	1	94.2	478	9.42	47.8
10	26	m	2	38.0	255	3.80	25.5
10	52	m	2	80.3	312 [#]	8.03	31.2 [#]
30	26	f	1	56.7	442	1.89	14.7
30	52	f	2	125	952	4.17	31.7
30	26	m	2	77.2	494	2.57	16.5
30	52	m	2	147	836	4.90	27.9
100	26	f	2	90.9	856	0.909	8.56
100	52	f	2	237	2460	2.37	24.6

[#]AUC0-8h, more than 50% of samples were BLQ at 24h

Increase in plasma exposure (C_{max} and AUC) to metabolite ACT-338375 was generally less than dose-proportional, with plasma exposure greater in M. T_{max} was at 2-4 hours. Accumulation was observed between Weeks 26 and 52.

Table: ACT-338375 (M13) Plasma Levels (Sponsor's)

Dose (mg/kg/day)	Week	Sex	Tmax (h)	Cmax (ng/mL)	AUC0-24h (h*ng/mL)	Cmax/D (ng/mL)/ (mg/kg/day)	AUC0-24h/D (h*ng/mL)/ (mg/kg/day)
3	26	m	2	18.8	86.7 [#]	6.27	28.9 [#]
3	52	m	2	20.8	109 [#]	6.93	36.2 [#]
10	26	f	2	36.5	162 [#]	3.65	16.2 [#]
10	52	f	2	67.6	457	6.76	45.7
10	26	m	2	51.6	324	5.16	32.4
10	52	m	2	74.6	438	7.46	43.8
30	26	f	4	64.5	359 [#]	2.15	12.0 [#]
30	52	f	2	131	1110	4.37	37.0
30	26	m	2	104	715	3.47	23.8
30	52	m	2	152	1020	5.07	33.9
100	26	f	2	156	1250	1.56	12.5
100	52	f	2	332	3490	3.32	34.9

[#]AUC0-8h, more than 50% of samples were BLQ at 24h

Dosing Solution Analysis

All ponosimod samples analyzed during Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 104 were within $\pm 10\%$ (range: 90% to 110%) of nominal concentrations.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Female fertility study in the rat

Study no.: T-07.108

Study report location: EDR

Conducting laboratory and location:

(b) (4)

Date of study initiation: June 21, 2007

GLP compliance: Yes, (b) (4)

QA statement: Yes

Drug, lot #, and % purity: ACT-128800, Batch No AA-016498-Batch- (b) (4)
99.4%

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 pre-mating period at all doses.

- No drug-related changes were observed in F fertility or early embryonic development at any dose.
- The NOAEL was 100 mg/kg/day.

Methods

Doses: 0, 10, 30, and 100 mg/kg
Frequency of dosing: Daily
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: 0.25 % (w/w) Methylcellulose (400 mPa.s.) containing 0.05 % (v/v) Tween 80
Species/Strain: Rat/Wistar Han
Number/Sex/Group: 20
Age: F: 6-7 weeks
M: 9-10 weeks
Study design: F were dosed for 14 days prior to mating, during the mating period, and then until Gestation Day (GD) 6. One F was paired with 1 M, with the cohabitation lasting for 10 days and if pregnancy did not result were re-paired for another 10 days. F were sacrificed on GD 13

Observations and results

Mortality

Observations for mortality and morbidity were conducted twice daily.

No deaths occurred during the study.

Clinical Signs

Cage-side observations of F were conducted once daily from the start of dosing days.

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

Body Weight

F were weighed twice weekly on Days 1, 4, 8, 11, and 15 pre-mating, daily from mating until GD 7, and then on GDs 10 and 13.

Body weight gain was decreased by 11% during the pre-mating period (Days 0-15) at the HD. No change in body weight gain was observed during gestation.

Food Consumption

F food consumption was recorded twice weekly during dosing and on GD 0-4, 4-7, 7-10, and 10-13.

Food consumption was dose-dependently decreased during the first week of pre-mating at the LD (6%), MD (10%), and HD (15%) but was similar during gestation.

Vaginal cytology

Vaginal cytology was determined, and estrous cycles assessed. Samples were collected daily for 10 days prior to pairing.

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

Gross findings

F were terminated on post coitum Day 13. Major organs were examination.

There were no adverse drug-related macroscopic findings.

Ovarian and uterine examinations

Cervix, ovaries, uterus, pituitary, and vagina were removed at necropsy and the following observations conducted: pregnancy status, number of corpora lutea, implantation sites, resorptions, and live and dead embryos.

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.

Study title: ACT-128800: Toxicity study on male fertility in the Han Wistar rat

Study no.: T-08.429

Study report location: EDR

Conducting laboratory and location:



Date of study initiation: August 26, 2008

GLP compliance: Yes,  (b) (4)

QA statement: Yes

Drug, lot #, and % purity: ACT-128800, Batch № 4, 99.97%

Key Study Findings

- There were no deaths or adverse clinical signs.
- There were decreases in body weight gain and food consumption.
- The NOAEL for M fertility was 100 mg/kg/day.

Methods

Doses: 0, 10, 30, and 100 mg/kg
Frequency of dosing: Daily
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: 0.25% (w/w) Methylcellulose (MC) and 0.05% (v/v)
Tween 80 in Milli-Q-Water
Species/Strain: Rat/Wistar Han
Number/Sex/Group: 22
Age: M: 8 weeks
F: 10 weeks
Weight: Males - 213-268 g; Females - 179-222 g
Study design: M were dosed for 10 weeks prior to mating, during the 28-days mating period (maximum; 2 pairing periods with different F), and then until termination. F were not dosed

Observations and results

Mortality

Observations for mortality were conducted twice daily.

No deaths occurred during the study.

Clinical Signs

Clinical observations were conducted once daily prior to dosing and during dosing M were observed twice daily and F were observed once daily. Detailed examinations were conducted once prior to dosing for M and F and weekly during the dosing period for M only.

No adverse clinical signs were noted.

Body Weight

During dosing, M body weights were recorded daily until termination. Body weights were recorded in F daily during mating and gestation.

Body weight gain in M was decreased during the first week of the pre-mating period (Days 1-8) at the HD (16%) and over the full pre-mating period (Days 1-70) at the LD (11%), MD (13%), and HD (15%). No change in body weight gain was observed during the mating period or after the mating period. There was no change in F body weight.

Food Consumption

Food consumption was recorded weekly for M during pre-mating and after the mating period and for F during the gestation period.

Food consumption in M was dose-dependently decreased during the first week (Days 1-8) of dosing at the LD (4%), MD (5%), and HD (9%) and was decreased by 4%

for all groups over the pre-mating period (Days 1-70). Similarly, food consumption was decreased by 3-5% after the pairing period. Mean food consumption was similar in all F groups.

Gross findings

F were terminated on GD 21 and M were terminated after the F at the end of dosing. All animals were examined macroscopically.

There were no drug-related macroscopic findings in M and F.

Reproductive Parameters

The pregnancy status, number of corpora lutea, implantation sites, status of implantation (early/late intrauterine deaths, live fetuses), and gravid uterus weight were recorded and fetuses evaluated.

There were no drug-related effects on mating performance of F. There were no changes in the mean day to mating, fertility and mating indices, and number of corpora lutea. There was slight pre-implantation loss in LDF and HDF, resulting in a decrease in implantation sites. There were no differences in number of live embryos, dead embryos, resorptions, embryo weight, sex ratio, and placenta weight.

Table: Summary of cesarean data (Sponsor's)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4
NUMBER OF DAMS	21	21	21	22
CORPORA LUTEA	296	309	295	329
MEAN (+)	14.1	14.7	14.0	15.0
ST.DEV.	1.3	1.4	1.1	1.4
PRE-IMPLANTATION LOSS	9	24	8	22
% OF CORP. LUTEA (#)	3.0	7.8 ##	2.7	6.7 #
MEAN (+)	0.4	1.1	0.4	1.0
ST.DEV.	0.6	1.5	0.9	1.2
NUMBER OF DAMS AFFECTED	8	9	5	13
IMPLANTATION SITES	287	285	287	307
% OF CORP. LUTEA (#)	97.0	92.2 ##	97.3	93.3 #
MEAN (+)	13.7	13.6	13.7	14.0
ST.DEV.	1.5	2.2	1.5	1.6
POST-IMPLANTATION LOSS	20	29	11	13
% OF IMPL. SITES (#)	7.0	10.2	3.8	4.2
MEAN (+)	1.0	1.4	0.5	0.6
ST.DEV.	0.9	1.8	0.7	0.7
NUMBER OF DAMS AFFECTED	14	13	9	11
IMPLANTATION SITE SCARS	0	0	0	0
EMBRYONIC/FETAL DEATHS TOTAL	20	29	11	13
EMBRYONIC RESORPTIONS	20	29	10	13
% OF IMPL. SITES (#)	7.0	10.2	3.5 #	4.2
MEAN (+)	1.0	1.4	0.5	0.6
ST.DEV.	0.9	1.8	0.6	0.7
NUMBER OF DAMS AFFECTED	14	13	9	11
FETAL RESORPTIONS	0	0	1	0
% OF IMPL. SITES (#)			0.3	
MEAN (+)			0.0	
ST.DEV.			0.2	
NUMBER OF DAMS AFFECTED			1	
FETUSES				
TOTAL FETUSES	267	256	276	294
% OF IMPL. SITES (#)	93.0	89.8	96.2	95.8
MEAN (+)	12.7	12.2	13.1	13.4
ST.DEV.	1.9	2.8	1.4	1.9
LIVE FETUSES	267	256	276	294
DEAD FETUSES	0	0	0	0
ABNORMAL FETUSES				1
% OF FETUSES (#)				0.3
MEAN (+)				0.0
ST.DEV.				0.2
NUMBER OF DAMS AFFECTED				1

*/** : Dunnett-Test based on pooled variance significant at level 5% (*) or 1% (**)

#/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)

+ : Steel Test significant at level 5%

Semenology and Spermatic Count

Sperm analysis was conducted on the first 10 M/group. Epididymal sperm samples were collected from left causal epididymis and sperm counted microscopically for determination of percentage of not motile, stationary motile, and progressively motile sperm. Sperm samples from the left vas deferens were used for morphological assessment.

There was a non-statistically significant change in sperm motility in all dose group, but no change in morphology. A statistically significant decrease in sperm head count from the testis was noted at all doses, but not at the HD in the epididymides.

Table: Summary of sperm motility (Sponsor's)

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 30 MG/KG	GROUP 4 100 MG/KG
Progressive motile %	MEAN	68	61	62	61
	ST.DEV.	8	11	11	11
	N	11	10	10	10
Stationary motile %	MEAN	22	27	23	25
	ST.DEV.	7	6	5	9
	N	11	10	10	10
Not motile %	MEAN	10	12	15	15
	ST.DEV.	2	5	9	3
	N	11	10	10	10

Table: Summary of sperm head count (Sponsor's)

		GROUP 1 0 MG/KG	GROUP 2 10 MG/KG	GROUP 3 30 MG/KG	GROUP 4 100 MG/KG
Testis Millions/ Gram organ	MEAN	130.84	112.00 **	110.04 **	115.27 **
	ST.DEV.	10.40	11.75	8.49	10.73
	N	11	10	10	10
Cauda epidid. Millions/ Gram organ	MEAN	774.01	684.50 *	650.70 **	747.57
	ST.DEV.	89.70	47.60	77.48	36.58
	N	11	10	10	10

Organ Weight

The testis and epididymis were weighed.

There was a no change in testis or epididymis weights.

Tissues

At necropsy, the following organs from M, as listed in the study report, were prepared for histopathology examination. All organs in control and HD groups were examined.

Pituitary	Testes* (in modified DAVIDSON)
Prostate	Epididymides* (in modified DAVIDSON)
Seminal vesicles with coagulating gland	Animal identification
Gross lesions	

* The left epididymis and left testis were allocated for the seminology.

There were no drug-related microscopic findings.

9.2 Embryonic Fetal Development

Study title: ACT-128800: Preliminary embryo-fetal toxicity study in Han Wistar rats by oral gavage administration

Study no: T-05.110
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: November 17, 2005
 GLP compliance: Yes, (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch № AA-016498-BATCH- (b) (4)
 99.4%

Key Study Findings included decreased body weight gain and food consumption at the MD and HD. Litter resorption was observed in 2 HD dams and increased post-implantation loss noted in the other 4 HD dams, resulting in a decrease in live fetuses at the HD and decrease in HD fetal weight. Major abnormalities in HD fetuses included fistula ascending aorta trunk, muscular ventricular septal defected, short fibula, misshapen tibia, and malrotated hindlimbs. No premature deaths, adverse clinical signs, or differences in numbers of corpora lutea, implantations, and pre-implantation losses were noted. The sponsor considered the MD (10 mg/kg/day) the maternal NOEL. The sponsor's assessment appears appropriate. The NOEL is associated with ponesimod plasma exposures (AUC) on Day 17 of 10600 ng*h/mL in F dams. The fetal NOAEL was the MD due to increased embryofetal lethality, resorption and high post-implantation loss, and teratogenicity, abnormalities of the cardiovascular and limb systems.

Methods

Doses: 0, 1, 10, and 100 mg/kg
 Frequency of dosing: Daily
 Dose volume: 10 mL/kg
 Route of administration: Oral, gavage
 Formulation/Vehicle: 0.25% (w/w) methylcellulose containing 0.05% (v/v) Tween 80
 Species/Strain: Rat/Wistar Han
 Number/Sex/Group
 Main: 6
 Toxicokinetics: 5
 Age: 74-81 days
 Weight: Females - 176-213 g at GD 0
 Study design: Ponesimod was administered daily on GDs 6-17

Table: Toxicokinetic data of ponesimod (Sponsor's)

Dose (mg/kg/day)	C _{max} (ng/mL)		AUC ₂₄ (ng.h/mL)	
	Day 6	Day 17	Day 6	Day 17
1	76.0	121	448	661
10	1250	1650	7940	10600
100	6850	10900	80200	113000

Study title: ACT-128800: Embryo-fetal toxicity study in the Han Wistar rat by gavage (once daily) administration

Study no: T-06.093
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: May 23, 2006
 GLP compliance: Yes,  (b) (4)
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch № AA-016498-BATCH- (b) (4)
 99.4%

Key Study Findings

- No dams died or had adverse clinical signs.
- The body weight gain, adjusted and unadjusted for gravid uterine weight, and food consumption of dams were decreased at the HD.
- There were no differences in numbers of corpora lutea, implantations, or pre-implantation losses. There was an increase in post-implantation loss that resulted in a decrease in mean live fetuses at the HD and decrease in HD mean fetal weight.
- Major abnormalities in MD and HD fetuses included fused ascending aorta trunk and muscular ventricular septal defect. Other major abnormalities observed in HD fetuses included membranous ventricular septal defects, syndactyly, ectrodactyly, malrotated hindlimbs, and eye microphthalmia.
- The sponsor considered the HD (40 mg/kg/day), the maternal NOAEL. The sponsor's assessment appears appropriate.
- The fetal NOAEL was the LD due to increased embryofetal lethality and fetal teratogenicity. The ponesimod plasma exposures (AUC) in dams on Day 17 associated with 1 mg/kg/day was 661 ng*h/mL (taken from the dose ranging study).

