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APPLICATION NUMBER:

213498Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY

NDA- 213498 (PONVORY)

CLINICAL PHARMACOLOGY REVIEW

NDA Number	213498
Link to EDR	\\CDSESUB1\evsprod\NDA213498\0001
Submission Date(s)	March 18, 2020
Submission Type	Type 1- New Molecular Entity (Standard Review)
Brand Name	PONVORY
Generic Name	Ponesimod
Formulation and Strength	Film-coated Tablets (2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, and 20 mg)
Route of Administration	Oral
Proposed Indication	Relapsing forms of multiple sclerosis
Applicant	Janssen Pharmaceuticals Inc.
Associated IND	IND-101722
Reviewers	Dawei Li, Manuela Grimstein, Vishnu Sharma
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1 Executive Summary

The Applicant is seeking approval for ponesimod (2 mg (b) (4) 20 mg film coated tablets), for the treatment of relapsing forms of multiple sclerosis. Ponesimod is a sphingosine 1-phosphate (S1P) receptor 1 modulator. Ponesimod binds with high affinity to S1P receptor 1 and blocks the capacity of lymphocytes to egress from lymph nodes, reducing the number of lymphocytes in peripheral blood.

The Applicant proposed dose titration starts with 2 mg once daily on Day 1 and Day 2, followed by once daily doses of 3 mg on Day 3 and Day 4, 4 mg on Day 5 and Day 6, 5 mg on Day 7, 6 mg on Day 8, 7 mg on Day 9, 8 mg on Day 10, 9 mg on Day 11, 10 mg on Day 12, Day 13 and Day 14. After dose titration is complete, the recommended maintenance dosage of ponesimod is one 20 mg taken orally once daily with or without food.

The efficacy and safety of ponesimod in patients with relapsing MS is supported by a single pivotal phase 3 randomized, double blind, parallel group active controlled superiority study. The primary endpoint is the annualized relapse rate (ARR) from baseline up to end of study (EOS). The Applicant reports statistically significantly superior to teriflunomide 14 mg (active control) in reducing the annualized relapse rate (ARR) up to EOS by 30.5% (p=0.0003).

Ponesimod is extensively metabolized. Two inactive circulating metabolites, M12 and M13, have been identified in human plasma. M13 is approximately 20% and M12 is 6% of total drug related exposure. In vitro studies indicate that metabolism of ponesimod to M13 occurs primarily through a combination of non-Cytochrome P450 (CYP450) enzymatic activities. Multiple CYP450 (CYP2J2, CYP3A4, CYP3A5, CYP4F3A, and CYP4F12) and non-CYP450 enzymes catalyze the oxidation of ponesimod to M12. Ponesimod also undergoes direct glucuronidation (mainly UGT1A1 and UGT2B7). Ponesimod and M13 are unlikely to inhibit or induce CYP450 enzymes, nor expected to inhibit major efflux and uptake transporters at clinically relevant doses. Currently, limited data showed that concomitant use of strong PXR agonists may decrease the systemic exposure of ponesimod. It is unclear whether the impact of strong PXR agonists (e.g. rifampin, phenytoin, carbamazepine) on ponesimod systemic exposure would be considered of clinical relevance.

The key review focuses on the acceptability of the proposed dosing regimen including both titration and maintenance doses and appropriateness of dosing recommendations for ponesimod in specific populations and patients taking concomitant medications.

1.1 Recommendation

The Office of Clinical Pharmacology has reviewed the clinical pharmacology and biopharmaceutical information submitted in NDA 213498 and considers it acceptable from a clinical pharmacology perspective. The key review issues with specific clinical pharmacology recommendations and comments are summarized below.

Review Issues	**Recommendations and Comments**
Supportive evidence of effectiveness	A single pivotal trial in patients with relapsing MS was submitted in

	NDA 213498 as primary evidence of effectiveness.
General dosing instructions	Titration dosing regimen: 2 mg once daily on Day 1 and Day 2, 3 mg on Day 3 and Day 4, 4 mg on Day 5 and Day 6, 5 mg on Day 7, 6 mg on Day 8, 7 mg on Day 9, 8 mg on Day 10, 9 mg on Day 11, 10 mg on Day 12, Day 13 and Day 14. Maintenance dose: 20 mg/day with or without food
Dosing in patient subgroups (intrinsic and extrinsic factors)	Ponesimod is not recommended in patients with moderate and severe hepatic impairment. No therapeutic individualization for intrinsic or extrinsic factors is recommended
Bridge between the "to-be-marketed" and clinical trial formulations	Not applicable. To-be-marketed formulation was used in phase 3 clinical trial.

1.2 Post-marketing Requirement

Given that the impact of strong PXR agonists (e.g. rifampin, phenytoin, carbamazepine) on the PK of ponesimod is inconclusive.

PMR XX_X: Requirement to conduct Drug-drug Interaction study to evaluate the impact of strong PXR agonists on the pharmacokinetics of ponesimod.

Rationale: *In vitro* assessments and limited clinical data indicated that concomitant use of strong PXR agonists (e.g. rifampin, phenytoin, carbamazepine) may decrease the systemic exposure of ponesimod. It is unclear whether this decrease in ponesimod systemic exposure would be considered of clinical relevance.

2 Summary of Clinical Pharmacology Assessment

2.1 The Pharmacology and Clinical Pharmacokinetics

Mechanism of Action

Ponesimod is a sphingosine 1-phosphate (S1P) receptor 1 modulator. Ponesimod binds with high affinity to S1P receptor 1 located on lymphocytes. 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 may involve reduction of lymphocyte migration into the central nervous system.

Pharmacokinetics

Absorption: Ponesimod exposure increases in an apparent dose proportional manner at dose range from 1 to 75 mg/day. The time to reach maximum plasma concentration of ponesimod is 2 to 4 hours post-dose. The absolute oral bioavailability of a 10 mg dose is 83.8%. Food does not have a clinically relevant effect on ponesimod pharmacokinetics.

Distribution: Following IV administration in healthy subjects, the steady state volume of distribution of ponesimod is 160 L. Ponesimod is highly bound to plasma proteins (> 99%).

Metabolism: Ponesimod is extensively metabolized prior to excretion in humans, though unchanged ponesimod was the main circulating component in plasma. Two inactive circulating metabolites, M12 and M13, have also been identified in human plasma. M13 is approximately 20% and M12 is 6% of total drug related exposure. *In vitro* studies indicate that metabolism of ponesimod to M13 occurs primarily through a combination of non-Cytochrome P450 (CYP450) enzymatic activities. Multiple CYP450 (CYP2J2, CYP3A4, CYP3A5, CYP4F3A, and CYP4F12) and non-CYP450 enzymes catalyze the oxidation of ponesimod to M12. Ponesimod also undergoes direct glucuronidation (mainly UGT1A1 and UGT2B7).

Elimination: After a single IV administration, the total clearance of ponesimod is 3.8 L/hour. The elimination half-life after oral administration is approximately 33 hours.

Following a single oral administration of 14C ponesimod, 57% to 80% of the dose was recovered in feces (16% as unchanged ponesimod), and 10% to 18% in urine (no unchanged ponesimod).

Drug-Drug Interaction (DDI) potential:

Overall, at clinically relevant dose, the major metabolic enzyme and transporter mediated DDI potential of ponesimod was low. The impact of strong PXR agonists (e.g. rifampin, phenytoin, carbamazepine) on the PK of ponesimod is inconclusive.

Population pharmacokinetic analysis did not reveal a significant impact of body weight, age, gender, race and disease status on the PK exposures of ponesimod.

2.2 Dosing and Therapeutic Individualization

2.2.1 General Dosing

The Applicant proposed dose titration starts with 2 mg once daily on Day 1 and Day 2, followed by once daily doses of 3 mg on Day 3 and Day 4, 4 mg on Day 5 and Day 6, 5 mg on Day 7, 6 mg on Day 8, 7 mg on Day 9, 8 mg on Day 10, 9 mg on Day 11, 10 mg on Day 12, Day 13 and Day 14. After dose titration is complete, the recommended maintenance dosage of ponesimod is one 20 mg taken orally once daily with or without food.

2.2.2 Therapeutic Individualization

Ponesimod is not recommended in patients with moderate and severe hepatic impairment. No therapeutic individualization for intrinsic or extrinsic factors is recommended.

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

The Office of Clinical Pharmacology recommends the following labeling concepts to be included in the final package insert:

"7. DRUG INTERACTIONS

7.5 (b) (4)

In vitro assessments and limited clinical data indicated that concomitant use of strong (b) (4) (e.g. rifampin, phenytoin, carbamazepine) may decrease the systemic exposure of TRADENAME. It is unclear whether this decrease in ponesimod systemic exposure would be considered of clinical relevance. (b) (4)

"12.3

Effect of Other Drugs on Ponesimod

(b) (4) In vitro studies with human liver preparations indicate that metabolism of ponesimod occurs through multiple, distinct enzyme systems, including multiple CYP450 (CYP2J2, CYP3A4, CYP3A5, CYP4F3A, and CYP4F12), UGT (mainly UGT1A1 and UGT2B7) and non-CYP450 oxidative enzymes, without major contribution by any single enzyme."

3 Comprehensive Clinical Pharmacology Review

3.1 Overview of the Product and Regulatory Background

Ponesimod is developed to be available as film coated tablets (2-20 mg) for oral administration. It is proposed for the treatment of patients with relapsing forms of multiple sclerosis. The approval is based on the clinical effectiveness obtained from one pivotal phase 3 randomized, double blind, parallel group active controlled superiority study.

The key regulatory correspondences are summarized in the Table below.

Date	FDA Correspondence
2-Mar-2009	IND Study May Proceed Letter
6-Dec-2011	Type B End of Phase 2 Meeting to discuss the Phase 3 development plan of ponesimod for the treatment of relapsing-remitting multiple sclerosis (RRMS)
4-May-2012	FDA Email - FDA feedback regarding the clinical pharmacology program to support Phase 3 and an NDA
4-Sep-2019	Type B Pre-NDA Meeting

3.2 General Pharmacology and Pharmacokinetic Characteristics

The clinical pharmacology and pharmacokinetics information of ponesimod are summarized below.

Table 1: Summary of Ponesimod Clinical Pharmacology Information

Pharmacology	
Mechanism of Action	Ponesimod binds with high affinity to S1P receptor 1 located on lymphocytes. Ponesimod blocks the capacity of lymphocytes to egress from lymph nodes, reducing the number of lymphocytes in peripheral blood.
Active Moieties	Ponesimod is the active moiety circulating in plasma accounted for 66.1% of total radioactivity in a mass balance study.
QT Prolongation	Exposure-response analysis indicates that at the therapeutic dose of 20 mg, no clinically relevant effect on QTc interval is expected, with the upper bound of the 2-sided test 90% CI being 5.9 ms.
General Information	
Bioanalysis	Ponesimod and M12, M13 metabolites were measured using validated LC/MS/MS methods. The accuracy, precision, and other relevant parameters for the assay are sufficient to meet the requirements of the submitted studies. A summary of the analytical method is included in Appendix.
Healthy Volunteers vs. Patients	The pharmacokinetics of ponesimod is similar in healthy subjects and subjects with multiple sclerosis.
Drug total exposure following the therapeutic dosing regimen	In patients with RMS receiving ponesimod 20 mg QD, the mean trough concentrations were 126 ng/mL and 125 ng/mL on week 12 and week 108, respectively, in the pivotal trial (study AC-058B301).
Dose Proportionality	The systemic exposures increased approximately proportionally with an increase in dose from 1 mg to 75 mg.
Accumulation	A mean accumulation ratio of 2.0 to 2.6 was observed at steady-state
Variability	The pharmacokinetic profile of ponesimod is characterized by the inter subject variability of approximately 25% across studies.
Inhibitor/Inducer [in vitro]	Ponesimod and M13 are unlikely to inhibit any evaluated CYPs or UGTs (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4 and UGT2B7 or UGT1A1) or induce CYP1A2, CYP2B6, CYP2C9, or CYP3A4 at clinically relevant doses.
Transporter Systems [in vitro]	Ponesimod is not identified as substrate of P-gp, BCRP, OATP1B1 or OATP1B3; ponesimod and M13 are unlikely to inhibit major efflux and uptake transporters at clinically relevant doses.
Absorption	
Bioavailability [oral]	83.8%

Tmax [oral]	2 to 4 hours post-dose	
Food effect (high-fat) GMR (90% CI)	AUCinf	Cmax
	1.2 [1.1-1.3]	1.1 [1.0-1.3]
Distribution		
Volume of Distribution	The estimated steady state volume of distribution of ponesimod is 160 L.	
Plasma Protein Binding	> 99% (mainly binding to albumin and alpha-1-acid glycoprotein)	
Elimination		
Terminal Elimination Half-life	Approximately 33 -hours.	
Metabolism / Excretion	*Metabolism:* Ponesimod is extensively metabolized. Two inactive circulating metabolites, M12 and M13, have been identified in human plasma. M13 is approximately 20% and M12 is 6% of total drug related exposure. *In vitro* studies indicate that metabolism of ponesimod to M13 occurs primarily through a combination of non-Cytochrome P450 (CYP450) enzymatic activities. Multiple CYP450 (CYP2J2, CYP3A4, CYP3A5, CYP4F3A, and CYP4F12) and non-CYP450 enzymes catalyze the oxidation of ponesimod to M12. Ponesimod also undergoes direct glucuronidation (mainly UGT1A1 and UGT2B7 *Excretion:* Following a single oral administration of 14C ponesimod, 57% to 80% of the dose was recovered in feces (16% as unchanged ponesimod), and 10% to 18% in urine (no unchanged ponesimod).	

3.3 Clinical Pharmacology Review Questions

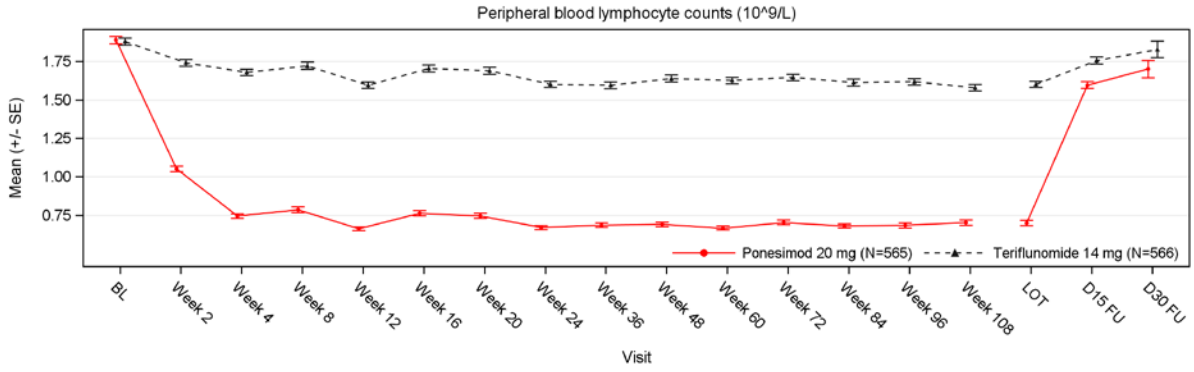
3.3.1 Does the clinical pharmacology program provide supportive evidence of effectiveness?

The evidence of effectiveness of ponesimod is primarily based on one Phase 3 pivotal, multicenter, randomized, double-blind (DB), active-controlled, parallel-group, superiority study (AC-058B301). This study met its primary endpoint: subjects treated with ponesimod 20 mg statistically significantly reduced ARR (confirmed relapses) up to end of study (EOS) by 30.5% compared to subjects treated with teriflunomide 14 mg (ARR ratio: 0.695; 99% confidence limits [CLs]: 0.536, 0.902; p=0.0003) (Please refer to the clinical review and biostatistics review for further details).

Peripheral blood lymphocyte counts were measured to evaluate the pharmacological effect of ponesimod. In study AC-058B301, there was a rapid decrease in lymphocyte count from baseline to Week 2 and Week 4 in the ponesimod 20 mg group (mean percent change from baseline of −42.34% and −59.15%, respectively) compared to the teriflunomide 14 mg group (−5.21% and −8.45%, respectively). In the ponesimod 20 mg group, the largest mean percent

decrease was observed at Week 12 (−63.61%) compared to −12.88% in the teriflunomide 14 mg group. From Week 12 onwards up to Week 108, mean lymphocyte count in the ponesimod 20 mg group remained stable. (see **Figure 1**).

Figure 1: Mean (plus/minus SE) Peripheral Blood Lymphocyte Count by Visit (Study B301)



Number of Subjects

Ponesimod 20 mg	563	550	540	403	533	420	433	520	504	497	495	487	476	463	464	560	484	101
Teriflunomide 14 mg	566	554	551	429	541	448	443	535	523	503	492	490	481	474	466	564	495	100

Source: Applicant's Clinical Study Report – csr-full-ac-058b301, Figure 41 on page 202

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

To reduce first-dose effects of ponesimod on heart rate (HR) and rhythm, a titration dosing regimen was proposed based on results from dose titration study (AC-058-115). The proposed titration dosing regimen (2 mg once daily on Day 1 and Day 2, followed by once daily doses of 3 mg on Day 3 and Day 4, 4 mg on Day 5 and Day 6, 5 mg on Day 7, 6 mg on Day 8, 7 mg on Day 9, 8 mg on Day 10, 9 mg on Day 11, 10 mg on Day 12, Day 13 and Day 14) was evaluated in the pivotal efficacy/safety study (AC-058B301).

The proposed maintenance dosing regimen is the same that was evaluated in the pivotal efficacy/safety study (AC-058B301). Maintenance dose and dosing regimen for the pivotal study were selected based on a phase 2 dose finding study (AC-058B201) in patients with relapsing-remitting multiple sclerosis.

Efficacy of ponesimod was assessed in patient with relapsing multiple sclerosis in Study AC-058B301. As per applicant's analyses, the mean ARR (number of confirmed relapses per year) was 0.202 and 0.290 in the ponesimod 20 mg and teriflunomide 14 mg groups, respectively (Table 1). Ponesimod 20 mg statistically significantly reduced ARR (confirmed relapses) up to EOS by 30.5% compared to teriflunomide 14 mg (ARR ratio: 0.695; 99% confidence limits [CLs]: 0.536, 0.902; p=0.0003) (**Figure 2, Table 2**).

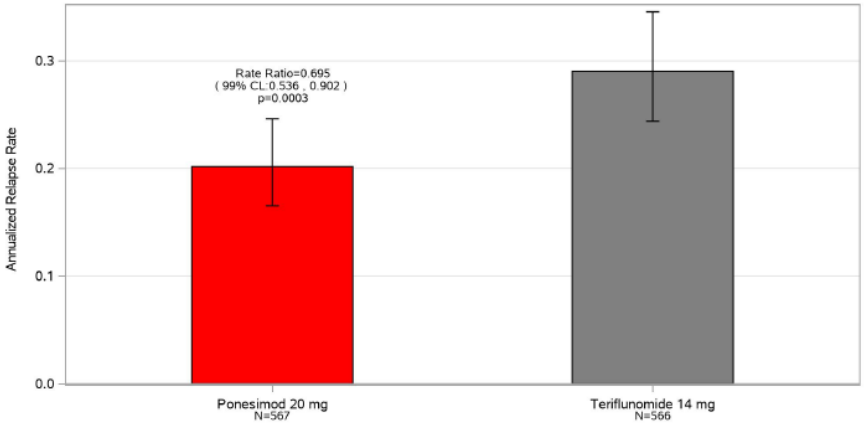
Table 2: Confirmed Relapses up to EOS - Annualized Relapse Rate From Negative Binomial Regression (Primary Analysis)

	Ponesimod 20 mg N=567	Teriflunomide 14 mg N=566
Mean estimate (ARR)	0.202	0.290
99% CL	(0.165 , 0.246)	(0.244 , 0.345)
95% CL	(0.173 , 0.235)	(0.254 , 0.331)
Treatment effect (rate ratio)	0.695	
99% CL	(0.536 , 0.902)	
95% CL	(0.570 , 0.848)	
p-value	0.0003	
Dispersion estimate	0.765	
Number of subjects included in analysis	567	566
Total number of relapses	242	344
Total time (years)	1119	1137
Raw ARR	0.216	0.303

ARR=annualized relapse rate (confirmed relapses per year), rate ratio: ponesimod versus teriflunomide.
Negative binomial model was applied with Wald confidence limits and p-value.
Offset: log time (years) up to end-of-study (EOS).
Covariates: EDSS strata (<=3.5,>3.5), DMT in last 2 years prior to randomization strata (Y, N), number of relapses in year prior study entry (<=1, >=2).
EDSS=expanded disability status scale; DMT=disease modifying therapy for MS.

Source: Applicant's Clinical Study Report – csr-full-ac-058b301, Table 11 on page 93

Figure 2: Confirmed Relapses up to EOS - Annualized Relapse Rate From Negative Binomial Regression (Primary Analysis)



ARR=annualized relapse rate (confirmed relapses per year), rate ratio: ponesimod vs. teriflunomide.
Negative binomial model is applied with Wald confidence limits and p-value.
Offset: log time (years) up to EOS.
Covariates: EDSS strata (<=3.5,>3.5), DMT in last 2 years prior to randomization strata (Y,N), number of relapses in year prior study entry (<=1, >=2).

Source: Applicant's Clinical Study Report – csr-full-ac-058b301, Figure 8 on page 92.

In terms of safety, the proportion of subjects who experienced at least 1 TEAE during the study was similar in the 2 treatment groups (88.8% ponesimod 20 mg versus 88.2% teriflunomide 14 mg). The proportion of subjects who experienced at least 1 SAE was similar in both treatment groups (8.7% and 8.1% of subjects in the ponesimod 20 mg and the teriflunomide 14 mg groups, respectively). TEAEs leading to premature treatment discontinuation were reported in

8.7% of subjects in the ponesimod 20 mg group compared to 6.0% in the teriflunomide 14 mg group. The safety profile of ponesimod appears to be consistent with the known safety profile of other S1P receptor modulators, such as bradycardia etc.

Overall, the proposed dosing regimen is supported by the efficacy and safety results in Study AC-058B301. Please refer to the clinical review and statistical review for additional details.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

Dose adjustment is not necessary based on intrinsic factors such as age, gender, body weight, race, renal impairment and mild hepatic impairment. Population pharmacokinetic analysis did not reveal a significant impact of body weight, age, gender, race and disease status on the PK exposures of ponesimod. Please refer to **4.2 Pharmacometric review** for details. The applicant conducted dedicated clinical studies assessing the impact of renal function and hepatic function on the PK of ponesimod (see below).

Renal Impairment (Study AC-058-113)

Ponesimod Cmax increased by 9% and AUC0-∞ increased by 14% in severe renal impaired subjects compared to matched healthy subjects. These differences were not clinically meaningful, and no dose adjustment is recommended for patients with renal impairment.

Hepatic Impairment (Study AC-058-112)

Ponesimod AUC0-∞ (geometric mean) in the mild, moderate, and severe hepatic impairment groups was 1.3-fold (1.0, 1.8), 2.0-fold (1.5, 2.7), and 3.1-fold (2.2, 4.3) greater, respectively, compared to healthy subjects. The elimination t½ (geometric mean) was 1.5-fold (90% CI: 1.2, 1.8), 1.8-fold (1.3, 2.5), and 2.6-fold (2.0, 3.3) greater, in the mild, moderate, and severe liver impairment groups, respectively, compared to healthy subjects. Based on the PK results of this study, no dose adjustment of ponesimod is needed for patients with mild hepatic impairment. It is not feasible to have dose adjustment for moderate and severe hepatic impairment due to the designated distribution with fixed titration package of ponesimod. Therefore, ponesimod is not recommended in patients with moderate, and severe hepatic impairment.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

Food-drug interactions

Food-effect study conducted in healthy subjects indicated that high fat food increased the Cmax and AUC of ponesimod by 10% and 20%, respectively (AC-058-101). Tmax was slightly delayed (approximately 2.5 hours delay) in the presence of high fat food. The changes in Cmax and AUC are not considered clinically relevant and ponesimod is recommended to be administered without regard to food.

Drug-drug interaction

DDI of ponesimod as victim

In vitro studies suggested that metabolism of ponesimod to M13 occurs primarily through a combination of non-CYP enzymatic activities. Multiple CYPs (2J2, 3A4, 3A5, 4F3A, and 4F12) and non-CYP enzymes catalyze the oxidation of ponesimod to M12. Ponesimod also undergoes direct glucuronidation (mainly UGT1A1 and UGT2B7), in addition to other minor direct metabolic pathways involving oxidation or reduction. The sponsor claimed that this metabolism profile of multiple independent pathways involving multiple enzymes and without major contribution from any single enzyme, ponesimod is unlikely to be a victim of DDI involving metabolic enzyme inhibitors or inducers.

Considering some inducers (e.g. rifampin) may co-induce multiple metabolic enzymes, which may potentially affect the PK of ponesimod, information request (IR) was sent to the sponsor during the review cycle, requesting for additional assessments of the impact of inducers on the PK of ponesimod. To address the Agency's concern, the sponsor reassessed the potential contribution of PXR-inducible enzymes to the metabolic clearance of ponesimod based on *in vitro* and clinical data.

The potential impact of strong, moderate, and weak PXR agonists on ponesimod AUC was then estimated using a literature-based approach (Asaumi R, 2018; Ohno Y, 2008*). The estimated AUC ratios for ponesimod in the induced vs. non-induced conditions ranged from 0.51 to 0.95 (**Table 3**).

Table 3: Estimated AUC Ratios with Coadministration of PXR Agonists Based on In Vitro Metabolism and Enzyme Phenotyping Data

PXR Agonist	Ponesimod $AUC_{induced}/AUC_{non\text{-}induced}$
Rifampicin	0.51
Phenytoin	0.63
Carbamazepine	0.72
Efavirenz	0.85
St. John's wort	0.87
Bosentan	0.94
Pioglitazone	0.95

The sponsor also reviewed clinical studies AC-058B201 and AC-058B301 for experience with subjects receiving PXR agonist co-medication. Only 4 subjects on ponesimod were exposed to strong PXR agonists (carbamazepine) during treatment (Study AC-058B201).

***Asaumi R**, Toshimoto K, Tobe Y, et al. Comprehensive PBPK Model of Rifampicin for Quantitative Prediction of Complex Drug-Drug Interactions: CYP3A/2C9 Induction and OATP Inhibition Effects. CPT Pharmacometrics Syst Pharmacol. 2018;7(3):186-196.*

***Ohno Y,** Hisaka A, Ueno M, Suzuki H. General framework for the prediction of oral drug interactions caused by CYP3A4 induction from in vivo information. Clin Pharmacokinet. 2008;47(10):669-680.*

The plasma concentrations in subjects receiving the strong PXR agonists were typically in the lower range (similar or lower than the first quartile) of the population never exposed to PXR agonists. Due to limited available data, it is unclear whether the expected decrease in ponesimod systemic exposure would be considered of clinical relevance.

Overall, current available data is not sufficient to exclude the potential impact of strong PXR agonists (e.g. rifampin, phenytoin, carbamazepine) on the PK and efficacy of ponesimod.

Based on results from *in vitro* studies, ponesimod is not identified as substrate of P-gp, BCRP, OATP1B1 or OATP1B3.

DDI of ponesimod and M13 as perpetrators

In vitro studies suggested that ponesimod and M13 are unlikely to inhibit major CYPs or UGTs (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4 and UGT2B7 or UGT1A1) or induce CYP1A2, CYP2B6, CYP2C9, or CYP3A4 at clinically relevant doses. Ponesimod and M13 are not expected to inhibit major efflux (P-gp, BCRP, MATE1, and MATE2K) and uptake transporters (OATP1B1, OATP1B3, OAT1, OAT3, OCT1, and OCT2) at clinically relevant doses.

PBPK analyses were conducted to evaluate ponesimod's potential for clinical DDI mediated by inhibition and/or induction of CYP3A, CYP2C9 and CYP2C19. PBPK analyses predicted that ponesimod (20 mg QD) has low potential for a clinically relevant interaction with a sensitive CYP3A substrate (such as midazolam), a CYP2C9 substrate (such as tolbutamide and S-warfarin) or a CYP2C19 substrate (such as omeprazole).

Other DDIs

Beta-Blockers: In a drug-drug interaction study (AC-058-117), the up-titration regimen of ponesimod was administered to subjects receiving propranolol (80 mg) once daily at steady state No significant changes in pharmacokinetics of ponesimod or propranolol were observed. Concomitant administration of ponesimod with propranolol resulted in an additive effect on HR. Compared to ponesimod alone, the combination with propranolol after the first dose of ponesimod (2 mg) had a 12.4 bpm (90% CI: -15.6 to -9.1) decrease in mean hourly heart rate and at the first dose of ponesimod (20 mg) after up titration a 7.4 bpm (90% CI: -10.9 to -3.9) decrease in mean hourly heart rate.

Oral Contraceptives: In study AC-058-104, co-administration of ponesimod with an oral hormonal contraceptive (containing 1 mg norethisterone/norethindrone and 35 µg ethinyl estradiol) showed no clinically relevant pharmacokinetic interaction with ponesimod.

3.3.5 Is the to-be-marketed formulation the same as the clinical trial formulation, and if not, are there bioequivalence data to support the to-be-marketed formulation?

The to-be-marketed (TBM) formulation of ponesimod is film coated tablet (2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, and 20 mg). Ponesimod capsules of the polymorphic Forms [redacted] (b) (4) were used in the early stage of clinical development of ponesimod. Overall, inter- and intra-study comparisons suggest that, at equivalent doses, exposure to ponesimod is comparable among

oral formulations evaluated in the clinical program. Population PK analyses indicated that polymorphic [redacted] (b) (4) tablet or capsule formulation, did not affect the overall systemic exposure to a significant extent. TBM tablets were used in the pivotal Phase 3 clinical trial (AC-058B301).

4 Appendices

4.1 Summary of Bioanalytical Method Validation and Performance

Bioanalytical methods used throughout the clinical development of ponesimod and its metabolites are summarized in Table 4 and Table 5.

Table 4: Summary of Bioanalytical Method Measuring ponesimod in Plasma.

Method ID	SBA_S_06009	SBA_S_09135	BA-13.007	BA13059
Study Number Supported	AC-058-101, AC-058-102, AC-058-103, AC-058-104, AC-058-105, AC-058-106, AC-058-107, AC-058-108, AC-058-111, and AC-058B201	AC-058-109, AC-058-110, AC-058-112, and AC-058-113	AC-058-115 and AC-058B301	AC-058-117
LLOQ (ng/mL)	1	1	1	1
Linear Range (ng/mL)	1.00-2000	1.00-1000	1.00-1000	1.00-1000
Inter-day Accuracy for QC	-3.7% - 14.0%	-0.3% - 3.8%	-4.1% - 0.8%	-4.3% - -0.6%
Inter-day Precision for QC	3.6% - 6.2%	3.2% - 7.1%	4.6% - 10.4%	1.3% - 9.0%

Table 5. Summary of Bioanalytical Method Measuring ponesimod Major Metabolite (M13) in Plasma.

Method ID	SBA_S_09135	BA-13.232
Study Number Supported	AC-058-109, AC-058-110, AC-058-112, and AC-058-113	AC-058-114
Analytes	M13	M13
LLOQ (ng/mL)	1	1
Linear Range (ng/mL)	1.00-1000	1.00-1000
Inter-day Accuracy for QC	2.9% - 5.9%	-3.1% - 2.2%

Inter-day Precision for QC	3.2% - 7.0%	1.4% - 7.0%

Bioanalytical method performance was summarized in each individual study review.

Stability in plasma

Ponesimod is stable in plasma for up to 1233 days (QC samples) when stored in freezer. M13 is stable in plasma up to 98 days when stored in ultra-freezer. All samples were stored and processed in the time frame supported by the stability data.

4.2 Pharmacometric review

EXECUTIVE SUMMARY

This document is a review of the sponsor's population pharmacokinetic (PK) analysis and PK/pharmacodynamic (PK/PD) model-based simulations analysis which support labeling statements.

SPONSOR'S ANALYSIS

Population PK analysis

Objectives

- To describe the population PK characteristics of ponesimod including between-subject variability.
- To investigate the relationship between covariates and model parameters.
- To assess the goodness-of-fit of the model and visualize the influence and relevance of identified covariates.

Data: Th PK data of 680 subjects from a total of 13 studies (AC-058-101, -102, -103, -105, -107, -108, -109, -110, -112, -113, -115, AC-058A200, and AC-058B201) were used to develop population PK models for ponesimod. The covariate characteristics of the data is provided in **Table 6** .

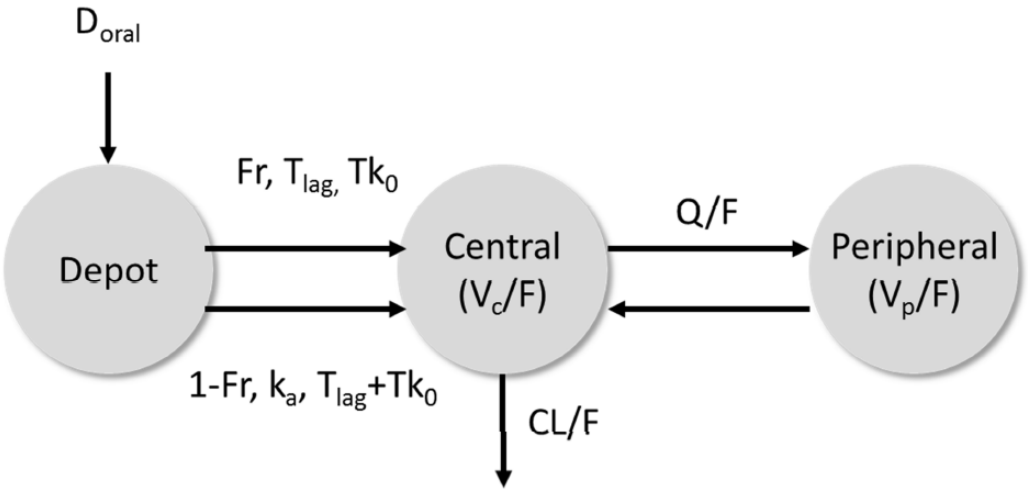
Table 6: Summary statistics of the 680 subjects included in the PK dataset

Covariates	**Mean (Min, Max)**
Demographics	
Age (years)	36.8 (17, 65)
Body weight (Kg)	74.9 (42, 129.3)
Male N (%)	336 (49)
Race N (%)	
White	601 (88)
Asian	16 (2)
Black	53 (8)
Others	10 (2)
Disease Status *N (%)*	
Healthy	255 (37)
Hepatic Impairment (Mild / Moderate / Severe)	8 (1.2) / 8 (1.2) / 8 (1.2)
Renal Impairment (Moderate / Severe)	8 (1.2) / 8 (1.2)
Psoriasis	45 (7)
MS	340 (50)
Formulations *N (%)*	
Capsule A	85 (12)
Capsule B	431 (61)
Tablet C	190 (27)

Method: Nonlinear mixed effect PK modeling was conducted using Monolix® software. The base structural model was first developed to describe ponesimod PK profiles. Covariate modeling was done using forward addition ($p < 0.01$) and backward elimination ($p < 0.001$). Covariates including age, weight, sex, race, food, formulation and disease status were tested. Continuous covariates were included into the model as a power relationship, while categorical covariates were implemented as a difference to a reference group. The final model was evaluated using goodness-of-fit plots, individual-predicted PK profiles, parameter distribution, and visual predictive checks.

Results: The PK of ponesimod was described by a two compartments model with sequential zero/first-order absorption and first-order elimination (**Figure 3**). Body weight was added on clearance and volume of distribution (central and peripheral); race was added on clearance and peripheral volume of distribution; food status and formulation were added on lag time; and disease status (including psoriasis, MS, hepatic impairment (HI)) was added on clearance and central volume of distribution. The parameter estimates of the final pop PK model for ponesimod are shown in **Table 7**.

Figure 3: Schematic representation of the PK model for ponesimod



CL/F=apparent plasma clearance of elimination; Fr=fraction of dose absorbed via zero-order process; ka=first-order absorption rate constant; Q/F=apparent inter-compartmental drug flow; Vc/F=apparent central volume of distribution; Vp/F=apparent peripheral volume of distribution; T_{lag}=absorption lag time; Tk_0=duration of the zero-order absorption.
Circles indicate compartments, solid lines denote drug flow with associated PK parameters.

Source: Janssen population PK/PD report: Figure 10, Page 29.

