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Translation of In Vitro Transport Inhibition Studies to Clinical Drug-Drug Interactions for Glecaprevir and Pibrentasvir[S]

Matthew P. Kosloski, Daniel A.J. Bow, Ryota Kikuchi, Haoyu Wang, Elaine J. Kim, Kennan Marsh, Federico Mensa, Jens Kort, and Wei Liu

AbbVie Inc., North Chicago, Illinois

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ABSTRACT

Glecaprevir and pibrentasvir are oral direct-acting antiviral agents approved in combination for treatment of chronic hepatitis C viral infection. In vitro studies identified the combination as potentially clinically relevant inhibitors of the efflux transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and the hepatic uptake transporters organic anion transporting polypeptide (OATP) 1B1 and OATP1B3. Glecaprevir inhibited P-gp, BCRP, OATP1B1, and OATP1B3 with IC_{50} values of 0.33, 2.3, 0.017, and 0.064 μM, respectively. Pibrentasvir inhibited P-gp, BCRP, and OATP1B1 with IC_{50} values of 0.036, 14, and 1.3 μM, respectively. Neither agent inhibited organic cation transporter (OCT) 1, OCT2, organic anion transporter (OAT) 1, OAT3, multidrug and toxin extrusion (MATE) 1, or MATE2K. Open-label phase 1 clinical drug-drug interaction studies were conducted in healthy subjects to evaluate interaction potential of glecaprevir/pibrentasvir and coadministered selective substrates for P-gp

(digoxin, dabigatran etexilate, and sofosbuvir), BCRP (rosuvastatin and sofosbuvir), and OATP1B1/3 (pravastatin and rosuvastatin). The pharmacokinetic maximum plasma concentration (C_{max}) and area under the concentration-time curve (AUC) parameters were evaluated for probe substrates alone and in combination with glecaprevir/pibrentasvir. The C_{max} central values increased by 72%, 105%, 123%, 462%, and 66% for digoxin, dabigatran, pravastatin, rosuvastatin, and sofosbuvir, respectively, and the AUC central values increased by 48%, 138%, 130%, 115%, and 125% for digoxin, dabigatran, pravastatin, rosuvastatin, and sofosbuvir, respectively. Exposure of sofosbuvir metabolite GS-331007 (nucleoside analog) was similar with or without glecaprevir/pibrentasvir. The outcomes of the clinical drug-drug interaction studies confirmed clinically relevant inhibition of P-gp, BCRP, and OATP1B1/3, and were used to provide dosing guidance for the concomitant use of glecaprevir/pibrentasvir with relevant transporter substrates.

Introduction

The adoption of direct-acting antiviral agents (DAAs) has transformed the clinical treatment of chronic hepatitis C virus (HCV) infection. Long the standard of care, pegylated interferon and ribavirin have been replaced with all-oral DAA combination regimens offering increased efficacy, better tolerability and safety, and shorter treatment durations. These DAA regimens consist of two or more inhibitors of hepatitis C nonstructural protein (NS) 3/4A protease, NS5A, and NS5B polymerase and achieve sustained virological response rates of ≥95% across the six common HCV genotypes with as short as 8 weeks of treatment (Bourlière and Pietri, 2019). The

fixed-dose combination of glecaprevir (formerly ABT-493), a NS3/4A protease inhibitor, and pibrentasvir (formerly ABT-530), a NS5A inhibitor, is approved as a once-daily regimen for the treatment of chronic HCV infection with HCV genotypes 1–6 (Mavyret, 2019). Both agents exhibit a high barrier to development of resistance and maintain potent antiviral activity against common HCV variants that confer resistance to NS3/4A protease and NS5A inhibitors (Ng et al., 2017, 2018). Glecaprevir and pibrentasvir are 97.5% and >99.9% bound to plasma proteins, respectively (Gane et al., 2017; Mavyret, 2019). Metabolism by CYP3A plays a secondary role in the disposition of glecaprevir and <1% of drug is recovered unchanged in the urine. Pibrentasvir is not metabolized or eliminated in urine.

Chronic HCV infection is frequently complicated by comorbid conditions (Louie et al., 2012) that require therapeutic interventions. Review of a real-world HCV-infected patient population found 31%–66% of the patients are at risk for potentially clinically significant drug-drug interactions between their concomitant medications and HCV DAA regimens containing a NS3/4A protease inhibitor and/or NS5B inhibitor

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ABBREVIATIONS: AUC, area under the concentration-time curve; AUC_{0-24}, area under the concentration-time curve from time 0 to 24 hours after dosing; BCRP, breast cancer resistance protein; BSA, bovine serum albumin; DAA, direct-acting antiviral agent; DDI, drug-drug interaction; HCV, hepatitis C virus; I_{gut}, theoretical drug concentration in the gastrointestinal tract; $I_{in,max}$, estimated maximum plasma inhibitor concentration at the inlet to the liver; $I_{max,u}$, maximum unbound systemic plasma concentration of inhibitor; MATE, multidrug and toxin extrusion; NS, nonstructural protein; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; P-gp, P-glycoprotein.

(Höner Zu Siederdissen et al., 2016). Current DAA regimens are often associated with clinically relevant inhibition of intestinal efflux and/or hepatic uptake drug transporters. Potential drug-drug interactions may be significant enough to require dose adjustment or use of alternative therapies, and particular attention must be given to the clinical management of such interactions (Back and Else, 2013; Serfaty, 2013).

P-glycoprotein (P-gp) is an efflux transporter expressed in a variety of tissues including the apical membrane of intestinal epithelial cells, renal proximal tubular cells, brain capillary endothelial cells, and the canalicular membrane of hepatocytes (Giacomini et al., 2010). Efflux across the apical membrane of intestinal epithelium regulates oral bioavailability of many P-gp substrates, and due to high local drug concentrations in intestine following dissolution of oral dosage forms is a frequent site of P-gp–related drug-drug interactions. Digoxin (a cardiac glycoside), dabigatran etexilate (an oral direct thrombin inhibitor), and sofosbuvir (a HCV NS5B inhibitor) are all sensitive substrates of P-gp (Greiner et al., 1999; Härtter et al., 2012; Kirby et al., 2015); dabigatran etexilate is also selective for P-gp localized in the intestine due to rapid conversion to the non-P-gp substrate dabigatran following absorption (Chu et al., 2018). Breast cancer resistance protein (BCRP) is another efflux transporter with substantial overlap in localization and function to P-gp (Giacomini et al., 2010). No selective probes of BCRP activity are available for the evaluation of clinical drug-drug interaction potential, but rosuvastatin is often used as a probe for BCRP as well as the hepatic uptake transporters organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 (Elsby et al., 2012). Sofosbuvir is also a substrate of BCRP in addition to P-gp. Cumulative evidence demonstrates the importance of OATP1B1/3 in the disposition of a variety of clinically used drugs—notably including statins (Giacomini et al., 2010). Pravastatin is a selective substrate of OATP1B1 and OATP1B3 (Elsby et al., 2012). In addition to utility as probe mechanistic substrates for P-gp, BCRP, and OATP transporters, clinical drug-drug interaction studies help formulate appropriate dosing recommendations to manage potential safety risks associated with elevated concentrations of studied drugs and other substrates of these transporters.

In vitro studies in membrane vesicles and transporter overexpressing cell lines were conducted to evaluate the inhibition potential of glecaprevir and pibrentasvir for clinically relevant transporters, namely, P-gp, BCRP, OATP1B1/3, organic cation transporter (OCT) 1, OCT2, organic anion transporter (OAT) 1, OAT3, multidrug and toxin extrusion (MATE) 1, and MATE2K. The results of in vitro studies were used to predict clinical drug-drug interaction (DDI) potential of glecaprevir/pibrentasvir, which were then confirmed in a series of clinical studies conducted in healthy volunteers with digoxin, dabigatran etexilate, sofosbuvir, rosuvastatin, and pravastatin.

