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ORIGINAL RESEARCH ARTICLE

Evaluation of the Potential for Cytochrome P450 and Transporter-Mediated Drug–Drug Interactions for Firsocostat, a Liver-Targeted Inhibitor of Acetyl-CoA Carboxylase

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Abstract

Background and Objective Firsocostat is an oral, liver-targeted inhibitor of acetyl-CoA carboxylase in clinical development for the treatment of metabolic dysfunction-associated steatohepatitis. This work evaluated the potential drug–drug interactions (DDIs) of firsocostat as a victim and as a perpetrator, to inform concomitant medication use.

Methods In this phase I study, healthy participants ($n = 13$–30 in each of four cohorts) received firsocostat alone or in combination with either victims or perpetrators of cytochrome P450 (CYP) enzymes and drug transporters to evaluate firsocostat as both a victim and perpetrator of DDIs, respectively.

Results Overall, 80 participants completed the study. As a victim of DDI, firsocostat plasma exposure (area under the plasma concentration-time curve [AUC] from 0 to infinity [AUC_{α}]) was 19-fold, 22-fold, 63%, and 38% higher when administered with single-dose rifampin 600 mg (organic anion transporting polypeptide [OATP] 1B1/B3 inhibitor), single-dose cyclosporine A 600 mg (OATP/P-glycoprotein/CYP3A inhibitor), multiple-dose probenecid 500 mg twice daily (evaluated as a uridine diphosphate glucuronosyltransferase [UGT] inhibitor), and multiple-dose voriconazole 200 mg twice daily (CYP3A inhibitor), respectively, compared with the administration of firsocostat alone. As a perpetrator of DDI, multiple-dose administration of firsocostat did not affect the exposure of midazolam 2 mg (CYP3A substrate) or drospirenone/ethinylestradiol 3 mg/0.02 mg (combined oral contraceptive). Study treatments were well-tolerated and all adverse events were mild.

Conclusions Firsocostat can be administered with CYP3A and UGT inhibitors without dose adjustment. However, firsocostat should not be coadministered with strong OATP1B/3 inhibitors, such as rifampin and cyclosporine A. Firsocostat can be administered with CYP3A substrates or combined oral contraceptives without dose modification.

1 Introduction

Metabolic dysfunction-associated steatohepatitis (MASH, previously known as nonalcoholic steatohepatitis or NASH) is a chronic liver disease associated with increased morbidity and mortality [1]. Owing to the accumulation of excess lipids in the liver, MASH may result in progressive fibrosis and, consequently, cirrhosis, which has been reported in 10–15% of affected patients [2]. The estimated prevalence of MASH is between 2 and 6% globally [3]. Due to the increasing prevalence of MASH coupled with a lack of approved therapies, this disease constitutes a growing unmet medical need [4–6].

Firsocostat is an oral, liver-targeted inhibitor of acetyl-coenzyme A carboxylase (ACC) 1 and ACC2 that is under clinical development for the treatment of MASH (at a 20 mg dose) in combination with cilofexor (a nonsteroidal farnesoid X receptor agonist, at a 30 mg dose) and semaglutide (a glucagon-like peptide-1 receptor agonist, at a 2.4 mg dose) [7]. Firsocostat has a passive permeability of 1.15×10^{-6} cm/s in Caco-2 cells (data on file, Gilead Sciences, Inc.). In humans, firsocostat inhibits de novo hepatic lipogenesis, resulting in decreases of lipid accumulation in the liver [8]. Moreover, in participants with noncirrhotic MASH (F2/F3 fibrosis), treatment with firsocostat 20 mg significantly improved hepatic steatosis compared with placebo [9].

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Key Points

Data from this study suggest that firsocostat may be coadministered with inhibitors of cytochrome P450 (CYP) 3A or uridine diphosphate glucuronosyltransferase without the need for dose modification.

Firsocostat may be coadministered with CYP3A substrates and combined oral contraceptives without dose modification; however, coadministration of firsocostat with strong hepatic organic anion transporting polypeptide inhibitors is not recommended.

Prior clinical studies in healthy participants have shown that firsocostat exhibits dose-proportional pharmacokinetics across the dose range of 20–500 mg [8, 10, 11], and more than 90% of firsocostat and its metabolite GS-834773 are eliminated in feces [12]. Preclinical in vitro data indicate that firsocostat is a substrate for drug transporters, such as P-glycoprotein (P-gp) and organic anion transporting polypeptides (OATPs), and metabolizing enzymes, such as uridine diphosphate glucuronosyltransferase (UGT) 1A3, 1A8, and 1A1, and cytochrome P450 (CYP) 3A (data on file, Gilead Sciences, Inc.). Additionally, in vitro data suggest that firsocostat does not act as an inhibitor of CYP isoforms, including CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4; however, at higher than clinically relevant exposures, firsocostat may induce CYP3A (via pregnane X receptor [PXR] activation; data on file, Gilead Sciences, Inc.).

The objective of this study was to characterize the drug–drug interaction (DDI) profile of firsocostat when administered with medications classified as either victims or perpetrators of CYP enzymes and drug transporter-mediated DDIs. To inform firsocostat dosing recommendations, firsocostat was evaluated as a victim of DDI when administered with rifampin (a selective OATP1B1/1B3 inhibitor) [13], cyclosporine A (mixed OATP/P-gp/multidrug resistance-associated protein 2 [MRP2]/CYP3A inhibitor) [14], probenecid (UGT inhibitor) [15], or voriconazole (CYP3A inhibitor) [16], and as a perpetrator of DDI when administered with midazolam (CYP3A substrate) [13, 17] or drospirenone/ethinylestradiol combination (combined oral contraceptive) [18].

2 Methods

2.1 Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice guidelines, and was conducted at PPD Development (7551 Metro Center Drive, Suite 200, Austin, TX 78744, USA). The study protocol was reviewed and approved by Salus Institutional Review Board (IRB) on 10 October 2016 (approval number 3072889; 2111 West Braker Lane, Suite 400, Austin, TX 78758, USA). All study participants provided written informed consent before study participation.

