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

Drug Transporters in Xenobiotic Disposition and Pharmacokinetic Prediction

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ABSTRACT

Drug transporters are widely expressed in organs and tissue barriers throughout human and animal bodies. Studies over the last two decades have identified various ATP-binding cassette and solute carrier transporters that play critical roles in the absorption, distribution, metabolism, and elimination of drugs and xenobiotics. This special section contains more than 20 original manuscripts and reviews that cover the most recent advances in the areas of drug transporter research, including the basic biology and function of transporters, expression of drug transporters in organ and tissue barriers, the mechanisms underlying regulation of transporter expression, transporter-mediated drug disposition in animal models, and the development and utilization of new technologies in drug transporter study, as well as pharmacokinetic modeling and simulation to assess transporter involvement in drug disposition and drug-drug interactions. We believe that the topics covered in this special section will advance our understanding of the roles of transporters in drug disposition, efficacy, and safety.

Introduction

Transporters expressed on the plasma membrane of cells belong to a large superfamily of membrane proteins that mediate the influx of nutrients and elimination of toxic wastes, which are essential for cell development and survival. Besides endogenous substrates, such as sugars, amino acids, and nucleotides, xenobiotics (e.g., drugs and environmental chemicals) also can be substrates and inhibitors of transporters. Most drug transporters can be mechanistically classified into two families with collectively >500 members, namely, ATP-binding cassette (ABC) and solute carrier (SLC) transporters (Benadiba and Maor, 2016; Colas et al., 2016; Giacomini et al., 2010).

Because of the substantial and strategic localization of ABC and SLC transporters in organs important for drug disposition, studies in the last two decades have provided convincing evidence that transporters play pivotal roles in the absorption, distribution, and elimination of drugs and xenobiotics (Giacomini et al., 2010; Benadiba and Maor, 2016; Colas et al., 2016). Transporters also interplay with metabolizing enzymes to affect drug metabolism by altering the access of drugs to metabolizing enzymes (Varma and El-Kattan, 2016). Over the years, drug transporters have been found to be involved in the disposition and drug-drug interactions (DDIs) of an increasing number of drugs and new molecular entities approved by the Food and Drug Administration (FDA) (Agarwal et al., 2013; Lee et al., 2017). As a result, the International Transporter Consortium has published white papers (Giacomini et al., 2010; Tweedie et al., 2013) emphasizing the importance of evaluating the most important drug transporters [e.g., P-glycoprotein, breast cancer resistance protein (BCRP), organic anion transporter (OAT)1, OAT3, organic cation transporter (OCT)2, Organic anion transporting polypeptide (OATP)1B1, and OATP1B3] in vitro and in vivo for clinically relevant impacts on drug disposition and DDIs. Accordingly, the FDA recommended specific considerations for transporter-mediated interactions as part of overall DDI evaluation (https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf) and in vitro transporter-mediated DDI studies (https://www.fda.gov/downloads/Drugs/GuidanceCompliance-RegulatoryInformation/Guidances/UCMS81965.pdf) for the pharmaceutical industry. The FDA revises their guidance for industry periodically.
based on the most recent advances in transporter research. The European Medicines Agency also published similar guidance with respect to clinically important transporters in DDI evaluation (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf). Transporter research has now become an integral part of drug development.

We strongly believe that further understanding of the roles of transporters in drug disposition rely on progress in transporter research in the areas outlined as follows. Transporter expression has been extended to organs or tissue barriers beyond the liver, kidney, small intestine, and brain that are not usually studied in the context of drug development, such as human skin (Osman-Ponchet et al., 2014) and placenta (Koren and Ornoy, 2018). Such studies will broaden our knowledge about in vivo transporter functions in drug disposition and distribution in the human body. Clinical studies have shown that changes in transporter expression resulting from dysregulation can impact drug disposition and disease phenotypes; therefore, understanding the mechanisms underlying the regulation of transporter expression is an important aspect of transporter research (Ho and Kim, 2005). Recent studies suggest that the molecular mechanisms underlying the regulation of transporter expression are quite diverse, ranging from nuclear receptor-mediated transcriptional regulation to translational, post-translational and epigenetic regulation, such as those illustrated for ABC transporters (Miller, 2015). Development and utilization of novel animal models, including nonrodent species (Dalggaard, 2015) and transporter knockout or humanized mice or rats (Durmus et al., 2016), have greatly facilitated our understanding of the roles of transporters in drug disposition in vivo. As always, the application of new technologies in transporter study will greatly advance the transporter research field. In this regard, the use of quantitative proteomics to measure the absolute protein abundance of transporters in human tissues has now become a fundamental aspect in in vitro-in vivo extrapolation (IVIVE) of drug transport and pharmacokinetics (PK) prediction (Qiu et al., 2014; Al Feteisi et al., 2015).

