

## ARTICLE

# Quantitative Prediction of OATP-Mediated Drug-Drug Interactions With Model-Based Analysis of Endogenous Biomarker Kinetics

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Quantitative prediction of the magnitude of transporter-mediated clinical drug-drug interactions (DDIs) solely from *in vitro* inhibition data remains challenging. The objective of the present work was to analyze the kinetic profile of an endogenous biomarker for organic anion-transporting polypeptides 1B (OATP1B), coproporphyrin I (CPI), and to predict clinical DDIs with a probe OATP1B substrate (pravastatin) based on “*in vivo*” inhibition constants ( $K_i$ ). The CPI kinetics in the presence and absence of strong and weak OATP1B inhibitors (rifampin and GDC-0810) were described well with a one-compartment model, and *in vivo*  $K_i$  were estimated. Clinical DDIs between pravastatin and these inhibitors were predicted using physiologically based pharmacokinetic (PBPK) models coupled with the estimated *in vivo*  $K_i$  and predicted magnitude matched well with the observed DDIs. In conclusion, model-based analysis of the CPI profile has the potential to quantitatively predict liability of a new molecular entity (NME) as an OATP1B inhibitor early in drug development.

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## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Endogenous biomarkers of drug transporters have recently been evaluated extensively as alternative tools to assess the risk of clinical DDIs.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study aimed to demonstrate that PBPK model-based analysis of biomarker kinetics allows for quantitative prediction of clinical transporter-mediated DDIs, regardless of the magnitude of interaction, using CPI as an example.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ One-compartment model allowed for sufficient description of CPI kinetics in the presence of

inhibitors. PBPK model-based prediction of clinical DDIs, using the estimated “*in vivo*  $K_i$ ” from CPI analyses, accurately predicted the reported magnitudes of interactions, both for strong and weak inhibitors of OATP1B (rifampin and GDC-0810).

### HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ This study presents a generalizable modeling framework to predict OATP1B inhibition liability of an NME early in drug development based on biomarker data. The framework may ultimately replace the need for dedicated clinical DDI studies between NME and an OATP1B substrate.

Inhibition of drug transporters can alter pharmacokinetics of transporter substrates and, thus, affect their efficacy and safety profiles. Because of the importance of transporter-mediated drug-drug interactions (DDIs) in clinical drug use, the US Food and Drug Administration (FDA; in the United States), the European Medicines Agency (EMA; in Europe), and the Pharmaceuticals and Medical Devices Agency (PMDA; in Japan) recommend conducting a clinical DDI study if an investigational drug has certain transporter inhibition potency *in vitro*.<sup>1–3</sup> In all of the guidance documents, the recommended first-step of evaluation is to calculate the ratio of maximum inhibitor concentration and *in vitro* inhibition constant ( $K_i$ ) and compare that with a predefined cutoff value (also called “basic models”). Despite the effort in the field to refine such *in vitro-in vivo* extrapolation strategies for the risk-assessment of transporter-mediated DDIs, a general discordance in the *in vitro-in vivo* extrapolation is

still prevalent.<sup>4,6</sup> The empirical cutoff values in these guidance documents reduce the risk of false-negative predictions to minimize the risk of unexpected clinical DDIs. However, this in turn can cause higher rates of false-positive predictions and may lead to unnecessary evaluation of transporter-mediated DDIs in clinical studies.

Recently, there has been a growing interest to replace or supplement dedicated transporter-related clinical DDI studies with measurement of endogenous biomarker kinetics in plasma or urine samples from clinical studies. For example, the coproporphyrin I (CPI) and III (CPIII), byproducts of heme synthesis, have been reported as promising biomarkers of organic anion transporting polypeptides 1B (OATP1B) transporters. These have been used as clinical diagnosis markers for Rotor syndrome, and a recent study demonstrated that genetic OATP1B deficiency causes Rotor syndrome.<sup>7</sup> Lai *et al.*<sup>8</sup> first demonstrated that CPI

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and CP111 showed comparable levels of increases in exposures as that of rosuvastatin, an OATP1B probe substrate, in the presence of OATP1B inhibitor rifampin in humans. Liu *et al.*<sup>9</sup> later demonstrated that these biomarkers are also sensitive to detect weak inhibition of OATP1B by GDC-0810, an orally bioavailable selective estrogen receptor downregulator, suggesting CPI and CP111 as promising alternatives to probe substrates in evaluating OATP1B-mediated DDIs. Other OATP1B biomarkers, as well as biomarkers for other transporters, have also been reported recently in humans, including bile acid conjugates for OATP1B,<sup>10,11</sup> taurine for organic anion transporter (OAT)1,<sup>12</sup> glycochenodeoxycholate-3-sulfate, and 6 $\beta$ -hydroxycortisol for OAT3,<sup>12,13</sup> or N-methylnicotinamide for multidrug and toxin extrusion protein.<sup>14</sup>