Table 7: Parameter estimates of sponsor's final population PK model for ponesimod

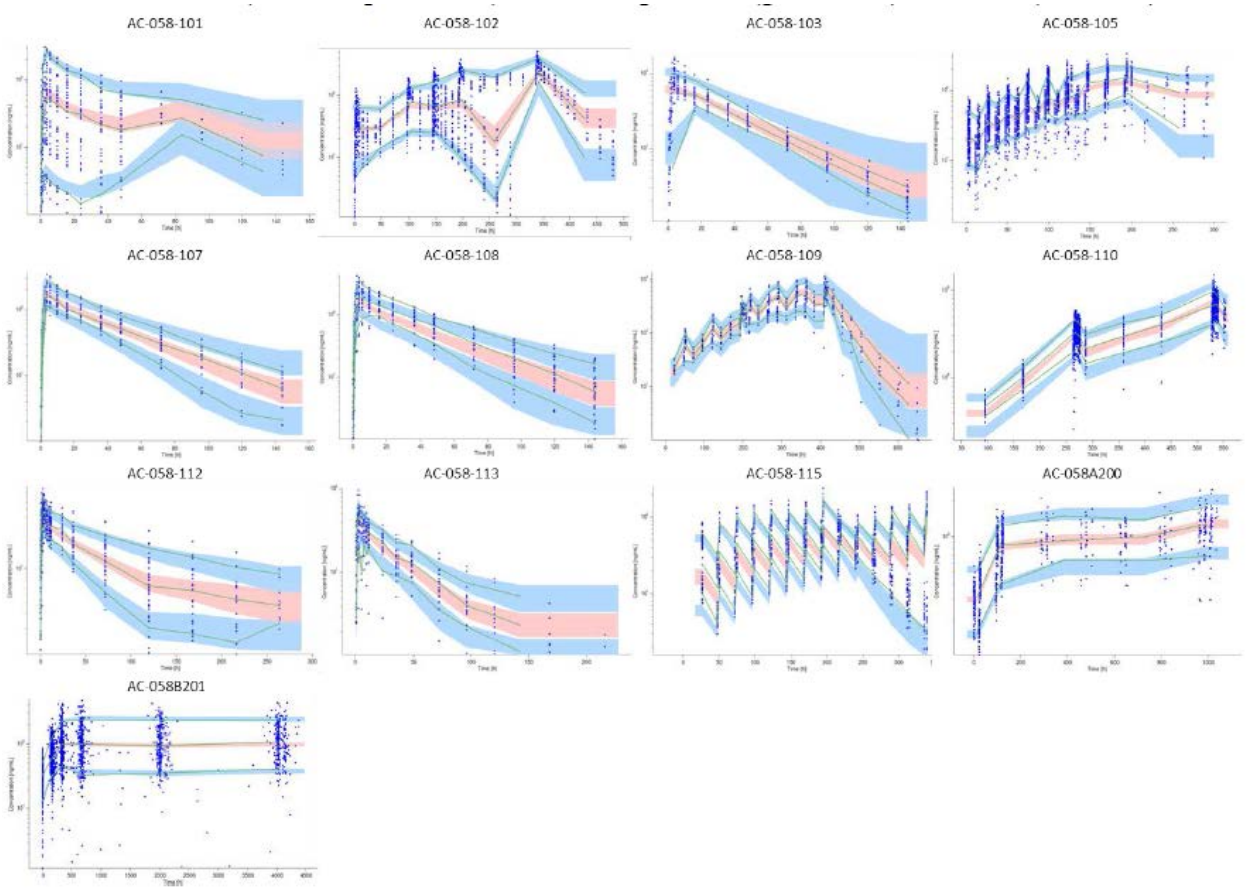
Parameter		Estimate	r.s.e. (%)	p-value
T_{lag} (h)	Absorption lag time	0.396	6	
Formulation Capsule A on T_{lag}	Covariate effect	−0.001	3600	0.9800
Formulation Capsule C on T_{lag}	Covariate effect	0.397	12	< 0.0001
Food status fed on T_{lag}	Covariate effect	0.483	26	0.0002
Food status uncontrolled on T_{lag}	Covariate effect	0.047	306	0.7400
Tk_0 (h)	Duration of zero-order absorption process	0.578	5	
Fr	Fraction absorbed via zero order	0.147	8	
k_a (1/h)	Absorption rate constant	0.932	7	
V_{cent}/F (L)	Apparent volume of distribution, central compartment	165	4	
Body weight on V_{cent}	Covariate effect	0.847	8	< 0.0001
Psoriasis on V_{cent}	Covariate effect	0.368	12	< 0.0001
Multiple sclerosis on V_{cent}	Covariate effect	0.189	17	< 0.0001
V_{per}/F (L)	Apparent volume of distribution, peripheral compartment	107	6	
Body weight on V_{per}	Covariate effect	0.691	21	< 0.0001
Race Asian on V_{per}	Covariate effect	−0.217	63	0.1100
Race Black on V_{per}	Covariate effect	−0.475	19	< 0.0001
Race Other on V_{per}	Covariate effect	−0.620	39	0.0100
	Covariate effect			
Q/F (L/h)	Apparent inter-compartmental clearance for ponesimod	20.90	11	
CL/F (L/h)	Apparent clearance	6.64	1	
Body weight on CL	Covariate effect	0.422	10	< 0.0001
Race Asian on CL	Covariate effect	−0.145	46	0.0310
Race Black on CL	Covariate effect	−0.162	23	< 0.0001
Race Other on CL	Covariate effect	−0.238	36	0.0056
HI (mild) on CL	Covariate effect	−0.355	25	< 0.0001
HI (moderate) on CL	Covariate effect	−0.737	11	< 0.0001
HI (severe) on CL	Covariate effect	−1.14	8	< 0.0001
Between-subject variability (standard deviation)				
Omega_T_{lag}		0.414	8	
Omega_Tk_0		0.518	8	
Omega_Fr		0.574	10	
Omega_k_a		0.556	6	
Omega_V_{cent}		0.218	6	
Omega_V_{per}		0.277	9	
Omega_Q		0.103	244	
Omega_CL		0.258	3	
Residual error terms				
a	Additive error	0.006	28	
b	Proportional error	0.206	1	

Source: Janssen population PK report: Table 18, Page 38

The population PK model for ponesimod was assessed with diagnostics plots including goodness-of-fit (**Figure 10**) and visual predictive checks (**Figure 4**). Overall, the PK model adequately describes the PK of ponesimod. The final population PK model was used to simulate

steady-state PK profiles of ponesimod 20 mg QD to evaluate the impact of covariates on ponesimod PK profiles and exposures (C_{max}, C_{trough}, and AUC_{tau}) (**Figure 5**). While the effects of body weight, race, food status, formulation, MS, and psoriasis were within the range of the between-subject variability (simulated $C_{max,ss}$ values range from 132 (10th percentile) to 221 ng/mL (90th percentile)), the influences of moderate and severe HI were larger than the between-subject variability ($C_{max,ss}$ >= 221 ng/mL).

Figure 4: Visual Predictive Checks stratified by study. Ranges for median, and 10 and 90% quantiles of simulated values (blue and pink areas), observed quantiles (green lines), and data (blue dots)



Source: Janssen population PK report: Figure 15, Page 44.

Figure 5: Covariate effects on ponesimod PK profiles at steady-state doses of 20 mg QD (top) and the corresponding change in exposure compared to the reference subject (bottom)

	$C_{max, ss}$ (ng/mL)	$C_{trough, ss}$ (ng/mL)	AUC_{τ} (h*ng/mL)	+/− compared to the reference subject*		
				$C_{max, ss}$	$C_{trough, ss}$	AUC_{τ}
Reference subject*	172	91.9	3014			
Body weight						
• 50 kg	211	105.0	3577	+23%	+14%	+19%
• 100 kg	148	83.3	2670	−14%	−9%	−11%
Psoriasis	158	98.9	3014	−8%	+8%	+/−0%
MS	164	95.6	3014	−5%	+4%	+/−0%
Food status						
• Fed	172	91.5	3012	+/−0%	+/-0%	+/−0%
Formulation						
• Capsule C	172	91.9	3014	+/−0%	+/−0%	+/−0%
Race (Black)	191	111.0	3544	+11%	+20%	+18%
HI						
• Mild	225	144.0	4298	+31%	+57%	+43%
• Moderate	308	226.0	6296	+79%	+146%	+109%
• Severe	437	355.0	9412	+154%	+287%	+212%

MS: multiple sclerosis, HI: hepatic impairment.

* The reference subject is fasted, White, healthy (no psoriasis, no HI, etc.), and has a body weight of 75 kg and received tablet C.

Source: Janssen population PK report: Figure 18 and Table 20, Page 48-49.

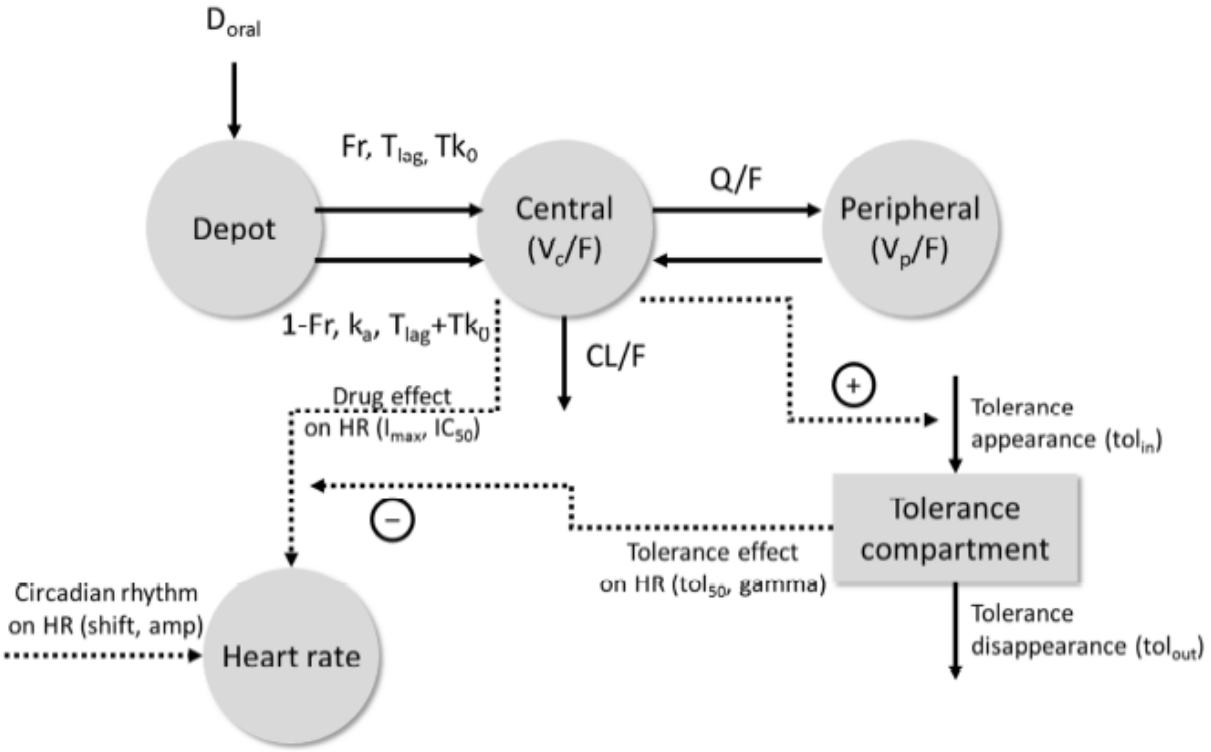
Population PK/PD simulation analysis

Objectives

- Effect of the proposed up-titration regimen (with the daily doses of 2-2-3-3-4-4-5-6-7-8-9-10-10-10-20 mg given for 15 days) on the bradycardia (<40 beats per minute (bpm)) incidence in subjects with normal hepatic function and in subjects with mild HI.
- To evaluate the impact of the baseline heart rate (HR_{BL}) on the incidence of bradycardia (HR<40 bpm) during the ponesimod up-titration phase in subjects with normal hepatic function.
- To provide guidance on the re-initiation of the ponesimod up-titration in case of treatment interruptions following either the maintenance treatment at 20 mg dose or during the up-titration period in subjects with normal hepatic function.
- To estimate the required time to recover total lymphocytes count to normal values after ponesimod treatment interruption in subjects with normal liver function and in subjects with mild HI.

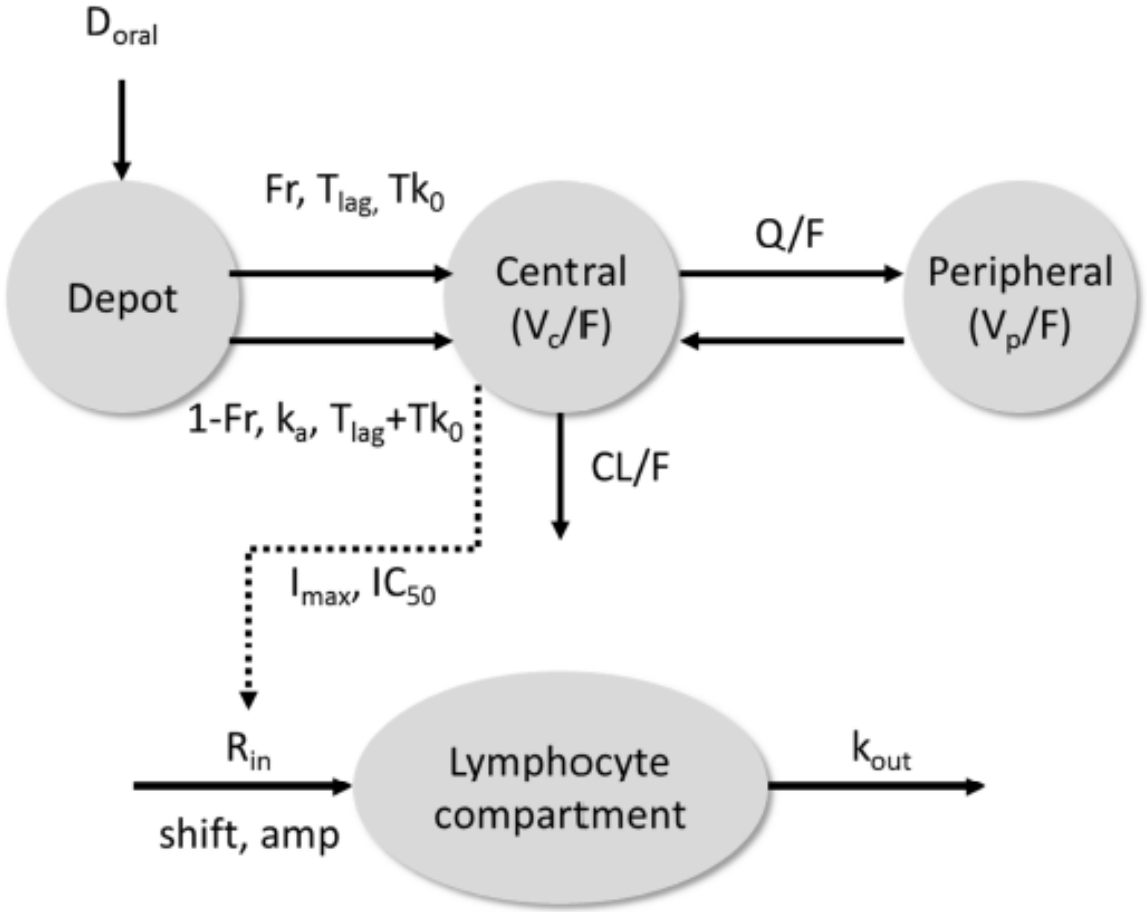
Method: The available PK model developed based on data in healthy subjects and in patients with MS, and the PK/PD models for describing the effects of ponesimod on HR and total lymphocyte counts, developed based on Phase 1 studies in healthy subjects, were used in a simulation-based approach to accomplish the objectives. Briefly, the PK/PD model for HR comprised a circadian rhythm, a tolerance compartment, and a drug effect modeled with a maximal effect (Imax) function, with Imax decreasing with increasing tolerance (**Figure *6***). This model was able to describe the effect of ponesimod on HR and its variability (Lott 2018). For PK/PD of total lymphocyte counts, an indirect response model with effect of circadian variation and concentration-dependent inhibition on the lymphocyte zero-order production rate described the effect of ponesimod on lymphocyte count (**Figure -7**)(Lott 2017).

Figure 6: Schematics of the population PK/PD model for heart rate reduction



Source: Janssen population PK/PD report: Figure 11, Page 32.

Figure -7: Schematics of the population PK/PD Model for lymphocytes



Source: Janssen population PK/PD report: Figure 12, Page 34.

Results:

Effect of proposed up-titration regimen on bradycardia events: In subjects with normal liver function and in subjects with mild HI, model-based simulations suggested a mean daily bradycardia incidence of <1% during the first 15 days of therapy following the up-titration dosing (**Table 8**). The mean cumulative (Day 1-15) incidence of bradycardia was 2.922% and 2.979% in subjects with normal liver function and subjects with mild HI, respectively. For the subjects with HR_{BL} > 55 bpm, the mean cumulative incidences of bradycardia were 0.8%. These results supported the proposed gradual up-titration regimen of ponesimod in healthy subjects and subjects with mild HI. In the Phase 3 study, four subjects (out of 565 subjects, or 0.7%) have reported bradycardia with the proposed up-titration regimen of ponesimod. All these events were reported on Day 1 after treatment initiation.

Table 8: Bradycardia incidence (%) per day and overall (day 1-15) estimated during the proposed up-titration regimen in subjects with normal hepatic function and in subjects with mild hepatic impairment.

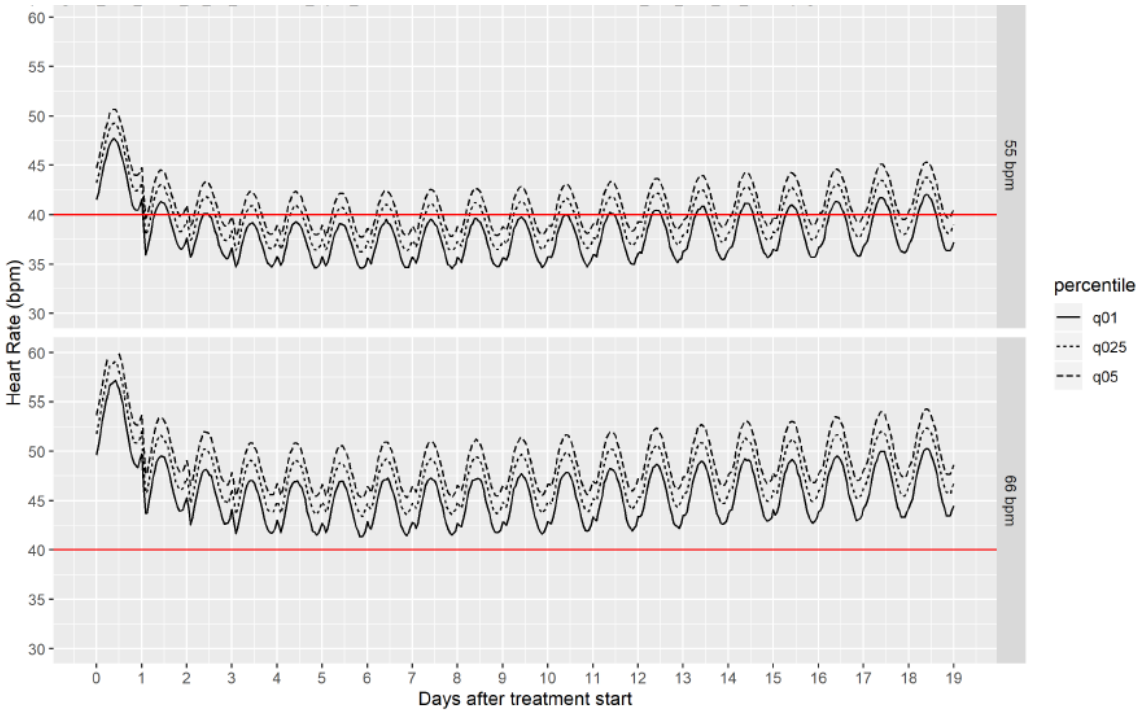
Titration Day	Dose (mg)	Mean Bradycardia <40 bpm Incidence (%)	
		Subjects With Normal Hepatic Function	Subjects With Mild Hepatic Impairment
1	2	0.369	0.410
2	2	0.541	0.604
3	3	0.835	0.979
4	3	0.765	0.894
5	4	0.853	0.906
6	4	0.753	0.788
7	5	0.753	0.727
8	6	0.700	0.662
9	7	0.637	0.544
10	8	0.578	0.465
11	9	0.490	0.417
12	10	0.406	0.317
13	10	0.343	0.262
14	10	0.284	0.215
15	20	0.361	0.238
Overall (Day 1-15)			
All Subjects		2.922 (2.199-3.760)	2.979 (2.000-3.745)
Subjects with HR at baseline ≥55 bpm		0.807 (0.323-1.473)	0.845 (0.325-1.512)

HR=heart rate
Overall bradycardia reported as median (5^{th} and 95^{th} percentiles)

Source: Janssen population PK/PD report: Table 4, Page 15.

Effect of HR at baseline on bradycardia: The PK/PD simulation indicated that ponesimod administration to subjects with HR_{BL} of 55 bpm would result in increased bradycardia incidence i.e. cumulative bradycardia incidence was ~28% as compared to <1% incidences predicted in subjects with HR_{BL} >55 bpm (***Figure 8***). These results supported the recommendations for first-dose HR monitoring in subjects with HR_{BL}<55 bpm.

Figure 8: Top row: Time course of the heart rate for the up-titration regimen in population of subjects with different values of HR_{BL}. Bottom row: Overall bradycardia incidences (%) estimated for the proposed up-titration scheme in population of subjects with different values of HR_{BL}.



HR_{BL}=baseline heart rate.
Note: Solid, short-dashed, and long-dashed black lines indicates the 1st, 2.5th and 5th percentiles of 50 replicates. Solid horizontal red line indicates the limit value to consider bradycardia (40 bpm). Upper panel: HR_{BL} 55 bpm; lower panel: HR_{BL} 66 bpm.

HR_{BL} (bpm)	Mean bradycardia <40 bpm incidence (%) median (5^{th}-95^{th} percentile)
55	28.18 (25.87-30.33)
66	0.59 (0.27-0.96)

HR_{BL}=baseline heart rate

Source: Janssen population PK/PD report: Figure 3, Page 17.

Effect of Treatment Discontinuation on bradycardia: The PK/PD **s**imulation results indicated that for treatment discontinuation lasting up to 3 days during maintenance dosing resulted in <1% bradycardia incidences (**Table 9**). In case of treatment interruption during up-titration, the overall bradycardia incidences were like that of the uninterrupted up-titration regimen (**Table 10**). These results suggested that for if less than 4 consecutive doses are missed, treatment can be resumed with the first missed dose. However, if 4 or more consecutive doses are missed, treatment should be reinitiated with Day 1 of the titration regimen.

Table 9: Top row: Description of doses (in mg QD) used in the different scenarios following the steady state in the maintenance phase. Bottom row: Overall bradycardia incidence (%) estimated after maintenance treatment discontinuation as a function of the duration of treatment discontinuation.

Number of Missed Doses (Yellow Cells)	Treatment Day (After Drug Discontinuation)											
	Ponesimod doses (mg)											
0 (reference)	20	20	20	20	20	20	20	20	20	20	20	20
1	20	0	20	20	20	20	20	20	20	20	20	20
2	20	0	0	20	20	20	20	20	20	20	20	20
3	20	0	0	0	20	20	20	20	20	20	20	20
4	20	0	0	0	0	20	20	20	20	20	20	20
5	20	0	0	0	0	0	20	20	20	20	20	20
6	20	0	0	0	0	0	0	20	20	20	20	20
7	20	0	0	0	0	0	0	0	20	20	20	20

Discontinuation duration (days)	Mean Bradycardia <40 bpm incidence (%)
0	0.469
1	0.529
2	0.714
3	0.741
4	1.076
5	1.473
6	1.790
7	2.500

Source: Janssen population PK/PD report: Table 6, Page 19.

Table 10: Top row: Description of doses (in mg QD) used in the different scenarios during the up-titration phase. Bottom row: Overall bradycardia incidence (%) estimated after up-titration treatment discontinuation period as a function of the duration of treatment discontinuation.

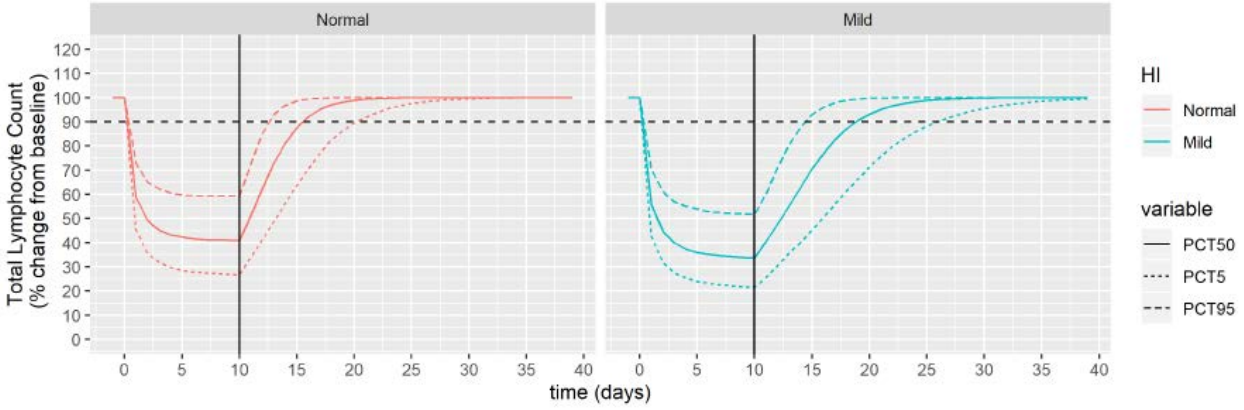
Scenario	Number of Missed Doses (Yellow Cells)	Treatment Day																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
		Ponesimod Doses (mg)																		
0 (reference)	0	2	2	3	3	4	4	5	6	7	8	9	10	10	10	20	20	20	20	20
1a	1	2	2	0	3	3	4	4	5	6	7	8	9	10	10	10	20	20	20	20
1b	2	2	2	0	0	3	3	4	4	5	6	7	8	9	10	10	10	20	20	20
1c	3	2	2	0	0	0	3	3	4	4	5	6	7	8	9	10	10	10	20	20
2a	1	2	2	3	3	4	4	5	0	6	6	7	8	9	10	10	10	20	20	20
2b	2	2	2	3	3	4	4	5	0	0	6	6	7	8	9	10	10	10	20	20
2c	3	2	2	3	3	4	4	5	0	0	0	6	6	7	8	9	10	10	10	20
3a	1	2	2	3	3	4	4	5	6	7	8	9	10	10	10	0	20	20	20	20
3b	2	2	2	3	3	4	4	5	6	7	8	9	10	10	10	0	0	20	20	20
3c	3	2	2	3	3	4	4	5	6	7	8	9	10	10	10	0	0	0	20	20

Discontinuation duration (days)	Up-titration Day	Up-titration doses	Mean Bradycardia <40 bpm incidence (%)
1	3	2 to 3	2.851
2	3	2 to 3	2.726
3	3	2 to 3	2.761
1	8	5 to 6	1.754
2	8	5 to 6	1.843
3	8	5 to 6	1.992
1	15	10 to 20	0.966
2	15	10 to 20	1.168
3	15	10 to 20	1.478

Source: Janssen population PK/PD report: Table 7, Page 22.

Effect of treatment discontinuation on total lymphocytes: The PK/PD **s**imulations suggested that after stopping ponesimod maintenance treatment of 20 mg in subjects with normal hepatic function, the total lymphocyte count returns above the lower limit of the normal value within 2, 4 and 8 days in 50%, 90%, and 99% of subjects, respectively (**Figure 9**).

Figure 9: **Top row: Total lymphocytes counts following ponesimod 20 mg QD for 10 days in subjects with normal hepatic function and mild hepatic impairment.; Bottom row: Total lymphocyte count nadir and time to recovery after stopping maintenance treatment (20 mg daily) in subjects with normal hepatic function and with mild hepatic impairment.**



Hepatic Function	Median (5^{th}-95^{th} percentile)	
	Nadir (fraction of baseline)	Time to recovery to normal values (days)
Normal	0.41 (0.27-0.59)	2 (0-6)
Mild Hepatic Impairment	0.34 (0.21-0.52)	3 (0-9)

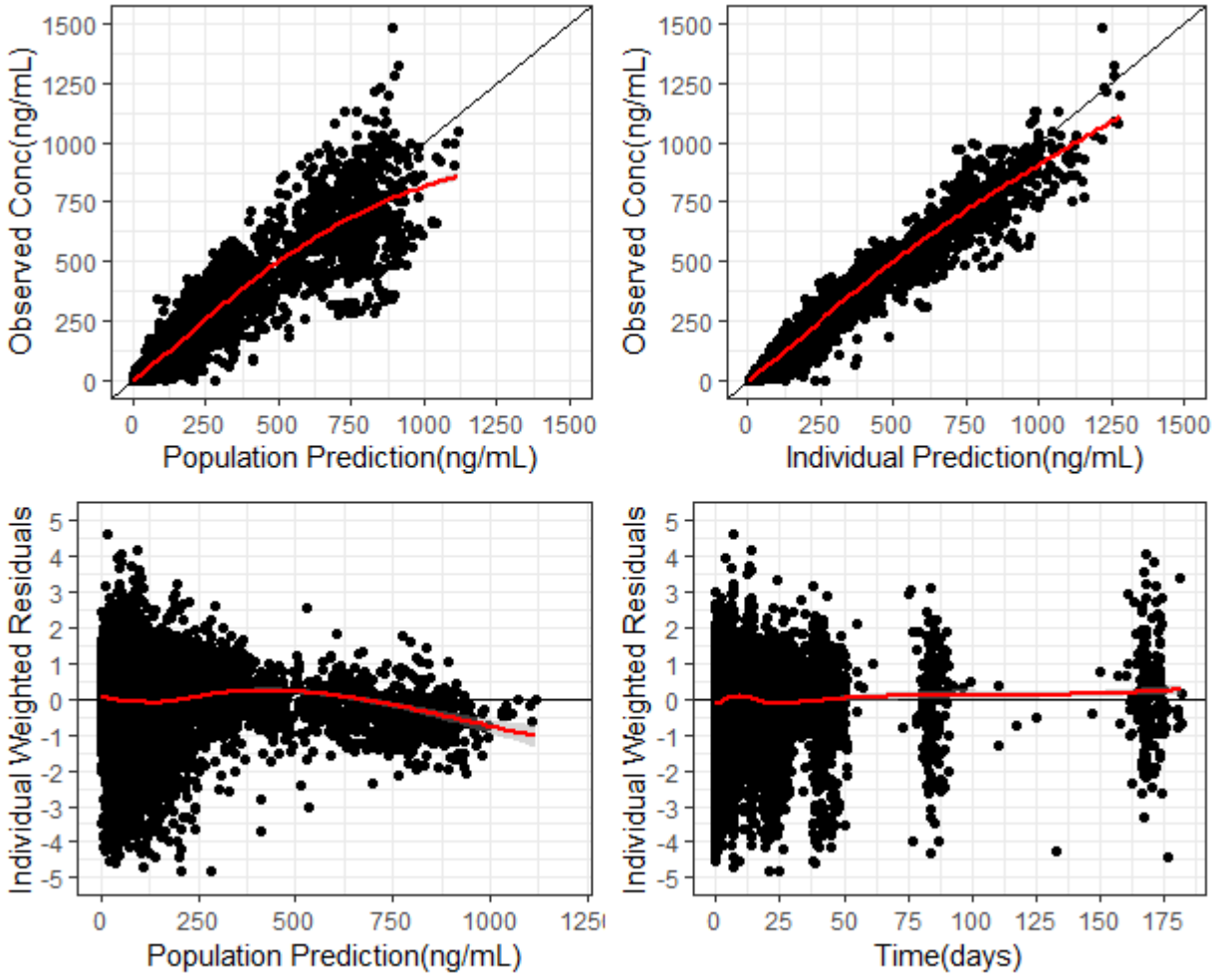
Source: Janssen population PK/PD report: Figure 9 and Table 8, Page 23.

REVIEWER'S ANALYSIS

Sponsor's population PK model evaluation

The reviewer was able to run the sponsor's final PK model and obtained similar results. Model diagnostics for ponesimod is shown in **Figure 10** respectively.

Figure 10: Goodness-of-fit plots of sponsor's population PK model for ponesimod



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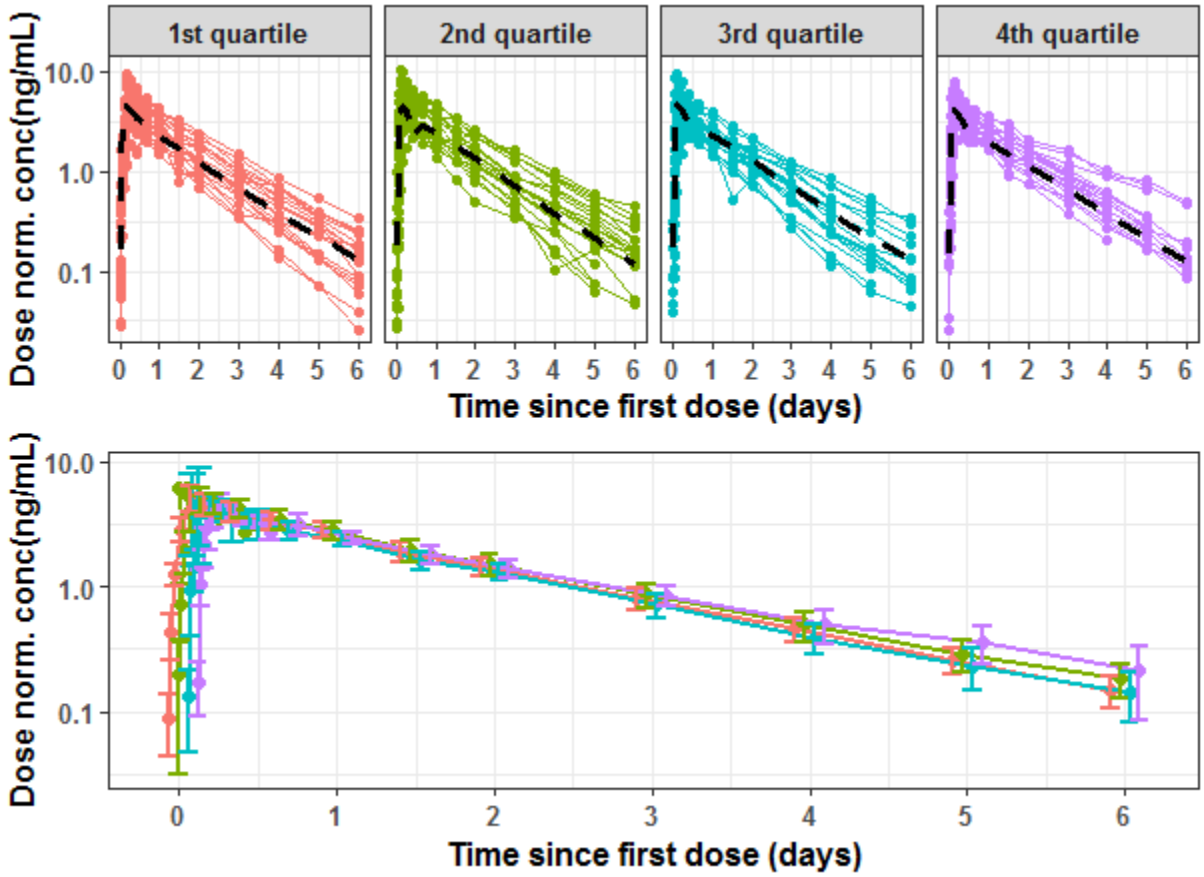
The clinically relevant impact of the covariates on the PK of ponesimod was evaluated based on both pop PK modeling results and the observed data (by evaluating the overlap of 95% CI of PK profiles stratified by the covariate-of-interest). For the observed data, the rich PK sampling data from six studies (AC-058-101, -103, -107, -108, -112 and -113) were pooled together to evaluate the impact of covariates on ponesimod.

Age effect: The PK profiles of ponesimod were distributed into quartiles (i.e. 1st quartile: n=13, 18-24; 2nd quartile: n=15, 24-36; third quartile: n=18, 36-45; and 4th quartile: n=16, 45-59) based on their weight distribution and plotted along with the corresponding median population predictions (**Figure 11**). Overall, overlap of 95% confidence intervals of the PK profiles by age quartiles suggested lack of clinically relevant impact of age on the PK of ponesimod. Additionally, the pop PK model developed from the data of 680 subjects (range: 17-65 years) did not identify age as a significant covariate affecting ponesimod PK.

Figure 11: Top row: Individual PK profiles of ponesimod by age quartiles. Black dashed line indicates median population predictions from sponsor's final PK model; Bottom row: Mean (95% CI) PK profiles of ponesimod by age quartiles. Red, green, cyan and purple color indicates 1st, 2nd, 3rd and 4th quartile of age respectively.



