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EXPERT REVIEW

Drug Disposition Classification Systems in Discovery and Development: A Comparative Review of the BDDCS, ECCS and ECCCS Concepts

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ABSTRACT BDDCS, ECCS and ECCCS are compound disposition classification concepts that aim to streamline, de-risk and speed-up drug development. Although all three systems have the same purpose and are based on classifying drugs into four main categories, they have different backgrounds and contrast in their criteria. Here the details, differences and most important applications of the three systems are reviewed with particular emphasis of their roles for drug discovery and development.

KEY WORDS BDDCS · drug disposition classification · ECCCS · ECCS · enzyme/transporter interplay

ABBREVIATIONS

BCS	Biopharmaceutics classification system
BDDCS	Biopharmaceutics drug disposition classification system
$CL_{int,h}$	Total hepatic *intrinsic* clearance
$CL_{int,met}$	*Intrinsic* metabolic clearance
$CL_{int,sec}$	*Intrinsic* biliary clearance
$CL_{int,tot}$	Total *intrinsic* clearance by metabolism and biliary excretion
CYP	Cytochrome P450
D	Clinical dose
DDI	Drug-drug interaction
ECCS	Extended clearance classification system
ECCCS	EC3S extended clearance concept classification system
ECM	Extended clearance model
fn_h	Fraction of hepatic elimination
fn_{met}	Fraction of metabolic elimination
$[I]_u$	Unbound inhibitor concentration
IVIVC	*In vitro-in vivo* correlation
IVIVE	*In vitro-in vivo* extrapolation
K_i	Inhibition constant
Kp_{uu}	Unbound liver-to-capillary blood concentration ratio
LLC-PK1	Porcine kidney proximal tubule cell line
LogP	Octanol-water partition coefficient or lipophilicity
MDCK-LE	Canine kidney low efflux cell line
MW	Molecular weight
OATP	Organic anion transporting polypeptide
Q_h	Hepatic blood flow
PAMPA	Parallel artificial membrane permeability assay
PCA	Principal component analysis
$Perm_{pas}$	Passive intestinal permeability
PK	Pharmacokinetics
PS_{eff}	Sinusoidal efflux clearance or membrane permeability
$PS_{inf,act}$	Sinusoidal influx clearance or membrane permeability by active uptake
$PS_{inf,pas}$	Sinusoidal influx clearance or membrane permeability by passive diffusion
$PS_{inf,tot}$	Total sinusoidal influx clearance or membrane permeability

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INTRODUCTION

The BDDCS (Biopharmaceutics Drug Disposition Classification System), based on aqueous solubility and extent of metabolism, has been widely used since 2005 to predict drug disposition in liver and intestine (1). Defining the extent of metabolism relies upon clinical data unless intestinal permeability rates are used as a substitute. Possible applications of the approach proposed by *Wu and Benet* are the anticipation of

transporter effects in drug elimination and absorption, the prediction of the transporter-enzyme interplay and of potential drug-drug interactions (DDIs) in the intestine and liver, the likelihood for absence or presence of central drug effects, and the assessment of the effects of high fat meals on the extent of bioavailability (2). In recent years, the ECCCS (Extended Clearance Concept Classification System) framework, based solely on *in vitro* data of passive sinusoidal uptake and total (metabolic + biliary) intrinsic clearance, was introduced by *Camenisch et al.* to predict drug elimination and potential drug-drug interactions in the liver and/or kidney (3–6). V*arma et al.* developed in 2015 the Extended Clearance Classification System (ECCS) targeting the prediction of the predominant clearance mechanism based on physicochemical properties and passive membrane permeability (7).

This expert review aims to revisit the concepts of BDDCS, ECCS and ECCCS (to avoid any mix-up with ECCS from here on referred as EC3S), to demonstrate the comparative value of the three drug classification systems in identifying and predicting drug disposition and related enzyme/transporter interplay and, with respect to these points, exemplify their utility for early and late drug development.

DRUG DISPOSITION CLASSIFICATION SYSTEMS

BDDCS

The development of the Biopharmaceutics Classification System (BCS) provided a framework for determining conditions under which *in vitro-in vivo* correlations (IVIVC) are expected (Fig. 1a) (8,9). BCS recognizes that drug intestinal permeability can predict the extent of absorption and its main purpose is for biowaivers of *in vivo* bioequivalence studies (10). BDDCS acknowledged later that for drugs exhibiting high intestinal permeability rates the major route of elimination is via metabolism, while drugs exhibiting poor intestinal permeability are primarily eliminated as unchanged drug in the urine and bile (1). Consequently, BDDCS classifies drug substances into four classes based on aqueous solubility and the extent of metabolism from clinical data and can therefore be viewed as a further development of BCS (Fig. 1b): Class 1 (high solubility, extensive metabolism), Class 2 (low solubility, extensive metabolism), Class 3 (high solubility, poor metabolism), and Class 4 (low solubility, poor metabolism). A drug substance is considered "highly soluble" when the highest dose strength is soluble in 250 mL or less of aqueous media over a pH range of 1–7.5 at 37°C. If a compound is ≥70% metabolized, it is considered as "highly metabolized". This 70% threshold was chosen due to the observational fact that very few marketed drugs fall into the 30 to 70% metabolism category and that most compounds are either extensively (≥70%) or poorly (<30%) metabolized (11). A compilation of the BDDCS classification for more than 900 drug compounds can be found in (12). Based on BDDCS, the effects of efflux and solute-carrier transporters on drug absorption, distribution and elimination were evaluated and generalized as follows: While transporter involvement for Class 1 compounds is generally minimal, efflux transporter effects in the intestine and/or liver together with uptake transporter involvement in the liver are considered possible for Class 2 compounds. Uptake transporter effects in the intestine and liver will predominate for Class 3 compounds (efflux effects are also possible) whereas for Class 4 compounds ubiquitous uptake and efflux transporter effects have to be taken into consideration. However, there is not a definite explanation provided for why uptake and efflux involvement in the liver can be anticipated for certain (but by far not all) BDDCS Class 2 drugs (11). One might actually expect, similar to BDDCS Class 1 compounds, that for highly permeable BDDCS Class 2 drugs the hepatocytes (at least at the sinusoidal membrane) become sufficiently "leaky" so that access to transporters will be insufficient to allow relevant active transport. Fagerholm postulated that differences in the degrees of "high" permeability dictate whether the compound will be a substrate for transporters, an idea that was further substantiated with the introduction of the EC3S concept as discussed in more detail below (3,13).

