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APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA Number	209884 (IND 076122)
Link to EDR	\\CDSESUB1\evsprod\NDA209884\0000
Submission Date	July 26, 2018
Submission Type	Priority (Rolling Submission)
Brand Name	Mayzent
Generic Name	Siponimod (BAF312)
Dosage Form and Strength	Film coated tablets, 0.25 mg and 2 mg
Route of Administration	Oral
Proposed Indication	Secondary Progressive Multiple Sclerosis (SPMS)
Applicant	Novartis Pharmaceuticals
OCP Review Team	Dawei Li, Angela Men, Manuela Grimstein, Xinyuan Zhang, Simbarashe Zvada, Kevin Krudys, Jeffrey Kraft, Christian Grimstein
OCP Final Signatory	Mehul Mehta, PhD., FAAPS Division Director Division of Clinical Pharmacology I

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1. EXECUTIVE SUMMARY

The applicant is seeking approval for siponimod 0.25 mg and 2 mg film coated tablets, for the treatment of secondary progressive multiple sclerosis (SPMS). Siponimod acts as an antagonist on sphingosine-1-phosphate subtype 1 (S1P1) receptors on lymphocytes, prevents T cell egress from lymph nodes, and limits central inflammation. The applicant proposed dose titration starts with 0.25 mg once daily on Day 1 and Day 2, followed by once daily doses of 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, to reach the maintenance dose of siponimod starting on Day 6. The proposed maintenance dose of siponimod is 2 mg taken orally once daily with or without food. The proposed maintenance dose for patients with CYP2C9 *1/*3 or *2/*3 genotypes is 1 mg/day. The efficacy and safety of siponimod in SPMS patients is supported by a single pivotal phase 3 randomized, double-blind, placebo controlled, multicenter trial. The primary endpoint is time to 3-month confirmed disability progression (CDP). The Applicant reports statistically significant effect in the placebo-controlled pivotal trial for time to 3-month CDP.

Siponimod is metabolized primarily by CYP2C9 and to a lesser extent CYP3A4. Additionally, CYP2C9 genotype had a significant effect on the exposure of siponimod, with CYP2C9 *3/*3, CYP2C9 *1/*3, and CYP2C9 *2/*3 individuals having the largest effect. The key review questions focus on acceptability of the proposed dosing regimen including both titration and maintenance doses and appropriateness of dosing recommendations for siponimod in specific populations and patients taking concomitant medications.

1.1 Recommendations

The Office of Clinical Pharmacology Divisions of Clinical Pharmacology I, Pharmacogenomics, and Pharmacometrics, have reviewed the clinical pharmacology and biopharmaceutical information submitted in NDA 209884 and consider it acceptable from a clinical pharmacology perspective. The approvability of siponimod specifically for SPMS will be deferred to clinical and statistical review teams. The key review issues with specific recommendations /comments are summarized below:

Review Issues	**Recommendations and Comments**
Supportive evidence of effectiveness	A single pivotal trial in SPMS patients was submitted in NDA 209884 as primary evidence of effectiveness. No additional supportive evidence of effectiveness in SPMS is available.
General dosing instructions	Titration dosing regimen: 0.25 mg once daily on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5. Maintenance dose: 2 mg/day

Dosing in patient subgroups (intrinsic and extrinsic factors)	For patients with CYP2C9 *1/*3 or *2/*3 genotypes: - Titration dosing regimen: 0.25 mg once daily on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1 mg on Day 5. - Maintenance dose: 1 mg
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 Requirements and Commitments

The sponsor has proposed not giving siponimod to individuals with the CYP2C9 *3*/*3 genotype, as well as dose adjustments for individuals with CYP2C9 *1/*3, *2/*3, (b) (4) genotypes. Given that the proposed labeling language requires testing prior to administration of siponimod, such a device would be considered essential to the safe and effective use of siponimod and qualify as an in-vitro companion diagnostic device. The FDA is asking the sponsor to complete the post-marketing commitment listed below.

Post-Marketing Commitment: Commitment to establish an in-vitro diagnostic device that is essential to the safe and effective use of siponimod for the approved indication. Such a device should detect, at a minimum, the presence of the *2 and *3 alleles in cytochrome P450 2C9 (CYP2C9). The device should detect patients homozygous for the CYP2C9 *3/*3 genotype with statistical confidence, given that currently marketed devices to detect CYP2C9 alleles do not detect these individuals with high confidence.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action (MOA): Siponimod binds selectively on two out of five G-protein-coupled receptors (GPCRs) for S1P, namely S1P1 and S1P5. By acting as a functional antagonist on S1P1 receptors on lymphocytes, siponimod prevents egress from lymph nodes. This reduces the recirculation of T-cells into the central nervous system (CNS) to limit central inflammation.

Absorption: Siponimod exposure increases in an apparent dose proportional manner at dose range from 0.3 to 20 mg/day. The median siponimod Tmax ranged from 3 - 8 hours. Absolute bioavailability is approximately 84%. Food intake has no significant effect on the systemic exposure of siponimod.

Distribution: The estimated volume of distribution in healthy volunteers is 124L. High plasma protein binding (i.e., >99.9 %) is observed for siponimod.

Metabolism: Siponimod is extensively metabolized, primarily by CYP2C9 and to a lesser extent CYP3A4. The major circulating metabolites M3 and M17 are found to be inactive and are not expected to contribute to the clinical effect and the safety of siponimod in humans.

Elimination: The mean elimination half-life is approximately 30 hours. Following a single oral dose of ^{14}C-siponimod at 10 mg, radioactivity is excreted predominantly via the fecal route (86.7%), only a minor amount of radioactivity is excreted in the urine (<3%).

Steady state was reached after approximately 6 days of multiple once daily administration of siponimod.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The applicant proposed dose titration starts with 0.25 mg once daily on Day 1 and Day 2, followed by once daily doses of 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, to reach the maintenance dose of siponimod starting on Day 6. The proposed maintenance dose of siponimod is 2 mg taken once daily with or without food orally. The proposed maintenance dose for patients with CYP2C9 *1/*3 or *2/*3 genotypes is 1 mg/day.

(b) (4)

the review team recommended a reduced titration dose from 1.25mg to 1 mg on day 5 for patients with CYP2C9 *1/*3 or *2/*3 genotypes.

2.2.2 Therapeutic individualization

The general recommended maintenance dose is 2 mg/day. A dose reduction to 1 mg/day is recommended for patients with CYP2C9 *1/*3 or *2/*3 genotypes.

CYP2C9 and CYP3A4 Inhibitors: In a dedicated drug-drug interaction (DDI) trial, concomitant fluconazole (a moderate CYP2C9/CYP3A4 inhibitor) increased siponimod's Cmax by 10% and the AUC 2-fold. Physiologically based pharmacokinetic (PBPK) modeling predicted that moderate inhibitor of CYP3A4 and CYP2C9, such as fluconazole, can increase siponimod exposure 2-to 4-fold among the different CYP2C9 genotypes compared to the exposure in subjects with CYP2C9*1/*1 without a perpetrator. For patients on siponimod treatment, taking moderate CYP2C9/3A4 dual inhibitor (e.g. fluconazole) or a moderate CYP2C9 inhibitor concomitantly with a strong or moderate CYP3A4 inhibitor are not recommended. Caution should be exercised for concomitant use of moderate CYP2C9 inhibitors.

CYP2C9 and CYP3A Inducers: In a dedicated DDI trial, concomitant rifampicin (a moderate CYP2C9/strong CYP3A4 inducer) decreased siponimod's Cmax by 45% and the AUC by 57%. PBPK modeling predicted that concomitant administration of a strong CYP3A4/moderate CYP2C9 inducer,

such as rifampicin, can decrease siponimod exposure by approximately 60-80%. The concomitant use of strong CYP3A4/moderate CYP2C9 inducers with siponimod is not recommended. The concomitant use of moderate CYP3A4 inducer with siponimod is not recommended for patients with CYP2C9 *1/*3 or *2/*3 genotypes.

The recommended dosing regimen for patients with different CYP2C9 genotypes are summarized below:

	CYP2C9*1/*1	CYP2C9*1/*2	CYP2C9*2/*2	CYP2C9*1/*3	CYP2C9*2/*3
Titration dosing regimen	0.25 mg once daily on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5.			0.25 mg once daily on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1 mg on Day 5.	
Maintenance dose	2 mg/day			1 mg/day	
CYP2C9 and CYP3A4 Inhibitors	Concomitant use of CYP2C9/3A4 dual inhibitor (e.g. fluconazole) or moderate CYP2C9 inhibitor concomitantly with a strong or moderate CYP3A4 inhibitor are not recommended. Caution should be exercised for concomitant use of moderate CYP2C9 inhibitors.				
CYP2C9 and CYP3A Inducers	Concomitant use of strong CYP3A4/moderate CYP2C9 inducers (e.g. rifampicin or carbamazepine) is not recommended.				
				Concomitant use of moderate CYP3A4 inducer is not recommended.	

2.3 Outstanding Issues

None identified.

2.4 Summary of Labeling Recommendations

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

- The recommended titration dosing regimen for patients with CYP2C9 *1/*3 or *2/*3 genotypes is 0.25 mg once daily on Day 1 and Day 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1 mg on Day 5, to reach the maintenance dose of siponimod starting on Day 6.
- Concomitant use of CYP2C9/3A4 dual inhibitor (e.g. fluconazole) or moderate CYP2C9 inhibitor concomitantly with a strong or moderate CYP3A4 inhibitor are not recommended for all patients. Caution should be exercised for concomitant use of moderate CYP2C9 inhibitors.

- Concomitant use of strong CYP3A4/moderate CYP2C9 inducers (e.g. rifampicin or carbamazepine) with siponimod is not recommended for all patients. The concomitant use of moderate CYP3A4 inducer with siponimod is not recommended for patients with CYP2C9 *1/*3 or *2/*3 genotypes.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Siponimod is developed to be available as 0.25 mg and 2 mg film coated tablets for oral administration. It is proposed for the treatment of patients with SPMS. The approval is based on the clinical effectiveness, time to 3-month CDP, obtained from one pivotal trial. (b) (4) Fast Track Designation for siponimod was granted for SPMS indication in September 2012. (b) (4)

3.2 General Pharmacological and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Siponimod promotes internalization and degradation of S1P1 receptors, thereby acting as a functional antagonist on S1P1. This reduces the recirculation of T-cells into the central nervous system (CNS) to limit central inflammation
Active Moieties	Siponimod is the active moiety circulating in plasma accounted for 57.1% of total radioactivity in a mass balance trial.
QT Prolongation	Siponimod increased the mean placebo-corrected baseline-adjusted mean QTcF (ΔΔQTcF) by more than 5 ms with a maximum mean effect of 7.8 ms (2 mg, therapeutic dose) at 3 h post-dose. The upper bound of the one-sided 95% CI for the ΔΔQTcF at all time points remained below 10 ms. The results did not suggest an arrhythmogenic potential related to QT prolongation with siponimod.
General Information	
Bioanalysis	Siponimod and M3, M17 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	PK of siponimod in MS patients is comparable to that in healthy subjects.	
Drug exposure at steady state following the therapeutic dosing regimen	In SPMS patients receiving siponimod 2 mg qd, the geometric trough concentrations were 23.2 ng/mL and 28.9 ng/mL on day 28 and month 24, respectively, in the pivotal trial (study A2304).	
Dose Proportionality	Siponimod exposure increases in an apparent dose-proportional manner over the multiple dose range of 0.3 to 20 mg/day in healthy subjects.	
Accumulation	A mean accumulation ratio of 1.88 to 2.72 was observed at steady-state.	
Variability	CV% for Cmax,ss: 10-37% and AUC0-24: 16-45%	
Absorption		
Bioavailability [oral]	Absolute bioavailability is approximately 84%.	
Tmax [oral]	The median siponimod Tmax ranged from 3 - 8 hours.	
Food effect (high-fat)	AUCinf	Cmax
GMR (90% CI)	0.96 (0.92-1.00)	0.91 (0.86-0.97)
Distribution		
Volume of Distribution	The estimated volume of distribution in healthy volunteers is 124L.	
Plasma Protein Binding	>99.9% (Lipoprotein and albumin).	
Elimination		
Mean Terminal Elimination half-life	Approximately 30 hours.	
Metabolism		

Primary metabolic pathway(s) [*in vitro*]	Siponimod is extensively metabolized, primarily by CYP2C9 and to a lesser extent CYP3A4. The major circulating metabolites M3 and M17 are found to be inactive.
Inhibitor/Inducer [*in vitro*]	Siponimod, M3 and M17 are unlikely to inhibit any major CYPs or induce CYP1A2, 2B6, 2C9, and 3A4 at clinically relevant doses.
Transporter Systems [*in vitro*]	Siponimod is not identified as substrate of P-gp, BCRP or MRP2 transporters. Siponimod, M3 and M17 are unlikely to inhibit major efflux, uptake and SLC transporters at clinically relevant doses.
Excretion	
Primary excretion pathways (%dose) ±SD	Following a single oral dose of 14C-siponimod at 10 mg, radioactivity was excreted predominantly via the fecal route (86.7%), only a minor amount of radioactivity was excreted in the urine (<3%).

3.3 Clinical Pharmacology Questions

3.3.1 To what extent does the available clinical pharmacology information provide supportive evidence of effectiveness?

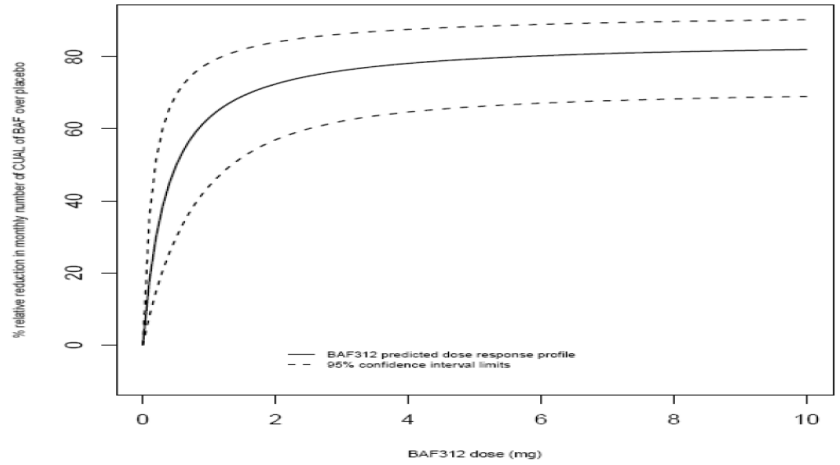
The primary evidence of effectiveness of siponimod for the treatment of SPMS is based on results obtained from the pivotal, placebo-controlled phase 3 trial (study A2304). In study A2304, the efficacy and safety of a fixed dose level of 2 mg were evaluated in 1651 patients with SPMS. See the response to Question 3.3.2 for further details.

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

To attenuate negative chronotropic effect of the initial dose of siponimod, a titration dosing regimen was proposed based on results from dose titration study (Study A2107). In dose-titration group, the mean HR values reach, at the end of the dose-titration period, values similar to those observed in the placebo group. The proposed titration dosing regimen (0.25 mg once daily on Day 1 and Day 2, followed by once daily doses of 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5) was evaluated in the pivotal efficacy/safety study (A2304). (b) (4) the review team recommended a reduced titration dose from 1.25mg to 1 mg on day 5 for patients with CYP2C9 *1/*3 or *2/*3 genotypes. From a clinical pharmacology perspective, the reduced titration dose on day 5 is not expected to affect the exposure and efficacy of siponimod significantly.

The proposed maintenance dosing regimen is the same that was evaluated in the pivotal efficacy/safety study (A2304). Maintenance dose and dosing regimen for the pivotal study were selected based on a phase 2 dose finding study (Study A2201) in patients with Relapsing-Remitting Multiple Sclerosis (RRMS). Based on the MRI dose-response curve (Figure 1), near-maximal efficacy at the 2 mg dose was observed.

Figure 1 Dose response curve at Month 3 estimated by Bayesian longitudinal analysis



Combined unique active lesions (CUAL) are defined as new Gd-enhanced T1 lesions or new or enlarging T2 lesions, without double counting of lesions at any specific point in time.

The observed near-maximal effectiveness for the 2 mg dose, together with the observation that the higher 10 mg dose did not provide significantly better efficacy but was associated with higher incidences of the more frequently reported adverse events, the 2 mg dose was selected for further Phase 3 study. However, the primary endpoint (combined unique active [MRI] lesions, CUAL) used for this dose-response analysis and patients population were different from those in Phase 3 study, in which the primary endpoint was the evaluation of time to 3-month CDP in patients with SPMS. Therefore, observed efficacy for the 2 mg dose in patients with RRMS may not necessarily result in similar near-maximal efficacy in patients with SPMS.

Efficacy of siponimod was assessed in patient with SPMS in Study A2304. As per applicant's analyses, siponimod showed a 21.2% risk reduction compared to placebo for time to 3-month CDP, the primary endpoint, based on Expanded Disability Status Scale (EDSS) that was statistically significant (hazard ratio 0.79, p=0.0134), as summarized in Table 1. Key secondary endpoints were also assessed. The results for time to 3-month confirmed worsening in Timed 25-Foot Walk Test (T25W) of at least 20% from baseline are summarized in Table 2. This key secondary endpoint did not reach statistical significance, with an observed risk reduction of 6.2% in favor of the siponimod group (p=0.4398). For the other key secondary endpoint, the change from baseline in T2 lesion volume at both Month 12 and Month 24 was statistically significant (p-values <0.0001). The results for change from baseline in T2 lesion volume at Month 12 and 24 are summarized in Table 3.

Table 1. Time to 3-month CDP based on EDSS – Cox proportional hazards model (FAS)

			Comparison: BAF312 vs Placebo [#]		
Treatment	n/N'	(%)	Risk reduction	Hazard ratio (95% CI)	p-value
BAF312 (N=1099)	288/1096	(26.3)	21.2%	0.79 (0.65; 0.95)	0.0134
Placebo (N=546)	173/545	(31.7)			

n/N': n= number of subjects with events/N'=number of subjects included in the analysis (i.e. with non-missing covariates).
[#] Using a Cox proportional hazards model with treatment, country/region, baseline EDSS, and SPMS group (with/without superimposed relapses, baseline definition) as covariates. Risk reduction is derived as (1-hazard ratio) * 100.

Table 2. Time to 3-month confirmed worsening in T25W of at least 20% from baseline – Cox proportional hazards model (FAS)

			Comparison: BAF312 vs Placebo[£]		
Treatment	n/N'	(%)	Risk reduction	Hazard ratio (95% CI)	p-value
BAF312 (N=1099)	432/1087	(39.7)	6.2%	0.94 (0.80; 1.10)	0.4398
Placebo (N=546)	225/543	(41.4)			

n/N': n= number of subjects with events/N'=number of subjects included in the analysis (i.e. with non-missing covariates)
£ Using a Cox proportional hazards model with treatment, country/region, baseline EDSS, baseline T25W, and SPMS group (with/without superimposed relapses, baseline definition) as covariates. Risk reduction is derived as (1-hazard ratio) * 100.

Table 3. Change from baseline in T2 lesion volume (mm3) by time point (Month 12 and 24) – repeated measures model (FAS)

	Adjusted means (SE)		Comparison of adjusted means BAF312 vs Placebo			
Time point	BAF312 (N=1099) (N'=995)	Placebo (N=546) (N'=495)	Difference	SE	95% CI	p-value
Month 12	204.9 (67.47)	818.0 (87.29)	-613.1	95.39	(-800.2 ; -426.0)	<0.0001
Month 24	162.9 (73.90)	940.4 (97.20)	-777.5	108.62	(-990.6 ; -564.4)	<0.0001
Average over Month 12 and Month 24	183.9 (66.33)	879.2 (85.43)	-695.3	92.79	(-877.3; -513.3)	<0.0001

N'=number of subjects included in the analysis (i.e. with at least MRI scan post-baseline and non-missing covariates)

[redacted] (b) (4)

Preliminary analysis conducted by biostatistics reviewer also suggested that

the efficacy of siponimod showed in the applicant's analysis was driven by its effect on inflammation and relapses.

Furthermore, per OSI report, eighty-two sites in the A2304 study experienced issues with "dual database access", in which 32 users were identified to have had inappropriate access to both the first-dose database and the main database. Dual database access compromised the data of 101 subjects, accounting for 6.1% of study population. This could be a potential source of unblinding and affect efficacy analysis. If the compromised data were excluded, both the clinical reviewer's analysis and the applicant's analysis did not achieve statistical significance on the primary endpoint.

Overall, based on the available data, it is not clear whether the 2mg dose of siponimod utilized in Phase 3 is effective for patients with SPMS. 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?

Yes. CYP2C9 genotype had a significant effect on the AUCinf of siponimod, with CYP2C9 *3/*3, CYP2C9 *1/*3, and CYP2C9 *2/*3 individuals having the largest effect. Individuals with CYP2C9 *3/*3 genotype should not take siponimod and maintenance doses should be reduced from 2 mg/day to 1 mg/day in individuals with CYP2C9 *1/*3 and CYP2C9 *2/*3 genotypes.

Dose adjustment is not necessary based on intrinsic factors such as age, gender, bodyweight, race, renal or hepatic impairment (see details of renal/hepatic impairment studies below). Population pharmacokinetic analysis did not reveal a significant impact of age, gender, BMI, health status (healthy subject or RMS patient), creatinine CL, bilirubin, AST and ALT on the PK of siponimod. Body weight was shown to influence siponimod CL/F and Vc/F in the PopPK analyses. However, considering the limited effect on siponimod exposure, no dose adjustment based on body weight is recommended.

CYP2C9 Genotype

Based on the popPK analysis, the CL/F of siponimod was 38%, 48%, and 74% lower in CYP2C9 *1/*3 (N=148), CYP2C9 *2/*3 (N=32), and CYP2C9 *3/*3 (N=2) individuals, respectively, as compared to CYP2C9 *1/*1 (N=740) (see 4.2 Population PK and/or PD Analyses). The magnitude of this effect is similar to what was seen in the dedicated genotype study (study A2128) comparing CYP2C9 *1/*1 (N=12) with CYP2C9 *2/*3 (N= 6) and CYP2C9 *3/*3 (N=6) genotype groups (see 4.5 Pharmacogenomics Summary).

The sponsor has proposed excluding CYP2C9 *3/*3 individuals from receiving siponimod because in clinical trials, up to 4-fold higher exposure compared to normal CYP2C9 metabolizers (CYP2C9 *1/*1) has been observed.

Renal Impairment (Study A2129)

Siponimod Cmax decreased by 8% and AUC increased by 23% to 24% in severe renal impaired subjects compared to healthy matched subjects. These differences were not clinically meaningful and no dose adjustment is recommended for patients with renal impairment.

Hepatic Impairment (Study A2122)

The PK of siponimod was not significant affected by hepatic impairment. Mean Cmax increased by 16% for the mild impairment group and decreased by approximately 13% and 16% for the moderate and severe impairment groups, respectively. Mean AUCinf and AUClast increased by 5% for the mild impairment group and 15% for the severe impairment group, and a decrease in the mean AUC of about 13% were observed in the moderate impairment group. Similar mean CL/F values were observed for the hepatic impairment groups (3.56 to 4.68 L/h) and their matched healthy subjects (4.07 to 4.11 L/h).

In addition, no significant correlation was observed between Child-Pugh score, total bilirubin and exposure (Cmax and AUCinf) of siponimod.

These differences were not clinically meaningful and no dose adjustment is recommended for patients with 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 studies conducted in healthy subjects indicated that food decreased the Cmax and AUC of siponimod by 10% and 4%, respectively (Study A2111). Tmax was slightly delayed (approximately 2 to 3 hours delay) in the presence of food. The changes in Cmax and AUC are not considered clinically relevant and siponimod is recommended to be administered without regard to food.

Drug-drug interaction

Three dedicated DDI trials were conducted to evaluate the effect of concomitant medication (CYP450 enzyme inducer/inhibitor) on the PK of siponimod (summarized in Table 4).

Overall, co-administration of rifampicin decreased exposure of siponimod (Cmax, AUC) by 45% to 57%. In presence of fluconazole, siponimod AUC was increased by 2-fold and Cmax was increased by 10%. Co-administration of siponimod with itraconazole resulted in a 10% and 24% decrease in AUCs in the subjects with the CYP2C9*1/*2 and *1/*3 genotypes, respectively, indicating a possible CYP2C9 genotype influence on the itraconazole effect due to the different extent of CYP3A4 involvement in the metabolism of siponimod across different CYP2C9 genotypes.

Table 4: Summary of drug-interaction studies

Study #	Concomitant medication	PK parameters	
		AUCtau,ss	Cmax,ss
A2125 n=16	Rifampicin (600 mg qd), moderate CYP2C9/strong CYP3A4 inducer	Decreased 57%	Decreased 45%
A2108 n=14	Fluconazole (200 mg qd), moderate CYP2C9/CYP3A4 inhibitor	Increased approximately 2-fold	Increased 10%
A2124* n=30	Itraconazole (100 mg bid), strong CYP3A4 inhibitor	Decreased 10-24%	No effect

* CYP2C9*1/*2 and CYP2C9*1/*3 genotype subjects

PBPK analyses were conducted to predict the exposure to siponimod in subjects co-administered with CYP3A4/CYP2C9 inhibitors or inducers in sub-populations carrying polymorphic CYP2C9 variants (see Appendix 4.4).

Based on the PBPK predictions, co-administration of siponimod (maintenance dose of 2 mg qd for patients with CYP2C9*1/*1, *1/*2, and *2/*2, and 1 mg qd for CYP2C9*1/*3 and *2/*3 genotypes) with ketoconazole and erythromycin, a strong and moderate CYP3A4 inhibitor, respectively, or fluvoxamine, a weak CYP2C9 and CYP3A4 inhibitor, resulted in less than 2-fold increase in siponimod AUC, compared to a wild-type patient population without the inhibitor, for all different CYP2C9 genotypes (Table 5).

Consistent with results obtained from dedicated drug-drug interaction studies, the PBPK model predicted that siponimod AUC was likely to increase approximately 2-to 4-fold in the presence of a dual inhibitor of CYP3A4 and CYP2C9, such as fluconazole, and to decrease approximately 60-80% in the presence of a strong CYP3A4 and moderate CYP2C9 inducer, such as rifampicin. Concomitant administration of a moderate CYP3A4 inducer, such as efavirenz, resulted in approximately 50% decrease in siponimod AUC in patients with CYP2C9 *1/*3 or *2/*3 genotypes (Table 5).

Table 5. PBPK predicted Cmax and AUC ratios for siponimod in the presence of CYP3A4/CYP2C9 perpetrators in CYP2C9 polymorphic population relative to wild-type

Perpetrator	CYP2C9 Genotype									
	*1/*1		*1/*2		*2/*2		*1/*3		*2/*3	
	CmaxR	AUCR	CmaxR	AUCR	CmaxR	AUCR	CmaxR	AUCR	CmaxR	AUCR
fluconazole 200 mg qd	1.88	2.18	1.97	2.30	2.43	2.92	1.42	1.74	1.62	2.00
fluconazole 400 mg qd	2.60	3.15	2.72	3.32	3.29	4.09	1.89	2.38	2.10	2.67

ketoconazole 200 mg bid	1.18	1.24	1.27	1.37	1.62	1.84	0.98	1.16	1.19	1.44
ketoconazole 400 mg qd	1.17	1.22	1.25	1.34	1.58	1.79	0.95	1.12	1.14	1.38
erythromycin 500 mg bid	1.11	1.14	1.19	1.25	1.49	1.66	0.89	1.03	1.06	1.26
erythromycin 500 mg qid	1.13	1.17	1.21	1.28	1.53	1.72	0.92	1.07	1.10	1.32
fluvoxamine 100 mg qd	1.33	1.42	1.40	1.53	1.67	1.89	0.96	1.11	1.07	1.27
rifampicin 600 mg qd	0.46	0.29	0.48	0.29	0.54	0.37	0.29	0.22	0.32	0.25
efavirenz 600 mg qd	0.77	0.69	0.80	0.73	0.90	0.86	0.49	0.48	0.52	0.53

Data are presented as population geometric mean values for exposure ratios (AUCtau and Cmaxss) at steady-state expressed as the fold change in the CYP2C9 polymorphic populations with perpetrator versus the wild-type population (CYP2C9*1/*1) without perpetrator. Siponimod dose of 2 mg qd for the genotypes CYP2C9*1/*1, CYP2C9*1*2, CYP2C9*2*2 and dose of 1 mg qd for CYP2C9*1/*3 and CYP2C9*2/*3.

Results from genomic study, drug interaction studies and PBPK prediction suggest that the effect of concomitant CYP2C9/3A4 inhibitors or inducers results in different magnitudes of change in siponimod exposure across different genotypes. Additionally, current available strengths of siponimod and dose titration regimen limit the dosing adjustment for genotype based DDI management. To simplify the dosing regimen under concomitant medication and keep the recommendation to be consistent when co-administrated with inducer and inhibitor, the review team recommend that:

- Taking moderate CYP2C9/3A4 dual inhibitor (e.g. fluconazole) or moderate CYP2C9 inhibitor concomitantly with a strong or moderate CYP3A4 inhibitor are not recommended for all patients.
- Concomitant use of strong CYP3A4/moderate CYP2C9 inducers (e.g. rifampicin or carbamazepine) is not recommended for all patients. Caution should be exercised for concomitant use of moderate CYP2C9 inhibitors.
- Concomitant use of moderate CYP3A4 inducer with siponimod is not recommended for patients with CYP2C9 *1/*3 or *2/*3 genotypes.

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 will be 0.25 mg and 2 mg film coated tablets. Clinical service formulation (CSF) liquid and capsule, market formulation (MF) tablet and final market image (FMI) tablet were used in the clinical development of siponimod. Overall, inter- and intra-study comparisons suggest that, at equivalent doses, exposure to siponimod is comparable among oral formulations (CSF liquid and capsule, MF and FMI tablets) evaluated in the clinical program. Bioequivalence between MF and FMI formulations of siponimod (compared after single 0.25- and 4-mg doses) was demonstrated for Cmax, AUClast and AUCinf. FMI tablets (same as TBM tablets) were used in the pivotal Phase 3 clinical trial (Study A2304).

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

Bioanalytical methods used throughout the clinical development of siponimod and its metabolites are summarized in Table 6 and Table 7.

Table 6. Summary of Bioanalytical Method Measuring Siponimod in Plasma.

Method ID	BAF-A	BAF-B	BAF-C	BAF-D
Study Number Supported	StudyA2101, StudyA2102, StudyA2105, StudyA2107, StudyA2119, StudyA2108, StudyA2110, StudyA2111, StudyA2121, StudyA2118, StudyA2116, StudyA2122, StudyA2128, StudyA2129	StudyA2101, StudyA2102, StudyA2105, StudyA2107, StudyA2104, StudyA1101, StudyA2119, StudyA2108, StudyA2110, StudyA2111, StudyA2121, StudyA2118, StudyA2116, StudyA2304	StudyA2101, Study A2102	StudyA2130, StudyA2126, StudyA2125, StudyA2124, StudyA2304
LLOQ (ng/mL)	0.02	0.25	2.5	0.05
Linear Range (ng/mL)	0.02 - 20	0.25 - 500	2.5 - 2500	0.05 - 100
Inter-day Accuracy for QC	-2.7% - 8.7%	-2.3% - 8.0%	-5.5% - 5.6%	-7.6% to -2.0 %
Inter-day Precision for QC	3.7% - 13.1%	2.0% - 7.5%	3.4% - 10.3%	1.7% - 6.9%

Table 7. Summary of Bioanalytical Method Measuring Siponimod Major Metabolites in Plasma.

Method ID	LNL-A		LNL-B	BAF-D
Study Number Supported	Study A2118, Study A2122, Study A2128, Study A2129		Study A2125, Study A2124	StudyA2126, StudyA2124, StudyA2304
Analytes	M3	M3	M3	M17
LLOQ (ng/mL)	0.01	0.25	0.01	0.02
Linear Range (ng/mL)	0.01 -10	0.25 - 250	0.01 -10	0.1 - 50

Inter-day Accuracy for QC	-2.5%-17.0%*	-2.5% - 6.4%	0.3% - 6.0%	-2.7% to -0.8%
Inter-day Precision for QC	4.8% - 11.2%	2.3% - 9.4%	2.7% - 7.7%	2.0% - 5.5%

*intra-day bias at LLOQ was 17.0%

Bioanalytical method performance was summarized in each individual study review.

Stability in plasma

Siponimod is stable in plasma for up to 426-1302 days (QC samples) when stored below -20°C to -70°C. M3 and M5 (minor metabolite) in plasma are stable in QC samples for up to 704 days when stored below -60°C, and up to 326 days in incurred samples when stored below -60°C. M17 in plasma is stable for up to 266 days (QC samples) when stored below -80°C. All samples were stored and processed in the time frame supported by the stability data.

4.2 Population Pharmacokinetic Analyses

The Applicant performed population PK analyses using siponimod concentration time data from SPMS patients (Study A2304). The Applicant updated a population PK model that has been previously developed using data from studies in healthy volunteers and RRMS patients. The structural form of the previous PK model was retained, and some parameters were re-estimated in the analysis using the data from SPMS patients only. The remaining parameters were fixed as there was a sparse PK sampling schedule in Study A2304 (~3h and trough samples). The typical and interindividual (IIV) values of CL/F and Vc/F, covariate effects for CL/F (body weight and CYP2C9 genotype), as well as residual variability were estimated. Parameter estimates obtained for SPMS patients were compared with those for healthy volunteers and RRMS patients to assess potential differences.

Study design and datasets

Table 8 provides a brief description of the study population, duration of dosing, dosage regimens and PK sampling times for studies included in the population PK and population PK/PD analysis. A summary of the demographics for the subjects included in the population PK analysis are summarized in Table . There were more female patients (60%) than male (40%). Most of the population was Caucasian (95%). There were only 14 Chinese patients and 14 Japanese patients. The majority of the population had CYP2C9 genotype *1/*1 (WT/WT)(64%), with 19% having CYP2C9 genotype *1/*2 (WT/*2), and 13% having CYP2C9 genotype *1/*3 (WT/*3). There were few patients with CYP2C9 genotypes *2/*2 (2%) and *2/*3 (3%) and no patients with CYP2C9 genotype *3/*3.

Table 8: Details of Study Design and Pharmacodynamic Sampling Times

Study	Population	Treatment	Lymphocyte Sampling times
A2101	Healthy Volunteers	Single dose of placebo, 0.1, 0.3, 1, 2.5, 5, 10, 17.5, 25 and 75 mg	Pre-dose, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96 h, 7, 10 and 14 days post dose.
A2105	Healthy Volunteers	Placebo on run-in (day -1); placebo, 0.3, 1, 2.5, 10, 20 mg/day for 28 days	SCR; Run-in (Day -1): pre-dose, 1, 2, 3, 4, 5, 6, 8, and 12 h; Day 1: pre-dose, 1, 2, 3, 4, 5, 6, 8, and 12 h Days 3, 5, 7, 14, and 21: pre-dose; Day 28: pre-dose, 1, 2, 3, 4, 5, 6, 8, and 12 h; Days 35, 42 (only cohorts of 10 and 20 mg) and EOS.
A2107	Healthy Volunteers	One of the four studied regimens: placebo, 10 mg, Titration #1, Titration #2, once a day in the morning for 12 days.	SCR, BSL pre-dose, Days 12 and 22.
A1101	Healthy Volunteers (Japanese)	Single dose of placebo, 0.5, 2.5, 10 and 20 mg.	Run-in (Day-1): pre dose, 1, 2, 3, 4, 5, 6, 8, 12 h; Day 1: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 h; Days 2-7: 24, 48, 72, 96, 144 h; Days 10, 14: pre-dose.
A2110	Healthy Volunteers	Placebo, 0.5, 1, 2, and 4 mg with five drug discontinuation periods (48, 72, 96, 120 and 192 h) corresponding to 1, 2, 3, 4 and 7 missed doses.	SCR, BSL, Day 10 of daily dosing, the last day of the drug discontinuation period, pre-dose on the day of restarting dosing, study completion.
A2118	Healthy Volunteers	2 mg on Days 1-10, 10 mg on Days 11-18.	BSL, Days 1, 7, 10, 18, 32-39.
A2201	RRMS patients	1st patient group: Placebo, 10 mg, 2 mg, 0.5 mg. 2nd patient group: Placebo, 0.25 mg, 1.25 mg. It was taken once a day with or without food. For both patient groups in period 2, first 10 days of treatment had specified dose titration schedule.	SCR, BSL, Day 7 and at monthly visits until the end of double-blind treatment
A2304	SPMS	Core Part: BAF312 or placebo BAF312 2 mg daily oral dose, starting with a six-day dose uptitration period. Dose adjustment (to 1 mg/day without possible increase) is required for patients whose circulating lymphocyte level remains below 0.2 $x10^9$/L approximately for a week. Extension Part: open-label BAF312	SCR, BSL, Day 28, Months 3, 6, 9, 12, 15, 18, 21, 24, 27, 30/42/54, 33/39/45/51/57, 36/48, EOT, EOS and FO

SCR: screening (Day -45 to Day -8); BSL: baseline (Day -7 to Day 1 pre-treatment); EOT: end of treatment ; EOS: end of study; FO: follow-up

Source: Applicant's population PK/PD report (Table 3-2, Page 29).

Table 9: Summary of Subject Demographics, Population PK Analysis

	Statistic	Study 2304
Demographic	**Number of Subjects**	**1045**
Age (yrs)	Mean (SD)	47.9 (7.84)
	Median (Min-Max)	49.0 (23.0-61.0)
Body Weight (kg)	Mean (SD)	71.6 (15.7)
	Median (Min-Max)	70.0 (40.0-142)
Gender N (%)	Female	632 (60)
	Male	413 (40)
Race N (%)	Asian	29 (3)
	Black	7 (1)
	Caucasian	993 (95)
	Other	11 (1)
	Unknown	5 (0)
Ethnicity N (%)	Chinese	14 (1)
	Hispanic/Latino	68 (7)
	Japanese	14 (1)
	Mixed Ethnicity	17 (2)
	Other	657 (63)
	Russian	65 (6)
	Unknown	192 (18)
	West Asian	18 (2)
CYP2C9 Genotype N (%)	*2/*2	18 (2)
	*2/*3	29 (3)
	WT/*2	203 (19)
	WT/*3	131 (13)
	WT/WT (wild-type)	664 (64)

Source: Applicant's population PK/PD report (Table 5-1, Page 49).

Methods

- Population PK model development

The Applicant developed the population PK base model for siponimod based on the previous population PK model developed in healthy volunteers and RRMS patients, a two-compartment disposition model with first-order elimination and combined zero- and first-order absorption. As in the previous model, IIV was included on CL/F(clearance), Vc/F (central volume of distribution), Vp/F (peripheral volume of distribution), Q/F (between compartment clearance), absorption parameters Ka (constant of first-order absorption) and D1 (duration of the zero-order absorption). Correlations between random effects at the individual level (ETA) terms were modeled using a full block covariance matrix. Body weight on CL/F and Vc/F were in the previous final model and were included in the base model. Formulation on D1 was also included in the previous model, but only the tablet siponimod formulation was used in the SPMS study which was the reference formulation in the Applicant's previous model. Although CYP2C9 genotype on CL/F and food (FDA standardized high fat breakfast) on D1 were found to be significant in the previous analysis, they were not in the previous final model

as the effects of these covariates were estimated on reduced datasets; consequently, CYP2C9 genotype and food were not included in the base model. In addition, siponimod was given without regards to food in the SPMS study; consequently, the Applicant did not evaluate food effect. CYP2C9 genotype was evaluated in the covariate analysis. The plasma concentration data of siponimod were log-transformed prior to analysis, and an additive residual error structure was used.

The development of the population PK analysis for siponimod in only SPMS patients was based on Study A2304. Of the 3454 concentrations from Study A2304 included in the population PK analysis, 92 were flagged with a comedication affecting CYP2C9 or CYP3A4 enzymes. These concentrations were firstly not included in the estimation of parameters in the population PK analysis, but a prediction was obtained using missing dependent variable (MDV =1) and event identifier (EVID =2) flags in NONMEM. Consequently, 3362 concentrations were included in the estimation of parameters in the population PK analysis. Secondly, parameters estimates were obtained after including the concentrations flagged as comedications, then parameters estimates were compared for models with and without comedication effects.

Covariates available for evaluation in the population PK analysis included the following continuous and categorical covariates: age (yr) at baseline; body weight (kg) at baseline; CYP2C9 genotype (*1/*1 (wild-type), *1/*3, *1/*2, *2/*2, and *2/*3); ethnicity (only for Japanese and Chinese); gender, co-medications affecting CYP2C9 or CYP3A4 enzymes. Baseline covariates were obtained from observations on the first day of dosing or at screening if this value was not available. A backward deletion was performed at the p=0.001 (increased objective function value [OFV] less than 10.83 points, degrees of freedom [d.f.]=1) significance level where the relative influence of each covariate on the model was re-evaluated by deleting it from the full model on an individual basis. Where significant covariate effects are identified, assessment of effect magnitude over a relevant range, along with confidence interval (CI), was provided. A covariate may be retained in the final model, despite not meeting the criteria above, if there is a strong pharmacological or physiological rationale for its inclusion. The clinical relevance of covariates in the population PK model were evaluated using non-parametric bootstrap analysis by generating 1000 data sets through random sampling with replacement from the original data using the individual as the sampling unit.

Relevant covariates (e.g., CYP2C9 genotype for population PK model) was considered during the sampling process to ensure that the bootstrap datasets adequately represented the original data (with respect to distributions of covariates). Stratification by a categorical covariate meant that each bootstrap sample was created by sampling separately from populations with each value of the covariate, where each bootstrap sub-sample had the same number of individuals as the original dataset. Population parameters for each data set were estimated using NONMEM. This resulted in a distribution of estimates for each population model parameter. Empirical 95% CIs were constructed by obtaining the 2.5th and 97.5th quantiles of the resulting parameter distributions.

- Co-medications

A summary of the number of subjects administered comedications affecting CYP2C9 or CYP3A4 enzymes in studies 2304 and 2201 are summarized in Table . There were very few subjects receiving comedications affecting either CYP2C9 or CYP3A4 enzymes; 3, 0, 12, 5, 4 and 38 subjects were administered a CYP2C9-inducer, CYP2C9-inhibitor, CYP3A4-inducer, CYP3A4 strong inhibitor, CYP3A4 moderate inhibitor and CYP3A4 weak inhibitor, respectively. Of the 3933 concentrations from Studies A2304 and A2201 (3454 and 479, respectively), less than 1% were flagged as being associated with CYP2C9-inducer, CYP2C9-inhibitor, CYP3A4-inducer, CYP3A4 strong inhibitor or CYP3A4 moderate inhibitor, and only 2% were flagged as being associated with CYP3A4 weak inhibitor. Consequently, comedications affecting CYP2C9 or CYP3A4 enzymes were not formally tested in the covariate analysis, but a prediction was obtained as by using flags. To evaluate the effect of comedications, the Applicant compared the parameter estimates from models with comedications and the model where comedications were excluded.

Table 10: Summary of Subject Administered Comedications Affecting CYP2C9 or CYP3A4 Enzymes, Population PK Analysis

	Statistic	**Study 2201**	**Study 2304**	**Overall**
Demographic	**Number of Subjects**	**212 (17)**	**1045 (83)**	**1257**
CYP2C9-inducer N (%)	No	211 (100)	1033 (99)	1244 (99)
	Yes	1 (0)	12 (1)	13 (1)
CYP2C9-inhibitor N (%)	No	212 (100)	1044 (100)	1256 (100)
	Yes	.	1 (0)	1 (0)
CYP3A4-inducer N (%)	No	207 (98)	1012 (97)	1219 (97)
	Yes	5 (2)	33 (3)	38 (3)
CYP3A4 strong inhibitor N (%)	No	208 (98)	1037 (99)	1245 (99)
	Yes	4 (2)	8 (1)	12 (1)
CYP3A4 moderate inhibitor N (%)	No	209 (99)	1027 (98)	1236 (98)
	Yes	3 (1)	18 (2)	21 (2)
CYP3A4 weak inhibitor N (%)	No	196 (92)	869 (83)	1065 (85)
	Yes	16 (8)	176 (17)	192 (15)

Source: Applicant's population PK/PD report (Table 5-2, Page 50).

- Model diagnostics

The goodness-of-fit (GoF) was assessed by a variety of plots and computed metrics. The GoF plots included data points for observed and/or predicted data, reference lines (identity, zero line, etc.), and smooth lines through the data. Model diagnostics included a posterior prediction-corrected visual

predictive check (pc-VPC) which was performed for the final population PK model by simulating 500 datasets using the final models including covariates if applicable, sampling times and the dosing histories contained in the dataset. From these 500 simulations, median of the observed data (siponimod concentration) values for the PK model, 2.5th and 97.5th percentiles of the observed data, 95% prediction intervals (PI) for median of predicted data and 95% PI for the 2.5th and 97.5th percentiles of predicted data were plotted. The observed and simulated observations were normalized by the ratio between the median typical population predictions for the specific bin and the typical population prediction for the observation. This comparison was used to evaluate whether the derived model and associated parameters were consistent with the observed data.

Results

A total of 3454 siponimod concentrations (on average, 3 per subject) from 1045 SPMS patients were included in the population PK analysis. All patients received 2 mg oral daily dose and only 5 of them had a subsequent dose adjustment to 1 mg during the double-blind treatment period (with the majority of the doses being 2 mg for 3 of them). Parameters estimates for the final model in SPMS patients are displayed in Table . The effects of weight on CL/F and Vc/F were modeled using power functions where the exponents were fixed to 0.75 and 1 for CL/F and Vc/F, respectively. All the parameters were estimated with good precision (RSE% ranged from 1 to 7%). The estimates of Q/F, Vp/F, KA and D1 were fixed to the estimates from the model developed in healthy volunteers and RRMS patients. For the range of weights in the present analysis population (40-142 kg), Vc/F ranged from 71.5-254 L which was 43% lower to 101% higher compared to the typical value for a 70.5-kg subject and CL/F ranged from 2.03-5.26 L/hr which was 35% lower to 69% higher compared to the typical value for a 70.5-kg subject (with CYP2C9 genotype *1/*1 or *1/*2). Compared to CYP2C9 genotypes *1/*1 and *1/*2 in the analysis population, siponimod CL/F was 20% lower (95% CI: 9% to 30% lower) for CYP2C9 genotype *2/*2, 38% lower (95% CI: 35% to 41% lower) for CYP2C9 genotype *1/*3, and 48% lower (95% CI: 42% to 53% lower) for CYP2C9 genotype *2/*3. The goodness of fit plots and pc-VPC of the final siponimod population PK model are displayed in Figure and Figure 3, respectively.

Table 11: Parameter estimates for the final population model for siponimod in SPMS patients

Parameter [Units]	NONMEM Estimates Point Estimate	%RSE	95% CI	
CL /F [L/hr]	3.11	1.06	3.05-3.17	
V_c/F [L]	126	3.33	118-134	
Q /F [L/hr]	0.212 fixed	-	-	
V_p/F [L]	27.9 fixed	-	-	
KA [1/hr]	0.685 fixed	-	-	
D1 [hr]	1.72 fixed	-	-	
CL/F~Weight	0.750 fixed	-	-	
V_c/F~Weight	1.00 fixed	-	-	
CL/F~WT/*3	0.620	2.66	0.588-0.652	
CL/F~*2/*2	0.803	6.61	0.699-0.907	
CL/F~*2/*3	0.524	5.38	0.469-0.579	
Inter-individual				CV%* or R
$\omega^2_{CL/F}$	0.0584	7.50	0.0498-0.0670	24.2%
Covar $\eta_{CL/F}$, $\eta_{Vc/F}$	0.0398	26.1	0.0194-0.0602	0.499
$\omega^2_{Vp/F}$	0.109	30.2	0.0445-0.173	33.0%
$\omega^2_{Q/F}$	0.0999 fixed	-	-	31.6%
$\omega^2_{Vp/F}$	0.0694 fixed	-	-	29.9%
ω^2_{KA}	0.285 fixed	-	-	57.4%*
ω^2_{D1}	0.273 fixed	-	-	56.0%*
Residual variability				CV%
σ^2_{prop}	0.302	1.60	0.293-0.311	30.2

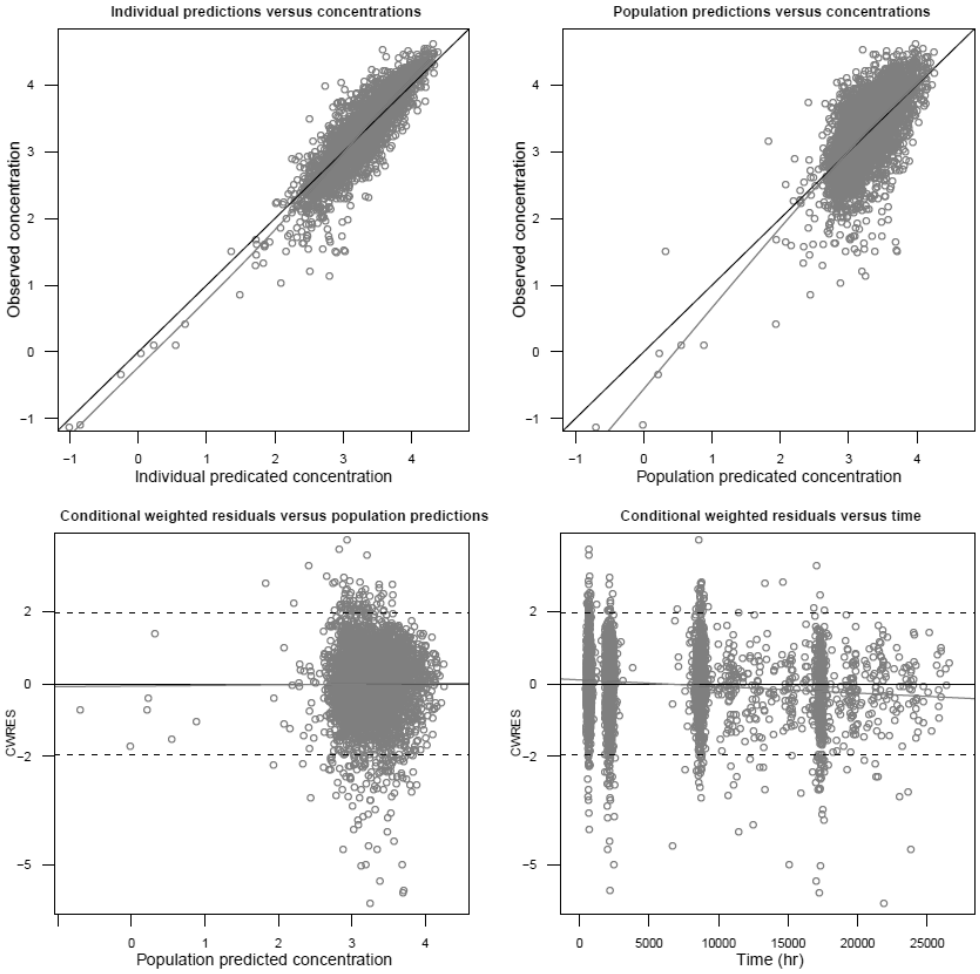
Abbreviations: %RSE: percent relative standard error of the estimate = SE/parameter estimate * 100; CL/F = apparent clearance, V_c/F = apparent volume of central compartment, Q/F = inter-compartmental exchange flow rate, V_p/F = apparent volume of peripheral compartment, D1= duration of the zero-order absorption, KA= absorption rate constant, σ^2_{prop} = proportional component of the residual error model,95% CI= 95% confidence interval on the parameter; R= correlation coefficient; $\omega^2_{CL/F}$, $\omega^2_{Vc/F}$, $\omega^2_{Q,/F}$, $\omega^2_{Vp/F}$, ω^2_{KA} and ω^2_{D1} = variance of random effect of CL/F, V_c/F, Q/F, V_p/F, KA and D1, respectively; Covar $\eta_{CL/F}$, $\eta_{Vc/F}$ = covariance of random effect of CL/F and Vc/F

The reference population for the PK parameters CL/F and V_c/F are a 70.5-kg subject with CYP2C9 genotype WT/WT or WT/*2.

