



Siponimod pharmacokinetics, safety, and tolerability in combination with rifampin, a CYP2C9/3A4 inducer, in healthy subjects

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Abstract

Purpose To assess the potential pharmacokinetic (PK) interactions between siponimod and rifampin, a strong CYP3A4/moderate CYP2C9 inducer, in healthy subjects.

Methods This was a confirmatory, open-label, multiple-dose two-period study in healthy subjects (aged 18–45 years). In Period 1 (Days 1–12), siponimod was up-titrated from 0.25 to 2 mg over 5 days (Days 1–6) followed by 2 mg once daily on days 7–12. In Period 2, siponimod 2 mg qd was co-administered with rifampin 600 mg qd (Days 13–24). Primary assessments included PK of siponimod (Days 12 and 24; maximum steady-state plasma concentration [$C_{\max,ss}$], median time to achieve $C_{\max,ss}$ [$T_{\max,ss}$], and area under the curve at steady state [$AUC_{\tau,ss}$]). Key secondary assessments were PK of M3 and M5 metabolites, and safety/tolerability including absolute lymphocyte count (ALC).

Results Of the 16 subjects enrolled (age, mean \pm standard deviation [SD] 31 ± 8.3 years; men, $n = 15$), 15 completed the study. In Period 1, siponimod geometric mean $C_{\max,ss}$ (28.6 ng/mL) was achieved in 4 h (median $T_{\max,ss}$; range, 1.58–8.00) and the geometric mean $AUC_{\tau,ss}$ was $546 \text{ h} \times \text{ng/mL}$. In Period 2, the siponimod geometric mean $C_{\max,ss}$ and $AUC_{\tau,ss}$ decreased to 15.7 ng/mL and $235 \text{ h} \times \text{ng/mL}$, respectively; median T_{\max} remained unchanged (4 h). Rifampin co-administration increased M3 $C_{\max,ss}$ by 53% while M5 $C_{\max,ss}$ remained unchanged. The $AUC_{\tau,ss}$ of M3 and M5 decreased by 10% and 37%, respectively. The majority of adverse events reported were mild, with a higher frequency during Period 2 (86.7%) versus Period 1 (50%). The mean ALC increased slightly under rifampin co-administration but remained below $1.0 \times 10^9/L$.

Conclusions The study findings suggest that in the presence of rifampin, a strong CYP3A4/moderate CYP2C9 inducer, siponimod showed significant decrease in $C_{\max,ss}$ (45%) and $AUC_{\tau,ss}$ (57%) in healthy subjects.

Keywords Siponimod · Rifampin · Pharmacokinetics · Drug–drug interactions · Healthy subjects

Introduction

Multiple sclerosis (MS) is a chronic immune-mediated inflammatory disease of the central nervous system (CNS) accompanied by axonal damage, neurodegeneration, and demyelination [1]. The majority of patients (80%) are diagnosed with

the relapsing–remitting form (RRMS) of the disease and approximately 50% of these patients eventually develop secondary progressive multiple sclerosis (SPMS) within 10–15 years of disease onset [2].

Siponimod (BAF312) is a potent, oral, and selective modulator of sphingosine 1-phosphate receptor subtypes 1 and 5 (S1P_{1,5}) [3], currently under advanced phase of clinical development for the treatment of SPMS. Siponimod is the first disease modifying therapy showing a robust effect on disability progression in a representative SPMS population [3, 4]. Sphingosine 1-phosphate signalling plays a key role in neuro-inflammatory processes [5, 6]. Siponimod crosses the blood-brain barrier [6]; its interaction with S1P₁ limits the inflammatory effects mediated by B cells and T cells [3, 7]. In addition, modulation of S1P receptors is known to limit central inflammation and promote CNS repair in animal

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models, independent of peripheral immune effects, which may be relevant to SPMS pathology [5, 8–10].

In healthy subjects, siponimod has a dose-linear and time-independent pharmacokinetic (PK) profile. The steady state of plasma siponimod is reached after ~6 days, with a mean accumulation ratio of 1.9–2.7 and an effective half-life ($T_{1/2}$) of approximately 30 h (range 22–38 h) [3]. Studies in humans indicate that siponimod is eliminated from the systemic circulation mainly due to metabolism and subsequent biliary/faecal excretion. The polymorphic enzyme CYP2C9 is primarily responsible for the clearance of siponimod (79.2%) with some contribution from CYP3A4 (18.5%) [11]. Metabolite M3 is one of the main circulating metabolites of siponimod in humans [12]; it is formed by glucuronidation of the hydroxylated M5.

Rifampin is a potent activator of the human pregnane X receptor and is classified as a moderate CYP2C9 and a strong CYP3A4 inducer [13]. Moreover, rifampin induces certain drug transporter proteins, such as intestinal and hepatic P-glycoprotein (P-gp) [14]. Rifampin is recommended in regulatory guidelines by the US Food and Drug Administration (FDA) as a prototype inducer to use in human drug–drug interaction (DDI) studies for drugs that are substantially metabolised by CYP2C9/3A4 enzymes [15]. Rifampin is readily absorbed from the gastrointestinal tract. Peak serum concentrations in healthy adults show a wide inter-individual variability. Absorption of rifampin is reduced by approximately 30% when the drug is ingested with food. Rifampin is eliminated via urine and faeces; with repeated administration, the mean $T_{1/2}$ of rifampin in healthy subjects is 2 to 3 h [15].

Based on the identified CYP enzymes involved in the metabolism of siponimod and in accordance with pertinent regulatory guidelines, the PK, safety, and tolerability of multiple doses of siponimod 2 mg once daily (qd) and its selected metabolites (M3 and M5) were investigated when administered with or without the potent CYP2C9/3A4 inducer rifampin in healthy subjects.