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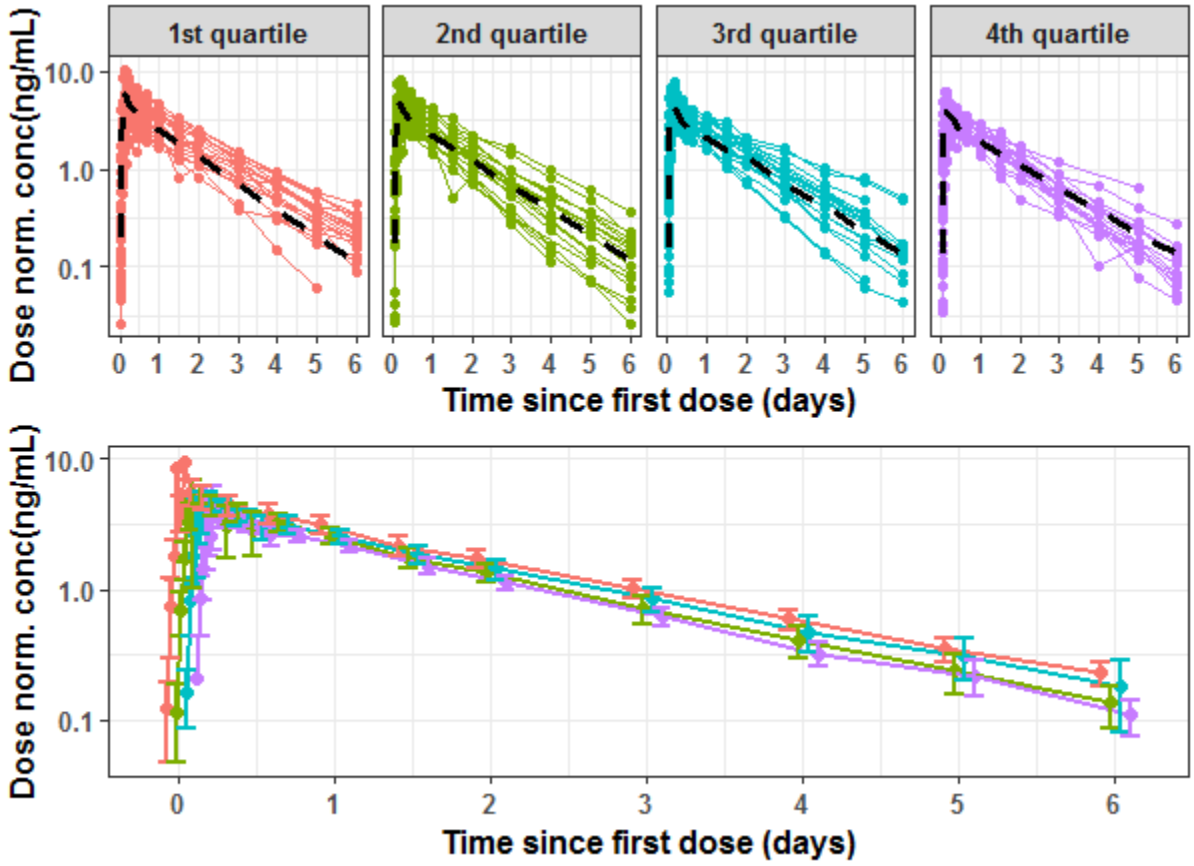
Body weight effect: The PK data of ponesimod were distributed into quartiles (i.e. 1st quartile: n=14, 47-62; 2nd quartile: n=17, 62-69; third quartile: n=16, 69-78; and 4th quartile: n=15, 78-96) based on their weight distribution and plotted along with the corresponding median

population predictions (**Figure 12**). Overall, increase in body weight resulted in lower AUC and C_{max} of ponesimod. Based on the the sponsor's PK simulations, 23% increase in $C_{max,ss}$ and 19% increase in $AUC_{tau,ss}$ was predicted for subjects weighing 50 kg as compared to the reference subject weighing 75 kg (**Figure 5**). These changes in exposures do not suggest clinically relevance impact of body weight on ponesimod PK.

Figure 12: Top row: Individual PK profiles of ponesimod by body weight quartiles. Black dashed line indicates median population predictions from sponsor's final PK model; Bottom row: Mean (95% CI) PK profiles of ponesimod by body weight quartiles. Red, green, cyan and purple color indicates 1st, 2nd, 3rd and 4th quartile of body weights respectively.



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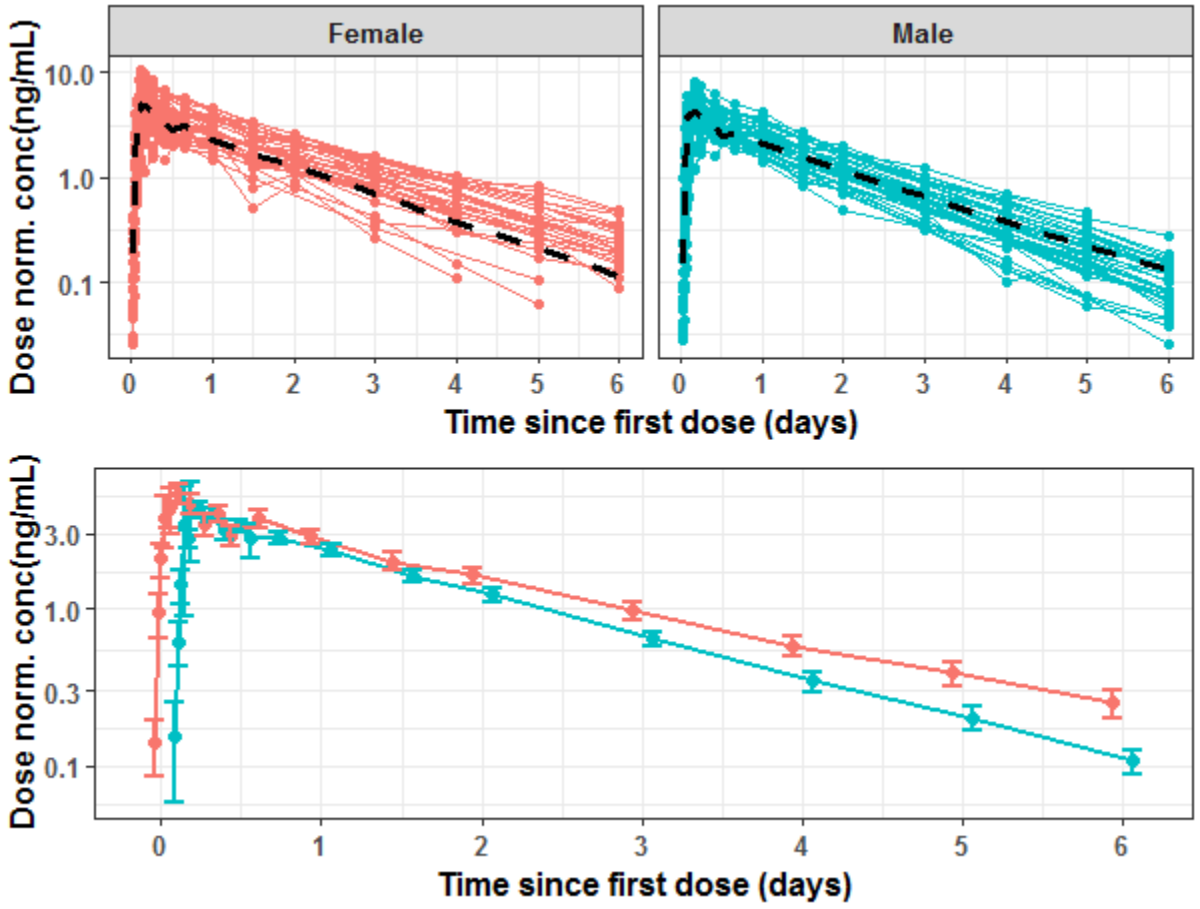
Sex effect: The PK profiles of 28 males and 34 females dosed with ponesimod were compared and plotted along with the corresponding population predictions (**Figure 13**). Overall, the median PK profiles of male subjects has shown a decrease of 25% in C_{max} and 24% in AUC_{0-t}. The body weight differences in male and female (i.e. median weight of 76 kg in male vs. 61 kg in female) appears to account for most of these PK exposures differences. Also, the pop PK model developed from the data of 680 subjects (336 females and 344 females) did not identify sex as a significant covariate of ponesimod PK.

Figure 13: **Top row: Individual PK profiles of ponesimod by sex. Black dashed line indicates median population predictions from sponsor's final PK model; Bottom row: Mean (95% CI) PK profiles of ponesimod by sex. Red color indicates males and strong cyan color indicates female subjects.**



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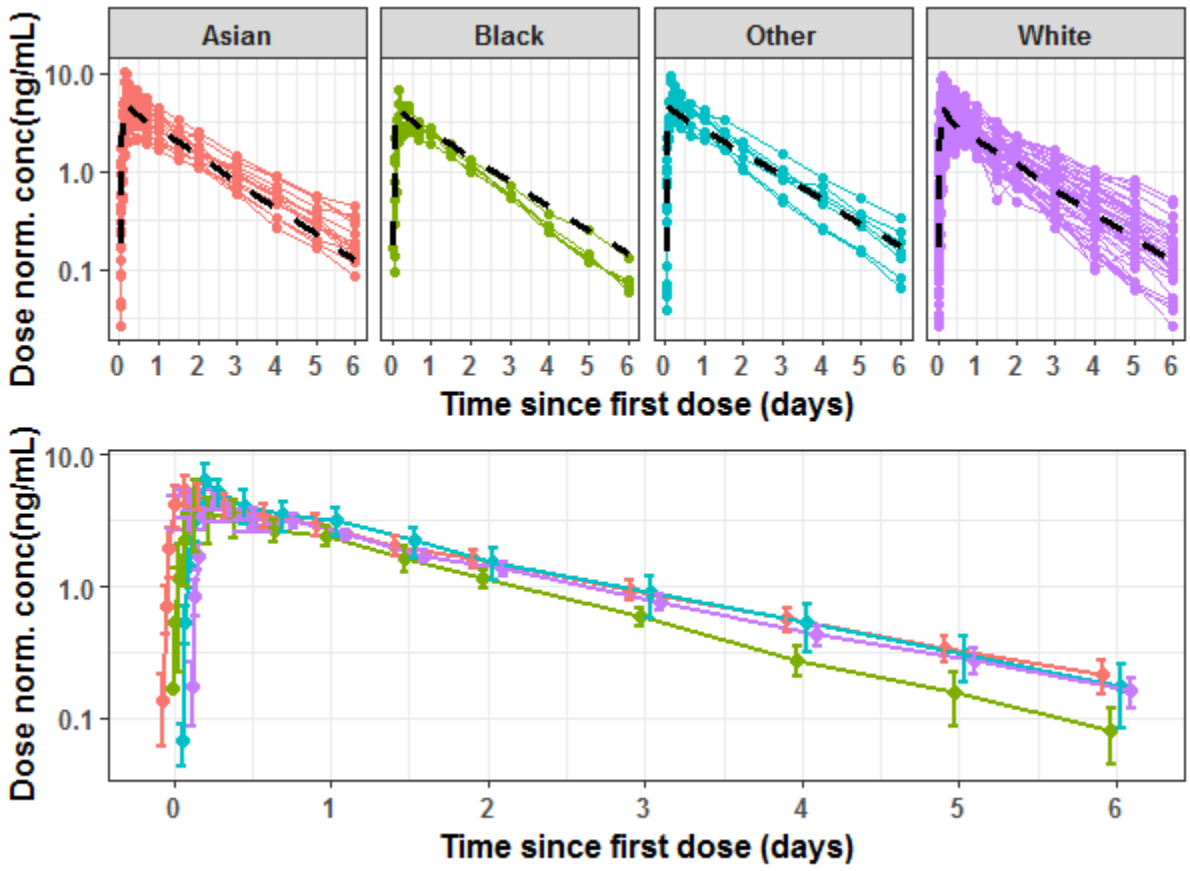
Race effect: The PK data of 41 white, 12 asian, five black and four other subjects dosed with ponesimod were compared and plotted along with the corresponding median population predictions (***Figure 14***). The data was limited for black and other categories. For white and asian category, overlap of 95% confidence intervals of the PK profiles suggested lack of clinically relevant impact of race on the PK of ponesimod. The population PK model which was developed from a larger dataset (white [n=601], asian [n=16], black [n=53], and others (n=10)] confirmed the above findings (**Figure 5**).

Figure 14: Top row: Individual PK profiles of ponesimod by race. Black dashed line indicates median population predictions from sponsor's final PK model; Bottom row: Mean (95%CI) PK profiles of ponesimod by race. Red, green, cyan and purple color indicates asian, black, other and white respectively.



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Multiple sclerosis effect: The Sponsor's pop PK simulations estimated 5% difference in $C_{max,ss}$ and negligible changes in $AUC_{tau,ss}$ between healthy subjects and subjects with multiple sclerosis, which suggested lack of clinically relevant impact of multiple sclerosis on the PK of ponesimod (**Figure 5**).

Sponsor's population PK/PD simulation evaluation

The sponsor's PK/PD simulations were used to support (i) the proposed gradual up-titration regimen of ponesimod; (ii) first-dose HR monitoring in subjects with HR_{BL}<55 bpm; (iii) missed doses strategy; and (iv) caution with immunosuppressants. Reviewers agrees with the sponsor's PK/PD simulations analysis and conclusion described in section **Population PK/PD simulation analysis**.

LISTING OF ANALYSIS CODES AND OUTPUT FILES

File Name	Description	Location
pk_analysis_ponesimod.R	Exploratory PK analysis	\\Reviews\Ponesimod_NDA213498_VS\Reviewer\Rscripts

REFERENCES

1. Janssen population PK report: Population pharmacokinetics of ponesimod, 2 September 2016.
2. Janssen population PK/PD report: Pharmacokinetic & pharmacodynamic model-based simulations supporting ponesimod dosing in multiple sclerosis subjects, 13 September 2019.
3. Lott D. Modeling and simulation report: Modelling the effect of ponesimod on heart rate. Doc No. D-17623 Actelion. 23 April 2018.
4. Lott D, Krause A, Seemayer CA, Strasser DS, Dingemanse J, Lehr T. Modeling the effect of the selective S1P receptor modulator ponesimod on subsets of blood lymphocytes. Pharm Res. 2017c; 34(3):599-609.

4.3 Physiologically based Pharmacokinetic Modeling Review

Executive Summary

The aim of this review is to evaluate the adequacy of physiologically-based pharmacokinetic (PBPK) modeling to predict the drug-drug interaction (DDI) potential of ponesimod as a CYP perpetrator. Specifically, the Applicant applied the PBPK modeling approach to assess the effects of ponesimod on the exposures of midazolam (CYP3A), warfarin (CYP2C9), tolbutamide (CYP2C9) and omeprazole (CYP2C19).

The Division of Pharmacometrics has reviewed the PBPK reports (FK13357 and FK13637) and supporting modeling files to conclude the following:

- There is low potential for a drug-drug interaction (considered as less than 0.8-fold or greater than 1.25-fold change in AUC or Cmax), between ponesimod and a substrate for CYP3A, CYP2C9 or CYP2C19.

Background

Ponesimod is provided as film-coated tablets for oral use at a maintenance dose of 20 mg once daily (QD), after a gradual 14-day up-titration regimen. The ponesimod metabolites identified in human plasma are ACT-204426 (M12) and ACT-338375 (M13), with only M13 considered as major (defined as >10% of total drug exposure). The mean exposure to ponesimod, M12 and M13 increased in a dose-proportional manner following multiple-dosing in healthy adults [Clinical Pharmacology Summary]. Also, ponesimod showed a time-independent PK after multiple dosing (5 to 20 mg/day for 7 days) in healthy adults [Study AC-058-102]. The multiple-dose PK of ponesimod (10 mg, 20 mg and 40 mg QD for 24 weeks) in the target patient population (relapsing-remitting MS) were consistent with those established in healthy subjects [Study AC-058B201]. Food had no clinically relevant effect on ponesimod PK [Study AC-058-101].

Ponesimod was extensively metabolized with several (>30) minor metabolites recovered in feces and urine [Study AC-058-106]. The metabolism of ponesimod to M13 is assumed to occur primarily via non-CYP metabolic pathway(s); while multiple CYPs (2J2, 3A4, 3A5, 4F3A, and 4F12) and non-CYP enzymes catalyze the oxidation of ponesimod to M12 [FK13520, B-18.004]. Ponesimod may undergo direct glucuronidation (UGT1A1 and UGT2B7, based on in vitro data [FK13537]. In the human mass balance study [Study AC-058-106], unchanged ponesimod in feces represented 16% of total dose. This may represent either unabsorbed dose, biliary excretion, and/or elimination as glucuronides with subsequent hydrolysis back to parent in the gastrointestinal tract. Glucuronides were not detected in plasma and feces, with minor amount (<2%) detected in urine. The potential for entero-hepatic circulation of ponesimod has not been investigated clinically. Ponesimod is not a substrate for the transporters P-gp, BCRP, or OATP1B1/3 [DD19014, FK13518, FK13541, FK13542].

Ponesimod showed potential for inhibition of CYP3A activity in human liver microsomes. The CYP3A reversible inhibition interaction parameter Ki (concentration causing half- maximal inhibition) was 15 μM [B-05.078]. Neither ponesimod nor M13 metabolite were

time-dependent inhibitors of CYP3A activity in pooled human liver microsomes [B-08.581; B-13.080]. As the basic static models did suggest a potential for intestinal CYP3A inhibition (Rgut value ≥11), further analysis was conducted using PBPK modeling herein described.

In vitro, ponesimod, but not M13 metabolite, showed induction potential towards CYP3A mRNA in all three human hepatocyte donors [FK13519]. The calculated nominal maximal effect (Emax) values for ponesimod were 12.0, 14.6, and 11.6-fold, with corresponding EC50 values of 3.11, 4.61, and 3.71 µM. Applying the basic model to the nominal Emax and EC50 values indicated low potential for clinical inductive effect (R3 values>0.8) by ponesimod. Nonetheless, PBPK modeling was also conducted as a confirmatory analysis.

Ponesimod showed potential for inhibition of the activity of CYP2C19 and CYP2C9, with respective Ki values of 5.9 and 6.5 µM, determined using human liver microsomes. Neither ponesimod nor M13 showed evidence of time-dependent inhibition of CYP2C9 and CYP2C19 activities [B-08.581; B-13.080; FK13536]. Ponesimod did not have an inductive effect on CYP2C9 activity or mRNA in hepatocyte cultures [B-08-466]. The basic static model indicated no potential for interaction with CYP2C9 or CYP2C19 (R1 values <1.02). Nonetheless, PBPK analysis was also conducted to further explore the clinical interaction potential of ponesimod on CYP2C9 and CYP2C19.

Regarding the M13 metabolite, it most potently inhibited the CYP2C8 enzyme (Ki = 6.4 µM). However, M13 has low potential for clinical inhibition based on the basic static model (R1 value <1.02) [FK13357]. Thus, the DDI potential of M13 was not investigated with a PBPK model.

The Applicant's proposed label stated, "In vitro investigations indicate that at the (b) (4) dose of 20 mg once-daily, ponesimod and its metabolite M13 do not show any clinically relevant drug-drug interaction potential for CYP or UGT enzymes, or transporters" (Section 12.3, Drug Interaction).

The aim of this review is verifying the adequacy of the PBPK analysis related to the ponesimod potential for clinical DDI mediated by inhibition and/or induction of CYP3A, CYP2C9 and CYP2C19.

Methods

The PBPK analyses were performed using the population-based PBPK software Simcyp® (V18, Simcyp Ltd., a Certara Company, Sheffield, United Kingdom). Predictions of plasma concentration-time profiles and drug-drug interactions were conducted using the software's default healthy volunteer virtual population.

The PBPK model of ponesimod was developed based on physicochemical properties, preclinical, in vitro, and clinical PK data. Key model parameters are described as follows. A mechanistic absorption model (ADAM) was developed based on in vitro permeability data. The absorption of ponesimod following oral tablet was described using the ADAM (Advanced Dissolution Absorption and Metabolism) model and in vitro permeability data (Mean A-B Caco-2 Papp=11.5

$x10^{-6}$ cm/s) [B-05.105]. The fraction absorbed (fa) was predicted to be 0.88 and the absolute bioavailability predicted to be around 90%, which is in line with the observed value of 84% [Study AC-058-114]. The unbound fraction in enterocytes (fugut) was assumed to be 1. The mean observed human clearance (CL=3.8 L/h) and volume of distribution (Vss=2.11 L/kg =161 L) following intravenous administration (5 mg, Study AC-058-114) were used as input parameters. The unbound fraction of ponesimod in plasma (fup) is 0.004 [B-05.075] and albumin is the main plasma binding protein [FK13292]. The blood-plasma partitioning ratio of ponesimod is 0.68.

The in vitro reversible inhibition Ki values for CYP2C9, CYP2C19 and CYP3A, used in ponesimod PBPK model, was 6.5, 5.9 and 15 µM, respectively [B-13.080]. The unbound fraction in in vitro incubations (fumic) was 0.18. This value was determined under the conditions at 0.25 mg/mL microsomes as it provided a conservative approach. CYP2C9 and CYP2C19 assays have used lower amounts of microsomal protein (0.1-0.15 mg/mL).

The in vitro CYP3A induction parameters Emax and EC50 values were 12-fold and 3.11 µM, respectively [FK13519]. The Applicant used the data from a donor (HC10-23) which had the largest Emax/EC50 ratio, thus it would represent the most conservative estimate for CYP3A induction from in vitro study. The data was calibrated against rifampicin control data (Emax and EC50 values were 155-fold and 0.919 µM, respectively) from the same donor. The unbound fraction in in vitro incubations (fuinc) was assumed to be equal to 1.

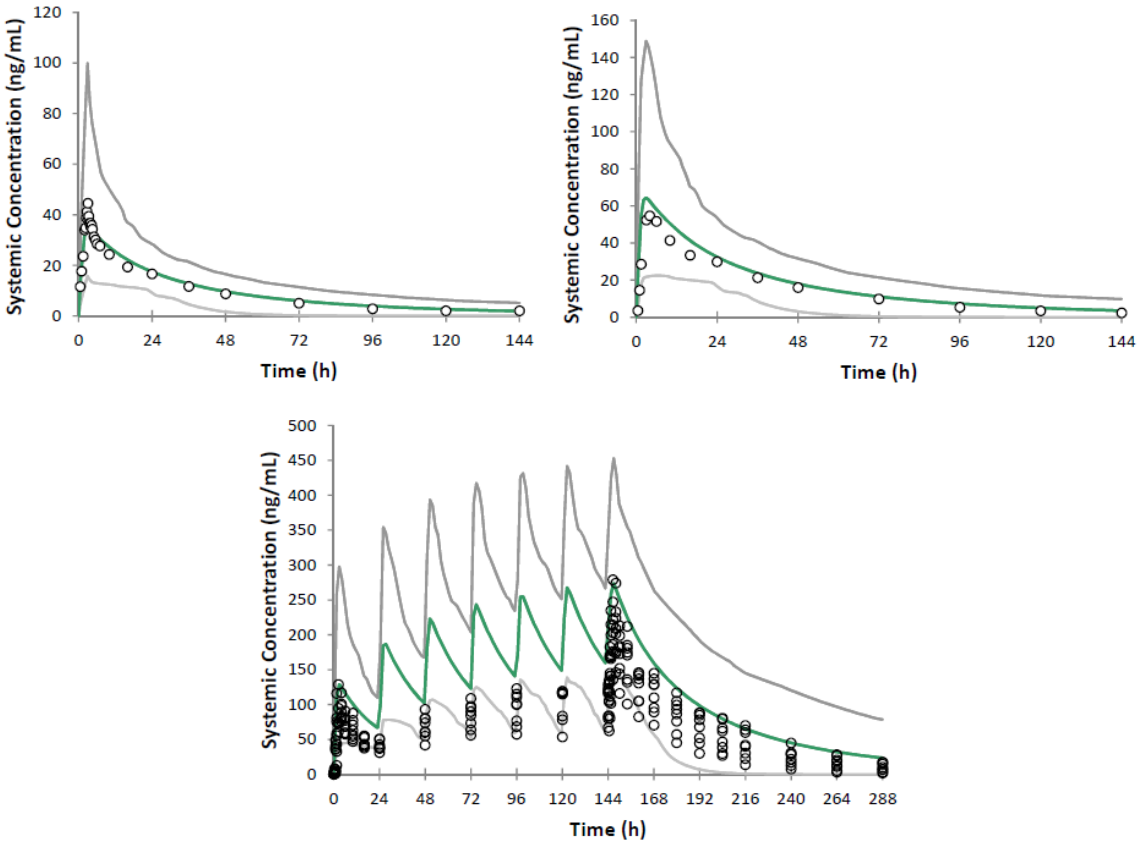
The default compound files (software's library V18) for the CYP2C9, CYP2C19 or CYP3A substrates SV-Tolbutamide, Sim-S-Warfarin, SV-Omeprazole_Solution, and Sim-Midazolam were used in the DDI simulations. Simulations were conducted under fasting conditions.

Results

Q1. Can PBPK analysis provide a reasonable description of the PK of ponesimod in adult healthy population?

There was a reasonable agreement between PBPK predicted and observed PK profile of ponesimod following 5 mg IV (3 h infusion, SD), 10 mg SD PO and 20 mg QD administration in healthy fasted subjects [Studies AC-058-114 and AC-058-102] (**Figure 15** and **Table 11**).

Figure 15. PBPK predicted and observed PK profiles of ponesimod



Plasma concentration time-profiles of ponesimod. Mean PBPK predicted (green lines, n= 10 x 10), observed (open circles-mean or individual data) and 95% prediction interval (gray lines). (A) single IV dose of ponesimod 5 mg (3 h infusion), n=14 (Study AC-058-114). (B) single oral dose of ponesimod 10 mg in fasted state, n=14 (Study AC-058-114). (C) multiple oral dose of ponesimod 20 QD for 7 days, n=8 (Study AC-058-102). (Source: FK13357, Figures 1, 2 and 3).

Table 11. Comparison between PBPK predictions and observed PK of ponesimod

Ponesimod dose regimen	Observed			Predicted		
	Cmax (ng/mL)	AUC (ng.h/mL)	Tmax (h)	Cmax (ng/mL)	AUC (ng.h/mL)	Tmax (h)
5 mg IV SD	48.5	1246	NA	37.2	1284	NA
10 mg tablet SD	61.4	2124	4.0 (3-6)	54.1	2354	3.0
20 mg QD Day 1	91.7	1318	4.0 (2.5-4)	105	1939	3.0
20 mg QD Day 7	207	3473	2.5 (2.5-4)	256	4829	2.6

PK data are geometric means, except median values are listed for Tmax. Observed: Studies AC-058-114 and AC-058-102. NA: Not applicable. (Source: Simulation output files, Study Reports, Reviewer's Analysis).

Ponesimod model reasonably recovered drug accumulation at steady-state following 20 mg QD dosing: 2.7 vs 2.5 (observed vs predicted, respectively).

Reviewer's comment:
The Applicant acknowledged that the PBPK model overpredicted ponesimod plasma exposure (AUC and Cmax) following the therapeutic dose level of 20 mg QD compared to observed in Study AC-058-102 (PE=25-40%, Table 1). Nonetheless, the prediction error was within a reasonable range (PE<50%). The misprediction of ponesimod exposure will not negatively impact the intended application- DDI simulations of ponesimod as perpetrator- due to the higher predicted plasma concentrations.

Q2. Can PBPK analysis be used to estimate the effects of ponesimod on a CYP3A substrate?
Yes. PBPK DDI simulations were conducted between repeated doses of ponesimod (20 mg QD for 10 days) and single doses of midazolam (sensitive CYP3A substrate, 5 mg on Day 10), using the healthy volunteer population model.

DDI simulations were performed with only CYP3A inhibition or induction parameters to determine the impact from each mechanism separately. This approach maximized the sensitivity of the simulation for potential induction effects or inhibitory effects. Therefore, it would represent a conservative scenario for the DDI potential compared to net-effect of the inhibition/induction mechanism simultaneously.

Considering DDI potential of ponesimod as a CYP3A inhibitor only, the interaction effect on midazolam in terms of predicted geometric mean ratios for Cmax and AUC were 1.05 and 1.06, respectively. While considering ponesimod as a CYP3A inducer only, the model predicted a decrease of 9% (0.91-fold) on midazolam exposure in the presence of steady-state ponesimod (**Table 12**).

Table 12. Predicted changes in midazolam's Cmax and AUC in the presence of ponesimod

Perpetrator ponesimod	**CYP3A substrate midazolam 5 mg SD**	
CYP3A interaction	**Cmax ratio [5th-95th percentile][a]**	**AUC ratio [5th-95th percentile][a]**
Reversible inhibition only	1.05 [1.02, 1.09]	1.06 [1.02, 1.10]
Induction only	0.91 [0.81, 0.99]	0.91 [0.81, 0.99]

[a]Geometric mean ratios expressing the fold change in Cmax and AUC calculated as follows: PK with ponesimod/ PK without ponesimod. Simulation design: 10 groups of 10 subjects (n=100) after a single oral dose of 5 mg on day 10 with or without 20 mg QD ponesimod administration for 10 days in healthy subjects. Simulations considering inhibition or induction interaction mechanism only. Source: Applicant's simulation output files.

In the sensitivity analyses for the in vitro CYP3A interaction parameters Ki and EC50 of ponesimod, the geometric mean ratios for Cmax and AUC of midazolam were around the thresholds for weak interaction, in all cases. The 95th percentiles were below 2-fold for inhibition and above 0.5-fold for induction. Results suggested minor risk of underestimation of interaction potential of ponesimod treatment towards midazolam (**Table 13**).

Overall, PBPK analysis suggested the DDI risk between ponesimod treatment and a sensitive

CYP3A substrate is low.

Table 13. Sensitivity Analysis of the CYP3A interaction parameters of ponesimod

Parameter	Fold-change from baseline	Value	Midazolam AUC ratio[a]	Midazolam Cmax ratio[a]
CYP3A Ki (μM)	0.1	1.5	1.34 [1.15-1.62]	1.32 [1.14-1.61]
CYP3A EC50 (μM)	0.1	0.108	0.76 [0.59-0.96]	0.77 [0.59-0.96]
CYP3A Emax (fold)	6*	12	0.69 [0.51-0.89]	0.69 [0.51-0.89]

[a]Geometric mean [5^{th}-95^{th} percentiles] ratios of midazolam AUC and Cmax in the presence/absence of ponesimod. Abbreviations: Ki, drug concentration supporting half maximal inhibition; EC50, drug concentration supporting half maximal induction. Emax, maximal fold induction. *Value determined in vitro without rifampin calibration, using EC50=3.11 μM. Source: Reviewer's independent analyses.

Q3. Can PBPK analysis be used to estimate the effects of ponesimod on a CYP2C9 substrate?
Yes. PBPK DDI simulations were conducted between repeated doses of ponesimod (20 mg QD for 15-25 days) and single doses of warfarin (10 mg on Day 10) or tolbutamide (500 mg on Day 10), as sensitive CYP2C9 substrates, using the healthy volunteer population model.

Ponesimod interaction effect on tolbutamide in terms of predicted geometric mean [5^{th}-95^{th} percentiles] ratios for Cmax and $AUC_{216\text{-}336h}$ were 1.00 [1.00-1.01] and 1.00 [1.00-1.01], respectively. Sensitivity analysis of CYP2C9 Ki value showed less than 1.25-fold increase in tolbutamide exposure with up to 10-fold lower Ki value than that reported in vitro.

The interaction effect on warfarin represented by geometric mean [5^{th}-95^{th} percentiles] ratios for Cmax and $AUC_{216\text{-}576h}$ ratios were 1.00 [1.00-1.01] and 1.00 [1.00-1.01], respectively. Sensitivity analysis of CYP2C9 Ki value suggested minor risk of underprediction of inhibition potential of ponesimod towards S-warfarin. The Cmax and AUC ratios of S-warfarin were below the threshold of 1.25-fold with up to a10-fold lower Ki value than that reported in vitro (Reviewer's Analysis).

Therefore, PBPK analysis suggested a clinically relevant DDI between ponesimod and a CYP2C9 substrate is unlikely.

Q4. Can PBPK analysis be used to estimate the effects of ponesimod on a CYP2C19 substrate?
Yes. PBPK DDI simulations were conducted between repeated doses of ponesimod (20 mg QD for 10 days) and single doses of S-mephenytoin (200 mg on Day 9) or omeprazole (20 mg on Day 10), using the healthy volunteer population model.

Ponesimod interaction effects on omeprazole represented by Cmax and AUC ratios were 1.05 [5^{th}-95^{th} percentiles:1.01-1.11] and 1.06 [5^{th}-95^{th} percentiles:1.02-1.12], respectively. Sensitivity analysis of CYP2C19 Ki value showed less than 1.25-fold increase in omeprazole exposure with up to 10-fold lower Ki value than that reported in vitro. (Reviewer's Analysis).

The Applicant also evaluated the potential of ponesimod inhibition towards S-mephenytoin, which showed no DDI effect.

Conclusions
PBPK analysis predicted there is a low potential for a clinically relevant interaction between ponesimod (20 mg QD) and a sensitive CYP3A substrate (such as midazolam).

Similarly, PBPK analyses predicted that ponesimod (20 mg QD) has low potential for a clinically relevant interaction with a CYP2C9 substrate (such as tolbutamide and S-warfarin) or a CYP2C19 substrate (such as omeprazole).

4.4 Individual Study Review

4.4-1. BIOPHARMACEUTICS STUDIES

4.4-1.1 Bioavailability

Study AC-058-114: Single-center, open-label, randomized, two-way crossover study to investigate the absolute bioavailability of a single oral dose of ponesimod in healthy male subjects.

Objectives:

1) To investigate the absolute bioavailability of a single dose of oral ponesimod (tablet) compared to an intravenous (i.v.) formulation

2) To investigate the pharmacokinetics (PK), safety, and tolerability of ponesimod and its metabolites ACT-204426 (M12) and ACT-338375 (M13) after a single-dose treatment (i.v. and oral formulation) in healthy male subjects.

Methodology:

The study consisted of two parts: a pilot phase and the main study. The pilot phase was completed prior to the start of the main study.

Pilot phase: Subjects arrived at the site on Day −1. The study drug was administered on the morning of Day 1 and was followed by a 144-hour (6 days) observation period. On Day 2, i.e., 24 hours after study drug administration (start of the infusion), the subjects were discharged from the study site. The subjects returned to the study site 36, 48, 72, 96, 120, and 144 hours after study drug administration for additional tolerability, safety, and PK assessments.

Main study (Treatments A and B): Each subject received both Treatment A (ponesimod i.v.) and Treatment B (ponesimod oral). Subjects were randomized to one of two possible sequences of treatments A and B: A-B or B-A (1:1 ratio).

Washout period between doses was 14 days for all subjects.

Number of subjects:

Pilot phase: 3 healthy male subjects.

Main study: 14 healthy male subjects.

Main criteria for inclusion:

Healthy males aged 18–45 years (inclusive) with body mass index of 18–28 kg/m2 (inclusive) at screening, supine systolic blood pressure (SBP) 100–145 mmHg and diastolic blood pressure (DBP) 50–90 mmHg, heart rate (HR) 50–95 bpm (inclusive) and PR interval < 200 ms measured by 12-lead electrocardiogram (ECG).

Test product, dose and mode of administration:

Pilot phase: A 3-hour i.v. infusion of 5 mg ponesimod in sterile 0.9% sodium chloride (NaCl) administered to subjects in the fasted state. A total volume of 50 mL was infused.

Main study: Treatment A: a 3-hour i.v. infusion of 5 mg ponesimod in sterile 0.9% NaCl administered to subjects in the fasted state. A total volume of 50 mL was infused. Treatment B: a single oral dose of 10 mg ponesimod administered as 1 tablet to subjects in the fasted state.

Analytical assay:

Ponesimod and metabolites (M12 and M13) were determined by validated liquid chromatography with tandem mass spectrometry methods with an LLOQ of 1.00 ng/mL for ponesimod, 1.00 ng/mL for ACT-204426 (M12), and 1.00 ng/mL for ACT-338375 (M13).

PK Evaluations:

For each treatment period (including pilot phase), the following plasma PK parameters of ponesimod and its metabolites ACT-204426 (M12) and ACT-338375 (M13) were derived by non-compartmental analysis of the concentration-time data.

- After oral administration: Cmax, Tmax, AUC0-t, AUCinf, T1/2,
- After i.v. administration: AUC0-t, AUCinf, T1/2, CL, Vss.
- The absolute bioavailability (F) was defined as the oral to i.v. ratio of AUCinf values.

PD Evaluations:

Absolute lymphocyte count, absolute change from baseline, and percentage change from baseline for each timepoint of assessment.

RESULTS

Pharmacokinetics

Ponesimod

Summary of PK parameters of ponesimod (main study)

	t_{max} (h)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-t} (h.ng/mL)	$AUC_{0-\infty}$ (h.ng/mL)	CL (L/h)	V_{ss} (L)	F (%)
5 mg ponesimod i.v. (n=3), pilot		27.6 (18.3-41.4)	37.1 (27.2-50.6)	976 (811-1175)	1028 (895-1181)	4.9 (4.2-5.6)	176 (106-293)	
5 mg ponesimod i.v. (n=14), main		32.9 (28.5-38.1)	48.5 (43.9-53.6)	1247 (1102-1411)	1335 (1173-1519)	3.8 (3.3-4.3)	160 (146-174)	83.8 (80.2–87.5)
10 mg ponesimod oral (n=14), main	4 (3-6)	31.7 (27.9-36.0)	61.4 (55.3-68.3)	2124 (1875-2406)	2236 (1957-2554)			

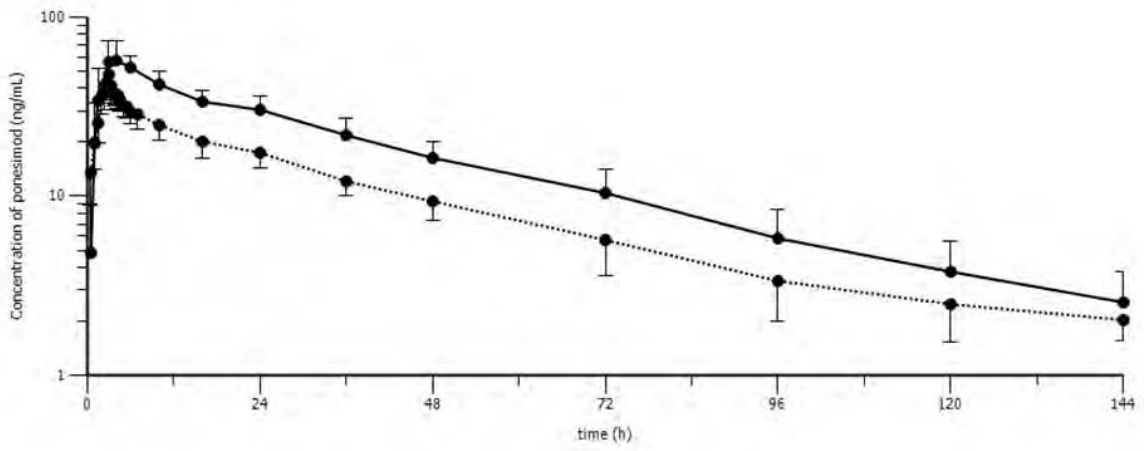
Data are expressed as median (range) for t_{max}, geometric mean (95% confidence interval) for $t_{1/2}$, C_{max}, AUC, CL, and V_{ss}, and percentage of dose-corrected ratio of the geometric mean $AUC_{0-\infty}$ (oral) / $AUC_{0-\infty}$ (i.v.) (90% confidence interval) for F.

