Cytochrome P450 3A Induction Predicts P-glycoprotein Induction; Part 2: Prediction of Decreased Substrate Exposure After Rifabutin or Carbamazepine

Justin D. Lutz[1], Brian J. Kirby[1], Lu Wang[2], Qinghua Song[2], John Ling[1], Benedetta Massetto[3], Angela Worth[4], Brian P. Kearney[1] and Anita Mathias[1]

Rifampin demonstrated dose-dependent relative induction between cytochrome P (CYP)3A and P-glycoprotein (P-gp), organic anion transporting polypeptides (OATPs), or CYP2C9; P-gp, OATP, and CYP2C9 induction was one drug–drug interaction (DDI) category lower than that observed for CYP3A across a wide range of pregnane X receptor (PXR) agonism. The objective of this study was to determine if these relationships could be utilized to predict transporter induction by other CYP3A inducers (rifabutin and carbamazepine) and of another P-gp substrate, sofosbuvir. Healthy subjects received sofosbuvir and a six-probe drug cassette before and after 300 mg q.d. rifabutin or 300 mg b.i.d. carbamazepine. Induction of P-gp, CYP2C9, and decreased sofosbuvir exposure were successfully predicted by observed CYP3A induction. Carbamazepine induction of OATP was underpredicted, likely due to reported additional non-PXR agonism. The results demonstrate that the effect of a PXR agonist on CYP3A can be leveraged to inform on induction liability for other primarily PXR-regulated P450s/transporters, allowing for prioritization of targeted DDI assessments during new drug development.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ It was recently demonstrated that varying levels of PXR agonism will elicit the same induction DDI category for P-gp, OATP, and CYP2C9, which will be one DDI category less than CYP3A induction.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study determined if induction relationships between P450s/transporters, established with rifampin, can predict the effect of 1) other inducers on these same P450s/transporters, 2) inducers on nonprobe drugs that are transporter substrates.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study demonstrated that the level of CYP3A induction for a compound can be leveraged to inform on its potential DDI liability for induction of P-gp, OATP, and CYP2C9. Accurate prediction of sofosbuvir exposure changes after inducer coadministration confirms that changes in exposure of nonprobe drugs transported by P-gp can be predicted based on CYP3A probe drug exposure changes.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The presented data and DDI liability assessment methods allow for focused DDI assessments supporting the clinical pharmacology and broader development of new chemical entities.

Drug-metabolizing enzymes and drug transporters, including cytochrome P450 3A (CYP3A), cytochrome P450 2C9 (CYP2C9), cytochrome P450 1A2 (CYP1A2), P-glycoprotein (P-gp), organic anion transporting polypeptides (OATP) 1B1 and 1B3, and breast cancer resistance protein (BCRP) play a major role in the detoxification and elimination of drugs from the body and can be involved in drug–drug interactions (DDIs) that may have clinical relevance. The expression of many of these P450s and transporters is regulated by pregnane X receptor (PXR), a nuclear receptor with broad substrate specificity.[1–5]

Induction of P450s has been extensively characterized *in vivo*; however, little is known about how inducers affect drug transporters. As such, due to their shared regulatory mechanisms, information on P450 is often leveraged to predict transporter induction, e.g., a strong inducer of CYP3A is assumed to also be a strong inducer of P-gp and OATP. This assumption was

[1]Department of Clinical Pharmacology, Gilead Sciences, Inc., Foster City, California, USA; [2]Department of Biometrics, Gilead Sciences, Inc., Foster City, California, USA; [3]Department of Clinical Operations, Gilead Sciences, Inc., Foster City, California, USA; [4]Department of Clinical Research, Gilead Sciences, Inc., Foster City, California, USA. Correspondence: Justin D. Lutz (justin.lutz@gilead.com)

Received 22 December 2017; accepted 14 March 2018; advance online publication 26 April 2018. doi:10.1002/cpt.1072

Table 1 Observed probe drug AUC_{inf} and C_{max} GMR (90% CI) for after coadministration with rifabutin and carbamazepine

Probe		RBT q.d. 300 mg	CBZ b.i.d. 300 mg
TDAB (P-gp)	AUC_{inf}	0.809 (0.652, 1.002)	0.714 (0.593, 0.859)
	C_{max}	0.867 (0.678, 1.110)	0.666 (0.521, 0.852)
PRA (OATP)	AUC_{inf}	0.876 (0.726, 1.057)	0.382 (0.321, 0.455)
	C_{max}	0.920 (0.740, 1.143)	0.345 (0.280, 0.424)
ROS (OATP/BCRP)	AUC_{inf}	0.937 (0.841, 1.044)	0.388 (0.353, 0.427)
	C_{max}	1.26 (1.11, 1.43)	0.390 (0.348, 0.438)
MDZ (CYP3A)	AUC_{inf}	0.310 (0.273, 0.351)	0.211 (0.183, 0.244)
	C_{max}	0.465 (0.423, 0.510)	0.318 (0.281, 0.361)
TOL (CYP2C9)	AUC_{inf}	0.628 (0.600, 0.658)	0.639 (0.616, 0.664)
	C_{max}	0.804 (0.780, 0.829)	0.935 (0.887, 0.985)
CAF (CYP1A2)	AUC_{inf}	0.975 (0.908, 1.048)	0.728 (0.684, 0.775)
	C_{max}	1.17 (1.10, 1.25)	1.02 (0.96, 1.09)

