Prediction of the Impact of Cytochrome P450 2C9 Genotypes on the Drug–Drug Interaction Potential of Siponimod With Physiologically-Based Pharmacokinetic Modeling: A Comprehensive Approach for Drug Label Recommendations

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We predicted the drug–drug interaction (DDI) potential of siponimod in presence of cytochrome P450 (CYP)2C9/CYP3A4 inhibitors/inducers in subjects with different CYP2C9 genotypes by physiologically-based pharmacokinetic (PK) modeling. The model was established using *in vitro* and clinical PK data and verified by adequately predicting siponimod PK when coadministered with rifampin. With strong and moderate CYP3A4 inhibitors, an increased DDI risk for siponimod was predicted for *CYP2C9*3/*3* genotype vs. other genotypes area under the curve ratio (AUCR): 3.03–4.20 vs. ≤ 1.49 for strong; 2.42 vs. 1.14–1.30 for moderate. AUCRs increased with moderate (2.13–2.49) and weak (1.12–1.42) CYP3A4/CYP2C9 inhibitors to the same extent for all genotypes. With strong CYP3A4/moderate CYP2C9 inducers and moderate CYP3A4 inducers, predicted AUCRs were 0.21–0.32 and 0.35–0.71, respectively. This complementary analysis to the clinical PK-DDI studies confirmed the relevant influence of CYP2C9 polymorphism on the DDI behavior of siponimod and represented the basis for the DDI labeling recommendations.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The clinical relevance of the different cytochrome P450 (CYP)2C9 genotypes is well described. However, there are few examples of dedicated drug–drug interaction (DDI)–physiologically-based pharmacokinetic (PBPK) simulations published that support the DDI labeling recommendations for the different genotypes.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Do CYP3A4/CYP2C9 perpetrators impact the exposure of siponimod in the various CYP2C9 genotypes, and to what extent?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ PBPK simulations are valuable tools to translate clinical observations to untested perpetrators and/or subpopulations with different genotypes.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ For drugs with polymorphic enzymes as major clearance factors, PBPK simulations can be utilized to give a genotype-specific dose and dose adjustment recommendation, which is a step in personalized medicine without performing DDI studies for all relevant genotypes.

Siponimod is a potent, oral, selective sphingosine 1-phosphate receptor subtypes 1 and 5 modulator.[1] In a recently published phase III study, siponimod 2 mg (s.d.) reduced the risk of disability progression in patients with secondary progressive multiple sclerosis.[2] Siponimod has recently been approved by the US Food and Drug Administration (FDA) for the treatment of relapsing forms of multiple sclerosis, including clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease, in adults.[3]

Siponimod exhibits time-independent pharmacokinetics (PKs). The rate and extent of systemic exposure increase in a dose-proportional manner after single (0.1–75 mg; **Table 1**) and multiple (0.3–20 mg, **Table 1**) doses of siponimod.[1] The mean elimination half-life ($t_{1/2}$) of siponimod is ~ 30 hours (range:

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Table 1 Observed and simulated siponimod PK parameters

Dose (mg) and form	N		C_{max} (ng/mL)		AUC_{inf} (ng·hour/mL)		T_{max} (h)		$t_{1/2}$ (h)	
	SimCYP	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
Summary of siponimod PK after single intravenous and oral administration of 0.25 mg[a]										
i.v.	100	15	3.02 (13; 2.95–3.09)	3.22 (19; 1.88–3.81)	82.1 (37; 76.9–87.3)	80.1 (25.6; 47.7–125)	3.00 (2.95–3.00)	2.92 (2.92–2.92)	25.5 (36; 23.8–27.1)	27.4 (22.5; 20.1–47.7)
p.o.	100	15	1.88 (14; 1.83–1.92)	1.71 (24; 0.920–2.30)	69.7 (38; 65.1–74.3)	67.4 (26; 39.8–105)	3.25 (2.35–5.05)	8.00 (4.00–8.03)	25.5 (36; 23.8–27.1)	26.7 (22; 17.2–43.8)
Summary of observed and simulated siponimod PK after single oral administration[b]										
0.1 Solution	340		0.78 (20; 0.76–0.79)		29.1 (48; 27.7–30.5)	—	3.45 (2.31–5.60)	—	25.6 (49; 24.4–26.8)	—
0.3 Solution	340	8	2.33 (20; 2.29–2.37)	2.26 (6; 2.17–2.34)	87.2 (48; 83.1–91.4)	89.3 (20; 79.2–99.4)	3.45 (2.30–5.60)	5 (4.00–8.00)	25.6 (49; 24.4–26.8)	33.21 (28)
1 Solution	340	8	7.76 (20; 7.62–7.91)	7.43 (42; 5.37–9.50)	291 (48; 277–305)	349 (28; 292–406)	3.45 (2.30–5.60)	5 (4.00–15.7)	25.6 (49; 24.4–26.8)	45.7 (26)
2.5 Solution	340	6	19.4 (20; 19.1–19.8)	21.9 (35; 16.7–27.0)	727 (48; 693–761)	766 (36; 598–935)	3.45 (2.30–5.60)	4 (3.00–8.00)	25.6 (49; 24.4–26.8)	27.0 (22)
2.5 Capsule	340	7	19.4 (20; 19.1–19.8)	19.3 (19; 17.1–21.5)	727 (48; 693–761)	745 (25; 632–858)	3.45 (2.30–5.60)	6 (4.00–8.00)	25.6 (49; 24.4–26.8)	29.3 (17)
5 Capsule	340	8	38.8 (20; 38.1–39.5)	38.5 (21; 33.5–41.5)	1,454 (48; 1,385–1,523)	1,260 (20; 1,149–1,384)	3.45 (2.30–5.60)	3 (2.00–8.00)	25.6 (49; 24.4–26.8)	31.3 (19)
10 Capsule	340	8	77.7 (20; 76.2–79.1)	77.3 (25; 64.8–89.7)	2,908 (48; 2,770–3,045)	2,710 (14; 2,507–2,915)	3.45 (2.30–5.60)	5 (3.82–16.0)	25.6 (49; 24.4–26.8)	32.0 (17)
17.5 Capsule	340	8	136 (20; 133–138)	111 (32; 93.7–128.4)	5,088 (48; 4,847–5,329)	4,230 (25; 3,666–4,792)	3.45 (2.30–5.60)	4 (2.00–8.00)	25.6 (49; 24.4–26.8)	42.3 (38)
25 Capsule	340	8	194 (20; 191–198)	217 (29; 180–254)	7,269 (48; 6,925–7,613)	8,140 (24; 6,987–9,302)	3.45 (2.30–5.60)	3.50 (1.50–12.0)	25.6 (49; 24.4–26.8)	47.7 (20)
75 Capsule	340	8	582 (20; 572–593)	491 (51; 346–637)	21,807 (48; 20,774–22,840)	18,600 (51: 11,831–25,397)	3.45 (2.30–5.60)	6 (2.00–24.00)	25.6 (49; 24.4–26.8)	56.7 (12)

(Continues)

Table 1 (Continued)

