When Does the Rate-Determining Step in the Hepatic Clearance of a Drug Switch from Sinusoidal Uptake to All Hepatobiliary Clearances? Implications for Predicting Drug-Drug Interactions

Gabriela I. Patilea-Vrana and Jashvant D. Unadkat

Department of Pharmacetics, University of Washington, Seattle, Washington

Received March 7, 2018; accepted August 10, 2018

ABSTRACT

For dual transporter-enzyme substrate drugs, the extended clearance model can be used to predict the rate-determining step(s) (RDS) of a drug and hence predict its drug-drug interaction (DDI) liabilities (i.e., transport, metabolism, or both). If the RDS of the hepatic clearance of the drug is sinusoidal uptake clearance (CL$_{s,uptake}$), even if the drug is eliminated mainly by hepatic metabolism, its DDI liability (as viewed from changes to systemic drug concentrations) is expected to be inhibition or induction of uptake transporters but not hepatic enzymes; however, this is true only if the condition required to maintain CL$_{s,uptake}$ as the RDS is maintained. Here, we illustrate through theoretical simulations that the RDS condition may be violated in the presence of a DDI. That is, the RDS of a drug can switch from CL$_{s,uptake}$ to all hepatobiliary clearances [i.e., metabolic/biliary clearance (CL$_{met+bile}$) and CL$_{s,all}$], leading to unexpected systemic DDIs, such as metabolic DDIs, when only transporter DDIs were anticipated. As expected, these analyses revealed that the RDS switch depends on the ratio of CL$_{met+bile}$ to sinusoidal efflux clearance (CL$_{s,eff}$). Additional analyses revealed that for intravenously administered drugs, the RDS switch also depends on the magnitude of CL$_{s,uptake}$: We analyzed published in vitro quantified hepatobiliary clearances and observed that most drugs have a CL$_{met+bile}$/CL$_{s,uptake}$ ratio < 4; hence, in practice, the magnitude of CL$_{s,uptake}$ must be considered when establishing the RDS. These analyses provide insights previously not appreciated and a theoretical framework to predict DDI liabilities for drugs that are dual transporter-enzyme substrates.

Introduction

Identifying liabilities with respect to drug-drug interactions (DDI) is important in drug development. In 2015, 25 of the 33 new drug applications contained in vitro transporter data, and of 20 clinical trials using the new molecular entities (NMEs) as victim drugs, only nine resulted in a significant area under the curve (AUC) change (Yu et al., 2017). These data acknowledge that drug transporters are important in determining drug disposition (Giacomini et al., 2010; Hillgren et al., 2013; Patel et al., 2016).

As shown by the hepatic extended clearance model (ECM), when a drug is both transported into and metabolized by or excreted by the liver, the rate-determining step (RDS) in the systemic clearance of the drug can be its hepatic uptake clearance, metabolic clearance, biliary (canalicular efflux) clearance, or all hepatobiliary clearances (Miyazaki et al., 1987; Stevens and Pang, 1997; Sihira et al., 2006; Kusuhara and Sugiyama, 2009; Li et al., 2014; Patilea-Vrana and Unadkat, 2016). The RDS of a drug can be identified using models such as the Extended Clearance Concept Classification System and the Extended Clearance Classification System, which use the drug’s in vitro quantified hepatobiliary clearance values or the drug’s physicochemical properties, respectively (Camenisch and Umehara, 2012; Varma et al., 2015). Using such models is advantageous since the RDS of a drug helps identify where the DDI liabilities lie. Of note, unless indicated otherwise, all subsequent references to DDI should be interpreted as those DDIs that can be observed from measurement of the systemic concentrations of the victim drug. For example, if the RDS of a drug is its hepatic uptake clearance (RDS$_{uptake}$), then the focus of the DDI studies should be transporter-based [e.g., hepatic organic anion-transporting polypeptide (OATP)-mediated uptake of atorvastatin] (Maeda et al., 2011) or if the RDS is both hepatic uptake and metabolic or biliary clearance (RDS$_{uptake+bile}$), the focus of DDI studies should be all hepatobiliary pathways (e.g., OATP and cytochrome P450-mediated clearance of cerivastatin) (Mück et al., 1999; Backman et al., 2002).

Here, we asked whether knowledge of the RDS of a drug is enough to predict DDI liabilities for drugs that are dual transporter-enzyme substrates. If not, the focus of DDI studies will be misdirected and will result in either a negative or unexpected DDI and therefore toxicity. Under the worst-case scenario, the latter will lead to discontinuation of drug development and the end result is that both outcomes will increase drug development cost (Paul et al., 2010). For these reasons, it is important to ask whether the RDS can switch from hepatic uptake clearance to all hepatobiliary clearance pathways thus resulting in unexpected systemic DDIs. Using the ECM theory and simulations, we aimed to: 1) provide a theoretical framework of when the RDS$_{uptake}$ switches to RDS$_{all}$ in the presence of a DDI and 2) apply the RDS
framework to predict DDI liabilities through theoretical and practical examples. The resulting analyses and simulations provide novel insights, hitherto not appreciated, into factors that determine when a victim drug experiences the RDSuptake switch to RDSall and elucidate important considerations for predicting DDI liabilities for drugs that are substrates of both hepatic transporters and enzymes.