TDAB, total dabigatran; PRA, pravastatin; ROS, rosuvastatin; MDZ, midazolam; TOL, tolbutamide; CAF, caffeine; RBT, rifabutin; CBZ, carbamazepine.

recently tested *in vivo* where healthy subjects received a cassette of six probe drugs of P450s and transporters over a range of rifampin doses, a prototypical PXR agonist.[6] It was demonstrated that, unlike CYP3A, strong induction of P-gp, OATP, and CYP2C9 is unlikely to occur due to PXR agonism and will be one DDI category lower than that of CYP3A induction. The results of that study provided proof-of-concept that the effect of a PXR agonist on CYP3A can inform on its induction liability for other transporters/P450s.

A theoretical extension of these findings, established with rifampin (RIF), is that CYP3A induction data should be able to predict the effect of other inducers on primarily PXR regulated transporters/P450s. Establishing this to be true may offer the opportunity to proactively employ targeted CYP3A assessment to broadly inform the clinical pharmacology programs supporting development of new chemical entities. The primary objective of this study was twofold: 1) to test if the induction relationships, established with RIF, can be utilized to predict P-gp, OATP, and CYP2C9 induction by other CYP3A inducers, such as rifabutin (RBT) and carbamazepine (CBZ), and 2) to broaden the applicability of the methodology by applying it to predict the effect of inducers on nonprobe drugs (e.g., new chemical entities, NCEs). For the latter purpose, sofosbuvir (SOF, a nucleotide hepatitis C virus NS5B polymerase inhibitor), a sensitive substrate of P-gp, was selected.[7]

RESULTS

Subject demographics

All 44 subjects received all doses of study drugs. Administration of probe cassette and SOF was generally safe and well tolerated with or without RBT or CBZ. Overall, the majority of the subjects were male ($n = 40$; 90.9%), white ($n = 29$; 65.9%), and Hispanic or Latino ($n = 35$; 79.5%). The mean body mass index (BMI) at baseline was 25.7 kg/m^2 (range: 19.6–29.4 kg/m^2). The mean estimated creatinine clearance at baseline was 117.7 mL/min (range: 88.5–150.6).

Transporter/P450 induction after rifabutin and carbamazepine coadministration

In general, probe drug plasma exposures decreased after RBT and CBZ coadministration. The observed area under the curve from time zero to infinity (AUC_{inf}) geometric mean ratios (GMRs) (90% confidence interval (CI)) are presented in **Table 1**. Tabulated probe drug AUC_{inf}, AUC_{last}, and maximum plasma concentration (C_{max}) values, with and without inducer coadministration, are provided in the **Supplemental Materials.** Rifabutin was not an inducer of P-gp, or OATP, but induced CYP2C9 (weak) and CYP3A (moderate). Carbamazepine demonstrated weak induction of P-gp, CYP2C9, and CYP1A2, and moderate induction of OATP and CYP3A. Consistent with previous findings after multiple ascending dose levels of RIF,[6] no additional decrease in rosuvastatin (ROS) AUCR (AUC ratio) was noted when compared to pravastatin (PRA) AUCR, indicating that BCRP is not induced by RBT and CBZ.

Rifabutin and CBZ elicited only weak-to-no induction (AUCR >0.5) of CYP1A2. These results were expected, as CYP1A2 is primarily regulated by aryl hydrocarbon receptor (AHR) [8] and not PXR. These data suggest that PXR-mediated induction should not be used to predict AHR-mediated induction. Induction of BCRP and CYP1A2 will not be discussed further. For all probes, similar decreases in C_{max} values were observed as with AUC_{inf} (**Table 1**).

Predicted transporter/P450 induction after rifabutin and carbamazepine coadministration

P-gp and CYP2C9 induction was predicted by the effect of RBT and CBZ on CYP3A (**Figure 1a,c**). P-gp induction was predicted by the effect of RBT and CBZ on CYP2C9 (**Figure 1b**).