Methods

Subjects

Healthy men and women aged between 18 and 45 years, with a body mass index (BMI) between 18 and 30 kg/m², total body weight ≥ 50 kg, and a CYP2C9*1*1 (wild type) genotype, were enrolled into the study. Subjects were not eligible for inclusion in the study if they were using any other investigational drugs at the time of enrolment or had a history of hypersensitivity to the study drugs or any contraindication to rifampin. Subjects using any prescription drugs (including vaccines) or herbal supplements within 2 weeks before initial dosing (day 1), and/or over-the-counter medication or dietary

supplements (including vitamins) within 1 week before initial dosing, and/or potent inducers of CYP2C9/3A4 within 4 weeks or five half-lives whichever is greater, before initial dosing, and/or potent inhibitors of CYP3A4/2C9 within 5 half-lives before initial dosing, were not enrolled in the study. In addition, subjects with a clinically significant disease in any major system organ class and women of childbearing potential were excluded from the study. Additional subjects were to be enrolled to replace subjects who discontinued the study for reasons other than safety. Except for medication which may be required to treat adverse events, no medication other than study drug was allowed from the first dosing until all of the study completion evaluations were conducted.

Study design

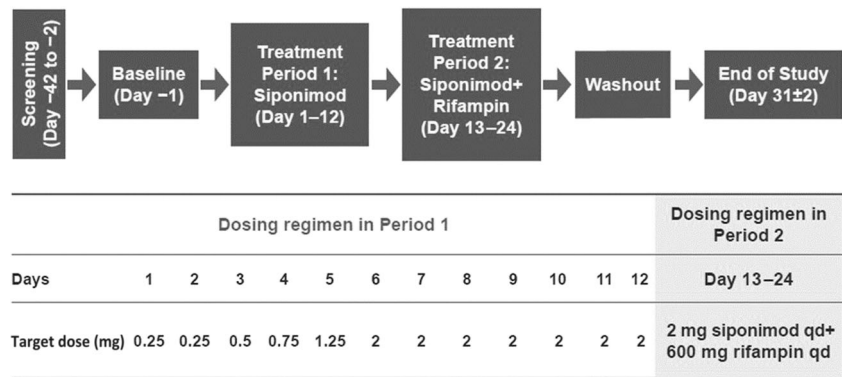
This was a confirmatory, open-label, multiple-dose, two-period, single-sequence study, consisting of a screening epoch (days –42 to –2), a baseline period (day –1), and two treatment epochs (Fig. 1): treatment period 1, where siponimod treatment alone was given on days 1 through 12 (including up-titration) and treatment period 2, where siponimod 2 mg qd was co-administered with rifampin 600 mg qd on days 13 through 24.

Siponimod was initiated using the established dose titration regimen. During period 1, subjects received 0.25 mg on the first 2 days, followed by 0.5 mg, 0.75 mg, and 1.25 mg on days 3, 4, and 5, respectively, and a stable dose of 2 mg from day 6 to day 12. Subjects remained at the clinic for the first 6 days for drug administration according to the titration scheme and PK sampling. Subsequently, they continued to visit the clinic for drug administration and any scheduled assessment. During period 2, subjects received concomitantly rifampin 600 mg qd and siponimod 2 mg qd in a fasted state (1 h prior to meals) from days 13 through 24. A study completion evaluation was conducted approximately 7 days (\pm 2 days) after the last dose of siponimod.

Screening assessments included physical examination, evaluation of medical history, and vital signs, single 12-lead electrocardiogram (ECG), 24-h Holter ECG, and laboratory assessments. Eligible subjects were domiciled at the study site for baseline evaluations approximately 24 h before dosing. After a final assessment of safety and eligibility per protocol, subjects received the study drugs in a fixed sequence of periods 1 and 2.

All study subjects provided written informed consent before any study-specific procedures were performed. The study protocol and subject consent forms were reviewed by an institutional review board. The study was conducted in accordance with the International Council for Harmonisation-Good Clinical Practice Guidelines E6 (R1), with applicable local regulations (including European Directive 2001/20/EC, United States Title 21 Code of Federal Regulations, and

Fig. 1 Study design. qd, once daily



Japanese Ministry of Health, Labour, and Welfare) and the ethical principles outlined in the Declaration of Helsinki [16].

Pharmacokinetic assessments

Primary assessments included multiple-dose PK of siponimod alone or in combination with rifampin: maximum steady-state plasma concentration ($C_{\max,ss}$), area under the curve at steady state ($AUC_{\tau,ss}$), and median (range) time to achieve $C_{\max,ss}$ ($T_{\max,ss}$). Secondary assessments included multiple-dose PK of the metabolites, M3 and M5, when siponimod was administered alone or in combination with rifampin: $C_{\max,ss}$ and $AUC_{\tau,ss}$. All PK parameters were calculated using noncompartmental methods with Phoenix® WinNonlin® version 6.4 (Certara USA, Inc., Princeton, NJ, USA).

Safety assessments

Safety assessments included the recording of all adverse events (AEs) and serious AEs (SAEs) with their severity and relationship to the study drug. In addition, safety monitoring also included physical examination, vital signs, clinical laboratory examinations, 12-lead ECG, and 24-h Holter ECG recording (at screening only; no on-treatment Holter ECG recording was performed). Haematology, clinical chemistry, and urinalysis assessments were performed at predefined time points. Absolute lymphocyte count (ALC) levels were assessed separately or as part of the haematology investigations.

Pharmacokinetic blood sampling schedule and bioanalysis

Blood samples were collected by direct venepuncture or an indwelling cannula inserted in a forearm vein. The PK of siponimod and its metabolites M3 and M5 were studied over 24 h on day 12 after multiple administration of siponimod alone (period 1) and on day 24 after co-administration of siponimod and rifampin (period 2). The PK sampling and bioanalysis were conducted per dose on days 1, 2, 3, 4, 5, 6,

8, 10, 12, 13, 15, 17, 19, 21, 22, 23, 24, and 25 (a 24-h post dose sample after the last dose on day 24). Plasma concentrations of siponimod and its metabolites (M3 and M5) were determined by validated liquid chromatography with tandem mass spectrometry methods with a lower limit of quantification of 0.05 ng/mL for siponimod and 0.01 ng/mL for metabolites M3 and M5, respectively. The details of the analytical methods have been described previously [17].

