Pharm Res (2015) 32:3785–3802
DOI 10.1007/s11095-015-1749-4

PERSPECTIVE

Predicting Clearance Mechanism in Drug Discovery: Extended Clearance Classification System (ECCS)

Manthena V. Varma[1] • Stefanus J. Steyn[2] • Charlotte Allerton[1] • Ayman F. El-Kattan[2]

Received: 17 April 2015 / Accepted: 29 June 2015 / Published online: 9 July 2015

ABSTRACT Early prediction of clearance mechanisms allows for the rapid progression of drug discovery and development programs, and facilitates risk assessment of the pharmacokinetic variability associated with drug interactions and pharmacogenomics. Here we propose a scientific framework – Extended Clearance Classification System (ECCS) – which can be used to predict the predominant clearance mechanism (rate-determining process) based on physicochemical properties and passive membrane permeability. Compounds are classified as: Class 1A – metabolism as primary systemic clearance mechanism (high permeability acids/zwitterions with molecular weight (MW) ≤400 Da), Class 1B – transporter-mediated hepatic uptake as primary systemic clearance mechanism (high permeability acids/zwitterions with MW >400 Da), Class 2 – metabolism as primary clearance mechanism (high permeability bases/neutrals), Class 3A –renal clearance (low permeability acids/zwitterions with MW ≤400 Da), Class 3B – transporter mediated hepatic uptake or renal clearance (low permeability acids/zwitterions with MW >400 Da), and Class 4 – renal clearance (low permeability bases/neutrals). The performance of the ECCS framework was validated using 307 compounds with single clearance mechanism contributing to ≥70% of systemic clearance. The apparent permeability across clonal cell line of Madin−Darby canine kidney cells, selected for low endogenous efflux transporter expression, with a cut-off of 5×10^{-6} cm/s was used for permeability classification, and the ionization (at pH7) was assigned based on calculated pKa. The proposed scheme correctly predicted the rate-determining clearance mechanism to be either metabolism, hepatic uptake or renal for ~92% of total compounds. We discuss the general characteristics of each ECCS class, as well as compare and contrast the framework with the biopharmaceutics classification system (BCS) and the biopharmaceutics drug disposition classification system (BDDCS). Collectively, the ECCS framework is valuable in early prediction of clearance mechanism and can aid in choosing the right preclinical tool kit and strategy for optimizing drug exposure and evaluating clinical risk of pharmacokinetic variability caused by drug interactions and pharmacogenomics.

KEY WORDS extended clearance classification system (ECCS) · hepatic uptake · metabolism · permeability · renal clearance

INTRODUCTION

To increase the flow of new drugs to patients, the pharmaceutical industry is focused on designing and developing molecules to achieve systemic drug exposures capable of robustly modulating disease relevant biological mechanisms in humans [1, 2]. Clearance rate is a critical determinant of drug exposure in the systemic circulation and consequently at the pharmacological target compartment. Hence, it is a key component in determining the therapeutic efficacious dose. Definitive assessment of a compound's clearance mechanisms takes place during a human radiolabel ADME mass balance study in clinical development stage, while preliminary information can be obtained via first in human studies [3].

Electronic supplementary material The online version of this article (doi:10.1007/s11095-015-1749-4) contains supplementary material, which is available to authorized users.

✉ Ayman F. El-Kattan
ayman.el-kattan@pfizer.com

[1] Pfizer Global Research and Development, Pfizer Inc. Groton, Connecticut 06340, USA

[2] Pfizer Global Research and Development, Pfizer Inc. Cambridge, Massachusetts 02139, USA

However, given the focus on bringing forward development compounds with a high probability of testing the biological mechanism in humans, an earlier understanding of rate-determining clearance mechanism is required to design compounds with optimized disposition properties. Currently significant investment is required to predict the human clearance mechanism and the clearance rate from human *in vitro* systems, as well as pre-clinical experiments. Understanding of the predominant clearance mechanism is essential in ensuring the correct ADME screen sequences are selected to guide medicinal chemistry design and to identify molecules capable of achieving the systemic and target exposure required. In the absence of this, molecular design may be optimized on parameters which are not relevant to the systemic clearance of a compound. As such, a framework to provide prospective guidance as to the predominant clearance mechanism for a given series or compound, would increase the efficiency of medicinal chemistry design in bringing forward "best-in-class" compounds. In addition, an early understanding of the clearance mechanism enables successful prediction of the changes in the systemic exposure caused by drug-drug interactions (DDIs) or genetic polymorphisms of enzymes and/or transporters, which are important considerations in the nomination of a clinical development candidate [4].

The major drug elimination routes in human are metabolism, biliary, and renal, dominated by the liver and kidney [4]. It should be emphasized that a drug's predominant elimination process such as metabolism, biliary or renal excretion may not be always its rate-determining step in systemic clearance – the latter being the determinant of systemic drug exposure [5–11]. For example, atorvastatin has a high extent of metabolism (>90% of parent eliminated as metabolites), however, active uptake mediated by organic anion transporting polypeptide (OATP) transporters is the rate-determining step of its clearance [12]. Similar evidences were reported with bosentan, cerivastatin, fluvastatin, and repaglinide [5–11]. In addition, compounds such as valsartan and rosuvastatin are predominantly excreted unchanged in bile while active uptake mediated by OATP transporters is the rate-determining step of their clearance. Aligning the selection of *in vitro* tools to characterize the rate-determining process in the clearance mechanism of a compound series is essential in ensuring success in efficiently modulating clearance through design iterations. It is well understood that the physiochemical properties of a drug play a major role in guiding its clearance mechanism (rate-determining process), as well as elimination from the body.

In this article, we discuss the physiological basis of clearance and the associated drug-related descriptors. Bringing together these multiple facets, we propose and validate a novel classification system to enable prediction of the predominant clearance mechanism (rate-determining process) that can be used in the drug discovery and development settings.

HEPATIC CLEARANCE

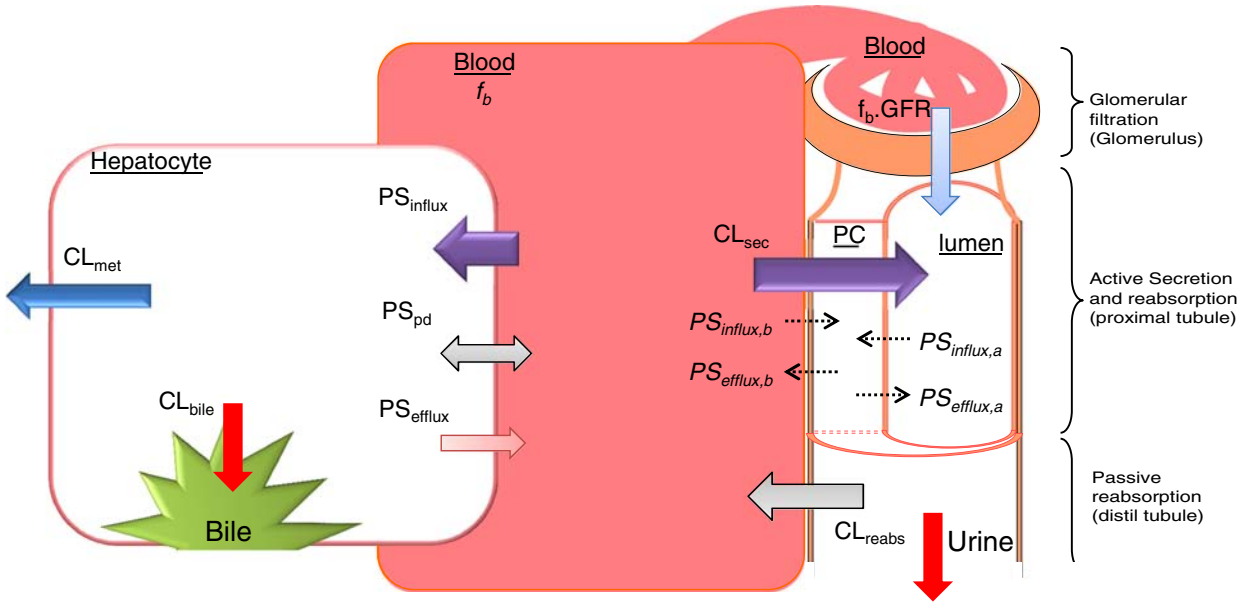
Hepatic clearance is determined by the liver blood flow, drug binding in blood, and the intrinsic capability of the liver to clear the drug, which is dependent on drug interactions with specific drug metabolizing enzymes and membrane transporters [8, 10, 13]. Recognition of the involvement of the transporter-enzyme interplay in defining hepatic clearance is needed to achieve accurate clearance predictions (for reviews, [5, 8, 9, 14]). The mathematical expression defining the overall hepatic intrinsic clearance comprising of membrane transport and metabolism is given by (Eq. 1) [10, 15, 16]:

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

Based on this "extended clearance term", total hepatic clearance (CL_h) assuming well-stirred conditions is given by (Eq. 2) [13]:

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

PS_{influx} and PS_{efflux} are the active (transporter-mediated) sinusoidal influx and efflux clearances, respectively. PS_{pd} represents passive diffusion clearance (Fig. 1). CL_{int} is the sum of the metabolic and biliary intrinsic clearances ($CL_{int,met}$+$CL_{int,bile}$), Q_h is the liver blood flow, E_h is the hepatic extraction ratio, and f_b is the unbound fraction in blood. For drugs subjected to metabolic clearance and hepatic influx or efflux clearance, consideration of one process alone will not provide an all-inclusive understanding of hepatic clearance and thus the systemic pharmacokinetics [5, 9, 10, 17–20]. However, Eq. 2 is greatly simplified to Eq. 3 when both active transport is negligible (PS_{influx} & PS_{efflux}<< PS_{pd}) and passive diffusion clearance greatly exceeds the sum



Hepatic clearance

$$CL_{int,h} = \frac{(PS_{influx} + PS_{pd}).CL_{met+bile}}{(PS_{efflux} + PS_{pd} + CL_{met+bile})}$$

Rapid-equilibrium	Uptake-determined
if, $CL_{met+bile} << PS_{pd}$ & PS_{influx} and $PS_{efflux} << PS_{pd}$	if, $CL_{met+bile} >> PS_{pd} + PS_{efflux}$
$CL_{int,h} = CL_{met+bile}$	$CL_{int,h} = (PS_{active} + PS_{passive})$
Metabolism+biliary CL are rate-determining steps	Hepatic uptake is the rate-determining step

Renal clearance

$$CL_{renal} = (f_b.GFR + CL_{sec}).(1 - F_{reabs})$$

Low passive permeable	High passive permeable
if, $F_{reabs} = 0$	if, $F_{reabs} = 1$
$CL_{renal} = (f_b.GFR + CL_{sec})$	$CL_{renal} = 0$
Renal CL is likely predominant mechanism	No renal CL expected

Fig. 1 Schematic representation of the processes involved in the hepatic and renal clearance of drugs. Transporter-enzyme interplay in the hepatic clearance can be mechanistically described by the extended-clearance concept, where the rate-determining process for hepatic clearance is defined by metabolic/biliary clearance or uptake clearance or a combination of both. Renal clearance is a function of glomerular filtration, active secretion and tubular reabsorption. When the passive permeability is high, renal clearance tends to be negligible due to potentially complete reabsorption. However, when passive permeability is low, renal clearance can be a significant contributor to systemic clearance driven by glomerular filtration and active secretion. *PC* – proximal nephron cell. See the text for definitions of the parameters.

of intrinsic metabolic and biliary clearance ($PS_{pd} >> CL_{int,met} + CL_{int,bile}$) [13]:

$$CL_h = Q_h \cdot \frac{f_b \cdot CL_{int}}{Q_h + f_b \cdot CL_{int}} = Q_h \cdot E_h \qquad \text{(Eq.3)}$$

This expression has been routinely applied in predicting clearance of cytochrome P450 (CYP) and other enzyme substrates [21–23].