2.2 Study Participants

Adults who were 18–45 years of age, nonsmokers, and had not used nicotine or nicotine-containing products in the 90 days before study drug administration were eligible for study inclusion. Only female participants were eligible in the cohort that assessed the effects of firsocostat and oral contraceptives (Cohort 4). At the time of screening (≤ 28 days before the first dose of study drug), all participants were required to have had a body mass index (BMI) of 19–30 kg/m^2, creatinine clearance rate of ≥ 90 mL/min (measured by the Cockcroft–Gault equation), normal laboratory evaluations, normal or clinically insignificant 12-lead electrocardiogram (ECG) findings, and no significant medical history. Participants were also required to be in general good health, as determined by the investigator. For all cohorts, participants were excluded if they were pregnant or breastfeeding; had any serious or active medical or psychiatric illness; had a positive test result for human immunodeficiency virus 1 antibody, hepatitis B surface antigen, or hepatitis C antibody; had liver disease (including Gilbert's syndrome); had an implanted defibrillator or pacemaker; had current alcohol or substance abuse with the potential to interfere with participant safety or compliance; had poor venous access; used any prescription or over-the-counter medications (except vitamins, acetaminophen, ibuprofen, and/or hormonal contraceptive medications) or herbal products within 27 days of commencing study drug dosing; had received any systemic steroids, immunosuppressant therapies, or chemotherapeutic agents in the 3 months before screening; or had received any investigational drugs in the 30 days before screening.

2.3 Study Design

This was a phase I, open-label, single-center, multiple-cohort study assessing the DDI potential for firsocostat in healthy participants. After screening and following the completion of assessments on Day − 1, eligible participants were enrolled into one of four cohorts.

Cohort 1 assessed the effects of single doses of rifampin (OATP1B1/1B3 inhibitor) or cyclosporine A (OATP/P-gp/MRP2/CYP3A inhibitor) on the exposure of a single dose of firsocostat. Participants in Cohort 1 (n = 30) were randomized to one of six treatment sequences and received each of three following treatments. On Days 1, 7, and 15, participants received either (1) a single dose of firsocostat 20 mg; (2) a single dose of rifampin 600 mg coadministered with a single dose of firsocostat 20 mg; or (3) a single dose of cyclosporine A 600 mg coadministered with a single dose of firsocostat 20 mg. There was a washout period between each treatment: between Days 2 and 6 (5 days) and between Days 8 and 14 (7 days).

Cohort 2 assessed the effects of multiple doses of probenecid (UGT inhibitor) or voriconazole (CYP3A inhibitor) on the exposure of a single dose of firsocostat. Participants in Cohort 2 (n = 21) received a single dose of firsocostat 20 mg on Day 1, followed by probenecid 500 mg twice daily during Days 7–11 (5 days), and coadministered with a single dose of firsocostat 20 mg in the morning on Day 8. The final dose of probenecid was administered in the evening on Day 11. Voriconazole 200 mg twice daily was administered during Days 19–23 (5 days), and coadministered with a single dose of firsocostat 20 mg on Day 20. The final dose of voriconazole was administered in the evening on Day 23. There was a washout period between each treatment: between Days 2 and 6 (5 days) and between Days 12 and 18 (7 days).

Cohort 3 assessed the effect of multiple doses of firsocostat on the single-dose exposure of midazolam (CYP3A substrate). Participants in Cohort 3 (n = 13) received a single dose of midazolam 2 mg on Day 1. Firsocostat 50 mg was administered once daily during Days 7–19 (13 days), and coadministered with a single dose of midazolam 2 mg on Days 7 and 16. The final dose of firsocostat was administered in the morning on Day 19. There was a washout period between treatments during Days 2–6 (5 days).

Cohort 4 assessed the effect of multiple doses of firsocostat on the single-dose exposure of drospirenone/ethinylestradiol (oral contraceptive). Participants in Cohort 4 (n = 16, all women) received a single dose of drospirenone/ethinylestradiol 3 mg/0.02 mg on Day 1. Firsocostat 50 mg was administered once daily during Days 7–19 (13 days), and coadministered with a single dose of drospirenone/ethinylestradiol 3 mg/0.02 mg on Days 7 and 16. The final dose of firsocostat was administered in the morning on Day 19. There was a washout period between treatments during Days 2–6 (5 days).

A summary of cohorts and a treatment schematic are presented in Fig. 1. Participants were confined to the clinic from Day −1 until completion of assessments on Day 19 (Cohort 1), Day 24 (Cohort 2), or Day 20 (Cohorts 3 and 4). In all cohorts, study drugs were administered following an overnight fast (no food or drink except water) for at least

Treatment information

Cohort 1 (n = 30)	Cohort 2 (n = 21)	Cohort 3 (n = 13)	Cohort 4 (n = 16)
Firsocostat 20 mg (single dose) Cyclosporine A 600 mg (single dose) Rifampin 600 mg (single dose)	Firsocostat 20 mg (single dose) Probenecid 600 mg BID Voriconazole 200 mg BID	Midazolam 2 mg (single dose) Firsocostat 50 mg QD	Drospirenone/ethinylestradiol 3 mg/0.02 mg (single dose) Firsocostat 50 mg QD

Treatment schedule

Study day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Cohort 1	FIR	Washout					FIR+ CSA	Washout							FIR+ RIF								
	FIR						FIR+ RIF								FIR+ CSA								
	FIR+ CSA						FIR+ RIF								FIR								
	FIR+ CSA						FIR								FIR+ RIF								
	FIR+ RIF						FIR+ CSA								FIR								
	FIR+ RIF						FIR								FIR+ CSA								
Cohort 2	FIR	Washout					PBC	FIR+ PBC	PBC			Washout							VOR	FIR+ VOR	VOR		
Cohort 3	MDZ	Washout					MDZ+ FIR	FIR								MDZ+ FIR	FIR						
Cohort 4	DEE	Washout					DEE+ FIR	FIR								DEE+ FIR	FIR						

Fig. 1 Study schematic and treatment schedule. *BID* twice daily, *CSA* cyclosporin A, *DEE* drospirenone/ethinylestradiol, *FIR* firsocostat, *MDZ* midazolam, *PBC* probenecid, *QD* once daily, *RIF* rifampin, *VOR* voriconazole

10 h, and participants continued to fast until 4 h post-dose on days requiring pharmacokinetic sample collection and 2 h post-dose on all other days. Participants were restricted from consuming water from 1 h before until 2 h after study drug administration (with the exception of 240 mL of water given with the study drug).

2.4 Pharmacokinetic Sampling

Intensive pharmacokinetic sampling occurred before study drug administration (< 5 min) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, 72, and 96 h following the administration of each study drug on Days 1, 7, and 15 (Cohort 1); Days 1, 8, and 20 (Cohort 2); and Days 1, 7, and 16 (Cohorts 3 and 4).