Statistical methods

A total of 16 subjects were recruited to obtain 12 completers in this study. Using the SimCYP physiologically based pharmacokinetics (PBPK) model as described previously [11], siponimod C_{\max} and AUC were predicted to decrease by 60% and 73%, respectively, when co-administered with rifampin. In order to control the width of confidence intervals (CI) for the geometric mean ratio for siponimod AUC and C_{\max} in the presence or absence of rifampin, a target was established that the relative half-width was at most 1.25 (0.223 in log scale, 20% from the reference treatment). With the chosen sample size of 12 subjects and an assumed intra-subject coefficient of variation (CV) for AUC of 20% based on previous siponimod studies [18], the relative half-width of 12 subjects is 1.14 (0.131 in log scale, 12% from the reference treatment). The 90% CIs at an observed geometric mean ratio can be evaluated as $\text{ratio} \cdot \exp(-0.161)$, $\text{ratio} \cdot \exp(+0.161)$. The screening for the CYP2C9 genotype and exclusion of subjects that were not homozygous for the CYP2C9*1*1 allele (wild type) helped to reduce the inter-subject variability in PK parameters and thereby reduced the minimum sample size required.

The safety analysis set included subjects who received any study drug, whereas the PK analysis set included those subjects who received any study drug with at least one available valid PK concentration measurement, and with no protocol deviations that impacted the PK.

The effect of rifampin on the PK of siponimod and its metabolites in healthy subjects was analysed by using a mixed-effects model. Log-transformed PK parameters

$C_{\max,ss}$ and $AUC_{\tau,ss}$ were analysed using a mixed-effects model with treatment as a fixed effect and subject as a random effect. The results were back-transformed to the original scale to obtain adjusted geometric mean ratios and the corresponding 90% CIs for treatment comparison (siponimod + rifampin vs. siponimod alone).

All plasma concentrations that were below the limit of quantification were set to zero for the calculation of the PK parameters. Missing values for any PK parameters were not imputed and were handled as missing values. Descriptive summary statistics were provided for all PK parameters including mean (arithmetic and geometric), standard deviation (SD), CV (arithmetic and geometric), median, minimum, and maximum. Summary tables of treatment-emergent AEs (TEAEs), their severity, and relationship to the study drug, were also provided.

Results

Subject disposition and demographics

A total of 16 subjects were enrolled, and 15 completed the study. One subject was discontinued from the study because of a viral infection during period 1. All 16 subjects (100%) were included in the safety and PK analysis sets. The mean (SD) age of the study population was 31.1 (8.3) years, mean (SD) weight was 79.2 (9.4) kg, and mean (SD) BMI was 25.8 (3.0) kg/m². The majority of the subjects enrolled were men ($n = 15$, 94%) with African-American ($n = 8$, 50%) and Caucasian ($n = 6$, 37.5%) origin.

Pharmacokinetics of siponimod

Pharmacokinetic parameters of siponimod during both treatment periods have been summarised in Table 1. Following daily oral doses of siponimod, mean trough concentrations reached steady state by day 10. Mean trough concentrations decreased over period 2 and appeared to reach a new steady-state level on day 24 (Fig. 2a).

After multiple doses of siponimod alone, the geometric mean $C_{\max,ss}$ was 28.6 ng/mL and the $AUC_{\tau,ss}$ was 546 h × ng/mL. The median (range) $T_{\max,ss}$ was 4 (1.5–8) hours. In the presence of rifampin, the geometric mean $C_{\max,ss}$ reduced by approximately 45% (15.7 h × ng/mL) while the $AUC_{\tau,ss}$ of siponimod decreased by 57% (235 h × ng/mL). The reduced concentrations in period 2 were associated with a higher fluctuation (defined as the degree of fluctuation of the observed minimum concentration to the observed maximum concentration over a dosing interval; 114%) compared with period 1 (56.9%). The median T_{\max} (4 h) of siponimod was unaltered during both treatment periods.

Overall, the variability (CV%) of PK parameters was comparable between the two periods with the exception of the minimum steady-state plasma concentration ($C_{\min,ss}$; period 1 CV%: 20.6%; period 2 CV%: 32.9%).

The geometric mean ratios (90% CIs) for siponimod PK parameters indicated that rifampin significantly decreased the exposure parameters of siponimod (Fig. 3a). The ratio of geometric means for $C_{\max,ss}$ and $AUC_{\tau,ss}$ of rifampin co-administered with siponimod versus siponimod alone were 0.55 (0.52; 0.58) and 0.43 (0.41; 0.45), respectively.

Pharmacokinetics of M3 and M5

The plasma PK parameters of both M3 and M5 are presented in Table 1. Following daily oral doses of siponimod, the trough concentrations of both M3 and M5 reached steady state by day 10 and decreased over treatment period 2 (Fig. 2b, c).

M3 In the presence of rifampin, the M3 $C_{\max,ss}$ significantly increased by 53%, with a geometric mean ratio (90% CI) of 1.53 (1.30; 1.81). The $AUC_{\tau,ss}$ of M3 decreased by 10%, corresponding to a geometric mean ratio (90% CI) of 0.90 (0.78; 1.03), indicating that the total exposure of M3 remained stable in the absence or presence of rifampin (Fig. 3b). Overall, the variability (CV%) of PK parameters was higher in period 2, but less noticeable for $C_{\min,ss}$ (period 1 CV%: 37.8%; period 2 CV%: 38.1%). The molecular weight-adjusted M3/parent ratios (MR) for both $C_{\max,ss}$ and $AUC_{\tau,ss}$ increased from day 12 (geometric mean [CV%] 0.422 [51.4%] and 0.388 [47.2%]), respectively) to day 24 (1.05 [50.9%] and 0.808 [51.0%]), respectively).