Dose (mg) and form	N		C_{max} (ng/mL)		$AUC_{(0-24\ h)}$ (ng·hour/mL)		C_{min} (ng/mL)	
	SimCYP	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
Summary of observed and simulated siponimod PK after multiple oral administration at day 28[c]								
0.3 Solution	340	6	4.00 (38; 4.63–4.97)	5.13 (16; 4.76–5.86)	86.2 (48; 82.2–90.3)	97.9 (19)	2.62 (69; 2.46–2.78)	2.83 (27)
1 Solution	340	6	16.0 (38; 15.4–16.6)	14.9 (10; 14.0–15.9)	287 (48; 274–301)	282 (16)	8.74 (69; 8.20–9.27)	7.74 (20)
2.5 Capsule	340	7	40.0 (38; 38.6–41.5)	38.3 (37; 29.1–47.4)	719 (48; 685–752)	692 (45)	21.9 (69; 20.5–23.2)	15.2 (120)
10 Capsule	340	9	160 (38; 154–166)	147 (24; 126–168)	2,874 (48; 2,740–3,009)	2,580 (24)	87.4 (69; 82.0–92.7)	70.6 (29)
20 Capsule	340	9	320 (38; 309–332)	359 (17; 325–394)	5,749 (48; 5,480–6,017)	6,370 (23)	175 (69; 164–186)	166 (88)

Pharmacokinetic data are presented as geometric means with percentage of coefficient of variation geometric mean and/or 90% confidence interval range in parentheses except for T_{max} (median with range). $AUC_{0-24\ h}$, area under the curve from time 0–24 hours; AUC_{inf}, area under the curve from time zero to infinity; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration;h, hour;PK, pharmacokinetic; $t_{1/2}$, terminal elimination half-life; T_{max}, time to reach maximum systemic concentration.
[a]Observed values were taken from Novartis data on file. [b]Observed values were taken from single ascending dose study. [c]Observed values were taken from multiple ascending dose study.[1]

22–38 hours) with a washout period of 6 days.[1] Siponimod is eliminated from systemic circulation primarily through hepatic oxidative metabolism with subsequent fecal/biliary excretion (**Figure 1**).[4] Cytochrome P450 (CYP)2C9 is the major hepatic enzyme involved in the metabolism (79.3%) of siponimod, followed by CYP3A4 (18.5%).[4,5] Siponimod did not exhibit clinically relevant inhibition potential on CYP enzymes and transporters based on static-mechanistic model calculations,[6] and it was not identified as transporter substrate *in vitro* (**Table S1**).

CYP2C9 is a polymorphic enzyme with >50 single nucleotide polymorphisms (SNPs). Of these, the CYP2C9*2 and CYP2C9*3 SNPs are the primary causes of clinically relevant reductions in enzyme activity.[5] These SNPs lead to six most prominent CYP2C9 genotypes conferring to three functionally different phenotypes: extensive metabolizers, intermediate metabolizers, and poor metabolizers (PMs).[5,7–10] The prevalence of the six clinically relevant CYP2C9 genotypes in the white population ranges from 62–65% for *CYP2C9*1/*1*, 20–24% for *CYP2C9*1/*2*, 9–12% for *CYP2C9*1/*3*, 1–2% for *CYP2C9*2/*2*, 1.4–1.7% for *CYP2C9*2/*3*, and 0.3–0.4% for *CYP2C9*3/*3*.[5,9,11] CYP2C9 polymorphism has shown a significant impact on siponimod metabolism.[5] Regarding siponimod clearance, population PK analyses (PopPK; **Table S2**) and a CYP2C9 pharmacogenetic study[5] indicated that subjects with *CYP2C9*1/*1* and *CYP2C9*1/*2* genotypes behave as extensive metabolizers, *CYP2C9*2/*2* and *CYP2C9*1/*3* genotypes behave as intermediate metabolizers, and *CYP2C9*2/*3* and *CYP2C9*3/*3* genotypes as PMs. Compared with subjects with the *CYP2C9*1/*1* genotype, those with the *CYP2C9*2/*2*, **1/*3*, **2/*3*, and **3/*3* genotypes have 20%, 35–38%, 45–48%, and 74% lower total body clearance of the drug from plasma values, respectively (Novartis data on file, **Table S2**).[5] The recommended maintenance dose of siponimod as approved by the FDA is 2 mg taken orally once daily. In patients with a *CYP2C9*1/*3* or **2/*3* genotype, a lower maintenance dose of 1 mg taken orally once daily is recommended, whereas siponimod is contraindicated in patients homozygous for CYP2C9*3 (i.e., *CYP2C9*3/*3* genotype), because of the substantially elevated siponimod plasma levels.[3]

In subjects with the *CYP2C9*1/*1* genotype, upon coadministration with fluconazole (a moderate CYP3A4/CYP2C9 inhibitor), siponimod area under the curve (AUC) increased approximately two fold and the maximum plasma concentration (C_{max}) increased by ~ 10%.[5] Coadministration of rifampin (a strong CYP3A4/moderate CYP2C9 inducer) in *CYP2C9*1/*1* subjects reduced siponimod C_{max} by 45% and AUC by 57%.[12] Although an increased siponimod exposure was expected, coadministration of itraconazole (a strong CYP3A4 inhibitor) in *CYP2C9*1/*2* and *CYP2C9*1/*3* subjects led to a decrease in siponimod AUC by 9–10% and 24%, respectively (unpublished data[13]).

It is expected that the CYP2C9 genotype influences the effects of CYP3A4 and CYP2C9 inhibitors and inducers on siponimod PK. Physiologically-based pharmacokinetic (PBPK) modeling can help in predicting *in vivo* PK changes and drug–drug interactions (DDIs) caused by CYP perpetrators for the six different CYP2C9 genotypes while waiving a large

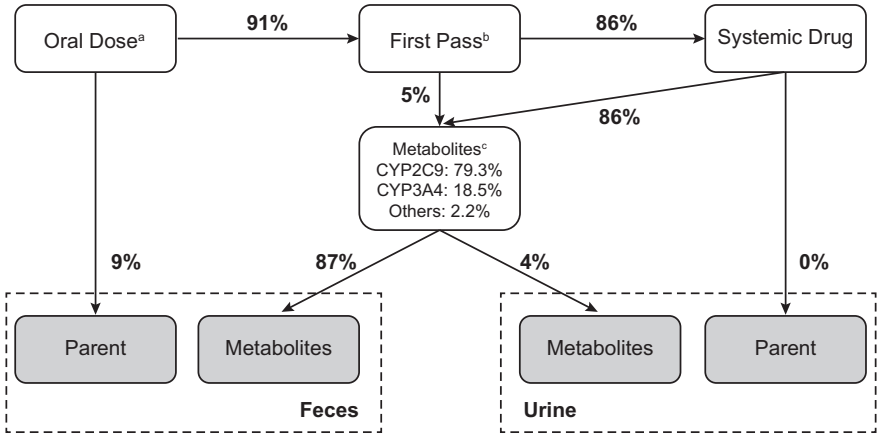

Figure 1 Drug disposition pathways for siponimod. [a]Oral absorption of siponimod was estimated to be ~ 91% based siponimod excreted to feces in the human absorption, distribution, metabolism, and excretion study,[4] assuming that the drug and metabolites are stable against intestinal bacterial enzymes. [b]Based on SimCYP simulations. [c]Fractional contribution of CYP enzymes based on phenotyping data.[5] Values represent normalized values scaled to 100%. CYP, cytochrome P450.

number of additional clinical DDI studies.[14,15] This simulation study aimed to establish a middle-out siponimod PBPK model reflecting the *in vitro* fractional enzyme contributions confirmed by clinical DDI data. Subsequently, the DDI potential of siponimod as a substrate in the presence of typical CYP2C9 and CYP3A4 perpetrators was evaluated for the six clinically relevant CYP2C9 genotypes in the white population.