The use of an endogenous biomarker for transporter-mediated DDI assessment has numerous advantages over a dedicated clinical DDI study using probe substrates (X. Chu *et al.*, personal communication). Specifically, if a biomarker evaluation can replace dedicated DDI studies with probe substrates, one can avoid unnecessary exposure of subjects to the study drug and can offer cost and time efficiency in drug development. Assessment of DDI potential early in development will also help in clinical development strategies, such as appropriate concomitant medication guidance for phase II and III studies, timing of DDI studies, and reprioritization of other DDI studies. Furthermore, by measuring biomarker kinetics in dose-ranging studies, DDI potential in broader range of exposures can be evaluated that cannot be done in typical clinical DDI studies, which usually evaluates only one dose level of the perpetrators. On the other hand, endogenous biomarkers also have certain limitations for their reliable use in DDI assessment. These include paucity of clinical data to verify their performances in the presence of a variety of perpetrators and lack of experience in quantitatively linking changes in biomarker levels to the magnitude of clinical DDI, partly due to biomarkers' complex physiological distributions. Conventional exposure metrics for probe substrates, such as peak plasma concentration ( $C_{max}$ ) or area under the curve (AUC), are often inappropriate for describing the effect of inhibitors on biomarker kinetics, and model-based approaches are needed for the quantification of *in vivo* DDI magnitudes (X. Chu *et al.*, personal communication).

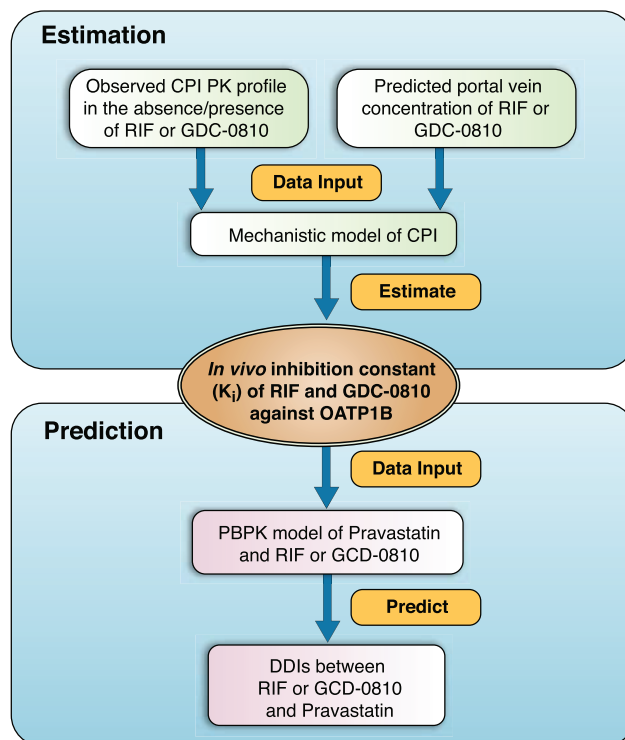
Recently, Barnett *et al.*<sup>15</sup> reported model-based analyses of CPI kinetic profiles in the presence of rifampin and demonstrated that similar *in vivo*  $K_i$  values can be obtained from CPI and rosuvastatin kinetic profiles. However, the clinical DDI data in their model analysis only involved one inhibitor, rifampin, and applicability of the approach to inhibitors with different potency was not fully evaluable. In this study, we aimed to demonstrate the practical utility of model-based approach to extrapolate biomarker observation into clinical DDI predictions using inhibitors with different potencies of inhibition, and to provide a framework for such translation in drug development scenarios. For these purposes, we analyzed kinetic profiles of CPI to quantify the effect of two OATP1B inhibitors with varying potency, rifampin and GDC-0810, and applied the estimated "*in vivo*"

$K_i$  for quantitative prediction of clinical DDIs with a probe substrate, pravastatin.