M5 The geometric mean ratio of 0.96 (0.86; 1.07) for M5 $C_{\max,ss}$ was within the default no-effect limits of 0.80 to 1.25, suggesting that the M5 $C_{\max,ss}$ was stable in the presence of rifampin (Fig. 3c). Rifampin significantly decreased the $AUC_{\tau,ss}$ of M5 by 37% with a geometric mean ratio (90% CI) of 0.63 (0.58; 0.68). Overall, the variability (CV%) of PK parameters ($AUC_{\tau,ss}$, $C_{\max,ss}$, $C_{\min,ss}$, and the average plasma concentration during a dosing interval at steady state $C_{av,ss}$) was slightly greater in period 2 than in period 1 (Table 1). The molecular weight-adjusted M5/parent ratios for both $C_{\max,ss}$ and $AUC_{\tau,ss}$ were comparable between day 12 (geometric mean [CV%] 0.0225 [19.5%] and 0.0231 [22.2%]), respectively) and day 24 (0.0329 [28.4%] and 0.0392 [22.6%]), respectively).

Safety

Overall, 15 of the 16 subjects enrolled (93.8%) experienced at least one TEAE during the study (Table 2). All reported TEAEs, except for one moderate TEAE, were mild in severity and all TEAEs resolved by the end of the study. No serious AEs or deaths were reported during the study. Overall, the most

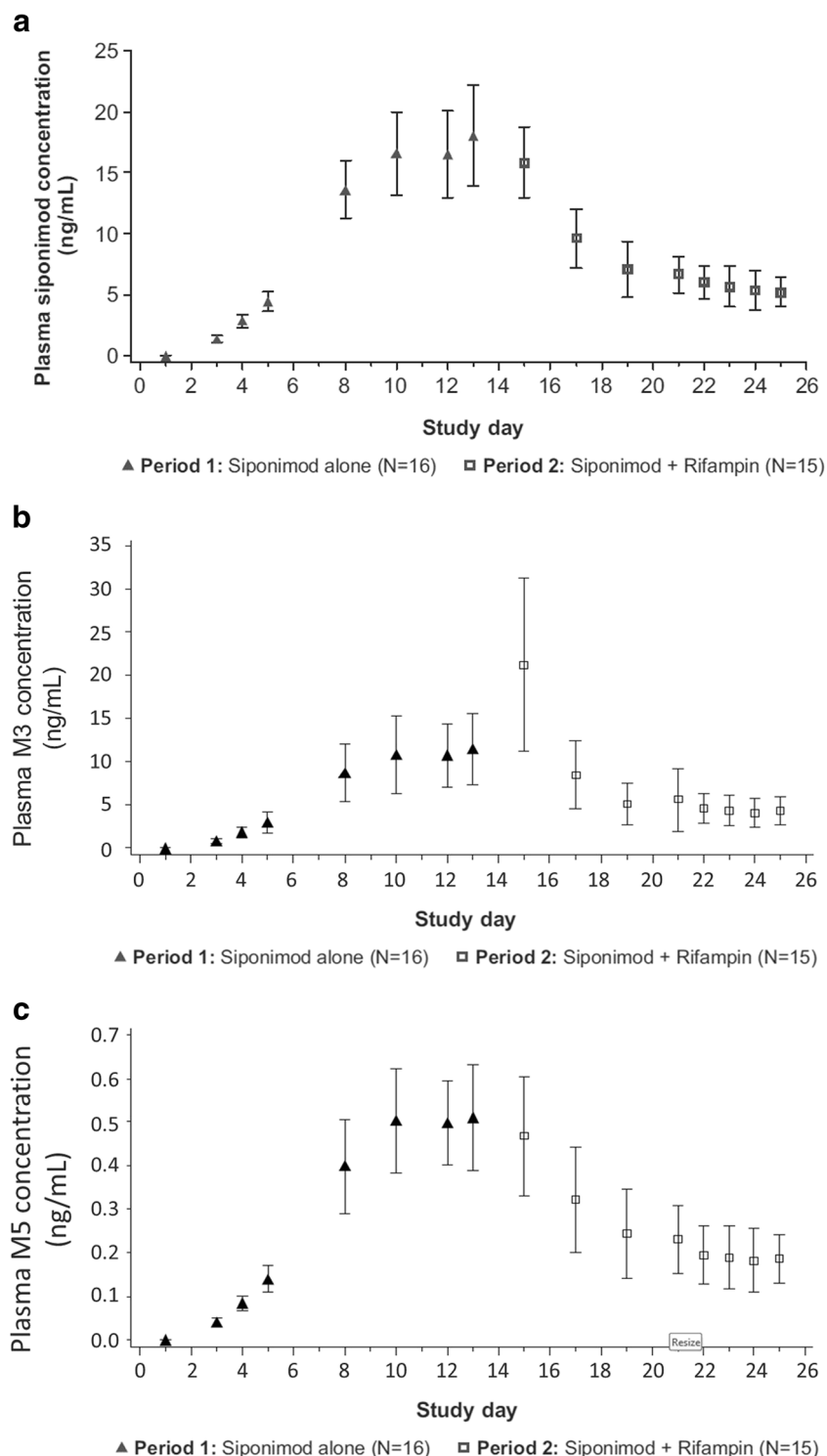
Table 1 Plasma pharmacokinetic parameters for siponimod (PK analysis set)