*$CV_{TV_p} = \sqrt{\omega^2_p}$ when ω^2_P less than 0.15 and $CV_{TV_p} = \sqrt{e^{\omega^2_p} - 1}$ when ω^2_P exceeds 0.15

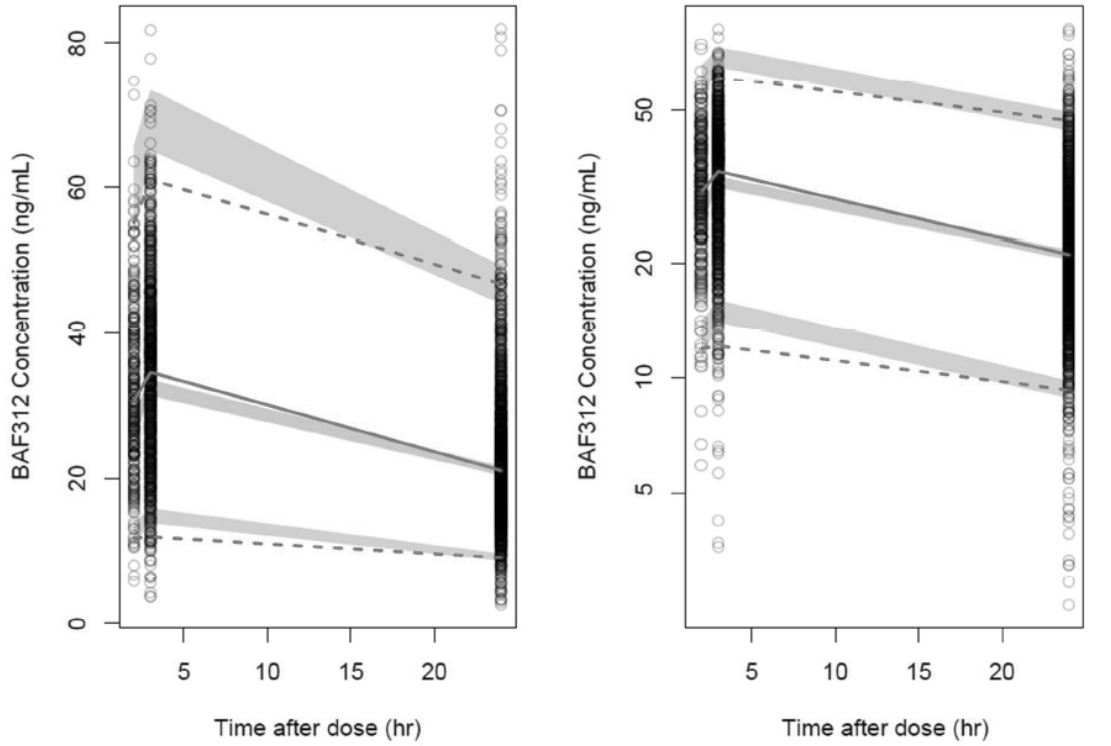
Source: Applicant's population PK/PD report (Table 5-9, Page 65-66).

Figure 2: Goodness of fit plots for the final population PK model for siponimod



Source: FDA analysis

Figure 3: Prediction-corrected visual predictive check for the final siponimod population PK model



Open Circle: Observed Concentrations; Solid Line: Median of Observed Concentrations; Dashed Lines: 2.5th and 97.5th Percentile of Observed Concentrations. Red Shaded Region: 95% Prediction Interval for Median of Predicted Concentrations; Blue Shaded Regions: 95% Prediction Intervals for the 2.5th and 97.5th Percentiles of Predicted Concentrations

Source: Applicant's population PK/PD report (Figure 5-9, Page 69).

Co-medication

Concentrations flagged with comedication CYP3A4 weak inhibitors and CYP3A4 inducers had the most data, with all other comedications affecting CYP2C9 or CYP3A4 enzymes having four concentrations or less. The final model excluded flagged concentrations. In study A2304, CYP2C9-inducer, CYP2C9-inhibitor, CYP3A4-inducer, CYP3A4 strong inhibitor and CYP3A4 moderate inhibitor were; 12, 1, 33, 8 and 18, respectively. Of the 3454 concentrations from Study A2304, less than 1% were flagged as being associated with CYP2C9-inducer, CYP2C9-inhibitor, CYP3A4 strong inhibitor or CYP3A4 moderate inhibitor, 2% were flagged as with CYP3A4-inducer and 13% were flagged as with CYP3A4 weak inhibitor in Study A2304. The incorporation of data from Study A2201 did not substantially increase the overall percentage of subjects or the percentage of concentrations flagged as being associated with a comedication of interest. Of the 3933 concentrations from studies A2201 and A2304, less than 1% were flagged as with CYP2C9-inducer, CYP2C9-inhibitor, CYP3A4 strong inhibitor or CYP3A4 moderate inhibitor, 2% were flagged as with CYP3A4-inducer and 13% were flagged as with

CYP3A4 weak inhibitor. CYP3A4 inhibitor on CL/F, where concentrations were flagged as on a CYP3A4 weak inhibitor if on a CYP3A4 weak inhibitor regardless if on another comedication (CYP2C9-inducer, CYP2C9-inhibitor and CYP3A4-inducer), was found not to be significant. It should be noted, that a decrease in CL/F is expected with CYP3A4 inhibitors and all the above estimates indicated an increase in CL/F. It was concluded that comedication affecting CYP2C9 or CYP3A4 enzymes did not impact siponimod in the Applicant's analysis.

Summary of Applicant's findings

- The PK of siponimod in SPMS patients was well described by a two-compartment model with a combined zero- and first-order absorption process and first-order elimination.
- Siponimod PK in SPMS patients was comparable to that in healthy volunteers and RRMS patients and do not have significant differences.
- Body weight was found to have a significant impact on siponimod PK. Vc/F ranged from 43% lower to 101% higher and CL/F ranged from 35% lower to 69% higher compared to a 70.5-kg individual for the range of weights in the present analysis population (40-142 kg).
- CYP2C9 genotype was found to have a significant impact on siponimod PK. Compared to *1/*1 and *1/*2, BAF312 CL/F was 48% (95% CI: 42% to 53%), 38% (95% CI: 35% to 41%) and 20% (95% CI: 9% to 30%) lower for genotypes *2/*3, *1/*3 and *2/*2.
- Based on a small number of subjects (n=14 for each), Japanese and Chinese ethnicities had no significant impact on siponimod PK.

Reviewer's comments: *Applicant's population PK analysis reasonably described the PK of siponimod in adult SPMS patients. The submitted final population PK model was reproducible and FDA reviewer agreed that it was appropriate. The population PK supports that no dose adjustment is necessary based on gender, body weight and age. However, the analysis was not conclusive on the use of comedications due to small samples sizes and circumstances where inhibitors and inducers were taken concurrently. Also, there was no data beyond 65 years of age.*

4.3 Exposure Response Analyses and Alternative Dosing Regimen Simulations

Not applicable

4.4 Physiologically based Pharmacokinetic Modeling Review

Executive Summary

The purpose of this review is to document the clinical pharmacology evaluation of the applicant's PBPK analyses submitted to support dosing recommendations of siponimod with concomitant administration of CYP3A4/CYP2C9 inhibitors or inducers.

The following PBPK modeling and simulation reports and updates were submitted and reviewed:

- PBPK Report entitled “Predictions of Siponimod (BAF312) PK and DDI interactions of typical CYP perpetrators using SimCYP” (DMPK R1600759-01)
- Response to FDA Information Request (Clinical Pharmacology) dated August 03, 2018
- Response to FDA Information Request (Clinical Pharmacology) dated October 19, 2018

The applicant’s PBPK analyses were considered adequate to predict the exposure of siponimod in subjects co-administered with CYP3A4/CYP2C9 inhibitors or inducers and carrying polymorphic CYP2C9 genotypes.

The PBPK analyses are adequate to support labeling language for siponimod genotype-based drug-drug interaction with CYP3A4/CYP2C9 modulators.

Methods

Model development

A population-based PBPK software Simcyp® (V16, Simcyp Ltd., a Certara Company, Sheffield, United Kingdom) was used by the applicant to develop the PBPK model for siponimod. The final siponimod model parameters and their sources are summarized in Supplementary Table 1.

Siponimod PBPK model was developed and verified using in vitro, human PK and mass balance data ([14C] human ADME study CBAF312A2104). A brief description of the workflow of model development for siponimod follows below:

A full PBPK model for siponimod was developed using a Kp scalar of 0.574 to allow recovery of the observed Vss value of 1.45 L/kg after siponimod intravenous administration (study CBAF312A2126-part 1). The blood-to- plasma concentration ratio of siponimod was determined to be 0.765 (DMPK R0400881-01). Unbound fraction in plasma (fu) determined in vitro was 0.02% (DMPK R0400881-01).

Mass balance data demonstrated no significant excretion of unchanged siponimod in urine (study CBAF312A2104). Therefore, siponimod model assumed no renal elimination and the renal clearance of siponimod was set to zero in the PBPK model.

Siponimod model assumed first order absorption. Based on mass balance data (study CBAF312A2104) siponimod fraction absorbed (fa) was at least 0.91. Siponimod absolute bioavailability was determined to be 84% (study CBAF312A2126). The model assumed gut secretion or direct excretion of siponimod via the bile unlikely because siponimod was not a substrate for efflux transporters (P-gp, BCRP or MRP2) (study DMPK R1300921) and the observed linear pharmacokinetics in the dose range of 0.1-75 mg single dose and 0.3-20 mg qd. The ka value was estimated by population PK analysis (BAF312A population PK 2014). A sensitivity analysis was performed to assess the impact of lag time on Tmax and to derive a value that allowed recovery of the observed Tmax (median 4 hours, range 2-12 hours) at 2 mg q.d, in healthy subjects (study CBAF312A2101). Fugut was set to be equal to unbound fraction in plasma (fugut=0.0002). Sensitivity analysis was used to support validity of the fugut value.

Oxidative metabolism was identified as the major contributor to siponimod clearance. The phase I metabolic reactions in the biotransformation of siponimod involved C-hydroxylation (M5, M6 and M7), cleavage/hydrolysis at the oxime ether bound (M1, M2) and further reduction yielding metabolite M8. Phase II reactions of hydroxylated metabolites involved sulfation to yield M4a, M4b and M4c (from M5, M6 and M7, respectively) and glucuronidation to yield M3 (from M5) and M12 (formed by hydroxylation followed by glucuronidation) (study CBAF312A2104). In vitro metabolism of siponimod was determined using in human liver microsomes (HLM) and recombinant human CYPs (rhCYPs). Enzyme kinetics parameters for the CYPs were determined using rhCYPs. Based on kinetic results with rhCYPs and the relative abundance of the CYP isoforms in HLM, CYP2C9 was estimated to be the main enzyme responsible for siponimod metabolism (fm,CYP2C9=79.3%), followed by a partial contribution from CYP3A (fm,CYP3A4=18.5%), and minor contribution from CYP2B6 (fm,CYP2B6=0.32%), CYP2C8 (fm,CYP2C8=1.74%), CYP2C19 (fm,CYP2C19=0.16%) and possibly CYP1A1 (DMPK R0500432). Inhibition of siponimod metabolism by selective CYP chemical inhibitors in HLMs demonstrated significant effect with a selective inhibitor for CYP2C9, and lesser inhibition with a selective inhibitor for CYP3A4. Siponimod main metabolite in feces, M5, was determined to be mainly catalyzed by CYP2C9 (DMPK R0500432). Collectively, in vitro data and mass balance data suggest that CYP2C9 contributes predominantly to the metabolism of siponimod with partial contribution from CYP3A.

A retrograde approach was used to estimate the total hepatic metabolic intrinsic clearance (CLuH,int) value from the mean observed CL of 3.12 L/h following single intravenous administration of siponimod (study CBAF312A2126). The CLuH,int value calculated was then assigned to the respective CYP metabolic pathways based on the relative contribution (fm,CYP), as stated above. Intrinsic clearance of allelic CYP2C9 variants was calculated relative to wild-type value (Clint= 45.1 µL/min/mg) based on clearances values estimated from population PK analysis for each genotype (Supplementary Table 2). Genotype-specific reduction in clearance relative to the wild-type genotype (CYP2C9*1/*1, CL= 3.12 L/h)) were observed. While subjects carrying CYP2C9*1/*2 had no difference in clearance (3.16 L/h), subjects carrying *2/*2, *1/*3, *2/*3, and *3/*3 had approximately 20%, 35%, 45% and 74% reduced clearance values (2.5 L/h, 2.0 L/h, 1.7 L/h and 0.8 L/h), respectively (BAF312A Population PK).

Siponimod exhibited no significant modulatory potential toward CYP enzymes. The most potent inhibition is towards CYP2C9 with a Ki,u value of 0.26 µM which is approximately 430-fold higher than the observed unbound Cmax value at multiple doses of 2 mg ($C_{max,u_{ss}}$) of 0.6 nM (fu value of 0.01). Thus, interaction kinetic parameters for siponimod were not incorporated in the model.

Model verification

The developed siponimod model was verified by comparing the simulated plasma PK profile after single dose and multiple dose administration of siponimod with those observed in the phase 1 clinical studies CBAF312A2126, CBAF312A2101, and CBAF312A2105. The ability of siponimod PBPK to predict

the pharmacokinetics in subjects carrying different genotypes for CYP2C9 was verified comparing the observed data from the phase 1 PK study CBAF312A2128-part 1 and simulated results. Clinical DDI studies were also used to verify the fractional contribution of CYP3A and CYP2C9 to siponimod metabolism. PBPK simulations were performed according to the trial designs presented in Table 12.

Table 12. PBPK simulation design parameters for siponimod model verification

#	Trials x N	Clinical Study	Siponimod Dose Regimen*	Perpetrator	Dose Regimen	Demographics	CYP2C9 Genotype	PBPK Model Objective
8	10x11	Not available	2 mg qd for 18 days	fluconazole	200 mg qd for 18 days	Age 18-33y 0 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
9	10x11	Not available	2 mg qd for 18 days	fluconazole	400 mg qd for 18 days	Age 18-33y 0 female	*1/*1; *1/*2; *1/*3; *2*2; *2/*3	Prediction
10	10x10	Not available	2 mg qd for 17 days	ketoconazole	200 mg bid for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
11	10x10	Not available	2 mg qd for 17 days	ketoconazole	400 mg qd for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1*/3; *2/*2; *2/*3	Prediction
12	10x10	Not available	2 mg qd for 17 days	erythromycin	500 mg bid for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
13	10x10	Not available	2 mg qd for 17 days	erythromycin	500 mg qid for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
14	10x10	Not available	2 mg qd for 17 days	fluvoxamine	100 mg qd for 18 days	Age 18-55y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
15	10x10	Not available	2 mg qd for 36 days	efavirenz	600 mg qd, for 36 days	Age 18-45y 0.5 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
16	10x16	Not available	2 mg qd for 36 days	rifampicin	600 mg qd for 36 days	Age 18-45y 0.5 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction

*Software's virtual healthy volunteer populations were used for all simulations. (Source: Tables 3-1, 3-2, 3-3 and 3-4 from PBPK report)

All simulations were performed using the software's library healthy volunteer population. This assumption was considered adequate because no clinical relevant difference in the pharmacokinetics of siponimod was noted between healthy volunteer and multiple sclerosis patient population based on population PK analysis (BAF312A Population PK report). All simulations were conducted in the fasted state as food had no significant effect on siponimod exposure regarding Cmax and AUC at 4 mg dose (CBAF312A2111).

The default perpetrator models for itraconazole "SV-Itraconazole_Fed Capsule", and rifampicin "SV-Rifampicin-MD" from the software's compound library (V16) were used in the PBPK simulations for the respective DDIs. Internal verification of the compound files was performed using respective DDI studies with midazolam. The library fluconazole model "SV-Fluconazole" (V16) was modified by

changing the CYP2C9 inhibition constant Ki value from 7.92 to 20.4 µM based on published data[1]. Verification of the modified fluconazole model was performed by comparing observed and predicted DDI effect between fluconazole with the index CYP2C9 substrate S-warfarin with both the default and modified models (data not shown). A less potent inhibition constant improved predictability of the clinical DDI effect (AUCR). The applicant's modified fluconazole model was considered adequate to verify the contribution of the CYP2C9 pathway using clinical DDI studies with fluconazole. Of note, this proposed changed in the inhibition constant of fluconazole has been verified by the software developer and software's library model for fluconazole has been updated in version 17.

Model application

Siponimod PBPK model was used to predict the effect of CYP3A/2C9 modulators on the PK of siponimod in subjects with different CYP2C9 genotypes, according to the trial designs presented in Table 13.

Table 13. PBPK simulation design parameters for siponimod model application

#	Trials x N	Clinical Study	Siponimod Dose Regimen*	Perpetrator	Dose Regimen	Demographics	CYP2C9 Genotype	PBPK Model Objective
8	10x11	Not available	2/1 mg qd for 18 days	fluconazole	200 mg qd for 18 days	Age 18-33y 0 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
9	10x11	Not available	2/1 mg qd for 18 days	fluconazole	400 mg qd for 18 days	Age 18-33y 0 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
10	10x10	Not available	2/1 mg qd for 17 days	ketoconazole	200 mg bid for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
11	10x10	Not available	2/1 mg qd for 17 days	ketoconazole	400 mg qd for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
12	10x10	Not available	2/1 mg qd for 17 days	erythromycin	500 mg bid for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
13	10x10	Not available	2/1 mg qd for 17 days	erythromycin	500 mg qid for 17 days	Age 18-50y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
14	10x10	Not available	2/1 mg qd for 17 days	fluvoxamine	100 mg qd for 18 days	Age 18-55y 0.25 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
15	10x10	Not available	2/1 mg qd for 36 days	efavirenz	600 mg qd, for 36 days	Age 18-45y 0.5 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction
16	10x16	Not available	2/1 mg qd for 36 days	rifampicin	600 mg qd for 36 days	Age 18-45y 0.5 female	*1/*1; *1/*2; *1/*3; *2/*2; *2/*3	Prediction

[1] Neal JM, Kunze KL, Levy RH, et al (2003) Ki iv, an in vivo parameter for predicting the magnitude of a drug interaction arising from competitive enzyme inhibition. Drug Metab Dispos 31:1043–1048.

**Siponimod dose of 2 mg qd for the genotypes CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2/*2 and dose of 1 mg qd for CYP2C9*1/*3 and CYP2C9*2/*3. Software's virtual healthy volunteer populations were used for all simulations (Source: Tables 3-3 and 3-4 from PBPK report and Response to IR dated 10/19/18)

The default perpetrator models for ketoconazole, erythromycin, fluvoxamine, rifampicin, and efavirenz from the software's compound library (V16) were used in the PBPK simulations for the respective DDIs. Internal verification of the compound files was performed using respective DDI studies with midazolam or simvastatin.

Results

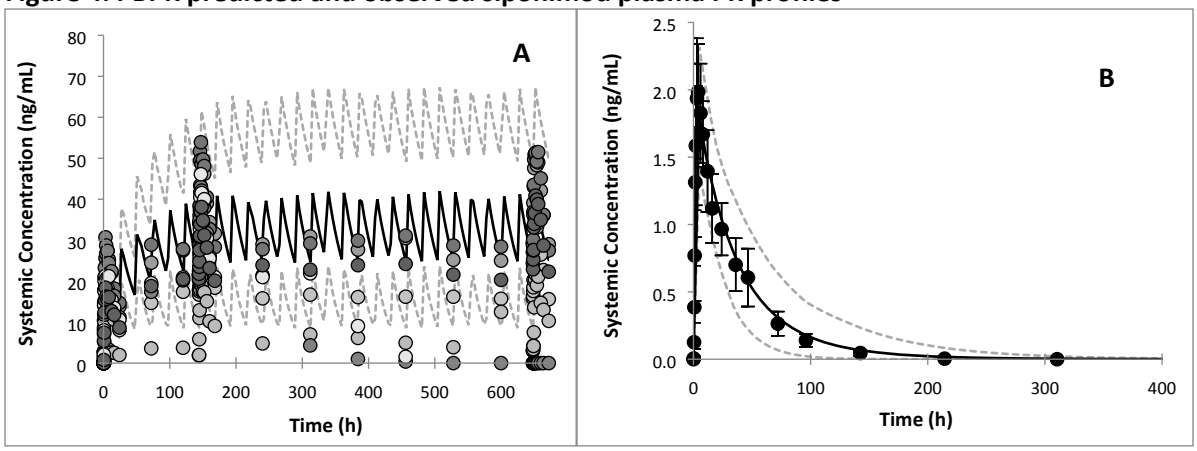
Does PBPK model provide a reasonable description of the PK of siponimod?

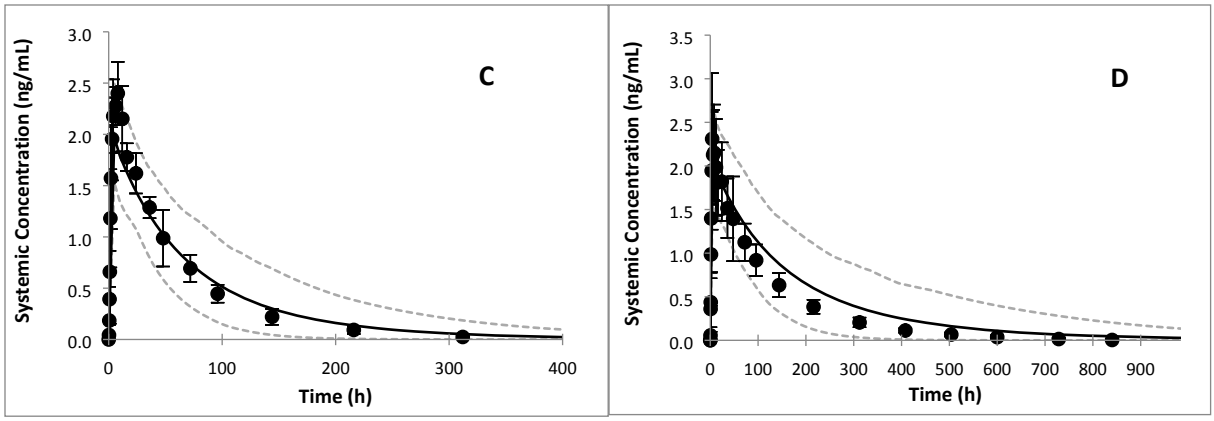
Yes, PBPK simulations reasonably described siponimod plasma PK profile in healthy subjects (studies CBAF312A2126; CBAF312A2101; CBAF312A2105) both after single dosing (0.1-75 mg) and at steady-state (0.3-20 mg qd for 28 days) (Simulations #1-3, Table 12). The predicted geometric mean values for AUC (AUCinf or AUC24h), Cmax, and Cmin were within 80-130% of the observed values. Siponimod linear PK observed clinically (up to 75 mg single dose and 20 mg qd) was captured by the model, with a mean accumulation ratio (Racc, AUC at Day 28 and at Day 1 following 0.3-20 mg qd) approximately 2.0, compared to observed Racc range of 1.9-2.8.The PK profile of siponimod following multiple dose is illustrated in Figure 4A and listed in Table 14.

The model assumed that the fractional contribution of CYP2C9 (fm,CYP2C9) and CYP3A4 (fm,CYP3A4) to siponimod metabolism were approximately 80% and 18%, respectively (Supplementary Table 1). This assumption was verified using genotype PK data (study CBAF312A2128) and clinical DDI data (see section below).

Siponimod fraction metabolized via CYP2C9 was initially verified by comparing PBPK predictions (Simulations # 4, Table 12) with observed PK data from a genotype PK study (CBAF312A2128) conducted in subjects carrying the CYP2C9 alleles *1/*1, *2/*3, and *3/*3. The predicted geometric mean values for AUCinf, Cmax, and half-life (t½) were within 90-128% of the observed values (Table 14).

Figure 4. PBPK predicted and observed siponimod plasma PK profiles





Simulated mean (lines) and observed individual and mean (circles) plasma concentration-time profiles of siponimod following a single or multiple oral dose. The solid black line and dashed grey lines represent simulated mean time-plasma concentration profiles and the 5th/95th percentile of the total virtual population. In Figure 4A, siponimod dose of 2.5 mg qd for 28 days (Simulation # 3, Table 12. Observed data from study CBAF312A2105). In Figures 4B, C and D, siponimod single dose of 0.25 mg on Day 1 in sub-populations carrying the three CYP2C9 genotypes *1*1 (Figure 4B) *2*3 (Figure 4C) and *3*3 (Figure 4D) (Simulations # 4, Table 12. Observed data from study CBAF312A2128) (Source: Simulation output files).

Table 14. Comparison of observed and predicted PK parameters of siponimod following single and multiple oral administration

Dose	CYP2C9 genotype	Cmax (ng/mL)		AUC (ng.h/mL)		Cmin (ng/mL)		t1/2 (h)	
		Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
2.5 mg qd for 28 days	No exclusion (N=7)	40.0 (38)	38.3 (37)	719 (48)	692 (45)	20.9 (61)	15.2 (120)	NA	NA
0.25 mg SD	*1/*1 (N=12)	1.90 (14)	2.03 (17.2)	70.6 (37)	70.5(21.2)	NA	NA	25.5 (37)	28.1 (18.5)
	*2/*3 (N=6)	2.02 (14)	2.45 (13.1)	142 (39)	144 (15.7)	NA	NA	49.2 (42)	50.9 (31.7)
	*3/*3 (N=6)	2.10 (15)	2.35 (29.0)	342 (42)	271 (22.4)	NA	NA	118 (49)	126 (12.7)

Data are presented as geometric means (%CV). Observed values from studies CBAF312A2105 and CBAF312A2128. Trial design parameters: simulations # 3 and 4 from Table 12 (Source: Tables 6-8 and 6-10 of PBPK reference and reviewer's analysis).

The predicted impact of carrying polymorphic CYP2C9 variants (reduced CYP2C9 metabolism) in siponimod clearance is shown in Table 15 (predicted PK ratios expressed as the fold change in a polymorphic population versus the wild-type (CYP2C9*1/*1) population). Approximately 40% and 50% reduction in clearance was predicted in *1/*3 and *2/*3 populations, respectively. Genotype-based dose management (i.e., use of half- maintenance dose (1 mg qd) in subjects carrying the *3 allele) provided comparable exposure in this sub-population compared to wild-type.

Table 15. PBPK predicted state-state PK parameters for siponimod in CYP2C9 polymorphic population relative to wild-type

CYP2C9 Genotype	Dose (mg)	Cmax,ss (ng/mL)	Cmax ratio	AUCtau (ng/mL.h)	AUC ratio	Cmin,ss (ng/mL)	Cmin ratio	CL (L/h)	CL ratio
***1/*1**	2	33.6	1	587	1	16.3	1	3.40	1
***1/*2**	2	35.4	1.06	632	1.08	18.1	1.10	3.16	0.93
***2/*2**	2	42.5	1.27	803	1.37	24.9	1.53	2.49	0.73

***1/*3**	1	24.6	0.73	483	0.82	15.7	0.96	2.07	0.61
***2/*3**	1	28.6	0.85	576	0.98	19.4	1.19	1.74	0.51

Data presented as population geometric mean values for PK parameters (Cmax, AUCtau, Cmin and CL) at steady-state and ratios expressed as the fold change in the CYP2C9 polymorphic populations versus the wild-type population (CYP2C9*1/*1)**.** Siponimod dose of 2 mg qd for the genotypes CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2*2 and dose of 1 mg qd for CYP2C9*1/*3 and CYP2C9*2/*3. Trial design parameters: Control group- simulations #8-17, Table 13. (Source: Simulation output files- Response to IR dated 10/19/18- Reviewer's analysis)

Does PBPK model provide a reasonable prediction of the effects of CYP3A4/ CYP2C9 modulators on the PK of siponimod?

Yes. The predicted effects of the strong CYP3A4 inhibitor itraconazole (study CBAF312A2124); the moderate CYP3A4/CYP2C9 inhibitor fluconazole (study CBAF312A2108), and the strong CYP3A4/ moderate CYP2C9 inducer rifampicin (study CBAF312A2125) on the systemic exposure of siponimod were retrospectively evaluated to verify the fractional contribution of CYP2C9 and CYP3A4 in subjects carrying CYP2C9*1/*1 genotype.

The effect of fluconazole inhibition (Simulation #5, Table 12) or rifampicin induction (Simulation #6, Table 12) on both CYP3A4 and CYP2C9 metabolic pathways were reasonably predicted by PBPK modeling. The predicted geometric mean Cmax and AUC ratios for siponimod following co-administration of fluconazole (Table 16) or rifampicin (Table 17), relative to the control in the absence of the inhibitor or inducer, were within 80-125% of the observed ratios. The predictions of the effect of fluconazole and rifampicin on the PK of siponimod indicated that the relative contributions of CYP2C9 and CYP3A4 to siponimod metabolism are adequately assigned in siponimod PBPK model.

Table 16. Comparison of observed and predicted Cmax and AUC values of siponimod (4 mg single oral dose on day 3) in the absence and presence of fluconazole (200 mg, day 1 bid, day 2-19 qd)

CYP2C9 genotype	Inhibition	Cmax (ng/mL)		AUCinf (ng.h/mL)		Cmax ratio (90%CI)		AUC ratio (90%CI)	
		Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
***1/*1**	Control	28.8	31.2	1088	1120	1.07	1.10	2.10	1.98
	With fluconazole	30.8	34.0	2288	2190	(1.06-1.07)	(1.04-1.16)	(2.06-2.14)	(1.87-2.10)

PK data are presented as geometric means. Observed values (N=11) from study CBAF312A2124. Trial design parameters: simulation #5, Table 12 (Source: Table 6-12 of PBPK report and reviewer's analysis).

Table 17. Comparison of observed and predicted Cmax and AUC values of siponimod (2 mg qd, 12 days) in the absence and presence of rifampicin (600 mg bid, 12 days)

CYP2C9 genotype	Induction	Cmax (ng/mL)		AUCinf (ng.h/mL)		Cmax ratio (90%CI)		AUC ratio (90%CI)	
		Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
***1/*1**	Control	32.5	29.0	565	554	0.50	0.55	0.32	0.43
	With rifampicin	16.3	16.0	181	239	(0.49-0.51)	(0.52-0.58)	(0.31-0.33)	(0.41-0.45)

PK data are presented as geometric means. Observed values (N=15) from study CBAF312A2125. Trial design parameters: simulation #6, Table 12 (Source: Table 6-16 of PBPK report and reviewer's analysis).

PBPK simulation results (Simulation #7, Table 12) were not consistent with the results of the clinical itraconazole DDI study (Table 18). The underlying mechanism leading to a reduced exposure of

siponimod in the presence of itraconazole is currently unknown. Thus, the mechanism of this interaction could not be incorporated in the PBPK model. Correspondingly, the clinical DDI observation with itraconazole should not be extrapolated to other selective CYP3A4 inhibitors.

Table 18. Comparison of observed and predicted Cmax and AUC values of siponimod (0.25 mg single oral dose at day 5) in the absence and presence of itraconazole (100 mg bid, 17 days)

CYP2C9 genotype	Inhibition	Cmax (ng/mL)		AUCinf (ng.h/mL)		Cmax ratio (90%CI)		AUC ratio (90%CI)	
		Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
*1/*2	Control	1.98	1.91	75.9	75.0	1.02 (1.02-1.02)	1.01 (0.96-1.06)	1.18 (1.17-1.18)	0.90 (0.84-0.96)
	With itraconazole	2.02	1.91	89.4	66.2				
*1/*3	Control	2.06	1.81	115	96.8	1.02 (1.02-1.02)	0.94 (0.91-0.97)	1.28 (1.26-1.30)	0.76 (0.69-0.82)
	With itraconazole	2.10	1.70	147	73.2				

PK data are presented as geometric means. Observed values (N=16 [CYP2C9*1*2] and 13 [*1*3]) from study CBAF312A2124. Trial design parameters: simulation #7, Table 12 (Source: Table 6-11 of PBPK report and reviewer's analysis).

Overall, the Applicant's siponimod PBPK model provided a reasonable description of siponimod PK, including the impact of polymorphic CYP2C9 variants, and was considered adequate to predict the effects of CYP3A4/CYP2C9 modulators on the steady state pharmacokinetics of siponimod.

What are the effects of CYP3A4/CYP2C9 modulators on siponimod PK?

To evaluate the DDI liabilities of siponimod as a victim, the DDI effects of strong, moderate and weak inhibitors for CYP3A4/CYP2C9 and strong or moderate inducers of CYP3A4/CYP2C9 on the PK of siponimod were simulated in sub-populations carrying polymorphic CYP2C9 variants, namely CYP2C9*1/*1, *1/*2, *1/*3, *2/*2 and *2/*3 (Simulations #8-17, Table 13).

The potential combined effect of CYP2C9 polymorphism and concomitant administration of CYP3A4/CYP2C9 inhibitors or inducers, as predicted by PBPK simulations (predicted PK ratios expressed as the fold change in a polymorphic population with a perpetrator versus the wild-type population without a modulator), is shown in Table 19.

Table 19. PBPK predicted Cmax and AUC ratios for siponimod in the presence of CYP3A4/CYP2C9 modulators in CYP2C9 polymorphic population relative to wild-type

Perpetrator	CYP2C9 Genotype									
	*1/*1		*1/*2		*2/*2		*1/*3		*2/*3	
	CmaxR	AUCR	CmaxR	AUCR	CmaxR	AUCR	CmaxR	AUCR	CmaxR	AUCR
fluconazole 200 mg qd	1.88	2.18	1.97	2.30	2.43	2.92	1.42	1.74	1.62	2.00
fluconazole 400 mg qd	2.60	3.15	2.72	3.32	3.29	4.09	1.89	2.38	2.10	2.67
ketoconazole 200 mg bid	1.18	1.24	1.27	1.37	1.62	1.84	0.98	1.16	1.19	1.44
ketoconazole 400 mg qd	1.17	1.22	1.25	1.34	1.58	1.79	0.95	1.12	1.14	1.38
erythromycin 500 mg bid	1.11	1.14	1.19	1.25	1.49	1.66	0.89	1.03	1.06	1.26

erythromycin 500 mg qid	1.13	1.17	1.21	1.28	1.53	1.72	0.92	1.07	1.10	1.32
fluvoxamine 100 mg qd	1.33	1.42	1.40	1.53	1.67	1.89	0.96	1.11	1.07	1.27
rifampicin 600 mg qd	0.46	0.29	0.48	0.29	0.54	0.37	0.29	0.22	0.32	0.25
efavirenz 600 mg qd	0.77	0.69	0.80	0.73	0.90	0.86	0.49	0.48	0.52	0.53

Data are presented as population geometric mean values for exposure ratios (AUCtau and Cmaxss) at steady-state expressed as the fold change in the CYP2C9 polymorphic populations with perpetrator versus the wild-type population (CYP2C9*1/*1) without perpetrator. Data without inhibitor are presented in Table 15. Siponimod dose of 2 mg qd for the genotypes CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*2/*2 and dose of 1 mg qd for CYP2C9*1/*3 and CYP2C9*2/*3. Trial design parameters: simulations #8-16, Table 13.
(Source: Simulation output files- Response to IR dated 10/19/18)

Based on the PBPK predictions, co-administration of siponimod (maintenance dose of 2 mg qd for the genotypes CYP2C9*1/*1, *1/*2, and*2/*2, and 1 mg qd for CYP2C9*1*3 and *2*3) with ketoconazole and erythromycin, a strong and moderate CYP3A4 inhibitor, respectively, or fluvoxamine, a weak CYP2C9 and CYP3A4 inhibitor, resulted in less than 2-fold increase in siponimod Cmax and AUC, compared to a wild-type patient population without the inhibitor, for all different CYP2C9 genotypes (Table 19).

The concomitant use of siponimod with 200 mg qd fluconazole, a moderate inhibitor of CYP3A4 and CYP2C9, resulted in 1.4- to 3-fold increase in siponimod Cmax and AUC, compared to a wild-type patient population without the inhibitor, across the different CYP2C9 genotypes. The concomitant use of the highest fluconazole dose (400 mg qd) resulted in an increase of approximately 2- to 4-fold in siponimod AUC for all different CYP2C9 genotypes (Table 19).

The concomitant use of siponimod with 600 mg qd rifampicin, a strong inducer of CYP3A4 and moderate inducer of CYP2C9, resulted in a predicted decrease around 60-80% in siponimod AUC across the CYP2C9 genotypes. Induction of CYP3A pathway only by co-administration of the moderate CYP3A4 inducer efavirenz (600 mg qd) resulted in approximately 50% decrease in siponimod AUC in the CYP2C9 *1/*3 and *2/*3 genotypes (Table 19).

Conclusions

The PBPK model of siponimod was considered adequate to predict siponimod exposure in subjects co-administered with CYP3A4/CYP2C9 modulators and carrying polymorphic CYP2C9.

The PBPK analyses are adequate to support labeling language for siponimod interaction with CYP3A4/CYP2C9 modulators.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Input parameters for siponimod PBPK model (Simcyp®, V16)

INPUT PARAMETERS	VALUE	UNITS	COMMENT/SOURCE
Physicochemical and binding properties			
MW	516.6	g/mol	internal data
Log P	1.8	-	internal data
Compound type	Ampholyte	-	internal data
pKa	3.1/8.1	-	internal data
B/P	0.765	-	in-vitro, DMPK R0400881-01
fu,p	0.0002	-	in-vitro, DMPK R0400881-01
Absorption	First order		
fa	0.91	-	Assumed based on clinical observation, CBAF312A2104
CV fa	8.6	%	Population PK report
Ka	0.687	1/h	Population PK report
CV ka	7.8	%	Population PK report
Lag time	1.5	h	Optimized
PAMPA	10	10-6 cm/s	internal data
fugut	0.0002	-	Assumed, SA
Qgut	9.851	L/h	Predicted
Distribution	Full PBPK, Method 2		
Vss	1.45	L/h	CBAF312A2126
CV Vss	3.4	%	Population PK report
Kp scalar	0.574	-	Optimized
Elimination	Enzyme kinetics		
CLint,u (CYP2B6)	0.7743	µL/min/pmol	Calculated based on retrograde method using CLiv of 3.12 L/h (CBAF312A2126, DMPK R0500432) and fractional contribution based on in vitro data
Clint,u (CYP2C8)	3.0138	µL/min/pmol	
Clint,u (CYP2C19)	0.4686	µL/min/pmol	
Clint,u (CYP3A4)	5.6151	µL/min/pmol	
Clint,u (CYP2C9*1/*1)	45.0799	µL/min/pmol	Calculated based on Population PK CLearance data (see Supplementary Table 2)
Clint,u (CYP2C9*1/*2)	45.8599	µL/min/pmol	
Clint,u (CYP2C9*1/*3)	24.5911	µL/min/pmol	
Clint,u (CYP2C9*2/*2)	33.2528	µL/min/pmol	
Clint,u (CYP2C9*2/*3)	18.9130	µL/min/pmol	
Clint,u (CYP2C9*3/*3)	2.86692	µL/min/pmol	
CLr	0	L/h	Assumed based on clinical observation, CBAF312A2104

(Source: Table 4 of PBPK report and Response to IR dated 10/19/18). Abbreviations: B/P: blood-to-plasma ratio; CLr: renal clearance; CLu,H,int: hepatic metabolic intrinsic clearance; CV: coefficient of variation; fa: fraction absorbed; fu: fraction unbound; fugut: Unbound fraction in enterocytes; ka: first order absorption rate constant; Kp scalar: Extent of tissue Kp; log P: partition coefficient; MW: molecular weight; pka: acid dissociation constant; Qgut: nominal flow in the gut model; Vss: volume of distribution at steady-state.

Supplementary Table 2. Intrinsic clearance values of allelic CYP2C9 genotypes calculated based on genotype specific clearance data from Population PK analysis

Genotype	Total CL (L/h)[a]	CL driven by CYP2C9 (L/h) [b]	Clint CYP2C9 multiplied by scaling factor A [c]	Relative contribution compared to CYP2C9*1*1[d]	CLint,u CYP2C9 (µL/min/pmol)[e]
***1/*1**	3.12	2.48	0.6212	1	45.0799[f]

***1/*2**	3.16	2.51	0.6320	1.0173	45.8599
***1/*3**	2.028	1.38	0.3389	0.5455	24.5911
***2/*2**	2.496	1.85	0.4582	0.7376	33.2528
***2/*3**	1.716	1.07	0.2606	0.4195	18.9130
***3/*3**	0.8112	0.165	0.0395	0.06359	2.86692

[a] Population PK CLearance data (report CBAF312A-Phase 1-2-PopPK). Genotype-specific CL reduction towards *1/*1 reported were 1.3% increased for CYP2C9*1/*2, and 35%, 20%, 45% and 74% decreased for *1/*3, *2/*2, *2/*3, and *3/*3, respectively.
[b] Calculation of plasma clearance driven by CYP2C9: CLp(2C9) = total CL –total CL x 0.207, where fm,CYP2C9= 0.793 and fm,other=0.207.
[c] CLint,CYP2C9 calculated by solving well-stirred model equation: CLint,CYP2C9 * Factor A = CLp(2C9)*Qp / (Qp-CLp(2C9)), with CLp and Qp as plasma clearance and liver plasma flow, respectively. Factor A correction factor accounting for the product of fup/fumic*hepatic enzyme abundance*scaling factor to account for unit change.
[d]Calculated by dividing the CLint,CYP2C9*Factor A of each respective genotype by the Clint,CYP2C9*Factor A of the genotype CYP2C9*1/*1.
[e] CLint,u CYP2C9 for each genotype was calculated by multiplying the CLint,u CYP*2C9*1/*1 with the relative contribution compared to CYP2C9*1*1.[f] CLint,u CYP2C9 calculated by retrograde calculator in SimCYP.
(Source: Table 6-4 of PBPK report and Response to IR dated 10/19/18).

4.5 Pharmacogenomics Summary

Executive Summary

The sponsor's classification of subject's CYP2C9 genotype assignments is acceptable. CYP2C9 genotype calls assigned by the sponsor were confirmed by the reviewer. In the reviewer's assessment, the CYP2C9 genotype calls of subjects can be reliably utilized in the PopPK and PBPK analyses, which demonstrates that CYP2C9 genotype status has a significant impact on siponimod exposures. The sponsor has proposed not giving CYP2C9 *3/*3 subjects siponimod and adjusting doses in *1/*3, (b) (4), and *2/*3 subjects in their proposed label.

Background

Siponimod is a new member of a class of oral compounds referred to as sphingosine-1-phosphate (S1P) receptor modulators. Sphingosine 1-phosphate (S1P) is a well-described natural ligand with key roles in the immune, cardiovascular, and central nervous systems through its action on five G-protein-coupled receptors (S1P1-5). The sponsor is seeking approval for the use of siponimod film coated tablets for treatment of patients with SPMS.

The sponsor has proposed not dosing *3/*3 subjects and dosing recommendations for CYP2C9 *1/*3, *2/*3, and (b) (4) subjects lowering them from a maintenance dose of 2 mg/day down to 1 mg/day, (b) (4). A dedicated genotype study (BAF312A2128) and PopPK analyses showed CYP2C9 *2/*3 (N=35) and CYP2C9 *1/*3 subjects (N=131) have an approximately 2-fold higher AUC, CYP2C9 *3/*3 subjects (N=6) had an approximately 4-fold increase in AUC, and CYP2C9 (b) (4) subjects (N=18) had an approximate 20% increase in AUC. No other dosing recommendations based on CYP2C9 metabolizer status are proposed by the sponsor.

The purpose of this review is to evaluate the CYP2C9 genotype information submitted by the sponsor and confirm the genotypes of subjects utilized in the dedicated genotype study and PopPK analyses for siponimod.

Submission Contents Related to Genomics

The sponsor submitted the following reports and datasets related to the pharmacogenetic (PGx) analysis of siponimod:

Table 20: Reports and Datasets Pertaining to PGx Analyses of Siponimod

Report Description	***Datasets***
Subject Level Genotypes for Study BAF312A2128	BAF312A2128-genotyping.xlsx
Subject Level Genotypes for Study BAF312A2304	BAF312A2304-genotyping.xlsx

A summary of the studies utilized to investigate the effects of CYP2C9 genotype on siponimod PK is provided in Table 21 below. Of note, study BAF312A2128 was a dedicated study to investigate CYP2C9 poor metabolizer (PM) and normal metabolizer (NM) phenotypes and the effect on siponimod PK; study BAF312A2304 was utilized for popPK analyses. Normal metabolizer phenotype is also referred to as extensive metabolizer phenotype in the submission.

Table 21: Clinical Trials Where Genotypes Were Verified by Reviewer

Study	***Description***	***Number of Subjects***
BAF312A2128	Open-label study to assess the pharmacokinetics, safety and tolerability of siponimod in healthy subjects with CYP2C9 extensive (EM) and poor metabolizer (PM) phenotype	24
BAF312A2304	A multicenter, randomized, double-blind, parallel-group, placebo-controlled variable treatment duration study evaluating the efficacy and safety of Siponimod (BAF312) in patients with secondary progressive multiple sclerosis followed by extended treatment with open-label BAF312	1045

The pivotal phase 3 trial (BAF312A2304) included 1651 randomized subjects, of which 1045 were included in popPK analyses. Genotyping was performed in a CLIA environment by (b) (4) using TaqMan and Sanger sequencing methods. DNA samples were collected from all subjects as consent to CYP2C9 genotyping was required for study participation. Table 22 lists the variants investigated, methods utilized, and functional consequences of these variants.

Table 22: Polymorphisms Tested and Genotyping Methods Utilized for CYP2C9

Polymorphism	*Identifier*	*Genotyping Method*	*CYP2C9 Enzymatic Activity*
CYP2C9*2	rs1799853	TaqMan; Sanger Sequencing	Slightly Decreased
CYP2C9*3	rs1057910	TaqMan; Sanger Sequencing	Severely Decreased

Source: Responses to Questions Received on January 15, 2019; Sections 2.2

Key Questions and Summary of Findings

Is the sponsor's CYP2C9 genotype classification accurate based on reported genotype data?

Yes. While the sponsor genotyped only alleles associated with lack of functional CYP2C9, the assigned genotypes were concordant with the reviewer's assignments.

Sponsor's Analyses:

Subjects were classified by CYP2C9 genotype based on the results of genotyping for 2 alleles, rs1799853 (*2) and rs1057910 (*3). A summary of the sponsor's assigned CYP2C9 genotypes for subjects from study BAF312A2304 included in the popPK analyses and all subjects from study BAF312A2128 are below in Table 23.

Table 23: Sponsor's Assignment of CYP2C9 Genotype in Study BAF312A2304

Study	*CYP2C9 Genotype*	*Number of Subjects*	*Percentage of Subjects*
BAF312A2304			***(N=1045)***
	**1/*1*	664	64%
	**1/*2*	203	19%
	**1/*3*	131	13%
	**2/*2*	18	2%
	**2/*3*	29	3%
	**3/*3*	0 (Excluded from study)	N/A
BAF312A2128			***(N=24)***
	**1/*1*	12	50%
	**2/*3*	6	25%

	*3/*3	6	25%

Source: Applicant's population PK/PD report (Table 5-1, Page 49).

Reviewer's Analyses:

Subjects were re-classified using identical methodologies as listed above using the sponsor provided subject level genotype datasets. The reviewer notes that all sponsor calls were concordant with the reviewer's calls for both study BAF312A2304 and BAF312A2128.

Does the dedicated CYP2C9 genotype study (BAF312A2128) show an effect of CYP2C9 genotype on siponimod PK?

*Yes. AUC_{inf} exposure levels are approximately 2-fold higher in *2/*3 subjects and approximately 4-fold higher in *3/*3 subjects.*

Sponsor's Analyses:

The sponsor's analyses show that exposure levels (AUC_{inf}) in *2*/3 and *3/*3 subjects were approximately 2-fold and 4-fold higher than *1/*1 subjects, respectively. Figure 5 below shows the effects of genotype on siponimod PK as assessed in the formal genotype study and that this effect is consistent with those seen in the popPK analyses.