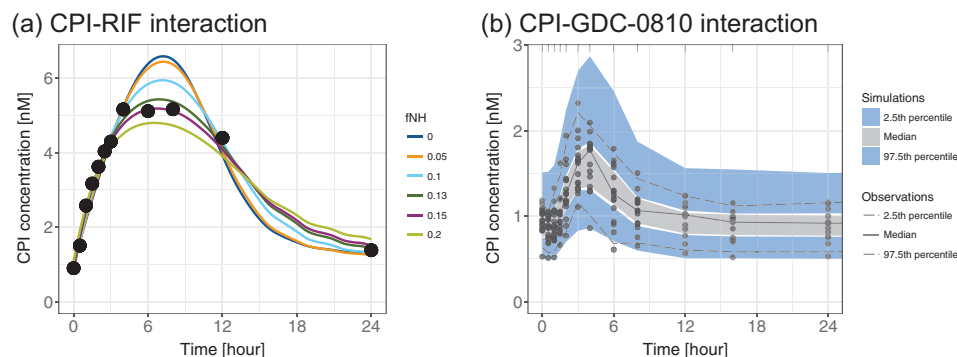
## METHODS

### Model-based analysis of CPI interactions with inhibitor kinetics

Plasma concentration-time profiles of CPI in the presence of rifampin<sup>8</sup> or GDC-0810<sup>9</sup> were used for model-based analysis. General workflow of the model-based analysis of CPI kinetics and the prediction of clinical DDIs is described in **Figure 1**. For the model-based analysis of CPI, observed CPI concentrations and predicted portal vein unbound inhibitor concentrations predicted from Simcyp were used as data input. For rifampin, the default Simcyp model file for single-dose rifampin was used for simulation. Development of the physiologically-based pharmacokinetic (PBPK) model for GDC-0810 is reported separately (Y. Chen *et al.*, personal communication). CPI disposition parameters and *in vivo* unbound  $K_i$  ( $K_{i,u}$ ) for each of the two inhibitors were estimated with a one-compartment model of CPI (**Figure S1b**) in nonlinear mixed-effect modeling (NONMEM). Model code is available in **Text S1**. Sensitivity analysis was performed to evaluate the influence of contribution of nonhepatic clearance ( $f_{NH}$ ) on the estimated values of other parameters, including  $K_i$ .



**Figure 1** Workflow of model-based analysis of coproporphyrin I (CPI) kinetic profiles and prediction of clinical drug-drug interactions (DDIs). OATP, organic anion-transporting polypeptide; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; RIF, rifampin.



**Figure 2** Observed and simulated concentration-time profiles of coproporphyrin I (CPI) in the presence of (a) rifampin (RIF) and (b) GDC-0810. Points represent observed concentration-time profiles. For a, colors represent simulated curves with optimized parameters using different  $f_{NH}$  values. For b, simulated profiles are represented as visual predictive check plot.  $f_{NH}$ , fraction of non-hepatic clearance to overall CPI clearance.

### Evaluation of urinary CPI elimination in Rotor syndrome patients

Urinary elimination profiles of CPI in patients with Rotor syndrome were queried in PubMed with the keyword “coproporphyrin AND urine AND Rotor’s syndrome.” Ratio of urinary elimination between healthy control subjects and patients with Rotor syndrome were calculated to estimate  $f_{NH}$ , as described in the Discussion section (Table 1).

### Prediction of clinical DDIs with physiologically based pharmacokinetic model

Estimated *in vivo*  $K_{i,u}$  of inhibitors were incorporated into the physiologically based pharmacokinetic (PBPK) models of rifampin and GDC-0810. Clinical DDIs between these inhibitors and an OATP1B probe substrate (pravastatin) were predicted using Simcyp default model file for pravastatin. Sensitivity analysis was performed to evaluate the influence of *in vivo*  $K_{i,u}$  values on the predicted magnitude of DDIs. For rifampin, a range of  $K_{i,u}$  were selected based on the ranges observed in the above sensitivity analysis for the influence of  $f_{NH}$  on  $K_{i,u}$  estimation. For GDC-0810,  $K_{i,u}$  values were increased/decreased by threefold, as there were little variation in the estimated  $K_{i,u}$  values in the above sensitivity analysis. The simulated AUC ratios (AUCRs) were compared with reported AUCR in clinical DDI studies.<sup>9,16,17</sup>

### Software

Simcyp simulator version 16 release 1 (Certara USA, Princeton, NJ) was used for the prediction of inhibitor portal vein concentrations and the prediction of clinical DDIs. All PBPK simulations were performed with virtual populations of 80 to 100 virtual subjects. NONMEM version 7.3 (ICON Development Solutions, Ellicott City, MD) was used for model-based analysis of CPI kinetics. R statistical software<sup>18</sup> was used for data assembly, exploratory data analysis, and model diagnosis.