$AUC_{0-\infty}$: area under the plasma concentration-time curve from zero to infinity, AUC_{0-t}: area under the plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification, CL: clearance, C_{max}: maximum plasma concentration, F: absolute bioavailability, i.v.: intravenous, $t_{1/2}$: terminal half-life, t_{max}: time to reach C_{max}, V_{ss}: volume of distribution at steady state.

Source: Table 18, Table 19, and Table 24.

Mean (±SD) concentration-time profile (semilogarithmic scale) of ponesimod after a single oral dose of 10 mg (solid line) and after a single 3-hour i.v. infusion of 5 mg is presented graphically by treatment in the Figure below (main study):

Mean (± SD) plasma concentration (semilogarithmic scale) vs time profiles of ponesimod after a single oral dose of 10 mg (solid line) and after a single 3-hour i.v. infusion of 5 mg (dotted line) in healthy subjects (n=14)



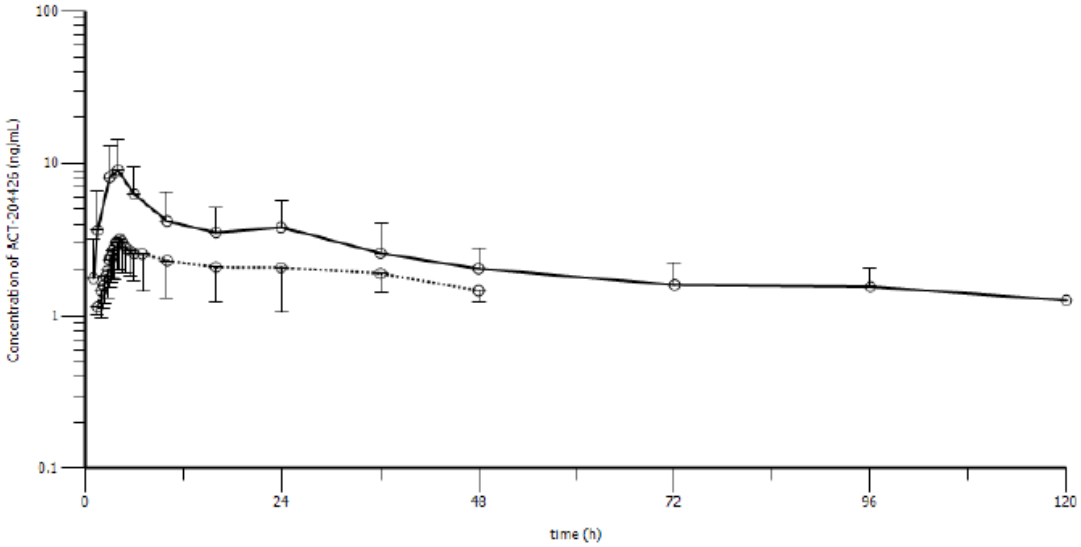
M12 metabolite

Summary of PK parameters of M12 (ACT-204426).

	t_{max} (h)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-t} (h.ng/mL)	$AUC_{0-\infty}$ (h.ng/mL)
5 mg ponesimod i.v. (n=14)		35.3 (27.9–44.8)	3.3 (2.7–3.9)	63.5 (44.8–90.0)	146.8 (115.9–185.9)
10 mg ponesimod oral (n=14)	4.0 (3.0–6.0)	31.7 (26.5–38.0)	8.4 (6.4–11.0)	171.4 (119.5–245.9)	242.4 (185.9–316.2)

Data are expressed as median (range) for t_{max} and geometric mean (95% confidence interval) for $t_{1/2}$, C_{max}, and AUC.
Following i.v. infusion, $t_{1/2}$ was calculated in 13 subjects.
$AUC_{0-\infty}$: area under the plasma concentration-time curve from zero to infinity, AUC_{0-t}: area under the plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification, C_{max}: maximum plasma concentration, i.v.: intravenous, $t_{1/2}$: terminal half-life, t_{max}: time to reach C_{max}.
Source: Table 19.

Mean (± SD) plasma concentration (semilogarithmic scale) vs time profiles of M12 (ACT-204426) after a single oral dose of 10 mg (solid line) and after a single 3-hour i.v. infusion of 5 mg (dotted line) in healthy subjects (n=14, main study) is presented in the Figure below:



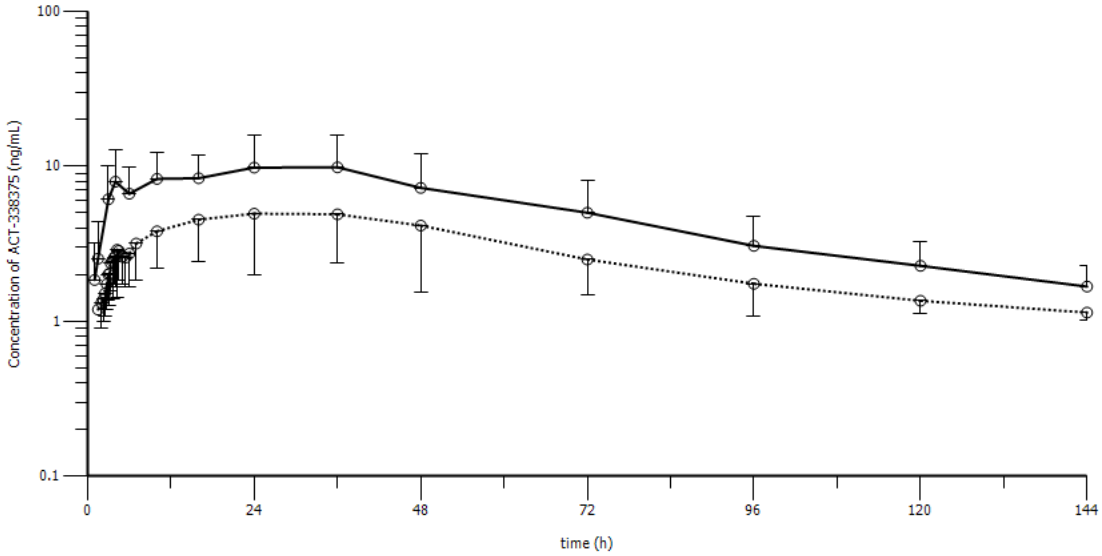
M13 metabolite

A summary of the PK parameters for M13 is presented by treatment in the table below.

	t_{max} (h)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-t} (h.ng/mL)	$AUC_{0-\infty}$ (h.ng/mL)
5 mg ponesimod i.v. (n=14)		39.2 (30.9–49.6)	5.1 (3.9–6.6)	306 (222–420)	386 (292–511)
10 mg ponesimod oral (n=14)	24 (4.0–36)	38.2 (31.0–47.0)	9.7 (7.4–12.7)	634 (466–862)	737 (563–965)

Data are expressed as median (range) for t_{max} and geometric mean (95% confidence interval) for $t_{1/2}$, C_{max}, and AUC.
$AUC_{0-\infty}$: area under the plasma concentration-time curve from zero to infinity, AUC_{0-t}: area under the plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification, C_{max}: maximum plasma concentration, i.v.: intravenous, $t_{1/2}$: terminal half-life, t_{max}: time to reach C_{max}.
Source: Table 19.

Mean (± SD) plasma concentration (semilogarithmic scale) vs time profiles of M13 (ACT-338375) after a single oral dose of 10 mg (solid line) and after a single 3-hour i.v. infusion of 5 mg (dotted line) in healthy subjects (n=14, main study) is presented in the Figure below:



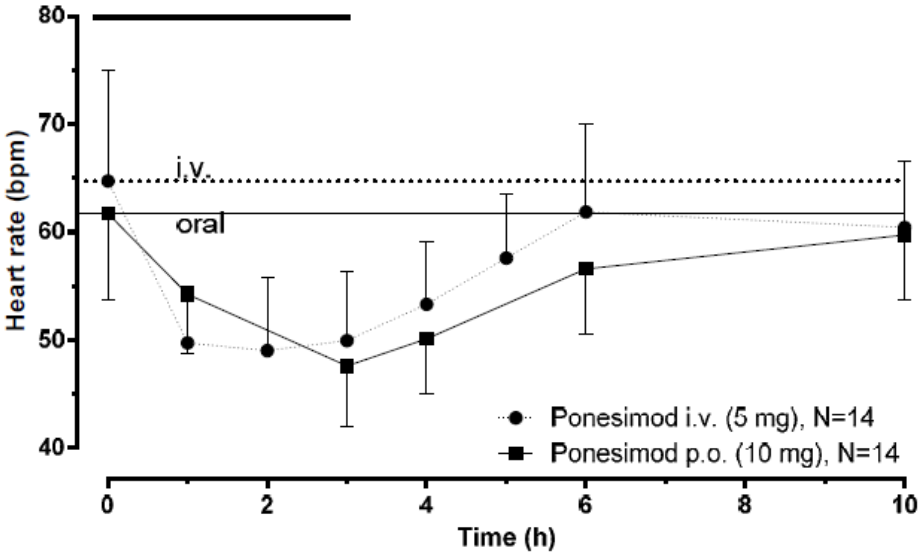
Pharmacodynamics

A transient decrease in total lymphocyte count was observed following both i.v. and oral administration of ponesimod. In the main study, the maximum decrease from baseline was 0.56 ± 0.45 x 10^9 cells/L (baseline: 1.98 ± 0.35 x 10^9 cells/L) and 0.70 ± 0.30 x 10^9 cells/L (baseline: 1.90 ± 0.34 x 10^9 cells/L) for the i.v. and the oral route, respectively, observed 6 h post-dose (i.e., 6 hours after the start of infusion for Treatment A).

ECG recording

Following i.v. infusion (5 mg) in both phases of the study, a similar transient decrease of HR was observed when compared to oral administration (10 mg) of ponesimod. The HR effect of ponesimod in the main study during the first 10 hours following administration was showed in the Figure below,

Mean (± SD) HR following i.v. (dotted line) or oral (solid line) administration of ponesimod - main study (n=14)



Safety

In both the pilot phase and the main study, no AEs leading to discontinuation and no SAEs were observed. No clinically significant treatment-emergent abnormalities in clinical laboratory variables were observed. In addition to the events of sinus bradycardia, analysis of treatment-emergent 12-lead ECG abnormalities revealed one finding of short PQ interval (Treatment B), two findings of QTc prolongation (Treatment A), and one finding of short QTc (Treatment A). All these findings were observed in the main study only.

CONCLUSIONS

Pharmacokinetics

The absolute bioavailability of ponesimod was estimated to be 83.8% (90% CI: 80.2–87.5).

Clearance and volume of distribution of ponesimod (following a single i.v. infusion of 5 mg during 3 hours) were 3.8 L/h (95% CI: 3.3 –4.3) and 160 L (95% CI: 146.1–174.2), respectively.

Safety and tolerability

Both i.v. and oral ponesimod formulations were well tolerated. The most frequently reported AEs (main study and pilot) included headache and sinus bradycardia. Apart from one severe AE of headache, all AEs were of mild to moderate intensity. There were no SAEs and no AEs leading to discontinuation.

No clinically significant treatment-emergent abnormalities in clinical laboratory, vital signs, or ECG variables were observed.

4.4-2. IN VITRO STUDIES PERTINENT TO PK USING HUMAN BIOMATERIALS

4.4-2.1 *In Vitro* Metabolism

Study B-18.004: *Identification of the human enzymes involved in ponesimod metabolism*

Objectives: identification of the human enzymes involved in the formation of M12 and M13.

METHODS

1) incubation of ponesimod with human hepatocytes in the absence or presence of the broad non-selective P450 inhibitor ABT to unravel the respective roles of P450 and non-P450 enzymes.

Cryopreserved hepatocytes were thawed at 37 °C and purified on a P ercoll cushion (15 %) at 50 g for 4 min at 4 °C. The cell pellet was resuspended in 1 mL culture medium. The cell suspension was then adjusted with culture medium at a nominal density of 5 x 105 viable cells/mL. The cells were pre-incubated 30 minutes in the presence or absence of 1 mM ABT followed by the addition of 5 µM ACT-128800C for a further 24 h incubation. Duplicate wells were sampled after 24 h of incubation by addition of 200 µL of a cetonitrile and the entire well was transferred into Eppendorf vials. Samples were stored frozen at -20 °C pending analysis. Samples were centrifuged at 20800 g for 5 min at 10 °C and the supernatant was submitted to HPLC with radiochemical detection.

2) incubations of ponesimod with human liver or intestinal microsomes, cytosol, mitochondria and S9 to characterize the subcellular localization of ponesimod metabolic pathways.

Incubations of ACT-128800C were performed for 24 h at a single substrate concentration of 5 uM in a total volume of 200 uL. A 1 uL-aliquot of the compound stock solution was added to 100 mM phosphate buffer (pH 7.4) containing the subcellular fraction at a concentration of 3 mg/mL or 1 mg/mL and the mixture was incubated at 37 °C in an Eppendorf thermomixer at 550 rpm. The reaction was initiated by addition of 20 uL of freshly prepared NAD and NADPH stock solutions and terminated by addition of 200 uL of ice-cold acetonitrile after 24 h. Samples were centrifuged at 20800 g for 5 min at 10 °C and the supernatants were submitted to HPLC analysis with radio detection.

3) incubations of ponesimod with recombinant human enzymes.

The identification of the individual enzymes involved in ponesimod metabolism was performed using recombinant human enzymes. Unstable intermediate metabolites were put into evidence using the carbonyl trapping agent methoxyamine and co-incubation of relevant recombinant enzymes was performed to unravel sequential metabolic steps.

RESULTS

<u>P450 versus non-P450 contributions using the P450 non-selective inhibitor ABT</u>

Incubations of ACT-128800C with human hepatocytes were performed in the presence of the non-selective P450 inhibitor ABT. Four metabolites were seen in hepatocytes, i.e. the [redacted] (b) (4) M10 and M11, and the oxidation products M12 and M13. The following Table gives an overview on the number of metabolites formed together with their individual relative contributions.

Ponesimod Metabolism by Human Hepatocytes in the Presence or Absence of the Pan-CYP Inhibitor 1-ABT

		metabolite [1]					
hepatocytes batch	ABT	M10 [2]	M11	M12	M13	M32	ACT-128800C
1	without	12	27	8.7	32		21
	with	13	19	2.0	34		26
2	without	3.7	16	19	26		34
	with	8.8	20	2.4	32		37
3	without		6.9	7.0	12		74
	with	1.2	3.9	4.5	14		74
no cell control	without					1.9	98

The formation of M10, M11 and M13 was not affected by the presence of ABT, with contributions remaining in the same range, i.e. 1.2-13 %, 3.9-20 % and 14-34 %, respectively. M12 formation was reduced to a 2.0-4.5 % range which represented between one third to nine tenth of the respective contributions without ABT. As M13 formation was undiminished, indicating that M13 formation was independent of both CYPs and M12 formation. Overall, using ABT in hepatocytes, ponesimod turnover was at most reduced by 5 %. Ponesimod turnover was only marginally affected by the presen ce of ABT, suggesting that P450 enzymes were overall playing a minor role in ponesimod metabolism.

Subcellular localization of ponesimod metabolism

ACT-128800C was incubated with human liver microsomes, cytosol, mitochondria and S9 fraction to identify the subcellular localization of the hepatic enzymes involved in ponesimod metabolism. The following Table gives an overview on the number of metabolites formed in the different incubations together with their individual relative contributions.

Metabolic profiles of ACT-128800C after 24 h incubations with human liver microsomes, cytosol, mitochondria, S9 fraction and hepatocytes

	metabolite (1)						
subcellular fraction	M6 (2)	M8	M12	M13	M32	sum of metabolites	ACT-128800C
microsomes					9.6	9.6	81
cytosol	1.7	2.1	0.8		1.2	5.8	83
mitochondria					3.3	3.3	84
S9			2.3	0.9	4.4	7.6	81
microsomes without cofactor					0.7	0.7	89
without subcellular fraction					0.9	0.9	93

(1) numbers are expressed as percent of total radioactivity in the respective chromatograms; (2) empty cells indicate the absence of a metabolite in the incubation.

Ponesimod metabolism using recombinant human enzymes

ACT-128800C was incubated with a panel of recombinant human P450 and non-P450 enzymes to identify which could metabolize ponesimod. The following Table gives an overview on the number of observed metabolites in the relevant incubations together with their individual relative contributions.

recombinant enzymes	metabolite (1)					ACT-128800C
	M1(2)	M9	M12	M13	M32	
CYP2J2			3.4*	3.8	74	17*
CYP3A4		6.5			7.4	85
CYP3A5	1.7	1.6			4.0	91
CYP4F3A	2.6				0.8	95
CYP4F12	2.3				0.6	97
CYP2J2 + ALDH1A1			73 *	3.9	8.1	12 *
ALDH1A1 (no P450 control)						98
no enzyme control						98

(1) numbers are expressed as percent of total radioactivity in the respective chromatograms; (2) empty cell indicate the absence of a metabolite in the incubation, * co-eluting peaks on the radiochromatogram.

ACT-128800C turnover was only seen using CYP2J2, CYP3A4, CYP3A5, CYP4F3A and CYP4F12. CYP2J2 was the most productive enzyme, yielding 74% of M32, 3.4 % of M12 and 3.8 % of M13.

CONCLUSIONS

- M13 formation was independent of both CYPs and M12 formation.
- P450 and non-P450 enzymes catalyze the initial formation of a glyceraldehyde intermediate metabolite which is subsequently metabolized to M12 in the cytosol. The formation of the glyceraldehyde intermediate was catalyzed by CYP2J2, CYP3A4, CYP3A5, CYP4F3A and CYP4F12.
- P450 enzymes were playing a limited role in overall metabolic clearance of ACT-128800C

Study DMPK FK13537: *In Vitro* Uridine Diphospho Glucuronosyltransferase (UGT) Reaction Phenotyping of JNJ-67896153 (Ponesimod)

Objectives: To determine the uridine diphosphate glucuronosyltransferases (UGTs) involved in the formation of the glucuronide conjugates of JNJ -67896153 using recombinant single enzyme systems.

METHODS

Recombinant UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15 and 2B17 (0.15 mg/mL) prepared from baculovirus infected insect cells, HLM and HIM (0.2 mg/mL) were incubated with 1 µM JNJ-67896153 for up to 90 minutes at 37°C. Samples were preincubated with 25 µg/mL alamethicin at room temperature for 10 minutes to minimize latency in catalytic activity. Reactions were started by the addition of 2 mM uridine 5'-diphosphoglucuronic acid (UDPGA) as the cofactor. Aliquots were removed and quenched with acetonitrile at 0, 30 and 90 minutes. Umbelliferone and chenodeoxycholic acid were selected as control substrates and were incubated under the same conditions as JNJ-67896153.Incubations were carried out at protein concentrations of 0.15 mg/mL for the UGT enzymes, and 0.2 mg/mL for the human liver and intestinal microsomes.

RESULTS

The following Table summarized the relative amounts formed in the individual enzyme incubations.

Phenotyping Screen: Relative Fold-Increases in JNJ-67896153 Glucuronide Formation in Recombinant Human UGT Enzymes, HLM and HIM.

UGT	Relative Fold-Increases in JNJ-67896153 Glucuronide Formation After 90 Minutes	Relative Fold-Increases in Umbelliferone Glucuronide Formation After 90 Minutes	Relative Fold-Increases in CDCA Glucuronide Formation After 90 Minutes
Control	*	*	*
HLM	51	1140	13
HIM	51	1020	*
1A1	19	30	*
1A3	4	*	93
1A4	7	*	*
1A6	*	1370	*
1A7	*	260	*
1A8	*	58	30
1A9	*	1180	*
1A10	*	13	*
2B4	3	*	6
2B7	34	24	*
2B10	*	*	*
2B15	*	32	*
2B17	*	*	*

Note: * = No Product Detected/Trace Amounts (< 3-fold compared to Time 0)

In single recombinant UGT incubations, time-dependent product formation was seen in several isoforms. UGT2B7 and UGT1A1 catalyzed the highest rates of formation (34- and 19-fold increases compared to time 0, respectively) with lower rates (3- to 7-fold increases) observed with other isoforms (UGT1A3, 1A4 and 2B4). JNJ-67896153 turnover in all UGTs, HLM and HIM (normalized to the UGT insect control samples) was minimal (less than 10%).

CONCLUSIONS

- The UGTs involved in M10 and M11 formation were identified.
- UGTs 1A1 and 2B7 catalyzed the highest rate of formation of M10/M11, with lower rates observed for UGTs 1A3, 1A4, and 2B4.

Study B-05.078: Assessment of the inhibition potential of human cytochrome P450 enzymes by the S1P1 receptor agonist ACT-128800 *in vitro*

Objectives: to assess the potential of ACT-128800 for inhibition of the human cytochrome P450 isoforms 1A2, 2A6, 2B6, 2C9, 2Cl 9, 2D6, 2El, and 3A4 using either human liver microsomes and isoform-specific marker reactions or recombinant cytochrome P450 enzymes.

METHODS

Inhibition of CYPs 1A2, 2A6, 2C9, 2D6, 2E1 and 3A4 was tested using human liver microsomes and isoform-specific marker transformations. Inhibition of CYP2B6 and 2C19 activities was done with the recombinant P450 enzymes expressed in baculovirus- infected Sf9 cells. Two different probes were used to assess the inhibitory potential of ACT-128800 on CYP3A4 activity, i.e. midazolam 1'-hydroxylation and testosterone 6β-hydroxylation. For the determination of IC50 values, all marker substrates were used at one single concentration around their respective Km values. Seven to eight different ACT-128800 concentrations up to 100 µM were used for inhibition. Metabolite formation was monitored using either 14C-radiodetection (CYPs 2B6, 2C19), UV detection at an appropriate selective wavelength (CYPs 2A6 and 2E1), or LC-MS/MS (CYPs 1A2, 2C9, 2D6, and 3A4). IC50 values were determined for all P450 isoforms, where appropriate. In addition, the Ki values of ACT-128800 on the activities of CYPs 2C9, 2Cl 9, 2D6 and 3A4 (midazolam 1'-hydroxylation) were determined using four different substrate concentrations. The Ki values were estimated by re-plotting the slopes derived from the Lineweaver-Burk plots against the ACT-128800 concentrations. Literature-known reference inhibitors for all P450 isoforms were run in parallel.

RESULTS

The overview on the marker transformations and the estimated IC50 and Ki values was presented in the Table below:

Overview on the inhibition data of ACT-128800 on the different human cytochrome P450 isoforms

P450 isoform	marker transformation	IC_{50} (µM)	K_i (µM)
1A2	phenacetin-O-deethylation	> 50	n.d. (1)
2A6	coumarin 7-hydroxylation	> 100 (2)	n.d.
2B6	(S)-mephenytoin N-demethylation	> 50 (3)	n.d.
2C9	diclofenac 4'-hydroxylation	14	6.5
2C19	(S)-mephenytoin 4'-hydoxylation	9.6	5.9
2D6	dextromethorphan N-demethylation	36	10
2E1	chlorzoxazone 6-hydroxylation	> 50 (4)	n.d.
3A4	midazolam 1'-hydroxylation	19	15
3A4	testosterone 6β-hydroxylation	22	n.d.

(1) n.d. = not determined; (2) 35 % inhibition at 100 µM (3) 34 % inhibition at 50 µM; (4) 13 % inhibition at 50 µM

In these in vitro experiments, ACT-128800 had only a weak effect on the activities of CYPs 1A2, 2A6, 2B6 and 2E1. ACT-128800 elicited a moderate inhibition of CYP2D6 activity with IC50 and

Ki values of 36 µM and 10 µM, respectively. A similar effect of ACT-128800 was observed in both assays probing for CYP3A4 activity with IC50 values of 19 and 22 µM using midazolam or testosterone as substrates. A more significant inhibition was observed on both members of the CYP2C family, i.e. CYP2C9 and CYP2C19. IC50 and Ki values on CYP2C9-mediated diclofenac 4'-hydroxylation were 14 µM and 6.5 µM.

In preclinical animal models, ACT-128800 is fully active at an oral dose of 3.0 mg/kg. Total and free peak plasma concentrations at this dose in the rat are around 750 nM and 5 nM [1], respectively, taking into account a plasma protein binding of 99.4%.

CONCLUSIONS

At clinically relevant concentrations, the potential of ACT-128800 to significantly inhibit the cytochrome P450-mediated clearance of co-administered drugs should be limited.

Study B-13.080: Assessment of the inhibition potential of the S1P1 receptor agonist ponesimod and its metabolite ACT-338375 on human cytochrome P450 and UGTs enzymes *in vitro*: complementary data

Objectives: to assess the inhibition potential of ponesimod on the CYP2C8, CYP2D6, UGT1A1, UGT2B7, and the inhibition potential of metabolite ACT-338375 on the CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, UGT1A1 and UGT2B7 using either human liver microsomes and isoform-specific marker reactions or recombinant enzymes.

METHODS

Inhibition of CYP2D6 and CYP2C8 for ponesimod, and of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2D6 and CYP3A4 for ACT-338375 was tested using human liver microsomes and P450 isoform-specific marker transformations. Inhibition of CYP2B6 and CYP2C19 activity was assessed with recombinant P450 enzyme expressed in baculovirus-infected Sf9 cells. Two different probes, i.e. midazolam and testosterone, were used to assess the inhibitory potential of ACT-338375 on CYP3A4 activity. For the determination of IC50 values, all marker substrates were used at a single concentration around their respective Km values. Different ranges of ponesimod or ACT-338375B concentrations were used for the various inhibition assays and are described in detail in the Methods section above. Metabolite formation was monitored using LC-MS/MS. IC50 values were determined, where appropriate. In addition, the Ki value of ponesimod and ACT-338375 on CYP2C8 inhibition was determined using four different substrate concentrations.

RESULTS

The overview on the marker transformations and the estimated IC50 and Ki values of ACT-338375 was presented in the Table below:

Overview on the inhibition data of ACT-338375 on the different human cytochrome P450 isoforms

P450 isoform	marker transformation	IC_{50} (µM)	K_i (µM)
CYP1A2	phenacetin-O-deethylation	> 100	ND (1)
CYP2A6	coumarin 7-hydroxylation	> 100 (2)	ND
CYP2B6	(S)-mephenytoin N-demethylation	> 100 (3)	ND
CYP2C8	paclitaxel 6α-hydroxylation	9.7	6.4
CYP2C9	diclofenac 4'-hydroxylation	43	ND
CYP2C19	(S)-mephenytoin 4'-hydoxylation	> 100 (4)	ND
CYP2D6	dextromethorphan N-demethylation	> 100	ND
CYP3A4	midazolam 1'-hydroxylation	160	ND
CYP3A4	testosterone 6β-hydroxylation	200	ND

(1) ND = not determined; (2) 30 % inhibition at the highest concentration of 100 µM; (3) 31 % inhibition at the highest concentration of 100 µM; (4) 34 % inhibition at the highest concentration of 100 µM;

ACT-338375 had only weak inhibitory effects on CYP1A2, CYP2D6, CYP2A6, CYP2B6, CYPC19 and CYP3A4 with IC50 values above 100 µM. More pronounced inhibition was observed on CYP2C8 and CYP2C9 with IC50 values of 9.7 and 43 µM, respectively. The corresponding Ki value for CYP2C8 inhibition was 6.4 µM with mixed-type inhibition.

Ponesimod had an inhibitory effect on CYP2C8 with an IC50 value of 28 µM. The corresponding Ki value for CYP2C8 inhibition was 19 µM with mixed-type inhibition as the mode of inhibition.

The potential of ponesimod to cause changes in the enzymatic activity of CYP2C8 or CYP2D6, and of ACT-338375 on CYP3A4, CYP2C9 and CYP2D6 due to time-dependent inhibition was also investigated using human liver microsomes. The estimated IC50 and shift values in these inhibition experiments are summarized in Tables below:

Overview on the time-dependent inhibition data of ponesimod on different human cytochrome P450 isoforms

P450 enzyme	compound	IC_{50} without pre-incubation (µM)	IC_{50} with pre-incubation (µM)	shift
CYP2C8	ponesimod	49	45	1.1
	gemfibrozil 1-O-ß glucuronide	148	5.9	25
CYP2D6	ponesimod	68	69	<1
	paroxetine	6.4	0.2	30

Overview on the time-dependent inhibition data of ACT-338375 on different human cytochrome P450 isoforms

P450 enzyme	compound	IC_{50} without pre-incubation (µM)	IC_{50} with pre-incubation (µM)	shift
CYP2C9	ACT-338375B	>100	>100	n.d.
	tienilic acid	5.2	0.09	58
CYP2D6	ACT-338375B	>100	>100	n.d.
	paroxetine	4.8	0.2	24
CYP3A4	ACT-338375B	>100	>100	n.d.
	mibefradil	16	0.09	180

n.d. = not determined

Based on these data, ponesimod is not a time-dependent inhibitor of CYP2C8 and CYP2D6, and ACT-338375 is not a time-dependent inhibitor of CYP3A4, CYP2C9 and CYP2D6 enzymes.

The assessment of the inhibitory potential of ponesimod and ACT-338375 on the human UGT1A1 and UGT2B7 in vitro was performed using recombinant UGT enzymes expressed in

baculovirus-infected cells. The results of these inhibition experiments are summarized in Tables below:

Overview on the inhibition data of ponesimod on UGT1A1 and UGT2B7

UGT isoform	marker transformation	IC_{50} (µM)
UGT1A1	estradiol 3-β-glucuronidation	> 50 (1)
UGT2B7	3'-azido-3'-deoxythymidine 5-β-glucuronidation	17

(1) 77 % inhibition at the highest concentration of 50 µM,

Overview on the inhibition data of ACT-338375 on UGT1A1 and UGT2B7

UGT isoform	marker transformation	IC_{50} (µM)
UGT1A1	estradiol 3-β-glucuronidation	24
UGT2B7	3'-azido-3'-deoxythymidine 5-β-glucuronidation	> 50 (2)

(2) 73 % inhibition at the highest concentration of 50 µM.

Reviewer's comments:

At clinically relevant dosing regimen (20 mg/day), estimated maximum unbound plasma concentrations were 0.0046 µM for ponesimod and 0.0011 µM for M13, respectively. These clinically relevant concentrations were significantly lower than the estimated IC50 values for the evaluated enzyme isoforms.

CONCLUSIONS

Ponesimod is not expected to demonstrate potential to inhibit the CYP2C8, CYP2D6, UGT1A1, UGT2B7 at clinically relevant concentrations.

ACT-338375 is not expected to demonstrate potential to inhibit CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, UGT1A1 and UGT2B7 at clinically relevant concentrations.

Study FK13536: An *In Vitro* Investigation of the Potential of JNJ-67896153 (Ponesimod) and JNJ-70051540 (M13 Metabolite) as Time Dependent Inhibitors of CYP1A2, 2B6, 2C8 and 2C19

Objectives: To evaluate the potential of JNJ-67896153 and JNJ-70051540 to act as time-dependent inhibitors (TDI) of cytochrome P450 (CYP450) 1A2, 2B6, 2C8 and 2C19 isoforms in pooled, mixed gender human liver microsomes.

METHODS

The potential of JNJ-67896153 (Ponesimod) and JNJ-70051540 (M13 metabolite) as time-dependent inhibitors was assessed against 4 of the major human CYP450 isoforms (CYP1A2, 2B6, 2C8 and 2C19) in pooled liver microsomes. Residual CYP-specific activity following 60-minute incubation of five concentrations of the test compounds (0.05 to 50 µM) with and without NADPH was normalized to the residual activity from the blank vehicle control. Any significant, NADPH-dependent reduction in activity over time could indicate possible irreversible (via reactive intermediate formation) or quasi-irreversible (via metabolic intermediate complex formation) inactivation of the CYP isoform. Positive control time-dependent inhibitors and reversible inhibitors for each isoform were treated in the same way.

RESULTS

In samples pre-incubated with JNJ-67896153 or with JNJ-70051540, none of the isoforms were inhibited in an NADPH- and concentration-dependent fashion up to 50 µM (see the Table below).

Apparent Partition Ratios (APR), Residual Activity Ratios and Percent Loss of Activity Following Pre-Incubation of JNJ-67896153, JNJ-70051540 and Control Compounds in Human Liver Microsomes with and without NADPH

CYP (Content pmol/mg protein)	Probe Substrate (µM)	Inhibitor (PC, NC, JNJ1, JNJ2)	APR (Inh. Conc., µM) [a]	Residual Activity Ratio (+/- NRS) [b]	Maximum % Loss of Activity [c]	TDI Assessment
1A2 (50)	Phenacetin (400)	Furafylline	101 (2.5)	0.60	34	Strong
		α-NF [d]	N.D.	> 1.0	31	No TDI
		JNJ-67896153	N.D.	1.0	< 5	No TDI
		JNJ-70051540	N.D.	1.0	< 5	No TDI
2B6 (34)	Bupropion (125)	Phencyclidine	351 (6.0)	0.33	63	Strong
		Ketoconazole	N.D.	> 1.0	23	No TDI
		JNJ-67896153	> 3000 (50)	0.90	31	No TDI
		JNJ-70051540	> 3000 (50)	0.90	18	No TDI
2C8 (51)	Amodiaquine (10)	Gemf. Gluc. [e]	282 (7.2)	0.29	66	Strong
		Quercetin	N.D.	> 1.0	35	No TDI
		JNJ-67896153	N.D.	0.92	5	No TDI
		JNJ-70051540	N.D.	0.93	14	No TDI
2C19 (7)	S-Mephenytoin (150)	Ticlopidine	220 (0.77)	0.31	76	Strong to Very Strong
		N-3-BPb [f]	N.D.	0.89	63	No TDI
		JNJ-67896153	N.D.	0.97	5	No TDI
		JNJ-70051540	N.D.	0.91	8	No TDI

[a] – Inhibitor Concentration corresponding to the determined APR value, based on Molar Ratio of Inhibitor to CYP Isoform
[b] – Maximum % loss compared to vehicle control
[c] – Ratio of residual activity with and without NADPH at maximum inhibitor concentration
[d] – α-NF = α-Naphthoflavone
[e] – Gemf. Gluc. = Gemfibrozil Glucuronide
[f] – N-3-BPb = N-3-Benzylphenobarbital
MRM = Multiple Reaction Monitoring; ESI = Electrospray Ionization
APR = Apparent Partition Ratio
TC = Test Compound, PC = Positive Control, NC = Negative (Reversible) Inhibitor
N.D. – Apparent Partition Ratio was not determined due to lack of time-dependent inhibition

CONCLUSIONS

JNJ-67896153 (Ponesimod) and JNJ-70051540 (M13 metabolite), incubated at concentrations up to 50 µM, were not found to be time-dependent inhibitors of CYP1A2, 2B6, 2C8 or 2C19.

Study FK13543: An *In Vitro* Investigation of the Potential of JNJ-67896153 (Ponesimod) and JNJ-70051540 (M13 Metabolite) to Inhibit CYP2J2

Objectives: To evaluate the reversible inhibitory potentials of JNJ-67896153 and JNJ-70051540 against the cytochrome P450 2J2 (CYP2J2) isoform in the human liver.

METHODS

JNJ-67896153 and JNJ-70051540 were incubated at 8 serially diluted concentrations of (0, 0.05, 0.15, 0.5, 1.5, 5, 15 and 50 uM) in the presence of 1 µM astemizole in 0.1 mg/mL HLM, 10 pmol/mL rhCYP3A4 or rhCYP2J2. The formation of O-desmethylastemizole (catalyzed by CYP2J2) and 6-hydroxyastemizole (catalyzed by CYP3A4) were monitored and IC50 values determined in each enzyme system. Danazol (CYP2J2 selective inhibitor) and ketoconazole were also incubated as controls.

RESULTS

As shown in the Table below, JNJ-67896153 was found to be a moderately strong inhibitor with IC50 values of 22 and 7.8 µM in HLM and rhCYP2J2, respectively. JNJ-70051540 was a weak CYP2J2 inhibitor with IC50 values greater than the highest incubation concentration tested, 50 µM.