Positron emission tomography (PET) imaging is a noninvasive method that has been used to evaluate in vivo transporter function in organs such as the human brain that otherwise cannot be readily quantified (Kilbourn, 2017). Recent studies have identified endogenous substrates of transporters that may be used as biomarkers to evaluate transporter functions and transporter-mediated DDIs in vivo (Chu et al., 2017; Mariappan et al., 2017). Since it is not always feasible to conduct experiments to evaluate the involvement of transporters in drug disposition and DDIs in vivo, computational modeling and simulation such as physiologically based pharmacokinetic (PBPK) modeling and simulation are an emerging area to predict transporter functions in DDIs, renal and hepatic clearance, and special populations (Hsu et al., 2014; Pan et al., 2016). Finally, more effort is needed in basic transporter biology to analyze the structure-function of transporters, identify additional drugs and xenobiotics as transporter substrates and inhibitors, and understand novel transporter-protein or transporter-ligand interactions that may affect transporter expression and functions. This special issue highlights recent advances in several of the aforementioned areas in transporter research.

**Transporter Biology and Function.** Although dozens of transporters have been discovered over recent decades, their basic biology, function, and transport mechanisms are still not fully understood. Therefore, there have been continuous efforts to explore various aspects in basic transporter biology and function, which is essential for understanding both the mechanisms and the implications of transporter-mediated drug disposition and DDIs. In this issue, Crawford et al. (2018) have extensively reviewed the importance of interactions of ABC transporters, including ABCB1, ABCB11, ABCC1, ABCC4, and ABCG2, with kinases and regulatory proteins to influence the transcription of ABC transporters, modify their activity, or alter cellular localization beyond competitive inhibition. ABC transporter gene transcription or protein trafficking can be regulated or affected by epidermal growth factor through complex kinase signaling pathways, such as the MAPK-ERK and PI3K-Akt pathways. Some kinases can directly influence ABC transporter activity by phosphorylation, regulation of transporter expression, or altering transporter trafficking. ABC transporter expression or activity may also be affected by interacting with regulatory proteins such as ezrin-radixin-moesin (ERM) proteins and postsynaptic density proteins. Protein-protein interactions with such proteins may be critical for proper localization and stabilization and thus functions of ABC transporters at the plasma membrane. Understanding of drug disposition and DDIs will benefit from further knowledge of how kinases and protein-protein interactions affect ABC transporter expression, function, and cellular localization. Gao et al. (2018) demonstrated that the glucuronide conjugate of 25-hydroxyvitamin D$_3$ is a substrate of multidrug resistance protein (MRP)2, MRP3, OATP1B1, and OATP1B3 and that the sulfate conjugate of 25-hydroxyvitamin D$_3$ is likely a substrate of BCRP, OATP2B1, and OATP1B3 using cells overexpressing these transporters and sandwich-cultured human hepatocytes (SCHs). This is the first study to demonstrate that transporters could be important in the enterohepatic circulation of 25-hydroxyvitamin D$_3$ conjugates, providing a mechanism of 25-hydroxyvitamin D$_3$ delivery to the intestinal tract, which could be critical for vitamin D receptor-dependent regulation of genes in enterocytes, including intestinal CYP3A, which is important for intestinal drug metabolism and disposition. Several key questions remain to be answered in future studies. For example, can 25OHD$_3$-S and 25OHD$_3$-G be deconjugated to 25OHD$_3$ by intestinal sulfatases or glucuronidas? Would this occur in the lumen of the gastrointestinal tract or after transporter-mediated uptake into enterocytes? What is the contribution of enterohepatic circulation of the 25OHD$_3$ conjugates to the overall disposition and biologic activity of vitamin D? In this issue, Nieskens et al. (2018) evaluated the toxicity of cisplatin in human-derived proximal tubule epithelial cells in the presence and absence of OAT1 or OAT3 overexpression and showed that overexpression of OAT1 or OAT3 reduced cisplatin toxicity as a result of downregulation of expression and activity of OCT2, which mediates the cellular uptake of cisplatin (Nieskens et al., 2018). The results suggest that caution should be taken when conducting drug-induced toxicity studies in cells in which both OAT and OCT are expressed because expression of one transporter may alter expression of another. Rahman et al. (2018) identified the molecular determinants within the protein sequence of human equilibrative nucleoside transporter 3 (hENT3) that are critical for the transport of 3’-deoxy-nucleosides using mutagenesis analysis. They showed that the N-terminal half of hENT3 is primarily responsible for transport of 3’-deoxy-nucleosides, and the amino acids at positions 225 and 231 in the N-terminal half contribute to the ability of hENT3 to transport 3’-azido-3’-deoxythymidine and 2’3’-dideoxyinosine. Such studies may help develop strategies to overcome adverse toxicities of 3’-deoxy-nucleoside-based drugs and guide the development of novel nucleoside drugs with less toxicity. Overall, these studies will enhance the mechanistic understanding of basic biology and functions of various transporters important for drug transport, efficacy, and toxicity.

