DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

Navigating Transporter Sciences in Pharmacokinetics Characterization Using Extended Clearance Classification System (ECCS)

Ayman F. El-Kattan and Manthena V. S. Varma

Pharmacokinetics Dynamics and Metabolism, Medicine Design, Pfizer Global Research and Development, Pfizer Inc., Cambridge, MA (AFE) and Groton, CT (MVS).

Running title: Drug transporters and ECCS

Corresponding Author: Manthena V. Varma, Pharmacokinetics, Dynamics, and Metabolism, MS 8220-2451, Pfizer Global Research and Development, Pfizer Inc., Groton, CT 06340; Phone:+1-860-715-0257. Fax: +1-860-441-6402. E-mail: manthena.v.varma@pfizer.com

Number of text pages: 25

Number of tables: 0

Number of Figures: 5

Number of references: 102

Number of words in Abstract: 226

ABBREVIATIONS: ADCE, absorption, distribution, clearance and elimination; AUC, area under the plasma concentration-time curve; BCRP, breast cancer resistance protein; CYP, Cytochrome P-450; DDI, drug-drug interaction; ECCS, extended clearance classification system; IC_{50}, inhibitory potency; Kp_{uu}, liver-to-plasma unbound concentration ratio; MRP, multidrug resistance protein; NTCP, Na+-taurocholate cotransporting polypeptide; MATE, multidrug and toxin extrusion protein; MDCK, Madin-Darby Canine Kidney; MW, molecular weight; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; PBPK, physiologically-based pharmacokinetic; P-gp, P-glycoprotein; SLC, solute carrier.

DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

Membrane transporters play an important role in the absorption, distribution, clearance and elimination (ADCE) of the drugs. Supported by the pharmacokinetics data in human, several transporters including organic anion transporting polypeptide (OATP)1B1, OATP1B3, organic anion transporter (OAT)1, OAT3, organic cation transporter (OCT)2, multidrug and toxin extrusion proteins (MATEs), P-glycoprotein and breast cancer resistance protein (BCRP) are suggested to be of clinical relevance. An early understanding of transporters role in the drug disposition and clearance allows reliable prediction/evaluation of the pharmacokinetic changes due to drug-drug interactions (DDIs) or genetic polymorphisms. We recently proposed extended clearance classification system (ECCS) based on simple drug properties (i.e., ionization permeability and molecular weight) to predict predominant clearance mechanism. According to this framework, systemic clearance of class 1B and 3B drugs is likely determined by the OATP-mediated hepatic uptake. Class 3A, 4 and certain class 3B drugs are predominantly cleared by renal, wherein, OAT1, OAT3, OCT2 and MATEs could contribute to their active renal secretion. Intestinal efflux and uptake transporters largely influence the oral pharmacokinetics of class 3A, 3B and 4 drugs. We discuss the paradigm of applying ECCS framework in mapping the role of clinically relevant drug transporters in early discovery and development; and thereby, implementing the right strategy to allow optimization of drug exposure and evaluation of clinical risk due to DDIs and pharmacogenomics.

INTRODUCTION

Poor pharmacokinetics was attributed to almost 40% of the overall attrition during drug development during 1990s (Kola and Landis, 2004). This is largely due to limitations in quantitative predictive tools resulting in unexpected high intestinal and hepatic extraction. To overcome high first-pass liabilities and filter out compounds with high metabolic clearance, drug discovery teams adopted evolving *in vitro* tools such as human liver microsomes (HLM) and hepatocytes (HHEP) to facilitate clearance optimization in early discovery. Improvements in human reagents and translation methodologies further allowed the successful prediction of human hepatic clearance mediated by drug metabolizing enzymes for new molecular entities (NMEs) (Houston, 1994; Obach, 1999; Hosea et al., 2009; Ring et al., 2011; Di et al., 2013).

It has been recognized that membrane transporters expressed in a variety of body organs such as liver, brain, intestine, and kidney play an important in the absorption, distribution, clearance and elimination (ADCE) of drugs and metabolites (International Transporter Consortium et al., 2010). About 400 membrane proteins categorized into two superfamilies of ATP-binding cassette (ABC) and solute carrier (SLC) transporters have been identified in human body (International Transporter Consortium et al., 2010). However, less than 20 of them are considered relevant in ADCE of drugs. In several cases, the clinical significance of the drug transporters was ascertained by transporter genetic polymorphism and drug-drug interaction (DDI) studies, which demonstrated major changes in the pharmacokinetics and/or consequent clinical responses of the substrate drugs (Shitara and Sugiyama, 2006; Niemi et al., 2011; Elsby et al., 2012; Lai et al., 2012).

Organic anion transporting polypeptides (OATP)1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*), and OATP2B1 (*SLCO2B1*), the organic anion transporter (OAT2, *SLC22A7*), and the organic cation

transporter (OCT1, *SLC22A1*), sodium taurocholate cotransporting polypeptide (NTCP, *SLC10A1)* are hepatic sinusoidal transporters shown to drive hepatic uptake of a wide variety of drugs and metabolites. On the other hand, canalicular membrane transporters, such as multidrug resistance protein (MRP2, *ABCC2*), breast cancer resistance protein (BCRP, *ABCG2*), and P-glycoprotein (P-gp, *ABCB1*) mediate biliary secretion (Muller and Jansen, 1997; Chandra and Brouwer, 2004; International Transporter Consortium et al., 2010; Shitara et al., 2013; Pfeifer et al., 2014). In kidney, organic anion transporter 1 and 3 (OAT1 and OAT3) and organic cation transporter 2 (OCT2) localized on the basolateral membrane and multidrug and toxin extrusion proteins (MATE1/2-K) expressed on the apical membrane of the proximal tubule cells are of relevance in active renal secretion and reabsorption of drugs. While many ABCs and SLCs have been identified in human intestine, efflux transporters including P-gp and BCRP are often implicated in limiting oral drug absorption (Kim et al., 1998; Varma et al., 2003; Kunta and Sinko, 2004; International Transporter Consortium et al., 2010; Varma et al., 2010a; Estudante et al., 2013). Collectively, clinical evidence point to the need for understanding the role of OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K, P-gp and BCRP in the disposition of investigational drug (International Transporter Consortium et al., 2010; EMA, 2012; USFDA, 2012). Nevertheless, other transporters could be of high relevance in the pharmacokinetics of certain chemotypes and warrants characterization on case-by-case basis.

Here, we review the mechanistic aspects of major clearance mechanisms, namely hepatic uptake, metabolism and renal clearance; and present the scope and utility of the Extended Clearance Classification System (ECCS) as a framework for navigating through transporter sciences in the process of characterizing clearance and disposition mechanism(s) and predicting transporter-mediated DDIs in drug discovery and development.

DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

Extended Clearance Classification System

On the premises that early identification of clearance mechanism can facilitate adopting the 'right' strategy and tools for quantitative pharmacokinetic predictions, we recently proposed a framework called the extended clearance classification system (ECCS) (Varma et al., 2015b). The primary scope of this classification system is to identify the clearance mechanism (rate-determining step) of NMEs using physicochemical properties and *in vitro/in silico* data readily available in early drug discovery. According to ECCS, NMEs are classified based on permeability, molecular weight (MW), and ionization state, which are previously shown to be strongly associated with major clearance mechanisms – hepatic uptake, metabolism and renal clearance. For example, Benet and co-workers proposed that high permeable compounds shown high extent of metabolism (>70%) and vice versa (Wu and Benet, 2005). On the other hand, Pfizer colleagues demonstrated that high MW (≥400 Dalton) acids/zwitterions undergo hepatic uptake via OATP transporters, which is often the rate-determining step in their clearance (Varma et al., 2012a). Additionally, compounds undergoing biliary excretion often involve hepatic uptake as the rate-determining step in their systemic clearance. Subsequently, we established a permeability cut-off of $5x10^{-6}$ cm/s using in-house low-efflux Madin-Darby Canine Kidney (MDCK) cell lines in the process of implementing ECCS right from the early stages of drug discovery (Varma et al., 2012b). Extensive validation of ECCS resulted in overall good predictive rates (Varma et al., 2015b; El-Kattan et al., 2016; Varma et al., 2017a). The general characteristics of the six classes with respect to the clearance mechanism are as follows (Figure 1):

ECCS Class 1A: Acids/zwitterions with high permeability and small MW (<400 Dalton). The clearance of class 1A compounds is determined by metabolic rates with extent of

DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

metabolism ≥70%. These tend to be metabolized by UGT ≅ CYP2C enzymes >> esterases >> CYP3A4 enzymes.

ECCS Class 1B: Large MW (≥400 Dalton) acids/zwitterions and with high permeability. These compounds involve hepatic uptake mediated by OATP1B1/1B3 in their systemic clearance. Once in the liver, they are generally metabolized by CYP2C > esterases > UGT > CYP3A enzymes and excreted in bile/urine as metabolites. The extent of metabolism is high (≥70%).

ECCS Class 2: Bases/neutrals with high membrane permeability. Similar to class 1A and 1B, class 2 compounds are cleared by metabolism [high extent of metabolism ≥ 70%]. They are primarily metabolized by enzymes such as CYP3A4>> UGT > CYP2D6 > esterases = CYP2C. The high contribution of CYP3A4, CYP2D6, and UGT enzymes is in agreement with the basic nature of many of these drug molecules and their higher lipophilicity (Log $D_{pH7.4}$).

ECCS Class 3A: Acids/zwitterions with low permeability and small MW (<400 Dalton). Class 3A compounds are renally cleared, where OAT1 and OAT3 transporters are potentially involved in their active renal secretion. These are also potential substrates for efflux transporters such as BCRP, MRP2 and P-gp, which facilitate active secretion of hydrophilic compounds across the apical membrane of proximal tubule cells.

ECCS Class 3B: Acids/zwitterions with low permeability and large MW (≥400 Dalton). Their mechanism of clearance eliminationis either active hepatic uptake and/or renal elimination. The hepatic uptake is typically mediated by OATPs transporters; once in the liver, they tend to be eliminated in bile as unchanged drug. Renal secretion is primarily mediated by OAT transporters.

ECCS Class 4: Bases/neutrals with low permeability. They are primarily eliminated renally with low extent of metabolism <30%. Their renal elimination is mediated by OAT1, OAT3, and/or OCT2; and P-gp and MATE1/2K appears to be the major efflux transporters affecting the renal elimination.

Permeability categorization here is based on the MDCK cells with low efflux activity (Di et al., 2011). Tools based on artificial membranes (eg. phospholipid derived PAMPA membrane) (Yu et al., 2015) or other cell types (eg. Caco-2 cells with chemical inhibition of transporters (Fredlund et al., 2017)) could be validated to serve this purpose. Since many of the cell types express a wide range of transporters to varying degree, validation should also focus on assessing the risk of misclassification (high vs low permeability) due to active mechanisms. MDCK cell expression of known renal uptake transporters such as OATs and OCTs is very low (Aslamkhan et al., 2003), thus may provide good measure of passive transcellular permeability.