RESULTS

Siponimod model verification

Systemic exposure. The simulated AUC from time 0 to infinity (AUC_{inf}) or from time 0–24 hours ($AUC_{0-24\ h}$), C_{max}, and minimum plasma concentration (C_{min}) after single i.v. (0.25 mg), single oral (s.d.; 0.1–75 mg), and multiple oral (0.3–20 mg q.d. for 28 days[1]) administration of siponimod were compared with the observed values (**Table 1**). Good model predictability of AUC, C_{max}, and C_{min} was achieved. The calculated percentage of residuals (=[predicted−observed]/observed) were within 30% (**Figure S1**). The residual plots for the PK parameters were well distributed, indicating that the established siponimod PBPK model was not biased by any arbitrary parameter optimization process. Linear PK observed clinically for siponimod single doses up to 75 mg (p.o., s.d.; **Figure 2a,b**) and up to 20 mg for the multiple doses (p.o., q.d. for 28 days; **Figure S2**) were reflected by the siponimod model.

A sensitivity analysis within the absorption rate constant (K_a) range of 0.1–2 showed a moderate change in siponimod (0.1 mg p.o., s.d. at day 1) C_{max} from 0.4 to 1.1 ng/mL. Therefore, small changes in K_a would not result in a significant C_{max} change. Time to reach the highest systemic drug concentration (T_{max}) of siponimod was sensitive to K_a changes below K_a values of 0.5 per hour. A sensitivity analysis within the fraction unbound in the enterocytes (f_{ugut}) range from 0.0002–0.01 revealed little impact on siponimod (0.1 mg p.o., single dose at day 1) C_{max} with changes from 0.69 to 0.65 ng/mL. Thus, any value within this range could be used. The sensitivity analysis results for the above siponimod model PK parameters are presented in **Figure S3**.

Impact of different CYP2C9 genotypes. The simulation trial parameters used to demonstrate the predictability of the siponimod model in different CYP2C9 genotypes (*1/*1, *2/*3, and *3/*3) were similar to the actual clinical study[5] conditions (e.g., female proportionality and age ranges in populations; **Table S3**). The simulated siponimod PK after single oral administration to subjects of these three different genotypes was comparable to the observed values (**Table 2, Figure 2c,d**), verifying the PK and the fraction metabolized via CYP2C9 ($f_{m,CYP2C9}$).

Predicted vs. observed siponimod DDI potential with CYP3A4 and CYP2C9 perpetrators. *Moderate CYP3A4/CYP2C9 inhibitor (fluconazole).* The geometric mean AUC_{inf} and C_{max} ratios of siponimod (4 mg p.o., s.d. on day 3) following oral administration of fluconazole (200 mg b.i.d. on day 1 and 200 mg q.d. on days 2–19) was predicted to be 2.15 and 1.07, respectively, for the *CYP2C9*1/*1* genotype (**Table 3**). The predicted ratios were in line with the corresponding clinical observation (AUC ratio (AUCR): 1.98; C_{max} ratio: 1.10).[5] For siponimod and fluconazole at steady state, the predicted AUCRs ranged from 2.18 for *CYP2C9*1/*1* to 2.09 for *CYP2C9*2/*3* (**Table 3**). An increase in AUCR was predicted for the *CYP2C9*3/*3* genotype (2.49).

The sensitivity analysis for the fluconazole CYP2C9 unbound inhibition constant range from 5–30 μM, predicted an AUCR change from 4.17 to 1.84, respectively, suggesting that the predictions of AUC changes for siponimod in the presence of fluconazole were sensitive to CYP2C9 inhibition constant (K_i) changes for the *CYP2C9*1/*1* genotype (**Figure S3**). However, the higher CYP2C9 K_i value has been published and successfully verified by the SimCYP team.[16]

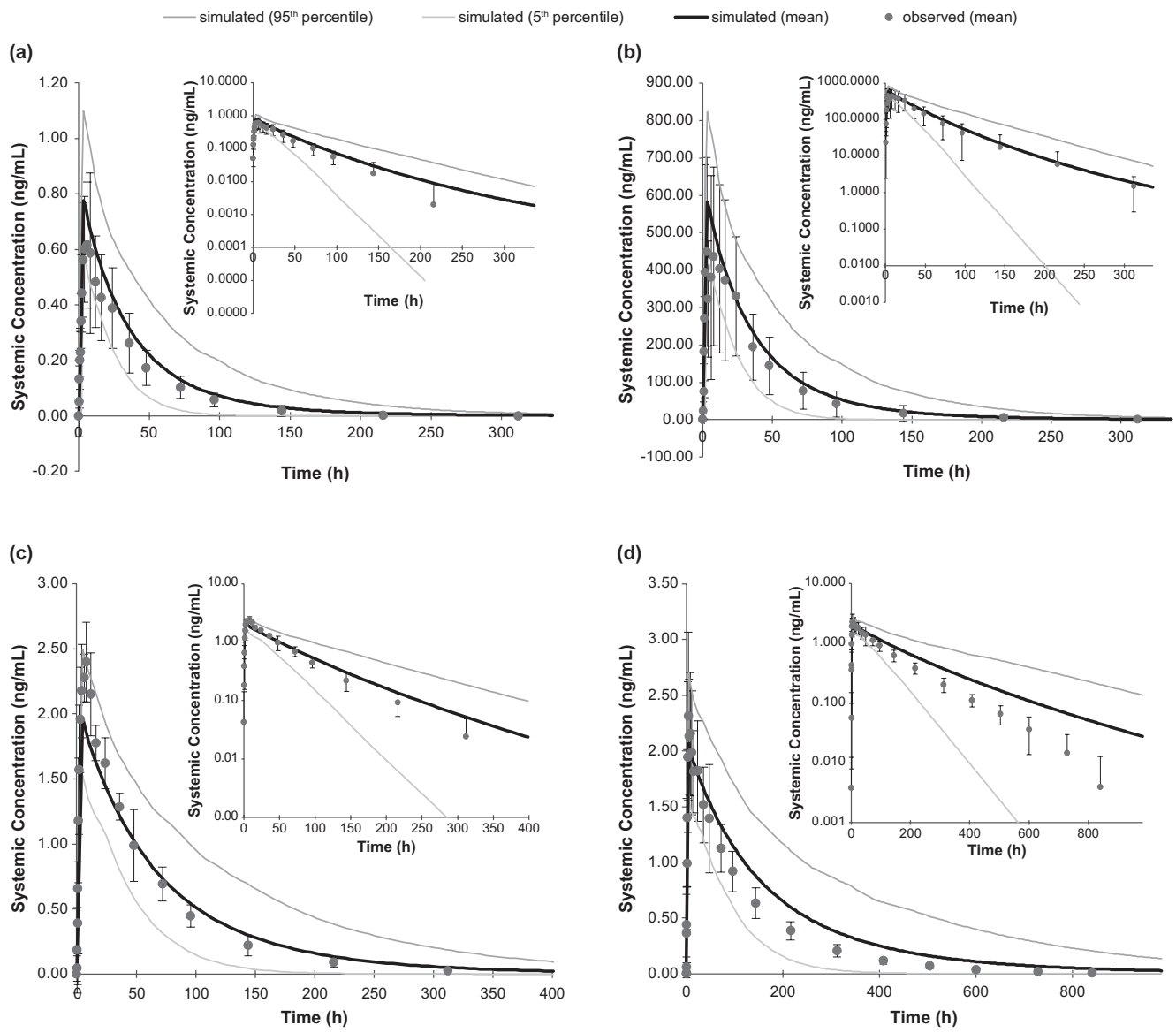

Figure 2 SimCYP simulation for siponimod pharmacokinetics following single dose oral administration. (**a**) Day 1, 0.1 mg. (**b**) Day 1, 75 mg for the *CYP2C9*1/*1* genotype (n = 11 at 0.1 mg and n = 8 at 75 mg) and 0.1 mg (**c, d**) for the *CYP2C9*2/*3* and *CYP2C9*3/*3* genotypes (n = 6 [*2/*3]; n = 66 [*3/*3]). The black and gray lines represent simulated mean time-plasma concentration profiles and the 5th/95th percentile of the total virtual population, respectively (n = 340). Full circles are the measured values after oral administration. CYP, cytochrome P450.