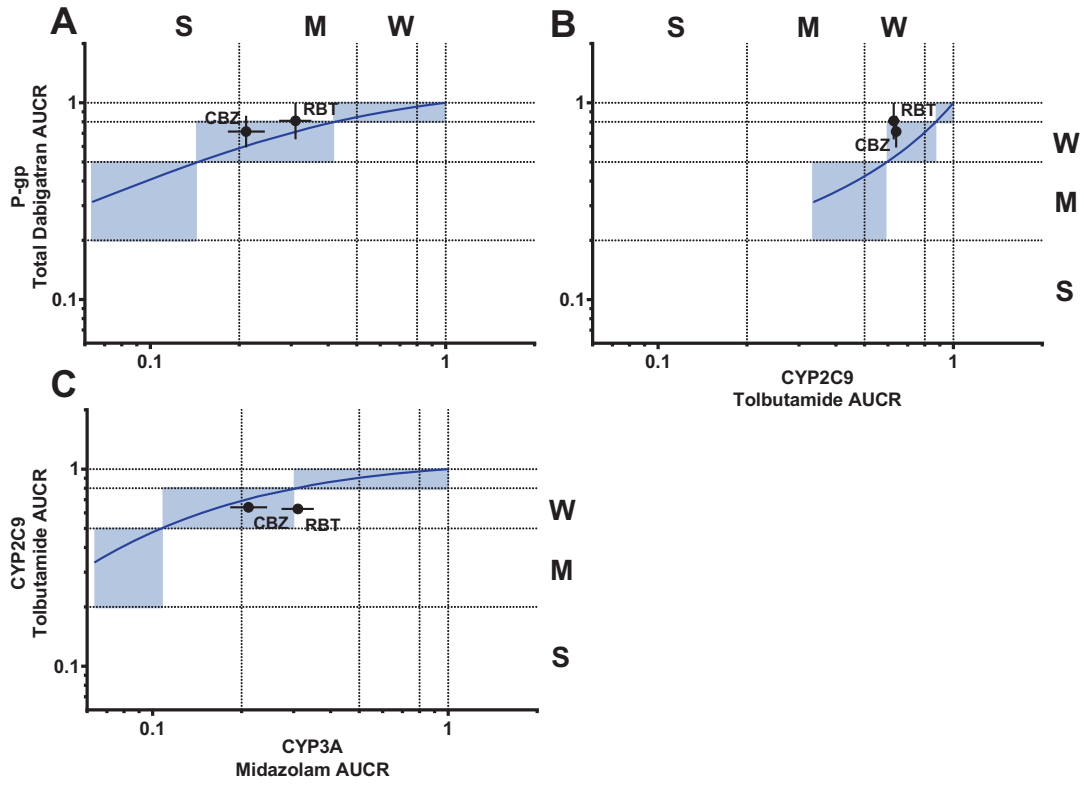

Figure 1 Prediction of induction between P-gp, CYP2C9, and CYP3A. The black points and error bars are observed AUCR mean and 90% CI, respectively, after coadministration with the indicated inducer. The blue lines are the induction relationship between P-gp and CYP2C9 and CYP3A. Blue areas are predicted P-gp and CYP2C9 DDI category based on CYP3A or CYP2C9 induction. Weak, moderate, and strong induction classification is denoted as W, M, and S, respectively.

OATP induction was predicted by the effect of RBT and CBZ on CYP3A or CYP2C9 (**Figure 2**). P-gp and OATP (as probed by ROS) induction was predicted by the effect of RBT and CBZ on OATP (as probed by PRA) (**Figure 3**). The blue lines in each figure are the identical previously characterized induction relationships for the respective transporter/P450 pairing.[6] The blue areas depict the DDI category (none, weak, moderate, or strong) for the transporter/P450 on the y-axis as predicted by the relevant induction relationship.

Weak induction of P-gp (**Figure 1a**) and CYP2C9 (**Figure 1c**) is well predicted based on CYP3A induction after CBZ coadministration. Similarly, weak induction of P-gp (**Figure 1b**) exposure is well predicted based on CYP2C9 induction after CBZ coadministration. P-gp was technically not induced by RBT (TDAB AUCR = 0.81), but CYP3A and CYP2C9 induction predicted weak P-gp induction (predicted TDAB AUCR of 0.71 and 0.53, respectively). The predictions of RBT-mediated induction are considered inaccurate from a DDI classification perspective, but the observed/predicted TDAB AUCR ratio for prediction from CYP3A and CYP2C9 induction was 1.13 and 1.53, respectively, indicating good numerical agreement between predicted and observed P-gp induction. Slight overprediction of induction is of little concern, if this methodology is to be applied to DDI liability assessment, as it results in a conservative overestimation of risk. In contrast, no RBT-mediated induction of CYP2C9 was predicted by CYP3A induction (predicted tolbutamide (TOL) AUCR = 0.81), but weak CYP2C9 induction was observed (observed TOL AUCR = 0.63). Although this prediction is considered inaccurate based on observed vs. predicted DDI classification, the observed/predicted TOL AUCR was 0.78, indicating good numerical agreement between predicted and observed CYP2C9 induction by RBT when predicted from CYP3A induction. Overall, good agreement between predictions and clinical observations for P-gp, CYP3A, and CYP2C9 is likely due to a simple PXR coregulated system[3,5] across the enzymes/transporters of interest allowing for extrapolation, especially when anchored off CYP3A as the reference P450.

Induction of OATP (as evidenced by PRA or ROS AUCR) by CBZ is underpredicted based on CYP3A or CYP2C9 (**Figure 2a,c**). Conversely, induction of P-gp by CBZ, based on OATP induction, is overpredicted (**Figure 3a**). OATP induction is regulated by several non-PXR dependent pathways.[9] CBZ is an agonist of both PXR and constitutive androstane receptor (CAR).[10] These results suggest that CBZ may be inducing OATP via a relevant non-PXR pathway and, hence, caution should be taken when extrapolating results across differing regulatory systems. OATP induction (as evidenced by PRA or ROS AUCR) after RBT coadministration is not accurately predicted (weak DDI predicted but no DDI observed) by CYP3A (**Figure 2a,c**) and CYP2C9 induction (**Figure 2b,d**) from the perspective of DDI classification. The PRA or ROS observed/predicted AUCR ratios for RBT-mediated induction are 1.24 or 1.29,