Methods

Doses: 0, 1, 10, and 40 mg/kg
Frequency of dosing: Daily
Dose volume: 10 mL/kg
Route of administration: Oral, gavage
Formulation/Vehicle: 0.25% (w/w) methylcellulose containing 0.05% (v/v)
Tween 80
Species/Strain: Rat/Wistar Han
Number/Sex/Group
Main: 22
Age: 10-11 weeks
Weight: Females - 180-211 g at GD 0
Study design: Ponesimod was administered daily on GDs 6-17

Observations and results**Mortality**

No unscheduled deaths occurred in dams.

Clinical Signs

Observations were conducted at least twice daily. Detailed physical examinations were conducted on GDs 0, 6, 12, 18, and 20. Detailed observations were recorded daily immediately before dosing, immediately after dosing, on completion of dosing of each group, between 1-2 hours after completion of dosing of all groups, and as late as possible in the working day.

No adverse clinical signs were noted in dams in any group.

Body Weight

Body weight recordings were taken on GDs 0, 3, 6-18, and 20.

During dosing (Days 6-20) decrease in body weight gain was observed in dams at the HD (23%). When body weight gain was adjusted for the gravid uterine weight, there was decrease of 16% compared to control dams.

Food Consumption

Food consumption was recorded on GDs 0-2, 3-5, 6-9, 10-13, 14-17, and 18-19.

During dosing (Days 6-20) decrease in food consumption was observed in dams at the MD (3%) and HD (5%).

Necropsy

On GD 20, F were sacrificed, and external features, orifices, and visceral organs and placentas examined macroscopically. The uterus was removed for assessment of fertility parameters. The gravid uterus including the ovaries was weighed.

Uterine weight was decreased, compared to control values, by 25% at the HD. There were no adverse macroscopic findings noted in dams in any group.

Cesarean Section Data

The number of corpora lutea, implantation sites, live and dead fetus, and early and late resorptions were recorded. Viable fetuses were sexed and weighed.

There were no differences in numbers of corpora lutea, implantations, or pre-implantation loss. There was increased post-implantation loss primarily due to late resorption noted in HD dams. The increase in resorptions and post-implantation loss resulted in a decrease in live fetuses at the HD. There was a statistically significant decrease in HD fetal weights by 12%. There was a 6% and 10% decrease in placenta weight at the MD and HD, respectively. No adverse effects on embryofetal survival, litter size, or sex ratio was noted at the LD or MD.

Table: Cesarean section data (Sponsor's)

Group		Corpora Lutea	Implantations	Resorptions			Live Young Male	Live Young Female	Total	Sex ratio (%M)	Implantation Loss (%)	
				Early	Late	Total					Pre-	Post-
1	Mean	13.2	12.0	0.8	0.0	0.9	6.0	5.1	11.2	54.3	8.9	6.6
	SD	1.45	2.15				1.81	1.93	2.04			
	N	22	22	22	22	22	22	22	22	22	22	22
2	Mean	12.8	12.0	0.9	0.0	0.9	5.5	5.5	11.1	50.3	6.6	7.9
	SD	0.85	1.15				1.63	1.71	1.69			
	N	22	22	22	22	22	22	22	22	22	22	22
3	Mean	12.9	11.9	0.6	0.1	0.8	5.4	5.7	11.1	48.8	7.8	7.0
	SD	1.78	2.10				2.09	2.08	2.47			
	N	22	22	22	22	22	22	22	22	22	22	22
4	Mean	13.2	12.1	1.4	4.0 b	5.4 b	3.2 b	3.8 a	6.7 B	40.1	9.2	47.9 b
	SD	1.56	2.88				2.47	2.18	3.84			
	N	22	22	22	22	22	21‡	21‡	22	21‡	22	22

‡ Excludes female no. 72 with litter resorption

Significant when compared with Group 1: a - p<0.05; b - p<0.01

Significant when compared with Group 1: A - p<0.05; B - p<0.01

Group		Placental Weight	Litter Weight	Litter Size	Male Fetal Weight	Female Fetal Weight	Overall Fetal Weight
1	Mean	0.51	38.71	11.18	3.56	3.41	3.48
	SD	0.056	6.355	2.039	0.268	0.231	0.228
	N	22	22	22	22	22	22
2	Mean	0.50	38.04	11.09	3.52	3.33	3.44
	SD	0.040	6.192	1.688	0.260	0.236	0.241
	N	22	22	22	22	22	22
3	Mean	0.48 a	37.29	11.09	3.47	3.32	3.40
	SD	0.037	7.042	2.467	0.235	0.241	0.229
	N	22	22	22	22	22	22
4	Mean	0.46 b	22.18 B	7.05 B	3.17 b	3.01 b	3.07 b
	SD	0.051	12.417	3.626	0.208	0.205	0.208
	N	21	21	21	18	21	21

Significant when compared with Group 1: a - p<0.05; b - p<0.01
Significant when compared with Group 1: A - p<0.05; B - p<0.01

Offspring (Malformations, Variations, etc.)

All viable fetuses were 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.

Major findings included abnormalities in the cardiovascular system (fused ascending aorta trunk, muscular and membranous ventricular septal defects), in the limbs (syndactyly, ectrodactyly, malrotated hindlimbs), and in the eyes (microphthalmia) in HD fetuses. Fused ascending aorta trunk and muscular ventricular septal defect were also observed in MD fetuses. Minor abnormalities were observed in MD and HD fetuses, including incomplete ossification of bones such as cranial centers, sacral caudal vertebral arches, and sternbrae, and small or absent posterior caudate liver lobes.

Table: Major abnormalities (Sponsor's)

Group	Fetuses				Litters			
	1	2	3	4	1	2	3	4
Number examined	246	244	244	148	22	22	22	21
Number affected	6	11	6	25	4	5	4	12
Microphthalmia	-	-	-	2 ^d	-	-	-	2
Misshapen/disorganised retina	-	-	-	1 ^d	-	-	-	1
Multiple folded retina	-	-	-	1	-	-	-	1
Folded retina	2	3	1	1 ^a	1	1	1	1
Fused maxilla to jugal	1 ^a	1	-	-	1	1	-	-
Displaced nares, constricted nasal cavities/nasopharyngeal canal: disorganised palate, misshapen tongue, ankyloglossia: single upper incisor socket: small misshapen pituitary	1	-	-	-	1	-	-	-
Fused premaxillae/upper incisor sockets: displaced tympanic annula	1 ^a	-	-	-	1	-	-	-
Small/misshapen/fused mandibles	1 ^a	1 ^a	-	-	1	1	-	-
Brachygnathia	1 ^a	-	-	-	1	-	-	-
Short lower jaw	-	1 ^a	-	-	-	1	-	-
Misshapen basisphenoid/displaced pterygoid process	-	1 ^a	-	-	-	1	-	-
Cleft palate	-	-	-	1 ^h	-	-	-	1
Cervical vertebral arch fused to exoccipital	-	1	-	-	-	1	-	-
Dorsally displaced pulmonary trunk/absent ductus arteriosus	-	-	1 ^a	-	-	-	1	-
Partially fused ascending aorta and pulmonary trunk	-	-	1 ^b	2 ^g	-	-	1	2
Partially fused innominate/left carotid arteries	-	-	-	1 ^c	-	-	-	1
Displaced left subclavian artery arises from descending aorta	-	-	-	1 ⁱ	-	-	-	1
Muscular ventricular septal defect	-	-	4 ^{ab}	5 ^{dgi}	-	-	3	4
Membranous ventricular septal defect	-	-	-	3 ^{ej}	-	-	-	3
Distorted rib cage	-	-	-	1 ^h	-	-	-	1
Diaphragm hernia, marked	-	3	-	-	-	1	-	-
Bent clavicle/scapula/ulna	2 ^{bd}	-	-	2 ^{eh}	2	-	-	2
Short/bent/thickened humerus	2 ^{bd}	-	-	1 ^h	2	-	-	1

Displaced/misshapen/disorganised kidney, epididymis attached to kidney	-	-	-	1 ^b	-	-	-	1
Absent ureter	-	-	1 ^c	-	-	-	1	-
Displaced ureter	-	-	-	1 ^b	-	-	-	1
Malpositioned[marked] testis	-	-	1 ^c	-	-	-	1	-
Small/misshapen testis: constricted seminal vesicles	-	1 ^b	-	-	-	1	-	-
Misshapen epididymis	-	1 ^b	1 ^c	-	-	1	1	-
Duplicated/fused hindlimbs, duplicated/fused pubis, duplicated ilium, small/duplicated ischia	-	1	-	-	-	1	-	-
Fused/misshapen/disorganised hindpaws	-	-	-	3 ^{ij}	-	-	-	3
Syndactyly	-	-	-	11 ^{adfn}	-	-	-	7
Ectrodactyly	-	-	-	3 ^{cd}	-	-	-	3
Malrotated hindlimb(s)	-	-	-	1 ^f	-	-	-	1

Superscript denotes fetuses with more than one abnormality

Tissues

At necropsy, the following organs, as listed in the study report, were prepared for histopathology examination. All organs in control and HD groups were examined.

- Lungs
- Lymph nodes
 - mesenteric
 - popliteal
 - mandibular
- Ovaries‡
- Pituitary‡
- Placenta‡
- Spleen
- Thymus
- Uterus (following dissection)‡

‡ Retained from animals on the final day of necropsy, only.

There were no adverse microscopic findings noted in dams in any group.

Study title: ACT-128800: Pilot study in the rabbit by oral gavage administration

Study no: T-05.111

Study report location: EDR

Conducting laboratory and location:



Date of study initiation: November 2, 2005

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: ACT-128800, Batch No AA-016498-BATCH-- 99.4%

Key Study Findings included decreased body weight gain and food consumption at all doses. No dams died or had adverse clinical signs. The sponsor concluded that the MD, 60 mg/kg/day, was suitable as the HD for the preliminary EFD study.

Methods

Doses: 30, 60, and 100 mg/kg
 Frequency of dosing: Daily
 Dose volume: 5 mL/kg
 Route of administration: Oral, gavage
 Formulation/Vehicle: 0.25% (w/w) methylcellulose containing 0.05% (v/v) Tween 80
 Species/Strain: Rabbit/New Zealand White
 Number/Sex/Group: 3
 Age: 18-26 weeks
 Weight: Females – 4.14-5.55 kg at GD 0
 Study design: Ponesimod was administered daily for 14 Days

Table: Toxicokinetic data of ponesimod (Sponsor's)

Dose (mg/kg/day)	C _{max} (µg/mL)			AUC ₈ (µg.h/mL)			AUC ₂₄ (µg.h/mL)		
	Day 1	Day 5	Day 14	Day 1	Day 5	Day 14	Day 1	Day 5	Day 14
30	1.55	1.22	1.83	10.0	7.82	11.0	18.6	15.1	19.9
	(0.31)	(-)	(0.35)	(1.9)	(-)	(1.8)	(-)	(-)	(4.7)
60	2.14	3.22	3.31	14.0	22.3	22.7	37.6	53.3	53.0
	(0.44)	(0.34)	(0.35)	(2.8)	(2.2)	(2.6)	(6.1)	(5.0)	(6.9)
100	2.77	4.23	4.46	17.0	-	30.2	44.5 ^a	-	67.3
	(0.43)	(0.52)	(0.75)	(2.2)	(-)	(3.9)	(-)	(-)	(9.0)

^a Derived from one animal only

Study title: Oral (gavage) once daily dosing developmental toxicity dose range finding study in the rabbit

Study no: T-06.074
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)

Date of study initiation: May 15, 2006
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: ACT-128800, Batch No AA-016498-BATCH- (b) (4), 99.4%

Key Study Findings included the early sacrifice of several dams due to aborted litters, decreased body weight and food consumption at the MD and HD, and decrease in pre-implantation and post-implantation losses at all doses, resulting in a decrease in live fetuses at the LD and no live fetuses at the MD and HD. A major abnormality in a LD fetus was brachydactyly. The sponsor considered the LD, 6 mg/kg/day, the maternal NOAEL due to decreases in body weight and food consumption. The sponsor's assessment appears appropriate. The fetal NOAEL could not be established due to increased embryofetal lethality and teratogenicity at all doses.

Methods

Doses: 0, 6, 20, and 60 mg/kg
Frequency of dosing: Daily
Dose volume: 5 mL/kg
Route of administration: Oral, gavage
Formulation/Vehicle: 0.25% (w/w) methylcellulose containing 0.05% (v/v)
Tween 80
Species/Strain: Rabbit/New Zealand White
Number/Sex/Group: 5
Weight: Females: 3.1-4.0 kg at mating
Study design: Ponesimod was administered daily on GDs 6-19

Study title: Oral (gavage) once daily dosing developmental toxicity study in the rabbit

Study no: T-06.075
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: July 14, 2006
GLP compliance: YES,  (b) (4)
QA statement: No
Drug, lot #, and % purity: ACT-128800, Batch No AA-016498-BATCH- (b) (4)
 99.4%

Key Study Findings

- No dams died or had adverse clinical signs.
- Body weight gain and food consumption of dams were decreased at the HD.
- WBC and lymphocyte counts were decreased at all doses.
- There was a decrease in post-implantation loss at the HD that resulted in a decrease in live fetuses.
- Minor abnormalities in HD fetuses included fused sternebra, increased presacral vertebrae, and delayed ossification.
- The sponsor considered the MD (1 mg/kg/day), the maternal NOAEL, which appears appropriate. The NOAEL was associated with ponesimod plasma exposure (AUC) on Day 19 of 736 ng*h/mL in F dams.
- The fetal NOAEL was the MD due to increased embryofetal lethality and increased number of minor morphological changes in the cardiovascular and limb systems at the HD.

Methods

Doses: 0, 0.25, 1, and 4 mg/kg
 Frequency of dosing: Daily
 Dose volume: 5 mL/kg
 Route of administration: Oral, gavage
 Formulation/Vehicle: 0.25% (w/w) methylcellulose containing 0.05% (v/v) Tween 80
 Species/Strain: Rabbit/New Zealand White
 Number/Sex/Group
 Main: 20
 Toxicokinetic: 3
 Weight: Females: 3.1-4.1 kg at mating
 Study design: Ponesimod was administered daily on GDs 6-19

Observations and results

Mortality

Animals were examined twice daily for mortality and morbidity.

No unscheduled deaths occurred in dams.

Clinical Signs

Detailed observations were conducted daily.