**Mapping Transporter Expression and Variability in Organ and Tissue Barriers.** The spatial distribution, membrane localization, and interindividual expression of drug transporters in drug-eliminating organs and barrier tissues continue to be important areas of transporter research as they directly impact local tissue drug concentrations, pharmacokinetics, and interindividual variability in drug disposition. Emerging evidence indicates that, similar to the zonation of cytochrome P450s, hepatocyte expression of some drug transporters is not homogeneous across the liver parenchyma, suggesting that hepatocytes around the periportal (PP) and pericentral (PC) vein regions may differ in
their capacities for drug transport. In this issue, Tachikawa et al. (2018) examined liver zonation expression of drug transporters and metabolizing enzymes in mouse liver using quantitative targeted proteomics and laser microdissection. Their data revealed that the protein expressions of Oatp1a1, Oatp1b2, and Oct were greater in PC regions, whereas protein expression of Oat2 was greater in PP regions. Several other transporters (e.g., Ent1), on the other hand, showed no differences in expression levels between the PP and PC regions in the mouse liver. The expression patterns of Oatps and Ent1 in the mouse liver appear to be consistent with liver distribution of their respective substrates after intravenous bolus injections. Given the large species difference between human and rodent OATP/Oatps, evaluation of zonal distribution of major drug transporters in human liver is needed. Nevertheless, consideration of heterogenic expression of drug transporters and enzymes in the human liver could help to refine methods for IVIVE and prediction of the hepatic clearance of drugs.

The placenta serves as a major distribution barrier between the maternal circulation and fetal compartment, and transporters expressed in the syncytiotrophoblasts are a major determinant of fetal drug exposure (Koren and Ornoy, 2018). For instance, the breast cancer resistance protein (BCRP/ABCG2) efflux transporter is localized to the apical membrane of the placental syncytiotrophoblasts and plays an important role in limiting fetal drug exposure to and potentially harmful chemicals in the maternal circulation (Mao, 2008). In this issue, Bircsak et al. (2018) determined the mRNA and protein expression of the BCRP in 137 term placenta from uncomplicated pregnancies in a racially and ethnically diverse population. The authors observed up to 47-fold and 14-fold interplacenta variability in BCRP mRNA and protein expression, respectively. Interestingly, BCRP mRNA correlated significantly with the transcription factors NRF2 and AhR. Single nucleotide polymorphisms in the ABCG2 noncoding regions were not associated with placental BCRP expression, but the coding region polymorphism C421A/Q411K correlated with altered BCRP protein expression. These data suggested possible variations in fetal exposure to drugs and endogenous compounds resulting from ABCG2 genetic polymorphism.