**Materials and Methods**

**Theoretical Background.** The ECM describes complex hepatobiliary clearance in terms of transport at the sinusoidal membrane via sinusoidal influx \((CL_{in}^{s})\) and efflux \((CL_{ef}^{s})\), transport at the canalicular membrane via biliary efflux \((CL_{bile})\), metabolism \((CL_{met})\), hepatic blood flow \((Qh)\), and fraction unbound in blood \((fu)\) (eq. 1). \(CL_{in}^{s}\) and \(CL_{ef}^{s}\) terms incorporate both transport-mediated plus passive diffusion clearance, whereas \(CL_{bile}\) describes active transport only. The interrelationships between the hepatobiliary clearances defined by the ECM create the RDS in the hepatic clearance of a drug. As described by us and others (Miyachi et al., 1987; Sirianni and Pang, 1997; Shitara et al., 2006; Patilea-Vrana and Unadkat, 2016), these can be 1) RDSuptake when the metabolic and biliary efflux clearances of the drug are much less than sinusoidal efflux clearance \((CL_{in}^{s} < CL_{ef}^{s})\) and the drug is highly permeable (passive diffusion) and/or active transport, \(CL_{in}^{s} \approx CL_{ef}^{s}\) and thus can rapidly distribute across the sinusoidal membrane; 2) RDSuptake when the metabolic plus biliary efflux clearances are much greater than the sinusoidal efflux clearance \((CL_{in}^{s} + CL_{bile} > CL_{ef}^{s})\); or 3) RDSall when a drug has both active transport and metabolism, but the preceding two extreme scenarios do not apply (\(CL_{in}^{s} \neq CL_{ef}^{s}\)).

\[
CL_{in}^{h} = \frac{Qh \cdot fu \cdot CL_{in}^{s} \cdot (CL_{in}^{s} + CL_{bile})}{Qh \cdot (CL_{in}^{s} + CL_{met} + CL_{bile}) + fu \cdot CL_{in}^{s} \cdot (CL_{in}^{s} + CL_{bile})} \quad (1)
\]

Identifying the RDS of a drug can be used to predict the liability of transporter versus metabolic DDIs (see Patilea-Vrana and Unadkat, 2016, for simulations of systemic and hepatic AUC when hepatobiliary clearances are inhibited). For example, although a victim drug has RDSuptake, inhibition of \(CL_{met} + CL_{bile}\) will not result in a significant increase in the systemic AUC, even though such DDIs could result in significant drug accumulation in the liver and hence potentially enhance the hepatic efficacy or toxicity of the drug. That is, from the point of view of systemic (e.g., victim plasma concentrations) measurements, inhibition of \(CL_{met} + CL_{bile}\) will be incorrectly interpreted as negative because there will be no change in systemic concentrations of the drug. On the other hand, inhibition of \(CL_{in}^{s}\) will result in an increase in the drug’s systemic AUC (and therefore potentially nonhepatic efficacy and toxicity of the drug) but will result in no changes in the hepatic AUC, provided the liver is the primary eliminating organ (for examples, see Patilea-Vrana and Unadkat, 2016). Less appreciated, however, is the fact that experiences the RDSuptake switch to RDSall and elucidate important hitherto not appreciated, into factors that determine when a victim drug

**Quantifying When a Drug with RDSuptake Will Switch to RDSall from Metabolic/Biliary Efflux DDIs.** We defined the \(P_{\text{in}}\) as the percent inhibition of \(CL_{in}^{s}\) required for RDSuptake to switch to RDSall. This quantifies when a significant DDI (AUCR \(\geq 1.25\)) occurs from inhibition of \(CL_{met} + CL_{bile}\), even when uptake is the DDI in the absence of a DDI. For CLmet + bile/CLs ratios ranging from 1 to 100, CLmet + bile was inhibited 10%–99%. Simulations were performed for CLmet + bile/CLs values of 0.25, 1, 4, and 8. CLmet values were chosen to represent ER = 0.2, 0.5, and 0.8 [low-, mid-, and high-extraction ratio (ER), respectively] and were back-calculated from eq. 3 to eq. 4. The percent inhibition of \(CL_{in}^{s}\) at which the \(CL_{in}^{s}/CL_{in}\) ratio reaches the tipping point (i.e., \(P_{\text{in}}\)) and thus causes the RDSuptake to switch to RDSall was calculated as shown in eq. 5.
Applying the RDS Framework to In Vitro and In Vivo Examples. Published data sets in which all hepatobiliary clearance pathways (CL\textsubscript{met} + CL\textsubscript{bile}, CL\textsubscript{met}, CL\textsubscript{bile}) were quantified in vitro were collected. The in vivo hepatobiliary clearances must be used to identify the RDS of a drug. As such, the provided in vitro to in vivo extrapolated (IVIVE) clearances were used; otherwise, in vitro hepatobiliary clearance values were scaled to in vivo using IVIVE scaling factors as provided by the authors. For all drugs, \( t_{1/2}^{\text{CL} / Q_h} \) was used to calculate the tipping point using eq. 2 (see Results section to follow). RDS was labeled as RDS\textsubscript{uptake} and RDS\textsubscript{all} if the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio was above and below the tipping point, respectively. For drugs with RDS\textsubscript{uptake}, the PL\textsubscript{met} + bile was calculated using eq. 5. Finally, for selected drugs, the predicted DDI liabilities using the RDS and PL\textsubscript{met} + bile were compared with the observed in vivo data. To ensure that only the systemic clearance, and not the bioavailability of the victim drug, was affected, clinical DDI studies were included if the victim was a dual-transporter/enzyme substrate and coadministered with a selective enzyme inhibitor administered i.v. It should be noted that the availability of such studies was limited.

Results

Identifying the Tipping Point (i.e., When RDS\textsubscript{uptake} Switches to RDS\textsubscript{all} and Factors That Influence this Switch. As described in the Theoretical Background section, RDS\textsubscript{uptake} occurs when CL\textsubscript{met} + bile > CL\textsubscript{ef} and, as such, inhibition of CL\textsubscript{met} + bile will not manifest in the systemic AUC of a victim drug. When this condition is violated owing to extensive inhibition of CL\textsubscript{met} + bile, there will be a significant increase in the systemic AUC of the victim drug when CL\textsubscript{met} + bile is inhibited further. In other words, when CL\textsubscript{met} + bile is no longer > CL\textsubscript{ef}, then RDS\textsubscript{uptake} switches to RDS\textsubscript{all}. In Fig. 1A, 84%, 98%, and 99.8% inhibition of CL\textsubscript{met} + bile led to a clinically significant increase in the systemic AUC of the three theoretical victim drugs shown (AUCR ≥ 1.25). Even though the victim drugs had different preinhibition CL\textsubscript{met} + bile values (1, 10, 100 liters/min), the postinhibition CL\textsubscript{met} + bile values were all the same (0.2 liters/min). Since CL\textsubscript{ef} was kept constant (0.1 liters/min), an AUCR of 1.25 was observed when CL\textsubscript{met} + bile/CL\textsubscript{ef} was 2 for all three victim drugs. This simulation illustrates that the RDS\textsubscript{uptake} switch to RDS\textsubscript{all} depends on the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio, not on the extent of CL\textsubscript{met} + bile inhibition.