Table 2 Observed and simulated siponimod PK parameters after single oral administration to subpopulations carrying the different CYP2C9 genotypes

	N		C_{max} (ng/mL)		AUC_{inf} (ng·hour/mL)		$t_{1/2}$ (h)	
CYP2C9 genotype	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
*1/*1	100	12	1.90 (14)	2.03 (17.2)	70.6 (37)	70.5 (21.2)	25.5 (37)	28.1 (18.5)
*1/*2	100	NA	1.92 (14)	NA	76.2 (37)	NA	27.4 (40)	NA
*1/*3	100	NA	1.99 (14)	NA	117 (34)	NA	41.1 (36)	NA
*2/*2	100	NA	1.97 (15)	NA	98.7 (34)	NA	34.9 (36)	NA
*2/*3	100	6	2.02 (14)	2.45 (13.1)	142 (39)	144 (15.7)	49.2 (42)	50.9 (31.7)
*3/*3	100	6	2.10 (15)	2.35 (29.0)	348 (45)	271 (22.4)	118 (49)	126 (12.7)

AUC_{inf}, area under the curve from time zero to infinity; C_{max}, maximum plasma concentration; CYP, cytochrome P450; h, hour; NA, not applicable; PK, pharmacokinetic; $t_{1/2}$, half-life.

Table 3 Predicted siponimod multiple dose AUC and C_{max} ratios in the presence of CYP3A4 and CYP3A4/CYP2C9 inhibitors or inducers in the six clinical relevant CYP2C9 genotypes (simulated single dose DDI ratios and observed DDI ratios in bold)

	CYP inhibitor										CYP inducer			
	Strong 3A4 inhibitor				Moderate 3A4 inhibitor		Moderate 3A4/2C9 inhibitor		Weak 3A4/2C9 inhibitor		Strong 3A4/moderate 2C9 inducer		Moderate 3A4 inducer	
	Itraconazole		Ketoconazole		Erythromycin		Fluconazole		Fluvoxamine		Rifampin		Efavirenz	
CYP2C9 genotype	AUC_i/AUC	C_{maxi}/C_{max}	AUC_i/AUC	C_{maxi}/C_{max}	AUC_i/AUC	C_{maxi}/C_{max}	AUC_i/AUC	C_{maxi}/C_{max}	AUC_i/AUC	C_{maxi}/C_{max}	AUC_i/AUC	C_{maxi}/C_{max}	AUC_i/AUC	C_{maxi}/C_{max}
*1/*1	1.17 (1.16–1.18)	1.12 (1.12–1.13)	1.24 (1.23–1.26)	1.18 (1.17–1.19)	1.14 (1.13–1.16)	1.11 (1.10–1.12)	2.18 (2.14–2.22) **Simulated single dose: 2.15 Observed: 1.98**	1.88 (1.85–1.92) **Simulated single dose: 1.07 Observed: 1.10**	1.42 (1.39–1.46)	1.33 (1.30–1.35)	0.32 (0.31–0.33) **Observed: 0.43**	0.50 (0.49–0.51) **Observed: 0.55**	0.71 (0.69–0.73)	0.79 (0.77–0.80)
*1/*2	1.18 (1.17–1.19) **Simulated single dose: 1.18 Observed: 0.90**	1.14 (1.13–1.15) **Simulated single dose: 1.02 Observed: 1.01**	1.26 (1.25–1.28)	1.20 (1.19–1.21)	1.16 (1.15–1.17)	1.12 (1.11–1.13)	2.18 (2.14–2.22)	1.89 (1.86–1.92)	1.41 (1.38–1.45)	1.32 (1.30–1.35)	0.32 (0.31–0.33)	0.49 (0.48–0.50)	0.70 (0.68–0.71)	0.77 (0.76–0.79)
*1/*3	1.29 (1.28–1.31) **Simulated single dose: 1.30 Observed: 0.76**	1.24 (1.23–1.26) **Simulated single dose: 1.02 Observed: 0.94**	1.41 (1.38–1.44)	1.34 (1.31–1.36)	1.25 (1.23–1.27)	1.21 (1.19–1.22)	2.13 (2.09–2.16)	1.93 (1.90–1.96)	1.35 (1.32–1.38)	1.29 (1.27–1.32)	0.30 (0.29–0.31)	0.43 (0.42–0.44)	0.61 (0.59–0.63)	0.69 (0.67–0.71)
*2/*2	1.24 (1.22–1.25)	1.19 (1.18–1.21)	1.34 (1.32–1.36)	1.27 (1.25–1.29)	1.21 (1.19–1.22)	1.16 (1.15–1.18)	2.16 (2.12–2.20)	1.92 (1.89–1.95)	1.38 (1.34–1.41)	1.31 (1.28–1.33)	0.31 (0.30–0.32)	0.46 (0.45–0.47)	0.65 (0.63–0.67)	0.73 (0.71–0.74)
*2/*3	1.35 (1.33–1.37)	1.30 (1.28–1.31)	1.49 (1.45–1.52)	1.41 (1.38–1.44)	1.30 (1.27–1.33)	1.25 (1.23–1.27)	2.09 (2.05–2.12)	1.91 (1.88–1.94)	1.31 (1.28–1.34)	1.27 (1.24–1.29)	0.29 (0.28–0.30)	0.41 (0.40–0.42)	0.58 (0.56–0.60)	0.65 (0.63–0.67)
*3/*3	3.03 (2.83–3.22)	2.89 (2.71–3.08)	4.20 (3.94–4.46)	3.97 (3.74–4.19)	2.42 (2.26–2.58)	2.32 (2.17–2.47)	2.49 (2.43–2.55)	2.39 (2.33–2.45)	1.12 (1.11–1.13)	1.11 (1.10–1.12)	0.21 (0.20–0.22)	0.27 (0.27–0.29)	0.35 (0.33–0.38)	0.41 (0.39–0.43)

Data presented in geometric mean (90% confidence interval), for a single dose siponimod DDI simulations with fluconazole *CYP2C9*1*1* or itraconazole *CYP2C9*1*2* and **1*3* the AUC/C_{max} ratios were reported as clinical reference data exists for this study design.

AUC, area under the curve; AUC_i, area under the curve for inhibition/induction; C_{max}, maximum plasma concentration; C_{maxi}. maximum plasma concentration for inhibition/induction; CYP, cytochrome P450; DDI, drug–drug interaction.

Strong CYP3A4/moderate CYP2C9 inducer (rifampin). The steady-state geometric mean AUC (AUCτ) and C_{max} ratios of siponimod (2 mg p.o., q.d. for 12 days) with coadministration of rifampin (600 mg b.i.d. for 12 days) were predicted to be 0.32 and 0.50, respectively (**Table 3**), which were comparable to the clinical observation (AUCR: 0.43; C_{max} ratio: 0.55).[12] For siponimod and rifampin at steady state, the predicted AUCRs ranged from 0.32 for *CYP2C9*1/*1* to 0.29 for *CYP2C9*2/*3*; for the *CYP2C9*3/*3* genotype, an AUCR of 0.21 was predicted (**Table 3**). The induction effect of rifampin (600 mg b.i.d. for 12 days) on the PK of siponimod (2 mg p.o., q.d. for 12 days) in the *CYP2C9*1/*1* genotype was also predicted using the rifampin model without CYP2C9 induction parameters. The simulated AUCτ (0.59) and C_{max} ratios (0.71) showed a less pronounced DDI effect compared with the clinical observations[12] indicating that the contribution of CYP2C9 induction is important and cannot be neglected.