IC50 values for the Inhibition of CYP2J2 and 3A4-Catalyzed Astemizole Metabolism by JNJ-67896153 and JNJ-70051540 and Control Compounds in Human Liver Microsomes and Recombinant Human CYP2J2 and CYP3A4

		Astemizole O-demethylation (CYP2J2)			Astemizole 6-hydroxylation (CYP3A4)		
Inhibitor	Enzyme	IC_{50} (µM)	S.E.	Max. Inhibition (%)	IC_{50} (µM)	S.E.	Max. Inhibition (%)
Danazol	HLM*	> 1.1		36	> 1.1		40
	rh3A4	> 1.1		29	> 1.1		32
	rh2J2	0.12	0.015	97	n/a		n/a**
Ketoconazole	HLM	0.68	0.050	73	0.023	0.0021	90
	rh3A4	0.062***	0.0040	95	0.051	0.0020	98
	rh2J2	0.12	0.0093	96	n/a		n/a**
JNJ-67896153	HLM	22	1.5	72	8.0	0.60	86
	rh3A4	n/a		< 5	26	1.2	72
	rh2J2	7.8	0.51	94	n/a		n/a**
JNJ-70051540	HLM	> 50		21	20	1.3	67
	rh3A4	n/a		< 5	> 50		14
	rh2J2	> 50		49	n/a		n/a**

*- CYP2J2 expression levels in HLM are approximately 15-30 times lower than CYP3A4 (Solanki et al., 2018)
**- Only trace amounts formed
***- 80% lower turnover compared to rhCYP2J2
n/a = no concentration-dependent inhibition observed up to 50 µM

CONCLUSIONS

JNJ-67896153 (Ponesimod) and JNJ-70051540 (M13 Metabolite) are not expected to demonstrate potential to inhibit CYP2J2.

Study B-08.466, B-17.003, FK13519: Assessment of the human cytochrome P450 induction potential of the S1P1 receptor agonist ponesimod and its metabolite ACT-338375B (M13) *in vitro*

Objectives: To investigate the potential of the S1P1 receptor agonist ACT-128800 (ponesimod) to induce human CYP3A4, CYP2C9 and CYP1A2 transcriptional activation assay (B-08.466). To investigate the potential of the major metabolite of ponesimod, i.e. ACT-338375, to activate human PXR in a transcriptional activation assay in CV-1 cells. In addition, the capacity of ponesimod and ACT-338375 to induce human CYP1A2 and CYP2B6 mRNA was tested in primary human hepatocytes (B-17.003). To evaluate the effects of JNJ-67896153 (ponesimod) and JNJ-70051540 (M13) (0.01 to 30 µM) on the expression of CYP3A4 in cultured human hepatocytes.

METHODS

ACT-128800 and metabolite ACT-338375 were tested on its capacity to activate the human PXR (hPXR) receptor in a transcriptional activation assay. Changes in CYP1A2, CYP2B6, CYP3A4 and CYP2C9 mRNA production and enzyme activity were determined using primary human hepatocyte cultures. Changes in CYP1A2 activity were determined directly with primary human heptatocytes.

RESULTS

B-08.466

Ponesimod (0.03-30 µM in two separate assays) activated hPXR from 1 to 10 µM by approximately 2 to 5-fold over vehicle control, with increases of 60-70% verses rifampicin (positive control) at 3 µM and similar to rifampicin at 10 µM.

Ponesimod did not induce CYP2C9 (activity or mRNA) or CYP1A2 (activity only, mRNA not determined) in any of the three human hepatocyte cultures. CYP3A4 mRNA levels and enzyme activity were induced; maximum increases relative to vehicle were observed at the highest concentration of 10 µM and ranged from 2.3 to 6.2-fold (mRNA) and 1.86 to 3.22-fold (activity). This mRNA induction by 10 µM ponesimod was 23-51% relative to the maximum rifampicin mRNA induction. The effect was less pronounced at lower concentrations with 12-31% of maximum rifampicin response at 3 µM ponesimod, 9-21% at 1 µM, and 5-14% at 0.1 µM.

B-17.003

M13 (0.03-30 µM) activated hPXR in the CV-1 transactivation assay from 3 to 30 µM by 1.2 to 1.8-fold over control, with increases 21-35% of rifampicin.

CYP1A2 and 2B6 mRNA was not induced by ponesimod. CYP1A2 and 2B6 mRNA was induced by M13 greater than 2-fold in one of the three donors tested. For CYP1A2 mRNA, maximum fold induction was 2.67-fold at 10 µM, which translated into 3.6% of the positive control (100 µM omeprazole). For CYP2B6 mRNA, maximum fold induction was 2.36-fold at 1 µM, which translated into 12.6% of the positive control (3000 µM phenobarbital).

FK13519

Ponesimod resulted in concentration dependent increases in CYP3A4 mRNA expression. Increases were ≥2-fold starting at 1 µM, with maximum increases observed at concentrations of

10 or 20 uM (i.e., 13.2, 14.1, and 12.2-fold relative to the vehicle control [0.1% dimethyl sulfoxide] or 7.8%, 16.7%, and 10.6% relative to the positive control rifampicin). The calculated EC50 values for ponesimod were 3.11, 4.61, and 3.71 µM. The normalized Emax/EC50 ratios for ponesimod to those obtained with the same donor hepatocytes for rifampicin (i.e., (Emax/EC50) ponesimod/(Emax/EC50) rifampicin) indicate a relative inductive effect for ponesimod of <3% compared to rifampicin for all three donor hepatocytes.

M13 did not cause concentration-dependent increases in CYP3A4 mRNA expression in two of three donors, rather mRNA levels were generally <1 relative to vehicle control. In the third donor, ≥2-fold increases were observed at 20 and 30 µM (4.1 and 3.1-fold relative to the vehicle control or 2.0% and 1.3% relative to the positive control rifampicin, respectively).

CONCLUSIONS

Ponesimod and M13 metabolite are not expected to demonstrate potential to induce evaluated CYP enzyme activity at clinically relevant concentrations.

Study B-14.025, B-05.105, FK13541: An *In Vitro* Investigation of the Potential of JNJ-67896153 (Ponesimod) and JNJ-70051540 (M13 Metabolite) to Inhibit human transport proteins

Objectives: To evaluate the potential of ponesimod and its metabolite M13 to inhibit the human uptake transporters, organic anion transporting polypeptides (OATP) OATP1B1 and OATP1B3, organic anion transporters (OAT) OAT1 and OAT3, organic cation transporters (OCT) OCT1 and OCT2 and the human efflux transporters, multidrug and toxin extrusion (MATE) MATE1 and MATE2K, p-glycoprotein (P-gp), and breast cancer resistance protein (BCRP).

METHODS

OATP1B1, OATP1B3, OAT1 and OCT2 inhibition was tested in CHO cells expressing the respective transporter, whereas P-gp inhibition was tested in MDCKII-MDR1 cells. BCRP inhibition was assessed in Sf9 membrane vesicles expressing BCRP. Inhibition of OAT3, OCT1, MATE1, MATE2K were tested in HEK293 cells expressing the respective transporter. P-gp inhibition was also tested in Caco-2 cells (B-05.105). Time-dependent inhibition of OATP1B1 and OATP1B3 were also evaluated in HEK293 cells (FK13541).

For cellular assays non-specific binding of ponesimod and ACT-338375 was assessed in tissue culture plates containing CHO, HEK293 or MDCKII wild-type cells. Rapid equilibrium dialysis (RED) was used to determine the free fractions in membrane vesicles. In all cellular assays, 41-90 % of ponesimod and 84-98 % of ACT-338375 was recovered from incubation solutions.

RESULTS

The in vitro inhibition of human transport proteins by ponesimod and metabolite M13 is summarized in the Table below:

Inhibition of Human Uptake and Efflux Transporters

Transporter	Test System Expressing the Transporter	Marker Substrate	IC_{50} (μM) Ponesimod	IC_{50} (μM) M13
P-gp	Caco-2 or MDCKII-MDR1 cells	digoxin, rhodamine 123, or colchicine	>30 [a]	>100 [b]
BCRP	Sf9 insect membrane vesicles	methotrexate	7.1 [c]	5.0 [c]
OATP1B1	CHO cells	atorvastatin	~30 [d]	2.7 [d]
OATP1B3	CHO cells	taurocholate	~20 [d]	1.6 [d]
OATP1B1	HEK293 cells + pre-incubation	^{3}H-17β-estradiol-glucuronide	2.42 [e]	1.25 [e]
OATP1B3	HEK293 cells + pre-incubation	^{3}H-17β-estradiol-glucuronide	2.35 [e]	8.57 [e]
OAT1	CHO cells	p-aminohippuric acid	>100 [d]	48 [d]
OAT3	HEK293 cells	estrone 3-sulfate	>100 [d]	33 [d]
OCT1	HEK293 cells	1-methyl-4-phenyl pyridinium	>100 [d]	>100 [d]
OCT2	CHO cells	1-methyl 4-phenyl pyridinium	>100 [d]	>1000 [d]
MATE1	HEK293 cells	metformin	43 [d]	>100 [d]
MATE2K	HEK293 cells	metformin	1.4 [d]	42 [d]

BCRP=breast cancer resistance protein; CHO=chinese hamster ovary; $f_{u,inc}$=unbound fraction in incubation; HEK=human embryonic kidney; IC_{50}=concentration that causes 50% inhibition; MATE=multidrug and toxin extrusion protein; MDCK=Madin-Darby canine kidney; MDR1=multidrug resistance protein 1 (also known as P-glycoprotein); OAT=organic anion-transporter; OATP=organic anion-transporting polypeptide; OCT=organic cation-transporter.

[a] Caco-2 model for ponesimod with digoxin and rhodamine 123 as marker substrates; no effect at highest concentration of 30 μM (for digoxin) or 100 μM (for rhodamine 123).

[b] MDCKII-MDR1 model for M13 with digoxin and colchicine as marker substrates.

[c] $f_{u,inc}$ determined in a surrogate assay using equilibrium dialysis, ponesimod $f_{u,inc}$=0.095; M13 $f_{u,inc}$=0.562.

[d] $f_{u,inc}$ under mock transport assay conditions with CHO and HEK cells, ponesimod $f_{u,inc}$=0.45; M13 $f_{u,inc}$=0.90.

[e] Mod4.2.2.6/FK13541; effective unbound concentration based on compound recovery from the transport assay; experiments included a pre-incubation period.

The most potent in vitro inhibition of transporters by ponesimod or M13 were observed for MATE2K, OATP1B1, OATP1B3, and BCRP. Consideration of the inhibitory potencies in low local (systemic or hepatic inlet) unbound ponesimod or M13 concentrations indicates a lack of potential clinically significant interaction.

CONCLUSIONS

JNJ-67896153 (Ponesimod) and JNJ-70051540 (M13 Metabolite) are not expected to demonstrate potential to inhibit evaluated transporters at clinically relevant concentrations.

Study FK13518: An *in vitro* study to assess the transepithelial transport of JNJ-67896153 (ponesimod) across Caco-2 cells.

Objectives: To investigate the in vitro permeability of JNJ-67896153; to determine if JNJ-67896153 is a substrate of MDR1in Caco-2 cells.

METHODS

Test System

Caco-2 cells were seeded 21-days before the experiment on 24-well cell culture inserts (Millicell®-PCF, 0.4 µm, 13 mm diameter, 0.7 cm2) at a density of 63000 cells/cm2. Cell culture media consisted of DMEM supplemented with 10% fetal calf serum, 1% non-essential amino acids, 1% L-glutamine and 100 U/mL penicillin/streptomycin. The media was replaced one day after seeding and subsequently every 2 to 3 days. At the day of the transport experiments (day 22), the integrity of the cell monolayer in each insert was investigated by measuring TEER before the transport experiment and by determining the leakage of 14C-mannitol during the transport experiment.

Non-specific binding of JNJ-53718678 was observed, the nominal starting incubation concentrations were corrected to effective concentrations by taking the measured values in the starting solution into account (0.553 µM, 1.78 µM, 6.33 µM and 22.2 µM concentrations were utilised instead of 1 µM, 3 µM, 10 µM and 30 µM, respectively).

^{3}H-atenolol and lower to those of ^{3}HL- propranolol were used as reference compounds. The MDR1 inhibitor GF120918 was used to assess the efflux transport of JNJ-67896153.

Data analysis was performed Using Microsoft's Excel. Transport of the compound(s) across the monolayers was expressed as apparent permeability (Papp, cm/s) according to the following equation:

$$Papp = \frac{\text{amount transported}}{\text{surface area x concentration (start) x time}}$$

The efflux ratio (BA/AB) of the test article was calculated using the following equation:

$$Efflux\ Ratio = P_{app\ BA}/P_{app\ AB}$$

Where:

Papp BA: apparent permeability (cm/s) from the basolateral to apical side

Papp AB: apparent permeability (cm/s) from the apical to basolateral side

RESULTS

For all concentrations tested, the bidirectional Papp values of JNJ-67896153 determined through Caco-2 cell monolayers were found to be higher to those of ^{3}H-atenolol and lower to those of ^{3}HL- propranolol, indicating that JNJ-67896153 is an intermediate permeable compound in Caco-2 cells (see the Table below).

Transepithelial transport of JNJ-67896153 and reference compounds in Caco2 cells (n=3) after 120 min.

Compound	Inhibitor	P_{app} AB ($\times 10^{-6}$ cm/sec) Average		SD	P_{app} BA ($\times 10^{-6}$ cm/sec) Average		SD	Efflux Ratio BA/AB	Recovery after 120 min SUM of both compartments (%) AB	BA
0.553 µM JNJ-67896153	-	12.7	±	0.7	5.29	±	0.99	0.42	49.7	73.2
	5 µM GF120918	8.48	±	0.40	4.60	±	0.70	0.54	32.9	45.8
1.78 µM JNJ-67896153	-	12.1	±	2.4	5.48	±	0.53	0.45	38.5	48.2
	5 µM GF120918	8.30	±	1.43	3.92	±	0.32	0.47	33.2	40.6
6.33 µM JNJ-67896153	-	7.55	±	0.75	4.66	±	0.18	0.62	35.7	44.6
	5 µM GF120918	7.21	±	0.53	3.75	±	0.35	0.52	32.3	37.3
22.2 µM JNJ-67896153	-	8.46	±	1.00	4.82	±	0.66	0.57	33.9	41.7
	5 µM GF120918	8.67	±	1.36	4.12	±	0.26	0.48	35.9	40.8
0.03 µM ^{3}H-Digoxin	-	1.17	±	0.39	12.7	±	0.7	10.8		
	5 µM GF120918	3.60	±	0.02	3.13	±	0.22	0.87		
1 µM ^{3}H-Atenolol	-	0.47	±	0.08	0.67	±	0.23	-		
1 µM ^{3}H-L-Propranolol	-	22.0	±	0.5	16.6	±	1.9	-		

AB: apical to basolateral
BA: basolateral to apical

At tested effective concentrations of 0.533-22.2 µM, Papp values of JNJ-67896153 in A-B and B-A directions ranged from 7.55-12.7 and 4.66-5.48 x 10-6 cm/s, respectively, and efflux ratios ranged from 0.42-0.62. The presence of GF120918 did not significantly influence the permeability of JNJ-67896153 (Efflux ratios of 0.42 to 0.54 (0.553 µM); 0.45 to 0.47 (1.78 µM); 0.62 to 0.52 (6.33 µM) and 0.57 to 0.48 (22.2 µM)), indicating that JNJ-67896153 is not an MDR1 substrate in Caco-2 cells under the conditions tested.

CONCLUSIONS

JNJ-67896153 is an intermediate permeable compound in Caco-2 cells. JNJ-67896153 is not an MDR1 substrate in Caco-2 cells under the conditions tested.

4.4-2. IN VITRO STUDIES PERTINENT TO PK USING HUMAN BIOMATERIALS
4.4-2.2 Protein Binding

Study B-05.075: *In vitro* binding to plasma protein and plasma/blood cell partitioning in rat, dog and man

Objective: To determine the extent of *in vitro* binding of ACT-128800 to plasma proteins in rat, dog and man.

METHODS

Non-specific binding and equilibration time were determined by equilibrium dialysis at nominal concentrations of 100 and 500 ng/mL over 0, 0.5, 1, 2, 4 and 6 hours. The equilibration time for ACT-128800 was selected to be 4 hours, and subsequent plasma protein binding experiments were conducted for this length of time.

Plasma protein binding was determined by equilibrium dialysis at nominal concentrations of 100, 500, 3,000, 20,000, 100,000 and 200,000 ng/mL. Plasma/blood cell partitioning was determined at nominal concentrations of 500 and 20,000 ng/mL.

RESULTS

The results of ACT-128800 plasma protein binding, over a range of concentrations, to rat, dog and human plasma proteins are presented in Table below:

Mean recovery of radioactivity and in vitro binding of ACT-128800 to rat, dog and human plasma proteins following incubation at ca 37°C for 4 hours

Species	Nominal ACT-128800 concentration (ng/mL)	Plasma protein binding (%)	Recovery (%)
Rat	100	99.53	96.18
	500	99.39	94.48
	3,000	99.44	101.1
	20,000	99.36	94.40
	100,000	99.43	93.56
	200,000	99.42	93.00
	Overall mean ± SD	**99.43 ± 0.06**	**95.45 ± 2.97**
Dog	100	99.59	96.88
	500	99.57	99.00
	3,000	99.06	101.4
	20,000	99.58	95.74
	100,000	99.57	97.71
	200,000	99.56	97.79
	Overall mean ± SD	**99.49 ± 0.21**	**98.09 ± 1.94**
Human	100	99.69	97.22
	500	99.52	95.57
	3,000	99.47	96.49
	20,000	99.65	96.13
	100,000	99.53	96.12
	200,000	99.61	94.60
	Overall mean ± SD	**99.58 ± 0.09**	**96.02 ± 0.88**

The mean binding of ACT-128800 to rat plasma proteins was 99.4 ± 0.1%. The mean binding of ACT-128800 to dog plasma proteins was 99.5± 0.2%. In human, the mean binding of ACT-128800 to plasma proteins was 99.6% ± 0.1%.

The extent of partitioning of ACT-128800, over a range of concentrations, to rat, dog and human blood cells and the derived blood/plasma ratios are presented in the following Table:

Mean *in vitro* blood/plasma partitioning of ACT-128800 in rat, dog and human blood following incubation at ca 37°C for 120 minutes at 500 and 20,000 ng/mL

Species	Nominal ACT-128800 concentration (ng/mL)	Blood:plasma ratio	Partition coefficient (%)	% Associated with red blood cells
Rat	500	0.73	74.36	25.64
	20,000	0.74	72.60	27.40
	Overall mean	**0.73**	**73.48**	**26.52**
Dog	500	0.52	84.83	15.17
	20,000	0.56	78.79	21.21
	Overall mean	**0.54**	**81.81**	**18.19**
Human	500	0.70	76.19	23.81
	20,000	0.66	80.76	19.24
	Overall mean	**0.68**	**78.48**	**21.53**

The mean percent association of radioactivity with red blood cells across the concentration range was 26.5%, 18.2% and 21.5% in rat, dog and human, respectively. The corresponding

CONCLUSIONS

ACT-128800 was very highly bound (>99%) to rat, dog and human plasma proteins. The mean blood/plasma ratios were 0.73, 0.54 and 0.68, in rat, dog and human, respectively.

Study FK13292: Assessment of the Binding of JNJ-67896153 (Ponesimod) to Human Albumin and alpha 1-Acid Glycoprotein (AAG)

METHODS

Human serum albumin and alpha-1-acid glycoprotein (AAG) were prepared at final concentrations of 4% and 0.14%, respectively in Sorenson's buffer (0.067M), which were then spiked with JNJ-67896153 (20, 100, and 500 ng/mL) or controls (1µM). Binding studies were performed in triplicates using classical equilibrium dialyzer (DIANORM) at 37oC for 4 hours (JNJ-67896153 and warfarin) or 8 hours (UCN-01). Samples were analyzed by LC-MS/MS against calibration standards in each treated matrix. Binding results are expressed as mean percent free and percent bound (± standard deviation).

RESULTS

Mean percent free and bound values (± standard deviation) of JNJ-67896153 and the control compounds are summarized in the Table below

Binding of JNJ-67896153 and Controls to Human Albumin (4%) and AAG (0.14%) using Classical Equilibrium Dialysis (Dianorm)

Compound	Conc. (ng/mL)	Species	Matrix	Tested Matrix Conc.	% Free [a]	% Bound [a]	% Recovery [b]
JNJ-67896153 (Ponesimod)	20	Human	Albumin	4.0%	1.27 ± 0.22	98.73 ± 0.22	98.20
	100				1.23 ± 0.06	98.77 ± 0.06	99.86
	500				1.20 ± 0.16	98.80 ± 0.16	93.02
	20		AAG	0.14%	5.65 ± 0.18	94.35 ± 0.18	88.92
	100				5.16 ± 0.29	94.84 ± 0.29	84.76
	500				4.47 ± 0.37	95.53 ± 0.37	84.32
Warfarin (control)	308 (1 µM)		Albumin	4.0%	0.69 ± 0.02	99.31 ± 0.02	89.98
UCN-01 (control)	483 (1 µM)		AAG	0.14%	0.30 ± 0.02	99.70 ± 0.02	89.06

[a] Data expressed as mean of triplicate samples ± standard deviation
[b] Recovery measures percent compound remaining post dialysis relative to the T=0 hr dosing solutions.

Mean percent binding values of JNJ-67896153 (20, 100, and 500 ng/mL) in 4% human albumin were 98.73%, 98.77%, and 98.80%, respectively. Mean percent binding values of JNJ-67896153 (20, 100, and 500 ng/mL) in 0.14% human AAG were 97.35, 94.84%, and 95.53%, respectively.

CONCLUSIONS

JNJ-67896153 (Ponesimod) was highly bound (>95%) to human albumin and AAG.

4.4-3. HUMAN PK STUDIES

4.4-3.1 Healthy Subject PK

Study AC-058-106: Single-center, open-label study with ^{14}C-labeled ACT-128800 (ponesimod) to investigate the mass balance, pharmacokinetics, and metabolism following single oral administration to healthy male subjects

Objectives:

- To investigate the rate and routes of elimination of ACT-128800, and the mass balance in urine and feces
- To investigate the pharmacokinetics (PK) of total radioactivity in whole blood and plasma
- To investigate the PK of ACT-128800 in plasma
- To investigate the PK of total radioactivity in CO2 from expired air
- To identify and quantify ACT-128800 metabolites in plasma, urine, and feces
- To evaluate the safety and tolerability of a single oral dose of 40 mg ^{14}C-labeled ACT-128800 in healthy male subjects

Study design: A single-center, open-label, single-dose Phase 1 study. The maximum duration of the study for each subject was between 32–42 days (inclusive of screening).

Number of patients: The planned and actual sample size was six subjects.

Main criteria for inclusion/exclusion:

Healthy male subjects aged 45 to 65 years (inclusive) with a heart rate (HR) of 55 to 90 bpm at screening were enrolled.

Subjects with lymphopenia (< 1,100 lymphocytes/µL) or history of clinically relevant constipation in the 4-week period prior to screening were excluded from the study.).

Dose and mode of administration: Subjects received a single oral dose of 40 mg ^{14}C-labeled ACT-128800 (one capsule).

All administered capsules were from the same batch:

Batch Number: 302622; Retest date: April 2009.

Sample collection:

- Blood: Pre-dose (0), 30, 60, 90, 150mins, 4, 6, 10, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240 h post-dose.
- Urine: 0–8 hours post-dose, 8–16 hours post-dose, and 16–24 hours post-dose (Day 1). From Day 2 to morning of Day 11, urine was collected in a 24-hour interval.
- Feces: Between Day −3 and Day −1 for baseline feces sample, and all complete feces portions from Day 1 (post-dose) to Day 10 (inclusive).
- Expired Air Sampling: Pre-dose (0), 30, 60, 90, 150mins, 4, 6, 10, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240 h post-dose.

Analytes, sample matrices, and methods:

The radioactivity was determined using a PerkinElmer scintillation counter (TriCarb 2800TR). The measurements were performed for a counting time of 10 minutes.

The LOQ was 10 DPM for plasma, urine, feces, and expired CO2 and 20 DPM for whole blood.

Due to an insufficient volume of one plasma sample, a total of 139 human plasma samples were analyzed for ACT-128800 using LC-MS/MS. The LOQ was 1.00 ng/mL.

Pooled plasma, feces, and urine samples were prepared and evaluated for ACT-128800 and radiolabeled metabolites. The LOQ was 15 cpm/peak area for plasma and 25 cpm/peak area for urine and feces.

PK evaluations:

- PK of total radioactivity in urine and feces
- PK of $^{14}CO2$ in expired air
- Cmax, tmax, t½, AUC0-t, and AUC0-inf of ACT-128800 in plasma derived by non-compartmental analysis of the plasma concentration-time data
- Profiling, identification, and quantification of metabolites in plasma, urine, and feces

PD evaluations:

Total lymphocyte count and percent change from baseline.

RESULTS

Excretion of radioactivity:

The cumulative recovery of total radioactivity expressed as a percent of the administered dose (mass balance) in the urine and feces samples is summarized in the following table:

Subject	001	002	003	004	005	006
Time Interval (h)	0–288	0–240	0–288	0–360	0–360	0–360
Recovery Feces (%)	64.0	79.6	61.5	66.5	57.3	60.3
Recovery Urine (%)	12.2	11.7	10.4	10.3	18.4	15.3
Total Recovery (%)	**76.2**	**91.3**	**71.9**	**76.8**	**75.7**	**75.6**

Source listings: Appendix 16.2.5.2.5–7: Tables 1.1, 1.2, and 1.3.1

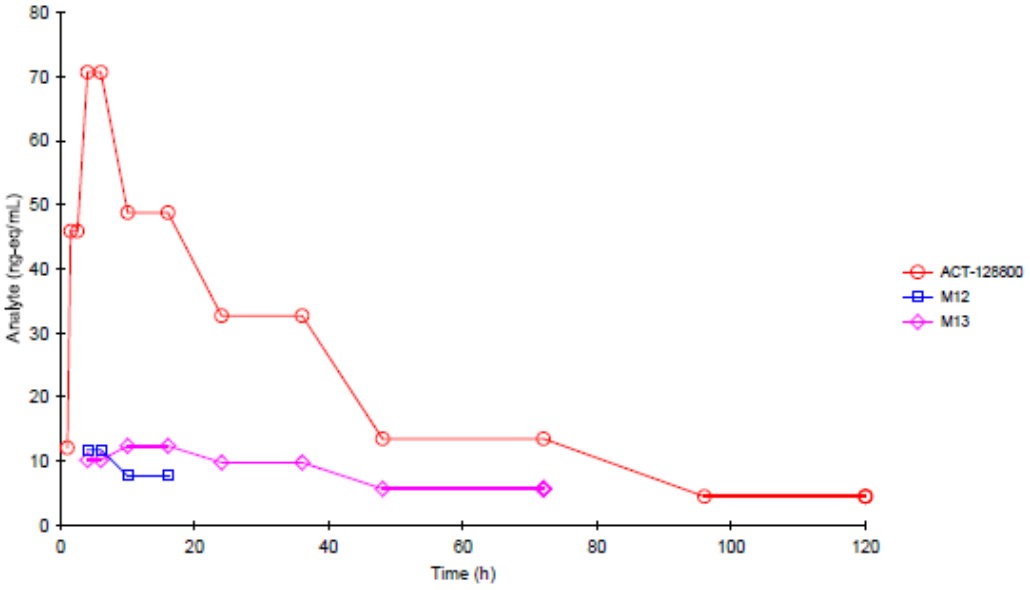
The majority of the radioactivity was excreted in the feces, ranging from 57.3%–79.6% of the administered radioactive dose. In urine, the recovered radioactivity accounted for 10.3–18.4% of the total administered radioactive dose.

The mean concentration of ^{14}C radioactivity in CO2 from expired air was relatively low. The total amount (semi-quantitative estimation) of radioactivity recovered in expired CO2 ranged from 0.6–1.9% of the administered dose.

Metabolism:

Metabolite profiles in plasma

The concentration-time profiles for the two ACT-128800 metabolites found in plasma, M12 (ACT-204426) and M13 (ACT-338375), and their relative radioactivity are shown in the Figure below:



Unchanged parent drug (ACT-128800) was the major component detected in all pooled plasma samples. Metabolites M12 (ACT-204426) and M13 (ACT-338375) were also detected and quantified (see the Table below).

PK parameters for ACT-128800, M12, and M13 in the plasma pools, Per-protocol set

Analyte	C_{max} (ng-eq/mL)	$t_{max}^{\#}$ (h)	AUC_{0-t} (ng-eq*h/mL)	AUC_{0-inf} (ng-eq*h/mL)	$t_{1/2}$ (ng-eq/mL)
ACT-128800	70.7	4	2,471	2,668	30.4
M12 (ACT-204426)	11.8	4	132	328*	17.6*
M13 (ACT-338375)	12.4	10	597	1,038*	53.7*

*Parameters could not be reliably assessed. $^{\#}t_{max}$ is calculated by default by WinNonlin at the first time point when C_{max} is first reached. Since pooled samples were used, actual t_{max} were 4-6, 4-6, and 10-16 hours, for ACT-128800, M12, and M12, respectively.

Based on AUC0-t values, exposure to M12, and M13, respectively, correspond to 5.3% and 24.2% of the exposure to ACT-128800. Using the more conservative AUC0-inf values, the percentages obtained for M12 and M13 are 12.3% and 38.9%, respectively. As a proportion of the total drug-related radioactive exposure (AUC0-inf), ACT-128800 corresponds to 66.1%, M12 to 8.1%, and M13 to 25.7%.

Reviewer's comments:

Clinical multiple ascending dose (MAD) study (AC-058-109) suggested that M12 represent only 6.1% and M13 represent 19.7% of total drug-related exposure.

Metabolites in urine

In human urine, 23 metabolites of ACT-128800 were detected and 12 of them were identified. The major component was an unidentified peak 'f' (RT: (b) (4) minutes), representing 7% of the radioactivity recovered in urine. In addition, seven identified metabolites (including M12) and six unidentified peaks were detected with an abundance between 1–5% of the total radioactivity recovered in urine. All other detected metabolites (including M13) accounted for less than 1% of the urinary radioactivity. Unchanged ACT-128800 was not found in urine samples.

Metabolites in feces

In human feces, 21 metabolites and the parent drug were quantified, and all but one were identified. Unchanged ACT-128800 and metabolite M12 were the major components detected, representing approximately 26% and 22% of the radioactivity recovered in feces, respectively. In addition, 12 identified metabolites were detected with an abundance between 1–5% of the radioactivity recovered in feces. All other metabolites (seven) accounted for less than 1% of the fecal radioactivity.

ACT-128800 and its metabolites (accounting for at least 1% of the radioactivity) identified in all evaluated biological matrices (plasma, feces, and urine) are summarized in the Table below.

Compound	Structure-Metabolism	Plasma (Yes/No)	% Feces radioactivity	% Urine radioactivity
ACT-128800	Parent (unchanged)	Yes	25.9	NF
M12 (ACT-204426)	Oxidation	Yes	22.3	2.5
M13 (ACT-338375)	Oxidation and hydrolytic cleavage	Yes	2.7	< 1%
M26	Oxidation and reduction	No	4.3	NF
M28	Oxidation and reduction	No	2.1	NF
M29	Oxidation (in two positions) and hydrolytic cleavage	No	2.0	3.6
M30	Oxidation, hydrolytic cleavage and reduction	No	1.6	NF
M31	Oxidation (in two positions) and hydrolytic cleavage	No	1.5	1.5
M33/M51	Oxidation (in two positions), hydrolytic cleavage and reduction	No	2.1	< 1%
M34	Oxidation (in two positions), hydrolytic cleavage and reduction	No	1.6	NF
M35/M36	Oxidation (in two positions), hydrolytic cleavage and reduction	No	3.2	< 1%
M32	O-dealkylation	No	1.7	NF
M38/M39/M40	Oxidation and glucuronidation	No	NF	2.1
M48	O-dealkylation, oxidation and reduction	No	NF	1.2

NF- Not found
Note: The metabolite M33 could not be separated from M51, as well as M35 from M36, and M38 from M39 and M40. For the calculation of the number of metabolites within a certain range (e.g., from 1–5% in feces), each metabolite was considered separately (e.g., M35/36 were considered as 2 metabolites with a % feces recovery of 3.2%). Therefore, the number of metabolites accounting for at least 1% of the radioactivity is based on a conservative approach.
Source: Appendix 16.2.4.2.2, Analytical study report (metabolites profiling).

Pharmacodynamic:

Following administration of 14C-ACT-128800, mean absolute lymphocyte counts declined rapidly, from a baseline value of 1.60 ± 0.16 × 10^9/L to a nadir of 0.51 ± 0.20 × 10^9/L at 6 h after dosing (range: 0.34 to 0.86 × 10^9/L).

Safety:

No deaths or SAEs were reported. All six subjects enrolled completed the study. Headache was the most frequently reported AE (83.3%), followed by dizziness (50%) and nasopharyngitis (33.3%). All AEs reported in this study were generally consistent with the known AE profile of ACT-128800.

Mean and median HR as measured by ECG decreased after administration of ACT-128800, with a maximal mean reduction of 25.7 bpm recorded at 2.5 h after drug administration (baseline: 67.2 bpm). No clinically relevant changes in mean or median PR and QRS values and no QTcB or QTcF prolongations greater than 450 ms were observed. Sinus bradycardia was the most frequently observed ECG abnormality.

CONCLUSIONS

- ACT-128800 and its metabolites are mainly excreted in the feces (recovery: 57.3–79.6% of the radioactive dose) and to a lesser extent in the urine (recovery 10.3–18.4% of the radioactive dose). M13 was the main metabolite in plasma.
- The observed safety profile was similar to the known profile of ACT-128800 with no new or unexpected safety findings in this study.

Study AC-058-101: Double-blind, placebo-controlled, randomized, single ascending dose study to investigate the tolerability, safety, pharmacokinetics (including food interaction), and pharmacodynamics of ACT-128800 (ponesimod) in healthy male subjects

A brief overview of some essential components of the study design is given below:

Study Design	Single center, double-blind, placebo-controlled, randomized, ascending dose, Phase I study.
Study Population	48 subjects were included (6 dose groups, 6 subjects on active drug and 2 subjects on placebo for each dose group) and analyzed. Age: 21–47 years Weight: 64–98 kg Race: Caucasian
Dosage and Administration	1, 3, 8, 20, 50, and 75 mg of ACT-128800, oral administration, one single administration. For the 20-mg dose group, the drug was administered twice; once in the fasted and once in the fed condition. The subjects will remain fasted from 10 hours prior to 4 h after drug intake (except 20 mg dose group, fed). The drug should be administered with 240 ml of water. Batch No. ACT-128800: 1 mg (PD05153, (b) (4) 5 mg (PD05154, (b) (4) 25 mg (PD05155, (b) (4) Placebo: PD05152, (b) (4)
PK Sampling:	Blood: Pre-dose, 0.5, 1.0, 1.5, 2.5, 4, 6, 10, 16, 24, 36 and 48 h post dose Urine: Prior to dosage, and 0-48h post-dose
PD Sampling:	Lymphocyte counts: Pre-dose, 0.5, 1.0, 2.5, 4, 6, 10, 16, 24, 36 and 48 h post dose.