To further emphasize the dependence on the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio, we simulated the systemic AUC of the victim drug (in the absence of DDI) for different CL\textsubscript{met} + bile and CL\textsubscript{ef} values while holding CL\textsubscript{in} constant. The systemic AUC remained unchanged when the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio remained fixed, even though the CL\textsubscript{met} + bile and CL\textsubscript{ef} values varied.

Applying the RDS Framework to In Vitro and In Vivo Examples. Published data sets in which all hepatobiliary clearance pathways (CL\textsubscript{met} + CL\textsubscript{bile}, CL\textsubscript{met}, CL\textsubscript{bile}) were quantified in vitro were collected. The in vivo hepatobiliary clearances must be used to identify the RDS of a drug. As such, the provided in vitro to in vivo extrapolated (IVIVE) clearances were used; otherwise, in vitro hepatobiliary clearance values were scaled to in vivo using IVIVE scaling factors as provided by the authors. For all drugs, \( t_{1/2}^{\text{CL} / Q_h} \) was used to calculate the tipping point using eq. 2 (see Results section to follow). RDS was labeled as RDS\textsubscript{uptake} and RDS\textsubscript{all} if the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio was above and below the tipping point, respectively. For drugs with RDS\textsubscript{uptake}, the PL\textsubscript{met} + bile was calculated using eq. 5. Finally, for selected drugs, the predicted DDI liabilities using the RDS and PL\textsubscript{met} + bile were compared with the observed in vivo data. To ensure that only the systemic clearance, and not the bioavailability of the victim drug, was affected, clinical DDI studies were included if the victim was a dual-transporter/enzyme substrate and coadministered with a selective enzyme inhibitor administered i.v. It should be noted that the availability of such studies was limited.

Results

Identifying the Tipping Point (i.e., When RDS\textsubscript{uptake} Switches to RDS\textsubscript{all} and Factors That Influence this Switch. As described in the Theoretical Background section, RDS\textsubscript{uptake} occurs when CL\textsubscript{met} + bile > CL\textsubscript{ef} and, as such, inhibition of CL\textsubscript{met} + bile will not manifest in the systemic AUC of a victim drug. When this condition is violated owing to extensive inhibition of CL\textsubscript{met} + bile, there will be a significant increase in the systemic AUC of the victim drug when CL\textsubscript{met} + bile is inhibited further. In other words, when CL\textsubscript{met} + bile is no longer > CL\textsubscript{ef}, then RDS\textsubscript{uptake} switches to RDS\textsubscript{all}. In Fig. 1A, 84%, 98%, and 99.8% inhibition of CL\textsubscript{met} + bile led to a clinically significant increase in the systemic AUC of the three theoretical victim drugs shown (AUCR ≥ 1.25). Even though the victim drugs had different preinhibition CL\textsubscript{met} + bile values (1, 10, 100 liters/min), the postinhibition CL\textsubscript{met} + bile values were all the same (0.2 liters/min). Since CL\textsubscript{ef} was kept constant (0.1 liters/min), an AUCR of 1.25 was observed when CL\textsubscript{met} + bile/CL\textsubscript{ef} was 2 for all three victim drugs. This simulation illustrates that the RDS\textsubscript{uptake} switch to RDS\textsubscript{all} depends on the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio, not on the extent of CL\textsubscript{met} + bile inhibition.

To further emphasize the dependence on the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio, we simulated the systemic AUC of the victim drug (in the absence of DDI) for different CL\textsubscript{met} + bile and CL\textsubscript{ef} values while holding CL\textsubscript{in} constant. The systemic AUC remained unchanged when the CL\textsubscript{met} + bile/CL\textsubscript{ef} ratio remained fixed, even though the CL\textsubscript{met} + bile and CL\textsubscript{ef} values varied.
demonstrating that the RDS in the hepatic clearance of a drug is dependent on the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio, not on the absolute value of these clearances (Fig. 1B). This was true for both when $\text{Cl}_{\text{met}+\text{bile}}$ was higher and lower than $\text{Cl}_{\text{ef}}$ (also see Supplementary Fig. 1). Since the systemic AUC decreased as the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio increased, only the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio needs to be considered when determining when the RDS$_{\text{uptake}}$ switches to RDS$_{\text{all}}$ for a victim drug.

Next, we identified the tipping point, defined here as the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio when RDS$_{\text{uptake}}$ switches to RDS$_{\text{all}}$. The RDS$_{\text{uptake}}$ switch to RDS$_{\text{all}}$ signifies when DDIs owing to inhibition of $\text{Cl}_{\text{met}+\text{bile}}$ start to become significant for a victim drug that has RDS$_{\text{uptake}}$. As demonstrated already, the RDS$_{\text{uptake}}$ switch to RDS$_{\text{all}}$ depends on the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio. As such, we identified the tipping point as the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio at which the systemic AUC increases significantly (AUCR = 1.25) owing to a decrease in the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio for a victim drug that has RDS$_{\text{uptake}}$ (Fig. 1C). As demonstrated in Fig. 1C, the tipping point for a low, mid, and high ER drug was 3.2, 2.0, and 0.8, respectively.

Since the tipping point varied for a low, mid, and high ER, the magnitude of $\text{Cl}_{\text{in}}$ is also an important factor in determining when the RDS$_{\text{uptake}}$ switches to RDS$_{\text{all}}$ (Fig. 1C). Extending the simulations to identify the tipping point across a range of $\text{Cl}_{\text{in}}$ values, we established a theoretical (eq. 2) and practical (Fig. 2) relationship between $\text{Cl}_{\text{in}}/Q_h$ and the tipping point. The tipping point decreases as $\text{Cl}_{\text{in}}$ increases. In other words, as a drug’s $\text{Cl}_{\text{in}}$ (and therefore its ER) increases, the drug is more likely to have RDS$_{\text{uptake}}$ and a larger $\text{PIm}_{\text{met+bile}}$, therefore making the drug more resistant to switching its RDS. In addition, as the influx across the sinusoidal membrane becomes larger, hepatic clearance becomes limited by blood flow and therefore less likely to result in a change in AUCR when either $\text{Cl}_{\text{met}+\text{bile}}$ (or for that matter $\text{Cl}_{\text{met}+\text{bile}}$) is inhibited. On the other hand, when $\text{Cl}_{\text{in}}$ (or ER) is small and the hepatic clearance becomes proportional to $\text{Cl}_{\text{in}}$, the victim drug becomes more susceptible to a change in RDS. This demonstrates that low ER drugs are more susceptible to RDS$_{\text{uptake}}$ switching to RDS$_{\text{all}}$, whereas high ER drugs are more resistant to the RDS switch.

