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

Drug Transporters in Xenobiotic Disposition and Pharmacokinetic Prediction

Qingcheng Mao, Yurong Lai, and Joanne Wang

Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, Washington (Q.M., J.W.), and Gilead Sciences, Inc., Foster City, California (Y.L.)

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/guidance/ucm292362.pdf) and in vitro transporter-mediated DDI studies (https://www.fda.gov/downloads/Drugs/GuidanceCompliance-RegulatoryInformation/Guidances/UCM581965.pdf) for the pharmaceutical industry. The FDA revises their guidance for industry periodically

ABBREVIATIONS: ABC, ATP-binding cassette; ABCB1, the first member of ATP-binding cassette transporter subfamily B; ABCG2, the second membrane of ATP-binding cassette transporter subfamily G; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; [^{11}C]DPV, [^{11}C]dehydropravastatin; DDI, drug-drug interaction; Dlg1, *Drosophila* disk large tumor suppressor; E6201, (3S,4R,5Z,8S,9S,11E)-14-(ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9,10-tetrahydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione); ECCS, extended clearance classification system; ERM, ezrin-radixin-moesin; FDA, Food and Drug Administration; hENT3, human equilibrative nucleoside transporter 3; IVIVE, in vitro-in vivo extrapolation; MRP, multidrug resistance protein; NFκB, nuclear factor-κB; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PBPK, physiologically based pharmacokinetic; PC, pericentral; PET, positron emission tomography; PK, pharmacokinetics; PP, periportal; SCH, sandwich-cultured hepatocyte; SLC, solute carrier; TMDD, target-mediated drug disposition; TPA, total protein approach; zo-1, Zonula occludens-1 protein.

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 (Dalgaard, 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 glucuronidases? Would this occur in the lumen of the gastrointestinal traction 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 OATPs/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 exposure to drugs 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 placentas 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/Q141K 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, *ABCC3* was expressed at the highest levels, followed by *OATP3A1, SLC22A3, SLC16A7, ABCA2, ABCC1*, and *OATP2B1*, and large interindividual variations (9.5-fold difference) in *ABCC3* mRNA expression were found among 48 samples from white female subjects (Takechi et al., 2018). Further analysis revealed that the large interindividual differences in *ABCC3* mRNA expression in human skin might be attributable to substantial variations in DNA methylation that may regulate transcription of the *ABCC3* gene (Takechi et al., 2018). Such variations in ABCC3 expression in human skin may affect transdermal delivery of drugs that are ABCC3 substrates. Eclov 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 (Eclov 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 $\alpha-$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 *SLCO1A2* 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 bile 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, Takano et al. (2018) used Slco1a4 gene knockout mice to assess the importance of Oatp1a4 in hepatic uptake of its substrates. In Slco1a4$^{-/-}$ 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 Slco1a4$^{-/-}$ mice. On the other hand, Gampa et al. (2018) assessed brain distribution in *Mdr1a/b*$^{-/-}$, *Bcrp1*$^{-/-}$, and *Mdr1a/b*$^{-/-}$*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-benzoxacyclotetradecine-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 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 OATP1B/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 canalicular efflux clearance of [^{11}C]DPV were obtained by analyses of the PET imaging data. Rifampicin treatment significantly reduced both hepatic uptake clearance and canalicular efflux clearance. This study demonstrated that PET imaging with [^{11}C]DPV can be used to quantitatively characterize OATP1Bs 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.