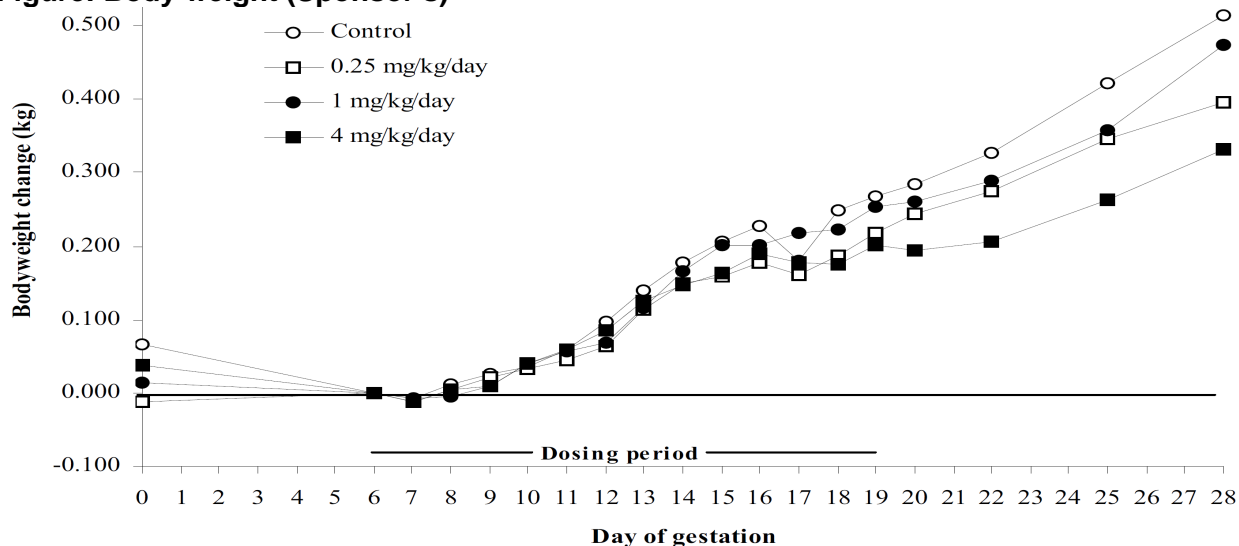
No adverse clinical signs were noted in dams in any group.

Body Weight

Body weight recordings were taken daily on GDs 0, 6-20, 22, 25, and 28.

During dosing (Days 6-20) a decrease in body weight gain was observed at the LD (14%), MD (8%), and HD (32%).

Figure: Body weight (Sponsor's)



Food Consumption

Food consumption recordings were taken on GD 4 and then every 2 days thereafter.

During dosing a decrease in food consumption was observed in dams at the HD (8%).

Hematology

Blood samples were taken from TK animals on GDs 6 and 19 at 4 hours post-dose.

Decreases in WBC were observed at the LD (11% and 31%), MD (33% and 35%), and HD (29% and 35%) on GDs 6 and 19, respectively and in lymphocytes at the LD (44% and 42%), MD (74% and 64%), and HD (82% and 80%) on GDs 6 and 19, respectively.

Necropsy

On GD 28, F were sacrificed, and the major organs examined macroscopically and placentas weighed.

There were no adverse macroscopic findings noted in dams.

Cesarean Section Data

The number of corpora lutea, implantation sites, live and dead fetus, and early and late resorptions were recorded. Viable fetuses were sexed and weighed.

There were no differences in numbers of corpora lutea, implantations, or pre-implantation losses. There was an increased post-implantation loss in HDF primarily due to an increase in early embryo deaths. The increase in early embryo deaths and post-implantation loss resulted in a decrease in live fetuses at the HD. There was a statistically significant decrease in HD fetal litter weight by 37%.

Table: Cesarean section data (Sponsor's)

	Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill	17	18	19	18
Number of corpora lutea	186	202	191	199
Mean number per female#	10.9	11.2	10.1	11.1
Standard deviation	1.9	2.2	2.4	2.3
Number of implantations	161	175	165	168
Mean number per female#	9.5	9.7	8.7	9.3
Standard deviation	2.3	2.8	2.6	2.4
Mean % pre-implantation loss#	13.6	14.5	12.7	14.3
Number of early embryo/foetal deaths	7	17	4	54
Number of late embryo/foetal deaths	7	5	3	11
Number of dead fetuses	0	0	0	0
Mean % post-implantation loss#	8.6	10.9	5.3	39.0***
Number of live fetuses	147	153	158	103
Mean number per female#	8.6	8.5	8.3	5.7**
Standard deviation	2.2	2.2	2.7	3.3
Mean % of implantations	91.4	89.1	94.7	61.0

Number of females with live foetuses at scheduled kill	17	18	19	18
Number of live foetuses	147	153	158	103
Mean number per female#	8.6	8.5	8.3	5.7
Standard deviation	2.2	2.2	2.7	3.3
Number of male foetuses	77	81	83	52
Number of female foetuses	70	72	75	51
Mean % male foetuses#	53.3	53.3	54.8	53.4
Mean litter weight#	331.5	304.9	298.1	208.7***
Standard deviation	78.4	67.0	91.7	118.5
Mean foetal weight#	38.8	36.7	36.2	37.1
Standard deviation	4.7	4.7	4.0	3.9
Mean foetal weight - males only#	39.2	36.3 (17)	36.3	37.7 (17)
Standard deviation	4.3	4.7	3.8	4.0
Mean foetal weight - females only#	38.7	36.9	36.8 (18)	37.1 (16)
Standard deviation	5.2	5.2	4.3	4.9
Mean placental weight#	3.96	3.66	3.70	4.09
Standard deviation	0.69	0.63	0.65	0.65
Mean gravid uterus weight#	511.4	467.6	455.8	343.9***
Standard deviation	112.9	99.2	131.4	171.8

() = N, where different from the original

= statistically analysed * = p < 0.05 ** = p < 0.01 *** = p < 0.001

Offspring (Malformations, Variations, etc.)

All viable fetuses were 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.

Variations in fetuses included additional blood vessel on the aortic arch in HD fetuses and changes in ossification at all doses that suggests a dose-dependent delay in development.

Table: Variations in fetuses (Sponsor's)

Finding	Type	Group 1 (group mean %)	Group mean percent of fetuses for statistically significant findings (group mean % of litters)	Level of significance	Background data range (% fetuses) (% litters)
Thoracic Cavity: Aortic Arch: additional blood vessel	Variant	5.1	24 (Grp 4) (66.7)	***	0.4 – 8.5 (5 - 47.4)
Sternum: 5 th sternebra: not ossified	Variant	16.9	34.7 (Grp 2) (77.8)	***	7.5 – 31.4 (26.3 – 77.8)
Sternum: 5 th sternebra: incomplete ossification	Variant	21.2	33.1 (Grp 3) (89.5)	*	3.8 – 27.8 (26.3 – 89.5)
Sternum: 6 th sternebra: not ossified	Variant	3.9	8.8 (Grp 4) (22.2)	*	0 – 11.1 (5.3 – 38.9)
Sternum: 6 th sternebra: incomplete ossification	Variant	12.3	21.6 (Grp 2)(77.8) 20.6 (Grp 3)(57.9)	* *	4.4 – 25.9 (26.3 – 84.2)
Forelimb: epiphyses: not ossified	Variant	18.7	29.3 (Grp 2)(77.8) 29.9 (Grp 3)(84.2) 37.5 (Grp 4)(66.7)	* * *	9.6 – 44.6 (36.8 – 100)
Forelimb: Proximal or distal epiphyses of humerus only: not ossified	Variant	22.2	37.9 (Grp 3)(94.7) 34.1 (Grp 4)(72.2)	** **	20.9 – 56.6 (66.7 – 100)
Forelimb: one or more phalange: incomplete ossification	Variant	30.2	46.3 (Grp 2)(94.4)	**	15.1– 60.6 (54.5 – 100)
Hindlimb: epiphyses: not ossified	Variant	37.0	46.3 (Grp.2)(88.9) 50.5 (Grp.3)(89.5)	* *	7.1 – 58.2 (42.1 – 100)
Hindlimb: one or more phalange: incomplete ossification	Variant	5.7	18.6 (Grp 2)(50.0) 18.4 (Grp 3)(47.4) 25.8 (Grp 4)(66.7)	*** * **	3.6 – 30.3 (15.0 – 73.7)

* = p<0.05 ** = p<0.01 *** = p<0.001

Parameters which are outside historical background data ranges are in bold type.

Abnormalities in fetuses included an increase in incidences of fused sternebra, increased presacral vertebrae, and delays in ossification that suggests a dose-dependent delay in development.

Table: Abnormalities in fetuses (Sponsor's)

Finding	Type	Group 1 (group mean %)	Group mean percent of fetuses for statistically significant findings (group mean % of litters)	Level of significance	Background data range (% fetuses) (% litters)
Sternum: one or more sternebra: fused	Minor	0	8.6 (Grp 4) (22.2)	*	0 – 5.1 (0 - 21.1)
Skull: Maxilla- uni- or bilateral: incomplete ossification	Minor	20.1	42.2 (Grp 4) (55.6)	*	1.3 – 33.8 (5.3 – 76.5)
Vertebra: Number of presacral vertebra: 27	Minor	12.3	24.2 (Grp 4) (55.6)	**	2.6 – 28.7 (16.7 – 55.6)
Forelimb: one or more metacarpal: not ossified	Minor	2.8	11.2 (Grp 2) (38.9) 12.8 (Grp 4) (33.3)	** *	0 – 12.8 (0 – 47.4)
Forelimb: one or more phalange: not ossified	Minor	0	4.1 (Grp 2) (33.3) 7.3 (Grp 3) (31.6) 8.8 (Grp 4) (27.8)	** ** *	0 – 10.9 (0 – 31.6)

* = p<0.05 ** = p<0.01 *** = p<0.001

Parameters which are outside historical background data ranges are in bold type.

Tissues

At necropsy, the following organs, as listed in the study report, were prepared in formaldehyde.

Lungs		Spleen	Pituitary glands
Lymph nodes	- mesenteric - popliteal - mandibular	Thymus	Placentae Ovaries Uterus

Toxicokinetics

Blood samples were taken from TK animals on GDs 6 and 19 at 1, 2, 4, 8, and 24 hours post-dose.

The increase in ponesimod C_{max} was less than dose-proportional between the LD and MD and greater than dose-proportional between the MD and HD. The increase in ponesimod AUC was less than dose-proportional between the LD and MD and approximately dose-proportional between the MD and HD. There was accumulation of ponesimod between Days 6 and 19.

Table: Toxicokinetic data of ponesimod (Sponsor's)

GD 6

Sampling Time		Day 6 of Gestation			
Dose		0.25 mg/kg/day	1 mg/kg/day	4 mg/kg/day	
Sex		Female	Female	Female	
C_{max}	(ng/mL)	Mean	18.2	51.1	229
AUC ₀₋₂₄	(ng.hr/mL)	Mean	221	672	2650
Dose Ratio ¹	Proportionality (C_{max})			2.8	12.6
Dose Ratio ¹	Proportionality (AUC _{0-24h})			3.0	12.0

GD 19

Sampling Time		Day 19 of Gestation			
Dose		0.25 mg/kg/day	1 mg/kg/day	4 mg/kg/day	
Sex		Female	Female	Female	
C_{max}	(ng/mL)	Mean	16.8	58.9	324
AUC ₀₋₂₄	(ng.hr/mL)	Mean	216	736	3170
Dose Ratio ¹	Proportionality (C_{max})			3.5	19.3
Dose Ratio ¹	Proportionality (AUC _{0-24h})			3.4	14.7
R_0^2	(AUC _{0-24h})		1.0	1.1	1.2

¹: Dose proportionality calculated versus lower dose

²: AUC₀₋₂₄ repeat dose / AUC₀₋₂₄ single dose

9.3 Prenatal and Postnatal Development

Study title: JNJ-67896153-AAA - Dose range finding pre- and post-natal development study by the oral route (gavage) in the rat

Study no: TOX13421

Study report location: EDR

Conducting laboratory and location:



Date of study initiation: August 2, 2018

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: ACT128800, Batch № LTT4AA1001, 99.7%

Key Study Findings included 2 delivery-related deaths, 1 LD with dead pups and 1 HMD that was unable to deliver, but no drug-related deaths. Decreases in body weight gain and food consumption, increase in pre-birth loss, and a decrease in pups delivered were noted at the HMD and HD. The NOAEL for toxicity and reproductive performance in F₀ dams and F₁ generation development was 10 mg/kg/day due to weight gain decrease in dams, increase in pre-birth loss, and a decrease in pups delivered. From the data, the sponsor decided to use 20 mg/kg/day as the HD in the pivotal pre- and postnatal development study in rats.

Methods

Doses: 0, 2.5, 10, 20, and 30 mg/kg
 Frequency of dosing: Daily
 Dose volume: 10 mL/kg
 Route of administration: Oral (gavage)
 Formulation/Vehicle: 0.25% (w/v) methylcellulose (400 cP) with 0.05% (v/v) Polysorbate 80 in water for injection
 Species/Strain: Rat/Wistar Han
 Number/Sex/Group: 6 Pregnant females/group
 Age: 9-12 weeks at mating
 Weight: 178-253 g
 Deviation from study protocol: Ponesimod was administered daily from GD 6 to lactation Day 6

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

Parameters		Dose Level			
		2.5 mg/kg	10 mg/kg	20 mg/kg	30 mg/kg
G6					
t_{last}	h	24	24	24	24
t_{max}	h	2(1-2) ²	2	4(2-4) ²	4(2-4) ²
C_{max}	ng/mL	241	798	1430	2140
C_{max}/D	kg·ng/mL/mg	96.3	79.8	71.5	71.3
$AUC_{(0-24h)}$ ¹	h·ng/mL	1270	5080	10800	14600
$AUC_{(0-24h)}/D$	h·kg·ng/mL/mg	508	508	538	485
AUC_{∞}	h·ng/mL	1280	5130	11000	14700
AUC_{∞}/D	h·kg·ng/mL/mg	512	513	549	490

/D: dose-normalized to 1 mg/kg.

¹: AUC_{last} is similar to $AUC_{(0-24h)}$, since t_{last} and tau are both 24 hours.

²: median (range).

Study title: JNJ-67896153-AAA - Pre- and post-natal development study by the oral route (gavage) in the rat (Segment III)

Study no: TOX13502

Study report location: EDR

Conducting laboratory and location:



Date of study initiation: October 22, 2018

GLP compliance: Yes, (b) (4)

QA statement: Yes

Drug, lot #, and % purity: ACT128800, Batch № LTT4AA1001, 99.7%

Key Study Findings

- No drug-related deaths, adverse clinical signs, or changes in body weight were observed in F₀ HD dams.
- Reproductive findings in F₀ 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.
- There was a decrease in viability and body weight gain in HD pups between lactation Days 0 and 4.
- Post-weaning, preputial cleavage was later in ponesimod-dosed M and vaginal opening was later in ponesimod-dosed F. Ambulation and fine movements were increased in MDF, HDM, and HDF. Fertility index and pregnancy were reduced and pre- and post-implantation loss increased, with numbers of live embryos decreased in HD ponesimod F₁ offspring.
- Ponesimod was well tolerated in F₀ dams. The NOAEL for postnatal development of offspring and reproductive performance in F₁ F was less than the LD due to delayed sexual maturation.