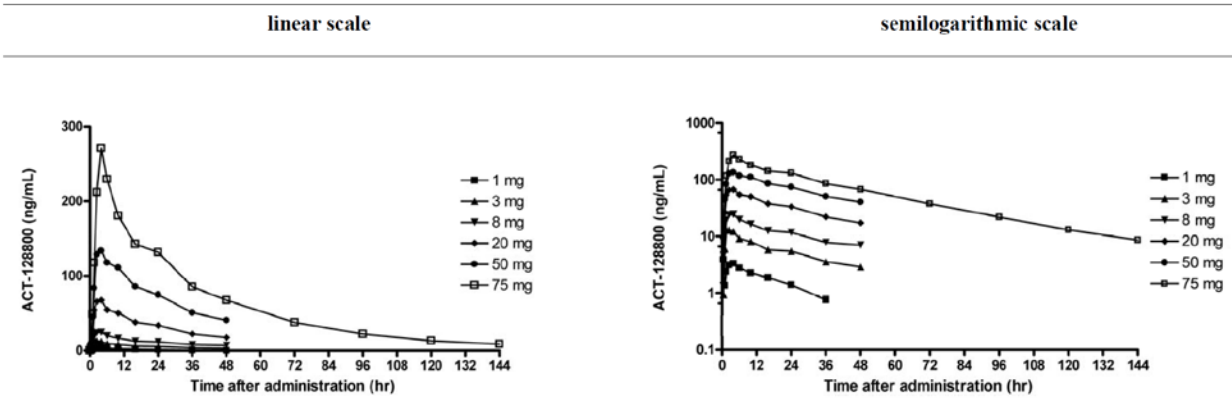
Analysis (Plasma)	Method: LC/MS/MS ACT-128800 (Ponesimod) LLOQ: 1 ng/mL Linear range: 1-2000 ng/mL Inter-batch Precision (%CV): 0.2-5.2% Inter-batch accuracy: -12.0 to -3.5%
PK Assessment	The following PK parameters for ACT-128800 were determined from plasma samples using noncompartmental methods: AUC0-t, AUC0-∞, Cmax, tmax, tlag, and t½. Concentrations of ACT-128800 in urine were determined (50-mg dose group).
PD Assessment	Absolute count-time profiles of lymphocytes, AUEC, time to nadir lymphocyte count, and nadir lymphocyte count.
Safety Assessment	AE, SAE, laboratory abnormalities, ECG, neurological assessments (75-mg dose group only), pulmonary tests (75 mg)

RESULTS

Pharmacokinetics

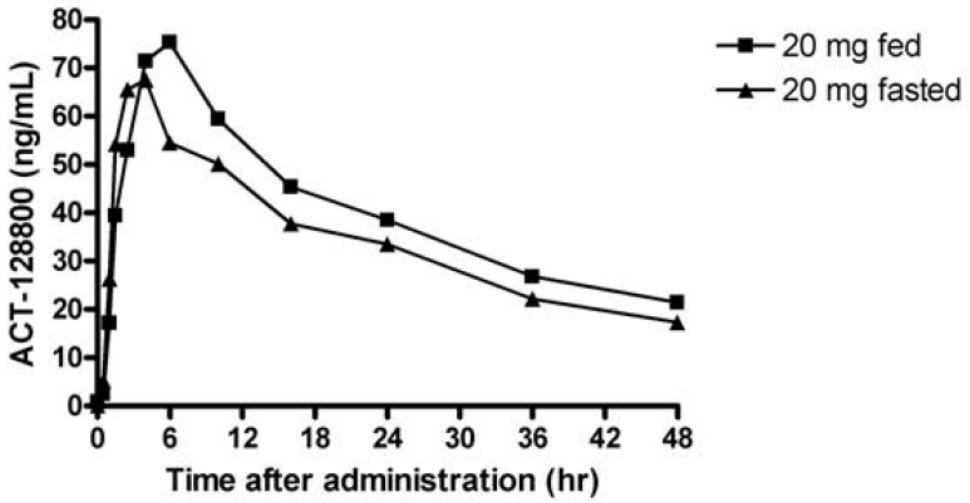
Mean plasma concentration versus time profiles of ACT-128800 after single ascending doses (1-75 mg) are shown in the following figure:

Arithmetic mean plasma concentration-time profiles of ACT-128800 in healthy subjects (n = 6 per group) after administration of a single dose of 1, 3, 8, 20, 50 or 75 mg of ACT-128800 in the fasted condition



Mean plasma concentration versus time profiles of ACT-128800 in the fed versus the fasted condition (20 mg) are shown in the figure below:

Arithmetic mean plasma concentration-time profiles of ACT-128800 in healthy subjects (n = 6) after administration of a single dose of 20 mg of ACT-128800 in the fed and fasted conditions (linear scale)



The pharmacokinetic parameters of ACT-128800 are given in the Table below:

Plasma pharmacokinetic parameters of ACT-128800 in healthy subjects after administration of a single dose of 1, 3, 8, 20, 50, and 75 mg of ACT-128800 in the fasted condition

Parameter	n	C_{max} (ng/mL)	t_{max} (hr)	$t_{1/2}$ (hr)	AUC_{0-t} (ng.hr/mL)	AUC_{0-inf} (ng.hr/mL)
Dose (mg)						
1	6	3.4	3.3	21.7	59.5	96.0
		(2.8- 4.2)	(1.5 - 4.0)	(18.1 - 26.0)	(42.5 - 83.2)	(75.9 - 121)
3	6	13.7	2.0	30.1	273	405
		(11.9 - 15.7)	(1.5 - 2.5)	(21.6 - 41.9)	(236 - 316)	(323 - 509)
8	6	27.2	3.3	33.4	573	913
		(23.7 - 31.2)	(1.5 - 6.0)	(28.1 - 39.7)	(484 - 679)	(751 - 1110)
20	6	71.0	2.5	27.7	1615	2344
		(56.0 - 90.1)	(1.5 - 4.0)	(19.2 - 39.8)	(1326 - 1968)	(1796 - 3058)
50	6	163	4.0	28.8	3539	5266
		(130 - 205)	(2.5 - 10.0)	(20.3 - 40.8)	(2952 - 4243)	(3942 - 7035)
75	6	274	4.0	31.4	8744	9153
		(256 - 292)	(2.5 - 6.0)	(24.4 - 40.5)	(7372 - 10372)	(7456 - 11238)

Data are geometric means (and 95% CI) or for t_{max} the median (and range).

ACT-128800 could be detected in plasma in all dose groups. Under fasting conditions, ACT-128800 was absorbed rapidly, with median tmax varying from 2 to 4 hr. Half-lives (geometric means) varied from 21.7 to 33.4 hr in the different dose groups. After single-dose

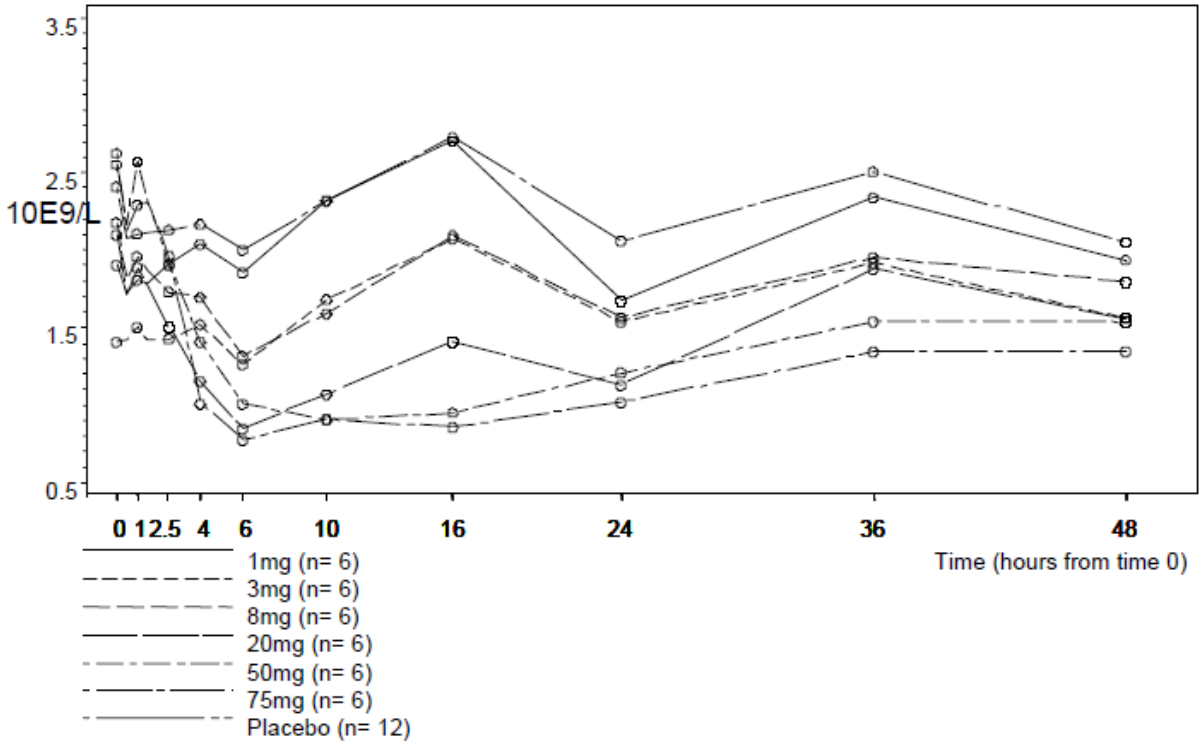
administration, the pharmacokinetics of ACT-128800 showed a dose-proportional increase for Cmax and AUC0–∞. Cmax and AUC0-∞ were slightly higher and tmax longer in the fed condition, but not considered as different to a clinically relevant extent.

Urine samples from the 50-mg dose group were analyzed for ACT-128800. The amount of unchanged ACT-128800 in urine collected over 48 hr was negligible (0.05%) and was therefore not determined in the other dose groups.

Pharmacodynamics

The graphical presentations of the absolute lymphocyte counts mean values over 48 hr for each dose group compared to placebo are displayed in the following figure:

Time course of absolute lymphocyte counts (mean) following administration of placebo or ACT-128800



A dose-dependent reduction in lymphocytes was observed after the administration of ACT-128800 at doses of 8 mg and higher. The reduction of circulating lymphocytes was most pronounced during the first 6 hours after administration. With the highest dose, 96 hr after study drug treatment, the effects on lymphocytes were comparable with the placebo-treated subjects.

Safety

In subjects receiving ACT-128800, no SAEs or severe AEs were reported. Most AEs were of mild intensity, with AEs of moderate intensity reported only at doses of 20 mg of ACT-128800 or higher. The number of AEs was apparently dose-related.

Starting with the dose of 8 mg, transient HR reduction was observed with the maximal effect at 2 hr 30 min post-dose, (mean reduction between 15 and 24 bpm [8–75 mg]) and with mean HR returning to over 50 bpm 6 to 10 hr post-dose. The maximal reduction in HR (33 bpm) and the lowest HR (31 bpm) were measured in two subjects in the 50-mg dose group (maximal HR reduction in placebo: 31 bpm and lowest HR: 43 bpm).

There were no apparent drug-related effects on clinical chemistry or coagulation parameters, with the exception of a transient CRP increase in the 50- and 75-mg dose groups.

Overall, ACT-128800 was well-tolerated after single oral doses of 1–75 mg in healthy male volunteers.

CONCLUSIONS

- ACT-128800 was rapidly absorbed (median tmax varied between 2 and 4 hr) and the mean terminal half-life varied between 21.7 and 33.4 hr. Drug exposure (AUC0-inf and Cmax) was shown to be dose-proportional.
- The first effects of the reduction of the peripheral lymphocytes were observed with a dose of 8 mg, and clear effects were observed with doses of 20 mg and above.
- Starting with the dose of 8 mg, a transient HR reduction was observed in all subjects treated with ACT-128800, with the maximal effect at 2 hr 30 min (mean reduction between 15 and 24 bpm).
- Single ascending doses of 1–75 mg ACT-128800 were well tolerated in healthy male volunteers.

Study AC-058-102: Single-center, double-blind, placebo-controlled, randomized, ascending multiple dose study to investigate the tolerability, safety, pharmacokinetics, and pharmacodynamics of ACT-128800 in healthy male and female subjects.

A brief overview of some essential components of the study design is given below:

Study Design	single-center, double-blind, placebo controlled, randomized, ascending multiple-dose. • In Part A, ACT-128800 was administered orally once daily for 7 days at doses of 5, 10, or 20 mg. • In Part B, ACT-128800 was administered orally once daily within an up-titration dosing scheme: 4 days at 10 mg, 4 days at 20 mg, and then 7 days at 40 mg.
Study Population	47 subjects were included (30 subjects in part A, 17 subjects in part B) and analyzed.
Dosage and Administration	**Part A:** ACT-128800 (b) (4) capsules 5 mg (1 capsule of 5 mg), 10 mg (2 capsules of 5 mg), or 20 mg (4 capsules of 5 mg) once per day/oral dosing/7 days. **Part B:** ACT-128800 (b) (4) capsules/up-titration: 4 days at 10 mg (2 capsules of 5 mg) once per day, 4 days at 20 mg (4 capsules of 5 mg) once per day, 7 days at 40 mg once per day (3 capsules of 5 mg and 1 capsule of 25 mg)/oral dosing/15 days. The batch numbers Placebo: Batch No. PD05152 / Retest date: (b) (4) Part A: ACT-128800 5 mg capsules Batch No. PD05154 / Retest date: (b) (4) Part B: ACT-128800 5 mg capsules Batch No. PD05154 / Retest date: (b) (4) ACT-128800 25 mg capsules

	Batch No. PD05155 / Retest date: (b) (4)
Analysis (Plasma)	Method: LC/MS/MS ACT-128800 (Ponesimod) LLOQ: 1 ng/mL Linear range: 1-2000 ng/mL Inter-batch Precision (%CV): 5.1-7.2 % Inter-batch accuracy: -2.5 to 1.0 %
PK Assessment	Part A: Plasma concentration-time profiles of ACT-128800 following drug administration on Days 1 and 7 and the following pharmacokinetic parameters derived by non-compartmental analysis: Cmax, tmax, t½, AUC0–24; accumulation factor (R). Part B: Plasma concentration-time profiles of ACT-128800 following drug administration on Days 1, 5, 9, and 15 and the following pharmacokinetic parameters derived by non-compartmental analysis: Cmax, tmax, t½ (Day 15 only), and AUC0–24. Trough levels were determined to assess attainment of steady-state conditions. Dose-proportionality was investigated at steady-state on Day 7 for 5, 10, and 20 mg (Part A) and Day 15 (40 mg, Part B).
PD Assessment	Absolute count-time profiles of lymphocytes, AUEC0-24, time to nadir lymphocyte count, and nadir lymphocyte count.
Safety Assessment	AE, SAE, laboratory abnormalities, ECG, pulmonary function, physical and neurological examination abnormalities

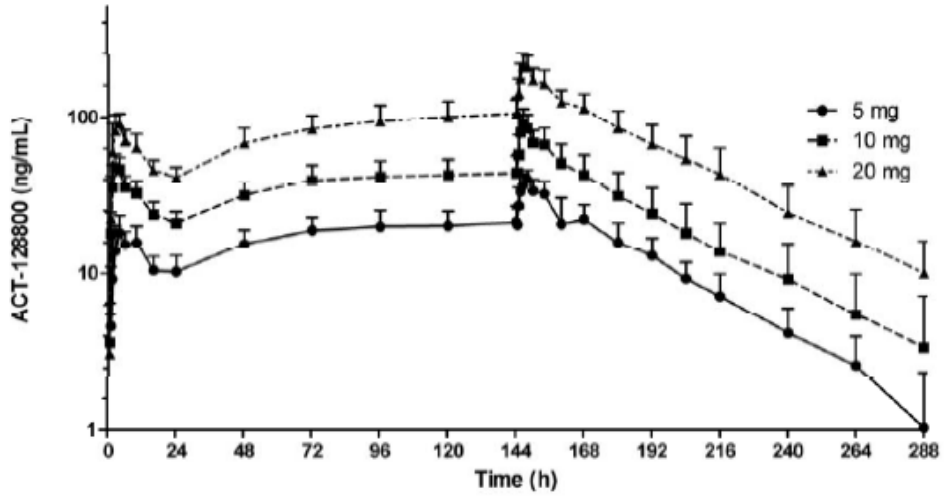
RESULTS

Pharmacokinetics

Part A

A graphical presentation of the mean plasma concentration-time course of ACT-128800 for all dose groups is given in the following figure:

Graphical presentation of the arithmetic mean plasma concentration (+ SD) of ACT-128800 for the dose groups 5, 10, and 20 mg over time (semilogarithmic, n = 8 per group)



A summary of all PK parameters is provided in Tables below (Days 1 and 7):

Summary of pharmacokinetic parameters on Day 1

Dose	N	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (ng·h/mL)
5 mg	8	20.3 (17.2, 24.0)	4.0 (1.5-10.0)	292 (244, 348)
10 mg	8	48.5 (40.5, 58.0)	2.5 (1.5-4.0)	684 (582, 805)
20 mg	8	91.7 (79.3, 106)	4.0 (2.5-4.0)	1318 (1150, 1511)

Summary of pharmacokinetic parameters on Day 7

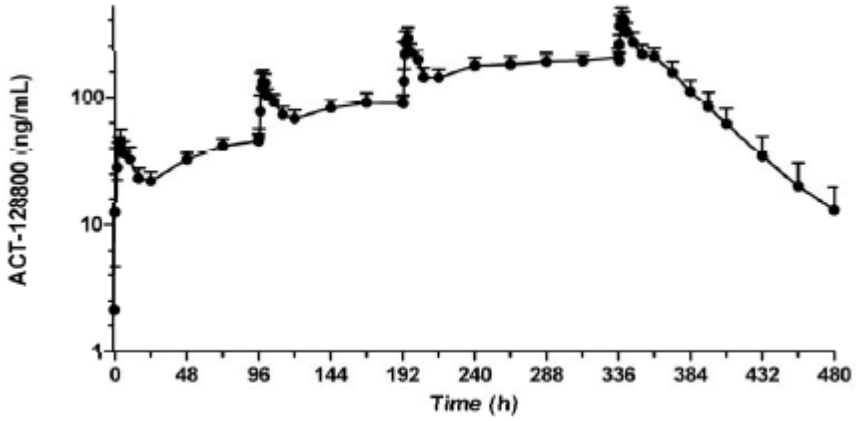
Dose	N	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-24} (ng.h/mL)	Accumulation factor
5 mg	8	40.3 (34.9, 46.4)	4.0 (1.5-4.0)	30.9 (27.2, 35.0)	643 (510, 809)	2.2 (2.0, 2.4)
10 mg	8	88.3 (72.1, 108)	2.5 (2.5-4.0)	32.1 (24.2, 42.6)	1400 (1119, 1752)	2.0 (1.7, 2.5)
20 mg	8	207 (175, 246)	2.5 (2.5-4.0)	32.7 (28.2, 38.0)	3473 (2895, 4166)	2.6 (2.3, 3.0)

The maximum concentration was reached between 2.5 and 4.0 h (median) and the half-life varied between 30.9 and 32.7 h (geometric mean). The accumulation factors ranged from 2.0 to 2.6. The PK of ponesimod were similar for female and male subjects in all dose groups.

Part B

A graphical presentation of the plasma concentration-time course of ACT-128800 in the 40 mg dose group is given in the following figure:

Graphical presentation of the arithmetic mean plasma concentration (+ SD) of ACT-128800 for the 40 mg dose group over time (semilogarithmic, n = 11)



The plasma concentrations of ponesimod reached close to steady-state levels before each up-titration (Day 5 and Day 9 pre-dose, with 10 and 20 mg of ponesimod, respectively). Steady-state concentration levels were reached starting on Day 13 with the dose of 40 mg.

The PK parameters of ponesimod in Part B are summarized in the Table below:

Summary of pharmacokinetic parameters on Days 1, 5, 9, and 15

Parameter	Geometric Mean (95% CI); t_{max}: Median (Range)			
	Day 1 10 mg	Day 5 20 mg	Day 9 40 mg	Day 15 40 mg
N	11	11	11	11
t_{max}, h	4.0 (1.5-4.0)	2.5 (1.5-4.0)	2.5 (1.5-4.0)	2.5 (1.5-6.0)
C_{max}, ng/mL	46.8 (40.8-53.6)	138 (121-157)	292 (256-332)	411 (359-471)
AUC_{0-24h}, ng.h/mL	667 (590-754)	2085 (1888-2302)	4277 (3886-4707)	6325 (5718-6997)
$t_{1/2}$, h	-	-	-	33.5 (29.5-37.9)

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

The mean t1/2 was 33.5 hours and the mean Cmax was reached at 2.5 hours on Day 15. PK were comparable between female and male subjects.

Dose proportionality and sex effect were tested using linear models with and without interactions. Regression lines from the linear models plotted together with the log-transformed values for Cmax and AUC0-24h showed a good fit of the linear models.

The results of the models without interaction are detailed below:

Dose proportionality of ACT-128800 for Cmax and AUC0-24

Parameter	Estimate of the power 90% Confidence interval
C_{max}	1.12 1.05 , 1.20
AUC_{0-24}	1.11 1.03 , 1.20

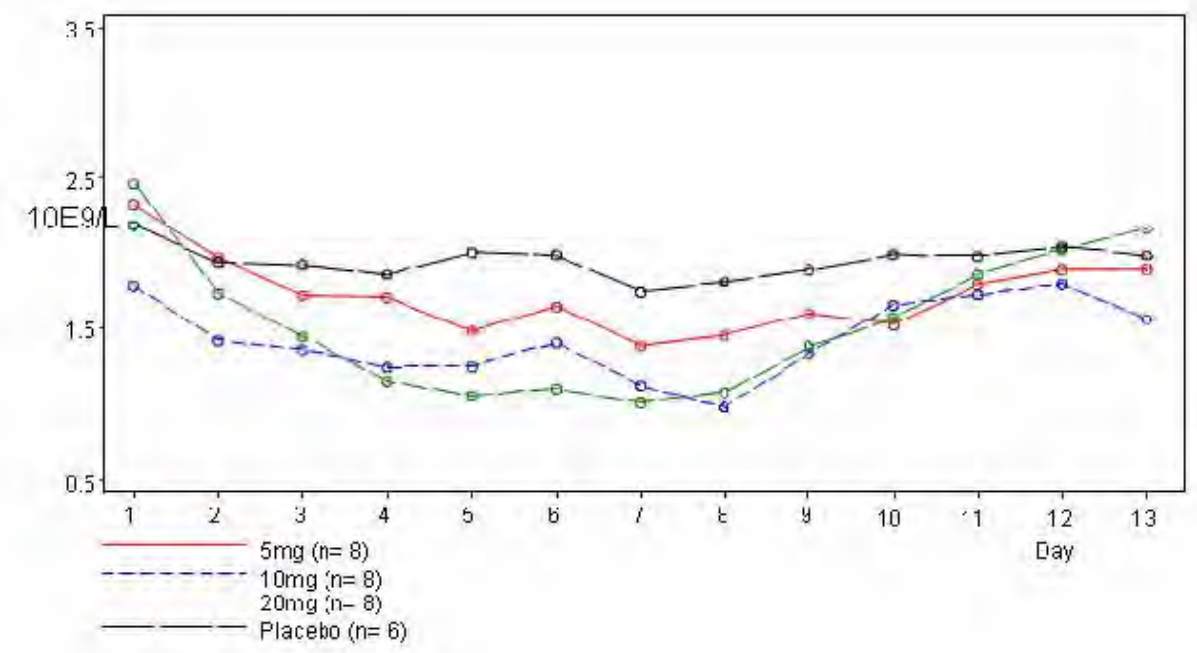
The lower confidence interval limits were above 1.00 suggesting that the pharmacokinetic parameters Cmax and AUC0-24 and increased more than proportionally with dose (5-40 mg).

Pharmacodynamics

Part A

The absolute total lymphocyte counts over time from Day 1 to Day 13 at trough are displayed in the following figure:

Mean total absolute lymphocyte counts at trough from Day 1 to Day 13 (Part A)



Ponesimod induced a dose-dependent reduction of the peripheral blood lymphocyte count from a dose of 5 mg onwards, with the greatest reduction observed 6 hours post dose. Dose-related effects on the total lymphocyte count were observed at both peak and trough.

In Part B, on Day 15 at the dose of 40 mg, the maximal mean lymphocyte reduction from baseline was attained at 6 hours post-dose (81%, to 19% of baseline value). On that day, the mean total ymphocyte reduction from baseline at trough was 70% (30% of baseline value). Total lymphocyte counts increased after the end of the treatment, returning to within normal range.

Safety

ACT-128800 was well tolerated except for mild to moderate AEs belonging to the three groups of respiratory, cardiovascular, and nervous systems. Two subjects were discontinued from study drug treatment due to AEs during the 20 mg up-titration period (edema mouth and granulocyte shift to the left) in Part B.

Dose-dependent mean and median HR reductions from baseline was observed in Part A dose groups, predominantly on Day 1. The peak effect was reached at 2.5 h after dosing on Day 1 in all dose groups. The ECG HR reductions were largest in the 20 mg dose group (mean decrease from baseline of 22 ± 6 bpm). The effect was still present at 4h post-dose and resolved 6–10 hours after dosing in all dose groups.

With the up-titration regimen used in Part B, the maximum ACT-128800 HR reducing effect showed a clear attenuation, with the mean change from baseline being 14 bpm for the 10 mg dose level, 9 bpm for the 20 mg dose level, and 4 bpm for the 40 mg dosing period. In addition, a gradual recovery of mean HR to baseline values could be identified during the treatment period at each dose level.

Dose-dependent disturbances of cardiac rhythm with sinus bradycardia and slowing of the AV conduction as reflected in AV-block were noticed at all dose levels and were most frequent at the 20 mg dose level of Part A. All cases of bradycardia and AV-block were transient did not require treatment.

CONCLUSIONS

Ponesimod was rapidly absorbed and t1/2 was approximately 32 hours. The accumulation factor of the drug (approximately 2-fold) was as expected. The increase was slightly more than dose-proportional for Cmax and AUC0-24 at doses of 5 to 40 mg. The PK profile was evaluated in female and male subjects and the parameters were similar in both sexes.

HR reductions were dose-dependent and were observed mainly after the first dose in Part A. At 10 and 20 mg, the effects on HR decreased with repeated administration of ACT-128800, indicating de-sensitization. The up-titration regimen implemented in Part B successfully reduced the expected effects on HR of the 20- and 40-mg doses of ACT-128800.

Dose-related PD effects on total lymphocyte counts were observed.

Treatment with ACT-128800 (dosing regimens evaluated in part A and B) was well tolerated and no severe or serious AEs were reported.

Study AC-058-115: Single-center, double-blind, placebo-controlled, randomized, two-way crossover, multiple-dose study to investigate the effects on heart rate and rhythm of two up-titration regimens of ponesimod in healthy male and female subjects.

Objectives: To investigate the effects of two different up-titration regimens of ponesimod on heart rate (HR) and rhythm. To evaluate the PK, PD, tolerability and safety of multiple-dose ponesimod during the up-titration regimens.

A brief overview of some essential components of the study design is given below:

Study Design	Double-blind, placebo-controlled, randomized, two-way crossover, multiple-dose up-titration
Study Population	N= 32 recruited and analyzed Age: 18-60 years (mean 34 years) Gender: 15 males (46.9%), 17 females (53.1%) Weight: 54.9- 92.2kg (mean 73.4kg) Race: 29 White (90.6%), 2 African American (6.3%) and 1 Asian (3.1%)
Dosage and Administration	Up-titration regimen A – the treatment started with placebo on Day 1, followed by ponesimod or matching placebo once daily (o.d.): Day 1: placebo; Day 2–3: 2 mg ponesimod or placebo; Day 4–5: 3 mg ponesimod or placebo; Day 6–7: 4 mg ponesimod or placebo; Day 8: 5 mg ponesimod or placebo; Day 9: 6 mg ponesimod or placebo; Day 10: 7 mg ponesimod or placebo; Day 11: 8 mg ponesimod or placebo; Day 12: 9 mg ponesimod or placebo; Day 13–14: 10 mg ponesimod or placebo; Day 15: 20 mg ponesimod or placebo. Up-titration regimen B – the treatment started with placebo on Day 1, followed by ponesimod or matching placebo (o.d.]): Day 1: placebo; Day 2–8: 10 mg ponesimod or placebo; Day 9: 20 mg ponesimod or placebo; Day 10–15: placebo. Ponesimod: 1 mg (PD12116, (b) (4)); 5 mg (PD11199, (b) (4) 10 mg (PD11058, (b) (4) 20 mg (PD11059, (b) (4) Matching placebo: (batch number: PD11057, retest date: (b) (4) The study drug was given with 240 mL of water in the morning to subjects in the sitting position. There was a washout period of 12 to 14 days between the last study drug administration.

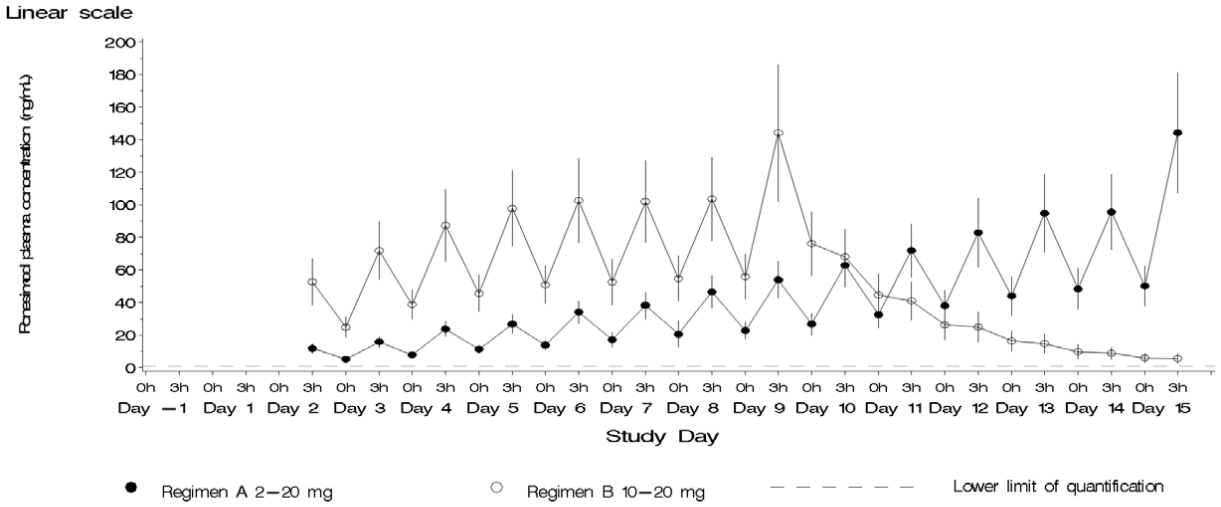
Analysis (Plasma)	Method: LC/MS/MS ACT-128800 (Ponesimod) LLOQ: 1 ng/mL Linear range: 1-1000 ng/mL Inter-batch Precision (%CV): 5.8-6.8% Inter-batch accuracy: -2.5 – 3.8 %
PK Assessment	Mean trough (pre-dose) and 3 h post-dose concentrations of ponesimod on each study day.
PD Assessment	Cardiodynamics variables derived from both 12-lead ECG and Holter, including HR, PR intervals, Mean hourly HR, HRnadir, time to HRnadir, and Emax.
Safety Assessment	Adverse event (AE), serious AE (SAE), vital signs, 12-lead ECG variables, clinical laboratory tests.

RESULTS

Pharmacokinetic Results

The concentration-time profiles of ponesimod are shown in showed in the figure below:

Arithmetic mean (± SD) plasma concentration-time profiles of ponesimod (n = 24)



If > 50% of the values at a given time point were BLQ (< 1 ng/mL), no mean value was calculated
0h and 3h represent Pre−dose and 3 hours post−dose timepoints respectively
Reference: Table 15.2.2−1
Program Location: /cvn/projects/prj/development/000000110343/dev/figures/fpconcSD.sas
Program Run: 11FEB14 cvn_vkiropla Program Status: FINAL

In up-titration regimen A, trough and 3 h plasma concentrations steadily increased and on Day 14 following administration of the second dose of 10 mg were similar to those achieved during up-titration regimen B at steady-state with the same dose (regimen A: Day 14, 3h: 93.1 ng/mL, regimen B: Day 6, 3h: 99.7 ng/mL).

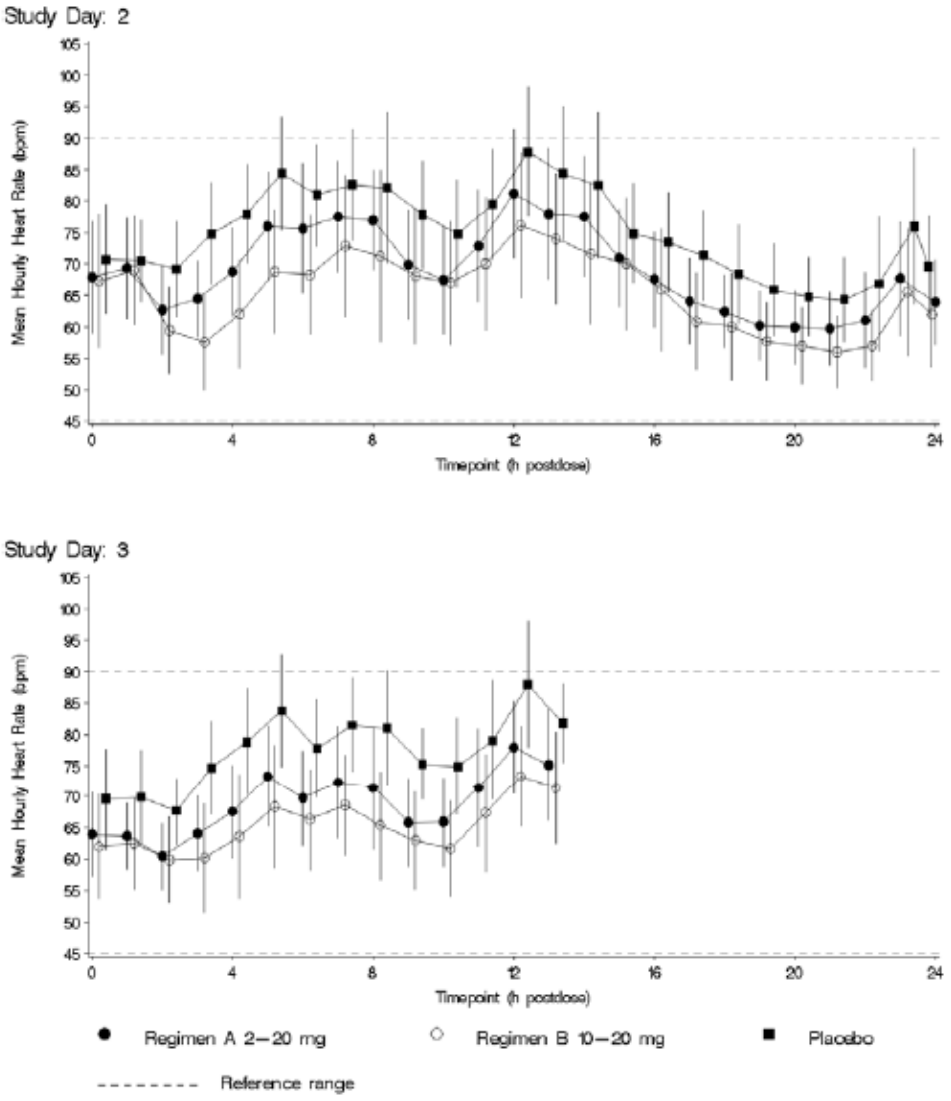
Pharmacodynamic Results

Effects on heart rate

The first dose of ponesimod (Day 2) resulted in a transient decrease in mean hourly HR from baseline as assessed by Holter. The decrease was greater with treatment regimen B (maximum mean decreases: 12 bpm) than with treatment regimen A (6 bpm) and placebo (0 bpm). These maximum mean decreases occurred 2–3 h after study drug administration and mean hourly HR had returned to pre-dose values by 4–5 h after administration. On Day 3, the decrease in mean hourly HR from pre-dose on Day 3 induced by ponesimod was similar or smaller when compared to Day 2, and there was no longer a difference between regimen A and regimen B (see Figure below).

Mean (SD) hourly Holter HR data over Days 2 to 3

Ponesimod, Protocol: AC—058—115 (Page 2 of 8)
Figure 15.3.4—5.1 Mean (SD) Hourly Holter HR Data
Per—protocol Set



For both treatment regimens, the pre-dose mean hourly HR values on Day 3 and later were 5 to 10 bpm lower when compared to baseline and remained lower during the complete ponesimod treatment period. HR < 45 bpm and decrease from baseline of > 20 bpm following the first ponesimod dose on Day 2 occurred more frequently during treatment regimen B than treatment regimen A. During treatment regimen A and placebo, HR values of interest occurred on all study days with similar frequency. In contrast, in treatment regimen B these values, most notably values of HR < 45 bpm, occurred more often on the first 4 days of ponesimod treatment.

In 3/24 (12.5%, 20 events), 4/24 (16.7%, 58 events), and 0/16 subjects in treatment regimen A, B, and in the placebo treatment group (both periods pooled together), respectively, a value for HR < 45 bpm was measured on 12-lead ECGs recorded throughout the study (mostly in male subjects). On recorded ECGs, 2/24 (8.3%, 15 events), 2/24 (8.3%, 15 events), and 1/16 (6.3%, 127 events) subjects in treatment regimen A, B, and the placebo treatment group, respectively, experienced a decrease from baseline in HR of > 20 bpm.

Effects on PR interval

In 7/24 (29.2%, 221 events), 7/24 (29.2%, 258 events), and 4/16 (25.0%, 112 events) subjects during treatment regimen A, B, and in the placebo treatment group, respectively, a value for PR ≥ 200 ms was measured on the 12-lead ECG recordings. These PR values of interest appeared to occur slightly more frequently in ponesimod-treated subjects when compared to placebo, without any obvious difference between treatment regimens.

Occurrences of AV-blocks

The proportion of subjects who experienced at least one AV-block was 25% for all treatment groups. However, the total number of occurrences of any AV-blocks was largest during treatment regimen B (143) followed by treatment regimen A (79) and the placebo treatment group (33).

Safety

The percentage of subjects, who experienced at least one AE was 83.3%, 91.7%, and 81.3% in regimen A, B, and placebo, respectively. The total number of AEs and the intensity of these AEs were similar across the different treatment groups. The percentage of AEs related to study drug was 66.7%, 83.3%, and 68.8% in regimen A, B, and placebo, respectively.

CONCLUSIONS

Overall, the safety and cardiodynamic profile of regimen A was qualitatively similar to that of regimen B but fewer and less pronounced ponesimod-related cardiodynamic effects were observed in treatment regimen A compared to treatment regimen B.

This study did not reveal any new safety findings related to multiple-dose administration of ponesimod.

4.4-3. HUMAN PK STUDIES

4.4-3.2 Intrinsic Factors

Study AC-058-112: A Single-center, open-label, single-dose Phase 1 study to investigate the pharmacokinetics (PK), tolerability, and safety of ponesimod in subjects with mild, moderate, or severe hepatic impairment due to liver cirrhosis, and in healthy subjects.

Objectives: To investigate the effects of varying degrees of hepatic impairment on the pharmacokinetics (PK) of ponesimod and its metabolites ACT-204426 (M12) and ACT-338375 (M13) after a single oral dose of ponesimod.