PK parameters	Siponimod		M3		M5	
	Siponimod alone: period 1 (day 12) N = 16	Siponimod + rifampin: period 2 (day 24) N = 15	Siponimod alone: period 1 (day 12) N = 16	Siponimod + rifampin: period 2 (day 24) N = 15	Siponimod alone: period 1 (day 12) N = 16	Siponimod + rifampin: period 2 (day 24) N = 15
AUC _{0-∞,ss} (h × ng/mL)	546 (17.6)	235 (16.9)	290 (33.4)	261 (45.0)	13.0 (18.7)	8.21 (24.1)
C _{max,ss} (ng/mL)	28.6 (17.1)	15.7 (17.9)	14.7 (30.3)	22.5 (46.2)	0.661 (19.1)	0.634 (29.1)
C _{min,ss} (ng/mL)	15.6 (20.6)	4.32 (58.6)	9.00 (40.5)	3.20 (67.2)	0.423 (20.2)	0.146 (56.0)
C _{av,ss} (ng/mL)	22.8 (17.6)	9.80 (16.9)	12.1 (33.4)	10.9 (45.0)	0.545 (18.7)	0.342 (24.1)
T _{max,ss} (h)	4.00 (1.50–8.00)	4.00 (1.50–4.05)	6.00 (1.50–12.0)	6.00 (4.00–8.00)	6.00 (1.50–12.0)	3.00 (2.00–6.00)
MR AUC _{last,ss}	–	–	0.388 (47.2)	0.808 (51.0)	0.0231 (22.2)	0.0338 (22.4)
MR C _{max,ss}	–	–	0.375 (43.3)	1.05 (50.9)	0.0225 (19.5)	0.0392 (22.6)

Data are presented as geometric mean (CV%), with the exception of T_{max} which is presented as median (range). N = number of subjects that received treatment in the study period

AUC_{0-∞,ss} area under the curve at steady state, C_{av} average plasma concentration, C_{max,ss} maximum steady-state plasma concentration, C_{min,ss} minimum steady-state plasma concentration, CV coefficient of variation, h hours, MR molecular weight-adjusted metabolic ratios, PK pharmacokinetic, T_{max} time to reach maximum concentration

Fig. 2 Arithmetic mean (SD) trough plasma concentration-time profiles of siponimod (**a**), metabolite M3 (**b**), and metabolite M5 (**c**) (PK analysis set). ▲ Period 1: siponimod alone ($N=16$). □ Period 2: siponimod + rifampin ($N=15$). Period 1: siponimod up-titrated from 0.25 to 2 mg qd (days 1 through 12) (reference). Period 2: siponimod 2 mg qd + rifampin 600 mg qd (days 13 through 24) (test). Values below the limit of quantification (<0.05 ng/mL) have been set to zero. PK pharmacokinetic, qd once daily, SD standard deviation



commonly reported TEAEs during the study were chromaturia ($n=8$, 50%), dizziness, headache, presyncope, and nausea (reported by three subjects each [18.8%]). The incidence of AEs reported during Period 2 was greater than in Period 1 (eight subjects [50%] vs. 13 subjects [86.7%]). The most common AE reported in Period 1 (three subjects: 18.8% vs. 0% in period 2) was presyncope (two mild and one moderate in severity),

mostly following a scheduled blood draw, and none were suspected to be related to study drug. The most common AE in period 2 was chromaturia (eight subjects, 53.3% vs. 0% in period 1), a known side effect of rifampin. One subject was discontinued from the treatment after the drug administration on day 9 (2 mg siponimod) due to a mild viral infection which was suspected to be related to siponimod administration.

