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DRUG METABOLISM & DISPOSITION

Special Section on Transporters in Drug Disposition and Pharmacokinetic Prediction—Perspective

Navigating Transporter Sciences in Pharmacokinetics Characterization Using the Extended Clearance Classification System

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ABSTRACT

Membrane transporters play an important role in the absorption, distribution, clearance, and elimination of 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 (MATE) proteins, P-glycoprotein and breast cancer resistance protein are suggested to be of clinical relevance. An early understanding of the transporter role in drug disposition and clearance allows reliable prediction/evaluation of pharmacokinetics and changes due to drug-drug interactions (DDIs) or genetic polymorphisms. We recently proposed an extended clearance classification system (ECCS) based on simple drug properties (i.e., ionization, permeability, and molecular weight) to predict the 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 and 4 drugs, and certain class 3B drugs, are predominantly cleared by renal, wherein, OAT1, OAT3, OCT2, and MATE proteins 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 the ECCS framework in mapping the role of clinically relevant drug transporters in early discovery and development; 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 the 1990s (Kola and Landis, 2004). This was 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 and hepatocytes 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).

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It is now recognized that membrane transporters expressed in a variety of body organs such as liver, brain, intestine, and kidney play an important role in the absorption, distribution, clearance, and elimination (ADCE) of drugs and metabolites (Giacomini 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 the human body. However, less than 20 of these are considered relevant in the ADCE of drugs (Giacomini et al., 2010). 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 [(OAT) OAT2, *SLC22A7*)], the organic cation transporter

ABBREVIATIONS: ABC, ATP-binding cassette; ADCE, absorption, distribution, clearance, and elimination; AUC, area under the plasma concentration-time curve; BCRP, breast cancer resistance protein; DDI, drug-drug interaction; ECCS, extended clearance classification system; MATE, multidrug and toxin extrusion; MRP, multidrug resistance protein; NME, new molecular entity; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; PBPK, physiologically based pharmacokinetic; PEPT1, peptide transporter 1; P-gp, P-glycoprotein; SLC, solute carrier; UGT, UDP glucuronosyltransferase.

[(OCT) OCT1, *SLC22A1*), and the sodium taurocholate cotransporting polypeptide (*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 [(MRP) MRP2, *ABCC2*], breast cancer resistance protein [(BCRP) *ABCG2*], and P-glycoprotein [(P-gp) *ABCB1*] mediate biliary secretion (Müller and Jansen, 1997; Chandra and Brouwer, 2004; Giacomini et al., 2010; Shitara et al., 2013; Pfeifer et al., 2014). In kidney, OAT1, OAT3, and OCT2 localized on the basolateral membrane and multidrug and toxin extrusion (MATE) proteins (MATE1/2-K) expressed on the apical membrane of the proximal tubule cells are of relevance in active renal secretion 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, 2010a; Kunta and Sinko, 2004; Giacomini et al., 2010; Estudante et al., 2013). Collectively, clinical evidence points to the need to understand the role of OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K, P-gp, and BCRP in the disposition of investigational drugs (Giacomini et al., 2010; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf; http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf). Nevertheless, other transporters could be of high relevance in the pharmacokinetics of certain chemotypes and warrants characterization on a 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.

Extended Clearance Classification System

Based on the premise that early identification of clearance mechanisms can facilitate adopting the right strategy and tools for quantitative pharmacokinetic predictions, we recently proposed a framework called the extended clearance classification system (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 the ECCS, NMEs are classified based on permeability, molecular weight, and ionization state, which have previously been shown to be strongly associated with major clearance mechanisms, i.e., hepatic uptake, metabolism, and renal clearance. For example, Benet and coworkers proposed that high permeable compounds show a high extent of metabolism (>70%) and vice versa (Wu and Benet, 2005). On the other hand, Pfizer and colleagues demonstrated that high molecular weight (≥400 Da) 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 permeability cutoff of 5×10^{-6} cm/s using in-house low-efflux Madin-Darby canine kidney cell lines in the process of implementing the ECCS right from the early stages of drug discovery (Varma et al., 2012b). Extensive validation of the ECCS resulted in overall good predictive rates (Varma et al., 2015b, 2017a; El-Kattan et al., 2016). The general characteristics of the six classes, with respect to the clearance mechanism, are as follows (Fig. 1):

ECCS class 1A: Acids/zwitterions with small molecular weight (<400 Da) and high permeability. The clearance of class 1A compounds is determined by metabolic rates with extent of metabolism ≥70%. These tend to be metabolized by UDP glucuronosyltransferase (UGT) ≅ CYP2C enzymes > > esterases > > CYP3A4 enzymes.