2.5 Bioanalytical Procedures

Concentrations of firsocostat, midazolam, and drospirenone/ethinylestradiol in human plasma samples were determined using fully validated high-performance liquid chromatography-tandem mass spectroscopy bioanalytical methods. The assays were performed and validated by Covance Bioanalytical Laboratory Services, Inc. (Madison, WI, USA) for firsocostat, midazolam, and ethinylestradiol, and by InVentiv Health Clinical Labs, Inc. (Princeton, NJ, USA) for drospirenone. Validation met the expectations presented in the US Food and Drug Administration guidance for bioanalytical method validation [19]. All samples were analyzed within the time frame supported by frozen stability storage data.

2.6 Pharmacokinetic Analyses

Pharmacokinetic parameters were estimated with Phoenix WinNonlin (version 7.0; Certara, LP, Princeton, NJ, USA) using standard noncompartmental methods. Samples with concentrations below the limit of quantitation of the bioanalytical assays that occurred before achieving the first quantifiable concentration were assigned a concentration value of zero, and at all other time points were treated as missing data in the noncompartmental analyses. Pharmacokinetic parameters included area under the plasma concentration-time curve (AUC) from 0 to infinity (AUC_{∞}), maximum observed plasma concentration (C_{max}), time to maximal concentration (T_{max}), and terminal-phase elimination half-life ($t_{½}$).

2.7 Statistical Methods

The selected sample size required 24 evaluable participants in Cohort 1, 18 evaluable participants in Cohort 2, 10 evaluable participants in Cohort 3, and 13 evaluable participants in Cohort 4. The selected evaluable sample size was projected to achieve at least 78% probability for Cohort 1 (n = 24, 4 per treatment sequence), at least 80% probability for Cohort 2 (n = 18), at least 90% probability for Cohort 3 (n = 10), and more than 90% probability for Cohort 4 (n = 13), so that the 90% confidence interval (CI) for the geometric least-squares mean (GLSM) ratio of AUC_{∞} and C_{max} in the test (the victim drug administered with the perpetrator drug) versus reference (the victim drug administered alone) treatments would be between 0.70 and 1.43 if the true GLSM ratio was 1.0. For each cohort, analyte, and pharmacokinetic parameter, a parametric (normal theory) mixed-effects analysis of variance model was fitted to the natural log-transformed values of the single-dose pharmacokinetic parameter being evaluated using SAS PROC MIXED (SAS software, version 9.4; SAS Institute, Inc., Cary, NC, USA). The statistical model included treatment, sequence, and period as fixed effects, and participant within sequence as a random effect for Cohort 1, and treatment as a fixed effect and participant as a random effect for Cohorts 2, 3, and 4. The test versus reference ratio and associated 90% CI were calculated by taking the exponential of the point estimate and the corresponding lower and upper limits, which was consistent with the two one-sided tests approach.

2.8 Safety Assessments

Safety was monitored throughout the study and evaluated by assessment of clinical laboratory tests, ECGs, periodic physical examinations (including vital sign measurements), and documentation of adverse events (AEs). Clinical and laboratory AEs were coded using the Medical Dictionary for Regulatory Activities (version 21.0).

3 Results

3.1 Participant Demographics

Overall, 80 participants were enrolled and received at least one dose of study drug (Cohort 1 [n = 30], Cohort 2 [n = 21], Cohort 3 [n = 13], and Cohort 4 [n = 16]). One participant from Cohort 2 prematurely discontinued the study drug owing to participant decision (family emergency) and one participant from Cohort 4 prematurely discontinued the study drug owing to a positive pregnancy test on Day 8.

The mean (range) age of participants was 32 (19–45) years. Most participants were female (n = 41, 51%; Cohort 4 enrolled only female participants by design), White (n = 48, 60%), and non-Hispanic or Latino (n = 50, 63%). The mean (range) BMI was 25.6 (20.2–30.3) kg/m^2.

Table 1 Demographics and baseline characteristics

	Cohort 1 [$n = 30$]	Cohort 2 [$n = 21$]	Cohort 3 [$n = 13$]	Cohort 4 [$n = 16$]
Median age, years (range)	30 (20–45)	31 (19–43)	31 (21–37)	32 (23–42)
Sex				
Male	22 (73)	9 (43)	8 (62)	0
Female	8 (27)	12 (57)	5 (38)	16 (100)
Race				
Black	11 (37)	7 (33)	4 (31)	7 (44)
White	19 (63)	13 (62)	8 (62)	8 (50)
Asian	0	0	1 (8)	0
American Indian or Alaskan Native	0	0	0	1 (6)
Other	0	1 (5)	0	0
Ethnicity: hispanic or latino	10 (33)	11 (52)	5 (38)	4 (25)
Median BMI, kg/m^2 (range)	24.5 (20.2–29.4)	27.1 (20.3–30.3)	25.2 (20.2–28.4)	26.2 (22.4–29.9)

Data are expressed as *n* (%) unless otherwise specified

BMI body mass index

Demographics and baseline characteristics for each cohort are presented in Table 1.

3.2 Pharmacokinetics

3.2.1 Firsocostat as a Victim of Drug–Drug Interactions (DDIs)

Firsocostat mean plasma concentration versus time profiles following the administration of a single dose of firsocostat 20 mg and with a single dose of rifampin 600 mg, single dose of cyclosporine A 600 mg, multiple doses of probenecid 500 mg, or multiple doses of voriconazole 200 mg are displayed in Fig. 2. Corresponding firsocostat pharmacokinetic parameters and GLSM ratios (90% CIs) are shown in Table 2.

Firsocostat AUC$_\infty$ and C_{max} were approximately 19- and 30-fold higher, respectively, following administration of firsocostat with rifampin compared with firsocostat alone (Table 2; Fig. 2). Firsocostat AUC$_\infty$ and C_{max} were 22- and 20-fold higher, respectively, following administration of firsocostat with cyclosporine A compared with firsocostat alone (Table 2; Fig. 2).

Firsocostat AUC$_\infty$ and C_{max} were 63% and 60% higher, respectively, following administration of firsocostat with multiple doses of probenecid compared with firsocostat alone (Table 2; Fig. 2). Firsocostat AUC$_\infty$ and C_{max} were 38 and 45% higher, respectively, following administration of firsocostat with multiple doses of voriconazole compared with firsocostat alone (Table 2; Fig. 2).

3.2.2 Firsocostat as a Perpetrator of DDIs

Mean plasma concentration versus time profiles of probe substrates (midazolam and drospirenone/ethinylestradiol) when administered alone or coadministered with firsocostat are shown in Fig. 3. Pharmacokinetic parameters of probe substrates and corresponding GLSM ratios (90% CIs) are presented in Table 3.