Hepatic Clearance and quantitative role of transport and metabolism

Increasing knowledge on the role of transporters in drug clearance led to the introduction of extended clearance concept by Sandy Pang (Sirianni and Pang, 1997), which was extensively investigated by Sugiyama and co-workers and several other research groups (Shitara and Sugiyama, 2006; Poirier et al., 2009; Watanabe et al., 2009; Jones et al., 2012; Varma et al., 2012c; Gertz et al., 2013; Jamei et al., 2014; Varma et al., 2015b; Patilea-Vrana and Unadkat, 2016). Considering the physiological components, extended clearance concept define the intrinsic hepatic clearance ($CL_{int,h}$) as an interplay of various processes namely, passive diffusion clearance (PS_{pd}) transporter-mediated sinusoidal influx clearance (PS_{influx}), basolateral efflux

DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

clearances (PS_{efflux}) and biliary and metabolic intrinsic clearances ($CL_{int} = CL_{int,bile} + CL_{int,met}$). The interplay of these four processes defines the rate-determining step in hepatic clearance. Mathematically expressed (Eq. 1) (Liu and Pang, 2005):

$$CL_{int,h} = \frac{\left(PS_{influx} + PS_{pd}\right) \cdot CL_{int}}{\left(PS_{efflux} + PS_{pd} + CL_{int}\right)} \quad \text{(Eq. 1)}$$

The total hepatic blood clearance (CL_h), assuming well-stirred conditions, can therefore be expressed using Eq. 2:

$$CL_h = Q_h \cdot \frac{f_{u,b} \cdot \left(PS_{influx} + PS_{pd}\right) \cdot CL_{int}}{Q_h \cdot \left(PS_{efflux} + PS_{pd} + CL_{int}\right) + f_{u,b} \cdot \left(PS_{influx} + PS_{pd}\right) \cdot CL_{int}} = Q_h \cdot E_h \quad \text{(Eq. 2)}$$

Where Q_h is the hepatic blood flow, and E_h is the hepatic extraction ratio. $f_{u,b}$ is the unbound fraction in blood. Over the last few years, extended clearance concept has captured significant attention due to its ability to address questions related to drug clearance, DDIs and pharmacogenomics, where the extent of metabolism was not able to successfully address (Watanabe et al., 2010; Jones et al., 2012; Varma et al., 2014).

The limiting conditions of the extended clearance term can be referred to as 'rapid-equilibrium' and 'uptake-determined' clearance. Extended clearance term (Eq. 2) is reduced to rapid-equilibrium condition (Eq. 3) when the compound is not a substrate for hepatic uptake transporters (e.g. OATP 1B1/1B3 and NTCP) and PS_{pd} is significantly higher than CL_{int}.

$$CL_h = Q_h \cdot \frac{f_{u,b} \cdot CL_{int}}{Q_h + f_{u,b} \cdot CL_{int}} \quad \text{(Eq. 3)}$$

It is generally acceptable to assume rapid-equilibrium condition for the compounds of ECCS class 2, where metabolism is typically the rate-determining step in their hepatic clearance (e.g. midazolam, propranolol, nifedipine, verapamil, indomethacin, and ibuprofen) (El-Kattan et al., 2016). For such compounds, hepatic clearance is expected to be well predicted using HLM for

DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

CYP substrates, and human hepatocytes for other drug metabolizing enzymes such as sulfotransferases, aldehyde oxidase, UDP glucuronosyltransferases (UGTs), and glutathione transferase (GST), etc (Houston, 1994; Obach, 1999; Williams et al., 2004; Hosea et al., 2009; Di et al., 2013).

On the other hand, 'uptake-determined' clearance can be assumed (Eq. 4) when a compound show active hepatic uptake, and PS_{pd} is significantly lower than CL_{int}.

$$CL_h = Q_h \cdot \frac{f_{u,b} \cdot PS_{influx}}{Q_h + f_{u,b} \cdot PS_{influx}} \quad \text{(Eq. 4)}$$

Most class 3B compounds and several class 1B compounds with hepatic uptake are shown to possess such characteristics (Watanabe et al., 2009; Maeda et al., 2011). Examples of drugs with hepatic uptake as rate-determining step for their systemic clearance include HMG-CoA reductase inhibitors (statins) and angiotensin II antagonists (sartans). *In vitro* metabolic clearance measured in HLM tends to underpredict hepatic clearance. However, predictions substantially improve if hepatic intrinsic uptake clearance measured in suspension or cultured human hepatocytes (eg. sandwich culture hepatocyte model) are considered (Watanabe et al., 2010; Jones et al., 2012; Ménochet et al., 2012; Varma et al., 2014; Bi et al., 2017; Kimoto et al., 2017). The role of active hepatic uptake as rate-determining step in the systemic clearance of class 1B and 3B drugs is substantiated by drug-drug interactions (DDIs) (Shitara et al., 2006; Maeda et al., 2011; Prueksaritanont et al., 2014; El-Kattan et al., 2016) and *SLCO1B1* polymorphism reports (encoding OATP1B1) (Nishizato et al., 2003; Niemi et al., 2005; Group et al., 2008; Ieiri et al., 2009).

ECCS and victim DDIs involving major hepatic transporters

We recently evaluated extensive and unbiased datasets of clinical DDIs of victim drugs with 'first-choice' clinical probe inhibitors recommended to investigate transporter activity involving OATP1B1/1B3 (rifampicin and cyclosporine), P-gp and BCRP (cyclosporine), OAT1/3 (probenecid), and OCT2 and MATEs (cimetidine) (Varma et al., 2017a). We identified a total of 276 interaction pairs (23 – rifampicin, 43 – cyclosporine, 62 – probenecid and 148 – cimetidine) with ECCS class assigned using our in-house permeability data, MW and ionization, and analyzed the DDI liability per ECCS class.

Clearly, co-administration of OATP1B1/1B3 probe inhibitor, rifampicin, caused moderate (AUC ratio 2-5) and high (AUC ratio >5) interactions for class 1B and 3B drugs. Consistent to ECCS predictions, only no/low (AUC ratio <2) interactions are evident for classes 1A/3A/2/4, although availability of clinical data for drugs in these classes – especially class 1A and 3A – is relatively sparse (Figure 2A). Nonetheless, very limited exceptions emerged following this exercise. For example, ambrisentan, a borderline class 3A drug, yielded ~2-fold interaction with rifampicin. This drug has been shown to be a substrate to OATPs, and the observed interaction can be ascribed to inhibition of OATP1B1/1B3-mediated hepatic uptake (Harrison et al., 2010). Overall, this analysis verifies that the clearance of class 1B and 3B drugs, but not others, is driven by OATP-mediated hepatic uptake. Further, note that the systemic clearance of the high permeable compounds with high extent of metabolism (class 1B) can be determined predominantly by OATPs. For example, Maeda *et al.* investigated the impact of a single dose rifampicin (OATP1B1/1B3 probe inhibitor) versus itraconazole (CYP3A4 probe inhibitor) on the pharmacokinetics of atorvastatin, a class 1B drug (Maeda et al., 2011). Atorvastatin systemic exposure was only altered in the presence of rifampicin, but not by itraconazole, suggesting that its systemic clearance is primarily determined by hepatic uptake alone, although atorvastatin

DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

show high extent of CYP3A metabolism (>70%). On the other hand, plasma exposure of class 1B drugs such as cerivastatin is influenced by both OATP and CYPs (Varma et al., 2015a). In case of atorvastatin, CL_{int} is 6-7x higher than PS_{pd}, and therefore its hepatic clearance is "uptake-determined" (Eq. 5), while cerivastatin is an example where CL_{int} and PS_{pd} are somewhat similar and thus their hepatic clearance is determined by "extended clearance" (Varma et al., 2014).

Hybrid parameters have been proposed to describe the predominant role of uptake and/or metabolic and biliary clearances for a given drug (Yoshikado et al., 2017a; Yoshikado et al., 2017b). Of particular interest are 'β' value and 'R_{DIF}' value. β value is defined as the fraction of metabolism + biliary clearance (CL_{int}) to all the intracellular fates of drug including basolateral (active + passive) efflux.

$$\beta = \frac{CL_{int}}{\left(PS_{basal\text{-}efflux} + CL_{int}\right)} \qquad \text{(Eq. 5)}$$

'R_{DIF}' is the ratio of passive to total hepatic uptake clearances (Eq. 6) – implied to describe the significance of active uptake to total hepatic clearance.

$$R_{DIF} = \frac{PS_{pd}}{PS_{inf}} \qquad \text{(Eq. 6)}$$

Accordingly, for compounds with low β value (<0.3), change in CL_{int} would lead to altered systemic exposure; however, compounds with high β value are expected to have uptake-determined clearance . In a recent clinical study, effect of single dose rifampicin (OATP inhibitor) or itraconazole (CYP3A inhibitor; 200 mg, intravenous dose) on the pharmacokinetics of subtherapeutic doses of bosentan (class 1B), repaglinide (class 1B), clarithromycin (class 2), simeprevir (class 1B), and midazolam (class 2, CYP3A probe substrate) administered orally as a

cocktail was evaluated (Yoshikado et al., 2017a). Rifampicin treatment significantly increased plasma AUCs of bosentan, repaglinide and simepravir (3.2-, 1.9- and 7.2-fold , respectively), while itraconazole showed a notable impact on clarithromycin, simeprevir and midazolam plasma exposure (2.3-, 2.2-, and 3.7-fold, respectively). Based on the relatively large estimated β value and small R_{DIF}, bosentan can be categorized as OATP-mediated uptake-determined clearance. However, low derived R_{DIF} and β values suggest that simeprevir follow extended clearance, where both uptake and metabolism are major contributors to its systemic clearance (Snoeys et al., 2016; Yoshikado et al., 2017a).

Collectively, impact of variation in the functional activity of hepatic metabolizing enzymes by drug inhibition or genetic variation on the systemic exposure of class 1B drugs depend on the interplay of transport and metabolism. In contrary, inhibition of biliary efflux should have a minimal effect on the plasma exposure of hepatic cleared class 3B drugs (Watanabe et al., 2009; Varma et al., 2012c; Jamei et al., 2014; Kimoto et al., 2017), as such drugs possess very low passive permeability to back diffuse from liver to plasma compartment. Therefore, OATP-mediated uptake is considered the rate-determining step in the class 3B drugs clearance. However, recent studies demonstrated significant role of basolateral efflux transporters such as MRP3 and MRP4 in the translocation of class 3B drugs from liver to plasma, suggesting that inhibition of biliary efflux may alter plasma exposure in such cases (Pfeifer et al., 2013; Pfeifer et al., 2014). Further quantitative understanding on the role of basolateral efflux in hepatic clearance is warranted in order to factor this mechanism in evaluating DDIs.

In case of interactions with cyclosporine, an inhibitor of hepatic OATP1B1/1B3 and intestinal P-gp/BCRP at clinically relevant doses, all class 1B and majority of the class 3B drugs showed moderate-to-high AUC ratios (AUC ratio 2-16) (Figure 2B). The interactions with cyclosporine

in the other classes are low-to-moderate (AUC ratio 1.25-5), and are likely associated with inhibition of intestinal P-gp and/or BCRP – particularly for class 4 drugs. For example, aliskiren and colchicine are known P-gp substrates with permeability-limited absorption and show upto 4.5-fold interaction with cyclosporine (Rebello et al., 2011; Terkeltaub et al., 2011). Inhibition of intestinal metabolism via CYP3A by cyclosporine could also be a contributing factor for class 2 drugs with low to moderate intestinal availability (Fg). Evidently, midazolam and ticagrelor, metabolized by CYP3A in the intestine, present a ~2-fold AUC change when co-administered with cyclosporine. Nevertheless, cyclosporine-induced change in presystemic disposition (Fa and Fg) may contribute to the DDIs for class 1B/3B compounds (Varma et al., 2012c). The analyses of exhaustive and unbiased clinical DDIs datasets involving rifampicin and cyclosporine clearly illustrate the utility of the ECCS in identifying DDI risk associated with OATPs (Varma et al., 2017a).