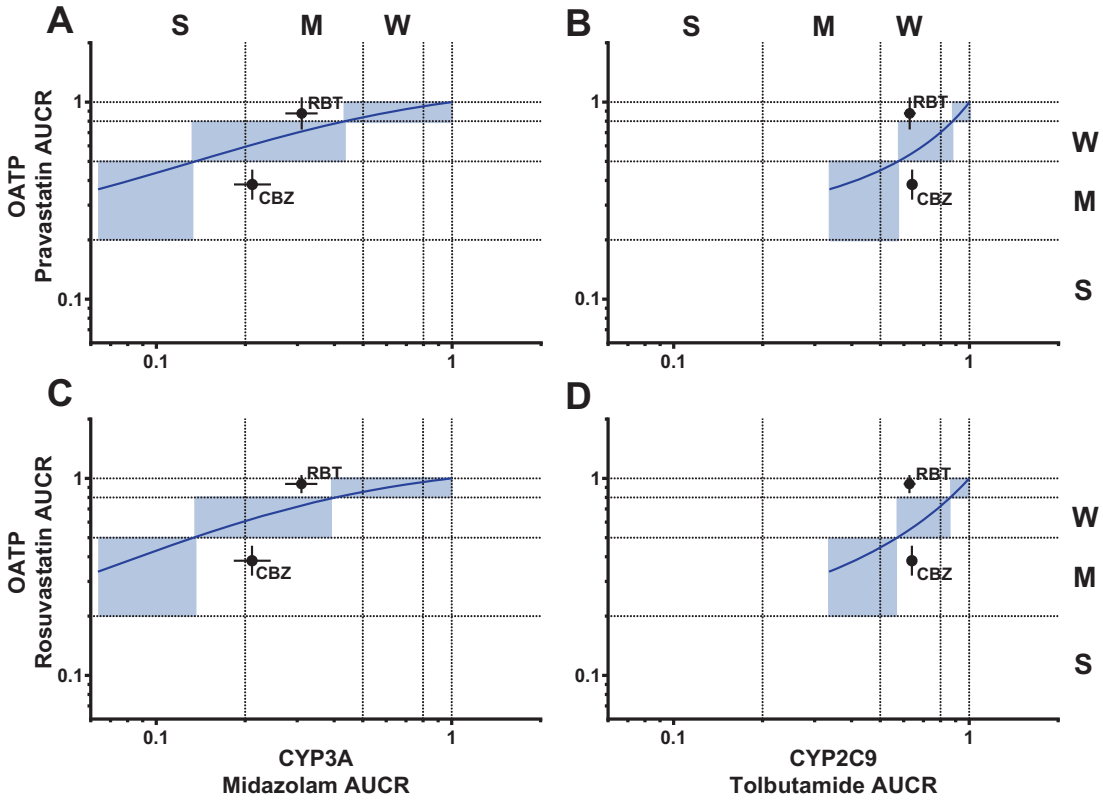

Figure 2 Prediction of OATP induction from CYP3A and CYP2C9 induction. The black points and error bars are observed AUCR mean and 90% CI, respectively, after coadministration with the indicated inducer. The blue lines are the induction relationships between CYP3A, CYP2C9, and OATP. Blue areas are predicted OATP DDI category based on CYP3A or CYP2C9 induction. Weak, moderate, and strong induction classification is denoted as W, M, and S, respectively.

respectively, when predicted from CYP3A induction, and 1.62 or 1.71, respectively, when predicted from CYP2C9 induction. These data indicate good numerical agreement between predicted and observed OATP induction by RBT based on CYP3A or CYP2C9 induction. Similar to the results obtained when predicting P-gp induction by RBT from CYP3A or CYP2C9 induction (**Figure 1a,b**), slight overprediction of induction is of little concern during DDI liability assessment, as it results in a conservative overestimation of risk. Pravastatin AUCR accurately predicts TDAB AUCR after RBT coadministration, but overpredicts TDAB AUCR after CBZ coadministration (**Figure 3a**). Conversely, PRA AUCR accurately predicts ROS AUCR after coadministration of CBZ or RBT (**Figure 3b**), demonstrating that accurate prediction of induction between probes of the same transporter (OATP) is possible.

Prediction of sofosbuvir exposure decrease after inducer coadministration

To further establish proof-of-concept that the induction of CYP3A can predict induction of drug transporters, specifically P-gp, the decrease in SOF exposure after coadministration with RIF, RBT, or CBZ were predicted and compared to observed data. Sofosbuvir mean (90% CI) AUCR after coadministration of RIF, RBT, or CBZ was 0.28 (0.24, 0.32),[7] 0.76 (0.63, 0.91), and 0.52 (0.46, 0.59), respectively. Tabulated SOF AUC_{inf}, AUC_{last}, and C_{max} values, with and without inducer coadministration, are provided in the **Supplemental Materials.** Rifampin, RBT, and CBZ elicit moderate, weak, and weak induction of SOF exposure *in vivo*. These induction classifications were accurately predicted based on observed strong, moderate, and moderate induction of CYP3A by the respective inducers (**Figure 4**).