## RESULTS

### Model-based analysis of CPI kinetics and estimation of *in vivo* unbound inhibition constant ( $K_{i,u}$ )

Figure 1 summarizes the workflow of model-based analysis of CPI kinetic profiles and prediction of clinical DDIs. Kinetic profiles of CPI in the presence of rifampin or GDC-0810 were well described by a one-compartment model (Figure 2; Figure S1b). For CPI-GDC-0810 interaction, both the central trend and variability were described with the estimated parameters including interindividual variability (IIV). Model parameters were reliably estimated with small to moderate parameter standard errors for most of parameters (Table 2). The estimated  $k_{deg}$  values were comparable between RIF-CPI and GDC-0810-CPI analysis (2.55 and 1.25 hours<sup>-1</sup>).

Sensitivity analysis of parameter estimates were performed by fixing  $f_{NH}$  to various numbers. The results showed that  $K_{i,u,RIF}$  estimate can reach as high as 10-fold with  $f_{NH}$  of 0% compared to the  $K_{i,u,RIF}$  estimate with optimized  $f_{NH}$  of 13% (Figure 3a). Likewise, sensitivity analysis was performed for CPI-GDC-0810 interaction, but  $f_{NH}$  had smaller influence on other parameters, including  $K_{i,u,GDC-0810}$ .

### Evaluation of urinary CPI elimination in patients with Rotor syndrome

A total of six publications were found to report urinary elimination of CPI in patients with Rotor syndrome (Table 1). Four of the reports included  $\leq 3$  patients with Rotor syndrome, whereas the other two reports included  $> 10$  patients with Rotor syndrome. Three publications reported CPI elimination as a ratio to creatinine elimination to normalize for urine volume, whereas the other three publications collected daily CPI elimination. The ratio of urinary elimination between healthy control subjects and patients with Rotor syndrome were comparable across different studies, ranging from 5–20% of healthy controls with the geometric mean of 9.8%. This value is comparable to the estimated  $f_{NH}$  of 13% from CPI-RIF analysis.

**Table 1** Reported urinary excretion of coproporphyrin I in subjects with and without Rotor's syndrome

Healthy control	Rotor syndrome	No. of patients with Rotor's syndrome	Unit	Healthy control/Rotor syndrome	Reference
13	370	2	nmol/day	0.036	31
<25	170	1	μg/day	<0.14	32
36	260	2	μg/day	0.14	33
6.6	35	3	μmol/mol creatinine	0.19	34
23	130	17	μg/g creatinine	0.18	35
13	230	11	μg/g creatinine	0.05	36
Geometric mean: <sup>a</sup>				0.098	

<sup>a</sup>Geometric mean excluded the study with one patient with Rotor syndrome.

### Prediction of clinical DDIs with pravastatin

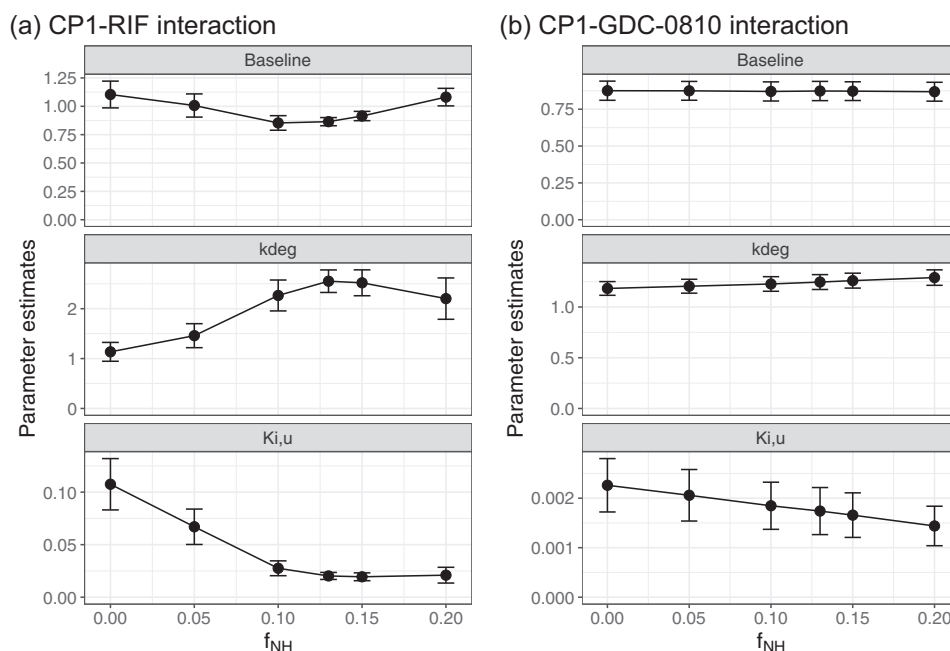
The predicted magnitude of DDIs (as represented by AUCR) between rifampin or GDC-0810 and pravastatin using the above estimated *in vivo*  $K_{i,u}$  values and the Simcyp pravastatin model were in good concordance with the observed AUCR (Figure 4). The effect of  $K_{i,u}$  on the predicted AUCR was then performed using sensitivity analysis with a range of  $K_{i,u}$ . For rifampin, the predicted AUCR was moderately sensitive to  $K_{i,u}$ ; larger  $K_{i,u}$  values, which were estimated with smaller  $f_{NH}$ , tend to predict lower AUCR. For GDC-0810, AUCR was sensitive to  $K_{i,u}$ , but the narrow range of  $K_{i,u}$  estimated from different  $f_{NH}$  (Figure 3b) was not likely to translate into appreciable differences in AUCR predictions.