Role of transporters in renal drug clearance

Renal blood clearance ($CL_{renal,b}$) is determined by glomerular filtration, tubular secretion, and reabsorption processes; and is mathematically described by (Russel et al., 2002; Lee and Kim, 2004; Feng et al., 2010; Morrissey et al., 2013):

$$CL_{renal,b} = (f_{u,b}.GFR + CL_{sec}) \cdot (1 - F_{reabs}) \quad \text{(Eq. 7)}$$

Where, $f_{u,b}$ is the unbound blood fraction, GFR is glomerular filtration rate, CL_{sec} is active renal secretory clearance and F_{reabs} is the reabsorbed fraction of filtered and secreted drug., CL_{sec} can be defined, assuming a well-stirred model, as (Eq. 8):

$$CL_{sec} = Q_r \cdot \frac{f_{u,b} \cdot CL_{int,sec}}{Q_r + f_{u,b} \cdot CL_{int,sec}} \quad \text{(Eq. 8)}$$

Where Q_r is the renal blood flow (15.7 mL/min/kg (Davies and Morris, 1993)) and $CL_{int,sec}$ is the intrinsic secretory clearance.

Localized on the basolateral membrane of the proximal tubules, OAT1, OAT3 and OCT2 are involved in the uptake of drugs, and are associated with clinical DDIs (Masereeuw and Russel, 2001; Lee and Kim, 2004; Feng et al., 2010; Morrissey et al., 2013).. On other hand, tubular reabsorption often depends on the passive permeability of compounds (Varma et al., 2009; Scotcher et al., 2016). ECCS framework suggests that drugs with low passive permeability are likely cleared by urinary route (>70% of the systemic clearance), with the exception of high MW acids or zwitterions (Class 3B), in which case, hepatic uptake may also be the rate-determining process to the systemic clearance (Varma et al., 2015b). Our group recently evaluated the role of OATs in the renal secretion of 31 compounds from ECCS 1A/3A/3B/4 classes (Mathialagan et al., 2017). Some trends emerged: class 1A and 3A compounds (low MW acids/zwitterions) showed major involvement of OAT1 or OAT3; class 4 compounds (low permeable bases/neutrals) are secreted by either OAT2 or OAT3; while, all class 3B compounds (high MW, low permeable acids/zwitterions) are predominantly secreted by OAT3 alone. Additionally, OAT3 emerged as a major contributor for the renal secretion for majority of the 31 compounds evaluated, implying its clinical significance for wide variety of drugs. OCT2 primarily transports organic cations and neutral compounds and many of the OCT2 substrates characterized to have significant renal secretion belong to ECCS class 4 (El-Kattan et al., 2016). Collectively, ECCS can indicate potential contribution of the $CL_{renal,b}$ to the total body clearance, as well as, a sense of likely transporters involved in renal secretion, which needs to be followed up with quantitative predictions.

Allometric scaling using animal data is widely applied for extrapolating the pharmacokinetic parameters, including renal clearance, to predict clinical pharmacokinetics (Paine et al., 2011). Such scaling methodology may be useful for drugs that are eliminated in the urine by glomerular filtration process as unchanged drug. However, allometry may not be a reliable methodology for drug cleared predominantly by transporter-mediated active process because of possible species difference in transporter expression and function (Chu et al., 2013). Prediction of active secretion is hindered by lack of established *IVIVE* methodologies – owing to the limitations in wider availability of primary cell systems (Brown et al., 2008). Nevertheless, approaches based on human kidney slices and transfected cell systems have be successfully applied for clearance and DDI predictions (Nozaki et al., 2007; Posada et al., 2015; Mathialagan et al., 2017).

ECCS and victim DDIs involving major renal transporters

Probenecid, a recommended probe inhibitor of OAT1/3, elicits a low-to-moderate AUC increase associated with decreased renal clearance for class 3A/3B/4 drugs (Figure 3A) (Varma et al., 2017a). For example, largest such interactions with probenecid involve furosemide and cephradine with about 3-fold AUC increase. On the other hand, cimetidine, an OCT2/MATEs probe inhibitor, show low (<2-fold) interaction due to inhibition of renal secretion for class 4 drugs only (Figure 3B). Also, DDIs involving inhibition of renal OAT1/3 are possible for class 3A/3B/4 drugs, while OCT2 mediated interactions are limited to class 4 drugs (El-Kattan et al., 2016; Mathialagan et al., 2017). Cimetidine inhibits OCT2 and MATE1/2K at clinically relevant concentrations and the majority of OCT2 substrates are also transported by MATE1/2K. Additionally, relatively selective MATE1/2K inhibitor, pyrimethamine, significantly increased plasma exposure of metformin (Kusuhara et al., 2011), therefore, contribution of MATE1/2K to

renal DDIs for class 4 drugs cannot be ruled out based on the available clinical data. However, given the overlapping substrate specificity between OCT2 and MATEs and basolateral uptake often being the rate-determining process for systemic clearance (Varma et al., 2015b), evaluating OCT2 activity alone could serve the purpose of DDI risk assessment in the clinic.

Oral absorption per ECCS class and impact of drug transporters

Following oral administration, absorption is the process that defines drug transfer from the site of administration in the gastrointestinal tract (GIT) to the enterocyte. Drug molecules can cross the apical intestinal membrane via various mechanisms following oral dosing. They include passive diffusion or active transport (Lennernäs, 1998). Passive diffusion involves two pathways: the paracellular pathway, where small MW hydrophilic drugs diffuses through the aqueous pores at the tight junctions between the enterocytes; and the transcellular (lipophilic) pathway, which requires lipophilic drug diffusion across the lipid cell membrane of the intestinal enterocyte. The active transport pathway is mediated by the interplay of influx and efflux transporters. The significance of each pathway is governed by the drug's physicochemical properties and its affinity for various efflux and influx transporters (Lipinski, 2000; Hurst et al., 2007; Varma et al., 2010a; Varma et al., 2010b; Yang and Smith, 2013). Typically, compounds that are absorbed by the transcellular pathway tend to have higher intestinal permeability and absorption relative to those absorbed via paracellular pathway. Understanding the dominant absorption pathway is key for predicting drug absorption and factors that may affect the overall process.

ECCS can provide initial guidance on the potential absorption liabilities of NMEs early on in drug discovery (Figure 4). Compounds with high permeability i.e. class 1A/1B/2 tend to have a high f_a ($\geq$ 85%) (El-Kattan et al., 2016). Interestingly, neither solubility nor efflux transporters

information provided additional perspective on the noted high absorption for majority of these high permeability molecules. These compounds are absorbed predominantly via passive transcellular pathway, which is consistent with their overall hydrophobicity. However, class 3A/3B/4 tend to have low oral absorption in humans ($f_a \leq 85\%$). Indeed, intestinal efflux transporters (e.g. BCRP, P-gp, and MRP2) have more profound impact in determining the extent of absorption of low permeability compounds (Tachibana et al., 2010). These observations are in concordance with pharmacogenomics and DDI data, where available. For example, rosuvastatin (ECCS Class 3B) show low f_a and is also a substrate for BCRP, expressed on the apical membrane of enterocytes (El-Kattan et al., 2016). Keskitalo *et al.* investigated the impact of *ABCG2* polymorphism (encoding BCRP transporter) on the pharmacokinetics of rosuvastatin in healthy volunteers following oral dosing, and demonstrated that the carriers of c.421AA genotype have 100% greater exposure than those with c.421CA, and 144% greater than with the c.421CC genotype. Similar changes were observed with the peak plasma concentrations (C_{max}), implying change in pre-systemic disposition as the major cause for altered pharmacokinetics of rosuvastatin in genetic variants (Keskitalo et al., 2009). Oral absorption of class 4 drugs tends to be impacted by intestinal P-gp. Clearly, inhibiting P-gp of digoxin is associated with major changes in its oral exposure but with minimal changes in plasma half-life, indicating the critical role of P-gp in reducing the oral absorption (Igel et al., 2007). Oral absorption of P-gp substrates is determined by key variables namely, effective intestinal permeability, solubility, oral dose, and affinity to P-gp (K_m) (Tachibana et al., 2012). Sensitivity analysis suggested that compounds with low permeability, low solubility/slow dissolution, and low oral dose, rate would not saturate the efflux transporter e.g. P-gp, at therapeutically relevant oral doses. Under these conditions, P-gp would play a key role in limiting the absorption of low permeability molecules. These

variables should be investigated to define the potential impact of P-gp DDIs and/or pharmacogenomics on the absorption of NMEs. These principles are also applicable to other intestinal efflux transporters and their potential impact on oral absorption e.g. BCRP and MRP2.

Intestinal uptake transporters such as OATP2B1, System L, monocarboxylate transporter 1, and PEPT1 transporters likely contribute to the drug absorption for compounds with low permeability i.e. ECCS Class 3A, 3B, and 4 drug molecules (Figure 4). These transporters can be divided into high affinity-low capacity transporters (e.g., System L and OATP2B1 transporters) and low affinity-high capacity transporters (e.g., monocarboxylate transporter and 1 PEPT1 transporters) (Varma et al., 2010a; Estudante et al., 2013; Filipski et al., 2013; Yang and Smith, 2013). High affinity-low capacity intestinal uptake transporters would improve the oral absorption of low permeability and low oral dose drug molecules (<100 mg). For example, gabapentin is an ECCS Class 3A molecule that is substrate for System L transporter (a high affinity-low capacity transporter). Increasing oral dose of gabapentin is associated with lower than proportional increase in systemic exposure in humans due to saturation in intestinal absorption process (Stewart et al., 1993). On the other hand, penicillins and cephalosporins are usually PEPT1 transporter substrates (low affinity/high capacity transporter) (Ganapathy et al., 1995). This transporter enabled a moderate f_a of these low permeability/high oral dose molecules (oral dose > 1 gm/day). From the drug molecules investigated, the average f_a for PEPT1 substrates is 70% with an average passive membrane permeability of 0.7×10^{-6} cm/sec (El-Kattan et al., 2016). The potential impact of pharmacogenomics and DDI involving uptake transporters is critical for low permeability ECCS Class 3A/3B/4 molecules. For example, grape fruit juice, a known inhibitor of OATP2B1, reduced the plasma exposure of oral rosuvastatin (OATP2B1 substrate) to 70% of control, suggesting the role of OATP2B1 in the absorption of rosuvastatin

(Kashihara et al., 2017). Overall, uptake intestinal transporters contribution is profound with compounds with low permeability.

Small and hydrophilic molecules (e.g. MW < 250 Dalton and cLog P < 0) with low permeability are likely absorbed via the paracellular pathway (Figure 4). This is particularly prominent for compounds in class 3A and 4. e.g. gabapentin, acyclovir, and cimetidine. This pathway accounts for < 0.01% of the intestinal membrane total surface area, it offers a limited window for drug absorption (Lennernas, 1995). In addition, the tight junctions between cells become tighter traveling from the jejunum towards the colon. Therefore, compounds that are absorbed via this pathway are not amenable for traditional controlled release formulation targeting the colon. Therefore, gastric retentive controlled release formulation technology were shown effective in extending the apparent half-life for these molecules (Berner and Cowles, 2006; Gordi et al., 2008).