A brief overview of some essential components of the study design is given below:

Study Design	Single-center, open-label, single-dose Phase 1 study
Study Population	32 subjects (14 females and 18 males) in 4 groups were recruited and data analyzed. • Group A: 8 subjects (4 males and 4 females) with mild hepatic impairment (Child-Pugh score 5–6). • Group B: 8 subjects (6 males and 2 females) with moderate hepatic impairment (Child-Pugh score 7–9). • Group C: 8 subjects (4 males and 4 females) with severe hepatic impairment (Child-Pugh score 10–15). • Group D: 8 healthy subjects (4 males and 4 females), matched to each subject with severe hepatic impairment.
Dosage and Administration	Ponesimod (ACT-128800) - 10 mg tablet. Single oral dose on Day 1 in fasted conditions Batch No.: PD11058; Retest date: (b) (4)
PK Sampling:	Blood: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 72, 120, 168, (for Gourp D, healthy subject) 216 and 264 hours post dose. Blood (for protein binding): 3, and 12 hours post dose.
Analysis (Plasma)	Method: LC/MS/MS LLOQ: 1 ng/mL Linear range: 1.00-1000 ng/mL

	<table><tr><th>Parameter</th><th>ACT-128800</th><th>ACT-204426</th><th>ACT-338375</th></tr><tr><td>Intra-run %CV</td><td>2.0 to 6.5</td><td>1.5 to 9.1</td><td>2.5 to 5.7</td></tr><tr><td>Inter-run %CV</td><td>≤ 7.1</td><td>≤ 8.6</td><td>≤ 7.0</td></tr><tr><td>Intra-run %Diff</td><td>-2.5 to 8.0</td><td>0.0 to 14.0</td><td>-1.8 to 11.0</td></tr><tr><td>Inter-run %Diff</td><td>-0.3 to 3.8</td><td>2.3 to 7.9</td><td>2.9 to 5.9</td></tr></table> (b) (4) assay LLOQ: 0.1 ng/mL Accuracy (inter-run): 98.0–101.6% (ponesimod); 99.3–101.6% (M12); 96.3–101.2% (M13) Precision (inter-run): 1.2–8.9% (Ponesimod); 1.5–7.5% (M12); 1.6–7.2% (M13)
PK Assessment	The following PK parameters of ponesimod and its metabolites were determined using non-compartmental method: Cmax, Tmax, AUC0-t, AUC0inf, t1/2, Ratio of free (unbound, Cu) to total (C) plasma concentrations (Cu/C) of ponesimod, M12, and M13, at 3 and 12 h post-dose.
PD Assessment	Absolute lymphocyte counts, absolute change from baseline, and percentage change from baseline at each time point of assessment
Safety Assessment	AEs, SAEs, Vital signs (supine blood pressure [BP] and HR), ECG, Clinical laboratory tests (hematology, clinical chemistry, and coagulation variables).

RESULTS

Pharmacokinetics of ponesimod

Descriptive statistics of ponesimod PK parameters, summarized by group, are displayed in the following tables:

Parameter [unit]		Group A (N = 8)	Group B (N = 8)	Group C (N = 8)	Group D (N = 8)
$AUC_{0-\infty}$ [h*ng/mL]	Geo. mean	2190	3320	5070	1650
	95% CI	1660, 2900	2440, 4520	3470, 7400	1280, 2130
AUC_{0-t} [h*ng/mL]	Geo. mean	2050	3040	4010	1550
	95% CI	1530, 2740	2350, 3930	3020, 5330	1190, 2010
C_{max} [ng/mL]	Geo. mean	54.7	51.4	49.2	48.2
	95% CI	41.1, 72.7	42.0, 62.9	40.1, 60.2	41.6, 56.0
t_{max} [h]	Median	3.50	3.00	4.00	4.00
	Min-Max	2.00, 4.00	1.50, 4.00	1.00, 12.00	3.00, 6.00
$t_{½}$ [h]	Geo. mean	45.7	55.6	80.5	31.6
	95% CI	35.7, 58.5	37.0, 83.7	60.3, 107	26.1, 38.1

Group A: mild hepatic impairment (Child-Pugh score 5–6); Group B: moderate hepatic impairment (Child-Pugh score 7-9); Group C: severe hepatic impairment (Child-Pugh score 10–15); Group D: healthy subjects. Per-protocol set. Source of data: Table 26 to Table 32.

The geometric mean ratios of AUC, Cmax, and t½, or difference of the medians for tmax, between subjects with mild, moderate, or severe hepatic impairment and healthy subjects, and their 90% CI are displayed in the table below:

Parameter [unit]	Statistics	A vs D	B vs D	C vs D
$AUC_{0-\infty}$ [ng*h/mL]	Ratio of Geo. means	1.33	2.01	3.07
	90% CI of the ratio	1.00, 1.76	1.50, 2.71	2.19, 4.32
AUC_{0-t} [ng*h/mL]	Ratio of Geo. means	1.32	1.97	2.59
	90% CI	0.989, 1.77	1.50, 2.58	1.95, 3.46
C_{max} [ng/mL]	Ratio of Geo. means	1.13	1.07	1.02
	90% CI	0.892, 1.44	0.883, 1.28	0.845, 1.23
t_{max} [h]	Difference of medians	−1.00	−1.00	0.00
	90% CI	−2.00, 0.00	−2.00, 0.00	-1.00, 2.00
$t_{½}$ [h]	Ratio of Geo. means	1.45	1.76	2.55
	90% CI	1.15, 1.83	1.26, 2.46	1.97, 3.30

Group A: mild hepatic impairment (Child-Pugh score 5–6), N = 8; Group B: moderate hepatic impairment (Child-Pugh score 7–9), N = 8; Group C: severe hepatic impairment (Child-Pugh score 10–15), N = 8; Group D: healthy subjects, N = 8. Source of data: Table 92.

Ponesimod AUC0-∞ (geometric mean) in the mild, moderate, and severe hepatic impairment groups was 1.3-fold (1.0, 1.8), 2.0-fold (1.5, 2.7), and 3.1-fold (2.2, 4.3) greater, respectively, compared to healthy subjects. The elimination t½ (geometric mean) was 1.5-fold (90% CI: 1.2, 1.8), 1.8-fold (1.3, 2.5), and 2.6-fold (2.0, 3.3) greater, in the mild, moderate, and severe liver impairment groups, respectively, compared to healthy subjects.

No major differences in Cmax and tmax were observed between Groups A, B, or C and Group D.

Pharmacokinetics of metabolites M12 (ACT-204426) and M13 (ACT-338375)

The summary statistics of PK parameters for M12 and M13 by group (healthy or mild, moderate, and severe hepatic impairment) are presented in the Table below.

Parameter [unit]	Statistics	Group A	Group B	Group C	Group D
		M12			
*$AUC_{0-\infty}$ [ng*h/mL]	Geo. mean	244	801	1050[a]	188
	95% CI	183, 324	524, 1220	431, 2540	138, 255
AUC_{0-t} [ng*h/mL]	Geo. mean	106	536	999	99.0
	95% CI	67.4, 167	306, 941	363, 2750	67.2, 146
C_{max} [ng/mL]	Geo. mean	6.34	10.5	13.9	5.82
	95% CI	4.21, 9.55	5.95, 18.6	5.30, 36.3	4.34, 7.80
t_{max} [h]	Median	3.50	4.00	24.00	4.00
	Min-Max	3.00, 4.00	3.00, 24.00	3.00, 72.00	3.00, 8.00
$t_{½}$ [h]	Geo. mean	57.7	81.5	93.5[a]	38.0
	95% CI	33.6, 99.0	50.2, 132	78.0, 112	25.9, 55.7
		M13			
*$AUC_{0-\infty}$ [ng*h/mL]	Geo. mean	729[b]	996[b]	1250[a]	586
	95% CI	451, 1180	775, 1280	792, 1980	392, 875
AUC_{0-t} [ng*h/mL]	Geo. mean	259	360	850	482
	95% CI	87.5, 769	115, 1130	567, 1270	305, 760
C_{max} [ng/mL]	Geo. mean	5.53	5.45	8.34	8.90
	95% CI	2.59, 11.8	2.47, 12.0	5.73, 12.1	6.27, 12.6
t_{max} [h]	Median	24.0	24.0	24.0	24.0
	Min-Max	4.00, 72.0	24.0, 168	24.0, 168	4.00, 36.0
$t_{½}$ [h]	Geo. mean	76.0[b]	84.7[b]	110[a]	36.0
	95% CI	48.2, 120	61.7, 116	75.4, 161	26.8, 48.3

Group A: mild hepatic impairment (Child-Pugh score 5–6), N = 8; Group B: moderate hepatic impairment (Child-Pugh score 7–9), N = 8; Group C: severe hepatic impairment (Child-Pugh score 10–15), N = 8; Group D: healthy subjects, N=8; Per-protocol set. Source of data: Table 40–Table 60

* M12: AUC_{extra} > 20% in: Group A, N = 8; Group B, N = 5; Group C, N = 4; Group D, N = 8. M13: AUC_{extra} > 20% in: Group A, N = 4; Group B, N = 5; Group C, N = 5; Group D, N = 3.[a] N = 7 [b] N = 6

The differences between the PK parameters of M12 and M13 across the different groups were explored and the results of the statistical analysis are summarized in the Table below.

Hepatic impairment affected the PK of M12 and M13 compared to healthy subjects. For M12, the elimination t½ (geometric means) was approximately 1.5-fold (90% CI: 0.9, 2.5), 2.1-fold (1.4, 3.4), and 2.5-fold (1.8, 3.4) greater in the mild, moderate, and severe hepatic impairment groups respectively, compared to healthy subjects. The longer elimination t½ in subjects with hepatic impairment resulted in AUC0-∞ values 1.3-fold (1.0, 1.8), 4.3-fold (2.9, 6.3), and 5.6-fold (2.9, 10.6) greater, respectively, compared to healthy subjects.

For M13, the elimination t½ (geometric means) was approximately 2.1-fold (90% CI: 1.5, 3.1), 2.4-fold (1.7, 3.2), and 3.1-fold (2.2, 4.3) greater in the mild, moderate, and severe hepatic

impairment groups respectively, compared to healthy subjects. The longer elimination t½ in subjects with mild, moderate, and severe hepatic impairment resulted in AUC0-∞ values 1.2-fold (0.8, 2.0), 1.7-fold (1.2, 2.5), and 2.1-fold (1.4, 3.4) greater, respectively, compared to healthy subjects.

		M12			M13		
Parameter [unit]	**Statistics**	**A vs D**	**B vs D**	**C[a] vs D**	**A[b] vs D**	**B[b] vs D**	**C[a] vs D**
$AUC_{0-\infty}$ [ng*h/mL]	Ratio of Geo. means	1.30	4.27	5.57	1.24	1.70	2.14
	90% CI of the ratio	0.95, 1.78	2.89, 6.30	2.92, 10.6	0.80, 1.96	1.16, 2.50	1.37, 3.35
AUC_{0-t} [ng*h/mL]	Ratio of Geo. means	1.07	5.42	10.1	0.54	0.75	1.76
	90% CI of the ratio	0.69, 1.67	3.26, 9.01	4.50, 22.6	0.22, 1.30	0.30, 1.87	1.12, 2.78
C_{max} [ng/mL]	Ratio of Geo. means	1.09	1.81	2.38	0.62	0.61	0.94
	90% CI of the ratio	0.75, 1.59	1.12, 2.92	1.13, 5.04	0.33, 1.16	0.32, 1.17	0.64, 1.37
t_{max} [h]	Median	−0.50	0.00	20.0	0.00	12.00	12.00
	90% CI of the median	−1.00, 0.00	−1.00, 4.00	0.00, 20.0	0.00, 12.00	0.00, 36.00	0.00, 48.00
$t_{½}$ [h]	Ratio of Geo. means	1.52	2.14	2.46	2.11	2.35	3.06
	90% CI of the ratio	0.93, 2.49	1.35, 3.40	1.77, 3.43	1.45, 3.07	1.71, 3.24	2.16, 4.33

Group A: mild hepatic impairment (Child-Pugh score 5–6), N = 8; Group B: moderate hepatic impairment (Child-Pugh score 7–9), N = 8; Group C: severe hepatic impairment (Child-Pugh score 10–15), N = 8; Group D: healthy subjects, N = 8; Per-protocol set.
* M12: AUC_{extra} > 20% in: Group A, N = 7; Group B, N = 5; Group C, N = 4; Group D, N = 8. M13: AUC_{extra} > 20% in: Group A, N = 4; Group B, N = 5; Group C, N = 6; Group D, N = 3.[a] $AUC_{0-\infty}$ and $t_{½}$ N = 7 [b] $AUC_{0-\infty}$ and $t_{½}$ N = 6.
Source of data: Table 92

Pharmacodynamics

Following administration of a single dose of 10 mg ponesimod, the maximum mean reduction from baseline in lymphocyte count in healthy subjects was 32%, reached 6 h post-dose. In the mild, moderate, and severe hepatic impairment groups, the greatest mean lymphocyte count reductions observed were 38% (at 6 h post-dose), 34% (at 6 h post-dose), and 23% (at 10 h post-dose), respectively.

Safety

In general, a single oral dose of 10 mg ponesimod was well tolerated. No new or unexpected safety findings were reported.

CONCLUSIONS

- AUC and t½ of ponesimod and its metabolites were increased in subjects with hepatic impairment compared to healthy subjects.
- Based on the PK results of this study, dose adjustment for subjects with moderate and severe hepatic impairment should be considered.
- Single-dose treatment with 10 mg of ponesimod was generally well tolerated.

Study AC-058-113 Single-center, open-label, single-dose Phase 1 study to investigate the pharmacokinetics, safety, and tolerability of ponesimod in subjects with moderate or severe renal function impairment.

Objectives: To compare the pharmacokinetics (PK) of ponesimod and its metabolites ACT-204426 (M12) and ACT-338375 (M13) after a single 10 mg oral dose in subjects with moderate or severe renal function impairment with those in healthy subjects.

A brief overview of some essential components of the study design is given below:

Study Design	Single-center, open-label, single-dose Phase 1 study
Study Population	A total of 24 subjects, male and female, 8 in each group: • Group A: 8 subjects with moderate renal function impairment • Group B: 8 subjects with severe renal function impairment • Group C: 8 healthy subjects.
Dosage and Administration	Ponesimod (ACT-128800) - 10 mg tablet. Single oral dose on Day 1 in fasted condition. Batch No.: 12AIMP014; Retest date: (b) (4)
PK Sampling:	Blood: pre-dose, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 168, (for Gourp C, healthy subject) 216 and 264 hours post dose. Blood (for protein binding): 3, and 12 hours post dose.
PK Assessment	The following PK parameters of ponesimod and its metabolites were determined using non-compartmental method: Cmax, Tmax, AUC0-t, AUCinf, t1/2, Ratio of free (unbound, Cu) to total (C) plasma concentrations (Cu/C) of ponesimod, M12, and M13, at 3 and 12 h post-dose.
PD Assessment	Absolute lymphocyte counts, absolute change from baseline, and percentage change from baseline at each time point of assessment
Safety Assessment	AEs, SAEs, Vital signs (supine blood pressure [BP] and HR), ECG, Clinical laboratory tests (hematology, clinical chemistry, and coagulation variables), urinalysis, eCLcr, body weight.

RESULTS

Pharmacokinetics of ponesimod

Descriptive statistics of ponesimod PK parameters, summarized by group, are displayed in the following tables:

Parameter [Unit]		Group A (n = 8)	Group B (n = 8)	Group C (n = 8)
C_{max} [ng/mL]	Geo. Mean	46.8	54.5	50.0
	95% CI	36.6, 59.9	49.4, 60.1	38.2, 65.4
t_{max} [h]	Median	3.00	4.00	3.50
	Min, Max	2.00, 12.00	2.00, 4.00	3.00, 10.00
AUC_{0-t} [ng·h/mL]	Geo. Mean	1486	1697	1435
	95% CI	1311, 1684	1183, 2432	1126, 1828
$AUC_{0-\infty}$ [ng·h/mL]	Geo. Mean	1587	1773	1554
	95% CI	1395, 1804	1242, 2533	1206, 2002
$t_{1/2}$ [h]	Geo. Mean	34.4	27.9	26.4
	95% CI	27.3, 43.4	19.4, 40.1	21.7, 32.1

Group A: moderate renal function impairment, Group B: severe renal function impairment, Group C: healthy subjects. Source of data: Table 26–Table 32.

$AUC_{0-\infty}$ = area under plasma concentration-time curve from zero to infinity; AUC_{0-t} = area under plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification; CI = confidence interval; C_{max} = maximum plasma concentration; $t_{1/2}$ = time to reach maximum plasma concentration; t_{max} = time to reach maximum plasma concentration

The geometric mean ratios of Cmax, AUC, and t½, or difference of the medians for tmax, between subjects with moderate or severe renal function impairment and healthy subjects, and their 90% CI are in the table below:

Parameter [Unit]	Statistics	A vs C	B vs C
C_{max}	Ratio of Geo. means	0.94	1.09
	90% CI	0.71, 1.23	0.88, 1.35
t_{max} [h]	Difference of medians	−0.50	0.00
	90% CI	−2.00, 1.00	−6.00, 1.00
AUC_{0-t}	Ratio of Geo. means	1.04	1.18
	90% CI	0.85, 1.27	0.86, 1.63
$AUC_{0-\infty}$	Ratio of Geo. means	1.02	1.14
	90% CI	0.83, 1.26	0.82, 1.58
$t_{1/2}$	Ratio of Geo. means	1.30	1.06
	90% CI	1.04, 1.63	0.78, 1.44

Group A: moderate renal impairment (n = 8), Group B: severe renal impairment (n = 8), Group C: healthy subjects (n = 8). Source of data: Table 47.

$AUC_{0-\infty}$ = area under plasma concentration-time curve from zero to infinity; AUC_{0-t} = area under plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification; CI = confidence interval; C_{max} = maximum plasma concentration; $t_{1/2}$ = time to reach maximum plasma concentration; t_{max} = time to reach maximum plasma concentration

No clinically relevant differences in Cmax, tmax, AUC and t1/2 were observed between Groups A, B and C.

Pharmacokinetics of metabolites M12 (ACT-204426) and M13 (ACT-338375)

The summary statistics of PK parameters for M12 and M13 by group (healthy or mild, moderate, and severe hepatic impairment) are presented in the Table below.

Parameter [Unit]		Group A (n = 8)	Group B (n = 8)	Group C (n = 8)
		M12 (ACT-204426)		
C_{max} [ng/ml]	Geo. Mean	4.9	6.2	5.2
	95% CI	3.4, 6.9	4.6, 8.5	3.6, 7.4
t_{max} [h]	Median	3.00	4.00	4.00
	Min, Max	2.00, 24.00	2.00, 4.00	3.00, 10.00
AUC_{0-t} [ng·h/ml]	Geo. Mean	96	152	83
	95% CI	61, 150	114, 203	56, 125
$AUC_{0-\infty}$ [ng·h/ml]	Geo. Mean	207[1]	278	200
	95% CI	138, 310	212, 365	119, 335
$t_{1/2}$ [h]	Geo. Mean	35.7[1]	45.2	47.9
	95% CI	15.5, 82.3	20.6, 99.0	23.4, 97.8
		M13 (ACT-338375)		
C_{max} [ng/ml]	Geo. Mean	7.8	12.8	8.5
	95% CI	5.7, 10.7	9.9, 16.4	6.0, 12.2
t_{max} [h]	Median	24.00	24.00	17.00
	Min, Max	4.00, 48.00	24.00, 48.00	3.00, 24.00
AUC_{0-t} [ng·h/ml]	Geo. Mean	485	839	451
	95% CI	308, 764	645, 1092	253, 804
$AUC_{0-\infty}$ [ng·h/ml]	Geo. Mean	585	925	658
	95% CI	403, 851	706, 1210	362, 1196
$t_{1/2}$ [h]	Geo. Mean	40.0	31.2	45.7
	95% CI	30.4, 52.6	22.4, 43.4	20.3, 102.9

Group A: moderate renal impairment, Group B: severe renal impairment, Group C: healthy subjects. [1]: n = 6 Source of data: Source data: Table 33–Table 46

$AUC_{0-\infty}$ = area under plasma concentration-time curve from zero to infinity; AUC_{0-t} = area under plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification; CI = confidence interval; C_{max} = maximum plasma concentration; $t_{1/2}$ = time to reach maximum plasma concentration; t_{max} = time to reach maximum plasma concentration

The differences between the PK parameters of M12 and M13 across the different groups were explored.

For M12, the ratio of geometric means of t½ was 0.75 (90% CI: 0.34, 1.66), and 0.95 (90% CI: 0.43, 2.08) for the comparison Group A:C and Group B:C, respectively. The ratio of geometric mean of AUC0-∞ was 1.04 (90% CI: 0.62, 1.73), and 1.39 (90% CI: 0.90, 2.15) for the comparison Group A:C and Group B:C, respectively.

For M13, the ratio of geometric means of t½ was 0.88 (90% CI: 0.46, 1.66) and 0.68 (90% CI: 0.35, 1.31) for the comparison Group A:C, and Group B:C, respectively. The ratio of geometric mean of AUC0-∞ was 0.89 (90% CI: 0.53, 1.50), and 1.41 (90% CI: 0.86, 2.29) for the comparison Group A:C, and Group B:C, respectively. (see table below)

Parameter [Unit]	Statistics	A vs C	B vs C
		M12 (ACT-204426)	
C_{max} [ng/ml]	Ratio of Geo. means	0.94	1.20
	90% CI	0.64, 1.37	0.84, 1.71
t_{max} [h]	Difference of medians	−1.00	0.00
	90% CI	−1.00, 1.00	−1.00, 1.00
AUC_{0-t} [ng·h/ml]	Ratio of Geo. means	1.15	1.83
	90% CI	0.73, 1.81	1.26, 2.65
$AUC_{0-\infty}$ [ng·h/ml]	Ratio of Geo. means	1.04	1.39
	90% CI	0.62, 1.73	0.90, 2.15
$t_{1/2}$ [h]	Ratio of Geo. means	0.75	0.95
	90% CI	0.34, 1.66	0.43, 2.08
		M13 (ACT-338375)	
C_{max} [ng/ml]	Ratio of Geo. means	0.92	1.50
	90% CI	0.64, 1.31	1.08, 2.08
t_{max} [h]	Difference of medians	14.00	20.00
	90% CI	0.00, 24.00	0.00, 24.00
AUC_{0-t} [ng·h/ml]	Ratio of Geo. means	1.08	1.86
	90% CI	0.62, 1.86	1.16, 2.99
$AUC_{0-\infty}$ [ng·h/ml]	Ratio of Geo. means	0.89	1.41
	90% CI	0.53, 1.50	0.86, 2.29
$t_{1/2}$ [h]	Ratio of Geo. means	0.88	0.68
	90% CI	0.46, 1.66	0.35, 1.31

Group A: moderate renal impairment, Group B: severe renal impairment, Group C: healthy subjects
Source of data: Table 47

$AUC_{0-\infty}$ = area under plasma concentration-time curve from zero to infinity; AUC_{0-t} = area under plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification; CI = confidence interval; C_{max} = maximum plasma concentration; $t_{1/2}$ = time to reach maximum plasma concentration; t_{max} = time to reach maximum plasma concentration

Pharmacodynamics

Mean lymphocyte count decreased from baseline in the 3 groups, without major differences among the different groups. The greatest mean percentage decreases from baseline in lymphocyte count were:

- Group A: 36.4% (from mean absolute value of 1.90 × 10^9/L at baseline to 1.21 × 10^9/L at 6 h post-dose)

- Group B: 45.1% (from mean absolute value of 1.84 × 10^9/L at baseline to 1.02 × 10^9/L at 6 h post-dose)

- Group C: 30.9% (from mean absolute value of 1.71 × 10^9/L at baseline to 1.17 × 10^9/L at 6 h post-dose).

The mean absolute lymphocyte count returned to baseline values within 2 days post-dose. Overall, the lymphocyte effects observed were consistent with previous studies with single doses of 10 mg ponesimod.

Safety

A single oral dose of 10 mg ponesimod was well tolerated.

CONCLUSIONS

- PK characteristics (Cmax, AUC0-∞, and t½) of ponesimod and M12 were not significantly increased in subjects with renal function impairment compared to healthy subjects. In subjects with severe renal impairment, systemic exposure to M13 was increased in a non-clinically significant manner as compared to subjects with normal renal function.
- Based on the PK results, dose adjustment is not needed in subjects with moderate or severe renal function impairment.
- No new or unexpected safety findings were reported. Single-dose treatment with 10 mg ponesimod was generally well tolerated.

Study AC-058-107: Single-center, open-label, parallel-group study to evaluate the pharmacokinetics, tolerability, and safety of a single dose of 40 mg ACT-128800 in Japanese and Caucasian healthy male and female subjects.

Objectives: To assess the relative pharmacokinetic (PK) properties of ACT-128800 in Japanese vs. Caucasian healthy male and female subjects after single-dose treatment.

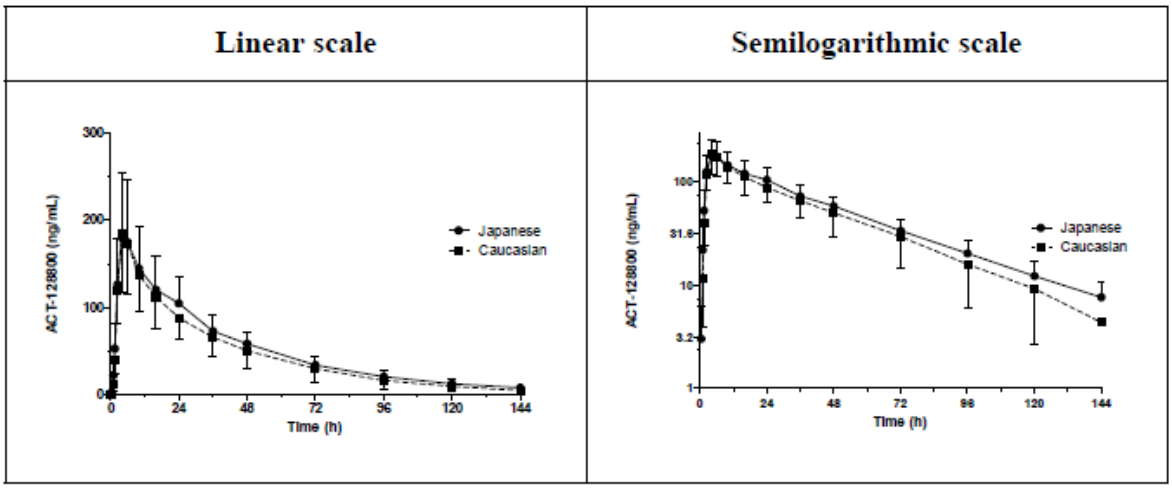
A brief overview of some essential components of the study design is given below:

Study Design	Single-center, open-label, parallel-group study
Study Population	10 Japanese and 10 Caucasian healthy male and female subjects (1:1 sex ratio), and matched for body weight
Dosage and Administration	A single, oral dose of 40 mg ACT-128800 (b) (4) capsule. Batch number PD07073 (BA1022716, expiry: August 2009).
PK Sampling:	pre-dose, 30, 60, 90, 150 mins, 4, 6, 10, 16, 24, 36, 48, 72, 96, 120, 144 hours post dose.
Analysis (Plasma)	Method: LC/MS/MS LLOQ: 1.00 ng/mL Linear range: 1.00-2000 ng/mL Inter-day Precision (%CV): 1.2-10.9% Inter-day accuracy: -10.5-11.0 %
PK Assessment	Cmax, tmax, AUC0-t, AUC0-inf, and t½ of ACT-128800 in both ethnic groups.
PD Assessment	Change from baseline to each measurement time point for lymphocyte counts
Safety Assessment	AEs, SAEs, Vital signs, Laboratory assessments, Pulmonary function tests, ECG, Physical examinations.

RESULTS

Pharmacokinetic (PK) results

The mean concentration time-profiles plotted on linear and logarithmic scales are shown in the Figure below:



PK parameters derived by non-compartmental analysis of the plasma concentration-time data for ACT-128800 are summarized by ethnic group in the following Table.

Ethnicity	Statistic	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-t} (ng·h/mL)	AUC_{0-inf} (ng·h/mL)	$t_{1/2}$ (h)
Caucasian	N	10	10	10	10	10
	Mean	194	4.2	6394	6719	28.9
	SD	65.5	0.63	2229	2431	7.3
	SE	20.7	0.20	705	769	2.3
	CV%	34	15	35	36	25
	95% CI of the Mean	147 , 241	3.7 , 4.7	4799 , 7989	4980 , 8458	23.6 , 34.1
	Median	184	4.0	5816	6060	29.1
	Min, Max	126 , 339	4.0 , 6.0	3930 , 11123	4021 , 11561	19.0 , 42.2
	Geo mean	185	4.2	6080	6353	28.1
	CV%	32	13	34	36	26
	95% CI of the Geo mean	147 , 232	3.8 , 4.6	4807 , 7691	4950 , 8154	23.4 , 33.6
Japanese	N	10	10	10	10	10
	Mean	196	4.3	7250	7627	33.1
	SD	76.9	1.3	2058	2167	4.1
	SE	24.3	0.42	651	685	1.3
	CV%	39	31	28	28	12
	95% CI of the Mean	141 , 251	3.4 , 5.2	5778 , 8722	6077 , 9177	30.2 , 36.0
	Median	188	4.0	6869	7116	34.0
	Min, Max	107 , 317	2.5 , 6.0	5194 , 11289	5399 , 11866	26.2 , 40.6
	Geo mean	182	4.1	7006	7368	32.9
	CV%	43	33	28	28	13
	95% CI of the Geo mean	136 , 244	3.3 , 5.2	5768 , 8510	6059 , 8962	30.1 , 36.0

The estimated geometric mean ratio and median difference (Japanese vs. Caucasian) with their 90% CIs for PK parameters of ACT-128800 are summarized. Exposure to ACT-128800 (AUC0-inf, AUC0-t) was slightly higher in Japanese subjects compared to the Caucasian subjects. (See Table below)

Comparison	Statistic	C_{max}	t_{max}	AUC_{0-t}	AUC_{0-Inf}	$t_{1/2}$
Japanese vs Caucasian	Ratio of geometric means	0.99		1.15	1.16	1.17
	90% confidence interval	0.74 , 1.31		0.91 , 1.46	0.91 , 1.48	1.00 , 1.37
	Median difference		1.0			
	90% confidence interval		-1.5 , 2.0			

Pharmacodynamics

The pharmacodynamic effect of ACT-128800 on lymphocytes (transient decrease in total lymphocyte counts) was similar in the two ethnic groups.

Safety

No SAEs were reported and no subject discontinued the study due to an AE. Frequently observed AEs were headache, bradycardia, and dizziness. The AE profile was similar in Caucasian and Japanese subjects, and in males and females. The reported AEs were in accordance with the known AE profile of ACT-128800 and no unexpected AE was reported in either ethnic group.

CONCLUSIONS

Overall, the PK profile of ACT-128800 was similar in Japanese and Caucasian subjects. The observed safety profile was also similar between the two ethnic groups and no unexpected safety findings were reported.

4.4-3. HUMAN PK STUDIES

4.4-3.3 Extrinsic Factors

Study AC-058-117: A Randomized, Double-blind, Parallel group, 2-period, Placebo-controlled, Phase 1 Study to Investigate the Effects on Heart Rate, Blood Pressure, and Pharmacokinetic Interactions of the Up-titration Regimen of Ponesimod in Healthy Adult Subjects Receiving Propranolol at Steady State

A brief overview of some essential components of the study design is given below:

Study Design	Randomized, double-blind, parallel-group, 2-period, placebo-controlled study
Study Population	52 subjects were enrolled Age: 23 - 54 years (mean 47 years) Gender: 24 female (51.1%), 23 male (48.9%) Weight: 49.90 - 91.40 kg (mean 72.69 kg) Race: 43 White (91.5%), 2 Asian (4.3%), 2 African American (4.3%)
Dosage and Administration	<u>Treatment Period 1</u> Subjects received a single dose of 2 mg ponesimod oral tablet under fed conditions. <u>Treatment Period 2</u> Treatment A: up-titration regimen of ponesimod once daily administered as oral tablets from Day 5 to Day 19 + placebo propranolol once daily, administered as a placebo oral capsule from Day 1 to Day 19. Treatment B: up-titration* regimen of ponesimod once daily administered as oral tablets from Day 5 to Day 19 + 80 mg propranolol once daily, administered as an 80-mg long-acting oral capsule from Day 1 to Day 19. Study drugs were taken orally, together with approximately 240 mL of noncarbonated water, under fed conditions.
PK Assessment	Ponesimod (Treatments A and B) Day 5: Cmax, tmax, and AUC24h Day 19: Ctrough, Cmax, tmax, and AUCτ

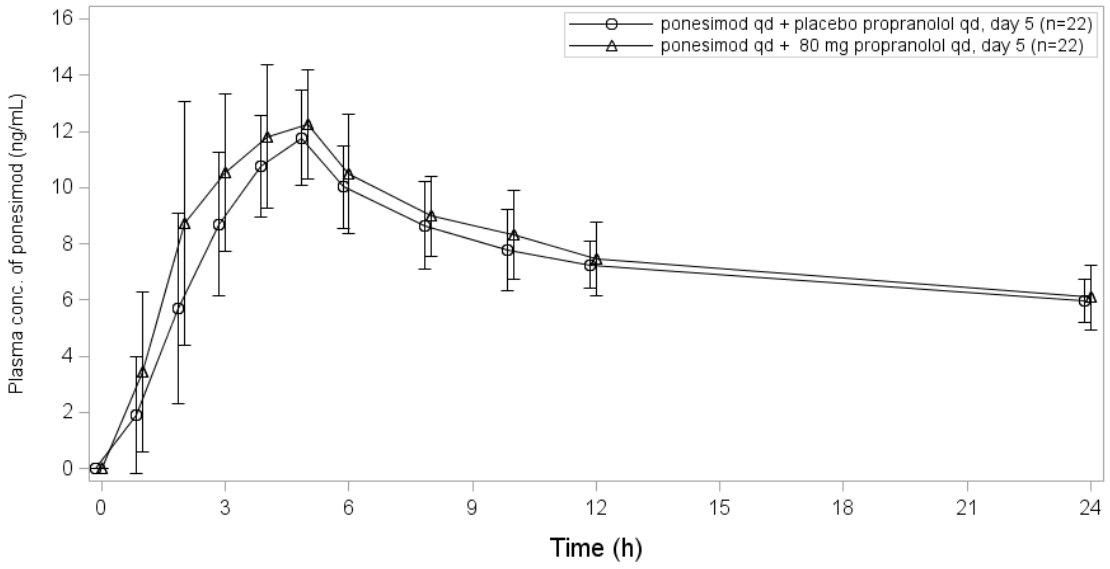
	Propranolol and 4-hydroxypropranolol (Treatment B only) Day 4 and Day 5: Ctrough, Cmax, tmax, AUCτ, and CLss/F* Day 19: Ctrough, Cmax, tmax, AUCτ, and CLss/F* * CLss/F was not determined for 4-hydroxypropranolol
PK Assessment	Heart rate and bradyarrhythmias were evaluated from the Holter ECG, lymphocyte count assessment.
Safety Assessment	AE, Clinical laboratory tests, 12-lead safety ECG (including telemetry), Vital signs, Physical examination, Ophthalmological examination, Neurological examination, PFTs, Specific toxicities, Incidence of adverse events of special interest.

RESULTS

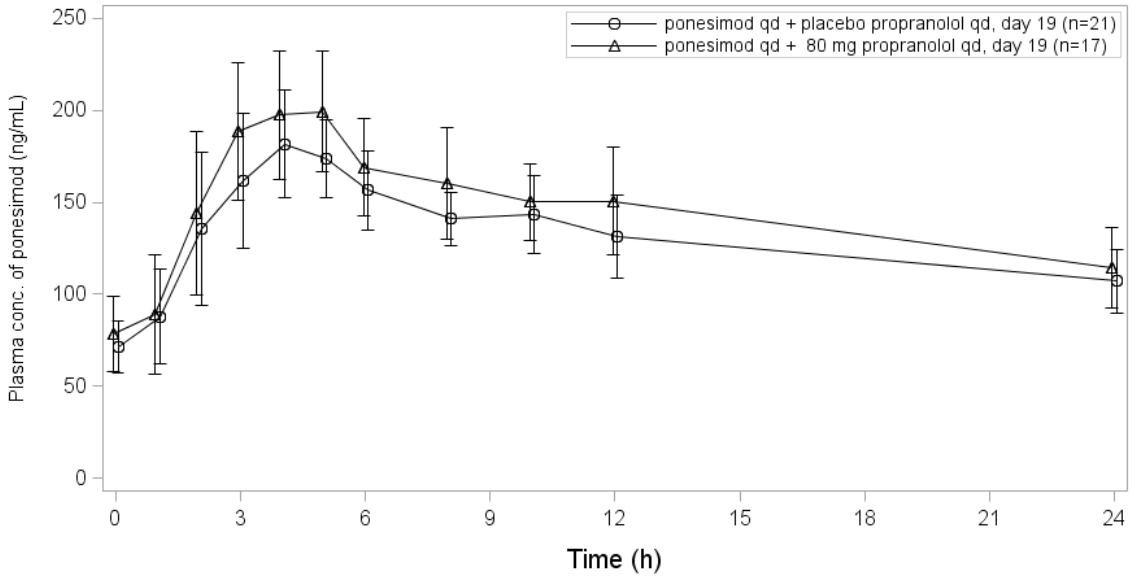
Pharmacokinetics of Ponesimod

The linear mean plasma concentration-time profiles of ponesimod of both treatments (Treatment A and Treatment B) are presented in the following figures:

Mean (SD) Plasma Concentration-Time Profiles of Ponesimod After Administration of an Up-titration Regimen of Ponesimod Alone Once Daily From Day 5 to Day 19 (Treatment A) and in Combination With Propranolol at 80 mg Once Daily From Day 1 to Day 19 (Treatment B), Day 5 (2 mg Ponesimod)



Mean (SD) Plasma Concentration-Time Profiles of Ponesimod After Administration of an Up-titration Regimen of Ponesimod Alone Once Daily From Day 5 to Day 19 (Treatment A) and in Combination With Propranolol at 80 mg Once Daily From Day 1 to Day 19 (Treatment B), Day 19 (20 mg Ponesimod)



On both Day 5 and Day 19, mean ponesimod concentrations were similar over the complete plasma concentration-time profile when ponesimod was administered alone and in combination with propranolol.