Materials and Methods

In Vitro Materials

Glecaprevir and pibrentasvir were synthesized by AbbVie Inc. (North Chicago, IL); working stock solutions were prepared in dimethyl sulfoxide. All other materials were purchased from commercial sources unless stated otherwise.

In Vitro Study Design

Inhibition of P-gp and BCRP. Inhibition of P-gp and BCRP by glecaprevir or pibrentasvir was explored via evaluation of transport for known probe substrates in isolated membrane vesicles expressing the efflux transporter of interest. Transporter assay kits (P-gp and BCRP) were obtained from Solvo Biotechnology (Budapest, Hungary). Assays were conducted according to the kit instructions. P-gp and BCRP probes were [³H]N-methylquinidine and Lucifer yellow, respectively. The final concentration of the substrates and the incubation times are summarized in Supplemental Table 1. ATP-dependent transport was calculated as the difference in values in the presence of AMP or ATP. Inhibition constants (IC_{50}) for glecaprevir or pibrentasvir were obtained by nonlinear least-squares fitting of log(inhibitor) versus normalized ATP-dependent transport of the reference substrate using a variable slope model in GraphPad Prism (La Jolla, CA). The IC_{50} values are presented as the geometric mean of two independent experiments.

Inhibition of OATP1B1/3, OCT1/2, OAT1/3, and MATE1/2K. Inhibition assessment for OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2K was conducted as described previously (Kikuchi et al., 2017). The final concentration of the probe substrates and incubation times are summarized in Supplemental Table 1. The inhibitory effect of the test compound on the uptake of the reference substrates is presented as the percentage of inhibition of the control. The IC_{50} values were determined assuming competitive inhibition in GraphPad Prism. Owing to excessive binding of pibrentasvir to plasma proteins, inhibition studies were conducted in the presence of 4% weight per volume of bovine serum albumin (BSA) to determine transporter inhibition under semiphysiologic conditions in addition to the conventional protein-free condition. The IC_{50} values are presented as the geometric mean of two independent experiments.

Prediction of Transporter Inhibition. Predictions of clinical drug-drug interaction with transporters were made according to the Food and Drug Administration's guidance in vitro metabolism- and transporter- mediated DDI studies (https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf). The pharmacokinetic parameters used in the calculation of the interaction potential are listed in Supplemental Table 2. For the prediction of P-gp and BCRP inhibition, $I_{gut}/IC_{50} > 10$ was considered potentially clinically relevant, where I_{gut} is the theoretical drug concentration in the gastrointestinal tract (dose/250 ml). The R-free and R-total values for the prediction of in vivo OATP1B1, OATP1B3, and OCT1 inhibition were calculated using the IC_{50} values obtained in the absence of BSA (R-free) or presence of 4% BSA (R-total) by the following equations (Kikuchi et al., 2017):

$$R - \text{free} = 1 + \frac{f_{u,p} \times I_{in,max}}{IC_{50}} \qquad (1)$$

$$R - \text{total} = 1 + \frac{I_{in,max}}{IC_{50,total}} \qquad (2)$$

$$I_{in,max} = I_{max} + \frac{F_a F_g \times k_a \times \text{Dose}}{Q_h \times R_B} \qquad (3)$$

where $f_{u,p}$ is the fraction of unbound inhibitor in plasma; $I_{in,max}$ is the estimated maximum plasma inhibitor concentration at the inlet to the liver; I_{max} is the maximum systemic plasma concentration of inhibitor; $F_a F_g$ is the fraction of the dose of inhibitor that is absorbed; k_a is the absorption rate constant of the inhibitor; Dose is the inhibitor dose; Q_h is the hepatic blood flow (97 L/h per 70 kg); and R_B is the blood-to-plasma concentration ratio. Values of $R \geq 1.1$ were considered potentially clinically relevant (https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf).

For the prediction of OCT2, OAT1, OAT3, MATE1, and MATE2K inhibition, $I_{max,u}/IC_{50}$ values were calculated, where $I_{max,u}$ is the maximum unbound systemic plasma concentration of inhibitor; $I_{max,u}/IC_{50} > 0.1$ (OCT2, OAT1, and OAT3) and $I_{max,u}/IC_{50} > 0.02$ (MATE1 and MATE2K) were considered potentially clinically relevant.

Clinical Study Design

Study Participants and Designs. Open-label, phase 1 clinical studies were conducted under Good Clinical Practice Guidelines and ethical principles that have their origin in the Declaration of Helsinki. All studies were conducted at the AbbVie Clinical Pharmacology Research Unit. Study protocols and amendments were approved by the Vista Health System institutional review boards (Waukegan, IL). A signed informed consent was obtained from each subject.

Adult male and female subjects between the ages of 18 and 55 years, with a body mass index between 18 and 30 kg/m² and in general good health, were enrolled. Female subjects were of nonchildbearing potential. The use of medications that might have inhibited or induced metabolic enzymes and drug transporters of interest was prohibited 1 month prior to dosing of study drugs through study completion. Twelve subjects participated in each of the digoxin, dabigatran etexilate, pravastatin, and rosuvastatin studies and 16 subjects participated in the sofosbuvir study. The study design schematics are depicted in Fig. 1. All study drugs were administered orally in the morning 30 minutes after a standardized breakfast each day.

Digoxin study. Single doses of digoxin 0.5 mg were administered in period 1 on day 1 and in period 2 on day 8. Glecaprevir 400 mg and pibrentasvir 120 mg were administered once a day in period 2 on days 1–12. The first dose in periods 1 and 2 was separated by a 10-day washout. Blood samples were collected for pharmacokinetic assessment of digoxin plasma concentrations up to 120 hours after doses in period 1 on day 1 (alone) and period 2 on day 8 (coadministered) and for glecaprevir and pibrentasvir plasma concentrations up to 24 hours after doses in period 2 on day 7 (alone) and day 8 (coadministered). Urine was collected for digoxin analysis up to 120 hours after doses in period 1 on day 1 and period 2 on day 8.

Dabigatran etexilate study. Single doses of dabigatran etexilate mesylate (dabigatran etexilate 150 mg equivalent) were administered on days 1 and 11. Glecaprevir 300 mg and pibrentasvir 120 mg were administered once a day on days 4–13. Blood samples were collected for pharmacokinetic assessment of dabigatran plasma concentrations up to 72 hours after doses on day 1 (alone) and day 11 (coadministered) and for glecaprevir and pibrentasvir plasma concentrations up to 24 hours after doses on day 10 (alone) and day 11 (coadministered).

Pravastatin study. Multiple doses of pravastatin 10 mg once a day were administered in period 2 on days 1–3 and period 3 on days 1–7. Glecaprevir 400 mg and pibrentasvir 120 mg were administered as a single dose in period 1 on day 1 and once a day in period 3 on days 1–7. The first doses in periods 1 and 2 were separated by an 8-day washout interval. No washout interval separated periods 2 and 3. Blood samples were collected for pharmacokinetic assessment of pravastatin plasma concentrations up to 24 hours after doses in period 2 on day 3 (alone) and period 3 on day 7 (coadministered) and for glecaprevir and pibrentasvir plasma concentrations up to 24 hours after doses in period 1 on day 1 (alone) and period 3 on day 1 (coadministered).

Rosuvastatin study. Multiple doses of rosuvastatin 5 mg once a day were administered in period 2 on days 1–7 and period 3 on days 1–7. Glecaprevir 400 mg and pibrentasvir 120 mg were administered as a single dose in period 1 on day 1 and once a day in period 3 on days 1–7. The first dose in periods 1 and 2 was separated by an 8-day washout interval. No washout interval separated periods 2 and 3. Blood samples were collected for pharmacokinetic assessment of rosuvastatin plasma concentrations up to 24 hours after doses in period 2 on day 7 (alone) and period 3 on day 7 (coadministered) and for glecaprevir and pibrentasvir plasma concentrations up to 24 hours after doses in period 1 on day 1 (alone) and period 3 on day 1 (coadministered).