References

Agarwal S, Chinn L, and Zhang L (2013) An overview of transporter information in package inserts of recently approved new molecular entities. *Pharm Res* 30:899–910.
Al Feteisi H, Achour B, Rostami-Hodjegan A, and Barber J (2015) Translational value of liquid chromatography coupled with tandem mass spectrometry-based quantitative proteomics for in vitro-in vivo extrapolation of drug metabolism and transport and considerations in selecting appropriate techniques. *Expert Opin Drug Metab Toxicol* 11:1357–1369.
Benadiba M and Maor Y (2016) Importance of ABC transporters in drug development. *Curr Pharm Des* 22:5817–5829.
Bircsak KM, Moscovitz JE, Wen X, Archer F, Yuen PYS, Mohammed M, Memon N, Weinberger BI, Saba LM, Vetrano AM, et al. (2018) Interindividual regulation of the BCRP/ABCG2 transporter in term human placentas. *Drug Metab Dispos* 46:619–627.
Brouwer KL, Keppler D, Hoffmaster KA, Bow DA, Cheng Y, Lai Y, Palm JE, Stieger B, and Evers R; International Transporter Consortium (2013) In vitro methods to support transporter evaluation in drug discovery and development. *Clin Pharmacol Ther* 94:95–112.
Chu X, Chan GH, and Evers R (2017) Identification of endogenous biomarkers to predict the propensity of drug candidates to cause hepatic or renal transporter-mediated drug-drug interactions. *J Pharm Sci* 106:2357–2367.
Colas C, Ung PM, and Schlessinger A (2016) SLC transporters: structure, function, and drug discovery. *MedChemComm* 7:1069–1081.
Crawford RR, Potukuchi PK, Schuetz EG, and Schuetz JD (2018) Beyond competitive inhibition: regulation of ABC transporters by kinases and protein-protein interactions as potential mechanisms of drug-drug interactions. *Drug Metab Dispos* 46:567–580.
Dalgaard L (2015) Comparison of minipig, dog, monkey and human drug metabolism and disposition. *J Pharmacol Toxicol Methods* 74:80–92.
Durmus S, van Hoppe S, and Schinkel AH (2016) The impact of organic anion-transporting polypeptides (OATPs) on disposition and toxicity of antitumor drugs: insights from knockout and humanized mice. *Drug Resist Updat* 27:72–88.
Eclov RJ, Kim MJ, Smith RP, Ahituv N, and Kroetz DL (2018) Rare variants in the ABCG2 promoter modulate in vivo activity. *Drug Metab Dispos* 46:636–642.
El-Kattan AF and Varma MVS (2018) Navigating transporter sciences in pharmacokinetics characterization using extended clearance classification system (ECCS). *Drug Metab Dispos* 46:729–739.
Follman KE and Morris ME (2018) Prediction of the effects of renal impairment on the clearance for organic cation drugs that undergo renal secretion: a simulation-based study. *Drug Metab Dispos* 46:758–769.
Futatsugi A, Toshimoto K, Yoshikado T, Sugiyama Y, and Kato Y (2018) Evaluation of alteration in hepatic and intestinal BCRP function in vivo due to ABCG2 c.421C>A polymorphism based on PBPK analysis of rosuvastatin. *Drug Metab Dispos* 46:749–757.
Gampa G, Kim M, Cook-Rostie N, Laramy J, Sarkaria JN, Paradiso L, DePalatis L, and Elmquist WF (2018) Brain distribution of a novel MEK inhibitor E6201: implications in the treatment of melanoma brain metastases. *Drug Metab Dispos* 46:658–666.

Gao C, Liao MZ, Han LW, Thummel KE, and Mao Q (2018) Hepatic transport of 25-hydroxyvitamin D$_3$ conjugates: a mechanism of 25-hydroxyvitamin D$_3$ delivery to the intestinal tract. *Drug Metab Dispos* **46**:581–591.

Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, et al.; International Transporter Consortium (2010) Membrane transporters in drug development. *Nat Rev Drug Discov* **9**:215–236.

Ho RH and Kim RB (2005) Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther* **78**:260–277.

Holmstock N, Oorts M, Snoeys J, and Annaert P (2018) MRP2 inhibition by HIV protease inhibitors in rat and human hepatocytes: a quantitative confocal microscopy study. *Drug Metab Dispos* **46**:697–703.

Hsu V, de L T Vieira M, Zhao P, Zhang L, Zheng JH, Nordmark A, Berglund EG, Giacomini KM, and Huang SM (2014) Towards quantitation of the effects of renal impairment and probenecid inhibition on kidney uptake and efflux transporters, using physiologically based pharmacokinetic modelling and simulations. *Clin Pharmacokinet* **53**:283–293.