When performing a sensitivity analysis, the AUCR of siponimod in the presence of rifampin within a degradation rate constant (K_{deg}), CYP3A4 range of 0.005–0.03 per hour changed from 0.294–0.275, suggesting a weak dependency. Similarly, within a K_{deg}, CYP2C9 range of 0.005–0.03 per hour, the AUCR changed from 0.293 to 0.252, indicating a weak dependency for the *CYP2C9*1/*1* genotype population (**Figure S3**).

Strong CYP3A4 inhibitor (itraconazole). The geometric mean AUC_{inf} and C_{max} ratios of siponimod (0.25 mg p.o., s.d. on day 5) with coadministration of itraconazole (100 mg p.o., b.i.d. from day 1 to day 17) were predicted to be 1.18 and 1.02, respectively, for the *CYP2C9*1/*2* genotype, and 1.29 and 1.02, respectively, for the *CYP2C9*1/*3* genotype. These predictions were different from the clinical observation (AUCR: 0.90; C_{max} ratio: 1.01 for the *CYP2C9*1/*2* genotype; AUCR: 0.76; C_{max} ratio: 0.94 for the *CYP2C9*1/*3* genotype). The coadministration of itraconazole and siponimod, the predicted AUCRs ranged from 1.17 for *CYP2C9*1/*1* to 1.35 for *CYP2C9*2/*3* at steady state, for the *CYP2C9*3/*3* genotype, an AUCR of 3.03 was predicted (**Table 3**).

DDI prediction of siponimod as a victim drug

Steady-state DDI effects of strong, moderate, and weak inhibition of CYP3A4 and/or CYP2C9 and moderate induction of CYP3A4 on the PK of siponimod were simulated for the six different CYP2C9 genotypes. Due to the longer $t_{1/2}$ of siponimod in subpopulations carrying *CYP2C9*3/*3*,[5] the simulation time was extended to 90 days for the inhibitors, and to 24 days for the inducer efavirenz, maintaining coadministration of perpetrators. The DDI effects of CYP3A4 and CYP2C9 perpetrators on siponimod exposure in subjects of the six clinically relevant genotypes are presented in **Table 3**.

Effect of inhibitors. *Strong CYP3A4 inhibitor (ketoconazole).* In the presence of the strong CYP3A4 inhibitor ketoconazole, predicted siponimod AUC_{inf} inhibition ratios tended to increase with reduced CYP2C9 metabolic activity in the different genotypes. The AUCRs increased from 1.24 in *CYP2C9*1/*1* up to 1.49 in *CYP2C9*2/*3*. The *CYP2C9*3/*3* genotype population showed a significantly higher DDI risk, with a predicted AUCR of 4.20 (**Table 3**).

Moderate CYP3A4 inhibitor (erythromycin). In the presence of the moderate CYP3A4 inhibitor erythromycin, an increase in predicted siponimod AUC_{inf} inhibition ratios was observed, increasing from 1.14 in *CYP2C9*1/*1* to 1.30 in *CYP2C9*2/*3*. The AUCR was larger for the *CYP2C9*3/*3* genotype (2.42) suggesting a higher DDI risk (**Table 3**).

Weak CYP3A4/2C9 inhibitors (fluvoxamine). In the presence of the weak CYP3A4/2C9 inhibitor fluvoxamine, the predicted AUCRs ranged between 1.42 in *CYP2C9*1/*1* genotype and 1.31 in *CYP2C9*2/*3* genotype. A further decrease in the AUCR was predicted in the *CYP2C9*3/*3* genotype (1.12) due to the dual inhibition of the metabolic pathways (**Table 3**).

Effect of inducers. *Strong CYP3A4/moderate CYP2C9 inducers.* In presence of strong CYP3A4 and moderate CYP2C9 inducer

Table 4 Net inhibition or induction effect ratios (inhibition or induction × CYP2C9 genotype exposure ratios)[a]

	CYP inhibitor					CYP inducer	
	Strong 3A4 inhibitor		Moderate 3A4 inhibitor	Moderate 3A4/2C9 inhibitor	Weak 3A4/2C9 inhibitor	Strong 3A4/ moderate 2C9 inducer	Moderate 3A4 inducer
	Itraconazole	Ketoconazole	Erythromycin	Fluconazole	Fluvoxamine	Rifampin	Efavirenz
CYP2C9 genotype	AUC_i/AUC	AUC_i/AUC	AUC_i/AUC	AUC_i/AUC	AUC_i/AUC	AUC_i/AUC	AUC_i/AUC
*1/*1	1.18	1.24	1.14	2.20	1.44	0.29	0.68
*1/*2	1.29	1.36	1.25	2.31	1.55	0.30	0.72
*2/*2	1.73	1.83	1.66	2.91	1.90	0.37	0.85
*1/*3	1.06	1.14	1.01	1.72	1.11	0.22	0.48
*2/*3	1.32	1.42	1.25	1.98	1.28	0.26	0.53

AUC, area under the curve; AUC_i, area under the curve for inhibition/induction; CYP, cytochrome P450.
[a]Calculated using drug–drug interaction ratios from **Table 3** multiplied by the genotype-specific exposure increase, and compared with the wild type (*CYP2C9*1/*1*, **Table 1**) where simulated exposures at a 2 mg siponimod dose were used for the genotypes *CYP2C9*1/*1*, **1/*2*, and **2/*2*, and a dose of 1 mg for *CYP2C9*1/*3* and **2/*3*. Calculations are based on geometric mean data.

(rifampin), the predicted AUCR ranged between 0.32 (*CYP2C9*1/*1*) and 0.21 (*CYP2C9*3/*3*) and the C_{max} ratios ranged between 0.5 and 0.27, respectively. Both metabolic pathways of siponimod were impacted, and only a small difference in the induction effect was predicted between CYP2C9 genotypes (**Table 3**).

Moderate CYP3A4 inducer (efavirenz). The predicted exposure change in presence of the moderate CYP3A4 inducer efavirenz was comparatively lower than with rifampin. The predicted AUCRs ranged between 0.35 and 0.71 and the C_{max} ratios ranged between 0.41 and 0.79 for the CYP2C9 genotypes (**Table 3**). The lowest DDI ratios were predicted for the *CYP2C9*3/*3* genotype (AUCR: 0.35 and C_{max} ratio: 0.41).

Siponimod CYP2C9 genotype-based DDI management

When considering the siponimod maintenance doses recommended by the FDA (2 mg daily in patients with *CYP2C9*1/*1*, **1/*2*, and **2/*2* genotypes and 1 mg daily in patients with *1/*3 and *2/*3 genotypes) and the contraindication for patients with the *CYP2C9*3/*3* genotype, the estimated net effects on siponimod exposure in the presence of CYP2C9/CYP3A4 inhibitors or inducers were estimated after multiple administrations (**Table 4**). The net effect is the simulated inhibition or induction ratio (**Table 3**) multiplied by the corresponding CYP2C9-genotype ratio ($AUC_{ss,tau}$ of the genotype vs. $AUC_{ss,tau}$ of wild type *CYP2C9*1/*1* at the FDA recommended dose for the respective genotypes without coadministration of any perpetrator).