The recognition of the correlation between intestinal permeability rate (not extent) and extent of metabolism allows the prediction of the BDDCS class for a new chemical entity to be based on passive membrane permeability (7,10,14). Measuring a surrogate of human intestinal absorption, such as Caco-2 permeability or even nonviable membranes like PAMPA, will therefore give correct BDDCS predictions (15). It needs to be emphasized that experimentally determined permeability rates can vary between laboratories and therefore should always be compared to a standard that is characterized to best differentiate between high and low permeability. Using labetalol as the differentiating drug for *in vitro* permeability measurements in Caco-2, zidovudine in MDCK and theophylline in PAMPA more than 80% extensively metabolized and more than 80% poorly metabolized drugs could be correctly classified. Lipophilicity also confidently relates to extent of metabolism but only for drugs outside an octanol-water partition interval (LogP) from 0 to 2 (12). This can surely be rationalized by the link between passive transcellular permeability, metabolism and degree of lipophilicity of the drug in question (10). Consequently, the correlation was hypothesized to fail when drug permeability is mainly determined by active uptake or paracellular passage (16).

ECCS

The Extended Clearance Classification System (ECCS) represents an extension of the BDDCS framework and is based

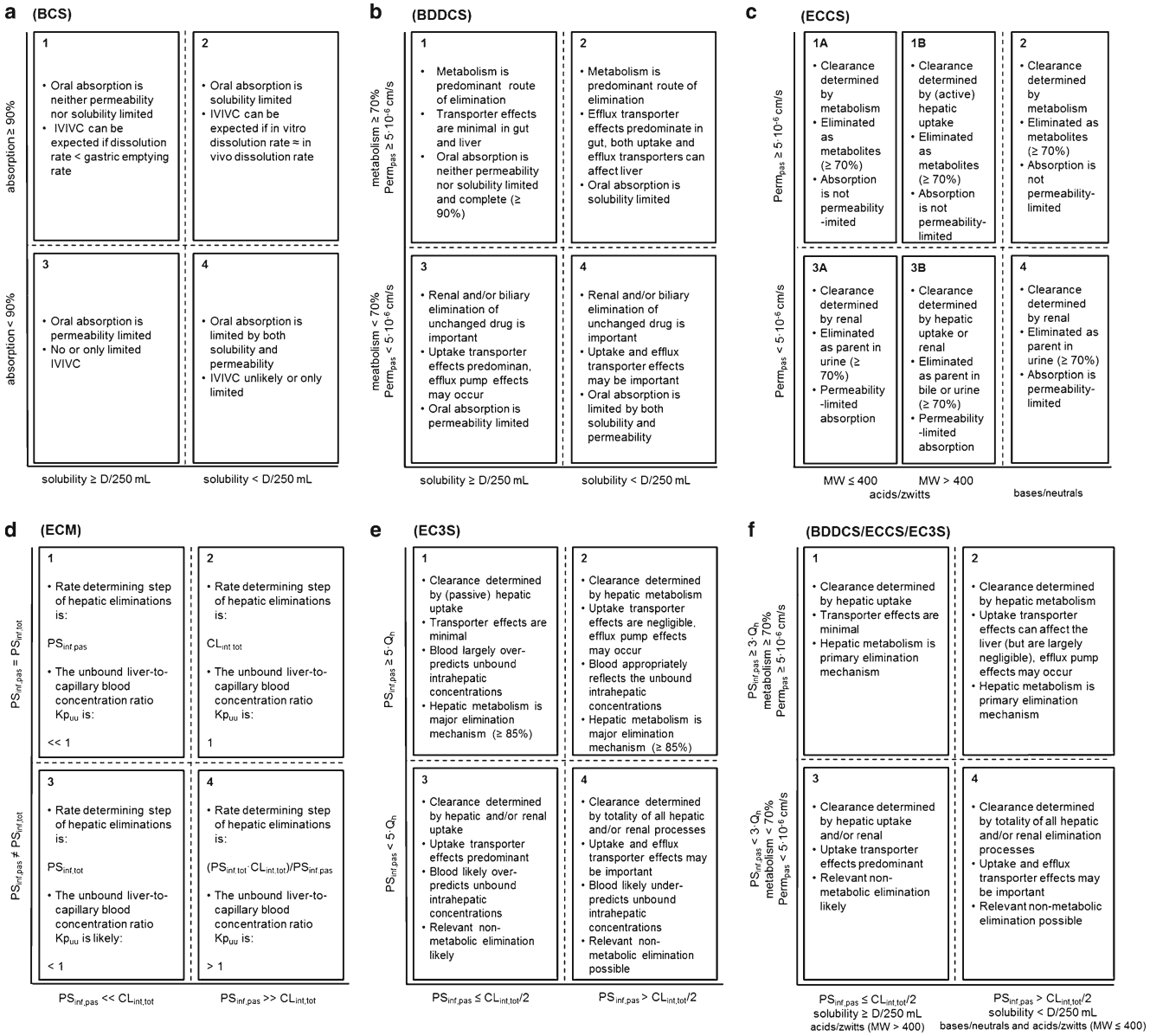

Fig. 1 Schematic representation of different drug categorization systems. Panel (**a**) illustrates the BCS classification based on extent of absorption and solubility (8,9). Panel (**b**) explains BDDCS using $Perm_{pas}$ (exemplified with a data threshold determined across the clonal MDCK-LE cell line according to (7)) or the extent of metabolism plus solubility as criteria (1). Panel (**c**) is the graphical representation of ECCS based on $Perm_{pas}$, MW and ionization whereas panel (**d**) shows the ECM class-dependent effect on $CL_{int,h}$ and Kp_{uu} according to Eqs. 2 and 3, respectively (6,7). Panel (**e**) illustrates EC3S using $CL_{int,tot}$ plus $PS_{inf,pas}$ for categorization (3,6). The combined BDDCS/ECCS/EC3S framework for the anticipation of predominate clearance and elimination mechanisms as ellaborated in this review is given in panel (**f**). For each panel, derived from the different criteria (as given on the *x*- and *y*-axes), the consequences/predictions for Class 1, 2, 3 and 4 compounds are summarized in the upper left, upper right, lower left and lower right boxes, respectively. $Perm_{pas}$, $PS_{inf,tot}$, $PS_{inf,pas}$, $CL_{int,tot}$, Q_h, D and MW are the passive membrane permeability (cm/s), the total sinusoidal influx clearance (mL/min/kg), the sinusoidal influx clearance by passive diffusion (mL/min/kg), the *intrinsic* clearance for metabolism and biliary excretion (mL/min/kg), the hepatic blood flow (mL/min/kg), the clinical dose strength (mg), and the compound's molecular weight (da), respectively.