ECCS class 1B: Large molecular weight (≥400 Da) acids/zwitterions 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. 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 small molecular weight (<400 Da) and low permeability. 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: Large molecular weight (≥400 Da) Acids/zwitterions with low permeability Their mechanism of clearance elimination is either active hepatic uptake and/or renal elimination. The hepatic uptake is typically mediated by OATP 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 appear to be the major efflux transporters affecting renal elimination.

The permeability categorization here is based on the Madin-Darby canine kidney cells with low efflux activity (Di et al., 2011). Tools based on artificial membranes (e.g., phospholipid-derived parallel artificial membrane permeability assay membranes) (Yu et al., 2015) or other cell types (e.g., 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 degrees, validation should also focus on assessing the risk of misclassification (high vs. low permeability) due to active mechanisms. Madin-Darby canine kidney 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 regarding the role of transporters in drug clearance led to the introduction of the extended clearance concept by Sirianni and Pang (1997), which was extensively investigated by Sugiyama and coworkers and several other research groups (Shitara and Sugiyama, 2006; Poirier et al., 2009; Watanabe et al., 2009; Jones

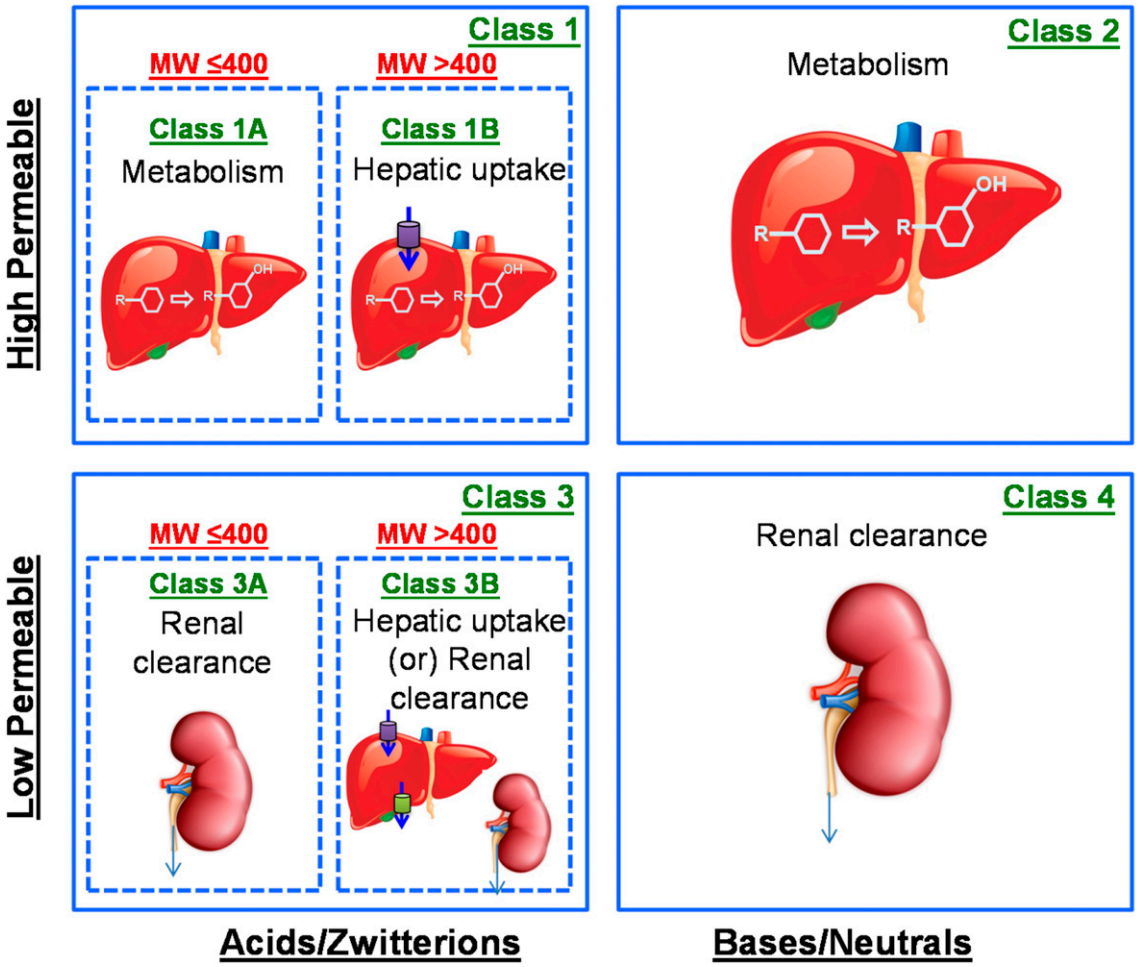


Fig. 1. ECCS for predicting the clearance mechanism (rate-determining process) (Varma et al., 2015b). Hepatic uptake mediated by OATPs is likely the rate-determining step in the clearance of class 1B and 3B compounds. Renal transporters, OAT1 and OAT3, contribute to the active secretion of class 3A,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

et al., 2012; Varma et al., 2012c, 2015b; Gertz et al., 2013; Jamei et al., 2014; Patilea-Vrana and Unadkat, 2016). Considering the physiological components, the extended clearance concept defines 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 clearances (PS_{efflux}), and biliary and metabolic intrinsic clearances ($CL_{int} = CL_{int,bile} + CL_{int,met}$). The interplay between these four processes defines the rate-determining step in hepatic clearance, which is mathematically expressed as follows (Liu and Pang, 2005):

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

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

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