DISCUSSION

The present siponimod PBPK modeling study was conducted as a complementary analysis to the clinical PK DDI studies to gather complete understanding of the DDI potential of siponimod with respect to the six clinically relevant CYP2C9 genotypes. These modeling results were used to guide the development of drug label recommendations. The study evaluated the DDI effect of CYP3A and CYP2C9 enzyme inducers or inhibitors on systemic exposure of siponimod through PBPK simulations in healthy subjects. The linear PK profiles of siponimod up to doses of 75 mg for the single dose and 20 mg for the multiple dose (q.d. for 28 days) were described well by the PBPK model. The systemic exposure at steady state after multiple oral administration was reached after 6 days, confirming validity of the reported $t_{1/2}$ of 30 hours for *CYP2C9*1/*1* genotypes.[1] The PK profiles of the two genotypes *CYP2C9*2/*3* and *CYP2C9*3/*3* were also well described by the established model.

Predicted effects of fluconazole (AUCR: 2.15; C_{max} ratio: 1.07) and rifampin (AUCR: 0.32; C_{max} ratio: 0.50) on the siponimod PK were comparable to the respective corresponding clinical data (fluconazole/rifampin; geometric mean AUC_{inf}R: 1.98/0.43 and C_{max} ratio: 1.10/0.55)[5,12] for the *CYP2C9*1/*1* genotype (**Table** 3), verifying the siponimod PK and fraction metabolized via CYP3A4 ($f_{m,CYP3A4}$). The fraction metabolized via CYP2C9 ($f_{m,CYP2C9}$, 0.79) was based on the *in vitro* phenotyping data,[4] and the selected CYP2C9 intrinsic clearance (CL_{int}) values for the different genotypes based on PopPK (**Table S2**) were verified by the correct PK estimation for the different genotypes (*CYP2C9*1/*1*, *CYP2C9*2/*3*, and *CYP2C9*3/*3*).

With decreased CYP2C9 metabolic activity, CYP3A4 becomes the dominant elimination pathway for the PMs (*CYP2C9*3/*3*) with an estimated fraction metabolized via CYP2C9 ($f_{m,CYP3A4}$) of 0.82; this genotype subpopulation is sensitive to strong CYP3A4 inhibitors (**Tables 3 and 5**). All other genotypes have <35% CYP3A4 metabolism contribution to total clearance (CL_{tot}). In the presence of strong CYP3A4 inhibitors, an increased DDI risk in subjects with *CYP2C9*3/*3* was predicted compared with other genotypes (predicted siponimod AUCR: 3.03–4.20 vs. <1.49), demonstrating the sensitivity, when both pathways CYP2C9 and CYP3A4 are less functional or inhibited.

For all genotypes, there was a moderate AUC increase (AUCR: 2.13–2.49) predicted with moderate dual CYP3A4/CYP2C9 inhibitors (fluconazole) and a weak AUC increase (AUCR: 1.12–1.42) with weak dual CYP3A4/CYP2C9 inhibitors (fluvoxamine). For the interaction with the strong CYP3A4 and moderate CYP2C9 inducer (rifampin), no relevant difference in AUCRs across all CYP2C9 genotypes was predicted (range: 0.21–0.32). The different CYP2C9 genotypes are equally inducible by a CYP2C9 inducer.[17] Therefore, we assumed that no adaptations were required for simulating CYP2C9-mediated induction effects on siponimod for the six clinically relevant genotypes.

The predicted geometric mean AUC_{inf} ratios of siponimod (0.25 mg, single dose at day 5) with a coadministration of the strong CYP3A4 inhibitor itraconazole (100 mg b.i.d.) in *CYP2C9*1/*2* (1.18) and *CYP2C9*1/*3* genotypes (1.29) were different from the clinical observations (0.90, 0.76, respectively; unpublished data[13]), indicating an apparent induction potential of itraconazole on the PK of siponimod rather than an inhibition effect. A potential explanation for the miss-predictions would be the involvement of another metabolizing enzyme of siponimod (i.e., CYP1A1), which was identified in the *in vitro* enzyme phenotyping study as a minor pathway.[4] CYP1A1 was not considered for the PBPK model due to its low abundance in the noninduced state.[18] However, additional investigations indicated that the potential involvement of CYP1A1 was not likely to be the root cause of the decreased siponimod exposure in the presence of itraconazole (unpublished data[13]), as the itraconazole *in vitro* CYP1A1 inhibition effect is stronger than its induction potential. The underlying mechanism leading to these clinical observations is currently unknown. However, it is considered that the effect observed was specific to itraconazole. It is concluded that other CYP3A4 inhibitors, without the effect observed with itraconazole (e.g., clarithromycin), should act as strong and selective CYP3A4 inhibitors, resulting in increased AUCRs of siponimod > 1 when coadministered. The basis for this assumption is the good correlation between observed and predicted DDI results of the dual perpetrators (CYP2C9/CYP3A4), fluconazole, and rifampin, verifying the fractional contribution of CYP3A4 to CL_{tot} of siponimod. The fraction metabolized via CYP2C9 ($f_{m,CYP2C9}$) was confirmed by the good match of the genotype-specific PK data for *CYP2C9*1/*1*, *CYP2C9*2/*3*, and *CYP2C9*3/*3*. Therefore, it is believed that the established siponimod model for

Table 5 SimCYP input parameters for the siponimod model

Input parameters	Siponimod	Fluconazole[a]
1. Physicochemical and binding properties		
Molecular weight (g/mol)	516.6	306.3
Log P: Octanol-water partition	1.8	0.20
Compound type	Ampholyte	Monoprotic base
pK_a	3.1/8.1	1.76
Blood to plasma drug concentration ratio	0.765	1
Fraction unbound in plasma	0.0002	0.89
Main plasma binding protein	HSA (assumption)	HSA
2. Absorption[b]		
Absorption model	First order	
Fraction available from dosage form	0.91[c]	0.988
CV f_a (%)	8.6[d]	30
Absorption rate constant (1/hour)	0.687[d]	1.863
CV K_a (%)	7.8	30
Lag time (h)	1.5 (optimized)	
Unbound fraction in enterocytes	0.0002[e]	0.89
Nominal flow in gut model (L/hour)	9.851	14.376
CV $Q_{(gut)}$ (%)	30	30
PAMPA permeability, PAMPA ($10-^6$ cm/s)	10	—
Permeability A-B, Caco-2 (cm/s*10^{-6})	—	0.89
Caco-2 reference (L/hour)	—	14.376
Permeability scalar	—	0.885
3. Distribution		
Distribution model	Full PBPK	Minimal PBPK model
Tissue model	Perfusion limited model	Perfusion limited model
Volume of distribution at steady state (L/kg)	1.45[f]	0.748
CV V_{ss} (%)	3.4[d]	30
Extent of tissue K_p, K_p scalar	0.574 (optimized)	—
4. Enzyme/transporter phenotyping		
Human liver microsomes		
In vitro intrinsic clearance (CYP2B6, µL/minutes/pmol) (fm_{CYP2B6})	0.733[g] (0.004)	—
CL_{int} (CYP2C8, µL/minutes/pmol) (fm_{CYP2C8})	2.941[g] (0.017)	—
CL_{int} (CYP2C19, µL/minutes/pmol) ($fm_{CYP2C19}$)	0.593[g] (0.001)	—
CL_{int} (CYP3A4, µL/minutes/pmol) (fm_{CYP3A4})	5.607[g] (0.185)	—
CL_{int} (allelic *CYP2C9*1/*1*, µL/minutes/pmol) (fm_{CYP2C9}/fm_{CYP3A4})	45.105[h] (0.804/0.175)	—
CL_{int} (allelic *CYP2C9*1/*2*, µL/minutes/pmol) (fm_{CYP2C9}/fm_{CYP3A4})	45.885[h] (0.788/0.189)	—
CL_{int} (allelic *CYP2C9*1/*3*, µL/minutes/pmol) (fm_{CYP2C9}/fm_{CYP3A4})	24.605[h] (0.678/0.287)	—
CL_{int} (allelic *CYP2C9*2/*2*, µL/minutes/pmol) (fm_{CYP2C9}/fm_{CYP3A4})	33.271[h] (0.727/0.244)	—
CL_{int} (allelic *CYP2C9*2/*3*, µL/minutes/pmol) (fm_{CYP2C9}/fm_{CYP3A4})	18.924[h] (0.616/0.343)	—
CL_{int} (allelic *CYP2C9*3/*3*, µL/minutes/pmol) (fm_{CYP2C9}/fm_{CYP3A4})	2.869[h] (0.074/0.822)	—
5. Other distribution and elimination property		
In vivo CL	—	—
In vivo renal clearance in a 20–30 year healthy man (L/hour)	0[i]	0.7
CL following i.v. administration (L/hour)	—	1.01
CV CL_{iv} (%)	—	24
In vitro CL	—	—