Nonetheless, solubility is a key parameter that should be duly investigated to ensure maximum oral bioavailable (Amidon et al., 1995; Wu and Benet, 2005). Earlier, we recommended the measurement of the equilibrium solubility of NMEs in either pH 1.2 medium for acidic molecules or fasted state simulated intestinal fluid (FaSSIF) medium (pH 6.5) for non-acidic molecules (Varma et al., 2012b). Using a cutoff value of 200 μg/mL, the data set suggested a 93% sensitivity and 86% specificity in predicting high and low solubility classification (Varma et al., 2012b). Therefore, NMEs with solubility in relevant matrices higher than 200 μg/mL are considered high solubility molecules and are of low likelihood for solubility-limited absorption. As the compounds progress into the advanced stages of preclinical/clinical development, a more thorough characterization of solubility and dissolution rate in physiologically relevant conditions is warranted to implement quantitative predictions via PBPK modeling and simulations.

DMD Fast Forward. Published on March 1, 2018 as DOI: 10.1124/dmd.117.080044
This article has not been copyedited and formatted. The final version may differ from this version.

Road map to integrated transporter sciences for pharmacokinetics characterization

Identifying ADCE attributes of compounds early in drug discovery is important in building strategy around clearance and dose optimization, de-risk pharmacokinetic variability due to intrinsic and extrinsic factors (e.g. DDIs and genetic mutations), and designing efficient clinical studies. Membrane transporters have a pivotal role in drug absorption, tissue distribution, regulating drug exposure at the site of metabolism and elimination from the organs, and eventually from the body. As discussed, ECCS is effective in predicting the role of clinically relevant transporters in the clearance of drugs. For instance, OATPs mediated uptake is often the rate-determining step for the hepatic clearance of class 1B and 3B drugs, while renal transporters, OAT1 and OAT3, are involved in the renal secretion of class 3A/3B/4 drugs; and OCT2 and MATEs drive renal secretion of class 4 drugs (Figure 1). Additionally, intestinal efflux pumps, P-gp and BCRP, could be of relevance in clinical pharmacokinetics of class 3A/3B/4 drugs.

Building a screening funnel for a chemical series in lead optimization stage based on ECCS could be beneficial to bring forward candidates with optimum ADCE and pharmacokinetic attributes for clinical development (Figure 5). The goal here would be to use ECCS framework to identify clearance mechanism (rate-determining step) and other key disposition characteristics of the chemical series and build structural activity relationship to reduce or eliminate the contributors to poor pharmacokinetics (i.e., absorption and/or high intestinal/hepatic extraction). For instance, a chemical series with low permeability and high MW acids (class 3B) likely have limited absorption and are cleared via OATP-mediated active hepatic uptake and/or OAT3-mediated renal secretion; and once in the liver they are eliminated in the bile. Therefore, the priorities of ADCE screening for this chemical series would involve screening compounds in

relevant transfected cell lines and primary human hepatocytes to investigate and quantify the uptake kinetics. Medicinal design rank-ordering efforts should target driving down uptake clearance and renal secretion, if the goal is to maximize plasma exposure. However, programs approaching pharmacological targets in the liver (hepatoselective) could benefit from increasing uptake clearance and reducing biliary efflux (Pfefferkorn et al., 2012). Clinical evidences suggest that alteration in metabolic or biliary efflux activity due to genetic polymorphism lead to change in pharmacodynamic response of statins, for which the pharmacological target resides in the liver (Varma and El-Kattan, 2016). Understanding absorption liability and the potential role of intestinal uptake and efflux transporters are also valuable at this stage. Crystal structures for most of the drug transporters are currently not available, but ligand-based quantitative structure-activity relationships (QSAR) using structure and molecular properties of the ligands can be developed to guide medicinal chemistry design and identify molecules that achieve the required systemic and target exposure (Varma et al., 2017c). Drug design applications can be further improved through uncovering transporter protein crystal structures and generation of quality data to refine and develop viable QSAR models.

During candidate selection stage, quantitative pharmacokinetic predictions are of utmost importance in order to inform study design of first-in-human dose-ranging studies, and more importantly, to avoid unexpected suboptimal exposure in clinic. 'Middle-out' PBPK modeling integrating mechanistic *in vitro* data has been suggested as an effective approach for pharmacokinetics predictions for OATP1B1/1B3 substrate drugs (Jones et al., 2012; Li et al., 2014a; Li et al., 2014b). However, when early clinical data is available such models may be verified and refined before application for the purposes of predicting DDIs, food-drug

interactions, and impact of transporter/enzyme pharmacogenomics, as well as, disease state on pharmacokinetics in humans. Our group and others have presented several examples of DDI predictions involving OATPs via mechanistic static and PBPK models (Varma et al., 2012c; Gertz et al., 2013; Varma et al., 2013; Varma et al., 2014; Yoshikado et al., 2016). Such mechanistic translational approaches for capturing disposition via renal and intestinal transporters are less evolved, and further work is warranted in these areas (Tachibana et al., 2012; Posada et al., 2015; Feng and Varma, 2016; Mathialagan et al., 2017; Scotcher et al., 2017).

It should be emphasized that the utility of ECCS is not limited for early drug discovery. Indeed, it allows us to revisit our understanding of ADCE characteristics of drug molecules in clinical development or marketplace and refine our knowledge where needed. For instance, montelukast (class 1B drug) has been routinely recommended as a potential *in vivo* CYP2C8 probe substrate (VandenBrink et al., 2011). However, being a class 1B drug, we hypothesized that hepatic uptake via OATPs is the major clearance mechanism, and investigated the quantitative role of hepatic uptake in its pharmacokinetics and DDIs (Varma et al., 2017b). On the basis of *in vitro* transport studies, *in vivo* DDI studies in preclinical animal models (eg. rat and monkey) and PBPK modeling and simulations of available clinical DDI data, OATPs-CYP2C8 interplay was noted as the major determinant of montelukast pharmacokinetics. This provides a case example for rationalizing the conduct of *in vitro* mechanistic studies and follow up clinical studies, so that clinical risk assessment is rationally made to support drug development. Transporter assays have inherent challenges and limitations that need to be considered on their application.. For example, non-specific binding to the cell surface, compound back-diffusion from cells during washing cycles and general variability of the cell systems used may pose issues when attempting to evoke

the role of transporters in drug disposition or reliably measure in vitro permeability. This may lead to the misclassification of the compounds, which should be carefully evaluated when designing *in vitro* ADCE screening strategy. Our initial validation set (Varma et al., 2015b) yielded about 8% mis-classification; and therefore, we suggest additional diligence particularly for compounds with values close to the cut-offs for the three parameters (i.e., Ionization, permeability and MW) defining ECCS class. Furthermore, if *in vitro* studies do not provide appropriate guidance for compounds due to technical challenges as discussed earlier, which would otherwise be assumed based on ECCS class, preclinical *in vivo* studies should be considered to support the qualitative assessment of the contribution of hepatic uptake to overall clearance, which in turn may provide impetus for early dedicated DDI studies using probe inhibitors and/or prioritized genotyping of subjects.

Finally, like any categorical frameworks, exceptions are seen with ECCS. For example, apixaban is binned in ECCS class 4 based on its poor in vitro permeability and neutral charge, which implies that renal clearance is the major pathway. However, interestingly, renal excretion accounts for only ~27% of total clearance, while biliary and direct intestinal secretion contributes to elimination of apixaban in the feces (Eliquis Label). This prominent biliary/intestinal secretion can be explained by high P-gp and BCRP efflux of Apixaban. Similar examples in class 4 (azithromycin and erythromycin) are apparent, wherein biliary/intestinal secretion rather than renal excretion of parent, was shown to be the major clearance mechanism for efflux substrates (internal Pfizer Data). Further work is needed in better understanding the molecular properties associated with such less known pathways.

In conclusion, we present ECCS as a useful framework that has been extensively validated and can be implemented at various stages of drug discovery and development with ease. Such a tool can outline the mechanistic *in vitro* and in vivo studies needed to best characterize ADCE attributes and pharmacokinetics.

CONFLICT OF INTEREST

Authors are full-time employees of Pfizer Inc. The authors have no conflicts of interest that are directly relevant to this study.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: El-Kattan, Varma

Performed data analysis: Varma

Wrote or contributed to the writing of the manuscript: El-Kattan, Varma

References

Amidon GL, Lennernäs H, Shah VP and Crison JR (1995) A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm Res* **12**:413-420.

Aslamkhan AG, Han Y-H, Yang X-P, Zalups RK and Pritchard JB (2003) Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. *Mol Pharmacol* **63**:590-596.

Berner B and Cowles VE (2006) Case studies in swelling polymeric gastric retentive tablets. *Expert Opin Drug Deliv* **3**:541-548.

Bi Y-a, Scialis RJ, Lazzaro S, Mathialagan S, Kimoto E, Keefer J, Zhang H, Vildhede AM, Costales C, Rodrigues AD and Varma MV (2017) Reliable Rate Measurements for Active and Passive Hepatic Uptake Using Plated Human Hepatocytes. *AAPS J* **19**:787-796.

Brown CD, Sayer R, Windass AS, Haslam IS, De Broe ME, D'haese PC and Verhulst A (2008) Characterisation of human tubular cell monolayers as a model of proximal tubular xenobiotic handling. *Toxicol Appl Pharmacol* **233**:428-438.

Chandra P and Brouwer KL (2004) The complexities of hepatic drug transport: current knowledge and emerging concepts. *Pharm Res* **21**:719-735.

Chu X, Bleasby K and Evers R (2013) Species differences in drug transporters and implications for translating preclinical findings to humans. *Exp Opin Drug Met Tox* **9**:237-252.

Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharm Res* **10**:1093-1095.

Di L, Feng B, Goosen TC, Lai Y, Steyn SJ, Varma MV and Obach RS (2013) A perspective on the prediction of drug pharmacokinetics and disposition in drug research and development. *Drug Metab Dispos* **41**:1975-1993.

Di L, Whitney-Pickett C, Umland JP, Zhang H, Zhang X, Gebhard DF, Lai Y, Federico JJ, Davidson RE, Smith R and Varma MV (2011) Development of a new permeability assay using low-efflux MDCKII cells. *J Pharm Sci* **100**:4974-4985.

El-Kattan AF, Varma MV, Steyn SJ, Scott DO, Maurer TS and Bergman A (2016) Projecting ADME Behavior and Drug-Drug Interactions in Early Discovery and Development: Application of the Extended Clearance Classification System. *Pharm Res* **33**:3021-3030.

Elsby R, Hilgendorf C and Fenner K (2012) Understanding the critical disposition pathways of statins to assess drug-drug interaction risk during drug development: it's not just about OATP1B1. *Clin Pharmacol Ther* **92**:584-598.

EMA (2012) Guideline on the investigation of drug interactions. *http://wwwemaeuropaeu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606pdf Committee for Human Medicinal Products (CHMP), London, UK*.

Estudante M, Morais JG, Soveral G and Benet LZ (2013) Intestinal drug transporters: an overview. *Adv Drug Deliv Rev* **65**:1340-1356.

Feng B, LaPerle JL, Chang G and Varma MV (2010) Renal clearance in drug discovery and development: molecular descriptors, drug transporters and disease state. *Exp Opin Drug Met Tox* **6**:939-952.

Feng B and Varma MV (2016) Evaluation and Quantitative Prediction of Renal Transporter-Mediated Drug-Drug Interactions. *J Clin Pharmacol* **56 Suppl 7**:S110-121.

Filipski KJ, Varma MV, El-Kattan AF, Ambler CM, Ruggeri RB, Goosen TC and Cameron KO (2013) Intestinal targeting of drugs: rational design approaches and challenges. *Curr Top Med Chem* **13**:776-802.