It should be noted that the relationship between $\text{Cl}_{\text{in}}/Q_h$ and the tipping point (eq. 2 and Fig. 2) depends on the chosen AUCR cutoff. Here, an AUCR of 1.25 was chosen based on Food and Drug Administration guidelines of what constitutes a positive DDI. If a higher AUCR cutoff were to be selected (Supplementary Fig. 2), this would lead to estimation of lower tipping points, thus making it more likely that drugs are labeled with RDS$_{\text{uptake}}$. Labeling a drug with RDS$_{\text{uptake}}$ when in fact it has RDS$_{\text{all}}$ can lead to underpredictions of DDI liabilities from metabolic enzymes and biliary transporters.

By understanding the relationship between $\text{Cl}_{\text{in}}$ and the tipping point, the RDS can be identified for any combination of a drug’s hepatobiliary clearance values (Fig. 2). For example, a high-ER drug with a $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio of 3 will have RDS$_{\text{uptake}}$, but a low-ER drug with the same $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio will have RDS$_{\text{all}}$. Furthermore, a drug will always have RDS$_{\text{uptake}}$ if the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio is greater than 4, irrespective of the value of $\text{Cl}_{\text{in}}$. It should be noted that for orally administered drugs, the tipping point will no longer depend on the magnitude of $\text{Cl}_{\text{in}}$ and therefore will always be 4 because blood flow limitations from systemic clearance are cancelled out by blood flow limitations of hepatic bioavailability.

Quantifying the $\text{PIm}_{\text{met+bile}}$ for Drugs with RDS$_{\text{uptake}}$. Identifying the RDS of a drug and when the RDS$_{\text{uptake}}$ to RDS$_{\text{all}}$ switch will happen identifies the drug’s DDI liabilities. We quantified the $\text{PIm}_{\text{met+bile}}$, defined here as the percent inhibition of $\text{Cl}_{\text{met}+\text{bile}}$ needed to cause the RDS$_{\text{uptake}}$ switch to RDS$_{\text{all}}$, to understand when inhibition of $\text{Cl}_{\text{met}+\text{bile}}$ starts to become a DDI liability for victim drugs that have RDS$_{\text{uptake}}$. As the $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio of the victim drug (before inhibition) increases, the $\text{PIm}_{\text{met+bile}}$ increases (Fig. 3A) because, as $\text{Cl}_{\text{met}+\text{bile}}$ becomes $>\text{Cl}_{\text{ef}}$, the victim drug become resistant to the RDS$_{\text{uptake}}$ switch to RDS$_{\text{all}}$. High-ER drugs have a higher $\text{PIm}_{\text{met+bile}}$ than low-ER drugs, demonstrating again that high-ER drugs are resistant to the RDS switch, whereas low-ER drugs are sensitive (Fig. 3A). Figure 3B illustrates that whereas a low-, mid-, and high-ER victim drug with $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ values of $\geq66\%$, $\geq66\%$, and $\geq87\%$, respectively, will cause the RDS$_{\text{uptake}}$ to switch to RDS$_{\text{all}}$. This translates to observing a positive DDI owing to inhibition of $\text{Cl}_{\text{met}+\text{bile}}$ for a victim drug that has been identified to have RDS$_{\text{uptake}}$ (before inhibition).

Without knowledge of the $\text{PIm}_{\text{met+bile}}$, such a DDI may not be expected. The purpose and conclusions of the simulations that have been used to establish the RDS framework up to this point are summarized in Fig. 4. As discussed, identifying the drug’s RDS is not enough to predict correctly the drug’s DDI liabilities. The tipping-point concept is an important consideration when identifying DDIs for victim drugs that are dual substrates of enzymes and transporters.

The flowchart in Fig. 5 can be used as a guide to identify the DDI liabilities for dual-transporter/enzyme substrates. All drugs with $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio $>4$ will have RDS$_{\text{uptake}}$ whereas drugs with $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio $<4$ will have RDS$_{\text{uptake}}$. So long as this ratio is greater than the tipping point. Drugs with $\text{Cl}_{\text{met}+\text{bile}}/\text{Cl}_{\text{ef}}$ ratio less than the tipping point will have RDS$_{\text{all}}$. If the drug has RDS$_{\text{uptake}}$, then uptake transporters will become a DDI liability, whereas if the drug has RDS$_{\text{all}}$, then transporters and enzymes will be a DDI liability. Even for drugs that have RDS$_{\text{uptake}}$, however, $\text{Cl}_{\text{met}+\text{bile}}$ can become a DDI liability if inhibition of $\text{Cl}_{\text{met}+\text{bile}}$ is greater than the predicted $\text{PIm}_{\text{met+bile}}$ and thus causes the RDS$_{\text{uptake}}$ switch to RDS$_{\text{all}}$. The flowchart identifies the

![](image-url)
**Fig. 3.** Identifying when drugs with RDS\textsubscript{uptake} will start to experience a DDI from inhibition of CL\textsubscript{met + bile}. (A) The P\textsubscript{imet + bile} identified as the % inhibition of CL\textsubscript{met + bile} required for the RDS\textsubscript{switch} to RDS\textsubscript{all} depends on the CL\textsubscript{met + bile}/CL\textsubscript{ef}' ratio (before inhibition) and the magnitude of CL\textsubscript{ef} (represented as low-, mid-, and high-ER drugs). The P\textsubscript{imet + bile} identifies when a positive DDI from inhibition of CL\textsubscript{met + bile}/CL\textsubscript{ef}' for a drug with RDS\textsubscript{uptake} would be expected. Lower CL\textsubscript{met + bile}/CL\textsubscript{ef}' ratios, as well as low ER drugs, are the most susceptible for the RDS\textsubscript{uptake} switch to RDS\textsubscript{all} owing to CL\textsubscript{ef} being the limiting factor in in vivo hepatobiliary transport. Such information may be used during drug development to select drug candidates if a certain RDS is desired.