Since a large number of drugs are metabolized by the CYP enzymes localized in the liver, human liver microsomal stability is typically assessed in early discovery to quantify and predict human clearance. Other major metabolizing enzymes of interest in drug discovery are UDP glucuronosyltransferases (UGTs), sulfotransferases, aldehyde oxidase and glutathione transferase (GST), among others [4, 24]. Drug uptake and efflux transporters expressed in a variety of organs including the intestine, liver, kidney and brain play a pivotal role in drug disposition, therapeutic efficacy, and toxicity [25]. Uptake and efflux transporters of interest in drug discovery include organic anion-transporting polypeptides (OATPs), organic anion transporters (OATs), organic cation transporters (OCTs), p-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) [25–27].

Hepatic uptake can be the rate-determining step for systemic clearance of drugs that are either eliminated unchanged in the bile or eliminated as phase I/II metabolites. In liver, uptake transporters OATP1B1, OATP1B3, and OATP2B1 are expressed on the sinusoidal membrane and play a pivotal role in the active uptake of many clinically important anionic drugs, including HMG-CoA reductase inhibitors (statins) and angiotensin II receptor antagonists (sartans) [17, 25, 28–30]. A

number of studies suggested that active uptake could be the rate-determining step in hepatic clearance, not only for compounds that are metabolically stable such as rosuvastatin [31] and pravastatin [32–34], but also for compounds that are extensively metabolized such as, atorvastatin [12, 35], glyburide [36, 37] and repaglinide [38, 39]. Clinically relevant DDIs leading to changes in systemic exposure of these drugs are primarily attributed to the inhibition of hepatic uptake mediated by members of the OATP family rather than the inhibition of hepatic metabolism or biliary efflux [9, 12, 40]. Furthermore, polymorphism in *SLCO1B1* (encoding OATP1B1) has been reported to lead to major changes in systemic exposure of statins, which in turn, regulates the relative exposure in peripheral tissue such as muscle and the risk of toxicity such as rhabdomyolysis [41–44]. Overall, these examples underscore the crucial role of hepatic uptake transporters (OATPs) in determining the hepatic clearance rate and plasma exposure of these molecules. While hepatic uptake could be the rate-determining process in the systemic clearance of several OATP transporter substrates, enzymatic metabolism and/or biliary efflux to a large extent dictate hepatic exposure and elimination from the body. For example, atorvastatin is metabolized primarily by the CYP3A4, while repaglinide and cerivastatin are metabolized by CYP2C8 and CYP3A4, with an overall extent of metabolism >90%.

Physicochemical Determinants of Active Hepatic Uptake

OATPs are the key transporters involved in active hepatic uptake [27]. Limited datasets are available to draw definite conclusions on the physicochemical attributes of the OATP substrates. In a recent analysis of 219 diverse commercial and proprietary compounds with human OATP substrate data, our group reported that the ionization state plays a key role in substrate interaction with OATPs [45]. Acids and zwitterions form the majority of the OATPs substrates, with almost no bases showing functional affinity. The majority of the OATPs substrates have molecular weight greater than 400 Da, and also possess high polar surface area. Lipophilicity may have limited association, which is reflected in a wide range of logD values for well-known OATP substrates (e.g., rosuvastatin, $logD_{7.4}$ -1.9; bosentan, $logD_{7.4}$ 2.4) [27]. Additionally, active uptake could be the rate-determining step in the hepatic clearance of compounds with low permeability (e.g., pravastatin, rosuvastatin, valsartan) and high permeability (e.g., atorvastatin, cerivastatin, glyburide, repaglinide and telmisartan) drugs, suggesting that membrane permeability alone is not an indicator in defining the role of uptake transport towards clearance – relative rate of active transport and passive transport is an important factor [6, 11, 27]. Collectively, evidence suggests that hepatic active uptake could be the predominant clearance mechanism for acids and zwitterions with a molecular weight >400 Da, irrespective of their membrane permeability.

Physicochemical Determinants of Hepato-Biliary Transport

Drug secretion into bile is predominantly driven by membrane transporters, with the involvement of canalicular efflux transporters, MRP2, BCRP, and P-gp [46, 47]. MRP2 and BCRP were shown to drive biliary excretion of various organic anions, including glutathione and glucuronide conjugates. For example, MRP2 and BCRP drive the biliary elimination of pravastatin and rosuvastatin, respectively [17, 27]. P-gp is typically known to efflux basic hydrophobic drugs. While substrate affinity towards canalicular efflux transporters is a prerequisite for active secretion into bile, it is evident that hepatic sinusoidal uptake is the rate-determining step in the hepatic clearance of compounds eliminated in bile (Eq. 2). For example, systemic clearance of valsartan is determined by OATP-mediated transporter, while about 80% of the dose is excreted in bile as parent [48, 49]. Since hepatic uptake is a key process in hepatobiliary transport, the dominant molecular features of drugs excreted in bile are (similar to those discussed in previous section): ionization state, molecular weight, lipophilicity, and polarity [45, 50–52]. Molecular weight has been commonly used as a physicochemical determinant of biliary elimination in several preclinical species and human, where higher molecular weight compounds show a greater propensity for biliary excretion, likely due to their active hepatic uptake [53, 54]. Yang *et al.*, analyzed a dataset compiled from published reports and suggested a molecular weight threshold of 400 and 475 for organic anions in rat and human, respectively [50]. The distinct molecular weight cut-off noted for biliary elimination (BE) may indicate that substrate specificity of the transporters involved in hepato-biliary transport is potentially associated with molecular size. Kato *et al.* studied the substrate affinity of the cephalosporins for MRP2 and BCRP and suggested involvement of efflux pumps in the molecular weight-dependent biliary excretion of β-lactam antibiotics in rats [55]. However, the fact that canalicular efflux transporters have much wider substrate specificity implies that secretion across the canalicular membrane is not selective to a certain molecular size [56–60]. Our recent analysis using a sizable dataset showed that human OATPs and rat Oatp1b2 substrates are acidic in nature and also tend toward larger molecular weight (>400 Da) [45]. Furthermore, predominant presence of large MW acids in the list of drugs with significant biliary elimination suggests that the substrate specificity of OATPs, which particularly transport acids with high molecular weight, is the key driver for hepatobiliary elimination. Overall, polar acidic drugs with high molecular weight are taken up by hepatoselective OATP transporters and consequently eliminated in bile. These properties are also associated

with low passive permeability [45, 61]. Nevertheless, rare exceptions to these general observations are seen with quaternary ammonium compounds (MW < 300 Da), which are extensively excreted in bile due to their affinity to OCT1 and P-gp [62].

Physicochemical Determinants of Metabolism

Generally, lipophilic drugs undergo metabolism to form hydrophilic metabolites, which have limited passive membrane permeability, to allow their removal from the body by excretion in urine and/or bile. Intuitively, this means drugs with good permeability are predominantly metabolized in the body. Benet and colleagues proposed the biopharmaceutics drug disposition classification system (BDDCS) on the basis of the apparent trend between permeability and extent of metabolism [63–65]. Generally, once absorbed, highly permeable drugs are extensively metabolized (≥70%) before being excreted/eliminated from the body as phase I and/or phase II metabolites. For such drugs, provided they are not substrates for hepatic active uptake transporters, hepatic clearance can be directly assessed from metabolic clearance alone (Eq. 3).

Lipophilicity (LogP or $LogD_{7.4}$) has been correlated with a wide array of ADME processes, including solubility, membrane permeability and affinity for drug metabolizing enzymes [66–68]. We previously studied the physicochemical determinants of multiple processes involved in the oral bioavailability of drugs [69, 70]. It is noted that gut and hepatic extraction via metabolism, are most influenced by lipophilicity, where drugs with $cLogD_{7.4} > 3$ demonstrate high extraction [69]. The ionic charge of the chemical substrate is also an important determinant, with bases tending to be more readily metabolized by the enzymes. Also ionic charge is linked to affinity for specific drug metabolizing enzymes. For example, CYP2C9 substrates are relatively acidic, while CYP3A and CYP2D6 substrates are either basic or neutral [71, 72]. However, no generalization can be made with respect to ionic charge dependency due to the presence of a wide variety of phase I and phase II metabolizing enzymes (CYPs, UGTs, etc.).

RENAL CLEARANCE

Renal clearance is determined by glomerular filtration, tubular secretion, and reabsorption processes, and can be mathematically described by:

$$CL_{renal} = (f_b.GFR + CL_{sec}) \cdot (1 - F_{reabs}) \quad \text{(Eq.4)}$$

where GFR is glomerular filtration rate, CL_{sec} is renal secretory clearance and F_{reabs} is the fraction of filtered and secreted drug that is reabsorbed. Assuming a well-stirred model, CL_{sec} can be expressed as (Eq. 5):

$$CL_{sec} = Q_r \cdot \frac{f_b \cdot CL_{int,sec}}{Q_r + f_b \cdot CL_{int,sec}} \quad \text{(Eq.5)}$$

Where Q_r is the renal blood flow and $CL_{int,sec}$ is the intrinsic secretory clearance that can be described by:

$$CL_{int,sec} = \frac{PS_{influx,b} \cdot PS_{efflux,a}}{(PS_{efflux,b} + PS_{efflux,a})} \quad \text{(Eq.6)}$$

$PS_{influx,b}$, $PS_{efflux,b}$, $PS_{influx,a}$, and $PS_{efflux,a}$ are influx and efflux intrinsic transport clearances across the basolateral and apical membranes of proximal tubule cells, respectively (Fig. 1). Glomerular filtration is the ultra-filtration of about 10% of total renal blood flow at the glomerulus of the nephron and is defined by the blood flow rate and f_b (unbound blood fraction). Glomerular filtration occurs for all drugs however, its contribution to total clearance is typically low due to low GFR (1.78 mL/min/kg). Tubular secretion facilitates transport of compounds from the plasma into the proximal tubular lumen, which is predominantly controlled by active transporters and is therefore dependent on transporter kinetics, f_b, and the blood flow rate [73, 74]. Many compounds undergo tubular reabsorption from urine into blood all along the nephron, due to the high concentration gradient created by the water reuptake process [75]. The degree of reabsorption mainly depends on passive permeability and is also influenced by urine flow and pH. Nevertheless, uptake and efflux transporters localized on the luminal (apical) membrane at the proximal tubuli may contribute to the reabsorption process [76, 77].

Active renal secretion involves transporters that facilitate drug uptake across the basolateral membrane of proximal tubule cells from blood and efflux across the apical membrane into lumen. Apparent low passive permeability across the basolateral membrane compared to the apical membrane [78] and a high counter concentration-gradient created by water reabsorption makes the contribution of passive tubular secretion negligible, if any. It is recognized that the polyspecific members of the organic ion transporter family (*SLC22*) primarily localized on the basolateral membrane of proximal tubuli play a pivotal role in the renal secretion process – the members consists of organic cation transporter 2 (OCT2) and organic anion transporters 1, 2 and 3 (OAT1, OAT2 and OAT3) [79–81]. Although, active secretion involves passage across two membranes of proximal tubule cells, drug uptake from the blood compartment to cell will be the rate-determining process towards systemic clearance [16], especially for hydrophilic drugs with minimum passive and active back flux. There may be exceptions for equilibrative facilitative transporters like OCT2, since their bidirectional function can reverse transport when the free intracellular

substrate concentration is substantially higher than free blood concentration.

Physicochemical Determinants of Renal Clearance

Drugs with sufficient passive permeability can be efficiently reabsorbed, as the passive reabsorption process occurs throughout the length of the nephron; unlike the active renal secretion that predominantly occurs at the proximal tubule. Therefore, the physicochemical determinants of passive membrane permeability may reasonably describe renal drug clearance [82]. Generally, renal clearance decreases with increasing lipophilicity and shows a positive correlation with polar descriptors. Our previous studies demonstrate a distinct inverse relationship between MDCK-LE (Madin-Darby canine kidney- low efflux cells) permeability, the percentage of parent excreted in urine, and human renal clearance rate [61]. Membrane permeability requires lipid solubility as well as desolvation of associated hydrogen-bonded water molecules [83, 84]. The relationship of renal clearance with lipophilicity and polar descriptors can therefore be attributed to their effect on the passive reabsorption process. Hydrophobicity and basicity are indicated to be the principal determinants of substrate interaction with OCTs, while hydrophobicity and acidity are associated with substrate affinity for OATs [85]. However, most of the high affinity substrates to these transporters are relatively hydrophilic (cLog $P<0$) [79, 86]. Furthermore, hydrogen bonding ability seems to be an advantageous mechanism to stabilize the substrate-transporter complex [85]. Taken together, ionized compounds with low permeability are predominantly renally secreted due to (i) an ability to interact with the renal transporters at the proximal tubuli and (ii) a limited ability for passive reabsorption process along the length of the nephron [82, 87].