### DISCUSSION

This study aimed to predict the magnitude of DDIs caused by the inhibition of OATP1B transporters via model-based

analysis of CPI kinetic profiles. Using a one-compartment model with hepatic and nonhepatic elimination routes, CPI kinetics in the presence and absence of inhibitors were described and “*in vivo*” inhibitory potency of two OATP1B inhibitors, rifampin (a strong inhibitor) and GDC-0810 (a weak inhibitor), were estimated (Figure 1) from their DDI with CPI. These estimated inhibitory potencies resulted in a reasonably accurate prediction of the observed magnitude of interaction with a probe drug, pravastatin, using PBPK modeling.

A one-compartment model described the observed PK profiles of CPI in the presence of OATP1B inhibitor rifampin and GDC-0810 well (Figure 2) and all parameters were estimated with reasonable precision, as noted by the low standard error of the parameter estimates (Figure 3, Table 2). Interestingly, estimated values of  $k_{deg}$  for CPI were similar regardless of inhibitors, suggesting that this estimate could be used universally for both model-based analysis and predictions



**Figure 3** Model-estimated parameter values with different  $f_{NH}$ . Points and bars represent point estimates of each parameter and standard error of the estimates, respectively. See Table 2 for the unit of each parameter. CPI, coproporphyrin I;  $f_{NH}$ , contribution of non-hepatic clearance to overall CPI clearance;  $k_{deg}$ , degradation rate constant of CPI;  $K_{i,u}$ , unbound inhibition constant of RIF or GDC-0810; RIF, rifampin.

**Table 2** Summary of estimated parameter values for CPI kinetics and inhibition constants of rifampin and GDC-0810 for OATP1B transporters

Parameters	Unit	RIF-CPI interaction	GDC-0810-CPI interaction
		Estimates, parameter SE (%)	Estimates, parameter SE (%)
Baseline	nM	0.863 (4.61)	0.873 (7.47)
$k_{deg}$	hr <sup>-1</sup>	2.55 (8.88)	1.25 (5.88)
$f_{NH}$ (fixed)	%	12.9 (6.66)	12.9 (fixed)
Proportional residual error	%CV	5.13 (20.4)	11.9 (14.9)
$K_{i,u}$	$\mu$ M	0.0203 (17.0)	0.00174 (27.3)
IIV on baseline	%CV	–	18.2 (23.2)
IIV on $K_{i,u}$	%CV	–	30.1 (54.3)

%CV, coefficient of variation; CPI, coproporphyrin I;  $f_{NH}$ , contribution of nonhepatic clearance to overall clearance of CPI; IIV, interindividual variability;  $k_{deg}$ , degradation rate constant of CPI; OATP, organic anion-transporting polypeptide; RIF, rifampin.

for trial designs. Moderate IIV was observed in  $K_{i,u,GDC-0810}$  (30.1%). In our CPI analysis, IIV in portal vein concentration of GDC-0810 was not considered. Although Simcyp allows for