**Applying the RDS Framework to In Vitro and In Vivo Examples.** To provide context to the theoretical framework presented, examples from literature, where available, were used. For drugs with in vitro–quantified hepatobiliary clearances that were extrapolated to in vivo via IVIVE, the tipping point and the P\textsubscript{imet + bile} were calculated using eq. 2 and eq. 5, and a subset of the analyzed data set, which includes primarily statin drugs, is shown in Fig. 6 (also see Supplementary Table 1) (Camenisch and Umehara, 2012; Jones et al., 2012; Varma et al., 2014; Kunze et al., 2015; Riede et al., 2017). If no empirical scaling factors (such as for active uptake clearance to match observed in vivo clearance) are required for the RDS\textsubscript{uptake} switch to RDS\textsubscript{all}, the RDS\textsubscript{uptake} switch (Supplementary Table 1). It further elucidates that current in vitro quantification techniques may underestimate CL\textsubscript{ef} which can lead to erroneous labeling of the RDS and thus incorrect DDI liability predictions (Fig. 7; Supplementary Fig. 3).

To illustrate more fully the applicability of the RDS framework, predicted DDI liabilities using the RDS framework were compared with in vivo DDI examples. As indicated in Table 1, when empirical scaling factors are used during the IVIVE process or hepatobiliary clearances were estimated from in vivo via PBPK, atorvastatin and repaglinide have RDS\textsubscript{uptake} and P\textsubscript{imet + bile} of 10%–51% and 15%–40%, respectively, whereas bosentan has RDS\textsubscript{all}. For atorvastatin and repaglinide, the in vitro data predicted that uptake transporters (OATPs) are the primary DDI liability, with the drugs’ major metabolic enzymes (CYP3A and CYP2C8, respectively) becoming a potential liability only if the in vivo hepatic metabolic inhibition is greater than the P\textsubscript{imet + bile}. For bosentan, in the in vitro data predicted that both OATPs and CYP3A4 are potential DDI liabilities. Clinically, for atorvastatin, coadministration of rifampin (an OATP inhibitor) leads to an AUCR of 12, whereas 33% inhibition of CYP3A4 due to intravenous itraconazole (as measured using CYP3A4 probe midazolam) did not change atorvastatin systemic AUC, even though inhibition of atorvastatin metabolism was observed via a decrease in the 2-hydroxyatorvastatin concentrations (Maeda et al., 2011). In a similarly conducted experiment, coadministration of rifampin resulted in an AUCR of 3.2 and 1.9 for bosentan or repaglinide, respectively, whereas 73% inhibition of CYP3A4 owing to intravenous itraconazole did not significantly change the systemic AUC of these drugs (Yoshikado et al., 2017). Furthermore, repaglinide coadministered with oral rifampin and trimethoprim (CYP2C8-selective inhibitor) resulted in AUCR 2.6 and 1.8, respectively (Kim et al., 2016). The in vivo DDI liability for OATPs was well predicted for all three victim drugs. The in vivo DDI liability for CYP3A4 was well predicted for atorvastatin. Since a probe was not used to assess the degree of CYP2C8 inhibition, it is difficult to determine whether the significant DDI when repaglinide was coadministered with trimethoprim was because RDS\textsubscript{uptake} Switched to RDS\textsubscript{all} or because repaglinide truly has RDS\textsubscript{all}. The in vitro metrics, as well as a whole-body PBPK DDI model, suggests that repaglinide has RDS\textsubscript{uptake} (Varma et al., 2013; thus, the repaglinide-trimethoprim DDI is likely due to the DDI switch. Lastly, since bosentan was predicted to have RDS\textsubscript{all}, a DDI was expected to result from CYP3A4 inhibition, but none was observed. It should be noted that the metabolic DDI liability prediction is assuming one main
drug-metabolizing enzyme and no significant biliary efflux (e.g., CLmet + bile = CLCYP3A4 for atorvastatin and bosentan). This assumption predicts the highest DDI risk owing to inhibition of CLmet + bile, and has a higher chance of predicting false-positive DDI results.

In the published in vitro data sets, discrepancies in the in vitro quantified values, particularly for CLs\textsubscript{in}, can be observed (Supplementary Table 1; Table 1). For example, in one report, the authors used empirical scaling factors for active sinusoidal uptake clearance to match hepatic clearance with clinically observed data that ranged from 1.1 to 101.8 with a geometric mean of 10.6 (Varma et al., 2014); however, the scaling factor used severely impacted the labeling of the RDS (e.g., fluvastatin, glyburide, pravastatin) or impacted the predicted PI\textsubscript{met + bile} of drugs (e.g., atorvastatin, rosuvastatin, fluvastatin, repaglinide) (Supplementary Table 1). Assumptions regarding CLs\textsubscript{ef} also caused discrepancies. In all reports, CLs\textsubscript{ef} was assumed to be equal to passive diffusion across the sinusoidal membrane, except in one report in which CLs\textsubscript{ef} was back-calculated from total sandwich cultured human hepatocytes CL\textsubscript{int} (Camenisch and Umehara, 2012). The assumptions surrounding CLs\textsubscript{ef} impacted the CLmet + bile/CLs\textsubscript{ef} ratio, which either changed how the RDS was labeled or the magnitude of the PI\textsubscript{met + bile} (e.g., aliskerin, ciprofloxacin, digoxin) (Supplementary Table 1). All in all, mispredictions of any of the hepatobiliary clearances impact the RDS labeling, magnitude of the PI\textsubscript{met + bile}, and DDI liability predictions.