No changes in midazolam exposure (AUC$_\infty$ and C_{max}) were observed when midazolam was coadministered with firsocostat compared with the administration of midazolam alone. The 90% CI of the GLSM ratios for midazolam AUC$_\infty$ on both Days 7 and 16 compared with Day 1 were within the strict equivalence boundaries of 0.80–1.25 (Table 3).

No changes in drospirenone exposure (AUC$_\infty$ and C_{max}) were observed when drospirenone/ethinylestradiol was coadministered with firsocostat compared with the administration of drospirenone/ethinylestradiol alone. The 90% CI of the GLSM ratios for drospirenone AUC$_\infty$ on both Days 7 and 16 compared with Day 1 were within the strict equivalence boundaries of 0.80–1.25.

Ethinylestradiol AUC$_\infty$ and C_{max} were 4 and 12% higher, respectively, on Day 7 compared with Day 1, and 34 and 21% higher, respectively, on Day 16 compared with Day 1 when drospirenone/ethinylestradiol was coadministered with firsocostat compared with administration of drospirenone/ethinylestradiol alone (Table 3). The 90% CI of the GLSM ratios for ethinylestradiol AUC$_\infty$ were within the strict equivalence boundaries of 0.80–1.25 on Day 7 compared with Day 1, but not on Day 16 compared with Day 1 (Table 3).

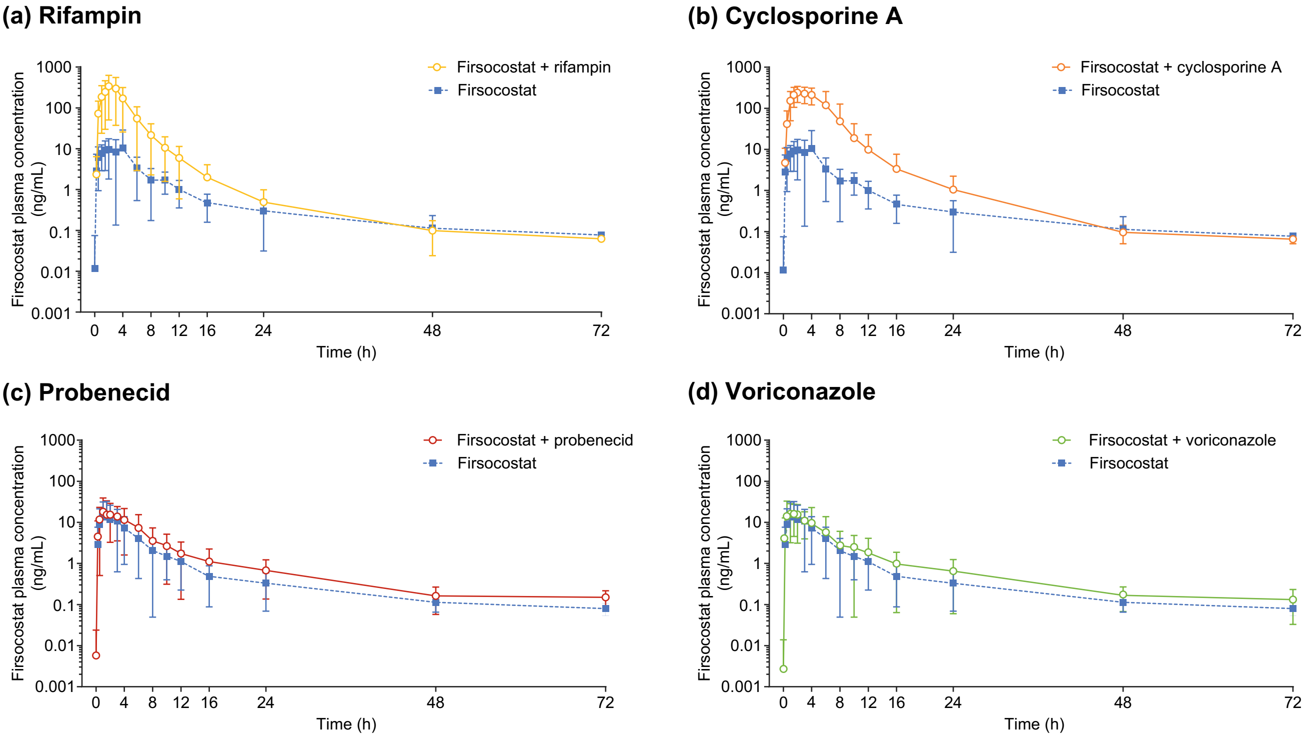

Fig. 2 Mean (±SD) plasma concentration of firsocostat with and without coadministration of **a** a single dose of rifampin, **b** a single dose of cyclosporine A, **c** multiple doses of probenecid, and **d** multiple doses of voriconazole over time. *SD* standard deviation

3.3 Safety

Study drugs were generally well tolerated. In total, 45/80 participants (56%) experienced at least one AE, and 10 participants (13%) experienced an AE that was assessed by the investigator to be related to a study drug. No Grade 3 or 4 AEs, serious AEs, or deaths were reported. A summary of reported AEs is presented in Table 4. The most common AEs were headache, nausea, flushing, diarrhea, dizziness, chromaturia, infrequent bowel movements, abdominal distension, and vomiting. All AEs were mild (Grade 1 in severity) except for AEs experienced by three participants in Cohort 1, who all had vomiting (Grade 2); two of these participants also experienced nausea (Grade 2) following the administration of firsocostat and cyclosporine A. Within Cohort 1, there was a higher incidence of AEs in participants who received firsocostat and cyclosporine A than those who received firsocostat alone or in combination with rifampin. Treatment-related AEs were reported for Cohort 1 (n = 5) and Cohort 4 (n = 5). No treatment-related AEs were reported for Cohorts 2 and 3, and no treatment-related AEs occurred during treatment with firsocostat alone. No notable changes in vital signs or clinically significant ECG abnormalities were reported.

4 Discussion

This phase I study evaluated the DDI profile of firsocostat as both a victim and a perpetrator of DDIs when administered with select victims or perpetrators of CYP enzymes and drug transporters. Firsocostat, in combination with cilofexor and semaglutide, is in clinical development for the treatment of MASH [7]. Characterizing the DDI profile of firsocostat is important to ensure safe administration with other medications in patients with MASH, a chronic disease with a high polypharmacy burden [20].