Figure 5: Effect of CYP2C9 Genotype of Siponimod PK

- Formal study (Study A2128)

PK parameter (unit)	CYP2C9 *1*1 (N = 12)	CYP2C9 *2*3 (N = 6)	CYP2C9 *3*3 (N = 6)
Cmax (ng/mL)[b]	2.03 (17.2)	2.45 (13.1)	2.35 (29.0)
Tmax (h)[a]	4.00 (2.00-6.00)	5.00 (4.00-8.00)	4.00 (4.00-16.00)
AUClast (h*ng/mL)[b]	68.6 (21.3)	140 (15.6)	266 (22.9)
AUCinf (h*ng/mL)[b]	70.5 (21.2)	144 (15.7)	271 (22.4)

- PopPK analysis

CYP2C9 Genotype	Estimated CL/F (L/h) Phase 1/Phase 2	Phase 3	% of CYP2C9*1*1 CL/F Phase 1/Phase 2	Phase 3
Extensive metabolizers				
CYP2C9*1*1	3.3	3.1	100	100
CYP2C9*1*2	3.3[a]	3.1	99[a]	100
Intermediate metabolizers				
CYP2C9*2*2	2.6[a]	2.5	80[a]	80
CYP2C9*1*3	2.1	1.9	65	62
Poor metabolizers				
CYP2C9*2*3	1.8	1.6	55	52
CYP2C9*3*3	0.9	-	26	-

Source: Adapted from PopPK study report and BAF312A2128 study report

Summary and Conclusions

Overall, the sponsor's classification of subjects based on genotyped alleles is acceptable. There was concordance between the sponsor's assignments and the reviewer's. The CYP2C9 genotype data, utilized by the sponsor in the PopPK and PBPK analyses demonstrating that CYP2C9 genotype has a significant impact on siponimod exposures, is robust and reliable.

4.6-1. BIOPHARMACEUTICS STUDIES

4.6-1.1 Bioavailability

Study A2126: A randomized, open-label study to measure the absolute bioavailability, safety, tolerability, and pharmacodynamics of oral and intravenous BAF312 (siponimod) final market image (FMI) in healthy subjects.

Objectives:

The primary objective of this study was to determine the absolute bioavailability of a single oral dose of 0.25 mg of siponimod in healthy subjects.

Methodology:

This was a randomized, open-label study in healthy subjects.

This study consisted of a maximum 42-day screening period, 2 baseline periods (1 before each treatment period), and 2 treatment periods followed by an end-of-study (EOS) evaluation approximately 14 days after the last study drug administration.

Eligible subjects were randomly assigned to 1 of the 2 different sequences:

Sequence 1: Treatment A (siponimod 0.25 mg, single oral dose) followed by Treatment B (siponimod 0.25 mg single i.v. infusion over 3 hours)

Sequence 2: Treatment B (siponimod 0.25 mg single i.v. infusion over 3 hours) followed by Treatment A (siponimod 0.25 mg, single oral dose)

Number of subjects:

A total of 16 subjects were enrolled and 14 subjects (87.5%) completed the study. All 16 subjects (100%) were included in the safety, PK, and PD analysis sets. Subjects (b) (6) (AB) and (b) (6) (BA) only completed 1 treatment and were not included in the statistical analysis of PK and PD data.

Main criteria for inclusion:

Healthy male and female subjects (non-childbearing potential), non-smoking, between 18 and 50 years of age, inclusive, body weight between 50 to 100 kg, inclusive, and a body mass index (BMI) between 18 to 30 kg/m2, inclusive.

Test product, dose and mode of administration:

- BAF312 (siponimod) 0.25 mg film-coated final market image (FMI) tablet, administered as a single oral dose (Batch number 1010004141)
- BAF312 (siponimod), 0.25 mg lyophilisate in vial, administered as a single 3 hour i.v. infusion (Batch number 1010006310)

PK Sampling:

Blood samples were taken before the BAF312 dose and then at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 216, 312, 336 hours after administering the oral dose or after starting the intravenous infusion.

Analytical assay:

Siponimod and metabolites (M16 and M17) were determined by validated liquid chromatography with tandem mass spectrometry methods with an LLOQ of 0.050 ng/mL for siponimod, 0.050 ng/mL for LYG778 (M16), and 0.100 ng/mL for LYS815 (M17).

Analyte	**Parameter**	**Quality Control Samples**	**Standard Curve Samples**
BAF312 (siponimod)	Quality Control or Standard Curve Concentration (ng/mL)	0.150, 2.00, 50.0, 75.0	0.0500-100
	Between Batch Precision (%CV)	3.4-5.2	2.6-4.8
	Between Batch Accuracy (%Bias)	-1.4-4.0	-4.5-4.0
	Linearity	Weighted linear equation (1/X2), mean r= 0.9972	
	Linear Range (ng/mL)	0.0500-100	
	Sensitivity (LLOQ, ng/mL)	0.0500	
LYG778 (M16)	Quality Control or Standard Curve Concentration (ng/mL)	0.150, 0.750, 2.00, 7.50	0.0500-10.0
	Between Batch Precision (%CV)	4.0-7.1	3.1-6.8
	Between Batch Accuracy (%Bias)	-2.3-0.7	-2.2-4.1
	Linearity	Weighted linear equation (1/X2), mean r= 0.9961	
	Linear Range (ng/mL)	0.0500-10.0	
	Sensitivity (LLOQ, ng/mL)	0.0500	
LYS815 (M17)	Quality Control or Standard Curve Concentration (ng/mL)	0.300, 2.50, 15.0, 37.50	0.100-50.0
	Between Batch Precision (%CV)	3.8-7.3	3.5-6.6
	Between Batch Accuracy (%Bias)	-2.8-0.3	-2.0-1.8
	Linearity	Weighted linear equation (1/X2), mean r= 0.9969	
	Linear Range (ng/mL)	0.100-50.0	

	Sensitivity (LLOQ, ng/mL)	0.100

PK Evaluations:

Plasma concentrations of siponimod and its metabolites, M16 and M17, were determined in plasma by validated liquid chromatography with tandem mass spectrometry methods. Pharmacokinetic parameters were determined using non-compartmental methods:

- After oral administration: Cmax, Tmax, AUClast, AUCinf, Lambda_z, T1/2, Vz/F (siponimod only), CL/F (siponimod only), adjusted R2, %AUCextrap, and MR Cmax (M16 and M17 only), MR AUClast (M16 and M17 only), and MR AUCinf (M16 and M17 only) from the plasma concentration-time data.
- After i.v. administration (Parts 1 and 2): Cmax, Tmax, AUClast, AUCinf, Lambda_z, T1/2, Vz (siponimod only), CL (siponimod only), adjusted R2, %AUCextrap, MR Cmax (M16 and M17 only), MR AUClast (M16 and M17 only), and MR AUCinf (M16 and M17 only) from the plasma concentration time data.
- The absolute bioavailability (F) was defined as the oral to i.v. ratio of AUCinf values.

PD Evaluations:

The PD effects of siponimod were determined through HR analysis (25-hour Holter ECGs) and ALC evaluation. Pharmacodynamic parameters of ALC included: area under the effect curve (AUEC), maximum effect (Emax), and time to maximum effect (TEmax). Heart rate analyses included 5-minute and hourly average data from 25-hour Holter ECGs in i.v.-treated subjects only. The 1-minute average HR data were stored in the database for potential retrospective analysis, if deemed necessary.

Statistical Methods:

Descriptive summary statistics of siponimod and metabolites (M16 and M17) plasma concentration data were provided by treatment and visit/sampling time point. Summary statistics included mean (arithmetic and geometric), standard deviation (SD), coefficient of variation (CV; arithmetic and geometric), median, minimum, and maximum.

Siponimod PK parameters AUClast, AUCinf and Cmax were compared between the 2 formulations, siponimod oral FMI tablet (test) and i.v. formulation (reference). For each of those PK parameters, the log-transformed data were analyzed using a linear model including treatment, period, sequence, and subject within sequence as fixed factors. A point estimate and a 90% confidence interval (CI) for the ratio of treatment geometric means on the original scale were provided for each comparison.

RESULTS

Pharmacokinetics

Siponimod

Following table represents PK parameters calculated for different treatments.

Treatment	Statistics	AUCinf (ng*h/mL)	AUClast (ng*h/mL)	Cmax (ng/mL)	Tmax (h)	T1/2 (h)	CL[a] (L/h)	Vz[a] (L)	F
Treatment A N=15	n	15	15	15	15	15	15	15	14
	Mean (SD)	69.4 (17.2)	65.7 (17.0)	1.75 (0.375)	.	27.3 (6.31)	3.82 (0.996)	146 (29.1)	0.843 (0.0698)
	CV% mean	24.8	25.9	21.4	.	23.1	26.1	20.0	8.3
	Geo-mean	67.4	63.6	1.71	.	26.7	3.71	143	0.841
	CV% geo-mean	25.6	26.8	24.4	.	22.1	25.6	20.0	8.6
	Median	70.5	67.3	1.75	8.00	25.7	3.55	149	0.859
	[Min; Max]	[39.8; 105]	[37.2; 102]	[0.920; 2.30]	[4.00; 8.03]	[17.2; 43.8]	[2.37; 6.28]	[97.9; 214]	[0.695; 0.939]
Treatment B N=15	n	15	15	15	15	15	15	15	.
	Mean (SD)	82.4 (20.1)	78.3 (19.7)	3.27 (0.535)	.	28.1 (6.99)	3.22 (0.848)	126 (25.9)	.
	CV% mean	24.4	25.2	16.4	.	24.9	26.3	20.6	.
	Geo-mean	80.1	76.0	3.22	.	27.4	3.12	124	.
	CV% geo-mean	25.6	26.6	19.4	.	22.5	25.6	19.8	.
	Median	84.2	79.0	3.42	2.92	25.5	2.97	131	.
	[Min; Max]	[47.7; 125]	[44.4; 119]	[1.88; 3.81]	[2.92; 2.92]	[20.1; 47.7]	[2.00; 5.24]	[95.9; 191]	.

n = number of subjects with non-missing values; N = number of subjects who received each treatment

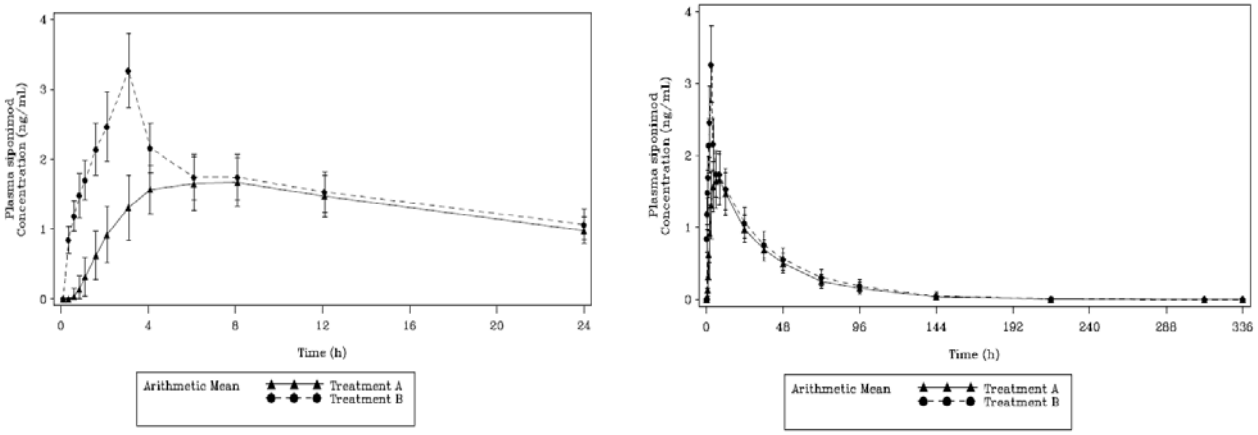
CV% = coefficient of variation (%) = SD/mean*100; CV% geo-mean = sqrt (exp(variance for log-transformed data)-1)*100.

Treatment A: siponimod 0.25 mg single oral dose; Treatment B: siponimod 0.25 mg/3 h single intravenous infusion.

[a] For Treatment A, CL/F and Vz/F are presented under CL and Vz, respectively.

Source: Table 14.2-5.2a

Arithmetic mean (SD) concentration-time profiles of siponimod for are presented graphically by treatment in the Figure below:

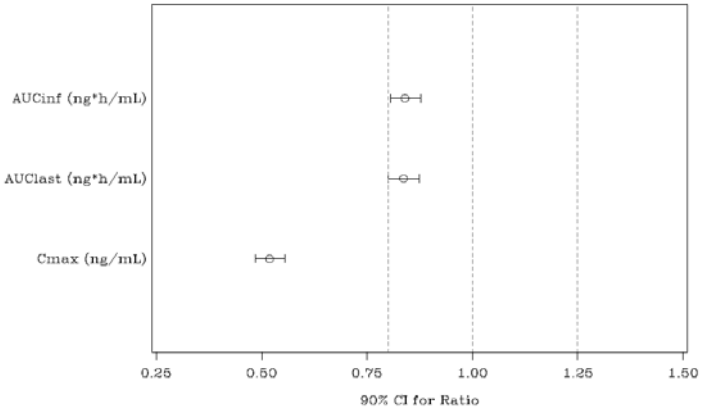


Treatment A: siponimod 0.25 mg single oral dose;

Treatment B: siponimod 0.25 mg/3 h single intravenous infusion.

The siponimod ratio of geometric means (90% CI) for oral to i.v. 0.25 mg siponimod (BAF312) for Cmax was 0.519 (0.486, 0.556), while the ratios for AUCinf and AUClast were 0.841 (0.806, 0.877) and 0.837

(0.801, 0.875), respectively (Figure below). The results indicate that a 0.25 mg oral dose of siponimod had an 84% bioavailability.



Geometric mean ratio (90 percent CI) for plasma siponimod PK parameters of Treatment A version Treatment B

M16 metabolite

Following a single 0.25 mg i.v. or oral dose of siponimod, plasma concentrations of LYG778 (M16) were BLQ for all subjects at all time points.

M17 metabolite

A summary of the PK parameters for M17 metabolite following a single 0.25 mg i.v. or oral dose of siponimod is presented by treatment in the table below.

Treatment	Statistics	AUCinf (ng*h/mL)	AUClast (ng*h/mL)	Cmax (ng/mL)	Tmax (h)	T1/2 (h)	MR AUCinf	MR AUClast	MR Cmax
Treatment A N=15	n	9	15	15	15	9	9	15	15
	Mean (SD)	114 (24.4)	72.6 (25.7)	0.366 (0.0912)	.	158 (35.7)	0.989 (0.183)	0.652 (0.188)	0.125 (0.0289)
	CV% mean	21.4	35.4	24.9	.	22.6	18.5	28.9	23.1
	Geo-mean	112	68.3	0.356	.	155	0.974	0.627	0.122
	CV% geo-mean	20.5	38.0	25.7	.	22.0	19.0	29.6	24.0
	Median	104	70.4	0.348	96.0	155	1.01	0.614	0.124
	[Min; Max]	[89.6; 158]	[36.4; 122]	[0.211; 0.568]	[6.00; 144]	[112; 231]	[0.736; 1.31]	[0.374; 0.961]	[0.0806; 0.173]
Treatment B N=15	n	8	15	15	15	8	8	15	15
	Mean (SD)	109 (33.0)	78.3 (30.5)	0.364 (0.101)	.	151 (18.5)	0.829 (0.177)	0.578 (0.133)	0.0656 (0.0156)
	CV% mean	30.3	39.0	27.8	.	12.3	21.4	23.0	23.8
	Geo-mean	105	73.3	0.352	.	150	0.811	0.564	0.0639
	CV% geo-mean	29.2	38.7	27.2	.	12.6	23.1	23.9	24.1
	Median	100	67.3	0.335	96.0	152	0.842	0.605	0.0666
	[Min; Max]	[76.6; 169]	[33.9; 155]	[0.218; 0.613]	[72.0; 144]	[121; 174]	[0.541; 1.06]	[0.358; 0.821]	[0.0452; 0.0976]

MR = Metabolite-to-parent ratio, calculated as (LYS815 parameter * BAF312 MW (516.61 D))/(BAF312 parameter * LYS815 MW (884.5 D)); n = number of subjects with non-missing values; N = number of subjects who received each treatment

CV% = coefficient of variation (%) = SD/mean*100; CV% geo-mean = sqrt (exp(variance for log-transformed data) 1)*100.

Treatment A: siponimod 0.25 mg single oral dose; Treatment B: siponimod 0.25 mg/3 h single intravenous infusion.

Source: Table 14.2-5.4a

The PK of LYS815 was comparable following oral and i.v. administrations. The geometric mean of the individual metabolite-to-parent molecular ratios were 0.974 and 0.811 for AUCinf following the single oral and i.v. dose of 0.25 mg siponimod, indicating siponimod is extensively metabolized to M17.

Pharmacodynamics

ALC

Following the single i.v. or oral dose of 0.25 mg siponimod, the observed nadir ALC (Emax) was comparable between the 2 routes of administration. The median TEmax was 6.17 hours for both 0.25 mg i.v. and oral siponimod.

Bradycardia/Bradyarrhythmia (HR, AV blocks, sinus pauses)

Heart rate analyses were based on averaged individual and mean, minimum, and maximum 1-minute, 5-minute, and hourly average HR data from 25-hour Holter ECG recordings in i.v.-treated subjects. No clinically relevant or symptomatic effect on mean hourly average HR was observed and mean hourly average HR remained above 50 bpm during the entire 25-hour Holter ECG recording period (from 1 hour before and until 24 hours after the start of infusion) period following i.v. administration of 0.25 mg siponimod over 3 hours.

Bradyarrhythmic events such as AV blocks and sinus pauses detected in the online cardiac monitoring/25-hour Holter ECG recording were asymptomatic and their frequency, duration, and diurnal pattern of occurrence was consistent with observations from previous clinical studies and yielded no new safety signals.

Safety

Overall, a single oral dose of 0.25 mg and single i.v. doses of 0.25 mg over 3 hours) of siponimod were safe and well tolerated by the healthy subjects in this study. No deaths or SAEs were reported during the study. Two subjects in Part 1 discontinued due to TEAEs (back pain and asymptomatic non-sustained ventricular tachycardia). No clinically relevant changes in clinical laboratory results and vital sign parameters were noted during the study and no individual value was reported as a TEAE by the Investigator.

CONCLUSIONS

- The absolute bioavailability of siponimod as a single 0.25 mg dose administered orally was 84%, as compared with a single 0.25 mg siponimod i.v. dose administered over 3 hours in healthy subjects.
- The geometric mean metabolite-to-parent molecular ratios for LYS815 (M17) ranged from 0.811 to 0.974 for AUCinf, indicating M17 was a prominent systemic metabolite of siponimod in human.
- A single oral dose of siponimod 0.25 mg and a single i.v. infusion of siponimod 0.25 mg over 3 hours were safe and well tolerated by healthy subjects in this study.

4.6-1. BIOPHARMACEUTICS STUDIES
4.6-1.2 Comparative BA/BE

Study A2111: A randomized, open-label, three-period crossover study to assess both the bioequivalence of the BAF312 final market image (FMI) tablet formulation as compared to the BAF312 market formulation (MF) and the effect of food on the relative bioavailability of the FMI after single 0.25 mg and 4 mg doses in healthy volunteers

Objectives:

Primary:

- To investigate whether 0.25 mg and 4 mg of BAF312 FMI are bioequivalent to the same dose strengths of BAF312 MF tablet.
- To investigate the effect of food on the pharmacokinetics of the 0.25 mg and 4 mg BAF312 FMI tablet.

Secondary:

- To investigate the safety and tolerability of the two BAF312 formulations following a single oral dose strength of 0.25 and 4 mg.

Methodology:

This was an open-label, randomized, study in healthy volunteers who were homozygous for the CYP2C9*1 (wild type) allele, using a three-period, three-treatment, six-sequence, single dose, crossover design at each of two dose levels. The study consisted of a 28-day screening period, three baseline periods (one before each treatment period), and three treatment periods, each separated by at least 14 days washout and a Study Completion evaluation approximately 14 days after the last drug administration.

Eligible subjects were randomized to one of the 12 treatment sequences (1-12) below:

Sequence	Period 1	Period 2	Period 3
1	A	B	C
2	B	C	A
3	C	A	B
4	A	C	B
5	B	A	C
6	C	B	A
7	D	E	F
8	E	F	D
9	F	D	E
10	D	F	E
11	E	D	F
12	F	E	D

Treatment A: 0.25 mg BAF312 MF tablet fasted

Treatment B: 0.25 mg BAF312 FMI tablet fasted

Treatment C: 0.25 mg BAF312 FMI tablet fed

Treatment D: 4 mg BAF312 MF tablet fasted

Treatment E: 4 mg BAF312 FMI tablet fasted

Treatment F: 4 mg BAF312 FMI tablet fed

Number of subjects:

A total of 62 subjects were enrolled in this study out of which 47 (75.8%) subjects completed the study. All 62 subjects were analyzed for safety and pharmacokinetics.

Main criteria for inclusion:

Non-smoking, healthy male and female subjects aged between 18 to 50 years (inclusive), who passed screening assessments, compliant with inclusion/exclusion criteria and provided written consent. Subjects were required to be CYP2C9 wild-type (CYP2C9*1 homozygous carriers) as determined during screening.

Test product, dose and mode of administration:

The BAF312 tablets were provided as 0.25 mg and 4 mg tablets for oral administration. The batch numbers of the test drug in various strengths is presented below:

BAF312 0.25 mg (MF), X139 0411;

BAF312MF 4 mg (MF), X141 0411;

BAF312 0.25 mg (FMI), X198 0811;

BAF312MF 4 mg (FMI), X199 0811.

Analytical assay:

Siponimod were determined by validated liquid chromatography with tandem mass spectrometry methods with an LLOQ of 0.0200 ng/mL.

Analyte	**Parameter**	**Quality Control Samples**	**Standard Curve Samples**
BAF312 (siponimod)	Low curve		
	Quality Control or Standard Curve Concentration (ng/mL)	0.0600, 0.400, 4.00 and 15.0	0.0200 - 20.0
	Between Batch Precision (%CV)	2.5-3.9	2.4-3.6
	Between Batch Accuracy (%Bias)	-0.8-1.8	-0.8-0.8
	Linearity	Weighted linear equation (1/X2), mean r= 0.9989	
	Linear Range (ng/mL)	0.0200 to 20.0	
	Sensitivity (LLOQ, ng/mL)	0.0200	
	High curve		

BAF312 (siponimod)	Quality Control or Standard Curve Concentration (ng/mL)	0.400, 4.00, 40.0 and 400	0.250 - 500
	Between Batch Precision (%CV)	5.9-8.1	2.3-5.1
	Between Batch Accuracy (%Bias)	-1.3-0.8	-2.4-2.0
	Linearity	Weighted linear equation (1/X2), mean r= 0.9980	
	Linear Range (ng/mL)	0.250 - 500	
	Sensitivity (LLOQ, ng/mL)	0.250	

PK Evaluations:

The pharmacokinetic blood samples were collected at the following time points for all 3 periods: pre-dose, then 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 216, and 312 hours after BAF312 intake.

Plasma concentrations of BAF312 were determined using a validated liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method with the lower limit of quantification (LLOQ) of 0.02 ng/mL.

Primary variables (also referred to as primary PK parameters) were AUCinf, AUClast, and Cmax. These were used in the statistical analysis of the bioequivalence and food effect. Secondary PK parameters were T1/2, Tmax, Tlag, CL/F and Vz/F.

Statistical Methods:

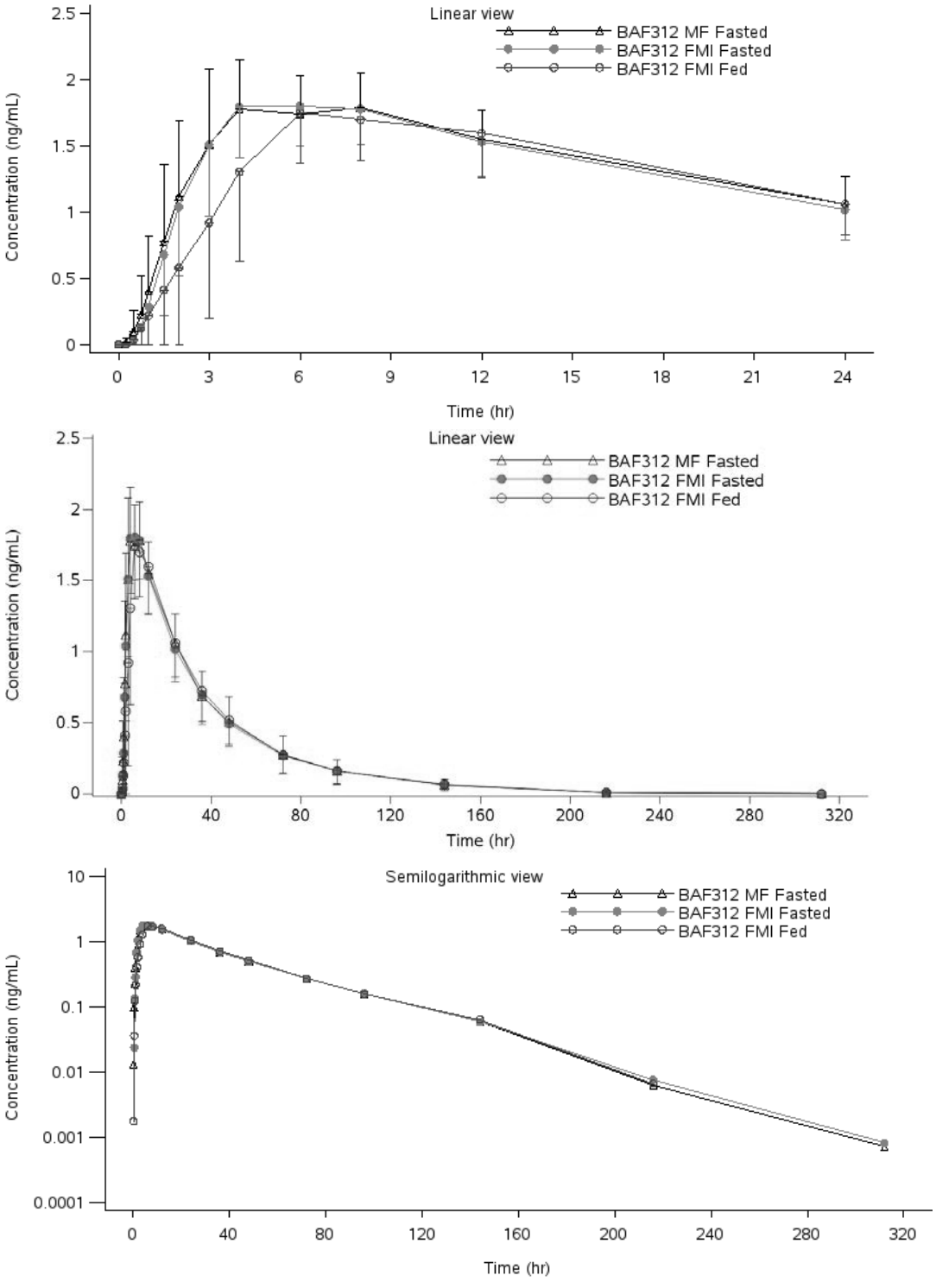
Descriptive statistics of PK parameters were calculated by treatment within dose, condition and period including mean, geometric mean, standard deviation (SD), and coefficient variance (CV), minimum, median, and maximum.

Geometric mean treatment ratios (test/reference) were reported along with their 90% confidence intervals, where reference for both ratios was FMI BAF312 fasted, and test was MF BAF312 fasted or FMI BAF312 fed.

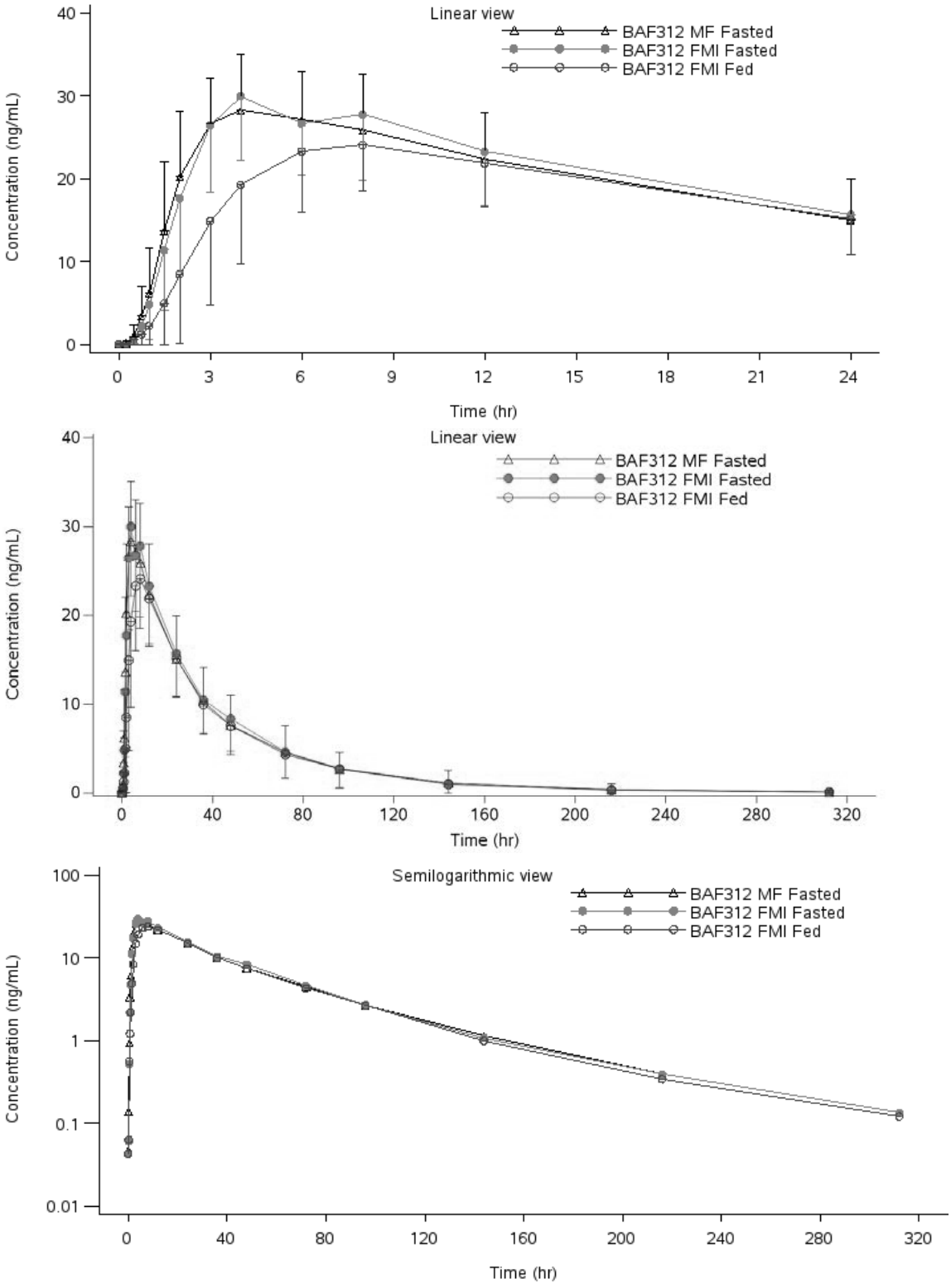
RESULTS

Pharmacokinetics

Arithmetic mean plasma concentration-time profiles of BAF312 are provided in the following figures:



Arithmetic mean (SD) concentration-time profiles by treatment for the 0.25 mg dose



Arithmetic mean (SD) concentration-time profiles by treatment for the 4 mg dose

Plasma PK parameters by dose are summarized in the following tables.

Summary of BAF312 plasma PK parameters by treatment for the 0.25 mg dose

Parameter [unit]	BAF312 MF Fasted	BAF312 FMI Fasted	BAF312 FMI Fed
Cmax [ng/mL]	1.98 ± 0.265 (13.4%) [30]	1.93 ± 0.271 (14.0%) [28]	1.95 ± 0.323 (16.6%) [30]
AUClast [hr*ng/mL]	71.7 ± 19.3 (26.8%) [29]	69.4 ± 21.4 (30.8%)[24]	69.8 ± 21.1 (30.2%) [28]
AUCinf [hr*ng/mL]	73.7 ± 19.2 (26.1%) [30]	72.7 ± 20.9 (28.7%) [28]	72.7 ± 21.2 (29.2%) [30]
Tmax [hr]	4 (2 - 12) [30]	4 (3 - 8) [28]	6 (2 - 12.1) [30]
Tlag [hr]	0.25 (0 - 0.75) [30]	0.5 (0.25 - 1.5) [28]	0.75 (0 - 3) [30]
T1/2 [hr]	31.1 ± 6.59 (21.1%) [30]	31.9 ± 6.64 (20.8%) [28]	30.8 ± 7.06 (22.9%) [30]
Vz/F [L]	156 ± 27.2 (17.5%) [30]	163 ± 27.4 (16.8%) [28]	157 ± 27.4 (17.4%) [30]
Cl/F [L/hr]	3.59 ± 0.864 (24.0%) [30]	3.7 ± 1.02 (27.7%) [28]	3.7 ± 1.01 (27.3%) [30]

Median (range) [N] for Tmax and Tlag parameters and Mean ± SD (CV%) [N] for remaining parameters
Source: PT-Table 14.2-1.5 and PT-Table 14.2-1.6

Summary of BAF312 plasma PK parameters by treatment for the 4 mg dose

Parameter [unit]	BAF312 MF Fasted	BAF312 FMI Fasted	BAF312 FMI Fed
Cmax [ng/mL]	30.4 ± 6.18 (20.3%) [26]	31.4 ± 8.09 (25.8%) [27]	27 ± 6.36 (23.6%) [28]
AUClast [hr*ng/mL]	1160 ± 502 (43.3%) [26]	1180 ± 484 (41.1%) [26]	1070 ± 438 (40.9%) [27]
AUCinf [hr*ng/mL]	1170 ± 521 (44.5%) [26]	1190 ± 500 (42.1%) [26]	1080 ± 442 (40.9%) [28]
Tmax [hr]	4 (2 - 8) [26]	4 (3 - 8.15) [27]	7.03(3 - 12) [28]
Tlag [hr]	0 (0 - 0.25) [26]	0 (0 - 0.25) [27]	0 (0 - 1) [28]
T1/2 [hr]	47.1 ± 9.41 (20.0%) [26]	48.4 ± 8.62 (17.8%) [26]	44.5 ± 11 (24.6%) [28]
Vz/F [L]	257 ± 79 (30.8%) [26]	265 ± 105 (39.5%) [26]	257 ± 83.5 (32.4%) [28]
Cl/F [L/hr]	3.91 ± 1.38 (35.3%) [26]	3.86 ± 1.45 (37.5%) [26]	4.17 ± 1.33 (32.0%) [28]

Median (range) [N] for Tmax and Tlag parameters and Mean ± SD (CV%) [N] for remaining parameters
Source: PT-Table 14.2-1.5 and PT-Table 14.2-1.6

The bioequivalence and the food effect analyzes are presented in the following tables.

Geometric mean, estimated geometric mean ratio and 90% confidence intervals for PK variables for formulation and fasting status for the 0.25 mg dose

Treatment	PK parameter (unit)	Adjusted geometric means*	Geometric mean ratio* Estimate (Test/Reference)	Lower 90% CI	Upper 90% CI
0.25 mg BAF312 MF Fasted (Test)	Cmax [ng/mL]	1.95	1.01	0.97	1.06
	AUClast [hr*ng/mL]	69.42	1.02	1.00	1.05
	AUCinf [hr*ng/mL]	71.48	1.02	0.99	1.05
0.25 mg BAF312 FMI Fed (Test)	Cmax [ng/mL]	1.92	1.00	0.95	1.04
	AUClast [hr*ng/mL]	67.31	0.99	0.97	1.02
	AUCinf [hr*ng/mL]	69.73	1.00	0.97	1.02
0.25 mg BAF312 FMI Fasted (Reference)	Cmax [ng/mL]	1.92			
	AUClast [hr*ng/mL]	67.74			
	AUCinf [hr*ng/mL]	70.03			

* Back-transformed from log scale

Model: The log transformed PK parameter data were analyzed using a fixed effect model with treatment, period and subject as fixed effects.

Source: PT-Table 14.2-1.1

Geometric mean, estimated geometric mean ratio and 90% confidence intervals for PK variables for formulation and fasting status for the 4 mg dose

Treatment	PK parameter (unit)	Adjusted geometric means*	Geometric mean ratio* Estimate (Test/Reference)	Lower 90% CI	Upper 90% CI
4 mg BAF312 MF Fasted (Test)	Cmax [ng/mL]	29.53	1.00	0.94	1.06
	AUClast [hr*ng/mL]	1045.99	0.98	0.94	1.02
	AUCinf [hr*ng/mL]	1052.84	0.98	0.94	1.02
4 mg BAF312 FMI Fed (Test)	Cmax [ng/mL]	27.02	0.91	0.86	0.97
	AUClast [hr*ng/mL]	1022.33	0.96	0.92	1.00
	AUCinf [hr*ng/mL]	1029.41	0.96	0.92	1.00
4 mg BAF312 FMI Fasted (Reference)	Cmax [ng/mL]	29.57			
	AUClast [hr*ng/mL]	1067.16			
	AUCinf [hr*ng/mL]	1074.10			

* Back-transformed from log scale

Model: The log transformed PK parameter data were analyzed using a fixed effect model with treatment, period and subject as fixed effects.

Source: PT Table-14.2-1.1

Plasma concentration-time profiles of BAF312 over 312 hours post dose were similar regardless of the treatment (MF fasted, FMI fasted, FMI fed) for each dose. Tmax was slightly delayed (approximately 2 to 3 hours delay) in FMI fed versus FMI fasted (6 to 7 hours versus 3 hours) for both doses, range of values overlapped between all treatments. Mean Cmax was about 10% lower in 4 mg FMI fed versus 4 mg FMI fasted.

The statistical analysis demonstrated that FMI fasted and MF fasted were bioequivalent for both 0.25 mg and 4 mg, as indicated by the fact that all 90% CI were in the prespecified range of [0.80 -1.25]. Similarly, FMI fasted and FMI fed fulfilled the bioequivalence criteria at both doses.

CONCLUSIONS

- The FMI and MF formulations of BAF312 were bioequivalent for both 0.25 mg and 4 mg doses.
- FMI fasted and FMI fed fulfilled the bioequivalence criteria at both doses. The slightly increased Tmax in both dose groups and the 10% lower Cmax in the 4 mg FMI fed group are considered clinically non relevant.

4.6-2. IN VITRO STUDIES PERTINENT TO PK USING HUMAN BIOMATERIALS

4.6-2.1 *In Vitro* Metabolism

Study DMPK R0500497: *In vitro* assessment of cytochrome P450 enzyme inhibition by BAF312

Objectives: To determine the potential of BAF312 to function as an *in vitro* inhibitor of cytochrome P450 (CYP)-mediated reactions in human liver microsomes.

METHODS

Following probe substrates were used for each CYP enzyme.

Evaluation of BAF312 as an Inhibitor of human P450 enzymes

CYP enzyme	Probe reaction
CYP1A2	phenacetin O-deethylation
CYP2A6	coumarin 7-hydroxylation
CYP2C8	paclitaxel 6α-hydroxylation
CYP2C9	diclofenac 4'-hydroxylation
CYP2C19	S-mephenytoin 4'-hydroxylation
CYP2D6	bufuralol 1'-hydroxylation
CYP2E1	chlorzoxazone 6-hydroxylation
CYP3A4	midazolam 1'-hydroxylation
CYP3A4	testosterone 6β-hydroxylation

Conditions used for *in vitro* studies below follow the Agency's guidance. These methods are acceptable.

- Evaluation of BAF312 as a metabolism-independent inhibitor of human P450 enzymes: Determination of IC50 values

RESULTS

The effect of increasing test substance concentrations on the CYP-selective metabolic probe reactions is provided in the table below:

Inhibitory effect of BAF312 on CYP isoenzyme-selective metabolic reactions

CYP enzyme	**Probe reaction**	**IC_{50} value [µM]**
CYP1A2	phenacetin O-deethylation	> 200
CYP2A6	coumarin 7-hydroxylation	> 200
CYP2C8	paclitaxel 6α-hydroxylation	> 200
CYP2C9	diclofenac 4'-hydroxylation	≈ 230 ± 30 *
CYP2C19	S-mephenytoin 4'-hydroxylation	> 100
CYP2D6	bufuralol 1'-hydroxylation	> 200
CYP2E1	chlorzoxazone 6-hydroxylation	> 100
CYP3A4	midazolam 1'-hydroxylation	> 200
CYP3A4	testosterone 6β-hydroxylation	100 ± 4

* estimated by extrapolation of experimental data

BAF312 displayed weak inhibition of CYP3A4-mediated testosterone 6β-hydroxylation (IC50 = 100 ± 4 µM) and very weak inhibition of CYP2C9-mediated diclofenac 4'-hydroxylation (IC50 ≈ 230 ± 30 µM). No significant inhibition of CYP1A2, CYP2A6, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and CYP3A4-mediated midazolam 1'-hydroxylation was observed at BAF312 concentrations up to 100 or 200 µM, respectively.

CONCLUSIONS

- BAF312 has a low potential for cytochrome P450-mediated drug-drug interactions.
- Inhibition of CYP3A4 or CYP2C9 may occur if human systemic concentrations would exceed 100 µM or 200 µM, respectively.
- BAF312 is unlikely to inhibit the metabolic clearance of co-medications metabolized by CYP1A2, CYP2A6, CYP2C8, CYP2C19, CYP2D6 and CYP2E1.

Study DMPK R1200710: Evaluation of BAF312 as inducer of drug metabolizing enzymes in human hepatocytes

Objectives: To evaluate the potential for BAF312 to induce cytochrome P450 (CYP) enzymes (activities and mRNA) in primary human hepatocytes *in vitro*.

METHODS

The effects of 48 h treatment with BAF312 were tested in primary human hepatocytes of three individual donors.

Evaluation of changes in CYP1A2, CYP2B6, CYP2C9 and CYP3A activity were assessed by measuring metabolism of CYP-selective probe substrates.

Following probe reaction were used for each CYP enzyme.

CYP enzyme	Probe reaction
CYP1A2	Acetaminophen formation
CYP2B6	OH-bupropion formation
CYP2C9	4'-hydroxydiclofenac formation
CYP3A	1'OH-midazolam formation

Changes of relative mRNA levels of CYP1A2, CYP2B6, CYP2C9 and CYP3A4 were determined by real-time PCR (RT-PCR).

RESULTS

CYP1A2 mRNA and activity

The maximal increase of CYP1A2 mRNA expression in Livers 1, 2 and 3 was 1.1-, 0.9- and 1.4-fold, respectively, which corresponds to 1%, 0% and 1%, respectively, of the maximal positive control effect.

The maximal increase of CYP1A2 activity in Livers 1, 2 and 3 was 1.06-, 0.77- and 0.54-fold, respectively, which corresponds to 1%, 0% and 0%, respectively, of the maximal positive control effect.

CYP2B6 mRNA and activity

The maximal increase of CYP1A2 mRNA expression in Livers 1 and 2 was 1.0- and 0.9-fold, respectively, which corresponds to 1% and 0%, respectively, of the maximal positive control effect. In Liver 3, BAF312 treatment led to an increase in CYP2B6 mRNA of up to 26%, which is slightly above the threshold value of 20% increase relative to the vehicle control. In this liver, however, only a single value for vehicle control treated cells could be measured and values calculated relative to vehicle control are uncertain.

The maximal increase of CYP1A2 activity in Livers 1, 2 and 3 was 1.15-, 0.87- and 1.17-fold, respectively, which corresponds to 3%, 0% and 6%, respectively, of the positive control effect.

CYP2C9 mRNA and activity

Treatment of hepatocytes with BAF312 up to 10 µM did not result in significant induction of CYP2C9 mRNA in all three livers tested. The maximal increase of CYP2C9 mRNA expression was 1.0-, 0.9- and

1.1-fold, respectively, which corresponds to 1%, 0% and 4%, respectively, of the maximal positive control effect.

BAF312 was shown to be a time-dependent inhibitor of CYP2C9. The induction readout for CYP2C9 enzyme activity in this study is expected to be affected by the inhibitory potential of BAF312. For CYP2C9, assessment of induction by BAF312 should be based on results of the mRNA readout.

CYP3A4 mRNA and activity

In Liver 1, Liver 2 and Liver 3, CYP3A4 mRNA was induced maximally 2.4-, 1.6- and 1.9-fold, respectively, which corresponds to 4%, 6% and 7% of the positive control response.

Incubation with BAF312 did not result in significant induction of CYP3A activity. The maximal increase in Liver 1, Liver 2 and Liver 3 was 0.97-, 1.00- and 1.03-fold, respectively, which corresponds to 0%, 0% and 1%, respectively, of the positive control effect.

CONCLUSIONS

BAF312 is not expected to induce CYP1A2, CYP2B6, CYP2C9 and CYP3A.

Study DMPK R1300932: *In vitro* assessment of cytochrome P450 enzyme inhibition by BAF312 metabolite LNL925 (M3)

Objectives: To determine the potential of LNL925 to function as an in vitro inhibitor of cytochrome P450 enzymes.

METHODS

Following probe substrates were used for each CYP enzyme.

CYP enzyme	Probe reaction
CYP1A2	Phenacetin O-deethylation
CYP2A6	Coumarin 7-hydroxylation
CYP2B6	Bupropion hydroxylation
CYP2C8	Amodiaquine N-deethylation
CYP2C9	Diclofenac 4'-hydroxylation
CYP2C19	S-mephenytoin 4'-hydroxylation
CYP2D6	Bufuralol 1'-hydroxylation
CYP2E1	Chlorzoxazone 6-hydroxylation
CYP3A4/5	Midazolam 1'-hydroxylation
CYP3A4/5	Testosterone 6β-hydroxylation

- Evaluation of BAF312 as a reversible inhibitor of human P450 enzymes: Determination of IC50 values

RESULTS

The effect of increasing test substance concentrations on the CYP-selective metabolic probe reactions is provided in the table below:

Inhibitory effect of LNL925 on CYP enzyme-selective metabolic reactions

CYP enzyme	Probe reaction	IC50 value (µM)	Time-dependent inhibition
CYP1A2	phenacetin O-deethylation	> 100	not observed
CYP2A6	coumarin 7-hydroxylation	> 100	n.i.
CYP2B6	bupropion hydroxylation	94 ± 6	n.i.
CYP2C8	amodiaquine N-deethylation	> 100	n.i.
CYP2C9	diclofenac 4'-hydroxylation	80 ± 3	not observed
CYP2C19	S-mephenytoin 4'-hydroxylation	> 100	n.i.
CYP2D6	bufuralol 1'-hydroxylation	> 100	not observed
CYP2E1	chlorzoxazone 6-hydroxylation	> 100	n.i.
CYP3A4/5	midazolam 1'-hydroxylation	> 100	not observed
CYP3A4/5	testosterone 6β-hydroxylation	> 100	n.i.

n.i.= not investigated

LNL925 showed potency for reversible inhibition of CYP2B6 (IC50 = 94 µM) and CYP2C9 (IC50 = 80 µM). Very little or no reversible inhibition of CYP1A2, CYP2A6, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5 was observed at LNL925 concentrations of up to 100 µM.

Time-dependent (irreversible) inhibition was not observed for CYP1A2, CYP2C9, CYP2D6 and CYP3A4/5 up to LML925 concentrations of 100 µM.

CONCLUSIONS

- LNL925 may inhibit the metabolic clearance of co-medications metabolized by CYP2B6 and CYP2C9, if sufficiently high concentrations are achieved in vivo.
- LNL925 is not expected to inhibit human CYP1A2, CYP2A6, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5.

Study DMPK R1300933: Evaluation of BAF312 metabolite LNL925 (M3) as inducer of drug metabolizing enzymes in human hepatocytes

Objectives: To evaluate the potential for the BAF312 metabolite LNL925 to induce cytochrome P450 (CYP) enzymes (activities and mRNA) in primary human hepatocytes in vitro.

METHODS

The effects of 48 h treatment with LNL925 were tested in primary human hepatocytes of three individual donors.

Evaluation of changes in CYP1A2, CYP2B6, CYP2C9 and CYP3A activity were assessed by measuring metabolism of CYP-selective probe substrates.

Following probe reaction were used for each CYP enzyme.

CYP enzyme	Probe reaction
CYP1A2	Acetaminophen formation
CYP2B6	OH-bupropion formation
CYP2C9	4'-hydroxydiclofenac formation
CYP3A	1'OH-midazolam formation

Changes of relative mRNA levels of CYP1A2, CYP2B6, CYP2C9 and CYP3A4 were determined by real-time PCR (RT-PCR).

RESULTS

CYP1A2 mRNA and activity

LNL925 induced CYP1A2 mRNA in Liver 1, Liver 2 and Liver 3 maximally 1.5-, 1.3- and 1.2-fold, respectively, which corresponds to 4%, 2% and 2% of the positive control response.

LNL925 induced CYP1A2 activity in Liver 1, Liver 2 and Liver 3 maximally 1.70-, 1.61- and 1.59-fold, respectively, which corresponds to 12%, 10% and 10% of the positive control response.

CYP2B6 mRNA and activity

LNL925 induced CYP2B6 mRNA in Liver 1, Liver 2 and Liver 3 maximally 2.4-, 1.2- and 1.6-fold, respectively, which corresponds to 12%, 2% and 7% of the positive control response.

LNL925 induced CYP2B6 activity in Liver 1, Liver 2 and Liver 3 maximally 1.71-, 1.29- and 1.48-fold, respectively, which corresponds to 23%, 7% and 11% of the positive control response. Since mRNA is the primary readout for induction of CYP2B6, LNL925 would not be considered an in vitro inducer of CYP2B6.

CYP2C9 mRNA and activity

LNL925 induced CYP2C9 mRNA in Liver 1, Liver 2 and Liver 3 maximally 1.3-, 1.1- and 1.3-fold, respectively, which corresponds to 19%, 12% and 20% of the positive control response.

Induction of CYP2C9 activity could not be evaluated in Liver 1 and Liver 3. LNL925 induced CYP2C9 activity maximally 1.97-fold, which corresponds to 24% of the positive control response. However, the

response did not show a concentration-dependency and is therefore not considered to indicate induction of CYP2C9 activity by LNL925.

CYP3A4 mRNA and activity

LNL925 induced CYP3A4 mRNA in Liver 1, Liver 2 and Liver 3 maximally 1.8-, 0.9- and 1.1-fold, respectively, which corresponds to 1%, 0% and 0% of the positive control response.

LNL925 induced CYP3A activity in Liver 1, Liver 2 and Liver 3 maximally 1.19-, 1.13- and 1.02-fold, respectively, which corresponds to 3%, 3% and 0% of the positive control response.