Errors from in vitro quantification of hepatobiliary clearances can propagate when establishing the RDS and the predicted DDI liabilities. Underprediction of both CLs\textsubscript{in} and CLmet + bile may erroneously label a drug with RDS\textsubscript{all} when it is truly RDS\textsubscript{uptake} (Fig. 7). CLmet + bile is the more sensitive parameter for determining the RDS because underpredictions of CLs\textsubscript{in} may mislabel the RDS only for drugs with CLmet + bile/CLs\textsubscript{ef} ratio >4 (Supplementary Fig. 4). For such drugs, even moderate (e.g., 2- to 5-fold) underpredictions of either clearance pathway will lead to RDS mislabeling (Supplementary Fig. 4). Furthermore, underpredictions of both CLs\textsubscript{in} and CLmet + bile leads to underprediction of PI\textsubscript{met + bile} resulting in predicting a larger DDI liability owing to CLmet + bile inhibition for a drug with RDS\textsubscript{all} (Fig. 7; Supplementary Fig. 4). Whereas underpredictions of hepatobiliary clearances will result in conservative DDI decisions, they also increase the chances of negative DDI studies.

Discussion

We built a theoretical RDS framework and identified important considerations when predicting DDI liabilities for dual transporter-enzyme

**How to predict DDI liabilities for dual enzyme/transporter substrates?**

1. Identify the drug’s RDS in the absence of DDI.
   - The CL\textsubscript{met + bile}/CL\textsubscript{ef} ratio in relation to the tipping point defines the RDS of the drug.

2. Identify when RDS\textsubscript{all} will switch to RDS\textsubscript{uptake} in the presence of DDI.
   - Inhibition of CL\textsubscript{met + bile} that results in the CL\textsubscript{met + bile}/CL\textsubscript{ef} ratio to be less than the drug’s tipping point, is defined as PI\textsubscript{met + bile}.
   - PI\textsubscript{met + bile} identifies when inhibition of CL\textsubscript{met + bile} starts to become a DDI liability for victim drugs that have RDS\textsubscript{uptake}.

Fig. 4. Summary of the purpose and conclusions for the simulations used to establish the RDS framework.

Fig. 5. Applying the RDS framework to identify DDI liabilities for dual transporter-enzyme substrate drugs. If CL\textsubscript{met + bile}/CL\textsubscript{ef} >4, then the drug will have RDS\textsubscript{uptake}, irrespective of the magnitude of CL\textsubscript{met + bile}. For drugs with RDS\textsubscript{all}, DDIs due to inhibition of CL\textsubscript{met + bile} can become significant, depending on the drug’s CL\textsubscript{met + bile}/CL\textsubscript{ef} ratio and the expected inhibition of CL\textsubscript{met + bile}. For example, 50% inhibition of CL\textsubscript{met + bile} may result in a significant DDI for a drug with RDS\textsubscript{all}, and CL\textsubscript{met + bile}/CL\textsubscript{ef} ratio <8 but no DDI will be observed if the drug has CL\textsubscript{met + bile}/CL\textsubscript{ef} ratio >8. The DDI liability owing to inhibition of CL\textsubscript{met + bile} increases as the CL\textsubscript{met + bile}/CL\textsubscript{ef} ratio decreases and the expected CL\textsubscript{met + bile} inhibition increases.
analyses corroborate and expand upon these results to provide a…

...CLmet + bile/CLs ratio < 4, indicating drugs primarily exist within the moderate RDS framework space. Furthermore, most drugs have fubCLs/AUC < 0.4, indicating severe underprediction of CLs. (B) When empirical scaling factors are used or hepatobiliary clearances are estimates from in vivo data using PBPK modeling, the RDS of the drugs is altered severely.

Our theoretical analysis demonstrates that the CLmet + bile/CLs ratio, and not the absolute magnitudes of the clearances, determines the RDS in the hepatic clearance of a drug. Previous publications allude to this relationship. The authors of the ECCCS observed through experimental data that when CLmet + bile is 2 × CLs, drugs that have RDSuptake can be separated from those that do not (Riede et al., 2016). Furthermore, the β value [β = CLmet + bile/(CLmet + bile + CLs)] introduced by Yoshikado et al. (2016) can be used to differentiate the RDS, such as when β approaches unity (i.e., CLmet + bile > CLs), a drug has RDSuptake. Our analyses corroborate and expand upon these results to provide a quantitative definition of the demarcation point between RDSuptake and RDSall (i.e., the tipping point) and illustrate that the magnitude of CLs, in addition to the CLmet + bile/CLs ratio, is an important factor in determining the RDS of a drug. That is, as a drug’s CLs value increases, the drug is more likely to have RDSuptake and to become resistant to the RDSuptake switch to RDSall.

We found good agreement for atorvastatin in vivo to predict DDI liabilities (Table 1). For bosentan, overprediction of expected DDI owing to inhibition of CLmet + bile may be due to errors in the quantification of the hepatobiliary clearances. Indeed, a study in cynomolgus monkeys, in which bosentan plasma and liver drug concentrations were quantified, found that the in vitro scaled CLs and CLmet were 28- and 13-fold underpredicted, whereas CLcf (assumed equal to passive diffusion) was overpredicted by 2-fold compared with the in vivo–fitted values (Morse et al., 2017). Combining the in vitro metrics that identify RDSuptake for repaglinide with in vivo repaglinide DDIs, it appears that CYP2C8 and OATP1B1 inhibitor led to an 8-fold increase in systemic AUC; coadministration of itraconazole or cyclosporine (OATP1B1 and CYP3A4 inhibitor) led to much more modest 1.4- and 2.4-fold increases in systemic AUC (Niemi et al., 2003; Kajosaari et al., 2005).

...RDSuptake to RDSall and hence result in an unexpected systemic DDI. Two metrics have been developed to identify when the RDS switch occurs: the tipping point, defined as the CLmet + bile/CLs ratio at which RDSuptake will switch to RDSall, and the βmet + bile, defined as the percent inhibition of CLmet + bile at which a significant AUC change (AUCR > 1.25) for a drug with RDSuptake will start to be observed. The tipping point depends on the drug’s CLmet + bile/CLs ratio and on the magnitude of CLs. The former but not the latter condition is relevant when victim drugs are administered orally. Second, we showed that the CLmet + bile/CLs ratio must be > 4 for any drug to have RDSuptake. Third, we applied the RDS framework to in vitro–quantified hepatobiliary clearances and observed that most drugs have CLmet + bile/CLs ratio < 4; hence, in practice, the magnitude of CLs must be considered when establishing the RDS.