PROPOSAL OF EXTENDED CLEARANCE CLASSIFICATION SYSTEM (ECCS)

From the above review, it is clear that a drug's predominant clearance mechanism is defined by its physiochemical properties – wherein ionization state, molecular weight and membrane permeability show a distinct association [45, 61, 70, 82]. We therefore hypothesized that these fundamental drug properties could be used to predict a molecule's predominant clearance mechanism, or rate-determining process towards its systemic clearance. Based on these fundamentals, we propose the extended clearance classification system (ECCS), where drugs can be classified into 6 classes (Fig. 2): Class 1A – low molecular weight, high permeability acids and zwitterions, for which metabolism is the predominant clearance mechanism; Class 1B – high molecular weight, high permeability acids and zwitterions, for which

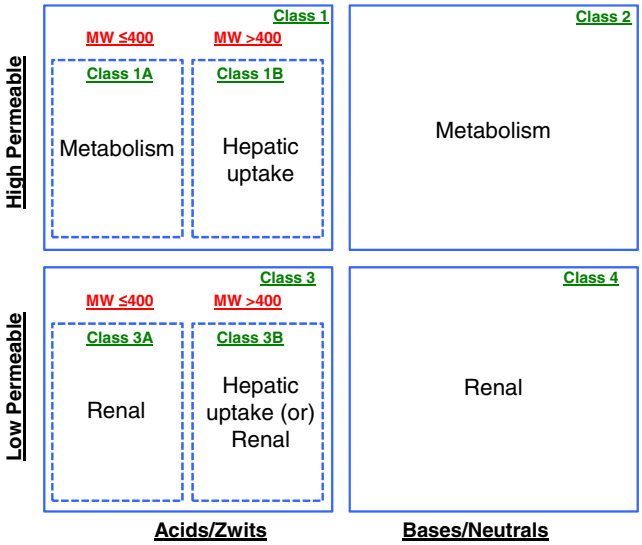
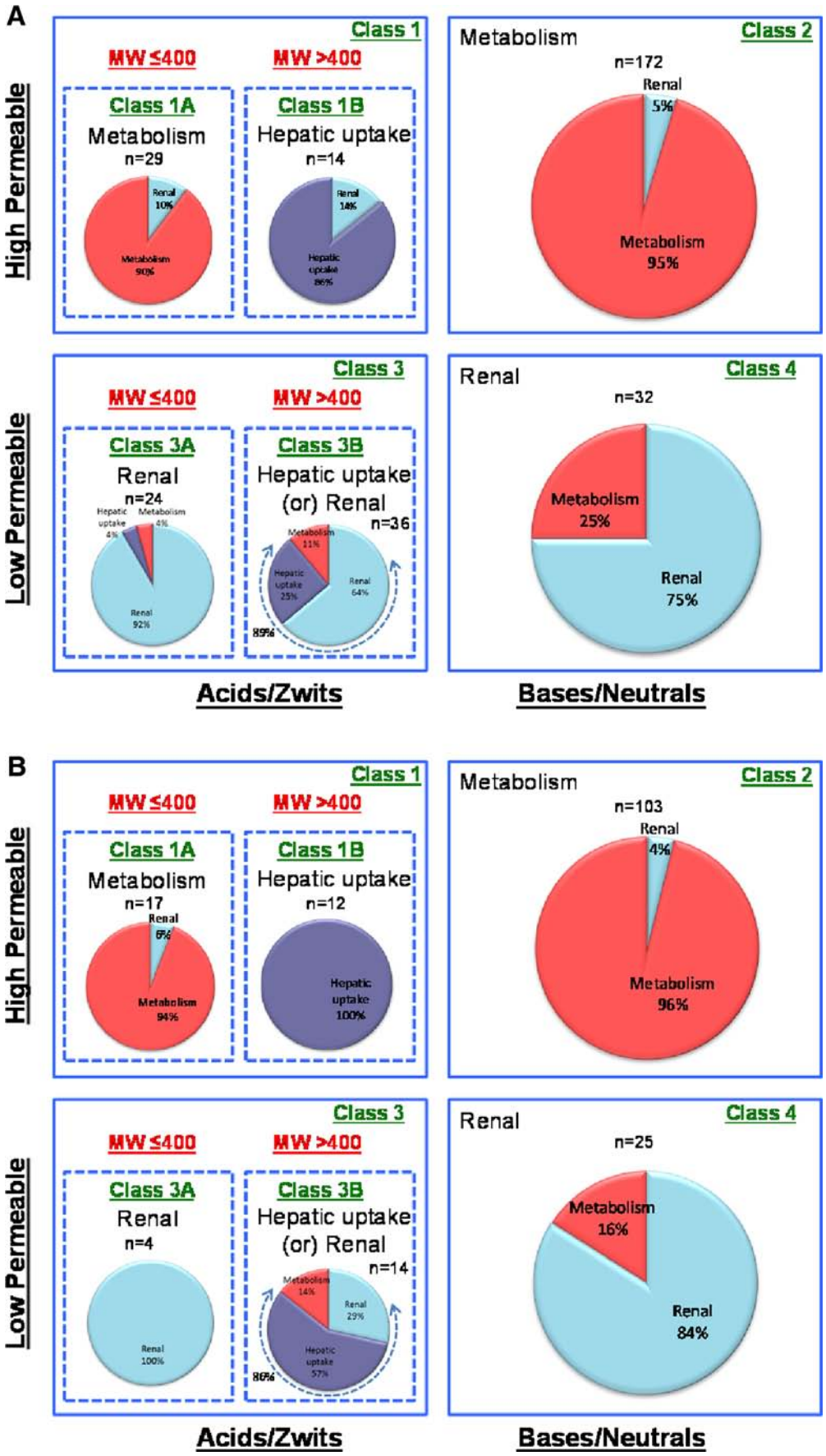


Fig. 2 The framework of extended clearance classification system (ECCS) for identifying the predominant mechanism that determines systemic clearance of drugs.

hepatic uptake is the predominant clearance mechanism; Class 2 – high permeability bases and neutrals, for which metabolism is the predominant clearance mechanism; Class 3A – low molecular weight, low permeability acids and zwitterions, for which renal clearance is the predominant clearance mechanism; Class 3B – high molecular weight, low permeability acids and zwitterions, for which hepatic uptake or renal elimination is the predominant clearance mechanism; and Class 4 – low permeability bases and neutrals, for which renal clearance is the predominant clearance mechanism. We derived a cut off value of 5×10^{-6} cm/s for the membrane permeability to define high and low permeability classes, based on a previous analysis conducted by our group [61]. Using a smaller set of compounds with apparent membrane permeability measured across a clonal cell line of MDCK-LE, this statistically derived cut-off value distinguished between high and low intestinal absorption (*Fa*) and renal clearance with high sensitivity and specificity. The molecular weight cutoff (400 Da) enables distinction between OATP substrates (Classes 1B and 3B) and non-substrates (Classes 1A and 3A) within the acids and zwitterions. For Class 1B compounds, their elimination is usually via excretion of phase I and II metabolites (≥70% of dose), while for Class 3B uptake substrates, their elimination is usually as parent in the bile, despite the rate-determining systemic clearance in both classes being dominated by hepatic uptake (Fig. 1).

Fig. 3 Prediction success of predominant clearance mechanism by ECCS, (**a**) when both the experimental and *in silico* permeability values were used ($n=307$), and (**b**) when only experimental permeability data was used ($n=175$). *Dotted line* in Class 3B represent percentage of drugs with hepatic uptake and renal as predominant clearance mechanism. ▶


A
High Permeable
Low Permeable
Class 1
MW ≤400
MW >400
Class 1A
Metabolism
n=29
Renal 10%
Metabolism 90%
Class 1B
Hepatic uptake
n=14
Renal 14%
Hepatic uptake 86%
Metabolism
Class 2
n=172
Renal 5%
Metabolism 95%
Class 3
MW ≤400
MW >400
Class 3A
Renal
n=24
Hepatic uptake 4%
Metabolism 4%
Renal 92%
Class 3B
Hepatic uptake (or) Renal
n=36
Metabolism 11%
Hepatic uptake 25%
Renal 64%
89%
Renal
Class 4
n=32
Metabolism 25%
Renal 75%
Acids/Zwits
Bases/Neutrals
B
High Permeable
Low Permeable
Class 1
MW ≤400
MW >400
Class 1A
Metabolism
n=17
Renal 6%
Metabolism 94%
Class 1B
Hepatic uptake
n=12
Hepatic uptake 100%
Metabolism
Class 2
n=103
Renal 4%
Metabolism 96%
Class 3
MW ≤400
MW >400
Class 3A
Renal
n=4
Renal 100%
Class 3B
Hepatic uptake (or) Renal
n=14
Metabolism 14%
Renal 29%
Hepatic uptake 57%
86%
Renal
Class 4
n=25
Metabolism 16%
Renal 84%
Acids/Zwits
Bases/Neutrals

DATA ANALYSIS AND RESULTS OF ECCS VALIDATION

A database of human clearance mechanisms was developed based on published data reported by Obach *et al.* [70], Varma *et al.* [69, 82] Berellini *et al.* [88] and Lombardo *et al.* [89]. Of the collated dataset of 1003 drugs, information on the clearance mechanism was sought for each drug and 739 drugs remained that had appropriate information available in the public domain. At this point, we separated out 307 drugs that had a single clearance mechanism (metabolism, renal or hepatic uptake) contributing to ≥70% of the total clearance. For the remaining drugs, the clearance either involved multiple mechanisms, lacked quantitative information on competing clearance mechanisms or could not be assigned due to conflicting information or did not meet our criteria (molecular weight ≤700 Da) of small molecule drugs. As discussed, for several drugs hepatic uptake mediated by OATPs is the rate-determining process in the hepatic clearance of drugs, although they may subsequently be metabolized or excreted unchanged in the bile [9, 11, 12, 27]. Similarly, biliary clearance is a two-step process primarily involving active sinusoidal uptake and active canalicular efflux and the systemic clearance is assumed to be determined by hepatic uptake [45]. The major difference between this dataset and what is reported by Lombardo *et al.* [89] is the addition of hepatic uptake as a rate-determining process of clearance [90]. Hepatic uptake was assigned as the primary clearance mechanism through the direct availability of transporter data, or from clinical drug-drug interactions and pharmacogenomic data [7, 27, 90–92]. For drugs that showed biliary elimination, the rate-determining step to their systemic clearance was assumed to be hepatic uptake as supported by their substrate affinity to hepatic uptake transporters particularly OATPs, as discussed above [45].

The apparent permeability across MDCK-LE was used for permeability classification and the ionization was assigned based on calculated pKa-values using MoKa (version 2.5.4, Molecular discovery). Experimental permeability data was available for 175 of the 307 clinical compounds for which the predominant clearance mechanism had been identified. For the remaining 132 drugs, permeability values were obtained using a validated continuous *in silico* model developed in Pfizer based on >100,000 data points (generated using the same experimental conditions) [93].

With 307 drugs, ECCS correctly predicted the predominant clearance mechanism on average for ~92% of the cases (Fig. 3a). Prediction success for individual classes was also high (>85%), except for in Class 4, where the prediction success was ~75%. For instance, high permeability bases and neutrals are expected to be cleared by metabolism (Class 2) and 95% of 172 drugs were correctly predicted. Similarly, the clearance mechanism of ~89% of 36 Class 3B drugs is correctly predicted with the rate-determining systemic clearance mechanism as either hepatic uptake or renal. Also notably, for 12 of the 14 high permeability acids and zwitterions with MW >400 Da (Class 1B), hepatic uptake primarily mediated by OATP transporters is the rate-determining step for their systemic clearance, although these drugs are known to be almost completely metabolized in the liver and eliminated/excreted as phase I and II metabolites. When only drugs with experimental permeability data (n= 175) were used, the overall prediction accuracy was unchanged, but there was some improvement for Class 1B and 4 predictions (Fig. 3b). Overall, ECCS provides an excellent framework for predicting the predominant clearance mechanism for compounds, and has been validated with a sizable set of drugs.