CONCLUSIONS

LNL925 is not expected to induce CYP1A2, CYP2B6, CYP2C9 or CYP3A4.

Study DMPK R1500796: *In vitro* assessment of cytochrome P450 enzyme inhibition by BAF312 metabolite LYS815 (M17)

Objectives: To determine the potential of LYS815 to function as an *in vitro* inhibitor of cytochrome P450 enzymes.

METHODS

Following probe substrates were used for each CYP enzyme.

CYP enzyme	Probe reaction
CYP1A2	Phenacetin O-deethylation
CYP2A6	Coumarin 7-hydroxylation
CYP2B6	Bupropion hydroxylation
CYP2C8	Amodiaquine N-deethylation
CYP2C9	Diclofenac 4'-hydroxylation
CYP2C19	S-mephenytoin 4'-hydroxylation
CYP2D6	Bufuralol 1'-hydroxylation
CYP2E1	Chlorzoxazone 6-hydroxylation
CYP3A4/5	Midazolam 1'-hydroxylation
CYP3A4/5	Testosterone 6β-hydroxylation

- Evaluation of BAF312 as a reversible inhibitor of human P450 enzymes: Determination of IC50 values

RESULTS

The effect of increasing test substance concentrations on the CYP-selective metabolic probe reactions is provided in the table below:

Inhibitory effect of LNL925 on CYP enzyme-selective metabolic reactions

CYP enzyme	Probe reaction	IC50 value (µM)	Time-dependent inhibition
CYP1A2	phenacetin O-deethylation	> 100	not observed
CYP2A6	coumarin 7-hydroxylation	> 100	n.i.
CYP2B6	bupropion hydroxylation	94 ± 6	n.i.
CYP2C8	amodiaquine N-deethylation	> 100	n.i.
CYP2C9	diclofenac 4'-hydroxylation	80 ± 3	not observed
CYP2C19	S-mephenytoin 4'-hydroxylation	> 100	n.i.
CYP2D6	bufuralol 1'-hydroxylation	> 100	not observed
CYP2E1	chlorzoxazone 6-hydroxylation	> 100	n.i.
CYP3A4/5	midazolam 1'-hydroxylation	> 100	not observed
CYP3A4/5	testosterone 6β-hydroxylation	> 100	n.i.

n.i.= not investigated

LNL925 showed potency for reversible inhibition of CYP2B6 (IC50 = 94 µM) and CYP2C9 (IC50 = 80 µM). Very little or no reversible inhibition of CYP1A2, CYP2A6, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5 was observed at LNL925 concentrations of up to 100 µM.

Time-dependent (irreversible) inhibition was not observed for CYP1A2, CYP2C9, CYP2D6 and CYP3A4/5 up to LML925 concentrations of 100 µM.

CONCLUSIONS

- LNL925 may inhibit the metabolic clearance of co-medications metabolized by CYP2B6 and CYP2C9, if sufficiently high concentrations are achieved in vivo.
- LNL925 is not expected to inhibit human CYP1A2, CYP2A6, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5.

Study DMPK R1500795: Evaluation of BAF312 metabolite LYS815 (M17) as inducer of drug metabolizing enzymes in human hepatocytes

Objectives: To evaluate the potential for the BAF312 metabolite LYS815 to induce cytochrome P450 (CYP) enzymes (activities and mRNA) in primary human hepatocytes *in vitro*.

METHODS

The effects of 48 h treatment with LYS815 were tested in primary human hepatocytes of three individual donors.

Evaluation of changes in CYP1A2, CYP2B6, CYP2C9 and CYP3A activity were assessed by measuring metabolism of CYP-selective probe substrates.

Following probe reaction were used for each CYP enzyme.

CYP enzyme	Probe reaction
CYP1A2	Acetaminophen formation
CYP2B6	OH-bupropion formation
CYP2C9	4'-hydroxydiclofenac formation
CYP3A	1'OH-midazolam formation

Changes of relative mRNA levels of CYP1A2, CYP2B6, CYP2C9 and CYP3A4 were determined by real-time PCR (RT-PCR).

RESULTS

CYP1A2 mRNA and activity

LYS815 induced CYP1A2 mRNA in Liver 1, Liver 2 and Liver 3 maximally 1.3-, 2.1- and 1.4-fold, respectively, which corresponds to 1%, 3% and 2% of the positive control response.

LYS815 induced CYP1A2 activity in Liver 1, Liver 2 and Liver 3 maximally 2.18-, 2.17- and 1.15-fold, respectively, which corresponds to 3%, 7% and 1% of the positive control response.

CYP2B6 mRNA and activity

LYS815 induced CYP2B6 mRNA in Liver 1, Liver 2 and Liver 3 maximally 1.2-, 0.8- and 0.7-fold, respectively, which corresponds to 2%, 0% and 0% of the positive control response.

LYS815 induced CYP2B6 activity in Liver 1, Liver 2 and Liver 3 maximally 1.20-, 1.09- and 1.01-fold, respectively, which corresponds to 2%, 3% and 0% of the positive control response.

CYP2C9 mRNA and activity

LYS815 induced CYP2C9 mRNA in Liver 1, Liver 2 and Liver 3 maximally 0.8-, 0.9- and 0.9-fold, respectively, which corresponds to 0%, 0% and 0% of the positive control response. LYS815 induced CYP2C9 activity in Liver 1, Liver 2 and Liver 3 maximally 1.02-, 1.13- and 0.85-fold, respectively, which corresponds to 1%, 6% and 0% of the positive control response.

CYP3A4 mRNA and activity

LYS815 induced CYP3A4 mRNA in Liver 1, Liver 2 and Liver 3 maximally 1.1-, 0.8- and 0.7-fold, respectively, which corresponds to 0%, 0% and 0% of the positive control response.

LYS815 induced CYP3A activity in Liver 1, Liver 2 and Liver 3 maximally 1.45-, 1.07- and 0.65-fold, respectively, which corresponds to 11%, 2% and 0% of the positive control response.

CONCLUSIONS

LYS815 is not expected to induce CYP1A2, CYP2B6, CYP2C9 or CYP3A4.

Study DMPK R1300921: Assessment of intestinal uptake mechanisms of BAF312 using the gastrointestinal Caco-2 cell line

Objectives: *In vitro* assessment of the mechanisms and, potentially, the kinetics of BAF312 uptake into the intestinal barrier using the human intestinal Caco-2 cell line.

METHODS

The uptake mechanism of BAF312 into the intestinal barrier was determined by testing the effect of increasing concentrations (nominal: 0.5-50 µM) on the in vitro permeability of BAF312 across the apical membrane of Caco-2 cells.

Conditions used in these studies are acceptable.

Analysis of uptake kinetics

To take into account the initial concentration loss that likely results from non-specific cellular binding events (Hassen, et al 1996), the overall (measured, apparent) membrane permeabilities PSapp for each substrate concentration at 37°C were corrected by the 4°C data as follows:

$$PS = PSapp_{37°C} - (PSapp_{4°C} - PSm_{4°C}) \qquad \text{Equation 1}$$

where PS is the effective (corrected) membrane permeability (µL/min/ mg protein), PSapp,37°C and PSapp,4°C are the apparent overall membrane permeabilities (µL/min/ mg protein) at 37 and 4°C, respectively, and PSm,4°C is the measured (nonsaturable, passive) membrane permeability (µL/min/ mg protein) at 4°C.

When the concentration-dependent uptake data are presented against the corresponding substrate concentration S, the kinetic uptake parameters are reflected by the Michaelis-Menten equation (Sasaki, et al 2004):

$$PSapp = PSm \pm PSc = PSm \pm \frac{Vc,max}{Km,app + S} = PSm \pm \frac{PSc,max \cdot Km,app}{Km,app + S} \qquad \text{Equation 2}$$

where PSc is the transporter-mediated (saturable) membrane permeability (µL/min/mg protein), PSc,max is the maximum transporter-mediated membrane permeability (µL/min/mg protein). Vc,max is the maximum transporter velocity/rate (pmol/min/mg protein), S is the applied substrate concentrations (µM), and Km,app is the apparent Michaelis-Menten constant (µM).

RESULTS

Membrane permeability characteristics of BAF312 are summarized in the table below:

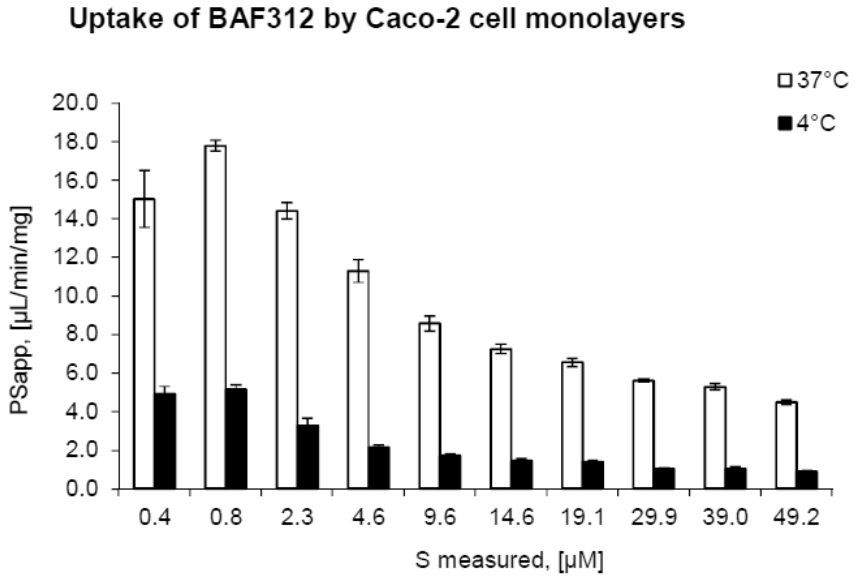
Species	PSm [a, b] (µL/min/mg)	PSc,max [a] (µL/min/mg)	(PSm+PSc,max) / PSm	Vc,max [a] (pmol/min/mg)	Km,app [a] (µM)
Human	13.5	0	1	NA	NA

[a] Estimated passive and active uptake parameters according to equation 2

[b] At a nominal BAF312 concentration of 1.0 µM

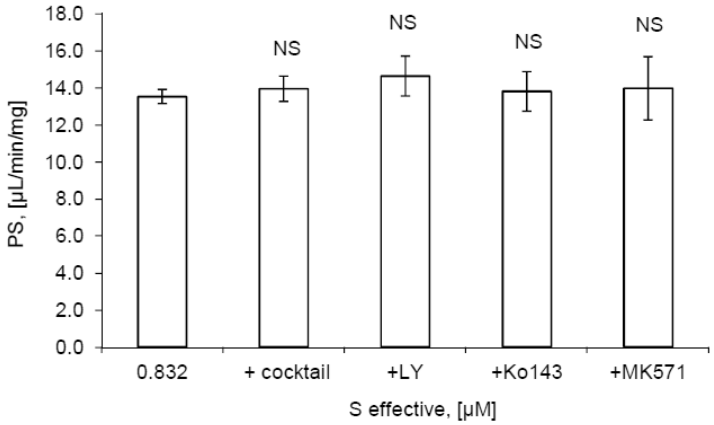
The results indicate that luminal membrane permeability of BAF312 in the intestine occurs most likely by a passive permeation process only.

The plot of BAF312 uptake data (nominal: 0.5-50 µM) at 4 and 37°C revealed decreasing cellular uptake with increasing incubation concentrations at both temperatures (See figure below).



This profile suggests saturation of non-specific binding to cellular compartments as same kinetic profile was observed at 37°C as well as at 4°C, where all active transport processes are inactivated.

BAF312 uptake into Caco-2 cells was evaluated in the presence of the well-known efflux-pump inhibitors LY, Ko143 and MK571 alone as well as applied as an inhibitor cocktail. No significant change in cellular BAF312 (measured: 0.8 µM) uptake could be determined in the presence of any of the inhibitors (showed in the following figure).



CONCLUSIONS

Uptake of BAF312 across the luminal membrane of the intestine occurs most likely solely by passive permeation without an involvement of drug efflux-transporters.

Study Title: Assessment of efflux transporter (BCRP, P-gp, BSEP, MRP2) inhibition by BAF312 and its main metabolites LNL925 (M3) and LYS815 (M17)

Study number: DMPK R1200722, DMPK R1300847, DMPK R1300849, DMPK R1300852 (BAF312); DMPK R1300852, DMPK R1300853 (M3); DMPK R1500825, DMPK R1500826 (M17)

Objective: The present studies were conducted to determine the potential of BAF312 and its main metabolites LNL925 (M3) and LYS815 (M17) to inhibit human ATP-binding cassette (ABC) transporter-mediated efflux via BCRP, P-gp, BSEP, MRP2.

Test System: MDCKII cells, LLC-PK1 cells and Sf9 derived vesicles

METHODS

The potential of BAF312 and its main metabolites LNL925 (M3) and LYS815 (M17) to inhibit human ATP-binding cassette transporter activity was assessed using recombinant MDCKII cells and Sf9 derived vesicles by determining the compound's ability to inhibit (sub) cellular uptake of the probe substrates.

Data analysis

Analysis of inhibition kinetics

The probe substrate transport in the presence of a competitive and non-competitive inhibitor can be described as follows (Gao et al 2001):

$$PSapp = PSm \pm \frac{V\max}{S + Km \cdot (1 + I / Ki)} \qquad \text{Eq. 1}$$

Where, PSapp represents the overall membrane permeability (nL/min/mg protein) of a probe substrate of choice (Table 3-1) at 37°C. S is the concentration (µM) of the probe compound in the medium, Vmax is the maximal uptake velocity/rate (pmol/min/mg protein), Km is the Michaelis-Menten constant (µM), PSm is the nonspecific (passive) membrane permeability (nL/min/mg protein), Ki is the inhibition constant (µM) and I is the concentration of the inhibitor in the medium (µM).

IC50 calculations

All absolute transporter uptake data were converted into relative inhibition values (y) by defining membrane permeability of the probe substrate without addition of inhibitor (baseline) as 0% inhibition and with addition of positive control inhibitor as 100% transporter inhibition and recalculating all the other uptake data relative to this number.

$$y = 100 - (100 / ((PSapp,p - PSapp,0) / (PSapp,p - PSapp,i))) \qquad \text{Eq. 2}$$

Where, PSapp,0 represents the initial membrane permeability without addition of inhibitor (=baseline) whereas PSapp,i and PSapp,p represent the membrane permeability in the presence of test inhibitor and positive control inhibitor, respectively.

Where applicable, IC50 values (inhibitor concentration that causes 50% inhibition of the maximal drug effect) were calculated using the following equation (Rautio et al 2006):

$$y = y0 + \frac{a \cdot I^n}{IC50^n + I^n} \qquad \text{Eq. 3}$$

Where, n is the slope factor (Hill coefficient), y0 is the relative baseline inhibition and a is the maximal transporter inhibition (%).

RESULTS

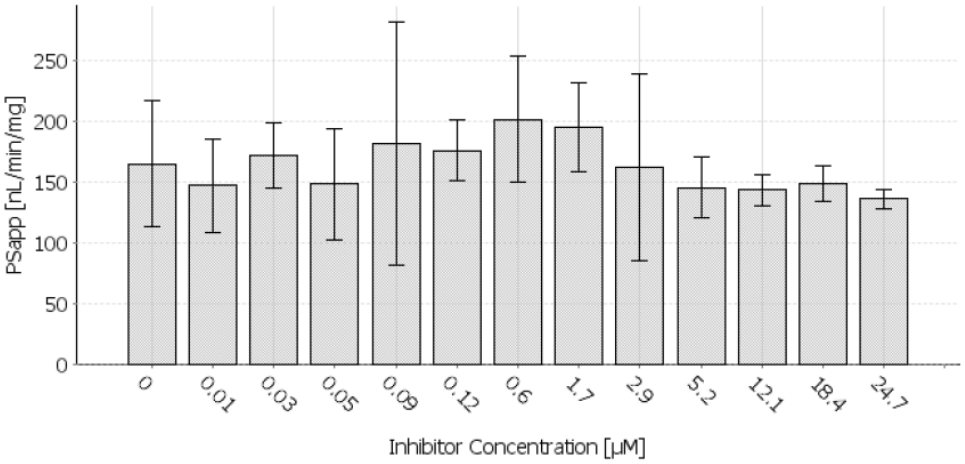
BAF312

The effect of increasing test substance concentrations on the uptake of probe substrate by inhibiting ABC transporter dependent efflux is summarized in the table below:

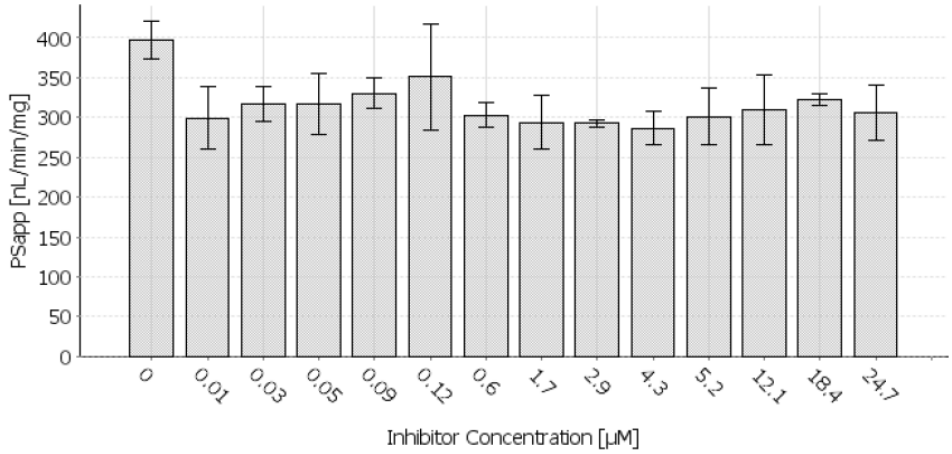
ABC transporter	Probe substrate	IC50 value (µM)	Ki (µM)	Max. inhibition (%)	Max conc. investigated (uM)
P-gp	Digoxin	not observed	not observed	not applicable	24.7
BCRP	PhIP	not observed	not observed	not applicable	24.7
BSEP	Taurocholate	not observed	not applicable	not observed	40
MRP2	E217βG	not observed	not applicable	not observed	52.6

BAF312 was not found to be an inhibitor of P-gp, BCRP, BSEP and MRP2 up to the highest concentration investigated (see the following figures).

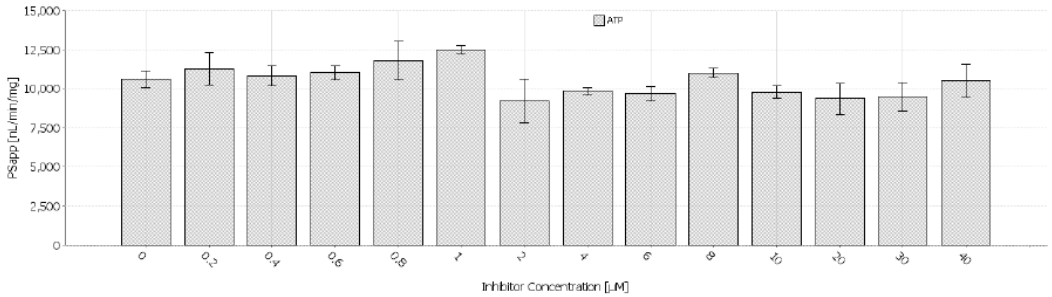
Effect of BAF312 on probe substrate uptake by P-gp-transporter expressing LLC-PK1 cells (Concentration-dependency)



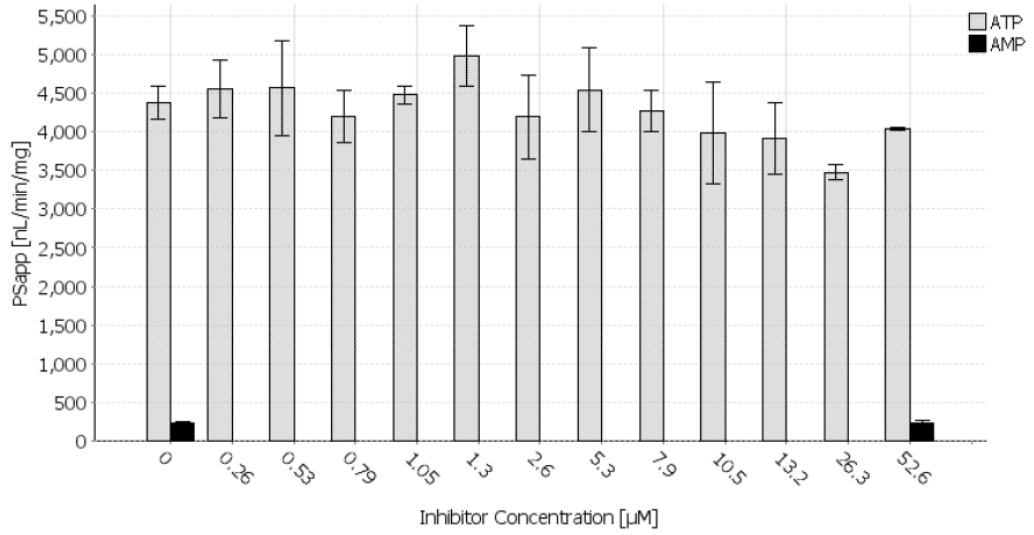
Effect of BAF312 on probe substrate uptake by P-gp-transporter expressing MDCKII cells (Concentration-dependency)



Effect of BAF312 on probe substrate uptake by Sf9 vesicles expressing human BSEP (Concentration-dependency)



Effect of BAF312 on probe substrate uptake by Sf9 vesicles expressing human MRP2 (Concentration-dependency)



LNL925 (M3)

The effect of increasing test substance concentrations on the uptake of probe substrate by inhibiting ABC transporter dependent efflux is summarized in the table below:

ABC transporter	Probe substrate	IC50 value (µM)	Ki (µM)	Max. inhibition (%)	Max conc. investigated (uM)
P-gp	Digoxin	not observed	not observed	not applicable	100
BCRP	PhIP	not observed	not applicable	not applicable	100
BSEP	Taurocholate	> 400	> 272	38.5 ± 11.3	400

LNL925 was not found to be an inhibitor of P-gp, BCRP up to 100 µM. LNL925 may inhibit BSEP if sufficiently high concentrations (> 400 uM) are achieved *in vivo*.

LYS815 (M17)

The effect of increasing test substance concentrations on the uptake of probe substrate by inhibiting ABC transporter dependent efflux is summarized in the table below:

ABC transporter	Probe substrate	IC50 value (µM)	Ki (µM)	Max. inhibition (%)	Max conc. investigated (uM)
P-gp	N-methyl quinidine	not observed	not applicable	not observed	1.35
BCRP	Estrone-3-sulfate	not observed	not applicable	not observed	1.35
BSEP	Taurocholate	not observed	not applicable	not observed	2.3
MRP2	E217βG	not observed	not applicable	not observed	1.35

LYS815 was not found to be an inhibitor of P-gp, BCRP, BSEP and MRP2 up to the highest concentration investigated.

CONCLUSIONS

- BAF312 was shown to be no inhibitor of P-gp, BCRP, BSEP and MRP2 up to 24.7 uM, 40 uM and 52.6 uM.
- LNL925 (M3) was shown to be no inhibitor of the transport activities of P-gp, BCRP, BSEP and MRP2 up to 100 uM *in vitro.*
- LYS815 (M17) was shown to be no inhibitor of the transport activities of P-gp, BCRP, BSEP and MRP2 up to 1.35 uM and 2.3 uM *in vitro.*

Study Title: Assessment of uptake transporters (OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K) inhibition by BAF312 and its main metabolites LNL925 (M3) and LYS815 (M17)

Study number: DMPK R1200723, DMPK R1200724, DMPK R1200725, DMPK R1300848 (BAF312); DMPK R1300854, DMPK R1300855, DMPK R1300856, DMPK R1300857 (M3); DMPK R1500827, DMPK R1500828, DMPK R1500829, DMPK R1500830 (M17)

Objective: The present studies were conducted to determine the potential of BAF312 and its main metabolites LNL925 (M3) and LYS815 (M17) to inhibit human solute carrier (SLC) transporter-mediated uptake via OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2, respectively.

Test System: HEK293 cells

METHODS

The potential of BAF312 and its main metabolites LNL925 (M3) and LYS815 (M17) to inhibit human solute carrier transporter activity was assessed using recombinant HEK293 cells by determining the compound's ability to inhibit (sub) cellular uptake of the probe substrates.

Data analysis

Analysis of inhibition kinetics

The probe substrate transport in the presence of a competitive and non-competitive inhibitor can be described as follows (Gao et al 2001):

$$PSapp = PSm \pm \frac{V\max}{S + Km \cdot (1 + I / Ki)} \qquad \text{Eq. 1}$$

Where, PSapp represents the overall membrane permeability (nL/min/mg protein) of a probe substrate of choice (Table 3-1) at 37°C. S is the concentration (µM) of the probe compound in the medium, Vmax is the maximal uptake velocity/rate (pmol/min/mg protein), Km is the Michaelis-Menten constant (µM), PSm is the nonspecific (passive) membrane permeability (nL/min/mg protein), Ki is the inhibition constant (µM) and I is the concentration of the inhibitor in the medium (µM).

IC50 calculations

All absolute transporter uptake data were converted into relative inhibition values (y) by defining membrane permeability of the probe substrate without addition of inhibitor (baseline) as 0% inhibition and with addition of positive control inhibitor as 100% transporter inhibition and recalculating all the other uptake data relative to this number.

$$y = 100 - (100 / ((PSapp,p - PSapp,0) / (PSapp,p - PSapp,i))) \qquad \text{Eq. 2}$$

Where, PSapp,0 represents the initial membrane permeability without addition of inhibitor (=baseline) whereas PSapp,i and PSapp,p represent the membrane permeability in the presence of test inhibitor and positive control inhibitor, respectively.

Where applicable, IC50 values (inhibitor concentration that causes 50% inhibition of the maximal drug effect) were calculated using the following equation (Rautio et al 2006):

$$y = y0 + \frac{a \cdot I^n}{IC50^n + I^n} \qquad \text{Eq. 3}$$

Where, n is the slope factor (Hill coefficient), y0 is the relative baseline inhibition and a is the maximal transporter inhibition (%).

RESULTS

BAF312

The effect of increasing test substance concentrations on the uptake of probe substrate by inhibiting SLC transporter dependent uptake is summarized in the table below:

SLC transporter	Probe substrate	IC50 value (µM)	Ki (µM)	Max. inhibition (%)	Max conc. investigated (uM)
OATP1B1	E217βG	1.65 ± 1.59	1.30	52.4 ± 12.4	24.3
OATP1B3	E217βG	2.88 ± 1.21	2.43	46.5 ± 5.1	24.3
OAT1	p-Aminohippuric acid	not observed	not observed	not applicable	24.3
OAT3	Estrone-3-sulfate	not observed	not observed	not applicable	24.3
OCT1	MPP+	not observed	not observed	not applicable	24.3
OCT2	MPP+	not observed	not observed	20.9	24.3
MATE1	MPP+	not observed	not observed	not applicable	40
MATE2K	MPP+	not observed	not observed	not applicable	40

BAF312 was found to be an inhibitor of OATP1B1 with an IC50 of 1.65 µM and a maximal inhibition of 52.4 % and of OATP1B3 with an IC50 of 2.88 µM and a maximal inhibition of 46.5%. Therefore, BAF312 may inhibit OATPs if sufficiently high concentrations are achieved *in vivo.* BAF312 was not found to be an inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K up to the highest concentration investigated (24.3uM or 40uM).

LNL925 (M3)

The effect of increasing test substance concentrations on the uptake of probe substrate by inhibiting SLC transporter dependent uptake is summarized in the table below:

SLC transporter	Probe substrate	IC50 value (µM)	Ki (µM)	Max. inhibition (%)	Max conc. investigated (uM)
OATP1B1	E217βG	3.7 ± 0.7	3.7	108.3 ± 8.0	400
OATP1B3	E217βG	4.1 ± 0.3	4.1	96.4 ± 2.4	400
OAT1	Cidofovir	not observed	not observed	not applicable	100
OAT3	Estrone-3-sulfate	not observed	not observed	not applicable	100
OCT1	MPP+	> 100	> 99.8	32.01 ± 1.75	100
OCT2	MPP+	not observed	not observed	not applicable	100
MATE1	MPP+	not observed	not observed	not applicable	100
MATE2K	MPP+	not observed	not observed	not applicable	100

LNL925 was not found to be an inhibitor of OAT1, OAT3, OCT2, MATE1 and MATE2K up to 100 µM. LNL925 was found to be an inhibitor of OCT1 with a maximal inhibition of 32.0 ± 1.7 % up to 100uM. LNL925 was shown to be an inhibitor of OATP1B1 and OATP1B3 with the IC50 values of 3.7 ± 0.4 µM for OATP1B1 and 4.1 ± 0.3 µM for OATP1B3, respectively.

LYS815 (M17)

The effect of increasing test substance concentrations on the uptake of probe substrate by inhibiting SLC transporter dependent efflux is summarized in the table below:

SLC transporter	Probe substrate	IC50 value (µM)	Ki (µM)	Max. inhibition (%)	Max conc. investigated (uM)
OATP1B1	E217βG	not observed	not observed	not applicable	2.3
OATP1B3	E217βG	not observed	not observed	not applicable	2.3
OAT1	Cidofovir	not observed	not observed	not applicable	2.3

OAT3	Estrone-3-sulfate	not observed	not observed	not applicable	2.3
OCT1	MPP+	not observed	not observed	not applicable	2.3
OCT2	MPP+	not observed	not observed	not applicable	2.3
MATE1	MPP+	not observed	not observed	not applicable	2.3
MATE2K	MPP+	not observed	not observed	not applicable	2.3

LYS815 was not found to be an inhibitor of OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K up to 2.3uM.

CONCLUSIONS

- BAF312 may inhibit OATPs if sufficiently high concentrations are achieved *in vivo*. BAF312 was not found to be an inhibitor of OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K up to the highest concentration investigated.
- LNL925 (M3) may inhibit OATPs with the IC50 values of 3.7 ± 0.4 µM for OATP1B1 and 4.1 ± 0.3 µM for OATP1B3, respectively. LNL925 is not likely to inhibit OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K up to 100uM *in vitro*.
- LYS815 (M17) was shown to be no inhibitor of OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K up to 2.3 uM *in vitro.*

4.6-2. IN VITRO STUDIES PERTINENT TO PK USING HUMAN BIOMATERIALS
4.6-2.2 Protein Binding

Study DMPK R0400881-01: *In vitro* blood distribution and plasma protein binding of [^{14}C]BAF312 including binding to individual human plasma proteins

Objective: To assess the *in vitro* blood distribution (concentration, time and temperature dependency) and the plasma protein binding of [^{14}C]BAF312 to individual human plasma proteins.

METHODS

Test System: Fresh heparinized blood and defrosted plasma; pools with n ≥ 3. Solutions of isolated human plasma proteins in PBS (human serum albumin, α1-acid glycoprotein, γ-globulins, high density lipoprotein, low density lipoprotein and very low-density lipoprotein).

Incubation at 37°C for 30 min after spiking; fraction in plasma (fp) obtained after cell separation by centrifugation.

Plasma protein binding was evaluated by equilibrium gel filtration in PBS containing [^{14}C]BAF312. Elution of protein was detected on-line (absorption at 280 nm), elution of radioactivity off-line by Liquid scintillation counting (LSC).

RESULTS

Binding of [^{14}C]BAF312 to individual human plasma proteins are summarized in the following table:

Protein binding of [14C]BAF312 to human plasma proteins measured by equilibrium gel filtration on a protein separating gel filtration column

The binding data were normalized to the amounts of the different proteins present in an identical volume of plasma

Protein	Amount of protein loaded [µg]	Physiological plasma concentration [mg/mL]	Free concentration [ng/ mL]	Bound / Run [ng]	Bound / mL plasma [ng]	f_b (%)	f_u (%)
HSA	2000	40	3.9	251	5015	99.92	0.08
			2.9	143	2855	99.90	0.10
AGP	50	1	2.9	67	1340	99.79	0.21
γ-globulin	500	12	5.2	2.2	52	90.93	9.07
HDL	50	3.9	5.7	165	12854	99.96	0.04
LDL	125	3.6	4.7	245	7068	99.93	0.07
VLDL	125	1.3	3.8	346	3594	99.89	0.11

Binding of [^{14}C]BAF312 to LDL, VLDL and HSA was similar and binding to HDL was highest. Binding of [^{14}C]BAF312 to α1-acid glycoprotein and particularly to γ-globulins was lower. This indicates that in human plasma the contribution of lipoproteins to the total binding is larger as compared to the contribution of albumin, the binding to α1-acid glycoprotein is even smaller and the binding to γ-globulin is negligible.

Fresh heparinized blood was spiked with different concentrations of [^{14}C]BAF312. Incubation was for 30 min at 37°C, before separation of blood cells and plasma by centrifugation. [^{14}C]BAF312 concentrations in blood and plasma are summarized in the table below:

Human (H = 0.48; mean f_p = 68 ± 2)					
Nominal concentration [ng/mL]	Actual concentration		f_p	C_b/C_p	C_{bc}/C_p
	Blood [ng/mL]	Plasma [ng/mL]	Mean ± SD [%]	Mean ± SD	Mean ± SD
10000	8866	11664	68 ± 2	0.76 ± 0.02	0.50 ± 0.05
1000	1035	1342	67 ± 1	0.77 ± 0.01	0.52 ± 0.01
100	99	132	69 ± 1	0.75 ± 0.01	0.49 ± 0.03
10	11.5	14.8	67 ± 2	0.78 ± 0.03	0.54 ± 0.06

CONCLUSIONS

[^{14}C]BAF312 was very highly bound (>99%) to all individual human plasma proteins, and lipoproteins contributed strongly to the overall binding.

The fraction of [14C]BAF312 in human plasma was 68%, corresponding to a ratio of blood-to-plasma concentrations (Cb/Cp) of 0.77.

Study DMPK R1300334: *Ex vivo* plasma protein binding of [^{14}C]BAF312 in hepatic impaired patients and healthy control subjects (study CBAF312A2122) by equilibrium gel filtration

Objective: To determine *ex vivo* binding of [^{14}C]BAF312 to plasma proteins in selected human plasma samples (4 h after dosing) from the clinical study CBAF312A2122.

METHODS

The free fraction of [^{14}C]BAF312 was measured by means of equilibrium gel filtration method in plasma samples from hepatic impaired patients (mild, moderate, and severe hepatic impaired groups, n=8/group) and healthy control subjects (n=14).

RESULTS

The mean plasma protein binding in different groups are summarized in the following table and figure:

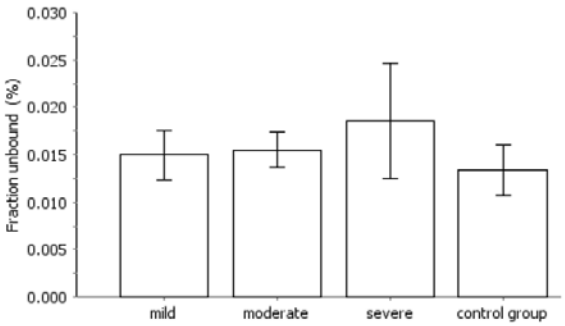
Mean *ex vivo* and *in vitro* plasma protein binding of [14C]BAF312

	Group	fu ± SD (%)
CBAF312A2122	Healthy subjects	0.0134 ± 0.00264 (n = 14)[b]
	Mild impairment	0.0150 ± 0.00258 (n = 8)
	Moderate impairment	0.0155 ± 0.00183 (n = 8)
	Severe impairment	0.0186 ± 0.00606 (n = 8)
In vitro	*In vitro* human plasma pool 1	0.0136 ± 0.00135 (n =1)[a]
	In vitro human plasma pool 2	0.0124 ± 0.000339 (n=1)[a]

a: one plasma pool from two different individuals, SD represents three runs; n: number of subjects/samples; b: 16 samples were received, but two samples were not analyzed (tube broke during centrifugation)

Mean *ex vivo* plasma protein binding of [^{14}C]BAF312 in hepatic impaired patients and healthy subjects

Gel filtration column was equilibrated with 10 ng/mL [^{14}C]BAF312. Error bars represent SD values.



The unbound fractions (mean ± SD) of [^{14}C]BAF312 from mild, moderate and severe hepatic impaired patients and from healthy subjects from all available samples amounted to 0.0150% ± 0.00258%, 0.0155% ± 0.00183, 0.0186% ± 0.00606 and 0.0134% ± 0.00264%, respectively.

CONCLUSIONS

[^{14}C]BAF312 was very highly bound (>99.9%) to human plasma proteins with no significant differences in fraction unbound (fu (%)) in healthy subjects, mild, moderate and severe hepatic impaired patients.

Study DMPK RCBAF312A2129-01: Clinical plasma protein binding of [^{14}C]BAF312 in renal impaired patients and healthy control subjects by equilibrium gel filtration

Objective: To determine *ex vivo* binding of [^{14}C]BAF312 to plasma proteins in selected human plasma samples (4 h after dosing) from the clinical study CBAF312A2129.

METHODS

The free fraction of [^{14}C]BAF312 was measured by means of equilibrium gel filtration method in plasma samples from severe renal impaired patients and healthy control subjects (n=8 / group).

RESULTS

Arithmetic mean plasma protein binding of [^{14}C]BAF312 from severe renal impairment patients and healthy subjects and *in vitro* (healthy human plasma donors) are summarized in the following table:

Mean *ex-vivo* and in vitro plasma protein binding of [^{14}C]BAF312

	Group	Fu (%) ± SD (%)
CBAF312A2129	Healthy subjects	0.0255 ± 0.00769 (n = 8)
	Severe impairment	0.0280 ± 0.0142 (n = 8)
In vitro	Blank human plasma pool 1	0.0201 [a]
	Blank human plasma pool 2	0.0261 [a]

a: one plasma pool from two different individuals; b: one plasma pool from three individuals, mean of 14 QC values; n: number of subjects.

[^{14}C]BAF312 was very highly bound (>99.9%) to human plasma proteins in all subjects. The unbound fractions (mean ± SD) of [^{14}C]BAF312 from severe renal impaired patients and from healthy subjects amounted to 0.0280% ± 0.0142% and 0.0255% ± 0.00769%, respectively.

CONCLUSIONS

[^{14}C]BAF312 was very highly bound (>99.9%) to human plasma proteins with no significant differences in fraction unbound (fu (%)) in healthy subjects, and severe renal impaired patients.

4.6-3. HUMAN PK STUDIES

4.6-3.1 Healthy Subject PK

Study A2104: An open label, single oral dose study to investigate the absorption, pharmacokinetics, distribution, metabolism, and elimination of 10 mg of [^{14}C] BAF312 in healthy male subjects

Objectives:

- Identify and quantify the metabolites of BAF312 in plasma, urine and feces
- Determine the rate and routes of excretion and the mass balance of total radioactivity in urine and feces
- Evaluate the absorption of unchanged drug and total radioactivity as feasible
- Elucidate the key biotransformation pathways and clearance mechanisms of BAF312 in man
- Determine the pharmacokinetics of total radioactivity of 14C-labeled BAF312, and of any important metabolites in plasma

Methodology: Single-center, open-label study in healthy male subjects administered a single oral ^{14}C-radiolabeled dose of 10 mg BAF312.

Number of patients: 4 healthy male volunteers were planned, recruited and completed the study.

Main criteria for inclusion: Healthy, non-smoking, male volunteers between 18 and 55 years of age, who were CYP2C9 wild-type (CYP2C9*1*1 carriers i.e. subjects without a *2 or *3 allele).

Dose and mode of administration: [^{14}C]BAF312 10 mg. Specific radioactivity: 0.37 MBq/mg (free base). The radiochemical purity was >96%.

The radiolabeled drug in solid from was provided by Novartis Pharma (Isotope Laboratory) in individual glass bottles each containing a dose of 10 mg/2 mL [^{14}C]BAF312 (batch no.: Y113 0609) as a concentrate for oral solution. Novartis also provided water for the dilution of the concentrate for on-site preparation of the drink solution of [^{14}C]BAF312. A pharmacist at study site prepared the drink solution prior to administration. Following dose administration by the Investigator, the vial was rinsed. The rinsing liquid was also swallowed by the subjects.

Sample collection:

- Blood : Pre-dose (0), 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240, 312, 480 and 816 h post-dose (22 timepoints in total).
- Urine: 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288, 288-312, 456-480, and 792-816 hours postdose, in a total of 17 fractions.
- Feces: Pre-dose blank obtained at screening or baseline, and all complete feces portions from 0-312 h post-dose. Additional feces samples were collected on Days 21 and 35.
- Vomitus: No vomiting occurred within the 12 hour interval specified for vomitus collection.

Analytes, sample matrices, and methods:

Total radioactivity in blood, plasma, urine and feces by liquid scintillation counting (LSC). Counting times urine, feces: 10 min; blood, plasma: 10 or 60 min;

LOQ in blood: 25 dpm, 2.31 ng-eq/mL;

LOQ in plasma: 7.5 dpm, 1.39 ng-eq/mL;

LOQ in urine: 10 dpm, 0.46 ng-eq/mL;

LOQ in feces 20 dpm 1.85 ng-eq/g.

BAF312 in plasma by a validated LC-MS-MS assay (LLOQ, 0.250 ng/mL using 0.0500 mL plasma).

AEB071 and metabolites in plasma, blood, urine and feces by LC with radioactivity detection (online or off-line by Topcount or LSC) with counting time up to 135 min/ well.

Characterization of BAF312 metabolites by LC-MS/(MS).

PK and ADME evaluations:

- Descriptive parameters, non-compartmental analysis
- PK parameters: AUCinf, AUC0-t, Cmax, Tmax, T½ , blood and plasma concentration-time data of ^{14}C radioactivity and/or BAF312
- Excretion / mass balance of 14C radioactivity in urine and/or feces
- Metabolite profiles in plasma, urine and feces fractions and fraction pools, AUC of main metabolites
- Identification of metabolites

Statistical methods:

Summary statistics (including mean and SD) for demographic and baseline characteristics, safety assessments, pharmacokinetic and ADME measurements. No formal inferential statistical analysis was performed. The number of subjects with adverse events was counted by body system and preferred term.

RESULTS

Excretion of radioactivity:

The excretion of total radiolabeled components (radioactivity) in urine and feces is summarized in the following table:

Time Period	^{14}C-Excretion [% of dose]					
[h]	Urine		Feces		Total	
0 - 216	3.61	(0.37)	84.1	(3.47)	87.7	(3.69)
0 - 312	3.70	(0.35)	86.7	(2.46)	90.4	(2.71)

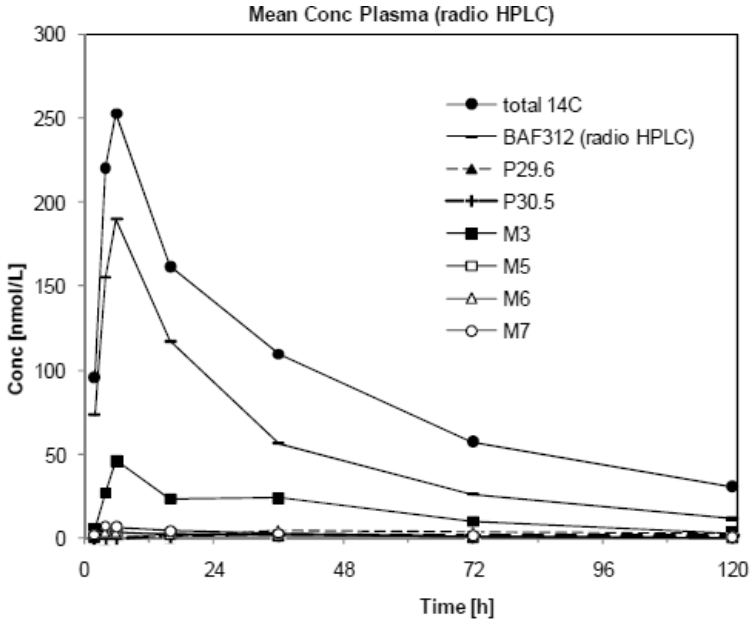
Means (SD) of n=4 subjects

Radioactivity was excreted mainly with feces (up to 86.7% of dose).

Metabolism:

Metabolite profiles in plasma

The concentration-time course of BAF312 and main metabolites in plasma between 0 and 120 hours post-dose are depicted in the following figure:



The AUC0-120h values of BAF312 and the plasma metabolites are listed in the following table:

Peak	Compound / Metabolite	AUC0-120h Min (nmol·h/L)	Max (nmol·h/L)	mean ± SD, N=4 [a,b] (nmol·h/L)		(% of total ^{14}C)	
P29.6	Unknown	267	491	378	± 103	3.72	±1.49
P30.5	Unknown	111	244	192	± 57.4	1.82	±0.467
M3	Glucuronide of M5	1690	2260	1850	± 268	18.4	±5.11
M5	Formed by hydroxylation	122	188	156	± 29.0	1.51	±0.343
M6	Formed by hydroxylation	135	213	170	± 39.9	1.63	±0.326
M7	Formed by hydroxylation	175	361	282	± 87.8	2.75	±1.11
BAF312	Parent drug	4350	10600	6320	± 2920	57.1 [c]	±5.91
P73.0	Formed during sample processing	91.0	275	190	± 91.7	1.74	±0.623
	Sum of unknown trace metabolites	176	231	204	± 29.8	2.02	±0.592
	Lost during sample processing and HPLC	622	1900	1040	± 578	9.30	±2.10
Total ^{14}C (total of radiolabeled components)		8200	16100	10800	± 3700	100	--

a: mean values of N=4 subjects.
b: mean values are means of individual values.
c: 58.8%, incl.P73 formed by methyl-esterification of parent drug during sample preparation.
--: not calculable, not meaningful.

Parent compound BAF312 represented the main proportion of radioactivity in plasma (57.1% of the plasma AUC0-120h). Metabolite (M3, formed by hydroxylation and glucuronidation) was the most prominent metabolite in plasma and amounted to 18.4% of the plasma AUC0-120h. Minor

proportions of other metabolite peaks were detected and attributed to the metabolites M5, M6, M7, P29.6 and P30.5, each accounting on average for 1.51-3.72% of the plasma AUC0-120h.

Relative exposure of metabolites in comparison to BAF312 is summarized in the following table:

Peak	AUCinf [a,b] (nmol·h/L)	Percentage of parent drug AUC (%)	T1/2 , mean [a] (h)
P29.6 [c]	378	5.8	--
P30.5 [c]	192	2.9	--
M3	1998	27.6	29.3
M5	173	2.4	34.0
M6	180	2.5	31.6
M7	320	4.4	35.2
BAF312 [d]	7240	100.0	33.3

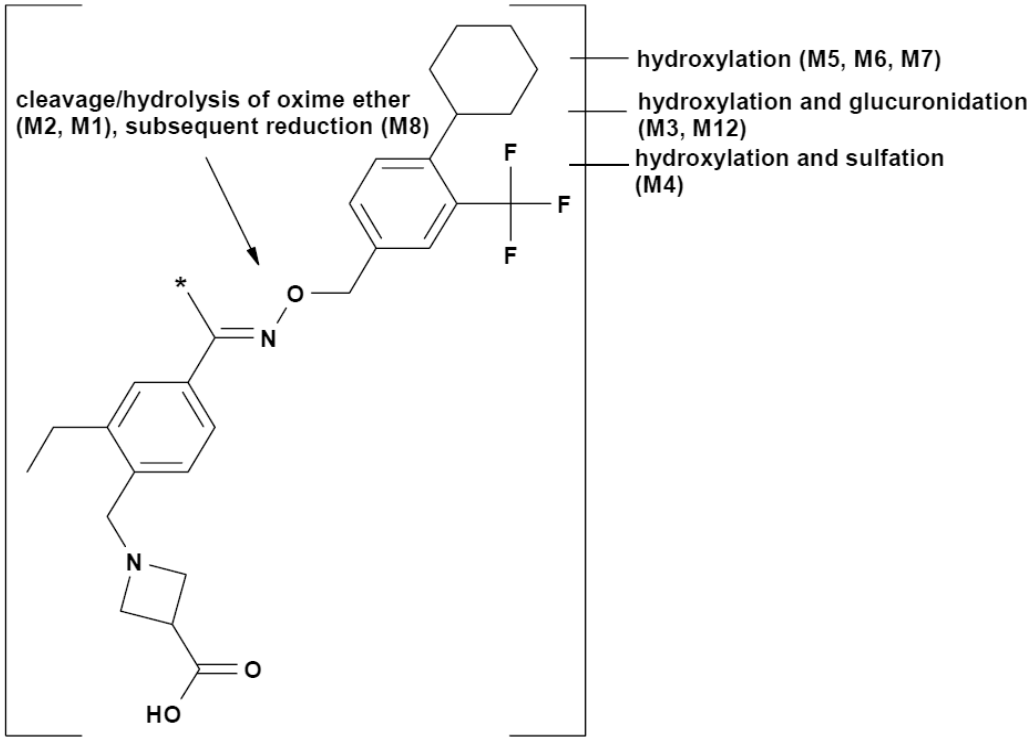
a: mean values of N=4 subjects.
b: AUCinf= AUClast+ AUCt-∞ ; AUCt-∞ = Clast ·T1/2/ ln2.
c: AUC0-120h used for metabolite and parent drug [d], AUCinf and T1/2 not calculable due to high percentage of extrapolation.
d: BAF312 and P73.0 summarized.
--: not calculable, not meaningful.

The AUCinf of metabolites M3 amounted to 27.6% of the parent drug AUC. The metabolites M5, M6 and M7 accounted each in average for 2.4-4.4% of the parent drug AUCinf. The terminal half-lifes of the metabolites M3, M5, M6 and M7 ranged between 29.3 and 35.2 hours.

Reviewer's note: The other major cholesterol ester metabolite M17 was not identified in this study.

Metabolism pathways

The biotransformation of BAF312 occurred by essentially by the following pathways:



The phase I metabolic reactions involved C-hydroxylations (M5, M6 and M7), cleavage/hydrolysis at the oxime ether bound (M1, M2) and further reduction yielding metabolite M8. Phase II reactions involved sulfation (M4) and glucuronidation (M3 and M12) of hydroxylated metabolites.