Fredlund L, Winiwarter S and Hilgendorf C (2017) In Vitro Intrinsic Permeability: A Transporter-Independent Measure of Caco-2 Cell Permeability in Drug Design and Development. *Mol Pharm* **14**:1601-1609.

Ganapathy ME, Brandsch M, Prasad PD, Ganapathy V and Leibach FH (1995) Differential recognition of β-lactam antibiotics by intestinal and renal peptide transporters, PEPT 1 and PEPT 2. *J Biol Chem* **270**:25672-25677.

Gertz M, Cartwright CM, Hobbs MJ, Kenworthy KE, Rowland M, Houston JB and Galetin A (2013) Cyclosporine inhibition of hepatic and intestinal CYP3A4, uptake and efflux transporters: application of PBPK modeling in the assessment of drug-drug interaction potential. *Pharm Res* **30**:761-780.

Gordi T, Hou E, Kasichayanula S and Berner B (2008) Pharmacokinetics of gabapentin after a single day and at steady state following the administration of gastric-retentive- extended-release and immediate-release tablets: a randomized, open-label, multiple-dose, three-way crossover, exploratory study in healthy subjects. *Clin Ther* **30**:909-916.

Group SC, Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M and Collins R (2008) SLCO1B1 variants and statin-induced myopathy--a genomewide study. *N Engl J Med* **359**:789-799.

Harrison B, Magee MH, Mandagere A, Walker G, Dufton C, Henderson LS and Boinpally R (2010) Effects of rifampicin (rifampin) on the pharmacokinetics and safety of ambrisentan in healthy subjects. *Clin Drug Invest* **30**:875-885.

Hosea NA, Collard WT, Cole S, Maurer TS, Fang RX, Jones H, Kakar SM, Nakai Y, Smith BJ, Webster R and Beaumont K (2009) Prediction of human pharmacokinetics from preclinical information: comparative accuracy of quantitative prediction approaches. *J Clin Pharmacol* **49**:513-533.

Houston JB (1994) Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. *Biochem Pharmacol* **47**:1469-1479.

Hurst S, Loi CM, Brodfuehrer J and El-Kattan A (2007) Impact of physiological, physicochemical and biopharmaceutical factors in absorption and metabolism mechanisms on the drug oral bioavailability of rats and humans. *Expert Opin Drug Metab Toxicol* **3**:469-489.

Ieiri I, Higuchi S and Sugiyama Y (2009) Genetic polymorphisms of uptake (OATP1B1, 1B3) and efflux (MRP2, BCRP) transporters: implications for inter-individual differences in the pharmacokinetics and pharmacodynamics of statins and other clinically relevant drugs. *Expert Opin Drug Metab Toxicol* **5**:703-729.

Igel S, Drescher S, Murdter T, Hofmann U, Heinkele G, Tegude H, Glaeser H, Brenner SS, Somogyi AA, Omari T, Schafer C, Eichelbaum M and Fromm MF (2007) Increased absorption of digoxin from the human jejunum due to inhibition of intestinal transporter-mediated efflux. *Clin Pharmacokinet* **46**:777-785.

International Transporter Consortium I, Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ and Zhang L (2010) Membrane transporters in drug development. *Nat Rev Drug Discov* **9**:215-236.

Jamei M, Bajot F, Neuhoff S, Barter Z, Yang J, Rostami-Hodjegan A and Rowland-Yeo K (2014) A mechanistic framework for in vitro-in vivo extrapolation of liver membrane transporters: prediction of drug-drug interaction between rosuvastatin and cyclosporine. *Clin Pharmacokinet* **53**:73-87.

Jones HM, Barton HA, Lai Y, Bi YA, Kimoto E, Kempshall S, Tate SC, El-Kattan A, Houston JB, Galetin A and Fenner KS (2012) Mechanistic pharmacokinetic modeling for the prediction of transporter-mediated disposition in humans from sandwich culture human hepatocyte data. *Drug Metab Dispos* **40**:1007-1017.

Kashihara Y, Ieiri I, Yoshikado T, Kazuya M, Fukae M, Kimura M, Hirota T, Matsuki S, Irie S, Izumi N, Kusuhara H and Sugiyama Y (2017) Small-dosing Clinical Study: Pharmacokinetic, Pharmacogenomic (SLCO2B1 and ABCG2), and Interaction (Atorvastatin and Grapefruit Juice) Profiles of Five Probes for OATP2B1 and BCRP. *J Pharm Sci*.

Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ and Niemi M (2009) ABCG2 polymorphism markedly affects the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther* **86**:197-203.

Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM and Wilkinson GR (1998) The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest* **101**:289.

Kimoto E, Bi Y-a, Kosa RE, Tremaine LM and Varma MV (2017) Hepatobiliary Clearance Prediction: Species Scaling from Monkey, Dog and Rat, and In Vitro-In Vivo Extrapolation of Sandwich Cultured Human Hepatocytes using 17 Drugs. *J Pharm Sci*.

Kola I and Landis J (2004) Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* **3**:711-715.

Kunta JR and Sinko PJ (2004) Intestinal drug transporters: in vivo function and clinical importance. *Curr Drug Metab* **5**:109-124.

Kusuhara H, Ito S, Kumagai Y, Jiang M, Shiroshita T, Moriyama Y, Inoue K, Yuasa H and Sugiyama Y (2011) Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at oral microdose and at therapeutic dose in healthy subjects. *Clin Pharmacol Ther* **89**:837-844.

Lai Y, Varma M, Feng B, Stephens JC, Kimoto E, El-Kattan A, Ichikawa K, Kikkawa H, Ono C, Suzuki A, Suzuki M, Yamamoto Y and Tremaine L (2012) Impact of drug transporter pharmacogenomics on pharmacokinetic and pharmacodynamic variability - considerations for drug development. *Expert Opin Drug Metab Toxicol* **8**:723-743.

Lee W and Kim RB (2004) Transporters and renal drug elimination. *Annu Rev Pharmacol Toxicol* **44**:137-166.

Lennernas H (1995) Does fluid flow across the intestinal mucosa affect quantitative oral drug absorption? Is it time for a reevaluation? *Pharm Res* **12**:1573-1582.

Lennernäs H (1998) Human intestinal permeability. *J Pharm Sci* **87**:403-410.

Li R, Barton HA and Varma MV (2014a) Prediction of pharmacokinetics and drug–drug interactions when hepatic transporters are involved. *Clin Pharmacokinet* **53**:659-678.

Li R, Barton HA, Yates PD, Ghosh A, Wolford AC, Riccardi KA and Maurer TS (2014b) A "middle-out" approach to human pharmacokinetic predictions for OATP substrates using physiologically-based pharmacokinetic modeling. *J Pharmacokinet Pharmacodyn*.

Lipinski CA (2000) Drug-like properties and the causes of poor solubility and poor permeability. *J Pharmacol Toxicol Meth* **44**:235-249.

Liu L and Pang KS (2005) The roles of transporters and enzymes in hepatic drug processing. *Drug Metab Dispos* **33**:1-9.

Maeda K, Ikeda Y, Fujita T, Yoshida K, Azuma Y, Haruyama Y, Yamane N, Kumagai Y and Sugiyama Y (2011) Identification of the rate-determining process in the hepatic clearance of atorvastatin in a clinical cassette microdosing study. *Clin Pharmacol Ther* **90**:575-581.

Masereeuw R and Russel FG (2001) Mechanisms and clinical implications of renal drug excretion. *Drug Metab Rev* **33**:299-351.

Mathialagan S, Piotrowski MA, Tess DA, Feng B, Litchfield J and Varma MV (2017) Quantitative Prediction of Human Renal Clearance and Drug-Drug Interactions of Organic Anion Transporter Substrates Using In Vitro Transport Data: A Relative Activity Factor Approach. *Drug Metab Dispos* **45**:409-417.

Ménochet K, Kenworthy KE, Houston JB and Galetin A (2012) Use of mechanistic modeling to assess interindividual variability and interspecies differences in active uptake in human and rat hepatocytes. *Drug Metab Dispos* **40**:1744-1756.

Morrissey KM, Stocker SL, Wittwer MB, Xu L and Giacomini KM (2013) Renal transporters in drug development. *Ann Rev Pharmacol Toxicol* **53**:503-529.

Muller M and Jansen PL (1997) Molecular aspects of hepatobiliary transport. *Am J Physiol* **272**:G1285-1303.

Niemi M, Neuvonen PJ, Hofmann U, Backman JT, Schwab M, Lutjohann D, von Bergmann K, Eichelbaum M and Kivisto KT (2005) Acute effects of pravastatin on cholesterol synthesis are associated with SLCO1B1 (encoding OATP1B1) haplotype *17. *Pharmacogenet Genomic* **15**:303-309.

Niemi M, Pasanen MK and Neuvonen PJ (2011) Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol Rev* **63**:157-181.

Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, Urasaki Y, Urae A, Higuchi S, Otsubo K and Sugiyama Y (2003) Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* **73**:554-565.

Nozaki Y, Kusuhara H, Kondo T, Hasegawa M, Shiroyanagi Y, Nakazawa H, Okano T and Sugiyama Y (2007) Characterization of the uptake of organic anion transporter (OAT) 1 and OAT3 substrates by human kidney slices. *J Pharmacol Exp Ther* **321**:362-369.

Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos* **27**:1350-1359.

Paine SW, Ménochet K, Denton R, McGinnity DF and Riley RJ (2011) Prediction of human renal clearance from preclinical species for a diverse set of drugs that exhibit both active secretion and net reabsorption. *Drug Metab Dispos* **39**:1008-1013.

Patilea-Vrana G and Unadkat JD (2016) Transport vs. Metabolism: What Determines the Pharmacokinetics and Pharmacodynamics of Drugs? Insights From the Extended Clearance Model. *Clin Pharmacol Ther* **100**:413-418.

Pfefferkorn JA, Guzman-Perez A, Litchfield J, Aiello R, Treadway JL, Pettersen J, Minich ML, Filipski KJ, Jones CS and Tu M (2012) Discovery of (S)-6-(3-cyclopentyl-2-(4-(trifluoromethyl)-1 H-imidazol-1-yl) propanamido) nicotinic acid as a hepatoselective glucokinase activator clinical candidate for treating type 2 diabetes mellitus. *J Med Chem* **55**:1318-1333.

Pfeifer ND, Hardwick RN and Brouwer KL (2014) Role of hepatic efflux transporters in regulating systemic and hepatocyte exposure to xenobiotics. *Annu Rev Pharmacol Toxicol* **54**:509-535.

Pfeifer ND, Yang K and Brouwer KL (2013) Hepatic basolateral efflux contributes significantly to rosuvastatin disposition I: characterization of basolateral versus biliary clearance using a novel protocol in sandwich-cultured hepatocytes. *J Pharmacol Exp Ther* **347**:727-736.

Poirier A, Cascais A-C, Funk C and Lavé T (2009) Prediction of pharmacokinetic profile of valsartan in human based on in vitro uptake transport data. *J Pharmacokinet Pharmacodynamic* **36**:585.

Posada MM, Bacon JA, Schneck KB, Tirona RG, Kim RB, Higgins JW, Pak YA, Hall SD and Hillgren KM (2015) Prediction of renal transporter mediated drug-drug interactions for pemetrexed using physiologically based pharmacokinetic modeling. *Drug Metab Dispos* **43**:325-334.

Prueksaritanont T, Chu X, Evers R, Klopfer S, Caro L, Kothare P, Dempsey C, Rasmussen S, Houle R and Chan G (2014) Pitavastatin is a more sensitive and selective OATP1B clinical probe than rosuvastatin. *Br J Clin Pharmacol*.