In this study, the effect of P-gp, OATP, UGT, and CYP3A inhibitors on the exposure of firsocostat (as a victim of DDI) were investigated, as firsocostat is a substrate for these transporters and metabolizing enzymes in vitro (data on file, Gilead Sciences, Inc.). The UGT inhibitor probenecid increased firsocostat AUC_{α} by approximately 60%, which indicates that UGT enzymes may partially contribute to the metabolism of firsocostat. Although probenecid inhibits several renal transporters (OAT1/OAT3/MRP2/MRP4) [21], firsocostat is not a substrate of renal transporters in vitro. Less than 1% of the firsocostat dose is eliminated unchanged in urine [22], which suggests that the observed small increase in firsocostat exposure with probenecid is likely mediated

Table 2 Pharmacokinetic parameters of firsocostat with and without coadministration of rifampin, cyclosporine A, probenecid, and voriconazole (Cohorts 1 and 2)

Effect of a single dose of rifampin on firsocostat (Cohort 1)			
Pharmacokinetic parameter	Firsocostat 20 mg + rifampin 600 mg [n = 30]	Firsocostat 20 mg [n = 30]	GLSM ratio (90% CI)
AUC_{α} [h·ng/mL]	1200 (53.8)	63.8 (56.6)	19.2 (16.5–22.3)
AUC_{last} [h·ng/mL]	1200 (53.9)	62.3 (58.1)	19.7 (16.9–23.0)
C_{max} [ng/mL]	489 (53.9)	19.3 (90.9)	29.5 (23.9–36.4)
T_{max} [h][a]	2.00 (1.52, 3.00)	2.00 (1.00, 3.00)	–
$t_{½}$ [h][a]	2.75 (2.60, 4.77)	7.01 (4.60, 10.5)	–
Effect of a single dose of cyclosporine A on firsocostat (Cohort 1)			
Pharmacokinetic parameter	Firsocostat 20 mg + cyclosporine A 600 mg [n = 30]	Firsocostat 20 mg [n = 30]	GLSM ratio (90% CI)
AUC_{α} [h·ng/mL]	1320 (53.5)	63.8 (56.6)	21.7 (18.7–25.2)
AUC_{last} [h·ng/mL]	1320 (53.6)	62.3 (58.1)	22.4 (19.2–26.1)
C_{max} [ng/mL]	313 (39.0)	19.3 (90.9)	20.3 (18.7–25.2)
T_{max} [h][a]	2.03 (2.00, 4.00)	2.00 (1.00, 3.00)	–
$t_{½}$ [h][a]	5.33 (3.92, 5.82)	7.01 (4.60, 10.5)	–
Effect of multiple doses of probenecid on firsocostat (Cohort 2)			
Pharmacokinetic parameter	Firsocostat 20 mg + probenecid 500 mg BID [n = 21]	Firsocostat 20 mg [n = 21]	GLSM ratio (90% CI)
AUC_{α} [h·ng/mL]	118 (61.2)	75.4 (80.4)	1.63 (1.45–1.82)
AUC_{last} [h·ng/mL]	115 (62.7)	73.5 (81.7)	1.62 (1.45–1.82)
C_{max} [ng/mL]	28.5 (68.7)	19.1 (94.9)	1.60 (1.32–1.95)
T_{max} [h][a]	2.00 (1.50, 3.00)	1.50 (1.07, 3.00)	–
$t_{½}$ [h][a]	14.8 (9.02, 20.1)	9.55 (6.20, 18.4)	–
Effect of multiple doses of voriconazole on firsocostat (Cohort 2)			
Pharmacokinetic parameter	Firsocostat 20 mg + voriconazole 200 mg BID [n = 21]	Firsocostat 20 mg [n = 21]	GLSM ratio (90% CI)
AUC_{α} [h·ng/mL]	103 (75.0)	75.4 (80.4)	1.38 (1.23–1.55)
AUC_{last} [h·ng/mL]	100 (77.0)	73.5 (81.7)	1.37 (1.23–1.54)
C_{max} [ng/mL]	26.8 (74.0)	19.1 (94.9)	1.45 (1.19–1.76)
T_{max} [h][a]	2.00 (1.00, 2.00)	1.50 (1.07, 3.00)	–
$t_{½}$ [h][a]	11.2 (8.27, 16.1)	9.55 (6.20, 18.4)	–

Unless otherwise stated, data are expressed as arithmetic means (%CV) rounded to 3 significant figures

%CV coefficient of variation, *AUC_{α}* area under the plasma concentration-time curve from 0 to infinity, *AUC_{last}* area under the plasma concentration-time curve from time zero to the last measurable concentration, *BID* twice daily, *CI* confidence interval, *C_{max}* maximum observed plasma concentration, *GLSM* geometric least-squares mean, *Q1* quartile 1, *Q3* quartile 3, $t_{½}$ terminal elimination half-life, *T_{max}* time to maximum concentration

[a]Data are expressed as median (Q1, Q3)

by UGT inhibition. Similarly, the strong CYP3A inhibitor voriconazole increased firsocostat AUC_{α} by approximately 40%, which suggests that CYP3A4 plays a minimal role in the metabolism of firsocostat. The small increases in exposure of firsocostat with CYP3A or UGT inhibition are not considered clinically relevant given all available safety and tolerability data for firsocostat from clinical trials to date (data on file, Gilead Sciences, Inc.). Firsocostat AUC_{α} increased by 22-fold following the coadministration of firsocostat with cyclosporine A (a mixed OATP/MRP2/P-gp inhibitor and a CYP3A4 inhibitor) and by 19-fold following the coadministration with rifampin (a strong OATP1B1/1B3