where Q_h is the hepatic blood flow; E_h is the hepatic extraction ratio; and $f_{u,b}$ is the unbound fraction in blood. Over the last few years, the 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 quantitatively rationalize (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. The extended clearance term (eq. 2) is reduced to the rapid-equilibrium condition (eq. 3) when the compound is not a substrate for hepatic uptake transporters (e.g., OATP1B1/1B3 and sodium taurocholate cotransporting polypeptide) and the value of PS_{pd} is significantly higher than the value of CL_{int}.

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

It is generally acceptable to assume the 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, and verapamil) (El-Kattan et al., 2016). For such compounds, hepatic clearance is expected to be well predicted using human liver microsomes for cytochrome P450 substrates and human hepatocytes for other drug metabolizing enzymes such as sulfotransferases, aldehyde oxidase, UGTs, glutathione transferase,

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 shows active hepatic uptake, and the value of PS_{pd} is significantly lower than the value of CL_{int}.

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

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 the rate-determining step for their systemic clearance include HMG-CoA reductase inhibitors (statins) and angiotensin II antagonists (sartans). In vitro metabolic clearance measured in human liver microsomes tends to underpredict hepatic clearance. However, predictions substantially improve if hepatic intrinsic uptake clearance measured in suspension or cultured human hepatocytes (e.g., the 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 the rate-determining step in the systemic clearance of class 1B and 3B drugs is substantiated by 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; Link et al., 2008; Ieiri et al., 2009).

ECCS and Victim DDIs Involving Major Hepatic Transporters. We recently evaluated extensive and unbiased data sets 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 MATE proteins (cimetidine) (Varma et al., 2017a). We identified a total of 276 interaction pairs (23 pairs for rifampicin, 43 pairs for cyclosporine, 62 pairs for probenecid, and 148 pairs for cimetidine) with the ECCS class assigned using our in-house permeability data, molecular weight, and ionization, and we analyzed the DDI liability per ECCS class.

Clearly, coadministration of the OATP1B1/1B3 probe inhibitor, rifampicin, caused moderate [area under the plasma concentration-time curve (AUC) ratio 2–5] and high (AUC ratio > 5) interactions for class 1B and 3B drugs. Consistent with ECCS predictions, only no/low (AUC ratio < 2) interactions are evident for classes 1A, 3A, 2, and 4, although availability of clinical data for drugs in these