Sofosbuvir study. This study consisted of two cohorts. In cohort 1, multiple doses of glecaprevir 400 mg and pibrentasvir 120 mg once a day were administered on days 1–7 of period 1 and then sofosbuvir 400 mg was administered with glecaprevir 400 mg and pibrentasvir 120 mg once a day on days 1–7 of period 2. In cohort 2, multiple doses of sofosbuvir 400 mg once a day were administered on days 1–7 of period 1 and then sofosbuvir 400 mg was administered with glecaprevir 400 mg and pibrentasvir 120 mg once a day on days 1–7 of period 2. Blood samples were collected for pharmacokinetic assessment of sofosbuvir and GS-331007 (nucleoside analog) plasma concentrations at up to 24 hours after dosing in period 1 on day 7 (alone) and period 2

Single-dose substrate design (digoxin)

Period 1		Period 2		
Day 1	Washout	Days 1 - 7	Day 8	Days 9 – 12
Digoxin			Digoxin	
		Glecaprevir + Pibrentasvir QD		

Single-dose substrate design (dabigatran)

Day 1	Days 2 to 3	Days 4 to 10	Day 11	Days 12 to 13
Dabigatran etexilate			Dabigatran etexilate	
		Glecaprevir + Pibrentasvir QD		

Multiple-dose substrate design (pravastatin, rosuvastatin)

Period 1		Period 2	Period 3
Day 1	Washout	Days 1 to 3 or 7[a]	Days 1 to 7
		Pravastatin QD or Rosuvastatin QD	
Glecaprevir + Pibrentasvir			Glecaprevir + Pibrentasvir QD

Multiple-dose substrate design (sofosbuvir)

Cohort	Period 1 (Days 1 to 7)	Period 2 (Days 1 to 7)
1		Sofosbuvir QD
	Glecaprevir + Pibrentasvir QD	
2	Sofosbuvir QD	
		Glecaprevir + Pibrentasvir QD

a. Doses were administered in Period 2 for 3 days (pravastatin) or 7 days (rosuvastatin)
QD = once daily

Fig. 1. Clinical DDI study designs used to evaluate interactions of glecaprevir and pibrentasvir with digoxin, dabigatran etexilate, pravastatin, rosuvastatin, and sofosbuvir.

on day 7 (coadministered) in cohort 2 and for glecaprevir and pibrentasvir plasma concentrations up to 24 hours after doses in period 1 on day 7 (alone) and period 2 on day 7 (combination) in cohort 1.

Sample Analysis. Plasma concentrations of all drugs were determined using validated liquid chromatography methods with tandem mass spectrometric detection. The lower limit of quantitation in plasma was established at ≤0.205 ng/ml (glecaprevir and pibrentasvir across studies), 1.00 ng/ml (total dabigatran), 0.0100 ng/ml (digoxin), 0.500 ng/ml (pravastatin), 0.100 ng/ml (rosuvastatin), 5 ng/ml (sofosbuvir), and 10 ng/ml (GS-331007). The lower limit of quantitation for digoxin in urine was 2.00 ng/ml. Drug concentrations below the respective lower limit of quantitation were reported as zero. A summary of the validated analytical methods is provided in Supplemental Table 3.

Pharmacokinetic and Statistical Analyses. For each analyte, where applicable, the maximum observed plasma concentration (C_{max}), the time to C_{max}, the predose trough concentration, and the trough concentration 24 hours after dosing were determined directly from the concentration-time data. Pharmacokinetic parameters including the area under the concentration-time curve (AUC) from time 0 to 24 hours after dosing (AUC_{0-24}), AUC from time 0 to the last measurable concentration, AUC from time 0 to infinity, terminal elimination half-life in plasma, the fraction of unchanged drug excreted in urine, and renal clearance were calculated via noncompartmental methods.

Pharmacokinetic and statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC). To assess the effect of coadministration with glecaprevir and pibrentasvir on each of the interacting medications, a repeated measures analysis was performed for the natural logarithms of the C_{max}, AUC, and/or the trough concentration 24 hours after dosing. Central value ratios and 90% confidence intervals were calculated. Repeated measures analyses were performed to assess the effect of coadministration with substrate drugs on glecaprevir and pibrentasvir C_{max}, AUC_{0-24}, and the trough concentration 24 hours after dosing. Pharmacokinetic parameters were considered similar with and without concomitant drugs if central value ratios were ≥0.80 and ≤1.25.

Safety and Tolerability Assessments. Safety and tolerability throughout each study were assessed via adverse event monitoring, laboratory tests, and vital signs measurements.

Results

In Vitro Study Results

In Vitro Inhibition of Drug Transporters. In vitro IC_{50} values for glecaprevir and pibrentasvir and the associated inhibition profiles are given in Table 1 and shown in Supplemental Fig. 1. Glecaprevir inhibited P-gp, BCRP, OATP1B1, and OATP1B3 with IC_{50} values of 0.33, 2.3, 0.017, and 0.064 μM respectively. Glecaprevir showed less than 50% inhibition of OCT2, OAT1, and OAT3 at 100 μM and less than 50% inhibition of OCT1, MATE1, and MATE2K at 30 μM; IC_{50} values for these transporters are considered as >100 μM (OCT2, OAT1, and OAT3) and >30 μM (OCT1, MATE1, and MATE2K). Pibrentasvir inhibited P-gp and BCRP with IC_{50} values of 0.036 and 14 μM, respectively. Pibrentasvir inhibited OATP1B1 in the absence of BSA in a concentration-dependent manner up to 3 μM; however, further inhibition was not observed at higher concentrations (30 and 300 μM). An IC_{50} value of 1.3 μM for pibrentasvir against OATP1B1 was determined when the assay was conducted in the presence of 4% BSA to mimic physiologic conditions. Pibrentasvir showed less than 50% inhibition at the concentrations tested (3 and 30 μM) for OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2K in the absence and presence of 4% BSA; IC_{50} values for these transporters are considered to be >30 μM.

Prediction of Transporter Inhibition. Inhibition of intestinal efflux transporters (P-gp and BCRP) was predicted based on the theoretical concentrations (dose/250 ml) for both glecaprevir and pibrentasvir in the gastrointestinal tract: values of $I_{gut}/IC_{50} > 10$ (Table 1). Using anticipated exposure of glecaprevir for the glecaprevir/pibrentasvir 300/120 mg dose combination in HCV-infected subjects, unbound drug exposure in the hepatic portal vein was estimated to be sufficient to inhibit OATP1B1 and OATP1B3: values of $R \geq$ 1.1 (Table 1). As for pibrentasvir, inhibition of OATP1B1, but not OATP1B3, was predicted using the IC_{50} value determined in the presence of 4% BSA: R-total = 1.4 (Table 1). Glecaprevir and pibrentasvir are not predicted to inhibit OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2K.

Clinical Study Results

Subject Demographics and Disposition. Sixty-four subjects received at least one dose of the study drugs across the studies. Of these 64 subjects, 83% (53/64) were male, 55% (35/64) were white, 42% (27/64) were black, and 5% (4/76) were of other races. Subjects ranged from 19 to 54 years of age and from 50 to 107 kg in body weight. Subjects enrolled in the

TABLE 1

In vitro inhibition of clinically relevant transporters

Glecaprevir: I_{gut} = 1430 μM, $I_{in,max}$ = 3.0 μM, and $I_{max,u}$ = 0.018 μM; Pibrentasvir: I_{gut} = 431 μM, $I_{in,max}$ = 0.56 μM, and $I_{max,u}$ = 0.00099 μM.