DISCUSSION

The relative induction of CYP3A vs. drug transporters or other P450s across a range of RIF dose levels was recently determined and the results of that study provided proof-of-concept that the effect of a PXR agonist on CYP3A can inform on induction liability for P-gp, OATP, and CYP2C9.[6] Although RIF is by far the most common and potent inducer evaluated in DDI studies, many inducers with varying degrees of induction exist. As such, this study aimed at extrapolating the results to other inducers to determine if the relationships between each P450 or transporter, established with RIF and probe drugs, could be broadened and utilized to quantitatively predict induction liability of other drugs, as either precipitants or objects of DDIs. To this purpose, two known CYP3A inducers, RBT and CBZ, were evaluated (**Table 1**) and it was confirmed that P-gp and CYP2C9 induction can be accurately predicted based on CYP3A induction (**Figure 1**). Therefore, in instances where *in vivo* CYP3A induction data are the only data available, these studies can be

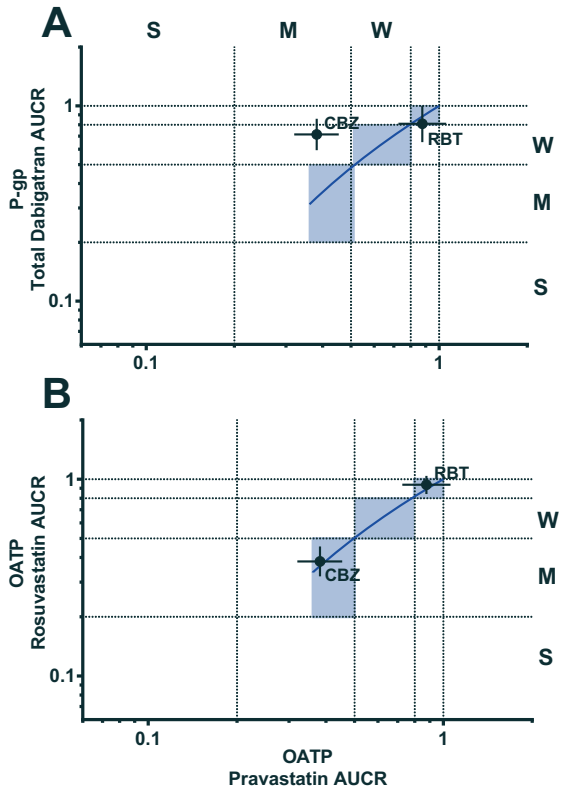

Figure 3 Prediction of P-gp or OATP (rosuvastatin) induction from OATP induction (pravastatin). The black points and error bars are observed AUCR mean and 90% CI, respectively, after coadministration with the indicated inducer. The blue line is the induction relationship between P-gp or OATP (ROS) and OATP (PRA). Blue areas are predicted P-gp DDI category based on OATP induction. Weak, moderate, and strong induction classification is denoted as W, M, and S, respectively.

leveraged to inform on P-gp and CYP2C9 induction magnitude for the same inducer, and can obviate the need to conduct dedicated DDI studies. For example, literature data demonstrates that oxcarbazepine (OXC) elicits weak induction of CYP3A, based on the probe drug felodipine (AUCR = 0.72 and CYP3A fraction metabolized f_m value of 0.84).[11,12] This magnitude of CYP3A induction is appreciably less than that observed with midazolam (MDZ) after CBZ coadministration (**Table 1**); hence, OXC is predicted to demonstrate weak-to-no induction of P-gp (based on **Figure 1a**) and would not be expected to result in clinically significant changes in the exposure of P-gp substrates.

Identification of the nuclear receptors involved with both inducer and transporter/P450 appears critical for accurate prediction by CYP3A induction (**Table 2**). If the NCE is primarily a PXR agonist,[6] CYP3A induction by the NCE will predict P-gp, CYP2C9, and OATP induction fairly well (as evidenced by the RBT results in **Figure 1a,c** as well as **Figure 2a,c**). If the NCE additionally agonizes non-PXR pathways, P-gp and CYP2C9 induction will be accurately predicted, but OATP induction may be underpredicted due to this fact. This was evidenced in predicting OATP (a transporter regulated by several non-PXR dependent mechanisms)[13] induction by CBZ (**Figure 2a,c**). In most instances, *in vitro* nuclear receptor selectivity data are available during development and should be considered prior to predicting *in vivo* induction liability.

To expand on relationships established with inducers on probe drugs to nonprobe drugs and NCEs that are transporter substrates, the effect of RIF, RBT, or CBZ on the exposure of SOF was predicted (**Figure 4**). SOF is a substrate of P-gp but not CYP3A; however, the assumption of induction potency parity for CYP3A and P-gp resulted in disallowing use of known or potential strong CYP3A inducers (assumed to be strong P-gp inducers) with SOF.[7,14] Accurate prediction of SOF exposure changes after coadministration of RIF, RBT, or CBZ confirms that decreased exposure of nonprobe drugs and NCEs that are transported by P-gp can be predicted based on CYP3A probe drug exposure decrease (**Figure 4**).

In conclusion, this study demonstrated that the level of CYP3A induction for a compound can be leveraged to inform on its potential DDI liability for induction for other P450s and transporters. The previously characterized relationships between the induction magnitude of CYP3A and P-gp, OATP, or CYP2C9 were used to successfully predict transporter/P450 probe drug induction by other known CYP3A inducers. The successful prediction of changes in SOF exposure after inducer coadministration establishes proof-of-concept that the effect of inducers on nonprobe drugs that are transporter substrates can be predicted from CYP3A induction data. These DDI prediction and liability assessment methods illustrated herein should prove to decrease the number of DDI studies that are conducted during new drug development via better leveraging of previously observed DDIs. Appreciation and application of these results will allow for more focused DDI assessments supporting the clinical pharmacology of new chemical entities as well as more informed labeling recommendations for new drugs that are transporter substrates or PXR agonists.