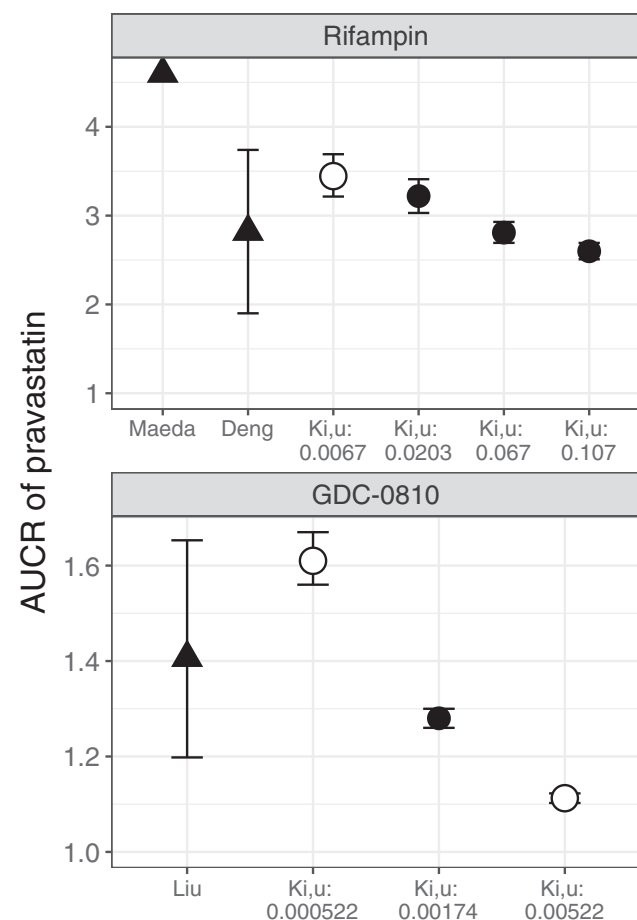
prediction of IIV, it is not designed to fit the observed concentration of each individual. Therefore, it was considered challenging to predict portal-vein concentrations for each individual in order to dissociate IIV in concentration from IIV in the observed magnitude of OATP1B inhibition. Therefore, the above IIV in  $K_{i,u,GDC-0810}$  includes both IIV in portal vein exposure as well as true IIV in inhibition constant. On the other hand, observed IIV in GDC-0810 plasma AUC was around 20%<sup>10</sup>. Thus, IIV of  $K_{i,u,GDC-0810}$  may be at least partly explained by the IIV in GDC-0810 portal-vein concentration, instead of sole contribution of inherent variability in inhibition constants.

The importance of the contribution of a hepatic elimination route of a substrate for accurately estimating the degree of inhibition by the inhibitor was highlighted in a previous study,<sup>15</sup> and the estimated  $f_{NH}$  in our study (13%) was comparable to the previously estimated contribution of renal elimination (12%). However, both of the two approaches have limitations. Our sensitivity analysis on  $f_{NH}$  of CPI did not result in a significant difference in overall model fit performance, suggesting that plasma concentration alone might not be sufficient to identify the contribution of each elimination route. Barnett *et al.*<sup>15</sup> made an assumption that nonhepatic clearance is dominated by urinary elimination. However, an earlier model-based analysis of CPI, which was based on <sup>15</sup>C-labeled coproporphyrin dosing, suggested some involvement of nonbiliary, nonrenal elimination pathways.<sup>19</sup> Overall, we currently have incomplete understanding of the elimination profiles of CPI.

To lend further support for the estimated contributions, we took another approach to estimate the contribution of hepatic elimination by utilizing CPI kinetics in patients with Rotor's syndrome. The rate of CPI urinary elimination ( $R_{urine}$ ) can be described as:

$$R_{urine} \approx \frac{K_{syn} \cdot CL_R}{CL_H + CL_{NH}} \quad (1)$$

where  $K_{syn}$ ,  $CL_H$ ,  $CL_{NH}$ , and  $CL_R$  represent synthesis rate, hepatic clearance, nonhepatic clearance, and renal clearance, respectively. If we assume  $CL_H$  becomes 0 in patients with Rotor's syndrome, ratio of  $R_{urine}$  in healthy control subjects and patients with Rotor's syndrome represent  $f_{NH}$ , even if  $CL_R$  is not the major nonhepatic elimination pathway:



**Figure 4** Observed and predicted area under the curve ratio (AUCR) of pravastatin in the presence of rifampin or GDC-0810. Triangles and circles represent observed<sup>9,16,17</sup> and predicted AUCR, respectively. Closed circles represents predictions with model-estimated  $K_{i,u}$  and open circles represents predictions with  $K_{i,u}$  values outside of the range of estimates from coproporphyrin I (CPI) analysis. Bars for observed and predicted data represent 90% confidence intervals and 95% prediction intervals, respectively.  $K_{i,u}$ , unbound organic anion-transporting polypeptide inhibition constant of rifampin or GDC-0810.



$$\frac{R_{urine,HC}}{R_{urine,RS}} \approx \frac{CL_{NH}}{CL_H + CL_{NH}} \quad (2)$$

Based on literature review, the contribution of nonhepatic clearance was estimated to be 4–19% (**Table 1**) and was comparable with model-estimated  $f_{NH}$  of 13% (**Table 2**). This strategy also has some limitations: (1) Eq. 1 ignores the fact that some of CPI is synthesized inside the hepatocyte and that part is not directly subject to hepatic and renal clearance; and (2) Eq. 2 assumes that  $CL_{NH}$  is not altered in the presence of Rotor syndrome. Nevertheless, estimated  $f_{NH}$  of ~10% is supported by all of the three analyses taking different approaches.