Transporter	Glecaprevir				Pibrentasvir			
	IC_{50}	I_{gut}/IC_{50}	R value	$I_{max,u}/IC_{50}$	IC_{50}	I_{gut}/IC_{50}	R value	$I_{max,u}/IC_{50}$
	μM				μM			
P-gp	0.33	4335	—	—	0.036	11,978	—	—
BCRP	2.3	622	—	—	14	31	—	—
OATP1B1	0.017	—	5.5	—	1.3[a]	—	1.4[b]	—
OATP1B3	0.064	—	2.2	—	>30	—	<1.1	—
OCT1	>30	—	<1.1	—	>30	—	<1.1	—
OCT2	>100	—	—	<0.1	>30	—	—	<0.1
OAT1	>100	—	—	<0.1	>30	—	—	<0.1
OAT3	>100	—	—	<0.1	>30	—	—	<0.1
MATE1	>30	—	—	<0.02	>30	—	—	<0.02
MATE2K	>30	—	—	<0.02	>30	—	—	<0.02

—, not applicable.
[a]Value determined in the inhibition studies in 4% BSA.
[b]R-total value.

digoxin, pravastatin, and sofosbuvir studies completed all study activities. One subject who withdrew consent in the dabigatran study and one subject who discontinued the rosuvastatin study after a panic attack were excluded from pharmacokinetic analyses. One subject discontinued the study drug in the dabigatran study prior to receiving the final dose of glecaprevir and pibrentasvir, but was included in all analyses.

Pharmacokinetics. Complete pharmacokinetic parameter data for glecaprevir, pibrentasvir, probe substrates, and coadministered drugs when administered alone or in combination are given in Table 2 and discussed subsequently. Concentration-time profiles in plasma are presented for probe substrates in Fig. 2 and for glecaprevir and pibrentasvir in Fig. 3. Central value ratios for the probe substrates when administered with glecaprevir and pibrentasvir relative to administration alone are summarized in Fig. 4.

Digoxin (P-gp). The C_{max} and AUC from time 0 to infinity central values for the P-gp substrate digoxin were 72% and 48% higher, respectively, when digoxin was coadministered with glecaprevir and pibrentasvir than for digoxin alone. The digoxin mean fraction excreted in urine increased from 57.8% to 68.1% and the mean renal clearance decreased from 9.68 to 6.99 L/h during coadministration. The glecaprevir and

pibrentasvir C_{max} and AUC_{0-24} central values were similar with or without digoxin ($\leq$16% difference).

Dabigatran etexilate (P-gp). Dabigatran etexilate is a prodrug for the pharmacologically active moiety dabigatran. When the P-gp substrate dabigatran etexilate was coadministered with glecaprevir and pibrentasvir, dabigatran C_{max} and AUC from time 0 to infinity central values were 105% and 138% higher, respectively, compared with when dabigatran etexilate was administered alone. The glecaprevir and pibrentasvir C_{max} and AUC_{0-24} central values were similar with or without dabigatran etexilate ($\leq$16% difference).

Pravastatin (OATP1B1/3). The C_{max} and AUC_{0-24} central values of pravastatin, a substrate of OATP1B1/3, were 123% and 130% higher, respectively, when coadministered with multiple doses of glecaprevir and pibrenatasvir compared with when pravastatin was administered alone. When coadministered with multiple doses of pravastatin, the first dose of glecaprevir and pibrentasvir exhibited 59% and 44% higher glecaprevir C_{max} and AUC_{0-24} values, respectively, but pibrentasvir exposure was similar ($\leq$24% difference) to when glecaprevir and pibrentasvir were administered alone as a single dose.

Rosuvastatin (OATP1B1/3 and BCRP). Following coadministration of rosuvastatin, a substrate of OATP1B1/3 and
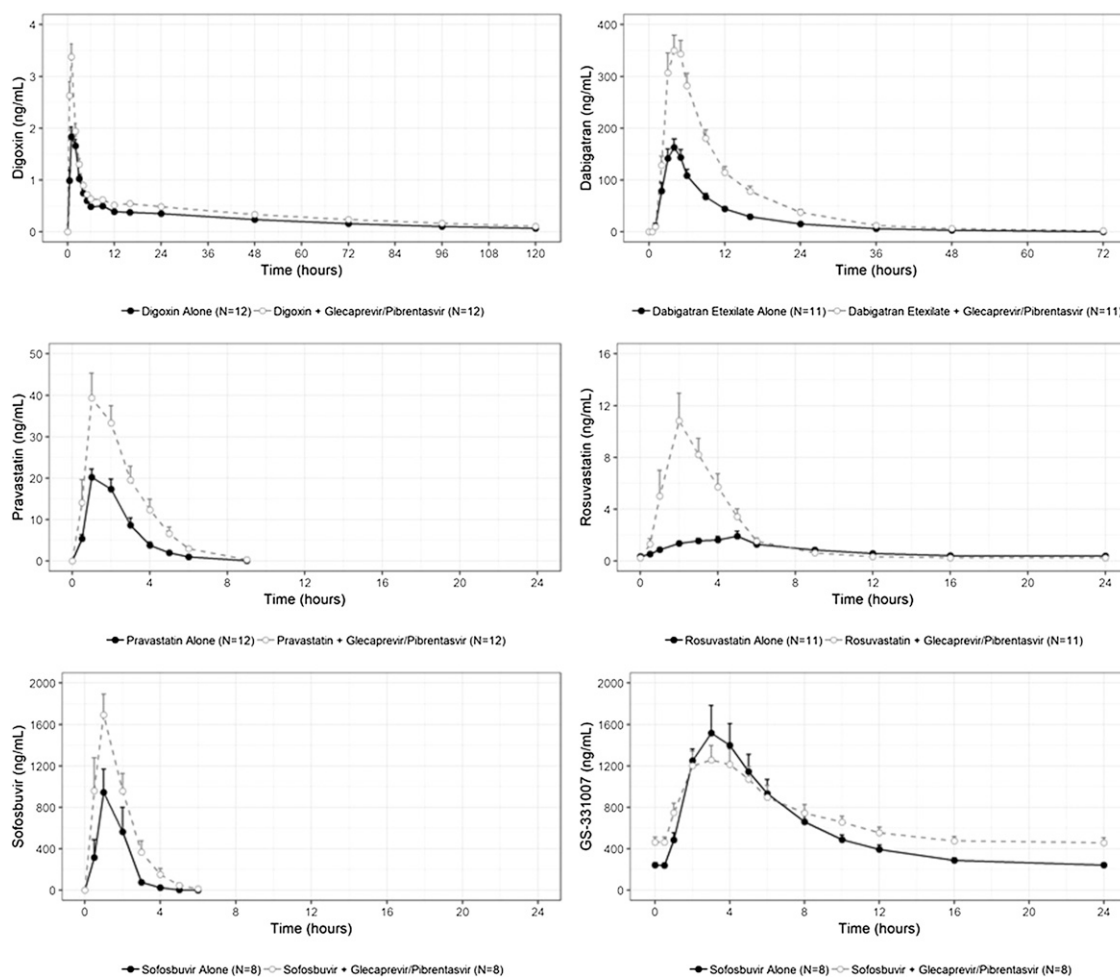

Fig. 2. Plasma concentration-time profiles of probe substrates administered alone (closed circles) or with glecaprevir/pibrentasvir (open circles) in healthy subjects. Data are presented as mean + S.E.

TABLE 2

Pharmacokinetic parameters for glecaprevir, pibrentasvir, and probe substrates in each study

The C_{max}, AUC, and C_{24} values are presented as the geometric mean (mean ± S.D.); the T_{max} values are presented as the median (minimum to maximum); and the $t_{1/2}$ values are presented as the harmonic mean (pseudo S.D.).