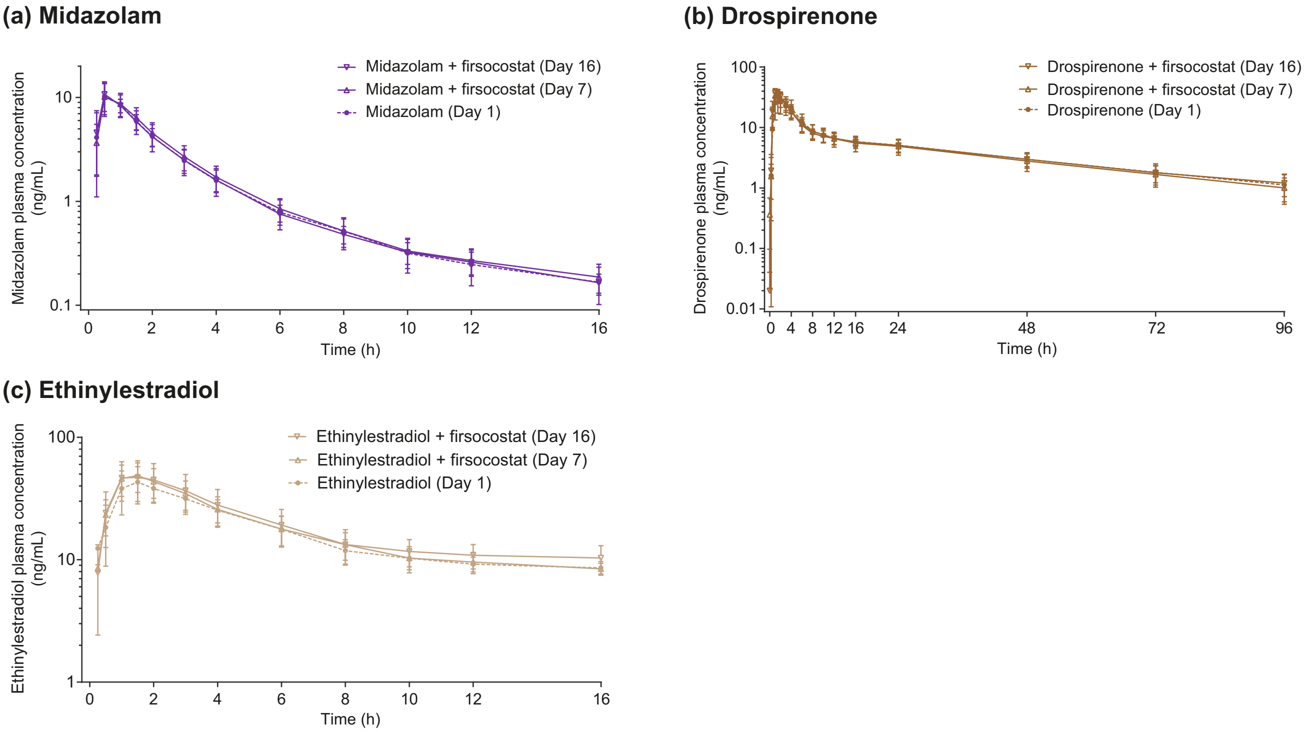

Fig. 3 Mean (± SD) plasma concentration of **a** midazolam, **b** drospirenone, and **c** ethinylestradiol over time, with and without coadministration of firsocostat. *SD* standard deviation

inhibitor). In addition to the high passive permeability of firsocostat, there were no observable differences in firsocostat T_{max} when administered with cyclosporine A. Therefore, intestinal P-gp inhibition is not likely to contribute to the observed interaction with cyclosporine A. These collective DDI data suggest that intestinal P-gp efflux may not play a significant role in the disposition of firsocostat and that firsocostat is highly sensitive to OATP inhibition. Results from this phase I study are consistent with a previous study that showed a 5-fold increase in firsocostat AUC_{∞} following coadministration of firsocostat with a lower dose of rifampin 300 mg [23]. Because the liver is a major site of firsocostat distribution, previous results suggest that the increase in firsocostat plasma exposure, primarily via OATP-mediated inhibition, is not expected to affect the hepatic exposure and pharmacodynamic effects (de novo lipogenesis) of firsocostat [23].

Preclinical data have suggested that firsocostat at higher than clinically relevant exposures may induce CYP3A (via PXR activation; data on file, Gilead Sciences, Inc.). This clinical study evaluated the perpetrator DDI effects of firsocostat on the exposure of the sensitive CYP3A substrate midazolam and a representative combined oral contraceptive drospirenone/ethinylestradiol (because drospirenone is the most sensitive progestin to CYP3A perpetration). The administration of firsocostat did not affect the exposure of midazolam or drospirenone. Although the increase in ethinylestradiol exposure when administered with firsocostat was outside the strict equivalence boundaries, the effect size was negligible and not clinically relevant. These suggest a lack of any clinically relevant effect of firsocostat on CYP3A.

The 20 mg dose of firsocostat used in the present study to evaluate the victim DDI potential of firsocostat is considered adequate because it is the same clinical dose of firsocostat under clinical evaluation in patients with MASH [7]. The 50 mg dose of firsocostat used to evaluate the DDI profile of firsocostat as a perpetrator is also considered adequate because it represents a worst case scenario (2.5-fold higher dose) of the firsocostat clinical dose under evaluation in patients with MASH [7]. The doses of all other interacting drugs investigated in this study are their approved therapeutic doses [24]. All perpetrator drugs, including firsocostat, were administered in multiple doses over a duration sufficient to reach steady state, allowing the observation of the maximum inhibition/induction effect. For rifampin, a single-dose administration was used to inhibit OATPs and to avoid induction associated with the administration of multiple doses of rifampin. Because

Table 3 Pharmacokinetic parameters of midazolam, drospirenone, and ethinylestradiol with and without coadministration of firsocostat (Cohorts 3 and 4)

Effect of multiple doses of firsocostat on midazolam (Cohort 3)

Pharmacokinetic parameter	Midazolam 2 mg Day 1 [n = 13]	Midazolam 2 mg + firsocostat 50 mg Day 7 [n = 13]	GLSM ratio (90% CI) Day 7/Day 1	Midazolam 2 mg + firsocostat 50 mg Day 16 [n = 13]	GLSM ratio (90% CI) Day 16/Day 1
AUC_α [h·ng/mL]	25.3 (27.3)	27.4 (20.1)	1.10 (0.995–1.22)	25.2 (17.5)	1.01 (0.915–1.12)
AUC_{last} [h·ng/mL]	24.1 (27.6)	26.0 (21.7)	1.10 (0.991–1.21)	24.1 (17.3)	1.02 (0.920–1.13)
C_{max} [ng/mL]	10.5 (29.7)	10.6 (28.2)	1.02 (0.917–1.15)	11.0 (27.7)	1.06 (0.949–1.19)
T_{max} [h][a]	0.50 (0.50, 0.52)	0.50 (0.50, 1.00)	–	0.50 (0.50, 0.50)	–
$t_{½}$ [h][a]	4.40 (3.70, 4.87)	5.65 (4.56, 7.35)	–	4.77 (4.40, 5.59)	–

Effect of multiple doses of firsocostat on drospirenone (Cohort 4)