Methods

Doses: 0, 5, 10, and 20 mg/kg
 Frequency of dosing: Daily
 Dose volume: 10 mL/kg
 Route of administration: Oral (gavage)
 Formulation/Vehicle: 0.25% (w/v) Methylcellulose (400 mPaS) with
 0.05% (v/v) Tween 80 in water
 Species/Strain: Rat/Wistar Han
 Number/Sex/Group: 22 Pregnant females/group (see tables below for F₁)
 Age: 9-12 weeks
 Weight: 178-258 g
 Deviation from study protocol: Ponesimod was administered daily from GD 6 to
 Lactation Day 20

F₁ generation selected for mating (Sponsor's)

Group number	First male mating		Group number	Second male mating with virgin naïve females	
	Males	Females		Males	Females
1	101 to 120	121 to 140	1	101 to 120	301 to 320
2	141 to 160	161 to 180	2	141 to 160	321 to 340
3	181 to 200	201 to 220	3	181 to 200	341 to 360
4	221 to 240	241 to 260	4	221 to 240	361 to 380

Observations and Results for F₀ Dams**Mortality**

Observations were conducted twice daily.

No drug-related deaths were observed. On lactation Day 2 one control dam was euthanized due to total litter loss on lactation Day 1. Two LD dams were euthanized moribund on GD 23 or GD 24 due to delivery difficulties, and 1 LD dam was euthanized after total litter resorption. One MD and 1 HD dam were not pregnant.

Clinical Signs

Observations were conducted once daily and twice daily during dosing, pre-dose and post-dose. Detailed examinations were conducted on GDs 0, 3, 6, 9, 12, 15, 18, and 20 and lactation Days 1, 4, 7, 10, 14, 17, and 21.

No adverse clinical signs were observed during the study.

Body Weight

Body weight recordings for F₀ dams were taken on GDs 0, 3, 6, 9, 12, 15, 18, and 20 and lactation Days 1, 4, 7, 10, 14, 17, and 21.

Body weight gain was comparable across the groups during gestation and lactation.

Food Consumption

Food consumption in F₀ dams were recorded on GDs 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20 and on lactation Days 1-4, 4-7, 7-10, 10-14, 14-17, and 17-21.

There was no drug-related change in food consumption during gestation, but a decrease (8%) was observed in HD dams during lactation.

Reproduction parameters

The following were recorded; date of mating, date of parturition, duration of gestation, number of implantation sites, and number, weight, and sex of pups (dead and alive), and external abnormalities.

Mean gestation length, gestation index, and implantation site numbers were similar among groups. There was a decrease in mean pre-birth loss at all doses, but similar mean number of pups were delivered compared with the control group. However, there was a decrease in viability of pups between lactation Days 0 and 4, with the viability index of 80% for the HD group compared to 100% for the control group. Mean pup weight gain PND 1-4 was decreased by 7% and 5% in HDM and HDF pups, respectively.

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

Sex: Female		0	5	10	20
Day(s) Relative to Littering (Litter: A)		mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
Females Completing Delivery [CHSQFS]	N+ve	22	19	21	21
with Liveborn Pups [CHSQFS]	N+ve	21	19	21	21
with Stillborn Pups [CHSQFS]	N+ve	3	1	2	2
with all Stillborn Pups [CHSQFS]	N+ve	0	0	0	0
with all Dead PND 21 [CHSQFS]	N+ve	1	0	0	0
Gestation Index	%	95.5	100.0	100.0	100.0
Gestation Length (Days) [GEN AN]	Mean	22.3 R ¹	22.1	22.3	22.4
	SD	0.6	0.3	0.5	0.5
	N	22	19	21	21
Number of Implantation Sites [GEN AN]	Mean	11.4 R ¹	11.8	10.8	11.6
	SD	2.7	1.9	2.0	1.9
	N	22	19	21	21
	Sum	251 R ¹	224	227	243
Pre-Birth Loss (%) [GEN AN]	Mean	11.73 R ¹	5.60	10.84	7.76
	SD	16.78	5.94	10.50	9.70
	N	22	19	21	21
Pups Delivered/Litter [GEN AN]	Mean	10.3 R ¹	11.1	9.6	10.7
	SD	3.1	1.8	2.0	2.1
	N	22	19	21	21
	Sum	227 R ¹	211	202	225

Sex: Female		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Day(s) Relative to Littering (Litter: A)					
Live Pups PND 0 [GEN AN]	Mean	10.5 I ¹	11.1	9.4	10.3
	SD	2.4	1.7	2.0	2.6
	N	21	19	21	21
	Sum	221 I ¹	210	197	216
Live Pups PND 1 [GEN AN]	Mean	10.5 I, a ²	11.1	9.3	9.3
	SD	2.4	1.7	2.0	2.6
	N	21	19	21	21
	Sum	221 I, a ²	210	196	195
Live Pups Precull [GEN AN]	Mean	10.5 I ¹	10.9	9.1 w ³	8.2 ww ⁴
	SD	2.4	1.7	2.0	2.6
	N	21	19	21	21
	Sum	221 I ¹	208	191 w ³	173 ww ⁴
Live Pups Postcull [GEN AN]	Mean	7.9 R ⁵	7.9	7.6	7.1 S ⁶
	SD	0.3	0.2	1.0	1.6
	N	21	19	21	21
	Sum	166 R ⁵	151	159	149 S ⁶
Live Pups PND 7 [GEN AN]	Mean	7.9 R ⁵	7.9	7.6	7.0 S ⁶
	SD	0.3	0.2	1.0	1.7
	N	21	19	21	21
	Sum	166 R ⁵	151	159	147 S ⁶

Sex: Female		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Day(s) Relative to Littering (Litter: A)					
Live Pups PND 10 [GEN AN]	Mean	7.9	7.9	7.6	7.0 I ¹
	SD	0.3	0.2	1.0	1.7
	N	21	19	21	21
	Sum	166	151	159	147 I ¹
Live Pups PND 14 [GEN AN]	Mean	7.9 R ²	7.9	7.6	7.0 S ³
	SD	0.5	0.2	1.0	1.7
	N	21	19	21	21
	Sum	165 R ²	151	159	147 S ³
Live Pups PND 21 [GEN AN]	Mean	7.8 R, k ⁴	7.9	7.6	7.0
	SD	0.6	0.2	1.0	1.7
	N	21	19	21	21
	Sum	163 R, k ⁴	151	159	147
Dead, Miss., Cannib. PND 0 [CHSQFS]	Sum	6	1	5	9
Dead, Miss., Cannib. PND 1-4 [CHSQFS]	Sum	0 cc ⁵	2	6 f ⁶	43 f ⁶
Dead, Miss., Cannib. PND 5-21 [CHSQFS]	Sum	3	0	0	2
Dead, Miss., Cannib. PND 0-21 [CHSQFS]	Sum	9	3	11	54
Live Birth Index (%)		97.4	99.5	97.5	96.0
Viability Index (PND 0-4) (%)		100.0	99.0	97.0	80.1
Weaning Index (PND 4-21) (%)		98.2	100.0	100.0	98.7
Sex Ratio PND 1 - % Males [CHSQFS]	Mean	52.3	55.4	57.5	52.2

Sex: Female		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Day(s) Relative to Littering (Litter: A)					
Sex Ratio PND 21 - % Males [CHSQFS]	Mean	52.0	53.7	54.7	51.8

[GEN AN] - Generalised Anova/Ancova Test

2 [I, a - Automatic Transformation: Identity (No Transformation), (All Groups) Test: Analysis of V

4 [ww - Test: Williams 2 Sided p < 0.01]

6 [S - Test: Shirley 2 Sided p < 0.05]

1 [I - Automatic Transformation: Identity (No Transformation)]

3 [w - Test: Williams 2 Sided p < 0.05]

5 [R - Automatic Transformation: Rank]

Necropsy

Necropsy of F₀ dams occurred at scheduled termination after weaning.

There were no adverse macroscopic findings in F₀ dams.

Observations and Results for F₁ Generation (pre-weaning)

On PND 4, the size of each litter was adjusted to yield, where possible, 4 M and 4 F per litter. Extra pups were sacrificed.

Mortality and Clinical signs

Observations were conducted on F₁ pups daily from birth. Detailed examinations were conducted weekly.

Deaths were noted in control (1 total litter on PND 1), and cannibalized pups were noted in control (1 pup), MD (2 pups from 1 litter), and HD (7 pups from 1 litter) groups. There was a decrease in viability at the HD, with 52 pup deaths noted prior to culling on PND 4 compared to 6, 3, and 11 in control, LD, and MD groups. Hematoma was observed in 7 control, 12 LD, 4 MD, and 7 HD pups. Pups in control (4 pups), LD (2 pups), MD (2 pups), and HD (9 pups) groups were observed as weak. One MD pup was cyanotic, 1 control, 1 LD, and 15 HD pups were pale, and 1 MD and 2 HD pups were cold to touch.

Body Weight

Body weight recordings for F₁ pups were taken on PNDs 1, 4, 7, 10, 14, 17, and 21.

Mean pup weight gain pre-weaning up to PND 21 was decreased by 8% and 5% in HDM and HDF pups, respectively.

Physical development

Observations and tests were conducted on all pups for righting reflex on PND 8, gripping reflex on PND 17, and pupillary and auditory reflexes on PND 21.

There were no drug-related changes in physical development parameters of righting reflex, gripping reflex, pupillary, and auditory reflexes.

Necropsy

Necropsy was conducted on F₁ pups and any abnormalities recorded.

No drug-related changes were observed.

Observations and Results for F₁ Generation (post-weaning)**Placement of F₁ adults (post-weaning animals)**

After weaning of the F₁ pups on PND 21, 1 M and 1 F pup per litter were selected for behavioral tests and mating to yield 20 M and 20 F per group. Unselected pups were sacrificed on PND 21

Mortality

Observations for mortality were conducted once daily for F₁ pups.

There were no F₁ deaths post weaning.

Clinical signs

Observations were conducted once daily for F₁ pups. Detailed examinations were conducted at least once weekly.

Pre-mating, chromodacryorrhea was observed in 1 MDM, 1 HDM, and 1 HDF, malocclusion in 1 MDM, 1 HDM, and 2 HDF, no palpable testis in 1 HDM. During gestation the following clinical signs were observed: desquamation in 1 control F, 1 MDF, and 3 HDF, chromodacryorrhea in 1 HDF, and malocclusion in 2 HDF.

Body Weight

Body weight recordings for M F₁ pups were taken at least twice weekly and for F F₁ pups twice weekly during pre-mating and mating periods and on GDs 0, 4, 8, and 13.

There were no drug-related decreases in body weight gain in M and in F pre-mating or during gestation.

Physical development

Vaginal opening was assessed from PND 28 and balanopreputial skinfold cleavage was assessed from PND 38.

Preputial cleavage was statistically significantly delayed by 2.7, 1.5, and 3.5 days in LDM, MDM, and HDM, and vaginal opening was statistically significantly delayed by 1.9, 0.6, and 1.7 days in LDF, MDF, and HDF.

Summary of sexual maturation findings

Day to cleavage	MALE				Day to opening	FEMALE			
	Control	LD	MD	HD		Control	LD	MD	HD
46-48	8	1	5	3	29-31	4	1	3	0
49-51	8	6	8	4	32-34	14	11	10	12
52-54	4	12	6	8	35-37	1	5	7	7
55-57	0	1	1	3	38-40	1	1	0	0
58-60	0	0	0	1	41-43	0	1	0	1
67	0	0	0	1	44-46	0	1	0	0
Average day	49.3	52.0*	50.8*	52.8*	Average day	33.0	34.9*	33.6*	34.7*
Difference	-	2.7	1.5	3.5	Difference	-	1.9	0.7	1.7

* Statistically significantly

Behavioral assessment

Tests conducted included locomotor activity at approximately 10 weeks of age, auditory startle at approximately 10 weeks of age, and water maze consisting of a multiple T-maze (learning and memory) between 8 and 9 weeks of age. In the T-water maze, performance (ability to swim) and learning and memory were assessed.

Motor activity: Ambulation and fine movements were statistically significantly increased in MDF, HDM, and HDF.

Table: Motor activity data in F₁ pups after maternal ponesimod dosing (Sponsor's)

Parameters	Dose Level					
	5 mg/kg		10 mg/kg		20 mg/kg	
	M	F	M	F	M	F
Fine movements	-	-	-	+ 20%**	+ 38%**	+ 65%***
Ambulatory movements	-	-	+ 32%	+34%**	+ 70%***	+86%***

** : p≤0.01; *** : p≤0.001; M: Male; F: Female.

Auditory startle response: Auditory startle habituation was comparable among groups in M and F.

Multiple T-maze: Swimming ability, completion times, and number of errors in the T-maze were comparable between ponesimod-dosed and control groups in M and F.

Reproduction parameters – male and female ponesimod offspring

Approximately 8 weeks post-weaning, mating pairs (1 M and 1 F) were set up for up to 2 weeks. 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 embryos, 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 LD and MD and control groups. However, differences were observed at the HD, i.e., decrease in the fertility index (78% compared to 95% for control); decrease in pregnant dams (13 HDF compared to 18 control), increase in pre-implantation and post-implantation loss, and a decrease in the number of live embryos.

Table: Caesarean data for F₁ females (Sponsor's)

Sex: Female		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Day(s) Relative to Mating (Litter: A)					
Females Pregnant	N	18	17	17	13
Dams with Viable Embryos		18	17	17	13
No. of Corpora Lutea [GEN AN]	Mean	14.5 R ¹	13.5	13.1	12.2
	SD	1.9	2.0	3.4	4.2
	Sum	261 R ¹	230	222	158
No. of Implantations [GEN AN]	Mean	13.7 R ¹	13.2	12.4	11.2
	SD	2.2	2.0	3.8	4.8
	Sum	247 R ¹	224	210	146
Pre-Implant Loss (%) [KWLWCX]	Mean	5.55	2.55	6.10	11.18
	SD	8.39	5.21	11.40	15.11
	N	18	17	17	13
Post-Implantation Loss [GEN AN]	Mean	1.1 R ¹	0.8	0.7	1.2
	SD	1.0	1.1	0.8	0.9
	Sum	19 R ¹	14	12	15
Post-Implantation Loss (%) [KWLWCX]	Mean	7.77	5.78	6.34	12.75
	SD	7.22	6.78	7.46	12.05
	N	18	17	17	13
No. of Live Embryos [GEN AN]	Mean	12.7 R ¹	12.4	11.6	10.1
	SD	2.3	1.6	3.8	4.8
	N	18	17	17	13

Reproduction parameters – male ponesimod offspring with naïve females

Following ponesimod F₁ F cesarean procedures, ponesimod F₁ M were mated with virgin untreated F (1:1) for up to 2 weeks. 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 embryos, and resorptions. The testes and epididymides of all M were retained.