Rebello S, Compain S, Feng A, Hariry S, Dieterich HA and Jarugula V (2011) Effect of cyclosporine on the pharmacokinetics of aliskiren in healthy subjects. *J Clin Pharmacol* **51**:1549-1560.

Ring BJ, Chien JY, Adkison KK, Jones HM, Rowland M, Jones RD, Yates JW, Ku MS, Gibson CR and He H (2011) PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: Comparative assessement of prediction methods of human clearance. *J Pharm Sci* **100**:4090-4110.

Russel FG, Masereeuw R and van Aubel RA (2002) Molecular aspects of renal anionic drug transport. *Ann Rev Physiol* **64**:563-594.

Scotcher D, Jones C, Rostami-Hodjegan A and Galetin A (2016) Novel minimal physiologically-based model for the prediction of passive tubular reabsorption and renal excretion clearance. *Eur J Pharm Sci*.

Scotcher D, Jones CR, Galetin A and Rostami-Hodjegan A (2017) Delineating the Role of Various Factors in Renal Disposition of Digoxin through Application of Physiologically Based Kidney Model to Renal Impairment Populations. *J Pharmacol Exp Ther* **360**:484-495.

Shitara Y, Horie T and Sugiyama Y (2006) Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci* **27**:425-446.

Shitara Y, Maeda K, Ikejiri K, Yoshida K, Horie T and Sugiyama Y (2013) Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles in hepatic clearance and intestinal absorption. *Biopharm Drug Dispos* **34**:45-78.

Shitara Y and Sugiyama Y (2006) Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. *Pharmacol Ther* **112**:71-105.

Sirianni GL and Pang KS (1997) Organ clearance concepts: new perspectives on old principles. *J Pharmacokinet Biopharm* **25**:449-470.

Snoeys J, Beumont M, Monshouwer M and Ouwerkerk-Mahadevan S (2016) Mechanistic understanding of the nonlinear pharmacokinetics and intersubject variability of

simeprevir: a PBPK-guided drug development approach. *Clin Pharmacol Ther* **99**:224-234.

Stewart BH, Kugler AR, Thompson PR and Bockbrader HN (1993) A saturable transport mechanism in the intestinal absorption of gabapentin is the underlying cause of the lack of proportionality between increasing dose and drug levels in plasma. *Pharm Res* **10**:276-281.

Tachibana T, Kato M and Sugiyama Y (2012) Prediction of nonlinear intestinal absorption of CYP3A4 and P-glycoprotein substrates from their in vitro Km values. *Pharm Res* **29**:651-668.

Tachibana T, Kato M, Takano J and Sugiyama Y (2010) Predicting drug-drug interactions involving the inhibition of intestinal CYP3A4 and P-glycoprotein. *Curr Drug Metab* **11**:762-777.

Terkeltaub RA, Furst DE, DiGiacinto JL, Kook KA and Davis MW (2011) Novel evidence-based colchicine dose-reduction algorithm to predict and prevent colchicine toxicity in the presence of cytochrome P450 3A4/P-glycoprotein inhibitors. *Arthritis Rheumatol* **63**:2226-2237.

USFDA (2012) Drug interaction studies - study design, data analysis, implications for dosing, and labeling recommendations. *http://wwwfdagov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362pdf Center for Drug Evaluation and Research (CDER), Rockville, MD*.

VandenBrink BM, Foti RS, Rock DA, Wienkers LC and Wahlstrom JL (2011) Evaluation of CYP2C8 inhibition in vitro: utility of montelukast as a selective CYP2C8 probe substrate. *Drug Metab Dispos* **39**:1546-1554.

Varma M, El-Kattan A, Feng B, Steyn S, Maurer T, Scott D, Rodrigues A and Tremaine L (2017a) Extended Clearance Classification System (ECCS) informed approach for evaluating investigational drugs as substrates of drug transporters. *Clin Pharmacol Ther* **102**:33-36.

Varma MV, Ambler CM, Ullah M, Rotter CJ, Sun H, Litchfield J, Fenner KS and El-Kattan AF (2010a) Targeting intestinal transporters for optimizing oral drug absorption. *Curr Drug Metab* **11**:730-742.

Varma MV, Ashokraj Y, Dey CS and Panchagnula R (2003) P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. *Pharmacol Res* **48**:347-359.

Varma MV, Bi YA, Kimoto E and Lin J (2014) Quantitative Prediction of Transporter- and Enzyme-Mediated Clinical Drug-Drug Interactions of Organic Anion-Transporting Polypeptide 1B1 Substrates Using a Mechanistic Net-Effect Model. *J Pharmacol Exp Ther* **351**:214-223.

Varma MV, Chang G, Lai Y, Feng B, El-Kattan AF, Litchfield J and Goosen TC (2012a) Physicochemical property space of hepatobiliary transport and computational models for predicting rat biliary excretion. *Drug Metab Dispos* **40**:1527-1537.

Varma MV and El-Kattan AF (2016) Transporter-Enzyme Interplay: Deconvoluting Effects of Hepatic Transporters and Enzymes on Drug Disposition Using Static and Dynamic Mechanistic Models. *J Clin Pharmacol*
56:S99-S109.

Varma MV, Feng B, Obach RS, Troutman MD, Chupka J, Miller HR and El-Kattan A (2009) Physicochemical determinants of human renal clearance. *J Med Chem* **52**:4844-4852.

Varma MV, Gardner I, Steyn SJ, Nkansah P, Rotter CJ, Whitney-Pickett C, Zhang H, Di L, Cram M, Fenner KS and El-Kattan AF (2012b) pH-Dependent solubility and permeability criteria for provisional biopharmaceutics classification (BCS and BDDCS) in early drug discovery. *Mol Pharm* **9**:1199-1212.

Varma MV, Kimoto E, Scialis R, Bi Y, Lin J, Eng H, Kalgutkar AS, El-Kattan AF, Rodrigues AD and Tremaine LM (2017b) Transporter-Mediated Hepatic Uptake Plays an Important Role in the Pharmacokinetics and Drug-Drug Interactions of Montelukast. *Clin Pharmacol Ther* **101**:406-415.

Varma MV, Lai Y and El-Kattan AF (2017c) Molecular properties associated with transporter-mediated drug disposition. *Adv Drug Deliv Rev* **116**:92-99.

Varma MV, Lai Y, Feng B, Litchfield J, Goosen TC and Bergman A (2012c) Physiologically based modeling of pravastatin transporter-mediated hepatobiliary disposition and drug-drug interactions. *Pharm Res* **29**:2860-2873.

Varma MV, Lai Y, Kimoto E, Goosen TC, El-Kattan AF and Kumar V (2013) Mechanistic modeling to predict the transporter- and enzyme-mediated drug-drug interactions of repaglinide. *Pharm Res* **30**:1188-1199.

Varma MV, Lin J, Bi YA, Kimoto E and Rodrigues AD (2015a) Quantitative Rationalization of Gemfibrozil Drug Interactions: Consideration of Transporters-Enzyme Interplay and the Role of Circulating Metabolite Gemfibrozil 1-O-beta-Glucuronide. *Drug Metab Dispos* **43**:1108-1118.

Varma MV, Obach RS, Rotter C, Miller HR, Chang G, Steyn SJ, El-Kattan A and Troutman MD (2010b) Physicochemical space for optimum oral bioavailability: contribution of human intestinal absorption and first-pass elimination. *J Med Chem* **53**:1098-1108.

Varma MV, Steyn SJ, Allerton C and El-Kattan AF (2015b) Predicting Clearance Mechanism in Drug Discovery: Extended Clearance Classification System (ECCS). *Pharm Res* **32**:3785-3802.

Watanabe T, Kusuhara H, Maeda K, Kanamaru H, Saito Y, Hu Z and Sugiyama Y (2010) Investigation of the rate-determining process in the hepatic elimination of HMG-CoA reductase inhibitors in rats and humans. *Drug Metab Dispos* **38**:215-222.

Watanabe T, Kusuhara H, Maeda K, Shitara Y and Sugiyama Y (2009) Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. *J Pharmacol Exp Ther* **328**:652-662.

Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR and Ball SE (2004) Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. *Drug Metab Dispos* **32**:1201-1208.

Wu CY and Benet LZ (2005) Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res* **22**:11-23.

Yang B and Smith DE (2013) Significance of peptide transporter 1 in the intestinal permeability of valacyclovir in wild-type and PepT1 knockout mice. *Drug Metab Dispos* **41**:608-614.

Yoshikado T, Maeda K, Furihata S, Terashima H, Nakayama T, Ishigame K, Tsunemoto K, Kusuhara H, Furihata KI and Sugiyama Y (2017a) A Clinical Cassette Dosing Study for Evaluating the Contribution of Hepatic OATPs and CYP3A to Drug-Drug Interactions. *Pharm Res* **34**:1570-1583.

Yoshikado T, Maeda K, Kusuhara H, Furihata KI and Sugiyama Y (2017b) Quantitative Analyses of the Influence of Parameters Governing Rate-Determining Process of Hepatic Elimination of Drugs on the Magnitudes of Drug-Drug Interactions via Hepatic OATPs and CYP3A Using Physiologically Based Pharmacokinetic Models. *J Pharm Sci* **106**:2739-2750.

Yoshikado T, Yoshida K, Kotani N, Nakada T, Asaumi R, Toshimoto K, Maeda K, Kusuhara H and Sugiyama Y (2016) Quantitative Analyses of Hepatic OATP-Mediated Interactions Between Statins and Inhibitors Using PBPK Modeling With a Parameter Optimization Method. *Clin Pharmacol Ther* **100**:513-523.

Yu H, Wang Q, Sun Y, Shen M, Li H and Duan Y (2015) A new PAMPA model proposed on the basis of a synthetic phospholipid membrane. *PloS one* **10**:e0116502.

Figure captions

Figure 1. Extended clearance classification system (ECCS) for predicting the clearance mechanism (rate-determining process) (Varma et al., 2015b). Hepatic uptake mediated by OATPs is likely the rate-determining step in the clearance of class 1B and 3B compounds. Renal transporters, OAT1 and OAT3, contribute to the active secretion of class 3A, 3B and 4 compounds, while OCT2 and/or MATEs are involved in renal secretion of class 4 compounds.

Figure 2. Victim DDIs per ECCS class with (A) OATP1B1/1B3 probe inhibitor, rifampicin, and (B) probe inhibitor of OATP1B1/1B3, P-gp and BCRP, cyclosporine. Schematics depicting the major transporters affected by these inhibitors are shown. Data points represent the mean AUC ratio of each victim-inhibitor pair from a single study or averaged value from multiple studies where available. Pink data points represent drugs with metabolism as the rate-determining clearance mechanism, blue data points represent drugs with hepatic uptake as the predominant clearance mechanism and aqua color data points represent drugs with predominant renal clearance. N is the number of interactions per ECCS class. Horizontal lines depict the categories of DDI magnitude – no (<1.25x), low (1.25x-2x), moderate (2x-5x) and high (>5x). Pie charts depict the percentage of interactions per ECCS class in the no (green), low (yellow), moderate (pink) and high (red) DDI magnitude categories. Data figures were adopted from (Varma et al., 2017a) with permission.

Figure 3. Victim DDIs per ECCS class with (A) OAT1 and OAT3 probe inhibitor, probenecid, and (B) OCT2 and MATEs probe inhibitor, cimetidine. Schematics depicting the major transporters affected by these inhibitors are shown. Data points represent the mean AUC ratio of each victim-inhibitor pair from a single study or averaged value from multiple studies where available. Only drugs with renal clearance as predominant clearance mechanism were presented here. Other legend details are similar as Figure 2. Data figures were adopted from (Varma et al., 2017a) with permission.