on the belief that solubility as an indirect measure of lipophilicity inter-correlates with permeability and, as a consequence, is not directly relevant for understanding clearance mechanisms or elimination routes (7). Therefore, different physicochemical properties, namely ionization state, molecular weight (MW) and membrane permeability are suggested as integral and independent variables for predicting a molecule's rate-determining process towards systemic clearance. Based on this theory ECCS classifies drugs into several categories as illustrated in Fig. 1c: Class 1A - low MW, high permeability acids or zwitterions for which clearance is mainly determined by metabolism, Class 1B - high MW, high permeability acids or zwitterions for which active hepatic uptake is the rate-determining clearance mechanism, Class 2 - high

permeability bases and neutrals which are rate-limited cleared by metabolism, Class 3A - low MW, low permeability acids or zwitterions for which clearance is predominantly determined by renal elimination, Class 3B – high MW, low permeability acids or zwitterions for which clearance is determined by hepatic uptake or renal elimination, and Class 4 - low permeability bases and neutrals for which renal elimination is the major clearance mechanism. A molecular weight cut-off of 400 Da distinguishes between uptake limited and non-uptake limited compounds within the acids and zwitterions. Ionization (at pH 7) can be assigned based on experimentally measured or calculated pKa values. To define high and low permeability classes intestinal membrane permeabilities in line with BDDCS are used. Passive diffusion ($Perm_{pas}$) measured across the clonal MDCK-LE cell line was demonstrated to differentiate best between high and low intestinal absorption with a threshold value of $5 \cdot 10^{-6}$ cm/s (it should be noted again that other laboratories will have different cut-offs that need to be self-determined or compared to published standards). Based on a dataset of 307 drugs with a single clearance mechanism contributing ≥70% of systemic clearance, ECCS correctly predicted the rate-determining clearance mechanism to be either metabolism, hepatic uptake or renal for about 92% of the cases. Prediction success for the individual Classes 1A, 1B, 2, 3A and 3B was also high (>85%), while it dropped significantly for Class 4 compounds (~75%) most likely due to the fact that 25% of the compounds assigned to this class are in fact predominantly metabolized. It needs to be emphasized here that a drug's predominant elimination process (such as metabolism, biliary secretion or renal excretion) does not necessarily correlate with its rate-determining step in systemic clearance. For example, valsartan (ECCS Class 3B compound) is predominantly excreted into bile while transporter-mediated uptake at the sinusoidal membrane is a major determinant of overall hepatic drug clearance (3,6). Hence, the major clearance mechanism is the determinant of systemic exposure and consequently of the overall drug-drug interaction risk of a victim compound (3,5). Using different ECCS Class 1B statins (namely cerivastatin, fluvastatin, pitavastatin and atorvastatin) as tool compounds, *Varma et al.* pulled data from different clinical interaction studies to exemplify the effect of inhibitors of the organic anion transporting polypeptide (OATP) solute-carrier family (namely cyclosporine A, rifampin and gemfibrozil) on plasma exposure as a consequence of the underlying rate limited uptake into hepatocytes (7). However, their overall interpretation needs to be assessed with caution as the selectivity of these inhibitors towards hepatic OATP uptake transporters is questionable and, as a consequence, the observed clinical interaction on these highly metabolized statins can also be attributed to inhibition of oxidative cytochrome P450 (CYP) enzymes as discussed in full detail elsewhere (5). It is also important to notice at this point that some ECCS Class 1B compounds with known uptake transporter affinity, e.g. fluvastatin, were demonstrated to lack significant interactions at the transporter level in clinics (17). This evident conflict between the ECCS class assignment and the clinical data has also been noticed by others and will be illustrated in more detail below (18). Nevertheless, as the permeability threshold for ECCS classification was derived based on the sigmoidal relationship between permeability and extent of human intestinal absorption it is also expected, in line with BCS, to provide an early indication as to whether absorption may be permeability-limited (likely for ECCS classes 3A, 3B and 4 (Fig. 1c)).

EC3S

The concept of EC3S was initially derived from the extended clearance model (ECM) for hepatic elimination, which describes overall *intrinsic* drug clearance in the liver ($CL_{int,h}$) as the interplay of active ($PS_{inf,act}$) and passive ($PS_{inf,pas}$) sinusoidal uptake clearance, metabolism ($CL_{int,met}$), biliary secretion ($CL_{int,sec}$) and sinusoidal efflux clearance (PS_{eff}) as follows (3,19):

$$CL_{int,h} = \frac{\left(PS_{inf,act} + PS_{inf,pas}\right) \cdot \left(CL_{int,sec} + CL_{int,met}\right)}{PS_{eff} + CL_{int,sec} + CL_{int,met}} \quad (1)$$

Efflux over the sinusoidal membrane from hepatocytes back into blood is frequently assumed to occur via passive diffusion only and passive sinusoidal efflux, in the absence of experimental data, is usually expected to be equal to $PS_{inf,pas}$ (6). Consequently, Eq. 1 can be simplified to:

$$CL_{int,h} = \frac{PS_{inf,tot} \cdot CL_{int,tot}}{PS_{inf,pas} + CL_{int,tot}} \quad (2)$$

Where, $CL_{int,tot}$ describes the sum of $CL_{int,met}$ and $CL_{int,sec}$ and $PS_{inf,tot}$ is the totality of $PS_{inf,act}$ and $PS_{inf,pas}$. Equation 2 permits the identification of the rate-determining hepatic clearance mechanism for any drug molecule as depicted in Fig. 1d, hence, allowing compound categorization into four (ECM) classes solely from hepatic clearance data. Derived from experimental observations using a diverse dataset of 29 drug compounds, it was shown that a $CL_{int,tot}$ value equivalent to about $2 \cdot PS_{inf,pas}$ can confidently separate the uptake limited ($PS_{inf,pas} << CL_{int,tot}$) from the non-uptake limited ($PS_{inf,pas} >> CL_{int,tot}$) compounds (6,20).