GENERAL CHARACTERISTICS OF THE ECCS CLASSES

Class 1A

These are acidic or zwitterionic compounds with high permeability (MDCK-LE P_{app} $\geq 5\times10^{-6}$ cm/s) and low molecular weight ≤400 Da. Class 1A compounds are cleared by metabolism to a greater extent than 70%. Neither uptake nor efflux transporters affect their systemic clearance and blood exposure. Examples of Class 1A drugs include non-steroidal anti-inflammatory drugs like ibuprofen and ketoprofen (Table I). Consistent with their acidic nature, they are generally metabolized by CYP2C enzymes [146], although other metabolic pathways like glucuronidation prevail for certain drugs (eg. valproic acid). From the 307 compounds investigated, 29 were classified as Class 1A compounds, with metabolism being identified as the predominant clearance mechanism for ~90% compounds (Fig. 3). Clinafloxacin and milrinone are examples of a misprediction, where the observed clearance mechanism is renal. Human *in vitro* tools aligned with the metabolic clearance mechanism, such as human liver microsomes (HLM) or human hepatocytes for CYPs and human UGTs for glucuronidation, can be effective tools for clearance prediction of Class 1A compounds [147].

Class 1B

These are acidic or zwitterionic compounds with high permeability (MDCK-LE P_{app} $\geq 5\times10^{-6}$ cm/s) and high molecular weight (≥400 Da). Class 1B compounds are predominantly systemically cleared by active hepatic uptake mediated by OATPs (Eq. 2; Fig. 1). Once cleared from the blood compartment to the liver compartment via these active uptake transporters, Class 1B compounds are metabolized and excreted in the bile and/or urine as phase I and phase II metabolites with

Table I Representative Examples per ECCS Class and the Major Transporters and Metabolizing Enzymes Involved in Their Clearance

ECCS class	Drug	Metabolic enzymes	Primary transporters	References
Class 1A	Acetyl salicylic acid	Carboxylesterase 1/2		[94]
	Diclofenac	CYP2C9, UGT2B7		[95, 96]
	Ibuprofen	CYP2C19,CYP2C9		[97, 98]
	Rosiglitazone	CYP2C8		[99]
	Valproic acid	UGT2B7, UGT1A6 and UGT1A9.		[100]
	Warfarin	CYP2C9		[101]
Class 1B	Atorvastatin	CYP3A4	OATPs	[102, 103]
	Bosentan	CYP2C9, CYP3A4	OATPs	[104]
	Cerivastatin	CYP2C8, CYP3A4	OATPs	[105]
	Fluvastatin	CYP2C9	OATPs	[106]
	Glyburide	CYP2C9, CYP3A4	OATPs	[36]
	Pitavastatin	UGT1A3 and UGT2B7.	OATPs	[107, 108]
	Repaglinide	CYP2C8, CYP3A	OATPs	[19, 39]
	Telmisartan	UGTs	OATPs	[109]
Class 2	Abacavir	Alcohol dehydrogenase		[110]
	Alfentanil	CYP3A4		[111, 112]
	Amitriptyline	CYP2D6 and CYP2C19		[113]
	Amlodipine	CYP3A4		[114]
	Budesonide	CYP3A4		[115]
	Caffeine	CYP1A2		[116]
	Diazepam	CYP3A4, CYP2C19		[117]
	Diltiazem	CYP3A4		[118]
	Erlotinib	CYP3A4		[119]
	Felodipine	CYP3A4		[120]
	Imipramine	CYP2D6		[121]
	Labetalol	UGT1A1		[122]
	Midazolam	CYP3A4		[123]
	Morphine	UGT2B7	p-glycoprotein	[124, 125]
	Nifedipine	CYP3A4		[126]
	Omeprazole	CYP2C19		[127]
	Propranolol	CYP2D6		[128]
	Sildenafil	CYP3A4		[129]
	Triazolam	CYP3A4		[112]
	Verapamil	CYP3A4		[120]
	Voriconazole	CYP2C19, 2C9, 3A4		[130, 131]
	Ziprasidone	Aldehyde Oxidase, CYP		[132]
Class 3A	Amoxicillin		OAT1	[133]
	Ceftizoxime		OAT1, OAT3	[134]
	Furosemide		OAT1, OAT3	[135]
	Penicillin_G		OAT1, OAT3	[136]
Class 3B	Piperacillin		OAT1, OAT3	
	Methotrexate		OAT1, OAT3	[137, 138]
	Rosuvastatin		OATPs, BCRP	[139]
	Valsartan		OATPs, MRP2	[48]
Class 4	Acyclovir		OAT1, OAT3	[140]
	Famotidine		OCT2	[141]
	Lamivudine		OCT2, MATEs	[142, 143]
	Ranitidine		OCT2	[144]
	Sitagliptin		OAT3	[145]

an extent of metabolism higher than 70%. A total of 14 compounds in the dataset fulfilled the Class 1B criteria, from which 12 (86%) are known to have active hepatic uptake as the rate-determining process for their systemic clearance. Some of the statins (e.g., atorvastatin, cerivastatin and fluvastatin,) and others (e.g., repaglinide and telmisartan) are examples of this class (Table I). These drugs are substrates to OATP1B1 and/or OATP1B3 transporters and are predominantly metabolized by CYP3A/CYP2C/UGT enzymes [6, 11, 27]. As illustrated in Fig. 4, OATP1B1/1B3 inhibitors such as cyclosporine, rifampin, and gemfibrozil show a significant impact on the plasma exposure of atorvastatin, cerivastatin, fluvastatin, and pitavastatin, while CYP inhibitors such as clarithromycin, erythromycin, itroconazole, fluconazole, and diclofenac show a lower impact [35, 148–159] – an indication of active transport being the predominant systemic clearance mechanism for these drugs. The two compounds of Class 1B that were mispredicted are levocabastine and sitafloxacin, which possessed a calculated permeability marginally above the cut-off (~6×10^{-6} cm/s), but show renal clearance as the predominant mechanism (Supplementary Table 1). Nevertheless, all the compounds with available experimental permeability data were correctly predicted in this class (Fig. 3b). This predominant clearance mechanism increases the reliance of Class 1B compounds on human *in vitro* systems such as suspension hepatocytes and sandwich culture human hepatocyte (SCHH) for predicting active hepatic uptake mediated clearance [27, 49, 91, 160–164]. *In vitro* tools aligned with predicting the metabolic components of drug elimination, such as human liver microsomes, will underestimate systemic clearance, although they can be critical for modelling liver concentrations [91, 165].

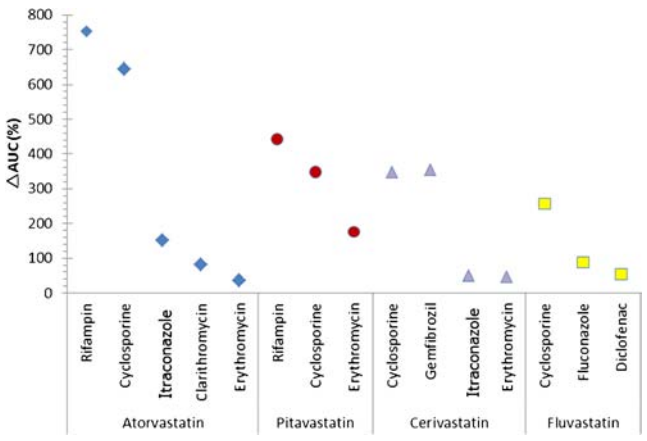


Fig. 4 The impact of OATP1B1 and CYPs inhibitors on the plasma AUC of ECCS Class 1B drugs atorvastatin, pitavastatin, cerivastatin, and fluvastatin [148–159]. Rifampicin and cyclosporine are OATPs inhibitors, gemfibrozil is an inhibitor of OATPs and CYP2C8, others are probe inhibitors for CYPs.

Class 2

These are neutral or basic compounds with high permeability (MDCK-LE P_{app} ≥5×10^{-6} cm/s). Class 2 compounds are predominantly cleared by metabolism. They cross the basolateral membrane of the hepatocytes via passive diffusion and are metabolized and excreted in the urine/bile as phase I or II metabolites. Inhibitors for metabolizing enzymes have a significant impact on the plasma exposure of Class 2 molecules, given the predominance of this clearance mechanism. For example, the CYP3A substrate midazolam, increased plasma exposure by 2541% when co-administered with the potent CYP3A4 inhibitor ritonavir [166]. A total of 172 of the compounds investigated are classified as Class 2 compounds, of which 95% are cleared by metabolism (Fig. 3). Representative examples of Class 2 include amitriptyline, diazepam, imipramine, midazolam, nifedipine, sildenafil, and voriconazole (Table I). Fluconazole and practolol are the examples of mispredictions, where the measured permeability is high but these drugs are cleared renally. Due to the expected rapid-equilibrium between blood and liver compartments, systemic clearance of Class 2 compounds can be described by Eq. 3; and HLM, human hepatocytes or other human *in vitro* systems aligned with the underlying metabolic process are effective in predicting the clearance of this class of compounds.

Class 3A

These are acids or zwitterions with low *in vitro* permeability (MDCK-LE P_{app} <5×10^{-6} cm/s) and low molecular weight (MW ≤400 Da). They are predominantly cleared by renal clearance and eliminated as unchanged drug in urine [45, 167, 168]. Renal uptake transporters such as OAT1 and OAT3 transporters may play a role in the renal elimination of Class 3A compounds. From the list of compounds investigated, 24 are classified as Class 3A, from which, ~92% fits the criteria of renal clearance as the major clearance mechanism (Fig. 3). Compounds such as amoxicillin, ciprofloxacin, furosemide and ofloxacin are examples on Class 3A molecules. However, nateglinide and torsemide were mispredicted to be Class 3A, while their predominant clearance mechanism is hepatic uptake and metabolism, respectively. Renal clearance can be predicted by single species scaling or physiologically based pharmacokinetic models [169].

Class 3B

These are acids or zwitterions with low permeability (MDCK-LE P_{app} <5×10^{-6} cm/s) and high molecular weight (>400 Da). Class 3B compounds are predominantly cleared by hepatic active uptake and/or renal clearance, followed by elimination as unchanged drug in bile and/or urine [45, 167,

168]. Unlike hepatic uptake compounds, renally cleared compounds span a wide molecular weight range; and therefore, Class 3A represent primarily renal clearance, but Class 3B compounds are cleared either by hepatic uptake or renal. Hepatic and renal uptake transporters such as OATP1B1, OATP1B3, OATP2B1, OAT1, and OAT3 transporters play a key role in the clearance of Class 3B compounds. Therefore, substrate specificity to OATPs *versus* OATs dictates the clearance pathway to be via hepatic or renal (Table I). From the list of compounds investigated, 36 are classified as Class 3B, from which, ~89% fits the criteria of hepatic uptake or renal as the major clearance mechanism (Fig. 3). Methotrexate, piperacillin, rosuvastatin and valsartan are representative examples. On the other hand, montelukast and tesaglitazar were wrongly predicted to be Class 3B compounds, while their observed clearance mechanism is metabolism. The mispredictions for these relatively lipophilic compounds could be due to underestimation of permeability caused by potential low solubility and/or non-specific binding artifacts within the permeability assay. Similar to Class 1B compounds, the prediction of active hepatic uptake clearance can be achieved via *in vitro* hepatocyte uptake studies [109, 160], whereas metabolic stability studies would underestimate the clearance of these molecules. Renal clearance can be predicted by single species scaling or physiologically based pharmacokinetic models [169].