Analyte	Administered Alone					Coadministered				
	C_{max}	T_{max}	AUC	$t_{1/2}$	C_{0-24}	C_{max}	T_{max}	AUC	$t_{1/2}$	C_{0-24}
	ng/ml	h	ng·h/ml	h	h	ng/ml	h	ng·h/ml	h	h
Digoxin study (N = 12)										
Digoxin	1.98 (2.06 ± 0.54)	1.5 (0.5–2.0)	33.4 (34.0 ± 6.9)[aa]	37.0 (4.4)	—	3.41 (3.51 ± 0.87)	1.0 (0.5–1.0)	49.4 (50.3 ± 10.4)[a]	42.4 (5.5)	—
Glecaprevir	2540 (3520 ± 2510)	3.0 (2.0–5.0)	8980 (11,500 ± 7600)[bb]	—	5.95 (6.64 ± 3.14)	2790 (3890 ± 3380)	3.0 (2.0–4.0)	9380 (12,100 ± 9600)[b]	—	5.60 (6.23 ± 2.79)
Pibrentasvir	208 (226 ± 96)	4.0 (3.0–6.0)	1730 (1880 ± 760)[b]	—	23.9 (26.5 ± 11.8)	242 (269 ± 128)	4.5 (3.0–5.0)	1860 (2030 ± 860)[b]	—	24.1 (26.9 ± 12.5)
Dabigatran Etexilate study (N = 11)										
Dabigatran	181 (184 ± 38)	4.0 (2.0–5.0)	1540 (1580 ± 420)[a]	9.9 (1.5)	—	370 (378 ± 90)	4.0 (3.0–5.0)	3670 (3820 ± 1240)[a]	12.4 (3.9)	—
Glecaprevir	672 (838 ± 821)	4.0 (2.0–5.0)	2760 (3480 ± 3490)[b]	—	4.62 (6.02 ± 5.15)	549 (660 ± 492)	4.0 (2.0–5.0)	2200 (2740 ± 2360)[b]	—	4.81 (6.72 ± 6.83)
Pibrentasvir	273 (283 ± 79)	5.0 (3.0–6.0)	2060 (2170 ± 737)[b]	—	30.7 (32.5 ± 12.3)	236 (248 ± 90)	5.0 (4.0–6.0)	1880 (2030 ± 950)[b]	—	29.1 (32.3 ± 17.6)
Pravastatin study (N = 12)										
Pravastatin	20.4 (23.0 ± 12.7)	1.0 (1.0–2.0)	48.6 (53.0 ± 25.8)[b]	—	—	45.6 (47.8 ± 15.1)	1.5 (1.0–4.0)	111 (115 ± 33)[b]	—	—
Glecaprevir	1790 (2190 ± 1380)	3.0 (2.0–5.0)	5870 (6890 ± 4510)[b]	—	3.75 (4.98 ± 4.77)	2850 (3410 ± 1980)	3.0 (2.0–5.0)	8480 (10,100 ± 6800)[b]	—	4.55 (6.41 ± 7.33)
Pibrentasvir	160 (168 ± 54)	4.5 (2.0–5.0)	906 (977 ± 385)[b]	—	8.34 (9.45 ± 4.86)	199 (205 ± 52)	4.0 (3.0–5.0)	1120 (1180 ± 390)[b]	—	10.6 (11.8 ± 45.8)
Rosuvastatin Study (N = 11)										
Rosuvastatin	1.89 (2.13 ± 1.14)	5.0 (1.0–5.0)	17.2 (18.8 ± 8.4)[b]	—	0.37 (0.40 ± .15)	10.6 (12.2 ± 6.2)	2.0 (2.0–4.0)	37.0 (41.4 ± 19.2)[b]	—	0.26 (0.25 ± 0.12)
Glecaprevir	1860 (2270 ± 1620)	3.0 (3.0–5.0)	6070 (7630 ± 5520)[b]	—	2.94 (3.96 ± 3.26)	2320 (2750 ± 1580)	4.0 (3.0–5.0)	7340 (8630 ± 4700)[b]	—	3.34 (3.88 ± 2.25)
Pibrentasvir	169 (176 ± 46)	5.0 (3.0–5.0)	1040 (1080 ± 270)[b]	—	8.93 (9.41 ± 2.93)	208 (213 ± 46)	5.0 (3.0–5.0)	1250 (1290 ± 340)[b]	—	12.3 (12.9 ± 4.4)
Sofosbuvir study (N = 16)[c]										
Sofosbuvir	1050 (1230 ± 640)	1.0 (1.0–2.0)	1410 (1540 ± 680)[b]	—	—	1750 (1860 ± 650)	1.0 (0.5–2.0)	3170 (3290 ± 940)[b]	—	—
GS-331007	1470 (1590 ± 740)	3.0 (2.0–4.0)	12,900 (13,500 ± 4100)	—	237 (244 ± 62)	1260 (1320 ± 410)	2.5 (2.0–4.0)	15,600 (16,200 ± 4600)	—	438 (457 ± 138)
Glecaprevir	3300 (3810 ± 2120)	3.0 (2.0–4.0)	10,600 (13,800 ± 11,500)[b]	—	4.65 (6.07 ± 4.78)	3230 (4010 ± 3030)	3.0 (2.0–5.0)	10,500 (15,100 ± 14,900)[b]	—	5.16 (8.23 ± 8.38)
Pibrentasvir	238 (263 ± 131)	4.0 (3.0–5.0)	1790 (2010 ± 1090)[b]	—	24.3 (27.3 ± 15.1)	235 (270 ± 162)	3.5 (3.0–5.0)	1860 (2190 ± 1430)[b]	—	28.1 (34.7 ± 25.3)

AUC_{0-inf}, AUC from time 0 to infinity; C_{24}, plasma trough concentration 24 h after dosing; $t_{1/2}$, terminal elimination half-life; T_{max}, time to C_{max}; —, not applicable.

[a] AUC_{0-inf}.

[b] AUC_{0-24}.