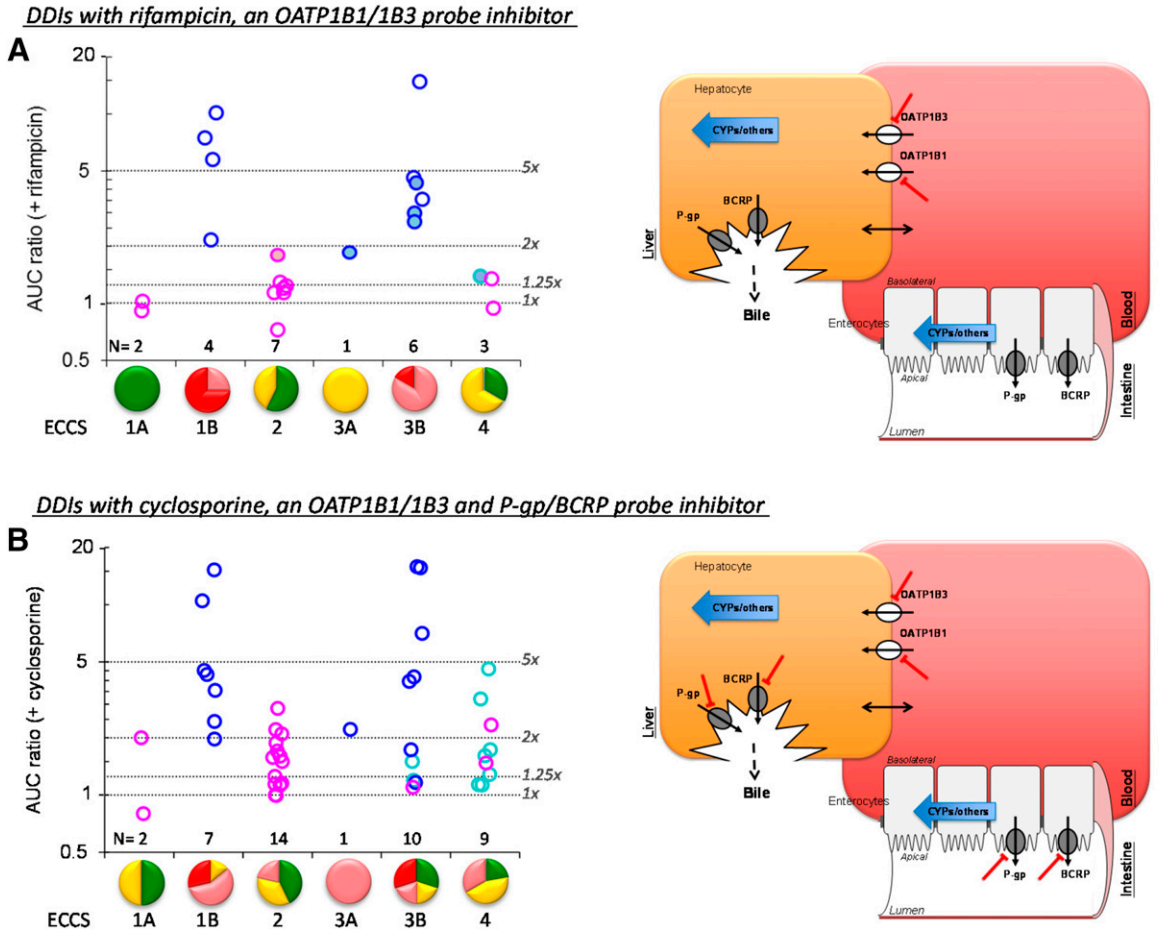


Fig. 2. Victim DDIs per ECCS class with OATP1B1/1B3 probe inhibitor, rifampicin (A), and OATP1B1/1B3, P-gp, and BCRP probe inhibitor, cyclosporine (B). Schematics depicting the major transporters affected by these inhibitors are shown. Data points represent the mean AUC ratio of each victim-inhibitor 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 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 (<1.25×), low (1.25×–2×), moderate (2×–5×), and high (>5×). 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 adapted from Varma et al. (2017a) with copyright statement from the original paper.

classes—especially classes 1A and 3A—are relatively sparse (Fig. 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. Furthermore, 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. (2011) 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. 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 showed a 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 cytochrome P450s (Varma et al., 2015a). In the case of atorvastatin, the CL_{int} value is six to seven times higher than the PS_{pd} value, and therefore its hepatic clearance is uptake determined (eq. 5), while cerivastatin is an example where the values of 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,b). Of particular interest are the β and R_{DIF} values. The β value is defined as the fraction of metabolism + biliary clearance (CL_{int}) to all the intracellular fates of a drug including basolateral (active + passive) efflux

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

The R_{DIF} value is the ratio of passive to total hepatic uptake clearances (eq. 6), implied to describe the significance of active uptake to total hepatic clearance

$$R_{DIF} = \frac{PS_{pd}}{PS_{inf}} \quad (6)$$

Accordingly, for compounds with low β values (<0.3), a change in the CL_{int} value would lead to altered systemic exposure; however, compounds with high β values are expected to have uptake-determined clearance. In a recent clinical study, the 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 the 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} value, bosentan can be categorized as OATP-mediated uptake-determined clearance. However, low derived R_{DIF} and β values suggest that simeprevir follows extended clearance, where both uptake and metabolism are major contributors to its systemic clearance (Snoeys et al., 2016; Yoshikado et al., 2017a).