(Continues)

Table 5 (Continued)

Input parameters	Siponimod	Fluconazole[a]
Hepatic uptake	1.0[j]	1
Overall biliary clearance, $CL_{int(hep)}$ (µL/minutes/10^6 cells)	0[i]	—
CV $CL_{int(hep)}$ (%)	30[i]	—
6. Interaction		
Inhibition constant (CYP2C9; µM)	—	20.4
Fraction unbound in human liver microsome (CYP2C9)	—	1
K_i (CYP2C19; µM)	—	2
$f_{u(mic)}$ (CYP2C19)	—	1
K_i (CYP3A4; µM)	—	10.7
$f_{u(mic)}$ (CYP3A4)	—	1
K_i (CYP3A5; µM)	—	84.6
$f_{u(mic)}$ (CYP3A5)	—	1

CL, clearance; $CL_{int,u,}$ unbound intrinsic clearance; $CL_{int(hep)}$, overall biliary clearance; CL_{iv}, clearance following intravenous administration; CV, coefficient of variation; CYP, cytochrome P450; f_a, fraction available from dosage form; f_m, fraction metabolized; $f_{u(mic)}$, fractions unbound in human liver microsome; HSA, human serum albumin; K_a, absorption rate constant; K_i, inhibition constant; K_p, partition coefficient; PAMPA, parallel artificial membrane permeability assay; PBPK, physiologically-based pharmacokinetic; $Q_{(gut)}$, nominal flow in gut model; V_{ss}, volume of distribution at steady state.
[a]All data taken from the fluconazole SimCYP compound file V16 except for a CYP2C9 K_i.[16] This was verified by showing improved predictability of the clinical drug–drug interaction effects on probe CYP2C9 substrates. [b]No involvement of intestinal efflux transporters in the absorption process (**Table S4**). [c]Based on amount of siponimod excreted to feces.[4] [d]Estimated based on population pharmacokinetic (PopPK) model. [e]Sensitivity analysis performed. [f]Based on mean V_{ss} observed (**Table 1**), converted to L/kg based on mean body weight of the study subjects (80.08 kg). [g]CL used in the retrograde calculator was the observed geometric mean CL = 3.12 L/hour (**Table 1**); due to the retrograde calculation $f_{u(mic)}$ was set to 1 for all enzymes. [h]Calculated based on PopPK CL data (**Table S2**). [i]Based on human absorption, distribution, metabolism, and excretion study[4] and enzyme phenotyping data,[4] it was concluded that hepatic metabolism is exclusively driving CL. [j]**Table S4**.

DDI simulations is still valid for typical CYP3A4 and CYP3A4/CYP2C9 perpetrators without CYP1A1 induction potential.

The recommended siponimod maintenance doses in the FDA approved prescribing information are 2 mg daily in patients with *CYP2C9*1/*1*, **1/*2*, and **2/*2* genotypes, and 1 mg daily in patients with *CYP2C9*1/*3* and **2/*3* genotypes, and siponimod is contraindicated for patients with a *CYP2C9*3/*3* genotype. Considering these doses, the predicted net effect on siponimod exposures ranged between 1.06 and 1.90 across CYP2C9 genotypes in presence of strong and moderate CYP3A4 inhibitors, and weak dual CYP3A4/2C9 inhibitors. A larger effect is predicted in the presence of moderate dual CYP3A4/2C9 inhibitors with ratios ranging between 1.72 and 2.91 (**Table 4**). Because of this significant increase in exposure of siponimod, the US prescribing information stipulated that concomitant use of siponimod and drugs that cause moderate CYP2C9 and moderate or strong CYP3A4 inhibition are not recommended. In addition, caution should be exercised for concomitant use of siponimod with moderate CYP2C9 inhibitors.[3]

Dual strong CYP3A4/moderate CYP2C9 inducers are predicted to reduce siponimod exposure by ~ 63% to 78%, and moderate CYP3A4 inducers by ~ 15–52% (**Table 4**). Therefore, concomitant use of siponimod with drugs that cause moderate CYP2C9 and strong CYP3A4 induction is not recommended for all patients irrespective of their CYP2C9 genotype.[3] Caution should be exercised for concomitant use of siponimod with moderate CYP2C9 inducers. In addition, concomitant use of siponimod and moderate (e.g., modafinil and efavirenz) or strong CYP3A4 inducers is not recommended for patients with *CYP2C9*1/*3* and **2/*3* genotypes.[3]

The current PBPK study indicated that the CYP2C9 genotype has a relevant influence on the DDI behavior of siponimod in the presence of different CYP3A4/CYP2C9 inducers and inhibitors. Moreover, this study demonstrated the potential of PBPK modeling to complement clinical DDI information with simulations of untested scenarios. These results served as the basis for the approved label recommendations[3] when siponimod is administered in combination with CYP2C9/CYP3A4 perpetrator drugs, to ensure that safety and the efficacy of siponimod are preserved in all patients.

METHODS

The population-based PKs simulator SimCYP version 16 (Certara, Princeton, NJ) was used to predict the DDI victim potential of siponimod in the presence of typical CYP3A4/CYP2C9 inhibitors and inducers.

All simulations within this study were performed with the SimCYP healthy volunteer population, as there was no clinical relevant PK difference between a healthy volunteer and a patient with multiple sclerosis population identified by PopPK.[12]

The fluconazole SimCYP version 16 model was adapted by changing the CYP2C9 K_i value from 7.92 to 20.4 µM[16] to increase predictability of the inhibition effects on probe CYP2C9 substrates (S-warfarin, tolbutamide, and phenytoin). The efavirenz model was built as a pure CYP3A4 inducer model based on SimCYP version 16, because negligible CYP2C9 induction is expected for a moderate CYP3A4 inducer.[19,20] Input values for the perpetrator drugs itraconazole, ketoconazole, erythromycin, fluvoxamine, and rifampin were provided by SimCYP version 16.

All systemic PK data were based on plasma concentrations. AUC, C_{max}, C_{min}, $t_{1/2}$, and T_{max} are reported as geometric means or median with range or as 90% confidence intervals. The exposure change in the presence of perpetrators was defined as the AUCR and/or C_{max} ratio.