**Mechanisms Underlying the Regulation of Transporter Gene Expression.** The ability of transporters to mediate drug transport across cellular membranes or tissue barriers depends on their levels of expression. Therefore, elucidating the mechanisms underlying the regulation of transporter expression at the transcriptional, translational, and post-translational levels and identification of factors that can affect transporter expression have always been important aspects of transporter research. In this issue, Tekechi et al. (2018) showed that, with respect to levels of gene expression of drug transporters in human skin, ABC3 was expressed at the highest levels, followed by OATP3A1, SLC22A3, SLC16A7, ABCA2, ABCCI, and OATP2B1, and large interindividual variations (9.5-fold difference) in ABC3 mRNA expression were found among 48 samples from white female subjects (Tekechi et al., 2018). Further analysis revealed that the large interindividual differences in ABC3 mRNA expression in human skin might be attributable to substantial variations in DNA methylation that may regulate transcription of the ABC3 gene (Tekechi et al., 2018). Such variations in ABC3 expression in human skin may affect transdermal delivery of drugs that are ABC3 substrates. Elov et al. (2018) identified four rare variants in the basal ABCG2 promoter region, and these variants displayed decreased promoter activity in hepatic, kidney, or intestinal cell lines, and two of the variants also showed significantly reduced in vivo liver promoter activity (Elov et al., 2018). Identification of such variants could possibly explain patient-level variations in ABCG2 expression in the kidney, liver, and intestine. Xiang et al. (2018) identified a nuclear factor-κB (NFκB) binding site in the proximal promoter region of human OATP1A2 and showed that this NFκB binding site is responsible for tumor necrosis factor α-mediated suppression of OATP1A2 expression in two different mammalian cell lines (Xiang et al., 2018), suggesting that the NFκB binding site may be a negative regulatory element that is involved in suppression of SLC01A2 gene expression in inflammation. Taken together, these studies further enrich our current knowledge about what could affect transporter gene expression, including genetic, epigenetic, and pharmacologic regulation of the promoter activity and hence transcription of transporter genes.

**Transporter-Mediated Drug Disposition in Animal Models.** Quantitative translation from animal models to humans remains a challenge, particularly when drug transporters are involved in drug absorption, disposition, elimination, and DDIs. For example, Kimoto et al. (2017) showed that cynomolgus monkeys and dogs are better predictive models for human biliary secretion, whereas rats tend to have higher activity levels of biliary secretion than humans. For hepatic transporters, rats display low amino acid homology and different expression levels with humans and often overpredict hepatobiliary clearance of drugs in humans. With that in mind, several single and combined Oatp1a and Oatp1b transporter gene knockout models have been generated and can be used to assess the roles of OATP transporters in the disposition of substrates. In this issue, Takeano et al. (2018) used Scolia4 gene knockout mice to assess the importance of Oatp1a4 in hepatic uptake of its substrates. In Scolia4 mice, the plasma exposure of ouabain and rosuvastatin increased, and the liver-to-plasma concentration ratios decreased. As a result, the quantitative contributions of Oatp1a4 to drug PK and tissue distribution were elucidated with the use of Scolia4 mice. On the other hand, Gampa et al. (2018) assessed brain distribution in Mdr1ab6/+, Bcrp1−/−, and Mdr1ab6+/+ Bcrp1−/− mice and found that the efflux transporters have minimal effects on the brain distribution of a potent synthetic MEK inhibitor, (3S,4R,5Z,8S,9S,11E)-14-(ethyl-amino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9,10-tetrahydro-1H-2-benzoxyacycletetracene-1,7(8H)-dione (E6201). The findings suggest that E6201 may be an attractive agent that can distribute into brain tissues targeting central nervous system tumors.

Based on gene alignment analysis across different species, the amino acid sequence identity of drug-metabolizing enzymes and transporters is generally high between humans and cynomolgus monkeys (Tahara et al., 2005; Yasunaga et al., 2008; Iwasaki and Uno, 2009). In 2013, Shen et al. demonstrated that the cynomolgus monkey can be a suitable model to assess OATP transporter-mediated DDIs in a nonclinical setting (Shen et al., 2013). In this issue, Karibe et al. (2018) tested the gastrointestinal function of BCRP in cynomolgus monkeys and characterized the impact of BCRP inhibitors on the bioavailability of BCRP probe substrates. The authors demonstrated that oral exposures of the BCRP substrates sulfasalazine and rosuvastatin were significantly increased by curcumin, but the changes in systemic clearance were minimal, suggesting that curcumin is a better in vivo selective BCRP inhibitor than lapatinib and pantoprazole for characterizing DDIs in the gastrointestinal tract. This finding exemplified that the cynomolgus monkey can be a useful preclinical model for assessing in vivo BCRP functions. Overall, these studies suggest that gene knockout models and cynomolgus monkeys can provide further insight into the roles of transporters in drug disposition and DDIs, although further evaluation is needed for quantitative translations to human PK.