$PS_{inf,tot}$ acts as differentiator between almost exclusive hepatic elimination (≥120 mL/min/kg) and combined hepatic and renal elimination (<120 mL/min/kg) (Fig. 2a) (4,6,20). Likewise, the fractional contribution of metabolic elimination to total *in vivo* clearance (fn_{met}) was found to highly correlate with $PS_{inf,pas}$ reaching a plateau ($fn_{met} = 1$) at a value of about 100 mL/min/kg, corresponding to 5-fold liver blood flow (Q_h) (Fig. 2b). Consequently, a passive sinusoidal permeability

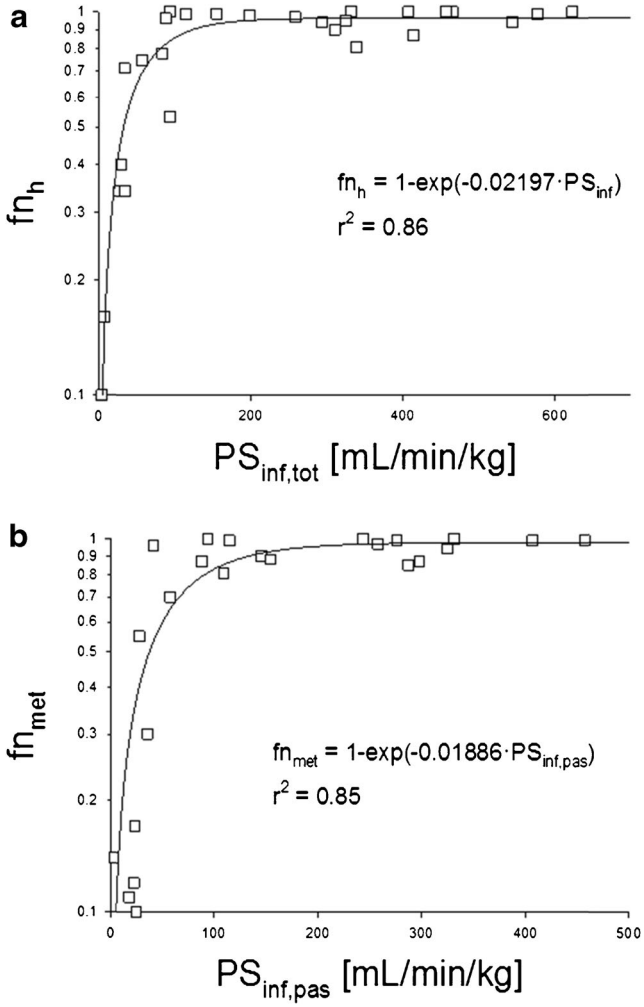

Fig. 2 Correlation between fraction of total (metabolic plus biliary) hepatic (fn_h, panel **a**) or metabolic (fn_{met}, panel **b**) elimination in human and *in vitro* sinusoidal membrane permeability ($PS_{inf,tot}$ (= $PS_{inf,act}$ + $PS_{inf,pas}$) or $PS_{inf,pas}$) for a heterogeneous set of 28 compounds (20). *Solid lines* show the non-linear regression fits to the exponential rise equation $y = 1\text{-}exp^{-b\cdot x}$ using OriginPro 9.1 (OriginLab Corporation, Northampton, USA).

above this threshold is proposed to translate into a complete hepatic metabolic elimination (i.e. ≥85%, no significant contribution of urinary and/or biliary elimination pathways), independent of potential active uptake transporter effects at the basolateral membrane of hepatocytes. Therefore, in conjunction with the above findings and introducing for compounds demonstrating a high intrinsic sinusoidal permeation potential (even if identified as hepatic uptake transporter substrates) the conceptual approximation of $PS_{inf,tot} \approx PS_{inf,pas}$, the mathematical principles of ECM can be combined with the gatekeeping role of passive sinusoidal uptake as illustrated in Fig. 1e (3,6). This new and universal (i.e. not just referring to hepatic elimination) drug classification system, categorizing drug substances into four classes based on $PS_{inf,pas}$ and CL_{int}, is referred as EC3S: Class1 ($CL_{int,tot}/2 \geq PS_{inf,pas} \geq 100$ mL/min/kg), Class 2 ($CL_{int,tot}/2 < PS_{inf,pas} \geq 100$ mL/min/kg), Class 3 ($CL_{int,tot}/2 \geq PS_{inf,pas} < 100$ mL/min/kg), and Class 4 ($CL_{int,tot}/2 < PS_{inf,pas} < 100$ mL/min/kg). EC3S Class 1 and 2 compounds are predicted to be primarily eliminated by hepatic metabolism, whereas the great majority of EC3S Class 3 and 4 compounds are additionally eliminated unchanged into the urine and/or bile to a relevant extent. Hepatic and/or renal transporter effects are expected to be minimal for Class 1 and 2 compounds whereas they may become predominant for compounds of Class 3 (mainly uptake effects) and 4 (uptake and efflux involvement).

All EC3S parameters, namely $PS_{inf,pas}$ (from hepatocyte uptake data), $CL_{int,met}$ (from microsomal incubations) and $CL_{int,sec}$ (determined in sandwhich-cultured hepatocytes assuming neglible metabolism in the system) can easily be determined *in vitro* (3,19). Assuming that metabolic intrinsic clearance significantly exceeds the biliary intrinsic clearance (i.e. $CL_{int,met} >> CL_{int,sec}$), EC3S categorization could be done with two parameters only (i.e. $PS_{inf,pas}$ and $CL_{int,tot} = CL_{int,met}$). In-house work with the intention to replace $PS_{inf,pas}$ with an experimentally easier accessible parameter such as e.g. Caco-2 or PAMPA permeability failed though (unpublished data). While the correlation between passive uptake clearance and passive cell permeability generally worked well up to a $PS_{inf,pas}$ threshold value of about 150 mL/min/kg (including all EC3S Class 3 and 4 and the lower uptake Class 1 and 2 compounds) it markedly collapsed above this cut-off such that $Perm_{pas}$ data significantly underestimated the measured $PS_{inf,pas}$ values (therefore predominantly affecting higher uptake Class 1 and 2 compounds). $PS_{inf,pas}$ represents a distributional clearance parameter across a single membrane (reflecting the ability of a compound to distribute from plasma into a tissue and vice versa) whereas $Perm_{pas}$ characterizes a permeation velocity across at least two cell membranes (reflecting the ability of a drug molecule to cross an entire cell layer). As such, above non-linearity can be rationalized by membrane trapping or cellular retention mainly affecting the higher lipophilic compounds (21,22).

BDDCS *vs* ECCS *vs* EC3S

Although derived from different theories (BCS for BDDCS, BDDCS for ECCS *vs.* ECM for EC3S), based on above comparative summary, the consequential and most fundamental drug elimination and clearance characteristics of the Classes 1–4 in BDDCS (Fig. 1b), ECCS (Fig. 1c) and EC3S (Fig. 1e) are largely comparable or complementary. The difference between the three approaches unmistakably comes from the parameters used for the compound classification (solubility plus extent of metabolism/intestinal permeability rate *vs.* ionization state plus MW plus intestinal permeability *vs.* intrinsic clearance plus sinusoidal membrane permeability). As a consequence, depending on the available data and the information required, one or the other system might be used.