Class 4

These are neutral or basic compounds with low permeability (MDCK-LE P_{app} $<5\times10^{-6}$ cm/s). Class 4 compounds are predominantly systemically cleared by glomerular filtration and active renal secretion that is mediated by basolateral transporters such as OCT2, OAT1, OAT2 and OAT3 (Table I). While OCT2 generally prefer basic compounds, OATs are also capable of transporting bases along with acids and zwitterions [82, 170]. With the current dataset, 32 compounds are classified as Class 4, from which 75% were predominantly cleared in urine (Fig. 3a). However, prediction accuracy improved to 84% when only compounds with experimental permeability data were assessed (Fig. 3b). Acyclovir, famotidine and sitagliptin are some examples of this class. Most of the Class 4 drugs which were mispredicted to be metabolically cleared (e.g., amiodarone, aprepitant and maraviroc) were found to be lipophilic in nature ($LogD_{7.4}>2$). We believe that the permeability of these compounds was underestimated using the current permeability assay, due to potential low solubility and/or non-specific binding issues. Renal clearance can be predicted by single species scaling or physiologically based pharmacokinetic models [169].

BCS, BDDCS AND ECCS: COMPARE AND CONTRAST

Solubility and membrane permeability are the fundamental properties determining the oral absorption (*Fa*) of drugs. Based on these properties, Amidon *et al.* [171] proposed the biopharmaceutics classification system (BCS), which conceptually explores dose number, dissolution number, and absorption number as key determinants of *Fa* (Fig. 5). This framework enabled the use of *in vitro* data rather than expensive *in vivo* human studies for establishing the bioequivalence of low risk (BCS Class I) compounds [172]. According to the US FDA guidance [172], a drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1–7.2. Tools including *in vitro* epithelial cell culture models, that are appropriately validated to predict the extent of drug absorption in humans are used for permeability classification [173–175]. While the pharmaceutical industry has taken advantage of BCS-based biowaivers, its principles are used throughout the drug discovery and development to drive orally active programs [173]. On the basis of the apparent correlation between intestinal permeability rate and extent of drug metabolism, Benet and coworkers proposed the biopharmaceutics drug disposition classification system (BDDCS) where drugs are categorized in terms of the extent of metabolism and solubility [65, 176, 177]. The group noted that the major route of elimination in humans for a majority of high-permeability BDDCS Class 1 and Class 2 drugs was an extent of metabolism ≥70%; while the major route of elimination for the poorly permeable (BDDCS Class 3 and 4) drugs was renal and/or biliary excretion of unchanged drug with an overall extent of metabolism ≤30%. Based on their dataset, which is also concurrent to this analysis, most drugs are either very highly metabolized or very poorly metabolized, and relatively few drugs showed extent of metabolism between 30 and 70% [63]. BDDCS provided an alternative framework for extending biowaivers for drugs with extensive metabolism (≥90% metabolized), but have no definitive human permeability or *Fa* information [26]. However, based on the established concordance between the membrane permeability and the extent of drug metabolism, if an efficient measure of permeability is obtained, it would be possible to use permeability to predict if the major route of elimination for NME is metabolism or renal/biliary [61].

Since 2005, BDDCS played a key role in shifting our understanding on the prediction of drug disposition and fate early on in drug discovery [65]. However, BDDCS classifies compounds based on their extent of elimination and solubility (Fig. 5), and has limited utility in predicting the rate-determining clearance mechanism. First, extent of metabolism is not a reflection of the clearance rate, as discussed in the previous sections. To reiterate, drugs such as atorvastatin,

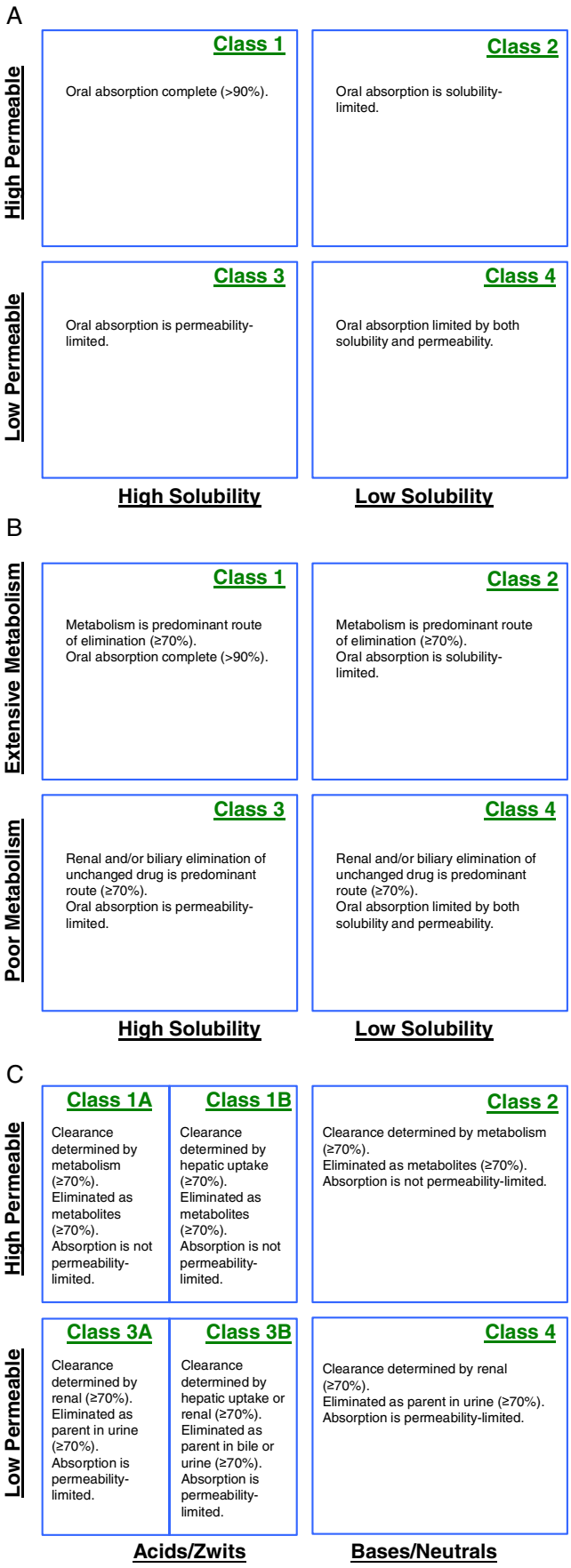


Fig. 5 Framework of (**a**) BCS for predicting oral drug absorption, (**b**) BDDCS for predicting drug disposition – major route of elimination, and (**c**) ECCS for predicting the predominant clearance mechanism.

fluvastatin, cerivastatin, and telmisartan, are extensively metabolized with an extent of metabolism higher than 70%, however, what determines their clearance rate is the active uptake mediated via hepatic uptake transporters, OATP1B1 and 1B3 [27]. Inhibitors of these transporters have a more profound impact on the pharmacokinetics compared to CYP inhibitors, which is a reflection that hepatic uptake is the rate-determining process (Fig. 4). Focusing on extent of metabolism would potentially misguide drug design to primarily focus on the metabolism as a clearance mechanism rather than uptake. Second, it is generally considered that BDDCS Class 1 compounds are not influenced by transporters located in the liver and/or intestine. It is interesting to note that drugs such as cerivastatin and fluvastatin are part of this class and inhibitors of OATP1B1 such as rifampicin and cyclosporine have more significant impact on their disposition relative to CYP inhibitors (Fig. 4). ECCS differentiates such drugs (Class 1B) from those metabolically cleared (Class1A and 2).

Thirdly, solubility is a fundamental principle for oral absorption as only drug in solution has the ability to permeate across enterocytes. Therefore, solubility classification is vital in identifying BCS and BDDCS Class1 drugs to support a biowaiver package for bioavailability and bioequivalence studies. However, it is not directly relevant to drug clearance. Aqueous solubility is an indirect measure of lipophilicity, which is also reflected in membrane permeability. Characterizing solubility classes for BCS and BDDCS at the early discovery stage is limited due to (i) the lack of availability of material in a crystalline form, resources involved and feasibility of high throughput solubility measures and (ii) uncertainty around the final salt form and maximum oral dose in the clinic. Additionally, we do not believe that solubility classes would provide any additional information to understand the clearance mechanisms or elimination routes. For instance, elimination route for both BDDCS Class 3 and 4 drugs are renal or biliary. Therefore, a fundamental principle, namely ionization state, is suggested as an integral variable in the ECCS framework. While ECCS Class 3B could not differentiate between renal and hepatic uptake mechanisms, compounds of ECCS Class 3A and 4 are generally renally cleared (Fig. 5c). As discussed earlier, ionization state is the key molecular property defining the ADME characteristics and is easily obtained from the chemical structure using either *in silico* tools or *in vitro* tools [178, 179]. Finally, unlike BCS and BDDCS, ECCS cannot be applied for biowaivers purposes as it does not consider solubility and formulation aspects of oral absorption. Nevertheless, the permeability cut-off for ECCS classification was initially derived based on sigmoidal relationship between

permeability and human intestinal absorption (*Fa*) [61], and thus provides an early indication as to whether absorption may be permeability-limited. Comparison of BCS and BDDS to ECCS are valuable analyses, since the latter represents a significant extension of these earlier frameworks by describing the impact of physiochemistry and permeability on predominant clearance mechanism. Other representations of the extended clearance concept [9, 11, 14, 27], such as the Hepatic Clearance Classification System [180] and Extended Clearance Model [20] have been published recently, and are different to the ECCS due to their focus on hepatic clearance and dependence on a range of *in vitro* measurements, and thus differ in the scope of application in early drug design.

CONCLUSIONS

In the drug design phase, an earlier understanding of the predominant clearance mechanism in a chemical series, increases efficiency by focusing on optimizing the right ADME parameters to enable the rapid identification of a "best-in-class" clinical candidate that is devoid of drug interaction risk and capable of robustly testing the mechanism in humans. Understanding the rate-determining clearance mechanism of a drug candidate is a pre-requisite for the successful prediction of clinical pharmacokinetics and DDIs, as well as determining the impact of genetic variations of metabolizing enzymes and transporters on drug exposure. The proposed ECCS framework enables the early identification of the clearance mechanism, based on membrane permeability and compound ionization, obtained using high throughput *in vitro* or *in silico* tools. This classification scheme successfully predicted the predominant clearance mechanism for approximately 92% of the compounds evaluated. Overall, ECCS increases efficiency by providing the basis to determine the right *in vitro* and *in vivo* experimental approach(es) to be conducted for timely and reliable prediction of clearance and pharmacokinetics in humans.

ACKNOWLEDGMENTS AND DISCLOSURES

The authors acknowledge Drs. R. Scott Obach, Tristan Maurer, David Tess, Shinji Yamazaki and Larry Tremaine for critical review of the manuscript and valuable suggestions.