**Technological Developments and Challenges.** The use of a wide array of methods and experimental systems, including in vitro transport assays in membrane vesicles and cell-based systems, as well as in vivo imaging and PK studies, have greatly advanced our understanding of transporter function and their roles in drug absorption, distribution, and elimination. Collective efforts from academia, industry, and regulatory agencies have led to considerable development in establishing criteria and standardizing in vitro methods and experimental systems to address key drug transport-related questions and assess transporter-based DDI potential during drug development (Giacomini et al., 2010; Brouwer et al., 2013). With appropriate IVIVE, quantitative prediction of transporter-mediated drug disposition and DDIs in vivo no longer sounds like a “castle in the sky.” Despite recent successes in the
application of PBPK modeling to predict transporter-mediated PK changes in vivo, however, there is still a tremendous need for new and innovative technologies and methods that can significantly facilitate and improve quantitative prediction of uptake and efflux transporters in the systemic exposure and organ- and tissue-specific disposition of drugs.

Several articles in this issue explore the development and/or application of new technologies to facilitate the in vivo and quantitative understanding of drug uptake and efflux transporters.

As a primary organ for drug elimination, the intrinsic clearance of the liver is governed by both intracellular drug metabolizing enzymes and transporters at the sinusoidal and canalicular membranes. Based on the extended clearance concept (Pang et al., 2007; Patilea-Vrana and Unadkat, 2016), mechanistic modeling of metabolic and transporter-mediated clearances can be used to construct PBPK models for the prediction of drug disposition and DDIs in humans. SCHs, which form proper canalicular networks and maintain both metabolic and transport activities, have been used widely to assess hepatobiliary disposition of drugs and drug metabolites (Brouwer et al., 2013). SCHs provide a unique tool for assessing the intrinsic hepatic clearance, which can be used to construct PBPK models. In this issue, Matsunaga et al. (2018) review the utility and applicability of SCHs for mechanistic understanding of the functional interplay between transporters and drug-metabolizing enzymes (Matsunaga et al., 2018). They also describe the utility of SCHs in simulating species-specific drug disposition in vivo and the application of SCHs to predict clinically relevant prediction DDIs. Of particular note, the usefulness of mathematical modeling in SCHs is highlighted for a quantitative understanding and improvement of prediction of in vivo hepatic disposition.

A key step in transporter IVIVE and mechanistic PBPK modeling requires the quantification of transporter proteins in drug elimination organs (e.g., liver) and comparison with that in in vitro systems to establish a proper scaling factor. In recent years, targeted protein quantification using liquid chromatography-tandem mass spectrometry with stable isotope-labeled standards has emerged as a major method for the identification and quantification of transporter proteins because of its reproducibility, high selectivity, and sensitivity. Such assays, however, can cover only a limited number of proteins (i.e., low throughput) and are costly if quantification is expected for a larger number of transporters. In this issue, Vildhede et al. (2018) applied a shotgun “total protein approach” (TPA) to simultaneously identify and quantify multiple transporter proteins in a single sample. This approach is achieved by operating the mass spectrometer in data-dependent acquisition mode and detecting fragmented peptides by searching a protein sequence database. Quantification of protein concentrations was achieved by a computational method that does not require a peptide standard. Using this approach, the authors determined the TPA-based quantification of seven liver uptake transporters (NTCP, OAT2, OAT7, OATP1B1, OATP1B3, OATP2B1, and OCT1) in human liver samples and compared the findings with data from targeted proteomic assays. The TPA-based approach showed good correlations with the targeted proteomics data, suggesting that the multiplexed global proteomics method may provide a fast and cost-effective approach to provide reasonable estimates of protein concentrations for hepatic transporters and drug metabolizing enzymes.

Qualitative fluorescence-based microscopy is nothing new to transport research; it has long been used as a standard tool to determine cellular and subcellular localization of transporter proteins using specific antibodies. With the development of transporter-specific fluorescent substrates and the availability of quantitative image analysis software, however, quantitative fluorescence microscopy is now offering a unique and unprecedented opportunity for transporter research. Differing from traditional assays that rely on measuring intracellular accumulation at the endpoint, this approach allows real-time observation of the fate of the transported molecule in live cells and intact animals at subcellular resolution. Coupled with quantification of fluorescence intensity of the substrate, much can be learned about the kinetics of uptake and efflux transporters and their interactions with inhibitors in live cells or in vivo.