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 among all groups.

Table: Cesarean data for F₁ naïve females (Sponsor's)

Sex: Female		0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Day(s) Relative to Mating (Litter: A)					
Females Pregnant	N	18	15	15	17
Dams with Viable Embryos		18	15	15	17
No. of Corpora Lutea [GEN AN]	Mean	13.0 R ¹	13.7	12.9	12.8
	SD	2.1	4.7	2.9	1.8
	Sum	234 R ¹	205	193	217
No. of Implantations [GEN AN]	Mean	12.3 R ¹	12.9	11.9	11.6
	SD	3.1	4.5	3.6	2.9
	Sum	221 R ¹	194	178	198
Pre-Implant Loss (%) [KWLWCX]	Mean	7.18	4.78	10.44	9.83
	SD	17.53	8.21	16.79	15.16
	N	18	15	15	17
Post-Implantation Loss [GEN AN]	Mean	1.0 R ¹	0.9	0.9	0.9
	SD	1.0	1.4	1.0	1.2
	Sum	18 R ¹	14	13	16
Post-Implantation Loss (%) [KWLWCX]	Mean	7.72	7.71	6.99	9.75
	SD	7.77	10.60	8.47	16.57
No. of Live Embryos [GEN AN]	Mean	11.3 R ¹	12.0	11.0	10.7
	SD	3.0	4.6	3.5	3.5
	N	18	15	15	17

Necropsy

Gross examination was conducted on all animals. The testes and epididymides of all M and ovaries, oviducts, uterus, cervix, and vagina of all F (ponesimod and naïve) were examined.

No adverse macroscopic findings were noted.

Toxicokinetics

Maternal blood samples were taken on lactation Days 4 and 12 at 0.5, 1, 2, 4, 8, and 24 hours post-dose. Blood samples were taken from 2 pups/dam on lactation Days 4 and 12 at 2, 4, 8, and 24 hours post-dosing of dams. Samples were analyzed for ponesimod.

In dams, increase in ponesimod plasma exposure (C_{max} and AUC) was generally dose-proportional and similar on lactation Days 4 and 12; T_{max} was 2 hours. In pups, increase in ponesimod plasma exposure (C_{max} and AUC) was generally dose-proportional between the LD and MD and was less than dose-proportional between the MD and HD; T_{max} was 8 hours. Plasma exposure in dams was greater than 10-fold the plasma exposure measured in pups.

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

Parameters		Dose Level		
		5 mg/kg	10 mg/kg	20 mg/kg
L4				
t_{last}	h	24	24	24
t_{max}	h	2	2	2
C_{max}	ng/mL	469	1200	1740
C_{max}/D	kg·ng/mL/mg	93.7	120	87.2
$AUC_{(0-24h)}^1$	h·ng/mL	2520	5160	8620
$AUC_{(0-24h)}^1/D$	h·kg·ng/mL/mg	503	516	431
L12				
t_{last}	h	24	24	24
t_{max}	h	2	2	2
C_{max}	ng/mL	395	772	1550
C_{max}/D	kg·ng/mL/mg	78.9	77.2	77.7
$AUC_{(0-24h)}^1$	h·ng/mL	1920	4240	7760
$AUC_{(0-24h)}^1/D$	h·kg·ng/mL/mg	383	424	388

/D: Dose-normalized to 1 mg/kg; ¹: AUC_{last} is similar to $AUC_{(0-24h)}$, since t_{last} is 24 hours.

Table: Pup toxicokinetic data for ponesimod (Sponsor's)

Parameters		Dose Level					
		5 mg/kg		10 mg/kg		20 mg/kg	
		M	F	M	F	M	F
PND4							
t_{last}	h	24	24	24	24	24	24
t_{max}	h	8	8	8	8	8	8
C_{max}	ng/mL	16.9	14.4	26.2	41.9	54.6	51.0
C_{max}/D	kg·ng/mL/mg	3.38	2.89	2.62	4.19	2.73	2.55
$AUC_{(0-24h)}^1$	h·ng/mL	207	200	406	427	858	816
$AUC_{(0-24h)}^1/D$	h·kg·ng/mL/mg	41.4	40.1	40.6	42.7	42.9	40.8
PND12							
t_{last}	h	24	24	24	24	24	24
t_{max}	h	8	8	8	8	8	8
C_{max}	ng/mL	13.8	13.3	17.6	17.1	54.0	54.3
C_{max}/D	kg·ng/mL/mg	2.77	2.65	1.76	1.71	2.70	2.72
$AUC_{(0-24h)}^1$	h·ng/mL	165	160	244	285	723	757
$AUC_{(0-24h)}^1/D$	h·kg·ng/mL/mg	33.0	32.1	24.4	28.5	36.1	37.9

/D: Dose-normalized to 1 mg/kg; ¹: AUC_{last} is similar to $AUC_{(0-24h)}$, since t_{last} is 24 hours.

M: Male; F: Female.

9.4 Juvenile Animal Studies

Study Title: Ponesimod: Oral (Gavage) Dose Range-Finding Toxicity Study in the Juvenile Rat

Study no.: T-15.063
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: November 2, 2015
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: ACT-128800, Batch No: 605930003, 100.8%

Key Study Findings included body weight decrease in HDM. Decreased WBCs and lymphocytes and medullary hypertrophy in the thymus were noted, which are consistent with the pharmacological action of ponesimod. Increased lung weight and incidences of incomplete lung collapse were noted at all doses and lung microscopic findings included osseous metaplasia, edema, and alveolar histiocytosis in the lungs. No premature deaths or adverse clinical signs were noted. The sponsor considered 100 mg/kg/day suitable HD for the pivotal juvenile study.

Methods

Doses: 0, 10, 30, and 100 mg/kg
 Frequency of dosing: Daily
 Number/Sex/Group
 Main: 6
 Toxicokinetic: 6 control and 18 LD, MD, and HD
 Dose volume: 10 mL/kg
 Formulation/Vehicle: 0.25 % (w/v) Methylcellulose containing
 0.05 % (v/v) Tween 80
 Route of administration: ORAL GAVAGE
 Species: Rat
 Strain: Wistar Han
 Age at start of experiment: 28 days old
 Comment on study design and conduct: Animals were dosed daily PND 28-42.

Histological Findings

The main microscopic findings were medullary hypertrophy in the thymus in HDM, MDF, and HDF. Alveolar histiocytosis and edema were observed in all groups, including control animals.

Summary of selected microscopic findings

Tissue	Number: Finding	MALE				FEMALE			
		6	6	6	6	6	6	6	6
		0	10	30	100	0	10	30	100
Lungs	Alveolar histiocytosis								
	minimal:	2	5	5	4	2	3	4	1
	slight:	0	0	1	1	0	1	2	3
	moderate:	0	0	0	1	0	0	0	2
	Alveolar edema								
	minimal:	0	4	1	4	0	2	3	3
	slight:	0	1	0	0	0	0	2	0
Thymus	Osseous metaplasia								
	Minimal:	0	0	0	3	0	1	1	1
	Medullary hypertrophy								
	minimal:	0	0	0	3	0	0	2	4
	slight:	0	0	0	0	0	0	0	1

Table: Ponesimod plasma levels (Sponsor's)

Day of dosing	Toxicokinetic parameter	Dose (mg/kg/day)					
		10	30	100	10	30	100
		M	M	M	F	F	F
Day 28	C _{max} (ng/mL)	1460	2690	6200	1650	2730	7080
	T _{max} (h)	2	2	2	2	2	4
	AUC _{last} (ng.h/mL)	5340	14500	44600	7330	16500	54600
	T _{last} (h)	8	24	24	24	24	24
Day 42	C _{max} (ng/mL)	682	2570	4900	836	3690	6930
	T _{max} (h)	1	2	2	1	2	2
	AUC _{last} (ng.h/mL)	1990	13000	30400	3230	22300	56600
	T _{last} (h)	8	24	24	24	24	24

Study Title: Ponesimod: 9 Week Oral (Gavage) Toxicity Study in the Juvenile Rat with a 4 Week Treatment Free Period

Study no.: T-16.018
 Study report location: EDR
 Conducting laboratory and location:



Date of study initiation: January 5, 2017
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: ACT-128800, Batch №: 605930003, 100.8%

Key Study Findings:

- There were 2 premature deaths; 1 control M and 1 HDF. Neither were considered drug-related. A single convulsive incidence was observed in 1 HMDF.
- There was a slight decrease in mean body weight gain at the LMD, HMD, and HD in M and F.
- WBC count, especially lymphocytes, was decreased in HMDM, HDM, LMDF, HMDF, and HDF, consistent with the pharmacological action of ponesimod. The decrease only partial recovered. Lymphocyte subset analysis demonstrated a dose-dependent decrease in T-cells and T-helper cells and an increase in cytotoxic T-cells and NK-cells.
- No apparent changes in auditory startle and learning and memory behaviors were observed at any dose. A slight increase in motor activity was observed in HDM
- The lymphoid organs had histological findings of decreased cellularity, consistent with the pharmacological action of ponesimod. Other microscopic findings were observed in liver (hypertrophy) at the HD and lungs (osseous metaplasia and alveolar histiocytosis); alveolar histiocytosis in lungs was observed at all doses including control animals with increased severity observed at the HMD and HD.
- The TDAR results suggest that there is immune suppression during dosing of ponesimod with partial recovery observed.
- No adverse changes were observed in developmental landmarks, reproduction, or behavior, so the NOAEL for these was concluded to be the HD (100 mg/kg/day) associated ponesimod plasma exposures (AUC) on PND 91 of 33200 and 61300 ng*h/mL in M and F, respectively.
- The sponsor considered ponesimod well tolerated at all doses and the HD the NOAEL. The sponsor noted that the increased lung weight and alveolar histiocytosis observed at the HMD and HD were expected from the adult data, were reversible, and did not appear to alter function. However, these types of histopathological changes were noted as adverse in adults. Therefore, the NOAEL is the LMD (10 mg/kg/day) associated with ponesimod plasma exposures (AUC) on PND 91 of 3790 and 6950 ng*h/mL in M and F, respectively.

Methods

	Doses:	0, 1, 10, 30, and 100 mg/kg
	Frequency of dosing:	Daily
	Number/Sex/Group	
	Main (Subset I):	12
	Recovery (Subset II):	6
Behavior and Reproduction (Subset III):		20
Toxicokinetic (Subset IV):		6 control and 18 LD, LMD, HMD, and HD
Immunotoxicity (Subset V):		10
	Dose volume:	10 mL/kg
	Formulation/Vehicle:	0.25 % (w/v) Methylcellulose containing 0.05 % (v/v) Tween 80
	Route of administration:	ORAL GAVAGE

Species: Rat
Strain: Wistar Han
Age at start of experiment: 28 days old
Period of development studied: PND 28-91
Comment on study design and conduct: Animals were dosed daily PND 28-91.

Observations and Results

Mortality

Animals were observed twice daily for mortality and morbidity.

Premature deaths that occurred during dosing included 1 control M on PND 40 and 1 HDF on PND 91. Neither death was drug-related.

Clinical Signs

Observations were conducted daily. Detailed examinations were conducted once weekly.

A single convulsive incidence was observed in 1 HMDF on PND 96.

Body Weight

In Subsets I, II, IV, and V, body weights were recorded on PNDs 28 and twice weekly thereafter. In Subset III, body weights were recorded twice weekly until pairing. During pairing Subset III F body weights were recorded weekly until mating was confirmed and following confirmation of mating on GDs 0, 3, 7, and 13. Subset III M body weights were recorded weekly during and after pairing.

In M, there was a decrease (11%) in mean body weight gain over the first week of dosing at the HD. Over the dosing period, a statistically significant decrease in mean body weight gain was observed at the LMD (5%), HMD (6%), and HD (9%). In F, there was a decrease in mean body weight gain over the first week of dosing of 7% at the HD. Over the dosing period, a statistically significant decrease in mean body weight gain was observed at the LMD (4%), HMD (6%), and HD (2%). Mean body weight gain of HDF during gestation (Days 0-13) was decreased by 5%. Mean body weight gain during the recovery period did not decrease in ponosimod dosed animals compared to control animals.

Food Consumption

In Subsets I and II, food consumption was recorded twice weekly from PND 28.

There was no drug-related effect on food consumption.

Ophthalmoscopy

Examination of Subsets I and II animals was conducted once prior to initiation of dosing (PND 25/26) and at the end of dosing for Subset I control and HD animals.

Generally, there were no adverse changes observed, although slight retina hyperreactivity was observed in 1 HDM.

Hematology

Blood samples were collected toward the end of dosing from Subset I and toward the end of recovery from Subset II animals.

There was a statistically significant increase in platelets in HDF and in reticulocytes in HMDM, HDM, and HDF. Decreases in WBC, lymphocytes, basophils, and LUCs were observed in HMDM, HDM, LMDF, HMDF, and HDF. These decreases only partially recovered.