However, due to the conceptual differences of the three approaches, a parameter switch across the different systems is not necessarily possible (e.g. use of solubility or ionization state instead of $CL_{int,tot}$ within EC3S framework) and, in consequence, BDDCS, ECCS and EC3S can occasionally provide different classification results (3). The most prominent example is likely atorvastatin which, using clinical elimination information (metabolism ≈ 70%, 80 mg dose), was initially classified as BDDCS Class 2 compound (12). Within the ECCS framework atorvastatin fulfills the Class 1B criteria (7). Based on its *in vitro* clearance parameters ($PS_{inf,pas}$ ≈ 58 mL/min/kg, $CL_{int,tot}$ ≈ 76 mL/min/kg), however, atorvastatin is categorized as EC3S Class 4 drug (3,6). BDDCS predicts that uptake and efflux transporter effects are possible, while envisaging that the elimination pathway is metabolism and ECCS expects that active hepatic uptake is the the rate-limiting step, while anticipating metabolism as the major elimination pathway. EC3S on the contrary forecasts that uptake and efflux transporter effects may be important and that the rate-limiting step of elimination is determined by the interplay of all processes involved in hepatic elimination (namely metabolism, uptake, and efflux), while expecting that metabolism possibly paired with non-metabolic clearance (biliary and/or renal secretion) would be the elimination pathway. Yet, for atorvastatin significant interactions with hepatic enzymes (mainly CYP3A4), efflux systems (mainly P-gp and BCRP) and uptake transporters (predominantly OATP1B1 and OATP1B3) are described in literature, while elimination is largely governed by extensive metabolism (≥70%) together with biliary secretion demonstrating that neither of the systems misinterprets atorvastatin with regards to the role of transporters and the potential relevance of the metabolic and non-metabolic elimination pathways (5,23,24). The most essential prediction difference between the three classification approaches is probably revealed best on the example of the well-known OATP uptake transporter substrate fluvastatin (BDDCS Class 1, ECCS Class 1B, EC3S Class 2). While all approaches correctly anticipate metabolism to be the main elimination pathway there is some misalignment with regards to the rate-determining clearance process and consequently the role of transporters (5,7,12). ECCS suggests that fluvastatin exhibits rate-limited uptake into hepatocytes as a consequence of basolateral solute-carrier transporters while EC3S predicts clearance to be determined by (hepatic) metabolism, principally resulting in the absence of any uptake transporter effects. BDDCS envisages transporter effects in gut and liver to be largely unimportant, ultimately implying metabolism to be the rate-determining step of hepatic elimination. In clinics, OATP1B1 polymorphisms have been proven not to affect the pharmacokinetics of fluvastatin suggesting active sinusoidal uptake not to be the rate-limiting clearance pathway (17,18). Hence, in agreement with the EC3S concept as discussed above, high passive membrane permeability making the uptake potential of a co-existing active component at the basolateral membrane of hepatocytes basically irrelevant might be proposed as the underlying cause of the evaluation differences between ECCS and EC3S or BDDCS (6). It should be noted here that classifications differences made by the various systems can similarly occur because of the underlying thresholds dissimilarities used for categorization (i.e. high metabolism equals ≥ 70% in BDDCS and ECCS frameworks whereas the metabolism limit in EC3S is defined at ≥ 85%) (1,7,20). According to the relationship given in Fig. 2b, a $PS_{inf,pas}$ value of 60 mL/min/kg ($\sim 3 \cdot Q_h$) is equivalent to a metabolism cut-off of ~70% used in BDDCS/ECCS while a value of about 100 mL/min/kg ($\sim 5 \cdot Q_h$) defines the 85% cut-off applied by EC3S. As such, it is inevitable that compound categorization on the basis of intestinal permeability rate (extent of *in vivo* metabolism) or sinusoidal uptake clearance will lead to framework-dependent binning discrepancies (i.e. Class 1/2 *vs* Class 3/4), especially for drugs with a $PS_{inf,pas}$ value between ~60–100 mL/min/kg (i.e. various EC3S class 3 and 4 compounds) and, as discussed above, for compounds with a $PS_{inf,pas}$ value exceeding 150 mL/min/kg (i.e. several EC3S class 1 and 2 compounds). Likewise, it is obvious that differences are particularly occurring for compounds at the boundaries of the classification groupings, where a simplified four group system does not necessarily allow a clear definition of the compound. Nevertheless, all three systems where shown to largely superimpose (~85%) mainly when comparing Class 1/2 *vs* Class 3/4 assignments (7,12,15,20). Figure 1f illustrates the naive attempt, following a careful integration of the above points, for unification of the three approaches into a single drug disposition classification well recognizing that the three independent frameworks do not perfectly collapse into one system and that some prediction communalities can only indirectly be implied from the other categorization systems (e.g. the clearance of BDDCS Class 1 compounds is not implicitly predicted to be determined by hepatic uptake). As a consequence, the three approaches remain alternatives and the choice which framework to use is largely subjective. However, as exemplified in full detail below, quantitative bottom-up anticipation of the clearance and elimination mechanism(s) according to ECM (or any other predictive method) could in the future mitigate this categorization "dilemma", as the question of the framework of choice and to which compound class a drug ultimately belongs may eventually become irrelevant.

COMPOUND CLASSIFICATION APPLICATIONS

The primary purpose of drug classification models such as BDDCS, ECCS and EC3S is to provide a predictive platform prior to any *in vivo* studies as to the potential characteristics of a

new chemical entity in terms of its disposition properties and the importance of transporters (3,6,7,11). All three classification systems do not exclude certain clearance mechanisms (e.g. compounds in EC3S classes 1 and 2 do not by definition lack affinity for uptake transporters). Rather, all methods predict the predominant processes which should be investigated further, as opposed to minor processes which can be neglected. The scope of application and the availability of data are evidently key drivers for selecting the framework of choice. As such, all three compound categorization approaches can jointly and complementarily help to identify possible hazards, to manage risk, to supplement or even substitute some of the animal models frequently used and to implement a tailor-made (compound-dependent) drug development process with regards to assay needs and tool selection.