In this special issue, two research articles have explored the application of quantitative fluorescence microscopy to characterize transporter function in vitro and in vivo. Using quantitative confocal microscopy, Holmstock et al. (2018) studied MRP2-mediated biliary excretion in SCHs by visualizing the biliary accumulation of a fluorescent substrate. They further demonstrated that the inhibitory effect of HIV protease inhibitors on MRP2 can be quantified based on quantitative analysis of changes in fluorescence intensity in the confocal images. Compared with conventional “offline” inhibition assays, the microscopy-based approach allowed investigation of the inhibitory effect of drugs on efflux transporters in a sensitive and nondestructive manner. Ryan et al. (2018) described a novel quantitative intravital microscopy method to characterize dose-dependent effects of inhibitors on bile salt export pump (BSEP)-mediated hepatocellular transport in vivo in living rats. BSEP expressed at the canalicular membrane of hepatocytes plays a vital role in the elimination of monovalent bile salts into the bile. Inhibition of BSEP is considered a susceptibility factor for drug-induced liver injury that often goes undetected during nonclinical testing (Morgan et al., 2010). Although in vitro assays exist for screening BSEP inhibition, they may not be readily translatable to in vivo BSEP inhibition and drug-induced liver injury because complex in vivo processes, such as metabolism, protein binding, and other physiologic components, are lacking in most in vitro BSEP models. The group previously developed quantitative multiphoton microscopy to quantify organic anion and bile acid transport in the liver of living rats at subcellular resolution (Ryan et al., 2014). In the present study, they extend this approach to characterize dose-dependent inhibition of BSEP in vivo using fluorescent probe substrates. These elegant studies demonstrated that quantitative intravital microscopy can detect BSEP inhibition at drug doses well below those that increase serum bile acid levels. Such an approach may be used to confirm the exposures needed to achieve in vivo BSEP inhibition and provide a better understanding of the relationship between in vitro data and in vivo outcome.

Imaging techniques, such as PET, provide unique opportunities to assess the contribution of transport proteins to drug disposition in vivo. PET is a noninvasive imaging method that is useful for quantitatively measuring drug concentrations in organs and tissues in vivo in humans and preclinical species, thus facilitating the in vivo assessment of transporter function and DDIs at tissue and organ levels. In this issue, Kaneko et al. (2018) performed a clinical PET study with a newly developed PET probe, $[^11]C$-dehydropravastatin ($[^11]C$DPV), to evaluate OATP1B1/MRP2-mediated hepatobiliary transport in healthy volunteers with and without an inhibitor, rifampicin (Kaneko et al., 2018). They showed that after intravenous injection, $[^11]C$DPV was rapidly distributed to the liver and kidney, followed by secretion into the bile and urine. Rifampicin significantly reduced the liver distribution and biliary excretion of $[^11]C$DPV. The in vivo hepatic uptake clearance and canalicul efflux clearance of $[^11]C$DPV were obtained by analyses of the PET imaging data. Rifampicin treatment significantly reduced both hepatic uptake clearance and canalicul efflux clearance. This study demonstrated that PET imaging with $[^11]C$DPV can be used to quantitatively characterize OATP1B1s and MRP2 functions in vivo in humans.

Pharmacokinetic Modeling and Prediction. In general, drug clearance mechanisms are definitively assessed in clinical development only after the proof-of-concept pharmacology studies are completed; however, there are increasing demands on gaining an early understanding...
of drug clearance mechanisms for prototypical human PK prediction to optimize drug-disposition properties during the molecule design phase. In 2015, Varma et al. proposed a framework named the extended clearance classification system (ECCS), which allows early identification of clearance mechanism using physiochemical properties and in vitro/in silico data readily available in the early drug discovery stage (Varma et al., 2015). In this issue, El-Kattan and Varma (2018) revisited the paradigm of applying ECCS framework to predict transporter roles in drug disposition and discussed the failed cases in ECCS predictions. It appears that the application of ECCS is beneficial for optimizing lead candidates in drug development through reducing or eliminating the most likely contributors to poor PK.