Our theoretical analysis demonstrates that the CLmet + bile/CLs ratio, and not the absolute magnitudes of the clearances, determines the RDS in the hepatic clearance of a drug. Previous publications allude to this relationship. The authors of the ECCCS observed through experimental data that when CLmet + bile is 2 × CLs, drugs that have RDSuptake can be separated from those that do not (Riede et al., 2016). Furthermore, the β value [β = CLmet + bile/(CLmet + bile + CLs)] introduced by Yoshikado et al. (2016) can be used to differentiate the RDS, such as when β approaches unity (i.e., CLmet + bile > CLs), a drug has RDSuptake. Our analyses corroborate and expand upon these results to provide a quantitative definition of the demarcation point between RDSuptake and RDSall (i.e., the tipping point) and illustrate that the magnitude of CLs, in addition to the CLmet + bile/CLs ratio, is an important factor in determining the RDS of a drug. That is, as a drug’s CLs value increases, the drug is more likely to have RDSuptake and to become resistant to the RDSuptake switch to RDSall.

We found good agreement for atorvastatin in vivo to predict DDI liabilities (Table 1). For bosentan, overprediction of expected DDI owing to inhibition of CLmet + bile may be due to errors in the quantification of the hepatobiliary clearances. Indeed, a study in cynomolgus monkeys, in which bosentan plasma and liver drug concentrations were quantified, found that the in vitro scaled CLs and CLmet were 28- and 13-fold underpredicted, whereas CLcf (assumed equal to passive diffusion) was overpredicted by 2-fold compared with the in vivo–fitted values (Morse et al., 2017). Combining the in vitro metrics that identify RDSuptake for repaglinide with in vivo repaglinide DDIs, it appears that CYP2C8 but not CYP3A4 inhibition may lead to RDSuptake switch to RDSall. Indeed, inhibition of repaglinide with gemfibrozil (CYP2C8 and OATP1B1 inhibitor) led to an 8-fold increase in systemic AUC; coadministration of itraconazole or cyclosporine (OATP1B1 and CYP3A4 inhibitor) led to much more modest 1.4- and 2.4-fold increases in systemic AUC (Niemi et al., 2003; Kajosaari et al., 2005).
The system used for in vitro quantification may be crucial since CLs-mediated clearance remains challenging (Chu et al., 2013; Feng et al., 2014). Scaling factors in Fig. 6 demonstrates that IVIVE of accurate transporter-CLmet + bile since the CLmet + bile/CLs ratio is one of the anchor points when establishing the RDS. Because CLmet is a difficult parameter to quantify in vitro, it is typically assumed to be equal to passive diffusion across the sinusoidal membrane; however, there are examples of active sinusoidal efflux transport, such as MRP3 efflux of rosuvastatin (Pfeifer et al., 2013). Active sinusoidal efflux would increase the magnitude of CLs and decrease the CLmet + bile/CLs ratio, making a drug more likely to have RDSs. One approach to measuring CLmet is to use an integrative temporal modeling approach in sandwich cultured hepatocytes (Pfeifer et al., 2013; Ishida et al., 2018).

Errors in the quantification of CLs and/or the CLmet + bile/CLs ratio can impact DDI liability predictions. For example, patients with OATP1B1 polymorphism c.521T>C have about a 2-fold greater atorvastatin AUC compared with the wild-type allele (Maeda, 2015). Because of the lower CLs, and therefore greater susceptibility to the RDSuptake to RDSall switch, patients with OATP1B1 polymorphism may experience a DDI attributable to inhibition of CYP3A, whereas patients with the wild-type allele may not. The same trend would be true for patients with polymorphic enzymes that result in lower CLmet + bile and thus lower CLmet + bile/CLs ratio. Polypharmacy use can also impact DDI liability predictions. For example, highly active antiretroviral therapy typically includes potent CYP3A4 and moderate OATP inhibitor roniviravir, among other drugs, which can impact the CLmet + bile/CLs ratio more severely than if only one drug is administered. Indeed, the systemic AUC of atorvastatin increased by 3.9- and 9.4-fold when coadministered with saquinavir/ritonavir and tipranavir/ritonavir, respectively (Fichtenbaum et al., 2002; Pham et al., 2009). Lastly, the saturation of enzymes, leading to a lower CLmet with increased dose, may lower the CLmet + bile/CLs ratio and cause DDIs owing to the RDSuptake to RDSall switch.

If a victim drug has RDSall, but it has been mislabeled as RDSuptake, then the DDI liability owing to inhibition of both transporter and metabolic activity could be underestimated. Considering potential DDI risks, it would be most conservative to assume a drug has RDSall; however, making such an assumption would lead to an increase in...
negative DDIs, particularly when conducting metabolic or biliary efflux DDI studies if the drug has DSS uptake. An analysis of the DDIs performed for a cohort of NMEs in 2013 showed a modest return on investment because 57% (n = 141) of all in vivo DDIs were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDIs, it may be more appropriate to make mechanistic-based rather than conservative decisions regarding DDI liabilities.

The RDS framework presented here should be used as a guide for identifying the DDI liabilities, whereas PBPK models should be used to develop the direction and magnitude of complex transporter-enzyme DDIs. Several examples of such models (e.g., repaglinide, simvastatin, rosuvastatin) exist that predict complex interactions resulting from chemical inhibition or genetic polymorphism (Varma et al., 2013; Rose et al., 2014; Tsamandouras et al., 2015). Even with PBPK models, there are limitations. For example, when a drug has DSS uptake, the CLm + bile is unidentifiable from plasma concentrations data since only CLm plays a significant role in determining hepatic clearance. Focusing on capturing the correct CLm + bile magnitude, and not the CLm + bile CLm ratio, can be misleading and will impact PBPK predictions. For instance, in an atorvastatin PBPK model, when cyclosporine CYP3A4 Ki was modulated 100-fold, a maximum 1.6 fold AUCR was achieved (Duan et al., 2017). Although the tendency is to run sensitivity analysis on the active investment because 57% (n = 141) of all in vivo DDIs were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDIs, it may be more appropriate to make mechanistic-based rather than conservative decisions regarding DDI liabilities.