[c] Pharmacokinetic parameters reported for $N = 8$ subjects in cohort 2 (sofosbuvir and GS-331007) or $N = 8$ subjects in cohort 1 (glecaprevir and pibrentasvir pharmacokinetic parameters).
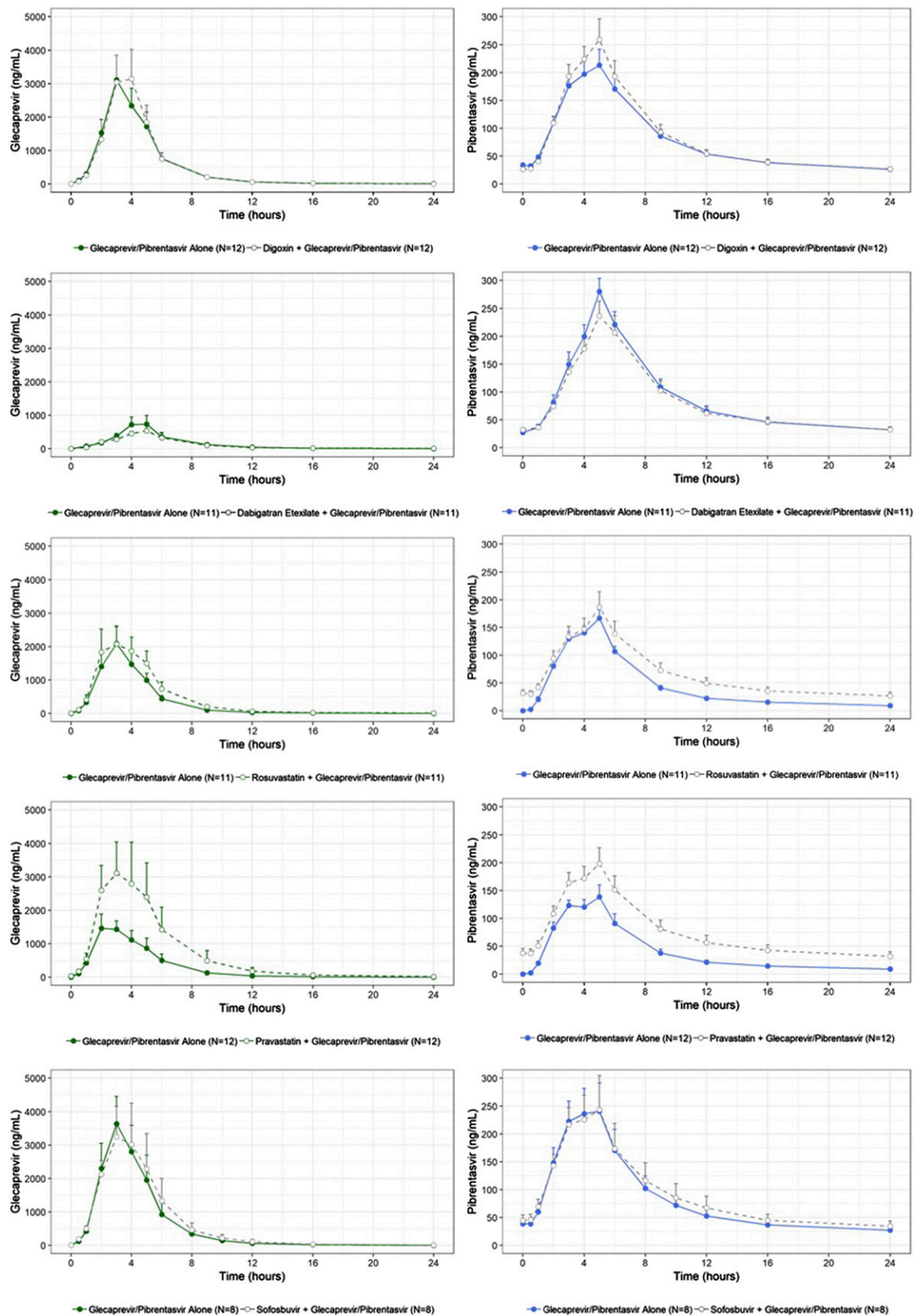
Fig. 3. Plasma concentration-time profiles of glecaprevir (blue) and pibrentasvir (green) when administered alone (closed circles) or with probe substrates (open circles) in healthy subjects. Data are presented as mean + S.E.
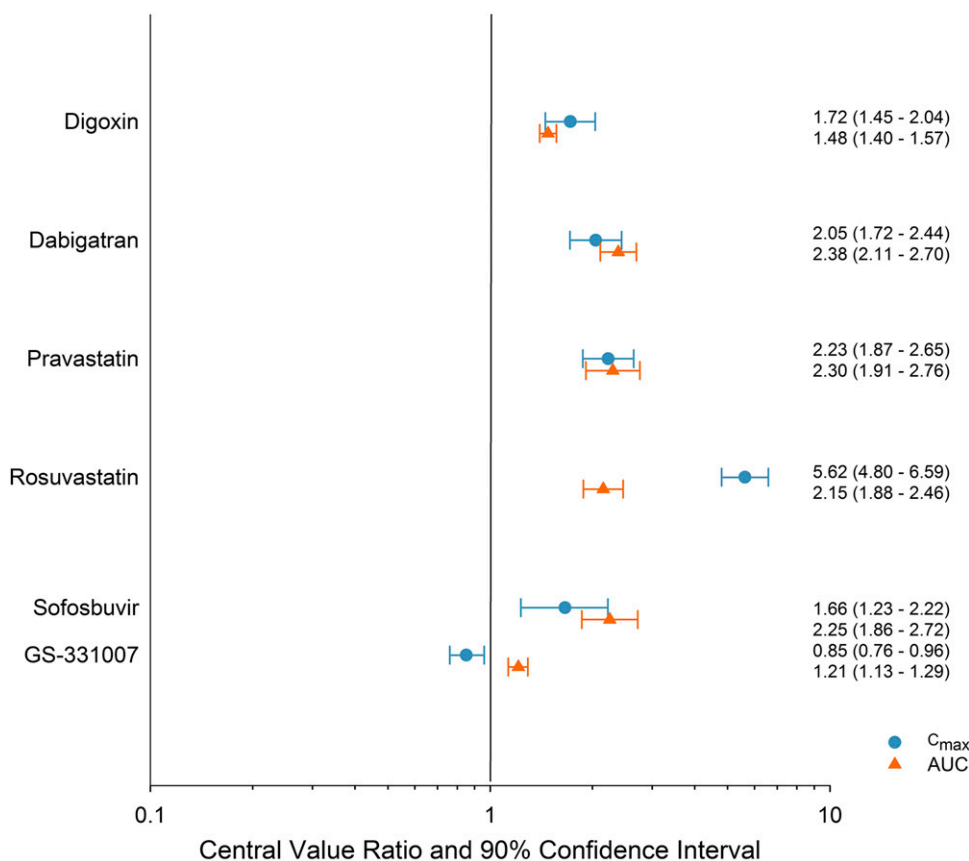
Fig. 4. Central value ratios and 90% confidence intervals for the effect of glecaprevir and pibrentasvir on concomitant medications.

BCRP, with glecaprevir and pibrentasvir, the rosuvastatin C_{max} and AUC_{0-24} central values were 462% and 115% higher, respectively, compared with when rosuvastatin was administered alone. Similar exposures were observed when the first glecaprevir and pibrentasvir dose was administered with rosuvastatin and when glecaprevir and pibrentasvir were administered alone (≤25% difference).

Sofosbuvir (P-gp and BCRP). Following coadministration of sofosbuvir, a substrate of P-gp and BCRP, with glecaprevir and pibrentasvir, the sofosbuvir C_{max} and AUC_{0-24} central values were 66% and 125% higher, respectively, compared with when sofosbuvir was administered alone; sofosbuvir metabolite GS-331007 C_{max} and AUC_{0-24} central values were similar to without glecaprevir and pibrentasvir (≤21% difference). Steady-state exposures of glecaprevir and pibrentasvir with sofosbuvir were minimally affected (≤16% change in central values) compared with administration of glecaprevir and pibrentasvir alone.

Safety. No serious adverse events were reported in any of the studies. One subject in the dabigatran etexilate study and one subject in the rosuvastatin study discontinued study drug due to adverse events that were assessed by the investigator as having no reasonable possibility of being related to the study drugs. No clinically significant vital signs, laboratory values, or electrocardiogram measurements were observed during the course of the studies.

Discussion

Direct-acting antiviral regimens containing NS3/4A protease inhibitors (e.g., paritaprevir, grazoprevir, and voxilaprevir) or NS5A inhibitors (e.g., ledipasvir, velpatasvir, elbasvir, and ombitasvir) have demonstrated potential interactions with a range of drug-metabolizing enzymes and transporters (Kiser et al., 2013; http://www.natap.org/2016/Pharm/Pharm_54.htm). In this paper, we characterized the potential for the combination of glecaprevir and pibrentasvir to increase exposure of the substrates for clinically relevant transporters.

In vitro inhibition of P-gp, BCRP, OATP1B1/3, OCT1/2, OAT1/3, and MATE1/2K was evaluated for glecaprevir and pibrentasvir. Owing to the high plasma protein binding (>99.9%) and poor solubility of pibrentasvir, an additional inhibition assessment was conducted for OATP1B1/3, OCT1/2, OAT1/3, and MATE1/2K in the presence of the physiologically relevant concentration of albumin, i.e., the total IC_{50} method (Kikuchi et al., 2017). Glecaprevir was identified as an inhibitor of P-gp, BCRP, OATP1B1, and OATP1B3, while pibrentasvir was an inhibitor for P-gp, BCRP, and OATP1B1. Glecaprevir and pibrentasvir did not inhibit OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2K.