CONCLUSIONS

- The metabolite M3 (formed by glucuronidation of the hydroxylated metabolite M5) was the main metabolite and accounted for 18.4% of the radioactivity AUC0-120h (27.6% of the exposure to BAF312).
- The biotransformation of BAF312 occurred by essentially by the following pathways: The phase I metabolic reactions involved C-hydroxylations (M5, M6 and M7), cleavage/hydrolysis at the oxime ether bound (M1, M2) and further reduction yielding metabolite M8. Phase II reactions involved sulfation (M4) and glucuronidation (M3 and M12) of hydroxylated metabolites.
- BAF312 was eliminated from the systemic circulation mainly due to metabolism, and subsequent biliary/fecal excretion.

Study A2101: Single center, randomized, double-blind, placebo-controlled ascending single dose study to explore the safety, tolerability, pharmacokinetics and pharmacodynamics of oral BAF312 in healthy volunteers

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

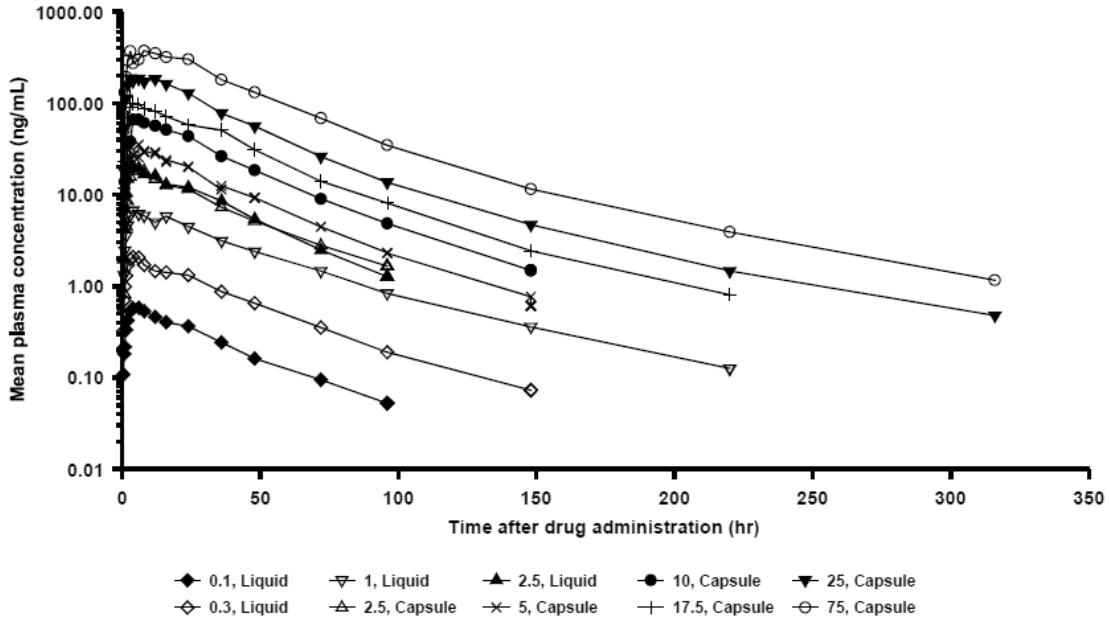
Study Design	Single-dose, ascending, randomized, double-blind, placebo-controlled
Study Population	N=98 recruited and analyzed (80 active, 14 placebo) Age: 18 - 55 years (mean 38.5 years) Gender: 73 males (74.5 %), 25 females (25.5%) Weight: 50-99 kg (mean 76.45 kg) Race: 55 Hispanic (56.1%), 28 Caucasian (28.6%), and 9 Black (9.2 %)
Dosage and Administration	10 cohorts Cohort 1: BAF312 0.1mg liquid (n=11) or placebo (n=2) Cohort 2: BAF312 0.3mg liquid (n=8) or placebo (n=2) Cohort 3: BAF312 1.0mg liquid (n=8) or placebo (n=2) Cohort 4: BAF312 2.5mg liquid/ capsule (n=13) or placebo (n=2) Cohort 5: BAF312 5mg capsule (n=8) or placebo (n=2) Cohort 6: BAF312 10mg capsule (n=8) or placebo (n=2) Cohort 7: BAF312 17.5 mg capsule (n=8) or placebo (n=2) Cohort 8: BAF312 25mg capsule (n=8) or placebo (n=2) Cohort 9: BAF312 75mg capsule (n=8) or placebo (n=2) Study drugs were administered with 240 mL of water between 07:20 and 09:00, after at least a 10-hour fast. Batch No. BAF312: F023GC BAF312 and F001BD BAF312 Placebo: X004 0104
PK Sampling:	Blood: Pre-dose, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, and 16 h post dose; Day 2 through Day 5 (24, 36, 48, 72, and 96 h post dose); Days 7, 10, and 14. Urine: Prior to dosage, and 0-24 h post-dose

<table>
<tr><td>PD Sampling:</td><td>Lymphocyte counts: Day -1 (pre-dose, 1, 2, 3, 4, 5, 6, 8, and 12 h post dose with placebo), Days 1- 5 (pre-dose, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 h post dose with study medication), Day 7, 10, and 14.</td></tr>
<tr><td>Analysis
(Plasma)</td><td>Method: LC/MS/MS
<table>
<tr><td>Parameters</td><td>Dynamic Range 1</td><td>Dynamic Range 2</td><td>Dynamic Range 3</td></tr>
<tr><td>LLOQ (ng/mL)</td><td>0.250</td><td>0.0200</td><td>2.50</td></tr>
<tr><td>Linear Range (ng/mL)</td><td>0.250-500</td><td>0.0200-20.0</td><td>2.50-2500</td></tr>
<tr><td>Inter-day Precision (%CV) for QCs</td><td>0.8-7.2</td><td>5.3-10.2</td><td>3.8-6.2</td></tr>
<tr><td>Inter-day Accuracy for QCs</td><td>-0.9-9.2</td><td>0.2-5.3</td><td>-0.5-6.3</td></tr>
</table></td></tr>
<tr><td>PK Assessment</td><td>The following PK parameters for BAF312 were determined from plasma samples using noncompartmental methods: AUC0-t, AUC0-∞, Cmax, tmax, tlag, and t½.</td></tr>
<tr><td>PD Assessment</td><td>Pharmacodynamic variables for the absolute lymphocyte counts were AUEC, Emax, and tmax. Descriptive summary statistics were given for these endpoints.</td></tr>
<tr><td>Safety Assessment</td><td>Physical examinations, vital signs, cystatin C and alphaglutathione S-transferase (α-GST), ECGs, clinical laboratory parameters (hematology, blood chemistry, and urinalysis), and adverse events monitoring.</td></tr>
</table>

RESULTS

Pharmacokinetics

The geometric mean concentrations of BAF312 in plasma after single ascending doses (0.1-75mg) under fasted conditions are shown in the following figure:



Source: Appendix 16.2.5-2, Post-text Figure 16.2.5-2.4

The main PK parameters are shown in the following figure:

BAF312 Treatment group	N	$AUC_{(0-\infty)}$ (h*ng/mL)		$AUC_{(0-t)}$ (h*ng/mL)		C_{max} (ng/mL)		t_{lag} (h)	t_{max} (h)	$t_{1/2}$ (h)	
		Arith Mean ± SD	Geo Mean [%CV geo mean]	Arith Mean ± SD	Geo Mean [%CV geo mean]	Arith Mean ± SD	Geo Mean [%CV geo mean]	Median (min-max)	Median (min-max)	Arith Mean ± SD	Geo Mean [%CV geo mean]
0.1 mg (L)	11	26.2 ± 9.73	24.6 [39]	24.6 ± 9.63	22.9 [41]	0.661 ± 0.265	0.618 [39]	0.25 (0.00-0.27)	4.00 (3.00-8.00)	34.12 ± 9.68	33.06 [26]
0.3 mg (L)	8	90.8 ± 17.4	89.3 [20]	88.6 ± 17.0	87.2 [20]	2.26 ± 0.141	2.26 [6]	0.00 (0.00-0.25)	5.00 (4.00-8.00)	34.30 ± 9.43	33.21 [28]
1.0 mg (L)	8	361 ± 98.0	349 [28]	357 ± 96.4	345 [27]	8.02 ± 3.56	7.43 [42]	0.00 (0.00-0.25)	5.00 (4.00-15.67)	46.91 ± 10.54	45.68 [26]
2.5 mg (L)	6	803± 251	766 [36]	779 ± 246	743 [36]	23.0 ± 7.70	21.9 [35]	0.13 (0.00-0.25)	4.00 (3.00-8.00)	27.58 ± 6.17	27.02 [22]
2.5 mg (C)	7	764 ± 181	745 [25]	741 ± 183	721 [26]	19.6 ± 3.53	19.3 [19]	0.75 (0.50-0.77)	6.00 (4.00-8.02)	29.67 ± 4.80	29.31 [17]
5.0 mg (C)	8	1290 ± 257	1260 [20]	1270 ± 258	1240 [21]	39.3 ± 8.60	38.5 [21]	0.25 (0.00-0.50)	3.00 (2.00-8.02)	31.76 ± 5.77	31.27 [19]
10.0 mg (C)	8	2730 ± 350	2710 [14]	2700 ± 340	2680 [13]	79.5 ± 21.4	77.3 [25]	0.25 (0.00-0.50)	5.00 (3.85-16.00)	32.43 ± 5.29	32.03 [17]
17.5 mg (C)	8	4340 ± 968	4230 [25]	4310 ± 961	4200 [25]	115 ± 29.8	111 [32]	0.25 (0.00-0.52)	4.00 (2.00-8.00)	44.72 ± 15.19	42.32 [38]
25.0 mg (C)	8	8350 ± 1990	8140 [24]	8310 ± 1980	8110 [24]	225 ± 63.6	217 [29]	0.25 (0.00-0.50)	3.50 (1.50-12.00)	48.47 ± 9.31	47.68 [20]
75.0 mg (C)	8	20800 ± 11700	18600 [51]	20700 ± 11600	18500 [51]	542 ± 250	491 [51]	0.25 (0.25-0.50)	6.00 (2.00-24.00)	57.05 ± 6.71	56.69 [12]

BAF312 was measurable in the plasma as early as 0.25 hr post-dose. The plasma concentration of BAF312 peaked between 3 and 6 hr post-dose (median) with a minimum value of 1.5 hr and a maximum value of 24 hr in individual subjects. Cmax and AUC0-24hr appeared to increase proportionally with rising dose intervals from 0.1 to 75 mg.

Dose proportionality

The relationship between dose and observed Cmax and AUC was estimated for the dose range of 0.1 - 75 mg and is summarized in the following table:

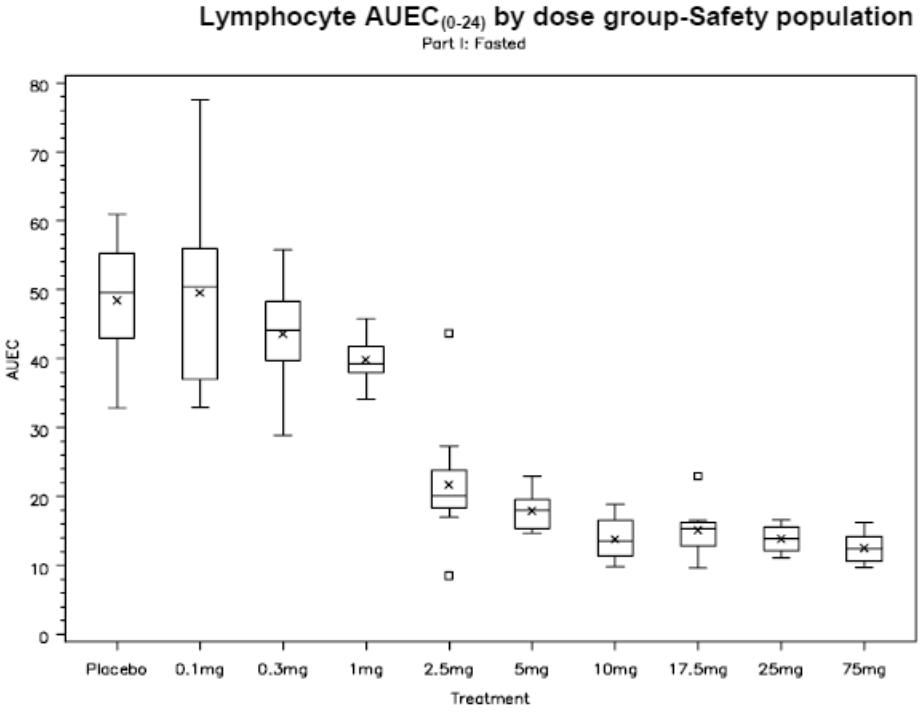
Parameter (Unit)	Intercept	Slope	90% CI (slope)	No-effect region	Conclusion
C_{max} (ng/mL)	1.97	1.01	(0.98, 1.04)	(0.97, 1.03)	No (proportion)
$AUC_{(0-24)}$ (h*ng/mL)	4.79	1.00	(0.97, 1.03)	(0.97, 1.03)	Yes (proportion)
$AUC_{(0-\infty)}$ (h*ng/mL)	5.65	1.00	(0.97, 1.02)	(0.97, 1.03)	Yes (proportion)
$AUC_{(0-t)}$ (h*ng/mL)	5.62	1.00	(0.98, 1.03)	(0.97, 1.03)	Yes (proportion)
Source: Post-text Table 14.2-1.3					

Proportionality was observed for the AUC calculations. Dose proportionality of Cmax was not seen over the whole dose range. However, in a slightly smaller dose range of 0.3-75 mg, dose proportionality of Cmax could be established with the no-effect region of 0.96 to 1.04.

Pharmacodynamics

Absolute lymphocyte counts were analyzed and summarized by dose group, changes from Day -1 to Day 1, and lymphocyte summary endpoints (AUEC(0-24), Emax(0-24), %Emax(0-24), TEmax(0-24) and %Emax) for either Day 1 or changes from Day -1.

AUEC(0-24) analysis of the absolute lymphocyte count is shown in the following figure:



The mean values for AUEC(0-24) showed a dose-dependent decline between the 0.3 mg dose and the 10 mg dose of BAF312. Further decreases in AUEC(0-24) values did not occur for doses above 10 mg. A similar trend was seen for Emax(0-24) values.

Median absolute lymphocyte counts at Time 0 on Day -1 ranged from 1.80 x 109/L to 2.75 x 109/L. When compared to Day-1 values, the absolute lymphocyte count declined with BAF312 administration in a dose-dependent manner (see table below).

Changes of lymphocyte count between Day -1 and Day 1

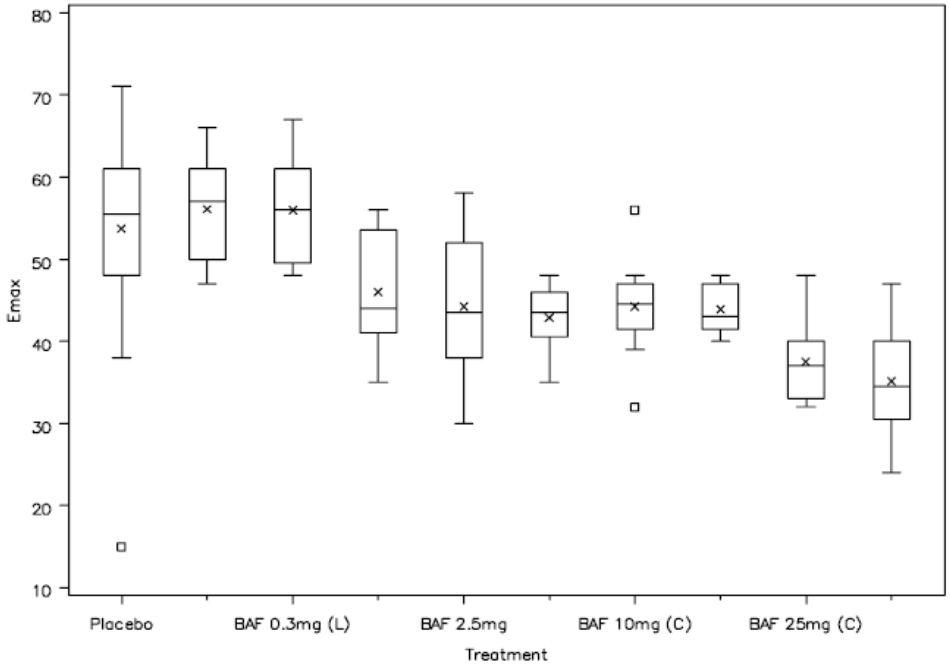
Treatment	Adjusted Mean ($x\ 10^9$/L)	Treatment Difference ($x\ 10^9$/L)
Placebo (all cohorts)	-0.026	
BAF312 0.1mg (L)	-0.188	-0.162
BAF312 0.3mg (L)	-0.246	-0.220
BAF312 1.0mg (L)	-0.590	-0.565
BAF312 2.5mg	-0.849	-0.823
BAF312 5.0mg (C)	-1.164	-1.139
BAF312 10.0mg (C)	-1.244	-1.218
BAF312 17.5mg (C)	-1.279	-1.253
BAF312 25.0mg (C)	-1.390	-1.364
BAF312 75.0mg (C)	-1.303	-1.277

Safety

Ventricular Heart Rate

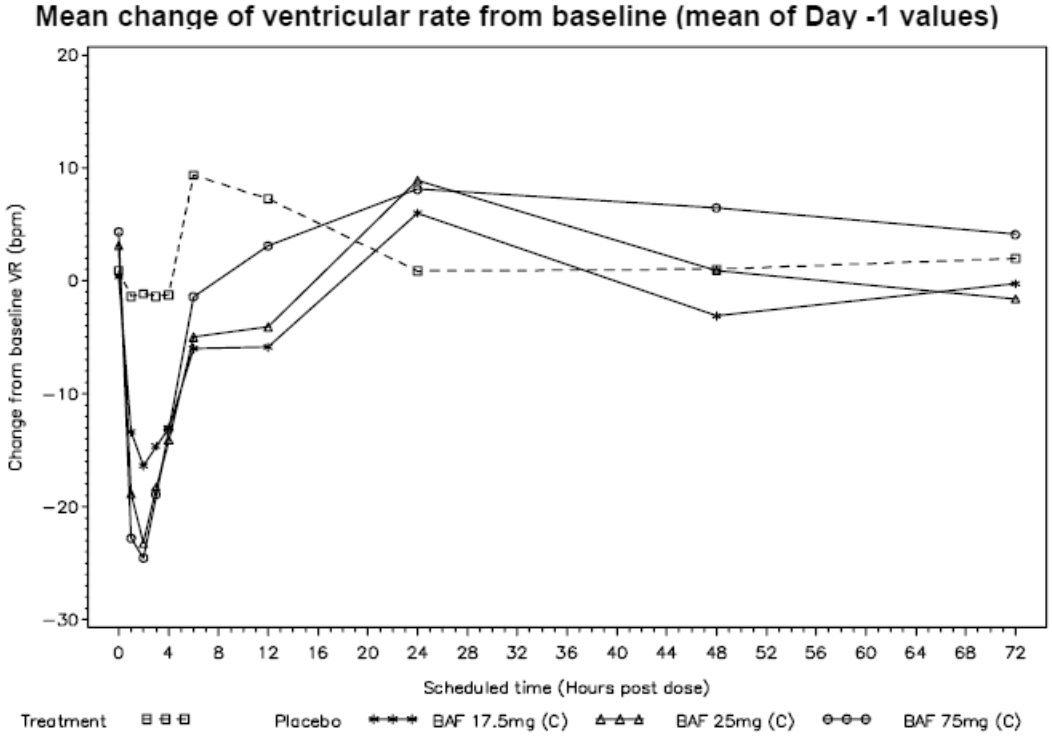
A dose-dependent decrease in mean ventricular heart rate was observed in fasting subjects over the first 24 hours post-dose. This effect reached a plateau at 5 mg of BAF312. The Emax(0-24) values by dose group are shown in the following figure:

Boxplot analysis of $Emax_{(0-24)}$ of heart rate by dose group



A similar trend was observed for AUEC(0-24) and TEmax(0-24). The peak effect was observed with the 2.5 mg and 5.0 mg doses.

The mean change in ventricular rate from baseline for placebo, 17.5 mg, 25.0 mg, and 75.0 mg of BAF312 were shown in the following figure:



Pulmonary Function

BAF312 did not produce a substantial or significant effect upon FEV1 or FEF25-75%. At doses of 10 mg and below, mean FEV1 values 6 hours post-dose ranged between 2.881 liters at the 2.5 mg dose and 3.668 liters at the 5.0 mg dose, in comparison to 3.41 liters observed among subjects receiving placebo. Similar results were noted for FEF25-75%. Consistent responses were not observed at doses below 5.0 mg. A trend towards a decline in FEV1 scores 6 hours post dose was noted at higher doses, with mean values of 3.16, 2.84, 2.95, 2.68 and 2.60 liters at the 10.0, 17.5, 25.0, and 75.0 mg doses, respectively.

Overall, the AEs seen in the study were as expected for this class of drug. There were no severe AEs, SAEs or deaths during the study. The AEs were mostly transient and either mild or moderate.

Discussion

As a potent S1P1/S1P5-selective receptor agonist, BAF312 was expected to avoid S1P3- mediated adverse effects such as acute, negative, chronotropic and pulmonary effects. Such effects were encountered with the less receptor-specific compound FTY720 (with S1P1, S1P3, S1P4, and S1P5 receptor activity). However, given the potential for cardiac and pulmonary AEs with FTY720, cardiac, pulmonary, neurological, and visual functions were closely monitored in this first-in-human BAF312 study. There were no significant pulmonary effects and minimal adverse effects upon other safety variables. However, acute, negative, chronotropic effects were demonstrated with BAF312.

The sponsor stated that the observed bradycardia did not require intervention and the effect on ventricular rate reached a plateau starting at 5 mg of BAF312.

CONCLUSIONS

- PK showed dose proportionality in the dose range of 0.1-75 mg, as analyzed by AUC. Median tmax was between 3 and 6 hr and an apparent terminal t1/2 of 27-57 hr was determined.
- BAF312 affected the peripheral lymphocyte count in a dose-dependent manner, with a plateau of effect starting at 5 mg.
- Transient dose-dependent decreases in ventricular rate and corresponding increases in PR intervals were observed.
- BAF312 did not produce clinically significant effects upon pulmonary function or neurological assessments.

Study A2105: A randomized, parallel, double-blind, placebo-controlled, time-lagged, ascending multiple-dose, pharmacokinetic, pharmacodynamic, safety and tolerability study of BAF312 in healthy volunteers

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

Study Design	Multiple -dose, ascending, randomized, double-blind, placebo-controlled
Study Population	N=50 enrolled and analyzed (37 active, 13 placebo) Age: 18 - 54 years (mean 34.7 years) Gender: 43 males (86.0%), 7 females (14%) Weight: 55.2-106.1 kg (mean 79.78 kg) Race: 39 Caucasian (78%), and 11 Black (22 %)
Dosage and Administration	5 cohorts Cohort 1: BAF312 0.3mg liquid (n=6) or placebo (n=3) Cohort 2: BAF312 0.1mg liquid (n=6) or placebo (n=2) Cohort 3: BAF312 2.5mg capsule (n=7) or placebo (n=2) Cohort 4: BAF312 10mg capsule (n=9) or placebo (n=3) Cohort 5: BAF312 20mg capsule (n=9) or placebo (n=3) Study drugs were administered with 240 mL of water at approximately 0900 h, after at least a 10-hour fast. Batch No. BAF312: F001BD / 6002122.003 (2.5 mg); F024GC / 6002123.002 (25 mg) Placebo: X0040104 / 3755667.015
Duration of treatment:	28 days
PK Sampling:	Blood samples (2 mL) Day 1: pre-dose, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h post dose Days 4, 6, 11, 14, 17, 20, 23, 26: pre-dose Day 7: pre-dose, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h post dose

<table>
<tr><td></td><td>Day 28 thru Day 29: pre-dose and 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36 h post-dose
Day 30: 48 h post-dose
Day 32: 96 h post-dose
Days 35, 38, 42 (Cohorts 4 & 5 only), and EOS.
Urine samples
Day 1: pre-dose, 0-6 h, 6-12 h; 12-24 h post-dose
Day 28: pre-dose, 0-6 h, 6-12 h; 12-24 h, 24-48 h, 48-72 h post-dose</td></tr>
<tr><td>PD Sampling:</td><td>Lymphocyte counts: Screening, Day -1 and Day 1(pre-dose, 1, 2, 3, 4, 5, 6, 8, and 12 h post dose), Days 3, 5, 7, 14, and 21 (pre-morning dose), Day 28 (pre-dose, 1, 2, 3, 4, 5, 6, 8, and 12 h post-dose), Days 35, 42 (Cohorts 4 & 5 only) and EOS.
Additional ALC data were obtained from safety samples: Baseline (Day -2) or pre-placebo on Run-in (Day -1), Days 14, 28, 42 (cohorts 4 and 5 only), and EOS.</td></tr>
<tr><td>Analysis</td><td>Method: LC/MS/MS
Plasma:
<table>
<tr><th>Parameters</th><th>Dynamic Range 1</th><th>Dynamic Range 2</th></tr>
<tr><td>LLOQ (ng/mL)</td><td>0.0200</td><td>0.250</td></tr>
<tr><td>Linear Range (ng/mL)</td><td>0.0200-20.0</td><td>0.250-500</td></tr>
<tr><td>Inter-day Precision (%CV)</td><td>2.3-8.9</td><td>4.8- 12.3</td></tr>
<tr><td>Inter-day Accuracy</td><td>-7.0-11.5</td><td>-3.5-4.8</td></tr>
</table>
Urine:
LLOQ (ng/mL): 0.0200; Linear Range (ng/mL): 0.0200-20.0
Inter-day Precision (%CV): 1.2-7.1;
Inter-day Accuracy: -7.3-9.8</td></tr>
<tr><td>PK Assessment</td><td>The following PK parameters for BAF312 were determined from plasma data using noncompartmental methods: AUClast, AUC0-24h, AUCτ, Cmax, Cmax,ss, Cmin,ss, Cav,ss, Tmax, Tmax,ss , Tlag, T1/2, Racc, Fluc and any other PK parameter deemed appropriate.</td></tr>
</table>

	The following PK parameters were calculated from urine data for BAF312: Ae0-t, CLR and any other PK parameters deemed appropriate.
PD Assessment	For ALC, time-matched change from baseline (placebo run-in Day -1) was calculated and the following summary endpoints determined: AUEC0-12h, Emax0-12h, and %Emax0-12h
Safety Assessment	Adverse events, laboratory evaluations, vital signs, ECG evaluations, cystatin C and alpha-GST, additional blood pressure assessments (24 hour ABPM), 24 hour Holter and telemetry monitoring, pulmonary function tests, ophthalmologic and neurological exams.

RESULTS

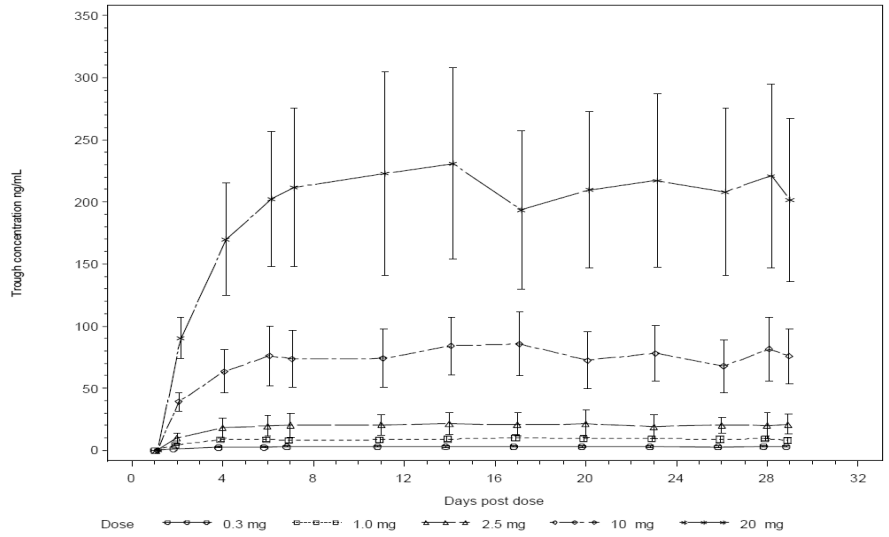
Pharmacokinetics

The PK parameters of BAF312 in plasma following a single oral dose administration on Day 1 are summarized in the table below (values are means and include ranges across all doses):

Parameter	Cohort 1 0.3 mg Daily (n=6)	Cohort 2 1 mg Daily (n=6)	Cohort 3 2.5 mg Daily (n=7)	Cohort 4 10 mg Daily (n=9)	Cohort 5 20 mg Daily (n=9)
Tlag (h)[1]	0.00 (0.00-0.25)	0.00 (0.00-0.00)	0.25 (0.25-1.00)	0.25 (0.25-0.25)	0.25 (0.00-0.25)
Tmax (h)[1]	3.00 (3.00-6.00)	4.50 (2.00-8.00)	3.00 (3.00-6.00)	3.00 (2.00-6.00)	4.00 (3.00-8.00)
Cmax (ng/mL)[2]	2.13 [13]	8.01 [6]	17.4 [88]	84.1 [11]	162 [20]
AUClast (h*ng/mL)[2]	36.2 [10]	136 [8]	293 [85]	1370 [11]	2740 [14]
AUC0-24h (h*ng/mL)[2]	36.2 [10]	136 [8]	293 [85]	1370 [11]	2740 [14]

[1]Median (min-max); [2]Geometric mean [%CV geo mean]

The following Figure represents the arithmetic mean (SD) pre-dose concentrations of BAF312 between Day 2 and Day 28.



Source: Post-text Table 14.2-1.3

The PK parameters of BAF312 in plasma following 28 days of daily oral administration are summarized in the table below (values are means and include ranges across all doses):

Parameters	Cohort 1 0.3 mg Daily (n=6)	Cohort 2 1 mg Daily (n=6)	Cohort 3 2.5 mg Daily (n=5)	Cohort 4 10 mg Daily (n=9)	Cohort 5 20 mg Daily (n=8)
Tlag (h)[1]	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Tmax,ss (h)[1]	3.50 (3.00-12.00)	3.00 (2.00-8.00)	4.00 (3.00-8.00)	3.00 (2.00-6.00)	3.00 (3.00-4.00)
Cmax,ss (ng/mL)[2]	5.31 [16]	14.9 [10]	38.3 [37]	147 [24]	359 [17]
AUClast (h*ng/mL)[2]	240 [38]	608 [28]	1450 [55]	5320 [35]	17000 [46]
AUCτ (h*ng/mL)[2]	97.9 [19]	282 [16]	692 [45]	2580 [24]	6370 [23]
CLz/F (L/h)[2]	3.06 [19]	3.55 [16]	3.61 [45]	3.89 [24]	3.21 (0.705)[3]
Vz/F (L)[2]	321 [22]	463 [40]	358 [64]	529 [36]	515 (148)[3]
Cavg (ng/mL)[2]	4.08 [19]	11.7 [16]	28.8 [45]	107 [24]	265 [23]
Cmin,ss (ng/mL)[2]	2.83 [27]	7.74 [20]	15.2 [120]	70.6 [29]	166 (88.0)[3]
T1/2(h)[2*]	71.5 [27]	90.4 [27]	68.8 [28]	93.6 [28]	110 [9]
Fluc (%)[2]	54.3 [29]	48.2 [30]	69.3 [39]	63.5 [14]	52.5 [41]
Racc[2]	2.71 [19.1]	2.07 [12.3]	2.72 [47.3]	1.88 [19.2]	2.28 [22.9]

[1]Median (min-max); [2]Geometric mean [%CV geo mean], [3]Arithmetic mean (SD)

*Corresponds to T1/2,β. The effective half-life is the half-life that reflects the observed drug accumulation; based on drug accumulation at steady-state, effective half-life is between 22 and 36 h

BAF312 trough concentrations measured from Day 1, 24 h post-dose to day 28, 24 h postdose, indicated that steady-state was reached in all 5 cohorts after approximately 6 days of multiple dosing for most of the subjects. Comparable PK parameters were defined on Days 7 and 28. Mean accumulation ratio (Racc), calculated as the ratio of AUCτ over AUClast (Day 1), was comprised between 1.88-2.72 on Day 28, with similar values determined for Day 7 (between 1.82-2.42).

Dose proportionality

Cmax, AUC0-24h/AUCτ and AUClast appeared to increase proportionally when the dose increased from BAF312 0.3 to 20 mg, on both Days 1 and 28. Dose proportionality, following a single oral dose on Day 1 and after multiple doses on Day 28, was assessed using a power model (see the table below):

Parameter	Day	Intercept	Slope	90% CI (slope)
Cmax	1	2.01	1.03	(0.98,1.09)
AUC0-24h	1	4.83	1.03	(0.97,1.09)
AUClast	1	4.83	1.03	(0.96,1.10)
Cmax,ss	28	2.79	0.99	(0.94,1.05)
AUCτ	28	5.69	0.98	(0.94,1.03)
AUClast	28	6.51	0.99	(0.92,1.06)

Dose PK relationship for AUClast and Cmax are analyzed with model: ln(PK)=ln(DOSE)*DAY
DAY and dose PK relationship for AUCτ and AUC0-24h are analyzed with model: ln(PK)=ln(DOSE)
Source: Post-text Table 14.2-1.1

Pharmacodynamics

On Day 1 the absolute lymphocyte counts (ALC) declined in a dose-dependent manner with the maximal reduction observed at approximately 4 to 6 hours post-dose. Changes in ALC (over days) showed a dose-dependent decline between the BAF312 0.3 mg to 10 mg dose. Recovery to baseline of ALC was observed at the end-of-study for all cohorts except cohort 5 (BAF312 20 mg) which remained below run-in Day -1 levels but showed a clear trend towards returning to run-in levels by the EOS visit. The effect of BAF312 to decrease the peripheral counts of different leukocyte subtypes was most prominent on CD3+ T cells and B cells with more pronounced response in CD4+ than in CD8+ T cells. Naïve CD4+ and CD8+ cells exhibited a more pronounced response than CD4+ and CD8+ peripheral effector memory cells, respectively. No substantial effects of BAF312 on the NK cells or on granulocytes were observed.

CONCLUSIONS

- Day 1 PK parameters were comparable to those calculated in the SAD study (CBAF312A2101 CSR), BAF312 steady-state concentrations were achieved following approximately 6 days of multiple once daily.
- Mean accumulation ratio Racc was in the range 1.89-2.83.
- An effective half-life between 22 and 38 h was calculated, which was comparable to the apparent terminal half-life determined in the single-dose study.

Study A2107: A double blind, placebo controlled, parallel group study to investigate two different dose-titration regimens of BAF312 on the negative chronotropic effect of BAF312 in healthy subjects.

Objectives: To evaluate the daily chronotropic effects of two dose-titration regimens of BAF312 (0.25 -10 mg) comparatively to the chronotropic effect of BAF312 10 mg and of placebo over 12 days.

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

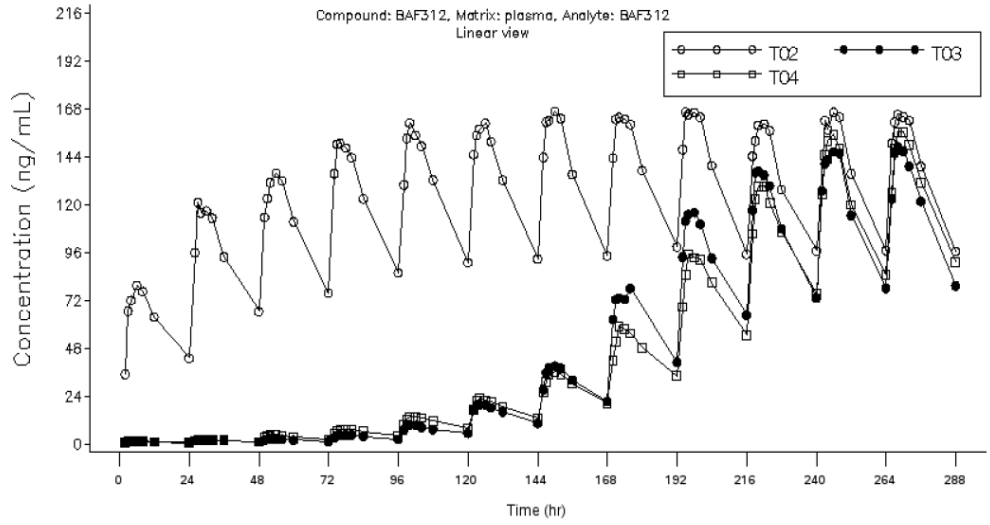
Study Design	Randomized double-blind parallel-group placebo-controlled study
Study Population	N= 56 recruited and analyzed Age: 18-64 years (mean 36.2 years) Gender: 51 males (91.1%), 5 females (8.9%) Weight: 53.0 - 100.2 kg (mean 80.14 kg) Race: 50 Hispanic (89.3%), 6 Other (10.7%)
Dosage and Administration	Dose-titration group 1: Starting with a 0.25-mg dose (Days 1-4) progressing to a 0.5-mg dose (Day 4) and a subsequent 1-mg dose (Day 5). The dose was then increased to 2 mg (Day 6), 4 mg (Day 7), 8 mg (Day 8), and 10 mg (Days 9-12). Dose-titration group 2: Starting with a 0.25-mg dose (Days 1 and 2) progressing to a 0.5-mg dose (Day 3) and a subsequent 0.75-mg dose (Day 4). The dose was then increased to 1.25 mg (Day 5), 2 mg (Day 6), 3 mg (Day 7), 5 mg (Day 8), 8 mg (Day 9) and 10 mg (Days 10-12). The non-titration group received a single dose of BAF312 10 mg on Days 1-12. The placebo group received placebo on Days 1-12. Siponimod 0.25 mg tablet Batch No. F021HE/6002636 Siponimod 1 mg tablet Batch No. FO22HE/6002630.001 Siponimod 4 mg tablet Batch No. FO25HE/6002702.001 Siponimod 5 mg tablet Batch No. F023HE/6002628.001 Matching placebo tablets Batch No. F02OHE/6002679.001 Study medication was administered orally with 240 mL of water between 8:00 AM and 9:00 AM after fasting.
PK Sampling:	• Days 1 to 11: pre-dose, 2, 3, 4, 6, 8 and 12 hours post dose • Day 12: pre-dose, 2, 3, 4, 6, 8, 12, and 24 hours post dose

Analysis (Plasma)	Method : LC/MS/MS BAF312 LLOQ: 0.02 ng/mL Linear range: 0.05-100 ng/mL Inter-day Precision (%CV): 2.0-5.2% Inter-day accuracy: -3.1 - 1.2 %
PK Assessment	The following plasma BAF312 PK parameters were determined for each individual using non-compartmental method(s): AUClast, AUC0-24h, AUCtau, Cmax, Cmax,ss, Cmin,ss, Cav,ss , Tmax, Tmax,ss , Tlag, Racc, Fluc and any other PK parameter deemed appropriate.
PD Assessment	12-lead digital Holter data, Cardiac rhythm data, Frequency and duration of atrial fibrillation and/or ventricular ectopy and sinus rhythm, Fraction from previous day (FFPD) in HR.
Safety Assessment	Adverse event (AE), serious AE (SAE), regular monitoring of hematology, blood chemistry, and urine for abnormalities, and regular assessments of vital signs, physical condition, and body weight.

RESULTS

Pharmacokinetic Results

The daily geometric mean concentrations of BAF312 in plasma after once daily administration of BAF312 over 12 days in fasted conditions were showed in the figure below:



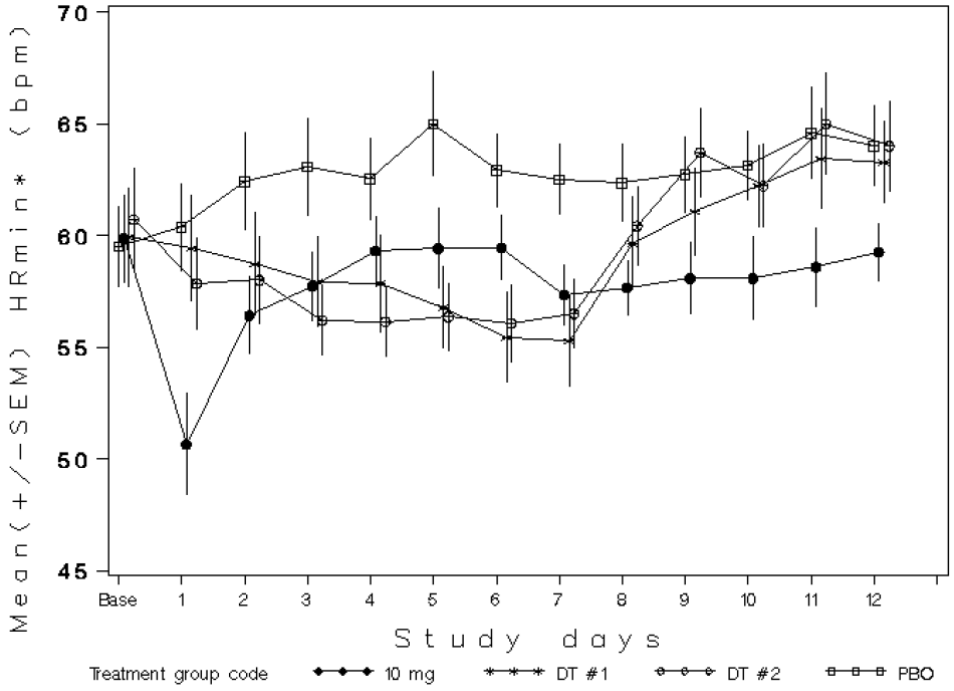
T02=BAF312 10 mg
T03=Dose Titration #1
T04=Dose Titration #2

Steady-state was reached in the 10 mg q.d. cohort after approximately 6 days of multiple dosing. The mean accumulation ratio (Racc), calculated for the 10 mg q.d. cohort, as the ratio of AUC0-24h on Day 12 over AUCl0-24h on Day 1, was 2.33. The time to steady state and the accumulation ratio estimated at steady state for the 10 mg q.d cohort are comparable to those observed in the multiple dose study A2105. On day 12, the concentrations-time profile in all three BAF312 treatment groups are comparable.

Pharmacodynamic Results

Mean daily minimum heart rate

The mean daily minimum HR for each treatment group is showed in the figure below:



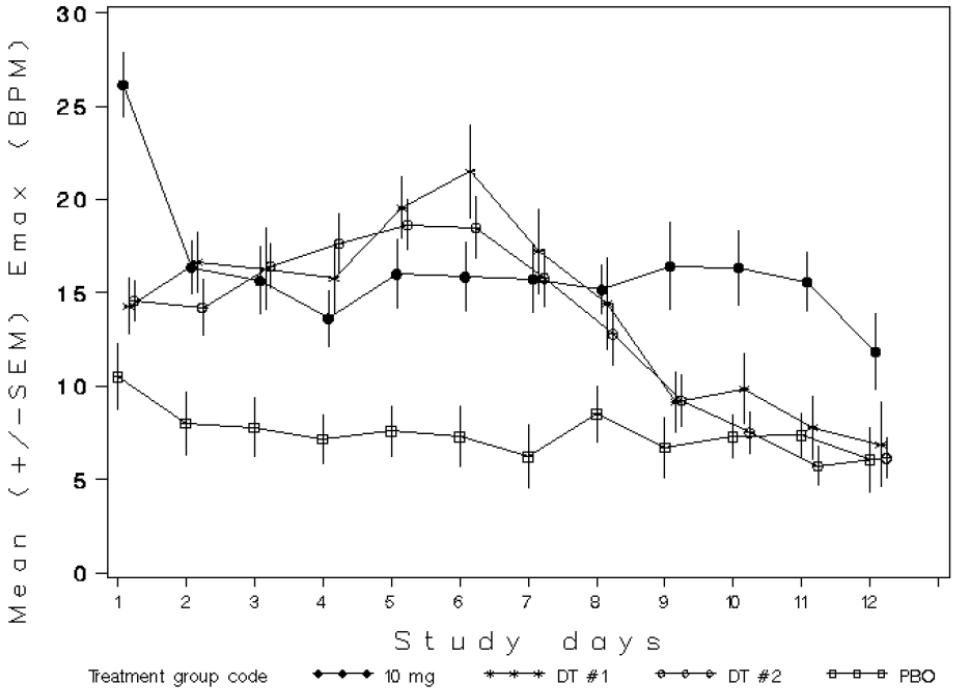
Source: PT-Figure 14.2-1.5

At no time did either dose-titration treatment group experience a clinically significant bradycardia as it did for 10-mg treatment group on Day 1. At Day 10, both dose-titration treatment groups were similar to placebo.

Maximum decrease from baseline in hourly average HR (Emax heart rate)

Emax is defined as the maximum decrease from baseline in hourly average HR on each day. A positive mean Emax corresponds to a decrease in HR from baseline.

The maximum decrease from baseline in hourly heart rate for each treatment group on Days 1-12 are showed in the following figure:



Note: A positive mean Emax corresponds to a decrease in HR from baseline.
Source: PT-Figure 14.2-1.7

The two dose-titration treatment groups did not experience a statistically significant difference from placebo in Emax on Day 1. The differences in the mean maximum decrease in the hourly average HR for the two dose-titration treatment groups versus placebo met statistical significance on Days 3-7 ($p \leq 0.0001$). From Day 8 to EOS, both dose-titration groups were similar to placebo. Neither dose-titration treatment group experienced bradycardia at any point in the study.

Atrioventricular block and sinus-pause events

The frequency and duration of sinus pauses (> 2 seconds) and atrioventricular (AV) blocks was determined through 24-hour Holter monitoring.

In dose-titration group 2, only one subject experienced at least one sinus pause compared to dose-titration treatment group 1 and the 10-mg treatment group where four subjects experienced at least one sinus pause. The placebo group experienced no pauses.

Safety

All three BAF312 treatment groups experienced more AEs than the placebo group, the AEs were generally of mild to moderate severity. There were no deaths or serious/severe adverse events and no

subject required medical treatment for adverse events. Overall, all three BAF312 dosing regimens were well tolerated in this study.

CONCLUSIONS

Both dose-titration schemes attenuate the negative chronotropic effect observed on Day 1 in the BAF312 10mg group. In both dose-titration schemes, the mean HR values reach, at the end of the dose-titration period, values similar to those observed in the placebo group. The results on the parameter “Pauses > 2 sec” would be in favor of a better safety profile with dose titration group2.

Study A2110: A randomized, partially double blind, placebo-controlled study to investigate the effect of the BAF312 treatment re-initiation on the initial negative chronotropic effect in healthy subjects.

Objectives: To measure the negative chronotropic effect of BAF312 re-initiation after periods of discontinuation from continued drug therapy.

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

<table>
<tr><td>Study Design</td><td>Partially double-blind, randomized, placebo-controlled study</td></tr>
<tr><td>Study Population</td><td>N= 138 recruited and analyzed
Age: 19 - 55 years (mean 40.8 years)
Gender: 81 males (58.7%), 57 females (41.3%)
Weight: 51.4 - 103.2 kg (mean 74.40 kg)
Race: 125 Caucasian (90.6%), 13 Black (9.4%)</td></tr>
<tr><td>Dosage and Administration</td><td>Four dose levels (0.5 mg, 1.0 mg, 2.0 mg, and 4.0 mg) were evaluated in combination with five drug discontinuation periods.
<table>
<tr><th>Sequence</th><th>Period 1</th><th>Period 2</th><th>Period 3</th></tr>
<tr><td>1</td><td>Placebo (192 h)</td><td>0.5 mg (120 h)</td><td>1.0 mg (72 h)</td></tr>
<tr><td>2</td><td>0..5 mg (48 h)</td><td>2 mg (96 h)</td><td>4.0 mg (72 h)</td></tr>
<tr><td>3</td><td>Placebo (96 h)</td><td>0.5 mg (72 h)</td><td>4.0 mg (192 h)</td></tr>
<tr><td>4</td><td>0.5 mg (96 h)</td><td>1.0 mg (120 h)</td><td>2.0 mg (192 h)</td></tr>
<tr><td>5</td><td>0.5 mg (192 h)</td><td>1.0 mg (96 h)</td><td>4.0 mg (120 h)</td></tr>
<tr><td>6</td><td>1.0 mg (48 h)</td><td>2.0 mg (72 h)</td><td>4.0 mg (96 h)</td></tr>
<tr><td>7</td><td>Placebo (48 h)</td><td>1.0 mg (192 h)</td><td>2.0 mg (120 h)</td></tr>
</table>
<table>
<tr><th>Study drug and strength</th><th>Formulation control number</th><th>Batch number</th></tr>
<tr><td>BAF312 0.25 mg</td><td>6002636.001</td><td>X186 0909</td></tr>
<tr><td>BAF312 1 mg</td><td>6002630.001</td><td>X065 0210</td></tr>
<tr><td>BAF312 4 mg</td><td>6002702.001</td><td>X188 0909</td></tr>
<tr><td>Placebo</td><td>6002679.003</td><td>X221 0909</td></tr>
</table>
All study drugs were administered between 8:00 AM and 10:00 AM in fasting conditions.</td></tr>
<tr><td>PK Sampling:</td><td>Visits 14, 26, 38 (pre-dose, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h post dose); Only pre-dose sample to be taken at Visit 6, 8, 10, 12, 18, 20, 22, 24, 30, 32, 34, 36.</td></tr>
<tr><td>Analysis
(Plasma)</td><td>Method : LC/MS/MS
<u>BAF312</u>
LLOQ: 0.02 ng/mL</td></tr>
</table>

	Linear range: 0.05-100 ng/mL Inter-day Precision (%CV): 2.0-5.2% Inter-day accuracy: -3.1 - 1.2 %
PK Assessment	Pharmacokinetic parameters (AUClast, Cmax, Clast, Tlag, Tmax) were calculated for phase C only (sampling over 24 h).
PD Assessment	Digital 12-lead Holter ECG, Heart Rate, Cardiac Rhythm, Absolute Lymphocyte Count
Safety Assessment	Adverse event (AE), serious AE (SAE), regular monitoring of clinical laboratory evaluations (hematology, clinical chemistry, urinalysis and liver function tests) performed at (study center laboratory) and regular assessments of vital signs, physical examination, body weight and height, electrocardiogram, continuous cardiac monitoring and meal records.