A summary list of key PK parameters of ponesimod for both treatments is presented in the Table below:

Pharmacokinetic Results of Ponesimod After Administration of an Up-titration Regimen of Ponesimod Once Daily From Day 5 to Day 19 Alone (Treatment A) and in Combination With Propranolol at 80 mg Once Daily From Day 1 to Day 19 (Treatment B) (Study AC-058-117: Pharmacokinetics Data Analysis Set)

Pharmacokinetics of ponesimod (mean [SD], t_{max}: median [range])	**Treatment A ponesimod alone**	**Treatment B ponesimod + propranolol**
Day 5 (2 mg ponesimod)		
n	22	22[a]
C_{max} (ng/mL)	12.3 (1.65)	12.9 (2.31)
t_{max} (h)	5.00 (2.00 – 8.00)	5.00 (2.00 – 5.07)
AUC_{24h} (ng.h/mL)	173 (16.4)	185 (29.6)
Day 19 (20 mg ponesimod)		
n	21	17
C_{trough} (ng/mL)	71.4 (13.9)	78.7 (20.6)
C_{max} (ng/mL)	188 (24.2)	211 (35.1)
t_{max} (h)	4.00 (3.00 – 5.00)	4.00 (3.00 – 5.00)
AUC_{τ} (ng.h/mL)	3126 (450)	3448 (534)

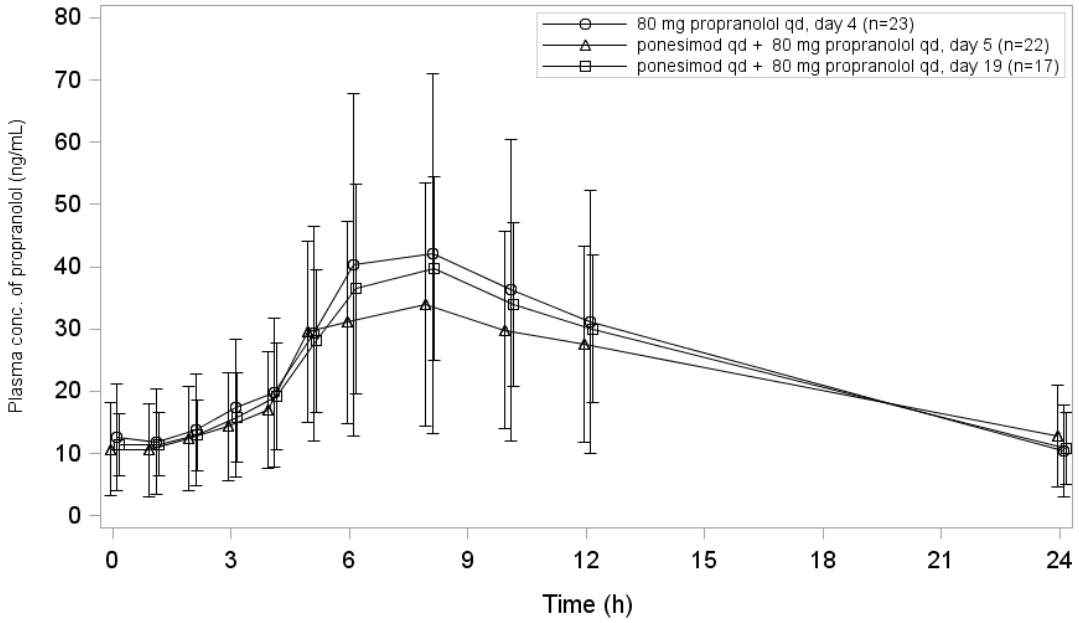
[a] n=21 for AUC_{24h}

Based on the geometric mean ratios (GMRs), on Day 5 ponesimod Cmax and AUC24h were similar when ponesimod was administered in the presence of propranolol (Treatment B) as compared to administration of ponesimod alone (Treatment A), as 100% was included in the 90% CI of the GMR. On Day 19, the GMRs of Treatment B versus Treatment A for ponesimod Cmax and AUCτ were 111% (90% CI: 102.98 – 120.48) and 110% (90% CI: 101.75 – 119.32), respectively.

Pharmacokinetics of Propranolol

The linear mean plasma concentration-time profiles of propranolol on Day 4, Day 5, and Day 19 are presented in the Figure below:

Mean (SD) Plasma Concentration-Time Profiles of Propranolol After Administration of an Up-titration Regimen of Ponesimod Once Daily From Day 5 to Day 19 in Combination With Propranolol at 80 mg Once Daily From Day 1 to Day 19 (Treatment B); Days 4 (Propranolol Alone), Day 5 (Propranolol With 2 mg Ponesimod), and 19 (Propranolol With 20 mg Ponesimod)



Mean propranolol concentrations were similar over the complete plasma concentration-time profile in the absence of ponesimod (ie, on Day 4) and in the presence of 2 mg or 20 mg ponesimod (ie, on Day 5 and Day 19), respectively.

Individual PK parameters of propranolol, including descriptive statistics, are presented in the table below:

Pharmacokinetic Results of Propranolol After Administration of an Up-titration Regimen of Ponesimod Once Daily From Day 5 to Day 19 in Combination With Propranolol at 80 mg Once Daily From Day 1 to Day 19 (Treatment B)

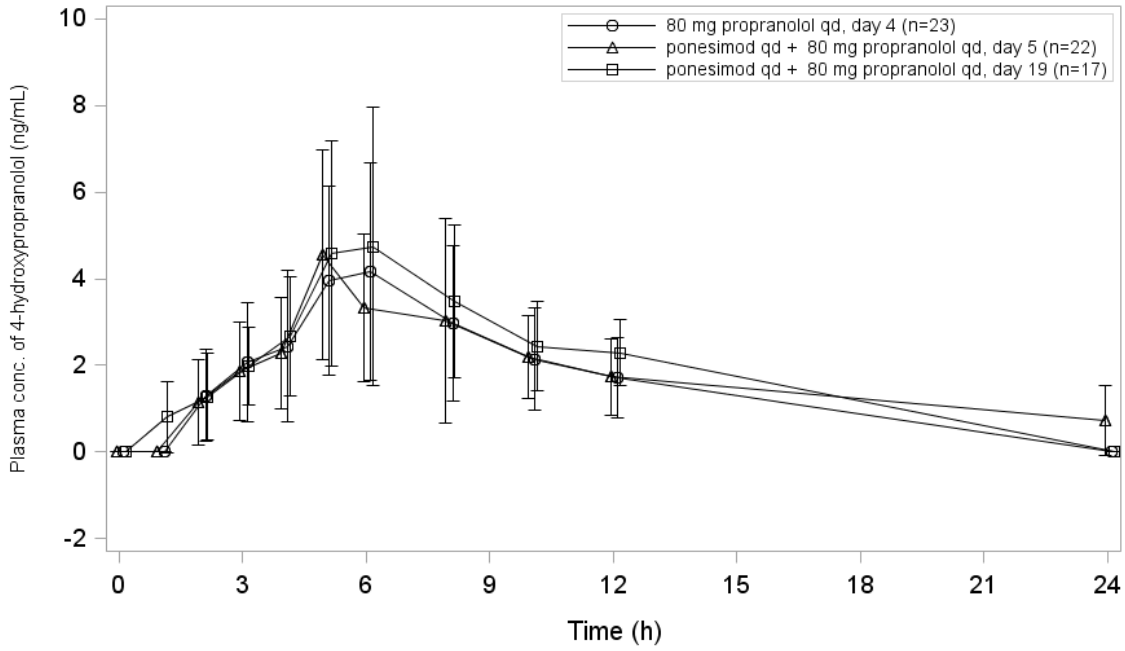
Pharmacokinetics of propranolol (mean [SD], t_{max}: median [range])	Treatment B		
	propranolol alone (Day 4)	propranolol + 2 mg ponesimod (Day 5)	propranolol + 20 mg ponesimod (Day 19)
n	23	22	17
C_{trough} (ng/mL)	12.6 (8.60)	10.7 (7.52)	11.5 (5.00)
C_{max} (ng/mL)	44.3 (29.5)	36.5 (18.8)	41.6 (16.9)
t_{max} (h)	8.00 (5.00 – 12.00)	6.18 (4.98 – 12.00)	8.00 (4.98 – 10.00)
AUC_{τ} (ng.h/mL)	583 (370)	534 (290)	566 (211)
CL_{ss}/F (L/h)	191 (162)	180 (127)	154 (98.1)

Based on the GMRs and 90% CI, propranolol Cmax and AUCτ can be concluded to be similar on Day 5 (propranolol coadministered with 2 mg ponesimod), and Day 19 (propranolol coadministered with 20 mg ponesimod) as compared to Day 4 (propranolol alone), as 100% was included in the 90% CI of the GMR.

Pharmacokinetics of 4-Hydroxypropranolol

The linear mean plasma concentration-time profiles of 4-hydroxypropranolol on Day 4, Day 5, and Day 19 are presented in the Figure below:

Mean (SD) Plasma Concentration-Time Profiles of 4-Hydroxypropranolol After Administration of an Up-titration Regimen of Ponesimod Once Daily From Day 5 to Day 19 in Combination With Propranolol at 80 mg Once Daily From Day 1 to Day 19 (Treatment B), Day 4 (Propranolol Alone), Day 5 (Propranolol With 2 mg Ponesimod), and Day 19 (Propranolol With 20 mg Ponesimod)



Mean 4-hydroxypropranolol concentrations were similar over the complete plasma concentration-time profile in the absence of ponesimod (ie, on Day 4) and in the presence of 2 or 20 mg ponesimod (on Day 5 and Day 19, respectively).

A summary list of key PK parameters of 4-hydroxypropranolol is presented in the Table below:

Pharmacokinetic Results of 4-Hydroxypropranolol After Administration of an Up-titration Regimen of Ponesimod Once Daily From Day 5 to Day 19 in Combination With Propranolol at 80 mg Once Daily From Day 1 to Day 19 (Treatment B)

Pharmacokinetics of 4-hydroxypropranolol (mean [SD], t_{max}: median [range])	Treatment B		
	propranolol alone (Day 4)	propranolol + 2 mg ponesimod (Day 5)	propranolol + 20 mg ponesimod (Day 19)
n	23	22	17
C_{trough} (ng/mL)	BQL (-)	BQL (-)	BQL (-)
C_{max} (ng/mL)	4.65 (2.42)	4.85 (2.82)	5.28 (3.39)
t_{max} (h)	6.00 (3.00 – 10.00)	5.00 (3.00 – 10.00)	5.98 (3.98 – 10.05)
AUC_{τ} (ng.h/mL)	41.4 (22.2)	42.8 (22.2)	49.6 (20.9)

BQL = below quantification limit

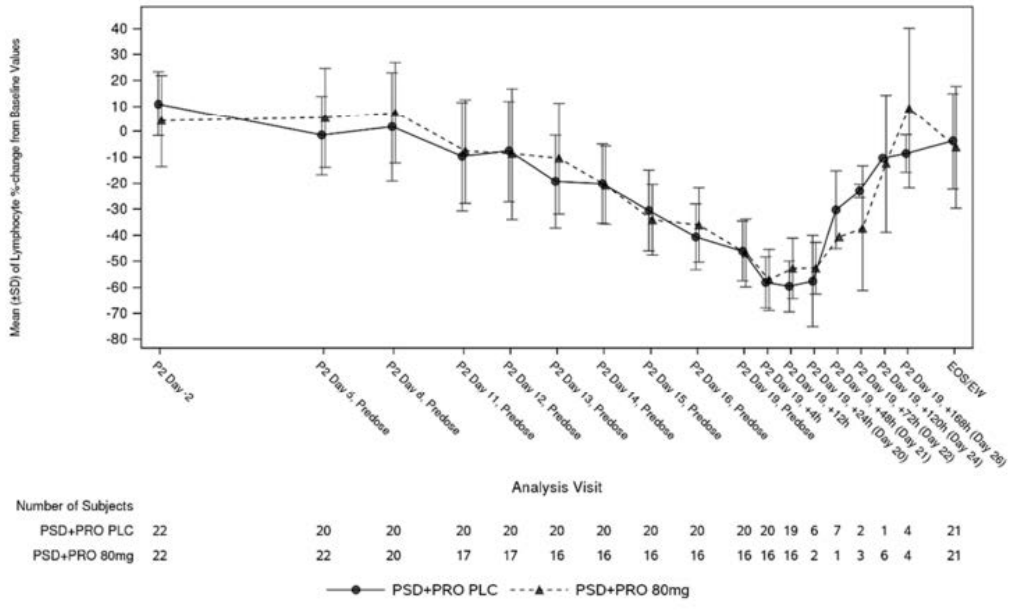
Based on the GMR and 90% CI, 4-hydroxypropranolol Cmax and AUC□ can be concluded to be similar on Day 5 (propranolol coadministered with 2 mg ponesimod), and Day 19 (propranolol coadministered with 20 mg ponesimod) as compared to Day 4 (propranolol alone), as 100% was included in the 90% CI of the GMR.

Pharmacodynamics

Effect on Lymphocytes Counts

Treatment with ponesimod resulted in a decrease in circulating lymphocytes. This decrease was similar during Treatment A and Treatment B (see Figure below).

Mean (SD) of Lymphocyte Count Percentage Change From Baseline Values by Treatment During Period 2; PD Analysis Set

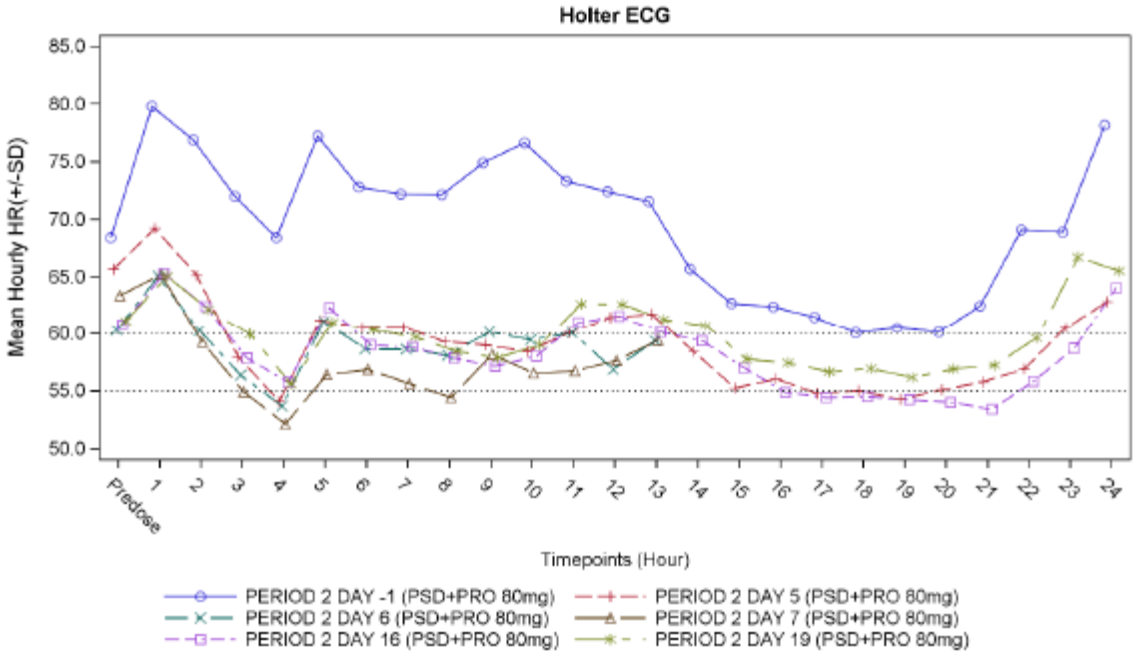


Effects on Heart Rate (HR)

Combination of ponesimod with propranolol: Treatment A (Ponesimod only) vs Treatment B (Ponesimod + propranolol)

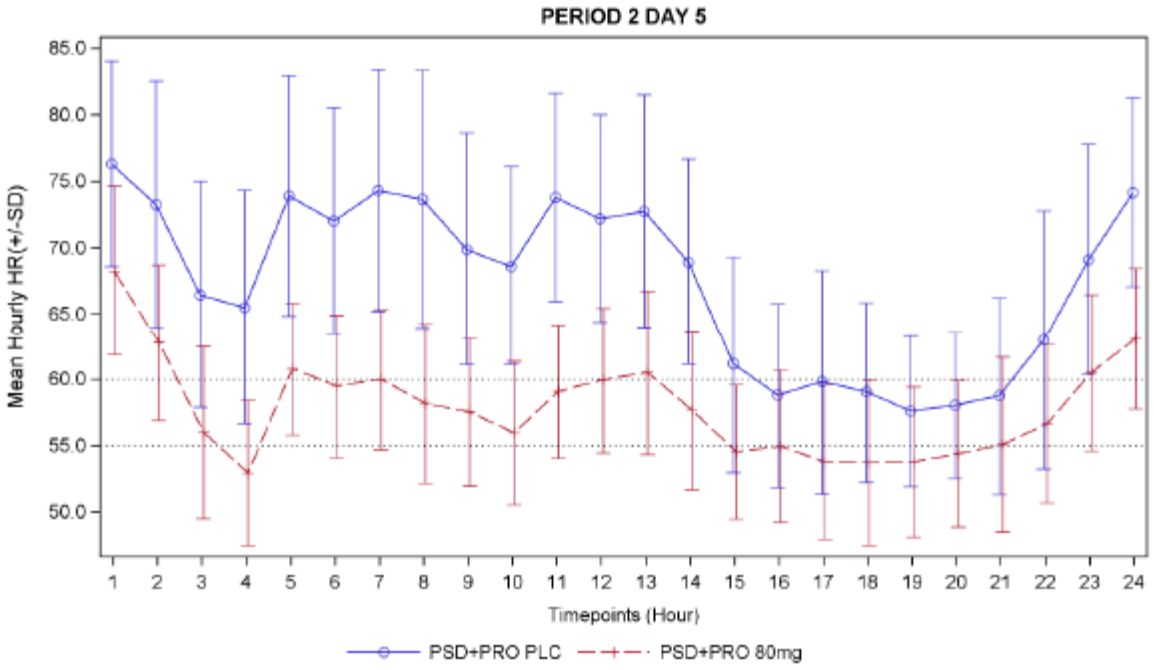
A graphical representation of the mean of the mean hourly HR over time for baseline (Treatment Period 2 Day -1), and ponesimod in combination with propranolol in Treatment B (Day 5, Day 6, Day 7, Day 16, and Day 19) is presented in the Figure below:

Mean of Mean Hourly HR Over Time for Treatment B with Baseline and Repeated Doses of Ponesimod in Combination with Propranolol: Period 2 Day -1, Period 2 Day 5, Day 6, Day 7, Day 16, and Day 19



The combination of propranolol with the first dose of 2 mg ponesimod on Day 5 in Treatment B gave a larger reduction in mean of the mean hourly HR than ponesimod alone (Treatment A). For Treatment B, the initial maximum reduction of the mean of the mean hourly HR of 16.0 bpm was observed at 5 hours postdose followed by another decrease of 20.1 bpm at 10 hours whereas for Treatment A, the single dose of 2 mg ponesimod alone on Day 5 produced an initial maximum decrease in mean of the mean hourly HR of 6.1 bpm at 3 hours postdose followed by another decrease of 6.6 bpm at 10 hours. The lowest value of the mean of the mean hourly HR for both treatments was observed at 4 hours postdose, with a value of 53.0 bpm for Treatment B and 65.5 bpm for Treatment A. The minimum value for mean hourly HR at 4 hours was 53 bpm for Treatment A and 44 bpm for Treatment B. A comparison of the mean hourly HR between Treatment A and Treatment B on Day 5 is present in the Figure below:

Mean of Mean hourly HR with SD over time for Day 5 of Treatment Period 2



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For the mean maximum decrease from time-matched baseline in mean hourly HR, (mean Emax HR over 12 hours), a comparison of Treatment B (combination of ponesimod + propranolol) versus Treatment A (ponesimod alone), showed that the combination exhibited the largest mean difference in Emax HR on Day 5 (after the first dose of ponesimod 2 mg) of -12.4 bpm (90% CI -15.61 to -9.14 bpm).

<u>Combination of ponesimod with propranolol: within Treatment B (propranolol only vs propranolol + ponesimod)</u>

The combination of propranolol with the first dose of ponesimod (2 mg) on Day 5 in Treatment B gave a larger reduction in HR than propranolol alone (Day 4), with an initial maximum reduction of the mean of the mean hourly HR of 16.0 bpm observed at 5 hours postdose followed by another decrease of 20.1 bpm at 10 hours versus 9.5 bpm and 16.5 bpm at 5 hours and 10 hours postdose, respectively, for propranolol alone. The lowest value of the mean HR was 53.0 bpm at 4 hours postdose for the combination on Day 5 and 59.6 bpm at 10 hours postdose for propranolol alone.

The largest maximum difference in time matched mean of the mean hourly HR was 19.3 bpm (at 10 hours postdose) when treatment with the combination of ponesimod 2 mg + propranolol was compared with ponesimod alone (Day 5 versus Day 1 of Period 1) and 8.8 bpm (at 3 hours postdose) when compared to propranolol alone (Day 5 vs Day 4).

Effects on Mean Arterial Pressure (MAP)

The maximum difference in time matched mean hourly MAP between Treatment B versus Treatment A was 5.8 mmHg (at 8 hours postdose), 6.6 mmHg (at 10 hours postdose), and 6.0 mmHg (at 10 hours postdose) on Day 5, Day 16, and Day 19, respectively.

Effects on PR interval from 12-lead ECG

No significant changes in the mean of PR values by day and treatment were observed during the study. In Treatment Period 1 Day 1 (ponesimod 2 mg), the mean PR interval values were ≤200 ms. In Treatment Period 2 Day -1, no difference between Treatment A and Treatment B groups was noted. Similarly, in Treatment Period 2 Day 1 and Treatment Period 2 Day 4 no differences in PR-values were noticed for both Treatment A (ponesimod with placebo) and Treatment B (ponesimod with propranolol). Adding ponesimod up-titration to steady state propranolol overall did not have an impact on PR values when the 2 treatments are compared.

Safety

A total of 6 subjects prematurely terminated the study: 1 subject in Treatment A, (ponesimod alone) and 5 subjects in Treatment B (combination of propranolol steady state and up-titration of ponesimod).

The total number of treatment-emergent 12-lead ECG abnormalities was significantly higher in the up-titration regimen of ponesimod at steady state of propranolol (Treatment B) compared to same up-titration regimen of ponesimod with placebo propranolol (Treatment A) during Treatment Period 2. These ECG abnormalities as seen on 12-lead ECG in period 2 were not clinically relevant. No second degree or higher AV block was observed.

Except for the expected decrease in lymphocytes (leukocytes), no relevant changes in clinical chemistry, hematology, or urinalysis variables were observed. No clinically relevant effects of any treatment on SBP and DBP were noted.

CONCLUSIONS

- There was no clinically relevant PK drug-drug-interaction between ponesimod and propranolol.
- Both Treatment A and Treatment B resulted in a maximum decrease of 57-59% from baseline in total lymphocytes.
- Overall concomitant administration of ponesimod with propranolol resulted in an additive effect on HR measured by Holter.
- No clinically significant changes in MAP have been reported.
- The combination of propranolol with ponesimod was associated with higher incidence of 12-lead ECG abnormalities compared to ponesimod alone.

Study AC-058-104: Single-center, open-label, two-period, two-treatment, randomized, crossover study to investigate the effect of multiple-dose ACT-128800 on the pharmacokinetics of a single dose of Ortho-Novum® 1/35 in healthy female subjects.

A brief overview of some essential components of the study design is given below:

Study Design	Open-label, two-period, two-treatment, randomized, crossover study
Study Population	24 subjects were enrolled Age: 29 - 60 years (mean 47.3 years) Weight: 50.6 – 76.5 kg (mean 63.7 kg) Race: 17 Caucasian (70.8%), 3 African American (12.5%), 2 Asian (8.3%), 1 Other (8.3%)
Dosage and Administration	Treatment period A: A single oral dose of Ortho-Novum was administered Treatment period B: The treatment commenced with a daily oral dose of 10 mg ACT-128800 for 3 days, followed by 20 mg for 3 days, and 40 mg for a further 8 days. A single oral dose of Ortho-Novum 1/35 was concomitantly administered with ACT-128800 on Day 14. There was a washout period of 2 weeks (± 2 days) between the two treatment periods. Study drugs were taken orally, together with approximately 240 mL of noncarbonated water, under fed conditions.
Analysis (Plasma)	Method: LC/MS/MS ponesimod LLOQ: 1 ng/mL Linear range: 1-2000 ng/mL Inter-day Precision (%CV): 7.6-8.2% Inter-day accuracy: -1.7- 0.3% Ethinyl estradiol LLOQ: 2 pg/mL

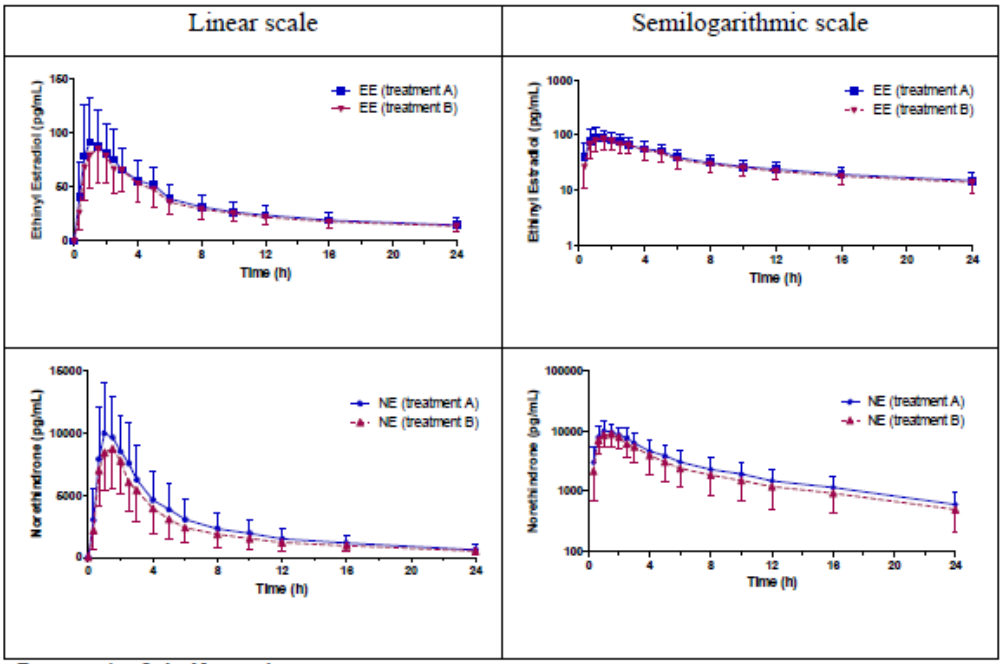
	Linear range: 2 - 500 pg/mL Inter-day Precision (%CV): 1.98 – 6.87% Inter-day accuracy: 1 – 5% <u>Norethindrone</u> LLOQ: 50 pg/mL Linear range: 50 - 25000 pg/mL Inter-day Precision (%CV): 1.53 – 6.15% Inter-day accuracy: 3-5%
PK Assessment	AUC0-24, Cmax, tmax, AUC0-∞, and t1/2 of ethinyl estradiol and norethisterone. Trough (pre-dose) plasma concentrations of ACT-128800 during treatment period B.
PK Assessment	Trough (pre-dose) total lymphocyte counts and percent change from baseline during treatment period B.
Safety Assessment	AE, SAEs, Laboratory tests, ECG, Vital signs, Physical examination, PFTs.

RESULTS

Pharmacokinetic results ethinyl estradiol and norethisterone

Plasma concentrations of ethinyl estradiol and norethisterone over the 24 h following Ortho-Novum administration in both treatment period A and treatment period B are shown in the following Figure:

Arithmetic mean (+/- SD) plasma concentration-time profiles of ethinyl estradiol and norethisterone administered to healthy subjects in the presence and absence of ACT-128800:



Treatment A = Ortho-Novum alone
Treatment B = Ortho-Novum + ACT-128800
EE = ethinyl estradiol, NE = norethisterone= norethindrone

The PK parameters derived by non-compartmental analysis of the plasma concentration-time data for ethinyl estradiol are summarized in Table below:

Summary of PK parameters for ethinyl estradiol

Treatment	Statistic	C_{max} (pg/mL)	t_{max} (h)	AUC_{0-24} (pg·h/mL)	AUC_{0-inf} (pg·h/mL)	$t_{1/2}$ (h)
A	N	22	22	22	22	22
	Mean	98.1	1.3	783	1235	18.9
	SD	45.4	0.52	267	564	7.8
	SE	9.7	0.11	56.9	120	1.7
	CV%	46	40	34	46	41
	95% CI of the Mean	78.0 , 118	1.0 , 1.5	665 , 901	985 , 1485	15.5 , 22.4
	Median	84.5	1.0	810	1077	16.9
	Min, Max	52.8 , 244	0.70 , 2.5	398 , 1276	497 , 2554	11.0 , 43.2
	Geo mean	90.5	1.2	740	1121	17.7
	CV%	41	38	36	48	37
	95% CI of the Geo mean	76.0 , 108	1.0 , 1.4	634 , 863	917 , 1370	15.1 , 20.8
B	N	22	22	22	22	22
	Mean	90.1	1.3	727	1187	20.8
	SD	31.8	0.38	200	514	11.3
	SE	6.8	0.081	42.6	110	2.4
	CV%	35	29	27	43	54
	95% CI of the Mean	76.0 , 104	1.1 , 1.5	639 , 816	959 , 1415	15.8 , 25.8
	Median	83.4	1.5	678	982	18.2
	Min, Max	50.0 , 165	0.70 , 2.0	392 , 1075	488 , 2750	11.1 , 64.7
	Geo mean	85.1	1.2	701	1096	19.0
	CV%	35	33	29	42	42
	95% CI of the Geo mean	73.2 , 99.0	1.1 , 1.4	619 , 794	916 , 1310	15.9 , 22.7

Treatment A = Ortho-Novum alone, Treatment B = Ortho-Novum + ACT-128800.
Note: more than 20% of AUC_{0-inf} is extrapolated.

The results show that the PK parameters for ethinyl estradiol were similar with concomitant administration of Ortho-Novum with ACT-128800 compared to Ortho-Novum alone. The 90% CIs of the estimated geometric mean ratio for Cmax and total exposure to ethinyl estradiol (AUC0-24) with and without co-administration of ACT-128800 were contained within the equivalence limits. This was also the case for AUC0-inf and t1/2 (see the Table below).

Geometric mean ratios of PK parameters of ethinyl estradiol

Comparison	Statistic	C_{max}	t_{max}	AUC_{0-24}	AUC_{0-inf}	$t_{1/2}$
B vs A	Ratio of geometric means	0.94		0.95	0.98	1.07
	90% confidence interval	0.86 , 1.03		0.89 , 1.01	0.87 , 1.10	0.92 , 1.25
	Median difference		-0.05			
	90% confidence interval		-0.25 , 0.50			

The PK parameters derived by non-compartmental analysis of the plasma concentration-time data for norethisterone are summarized in the Table below:

Summary of PK parameters for norethisterone

Treatment	Statistic	C_{max} (pg/mL)	t_{max} (h)	AUC_{0-24} (pg·h/mL)	AUC_{0-inf} (pg·h/mL)	$t_{1/2}$ (h)
A	N	22	22	22	22	22
	Mean	11026	1.4	60861	68797	9.0
	SD	3603	0.64	28027	32834	1.7
	SE	768	0.14	5975	7000	0.35
	CV%	33	45	46	48	18
	95% CI of the Mean	9429 , 12624	1.1 , 1.7	48434 , 73287	54239 , 83355	8.3 , 9.7
	Median	10800	1.0	56197	61812	9.0
	Min, Max	4460 , 16300	0.70 , 3.0	16307 , 108440	17948 , 135187	6.5 , 11.3
	Geo mean	10408	1.3	54126	61058	8.8
	CV%	37	44	56	56	19
	95% CI of the Geo mean	8868 , 12215	1.1 , 1.6	42934 , 68237	48447 , 76953	8.1 , 9.6
B	N	22	22	22	22	22
	Mean	9507	1.4	50340	57204	9.6
	SD	2974	0.55	21529	24797	2.0
	SE	634	0.12	4590	5287	0.43
	CV%	31	39	43	43	21
	95% CI of the Mean	8188 , 10825	1.2 , 1.7	40795 , 59885	46209 , 68198	8.7 , 10.5
	Median	10400	1.5	48796	54247	9.6
	Min, Max	4670 , 16100	0.70 , 3.0	15111 , 87110	18613 , 109058	6.4 , 13.9
	Geo mean	9023	1.3	45401	51713	9.4
	CV%	35	40	52	51	22
	95% CI of the Geo mean	7754 , 10500	1.1 , 1.6	36542 , 56408	41825 , 63938	8.6 , 10.3

Treatment A = Ortho-Novum alone, Treatment B = Ortho-Novum + ACT-128800.

The results show that the Cmax and exposure to norethisterone were slightly reduced with concomitant administration of ACT-128800 compared with Ortho-Novum alone. The 90% confidence intervals of the estimated geometric mean ratio for the AUC0-24 and Cmax of norethisterone with and without co-administration of ACT-128800 were not contained within the equivalence limits. For total exposure to norethisterone (AUC0-24), the lower CI of 0.76 was slightly below 0.80 and Cmax was 0.80. A similar lower CI of 0.76 was observed for AUC0-inf, while the CI of the t1/2 was within the equivalence limits (see the Table below).

Geometric mean ratios of PK parameters of norethisterone

Comparison	Statistic	C_{max}	t_{max}	AUC_{0-24}	AUC_{0-inf}	$t_{1/2}$
B vs A	Ratio of geometric means	0.87		0.84	0.85	1.06
	90% confidence interval	0.80 , 0.94		0.76 , 0.93	0.76 , 0.94	1.03 , 1.10
	Median difference		-0.05			
	90% confidence interval		-0.40 , 0.50			

Pharmacokinetics of ACT-128800

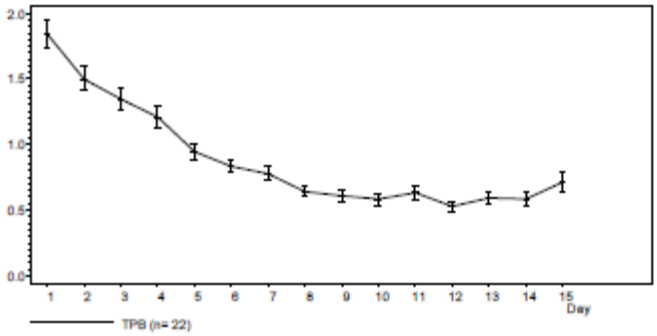
Trough plasma concentrations of ACT-128800 at steady state were comparable to those reported in previous study (study AC-058-102).

Pharmacodynamic results

Administration of ACT-128800 resulted in a clear reduction in the lymphocyte count in all exposed subjects. The mean total lymphocyte count determined pre-dose on Day 14 was0.58 ±

0.23 × 109/L (mean ± SD) compared to a baseline count of 1.84 ± 0.49 × 109/L (see the Figure below). The mean change from baseline on Day 14 pre-dose was -66.6% ± 14.5% (mean ± SD).

Total lymphocyte absolute counts (109/L) at trough from Day 1 to Day 15



Days 1–3: 10 mg ACT-128800, Days 4–6: 20 mg ACT-128800, Days 7–14: 40 mg ACT-128800

Safety

Concomitant administration of Ortho-Novum and ACT-128800 did not result in the occurrence of any AEs which were considered to be new or unexpected in comparison to the known AE profiles of the individual treatments. Further, concomitant administration was not associated with increased severity of any AE.

CONCLUSIONS

- The results of the study showed that there was no clinically relevant PK interaction between ACT-128800 and Ortho-Novum.
- Overall, the safety findings of this study were consistent with previous clinical studies of ACT-128800. No new or unexpected AEs were observed in concomitant administration of Ortho-Novum and ACT-128800.

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