Consistent with the anticipated inhibition of intestinal P-gp, the combination of glecaprevir and pibrentasvir increased the bioavailability of the P-gp substrates digoxin and dabigatran etexilate. Intestinal P-gp governs drug-drug interactions with many common orally administered drugs and the magnitude of interactions may depend on both the absolute bioavailability of individual substrates and the extent of P-gp inhibition (Greiner et al., 1999; Härtter et al., 2013; Pradaxa (dabigatran etexilate mesylate capsules), 2015). Digoxin exhibits 60%–80% absolute bioavailability when administered as a tablet, is predominantly eliminated

by renal excretion, and does not undergo significant enterohepatic circulation (Marcus et al., 1976; Lloyd et al., 1978). Thus, the digoxin AUC may only be increased a maximum of 25%–67% by inhibition of intestinal efflux, which affects bioavailability, not clearance—increases up to 150% have been reported upon coadministration with dronedarone and likely reflect additive inhibition of digoxin renal tubular secretion (Hori et al., 1993; Vallakati et al., 2013). The 48% increase in digoxin AUC observed with glecaprevir and pibrentasvir and accompanying modest impact on digoxin terminal elimination half-life in plasma are suggestive, but not conclusive, of an intestinal-based interaction. The prodrug dabigatran etexilate is a P-gp substrate, but it is rapidly converted to dabigatran, which is not transported by P-gp (Stangier and Clemens, 2009). Changes in dabigatran exposure following coadministration of dabigatran etexilate with P-gp inhibitors are thus restricted to interactions at the level of the intestinal epithelium, making it an ideal selective probe for intestinal P-gp inhibition. The low oral bioavailability of dabigatran etexilate also confers high sensitivity; dabigatran AUC increases up to 15% have been reported with the P-gp inhibitors ketoconazole and dronedarone (Pradaxa (dabigatran etexilate mesylate capsules), 2013; 2015; Zhao and Hu, 2014). Despite lower sensitivity, interactions of digoxin with P-gp inhibitors remain clinically relevant given the narrow therapeutic index of the drug (Liu et al., 2017).

As a drug class, statins are prototypical examples of clinically relevant OATP1B1/3 interactions since increased statin exposures are associated with the risk of myopathy or rhabdomyolysis (Giacomini et al., 2010). The magnitude of clinical statin exposure increases depends on the sensitivity of individual statins toward inhibition of OATP1B1/3-mediated hepatic uptake; however, many statins are also substrates of other drug-metabolizing enzymes or transporters that may also be inhibited by a concomitant medication (e.g., CYP3A, P-gp, and BCRP), making extrapolation among different statin drugs difficult. In vitro data suggest that glecaprevir and pibrentasvir may inhibit OATP1B1 and/or OATP1B3 (Table 1). In line with these projections, the glecaprevir and pibrentasvir combination increased clinical exposures of pravastatin and rosuvastatin. When evaluating the effect of genetic polymorphisms on statins, a reduced OATP1B1 function phenotype was associated with similar increases in AUC for pravastatin and rosuvastatin (61%–91%), but a reduced BCRP function phenotype was associated with higher rosuvastatin AUC increases (144%) than pravastatin (13%) (Kellick et al., 2014). Glecaprevir and pibrentasvir were identified as BCRP inhibitors in vitro, and the observed increases in rosuvastatin exposure may result from the contribution of both OATP1B1/3 and BCRP inhibition. It should be noted that genetic polymorphisms in BCRP may impact transporter expression and/or function throughout various tissues, and in the case of rosuvastatin may reduce active renal secretion (Verhulst et al., 2008). In contrast, clinically relevant inhibition of BCRP by glecaprevir and pibrentasvir is confined to the gut since systemic concentrations are insufficient to inhibit this transporter in other tissues, such as kidney.

Sofosbuvir is a substrate of P-gp and BCRP, but its predominant metabolite GS-331007 is not. GS-331007 does not exhibit anti-HCV activity, but concentrations are reflective of the pharmacologically active intracellular triphosphate

sofosbuvir metabolite. Systemic sofosbuvir concentrations may increase as a result of P-gp and/or BCRP inhibition without concurrent impact on GS-331007, and as per prescribing information sofosbuvir can be administered concomitantly with P-gp and BCRP inhibitors in a clinical setting (Kirby et al., 2015; Sovaldi (sofosbuvir), 2017). Thus, changes in sofosbuvir exposure are useful in confirming DDI mechanisms identified with other probe substrates, but are not expected to result in clinically significant interactions.

The selected substrates have limited potential to act as perpetrators of drug-drug interactions and were not expected to affect glecaprevir or pibrentasvir. Glecaprevir exposures were 44%–59% higher in the presence of pravastatin, but these increases are not considered clinically relevant. None of the other probe substrates evaluated here affected exposures of glecaprevir or pibrentasvir.

The Food and Drug Administration recommends that for clinical DDI studies, potential perpetrator drugs are administered at the highest approved dose and shortest dosing interval (https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf). Most of the clinical DDI studies presented here were conducted with glecaprevir 400 mg, a larger dose than the 300 mg used as part of the approved glecaprevir/pibrentasvir clinical regimen. In vitro projections were based on clinical exposures at the 300 mg dose level and suggest that transport inhibition remains relevant for the clinical regimen. When coadministered with glecaprevir, pibrentasvir exposures are higher than for pibrentasvir alone in a mechanism attributed to inhibition of intestinal P-gp and/or BCRP by glecaprevir. These increases were similar for glecaprevir 200–400 mg doses, increasing the pibrentasvir AUC to 3.0- to 3.5-fold and suggesting a saturation of this inhibition in the clinical dose range (Lin et al., 2017, 2018); potent inhibition of intestinal P-gp with glecaprevir 300 mg was confirmed in the dabigatran etexilate study. Similarly, substantial OATP1B1/3 interactions for glecaprevir 300 mg were observed in subsequent studies with simvastatin and lovastatin (Mavyret, 2019). No clinical drug-drug interaction studies with sensitive substrates of BCRP were conducted at a dose of glecaprevir 300 mg, but I_{gut}/IC_{50} values calculated for glecaprevir and pibrentasvir based on exposures for the approved 300/120 mg regimen remain several fold above the threshold for potential clinical relevance (https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf). For victim drugs where a potential increase in exposure is anticipated, utilization of the highest clinical dose in clinical DDI studies may be inadvisable. Dabigatran etexilate, digoxin, pravastatin, rosuvastatin, and sofosbuvir all exhibit linear pharmacokinetics at the study doses, in line with the Food and Drug Administration recommendations (https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf). Thus, the studies presented here are appropriate for informing clinical dosing guidance.

In conclusion, predictions based on in vitro results indicated the potential for clinically relevant inhibition of P-gp, BCRP, and OATP1B1/3 transporters by the glecaprevir and pibrentasvir combination. These predictions were confirmed in a clinical setting where the combination regimen increased exposure of probe transporter substrates. Health care providers should refer to glecaprevir/pibrentasvir prescribing information for appropriate dosing recommendations on concomitant use with these and other transport substrates in patients.

Authorship Contributions

Participated in research design: Kosloski, Bow, Kikuchi, Wang, Mensa, Kort, Liu.

Performed data analysis: Kosloski, Bow, Kikuchi, Wang, Liu, Kim, Marsh.

Wrote or contributed to the writing of the manuscript: Kosloski, Bow, Kikuchi, Wang, Mensa, Kort, Liu, Kim, Marsh.