Pharmacokinetic parameter	Drospirenone 3 mg Day 1 [n = 16]	Drospirenone 3 mg + firsocostat 50 mg Day 7 [n = 16]	GLSM ratio (90% CI) Day 7/Day 1	Drospirenone 3 mg + firsocostat 50 mg Day 16 [n = 15]	GLSM ratio (90% CI) Day 16/Day 1
AUC_α [h·ng/mL]	480 (25.4)	464 (27.4)	0.962 (0.864–1.07)	495 (22.5)	1.05 (0.938–1.17)
AUC_{last} [h·ng/mL]	421 (22.2)	404 (26.2)	0.949 (0.848–1.06)	430 (18.8)	1.03 (0.918–1.15)
C_{max} [ng/mL]	34.7 (23.4)	36.1 (25.0)	1.03 (0.915–1.16)	39.0 (15.8)	1.16 (1.02–1.31)
T_{max} [h][a]	1.52 (1.00, 3.00)	1.50 (1.01, 1.50)	–	1.07 (1.00, 1.52)	–
$t_{½}$ [h][a]	32.2 (27.9, 37.1)	30.4 (26.4, 37.7)	–	34.0 (28.2, 38.9)	–

Effect of multiple doses of firsocostat on ethinylestradiol (Cohort 4)

Pharmacokinetic parameter	Ethinylestradiol 0.02 mg Day 1 [n = 16]	Ethinylestradiol 0.02 mg + firsocostat 50 mg Day 7 [n = 16]	GLSM ratio (90% CI) Day 7/Day 1	Ethinylestradiol 0.02 mg + firsocostat 50 mg Day 16 [n = 15]	GLSM ratio (90% CI) Day 16/Day 1
AUC_α [h·ng/mL]	322 (33.3)	335 (32.5)	1.04 (0.89–1.21)	433 (37.1)	1.34 (1.14–1.56)
AUC_{last} [h·ng/mL]	235 (27.8)	257 (32.1)	1.07 (0.941–1.23)	308 (33.1)	1.31 (1.14–1.50)
C_{max} [ng/mL]	44.2 (31.9)	49.4 (27.0)	1.12 (1.04–1.22)	53.0 (23.4)	1.21 (1.11–1.31)
T_{max} [h][a]	1.50 (1.00, 1.79)	1.50 (1.05, 1.50)	–	1.50 (1.00, 1.50)	–
$t_{½}$ [h][a]	5.86 (4.32, 8.54)	5.86 (4.68, 8.05)	–	9.52 (4.79, 13.7)	–

Unless otherwise stated, data are expressed as arithmetic means (%CV) rounded to 3 significant figures

%CV coefficient of variation, AUC_α area under the plasma concentration–time curve from 0 to infinity, AUC_{last} area under the plasma concentration-time curve from time zero to the last measurable concentration, *CI* confidence interval, C_{max} maximum observed plasma concentration, *GLSM* geometric least-squares mean, *Q1* quartile 1, *Q3* quartile 3, $t_{½}$ terminal elimination half-life, T_{max} time to maximum concentration

[a]Data are expressed as median (Q1, Q3)

probenecid inhibits multiple UGT isoforms, the specific contribution of individual UGT isoform inhibition to the observed results cannot be determined in our DDI study.

Most observed AEs in the study were mild and no new safety signals were observed when firsocostat was administered with probe substrates and inhibitors of transporters and metabolizing enzymes. The higher incidence of AEs observed in Cohort 1 with firsocostat and cyclosporine A versus firsocostat alone may be consistent with the increased exposure of firsocostat with the combination treatment. However, the degree to which the AEs can be attributed to the combination treatment cannot be determined in the absence of data assessing the safety of cyclosporine A alone.

Table 4 Treatment-emergent AEs reported in at least two participants

	Cohort 1			Cohort 2			Cohort 3[a]		Cohort 4	
	FIR 20 mg [n = 30]	FIR 20 mg + CSA 600 mg [n = 30]	FIR 20 mg + RIF 600 mg [n = 30]	FIR 20 mg [n = 21]	FIR 20 mg + PBC 500 mg BID [n = 21]	FIR 20 mg + VOR 200 mg BID [n = 21]	MDZ 2 mg [n = 13]	MDZ 2 mg + FIR 50 mg [n = 13]	DPN 3 mg/EE 0.02 mg [n = 16]	DPN 3 mg/EE 0.02 mg + FIR 50 mg [n = 16]
Any AE	3 (10.0)	20 (66.7)	6 (20.0)	3 (14.3)	8 (38.1)	8 (38.1)	0	1 (7.7)	2 (12.5)	10 (62.5)
Abdominal discomfort	0	2 (6.7)	0	0	0	0	0	0	0	0
Abdominal distension	0	0	0	2 (9.5)	0	1 (4.8)	0	0	0	0
Abdominal pain	0	0	0	0	1 (4.8)	1 (4.8)	0	0	0	0
Acne	0	0	0	0	1 (4.8)	1 (4.8)	0	0	0	0
Anxiety	0	0	0	0	0	0	0	1 (7.7)	0	0
Chromaturia	0	0	4 (13.3)	0	0	0	0	0	0	0
Diarrhea	0	3 (10.0)	0	2 (9.5)	1 (4.8)	0	0	0	0	0
Dizziness	1 (3.3)	2 (6.7)	0	1 (4.8)	1 (4.8)	0	0	0	0	0
Flushing	0	8 (26.7)	0	0	0	0	0	0	0	0
Headache	0	8 (26.7)	0	1 (4.8)	1 (4.8)	0	0	0	0	7 (43.8)
Infrequent bowel movements	0	0	0	1 (4.8)	1 (4.8)	2 (9.5)	0	0	0	0
Menstruation irregular	0	0	0	0	1 (4.8)	1 (4.8)	0	0	0	0
Nausea	0	10 (33.3)	0	0	1 (4.8)	1 (4.8)	0	0	0	2 (12.5)
Photopsia	0	0	0	0	0	2 (9.5)	0	0	0	0
Presyncope	1 (3.3)	0	1 (3.3)	0	0	0	0	0	0	0
Vomiting	0	3 (10.0)	0	0	0	0	0	0	0	0

Data are expressed as n (%)

AEs adverse events, *BID* twice daily, *CSA* cyclosporin A, *DPN* drospirenone, *EE* ethinylestradiol, *FIR* firsocostat, *MDZ* midazolam, *PBC* probenecid, *RIF* rifampin, *VOR* voriconazole

[a]Only one AE was reported in Cohort 3 during midazolam 2 mg + firsocostat 50 mg treatment

5 Conclusion

The findings from this study suggest that firsocostat should not be administered with strong inhibitors of OATP, such as rifampin and cyclosporine A. Firsocostat can be administered with P-gp, UGT, and CYP3A4 inhibitors without dose modification based on the observed minimal impacts on firsocostat exposure. CYP3A substrates and combined oral contraceptives can be administered with firsocostat without any dose modifications.