RESULTS

Primary Pharmacodynamic Results

Statistical analysis of the maximum pooled placebo-adjusted heart rate difference after single dose challenge from pre-challenge heart rate average (entire PD population) is given in the table below:

Statistical analysis of maximum pooled placebo-adjusted heart rate decrease after single dose challenge from pre-challenge heart rate average (PD population)

Drug holiday	Statistics	BAF312 0.5 mg	BAF312 1 mg	BAF312 2 mg	BAF312 4 mg
48 h	N	15	17		
	Adjusted mean difference (SE)	2.67 (1.584)	3.72 (1.501)		
	90% CI	(0.052, 5.285)	(1.242, 6.203)		
	P-value	0.093	0.014		
72 h	N	16	13	16	15
	Adjusted mean difference (SE)	6.02 (1.540)	7.61 (1.664)	6.10 (1.535)	3.64 (1.584)
	90% CI	(3.478, 8.568)	(4.857, 10.357)	(3.565, 8.637)	(1.019, 6.252)
	P-value	<0.001	<0.001	<0.001	0.023
96 h	N	17	17	15	15
	Adjusted mean difference (SE)	4.65 (1.501)	6.40 (1.501)	9.39 (1.584)	6.57 (1.569)
	90% CI	(2.171, 7.132)	(3.923, 8.884)	(6.771, 12.004)	(3.980, 9.165)
	P-value	0.002	<0.001	<0.001	<0.001
120 h	N	16	16	16	16
	Adjusted mean difference (SE)	7.55 (1.540)	9.27 (1.532)	9.56 (1.535)	8.17 (1.532)
	90% CI	(5.008, 10.098)	(6.741, 11.804)	(7.024, 12.096)	(5.637, 10.699)
	P-value	<0.001	<0.001	<0.001	<0.001
192 h	N	17	17	15	13
	Adjusted mean difference (SE)	5.65 (1.501)	11.01 (1.501)	9.86 (1.566)	14.53 (1.664)
	90% CI	(3.171, 8.132)	(8.534, 13.495)	(7.271, 12.447)	(11.783, 17.284)
	P-value	<0.001	<0.001	<0.001	<0.001

Source: PT-Table 14.2-1.1

The magnitude of the negative chronotropic effects at BAF312 re-initiation appeared to be dependent on both the dose and the duration of treatment discontinuation.

The most pronounced HR decreases (> 30 bpm) were observed after drug discontinuation periods of 120 hours or more (1 mg/120 hours, 2 mg/120 hours and 2 mg/192 hours). Re-challenge at the dose

of 2 mg after the discontinuation period of 72 hours showed lower decrease in pooled placebo-adjusted HR (adjusted mean difference of 6.10 bmp) as compared to that after the discontinuation period of 96, 120 and 192 hours with the adjusted mean difference of 9.36, 9.56 and 9.86 bpm, respectively. The upper bound of the two-sided 90% confidence interval (CI) for mean maximum heart rate effect for all conditions were greater than 10 bpm (ranged from 12.004 to 12.447 bmp), except for the upper bounds at the condition 72 h (8.637 bpm). The results on the parameter of HR decrease would be in favor of a better safety profile for the discontinuation period of 72 hours.

Secondary Pharmacodynamic Results

AV blocks

First degree AV blocks (PR > 200 ms) were detected at all investigated dose levels and at all investigated drug discontinuation periods (not on all combinations) but no clear treatment dependent pattern could be identified.

A total of three events of second degree AV block were reported in this study:

- Two events of second degree AV blocks (Mobitz 1) were observed in two subjects (at 2 mg/72 h (05:14 PM) and placebo (06:34 AM, i.e. during resting hours associated with increased vagal tone), respectively).
- A second degree AV block with 2:1 conduction was detected at 01:40 AM (during periods associated with increased vagal tone) in 1 subject at 1 mg/120 h.

All observed first and second degree AV blocks were asymptomatic, based on their nature, diurnal pattern of occurrence and frequency were not considered to be of clinical relevance.

Sinus pauses

Single sinus pauses (RR > 2 s) were observed in:

- One subject (b) (6) at 0.5 mg/48 h within 3-4 h after dosing (RR: 2.03 s),
- One subject (b) (6) in the placebo group (RR: 2.08 s).

Two sinus pauses were detected in:

- One subject (b) (6) at 4 mg/96 h approx. 3 h after dosing (RR: 2.02 s).

More than three sinus pauses were detected in:

- One subject (b) (6) at 1 mg/96 h (total number: 42; 3 within 3-4 h and 39 within 4-5 h after dosing (08:54 AM), Longest RR: 2.26 s)

Overall, no trend for the incidence or duration of sinus pauses could be detected in relation to the investigated conditions (dose and number of missed doses).

Absolute Lymphocyte Count (ALC)

The observed reductions of ALC in this study show a similar pattern and magnitude compared to previous clinical studies in healthy subjects.

None of the subject in this study showed ALC values below the threshold of 0.2 x 109 cells/L and no subject had to be discontinued due to an exaggerated pharmacodynamic response.

Pharmacokinetic Results

PK steady-state had been reached in the majority of subjects just before dose discontinuation for the doses of 0.5 mg and 1 mg. For the 2 mg and 4 mg doses, concentrations were still increasing before dose discontinuation in most cases, with concentrations close to steady-state. BAF312 was almost completely cleared from plasma 120 h after discontinuation for all investigated dose levels. Exposure levels were as expected for each dose at re-challenge.

Safety

Overall, BAF312 was well tolerated and the majority of AEs were mild or moderate in severity. There was no major difference between the incidences of AEs between different dose levels and across all four dose discontinuation periods. No deaths were reported during this study. One SAE of atrial fibrillation of moderate severity was reported. The majority of AEs were mild or moderate in severity.

CONCLUSIONS

- The magnitude of the negative chronotropic effects at BAF312 re-initiation appeared to be dependent on both the dose and the duration of treatment discontinuation. In some of the investigated conditions BAF312 treatment re-initiation at the discontinued therapeutic dose level might be considered without the need for additional up-titration scheme.
- The frequency and nature of AV blocks observed in this study is similar to the observations in other studies with siponimod and is not considered to be of clinical relevance.
- Overall, no trend for the incidence or duration of sinus pauses could be detected in relation to the investigated conditions (dose and number of missed doses).
- Overall, BAF312 was well tolerated and the majority of AEs were mild or moderate in severity. There was no major difference in the frequency of AEs between different dose levels and across all four dose discontinuation periods.

4.6-3. HUMAN PK STUDIES

4.6-3.2 Intrinsic Factors

Study A2122: A single-dose, open-label, parallel-group study to assess the pharmacokinetics (PK) of BAF312 in subjects with mild, moderate and severe hepatic impairment compared to healthy control subjects

Objectives: To investigate the PK of BAF312 and selected metabolites, safety and tolerability after administration of a single dose of 0.25 mg of BAF312 in subjects with mild, moderate and severe hepatic impairment in comparison to healthy control subjects.

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

Study Design	Single-dose, open-label, parallel-group
Study Population	N= 40 recruited and analyzed (CYP2C9*1 homozygous carriers) Age: 40 - 63 years (mean 50.7 years) Gender: 26 males (65%), 14 females (35%) Weight: 50.2 - 112.0 kg (mean 76.35 kg) Race: 40 Caucasian (100%)
Dosage and Administration	4 groups Group 1: BAF312 0.25mg Mild Hepatic impairment n=8 Group 2: BAF312 0.25mg Moderate Hepatic impairment n=8 Group 3: BAF312 0.25mg Severe Hepatic impairment n=8 Group 4: BAF312 0.25mg Matched Healthy subjects n=16 Study drugs 0.25 mg was administered orally following fasting for 10 hours and continued to fast for at least 4 hours thereafter. Batch No. X009 0112; Packaging control No. 12-0439CH No grapefruit or grapefruit
PK Sampling:	Blood: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 144, 216, 312, 408 and 504 hours post dose.

Analysis (Plasma)	Method : LC/MS/MS BAF312 LLOQ: 0.02 ng/mL Linear range: 0.02-20 ng/mL Inter-day Precision (%CV): 4.3-7.8% Inter-day accuracy: -4.2- 2.5 % LNL925(M3) LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 4.3-11.5% Inter-day accuracy: -1.4-2.7 % LNL931 (M5) LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 3.1-8.0% Inter-day accuracy: -2.3- 3.3%
PK Assessment	The following PK parameters of siponimod and its metabolites were determined using non-compartmental method: Cmax, Tmax, AUClast, AUCinf, T1/2, Vz/F and CL/F from the plasma concentration-time data.
Safety Assessment	AEs, hematology, blood chemistry, coagulation, urinalysis, physical examinations, vital signs, body temperature, ECGs, cardiac monitoring, meal records, Columbia suicide severity rating scale (C-SSRS) and body weight.

RESULTS

Pharmacokinetics of siponimod

The descriptive statistics of primary and secondary pharmacokinetic parameters of siponimod in subjects with hepatic impairment and matched healthy subject groups are summarized in following tables:

Group	Statistics	AUCinf (h·ng/mL)	AUClast (h·ng/mL)	Cmax (ng/mL)
Child-Pugh severity - Mild	n	8	8	8
	Mean ± SD (CV%)	68.3 ± 24.5 (36.0)	66.8 ± 24.4 (36.5)	2.03 ± 0.532 (26.2)
	Geo-mean	64.3	62.8	1.96
	CV(%) geo-mean	39.4	39.9	28.6
Matched healthy subjects – Mild	n	8	8	8
	Mean ± SD (CV%)	64.2 ± 20.8 (32.3)	62.5 ± 20.9 (33.5)	1.74 ± 0.439 (25.2)
	Geo-mean	61.4	59.6	1.69
	CV(%) geo-mean	32.9	34.4	25.0
Child-Pugh severity – Moderate	n	7*	7*	7*
	Mean ± SD (CV%)	53.9 ± 7.57 (14.1)	52.3 ± 7.51 (14.4)	1.54 ± 0.191 (12.4)
	Geo-mean	53.4	51.9	1.53
	CV(%) geo-mean	13.2	13.5	13.0
Matched healthy subjects - Moderate	n	8	8	8
	Mean ± SD (CV%)	63.2 ± 18.6 (29.4)	61.7 ± 18.7 (30.2)	1.80 ± 0.417 (23.1)
	Geo-mean	61.1	59.6	1.76
	CV(%) geo-mean	27.4	28.3	21.9
Child-Pugh severity - Severe	n	8	8	8
	Mean ± SD (CV%)	73.7 ± 25.4 (34.5)	71.6 ± 24.5 (34.3)	1.58 ± 0.304 (19.3)
	Geo-mean	70.2	68.2	1.55
	CV(%) geo-mean	33.7	33.7	19.4
Matched healthy subjects - Severe	n	8	8	8
	Mean ± SD (CV%)	64.9 ± 23.6 (36.4)	63.4 ± 23.6 (37.3)	1.94 ± 0.603 (31.2)
	Geo-mean	60.8	59.1	1.86
	CV(%) geo-mean	41.8	43.1	31.8

*Valid PK parameters could not be calculated for one subject because the subject had maximum PK concentration at the pre-dose time point

Group	Statistics	Tmax** (h)	T1/2 (h)	CL/F (L/h)	Vz/F (L)
Child-Pugh severity – Mild	n	8	8	8	8
	Mean ± SD (CV%)	4.00 (2.00-8.00)	30.3 ± 15.1 (49.9)	4.15 ± 1.65 (39.7)	168 ± 85.8 (51.0)
	Geo-mean	-	27.4	3.89	154
	CV(%) geo-mean	-	49.4	39.4	44.8
Matched healthy subjects – Mild	N	8	8	8	8
	Mean ± SD (CV%)	4.00 (3.00-8.00)	27.6 ± 4.86 (17.7)	4.26 ± 1.35 (31.8)	167 ± 53.3 (31.9)
	Geo-mean	-	27.2	4.07	160
	CV(%) geo-mean	-	17.2	32.9	33.4
Child-Pugh severity – Moderate	N	7*	7*	7*	7*
	Mean ± SD (CV%)	4.00 (3.00-6.00)	27.0 ± 7.65 (28.3)	4.71 ± 0.577 (12.2)	179 ± 25.7 (14.4)
	Geo-mean	-	26.3	4.68	177
	CV(%) geo-mean	-	25.5	13.2	14.3
Matched healthy subjects – Moderate	N	8	8	8	8
	Mean ± SD (CV%)	4.00 (3.00-8.00)	27.8 ± 5.22 (18.8)	4.21 ± 1.03 (24.4)	167 ± 45.4 (27.2)
	Geo-mean	-	27.4	4.09	162
	CV(%) geo-mean	-	18.9	27.4	28.1
Child-Pugh severity - Severe	N	8	8	8	8
	Mean ± SD (CV%)	4.00 (3.00-12.00)	40.1 ± 22.6 (56.3)	3.73 ± 1.15 (30.9)	192 ± 48.4 (25.2)
	Geo-mean	-	36.3	3.56	187
	CV(%) geo-mean	-	46.0	33.7	27.1
Matched healthy subjects - Severe	N	8	8	8	8
	Mean ± SD (CV%)	4.00 (3.00-8.00)	25.7 ± 4.55 (17.7)	4.43 ± 1.90 (42.9)	156 ± 44.4 (28.5)
	Geo-mean	-	25.3	4.11	150
	CV(%) geo-mean	-	17.8	41.8	28.8

*Valid PK parameters could not be calculated for one subject because the subject had maximum PK concentration at the pre-dose time point

**Median (Min - Max) is displayed instead of Mean ± SD (CV%)

The statistical assessment (Geometric mean ratio and 90% confidence intervals) of siponimod primary harmacokinetic parameters for subjects with hepatic impairment vs. matched healthy subjects is shown in the following table:

Parameter (unit)	Subject group	n	Adjusted geometric mean*	Ratio of geometric means* (HI/Healthy subjects)	90% CI for ratio*
AUCinf (h ng/mL)	Child-Pugh severity - Mild	8	64.3	1.05	(0.768, 1.43)
	Matched healthy subjects - Mild	8	61.4	-	-
	Child-Pugh severity - Moderate	7**	54.7	0.895	(0.781, 1.03)
	Matched healthy subjects - Moderate	8	61.1	-	-
	Child-Pugh severity - Severe	8	70.2	1.15	(0.836, 1.59)
	Matched healthy subjects - Severe	8	60.8	-	-
AUClast (h ng/mL)	Child-Pugh severity - Mild	8	62.8	1.05	(0.768, 1.45)
	Matched healthy subjects - Mild	8	59.6	-	-
	Child-Pugh severity - Moderate	7**	53.2	0.893	(0.773, 1.03)
	Matched healthy subjects - Moderate	8	59.6	-	-
	Child-Pugh severity - Severe	8	68.2	1.15	(0.831, 1.60)
	Matched healthy subjects - Severe	8	59.1	-	-
Cmax (ng/mL)	Child-Pugh severity - Mild	8	1.96	1.16	(0.942, 1.42)
	Matched healthy subjects - Mild	8	1.69	-	-
	Child-Pugh severity - Moderate	7**	1.53	0.868	(0.720, 1.05)
	Matched healthy subjects - Moderate	8	1.76	-	-
	Child-Pugh severity - Severe	8	1.55	0.837	(0.666, 1.05)
	Matched healthy subjects - Severe	8	1.86	-	-

Model: The log transformed PK parameter data are analysed using a linear mixed effects model with subject group as a fixed effect and subject matched pair as a random effect. . Subjects that are not matched are excluded from this analysis.
* Back-transformed from log scale
** Valid PK parameters could not be calculated for one subject because the subject had maximum PK concentration at the pre-dose time point
Note: each group contains 8 subjects.

Mean Cmax of siponimod increased by 16% in mild hepatic impairment subjects as compared to matched healthy subject group, while mean Cmax decreased by approx. 13% and 16% in moderate and severe hepatic impairment subjects, respectively, as compared to their matched control groups. However, these differences were not statistically significant.

Mean AUCinf and AUClast increased by 5% in mild hepatic impairment group and 15% in severe hepatic impairment group compared to their matched healthy group. However, a decrease of the mean AUCs of about 13% was observed in the moderate hepatic impairment group compared to their matched healthy group. These differences were not statistically significant.

The mean elimination T1/2 of siponimod in subjects with mild and moderate hepatic impairment groups was comparable to their matched healthy subject groups, and ranged between 26 and 36 hours. The mean T1/2 increased by approx. 56% in severe hepatic impairment subjects, as compared to the matched control groups.

The mean systemic clearance (CL/F) of siponimod was comparable between hepatic impairment and their matched healthy control groups.

Reviewer's note: *56% increased mean T1/2 in severe hepatic impairment subjects may due to the large inter-subject variability (%CV of approx. 56%) observed in severe hepatic impairment subject group.*

Pharmacokinetics of siponimod metabolites

Following oral administration, siponimod is metabolized to M5 mainly by hydroxylation (CYP 2C9 and 3A4). M5 (hydroxylated metabolite) converts to M3 by glucuronidation and hydroxylation. M3 accounts for the major portion of the metabolites (approx. 28%).

Reviewer's note: *The other major metabolite of siponimod (M17, a cholesterol ester) was not evaluated in this study.*

PK of metabolite LNL925 or M3

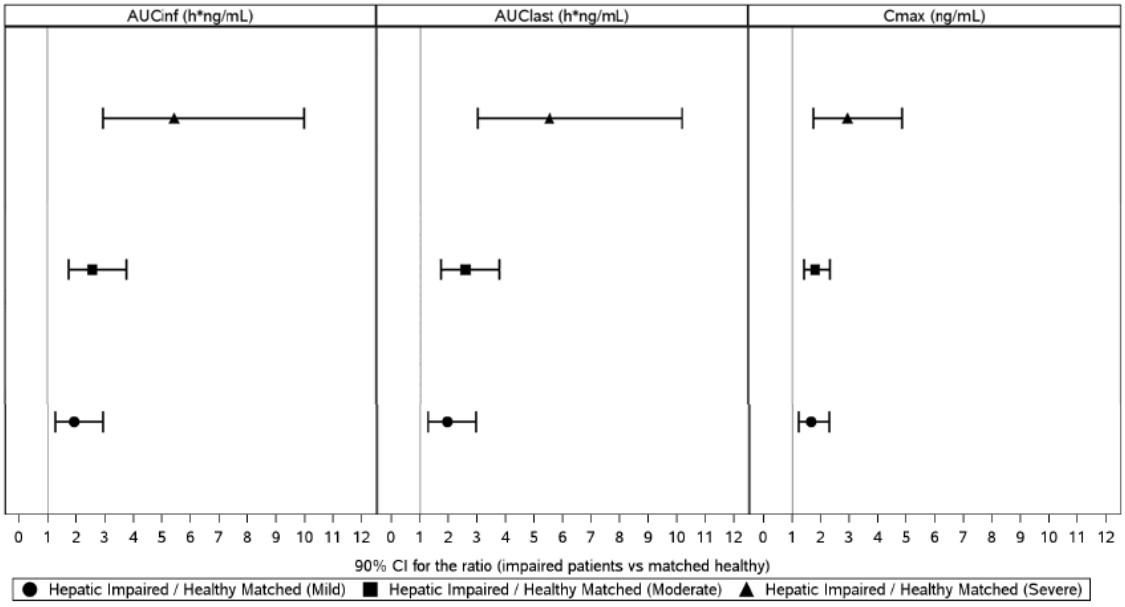
The descriptive statistics of primary and secondary pharmacokinetic parameters of M3 in subjects with hepatic impairment and matched healthy subject groups are summarized in the following table:

Summary statistics for plasma pharmacokinetic parameters of primary and secondary interest for LNL925 (M3) by group and subgroup (PK analysis set)

Subject group	AUCinf (h·ng/mL)	AUClast (h·ng/mL)	Cmax (ng/mL)	Tmax* (h)	T1/2 (h)	CL/F (L/h)	Vz/F (L)
Mild HI	8 77.8 ± 39.6 70.6 [48.2]	8 76.7 ± 39.6 69.5 [48.7]	8 1.20 ± 0.401 1.16 [29.7]	8 24.00 (8.00-36.00)	8 34.0 ± 14.2 31.7 [40.9]	8 3.86 ± 1.66 3.54 [48.2]	8 167 ± 44.6 162 [28.3]
Matched healthy to mild HI	8 41.0 ± 22.5 36.7 [52.3]	8 39.8 ± 22.0 35.6 [52.6]	8 0.751 ± 0.358 0.692 [43.6]	8 12.00 (4.00-24.00)	8 45.1 ± 38.1 37.6 [60.7]	8 7.55 ± 3.64 6.82 [52.3]	8 386 ± 125 370 [31.7]
Moderate HI	7** 128 ± 66.3 115 [52.2]	7** 127 ± 66.1 114 [52.5]	7** 1.61 ± 0.448 1.56 [26.6]	7** 24.00 (24.00-48.00)	7** 34.4 ± 10.3 33.1 [29.6]	7** 2.39 ± 1.10 2.16 [52.2]	7** 106 ± 23.8 104 [26.1]
Matched healthy to moderate HI	8 47.8 ± 19.4 45.1 [35.7]	8 46.7 ± 18.9 44.1 [35.8]	8 0.898 ± 0.300 0.861 [30.4]	8 9.00 (6.00-24.00)	8 44.1 ± 38.7 36.1 [64.1]	8 5.82 ± 1.76 5.55 [35.7]	8 307 ± 119 289 [38.4]
Severe HI	8 248 ± 152 196 [94.6]	8 246 ± 151 194 [95.1]	8 2.55 ± 1.42 2.14 [78.1]	8 30.00 (16.00-72.00)	8 50.8 ± 23.4 47.2 [39.8]	8 1.72 ± 1.56 1.27 [94.6]	8 107 ± 75.1 86.7 [79.7]
Matched healthy to severe HI	8 40.1 ± 22.2 36.0 [50.3]	8 39.0 ± 21.6 35.1 [50.3]	8 0.776 ± 0.332 0.729 [37.1]	8 7.00 (4.00-24.00)	8 44.1 ± 38.4 36.6 [60.5]	8 7.62 ± 3.53 6.94 [50.3]	8 384 ± 128 367 [32.9]

Results are presented as - number of subjects, arithmetic mean ± SD, geometric mean [%CV Geo mean] ; *: Median (Min - Max) is displayed instead of Mean ± SD (CV%); **Valid PK parameters could not be calculated for one subject because the subject had maximum PK concentration at the pre-dose time point; HI: Hepatic impairment

The graphical representation of geometric mean (90%CI) for LNL925 PK parameters is shown in the following figure:



M3 AUCs increased with the severity of the hepatic impairment or decreased hepatic function. Mean AUCinf and AUClast of M3 increased by 93% and 95% (approx. 1.9 fold), 156% and 159% (approx. 2.6 fold), 445% and 455% (approx. 5.5 fold) in mild, moderate and severe hepatic impairment subject groups, respectively, compared to their matched control groups.

Mean T1/2 of M3 in mild and moderate hepatic impairment groups was comparable to their matched control groups (between 31.7 hours and 33.1 hours in hepatic impairment vs 36.1 hours and 37.6 hours in healthy subjects). Mean T1/2 in severe hepatic impairment group was slightly increased (47.2 hours vs 36.6 hours) compared to matched control group. Mean CL/F of M3 was decreased by 48%, 56% and 81% in mild, moderate and severe hepatic impairment subject groups, as compared to their matched healthy control groups.

Pharmacokinetics of LNL931 or M5

The descriptive statistics of primary and secondary pharmacokinetic parameters of M5 in subjects with hepatic impairment and matched healthy subject groups are summarized in the following table:

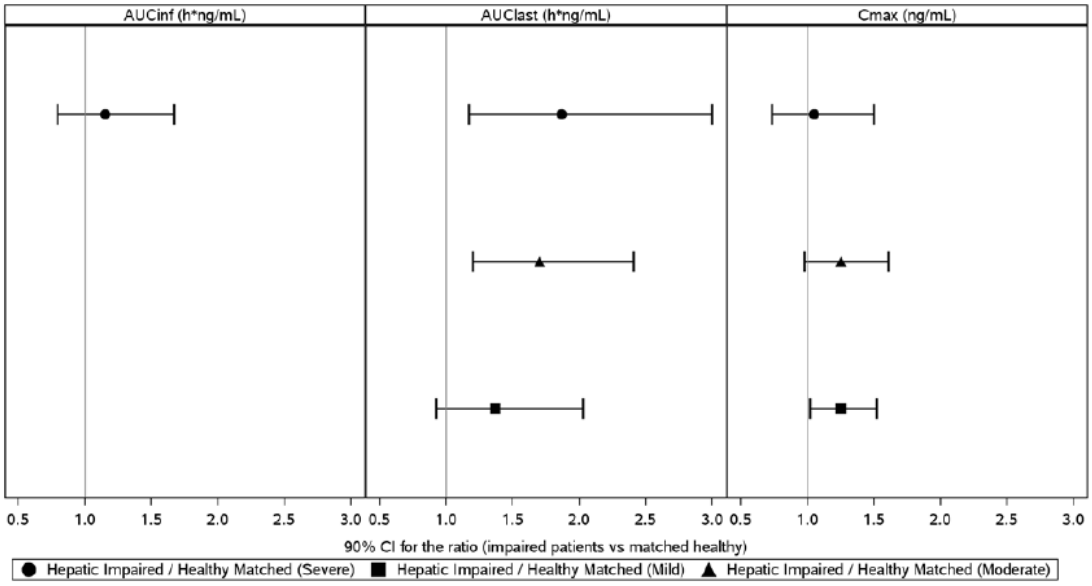
Subject group	AUCinf (h·ng/mL)	AUClast (h·ng/mL)	Cmax (ng/mL)	Tmax* (h)	T1/2 (h)	CL/F (L/h)	Vz/F (L)
Mild HI	2	8	8	8	2	2	2
	3.29 ± 1.66	1.50 ± 0.990	0.0434 ± 0.0175	12.00 (4.00-24.00)	26.0 ± 8.67	86.9 ± 43.7	2990 ± 551
	3.08 [56.4]	1.28 [64.0]	0.0403 [44.0]		25.3 [35.0]	81.3 [56.4]	2960 [18.7]
Matched healthy to mild HI	-	8	8	8	-	-	-
		1.03 ± 0.473	0.0329 ± 0.00687	4.00 (4.00-24.00)			
		0.938 [49.8]	0.0323 [19.7]				

Subject group	AUCinf (h·ng/mL)	AUClast (h·ng/mL)	Cmax (ng/mL)	Tmax* (h)	T1/2 (h)	CL/F (L/h)	Vz/F (L)
Moderate HI	1 5.26 5.26	7** 2.00 ± 1.20 1.77 [54.3]	7** 0.0467 ± 0.0155 0.0447 [32.4]	7** 4.00 (4.00-24.00)	1 48.4 48.4	1 47.5 47.5	1 3320 3320
Matched healthy to moderate HI	-	8 1.08 ± 0.304 1.04 [27.6]	8 0.0365 ± 0.00932 0.0356 [23.7]	8 4.00 (4.00-24.00)	-	-	-
Severe HI	3 3.43 ± 0.365 3.42 [11.0]	8 2.29 ± 0.782 2.10 [54.5]	8 0.0457 ± 0.0161 0.0426 [45.8]	8 20.00 (4.00-24.00)	3 32.1 ± 2.85 32.0 [8.7]	3 73.4 ± 8.30 73.1 [11.0]	3 3420 ± 703 3370 [19.8]
Matched healthy to severe HI	1 2.97 2.97	8 1.27 ± 0.661 1.12 [60.5]	8 0.0436 ± 0.0189 0.0407 [39.2]	8 4.00 (4.00-24.00)	1 25.9 25.9	1 84.1 84.1	1 3140 3140

Results are presented as - number of subjects, arithmetic mean ± SD, geometric mean [%CV Geo mean] ; *: Median (Min - Max) is displayed instead of Mean ± SD (CV%); HI: Hepatic impairment

**Valid PK parameters could not be calculated for one subject because the subject had maximum PK concentration at the pre-dose time point

The graphical representation of geometric mean (90%CI) for LNL931 PK parameters is shown in the following figure:



Mean peak plasma concentrations of siponimod metabolite M5 was slightly higher in subjects with hepatic impairment compared to respective matched healthy subjects. Mean Cmax of M5 increased by 25% in mild and moderate hepatic impairment subject groups and by 5% in severe hepatic impairment subject groups, as compared to their matched control groups.

Mean AUClast of siponimod metabolite M5 was higher in subjects with hepatic impairment compared to respective matched healthy subject groups. AUClast increased with the severity of the hepatic impairment or decreased hepatic function. Mean AUClast of M5 increased by 37%, 70%, and 87 % in mild, moderate and severe hepatic impairment subject groups, respectively, as compared to their matched control groups.

Hepatic function - exposure relationships

The correlation between pharmacokinetic parameters (in particular AUCinf) on log scale and the two laboratory parameters serum albumin and total bilirubin levels along with the Child-Pugh score was investigated and is summarized in the following table:

Covariate (units)	PK parameter (units)	Pearson correlation coefficient	P-value
Hepatic impaired			
Child-pugh score	Cmax(ng/mL)	-0.264	0.2261
	AUCinf(h ng/mL)	0.294	0.1754
Total bilirubin (µmol/L)	Cmax(ng/mL)	-0.256	0.2407
	AUCinf(h ng/mL)	0.394	0.0625
Serum albumin (g/L)	Cmax(ng/mL)	0.215	0.3280
	AUCinf(h ng/mL)	-0.218	0.3229

No significant ($p \leq 0.0001$) correlation was observed between the Child-Pugh score, hepatic function parameters and pharmacokinetic parameters in hepatic impairment and healthy subject groups.

Plasma protein binding of siponimod

Mean plasma protein binding data of [^{14}C] BAF312 from the current *ex vivo* study from subjects with impaired hepatic function (mild, moderate, and severe) and matching healthy subjects are listed in the table below (fu,p at 4 hours post dose):

Group	Unbound fraction (fu,p) (Unitless) Mean (n)
Child-Pugh severity - Mild	0.000150 (n=8)
Matched healthy subjects – Mild	0.000141 (n=7)
Child-Pugh severity – Moderate	0.000154 (n=8)
Matched healthy subjects - Moderate	0.000127 (n=6)
Child-Pugh severity – Severe	0.000186 (n=8)
Matched healthy subjects – Severe	0.000142 (n=7)

There was a slight tendency of an increase in unbound fraction of siponimod with a decrease in hepatic function. Mean fraction unbound (fu,p) at 4 hours post-dose was in the range of 0.000127-0.000141 in healthy subjects and 0.000150-0.000186 in subjects with impaired hepatic functions.

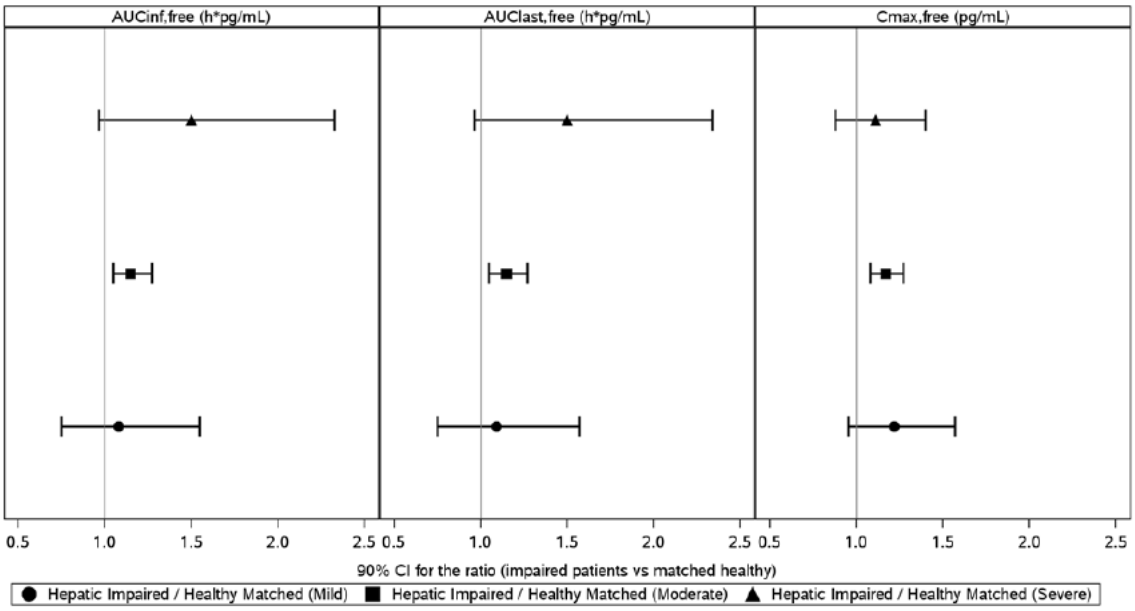
The summary statistics for unbound fraction PK parameters for siponimod are shown in the following table:

Group	Statistics	AUCinf,free (hr pg/mL)	AUClast,free (hr pg/mL)	Cmax,free (pg/mL)
Child-Pugh severity - Mild	n	8	8	8
	Mean ± SD (CV%)	10.4 ± 4.40 (42.2)	10.2 ± 4.35 (42.6)	0.302 ± 0.0851 (28.2)
	Geo-mean	9.53	9.31	0.291
	CV(%) geo-mean	50.3	50.6	30.9
Matched healthy subjects - Mild	n	7	7	7
	Mean ± SD (CV%)	9.12 ± 2.37 (26.0)	8.88 ± 2.42 (27.3)	0.244 ± 0.0570 (23.4)
	Geo-mean	8.84	8.57	0.238
	CV(%) geo-mean	28.4	30.3	23.4
Child-Pugh severity - Moderate	n	7**	7**	7**
	Mean ± SD (CV%)	8.50 ± 1.60 (18.8)	8.25 ± 1.57 (19.0)	0.242 ± 0.0399 (16.4)
	Geo-mean	8.38	8.13	0.239
	CV(%) geo-mean	18.0	18.2	17.5
Matched healthy subjects - Moderate	n	6	6	6
	Mean ± SD (CV%)	7.93 ± 1.77 (22.3)	7.74 ± 1.80 (23.2)	0.225 ± 0.0359 (15.9)
	Geo-mean	7.75	7.55	0.223
	CV(%) geo-mean	25.0	26.0	16.1
Child-Pugh severity - Severe	n	8	8	8
	Mean ± SD (CV%)	14.7 ± 10.6 (72.4)	14.3 ± 10.3 (71.9)	0.291 ± 0.106 (36.4)
	Geo-mean	12.5	12.1	0.276
	CV(%) geo-mean	62.0	61.7	36.2
Matched healthy subjects - Severe	n	7	7	7
	Mean ± SD (CV%)	8.71 ± 2.80 (32.1)	8.51 ± 2.81 (33.1)	0.266 ± 0.0608 (22.9)
	Geo-mean	8.29	8.07	0.259
	CV(%) geo-mean	35.6	37.2	25.0

**Valid PK parameters could not be calculated for one subject because the subject had maximum PK concentration at the pre-dose time point

The graphical

Representation of geometric mean (90%CI) for siponimod unbound PK parameters is shown in the following figure:



There was no significant increase in unbound AUC and Cmax in the subjects with mild hepatic impairment as compared to their matched healthy volunteers. In subjects with moderate hepatic impairment the increase was between 15% and 17% and was statistically significant. In severely impaired subjects the increase was 50% but did not reach statistical significance due to high variability (%CV of approx. 72%).

Safety

Siponimod, administered as a single oral dose of 0.25 mg was safe and well tolerated in subjects with mild, moderate, and severe hepatic impairment and demographically matched healthy subjects. All reported AEs were mild in intensity, there were no SAE or any AE-related study discontinuations and no significant difference could be observed in the overall AE incidence between subjects with hepatic impairment (n=2, 8.3%) compared to matched healthy control subjects (n=1, 6.3%).

Continuous online cardiac safety monitoring for up to 24 hours after administration of study drug to subjects with different levels of hepatic impairment did not reveal any significant bradycardia, bradyarrhythmic events or other cardiac rhythm abnormalities of clinical relevance.

Discussion

There was a slight tendency of an increase in unbound fraction of siponimod with a decrease in hepatic function. The increased unbound fraction is expected to be due to lower protein synthesis with progressive hepatic impairment. For PK parameters of unbound siponimod, there was a statistically significant increase in the moderate group as compared to their matched healthy subjects. However, the increase was 15% and 17% for AUC and Cmax, respectively. This is unlikely to be of clinical relevance. For the severe group, the increase was 50% for AUC (11% for Cmax) but did not reach statistical significance due to higher variability in this group.

The increased systemic exposure of the metabolites M3 and M5 associated with no significant changes in the parent drug exposure tends to indicate that the elimination pathway of the metabolites may be impacted in subjects with hepatic impairment.

The EC50 value of metabolites M5 and M3 tested on human sphingosine 1-phosphate receptor-1(S1P1) receptors were 470±71 nmol/L and >10000 nmol/L, respectively. M3 has shown very weak activity on S1P1 (EC50 >10000 nmol/L) compared to parent compound siponimod, which had shown the EC50 of 1.1±0.41 nmol/L in the same assay. Therefore, the observed systemic exposure increase for both M3 and M5 in hepatic impairment is unlikely to translate into a significant increase in pharmacological activity onS1P1 receptors. In addition, no significant correlation was observed between Child-Pugh score, total bilirubin and exposure (Cmax and AUCinf) of siponimod.

CONCLUSIONS

- The plasma exposure (Cmax and AUCinf) of total siponimod was comparable between subjects with mild, moderate and severe hepatic impairment and their matched healthy subjects.
- The unbound siponimod PK parameters were comparable in subjects with mild hepatic impairment, 15-17% increase for subjects with moderate hepatic impairment (which was statistically significant but not considered to be of clinically relevant), and 50% increase in subjects with severe hepatic impairment (which did not reach statistical significance due to high variability) in comparison with matched healthy subjects.
- The plasma exposure (Cmax and AUCinf) of M3 was approximately 3-5 folds higher in moderate to severe hepatic impairment subjects as compared to their matched healthy groups.
- The plasma exposure (AUClast) of M5 was approximately 2-fold higher in severe hepatic impairment subjects as compared to their matched healthy groups.
- Single oral dose administration of 0.25 mg siponimod in mild, moderate and severe hepatic impairment subjects and matched healthy subjects was safe and well tolerated.

Study A2129: A single-dose, open-label, parallel-group study to assess the pharmacokinetics (PK) of BAF312 in subjects with renal impairment (RI) compared to healthy subjects with normal renal function

Objectives: To investigate the PK of BAF312 and selected metabolites, safety and tolerability after administration of a single dose of 0.25 mg of BAF312 in subjects with severe renal impairment in comparison to healthy control subjects.

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

<table>
<tr><td>Study Design</td><td>Single-dose, open-label, parallel-group</td></tr>
<tr><td>Study Population</td><td>N= 16 recruited and analyzed (CYP2C9*1 homozygous carriers)
Individual demographic information is given in the table below:
<table>
<tr><th></th><th></th><th>Severe RI subjects N=8</th><th>Matched healthy subjects N=8</th></tr>
<tr><td>Age (years) +</td><td>Mean (SD)</td><td>57.9 (6.22)</td><td>54.9 (5.28)</td></tr>
<tr><td></td><td>Median</td><td>56.5</td><td>53.0</td></tr>
<tr><td></td><td>Range</td><td>51 – 68</td><td>49 – 66</td></tr>
<tr><td>Height (cm) ++</td><td>Mean (SD)</td><td>166.1 (12.36)</td><td>168.4 (7.45)</td></tr>
<tr><td></td><td>Median</td><td>168.3</td><td>169.3</td></tr>
<tr><td></td><td>Range</td><td>148 – 179</td><td>156 – 179</td></tr>
<tr><td>Weight (kg) ++</td><td>Mean (SD)</td><td>80.68 (13.987)</td><td>83.84 (13.727)</td></tr>
<tr><td></td><td>Median</td><td>84.45</td><td>82.70</td></tr>
<tr><td></td><td>Range</td><td>56.4 - 103.0</td><td>69.3 - 99.0</td></tr>
<tr><td>BMI (kg/m^2) ++</td><td>Mean (SD)</td><td>29.13 (3.276)</td><td>29.50 (3.762)</td></tr>
<tr><td></td><td>Median</td><td>28.46</td><td>30.09</td></tr>
<tr><td></td><td>Range</td><td>24.5 - 33.0</td><td>24.0 - 34.9</td></tr>
<tr><td>Sex - n(%)</td><td>Male</td><td>4 (50%)</td><td>4 (50%)</td></tr>
<tr><td></td><td>Female</td><td>4 (50%)</td><td>4 (50%)</td></tr>
<tr><td>Predominant race - n(%)</td><td>Caucasian</td><td>6 (75%)</td><td>6 (75%)</td></tr>
<tr><td></td><td>Black</td><td>2 (25%)</td><td>2 (25%)</td></tr>
<tr><td>Ethnicity - n(%)</td><td>Hispanic/Latino</td><td>1 (12.5%)</td><td>3 (37.5%)</td></tr>
<tr><td></td><td>Other</td><td>7 (87.5%)</td><td>5 (62.5%)</td></tr>
</table></td></tr>
<tr><td>Dosage and Administration</td><td>2 groups
Group 1: BAF312 0.25mg Severe Renal impairment n=8
Group 2: BAF312 0.25mg Matched Healthy subjects n=8
Study drugs 0.25 mg was administered orally with approximately 200 mL of water in the morning following an overnight fast of at least10 hours.
Batch No. X001 0212; Formulation control No. 6002636.010
No grapefruit or grapefruit</td></tr>
<tr><td>PK Sampling:</td><td>Blood: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 144, 216 and 312 (± 24 hours) hours post dose.</td></tr>
</table>

Analysis (Plasma)	Method : LC/MS/MS BAF312 LLOQ: 0.02 ng/mL Linear range: 0.02-20 ng/mL Inter-day Precision (%CV): 4.6 - 10.2% Inter-day accuracy: -4.4 - 3.0 % LNL925(M3) LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 2.0 - 9.2% Inter-day accuracy: -5.0 - 6.5 % LNL931 (M5) LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 1.6 - 7.3 % Inter-day accuracy: -4.8 - 3%
PK Assessment	The following PK parameters of siponimod and its metabolites were determined using non-compartmental method: Cmax, Tmax, AUClast, AUCinf, T1/2, Vz/F and CL/F from the plasma concentration-time data.
Safety Assessment	AEs, SAEs, hematology, blood chemistry, urinalysis, physical examinations, vital signs, ECGs, cardiac monitoring, and body weight.

RESULTS

Pharmacokinetics of total siponimod in plasma

The descriptive statistics of primary and secondary PK parameters of siponimod in subjects with renal impairment and matched healthy subject group are summarized in the following tables:

Subject group	Statistic	Cmax (ng/mL)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)
0.25 mg BAF312 (subjects with severe renal impairment)	n	8	8	8
	Mean (SD)	2.07 (0.360)	99.0 (45.9)	102 (46.6)
	CV% mean	17.4	46.4	45.8
	Geo-mean	2.04	91.0	93.7
	CV% geo-mean	18.2	45.1	44.4
	Median	2.12	87.2	88.4
	[Min; Max]	[1.56;2.53]	[52.0;193]	[53.7;197]
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	n	8	8	8
	Mean (SD)	2.27 (0.544)	76.4 (21.5)	78.2 (22.0)
	CV% mean	24.0	28.1	28.1
	Geo-mean	2.21	73.8	75.4
	CV% geo-mean	23.8	29.4	29.5
	Median	2.14	77.4	78.9
	[Min; Max]	[1.66;3.16]	[52.2;109]	[52.9;110]

Subject group	Statistic	Tmax# (h)	Tlag (h)	T1/2 (h)	CL/F (L/h)	Vz/F (L)
0.25 mg BAF312 (subjects with severe renal impairment)	N	8	8	8	8	8
	Mean (SD)		0.281 (0.209)	37.4 (11.0)	2.87 (1.12)	143 (37.0)
	CV% mean		74.2	29.4	38.8	25.8
	Geo-mean			36.2	2.67	139
	CV% geo-mean			27.6	44.4	24.9
	Median	6.00	0.250	34.4	2.90	127
	[Min; Max]	[4.00;8.00]	[0.00;0.750]	[24.4;60.1]	[1.27;4.66]	[109;206]
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	N	8	8	8	8	8
	Mean (SD)		0.406 (0.229)	26.3 (8.37)	3.44 (0.989)	126 (35.1)
	CV% mean		56.4	31.8	28.7	27.9
	Geo-mean		0.359	25.4	3.32	122
	CV% geo-mean		55.0	27.6	29.5	29.9
	Median	4.00	0.250	24.4	3.20	119
	[Min; Max]	[3.00;8.00]	[0.250;0.750]	[17.9;45.8]	[2.27;4.73]	[72.9;172]

The statistical assessment (Geometric mean ratio and 90% confidence intervals) of siponimod primary PK parameters for subjects with renal impairment vs. matched healthy subjects is presented in the table below:

PK parameter (Unit)	Subject group	N	Adjusted geometric mean*	Ratio of geometric means*	90% CI for ratio*
Cmax (ng/mL)	Severe RI subjects	8	2.04	0.92	(0.79, 1.08)
	Matched healthy to severe RI	8	2.21		
AUClast (h*ng/mL)	Severe RI subjects	8	91.02	1.23	(0.89, 1.71)
	Matched healthy to severe RI	8	73.76		
AUCinf (h*ng/mL)	Severe RI subjects	8	93.65	1.24	(0.90, 1.72)
	Matched healthy to severe RI	8	75.40		

RI- Renal Impairment subjects.

* back transformed from log-scale.

The PK of siponimod in healthy subjects was consistent with historical study data. Mean Cmax of siponimod decreased by 8%, while mean AUClast and AUCinf increased by 23% and 24%, respectively, in severe renal impairment subjects, as compared to their matched control group.

The mean elimination T1/2 of siponimod in subjects with severe renal impairment was comparable to their matched healthy subject group, and ranged between 25.4 and 36.2 hours, respectively. The mean systemic clearance (CL/F) of siponimod was comparable between the severe renal impairment group and the matched healthy control group (2.67 vs. 3.32 L/h).

Pharmacokinetics of unbound siponimod in plasma

One separate plasma sample was collected at 4 hours post-dose (expected siponimod Tmax) for each subject. Mean plasma protein binding data of [^{14}C] siponimod (4 hours post-dose) from subjects with severe impaired renal function and matching healthy subjects are listed in the following table:

Subject group	Statistic	BAF312 unbound fraction (fu) (unitless)
0.25 mg BAF312 (subjects with severe renal impairment)	n	8
	Mean (SD)	0.000280 (0.0000983)
	CV% mean	35.2
	Geo-mean	0.000268
	CV% geo-mean	29.3
	Median	0.000246
	[Min; Max]	[0.000211;0.000510]
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	n	8
	Mean (SD)	0.000255 (0.0000537)
	CV% mean	21.1
	Geo-mean	0.000250
	CV% geo-mean	21.5
	Median	0.000257
	[Min; Max]	[0.000168;0.000359]

[^{14}C] siponimod was very highly bound (>99.9%) to human plasma proteins in all subjects. The fraction unbound (fu (%)) ranged between 0.0168% and 0.0510% in individual subjects with no relevant differences between the two groups.

The descriptive statistics of primary PK parameters of unbound siponimod in subjects with renal impairment and matched healthy subjects are summarized in the following table:

Subject group	Statistic	Cmax (u) (ng/mL)	AUClast (u) (h*ng/mL)	AUCinf (u) (h*ng/mL)
0.25 mg BAF312 (subjects with severe renal impairment)	N	8	8	8
	Mean (SD)	0.000559 (0.000125)	0.0262 (0.0113)	0.0269 (0.0115)
	CV% mean	22.3	43.0	42.5
	Geo-mean	0.000548	0.0244	0.0251
	CV% geo-mean	21.6	40.5	40.2
	Median	0.000544	0.0241	0.0251
	[Min; Max]	[0.000437;0.000796]	[0.0152;0.0504]	[0.0158;0.0515]
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	N	8	8	8
	Mean (SD)	0.000571 (0.000165)	0.0192 (0.00587)	0.0196 (0.00602)
	CV% mean	28.9	30.6	30.7
	Geo-mean	0.000554	0.0185	0.0189
	CV% geo-mean	25.5	30.0	30.2
	Median	0.000524	0.0181	0.0184
	[Min; Max]	[0.000430;0.000944]	[0.0125;0.0305]	[0.0129;0.0310]

The statistical assessment (Geometric mean ratio and 90% confidence intervals) of siponimod primary unbound PK parameters for subjects with renal impairment vs. matched healthy subjects is shown in the table below:

PK parameter (Unit)	Subject group	N	Adjusted geometric mean*	Ratio of geometric means*	90% CI for ratio*
Cmax (u) (ng/mL)	Severe RI subjects	8	0.000548	0.99	(0.83, 1.17)
	Matched healthy to severe RI	8	0.000554		
AUClast (u) (h*ng/mL)	Severe RI subjects	8	0.0244	1.32	(1.00, 1.74)
	Matched healthy to severe RI	8	0.0185		
AUCinf (u) (h*ng/mL)	Severe RI subjects	8	0.0251	1.33	(1.02, 1.75)
	Matched healthy to severe RI	8	0.0189		
BAF312 unbound fraction (fu) (unitless)	Severe RI subjects	8	0.000268	1.07	(0.86, 1.34)
	Matched healthy to severe RI	8	0.00025		

RI- Renal Impairment subjects.

* back transformed from log-scale.

Similar mean siponimod Cmax(u) was observed for severe renal impairment subjects and their matched control group. Mean AUClast(u) and AUCinf(u) increased by 32% and 33%, respectively, in severe renal impairment subjects as compared to their healthy matched control group.