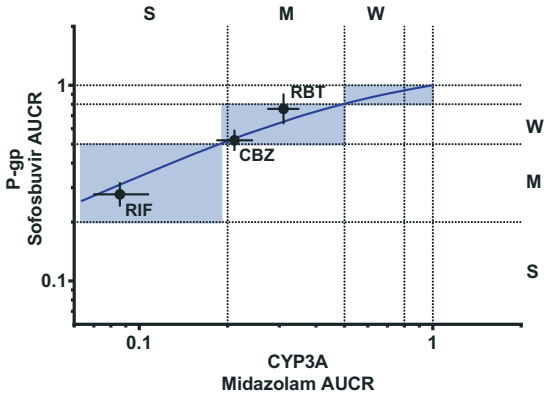

Figure 4 Prediction of sofosbuvir exposure decrease after inducer coadministration. The black points and error bars are observed AUCR mean and 90% CI, respectively, after coadministration with the indicated inducer. The blue line is the predicted induction relationship between SOF and MDZ AUCR. Blue areas are predicted SOF DDI category based on MDZ induction by the indicated inducer. Weak, moderate, and strong induction classification is denoted as W, M, and S, respectively

Table 2 Conditions under which CYP3A induction can confidently predict induction of other transporters and P450s

If NCE is an agonist of:	*Then* CYP3A induction can predict induction of:	
Primarily PXR	P-gp/CYP2C9	True
	OATP	
PXR and Non-PXR	P-gp/CYP2C9	True
	OATP	May be true; underprediction could occur
Primarily Non-PXR	P-gp/CYP2C9	Unknown if true or false; not tested but not recommended
	OATP	

METHODS

Study population

This was a phase I open-label, multiple-dose, single-center study. Eligible subjects were healthy, male, and nonpregnant, nonlactating female subjects of 18 to 45 years of age with a BMI between 19 and 30 kg/m^2. The study protocol and informed consent were approved by the study center's Institutional Review Board, and subjects provided written consent before study participation. Major inclusion criteria included healthy subjects based on medical history / physical examinations / laboratory evaluations, normal 12-lead electrocardiogram, estimated creatinine clearance ≥80 mL/min (Cockroft-Gault), no evidence of HIV, hepatitis B virus, or hepatitis C virus infection, and use of at least two forms of contraception, including an effective barrier method. Exclusion criteria included plasma and blood donation within 7 and 56 days of study enrollment, respectively, active medical illness, use of prescription drugs within 28 days of study drug dosing (except vitamins, acetaminophen, ibuprofen, and/or hormonal contraceptive).

Study design

Induction was assessed in two cohorts: RBT 300 mg q.d. ($N = 20$) and CBZ 300 mg b.i.d. ($N = 24$). Carbamazepine was initiated at 100 mg b.i.d. and escalated to final 300 mg over 1 week. Inducers were used for 10 days of the final dose level prior to and then continued through probe drug assessment. Rifabutin was administered in the evening, whereas CBZ was administered in the evening and morning (4 hours after probe drug administration). A cassette of six probe drugs were orally administered in the morning under fasted conditions, before and after inducer administration (2 days washout between doses of probe drugs, 7 days total probing): 75 mg dabigatran etexilate (DE), 20 mg PRA, 10 mg ROS, and a simultaneously administered cocktail of 2 mg MDZ, 500 mg tolbutamide (TOL), and 200 mg caffeine (CAF). These drugs were utilized to assess the activity of P-gp, OATP, OATP/BCRP, CYP3A, CYP2C9, and CYP1A2, respectively.[15–18] DE was administered as the probe drug, but changes in plasma total dabigatran (TDAB, free dabigatran + glucuronides) concentrations were measured (see Bioanalytical procedures, below) for P-gp activity assessment. The fraction transported (f_t) by OATP for PRA and ROS are similar[19,20] and, hence, it can be assumed that any further induction of ROS, relative to PRA, can be attributed to BCRP induction. The MDZ/TOL/CAF cocktail was previously validated to ensure its suitability for simultaneous P450 activity probing.[15] Additionally, 400 mg sofosbuvir was administered orally under fasting conditions in the morning 1 day prior to the start of the probe cassette. The study design is presented in **Table 3**.

Pharmacokinetic (PK) evaluation

Serial blood samples were collected after SOF administration on Days 1 and 20: predose (<5 minutes), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 96 hours postdose; after DE administration on Days 2 and 21: predose (<5 minutes), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48 hours postdose; after PRA administration on Days 4 and 23: predose (<5 minutes), 0.5, 1, 1.5, 2, 3, 4, 8, 12, and 24 hours postdose; after ROS administration on Days 6 and 25: predose (<5 minutes), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours postdose; after MDZ+TOL+CAF administration on Days 8 and 27: predose (<5 minutes), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24, and 48 hours postdose.