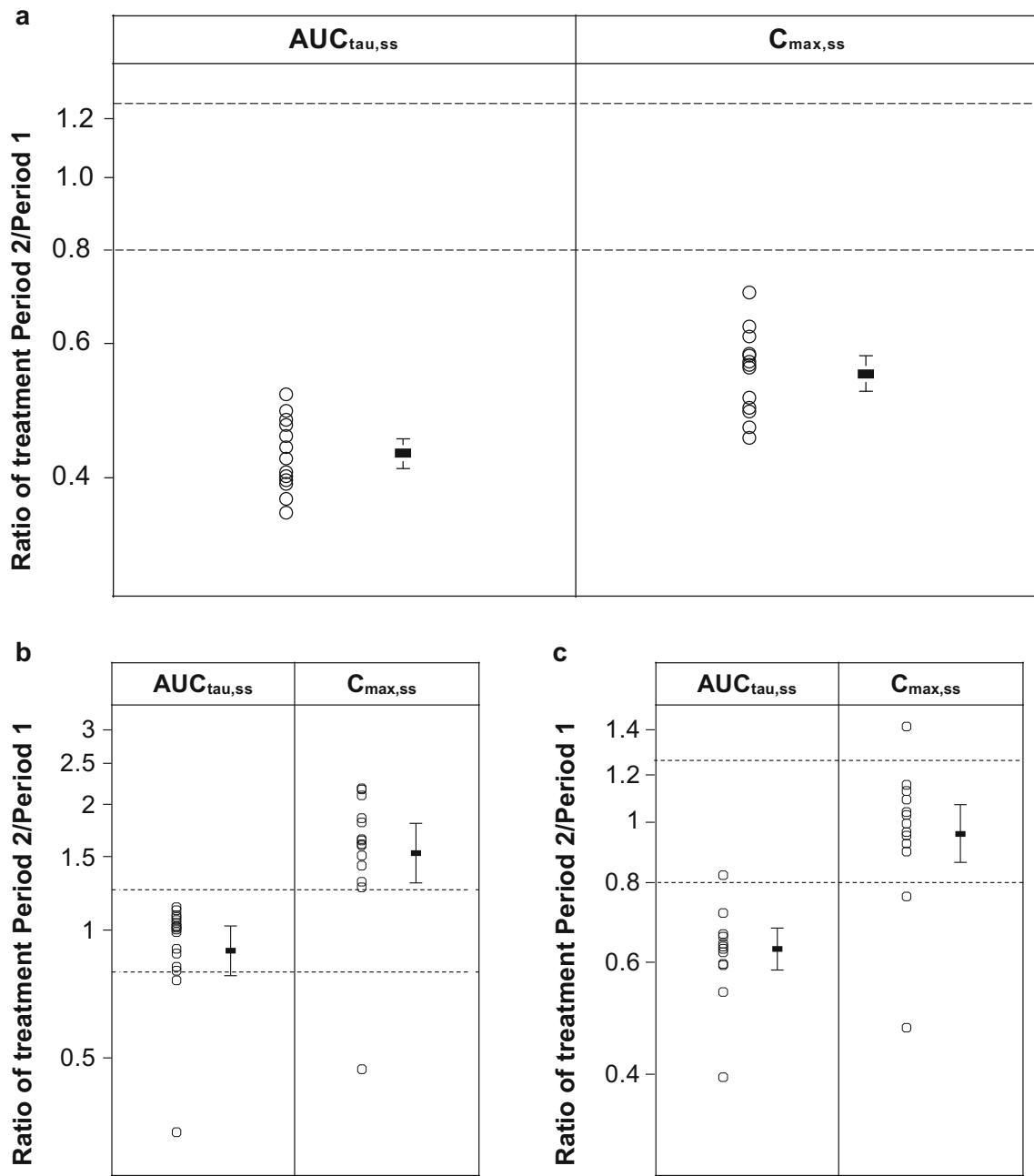


Fig. 3 Geometric mean ratios (90% CI) for plasma siponimod (a) and its metabolites M3 (b) and M5 (c). Period 1: siponimod alone (reference, day 12); Period 2: siponimod + rifampin (test, day 24). Closed squares and lines represent the geometric mean ratio and 90% CI; open circles

represent the individual treatment ratios. $AUC_{\tau,ss}$ area under the curve at steady state, CI confidence interval, $C_{max,ss}$ maximum steady-state plasma concentration

Overall, mean values for clinical chemistry parameters and vital signs remained within the normal ranges at all of the assessed time points. There was a mean increase in heart rate (HR) noted after co-administration of siponimod with rifampin, with a maximum value of 73.6 beats per minute (bpm) in day 25 of period 2 (mean change from baseline 10.2 bpm). No clinically significant abnormalities in 12-lead ECG results were observed in the subjects.

Absolute lymphocyte count

A treatment-emergent decrease in ALC was observed in all subjects, representing the desired primary pharmacodynamic effect of siponimod. The magnitude and time course of the mean ALC reduction in period 1 (siponimod alone) was similar to that reported previously during dose titration and at the 2 mg dose level on day 8 [19]. The

Table 2 Incidence of AEs by preferred term and treatment – n (%) of subjects (safety analysis set)

Preferred term	Siponimod alone: Period 1 N= 16, n (%)	Siponimod + rifampin: Period 2 N= 15, n (%)	Total N= 16, n (%)
Subjects with at least one AE	8 (50)	13 (86.7)	15 (93.8)
Anxiety	0	1 (6.7)	1 (6.3)
Back pain	1 (6.3)	0	1 (6.3)
Chromaturia	0	8 (53.3)	8 (50.0)
Diarrhoea	0	2 (13.3)	2 (12.5)
Dizziness	2 (12.5)	1 (6.7)	3 (18.8)
Excoriation	1 (6.3)	0	1 (6.3)
Fatigue	1 (6.3)	0	1 (6.3)
Headache	1 (6.3)	3 (20.0)	3 (18.8)
Medical device site reaction	0	1 (6.7)	1 (6.3)
Muscle spasms	1 (6.3)	0	1 (6.3)
Muscle tightness	1 (6.3)	0	1 (6.3)
Musculoskeletal pain	1 (6.3)	0	1 (6.3)
Myalgia	0	2 (13.3)	2 (12.5)
Nasal congestion	1 (6.3)	0	1 (6.3)
Nausea	1 (6.3)	2 (13.3)	3 (18.8)
Oral herpes	1 (6.3)	1 (6.7)	2 (12.5)
Oropharyngeal pain	0	1 (6.7)	1 (6.3)
Presyncope	3 (18.8)	0	3 (18.8)
Tooth fracture	0	1 (6.7)	1 (6.3)
Toxicity to various agents	0	1 (6.7)	1 (6.3)
Urine odour abnormal	0	1 (6.7)	1 (6.3)
Viral infection	1 (6.3)	0	1 (6.3)
Vision blurred	0	1 (6.7)	1 (6.3)

n = number of subjects with at least one AE in the category; N = number of subjects in the safety analysis set who took treatment for the respective period. The Medical Dictionary for Regulatory Activities Version 19.0 has been used for the reporting of AEs. Only TEAEs are included. Under one treatment, a subject with multiple occurrences of an AE is counted only once in the AE category

AE adverse event, TEAE treatment-emergent adverse event

mean ALC decreased from a baseline of $\sim 1.8 \times 10^9/L$ to $\sim 0.8 \times 10^9/L$ on day 8 (Fig. 4). The mean ALC increased slightly during combination treatment with rifampin in period 2 but remained at levels below $1.0 \times 10^9/L$ until the completion of period 2 or discontinuation of the study drug; these findings are consistent with the magnitude of steady-state ALC reduction in previous studies at the 1 mg dose level. The mean ALC recovered to near baseline levels ($1.73 \times 10^9/L$) by the end of the study (approximately 7 days after the last dose).

Discussion

The present DDI study assessed the effects of the strong CYP3A4/moderate CYP2C9 inducer rifampin on the PK of siponimod and its metabolites in healthy subjects. The simultaneous induction of two siponimod main metabolic

pathways, CYP2C9 and CYP3A4, significantly reduced the exposure of siponimod in the presence of rifampin.