Collectively, the 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 depends on the interplay of transport and metabolism. In contrast, 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) since such drugs possess very low passive permeability to back diffusion from the liver to plasma compartment. Therefore, OATP-mediated uptake is considered the rate-determining step in the class 3B drug clearance. However, recent studies demonstrated a 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, 2014). Further quantitative understanding on the role of basolateral efflux in hepatic clearance is warranted to factor this mechanism in evaluating DDIs.

In the case of interactions with cyclosporine, an inhibitor of hepatic OATP1B1/1B3 and intestinal P-gp/BCRP at clinically relevant doses, all class 1B and the majority of the class 3B drugs showed moderate-to-high AUC ratios (AUC ratio 2–16) (Fig. 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 up to 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 (F_g). Evidently, midazolam and ticagrelor, metabolized by CYP3A in the intestine, present a ~2-fold AUC change when coadministered with cyclosporine. Nevertheless, cyclosporine-induced changes in presystemic disposition (F_a and F_g) may contribute to the DDIs for class 1B and 3B compounds (Varma et al., 2012c). The analyses of exhaustive and unbiased clinical DDIs data sets 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):

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

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

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

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.

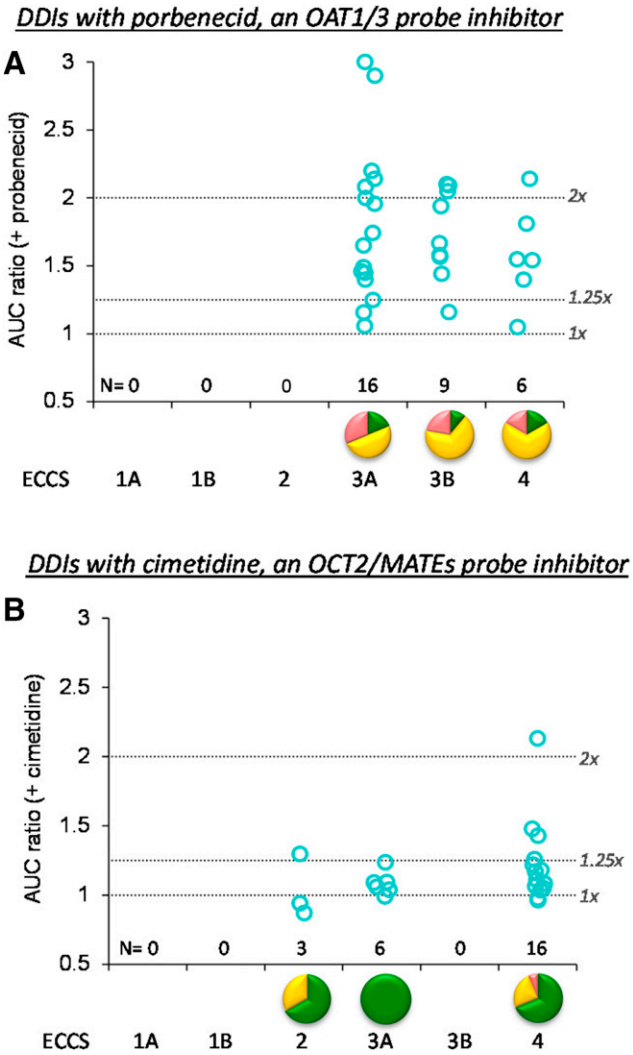
Localized on the basolateral membrane of the proximal tubules, OAT1, OAT3, and OCT2 are involved in the uptake of drugs, and are associated with clinical DDIs (Masereeuw and Russel, 2001; Lee and Kim, 2004; Feng et al., 2010; Morrissey et al., 2013). On other hand, tubular reabsorption often depends on the passive permeability of compounds (Varma et al., 2009; Scotcher et al., 2016). The 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 molecular weight acids or zwitterions (class 3B), in which case hepatic uptake may also be the rate-determining process in the systemic clearance (Varma et al., 2015b). Our group recently evaluated the role of OATs in the renal secretion of 31 compounds from ECCS classes 1A, 3A, 3B, and 4 (Mathialagan et al., 2017). Some trends emerged: class 1A and 3A compounds (low molecular weight acids/zwitterions) show 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 molecular weight, low permeable acids/zwitterions) are predominantly secreted by OAT3 alone. Additionally, OAT3 emerged as a major contributor in renal secretion for the majority of the 31 compounds evaluated, implying its clinical significance in a 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, the ECCS can indicate the potential contribution of $CL_{renal,b}$ 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 in extrapolating pharmacokinetic parameters, including renal clearance, in order to predict clinical pharmacokinetics (Paine et al., 2011). Such scaling methodology may be useful for drugs that are eliminated in the urine by the glomerular filtration process as unchanged drug. However, allometry may not be a reliable methodology for drugs cleared predominantly by transporter-mediated active processes because of possible species differences in transporter expression and function (Chu et al., 2013). Prediction of active secretion is hindered by lack of established in vitro-in vivo extrapolation 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 been successfully applied in 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, and 4 drugs (Fig. 3A) (Varma et al., 2017a). For example, the largest of such interactions with probenecid involve furosemide and cephradine with about a 3-fold AUC increase. On the other hand, cimetidine, an OCT2/MATE probe inhibitor, shows low interaction (<2-fold) due to inhibition of renal secretion for class 4 drugs only