Elimination Pathway Anticipation through BDDCS, ECCS and EC3S

Early knowledge of the predominant elimination pathway of a drug candidate in human may facilitate prediction of potential drug-drug interactions, pharmacokinetics, and toxicities (7,20,25). The so-called two-tier prediction method first uses, in line with BDDCS, a drug's *in vitro* permeability rate as compared to standard reference compounds to predict the extent of metabolism and then applies a previously published *in silico* logistic regression model (utilizing polarizability and predicted metabolic stability to estimate biliary or renal excretion) to envisage its likely major routes of elimination (15,25). This approach predicts about 74%, 85% and 73% of extensively metabolized, biliary eliminated and renally excreted parent drugs correctly. Varma and colleagues introduced an alternative method, built on an exhaustive data set ($n > 1000$) and only relying on computed structural descriptors (such as size, shape, lipophilicity, hydrophilicity, fraction unionized and flexibility), where a discriminant model based on principal component analysis (PCA) was used for elimination pathway prediction (26). Using this physicochemical approach the authors claim that PCA is highly predictive for the assignment of the primary elimination mechanism approaching 95% accuracy. Yet, in accordance with ECCS, all compounds in the dataset were selected having a single elimination mechanism contributing to ≥70% of systemic clearance and are therefore not really representative for the entire drug space. In contrast to the more qualitative BDDCS- and ECCS-based methods described above, use of the inverse exponential relationships between sinusoidal uptake (PS_{inf} or $PS_{inf,pas}$) and the fractional clearance pathway contributions allow more quantitative predictions of clearance pathways (Fig. 2) (6,20). Using this EC3S-based approach, including a diverse dataset of 28 compounds, the prediction for the fractional contributions of hepatic (fn_h), hepatic metabolic (fn_{met}) and renal elimination ($=1\text{-}fn_h$) in terms of r^2 was generally ≥ 0.85. The prediction for the hepatic biliary elimination pathway ($= fn_h\text{-}fn_{met}$) was weaker ($r^2 = 0.33$). However, considering that the *in vivo* biliary excretion by humans is often uncharacterized (i.e. indirectly estimated instead of directly measured) and/or biased (e.g. due to enterohepatic circulation events) this is not further surprising. Unpublished data, including more than 60 diverse developmental compounds, indicate that these relationships are similar in rat (i.e. analogous sinusoidal uptake thresholds can be applied), meaning that compound classifications derived from rat data (providing *in vivo* information on the extent of metabolism) in the majority of cases are also reflective for the human situation. It has to be mentioned at this point that compounds undergoing extrahepatic/renal excretion or extrahepatic metabolism to a relevant extent were initially excluded from an EC3S categorization as involvement of organs like lung or enzymes with prominent extrahepatic localization such as esterases are expected to impact a correct compound categorization. Yet, it was recently recognized that compounds with low metabolic clearance in human liver microsomes ($CL_{int,met}$ below ~30 mL/min/kg) frequently undergo significant non-CYP mediated metabolism (20). In contrast, drugs exhibiting a very high passive sinusoidal uptake ($PS_{inf,pas}$ above ~250 mL/min/kg) were experienced to regularly go through relevant intestinal secretion and/or extrahepatic metabolism (as e.g. in the lung). The reason behind these in-house observations is largely unknown. High cellular uptake paired with strong efflux activity (intestinal secretion) or intracellular trapping (extrahepatic metabolism) ultimately seems to foster these alternative elimination pathways though. Figure 3 illustrates the correlation between $PS_{inf,pas}$ and $CL_{int,met}$ by means of an area graph in which, in contrast to the standard EC3S representation (Fig. 1e), additional elimination and disposition (e.g. enterohepatic cycling) mechanisms have explicitly been incorporated (20). This illustration uncovers that within the main four class categorization framework several elimination pathway combinations can be described, allowing the definition of additional EC3S (sub)-classes. As a consequence, this approach not only provides more granularity with respect to elimination pathway anticipation (e.g. EC3S Class 2b compounds are not only predicted to be mainly eliminated by hepatic metabolism but are also expected to undergo predominantly CYP-mediated biotransformation) but likewise offers the opportunity for a numerical and hence comparative compound representation by reconciling the ratio between $PS_{inf,pas}$ and $CL_{int,met}$. Thus, by means of two easy to determine *in vitro* parameters and in line with the major (sub)class liabilities, evidence-based decision making with regards to possible *in vitro* or *in vivo* follow-up priorities/needs can be implemented (e.g. quantitative biliary secretion data for compounds with $PS_{inf,pas} < 60$ mL/min/kg, non-oxidative metabolic clearance data for compounds with microsomal $CL_{int,met} < 30$ mL/min/kg or extrahepatic elimination information for all compounds with a $PS_{inf,pas} > 250$ mL/