The estimated *in vivo*  $K_{i,u,RIF}$  was much lower than the reported *in vitro*  $K_{i,u,RIF}$  reported as discussed later in detail. To further evaluate the reliability of  $K_{i,u,RIF}$  estimates, sensitivity analysis for  $f_{NH}$  was performed, and  $K_{i,u,RIF}$  and  $K_{deg}$  were found to be sensitive to the  $f_{NH}$  of CPI (**Figure 3a**) for CPI-RIF interaction. This observation further supported the importance of clearly defining the elimination kinetic profiles of CPI in humans. Additional uncertainty in estimated  $K_{i,u}$  was the concentration of inhibitors used in simulations. The plasma concentration of rifampin used in our simulation was from the default Simcyp model compound file, which was verified using clinical data from Prueksaritanont *et al.*<sup>20</sup> However, the plasma concentration of rifampin measured in the study evaluating the interaction between CPI and rifampin<sup>8</sup> had twofold higher  $C_{max}$  and a longer terminal half-life. Preliminary analysis of CPI data with an updated rifampin model that matched observed time-profile in another paper<sup>9</sup> resulted in estimated  $K_{i,u,RIF}$  of 0.13  $\mu M$ , which was approximately five-times higher than the estimated value using the default rifampin model (results not shown). These observations highlight the importance of carefully choosing the inhibitor models and using the same model of inhibitors for parameter estimation with CPI and DDI predictions with probe substrates, as the use of different PBPK models can cause discrepancies in the predicted magnitude of inhibition.

Effects of the OATP1B inhibitors on pravastatin pharmacokinetics were predicted using estimated  $K_{i,u}$  values from the one-compartment model analysis. Predicted AUCR of pravastatin in the presence of rifampin was consistent with reported AUCR (**Figure 4**). Simulation with  $K_{i,u,RIF}$  from models with different  $f_{NH}$  showed that AUCR was not very sensitive to  $K_{i,u,RIF}$ . This is likely due to the relatively large contribution of the renal pathway to systemic elimination of pravastatin (47%).<sup>21</sup> Further validation of estimated  $K_{i,u,RIF}$  would be warranted using more sensitive OATP1B probes, such as pitavastatin.<sup>20</sup> Effect of GDC-0810 on pravastatin was also nicely recapitulated by using *in vivo*  $K_{i,u,GDC-0810}$  (**Figure 4**). Because  $f_{NH}$  did not have a large effect on  $K_{i,u,GDC-0810}$ , a threefold range of  $K_{i,u,GDC-0810}$  was tested to see how sensitive the predicted AUCR is to the  $K_{i,u,GDC-0810}$  estimate. The results demonstrated that AUCR was moderately sensitive, suggesting that accurate estimation of  $K_{i,u,GDC-0810}$  from model-based analysis is important for quantitatively extrapolating biomarker observation into prediction of clinical DDIs.

One of the important knowledge gaps in interpreting kinetic profiles of endogenous biomarkers is the involvement of other elimination pathways, such as other

transporters or biotransformation. For CPI, MRP2 is likely involved in the elimination, as suggested from *in vitro* studies<sup>22,23</sup> and altered kinetic profiles in patients with Dubin-Johnson syndrome (genetically linked disease with MRP2 deficiency) or subjects with ABCC2 polymorphisms.<sup>24</sup> It has implications on the inhibition potencies from clinical data. It is difficult, however, to quantify the contribution of MRP2 to overall elimination of CPI, because MRP2 is expressed in multiple organs, including the liver, kidneys, and intestines. As discussed earlier, some of the observed interactions between CPI and rifampin may come from inhibition of MRP2, whereas GDC-0810 is not expected to inhibit MRP2 at clinically relevant concentrations based on *in vitro* study (data not shown). One approach to address overlapping substrate specificities is to evaluate multiple endogenous biomarkers with different substrate specificities. For example, glycochenodeoxycholate-3-sulfate was recently shown to be another promising biomarker for OATP1B function.<sup>10</sup> Further studies in identifying contributions of multiple elimination pathways will help quantifying the impact on each transporter function using endogenous biomarkers.