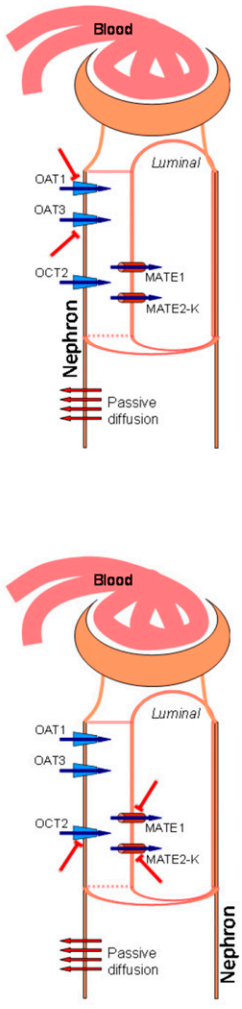


Fig. 3. Victim DDIs per ECCS class with OAT1 and OAT3 probe inhibitor, probenecid (A), and OCT2 and MATEs probe inhibitor, cimetidine (B). Schematics depicting the major transporters affected by these inhibitors are shown. Data points represent the mean AUC ratio of each victim-inhibitor pair from a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

(Fig. 3B). Also, DDIs involving inhibition of renal OAT1/3 are possible for class 3A, 3B, and 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, the relatively selective MATE1/2K inhibitor pyrimethamine significantly increased plasma exposure of metformin (Kusuhara et al., 2011); therefore, the 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 MATE proteins 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 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: 1) the paracellular pathway, where small molecular weight hydrophilic drugs diffuse through the aqueous pores at the tight junctions between the enterocytes; and 2) 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,b; 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 the paracellular pathway. Understanding the dominant absorption pathway is key to predicting drug absorption and factors that may affect the overall process.

The ECCS can provide initial guidance on the potential absorption liabilities of NMEs early on in drug discovery (Fig. 4). Compounds with high permeability (i.e., classes 1A, 1B, and 2) tend to have high f_a values (≥85%) (El-Kattan et al., 2016); interestingly, neither solubility nor efflux transporter information provided additional perspective on the noted high absorption for the majority of these high permeability molecules. These compounds are absorbed predominantly via the passive transcellular pathway, which is consistent with their overall hydrophobicity. However, classes 3A, 3B, and 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 a 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, when available. For example, rosuvastatin (ECCS class 3B) shows 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. (2009) 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 the c.421AA genotype have 100% greater exposure than those with the c.421CA genotype, and 144% greater exposure than those with the c.421CC genotype. Similar changes were observed with the peak plasma concentrations (C_{max}), implying change in presystemic 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 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 peptide transporter 1 (PEPT1) likely contribute to the drug absorption for compounds with low permeability, i.e., ECCS class 3A, 3B, and 4 drug molecules (Fig. 4). These transporters can be divided into high affinity/low capacity transporters (e.g., system L and OATP2B1) and low affinity/high capacity transporters (e.g., monocarboxylate and PEPT1) (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 a substrate for the system L transporter (a high affinity/low capacity