An open-label study design was chosen in accordance with the recommendations in the relevant FDA guidelines [15] as it was unlikely that the primary PK endpoint would be influenced by subject or investigator knowledge of the treatment. Healthy subjects were included in the study as no relevant differences are expected in the PK interaction between healthy subjects and MS patients. In order to ensure an accurate quantification of the siponimod (and metabolite) plasma concentration profile over time in case of a potent induction by rifampin, the therapeutic dose of 2 mg of siponimod was used in the study. However, in order to avoid the bradyarrhythmic effect of siponimod at treatment onset, a dose titration over 5 days has been used similar to other siponimod studies [20]. Consequently, this study is an artefactual multiple dose study which is not typically the case with PK-DDI studies with CYP inhibitors or inducers.

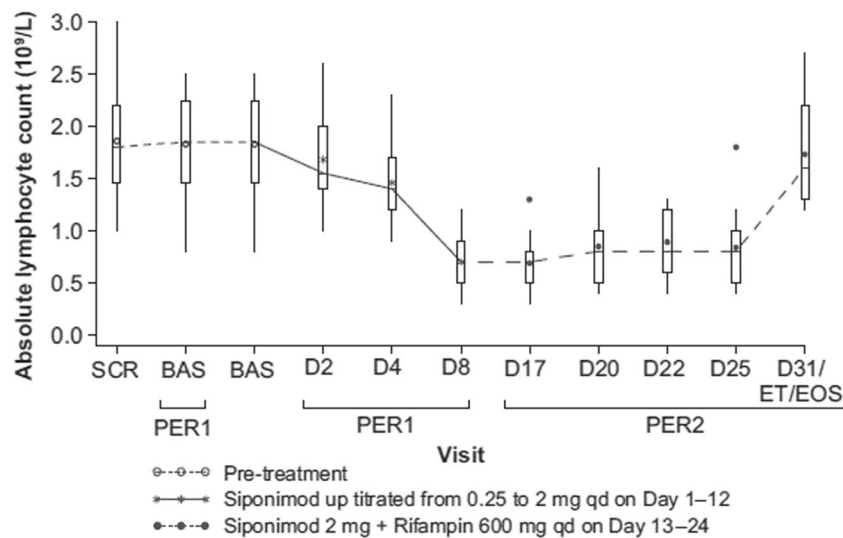


Fig. 4 Absolute lymphocyte count by visit (safety analysis set). The horizontal line in the box interior represents the median. The symbol in the box interior represents the mean. Values outside the whiskers are identified with symbols. The upper (lower) edge of the box represents the 75th (25th) percentile. A whisker is drawn from the upper (lower) edge of the box to the largest (smallest) value within $1.5 \times$ interquartile

range above (below) the edge of the box. BAS is the last non missing observation before the first study drug administration (including repeated measurements); PER1BAS is the scheduled baseline visit (Study day -1). BAS baseline, D day, EOS end of study, PER period, qd once daily, SCR screening

Rifampin, as one of the most potent inducers of CYP2C9/3A4, was predicted to significantly decrease the steady-state exposure of siponimod (60% decrease in C_{max} and 73% decrease in AUC). Our results showed that in the presence of rifampin, the $C_{max,ss}$ and $AUC_{tau,ss}$ of siponimod decreased by 45 and 57%, respectively, which corresponds to the exposure at 1 mg qd steady state. Siponimod trough concentrations decreased over period 2 when rifampin was co-administered and appeared to reach a new steady-state level between day 22 and day 24 in most subjects. This observation confirms that the maximum enzyme induction by rifampin was achieved on day 24 when PK assessments were performed.

The study also measured the PK of two siponimod metabolites, M3 and M5. Metabolite M3 is one of the main circulating metabolites of siponimod in humans [12]; it is formed by glucuronidation of the hydroxylated M5. Metabolite M5 results from metabolism via CYP2C9 mainly, with some contribution from CYP3A4 [17, 21].

They have been toxicologically and pharmacologically profiled in separate studies [12]. M3 and M5 have weak pharmacological activity on S1P₁R compared with the parent compound and did not show any signal for toxicity. Metabolite M3 accounts for about 28–39% of the plasma exposure to siponimod, while M5 represents only about 2% of the parent plasma exposure [12].

An increase in M3/M5 exposure is therefore expected when CYP2C9 and CYP3A4 enzymes are induced. Our results indicated that while the $C_{max,ss}$ of M3 increased significantly in the presence of rifampin, the $AUC_{tau,ss}$ of M3 displayed only a small change. The M5 $C_{max,ss}$ did not change

under co-administration of rifampin, but the $AUC_{tau,ss}$ of M5 decreased significantly. Therefore, while induction of CYP2C9 and CYP3A4 significantly increased the clearance of siponimod and increased the peak exposure of M3, rifampin did not increase the total exposure of the M3 and M5 metabolites of siponimod. It has been stated that rifampin also induces the glucuronidation pathway [22]. Since a decrease in M5 exposure was observed, the induced formation of M5 due to the enhanced hydroxylation of siponimod in the presence of rifampin may be counterbalanced by the induced glucuronidation of M5 by rifampin. While the induced glucuronidation is expected to lead to higher systemic levels of the glucuronidated metabolite M3, it was observed that M3 exposure was only marginally modified in the presence of rifampin. Its exposure is likely also influenced by the induction of transporter MRP2 (ABCC2) by rifampin [23], leading to an increase of its biliary excretion and renal elimination. The half-maximal effective concentration (EC_{50}) value of metabolites M5 and M3 tested on human S1P₁ receptors were 470 ± 71 nmol/L and $>10,000$ nmol/L, respectively. M3 has shown very weak activity on S1P₁ ($EC_{50} > 10,000$ nmol/L) compared with the parent compound, siponimod, which had shown an EC_{50} of 1.1 ± 0.41 nmol/L in the same assay [Novartis data on file]. Hence, the observed systemic exposure changes for both M3 and M5 in the presence of rifampin are unlikely to translate into significantly different pharmacological activity on S1P₁ receptors.