The RDS framework presented here should be used as a guide for identifying the DDI liabilities, whereas PBPK models should be used to develop the direction and magnitude of complex transporter-enzyme DDIs. Several examples of such models (e.g., repaglinide, simvastatin, rosuvastatin) exist that predict complex interactions resulting from chemical inhibition or genetic polymorphism (Varma et al., 2013; Rose et al., 2014; Tsamandouras et al., 2015). Even with PBPK models, there are limitations. For example, when a drug has DSS uptake, the CLm + bile is unidentifiable from plasma concentrations data since only CLm plays a significant role in determining hepatic clearance. Focusing on capturing the correct CLm + bile magnitude, and not the CLm + bile CLm ratio, can be misleading and will impact PBPK predictions. For instance, in an atorvastatin PBPK model, when cyclosporine CYP3A4 Ki was modulated 100-fold, a maximum 1.6 fold AUCR was achieved (Duan et al., 2017). Although the tendency is to run sensitivity analysis on the active investment because 57% (n = 141) of all in vivo DDIs were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDIs, it may be more appropriate to make mechanistic-based rather than conservative decisions regarding DDI liabilities.

The RDS framework presented here should be used as a guide for identifying the DDI liabilities, whereas PBPK models should be used to develop the direction and magnitude of complex transporter-enzyme DDIs. Several examples of such models (e.g., repaglinide, simvastatin, rosuvastatin) exist that predict complex interactions resulting from chemical inhibition or genetic polymorphism (Varma et al., 2013; Rose et al., 2014; Tsamandouras et al., 2015). Even with PBPK models, there are limitations. For example, when a drug has DSS uptake, the CLm + bile is unidentifiable from plasma concentrations data since only CLm plays a significant role in determining hepatic clearance. Focusing on capturing the correct CLm + bile magnitude, and not the CLm + bile CLm ratio, can be misleading and will impact PBPK predictions. For instance, in an atorvastatin PBPK model, when cyclosporine CYP3A4 Ki was modulated 100-fold, a maximum 1.6 fold AUCR was achieved (Duan et al., 2017). Although the tendency is to run sensitivity analysis on the active investment because 57% (n = 141) of all in vivo DDIs were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDIs, it may be more appropriate to make mechanistic-based rather than conservative decisions regarding DDI liabilities.

The RDS framework presented here should be used as a guide for identifying the DDI liabilities, whereas PBPK models should be used to develop the direction and magnitude of complex transporter-enzyme DDIs. Several examples of such models (e.g., repaglinide, simvastatin, rosuvastatin) exist that predict complex interactions resulting from chemical inhibition or genetic polymorphism (Varma et al., 2013; Rose et al., 2014; Tsamandouras et al., 2015). Even with PBPK models, there are limitations. For example, when a drug has DSS uptake, the CLm + bile is unidentifiable from plasma concentrations data since only CLm plays a significant role in determining hepatic clearance. Focusing on capturing the correct CLm + bile magnitude, and not the CLm + bile CLm ratio, can be misleading and will impact PBPK predictions. For instance, in an atorvastatin PBPK model, when cyclosporine CYP3A4 Ki was modulated 100-fold, a maximum 1.6 fold AUCR was achieved (Duan et al., 2017). Although the tendency is to run sensitivity analysis on the active investment because 57% (n = 141) of all in vivo DDIs were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDIs, it may be more appropriate to make mechanistic-based rather than conservative decisions regarding DDI liabilities.

The RDS framework presented here should be used as a guide for identifying the DDI liabilities, whereas PBPK models should be used to develop the direction and magnitude of complex transporter-enzyme DDIs. Several examples of such models (e.g., repaglinide, simvastatin, rosuvastatin) exist that predict complex interactions resulting from chemical inhibition or genetic polymorphism (Varma et al., 2013; Rose et al., 2014; Tsamandouras et al., 2015). Even with PBPK models, there are limitations. For example, when a drug has DSS uptake, the CLm + bile is unidentifiable from plasma concentrations data since only CLm plays a significant role in determining hepatic clearance. Focusing on capturing the correct CLm + bile magnitude, and not the CLm + bile CLm ratio, can be misleading and will impact PBPK predictions. For instance, in an atorvastatin PBPK model, when cyclosporine CYP3A4 Ki was modulated 100-fold, a maximum 1.6 fold AUCR was achieved (Duan et al., 2017). Although the tendency is to run sensitivity analysis on the active investment because 57% (n = 141) of all in vivo DDIs were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDIs, it may be more appropriate to make mechanistic-based rather than conservative decisions regarding DDI liabilities.

The RDS framework presented here should be used as a guide for identifying the DDI liabilities, whereas PBPK models should be used to develop the direction and magnitude of complex transporter-enzyme DDIs. Several examples of such models (e.g., repaglinide, simvastatin, rosuvastatin) exist that predict complex interactions resulting from chemical inhibition or genetic polymorphism (Varma et al., 2013; Rose et al., 2014; Tsamandouras et al., 2015). Even with PBPK models, there are limitations. For example, when a drug has DSS uptake, the CLm + bile is unidentifiable from plasma concentrations data since only CLm plays a significant role in determining hepatic clearance. Focusing on capturing the correct CLm + bile magnitude, and not the CLm + bile CLm ratio, can be misleading and will impact PBPK predictions. For instance, in an atorvastatin PBPK model, when cyclosporine CYP3A4 Ki was modulated 100-fold, a maximum 1.6 fold AUCR was achieved (Duan et al., 2017). Although the tendency is to run sensitivity analysis on the active investment because 57% (n = 141) of all in vivo DDIs were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDIs, it may be more appropriate to make mechanistic-based rather than conservative decisions regarding DDI liabilities.


---

**Address correspondence to:** Dr. Jashvant D. Unadkat, Department of Pharmaceutics, University of Washington, Box 357610, Seattle, WA 98195. E-mail: jash@uw.edu