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Declarations

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Conflict of interest Elijah J. Weber, Islam R. Younis, Cara Nelson, and Ahmed A. Othman were employees of Gilead Sciences, Inc. at the time of this work and may own stock in Gilead Sciences, Inc. Ann R. Qin and Timothy R. Watkins are employees of, and may own stock in, Gilead Sciences, Inc.

Ethics approval The study protocol was reviewed and approved by an IRB (Salus IRB; 2111 West Braker Lane, Suite 400, Austin, TX 78758, USA).

Consent to participate All participants provided written informed consent before study participation.

Consent for publication All authors approved this manuscript for publication.

Availability of data Gilead Sciences shares anonymized individual patient data upon request, or as required by law or regulation, with qualified external researchers based on submitted curriculum vitae and reflecting non-conflicts of interest. The request proposal must also include a statistician. Approval of such requests is at Gilead Science's discretion and is dependent on the nature of the request, the merit of the research proposed, the availability of the data, and the intended use of the data. Data requests should be sent to datarequest@gilead.com.

Author contributions CN and ARQ designed the study. EJW, IRY, CN, ARQ, TRW, and AAO analyzed and interpreted the data and drafted the manuscript. All authors revised and approved the final version of the manuscript submitted for publication.

References

1. Ekstedt M, Hagström H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61:1547–54.
2. Armandi A, Bugianesi E. Natural history of NASH. Liver Int. 2021;41(Suppl 1):78–82.
3. Henry L, Paik J, Younossi ZM. Review article: the epidemiologic burden of non-alcoholic fatty liver disease across the world. Aliment Pharmacol Ther. 2022;56:942–56.
4. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34:274–85.
5. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140:124–31.
6. Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2022;7:851–61.
7. Alkhouri N, Herring R, Kabler H, et al. Safety and efficacy of combination therapy with semaglutide, cilofexor and firsocostat in patients with non-alcoholic steatohepatitis: a randomised, open-label phase II trial. J Hepatol. 2022;77:607–18.
8. Stiede K, Miao W, Blanchette HS, et al. Acetyl-coenzyme A carboxylase inhibition reduces de novo lipogenesis in overweight male subjects: a randomized, double-blind, crossover study. Hepatology. 2017;66:324–34.
9. Loomba R, Kayali Z, Noureddin M, et al. GS-0976 reduces hepatic steatosis and fibrosis markers in patients with nonalcoholic fatty liver disease. Gastroenterology. 2018;155:1463-73.e6.
10. Westlin W, Harriman G, Harwood H, et al. NDI-010976, a potent, liver-directed, oral inhibitor of acetyl Coa carboxylase for non-alcoholic steatohepatitis: a phase 1 single ascending dose study in healthy volunteers. J Hepatol. 2016;64:S501–2.
11. Weber E, Younis IR, Nelson C, et al. Effect of renal impairment on the pharmacokinetics of firsocostat, an acetyl-coenzyme A carboxylase inhibitor, and cilofexor, a selective nonsteroidal farnesoid X receptor agonist. J Clin Pharmacol. 2023;63:560–8.
12. Hsueh C-H, Qin A, West S, Chung C, Othman A. A phase 1 mass balance study of firsocostat, a liver-targeted acetyl-CoA carboxylase inhibitor. Clin Pharmacol Ther. 2022;111:S24.
13. Tornio A, Filppula AM, Niemi M, Backman JT. Clinical studies on drug–drug interactions involving metabolism and transport: methodology, pitfalls, and interpretation. Clin Pharmacol Ther. 2019;105:1345–61.
14. Yang Y, Li P, Zhang Z, et al. Prediction of cyclosporin-mediated drug interaction using physiologically based pharmacokinetic model characterizing interplay of drug transporters and enzymes. Int J Mol Sci. 2020;21:7023.
15. Uchaipichat V, Mackenzie PI, Guo X-H, et al. Human UDP-glucuronosyltransferases: isoform selectivity and kinetics of 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. Drug Metab Dispos. 2004;32:413–23.
16. Czyrski A, Resztak M, Swiderski P, Brylak J, Glowka FK. The overview on the pharmacokinetic and pharmacodynamic interactions of triazoles. Pharmaceutics. 2021;13:1961.
17. Krupka E, Venisse N, Lafay C, et al. Probe of CYP3A by a single-point blood measurement after oral administration of midazolam in healthy elderly volunteers. Eur J Clin Pharmacol. 2006;62:653–9.
18. Ayala RC, Arya V, Younis IR. Design features of drug–drug interaction trials between antivirals and oral contraceptives. J Clin Pharmacol. 2016;56:541–7.
19. Bioanalytical method validation. Guidance for Industry. United States Food and Drug Administration; 2018. Available at: https://www.fda.gov/regulatory-information/search-fda-guida

nce-documents/bioanalytical-method-validation-guidance-industry. Accessed 28 Aug 2023.
20. Patel PJ, Hayward KL, Rudra R, et al. Multimorbidity and polypharmacy in diabetic patients with NAFLD: implications for disease severity and management. Medicine (Baltimore). 2017;96: e6761.
21. Weiss HM, Langenickel T, Cain M, et al. Clinical investigation of metabolic and renal clearance pathways contributing to the elimination of fevipiprant using probenecid as perpetrator. Drug Metab Dispos. 2021;49:389–94.
22. Devineni D, Vaccaro N, Murphy J, et al. Effects of rifampin, cyclosporine A, and probenecid on the pharmacokinetic profile of canagliflozin, a sodium glucose co-transporter 2 inhibitor, in healthy participants. Int J Clin Pharmacol Ther. 2015;53:115–28.
23. Kirby BJ, Lutz JD, Yue MS, et al. Organic anion transporting polypeptide inhibition dramatically increases plasma exposure but not pharmacodynamic effect nor inferred hepatic intracellular exposure of firsocostat. Clin Pharmacol Ther. 2021;109:1334–41.
24. Drugs. United States Food and Drug Administration. Available at: https://www.fda.gov/drugs. Accessed 20 Sep 2023.

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