REFERENCES

1. Arrowsmith J. Trial watch: phase II failures: 2008–2010. Nat Rev Drug Discov. 2011;10:328–9.
2. Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD, *et al.* Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. Drug Discov Today. 2012;17:419–24.
3. Roffey SJ, Obach RS, Gedge JI, Smith DA. What is the objective of the mass balance study? A retrospective analysis of data in animal and human excretion studies employing radiolabeled drugs. Drug Metab Rev. 2007;39:17–43.
4. Di L, Feng B, Goosen TC, Lai Y, Steyn SJ, Varma MV, *et al.* A perspective on the prediction of drug pharmacokinetics and disposition in drug research and development. Drug Metab Dispose Biol Fate Chem. 2013;41:1975–93.
5. Barton HA, Lai Y, Goosen TC, Jones HM, El-Kattan AF, Gosset JR, *et al.* Model-based approaches to predict drug-drug interactions associated with hepatic uptake transporters: preclinical, clinical and beyond. Expert Opin Drug Metab Toxicol. 2013;9:459–72.
6. Elsby R, Hilgendorf C, Fenner K. 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. 2012;92:584–98.
7. Lai Y, Varma M, Feng B, Stephens JC, Kimoto E, El-Kattan A, *et al.* Impact of drug transporter pharmacogenomics on pharmacokinetic and pharmacodynamic variability - considerations for drug development. Expert Opin Drug Metab Toxicol. 2012;8:723–43.
8. Pang KS, Maeng HJ, Fan J. Interplay of transporters and enzymes in drug and metabolite processing. Mol Pharm. 2009;6:1734–55.
9. Shitara Y, Horie T, Sugiyama Y. Transporters as a determinant of drug clearance and tissue distribution. Eur J Pharm Sci. 2006;27:425–46.
10. Liu L, Pang KS. The roles of transporters and enzymes in hepatic drug processing. Drug Metab Dispose Biol Fate Chem. 2005;33:1–9.
11. Li R, Barton HA, Varma MV. Prediction of pharmacokinetics and drug-drug interactions when hepatic transporters are involved. Clin Pharmacokinet. 2014;53:659–78.
12. Maeda K, Ikeda Y, Fujita T, Yoshida K, Azuma Y, Haruyama Y, *et al.* Identification of the rate-determining process in the hepatic clearance of atorvastatin in a clinical cassette microdosing study. Clin Pharmacol Ther. 2011;90:575–81.
13. Pang KS, Rowland M. Hepatic clearance of drugs. I. Theoretical considerations of a "well-stirred" model and a "parallel tube" model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. J Pharmacokinet Biopharm. 1977;5:625–53.
14. Yoshida K, Maeda K, Sugiyama Y. Hepatic and intestinal drug transporters: prediction of pharmacokinetic effects caused by drug-drug interactions and genetic polymorphisms. Annu Rev Pharmacol Toxicol. 2013;53:581–612.
15. Yamazaki M, Suzuki H, Sugiyama Y. Recent advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. Pharm Res. 1996;13:497–513.
16. Sirianni GL, Pang KS. Organ clearance concepts: new perspectives on old principles. J Pharmacokinet Biopharm. 1997;25:449–70.
17. Shitara Y, Sugiyama Y. 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. 2006;112:71–105.
18. Camenisch G, Umehara K. Predicting human hepatic clearance from *in vitro* drug metabolism and transport data: a scientific and pharmaceutical perspective for assessing drug-drug interactions. Biopharm Drug Dispos. 2012;33:179–94.
19. Varma MV, Lin J, Bi YA, Rotter CJ, Fahmi OA, Lam JL, *et al.* Quantitative prediction of repaglinide-rifampicin complex drug interactions using dynamic and static mechanistic models:

delineating differential CYP3A4 induction and OATP1B1 inhibition potential of rifampicin. Drug Metab Dispose Biol Fate Chem. 2013;41:966–74.
20. Camenisch G, Riede J, Kunze A, Huwyler J, Poller B, Umehara K. The extended clearance model and its use for the interpretation of hepatobiliary elimination data. ADMET DMPK. 2015;3:1–14.
21. De Buck SS, Sinha VK, Fenu LA, Nijsen MJ, Mackie CE, Gilissen RA. Prediction of human pharmacokinetics using physiologically based modeling: a retrospective analysis of 26 clinically tested drugs. Drug Metab Dispose Biol Fate Chem. 2007;35:1766–80.
22. Jones HM, Gardner IB, Collard WT, Stanley PJ, Oxley P, Hosea NA, *et al.* Simulation of human intravenous and oral pharmacokinetics of 21 diverse compounds using physiologically based pharmacokinetic modelling. Clin Pharmacokinet. 2011;50:331–47.
23. Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, Macintyre F, *et al.* The prediction of human pharmacokinetic parameters from preclinical and *in vitro* metabolism data. J Pharmacol Exp Ther. 1997;283:46–58.
24. Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, *et al.* Drug-drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. Drug Metab Dispos. 2004;32:1201–8.
25. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, *et al.* Membrane transporters in drug development. Nat Rev Drug Discov. 2010;9:215–36.
26. Shugarts S, Bene LZ. The role of transporters in the pharmacokinetics of orally administered drugs. Pharm Res. 2009;26:2039–54.
27. Shitara Y, Maeda K, Ikejiri K, Yoshida K, Horie T, Sugiyama Y. Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles in hepatic clearance and intestinal absorption. Biopharm Drug Dispos. 2013;34:45–78.
28. Fenner KS, Jones HM, Ullah M, Kempshall S, Dickins M, Lai Y, *et al.* The evolution of the OATP hepatic uptake transport protein family in DMPK sciences: from obscure liver transporters to key determinants of hepatobiliary clearance. Xenobiotica. 2012;42:28–45.
29. Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. Br J Pharmacol. 2009;158:693–705.
30. Shimizu K, Takashima T, Yamane T, Sasaki M, Kageyama H, Hashizume Y, *et al.* Whole-body distribution and radiation dosimetry of [11C]telmisartan as a biomarker for hepatic organic anion transporting polypeptide (OATP) 1B3. Nucl Med Biol. 2012;39:847–53.
31. Bergman E, Forsell P, Tevell A, Persson EM, Hedeland M, Bondesson U, *et al.* Biliary secretion of rosuvastatin and bile acids in humans during the absorption phase. Eur J Pharm Sci. 2006;29:205–14.
32. Yamazaki M, Kobayashi K, Sugiyama Y. Primary active transport of pravastatin across the liver canalicular membrane in normal and mutant Eisai hyperbilirubinemic rats. Biopharm Drug Dispos. 1996;17:607–21.
33. Varma MV, Lai Y, Feng B, Litchfield J, Goosen TC, Bergman A. Physiologically based modeling of pravastatin transporter-mediated hepatobiliary disposition and drug-drug interactions. Pharm Res. 2012.
34. Watanabe T, Kusuhara H, Maeda K, Shitara Y, Sugiyama Y. Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. J Pharmacol Exp Ther. 2009;328:652–62.
35. Lau Y, Huang Y, Frassetto L, Benet L. Effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. Clin Pharmacol Ther. 2006;81:194–204.
36. Varma MV, Scialis RJ, Lin J, Bi YA, Rotter CJ, Goosen TC, *et al.* Mechanism-based pharmacokinetic modeling to evaluate transporter-enzyme interplay in drug interactions and pharmacogenetics of glyburide. AAPS J. 2014;16:736–48.
37. Zheng H, Huang Y, Frassetto L, Benet L. Elucidating rifampin's inducing and inhibiting effects on glyburide pharmacokinetics and blood glucose in healthy volunteers: unmasking the differential effects of enzyme induction and transporter inhibition for a drug and its primary metabolite. Clin Pharmacol Ther. 2008;85:78–85.
38. Kalliokoski A, Backman JT, Kurkinen KJ, Neuvonen PJ, Niemi M. Effects of gemfibrozil and atorvastatin on the pharmacokinetics of repaglinide in relation to SLCO1B1 polymorphism. Clin Pharmacol Ther. 2008;84:488–96.
39. Varma MV, Lai Y, Kimoto E, Goosen TC, El-Kattan AF, Kumar V. Mechanistic modeling to predict the transporter- and enzyme-mediated drug-drug interactions of repaglinide. Pharm Res. 2013;30:1188–99.
40. Prueksaritanont T, Chu X, Evers R, Klopfer S, Caro L, Kothare P, Dempsey C, Rasmussen S, Houle R, Chan G. Pitavastatin is a more sensitive and selective OATP1B clinical probe than rosuvastatin. Br J Clin Pharmacol. 2014.
41. Group SC, Link E, Parish S, Armitage J, Bowman L, Heath S, *et al.* SLCO1B1 variants and statin-induced myopathy–a genomewide study. N Engl J Med. 2008;359:789–99.
42. Ieiri I, Higuchi S, Sugiyama Y. 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. Exp Opin Drug Metab Toxicol. 2009;5:703–29.
43. Niemi M, Neuvonen PJ, Hofmann U, Backman JT, Schwab M, Lutjohann D, *et al.* Acute effects of pravastatin on cholesterol synthesis are associated with SLCO1B1 (encoding OATP1B1) haplotype *17. Pharmacogenet Genomics. 2005;15:303–9.
44. Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, *et al.* Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. Clin Pharmacol Ther. 2003;73:554–65.
45. Varma MV, Chang G, Lai Y, Feng B, El-Kattan AF, Litchfield J, *et al.* Physicochemical property space of hepatobiliary transport and computational models for predicting rat biliary excretion. Drug Metab Dis Biol Fate Chem. 2012;40:1527–37.
46. Kusuhara H, Sugiyama Y. Pharmacokinetic modeling of the hepatobiliary transport mediated by cooperation of uptake and efflux transporters. Drug Metab Rev. 2010;42:539–50.
47. Lai Y. Identification of interspecies difference in hepatobiliary transporters to improve extrapolation of human biliary secretion. Exp Opin Drug Metab Toxicol. 2009;5:1175–87.
48. Yamashiro W, Maeda K, Hirouchi M, Adachi Y, Hu Z, Sugiyama Y. Involvement of transporters in the hepatic uptake and biliary excretion of valsartan, a selective antagonist of the angiotensin II AT1-receptor, in humans. Drug Metab Dis Biol Fate Chem. 2006;34:1247–54.
49. Poirier A, Cascais A-C, Funk C, Lavé T. Prediction of pharmacokinetic profile of valsartan in human based on *in vitro* uptake transport data. J Pharmacokinet Pharmacodyn. 2009;36:585–611.
50. Yang X, Gandhi YA, Duignan DB, Morris ME. Prediction of biliary excretion in rats and humans using molecular weight and quantitative structure-pharmacokinetic relationships. AAPS J. 2009;11:511–25.
51. Fagerholm U. Prediction of human pharmacokinetics-biliary and intestinal clearance and enterohepatic circulation. J Pharm Pharmacol. 2008;60:535–42.
52. Luo G, Johnson S, Hsueh MM, Zheng J, Cai H, Xin B, *et al.* In silico prediction of biliary excretion of drugs in rats based on