Related to the above point, it is still challenging to assess the appropriateness of the model structures for endogenous biomarkers, largely due to limited mechanistic understanding of biomarker kinetics. One example is the possible contribution of nonhepatic, nonrenal elimination of CPI, as highlighted above. Another point is that the current one-compartment model assumes that change in hepatic uptake clearance is directly translated into apparent hepatic clearance, which is a composite of multiple parameters, such as hepatic intrinsic clearance, blood flow rate, and enterohepatic circulations. We entertained the possibility of evaluating the appropriateness of the current one-compartment models by approximating a full PBPK model with the help of available CPI kinetic data in patients with Rotor's syndrome (**Text S2**). However, it is considered premature at this moment to draw solid conclusion on model appropriateness, especially given the lack of knowledge on mass-balance profiles of CPI and potential compensatory change in nonhepatic pathways in patients with Rotor's syndrome.

One of the major challenges in prediction of clinical DDIs has been *in vitro-in vivo* extrapolation of inhibition potencies. Multiple retrospective analyses of clinical DDI data with the PBPK model suggested that *in vivo*  $K_i$  values are smaller than *in vitro*  $K_i$  values.<sup>25–28</sup> Several mechanisms have been proposed, such as time-dependent inhibition of OATP by cyclosporine A, potentially via intracellular transinhibition,<sup>29</sup> low free fraction in the *in vitro* incubation medium, inhibition of OATPs by protein bound-form of inhibitors, inhibition of OATPs by metabolites, or the inhibition of hepatic or intestinal MRP2 by rifampin.<sup>30</sup> However, quantitative extrapolation for accurate clinical DDIs remains challenging. In the current study, estimated *in vivo*  $K_{i,u}$  from biomarker kinetic profiles (rifampin: 0.0203  $\mu M$ ; GDC-0810: 0.00174  $\mu M$ ; **Table 2**) were much smaller than reported *in vitro*  $K_i$  (rifampin: ~2  $\mu M$ ,<sup>4</sup> GDC-0810: 0.9  $\mu M$  for OATP1B3 and ~80% inhibition at 0.3  $\mu M$  for OATP1B1<sup>9</sup>), which were consistent with the above-mentioned retrospective analyses. Indeed, these *in vivo*  $K_{i,u}$  resulted in accurate prediction of clinical DDIs (**Figure 4**). Although generalizability need to be

further verified with other clinical OATP1B inhibitors and more sensitive OATP1B probes, such as pitavastatin,<sup>20</sup> these results suggest great potential of the proposed approach to provide reliable estimates of *in vivo*  $K_{i,u}$  for clinical DDI prediction, which may ultimately inform the need and extent of dose modifications of OATP1B substrates in the presence of new molecular entity (NME) without conducting a dedicated clinical DDI study on OATP1B. Even if clinical DDI studies are still needed, developed models can facilitate trial simulations to optimize study designs, such as the number of doses needed or the duration of the follow-up period.

The framework in **Figure 1** can be generalized to predict OATP1B inhibition potential of an NME, and data elements needed for such prediction are: (1) CPI plasma concentration-time profiles in the presence and absence of a NME; (2) NME concentration-time profiles (ideally at the site of interaction); and (3) PBPK models for victim drugs. Both (1) and (2) can readily be obtained from any clinical studies, including first-in-human studies, provided that sufficient coverage of exposures, including clinically relevant concentrations of NME is available. CPI has short terminal half-life (<1 hour; **Table 2**), therefore, samples from a single-dose study is sufficient, unless NME has long terminal-half life and accumulates after multiple doses (X. Chu *et al.*, personal communication). For (3), the selection of victim drugs for DDI prediction requires careful considerations, just as in the selection of victim drugs for a dedicated DDI study (X. Chu *et al.*, personal communication), as well as the availability of validated PBPK model for victim drugs. These data elements allow the use of the modeling framework, to understand early in drug development, the liability of an NME as an OATP1B inhibitor.

In conclusion, this study demonstrated that the magnitude of clinical DDIs due to OATP1B inhibition can be predicted using  $K_{i,u}$  identified using model-based analysis of endogenous biomarker profiles, in this case CPI. Importantly, clinical DDIs with the probe substrate, pravastatin, were accurately predicted for inhibitors with different potencies, rifampin and GDC-0810, suggesting that the proposed strategy is applicable for both strong and weak inhibitors. The process of estimating *in vivo*  $K_i$ , followed by PBPK simulations utilized in this study presents a general modeling framework to predict OATP1B inhibition liability of an NME using biomarker data early in drug development.

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