Pharmacokinetics of siponimod metabolites (LNL 925 or M3 and LNL931 or M5) in plasma

Pharmacokinetics of metabolite LNL925 or M3 in plasma

The descriptive statistics of primary and secondary PK parameters of M3 in subjects with severe renal impairment and matched healthy subject group are summarized in the following tables:

Subject group	Statistic	Cmax (ng/mL)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)
0.25 mg BAF312 (Subjects with severe renal impairment)	n	8	8	8
	Mean (SD)	0.666 (0.257)	44.5 (16.3)	45.8 (16.7)
	CV% mean	38.7	36.6	36.4
	Geo-mean	0.614	41.8	43.1
	CV% geo-mean	48.7	40.9	39.9
	Median	0.594	43.5	44.2
	[Min; Max]	[0.239;0.990]	[23.1;65.8]	[24.7;68.4]
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	n	8	8	8
	Mean (SD)	0.690 (0.150)	38.5 (8.53)	39.6 (8.51)
	CV% mean	21.8	22.2	21.5
	Geo-mean	0.674	37.7	38.8
	CV% geo-mean	23.6	21.9	21.1
	Median	0.716	37.3	38.7
	[Min; Max]	[0.454;0.915]	[27.8;54.2]	[28.9;55.5]

Subject group	Statistic	Tmax# (h)	Tlag (h)	T1/2 (h)	CL/F (L/h)	Vz/F (L)
0.25 mg BAF312 (Subjects with severe renal impairment)	N	8	8	8	8	8
	Mean (SD)		0.938 (0.291)	37.1 (8.66)	6.20 (2.44)	334 (178)
	CV% mean		31.1	23.4	39.3	53.3
	Geo-mean		0.898	36.2	5.81	303
	CV% geo-mean		32.8	23.6	39.9	46.3
	Median	20.0	1.00	35.4	5.73	277
	[Min; Max]	[8.00;36.0]	[0.500;1.50]	[25.9;50.4]	[3.65;10.1]	[199;735]
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	N	8	8	8	8	8
	Mean (SD)		0.938 (0.291)	29.7 (8.54)	6.56 (1.33)	285 (110)
	CV% mean		31.1	28.7	20.3	38.5
	Geo-mean		0.898	28.8	6.44	268
	CV% geo-mean		32.8	26.0	21.1	39.6
	Median	8.00	1.00	27.8	6.50	253
	[Min; Max]	[6.00;24.0]	[0.500;1.50]	[21.5;48.6]	[4.51;8.65]	[146;490]

The statistical assessment (Geometric mean ratio and 90% confidence intervals) of M3 primary PK parameters for subjects with renal impairment vs. matched healthy subjects is shown in the table below:

PK parameter (Unit)	Subject group	N	Adjusted geometric mean*	Ratio of geometric means*	90% CI for ratio*
Cmax (ng/mL)	Severe RI subjects	8	0.61	0.91	(0.66, 1.26)
	Matched healthy to severe RI	8	0.67		
AUClast (h*ng/mL)	Severe RI subjects	8	41.76	1.11	(0.84, 1.47)
	Matched healthy to severe RI	8	37.66		
AUCinf (h*ng/mL)	Severe RI subjects	8	43.06	1.11	(0.84, 1.46)
	Matched healthy to severe RI	8	38.80		

RI- Renal Impairment subjects.
* back transformed from log-scale.

Median Tmax of M3 in the severe renal impaired group was higher (20 hours) as compared to the matched healthy group (8 hours). Comparable exposure was observed between subjects with severe renal impaired and matched healthy subjects. Mean Cmax decreased by 9%, AUClast and AUCinf increased by 11% in the severe renal impairment subject group as compared to the matched healthy subjects group.

Mean elimination T1/2 of metabolite M3 in subjects with severe renal impairment was comparable to the matched healthy subject group, and ranged between 28.8 and 36.2 hours, respectively. Comparable T1/2 values were observed for the parent siponimod. The mean systemic clearance (CL/F) of siponimod was comparable between the severe renal impairment group and the matched healthy control group.

Pharmacokinetics of LNL931 or M5 in plasma

The descriptive statistics of primary and secondary PK parameters of M5 in subjects with severe renal impairment and matched healthy subject group are summarized in the following tables:

Subject group	Statistic	Cmax (ng/mL)	AUClast (h*ng/mL)	AUCinf (h*ng/mL)
0.25 mg BAF312 (subjects with severe renal impairment)	n	8	8	0
	Mean (SD)	0.0302 (0.0165)	1.20 (0.858)	-
	CV% mean	54.8	71.7	-
	Geo-mean	0.0273	0.978	-
	CV% geo-mean	47.9	78.7	-
	Median	0.0269	1.02	-
	[Min; Max]	[0.0148;0.0682]	[0.272;3.13]	-
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	n	8	8	0
	Mean (SD)	0.0395 (0.0156)	1.29 (0.671)	-
	CV% mean	39.3	51.9	-
	Geo-mean	0.0367	1.16	-
	CV% geo-mean	44.6	51.8	-
	Median	0.0363	0.982	-
	[Min; Max]	[0.0179;0.0626]	[0.650;2.32]	-

Subject group	Statistic	Tmax# (h)	Tlag (h)	T1/2 (h)	CL/F (L/h)	Vz/F (L)
0.25 mg BAF312 (Subjects with severe renal impairment)	n	8	8	4	0	0
	Mean (SD)		2.00 (0.707)	41.7 (19.1)	-	-
	CV% mean		35.4	45.7	-	-
	Geo-mean		1.89	38.6	-	-
	CV% geo-mean		38.1	46.9	-	-
	Median	6.00	2.00	37.7	-	-
	[Min; Max]	[4.00;24.0]	[1.00;3.00]	[23.1;68.1]	-	-
0.25 mg BAF312 (Healthy subjects matched to severe RI subjects)	n	8	8	5	0	0
	Mean (SD)		1.38 (0.354)	39.6 (12.2)	-	-
	CV% mean		25.7	30.7	-	-
	Geo-mean		1.34	38.1	-	-
	CV% geo-mean		26.3	33.2	-	-
	Median	4.00	1.50	41.9	-	-
	[Min; Max]	[4.00;16.0]	[1.00;2.00]	[24.6;55.5]	-	-

The statistical assessment (Geometric mean ratio and 90% confidence intervals) of M5 primary PK parameters for subjects with renal impairment vs. matched healthy subjects is shown in the table below:

PK parameter (Unit)	Subject group	N	Adjusted geometric mean*	Ratio of geometric means*	90% CI for ratio*
Cmax (ng/mL)	Severe RI subjects	8	0.03	0.74	(0.50, 1.10)
	Matched healthy to severe RI	8	0.04		
AUClast (h*ng/mL)	Severe RI subjects	8	0.98	0.84	(0.50, 1.43)
	Matched healthy to severe RI	8	1.16		

RI- Renal Impairment subjects.

* back transformed from log-scale.

Mean peak plasma concentrations of siponimod metabolite M5 and mean AUClast were slightly lower in subjects with renal impairment compared to respective matched healthy subjects. Mean Cmax of M5 decreased by 26% in severe renal impaired subjects group and mean AUClast decreased by 16% as compared to the matched control group (Table 11-14). AUCinf could not be reliably estimated for metabolite M5 since more than 20% of the AUC resulted from extrapolation. PK Parameters derived from AUCinf, such as CL/F and Vz/F could not be estimated either. Mean elimination T1/2 of metabolite M5 was similar in subjects with severe renal impairment and the matched healthy subject group (approx. 38 hours).

Safety

No AEs were reported in the subjects enrolled in this study. There were no clinically relevant alterations of laboratory, vital signs or ECG parameters that were attributable to drug effect. Cardiac safety monitoring within the first 24 h of administration in subjects with severe renal impairment did not reveal any significant bradycardia, bradyarrhythmic events or other cardiac rhythm abnormalities.

The HR results collected from 12-lead safety ECGs were comparable between subjects with severe renal impairment and demographically matched healthy subjects.

Discussion

Siponimod is predominantly metabolized, and therefore, cleared primarily via metabolic clearance. The current study data seem to indicate that the specific non-renal clearance pathways of siponimod are not affected in subjects with severe renal impaired functions.

Plasma protein binding is often altered in subjects with impaired renal function. The present data showed that neither siponimod plasma protein binding nor unbound siponimod PK were significantly affected by severe renal impairment.

CONCLUSIONS

- Total and unbound siponimod pharmacokinetics were marginally affected in subjects with severe renal impaired functions, with comparable Cmax and slightly increased AUCs compared to healthy matched subject group.
- M3 exposure was similar and M5 exposure was slightly lower in subjects with severe renal impairment compared with matched healthy subjects.
- Single oral doses of 0.25 mg of siponimod were safe and well tolerated in subjects with severe renal impairment and demographically matched healthy subjects.

Study A1101: A randomized, double-blind, placebo-controlled, ascending single dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of BAF312 in Japanese healthy male subjects.

Objectives: To assess the safety, tolerability pharmacokinetics and pharmacodynamics of single ascending oral dose of BAF312 in Japanese healthy male subjects.

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

<table>
<tr><td>Study Design</td><td>Double-blind, placebo-controlled, ascending single dose study</td></tr>
<tr><td>Study Population</td><td>Non-smoking, healthy Japanese male subjects, 20 and 45 years of age
N= 40</td></tr>
<tr><td>Dosage and
Administration</td><td>Cohort 1: BAF312 0.5mg n=8; placebo n=2
Cohort 2: BAF312 2.5mg n=8; placebo n=2
Cohort 3: BAF312 5mg n=8; placebo n=2
Cohort 4: BAF312 10mg n=8; placebo n=2
Study drugs was administered under fasted conditions.
<table>
<tr><th>Study drug</th><th>Formulation</th><th>Strength</th><th>Lot number</th><th>Expiry date</th></tr>
<tr><td>BAF312</td><td>tablet</td><td>0.25 mg</td><td>J09047</td><td>Mar-2011</td></tr>
<tr><td>BAF312</td><td>tablet</td><td>1 mg</td><td>J09048</td><td>Mar-2011</td></tr>
<tr><td>BAF312</td><td>tablet</td><td>5 mg</td><td>J09049</td><td>Mar-2011</td></tr>
<tr><td>Placebo</td><td>tablet</td><td>0 mg</td><td>J09046</td><td>Sep-2011</td></tr>
</table></td></tr>
<tr><td>PK Sampling:</td><td>Day 1 (pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 12 h post-dose); Day 2 through Day 7 (24, 36, 48, 72, 96 and 144 h post-dose); Day 10 (216 h) and Day 14 (312 h).</td></tr>
<tr><td>Analysis
(Plasma)</td><td>Method: LC/MS/MS
BAF312
LLOQ: 0.25 ng/mL
Linear range: 0.25-500 ng/mL
Inter-day Precision (%CV): 2.6-12.5%
Inter-day accuracy: -1.8 - 4.8%</td></tr>
<tr><td>PK Assessment</td><td>The following PK parameters of siponimod and its metabolites were determined using non-compartmental method: AUCinf, AUClast, AUC0-24h, Cmax, Tmax, Tlag, T1/2.</td></tr>
</table>

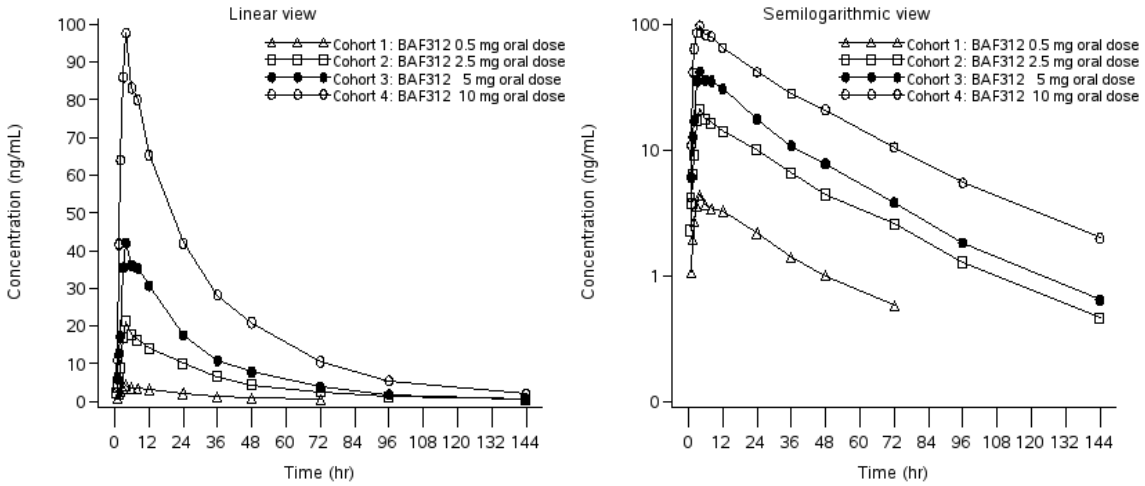
Safety Assessment	AEs, SAEs, hematology, blood chemistry, urinalysis, 12-leads ECG, 12-h 3-lead Digital Holter, 12-h Telemetry, pulmonary function test, physical examinations, vital signs, ECGs, cardiac monitoring, and body weight.

RESULTS

Pharmacokinetic (PK) results

BAF312 concentrations in plasma

Geometric mean concentrations of BAF312 in plasma after single dose under fasted conditions were showed in the Figure below:



The plasma concentration of BAF312 peaked between 3.5 and 4 h post-dose (median) with a minimum value of 2 h and a maximum value of 12 h in individual subjects. Cmax and AUCs appeared to increase dose proportionally from 0.5 to 10 mg. The decline of BAF312 plasma concentration was mono-exponential with a geometric mean T1/2 of between 28.5 and 39.7 h.

The main PK parameters were summarized in the following table:

Main PK parameters after BAF312 single dose administration to Japanese healthy subjects under fasted conditions

PK parameter		0.5 mg	2.5 mg	5 mg	10 mg
Cmax (ng/mL)	Mean (SD)	4.549 (0.71187)	21.46 (2.7281)	46.14 (9.9596)	101.8 (18.092)
	CV% mean	15.65	12.71	21.59	17.78
	Geo-mean	4.501	21.31	45.24	100.4
	CV% geo-mean	15.52	12.85	21.26	17.60
AUCinf (h·ng/mL)	Mean (SD)	161.5 (47.523)	686.3 (60.479)	1288 (261.64)	3326 (1000.0)
	CV% mean	29.43	8.81	20.31	30.06
	Geo-mean	155.9	684.1	1265	3197
	CV% geo-mean	28.60	8.67	20.64	30.89
AUClast (h·ng/mL)	Mean (SD)	146.8 (44.104)	666.2 (55.064)	1269 (261.05)	3295 (988.31)
	CV% mean	30.05	8.27	20.58	30.00
	Geo-mean	141.6	664.3	1245	3167
	CV% geo-mean	28.79	8.14	20.81	30.89
AUC0-24h (h·ng/mL)	Mean (SD)	72.64 (12.129)	323.7 (29.696)	688.6 (132.39)	1539 (254.87)
	CV% mean	16.70	9.17	19.22	16.56
	Geo-mean	71.82	322.6	678.0	1521
	CV% geo-mean	15.94	9.00	18.88	16.40
Tlag (h)	Median	0.3750	0.2500	0.2500	0.1250
	(Min; Max)	(0.250; 0.750)	(0.00; 0.500)	(0.00; 0.750)	(0.00; 0.500)
Tmax (h)	Median	4.000	4.000	3.500	4.000
	(Min; Max)	(3.00; 6.00)	(3.00; 4.00)	(2.00; 12.0)	(3.00; 6.00)
T1/2 (h)	Mean (SD)	30.23 (9.2286)	28.56 (2.1996)	29.88 (7.1618)	42.18 (14.283)
	CV% mean	30.53	7.70	23.97	33.86
	Geo-mean	28.96	28.49	29.14	39.68
	CV% geo-mean	32.57	7.62	24.29	40.81

Dose proportionality

The relationship between dose and observed Cmax and AUCs was investigated for the dose range of 0.5 - 10 mg and is summarized in the table below:

Estimate of the slope for the linear regression between log-PK parameter and log-dose

PK parameter (unit)	Slope estimate	90% CI for slope		Critical region for the 90% CI for slope	Dose proportionality across the whole range*	Proportionality dose range**
		Lower	Upper			
AUClast (h·ng/mL)	1.015	0.950	1.081	0.925 – 1.074	No	15.643
AUCinf (h·ng/mL)	0.985	0.919	1.052	0.925 – 1.074	No	15.566
AUC0-24h (h·ng/mL)	1.010	0.967	1.054	0.925 – 1.074	Yes	-
Cmax (ng/mL)	1.030	0.984	1.076	0.925 – 1.074	No	18.725

Source: PT-Table 14.2-1.1
*Dose range was 0.5 to 10 mg. Ratio highest to lowest dose is 20.
**Maximum dose range (rmax < r) within which the increase in the pharmacokinetic parameter can still be considered proportional to the increase in dose.

Dose proportionality was demonstrated only for the AUC0-24h. Cmax, AUClast and AUCinf increased with dose but dose proportionality across the entire dose range (dose range ratio = 20) could not be concluded from the statistical analysis because the 90% CIs of the slopes were slightly outside of the predefined ranges.

Safety

There was no severe and serious adverse event, or death. All adverse events were mild or moderate, most of which were transient and resolved spontaneously. Overall, single oral doses of BAF312 were tolerated in the investigated dose range between 0.5 and 10 mg.

CONCLUSIONS

Overall, the PK profile of BAF312 (0.5 to 10 mg) in Japanese subjects in was similar to the previous clinical studies in non-Japanese subjects.

BAF312 was to be safe and tolerated in Japanese healthy male subjects up to 10 mg.

4.6-3. HUMAN PK STUDIES

4.6-3.2 Extrinsic Factors

Study A2124: An open-label, three-period, single-sequence study to evaluate the effect of the CYP3A4 inhibitor itraconazole on siponimod (BAF312) single-dose pharmacokinetics, safety and tolerability in healthy subjects with CYP2C9*1*2 and *1*3 genotypes

Objectives: To evaluate the effect of itraconazole (ITR) on the pharmacokinetics of a single oral dose of 0.25 mg of siponimod in healthy subjects with the CYP2C9 genotypes *1*2 and *1*3.

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

Study Design	Open-label, single-dose, three-period, single-sequence crossover study
Study Population	N= 30 recruited and analyzed (CYP2C9 *1*2 and *1*3 carrier) Age: 18 - 50 years (mean 36.7 years) Gender: 28 males (93.3%), 2 females (6.7%) Weight: 55.8 - 95.0 kg (mean 75.71 kg) Race: 27 Caucasian (90%), 3 Black (10%)
Dosage and Administration	A single siponimod oral dose of 0.25 mg on Day 1; ITR 100 mg oral capsule twice daily (total daily dose 200 mg) from Day 15 until Day 18; A single siponimod oral dose of 0.25 mg on Day 19; ITR administration twice daily was continued from Day 19 until Day 31 (Cohort 1, *1*2 genotype), Day 35 (Cohort 2, *1*3 genotype). Siponimod 0.25 mg tablet Batch No. 1010004141 Administered under fastedcondition.
PK Sampling:	Blood: Treatment Period 1: up to 312 h post-dose (Day 14). Treatment Period 3: up to 312 h post-dose (Cohort 1); up to 408 h post-dose (Cohort 2).
Analysis (Plasma)	Method : LC/MS/MS <u>BAF312</u> LLOQ: 0.05 ng/mL Linear range: 0.05-100 ng/mL Inter-day Precision (%CV): 2.0-5.2% Inter-day accuracy: -3.1 - 1.2 %

	LYS815 (M17) LLOQ: 0.100 ng/mL Linear range: 0.100 - 50.0 ng/mL Inter-day Precision (%CV): 3.8 - 7.7% Inter-day accuracy: -5.8 - 3.3% LNL925(M3) LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 1.8 - 7.1% Inter-day accuracy: -2.3 - 2.0 % LNL931 (M5) LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 1.9 - 6.3% Inter-day accuracy: -2.9 - 2.1% Itraconazole LLOQ: 10.0 ng/mL Linear range: 10.0 -10000 ng/mL Inter-day Precision (%CV): 1.3 - 7.9% Inter-day accuracy: -2.5 - 1.0% Hydroxy Itraconazole LLOQ: 10.0 ng/mL Linear range: 10.0 -10000 ng/mL Inter-day Precision (%CV): 1.1 - 9.8% Inter-day accuracy: -2.0 - 1.7%
PK Assessment	The PK parameters determined for siponimod and metabolites M3, M5 and M17 were Cmax, Tmax, AUClast, AUCinf, T1/2, Vz/F (siponimod only) and CL/F (siponimod only) and other parameters as appropriate from the plasma concentration-time data.
Safety Assessment	Physical examinations, vital signs (body temperature, blood pressure, and pulse rate), height and weight, clinical laboratory evaluations (hematology, clinical chemistry, and urinalysis), 12-lead

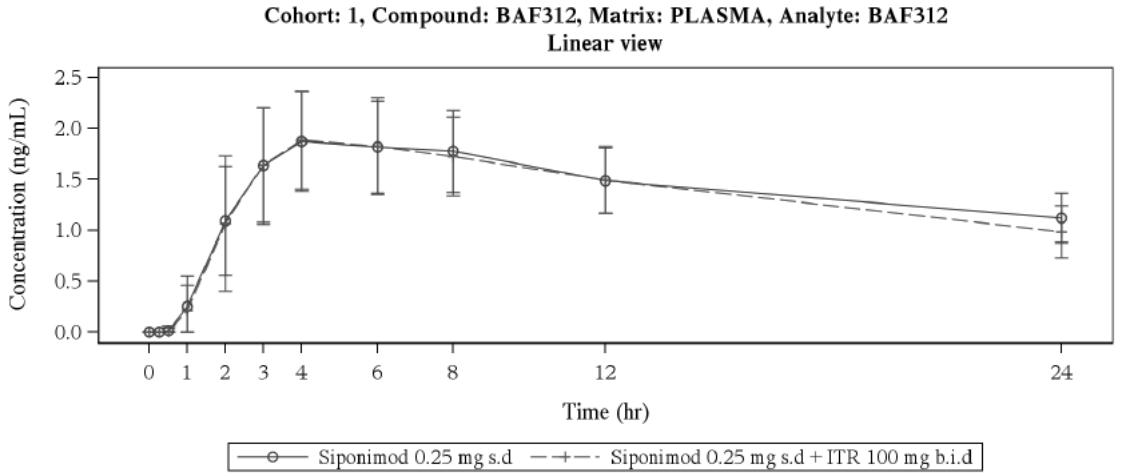
	electrocardiogram (ECG), 24-hour Holter ECG examinations, and adverse event (AE) and serious AE (SAE) monitoring. Additional safety evaluations included ECG (including single standard 12-lead ECG & continuous 25-hour online cardiac monitoring on Days 1 and 19) and pregnancy and assessments of fertility.

RESULTS

Primary pharmacokinetic results (Siponimod)

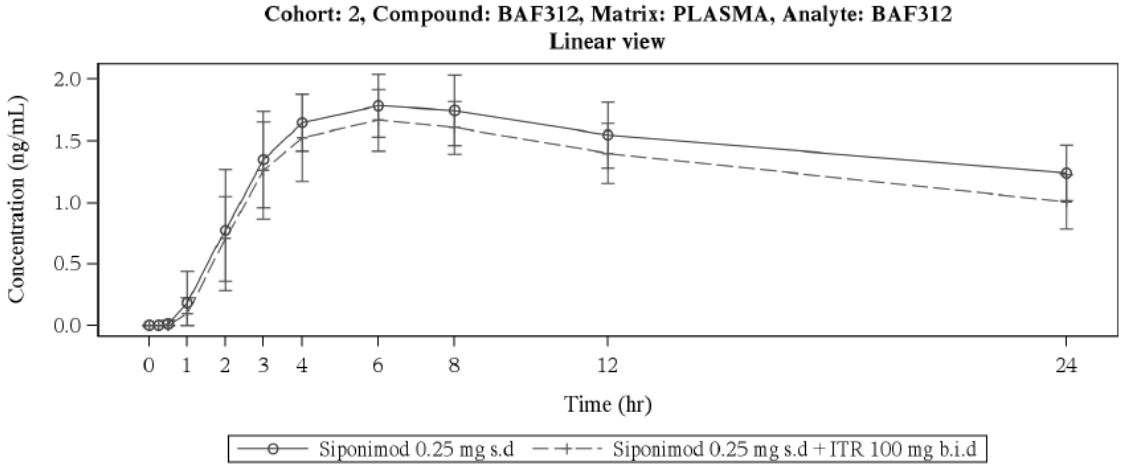
Arithmetic mean (SD) plasma concentration-time profiles of siponimod on Day 1 (0.25 mg alone) and on Day 19 (0.25 mg siponimod co-administered with ITR100 mg bid) are presented graphically in the following figures:

Arithmetic mean (SD) plasma concentration-time profiles of Siponimod 0.25 mg alone on Day 1 (Reference) and co-administered with itraconazole on Day 19 (Test) for Cohort 1 (Linear scale)



Source: Figure 14.2-1.2

Arithmetic mean (SD) plasma concentration-time profiles of BAF312 0.25 mg alone on Day 1 (Reference) and co-administered with itraconazole on Day 19 (Test) for Cohort 2 (Linear scale)



Source: Figure 14.2-1.2

A summary of the plasma PK parameters for siponimod is presented in the table below:

Summary statistics of siponimod plasma PK parameters on Day 1 (0.25 mg siponimod alone) and on Day 19 (0.25 mg siponimod coadministered with itraconazole)

Parameter (Unit)	Siponimod 0.25 mg s.d	Siponimod 0.25 mg s.d + ITR 100 mg b.i.d.
Cohort 1	**N=17**	**N=16**
AUC0-24 (h*ng/mL)	33.4 ± 7.42 [n=17] (22.2) 32.6 (21.5)	32.4 ± 7.74 [n=16] (23.9) 31.6 (22.8)
AUCinf (h*ng/mL)	77.4 ± 19.6 [n=17] (25.3) 75.0 (26.7)	68.8 ± 19.5 [n=16] (28.3) 66.2 (29.4)
AUClast (h*ng/mL)	73.8 ± 19.2 [n=17] (26.1) 71.4 (27.4)	66.2 ± 19.2 [n=16] (29.0) 63.6 (30.3)
CL/F (L/h)	3.44 ± 0.928 [n=17] (26.9) 3.33 (26.7)	3.93 ± 1.12 [n=16] (28.5) 3.78 (29.4)
Cmax (ng/mL)	1.97 ± 0.514 [n=17] (26.1) 1.91 (24.6)	1.96 ± 0.489 [n=16] (25.0) 1.91 (23.6)
T1/2 (h)	27.9 ± 7.00 [n=17] (25.1) 27.2 (23.1)	25.1 ± 4.98 [n=16] (19.8) 24.6 (20.0)
Tmax (h)#	4.00 (3.00-8.00) [n=17]	4.00 (3.00-8.00) [n=16]
Vz/F (L)	132 ± 20.4 [n=17] (15.4) 131 (16.4)	137 ± 24.8 [n=16] (18.2) 134 (19.9)
Cohort 2	**N=13**	**N=13**
AUC0-24 (h*ng/mL)	33.4 ± 5.18 [n=13] (15.5) 33.0 (17.1)	29.8 ± 4.95 [n=13] (16.6) 29.4 (16.7)
AUCinf (h*ng/mL)	99.5 ± 22.9 [n=13] (23.0) 96.8 (25.7)	75.6 ± 20.0 [n=13] (26.5) 73.2 (27.2)
AUClast (h*ng/mL)	95.0 ± 22.4 [n=13] (23.5) 92.2 (26.4)	72.1 ± 19.1 [n=13] (26.5) 69.8 (27.4)
CL/F (L/h)	2.66 ± 0.732 [n=13] (27.5) 2.58 (25.7)	3.53 ± 0.949 [n=13] (26.9) 3.42 (27.2)
Cmax (ng/mL)	1.83 ± 0.261 [n=13] (14.3) 1.81 (15.3)	1.72 ± 0.265 [n=13] (15.4) 1.70 (15.6)
T1/2 (h)	40.9 ± 10.3 [n=13] (25.1) 39.9 (23.9)	31.9 ± 7.52 [n=13] (23.6) 31.1 (23.5)
Tmax (h)#	6.00 (3.00-12.0) [n=13]	6.00 (3.00-8.00) [n=13]
Vz/F (L)	152 ± 39.1 [n=13] (25.6) 148 (23.1)	155 ± 22.5 [n=13] (14.5) 153 (15.2)

For each parameter, Mean±SD [N] (CV%mean) Geo-Mean (CV%Geo-mean) are provided
n=Number of non-missing observation with results/concentration.
N = number of subjects who received treatment.
Statistics are Mean ± SD [n] (CV%) Geometric mean (Geometric mean CV%)
CV% = coefficient of variation (%) = sd/mean*100
CV% geo-mean = (sqrt (exp (variance for log transformed data)-1))*100
For Tmax Statistics are Median (minimum-maximum) [n]
Source: Table 14.2-1.2

Statistical analysis of the effect of ITR on siponimod PK parameters is presented in the table below:

Statistical analysis of the effect of itraconazole on plasma PK parameters of siponimod

Parameter	Treatment	n*	Adjusted Geo-mean	Comparison	Treatment Comparison Geo-mean ratio	Treatment Comparison (90% CI)
Cohort 1						
Cmax (ng/mL)	Siponimod 0.25 mg alone	17	1.91	Siponimod + ITR vs. Siponimod alone	1.01	(0.96, 1.06)
	Siponimod + ITR	16	1.92			
AUClast (h*ng/mL)	Siponimod 0.25 mg alone	17	71.36	Siponimod + ITR vs. Siponimod alone	0.91	(0.85, 0.98)
	Siponimod + ITR	16	65.05			
AUCinf (h*ng/mL)	Siponimod 0.25 mg alone	17	75.03	Siponimod + ITR vs. Siponimod alone	0.90	(0.84, 0.96)
	Siponimod + ITR	16	67.67			
Cohort 2						
Cmax (ng/mL)	Siponimod 0.25 mg alone	13	1.81	Siponimod + ITR vs. Siponimod alone	0.94	(0.91, 0.97)
	Siponimod + ITR	13	1.70			
AUClast (h*ng/mL)	Siponimod 0.25 mg alone	13	92.25	Siponimod + ITR vs. Siponimod alone	0.76	(0.69, 0.83)
	Siponimod + ITR	13	69.80			
AUCinf (h*ng/mL)	Siponimod 0.25 mg alone	13	96.81	Siponimod + ITR vs. Siponimod alone	0.76	(0.69, 0.82)
	Siponimod + ITR	13	73.19			

- n* = number of subjects with non-missing values

- The log transformed PK parameters were analyzed using mixed model with treatment as fixed effect and subject as random effect.

Source: Table 14.2-1.3

When co-administered with ITR 100 mg bid, Siponimod geometric mean AUClast and AUCinf, were decreased by 9-10% in CYP2C9*1*2 subjects. A slightly larger decrease was observed for the CYP2C9*1*3 subjects (24%). Coadministration of siponimod with ITRdid not result in a change in geometric mean Cmax in either CYP2C9 genotype.

Secondary pharmacokinetic results (metabolites M3, M5, M17, itraconazole and OH-itraconazole)

M3: Following co-administered of siponimod 0.25 mg single oral dose with ITR 100 mg bid., M3 Cmax decreased by 19-20% and AUClast decreased by 23-25%, in both CYP2C9 genotypes.

M5: Following co-administered of siponimod 0.25 mg single oral dose with ITR 100 mg bid., M5 Cmax decreased by 11% and 16% and AUClast decreased by 21 and 36%, in subjects with the *1*2 and *1*3 genotypes, respectively.

M17: Following co-administered of siponimod 0.25 mg single oral dose with ITR 100 mg bid., M17 Cmax decreased by 28% and 38% and AUClast decreased by 58% and 77%, in subjects with the *1*2 and *1*3 genotypes, respectively.

Itraconazole and OH-itraconazole: The ITR and OH-ITR concentrations were comparable between both cohorts during the whole treatment period. These results are consistent with previous observations following a 100 mg bid administration.

Safety

Overall, single oral doses of siponimod 0.25 mg alone and in combination with ITR 100 mg (twice daily) were safe and well tolerated in healthy subjects in this study. The safety and tolerability profile of siponimod did not reveal any relevant differences between th e study cohorts or epochs as compared to the observations in previous studies with siponimod in healthy subjects. There were no SAEs and all reported AEs were mild in severity.

Discussion

Co-administration of siponimod 0.25 mg with the strong CYP3A4 inhibitor ITR at 100 mg bid. resulted in a 10% and 24% decrease in AUCs in the subjects with the CYP2C9*1*2 and *1*3 genotypes, respectively, indicating a possible 2C9 genotype influence on the ITR effect. These clinical results seem to indicate an induction rather than an inhibition effect of ITR on the metabolism of siponimod.

Currently available *in vitro* assessment data did support the mechanism leading to the clinical observations in this study. Since an idiosyncratic effect of ITR (possibly impacting an additional metabolic pathway) cannot be excluded, these results cannot be extrapolated to the other strong and specific CYP3A4 inhibitors. The current SimCYP model is still expected to be a reliable tool to provide good estimations of the effect of CYP3A4 inhibitors on siponimod exposure.

CONCLUSIONS

- Co-administration of siponimod 0.25 mg with ITR 100 mg bid. did not result in a change in Cmax in either CYP2C9 genotype, but decreased AUC by 10% and 24% in subjects with the *1*2 and *1*3 genotypes, respectively.
- Metabolites M3 and M5 Cmax decreased by 11-20% and AUCs were reduced by 23-36% in both CYP2C9 genotypes in presence of ITR. A slightly more pronounced decrease was observed for M17 (28% - 38% for Cmax; 58-77% for AUC).
- Single oral doses of siponimod 0.25 mg alone and in combination with ITR 100 mg (twice daily) were safe and well tolerated in healthy subjects in this study.

Study A2125: An open-label, multiple dose, 2-period, single-sequence study in healthy subjects with the CYP2C9*1*1 (wild type) genotype to evaluate the effect of the CYP2C9/3A4 inducer rifampin on siponimod pharmacokinetics.

Objectives: To assess the multiple-dose pharmacokinetics (PK) of siponimod in healthy subjects when given alone or in combination with the cytochrome P450 (CYP) 2C9/3A4 inducer rifampin.

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

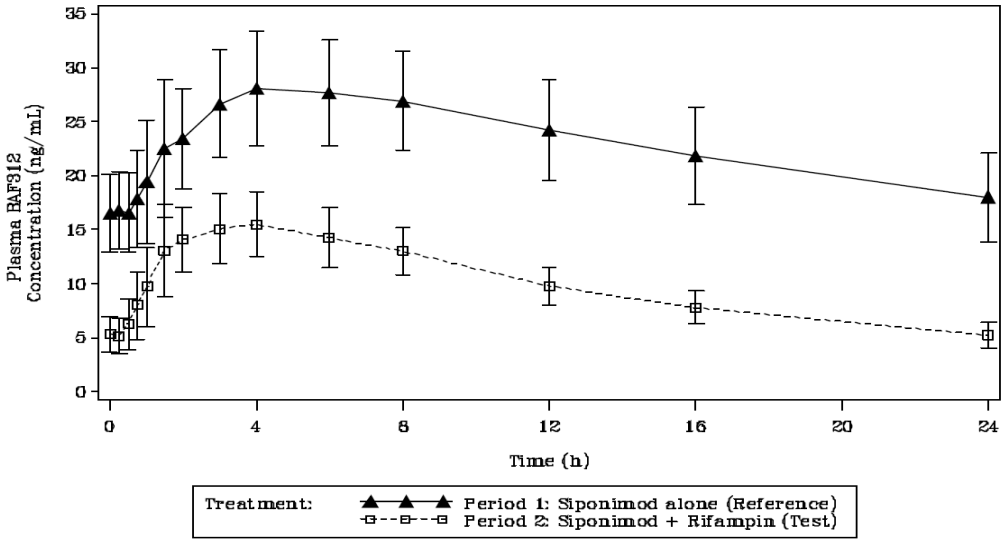
Study Design	Open-label, multiple-dose, 2-period, single-sequence study
Study Population	N= 16 recruited and analyzed (CYP2C9*1*1 genotype) Age: 18 - 43 years (mean 31.1 years) Gender: 15 males (93.8%), 1 females (6.3 %) Weight: 62.9 - 93.1 kg (mean 79.20 kg) Race: 6 Caucasian (37.5%), 8 Black (50.0%), 2 other (12.5%)
Dosage and Administration	Treatment Period 1: Day 1 = 0.25 mg, Day 2 = 0.25 mg, Day 3 = 0.5 mg (administered as one 0.5-mg tablet), Day 4 = 0.75 mg (administered as one 0.5-mg tablet and one 0.25-mg tablet), Day 5 = 1.25 mg (administered as five 0.25-mg tablets), Days 6 through 12 = 2 mg. Treatment Period 2: Siponimod 2 mg q.d. and rifampin 600 mg q.d. on Days 13 through 24. Siponimod 0.25 mg tablet Batch No. 1010004141 (Lot number 2016600); 1010004142 (Lot number 2016601) Siponimod 2 mg tablet Batch No. 1010004145 (Lot number 2016599) Rifampin 300 mg capsule Lot number: 3145675 Administered under fasted condition.
PK Sampling:	Blood: Treatment Period 1: up to 312 h post-dose (Day 14). Treatment Period 3: up to 312 h post-dose (Cohort 1); up to 408 h post-dose (Cohort 2).
Analysis (Plasma)	Method : LC/MS/MS <u>BAF312</u> LLOQ: 0.05 ng/mL Linear range: 0.05-100 ng/mL Inter-day Precision (%CV): 2.4-4.5%

	Inter-day accuracy: -2.0 - 2.0 % <u>LNL925(M3)</u> LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 3.1 - 6.9% Inter-day accuracy: -1.4 - 1.0 % <u>LNL931 (M5)</u> LLOQ: 0.01 ng/mL Linear range: 0.01-10 ng/mL Inter-day Precision (%CV): 2.4 - 6.6% Inter-day accuracy: -2.0 - 1.0%
PK Assessment	The PK parameters of primary interest were determined using non-compartmental methods and included Cmax,ss; AUClast,ss; and AUCtau,ss for total siponimod and metabolites, M3 and M5.
Safety Assessment	physical examinations, vital signs (body temperature, blood pressure, and pulse rate), height and weight, clinical laboratory evaluations (hematology, clinical chemistry, and urinalysis), 12-lead electrocardiogram (ECG), 24-hour Holter ECG examinations, and adverse event (AE) and serious AE (SAE) monitoring (including renal and liver events, prospective suicidality assessment using the Columbia-Suicide Severity Rating Scale (C-SSRS), and pregnancy).

RESULTS

Pharmacokinetic results Siponimod

Arithmetic mean (SD) plasma concentration-time profiles of BAF312 for Day 12 (Reference) and Day 24 (Test) are presented graphically in the following figure:



A summary of the plasma PK parameters for BAF312 is presented in the following table:

Summary statistics of plasma PK parameter values for BAF312-Day 12 and Day 24

PK Parameter (Unit)	Siponimod Alone, Study Day 12 N=16	Siponimod + Rifampin, Study Day 24 N=15
AUClast,ss (h*ng/mL)	554 ± 102 [n=15] (18.4%) 546 (17.6%)	239 ± 43.6 [n=15] (18.3%) 235 (16.9%)
AUCtau,ss (h*ng/mL)	554 ± 102 [n=15] (18.4%) 546 (17.6%)	239 ± 43.6 [n=15] (18.3%) 235 (16.9%)
Cmax,ss (ng/mL)	29.0 ± 5.37 [n=15] (18.5%) 28.6 (17.1%)	16.0 ± 3.13 [n=15] (19.6%) 15.7 (17.9%)
Cmin,ss (ng/mL)	15.9 ± 3.27 [n=15] (20.6%) 15.6 (20.6%)	4.75 ± 1.56 [n=15] (32.9%) 4.32 (58.6%)
Cav,ss (ng/mL)	23.2 ± 4.26 [n=15] (18.4%) 22.8 (17.6%)	9.94 ± 1.82 [n=15] (18.3%) 9.80 (16.9%)
Tmax,ss (h)#	4.00 (1.50 - 8.00) [n=15]	4.00 (1.50 - 4.05) [n=15]
Fluc (%)	56.9 ± 12.1 [n=15] (21.4%) 55.5 (23.4%)	114 ± 18.3 [n=15] (16.1%) 112 (15.9%)

n = number of subjects with non-missing values; N = number of subjects who received treatment in the study period; PK = pharmacokinetic; q.d. = once daily

Period 1: Siponimod up-titrated from 0.25 to 2 mg q.d. (Days 1 through 12) (Reference).

Period 2: Siponimod 2 mg q.d. + Rifampin 600 mg q.d. (Days 13 through 24) (Test).

Statistics are Mean ± SD [n] (CV%) Geometric mean (Geometric mean CV%)

CV% = coefficient of variation (%) = SD/mean*100

Geometric mean CV% = sqrt (exp (variance for log transformed data)-1)*100

For Tmax,ss, statistics are Median (minimum-maximum) [n]

Source: Table 14.2-2.1

Statistical analysis of the effect of rifampin on BAF312 PK parameters is presented in the table below:

Statistical analysis of the effect of rifampin on plasma PK parameters of BAF312

					Treatment Comparison		
			Adjusted		Geo-	90% CI	
PK Parameter (unit)	Study Day	n*	Geo-mean	Comparison (Test/Reference)	mean ratio	Lower	Upper
AUClast,ss (h*ng/mL)	Day 12	15	546				
	Day 24	15	235	Day 24/Day 12	0.43	0.41	0.45
AUCtau,ss (h*ng/mL)	Day 12	15	546				
	Day 24	15	235	Day 24/Day 12	0.43	0.41	0.45
Cmax,ss (ng/mL)	Day 12	15	28.6				
	Day 24	15	15.7	Day 24/Day 12	0.55	0.52	0.58

CI = confidence interval; Geo = geometric; n* = number of subjects with non-missing values; N = number of subjects who received treatment in the study period; PK = pharmacokinetic; q.d. = once daily

Model: Log-transformed PK parameters were analyzed using an analysis of variance model with a fixed effect for treatment and a random effect for subject.

Period 1: Siponimod up-titrated from 0.25 to 2 mg q.d. (Days 1 through 12) (Reference).

Period 2: Siponimod 2 mg q.d. + Rifampin 600 mg q.d. (Days 13 through 24) (Test).

Source: Table 14.2-3.1

Total exposure of siponimod, as measured by geometric mean AUCtau,ss, was decreased more than 50% from Period 1 (siponimod alone) to Period 2 when siponimod was co-administered with rifampin. Similarly, siponimod Cmax,ss was reduced by approximately 45% when siponimod was co-administered with rifampin. Co-administration of rifampin with siponimod did not alter the median Tmax (4 hours) of siponimod between Periods 1 and 2.

Pharmacokinetic results M3 metabolite (LNL925)

Total exposure of M3, as measured by geometric mean AUCtau,ss, was decreased by approximately 10% from Period 1 (siponimod alone) to Period 2 when siponimod was co-administered with rifampin. Conversely, M3 geometric mean Cmax,ss was increased by nearly 60% when siponimod was co-administered with rifampin. Co-administration of rifampin with siponimod did not alter the median Tmax (6 hours) of M3 between Periods 1 and 2.

Pharmacokinetic results M5 metabolite (LNL931)

Total exposure of M5, as measured by geometric mean AUCtau,ss, was decreased by 37% from Period 1 (siponimod alone) to Period 2 when siponimod was co-administered with rifampin. Conversely, M5 Cmax,ss was comparable when siponimod was co-administered with rifampin compared with siponimod alone. Co-administration of rifampin with siponimod displayed a shorter median Tmax for Period 2 (3 hours) than for Period 1 (6 hours).

Safety

Overall, multiple oral doses of siponimod (up-titrated from 0.25 to 2 mg on Days 1 through 12) alone and in combination with multiple oral doses of rifampin (600 mg q.d. on Days 13 through 24) were safe and well tolerated by the healthy subjects in this study. No deaths or SAEs were reported during the study.

Mean ALC increased slightly during the combination treatment with rifampin in Treatment Period 2. However, mean ALC remained at low levels below 1.0 × 109/L until the completion of Treatment Period 2 and the discontinuation of the study drug, consistent with the magnitude of steady-state ALC reduction in previous studies at the 1-mg dose level. Mean ALC recovered to near baseline levels by the EOS visit (approximately 7 days after the last dose), consistent with the ALC recovery pattern in previous studies at the same dose level.

No clinically relevant changes in clinical laboratory results, vital sign measurements, 12-lead ECG results, and C-SSRS findings were noted during the study, and no individual value was reported as a TEAE by the Investigator.

Discussion

The current study results indicated that while the Cmax,ss of M3 increased significantly in the presence of rifampin, the AUCtau,ss of M3 displayed little change only. The M5 Cmax,ss did not change under co-administration of rifampin, but the AUCtau,ss of M5 decreased significantly. Therefore, while induction of CYP2C9 and CYP3A4 did significantly increase the clearance of siponimod and increase the peak exposure to M3, rifampin did not increase the total exposure of the M3 and M5 metabolites of siponimod.

M3 has shown very weak activity on S1P1 (EC50 > 10 000 nmol/L) compared with parent compound siponimod (EC50~1.1 ± 0.41 nmol/L). Hence the observed systemic exposure changes for both M3 and M5 in presence of rifampin are unlikely to translate into a significantly different pharmacological activity on S1P1 receptors.

CONCLUSIONS

- Siponimod AUCtau,ss and Cmax,ss were decreased by 57% and 45%, respectively, in the presence of rifampin.
- M3 AUCtau,ss was decreased by 10% while Cmax,ss was increased by 53% in the presence of rifampin. M5 AUCtau,ss was decreased by 37% while Cmax,ss was comparable in the presence of rifampin.
- Multiple oral doses of siponimod (up-titrated from 0.25 to 2 mg on Days 1 through 12) alone and in combination with multiple oral doses of rifampin (600 mg q.d. on Days 13 through 24) were safe and well tolerated by the healthy subjects in this study.

Study A2108: An open-label, single-dose study, drug-drug interaction study to evaluate the pharmacokinetics, safety and tolerability of BAF312 when given alone and in combination with chronic fluconazole treatment in healthy volunteers.

Objectives: To characterize the pharmacokinetics (PK) of BAF312 following single dose alone and in combination with fluconazole in healthy volunteers.

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

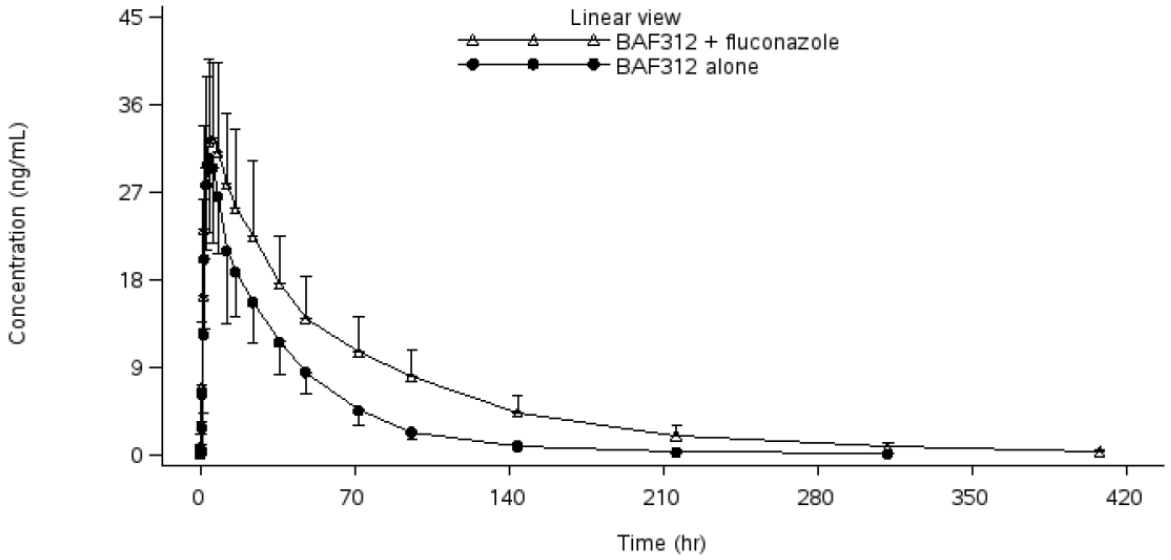
Study Design	Open-label, single dose, two periods and single sequence study
Study Population	N= 14 recruited and analyzed (CYP2C9*1*1 genotype) Age: 18-33 years (mean 24.1 years) Gender: 14 males (100%) Weight: 63.1-107.8 kg (mean 79.47 kg) Race: 13 Caucasian (92.9%), 1 Asian (7.1%)
Dosage and Administration	All subjects were to receive a single dose of 4 mg BAF312 in Period 1 (Day 1) and in Period 2 (Day 3). Fluconazole was to be administered at 200 mg b.i.d. on Day 1 and at 200 mg daily from Day 2 to Day 19. Washout period (min 14 days max 21 days) Siponimod 4 mg tablet Batch No. X188 0909 Control No. 6002702.001 Fluconazole (Diflucan) 200 mg, 28's Blister pack Batch No. 101492301; 814920131 Administered under fasted condition.
PK Sampling:	Blood: Period 1: Pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 144, 216, and 312 h post-dose Period 2: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 144, 216, 312, and 408 h post-dose
Analysis (Plasma)	Method : LC/MS/MS <u>BAF312</u> *Low Curve* LLOQ: 0.02 ng/mL Linear range: 0.02-20 ng/mL

	Inter-day Precision (%CV): 2.1-17.2% Inter-day accuracy: -2.2 - 13.0 % *High Curve* LLOQ: 0.250 ng/mL Linear range: 0.250- 500 ng/mL Inter-day Precision (%CV): 3.2-7.7% Inter-day accuracy: -2.0 - 5.3 %
PK Assessment	The PK parameters of primary interest were determined using non-compartmental methods and included AUCinf, AUClast, andCmax. Secondary PK parameters were T1/2, Tmax, Tlag, CL/F and Vz/F.
Safety Assessment	Adverse events (AEs), serious adverse events (SAEs), monitoring of hematology, blood chemistry and urine performed, regular assessments of vital signs, physical condition, body weight, and height and meal records.