physicochemical properties. Drug Metab Dis Biol Fate Chem. 2010;38:422–30.
53. Millburn P, Smith RL, Williams RT. Biliary excretion in foreign compounds. Sulphonamide drugs in the rat. Biochem J. 1967;105: 1283–7.
54. Millburn P, Smith RL, Williams RT. Biliary excretion of foreign compounds. Biphenyl, stilboestrol and phenolphthalein in the rat: molecular weight, polarity and metabolism as factors in biliary excretion. Biochem J. 1967;105:1275–81.
55. Kato Y, Takahara S, Kato S, Kubo Y, Sai Y, Tamai I, *et al.* Involvement of multidrug resistance-associated protein 2 (Abcc2) in molecular weight-dependent biliary excretion of beta-lactam antibiotics. Drug Metab Dis Biol Fate Chem. 2008;36:1088–96.
56. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, *et al.* Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science. 2009;323:1718–22.
57. Gandhi YA, Morris ME. Structure-activity relationships and quantitative structure-activity relationships for breast cancer resistance protein (ABCG2). AAPS J. 2009;11:541–52.
58. Matsson P, Pedersen JM, Norinder U, Bergstrom CA, Artursson P. Identification of novel specific and general inhibitors of the three major human ATP-binding cassette transporters P-gp, BCRP and MRP2 among registered drugs. Pharm Res. 2009;26:1816–31.
59. Varma MV, Ambler CM, Ullah M, Rotter CJ, Sun H, Litchfield J, *et al.* Targeting intestinal transporters for optimizing oral drug absorption. Curr Drug Metab. 2010;11:730–42.
60. Varma MV, Panchagnula R. pH-dependent functional activity of P-glycoprotein in limiting intestinal absorption of protic drugs: kinetic analysis of quinidine efflux *in situ*. J Pharm Sci. 2005;94: 2632–43.
61. Varma MV, Gardner I, Steyn SJ, Nkansah P, Rotter CJ, Whitney-Pickett C, *et al.* pH-Dependent solubility and permeability criteria for provisional biopharmaceutics classification (BCS and BDDCS) in early drug discovery. Mol Pharm. 2012;9: 1199–212.
62. Song IS, Choi MK, Jin QR, Shim WS, Shim CK. Increased affinity to canalicular P-gp via formation of lipophilic ion-pair complexes with endogenous bile salts is associated with mw threshold in hepatobiliary excretion of quaternary ammonium compounds. Pharm Res. 2010;27:823–31.
63. Benet LZ, Broccatelli F, Oprea TI. BDDCS applied to over 900 drugs. AAPS J. 2011;13:519–47.
64. Custodio JM, Wu C-Y, Benet LZ. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. Adv Drug Deliv Rev. 2008;60:717–33.
65. Wu C-Y, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res. 2005;22:11–23.
66. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2012;64:4–17.
67. Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002;45:2615–23.
68. Camenisch G, Alsenz J, van de Waterbeemd H, Folkers G. Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight. Eur J Pharm Sci. 1998;6:313–9.
69. Varma MV, Obach RS, Rotter C, Miller HR, Chang G, Steyn SJ, *et al.* Physicochemical space for optimum oral bioavailability: contribution of human intestinal absorption and first-pass elimination. J Med Chem. 2010;53:1098–108.
70. Obach RS, Lombardo F, Waters NJ. Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. Drug Metab Dis Biol Fate Chem. 2008;36: 1385–405.
71. Lewis DF. Homology modelling of human cytochromes P450 involved in xenobiotic metabolism and rationalization of substrate selectivity. Exp Toxicol Pathol. 1999;51:369–74.
72. Lewis DF, Modi S, Dickins M. Structure-activity relationship for human cytochrome P450 substrates and inhibitors. Drug Metab Rev. 2002;34:69–82.
73. Ho RH, Kim RB. Transporters and drug therapy: implications for drug disposition and disease. Clin Pharmacol Ther. 2005;78: 260–77.
74. Shitara Y, Sato H, Sugiyama Y. Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. 2005;45: 689–723.
75. Fagerholm U. Prediction of human pharmacokinetics - renal metabolic and excretion clearance. J Pharm Pharmacol. 2007;59: 1463–71.
76. Sun H, Frassetto L, Benet LZ. Effects of renal failure on drug transport and metabolism. Pharmacol Ther. 2006;109:1–11.
77. Jappar D, Hu Y, Keep RF, Smith DE. Transport mechanisms of carnosine in SKPT cells: contribution of apical and basolateral membrane transporters. Pharm Res. 2009;26:172–81.
78. Masereeuw R, Russel FG. Mechanisms and clinical implications of renal drug excretion. Drug Metab Rev. 2001;33:299–351.
79. Ahlin G, Karlsson J, Pedersen JM, Gustavsson L, Larsson R, Matsson P, *et al.* Structural requirements for drug inhibition of the liver specific human organic cation transport protein 1. J Med Chem. 2008;51:5932–42.
80. Inui KI, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. Kidney Int. 2000;58:944–58.
81. Launay-Vacher V, Izzedine H, Karie S, Hulot JS, Baumelou A, Deray G. Renal tubular drug transporters. Nephron Physiol. 2006;103:p97–106.
82. Varma MV, Feng B, Obach RS, Troutman MD, Chupka J, Miller HR, *et al.* Physicochemical determinants of human renal clearance. J Med Chem. 2009;52:4844–52.
83. Goodwin JT, Conradi RA, Ho NF, Burton PS. Physicochemical determinants of passive membrane permeability: role of solute hydrogen-bonding potential and volume. J Med Chem. 2001;44: 3721–9.
84. Varma MV, Sateesh K, Panchagnula R. Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport. Mol Pharm. 2005;2:12–21.
85. Ullrich KJ. Renal transporters for organic anions and organic cations. Structural requirements for substrates. J Membr Biol. 1997;158:95–107.
86. Bednarczyk D, Ekins S, Wikel JH, Wright SH. Influence of molecular structure on substrate binding to the human organic cation transporter, hOCT1. Mol Pharmacol. 2003;63:489–98.
87. Feng B, LaPerle JL, Chang G, Varma MV. Renal clearance in drug discovery and development: molecular descriptors, drug transporters and disease state. Exp Opin Drug Metab Toxicol. 2010;6:939–52.
88. Berellini G, Waters NJ, Lombardo F. In silico prediction of total human plasma clearance. J Chem Inf Model. 2012;52:2069–78.
89. Lombardo F, Obach RA, Varma MV, Stringer R, Berellini G. Clearance mechanism assignment and total clearance prediction in human based upon in silico models. J Med Chem. 2014.
90. Kusama M, Toshimoto K, Maeda K, Hirai Y, Imai S, Chiba K, *et al.* In silico classification of major clearance pathways of drugs with their physiochemical parameters. Drug Metab Dispos. 2010;38:1362–70.

91. Varma MV, Bi YA, Kimoto E, Lin J. 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. 2014;351: 214–23.
92. Niemi M, Pasanen MK, Neuvonen PJ. Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. Pharmacol Rev. 2011;63:157–81.
93. Gupta RR, Gifford EM, Liston T, Waller CL, Hohman M, Bunin BA, *et al.* Using open source computational tools for predicting human metabolic stability and additional absorption, distribution, metabolism, excretion, and toxicity properties. Drug Metab Dispos. 2010;38:2083–90.
94. Tang M, Mukundan M, Yang J, Charpentier N, LeCluyse EL, Black C, *et al.* Antiplatelet agents aspirin and clopidogrel are hydrolyzed by distinct carboxylesterases, and clopidogrel is transesterificated in the presence of ethyl alcohol. J Pharmacol Exp Ther. 2006;319:1467–76.
95. Davies NM, Anderson KE. Clinical pharmacokinetics of diclofenac. Therapeutic insights and pitfalls. Clin Pharmacokinet. 1997;33:184–213.
96. King C, Tang W, Ngui J, Tephly T, Braun M. Characterization of rat and human UDP-glucuronosyltransferases responsible for the *in vitro* glucuronidation of diclofenac. Toxicol Sci Off J Soc Toxicol. 2001;61:49–53.
97. Andersson TB, Bredberg E, Ericsson H, Sjoberg H. An evaluation of the *in vitro* metabolism data for predicting the clearance and drug-drug interaction potential of CYP2C9 substrates. Drug Metab Dis Biol Fate Chem. 2004;32:715–21.
98. McGinnity DF, Parker AJ, Soars M, Riley RJ. Automated definition of the enzymology of drug oxidation by the major human drug metabolizing cytochrome P450s. Drug Metab Dis Biol Fate Chem. 2000;28:1327–34.
99. Kirchheiner J, Thomas S, Bauer S, Tomalik-Scharte D, Hering U, Doroshyenko O, *et al.* Pharmacokinetics and pharmacodynamics of rosiglitazone in relation to CYP2C8 genotype. Clin Pharmacol Ther. 2006;80:657–67.
100. Chu XM, Zhang LF, Wang GJ, Zhang SN, Zhou JH, Hao HP. Influence of UDP-glucuronosyltransferase polymorphisms on valproic acid pharmacokinetics in Chinese epilepsy patients. Eur J Clin Pharmacol. 2012;68:1395–401.
101. Takahashi H, Echizen H. Pharmacogenetics of warfarin elimination and its clinical implications. Clin Pharmacokinet. 2001;40: 587–603.
102. Lennernas H. Clinical pharmacokinetics of atorvastatin. Clin Pharmacokinet. 2003;42:1141–60.
103. Lau YY, Okochi H, Huang Y, Benet LZ. Multiple transporters affect the disposition of atorvastatin and its two active hydroxy metabolites: application of *in vitro* and ex situ systems. J Pharmacol Exp Ther. 2006;316:762–71.
104. Dingemanse J, van Giersbergen PL. Clinical pharmacology of bosentan, a dual endothelin receptor antagonist. Clin Pharmacokinet. 2004;43:1089–115.
105. Shitara Y, Hirano M, Sato H, Sugiyama Y. Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. J Pharmacol Exp Ther. 2004;311:228–36.
106. Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. Clin Pharmacol Ther. 2006;80:565–81.
107. Hirano M, Maeda K, Shitara Y, Sugiyama Y. Contribution of OATP2 (OATP1B1) and OATP8 (OATP1B3) to the hepatic uptake of pitavastatin in humans. J Pharmacol Exp Ther. 2004;311:139–46.
108. Fujino H, Saito T, Tsunenari Y, Kojima J. Effect of gemfibrozil on the metabolism of pitavastatin–determining the best animal model for human CYP and UGT activities. Drug Metabol Drug Interact. 2004;20:25–42.
109. Li R, Ghosh A, Maurer TS, Kimoto E, Barton HA. Physiologically based pharmacokinetic prediction of telmisartan in human. Drug Metab Dis Biol Fate Chem. 2014;42:1646–55.
110. McDowell JA, Chittick GE, Stevens CP, Edwards KD, Stein DS. Pharmacokinetic interaction of abacavir (1592U89) and ethanol in human immunodeficiency virus-infected adults. Antimicrob Agents Chemother. 2000;44:1686–90.
111. Pea F, Furlanut M. Pharmacokinetic aspects of treating infections in the intensive care unit: focus on drug interactions. Clin Pharmacokinet. 2001;40:833–68.
112. Baneyx G, Parrott N, Meille C, Iliadis A, Lave T. Physiologically based pharmacokinetic modeling of CYP3A4 induction by rifampicin in human: influence of time between substrate and inducer administration. Eur J Pharm Sci. 2014;56:1–15.
113. Samer CF, Lorenzini KI, Rollason V, Daali Y, Desmeules JA. Applications of CYP450 testing in the clinical setting. Mol Diagn Ther. 2013;17:165–84.
114. Bailey DG, Dresser GK. Interactions between grapefruit juice and cardiovascular drugs. Am J Cardiovasc Drugs Drugs Devices Interv. 2004;4:281–97.
115. Edsbacker S, Andersson T. Pharmacokinetics of budesonide (Entocort EC) capsules for Crohn's disease. Clin Pharmacokinet. 2004;43:803–21.
116. Brosen K. Drug interactions and the cytochrome P450 system. The role of cytochrome P450 1A2. Clin Pharmacokinet. 1995;29(1):20–5.
117. Niwa T, Shiraga T, Ishii I, Kagayama A, Takagi A. Contribution of human hepatic cytochrome p450 isoforms to the metabolism of psychotropic drugs. Biol Pharm Bull. 2005;28:1711–6.
118. Sutton D, Butler AM, Nadin L, Murray M. Role of CYP3A4 in human hepatic diltiazem N-demethylation: inhibition of CYP3A4 activity by oxidized diltiazem metabolites. J Pharm Exp Ther. 1997;282:294–300.
119. van Erp NP, Gelderblom H, Guchelaar HJ. Clinical pharmacokinetics of tyrosine kinase inhibitors. Cancer Treat Rev. 2009;35: 692–706.
120. Ohno Y, Hisaka A, Suzuki H. General framework for the quantitative prediction of CYP3A4-mediated oral drug interactions based on the AUC increase by coadministration of standard drugs. Clin Pharmacokinet. 2007;46:681–96.
121. Bogni A, Monshouwer M, Moscone A, Hidestrand M, Ingelman-Sundberg M, Hartung T, *et al.* Substrate specific metabolism by polymorphic cytochrome P450 2D6 alleles. Toxicol In Vitro Int J Publ Assoc BIBRA. 2005;19:621–9.
122. Jeong H, Choi S, Song JW, Chen H, Fischer JH. Regulation of UDP-glucuronosyltransferase (UGT) 1A1 by progesterone and its impact on labetalol elimination. Xenobiotica. 2008;38:62–75.
123. Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, *et al.* First-pass metabolism of midazolam by the human intestine. Clin Pharmacol Ther. 1996;60:14–24.
124. Crowe A. The influence of P-glycoprotein on morphine transport in Caco-2 cells. Comparison with paclitaxel. Eur J Pharmacol. 2002;440:7–16.
125. Chau N, Elliot DJ, Lewis BC, Burns K, Johnston MR, Mackenzie PI, *et al.* Morphine glucuronidation and glucosidation represent complementary metabolic pathways that are both catalyzed by UDP-glucuronosyltransferase 2B7: kinetic, inhibition, and molecular modeling studies. J Pharmacol Exp Ther. 2014;349:126–37.
126. Foti RS, Rock DA, Wienkers LC, Wahlstrom JL. Selection of alternative CYP3A4 probe substrates for clinical drug interaction