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Address correspondence to: Wei Liu, AbbVie, Inc., Clinical Pharmacokinetics and Pharmacodynamics, Department R4PK, Building AP31-3, 1 North Waukegan Road, North Chicago, IL, 60064. E-mail: wei.liu@abbvie.com

Supplemental Materials

Supplemental Figure 1

Supplemental Figure 1
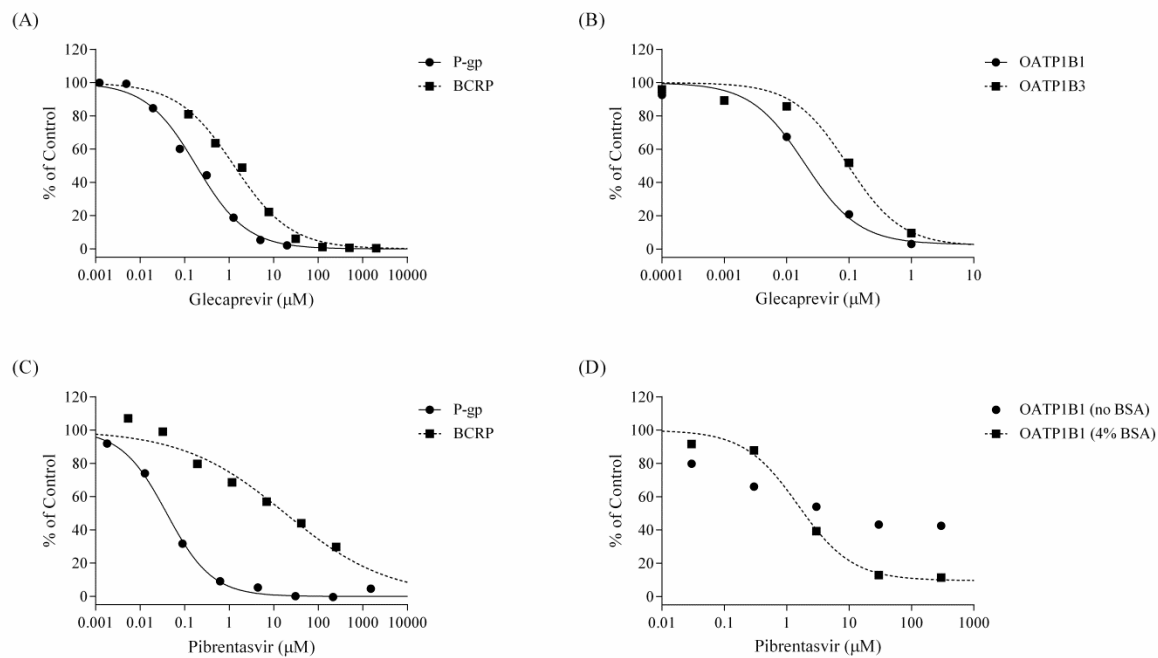
In vitro inhibition of P-gp, BCRP, OATP1B1 and OATP1B3. IC_{50} values of glecaprevir (A and B) and pibrentasvir (C and D) for P-gp, BCRP (A and C), OATP1B1 and OATP1B3 (B and D) were determined as described in Materials and Methods. The values are expressed as a percentage of transport in the absence of inhibitors. Solid and dashed lines represent the fitted line obtained by a nonlinear least-squares regression analysis. Representative IC_{50} curves are shown from two independent experiments.

Supplemental Table 1: Probe substrates and incubation times in transporter inhibition studies

Transporter	Test compound	Probe substrate	No BSA		4% w/v BSA		Incubation time (min)
			Substrate concentration (μM)	Radioactivity (μCi/mL)	Substrate concentration (μM)	Radioactivity (μCi/mL)	
P-gp	GLE or PIB	[^{3}H]NMQ	2.0	1.7	-	-	3
BCRP	GLE or PIB	Lucifer Yellow	50	-	-	-	10
OATP1B1	GLE	[^{3}H]E$_2$17βG	2.0	0.50	-	-	5
	PIB	[^{3}H]pitavastatin	0.20	0.10	1.0	0.50	2
OATP1B3	GLE	[^{3}H]E$_2$17βG	2.0	0.50	-	-	5
	PIB	[^{3}H]pitavastatin	0.20	0.10	1.0	0.50	2
OCT1	GLE	[^{3}H]MPP+	1.0	0.20	-	-	2
	PIB	[^{3}H]metformin	10	0.20	10	0.20	3
OCT2	GLE	[^{3}H]MPP+	1.0	0.20	-	-	1 or 2
	PIB	[^{3}H]metformin	10	0.20	10	0.20	3
OAT1	GLE	[^{3}H]PAH	1.0	0.20	-	-	1 or 2
	PIB	[^{3}H]methotrexate	1.0	0.50	2.0	1.0	2
OAT3	GLE	[^{3}H]ES	0.10	0.50	-	-	1 or 2
	PIB	[^{3}H]methotrexate	1.0	0.50	2.0	1.0	2
MATE1	GLE	[^{3}H]MPP+	1.0	0.20	-	-	1
	PIB	[^{3}H]metformin	10	0.20	10	0.20	1.5
MATE2K	GLE	[^{3}H]MPP+	1.0	0.20	-	-	1
	PIB	[^{3}H]metformin	10	0.20	10	0.20	1.5

GLE, glecaprevir; PIB, pibrentasvir; NMQ, N-methylquinidine; E217βG, estradiol 17-β glucuronide; MPP+, 1-methyl-4-phenylpyridinium; PAH, para-aminohippuric acid; ES, estrone-3-sulfate; -, not applicable

Supplemental Table 2: Parameters Used in Calculations to Predict Clinical Transporter Inhibition

Compound	Molecular Weight (AbbVie Inc., 2019)	Dose (mg)	$f_{u,p}$ (AbbVie Inc., 2019)	I_{max} (ng/mL) (AbbVie Inc., 2019)	Fa·Fg	K_a (hr^{-1}) (Center for Drug Evaluation and Research, 2016)	R_B
Glecaprevir	838.87	300	0.025	597	1	0.36	0.57
Pibrentasvir	1113.18	120	0.01**	110	1	0.26	0.62

**A fixed $f_{u,p}$ of 0.01 is used where the fraction unbound in plasma determined experimentally is <0.01

Authors: **Matthew P. Kosloski, Dan Bow, Ryota Kikuchi, Haoyu Wang, Elaine J. Kim,** Kennan Marsh, **Federico Mensa, Jens Kort, Wei Liu**

Supplemental Table 3: Summary of Validated Analytical Methods for Glecaprevir, Pibrentasvir, and Probe Substrates in DDI Studies Using LC-MS/MS

Compound	LLOQ (ng/mL)	Linear Range (ng/mL)	Inter-Run Accuracy %Bias[a]	Inter-Run Precision %CV[a]	Stability
Glecaprevir (dual range assay)	84.6	84.6 to 10,000 (high)	−1.5 to 0.0 (high)	2.6 to 3.3 (high)	1546d at −20°C, 282 d at −70°C
	0.198	0.198 to 102 (low)	−1.8 to 1.5 (low)	2.1 to 3.7 (low)	
Pibrentasvir (dual range assay)	84.1	84.1 to 1040 (high)	0.5 to 1.1 (high)	2.4 to 2.7 (high)	1546 d at −20°C, 282 d at −70°C
	0.197	0.197 to 101 (low)	−1.5 to 3.2 (low)	1.4 to 3.8 (low)	
Rosuvastatin	0.100	0.100 to 100	−2.3 to 3.32	1.62 to 10.9	485 d at −70°C
Pravastatin	0.500	0.500 to 250	−7.46 to 2.22	6.38 to 21.2	739 d at −20°C
Digoxin [plasma]	0.0100	0.0100 to 10.0	−3.99 to 2.05	3.71 to 19.9	286 d at −20°C
Digoxin [urine]	2	2 to 200	-4.72 to 1.84	3.93 to 7.53	152 d at −20°C
Sofosbuvir	5	5 to 5000	0.0 to 8.5	2.8 to 4.4	361 d at −20°C and −70°C
GS-331007	10	10 to 10000	-3.5 to 7.3	2.1 to 4.4	361 d at −20°C and −70°C
Total Dabigatran	1	1 to 800	1.19 to 6.15	4.39 to 6.05	95 d at −20°C

a.   Inter-run Accuracy % Bias and Inter-run Precision %CV were statistics from runs designated for precision and accuracy in validation.

Note: ISR passed at least once for each analyte

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