Table 3 Study design

	Days 1–9	10–26	27–35
	SOF 400 mg + cassette		SOF 400 mg + cassette
Cohort 1, $N = 24$		Escalated to CBZ 300 mg b.i.d.	
	Days 1–9	**10–20**	**21–29**
	SOF 400 mg + cassette		SOF 400 mg + cassette
Cohort 2, $N = 20$		RBT 300 mg q.d.	

Probe drug cassette		Dose	Abbreviation	P450/transporter	Cassette day
Dabigatran etexilate*		75 mg	DE	P-gp	2
Pravastatin		20 mg	PRA	OATP	4
Rosuvastatin		10 mg	ROS	OATP/BCRP	6
Cocktail	Midazolam	2 mg	MDZ	CYP3A	8
	Tolbutamide	500 mg	TOL	CYP2C9	
	Caffeine	200 mg	CAF	CYP1A2	

*DE was analyzed as total dabigatran (TDAB), the sum of conjugated and unconjugated active species.

Bioanalytical procedures

Bioanalysis was conducted at PPD Laboratories (Middleton, WI) and QPS (Newark, DE) using fully validated high-performance liquid chromatography-tandem mass spectroscopy (LC/MS/MS) bioanalytical methods. To determine the concentrations of TDAB, PRA, ROS, MDZ, TOL, CAF, and SOF in plasma samples, a 50–300 µL aliquot of plasma was spiked with isotopically labeled internal standard (^{2}H and/or ^{13}C). The sample was then processed by protein precipitation, liquid-liquid or solid phase extraction, followed by evaporation of the organic solvent. An aliquot of the reconstituted sample extract was injected onto the LC-MS/MS system. The calibrated ranges of the method were 1.00–500 ng/mL for TDAB, 0.100–100 ng/mL for PRA, 0.0500–50.0 ng/mL for ROS, 0.100–100 ng/mL for MDZ, 100–100,000 ng/mL for TOL, 20.0–20,000 ng/mL for CAF, and 5.00–2500 ng/mL for SOF. For all analytes, precision (%CV) was <15% (<20% at lower limit of quantitation (LLOQ)) and assay accuracy (% relative error) values were within ± 15% of 100% (±20% of 100% at limit of quantitation). All samples were analyzed in the timeframe supported by frozen stability storage data.

PK analyses

The AUC_{inf} and C_{max} were determined via noncompartmental analysis using Phoenix (v. 6, Certara USA, Princeton, NJ) for all analytes. For this analysis, actual plasma sampling times were utilized and a minimum of 3 points were used to define the terminal phase. All plasma concentrations <LLOQ and before the first observable timepoint were inputted as zero, whereas those after the first observable concentration were considered missing. Geometric mean treatment/control AUC_{inf} ratio (AUC_{inf} GMR or AUCR) with 90% CI was calculated.

Prediction of transporter/P450 induction by rifabutin and carbamazepine

Induction relationships between TDAB, PRA, MDZ, and TOL were previously determined using RIF as a prototypical PXR agonist.[6] These relationships were directly applied to predict the AUCR of a second probe drug (defined as the prediction object drug) based on the AUCR of the first probe drug (defined as the prediction reference drug) after coadministration of RBT or CBZ. For example, the induction relationship between CYP3A and P-gp, defined using multiple ascending dose levels of RIF,[6] predicts that if RBT or CBZ elicits a specific MDZ AUCR (reference drug), then RBT or CBZ would also elicit a specific TDAB AUCR (object drug) and corresponding weak (AUCR = 0.5–0.8), moderate (AUCR = 0.2–0.5) or strong (AUCR <0.2) P-gp induction classification.[21,22] Induction predictions from reference drug were determined to be accurate if both the predicted and observed DDI classification (weak, moderate, or strong) for the object drug were identical.

Prediction of sofosbuvir exposure changes after inducer coadministration

Utilizing the previously determined TDAB-MDZ induction relationship,[6] SOF AUCR after coadministration with RIF, RBT, or CBZ was predicted and compared to observed results. Observed SOF AUCR after coadministration of RBT and CBZ was determined in this study, whereas SOF AUCR after coadministration of RIF was determined previously.[7] Sofosbuvir is a P-gp substrate; a P-gp f_t value of 0.78 was estimated based on a 353% increase in exposure after strong P-gp inhibition.[7] To predict SOF AUCR based on observed MDZ AUCR, the maximum induction of SOF (E_{max}) was calculated using the estimated DE E_{max}[6] and the ratio of P-gp f_t values for SOF and DE (0.78 and 0.56, respectively).[17] The SOF-MDZ induction relationship was calculated using the previously described equations[6] and applied to predict the SOF AUCR based on MDZ AUCR after coadministration of RBT or CBZ. Sofosbuvir AUCR predictions were determined to be accurate if both the predicted and observed SOF DDI classification (weak, moderate or strong) were identical.

Statistical analyses

For each cohort and analyte, a parametric (normal theory) mixed-effects analysis of variance (ANOVA) model was fitted to the natural log-transformed values of the single-dose AUC_{inf} under evaluation using SAS PROC MIXED (Cary, NC). The ratio of geometric least-squares means (GLSMs) for test vs. reference treatments was calculated, as well as the associated 90% CI. The 2012 Food and Drug Administration (FDA) Drug–Drug Interaction (DDI) Guidance defines the severity of a given DDI as weak, moderate, or strong based on the mean treatment/control AUC_{inf} ratio range of 0.5 to 0.8, 0.2 to 0.5, and <0.2, respectively.[21,22]

Additional Supporting Information may be found in the online version of this article.