Table of changes in hematology parameters

Dose	Phase	WBC		Lymphocytes		Basophils		LUC		Platelets		Reticulocytes	
		Mean	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
MALE													
0	Dosing:	6.549	0%	5.436	0%	0.033	0%	0.046	0%	834.0	0%	0.1999	0%
	Recovery:	6.883	0%	5.815	0%	0.043	0%	0.030	0%	760.7	0%	0.2038	0%
LD	Dosing:	6.261	-4%	5.239	-4%	0.038	+15%	0.044	-4%	837.5	0%	0.1973	-1%
	Recovery:	5.987	-13%	4.812	-17%	0.020	-53%	0.030	0%	720.0	-5%	0.2107	+3%
LMD	Dosing:	6.354	-3%	5.380	-1%	0.029	-12%	0.040	-13%	826.0	-1%	0.2118	+6%
	Recovery:	6.478	-6%	5.433	-7%	0.032	-26%	0.030	0%	744.0	-2%	0.1998	-2%
HMD	Dosing:	4.385	-33%*	3.463	-36%*	0.020	-39%*	0.023	-50%*	902.1	+8%	0.2228	+11%*
	Recovery:	5.220	-24%	4.187	-23%	0.025	-42%	0.020	-33%	753.5	-1%	0.1973	-3%
HD	Dosing:	3.780	-42%*	2.880	-47%*	0.020	-39%*	0.019	-59%*	884.1	+6%	0.2435	+22%*
	Recovery:	6.310	-8%	5.147	-5%	0.030	-30%	0.023	-23%	783.2	+3%	0.1977	-3%
FEMALE													
0	Dosing:	4.622	0%	3.823	0%	0.028	0%	0.030	0%	757.3	0%	0.2080	0%
	Recovery:	5.275	0%	4.370	0%	0.027	0%	0.025	0%	696.2	0%	0.2108	0%
LD	Dosing:	4.843	+5%	4.018	+5%	0.028	0%	0.031	+3%	793.3	+5%	0.1974	-5%
	Recovery:	4.465	-15%	3.767	-14%	0.015	-44%	0.018	-28%	676.8	-3%	0.1942	-8%
LMD	Dosing:	3.671	-21%*	1.456	-62%*	0.018	-36%	0.021	-30%	886.3	+17%*	0.2115	+2%
	Recovery:	4.403	-17%	3.760	-14%	0.020	-26%	0.015	-40%	716.0	+3%	0.2057	-2%
HMD	Dosing:	2.016	-56%*	1.394	-64%*	0.008	-71%*	0.011	-63%*	843.2	+11%*	0.2091	+1%
	Recovery:	4.433	-16%	3.752	-14%	0.017	-37%	0.015	-40%	781.7	+12%	0.1940	-8%
HD	Dosing:	1.437	-69%*	0.638	-83%*	0.005	-82%*	0.005	-83%*	876.7	+16%*	0.2525	+21%*
	Recovery:	4.248	-19%	3.505	-20%	0.015	-44%	0.017	-32%	762.5	+10%	0.1758	-17%*

* Statistically significant from control

Clinical Chemistry

Blood samples were collected toward the end of dosing from Subset I and toward the end of recovery from Subset II animals.

There was a statistically significant increase in cholesterol, glucose, and phosphorus and decrease in triglycerides and calcium. Effects on triglycerides and calcium only partially recovered.

Table: Changes in selected clinical chemistry parameters

Dose	Sample	Creatinine		Cholesterol		Triglycerides		Glucose		Calcium		Phosphorus	
		Mean	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
MALE													
0	Dosing:	0.203	0%	64.7	0%	167.9	0%	177.4	0%	9.76	0%	4.84	0%
	Recovery:	0.217	0%	60.3	0%	145.5	0%	186.7	0%	10.18	0%	4.97	0%
LD	Dosing:	0.230	+13%*	58.6	-9%	153.7	-8%	196.2	+11%	9.53	-2%	5.18	+7%
	Recovery:	0.240	+11%	59.7	-1%	144.7	-1%	205.0	+10%	9.80	-4%*	4.90	-1%
LMD	Dosing:	0.232	+14%*	71.5	+11%	164.9	-2%	214.3	+21%*	9.34	-4%*	5.92	+22%*
	Recovery:	0.230	+6%	58.0	-4%	172.5	+19%	204.3	+9%	9.78	-4%*	5.02	+1%
HMD	Dosing:	0.233	+15%*	66.5	+3%	140.5	-16%*	210.8	+19%*	9.29	-5%*	5.21	+8%*
	Recovery:	0.250	+15%*	56.7	-6%	148.0	+2%	218.5	+17%	9.83	-3%*	5.02	+1%
HD	Dosing:	0.219	+8%*	83.9	+30%*	120.3	-28%*	207.3	+17%*	9.40	-4%*	5.66	+17%*
	Recovery:	0.258	+19%*	58.0	-4%	186.0	+28%	205.2	+10%	9.80	-4%*	5.07	+2%
FEMALE													
0	Dosing:	0.216	0%	52.3	0%	114.2	0%	173.2	0%	9.23	0%	4.40	0%
	Recovery:	0.227	0%	54.2	0%	131.2	0%	182.7	0%	9.70	0%	4.50	0%
LD	Dosing:	0.229	+6%	57.2	+9%	135.3	+18%	174.5	+1%	9.59	+4%	4.45	+1%
	Recovery:	0.240	+6%	57.0	+5%	151.3	+15%	184.8	+1%	9.60	-1%	4.45	-1%
LMD	Dosing:	0.224	+4%	59.0	+13%	112.8	-1%	174.6	+1%	9.18	-1%	4.60	+5%
	Recovery:	0.245	+8%*	45.2	-17%	138.5	+6%	180.5	-1%	9.68	0%	4.65	+3%
HMD	Dosing:	0.235	+9%	67.9	+30%*	103.0	-10%	188.0	+9%*	9.38	+2%	4.86	+10%
	Recovery:	0.252	+11%*	49.2	-9%	165.2	+26%	179.3	-2%	9.72	0%	4.08	-9%
HD	Dosing:	0.228	+6%	84.8	+62%*	91.0	-20%	193.5	+12%*	9.16	-1%	5.17	+18%*
	Recovery:	0.257	+13%*	59.7	+10%	169.8	+29%	194.7	+7%	9.50	-2%	3.95	-12%*

* Statistically significant from control

Urinalysis

Urine samples were collected toward the end of dosing from Subset I and toward the end of recovery from Subset II animals.

There were no adverse effects on urine parameters.

Sexual Maturation

In Subset III, vaginal opening was assessed in F from PND 25 until development, and balanopreputial separation was assessed in M from PND 35 until development.

No drug-related adverse changes were observed.

Reproductive Capacity

On PND 119/120, one F was mated, up to 10 days, with one M from the same dose group from Subset III. F were checked for mating by examination of the vaginal lavage for spermatozoa and the presence of a vaginal plug. F were euthanized on GD 13 and checked for pregnancy status, corpora lutea number, implantation number, resorptions, and dead and live embryos. M were euthanized after confirmation of F pregnancy status and sperm evaluated for motility and concentration. Sperm morphology was evaluated in control and HD M.

No drug-related adverse effects were observed on mating, fecundity, or fertility indices, and no statistically significant changes in all cesarean parameters and sperm parameters.

CNS/ Neurobehavioral Assessment

Tests were conducted on Subset III animals, for auditory startle, motor activity, and learning and memory (Morris water maze) toward the end of dosing and recovery in control and HD (15/sex/group).

No effects were observed on auditory startle or water maze. There was a statistically significant increase in motor activity in 1 of 6 ten-minute periods in HDM that did not fully recover.

Bone Evaluation

Length between the knee and heel of the left hindlimb was measured for 10/sex/group in Subset I and 6/sex/group in Subset II.

The bone length increase from Day 28 to 91 was decreased in HMDM (1%), HDM (5%), LMDF (5%), HMDF (1%), and HDF (9%).

Gross Pathology

Subsets I and II animals were euthanized on Days 93-97 and 121-124, respectively.

There were no adverse macroscopic findings noted.

Organ Weights

At necropsy, the following organs as listed in the study report were weighed from Subset I and II animals.

adrenal glands	pituitary
brain	popliteal lymph nodes
epididymides	prostate & seminal vesicles (incl.
heart	coagulating
	gland)
kidneys	spleen
liver	submandibular lymph nodes
lungs	testes
mesenteric lymph nodes	thymus
ovaries	thyroids (including parathyroids)

There was an increase in lung weight in HMDM, HDM, and HDF and in thyroid weight at all doses in M, and in liver weight in HDM and HDF. Changes in lymphoid organ weights included decreases in all lymph nodes at most doses in M and F, and at all doses in M and HDF. These changes were generally reversed or partial reversed at the end of the recovery period.

Table: Organ changes in mean absolute weight compared to control

	Adrnl	Brain	Epi	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prost/ Ovary	Spleen	Testes	Thym	Thyroid
								Sub	Me	P						
MALE																
LD:	-3% (+9%)	-1% (-4%)	-1% (+3%)	-7% (0%)	-5% (+1%)	-3% (-4%)	-7% (+3%)	-1% (+20%)	-12% (+60%)	+21% (-16%)	-12% (+12%)	-1% (+4%)	-2% (-5%)	+3% (+7%)	-1% (+8%)	+29%* (+1%)
LMD:	+3% (+11%)	-3% (-3%)	0% (+6%)	0% (+2%)	0% (+4%)	-3% (+4%)	0% (+7%)	-23% (+31%)	0% (+59%)	-23% (-11%)	-20% (+12%)	-3% (+15%)	-7% (0%)	+2% (+2%)	+4% (+16%)	+30%* (+6%)
HMD:	-7% (+1%)	0% (-3%*)	-1% (+1%)	-4% (+1%)	0% (0%)	0% (+2%)	+10%* (+5%)	-23% (-10%)	-8% (+63%)	-30% (+4%)	-9% (+2%)	-12% (+18%)	-9% (-2%)	+5% (-3%)	-9% (-5%)	+21%* (+4%)
HD:	-2% (+6%)	+1% (-4%*)	+4% (+8%)	+2% (-5%)	+4% (-11%)	+9% (-7%)	+20%* (-6%)	-20% (0%)	-14% (+19%)	-11% (-22%)	0% (-6%)	-3% (-2%)	-10% (-14%)	+7%* (+5%)	-2% (-17%)	+27%* (-3%)
FEMALE																
LD:	+5% (-10%)	0% (-3%)	-	+2% (-5%)	+4% (-3%)	+2% (+1%)	+2% (-3%)	+31%* (+12%)	+13% (-16%)	-24% (+5%)	+15% (-8%)	-3% (-7%)	+4% (-8%)	-	+7% (-18%)	+17% (-28%*)
LMD:	-2% (+14%)	0% (+2%)	-	0% (-6%)	+3% (+2%)	0% (0%)	+2% (+7%)	+1% (+22%)	-14% (+6%)	-22% (+31%)	+3% (-17%)	-3% (+2%)	0% (-4%)	-	+6% (-1%)	+20% (-27%*)
HMD:	-2% (+1%)	+1% (+1%)	-	+2% (-8%)	+1% (0%)	0% (+3%)	+5% (+5%)	-10% (+39%)	-14% (-2%)	-32% (-25%)	+12% (-21%)	-2% (+1%)	+3% (0%)	-	+7% (+1%)	+4% (-17%*)
HD:	-2% (+8%)	+4%* (0%)	-	0% (-1%)	+4% (+1%)	+13%* (+17%*)	+29%* (+12%)	-17% (+12%)	-32%* (-6%)	-19% (-14%)	+4% (0%)	0% (-6%)	-7% (+1%)	-	+10% (-4%)	+13% (-28%*)

* Statistically significant from control; recovery values in parenthesis; Adrnl – Adrenal; Epi – epididymides; Sub – mandibular; Me – mesenteric; P – popliteal; Pit – pituitary; Prost – prostate. Thym – thymus.

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

	Adrnl	Brain	Epi	Heart	Kidney	Liver	Lung	Lymph Nodes			Pit	Prost/ Ovary	Spleen	Testes	Thym	Thyroid
								Sub	Me	P						
MALE																
LD:	+1% (+11%)	+3% (-1%)	+3% (+6%)	-3% (+4%)	-1% (+2%)	+1% (-1%)	-3% (+5%)	+2% (+21%)	-7% (+65%)	+25% (-12%)	-8% (+15%)	-3% (+6%)	+2% (-2%)	+7% (+10%)	+3% (+11%)	+32%* (+6%)
LMD:	+5% (+7%)	0% (-5%)	+3% (+3%)	+3% (0%)	+3% (+1%)	0% (+1%)	+4% (+3%)	-23% (+27%)	+2% (+54%)	-22% (-15%)	-21% (+5%)	0% (+11%)	-5% (-3%)	+5% (0%)	+7% (+12%)	+32%* (+3%)
HMD:	-3% (+4%)	+5% (-1%)	+4% (+3%)	+1% (+3%)	+4%* (+1%)	+5% (+4%)	+16%* (+8%)	-19% (-9%)	-4% (+65%)	-30% (+8%)	-4% (0%)	-7% (+21%)	-5% (0%)	+11% (-1%)	-5% (-3%)	+26%* (+7%)
HD:	+1% (+17%)	+5% (+8%)	+7% (+22%*)	+5% (+6%)	+7%* (-1%)	+12%* (+4%)	+24%* (+5%)	-18% (+10%)	-12% (+32%)	-10% (-12%)	+4% (0%)	0% (+9%)	-7% (-4%)	+9% (+18%*)	+1% (-6%)	+30%* (+9%)
FEMALE																
LD:	+1% (-8%)	-3% (-1%)	-	-1% (-3%)	0% (0%)	-1% (+4%)	-1% (-1%)	+28% (+16%)	+9% (-15%)	-26% (+7%)	+12% (-6%)	-7% (-4%)	+1% (-5%)	-	+3% (-15%)	+14% (-26%*)
LMD:	-1% (+14%)	+2% (+2%)	-	+2% (-6%)	+5% (+3%)	+2% (+1%)	+4% (+7%)	+4% (+21%)	-12% (+4%)	-23% (+29%)	+5% (-17%)	-3% (+2%)	+1% (-4%)	-	+7% (-1%)	+21% (-26%*)
HMD:	+1% (+5%)	+5% (+4%)	-	+7% (-5%)	+6% (+4%)	+5% (+7%*)	+10%* (+9%)	-5% (+43%)	-10% (+2%)	-30% (-24%)	+17% (-19%)	+2% (+5%)	+8% (+3%)	-	+12% (+4%)	+8% (-14%*)
HD:	0% (+4%)	+8%* (-4%)	-	+3% (-5%)	+7% (-3%)	+17%* (+12%*)	+33%* (+7%)	-15% (+7%)	-29%* (-9%)	-18% (-19%)	+7% (-4%)	+3% (-10%)	-4% (-3%)	-	+13% (-9%)	+16% (-32%*)

* Statistically significant from control; recovery values in parenthesis; Adrnl – Adrenal; Epi – epididymides; Sub – mandibular; Me – mesenteric; P – popliteal; Pit – pituitary; Prost – prostate. Thym – thymus.

Histopathology

At necropsy, the following organs, as listed in the study report, were collected from Subset I and II animals. Microscopic examination included tissues from all unscheduled euthanized animals, all control and HD Main study animals, and target organs (liver, lung, mesenteric, mandibular, and popliteal lymph nodes, spleen, and thymus) from LD, LMD, and HMD Subset I animals.

adrenal glands	pancreas
aorta	pituitary
bone marrow smear	popliteal lymph nodes
brain (3 levels examined)	<i>preputial / clitoral glands</i>
caecum	prostate & seminal vesicles
colon	rectum
duodenum	salivary gland (mandibular/sublingual/ parotid)
epididymides	sciatic nerve
eyes (including optic nerves)	site of mammary gland
femur & joint (including marrow)	skeletal muscle
Harderian glands	skin
head ^(a) nasal cavity (1 to 4 levels) ^(b)	spinal cord (3 levels examined)
nasopharynx / nares ^(c)	spleen
Zymbal's gland ^(c)	stomach (glandular & non-glandular)
heart	submandibular lymph nodes
ileum	testes
jejunum (including Peyer's patch)	thymus
kidneys	thyroids (including parathyroids)
<i>lacrimal glands</i>	tongue ^(c)
<i>laryngeal pharynx</i>	trachea
larynx	ureters
liver	urinary bladder
lungs (including mainstem bronchi)	uterus (including horn/corpus/uterine cervix)
mesenteric lymph nodes	vagina
oesophagus	all gross lesions
ovaries	
<i>oviducts</i>	

^{a)} the head was fixed whole and stated tissues were taken during slide preparation

^{b)} level 3 examined only

^{c)} processed to slide but not microscopically examined

The tissues in *italics* were held in fixative and not processed further.