At later stages of drug discovery, the ability to accurately predict human PK and DDIs from preclinical in vitro or in vivo data remains a significant challenge. It is well documented that human intrinsic (e.g., genetic polymorphism, diseases) and extrinsic (e.g., comedications) factors can affect drug absorption, distribution, metabolism, and elimination. As such, modeling-based drug development has become a pivotal tool for pharmaceutical research to improve the accuracy of prediction. The compartmental modeling analyses, also known as "top-down" models, built predominantly on the observed clinical data allow the identification of human PK variations to narrow down to the range of the input data. In contrast, PBPK models are "bottom-up" models built based on the knowledge of body anatomic structures through mathematically transcribing predefined compartments (e.g., different tissues of the body, connected by systemic circulation). The inputs of a PBPK model are chemical properties and in vitro data to describe the PK variables of drugs through analyzing the effects of intrinsic and extrinsic factors on systemic and tissue exposure. PBPK modeling has rapidly advanced and become an integral part of pharmaceutical research (Sager et al., 2015; Zhuang and Lu, 2016).

Bosentan is a dual endothelin-receptor antagonist and cytochrome P450-metabolizing enzymes and hepatic OATP transporters are involved in bosentan disposition and elimination (Jones et al., 2012). When dosed intravenously in healthy volunteers, the systemic plasma clearance and volume of distribution of bosentan decreased with increasing doses from 10 to 750 mg (Weber et al., 1996). In 2001, Mager and Jusko (2001) described the nonlinear PK phenomenon using a target-mediated drug disposition (TMDD) model. The mechanism of nonlinear PK is proposed owing to dose-dependent saturable binding of the endothelin receptor with bosentan. Unfortunately, the TMDD model seemed unable to predict clinical DDIs associated with OATP transporter inhibition. In this issue, Sato et al. (2018) demonstrated in vitro saturable kinetics of hepatic uptake and metabolism of bosentan in experiments with primary hepatocytes. The authors further incorporated the in vitro kinetic values in different "bottom-up" PBPK models and found, through PBPK modeling, that the saturation of hepatic uptake, but not P450 metabolism of bosentan, accounts for the nonlinear intravenous PK observed in humans. The results explained the discrepancy between the receptor saturation and dose-dependent PK.

Also, in this issue, Futatsugi et al. (2018) describe their development of a PBPK model from clinical data of rosuvastatin and in vitro parameters for the quantitative prediction of the impact of altered activity of BCRP/ABCG2 c.421C>A in the intestine and liver. The model appears to be applicable to describing the variability of rosuvastatin PK resulting from a single nucleotide polymorphism of ABCG2. Likewise, Follman and Morris (2018) used a PBPK modeling-approach built-in SimCYP to assess the impact of altered intrinsic factors, such as changes in transporter expression and unbound fraction in renal impairment subjects on PK predictions). The authors showed that improved predictions can be achieved by adjusting transporter expression and protein binding in renal impairment subjects for the OCT/multidrug and toxin extrusion protein substrates metformin and ranitidine. Collectively, modeling and simulation approaches are advanced and have become useful tools that allow integrating chemical properties and intrinsic and extrinsic factors of human populations to explain and predict complex PK behaviors and DDIs.

Summary. It is now generally recognized that the impact of drug transporters on clinically relevant drug disposition and DDIs is equal to that of drug-metabolizing enzymes. Studies in the last two decades have identified various clinically important drug transporters (e.g., P-gp, BCRP, OAT1, OAT3, OCT2, OATP1B1, and OATP1B3) for which the FDA has recommended conducting research for drug development. It is expected that more transporters will be added to this list of transporters as this field advances and we learn more about transporters in determining drug disposition, DDIs, drug effects, and toxicity. This special issue focuses on several key areas in transporter research. Additional important areas for drug transporter research that are not covered in this special issue include, among others, applications of systems biology approaches and novel technologies such as "omics" approaches, in vitro 3D microtissue models, human organ-on-chips, and genome-wide association studies to assess transporter functions, evaluation of transporter-related interindividual variations in drug effects and toxicity, and identification of additional transporters as novel therapeutic targets. We recognize that transporter research is a rapidly growing field with many unanswered questions regarding the roles of transporters in human physiology, diseases, pharmacology, and drug disposition. Further studies in these areas to address the knowledge gap are critically important for improving drug treatment, efficacy, and safety.

Authorship Contributions
Wrote or contributed to the writing of the manuscript: Mao, Lai, Wang.

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