Figure 4. Oral absorption characteristics per ECCS class.

Figure 5. Schematic depiction of ECCS-informed approach for ADCE and pharmacokinetics characterization during various stages of drug discovery and development.

Figure 1.

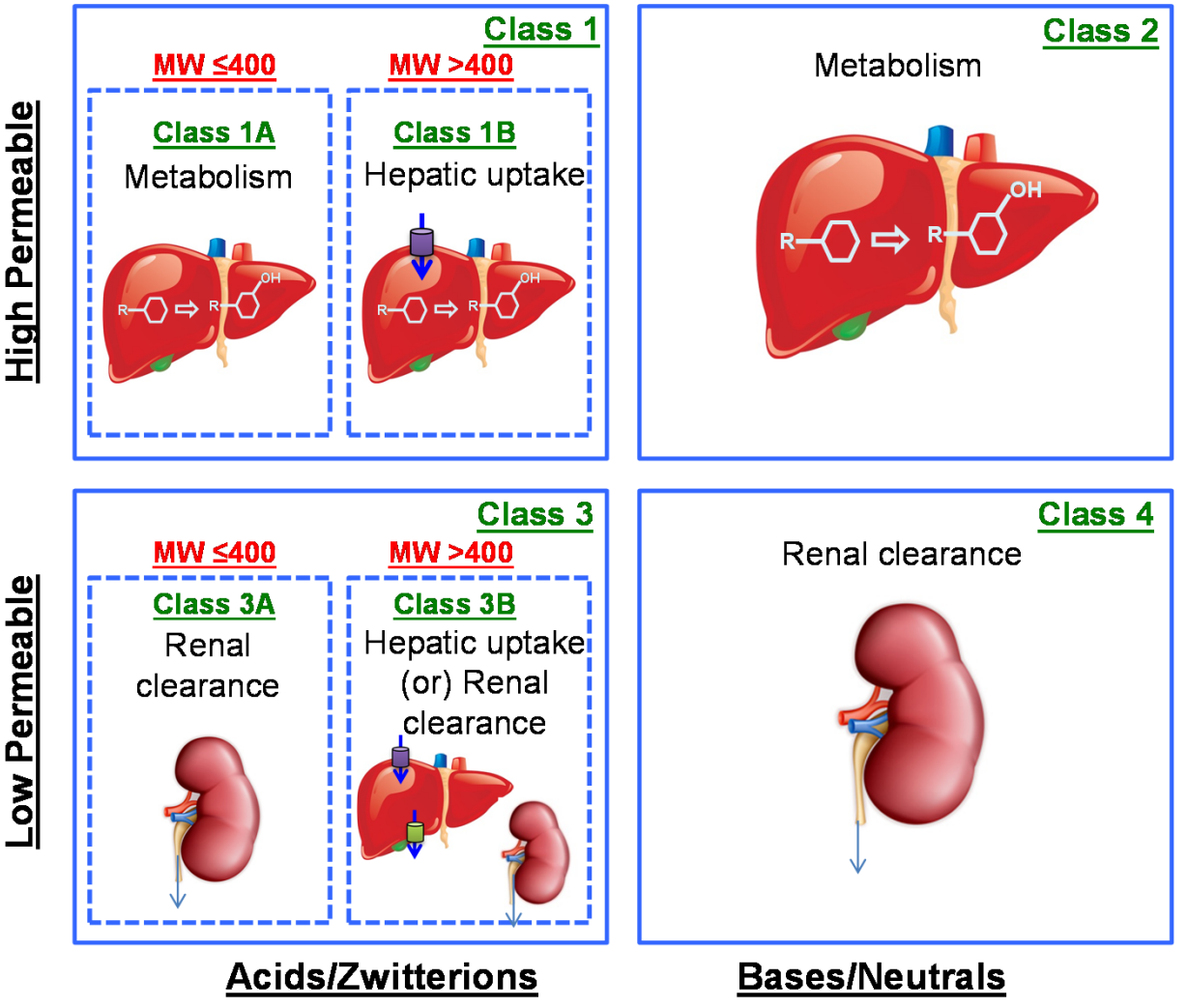

High Permeable
Low Permeable
Class 1
MW ≤400
MW >400
Class 1A
Metabolism
Class 1B
Hepatic uptake
Class 2
Metabolism
Class 3
MW ≤400
MW >400
Class 3A
Renal clearance
Class 3B
Hepatic uptake (or) Renal clearance
Class 4
Renal clearance
Acids/Zwitterions
Bases/Neutrals

Figure 2.

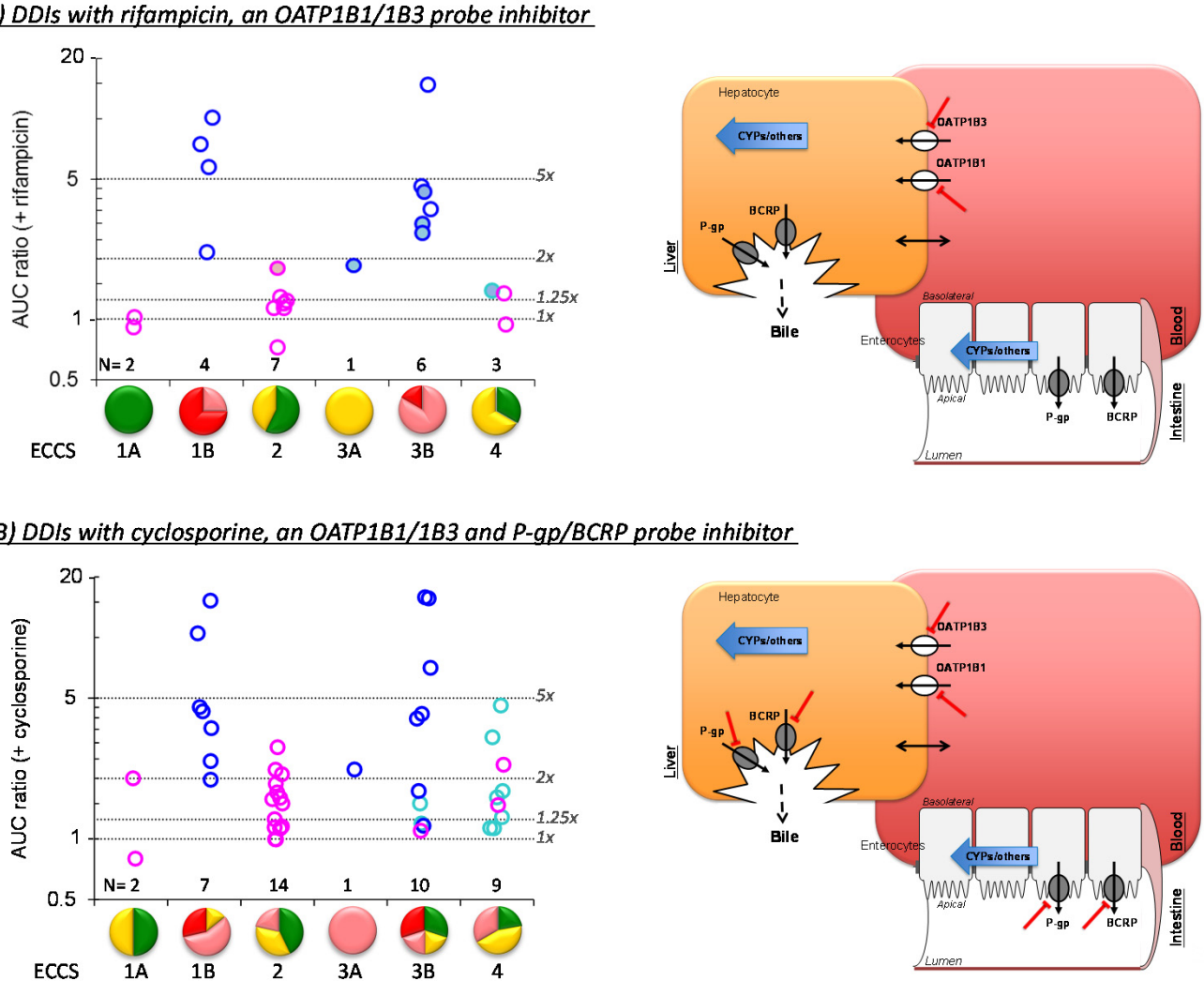

A) DDIs with rifampicin, an OATP1B1/1B3 probe inhibitor
AUC ratio (+ rifampicin)
20
5
1
0.5
5x
2x
1.25x
1x
N= 2
4
7
1
6
3
ECCS
1A
1B
2
3A
3B
4
Hepatocyte
CYPs/others
OATP1B3
OATP1B1
BCRP
P-gp
Liver
Bile
Basolateral
Blood
Enterocytes
CYPs/others
Apical
P-gp
BCRP
Intestine
Lumen
B) DDIs with cyclosporine, an OATP1B1/1B3 and P-gp/BCRP probe inhibitor
AUC ratio (+ cyclosporine)
20
5
1
0.5
5x
2x
1.25x
1x
N= 2
7
14
1
10
9
ECCS
1A
1B
2
3A
3B
4
Hepatocyte
CYPs/others
OATP1B3
OATP1B1
BCRP
P-gp
Liver
Bile
Basolateral
Blood
Enterocytes
CYPs/others
Apical
P-gp
BCRP
Intestine
Lumen

Figure 3.