ACKNOWLEDGMENTS

The authors thank Sunila Reddy, PharmD, for help in preparation of the article.

FUNDING

This study was funded by Gilead Sciences, Inc.

CONFLICT OF INTEREST

The authors declare no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.D.L., B.J.K., and A.M. wrote the article; J.D.L., B.J.K., Q.S., B.M., A.W., B.P.K., and A.M. designed the research; A.W. performed the research; J.D.L., B.J.K., L.W., Q.S., J.L., and B.M. analyzed the data.

1. Christians, U., Schmitz, V., Haschke, M. Functional interactions between P-glycoprotein and CYP3A in drug metabolism. *Expert Opin. Drug Metab. Toxicol.* **1**, 641–654 (2005).
2. Francis, G.A., Fayard, E., Picard, F., Auwerx, J. Nuclear receptors and the control of metabolism. *Annu. Rev. Physiol.* **65**, 261–311 (2003).
3. Gerbal-Chaloin, S. *et al*. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab. Dispos.* **29**, 242–251 (2001).
4. Lemmen, J., Tozakidis, I.E. & Galla, H.J. Pregnane X receptor upregulates ABC-transporter Abcg2 and Abcb1 at the blood-brain barrier. *Brain Res.* **1491**, 1–13 (2013).
5. Synold, T.W., Dussault, L. & Forman, B.M. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat. Med.* **7**, 584–590 (2001).
6. Lutz, J.D. *et al*. Cytochrome P450 3A induction predicts p-glycoprotein induction; Part 1: establishing induction relationships using ascending dose rifampin. *Clin. Pharmacol. Ther.* (2018) [Epub ahead of print].
7. Kirby, B.J., Symonds, W.T., Kearney, B.P. & Mathias, A.A. Pharmacokinetic, pharmacodynamic, and drug-interaction profile of the hepatitis C virus NS5B polymerase inhibitor sofosbuvir. *Clin. Pharmacokinet.* **7**, 677–690 (2015).
8. Zhou, S.F., Wang, B., Yang, L.P. & Liu, J.P. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. *Drug Metab. Rev.* **4**, 268–354 (2010).
9. Svoboda, M., Riha, J., Wlcek, K., Jaeger, W. & Thalhammer, T. Organic anion transporting polypeptides (OATPs): regulation of expression and function. *Curr. Drug Metab.* **12**, 139–153 (2011).
10. Faucette, S.R. *et al*. Relative activation of human pregnane X receptor versus constitutive androstane receptor defines distinct classes of CYP2B6 and CYP3A4 inducers. *J. Pharmacol. Exp. Ther.* **320**, 72–80 (2007).

11. Jalava, K.M., Olkkola, K.T. & Neuvonen, P.J. Itraconazole greatly increases plasma concentrations and effects of felodipine. *Clin. Pharmacol. Ther.* **61**, 410–415 (1997).
12. Zaccara, G., Gangemi, P.F., Bendoni, L., Menge G.P., Schwabe, S. & Monza, G.C. Influence of single and repeated doses of oxcarbazepine on the pharmacokinetic profile of felodipine. *Ther. Drug Monit.* **15**, 39–42 (1993).
13. Svoboda, M. *et al*. Organic anion transporting polypeptides (OATPs): Regulation of expression and function. *Curr. Drug Metab.* **12**, 139–153 (2011).
14. Sovaldi [package insert], Gilead Sciences, Foster City, CA. Revised April, 2017.
15. Blakey, G.E. *et al*. Pharmacokinetic and pharmacodynamic assessment of a five-probe metabolic cocktail for CYPs 1A2, 3A4, 2C9, 2D6 and 2E1. *Br. J. Clin. Pharmacol.* **57**, 162–169 (2004).
16. Hartter, S., Sennewald, R., Nehmiz, G., Reilly, P. Oral bioavailability of dabigatran etexilate (Pradaxa®) after co-medication with verapamil in healthy subjects. *Br. J. Clin. Pharmacol.* **75**, 1053–1062 (2012).
17. Maeda, K. *et al*. Identification of the rate-determining process in the hepatic clearance of atorvastatin in a clinical cassette microdosing study. *Clin. Pharmacol. Ther.* **90**, 575–581 (2011).
18. Simonson, S.G. *et al*. Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. *Clin. Pharmacol. Ther.* **76**, 167–177 (2004).
19. Deng, S. *et al*. Effects of a concomitant single oral dose of rifampicin on the pharmacokinetics of pravastatin in a two-phase, randomized, single-blind, placebo-controlled, crossover study in healthy Chinese male subjects. *Clin. Ther.* **31**, 1256–1263 (2009).
20. Prueksaritanont, T. *et al*. Pitavastatin is a more sensitive and selective organic anion-transporting polypeptide 1B clinical probe than rosuvastatin. *Br. J. Clin. Pharmacol.* **78**, 587–598 (2014).
21. European Medicines Agency. Concept paper on a revision of the Guideline on the investigation of drug interactions. EMEA/CHMP/694687/2016. March, 2017.
22. Food & Drug Administration (FDA). Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications — Guidance for Industry. October, 2017.