Adequate Battery: Yes.

Peer Review: No.

Signed Pathology report: Yes, by (b) (6)

Histological Findings

The main microscopic findings were noted in liver, lungs, and lymphoid tissues. Findings in lymphoid tissues included decreased cellularity in lymph nodes (mandibular, mesenteric, and popliteal) and spleen and medullary hypertrophy in the thymus at the HMD and HD. These changes are consistent with the pharmacological effect of ponesimod. Other microscopic findings included hypertrophy in the liver at the HD and alveolar histiocytosis and osseous metaplasia in the lungs at all doses; alveolar histiocytosis was also observed in control animals with the increase in severity observed in HMD and HD animals. In general, these findings recovered.

Summary of selected microscopic findings

Tissue	Finding	MALE						FEMALE							
		Main					Rec.	Main					Rec.		
		Number:	12 (1)	12	12	12	12	6	6	12	12	12	12 (1)	6	6
		0	1	10	30	100	0	100	0	1	10	30	100	0	100
Liver	Hypertrophy														
	minimal:	0 (0)	0	0	0	4	0	0	0	0	0	0	3 (0)	0	0

Tissue	Finding	MALE						FEMALE							
		Main				Rec.		Main				Rec.			
		12 (1)	12	12	12	12	6	6	12	12	12	12	12 (1)	6	6
Number:	0	1	10	30	100	0	100	0	1	10	30	100	0	100	
Lungs	Alveolar histiocytosis														
	minimal:	5 (0)	7	8	9	9	3	5	2	8	5	11	6 (1)	3	3
	slight:	0 (0)	0	0	1	2	0	0	0	0	0	1	3 (0)	0	0
	moderate:	0 (0)	0	0	0	0	0	0	0	0	0	0	2 (0)	0	0
	Osseous metaplasia														
	minimal:	0 (0)	2	1	0	4	1	2	0	0	2	0	2 (0)	0	0
	Alveolitis														
Minimal:	0 (0)	0	2	0	0	0	0	0	1	2	1	0 (0)	0	0	
Focal inflammation															
Minimal:	0 (0)	0	0	0	1	0	0	0	0	0	0	0 (0)	0	0	
Lymph Node - Mandibular	Decreased cellularity														
	minimal:	0 (0)	0	0	1	3	0	0	0	0	0	1	7 (0)	0	0
Lymph Node - Mesenteric	Decreased cellularity														
	minimal:	0 (0)	0	0	1	2	0	0	0	0	5	7	4 (1)	0	0
	slight:	0 (0)	0	0	0	0	0	0	0	0	0	3	8 (0)	0	0
	Congestion														
minimal:	3 (0)	2	1	1	5	1	0	1	0	0	1	9 (0)	0	0	
slight:	1 (0)	0	0	0	1	0	0	0	0	0	0	0 (0)	0	0	
Lymph Node - Popliteal	Decreased cellularity														
minimal:	0 (0)	0	0	0	8	0	0	0	0	0	0	0	6 (0)	0	0
Sciatic nerve	Nerve fiber degeneration														
minimal:	0 (0)	-	-	-	1	0	0	0	-	-	-	-	0 (0)	0	1
Spleen	Decreased cellularity														
	minimal:	0 (0)	0	0	2	6	0	0	0	0	0	4	1 (1)	0	0
	slight:	0 (0)	0	0	0	0	0	0	0	0	0	1	11 (0)	0	0
	Increased cellularity														
minimal:	0 (0)	0	0	0	0	0	0	0	0	0	0	0	7 (0)	0	0
Thymus	Medullary hypertrophy														
	minimal:	0 (0)	0	0	2	1	0	0	0	0	1	0	0 (0)	0	0
	slight:	0 (0)	0	0	5	4	0	0	0	0	3	0	0 (0)	0	0
	moderate:	0 (0)	0	0	5	7	0	0	0	0	0	10	6 (1)	0	0
	marked:	0 (0)	0	0	0	0	0	0	0	0	0	2	6 (0)	0	0

Early death data in parenthesis. Rec. – Recovery.

Toxicokinetics

Blood samples were collected on PNDs 28 and 91 from Subset IV animals, at 2 and 24 hours post-dose for control animals and at 0.5, 1, 2, 4, 8, and 24 hours post-dose from LD, LMD, HMD, and HD.

The increase in ponesimod plasma exposure (C_{max} and AUC) was approximately dose-proportional and was similar between the sexes on PND 28 but was greater in F on PND 91. T_{max} was at 1-2 hours in M and F. No accumulation was observed between PNDs 28 and 91.

Table: Ponesimod plasma levels (Sponsor's)

Dose (mg/kg/day)	Day	Sex	Tmax (h)	Cmax (ng/mL)	AUClast (h*ng/mL)	Tlast (h)	Cmax/D (ng/mL)/(mg/kg/day)	AUClast/D (h*ng/mL)/(mg/kg/day)
1	28	F	2	96.8	363	8	96.8	363
1	28	M	2	78.6	333	8	78.6	333
1	91	F	1	116	613	24	116	613
1	91	M	1	110	476	24	110	476
10	28	F	1	1040	5640	24	104	564
10	28	M	2	1130	5790	24	113	579
10	91	F	1	1190	6950	24	119	695
10	91	M	1	795	3790	24	79.5	379
30	28	F	2	3110	17700	24	104	592
30	28	M	2	2920	17900	24	97.3	597
30	91	F	2	3370	22200	24	112	741
30	91	M	2	2100	13100	24	70.1	438
100	28	F	2	7370	65500	24	73.7	655
100	28	M	2	6370	66400	24	63.7	664
100	91	F	1	4880	61300	24	48.8	613
100	91	M	2	3970	33200	24	39.7	332

Increase in ACT-304426 plasma exposure (C_{max} and AUC) was generally less than dose-proportional and was similar between the sexes. T_{max} was at 1-4 hours in M and F. No accumulation was observed between PNDs 28 and 91.

Table: ACT-204426 (M12) plasma levels (Sponsor's)

Dose (mg/kg/day)	Day	Sex	Tmax (h)	Cmax (ng/mL)	AUClast (h*ng/mL)	Tlast (h)	Cmax/D (ng/mL)/(mg/kg/day)	AUClast/D (h*ng/mL)/(mg/kg/day)
1	28	F	1	14.4	41.5	8	14.4	41.5
1	28	M	2	17.6	56.2	8	17.6	56.2
1	91	F	2	5.08	14.6	4	5.08	14.6
1	91	M	0.5	7.44	27.6	8	7.44	27.6
10	28	F	1	108	487	24	10.8	48.7
10	28	M	2	84.5	440	24	8.45	44.0
10	91	F	2	25.2	153	24	2.52	15.3
10	91	M	1	29.6	129	8	2.96	12.9
30	28	F	2	194	1050	24	6.46	35.1
30	28	M	2	156	1170	24	5.20	39.1
30	91	F	4	55.5	515	24	1.85	17.2
30	91	M	2	68.6	532	24	2.29	17.7
100	28	F	2	343	2620	24	3.43	26.2
100	28	M	2	266	3300	24	2.66	33.0
100	91	F	4	83.7	1200	24	0.837	12.0
100	91	M	2	123	921	24	1.23	9.21

Increase in ACT-338375 plasma exposure (C_{max} and AUC) was approximately dose-proportional and was similar between the sexes. T_{max} was at 2-4 hours. No accumulation was observed between PNDs 28 and 91.

Table: ACT-338375 (M13) plasma levels (Sponsor's)

Dose (mg/kg/day)	Day	Sex	Tmax (h)	Cmax (ng/mL)	AUClast (h*ng/mL)	Tlast (h)	Cmax/D (ng/mL)/(mg/kg/day)	AUClast/D (h*ng/mL)/(mg/kg/day)
1	28	F	2	8.90	41.9	8	8.90	41.9
1	28	M	2	12.9	46.3	8	12.9	46.3
1	91	F	2	5.02	12.6	4	5.02	12.6
1	91	M	2	5.30	15.9	4	5.30	15.9
10	28	F	2	82.2	625	24	8.22	62.5
10	28	M	2	74.8	609	24	7.48	60.9
10	91	F	2	27.6	188	24	2.76	18.8
10	91	M	2	35.3	176	8	3.53	17.6
30	28	F	2	226	1330	24	7.53	44.5
30	28	M	2	190	1780	24	6.33	59.3
30	91	F	4	71.4	581	24	2.38	19.4
30	91	M	2	96.0	772	24	3.20	25.7
100	28	F	2	450	4730	24	4.50	47.3
100	28	M	4	632	6770	24	6.32	67.7
100	91	F	4	147	2070	24	1.47	20.7
100	91	M	2	202	1730	24	2.02	17.3

T-cell dependent antibody response using KLH

The T-cell dependent antibody response (TDAR) was monitored using keyhole limpet hemocyanin (KLH). KLH was injected SC in Subset V animals (5/sex/group) on PNDs 77 and 84 and in Subset V recovery animals (5/sex/group) on PNDs 105 and 112. Blood samples were collected from Subset V animals on PNDs 77 (prior to KLH dose), 84 (prior to KLH dose), and 91 and from Subset V recovery animals on PNDs 105 (prior to KLH dose), 112 (prior to KLH dose), and 119. Samples were analyzed for IgG and IgM using an ELISA method.

IgM

No anti-KLH IgM was detected in blood samples collected on PND 77 and 105, prior to the KLH injection. In control animals, an increase in mean anti-KLH IgM response was observed on PND 84 (7 days post KLH injections) that continued to increase on PND 91 (14 days post KLH injections). In contrast, statistically significant lower increases in anti-KLH IgM was observed in HMDf and HDF on PND 84 and at all doses in M, HMDf, and HDF on PND 91. Anti-KLH IgM levels were similar in M at all doses on PND 84 but were lower on PND 91. In recovery animals, increase in anti-KLH IgM response was not statistically significant different among groups on PNDs 112 and 119.

Table of anti-KLH IgM titer counts

	77	DOSING 84	91	105	RECOVERY 112	119
Male						
Control:	136	167	11179	136	312	1643
LD:	136	136	2433*	136	719	4118
LMD:	136	136	3349*	136	402	2506
HMD:	136	334	3354*	136	611	1748
HD:	136	452	5278*	136	1044	3824
Female						
Control:	136	558	8883	136	532	3014
LD:	136	334	9013	136	275	1878
LMD:	136	519	4350	136	532	2869
HMD:	136	136*	2102*	136	1032	3105
HD:	136	136*	2741*	136	446	2277

136 = lower limit of quantitation (LLOQ)

IgG

No anti-KLH IgG was detected in blood samples collected on PND 77 and 105, prior to the KLH injection. In control animals, an increase in mean anti-KLM IgG response was observed on PND 84 (7 days post KLH injections) that continued to increase on PND 91 (14 days post KLH injections). In contrast, lower increases in anti-KLM IgG was observed at all doses in M and F on PNDs 84 and 91 that was statistically significant for HMD and HDF on PND 84 and all doses in M and F on PND 91. In recovery animals, increase in anti-KLM IgG response was not statistically significant different among groups on PNDs 112 and 119, apart from HDF on PND 112.

Table of anti-KLH IgG titer counts

	DOSING				RECOVERY	
	77	84	91	105	112	119
Male						
Control:	39	122	18300	39	66	5757
LD:	39	39	5983*	39	144	5051
LMD:	39	43	4880*	39	93	16648
HMD:	39	58	4969*	39	74	2373
HD:	39	89	8126*	39	105	4180
Female						
Control:	39	409	26262	39	174	7387
LD:	39	138	8313*	39	81	3925
LMD:	39	158	9977*	39	76	5847
HMD:	39	39*	2141*	39	215	11000
HD:	39	39*	811*	39	55*	4847

38.9 = lower limit of quantitation (LLOQ)

The TDAR results suggest that there is immune suppression during the dosing of ponesimod (≥ 1 mg/kg/day) with recovery observed during the recovery phase.

Immunophenotyping

Blood samples were collected at the end of dosing and recovery for lymphocyte subset analysis. Levels of CD3⁺ (T-lymphocytes), CD3⁺/CD4⁺ (T-helper cells), CD3⁺/CD8⁺ (cytotoxic T-cells), CD161a⁺ (NK-cells), and CD45RA⁺ cells (B-lymphocytes) were analyzed.

There was a dose-dependent decrease in T-cells and T-helper cells and an increase in cytotoxic T-cells and NK-cells. These changes were reversed at the end of the recovery period. B-lymphocytes were decreased in F at the end of the dosing and recovery periods.

Table: Changes in lymphocyte (cell population distribution; %)

	CD3 ⁺	CD3 ⁺ /CD4 ⁺	CD3 ⁺ /CD8 ⁺	CD161a ⁺	CD45RA ⁺
MALE					
Control:	41 (32)	72 (69)	28 (30)	3.4 (4.0)	26 (32)
LD:	41 (36)	72 (72)	28 (27)	2.8 (3.2)	26 (32)
LMD:	39 (36)	72 (71)	27 (29)	2.7 (3.6)	27 (34)
HMD:	26* (36)	63* (75)	36* (25)	4.0 (3.6)	29 (29)
HD:	20* (42*)	54* (72)	45* (28)	4.4 (4.1)	28 (26*)

	CD3 ⁺	CD3 ⁺ /CD4 ⁺	CD3 ⁺ /CD8 ⁺	CD161a ⁺	CD45RA ⁺
FEMALE					
Control:	43 (36)	76 (71)	24 (28)	2.5 (3.3)	26 (31)
LD:	46 (38)	76 (75)	24 (25)	2.5 (2.9)	22 (31)
LMD:	36* (42*)	69* (76)	29* (24)	3.1 (2.8)	23 (27)
HMD:	14* (45*)	56* (70)	41* (30)	3.3* (2.2)	21 (24*)
HD:	1* (47*)	- (71)	- (29)	5.5* (3.0)	14* (23*)

* statistically significant from control; recovery values in parenthesis.

10 Special Toxicology Studies

10.1 Local Tolerance

ACT-128800: Single Dose Intravenous (Infusion) Local Tolerance Study in the Rabbit. (Study No: T-13.001)

The possible local tolerance to IV ponesimod (24 mg in 0.1 mL) on the eye was evaluated in New Zealand White rabbits (n = 3/group) by infusing 0.149 and 0.447 mg/mL (0.3 mL/min) for approximately 28 minutes in the left marginal ear vein and simultaneously infusing vehicle in the right marginal ear vein. Skin reactions were assessed at up to 96 hours post-dose.