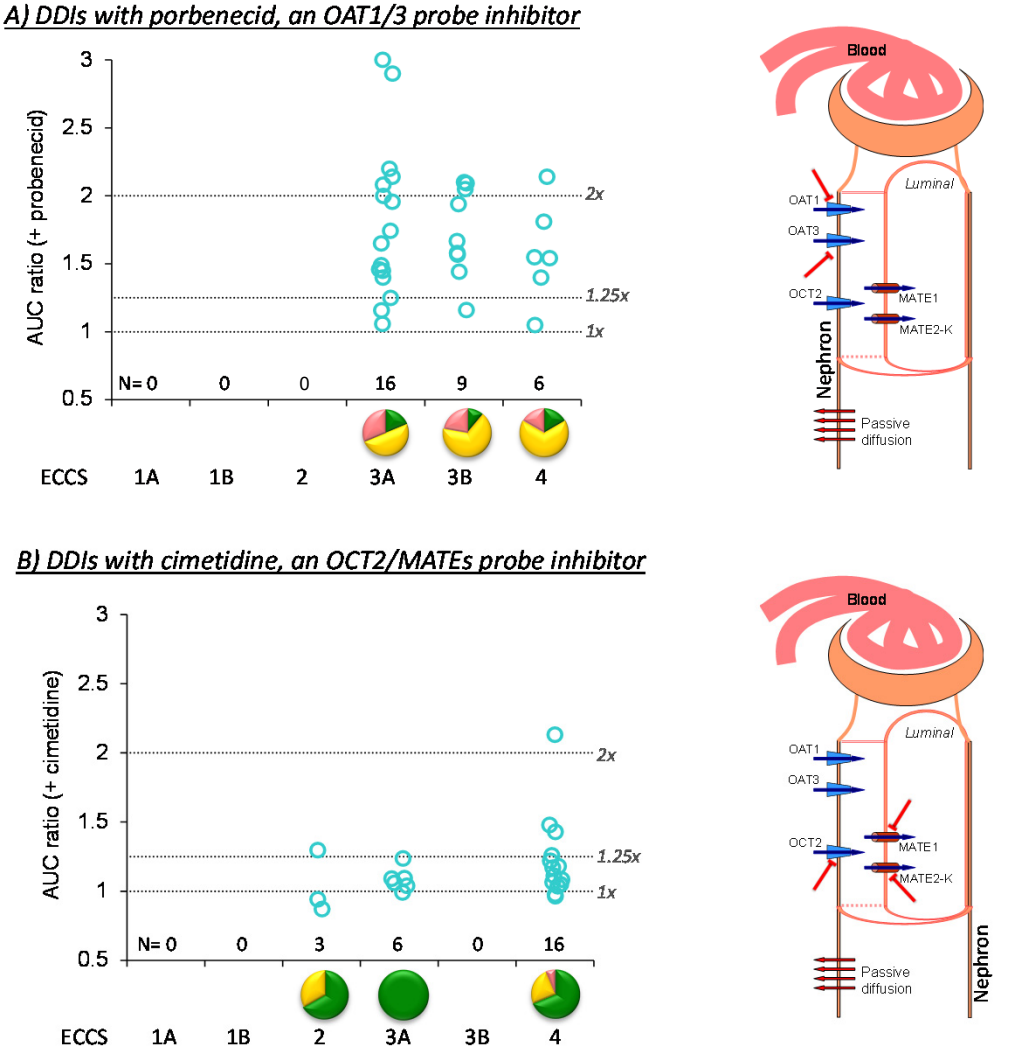

A) DDIs with porbenecid, an OAT1/3 probe inhibitor
AUC ratio (+ probenecid)
3
2.5
2
1.5
1
0.5
2x
1.25x
1x
N= 0
0
0
16
9
6
ECCS
1A
1B
2
3A
3B
4
Blood
Luminal
OAT1
OAT3
OCT2
MATE1
MATE2-K
Nephron
Passive diffusion
B) DDIs with cimetidine, an OCT2/MATEs probe inhibitor
AUC ratio (+ cimetidine)
3
2.5
2
1.5
1
0.5
2x
1.25x
1x
N= 0
0
3
6
0
16
ECCS
1A
1B
2
3A
3B
4
Blood
Luminal
OAT1
OAT3
OCT2
MATE1
MATE2-K
Passive diffusion
Nephron

Figure 4.

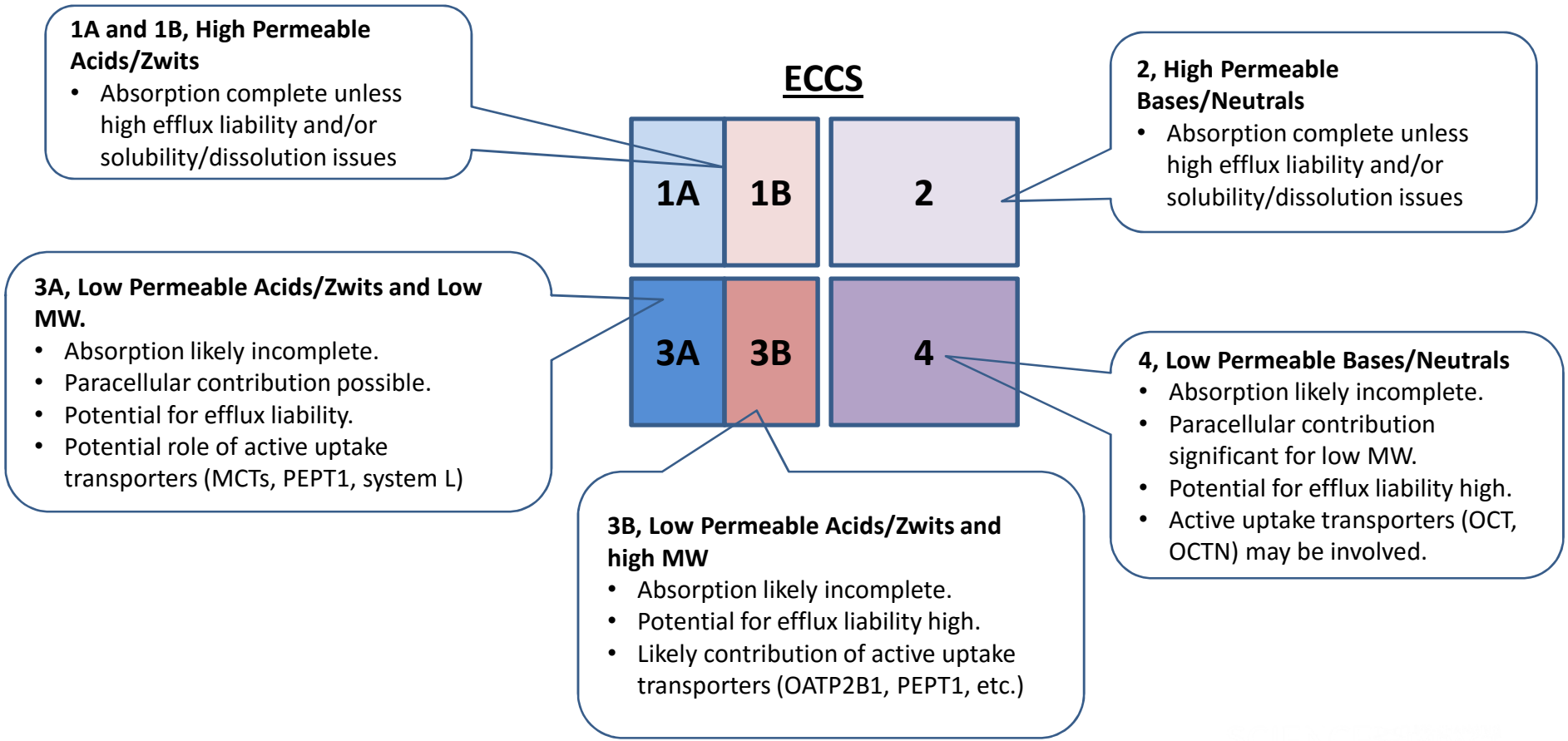
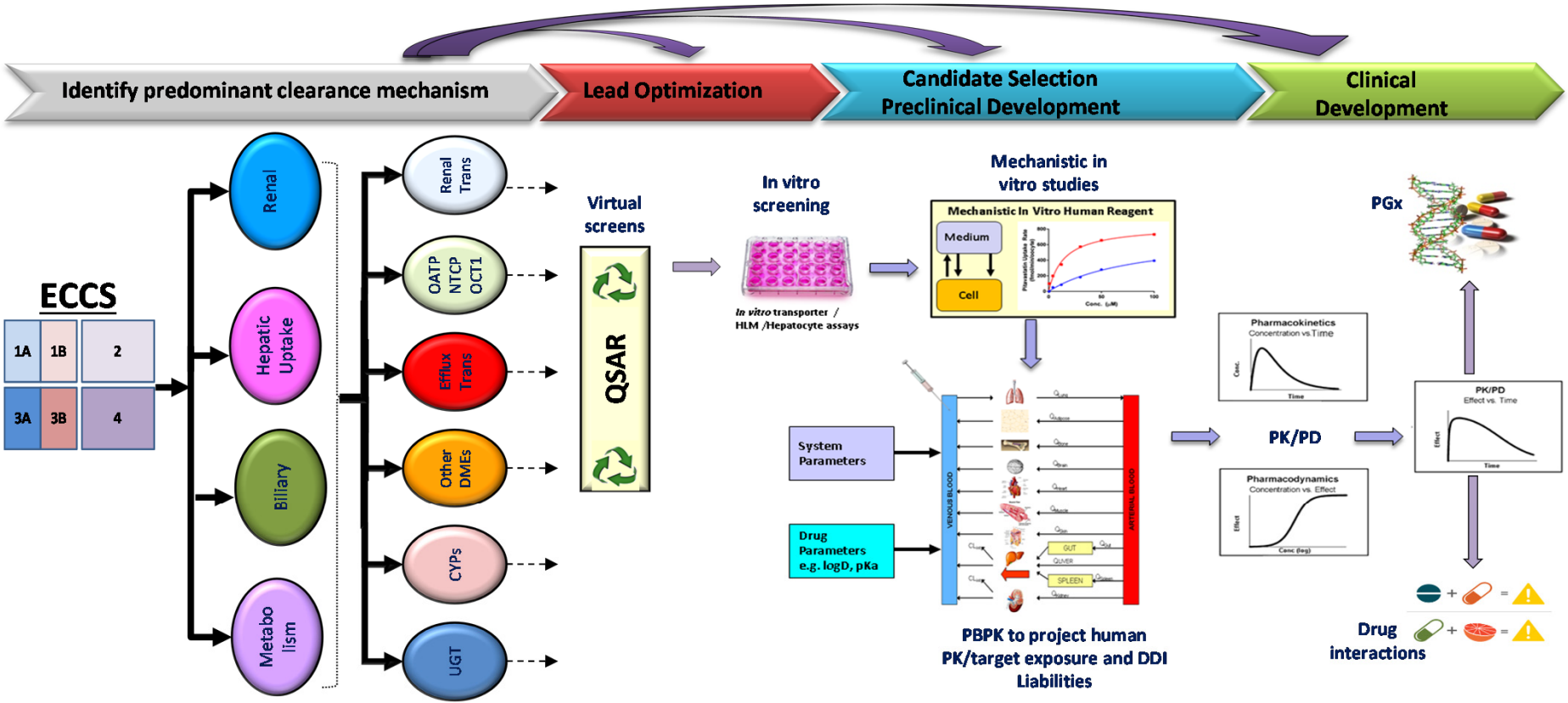

ECCS
1A
1B
2
3A
3B
4
1A and 1B, High Permeable Acids/Zwits
• Absorption complete unless high efflux liability and/or solubility/dissolution issues
2, High Permeable Bases/Neutrals
• Absorption complete unless high efflux liability and/or solubility/dissolution issues
3A, Low Permeable Acids/Zwits and Low MW.
• Absorption likely incomplete.
• Paracellular contribution possible.
• Potential for efflux liability.
• Potential role of active uptake transporters (MCTs, PEPT1, system L)
4, Low Permeable Bases/Neutrals
• Absorption likely incomplete.
• Paracellular contribution significant for low MW.
• Potential for efflux liability high.
• Active uptake transporters (OCT, OCTN) may be involved.
3B, Low Permeable Acids/Zwits and high MW
• Absorption likely incomplete.
• Potential for efflux liability high.
• Likely contribution of active uptake transporters (OATP2B1, PEPT1, etc.)

Figure 5.


Identify predominant clearance mechanism
Lead Optimization
Candidate Selection
Preclinical Development
Clinical
Development
ECCS
1A
1B
2
3A
3B
4
Renal
Hepatic Uptake
Biliary
Metabo lism
Renal Trans
OATP NTCP OCT1
Efflux Trans
Other DMEs
CYPs
UGT
Virtual screens
QSAR
In vitro screening
In vitro transporter / HLM /Hepatocyte assays
Mechanistic in vitro studies
Mechanistic In Vitro Human Reagent
Medium
Cell
System Parameters
Drug Parameters e.g. logD, pKa
VENOUS BLOOD
ARTERIAL BLOOD
GUT
LIVER
SPLEEN
PBPK to project human PK/target exposure and DDI Liabilities
Pharmacokinetics
Concentration vs Time
PK/PD
Pharmacodynamics
Concentration vs. Effect
PK/PD
Effect vs. Time
PGx
Drug interactions