A recently published study reported that the co-administration of siponimod with fluconazole, a CYP2C9/CYP3A4 inhibitor, resulted in a ~ 2 -fold increase in mean

AUC versus siponimod alone [24]. In addition, the CYP2C9 genotype has been shown to have a significant impact on siponimod metabolism [11]. The AUCs of siponimod were ~2- to-4-fold greater in healthy subjects with the CYP2C9*2/*3 and CYP2C9*3/*3 genotypes, with a minor increase in C_{max} versus the CYP2C9*1/*1 genotype. The mean $T_{1/2}$ was prolonged in CYP2C9*2/*3 (51 h) and CYP2C9*3/*3 (126 h) versus CYP2C9*1/*1 (28 h) genotypes [24]. These results further highlighted the relevance of the CYP2C9 pathway on siponimod metabolism.

Beside the induction of a number of drug-metabolising enzymes, rifampin additionally induces some drug transporter proteins, such as P-gp, MRP2, or OATP [14, 25]. In vitro investigations did not identify siponimod as a substrate of efflux transporters (P-gp, BCRP, or MRP2) or of transporters involved in sinusoidal uptake in the liver (OATPs, OCTs, and OATs) (data on file). The regulation of these transporter expression by rifampin is therefore not expected to translate into a clinically relevant changes in siponimod PK.

The CYP2C9 genotype influences the fractional contributions of the two oxidative metabolism pathways (CYP2C9 and CYP3A4) for overall elimination of siponimod: the hepatic CYP2C9 contribution to metabolism is anticipated to be 80.84 and 11.31% in the CYP2C9*1/*1 and *3*3 genotypes, respectively, according to physiological-based PK (PBPK) modelling [11]. CYP3A4 plays a minor role in siponimod metabolism for the extensive metaboliser (CYP2C9*1/*1 genotype), with a contribution of 17.23% to the overall elimination of siponimod; its contribution to the overall systemic clearance is expected to be greater in subjects with lower CYP2C9 activity associated with a reduced total clearance (78.91% contribution in the *3*3 genotype) [11]. Considering the variable CYP3A4 and CYP2C9 contributions to the overall clearance of siponimod in the different CYP2C9 genotypes, it is expected that the CYP2C9 genotype influences the effects of CYP3A4 and CYP2C9 inhibitors and inducers.

The frequency of the CYP2C9*1/*1 genotype is approximately 62 to 65% in Caucasians [26, 27]. The results of the current study therefore describe the effect of CYP2C9/CYP3A4 induction on siponimod PK in the largest portion of the Caucasian population. As rifampin is a dual (CYP3A4 and CYP2C9) inducer, impacting both siponimod main metabolic pathways, it is expected that the extent of rifampin induction observed in CYP2C9*1/*1 subjects in the current study can be extrapolated to all other CYP2C9 genotypes.

A SimCYP PBPK model has been established using a mixed approach combining in vitro data and PK parameters derived from clinical studies. This model was previously used to accurately predict the effect of fluconazole (a moderate CYP2C9/CYP3A4 inhibitor) co-administration and CYP2C9 genetic polymorphism on siponimod pharmacokinetics in healthy subjects [11, 24]. It is intended to perform complementary PBPK modelling to predict the DDI potential

of siponimod as substrate in the presence of typical CYP2C9/3A inducers for the 6 different CYP2C9 genotypes. Overall, multiple oral doses of siponimod alone and in combination with multiple doses of rifampin did not increase the risk of TEAEs in the study. A treatment-emergent decrease in ALC was observed in all subjects, representing the desired primary pharmacodynamic effect of siponimod. The ALC during combination treatment with rifampin was consistent with the magnitude of steady-state ALC reduction in previous studies at the 1-mg dose level [3]. There were no clinically relevant changes in laboratory results, vital signs, and ECG parameters. Minor increases in mean HR were not considered to be related to any study drug in this open-label study.

Conclusion

A clinically relevant decrease in siponimod PK exposure ($C_{max,ss}$ and $AUC_{tau,ss}$) was observed in the presence of rifampin. Multiple oral doses of siponimod alone and in combination with multiple oral doses of rifampin demonstrated a favourable safety profile and were well tolerated by healthy subjects in this study. These results highlight the relevance of the two main metabolic enzymes of siponimod, CYP2C9 and CYP3A4. Considering the expected influence of CYP2C9 genotype on the effects of CYP3A4 and CYP2C9 perpetrator drugs, further evaluations will be required to fully describe the DDI potential of siponimod as substrate in the presence of typical CYP2C9/3A inducers.

Final recommendations on the concomitant use of siponimod and CYP3A4/moderate CYP2C9 inducers including the effect in different genotypes will be made considering all pertinent preclinical, in silico predictions and clinical data.

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Author contributions Anne Gardin and Kasra Shakeri-Nejad conceptualised and designed the studies, analysed and interpreted the study data. Cathy Gray was involved in study design and study execution. Srikanth Neelakantham conceptualised and designed the studies, analysed and interpreted the study data, and conducted statistical analysis of the data. Felix Huth, Antonia M. Davidson, and Eric Legangneux were involved in conceptualising and designing of studies, and acquisition and analysis of data. All authors made significant contribution to data interpretation and review of the manuscript.

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Compliance with ethical standards

Conflicts of interest Antonia M. Davidson is a principal investigator at the PPD Phase 1 Clinic, Austin, TX, USA.

Anne Gardin, Cathy Gray, Srikanth Neelakantham, Felix Huth, Swati Dumitras, Eric Legangneux, and Kasra Shakeri-Nejad are employees of Novartis.

Research involving human participants All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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