Siponimod PBPK model

Model building. A siponimod PBPK model was established, in alignment with a drug disposition scheme of siponimod (**Figure 1**), using a mixed approach combining *in vitro* data, physicochemical parameters, and PK parameters derived from clinical studies. The clinical PK data from the single ascending dose (**Table 1**), multiple-ascending dose (**Table 1**), absolute bioavailability (**Table 1**), human absorption, distribution, metabolism, and excretion,[4] and fluconazole DDI study results[5,12,21] were used for building of the PBPK model. This model was verified for its PK and the fraction metabolized via CYP2C9 ($f_{m,CYP2C9}$) by the genotype PK data (**Table 2**)[5] and by clinical siponimod PK and DDI data in the absence and presence of rifampicin verifying the fraction metabolized via CYP3A4 ($f_{m,CYP3A4}$).[12] The final PBPK model was used to predict the siponimod DDI potential as substrate at steady state in the presence of typical CYP3A4/CYP2C9 inhibitors for the six clinically relevant CYP2C9 genotypes (*1/*1, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3). The model-building workflow for the siponimod PBPK model is illustrated in **Figure S4**.

Model input parameters

The siponimod SimCYP model was established based on the physicochemical properties from the *in vitro* absorption, distribution, metabolism, and excretion data. Fraction absorbed was determined from the human absorption, distribution, metabolism, and excretion study.[4] Fraction unbound in the enterocytes (f_{ugut}) was set to be equal to unbound fraction in plasma. Absorption rate constant (K_a) was taken from the PopPK analysis results (Novartis data on file). Lag time was optimized based on the clinical T_{max} data at steady state of the multiple-ascending dose study (**Table 1**). Volume of distribution at steady state and CL_{tot} were used as determined after single i.v. administration of siponimod in the clinical study (**Table 1**). Siponimod clearance is exclusively driven by CYP-mediated metabolism. The hepatic uptake of siponimod in human hepatocytes was purely passive with no contribution of active uptake transporters (**Table S1**). Therefore, the hepatic intrinsic clearance values ($CL_{int,h}$) for CYP2C9, CYP3A4, CYP2B6, CYP2C8, and CYP2C19 were determined by distributing the relative fractions metabolized (f_m) from the *in vitro* enzyme phenotyping study.[4] The respective $CL_{int,h}$ values for the six different CYP2C9 genotypes were calculated based on PopPK total body clearance of the drug from plasma results for each genotype (*1/*1, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3; **Table S3**). Total clearance (CL_{tot}) values were derived by multiplying CL_{tot}/F by F. There is no excretion of siponimod via urine and no biliary or intestinal secretion.[4] The siponimod model input parameters and all assumptions are summarized in **Table 5**.

DDI simulations used for model verification and prospective DDI predictions

Effects of CYP3A4 and CYP2C9 inhibitors on siponimod. Simulated DDI potential of fluconazole on the systemic exposure of siponimod after oral administration was compared with the respective clinical study results, matching the study design. This was used to verify estimations of f_m by CYP3A4 ($f_{m,CYP3A4}$) for siponimod. Fraction metabolized by CYP2C9 ($f_{m,CYP2C9}$) based on *in vitro* data was verified in model building based on the PK of the different genotypes *CYP2C9*1/*1*, *CYP2C9*2/*3* and *CYP2C9*3/*3*.[4] The model was further verified by the clinical rifampicin DDI study data (described in the next section). Using the siponimod model with the verified $f_{m,CYP3A4}$ value, the effects of itraconazole, ketoconazole, erythromycin, and fluvoxamine on the siponimod PK were simulated (**Table S4**).

Effects of CYP3A4 and CYP2C9 inducers on siponimod. Simulated DDI potential of rifampin on the systemic exposure of siponimod after oral administration was compared with the respective clinical study results,[12] matching the study design. The result supported validity of $f_{m,CYP3A4}$ for siponimod (i.e., estimated based on the clinical PK results and DDI data with fluconazole)[5] and the PK of the genotypes *CYP2C9*2/*3* and *CYP2C9*3/*3*. Subsequently, the effect of efavirenz on the siponimod PK was simulated (**Table S4**).

Sensitivity analysis

To investigate the impact of K_a changes on siponimod AUC, a sensitivity analysis within the range of 0.1–2 was performed. To investigate the impact of f_{ugut} changes on siponimod C_{max}, a sensitivity analysis was performed within the range of 0.0002–1. The fluconazole CYP2C9 K_i was revised by the SimCYP team. Therefore, to investigate the impact of CYP2C9 K_i changes on siponimod AUC in the presence of fluconazole, a sensitivity analysis was performed for the *CYP2C9*1/*1* genotype population. The CYP2C9 and CYP3A4 K_{deg} values were defined in the SimCYP version 16. A sensitivity analysis within the range of 0.005–0.03 per hour was performed to investigate the impact of $K_{deg,CYP2C9}$ and $K_{deg,CYP3A4}$ changes on siponimod AUCRs when coadministered with rifampin.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figure S1. Residual plot showing the predictability of C_{max}, C_{min}, and AUC; (**A**) C_{max} and AUC_{inf} after single oral administration of siponimod; (**B**) C_{min}, C_{max}, and $AUC_{0\text{-}24\ h}$ at days 28 after multiple oral administration of siponimod. $Area_{0\text{-}24h}$, area under the curve between 0-24 hours; AUC, area under the curve; AUC_{inf} : area under the curve extrapolated to infinity; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration.

Figure S2. Siponimod pharmacokinetics: SimCYP simulation for multiple dose oral administration.

Figure S3. (**A**) Effect of K_a on predicted T_{max} of siponimod. (**B**) Effect of f_{ugut} on predicted AUC and (**C**) C_{max} of siponimod. (**D**) Effect of $K_{i,CYP2C9}$ on the systemic AUC and (**E**) C_{max} ratio prediction of siponimod in the presence and absence of fluconazole. (**F**) Effect of mean turnover CYP2C9 and CYP3A4 on AUC and C_{max} ratios of siponimod in the presence of the CYP3A4/CYP2C9 inducer rifampin; mean turnover CYP2C9 on AUC and (**G**) C_{max} ratios for *CYP2C9*1/*1* genotype; mean turnover CYP3A4 on (**H, I**) AUC and (**J, K**) C_{max} ratio for *CYP2C9*1/*1* and *CYP2C9*3/*3* genotypes, respectively. AUC, area under the curve; C_{max}, maximum plasma concentrations; CYP2C9, Cytochrome P450 2C9; CYP3A4, Cytochrome P450 3A4; f_{ugut}, fraction unbound in enterocytes; K_a, absorption rate constant; $K_{i,CYP2C9}$, inhibition constant for CYP2C9; T_{max}, time at maximum plasma concentration.

Figure S4. Model building process for siponimod.

Table S1. In vitro investigation on siponimod as cytochrome P450 enzyme or transporter inhibitor and transporter substrate.

Table S2. Intrinsic clearance values of allelic cytochrome P450 2C9 genotypes calculated based on genotype specific clearance data from population pharmacokinetic analysis.†

Table S3. Pharmacokinetic simulation design parameters for the siponimod model verification and for predictions of subpopulations with the different cytochrome P450 2C9 genotypes.

Table S4. Drug–drug interaction simulation design parameters for predictions of siponimod as a victim drug with cytochrome P450 enzyme inhibitors and inducers.

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AUTHOR CONTRIBUTIONS
F.H., A.G., K.U., and H.H. wrote the manuscript. F.H. and A.G. designed the research. F.H. performed the research. F.H., A.G., K.U., and H.H. analyzed the data.

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