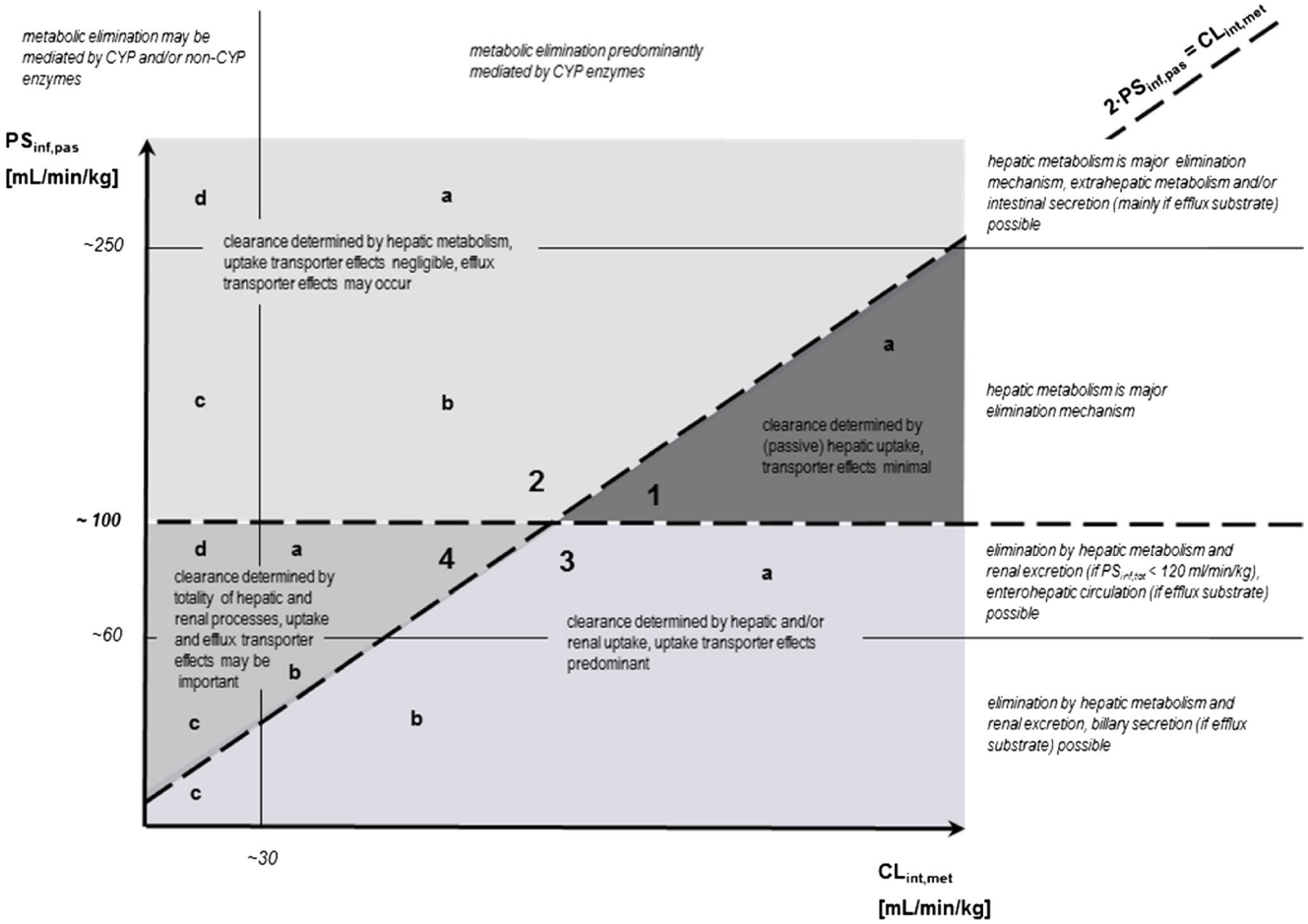

Fig. 3 Expanded human drug disposition prediction scheme according to EC3S allowing the description of additional subclasses (denoted as a, b, c and d) within the main compound categorization framework (denoted as classes 1, 2, 3 and 4) (20). From the graph representation, based on experimental $PS_{inf,pas}$ and microsomal $CL_{int,met}$ data, qualitative drug clearance (text inside graph axes) and elimination (text outside graph axes) characteristics for each subclass can be predicted (e.g. compounds assigned to EC3S Class 2b are expected to be eliminated predominantly by hepatic CYP-mediated metabolism, the clearance is rate-limited by hepatic metabolism, uptake transporter effects are negligible while efflux transporter effects might occur).

min/kg), which should make this graphical approach a valuable extension of the EC3S framework. As such, in line with the general compound categorization idea as outlined above, the diagram forms an alternative and more inclusive basis for a tailored compound-class dependent drug development process. Yet, this expanded drug disposition scheme was derived on a limited in-house data set ($n = 58$) and evidently further research will be required to confirm and adjust some of the thresholds given in this graphical prediction model. How (and if) this illustration can be extended with other disposition mechanisms such as absorption or CNS penetration needs to be explored.

Clearance Predictions against the Drug Classification Background

Allometric scaling is widely used to predict human pharmacokinetic (PK) parameters from preclinical species (27). Nevertheless, prediction errors are commonly observed in the practical application of simple allometry, for example, in cases where the overall clearance is mainly determined by enzyme or transporter activities which do not scale allometrically across species. So-called *in vitro-in vivo* extrapolation (IVIVE) methodologies, using *in vitro* data to predict *in vivo* parameters such as e.g. systemic clearance, provide an alternative to allometric scaling. Yet, the accuracy of the IVIVE approach is largely dependent on the rate-limiting clearance process *in vivo* and the fact how this mechanism is reflected in the *in vitro* system of choice (3,20). As such, the ultimate prediction success mainly depends on the selection of the most appropriate *in vitro* model.

As recently demonstrated, using *in vitro* transport data across the porcine kidney proximal tubule cell line LLC-PK1 and taking into consideration glomerular filtration, tubular secretion and reabsorption process contributions, a compound class dependent kidney clearance model could be introduced (4). It was demonstrated that for EC3S Class 1 and 2 compounds human renal clearance is generally irrelevant

(i.e. < 1 mL/min/kg) due to minor filtration and secretion contributions and predominant reabsorption of these highly permeable compounds. This general structure-pharmacokinetic relationship has also been noted by others (28–30). Consequently, it was shown that renal clearance of EC3S Class 1/2 compounds can be sufficiently well predicted by taking into consideration passive filtration only (average fold error of 1.4). In contrast, for EC3S Class 3 and 4 compounds renal clearance cannot be neglected as reabsorption becomes much less important and (active) tubular secretion develops to be the pre-dominant renal elimination process (average fold error of 2.54 based on passive filtration only). Thus, for these two compound classes all renal process clearances have to be taken into account resulting in an improved average fold error of 1.96. Including all twenty study compounds, the correlation between experimentally determined *in vivo* renal clearances and *in vitro* predicted filtration clearances in terms of threefold error was 70% ($R^2 = 0.17$, average fold error of 1.94). When correlating the *in vivo* renal clearances with *in vitro* clearance estimations including all process contributions 19 out of 20 compounds (95%) were correctly predicted within a threefold error ($R^2 = 0.83$, average fold error of 1.47).

In vitro-based *in vivo* hepatic clearance predictions can also significantly be improved when pursuing a compound class-dependent approach (3,6,20). Not astonishingly, referring to a dataset of 28 compounds, *in vitro* metabolism data from microsomes generally provided best results for EC3S Class 2 compounds (prediction accuracy within 2.5-fold error for 85% of the compounds). Hepatocyte uptake data seem to be highly predictive for the sinusoidal uptake rate-limited Class 1 and 3 compounds (2.5-fold error accuracy of 86%) whereas the *in vivo* hepatic clearance of Class 4 compounds generally needs to be assessed according to ECM taking all hepatic elimination pathways according to Eq. 1 into account (2.5-fold error accuracy of about 100%). The overall prediction accuracy of microsomes, hepatocyte uptake and ECM for hepatic clearance across all compound classes in terms of percentage within 2.5-fold error was 75%, 71% and 96%, respectively.

Evidently, overall (total) clearance could be assessed, confidently assuming absence of extra hepatic/renal clearance processes according to Fig. 3, as the sum of the individual IVIVE-based kidney and liver clearance predictions. Yet, considering above discussion on the newly emerged possibilities to quantitatively predict fractional clearance pathways, total clearance could also be estimated based on either renal or hepatic clearance predictions followed by an adjustment for the anticipated fractional clearance contributions. This alternative approach was recently described by *Riede et al.* (20). In their work they divided the ECM-based hepatic clearance predictions by projected fn_h estimates to account for extra-hepatic clearance pathway contributions. With this correction, 20 (71%) and 26 (93%) out of 28 compounds were predicted within two-fold and three-fold errors, respectively. While the accuracy of the total clearance prediction for EC3S Class 1 and 2 compounds was not significantly impacted by this approach (average fold error of 0.70 and 0.75 before and after correction, respectively) it was considerably improved for the EC3S Class 3 and 4 compounds in the dataset (average fold prediction error of 0.50 and 1.06 before and after correction, respectively). Including all 28 compounds the comparison of predicted and observed total clearance demonstrated overall a good predictability (average fold error of 0.88). Yet, on the basis of a more granular drug disposition scheme as depicted in Fig. 3, IVIVE-based total clearance predictions are likely to be improved even further (e.g. by inclusion of additional clearance mechanisms for compounds anticipated to undergo significant extra hepatic/renal clearance and/or non-oxidative metabolism). Further research is needed though to evaluate the compound (sub)class-dependent influence of these mechanisms on overall clearance prediction accuracy.