RESULTS

Plasma pharmacokinetics of BAF312

Arithmetic mean BAF312 plasma concentration-time profiles in both periods are shown in the figure blow:



Summary statistics of plasma PK parameters of BAF312 are presented in the table below:

Main plasma PK parameters per BAF312 treatment group

Period	N	AUClast (hr*ng/mL)	AUCinf (hr*ng/mL)	Cmax (ng/mL)	T1/2 (hr)	Tmax (hr)	Tlag (hr)	CL/F (L/hr)	Vz/F (L)
1		1110	1120	31.2	40.6	4.00	0.25	3.59	210
	14	(23.7)	(23.8)	(20.0)	(16.5)	(2.00-8.05)	(0-0.50)	(23.8)	(30.7)
2		2160	2190	34.0	61.6	4.00	0	1.83	162
	11	(31.6)	(32.1)	(19.8)	(12.3)	(3.00-8.00)	(0-0)	(32.1)	(23.2)

Geo-mean (CV%) except for Tmax and Tlag where median (range) is reported

CV% = coefficient of variation (%) = sd/mean*100

CV% geo-mean=(sqrt (exp(variance for log-transformed data)-1))*100

Source: PT-Table 14.2-1.4 and PT-Table 14.2-1.5

The geometric mean ratio and 90% confidence interval (CI) for geometric ratio of PK parameters for BAF312 alone and with fluconazole are summarized in the following table:

Geometric means, estimated geometric mean ratio and 90% CI for geometric ratio of PK parameters (Completers data)

PK Parameter (unit)	Treatment	N	Ratio (Test/Reference) *	90% CI for geometric mean ratio *
Cmax [ng/mL]	BAF312 alone	11	1.10	(1.04, 1.16)
	BAF312 + fluconazole	11		
AUCinf [hr*ng/mL]	BAF312 alone	11	1.98	(1.87, 2.10)
	BAF312 + fluconazole	11		
AUClast [hr*ng/mL]	BAF312 alone	11	1.97	(1.86, 2.08)
	BAF312 + fluconazole	11		

Reference: BAF312 alone – 4 mg BAF312;

Test: BAF312 + Fluconazole - 4 mg BAF312 (Day 3) + 200 mg Fluconazole (Bid Day 1, SD Day 2 to 19).

Model: Log-transformed PK parameter was analyzed by a fixed effect model, with fixed effects from treatment and subject.

* Back-transformed from log scale.

Source: PT-Table 14.2-1.1

Co-administration of BAF312 and fluconazole led to approximately two-fold increases of AUClast and AUCinf of BAF312 in plasma. Terminal half-life was increased by 50%. Cmax was only increased by 10% whereas Tmax remained unchanged, thus suggesting that fluconazole did not affect the absorption phase of BAF312.

Safety

Overall, all the AEs reported in this study were in line with those reported in previous clinical trials and no new significant AEs were reported during the study. No deaths or SAEs were reported during the study.

CONCLUSIONS

- The co-administration of fluconazole, a potent CYP2C9 inhibitor, led to approximately two fold increases of AUC and 10% increase of Cmax of BAF312.
- Overall administration of BAF312 was well tolerated with most of the AEs such as bradycardia, headache, abdominal discomfort, were in line with those reported in previous studies.

Study A2116: A randomized, double-blind, placebo-controlled study to evaluate pharmacodynamics and/or pharmacokinetic interaction of BAF312 (siponimod) and propranolol when co-administered in healthy subjects.

Objectives: To characterize the negative chronotropic effect of siponimod and propranolol co-administration after 10 days of combined treatment in healthy subjects.

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

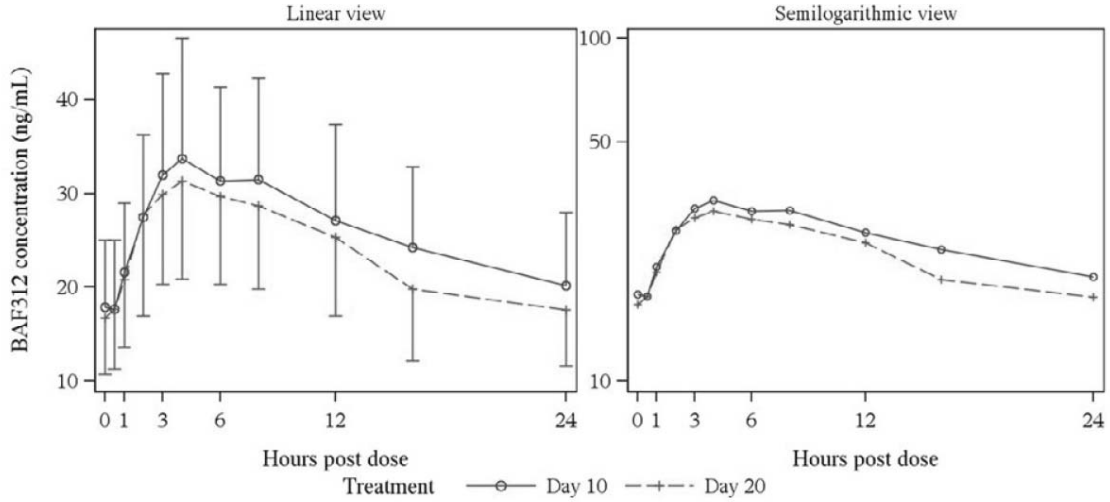
Study Design	Double-blind, randomized, placebo-controlled study
Study Population	N= 76 subjects were randomized Age: 19 - 55 years (mean 37.1 years) Gender: 61 males (80.3 %), 15 females (19.7 %) Weight: 53.1 - 104.7 kg (mean 77.9 kg) Race: 45 Caucasian (59.2%), 29 Black (38.2 %), 2 Other (2.6%)
Dosage and Administration	Group A: Siponimod dose titration regimen (Day 1-6) + siponimod 2 mg (Day 7-20) and propranolol placebo (Day 1-10) + propranolol 80 mg LA (Day 11-20) Group B: Siponimod-placebo (Day 1-10) + siponimod dose titration regimen (Day 11-16) + siponimod 2mg (Day 17-20) and propranolol 80 mg LA (Day 1-20) Group C: Siponimod-placebo (Day 1-20) and propranolol-placebo (Day 1-20) Group D: Siponimod-placebo (Day 1-20) and propranolol 80 mg LA (Day 1-20) Siponimod 0.25 mg tablet: Batch No. X0090112 Control No. 6002636.008 Siponimod 1 mg tablets: Batch No. 6002630.009 Control No. X0120112 Siponimod-placebo tablets Batch No. 6002679.003 Control No. X1380411 Generic placebo hard gelatin capsule 3755667.032 Control No. H503EE Administered under fasted condition.
PK Sampling:	Blood: • Day 1 (pre-dose): bioanalytical blank, • Day 9 (pre-dose),

	• Day 10 (pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h post-dose) : Siponimod and propranolol reference treatments (drugs alone), • Days 11, 15, 16, 17, and 19 (pre-dose, Day 11 pre-dose is in fact the Day 10 24 h sample), • Day 20 (pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h post-dose): siponimod and propranolol test treatments (drugs combined).
Analysis (Plasma)	Method : LC/MS/MS <u>BAF312</u> *Low Curve* LLOQ: 0.02 ng/mL Linear range: 0.02-20 ng/mL Inner-day accuracy: -9.5 - 6.4 % *High Curve* LLOQ: 0.250 ng/mL Linear range: 0.250- 500 ng/mL Inter-day Precision (%CV): 4.6 - 6.2% Inter-day accuracy: -1.3 - 1.6 % <u>Propranolol</u> LLOQ: 1 ng/mL Linear range: 1- 1000 ng/mL Inter-day Precision (%CV): 2.0 - 3.7% Inter-day accuracy: -4.5 - 2.4 %
PK Assessment	The PK parameters determined in this study included: Cmax,ss, Tmax,ss, Cmin,ss, Cav,ss, AUCtau,ss and Fluc,ss.
PD Assessment	12 Lead Holter recording and monitoring (HR, PR, bradyarrythmias), triplicate blood pressure measurement and pulmonary function test (spirometry).
Safety Assessment	AEs, SAEs, ECG, continuous cardiac monitoring (telemetry), Columbia-suicide severity rating scale (C-SSRS), hematology including absolute lymphocyte counts (ALC) and lymphocyte subsets (Flow Cytometry), blood chemistry, liver function tests, and urinalysis performed at the local laboratory, and regular assessments of vital signs, physical condition, body weight, height, and meal records.

RESULTS

Plasma pharmacokinetics of BAF312

The arithmetic mean (SD) plasma concentration time profiles of siponimod alone and in presence of propranolol are presented in the figure below,



Treatment group code: Group A, Compound/ Analyte: Siponimod, Group A: siponimod from Day 1-10, siponimod + propranolol from Day 11-20

Source: Figure 14.2-1.1

Summary statistics for PK parameters for siponimod administered alone or administered along with propranolol are given in the table below,

PK Parameter [unit]	Group A		Group B
	Day 10 (N= 19)	Day 20 (N=19)	Day 20 (N= 19)
Tmax,ss* (h)	4.00 (3.00, 8.00)	4.00 (2.00, 8.00)	4.00 (2.00, 6.00)
Cmax,ss# (ng/mL)	35.3 ± 12.0 [33.7; 30.6]	31.9 ± 10.2 [30.2; 36.7]	26.6 ± 10.0 [23.3; 72.1]
Cmin,ss# (ng/mL)	14.9 ± 5.53 [¥]	15.6 ± 6.71 [13.6; 71.6]	11.8 ± 5.81 [9.66; 88.6]
Cav,ss# (ng/mL)	26.3 ± 9.15 [25.0; 31.1]	23.7 ± 7.78 [22.4; 37.1]	19.3 ± 7.66 [17.0; 69.7]
AUCtau,ss# (h*ng/mL)	630 ± 220 [601; 31.1]	569 ± 187 [538; 37.1]	464 ± 184 [408; 69.7]
Fluc,ss# (%)	74.6 ± 18.4 [72.7; 22.8]	71.0 ± 16.8 [69.4; 22.2]	77.1 ± 23.9 [73.7; 31.8]

#: arithmetic mean ± standard deviation [geometric mean; % geometric mean CV]

*: median (minimum-maximum)

¥: Minimum value for Cmin, ss for Group A on Day 10 was zero, hence GM & CV% were not calculated.

Group A: Siponimod from Day 1-10, siponimod + propranolol from Day 11-20; Group B: Propranolol from Day 1-10, Prop + siponimod from Day 11-20

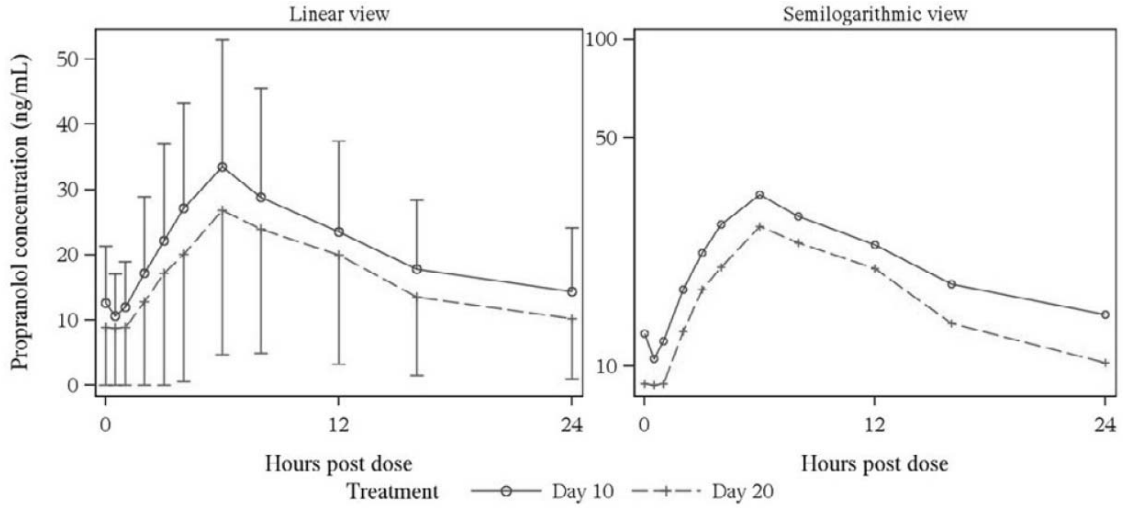
Source: Table 14.2-1.5; Table 14.2-1.7

Mean siponimod AUCtau,ss and Cmax,ss were approximately 7% lowered when coadministered with Propranolol. Siponimod Cmax, ss and inter-individual variability for exposure (Cmax,ss and AUCtau,ss) were comparable, no change in Tmax was observed when given alone and given with propranolol.

Siponimod steady state PK results of the current study were compared to a historical study (CBAF312A2118) data in which healthy subjects received siponimod 2 mg q.d for 10 days, without co-medications.

Plasma pharmacokinetics of Propranolol

The mean plasma concentration time profiles of propranolol alone and administered with siponimod are presented in the figure below,



Treatment group code: Group B, Compound/Analyte : Propranolol Group B: Prop from Day 1-10, Prop + siponimod from Day 11-20. Source: Figure 14.2-1.2

The summary statistics for PK parameters for propranolol are shown in the table below,

	Group A	Group B		Group D	
	Day 20	Day 10	Day 20	Day 10	Day 20
PK Parameter [unit]	(N=17)	(N= 19)	(N=17)	(N= 18)	(N=18)
Tmax,ss* (h)	6.00	6.00	6.00	6.00	6.00
	(0.00, 8.00)	(4.00, 8.00)	(0.517, 24.0)	(6.00, 8.00)	(4.00, 8.00)
Cmax,ss# (ng/mL)	30.8 ± 14.4 [27.2; 57.5]	33.6 ± 19.5 [28.0; 73.8]	29.1± 21.4 [22.5; 92.6]	28 ± 13.4 [25.2; 50.6]	29.8 ± 16.0 [26.1; 58.8]
Cmin,ss# (ng/mL)	8.25 ± 6.11	9.21 ± 6.92	7.87 ± 9.74	8.56 ± 4.78	8.41 ± 6.13
	[¥]	[¥]	[¥]	[7.24; 69.3]	[6.58; 86.9]
Cav,ss# (ng/mL)	17.6 ± 8.74	21.2 ± 12.4	17.7 ± 14.2	17.7 ± 8.69	18.2 ± 10.4
	[15.3; 63.1]	[17.3; 82.3]	[13.5; 91.6]	[15.8; 53.8]	[15.7; 61.7]
AUCtau,ss# (h*ng/mL)	422 ± 210 [366; 63.1]	509± 297 [416; 82.3]	424 ± 340 [324; 91.6]	425 ± 209 [379; 53.8]	436 ± 248 [377; 61.7]
Fluc,ss# (%)	138 ± 35.2	120 ± 47.5	133 ± 34.6	113 ± 20.8	123 ± 21.1
	[134; 25.8]	[113; 35.5]	[128; 29]	[111; 19.2]	[121; 16.3]

#: arithmetic mean ± standard deviation [geometric mean; % geometric mean CV]

*: median (minimum-maximum)

¥ CV% presented were CV% geometric mean; Minimum value for Cmin,ss for Group A and B on Day 10 and 20 are zeros, hence GM & CV% were not calculated.

Group A: siponimod from Day 1-10, Siponimod + propranolol from Day 11-20; Group B: propranolol from Day 1-10, Prop + Siponimod from day 11-20; Group D: propranolol from day 1-20

Ratios of geometric means for PK parameters of primary interest for siponimod and propranolol between treatment (siponimod given with and without propranolol and vice versa) are presented in the table below,

		Adjusted geometric mean		Ratio of geometric means	
Analyte	**Parameter (Unit)**	**Test**	**Reference**	**Estimate**	**90% CI (lower, Upper)**
Siponimod (N=18)	AUCtau,ss (h*ng/mL)	538	575	0.93	(0.85, 1.03)
	Cmax,ss (ng/mL)	30.2	32.3	0.93	(0.84, 1.04)
Propranolol (N=17)	AUCtau,ss (h*ng/mL)	324	394	0.82	(0.66, 1.03)
	Cmax,ss (ng/mL)	22.5	26.6	0.85	(0.69, 1.04)

Test: Propranolol (80 mg) + siponimod (2 mg)

Reference: Propranolol (or) siponimod alone

Source: Table 14.2-1.1

co-administration of siponimod and propranolol decreased the propranolol AUCtau,ss and Cmax,ss by approximately 18% and 15% respectively.

PD results

HR effect: The combination treatment at steady state (Group A and B combined on Day 20) showed an additional decrease of mean Emax HR by 6.21 bpm (95% CI: 2.32, 10.11; p=0.002) when compared to propranolol alone over 24 hours of evaluation. The mean minimum hourly average heart rate remained above 50 bpm during the combination treatment on all evaluation days.

BP Effect: The combination treatment (Groups A and B combined on Day 20) at steady state showed an additional Emax MABP decrease of 2.93 mmHg (95% CI: -0.28, 6.14; p= 0.0734) in comparison to propranolol alone over 24 hours of evaluation.

PR interval: The change from baseline in combination treatment at steady state (Groups A and B combined on Day 20) showed a mean increase of PR interval by 2.45 msec (95% CI: -5.32, 10.22; p=0.5341) at 2.5 hours post-dose and 7.06 msec (95% CI: 0.05, 14.07; p=0.0485) at 6.5 hours post-dose, in comparison to propranolol alone.

Bradyarrhythmias: In approximately, 76 x 6 days of 24 hour Holter recordings on study treatment days, only a few bradyarrythmias were reported. None of the events were associated with any clinical signs or symptoms.

Pulmonary function: The change from baseline in combination treatment at steady state (Day 19) showed an additional change of FEV1 by mean -0.07 L (95% CI: -0.17, 0.03; p=0.1804) when propranolol was administered on top of siponimod (after dose titration in Group A) and -0.05 L (95% CI: -0.15, 0.05; p=0.2957) when siponimod was administered on top of propranolol (Group B), in comparison to propranolol alone.

Safety

Siponimod was generally well tolerated in this study. There were no deaths or SAEs or AE of severe grade reported in this study. All the AEs were of mild to moderate grade.

CONCLUSIONS

- Concomitant administration of siponimod and propranolol had only minor effect on siponimod Cmax,ss and AUCtau,ss (7% decrease), while the propranolol Cmax,ss and AUCtau increased by 15 and 18% respectively.
- The combination treatment led to a mean Emax HR decrease of 6.21 bpm compared to propranolol alone and the mean minimum hourly average HR remained above 50 bpm. Addition of propranolol on top of siponimod had lesser negative chronotropic effects in comparison to addition of siponimod on top of propranolol. There was slight decrease in BP in the combination treatment in comparison to propranolol alone.
- There was a trend for slight prolongation of PR interval with the combination treatment. There was no 2nd degree AV block or sinus pause of more than 3 sec duration noted in the study during the combination treatment.
- There were no clinically significant changes in the pulmonary function with either drug alone or combination treatment.
- The combination treatment was generally well tolerated in a healthy subject population.

Study A2130: A randomized, double-blind, placebo-controlled, parallel-group study to evaluate the modulation of immune response to T-cell dependent and T-cell independent antigen stimuli by preceding, concomitant and interrupted administration of multiple therapeutic doses of BAF312 in healthy subjects.

Objectives: To compare the influence of preceding, concomitant and interrupted BAF312 administration on the efficacy of a T-cell dependent (influenza) and T-cell-independent (PPV-23) vaccination in healthy subjects relative to placebo.

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

<table>
<tr><td>Study Design</td><td>A randomized, double-blind, placebo-controlled, parallel group study</td></tr>
<tr><td>Study Population</td><td>N= 136 subjects were enrolled
Age: 18 - 55 years (mean 36.6 years)
Gender: 106 males (77.9%), 30 females (22.1%)
Weight: 56.1 - 101.3 kg (mean 77.3 kg)
Race: 86 Caucasian (63.2%), 43 Black (31.6%), 3 Asian (2.2%), 4 Other (2.9%)</td></tr>
<tr><td>Dosage and Administration</td><td>Group 1: Received 10 days of BAF312 followed by placebo, vaccination challenge on Day 21, again placebo for 14 days, and finally 14 days of BAF312.
Group 2: Received BAF312 for 13 days followed by 7-days of placebo, then a vaccination challenge on Day 21 again followed by placebo for 28 days.
Group 3: Starting with placebo for 10 days, the subjects received BAF312 for the next 38 days with a in between vaccination challenge on Day 21.
Group 4: Administered placebo for 48 days with a vaccination challenge on Day 21.
<table>
<tr><th>Study drug and strength</th><th>Formulation control number</th><th>Batch number</th></tr>
<tr><td>BAF312 0.25 mg</td><td>PCN 13-0312CH</td><td>13-0312CH/X274 1111</td></tr>
<tr><td></td><td>PCN 14-2383CH</td><td>14-2383CH/X002 0113</td></tr>
<tr><td>BAF312 0.5 mg</td><td>PCN 13-0312CH</td><td>13-0312CH/X275 1111</td></tr>
<tr><td></td><td>PCN 14-2383CH</td><td>14-2383CH/X004 0113</td></tr>
<tr><td>BAF312 1 mg</td><td>PCN 13-0312CH</td><td>13-0312CH/X273 1111</td></tr>
<tr><td></td><td>PCN 14-2383CH</td><td>14-2383CH/X005 0113</td></tr>
<tr><td>BAF312 2 mg</td><td>PCN 13-0312CH</td><td>13-0312CH/X276 1111</td></tr>
<tr><td></td><td>PCN 14-2383CH</td><td>14-2383CH/X008 0113</td></tr>
<tr><td>Pneumovax®</td><td>NDC 0006-4943-00</td><td>K004281</td></tr>
<tr><td>Influenza vaccine</td><td>NDC 66521-117-02</td><td>145202</td></tr>
</table>
</td></tr>
</table>

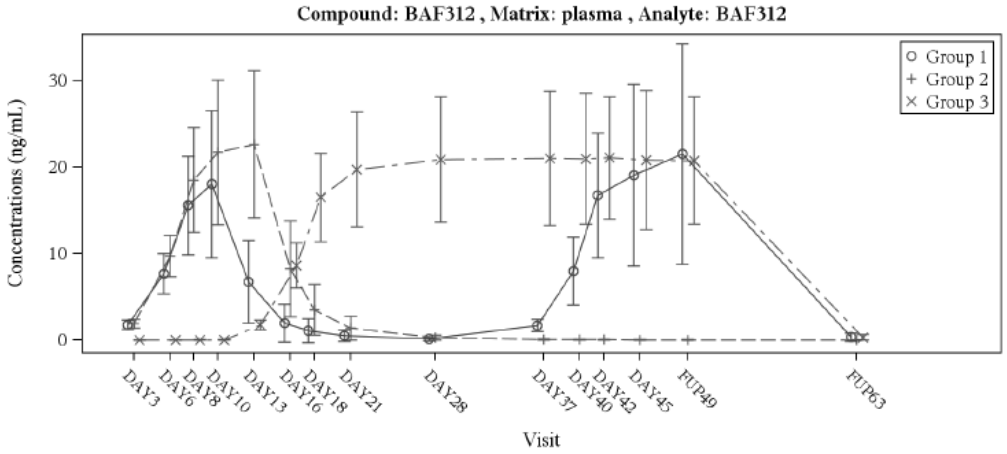
PK Sampling:	Blood: Days 3, 6, 8, 10, 13, 16, 18, 21, 28, 37, 40, 42, and 45 within 60 min prior to dosing. BAF312 post-dose PK samples were collected on Days 49 and 63.
Analysis (Plasma)	Method : LC/MS/MS BAF312 LLOQ: 0.05 ng/mL Linear range: 0.05 - 100 ng/mL Inter-day Precision (%CV): 2.8 - 6.2% Inter-day accuracy: -1.8 - 2.8 %
PK Assessment	Pre-dose (trough) samples were taken. Ctrough were used to confirm that PK steady state was reached after 10 days of BAF312 dosing and to confirm compliance by monitoring plasma concentrations during drug intake.
PD Assessment	Antibody titers obtained at four weeks after antigen challenge.
Safety Assessment	AEs, SAEs, the regular monitoring of hematology, blood chemistry and urine performed at (study center/central laboratory) and regular assessments of vital signs, physical condition, height and weight, and ECG.

RESULTS

Plasma pharmacokinetics of BAF312

The arithmetic mean concentration-time profiles of BAF312 per group are presented in the figure below,

Arithmetic mean (SD) BAF312 trough concentration-time profiles per group – PK analysis set



The unscheduled samples are not taken into account for the calculation of the statistics

Group 1: Day 1-10: BAF312, Day 11-34: Placebo, Day 35-48: BAF312;
Group 2: Day 1-13: BAF312, Day 14-48: Placebo;
Group 3: Day 1-10: Placebo, Day 11-48: BAF312.

Source: Figure 14.2-1.1

Steady state BAF312 plasma concentrations were comparable to historical observations and in most subjects achieved at 2 mg q.d as expected after approximately 10 days of treatment.

Pharmacodynamic results

The primary PD outcome variable was the proportion of responders to a vaccination induced by PPV-23 and influenza vaccine.

- For PPV-23, response was defined by a ≥2-fold increase of IgG concentrations at four weeks after vaccination compared to baseline.
- For influenza, response was defined by a ≥ 4-fold increase of Anti-hemagglutinin-inhibition titers at four weeks after vaccination compared to baseline (=seroconversion)

In addition, geometric mean data of PPV-23 IgG and IgM concentrations and influenza titers is provided.

Influenza

A quadrivalent influenza vaccine recommended for use in North America during the 2014/2015 season has been used. It contained the following four antigens:

- Influenza-A/California/7/2009 (A Cal)
- Influenza-A/Texas/50/2012 (A Tex)
- Influenza-B/Brisbane/60/2008 (B Bri)

- Influenza-B/Massachusetts/2/2012 (B Mas)

Responder analysis: Non-inferior responder rates with respect to each of the four antigens were determined for the preceding treatment group, but not for the interrupted and concomitant treatment groups (see table below) which showed responder rates that were approximately 15%-30% lower than on placebo (except for B-Bri with similar responder rates to placebo).

Proportion of responders to influenza at 4 weeks after vaccination by treatment group reported for each antigen

Antigen: A/California/7/2009

Treatment group	n	Responder (%)	Non-responder (%)	Proportion difference (95% CI)	P-value
Group 1 Interrupted (N=29)	28	20 (71.4)	8 (28.6)	-0.15 (-0.36, 0.06)	0.0827
Group 2 Preceding (N=30)	28	26 (92.9)	2 (7.1)	0.06 (-0.09, 0.22)	<.0001
Group 3 Concomitant (N=29)	27	20 (74.1)	7 (25.9)	-0.13 (-0.33, 0.08)	0.0482
Group 4 Placebo (N=30)	30	26 (86.7)	4 (13.3)		

Antigen: A/Texas/50/2012

Treatment group	n	Responder (%)	Non-responder (%)	Proportion difference (95% CI)	P-value
Group 1 Interrupted (N=29)	28	17 (60.7)	11 (39.3)	-0.16 (-0.40, 0.08)	0.1239
Group 2 Preceding (N=30)	28	20 (71.4)	8 (28.6)	-0.05 (-0.28, 0.17)	0.0157
Group 3 Concomitant (N=29)	27	13 (48.1)	14 (51.9)	-0.29 (-0.54, -0.03)	0.4539
Group 4 Placebo (N=30)	30	23 (76.7)	7 (23.3)		

Antigen: B/Brisbane/60/2008

Treatment group	n	Responder (%)	Non-responder (%)	Proportion difference (95% CI)	P-value
Group 1 Interrupted (N=29)	28	3 (10.7)	25 (89.3)	-0.03 (-0.19, 0.14)	0.0007
Group 2 Preceding (N=30)	28	4 (14.3)	24 (85.7)	0.01 (-0.17, 0.19)	0.0003
Group 3 Concomitant (N=29)	27	4 (14.8)	23 (85.2)	0.01 (-0.17, 0.20)	0.0003
Group 4 Placebo (N=30)	30	4 (13.3)	26 (86.7)		

Antigen: B/Massachusetts/2/2012

Treatment group	n	Responder (%)	Non-responder (%)	Proportion difference (95% CI)	P-value
Group 1 Interrupted (N=29)	28	8 (28.6)	20 (71.4)	-0.15 (-0.40, 0.10)	0.1138
Group 2 Preceding (N=30)	28	14 (50.0)	14 (50.0)	0.07 (-0.19, 0.32)	0.0026
Group 3 Concomitant (N=29)	27	7 (25.9)	20 (74.1)	-0.17 (-0.42, 0.07)	0.1599
Group 4 Placebo (N=30)	30	13 (43.3)	17 (56.7)		

n is the number of subjects in PD analysis set with non-missing/valid titers at both baseline (Day 21) and 4 weeks after influenza vaccination (Day 49).
N is the number of subjects in PD analysis set with valid measurements within the group.
Proportion difference (95% CI) and p-value are calculated based on the normal distribution and a non-inferiority margin of 0.3.
Comparison is conducted between groups with BAF312 (Group 1, 2, 3) and placebo (Group 4)
Group 1: Day 1-10: BAF312, Day 11-34: Placebo, Day 35-48: BAF312; (=interrupted treatment)
Group 2: Day 1-13: BAF312, Day 14-48: Placebo; (=preceding treatment)
Group 3: Day 1-10: Placebo, Day 11-48: BAF312; (=concomitant treatment)
Group 4: Day 1-48: Placebo.
Source: Table 14.2-2.4

Geometric mean titers: In all three treatment groups, there were relevant titer increases. At 4 weeks post influenza vaccination, geometric mean titers were ≥1:40 in all three BAF312 treatment groups with respect to A-Cal and A-Tex, but not with respect to B-Bri. In terms of B-Mas, the geometric mean titer was ≥1:40 in context of preceding and concomitant treatment, but not in context of concomitant treatment.

PPV-23

Responder analysis: Non-inferior responder rates have been determined for each of the three treatment regimens (p<0.001, see table below). In terms of preceding and concomitant BAF312 treatment, each of the subjects was identified as responder defined by an >2-fold increase of IgG

antibody concentrations four weeks post vaccination compared to baseline. There were only very few non-responders in the interrupted BAF312 treatment group (3 of 28) and placebo group (2 of 30).

Proportion of responders by treatment group

Treatment group	n	Responder (%)	Non-responder (%)	Proportion difference (95% CI)	P-value
Group 1 (N=29)	28	25 (89.3)	3 (10.7)	-0.04 (-0.19, 0.10)	0.0002
Group 2 (N=30)	28	28 (100)	0 (0.0)	0.07 (-0.03, 0.16)	<.0001
Group 3 (N=29)	27	27 (100)	0 (0.0)	0.07 (-0.03, 0.16)	<.0001
Group 4 (N=30)	30	28 (93.3)	2 (6.7)		

n is the number of subjects in PD analysis set with non-missing/valid values at both baseline (Day 21) and 4 weeks (Day 49) after PPV-23 vaccination.

N is the number of subjects in PD analysis set with valid measurements within the group.

Proportion difference (95% CI) and p-value are calculated based on the normal distribution with a non-inferiority margin of 0.3.

Comparison is conducted between groups with BAF312 (Group 1, 2, 3) and placebo (Group 4)

Group 1: Day 1-10: BAF312, Day 11-34: Placebo, Day 35-48: BAF312; (=interrupted treatment)
Group 2: Day 1-13: BAF312, Day 14-48: Placebo; (=preceding treatment)
Group 3: Day 1-10: Placebo, Day 11-48: BAF312; (=concomitant treatment)
Group 4: Day 1-48: Placebo.
Source: Table 14.2-2.5

Geometric mean antibody concentrations: In the placebo group, geometric mean antibody concentrations increased by approximately 8-fold and 5-fold compared to baseline with respect to IgG and IgM, respectively. In terms of BAF312, geometric mean IgG concentrations increased by approximately 9-fold, 10-fold and 7-fold, while IgM concentrations increased by approximately 5-fold, 5-fold and 4-fold in the interrupted, preceding and concomitant treatment group, respectively. Hence, IgG and IgM concentrations increased to a similar extent in the placebo and BAF312 treatment groups.

Safety

The overall incidence of adverse events was essentially similar in the BAF312 treatment groups as in the placebo group (35.3%-55.9% vs. 38.3%). There were no clinically relevant findings of laboratory, vital sign or ECG data noted. These data was similar across treatment groups or there were no marked trends over time. In this study, BAF312 was safe and tolerated at a dose of 2 mg q.d in healthy subjects.

CONCLUSIONS

Non-inferior responder rates were identified for the T-cell independent vaccination with PPV-23 with respect to each of the three BAF312 treatment groups. Hence, BAF312 treatment even when given concomitantly at a therapeutic multiple dose of 2 mg q.d. is not considered to compromise the efficacy of PPV-23 vaccination.

In terms of influenza, non-inferior responder rates have been determined with respect to the preceding treatment group. Hence, efficacy of an influenza vaccination as defined in the context of this study is not considered to be compromised in case BAF312 treatment is paused one week prior until four weeks after an influenza vaccination. Even though non-inferiority has not been established

with respect to the interrupted and concomitant treatment group, there was also a reasonable titer increase noted in both groups and responder rates were only approximately 15%-30% lower than on placebo.

Study A2121: An Open-label, multiple-dose, two-treatment period study to evaluate the effect of oral BAF312 on the pharmacokinetics and pharmacodynamics of a monophasic oral contraceptive in healthy female volunteers.

Objectives: To investigate whether BAF312 administered daily at a dose of 4 mg can affect exposure (Cmax,ss and AUCtau,ss) to a daily administered monophasic oral contraceptive (OC) regimen containing 30 µg of ethinylestradiol (EE) and 150 µg of levonorgestrel (LVG).

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

<table>
<tr><td>Study Design</td><td>An open-label, single center, single sequence, two- treatment period study</td></tr>
<tr><td>Study Population</td><td>N= 24 subjects were enrolled and analyzed (CYP2C9 *1*1)
Age: 18 - 27 years (mean 22.3 years)
Gender: 24 females (100%)
Weight: 50.4 - 82.6 kg (mean 64.4 kg)
Race: 20 Caucasian (83.3%), 3 Black (12.5%), 1 Other (4.2%)</td></tr>
<tr><td>Dosage and Administration</td><td>Monophasic OC was administered by the investigator or delegate from Day 1 to Day 49, and was dispensed to the subject for self-administration from Day 57 and up to 30 days after study completion visit.
BAF312 was administered from Day 23 to Day 49 with Titration period.
<table>
<tr><th>Study drug and strength</th><th>Formulation control number</th><th>Batch number</th></tr>
<tr><td>BAF312 0.25 mg</td><td>6002636.008</td><td>X139 0411</td></tr>
<tr><td>BAF312 1 mg</td><td>6002630.009</td><td>X140 0411</td></tr>
<tr><td>BAF312 4 mg</td><td>6002702.005</td><td>X141 0411</td></tr>
</table></td></tr>
<tr><td>PK Sampling:</td><td>EE/LVG PK: day 1 (pre-dose), day 16 (pre-dose), day 19 (pre-dose), day 21(pre-dose, 0.5; 1; 1.5; 2; 3; 4; 6; 8; 12; 16; 24 h post-dose), day 42 (pre-dose), day 44 (pre-dose), day 47 (predose), day 49 (pre-dose; 0.5; 1; 1.5; 2; 3; 4; 6; 8; 12; 16; 24 h post-dose).
BAF312 PK: day 23 (pre-dose), day 42 (pre-dose), day 44 (pre-dose), day 47 (pre-dose), day 49 (pre-dose, 0.5; 1; 1.5; 2; 3; 4; 6; 8; 12; 16; 24 h post-dose).</td></tr>
</table>

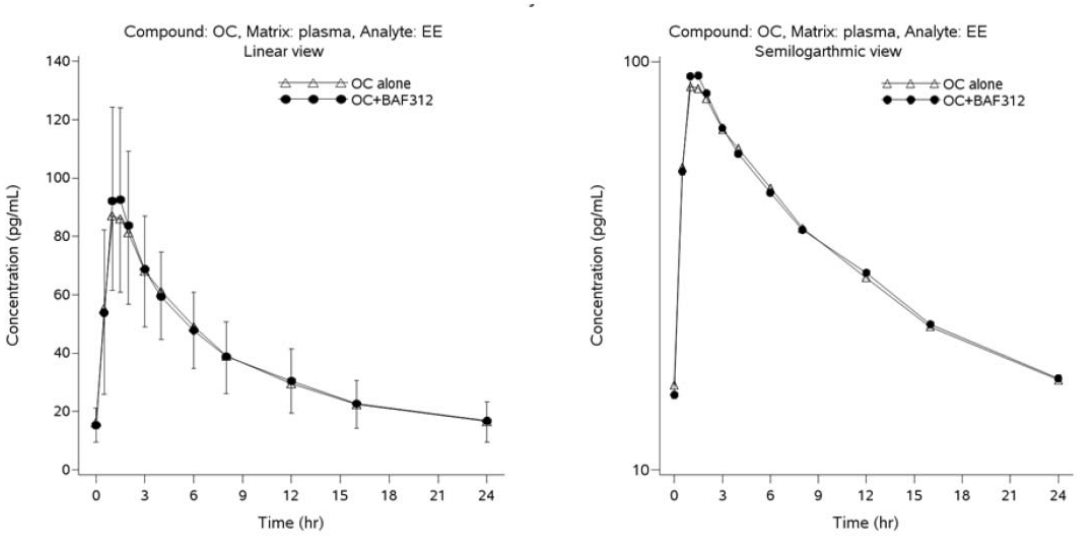
Analysis (Plasma)	Method: LC/MS/MS BAF312 *High curve* LLOQ: 0.250 ng/mL Linear range: 0.25 - 500 ng/mL Inter-day Precision (%CV): 2.5 - 5.2% Inter-day accuracy: -0.8 - 1.8 % *Low curve* LLOQ: 0.02 ng/mL Linear range: 0.02 - 20 ng/mL Inner-day accuracy: -4.8 - 4.8 % Ethinyl estradiol LLOQ: 5.00 pg/mL Linear range: 5.00 - 2500 pg/mL Inter-day Precision (%CV): 1.8 - 3.2% Inter-day accuracy: -3.5 - 5.0 % Levonorgestrel LLOQ: 50.0 pg/mL Linear range: 50.0 - 25000 pg/mL Inter-day Precision (%CV): 1.8 - 4.9% Inter-day accuracy: -5.2 - 5.0 %
PK Assessment	The following pharmacokinetic parameters of BAF312, EE and LVG were determined using non-compartmental method(s): Cmax,ss, Tmax,ss, Cmin,ss, Cav,ss, AUCtau, Fluc.
PD Assessment	• FSH, LH, progesterone and estradiol concentrations • SHBG concentration • Ovarian follicle size determined by transvaginal ultrasound (TVUS)
Safety Assessment	AEs, SAEs, Physical and gynecological examinations, Vital signs (Blood pressure, pulse rate), Routine laboratory analysis, Electrocardiogram (ECG), Columbia Suicidality Severity Rating Scale (C-SSRS)

RESULTS

Pharmacokinetic Results of Ethinylestradiol (EE) and Levonorgestrel (LVG)

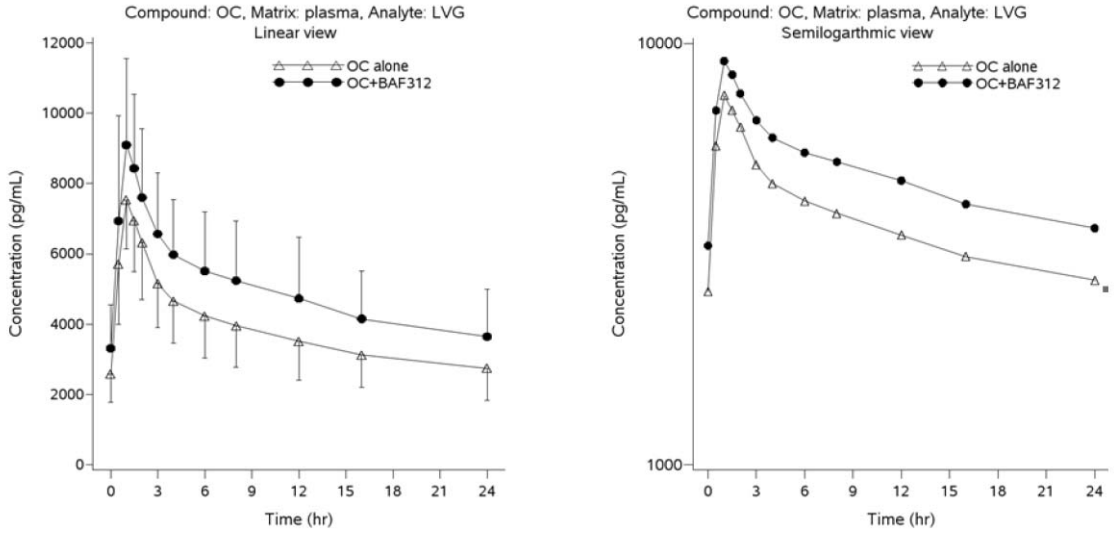
The mean plasma concentration time profiles of EE and LVG are presented in the following figures,

Arithmetic mean (SD) plasma concentration-time profiles for EE per treatment



OC: EE (30µg) /LVG (150µg); BAF312: BAF312 4mg; EE: Ethinylestradiol; LVG: Levonorgestrel;
Source: PT-Figure 14.2-1.1

Arithmetic mean (SD) plasma concentration-time profiles for LVG per treatment



OC: EE (30µg) /LVG (150µg); BAF312: BAF312 4mg; EE: Ethinylestradiol; LVG: Levonorgestrel;
Source: PT-Figure 14.2-1.1

Ratios of geometric means for PK parameters of primary interest for EE and LVG between treatment (OC given with and without BAF312A) are presented in table below,

Geometric mean ratio (Test/Reference) and 90% confidence intervals for PK variables of primary interest for OC

					Geometric mean ratio*	
Analyte	**PK parameter [unit]**	**Treatment**	**N**	**Adjusted geometric means***	**Estimate (Test/Reference)**	**90% CI for ratio**
Ethinylestradiol	Cmax,ss [pg/mL]	Test	23	90.6	1.02	(0.96,1.08)
		Reference	23	88.9		
	AUCtau [h*pg/mL]	Test	23	843	1.00	(0.96,1.05)
		Reference	23	840		
Levonorgestrel	Cmax,ss [pg/mL]	Test	23	9110	1.18	(1.11,1.26)
		Reference	23	7700		
	AUCtau [h*pg/mL]	Test	23	115000	1.29	(1.24,1.34)
		Reference	23	88900		

* Back-transformed from log scale

Model: The log transformed PK parameter data were analyzed using a fixed effect model with subject and treatment (Test:OC+BAF312 and Reference:OC alone) as fixed effects;

OC: EE (30µg) /LVG (150µg); BAF312: BAF312 4mg; EE: Ethinylestradiol; LVG: Levonorgestrel;

Source: PT-Table 14.2-1.1

The means of both AUCtau and Cmax,ss of EE were not changed significantly after administration of the combination treatment with BAF312A ccompared to OC administered alone. The means of AUCtau and Cmax,ss of LVG after administration of the combination treatment with BAF312A were increased by 28% and 18%, respectively, compared to OC administered alone.

Pharmacokinetic Results of BAF312

BAF312 for PK sample were collected over 24 hours after 21 days of 4 mg q.d BAF312 administration in Period 2.

There are no steady state BAF312 PK data at a dose of 4 mg available from the historical data. In phase 2 study A2201, the geometric mean BAF312 trough concentration at month 1, 3, 6 were range from 21.7-23.7 ng/mL in 2mg/day treatment group. In current study, the geometric mean Cmin,ss was 40.0 ng/mL. Considering the linear PK of BAF312, the steady state PK of BAF312A is expected to be similar when given with OC as compared to BAF312 alone.

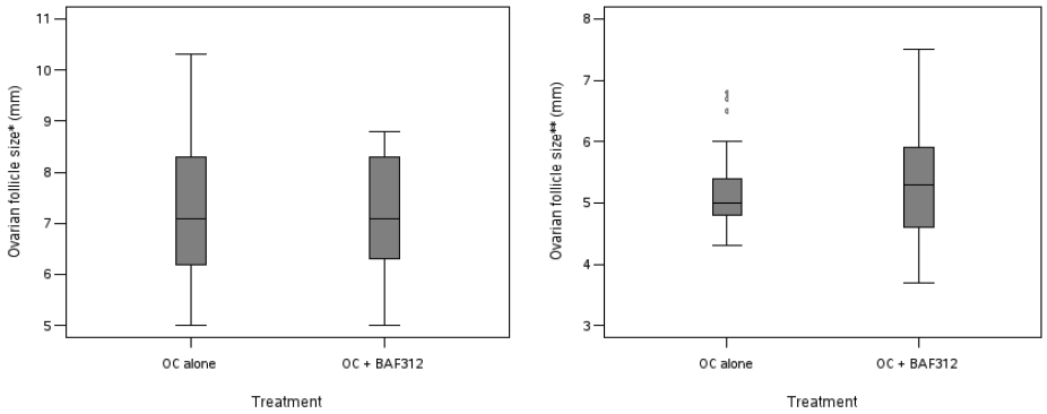
Pharmacodynamic Results

Follicle size assessed by Transvaginal Ultrasound (TVUS)

In Period 1, there was a decrease in the mean (SD) of the ovarian follicular size (mm) noted at Day 21 [5.19 (0.071)] from the Baseline [7.26 (1.378)]. In Period 2, there was a similar decrease in the mean (SD) of the largest ovarian follicular size at Day 49 [5.34 (0.951)] from Day 28 [7.18 (1.185)].

The following figure represents the box plot of the values for follicle size, compared between Period 1 and Period 2.

Box plot for PD parameter-ovarian follicle size



*Ovarian follicle size on baseline treatment period.
**On treatment period (Day 21)
Source: PT-Figure 14.2-2.2

FSH, LH, estradiol, progesterone and SHBG

The maximum concentration for estradiol, FSH, LH and progesterone throughout the population were summarized in the table below,

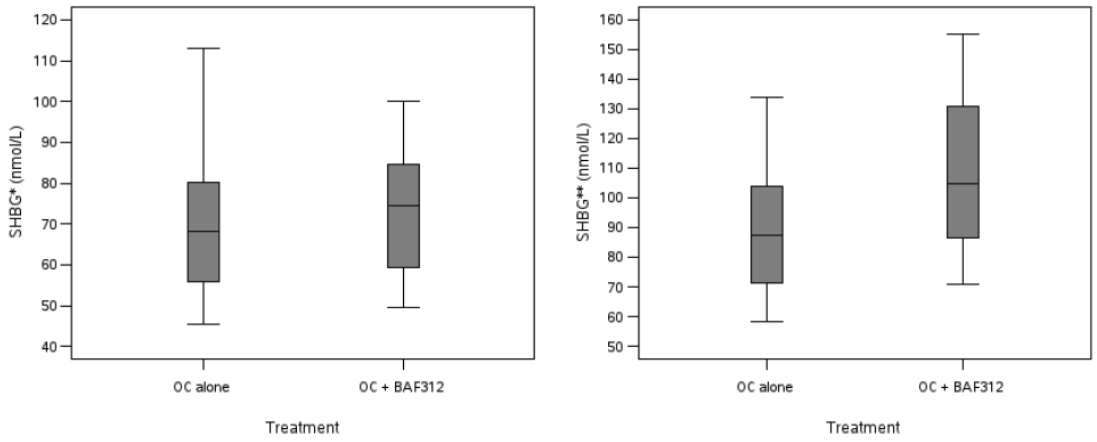
Summary statistics of maximum concentration for estradiol, FSH, LH, and progesterone

Treatment	Statistic	E2 (Estradio) (pmol/L)	Follicle Stimulating Hormone (U/L)	Luteinizing Hormone (U/L)	Progesterone (Blood) (nmol/L)
A	**n**	**23**	**23**	**23**	**23**
	Mean (SD)	132.90 (67.782)	4.9996 (2.00071)	5.2870 (3.84455)	1.170 (0.4777)
	CV% mean	51.0	40.0	72.7	40.8
	Geo-mean	122.23	4.5609	3.7576	1.098
	CV% geo-mean	40.5	49.0	141.1	35.9
	Median	120.70	5.0900	4.8200	1.030
	[Min; Max]	[73.4; 400.0]	[1.790; 8.720]	[0.102; 16.500]	[0.67; 2.73]
B	**n**	**23**	**23**	**23**	**23**
	Mean (SD)	205.09 (132.286)	4.7687 (1.99273)	4.5769 (3.73686)	1.255 (0.3595)
	CV% mean	64.5	41.8	81.6	28.6
	Geo-mean	178.74	4.3721	3.3717	1.205
	CV% geo-mean	53.8	46.1	102.4	30.3
	Median	172.90	4.4500	3.4400	1.270
	[Min; Max]	[73.4; 623.9]	[1.510; 9.580]	[0.455; 15.500]	[0.64; 2.08]

Treatment A: OC alone (21 days), BAF312 (0.25, 0.25, 0.5, 0.75, 1.25, 2 mg) (day 23 - 28); B: OC + 4 mg BAF312
OC: EE (30µg) /LVG (150µg); EE: Ethinylestradiol; LVG: Levonorgestrel;
CV% = coefficient of variation (%) = sd/mean*100.
CV% geo-mean = sqrt(exp(variance for log transformed data)-1) * 100.
Source: PT-Table 14.2-2.6.1

The values for SHBG showed a minor elevation during combination treatment compared to OC pill alone on day 21. The below figure represents the box plot of the values for SHBG, compared between Period 1 and Period 2.

Box plot for PD parameter- SHBG



*SHBG on baseline treatment period
**On treatment period (Day 21)
Source: PT-Figure 14.2-2.2

Co-administration of BAF312 with the OC showed no clinically significant alteration of PD markers (estradiol, FSH and LH) as compared to OC alone. Progesterone levels remained below 5 nmol/L, indicating that no ovulation occurred during the combination treatment. All follicle sizes remained below 10 mm on Day 21, indicating their lack of activity during the combination treatment. The minor elevation in SHBG levels during the combination treatment is not considered to be clinically significant.

Safety

The multiple oral doses of BAF312 when combined with daily administered EE (30 µg) and LVG (150 µg) were safe and well tolerated in healthy women in an outpatient setting. Dose titration regimen of BAF312 was well tolerated. No significant cardiovascular AEs were noted in the study. There were no discontinuations due to adverse events, no deaths, no serious and severe adverse events. Most AEs were mild in severity.

CONCLUSIONS

- BAF312 steady-state dosing of BAF312 4 mg had no effect on EE AUCtau and Cmax,ss. A weak effect on LVG Cmax,ss (18% increase), LVG AUCtau (28% increase) was observed when co-administered with BAF312.
- No clinically significant changes in SHBG in the combination treatment compared to OC pill alone were observed.
- The pharmacokinetics of EE and LVG were not altered to a clinical significant extent and the efficacy of OC pill was maintained with BAF312 co-administration.
- The combination treatment of OC pill (30 µg of ethinylestradiol and 150 µg of levonorgestrel) and BAF312 was found to be safe and well tolerated.

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