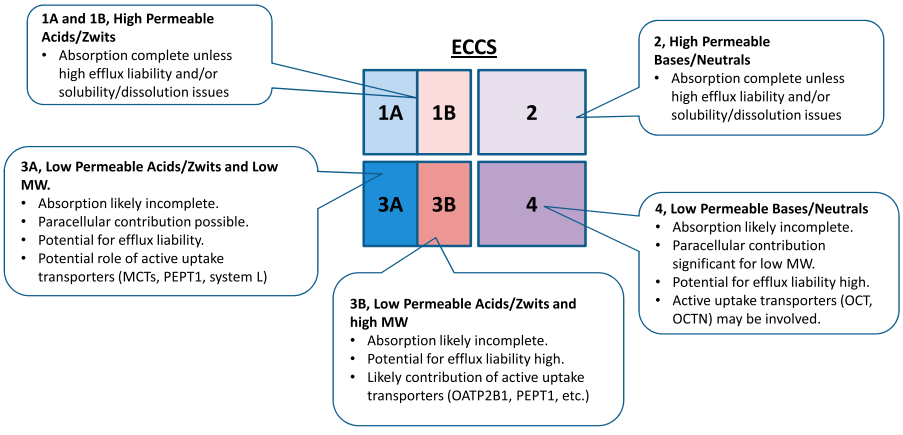


Fig. 4. Oral absorption characteristics per ECCS class.

transporter). Increasing the oral dose of gabapentin is associated with a lower than proportional increase in systemic exposure in humans due to saturation in the intestinal absorption process (Stewart et al., 1993). On the other hand, penicillin and cephalosporin drugs are usually PEPT1 substrates (low affinity/high capacity transporters) (Ganapathy et al., 1995). This transporter enabled a moderate f_a of these low permeability/high oral dose molecules (oral dose > 1 gm/d). From the drug molecules investigated, the average f_a value for PEPT1 substrates is 70% with an average passive membrane permeability of 0.7×10^{-6} cm/s (El-Kattan et al., 2016). The potential impact of the pharmacogenomics and DDIs involving uptake transporters is critical for low permeability ECCS class 3A, 3B, and 4 molecules. For example, grapefruit 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, the uptake intestinal transporter contribution is profound for compounds with low permeability.

Small and hydrophilic molecules (e.g., mol. wt. < 250 Da and clog P < 0) with low permeability are likely absorbed via the paracellular pathway (Fig. 4). This is particularly prominent for compounds in classes 3A and 4, e.g., gabapentin, acyclovir, and cimetidine. This pathway accounts for <0.01% of the intestinal membrane total surface area, and it offers a limited window for drug absorption (Lennernäs, 1995). In addition, the tight junctions between cells become tighter traveling from the jejunum toward 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 has been shown to be 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). Previously, 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 medium (pH 6.5) for nonacidic molecules (Varma et al., 2012b). Using a cutoff value of 200 μg/ml, the data set suggested 93% sensitivity and 86% specificity in predicting high and low solubility classifications (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 physiologically based pharmacokinetic (PBPK) modeling and simulations.

Road Map to Integrated Transporter Sciences for Pharmacokinetics Characterization

Identifying ADCE attributes of compounds early in drug discovery is important in building strategies around clearance and dose optimization, decreasing the risk of 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, and regulating drug exposure at the site of metabolism and elimination from the organs, and eventually from the body. As discussed, the ECCS is effective in predicting the role of clinically relevant transporters in the clearance of drugs. In a nutshell, OATP-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, and 4 drugs, and OCT2 and MATE proteins drive renal secretion of class 4 drugs (Fig. 1). Additionally, intestinal efflux pumps, P-gp and BCRP, could be of relevance in clinical pharmacokinetics of class 3A, 3B, and 4 drugs.