studies using *in vitro* data and *in vivo* simulation. Drug Metab Dis Biol Fate Chem. 2010;38:981–7.
127. Kanazawa H, Okada A, Higaki M, Yokota H, Mashige F, Nakahara K. Stereospecific analysis of omeprazole in human plasma as a probe for CYP2C19 phenotype. J Pharm Biomed Anal. 2003;30:1817–24.
128. Narimatsu S, Nakata T, Shimizudani T, Nagaoka K, Nakura H, Masuda K, *et al.* Regio- and stereoselective oxidation of propranolol enantiomers by human CYP2D6, cynomolgus monkey CYP2D17 and marmoset CYP2D19. Chem Biol Interact. 2011;189:146–52.
129. Gupta M, Kovar A, Meibohm B. The clinical pharmacokinetics of phosphodiesterase-5 inhibitors for erectile dysfunction. J Clin Pharmacol. 2005;45:987–1003.
130. Leveque D, Nivoix Y, Jehl F, Herbrecht R. Clinical pharmacokinetics of voriconazole. Int J Antimicrob Agents. 2006;27:274–84.
131. Jeu L, Piacenti FJ, Lyakhovetskiy AG, Fung HB. Voriconazole. Clin Ther. 2003;25:1321–81.
132. Beedham C, Miceli JJ, Obach RS. Ziprasidone metabolism, aldehyde oxidase, and clinical implications. J Clin Psychopharmacol. 2003;23:229–32.
133. Li M, Anderson GD, Phillips BR, Kong W, Shen DD, Wang J. Interactions of amoxicillin and cefaclor with human renal organic anion and peptide transporters. Drug Metab Dis Biol Fate Chem. 2006;34:547–55.
134. Ueo H, Motohashi H, Katsura T, Inui K. Human organic anion transporter hOAT3 is a potent transporter of cephalosporin antibiotics, in comparison with hOAT1. Biochem Pharmacol. 2005;70:1104–13.
135. Hasannejad H, Takeda M, Taki K, Shin HJ, Babu E, Jutabha P, *et al.* Interactions of human organic anion transporters with diuretics. J Pharm Exp Ther. 2004;308:1021–9.
136. Ye J, Liu Q, Wang C, Meng Q, Sun H, Peng J, *et al.* Benzylpenicillin inhibits the renal excretion of acyclovir by OAT1 and OAT3. Pharmacol Rep PR. 2013;65:505–12.
137. Liu Q, Wang C, Meng Q, Huo X, Sun H, Peng J, *et al.* MDR1 and OAT1/OAT3 mediate the drug-drug interaction between puerarin and methotrexate. Pharm Res. 2014;31:1120–32.
138. El-Sheikh AA, Greupink R, Wortelboer HM, van den Heuvel JJ, Schreurs M, Koenderink JB, *et al.* Interaction of immunosuppressive drugs with human organic anion transporter (OAT) 1 and OAT3, and multidrug resistance-associated protein (MRP) 2 and MRP4. Transl Re J Lab Clin Med. 2013;162:398–409.
139. Kitamura S, Maeda K, Wang Y, Sugiyama Y. Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. Drug Metab Dis Biol Fate Chem. 2008;36:2014–23.
140. Ye J, Liu Q, Wang C, Meng Q, Peng J, Sun H, *et al.* Inhibitory effect of JBP485 on renal excretion of acyclovir by the inhibition of OAT1 and OAT3. Eur J Pharm Sci. 2012;47:341–6.
141. Tahara H, Kusuhara H, Endou H, Koepsell H, Imaoka T, Fuse E, *et al.* A species difference in the transport activities of H2 receptor antagonists by rat and human renal organic anion and cation transporters. J Pharmacol Exp Ther. 2005;315:337–45.
142. Muller F, Konig J, Hoier E, Mandery K, Fromm MF. Role of organic cation transporter OCT2 and multidrug and toxin extrusion proteins MATE1 and MATE2-K for transport and drug interactions of the antiviral lamivudine. Biochem Pharmacol. 2013;86:808–15.
143. Choi CI, Bae JW, Keum SK, Lee YJ, Lee HI, Jang CG, *et al.* Effects of OCT2 c.602C > T genetic variant on the pharmacokinetics of lamivudine. Xenobiotica. 2013;43:636–40.
144. Bourdet DL, Pritchard JB, Thakker DR. Differential substrate and inhibitory activities of ranitidine and famotidine toward human organic cation transporter 1 (hOCT1; SLC22A1), hOCT2 (SLC22A2), and hOCT3 (SLC22A3). J Pharmacol Exp Ther. 2005;315:1288–97.
145. Chu XY, Bleasby K, Yabut J, Cai X, Chan GH, Hafey MJ, *et al.* Transport of the dipeptidyl peptidase-4 inhibitor sitagliptin by human organic anion transporter 3, organic anion transporting polypeptide 4C1, and multidrug resistance P-glycoprotein. J Pharmacol Exp Ther. 2007;321:673–83.
146. Lewis DF. Modelling human cytochromes P450 for evaluating drug metabolism: an update. Drug Metabol Drug Interact. 2000;16:307–24.
147. Hosea NA, Collard WT, Cole S, Maurer TS, Fang RX, Jones H, *et al.* Prediction of human pharmacokinetics from preclinical information: comparative accuracy of quantitative prediction approaches. J Clin Pharmacol. 2009;49:513–33.
148. He YJ, Zhang W, Chen Y, Guo D, Tu JH, Xu LY, *et al.* Rifampicin alters atorvastatin plasma concentration on the basis of SLCO1B1 521T>C polymorphism. Clin Chim Acta Int J Clin Chem. 2009;405:49–52.
149. Asberg A, Hartmann A, Fjeldsa E, Bergan S, Holdaas H. Bilateral pharmacokinetic interaction between cyclosporine A and atorvastatin in renal transplant recipients. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2001;1:382–6.
150. Mazzu AL, Lasseter KC, Shamblen EC, Agarwal V, Lettieri J, Sundaresen P. Itraconazole alters the pharmacokinetics of atorvastatin to a greater extent than either cerivastatin or pravastatin. Clin Pharmacol Ther. 2000;68:391–400.
151. Amsden GW, Kuye O, Wei GC. A study of the interaction potential of azithromycin and clarithromycin with atorvastatin in healthy volunteers. J Clin Pharmacol. 2002;42:444–9.
152. Siedlik PH, Olson SC, Yang BB, Stern RH. Erythromycin coadministration increases plasma atorvastatin concentrations. J Clin Pharmacol. 1999;39:501–4.
153. Prueksaritanont T, Chu X, Evers R, Klopfer SO, Caro L, Kothare PA, *et al.* Pitavastatin is a more sensitive and selective organic anion-transporting polypeptide 1B clinical probe than rosuvastatin. Br J Clin Pharmacol. 2014;78:587–98.
154. Muck W, Mai I, Fritsche L, Ochmann K, Rohde G, Unger S, *et al.* Increase in cerivastatin systemic exposure after single and multiple dosing in cyclosporine-treated kidney transplant recipients. Clin Pharmacol Ther. 1999;65:251–61.
155. Backman JT, Kyrklund C, Neuvonen M, Neuvonen PJ. Gemfibrozil greatly increases plasma concentrations of cerivastatin. Clin Pharmacol Ther. 2002;72:685–91.
156. Kantola T, Backman JT, Niemi M, Kivisto KT, Neuvonen PJ. Effect of fluconazole on plasma fluvastatin and pravastatin concentrations. Eur J Clin Pharmacol. 2000;56:225–9.
157. Park JW, Siekmeier R, Lattke P, Merz M, Mix C, Schuler S, *et al.* Pharmacokinetics and pharmacodynamics of fluvastatin in heart transplant recipients taking cyclosporine A. J Cardiovasc Pharmacol Ther. 2001;6:351–61.
158. Transon C, Leemann T, Vogt N, Dayer P. *in vivo* inhibition profile of cytochrome P450TB (CYP2C9) by (+/−)-fluvastatin. Clin Pharmacol Ther. 1995;58:412–7.
159. Muck W, Ochmann K, Rohde G, Unger S, Kuhlmann J. Influence of erythromycin pre- and co-treatment on single-dose pharmacokinetics of the HMG-CoA reductase inhibitor cerivastatin. Eur J Clin Pharmacol. 1998;53:469–73.
160. Li R, Barton HA, Yates PD, Ghosh A, Wolford AC, Riccardi KA, Maurer TS. A "middle-out" approach to human pharmacokinetic predictions for OATP substrates using physiologically-based pharmacokinetic modeling. J Pharmacokinet Pharmacodyn. 2014.
161. Jones HM, Barton HA, Lai Y, Bi YA, Kimoto E, Kempshall S, *et al.* Mechanistic pharmacokinetic modeling for the prediction of transporter-mediated disposition in humans from sandwich culture human hepatocyte data. Drug Metab Dis Biol Fate Chem. 2012;40:1007–17.

162. Soars MG, Grime K, Sproston JL, Webborn PJ, Riley RJ. Use of hepatocytes to assess the contribution of hepatic uptake to clearance *in vivo*. Drug Metab Dispos. 2007;35:859–65.
163. Ménochet K, Kenworthy KE, Houston JB, Galetin A. Use of mechanistic modeling to assess interindividual variability and interspecies differences in active uptake in human and rat hepatocytes. Drug Metab Dispos. 2012;40:1744–56.
164. Swift B, Pfeifer ND, Brouwer KL. Sandwich-cultured hepatocytes: an *in vitro* model to evaluate hepatobiliary transporter-based drug interactions and hepatotoxicity. Drug Metab Rev. 2010;42:446–71.
165. Watanabe T, Kusuhara H, Maeda K, Kanamaru H, Saito Y, Hu Z, *et al.* Investigation of the rate-determining process in the hepatic elimination of HMG-CoA reductase inhibitors in rats and humans. Drug Metab Dispos. 2010;38:215–22.
166. Greenblatt DJ, Peters DE, Oleson LE, Harmatz JS, MacNab MW, Berkowitz N, *et al.* Inhibition of oral midazolam clearance by boosting doses of ritonavir, and by 4,4-dimethyl-benziso-(2H)-selenazine (ALT-2074), an experimental catalytic mimic of glutathione oxidase. Br J Clin Pharmacol. 2009;68:920–7.
167. Tomita Y, Maeda K, Sugiyama Y. Ethnic variability in the plasma exposures of OATP1B1 substrates such as HMG-CoA reductase inhibitors: a kinetic consideration of its mechanism. Clin Pharmacol Ther. 2013;94:37–51.
168. Maeda K, Sugiyama Y. Transporter biology in drug approval: regulatory aspects. Mol Asp Med. 2013;34:711–8.
169. Paine SW, Ménochet K, Denton R, McGinnity DF, Riley RJ. 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. 2011;39:1008–13.
170. Feng B, Varma MV, Costales C, Zhang H, Tremaine L. *In vitro* and *in vivo* approaches to characterize transporter-mediated disposition in drug discovery. Exp Opin Drug Disc. 2014;9:873–90.
171. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res. 1995;12:413–20.
172. CDER/FDA. Guidance for industry: Waiver of *in vivo* bioavailability and bioequivalence studies for imidiate-release solid oral dosage forms based on a biopharmaceutics classification system. Center for Drug Evaluation and Research 2000.
173. Varma MV, Khandavilli S, Ashokraj Y, Jain A, Dhanikula A, Sood A, *et al.* Biopharmaceutic classification system: a scientific framework for pharmacokinetic optimization in drug research. Curr Drug Metab. 2004;5:375–88.
174. Lennernas H. Human intestinal permeability. J Pharm Sci. 1998;87:403–10.
175. Sandstrom R, Karlsson A, Knutson L, Lennernas H. Jejunal absorption and metabolism of R/S-verapamil in humans. Pharm Res. 1998;15:856–62.
176. Benet LZ, Amidon GL, Barends DM, Lennernas H, Polli JE, Shah VP, *et al.* The use of BDDCS in classifying the permeability of marketed drugs. Pharm Res. 2008;25:483–8.
177. Chen ML, Amidon GL, Benet LZ, Lennernas H, Yu LX, The BCS. BDDCS, and regulatory guidances. Pharm Res. 2011;28:1774–8.
178. Avdeef A. Absorption and drug development: solubility, permeability, and charge state. John Wiley & Sons, 2012.
179. Lee PH, Ayyampalayam SN, Carreira LA, Shalaeva M, Bhattachar S, Coselmon R, *et al.* In silico prediction of ionization constants of drugs. Mol Pharm. 2007;4:498–512.
180. Fan PW, Song Y, Berezhkovskiy LM, Cheong J, Plise EG, Khojasteh SC. Practical permeability-based hepatic clearance classification system (HepCCS) in drug discovery. Future Med Chem. 2014;6:1995–2012.