Slight or well-defined erythema at the administration site was noted between 24 and 72 hours post-dose in 1 animal in each group, and similar microscopic findings were observed in vehicle- and ponesimod-treated ears. Therefore, IV infusion of ponesimod up to 0.447 mg/mL did not result in adverse local reactions.

ACT-128800 : Evaluation of in vitro phototoxicity on Balb/c 3T3 fibroblasts using the Neutral Red uptake assay. (Study No: T-05.060)

The possible phototoxicity potential of ponesimod was evaluated in 2 experiments (0.316-1000 µg/mL and 1-50 µg/mL incubated for 1 hour) in Balb/c mouse 3T3 fibroblast cells using the Neutral Red uptake assay. Cells were irradiated in the presence of UV-A light for 105 and 96 minutes for experiments 1 and 2, respectively. Chlorpromazine (0.1-1000 µg/mL) was used as the positive control.

In experiment 1, the IC₅₀ values of Neutral Red uptake for ponesimod in the absence and presence of UV-A light were 20 and 7.6 µg/mL resulting in a PIF value of 2.6. However, due to the presence of precipitation (316 and 1000 µg/mL) and a very steep toxicity profile there was limited number of acceptable data points in experiment 1. In experiment 2, the concentration range was more focused allowing for a more accurate estimation. The IC₅₀ values of Neutral Red uptake for ponesimod in the absence and presence of UV-A light in experiment 2 were 14.6 and 7.8 µg/mL resulting in a PIF value of 1.9. In contrast, PIF values for chlorpromazine were 62 and 28 for experiment 1 and 2, respectively. The PIF for ponesimod in experiment 2 was <2; therefore, ponesimod is non-phototoxic.

Table: Data from Experiment 1 (Sponsor)

Test article	IC ₅₀ absence of UV-A (µg/mL)	IC ₅₀ presence of UV-A (µg/mL)	PIF Value
ACT-128800	19.973	7.640	2.614
Chlorpromazine	62.848	1.009	62.287*

* PIF ≥ 6, therefore positive control response was acceptable.

Table: Data from Experiment 2 (Sponsor)

Test article	IC ₅₀ absence of UV-A (µg/mL)	IC ₅₀ presence of UV-A (µg/mL)	PIF Value
ACT-128800	14.612	7.836	1.865
Chlorpromazine	62.582	2.272	27.545*

* PIF ≥ 6, therefore positive control response was acceptable.

(b) (4)



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11 Integrated Summary and Safety Evaluation

Ponesimod, an S1P modulator (selective for SIP1), is intended for the treatment of patients with relapsing multiple sclerosis to include clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease. Ponesimod acts at S1P receptors to promote internalization and degradation of S1P1 receptors. This effect of ponesimod 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. Ponesimod also had affinity at several off target human receptors, including endothelin ET_A receptor, monoamine oxidase MAO-B, and protein serine/threonine kinase Ca²⁺/calmodulin-dependent II. Functionally, oral ponesimod administered daily has been demonstrated to reduce adverse clinical signs of neurological impairment, inflammation, demyelination, and axonal damage in EAE rodent model.

In in vivo cardiovascular studies, hemodynamic and electrocardiographic effects of ponesimod were investigated in several species. Oral ponesimod had mixed effects in rat with no effect on arterial pressure or heart rate observed in normotensive rat (single doses 30-600 mg/kg), increased arterial pressure from Day 10 with repeat dosing (30 mg/kg, administered in food), and decrease in the normal increase in heart rate during early active phase in hypertensive rats (100 mg/kg). In guinea pig, single oral doses of ponesimod (0.3-30 mg/kg) resulted in dose-dependent decreases in blood pressure and heart rate and first-degree AV block followed by second-degree AV block and sometimes third-degree AV block; second-degree AV block was noted after repeat doses of oral ponesimod up to 3 mg/kg. After repeat doses, it was found that the second dose when administered 12-36 hours post first dose resulted in a much weaker effect and therefore, desensitization. However, if the second dose was administered at 48 hours post first dose a similar effect to the initial dose was observed suggesting resensitization with this time of delay in dosing. In conscious guinea pigs, an up-titration regimen of ponesimod, with doses every 12 hours, reduced the effects on heart rate and rhythm. In conscious M Beagle dogs, oral ponesimod (10-100 mg/kg) produced no effect on heart rate or ECG parameters at any dose; however, repeat doses of ponesimod (40 mg/kg/day) increased blood pressure at 0.5-2 hours post-dose.

Ponesimod evoked contraction of different arteries and in different species. Ponesimod induced a contractile response of the isolated rat basilar artery in the presence and absence of endothelium but did not induced a contractile response of the isolated aorta. Differences were also found using the coronary arteries from dogs and rats, with dog coronary arteries sensitive to the vasoconstrictor actions of ponesimod, in contrast, to rat coronary arteries that did not contract. The contractile effect by ponesimod is likely due to action of ponesimod at S1P3 receptors, as the ratio of s1p3:s1p1 mRNA levels is high in species that have arteries contracted by ponesimod.

In Wistar rats, single oral ponesimod (100 mg/kg) increased the enhanced pause (a marker of bronchoconstriction) at 3- and 6-hours post-dose and increased tidal volume and peak expiratory flow at 1.5-2.5 hours post-dose. These increases in enhanced pause, tidal volume, and peak flow were also observed up to Day 15 with repeat doses

of oral ponesimod (10-100 mg/kg/day). No effect of ponesimod (≥ 10 mg/kg) was observed on respiration in dogs.

Single oral doses of ponesimod (1-100 mg/kg) induced a dose-dependent increase in lung vascular permeability that corresponded to the level of lymphopenia. This suggests that the S1P1 receptor is responsible for both increased lung vascular permeability and reduced blood lymphocyte count. Lung permeability and increase in weight was also observed after repeat doses of oral ponesimod (10-100 mg/kg/day for 14 days) that was still increased at the end of dosing, although slightly less than after the initial dose. In M Wistar rats, lung vascular permeability and histopathology changes were assessed after a single dose and 7-day repeat doses of oral ponesimod. Distended perivascular spaces containing fluid with the presence of macrophages and eosinophils were observed in lungs 3-24 hours post-single dose. These changes correspond with an increase in lung weight. After 7-days of dosing there was a slight reduction in the increase in lung weight and severity of perivascular distension. Ponesimod induces a contractile response that is greater in the lower trachea compared to the upper tracheal segment, which is mediated by the S1P3 receptor, consistent with a higher expression ratio (3-fold) of s1p3:s1p1 receptor mRNA in the lower tracheal segment.

Oral bioavailability of ponesimod was 35-61%, 57-74%, and 84% in rats, dogs, and humans. Absorption of ponesimod in animals after oral administration was relatively fast, with T_{max} of 1-8 hours. In rats, plasma exposure to ponesimod was higher in F than M. Minimum accumulation of ponesimod was observed. Ponesimod distributed to most tissues in rodents and was detected in the brain, with exposures approximately 2-fold those in blood up to 4 hours post-dose. Ponesimod was detected in pups from dams dosed with ponesimod, suggesting placental and/or lactational transfer. In human plasma, the major circulating human metabolite is M13 a carboxylic acid, formed by non-CYP mediated oxidation through hydroxyketone. After oral ponesimod, the main components in plasma were ponesimod and metabolites M1, M7, M9, M10, M12-M16, and M24 in rat plasma and ponesimod and metabolites M12 and M13 in dog plasma. Adequate systemic exposure to M13 was demonstrated in mouse and rat. Therefore, human plasma exposure to M13 is covered adequately in nonclinical species.

In toxicology studies, deaths and early sacrificed were observed in rats (400 mg/kg) and dogs (≥ 75 mg/kg; due to deteriorating clinical signs). Clinical signs observed in toxicology studies included labored breathing in M rats at 10 mg/kg and in F rats at 100 mg/kg (carcinogenicity study) and incidences of tremor, collapse, uncoordination, impaired motility, and recumbency were noted in many dog studies at doses ≥ 40 mg/kg. WBCs and lymphocytes were greatly reduced in all species at most doses, with accompanying histopathological changes in lymphoid tissues that included lymph node hypocellularity, splenic reduction in cellularity and white pulp atrophy, and thymic medullary hypertrophy (enlargement). These changes in lymphocytes and lymphoid lesions, can be attributed to the pharmacological action of ponesimod.

Findings of lung toxicity were observed in mice, rats, and dogs. The most consistent microscopic finding was alveolar macrophage (histiocytosis) and statistically significant

increases in weight. Other microscopic findings in the lungs included bronchitis, pneumonitis, and inflammation. In mice, long duration dosing of ponesimod increased lung weight and incidences of alveolar hyalinoses consisting of an accumulation of eosinophilic amorphous material in alveolar cavities, alveolar macrophages, alveolar hematoidin, interstitial fibrosis, and osseous metaplasia. In toxicology studies in rats, alveolar histiocytosis was observed in the shorter duration studies (up to 4 weeks) but not in the chronic study possibly suggesting an adaptation. However, increase incidences and severity of hematoidin crystals, interstitial fibrosis, and osseous metaplasia, that can be a response to inflammation and injury, were more prevalent in some longer duration rodent studies (e.g. juvenile and carcinogenicity) and moderate fibrosing alveolitis was observed in the 52-week (low dose) dog study. Ponesimod was demonstrated to cause vascular leakage in mechanistic studies that corresponded with an increase in lung weight, this likely leads to alveolar edema, emphysema, hyalinoses, and histiocytosis noted in the toxicology studies, and change respiration function in the safety studies (increase in tidal volume and peak flow) and toxicology studies (tachypnea and labored breathing). This may suggest that lungs go through a damage and healing process that is adverse and will result in a pulmonary clinical risk. The safety margins, based on plasma exposure, are estimated at 15-, 0.5-, 0.7-, and 4.4-fold for mice, male rats, female rats, and dogs, respectively.

There were heart lesions in dogs with observations of arterial changes like arteriopathy, which was characterized by vascular wall thickening in the posterior and anterior papillary muscle and subendocardial fibrosis in the left ventricle value in the papillary muscle of the left ventricle that was observed from 13 weeks at doses equal and greater than 10 mg/kg and from 26 weeks at doses equal and greater than 5 mg/kg with 26 weeks; the NOAEL in the 52 week study was 3 mg/kg/day with an estimated safety margin of 4.4-fold on a plasma exposure basis.

In the toxicology studies that conducted TDAR assays, a decrease was observed in mean SRBC specific IgM and IgG titers at doses ≥ 10 mg/kg/day in adult rats (4-week), in mean anti-KLH IgM and IgG responses at doses ≥ 1 mg/kg/day in juvenile rats, and in mean anti-KLH IgM and IgM responses after administration of ponesimod- (b) (4) in dogs. The TDAR results suggest that there is immune suppression during the dosing of ponesimod that recovers when dosing is stopped.

In genetic toxicology studies, ponesimod did not demonstrate mutagenic potential in the Ames assay and human peripheral lymphocytes, or clastogenic potential in the *in vivo* mouse and rat micronucleus assays. The mouse study was positive for hemangiomas and hemangiosarcomas, with increase incidences at all doses in M and in HDF. In the rat carcinogenicity study, there were no drug-related increases in neoplasms in M or F.

Fertility was not affected in F or M (NOAEL was the HD, 100 mg/kg/day). In the rat DRF embryofetal development study, ponesimod was embryofetal lethal, with litter resorption in 2 dams administered 100 mg/kg/day and in the pivotal rat EFD study there was an increase in post-implantation loss resulting in a decrease in live fetuses at 40 mg/kg/day. Teratogenicity was noted in rats, with observed malformations including

fused ascending aorta trunk, muscular ventricular septal defect, membranous ventricular septal defects, syndactyly, ectrodactyly, malrotated hindlimbs, and eye microphthalmia. The fetal NOAEL was established at 1 mg/kg/day. In rabbits, no NOAEL was established in the dose range finding study (0, 6, 20, and 60 mg/kg/day) due to abortions, post-implantation loss, and limb abnormalities (e.g. brachydactyly). The low dose EFD (0, 0.25, 1, and 4 mg/kg) in rabbits established the fetal NOAEL at 1 mg/kg/day, due to increased post-implantation loss that resulted in a decrease in live fetuses at the HD; increased number of minor morphological changes in the cardiovascular and limb systems were noted at the MD and HD. Ponesimod plasma exposures (AUC) associated with the NOAELs in rats and rabbits were similar at 661 (rat DRF study) and 736 ng*h/mL, respectively, resulting in safety margins of approximately 0.2-fold. In the pivotal PPND study (0, 5, 10, and 20 mg/kg/day), sexual maturation was delayed at all doses, increased motor fine and ambulatory movements were noted at MD and HD, and decreased viability, decrease in body weight gain, and reduced reproductive performance (increased percentage of pre- and post-implantation loss and decrease in live embryos) were observed at the HD. Therefore, the NOAEL could not be established for postnatal development of offspring and reproductive performance. In the juvenile study (0, 1, 10, 30, and 100 mg/kg/day), the sponsor considered ponesimod well tolerated at all doses; however, statistically significant decrease in body weight at ≥ 10 mg/kg/day, slight increase in motor activity at 100 mg/kg/day in M, and increased lung weight and alveolar histiocytosis at ≥ 30 mg/kg/day were noted. The sponsor suggested the lunge changes were expected from the adult data, were reversible, and did not appear to alter function, but these types of changes were noted as adverse in adult studies. Therefore, the NOAEL in juvenile animals was 10 mg/kg/day, resulting in no safety margin (1.6-fold).

Table: NOAELs.

Toxicity	Species	Study	NOAEL (mg/kg)	Safety Margin Based on AUC*
Lungs	Mouse	13-week	90	Male - 16 Female - 15
		Carcinogenicity	150 30	Male - 11.9 Female - 2.4
	Rat	4-week	2	Male - 0.2 Female - 0.3
		26-week	4	Male - 0.5 Female - 0.7
		Juvenile	10	Male - 1.1 Female - 2.0
	Dog	52-week	3	4.4
Heart	Dog	52-week	3	4.4
Fertility	Rat	Fertility	100	Male - 18 [‡] Female - 32 [‡]
Teratogenicity	Rat	Embryo-fetal	1	0.2 [†]
Fetal lethality	Rabbit	Embryo-fetal	1	0.2
Pre- and postnatal	Rat	Pre- and postnatal	<1	ND
Juvenile Behavior	Rat	Juvenile 9-week	10	Male - 1.1 Female - 2.0

*AUC for 20 mg (7 days) was 3473 ng*h/ml (study №: AC-058-102); ‡ based on TK from rat 4-week study T-05.043; † based on TK from DRF EFD study T-05.110; ND – not determined.

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/s/

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