Building a screening funnel for a chemical series in the lead optimization stage based on the ECCS could be beneficial to bring forward candidates with optimum ADCE and pharmacokinetic attributes for clinical development (Fig. 5). The goal here would be to use the ECCS framework to identify the clearance mechanism (rate-determining step) and other key disposition characteristics of the chemical series and build structural-activity relationships 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 molecular weight acids (class 3B) likely has limited absorption and is cleared via OATP-mediated active hepatic uptake and/or OAT3-mediated renal secretion, and once in the liver is 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

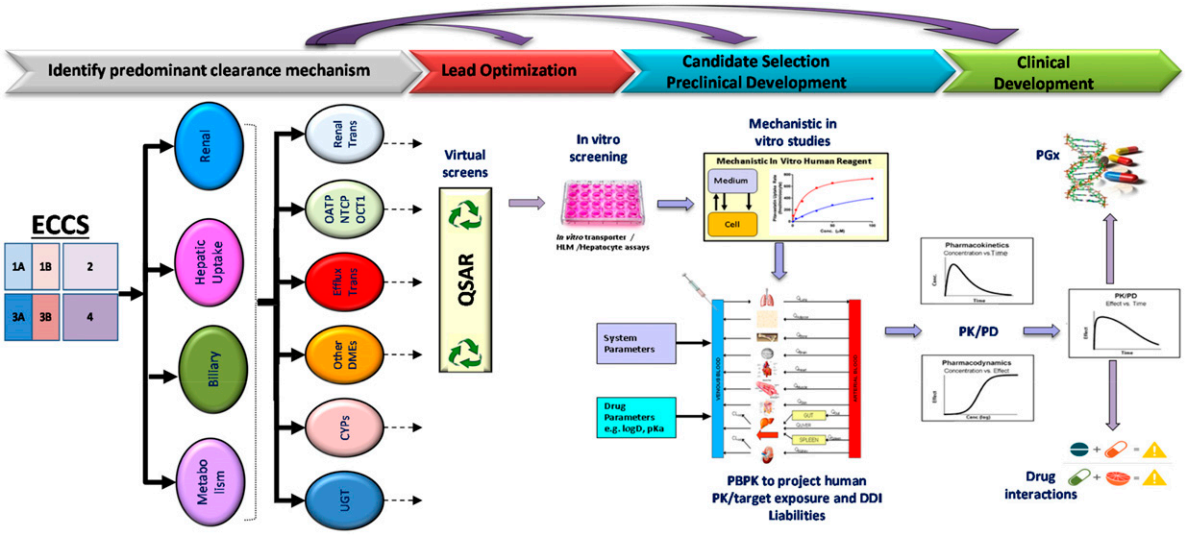


Fig. 5. Schematic depiction of ECCS-informed approach for ADCE and pharmacokinetics characterization during various stages of drug discovery and development.

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 (hepato-selective) could benefit from increasing uptake clearance and reducing biliary efflux (Pfefferkorn et al., 2012). Clinical evidence suggests that alteration in metabolic or biliary efflux activity due to genetic polymorphism leads to a change in the pharmacodynamic response of statins, in 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 is also valuable at this stage. Crystal structures for most of the drug transporters are currently not available, but ligand-based quantitative structure-activity relationships using the 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 exposures (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 quantitative structure-activity relationship models.

During the candidate selection stage, quantitative pharmacokinetic predictions are of utmost importance to inform the 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 to pharmacokinetics predictions for OATP1B1/1B3 substrate drugs (Jones et al., 2012; Li et al., 2014a,b). However, when early clinical data are available such models may be verified and refined before application for the purpose 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, 2013, 2014; Gertz et al., 2013; 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 the ECCS is not limited to early drug discovery. Indeed, it allows us to revisit our understanding of ADCE characteristics of drug molecules in clinical development or the 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 we investigated the quantitative role of hepatic uptake in its pharmacokinetics and DDIs (Varma et al., 2017b). Based on in vitro transport studies, in vivo DDI studies in preclinical animal models (e.g., 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, such that clinical risk assessment is rationally made to support drug development. Transporter assays have inherent challenges and limitations that need to be considered in their application. For example, nonspecific 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 misclassification of compounds, which should be carefully evaluated when designing in vitro ADCE screening strategies. Our initial validation set (Varma et al., 2015b) yielded about 8% misclassification; therefore, we suggest additional diligence, particularly for compounds with values close to the cutoff values for the three parameters (i.e., ionization, permeability, and molecular weight) defining the ECCS class. Furthermore, if in vitro studies do not provide appropriate guidance for compounds due to technical challenges as discussed previously, which would otherwise be assumed based on the ECCS class, preclinical in vivo studies should be considered to support the qualitative assessment of the contribution of transport mechanism to overall clearance. These studies, in turn, may provide the impetus for early dedicated DDI studies using probe inhibitors and/or prioritized genotyping of subjects.

Finally, as with any categorical framework, exceptions are seen with the 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 to better understand the molecular properties associated with such lesser known pathways.

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

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.

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