DDI Assessment using Compound Categorization Approaches

How metabolism and transporter effects in the intestine, liver or kidney can influence pharmacokinetic parameters (such as bioavailability, exposure, clearance, volume of distribution, and half-life) and consequently how many of these drug mechanisms are involved in victim DDIs is comprehensively discussed elsewhere (6,7,11). Based on this knowledge, drug classification schemes can be employed for rationalizing plasma and tissue DDIs with respect to metabolism alteration, transporter modulation and enzyme-transporter interplay. In a nutshell, ECCS is proposed to predict the major clearance mechanism responsible for causing plasma DDIs (Fig. 1c), while BDDCS predicts when transporter efflux, uptake and/or metabolism effects may be important and mediate a plasma or a tissue DDI, but not necessarily which process will primarily mediate the plasma interaction (or if it would involve both metabolism and a transporter) (Fig. 1b). EC3S on the other hand claims to anticipate all the underlying DDI mechanisms (Fig. 1e) while ECM provides the model-based (physiological) mathematical background to quantitatively describe the impact of these interactions, at least in the liver (3,6,11). The victim DDI effects of new chemical entities, in agreement with their EC3S assignment and in line with current belief that the rate-limiting clearance mechanism is the determinant for the overall DDI risk, can be generalized as follows (3,5,6): For Class 1 compounds (passive) hepatic uptake is anticipated as the rate-limiting clearance mechanism. Significant DDI effects with liver transporters and/or enzymes are therefore unlikely. Class 2 compounds on the other hand are predominantly cleared by hepatic metabolism and, at least in the absence of extrahepatic elimination pathways and/or non-oxidative

metabolism according to Fig. 3, the static ("worst-case") victim interaction potential can be estimated in a straightforward manner using microsomal clearance data only (3,6,20). Yet, for Class 3 and 4 compounds more elaborate DDI assessments are needed as uptake and efflux transporter effects (according to Eq. 1) as well as a renal and/or biliary elimination contributions have to be factored in. While for Class 3 compounds sinusoidal uptake data from hepatocytes work reasonably well for the anticipation of the hepatic DDI risk, for Class 4 compounds all process interdependencies according to ECM need to be considered. Applying such a compound-class dependent approach, the observed interaction effects on 8 marketed statins (namely lovastatin, simvastatin, cerivastatin, fluvastatin, pitavastatin, atorvastatin, pravastatin and rosuvastatin) in the presence of a variety of perpetrator drugs could be projected very well (out of 16 clinical DDI observations 14 were predicted within two-fold error, the average fold prediction error was 1.3) (5). In comparison, without applying a compound-class dependent approach and only using microsomal clearance data, the prediction accuracy within 2-fold error was only 56% with a clear under-prediction tendency primarily for the EC3S Class 4 compounds pravastatin and rosuvastatin in the dataset.

The outcome of DDI calculations for perpetrator drugs is likewise expected to be dependent on the compound classes especially when performing classical R-value (= $[I]_u/K_i$, where $[I]_u$ is the unbound inhibitor concentration in the relevant compartment and K_i is the inhibition constant on a particular pathway, respectively) risk assessments on active intrahepatic processes such as metabolism and/or canalicular efflux. Derived from Eq. 2, the unbound liver-to-capillary blood concentration ratio (Kp_{uu}) for any drug compound can be expressed as follows (6,31):

$$Kp_{uu} = \frac{[I]_{h,u}}{[I]_{b,u}} = \frac{PS_{inf,tot}}{PS_{inf,pas} + CL_{int,tot}} \quad (3)$$

Where, $[I]_{h,u}$ and $[I]_{b,u}$ represent the unbound intrahepatic and the unbound systemic blood/plasma concentrations, respectively. This ECM-based equation permits the assessment of $[I]_{h,u}$ from $[I]_{b,u}$ and Kp_{uu} for any drug molecule as depicted in Fig. 1d (where, $[I]_{h,u} = [I]_{b,u} \cdot Kp_{uu}$). Hence, the model demonstrates that experimental $[I]_{b,u}$ values are sufficiently representing the intrahepatic concentrations for Class 2 compounds only. Use of unbound blood/plasma concentrations as a substitute for unbound liver concentrations might however lead to considerable misjudgments of the DDI potential for the other three compound classes unless Kp_{uu} is used as a correction factor. While the perpetrator interaction potential of Class 1 and 3 compounds is typically over-predicted using unbound blood/plasma as reference matrix (i.e. $[I]_u$ in hepatocytes is significantly less than determined in blood), the risk for Class 4 is often under-estimated. In-house work is currently ongoing to quantitatively describe the impact of such corrections on the intracellular DDI prediction outcome of drug candidates.

CONCLUSIONS

1. In line with BDDCS and ECCS**,** EC3S recognizes that the fundamental parameter controlling drug disposition is the compound-class dependent interplay between transporters, enzymes and membrane permeability. All three frameworks provide a rational on the predominant routes of drug elimination and the potential effect of transporters on drug disposition and can therefore largely be used interchangeably. Yet, both ECCS and EC3S refer to *in vitro/in silico* data only and are therefore, in contrast to BDDCS, not relying on clinical dose estimates. The mathematical principles used to derive EC3S allow a numerical description of the different systemic elimination pathway contributions and the transporter-metabolism interplay in the liver and kidney. EC3S can therefore be viewed as a complementation or quantitative extension of the more qualitative BDDCS and ECCS principles.
2. Following thoughtful implementation, all three compound classification approaches may facilitate the compound class-dependent selection process in pharmaceutical research and foster a tailor-made profiling procedure in drug development. Timely knowledge of the qualitative and quantitative drug disposition relationships may be helpful for rapid progression of drug discovery programs (e.g. number of design iterations needed to modulate clearance) and development projects (e.g. increase in confidence level for human dose and DDI projections) or for the selection of the most appropriate process tools to be applied (e.g. sense or non-sense of dedicated metabolism-based DDI simulations, of pre-clinical studies examining the involvement of extra-metabolic elimination pathway contributions, or of clinical investigations in special populations).

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