Iwasaki K and Uno Y (2009) Cynomolgus monkey CYPs: a comparison with human CYPs. *Xenobiotica* **39**:578–581.

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

Kaneko K, Tanaka M, Ishii A, Katayama Y, Nakaoka T, Irie S, Kawahata H, Yamanaga T, Wada Y, Miyake T, et al. (2018) A clinical quantitative evaluation of hepatobiliary transport of [^{11}C] Dehydropravastatin in humans using positron emission tomography. *Drug Metab Dispos* **46**:719–728.

Karibe T, Imaoka T, Abe K, and Ando O (2018) Curcumin as an in vivo selective intestinal breast cancer resistance protein inhibitor in cynomolgus monkeys. *Drug Metab Dispos* **46**:667–679.

Kilbourn MR (2017) Small molecule PET tracers for transporter imaging. *Semin Nucl Med* **47**:536–552.

Kimoto E, Bi YA, Kosa RE, Tremaine LM, and Varma MVS (2017) Hepatobiliary clearance prediction: species scaling from monkey, dog, and rat, and in vitro-in vivo extrapolation of sandwich-cultured human hepatocytes using 17 drugs. *J Pharm Sci* **106**:2795–2804.

Koren G and Ornoy A (2018) The role of the placenta in drug transport and fetal drug exposure. *Expert Rev Clin Pharmacol* **11**:373–385.

Lee SC, Arya V, Yang X, Volpe DA, and Zhang L (2017) Evaluation of transporters in drug development: current status and contemporary issues. *Adv Drug Deliv Rev* **116**:100–118.

Mager DE and Jusko WJ (2001) General pharmacokinetic model for drugs exhibiting target-mediated drug disposition. *J Pharmacokinet Pharmacodyn* **28**:507–532.

Mao Q (2008) BCRP/ABCG2 in the placenta: expression, function and regulation. *Pharm Res* **25**:1244–1255.

Mariappan TT, Shen H, and Marathe P (2017) Endogenous biomarkers to assess drug-drug interactions by drug transporters and enzymes. *Curr Drug Metab* **18**:757–768.

Matsunaga N, Fukuchi Y, Imawaka H, and Tamai I (2018) Sandwich-cultured hepatocytes for mechanistic understanding of hepatic disposition of parent drugs and metabolites by transporter-enzyme interplay. *Drug Metab Dispos* **46**:680–691.

Miller DS (2015) Regulation of ABC transporters blood-brain barrier: the good, the bad, and the ugly. *Adv Cancer Res* **125**:43–70.

Morgan RE, Trauner M, van Staden CJ, Lee PH, Ramachandran B, Eschenberg M, Afshari CA, Qualls CW, Jr, Lightfoot-Dunn R, and Hamadeh HK (2010) Interference with bile salt export pump function is a susceptibility factor for human liver injury in drug development. *Toxicol Sci* **118**:485–500.

Nieskens TT, Peters JG, Dabaghie D, Korte D, Jansen K, Van Asbeck AH, Tavraz NN, Friedrich T, Russel F, Masereeuw R, et al. (2018) Expression of organic anion transporter 1 or 3 in human kidney proximal tubule cells reduces cisplatin sensitivity. *Drug Metab Dispos* **46**:592–599.

Osman-Ponchet H, Boulai A, Kouidhi M, Sevin K, Alriquet M, Gaborit A, Bertino B, Comby P, and Ruty B (2014) Characterization of ABC transporters in human skin. *Drug Metabol Drug Interact* **29**:91–100.

Pan Y, Hsu V, Grimstein M, Zhang L, Arya V, Sinha V, Grillo JA, and Zhao P (2016) The application of physiologically based pharmacokinetic modeling to predict the role of drug transporters: scientific and regulatory perspectives. *J Clin Pharmacol* **56** (Suppl 7):S122–S131.

Pang KS, Weiss M, and Macheras P (2007) Advanced pharmacokinetic models based on organ clearance, circulatory, and fractal concepts. *AAPS J* **9**:E268–E283.

Patilea-Vrana G and Unadkat JD (2016) Transport vs. metabolism: what determines the pharmacokinetics and pharmacodynamics of drugs? insights from the extended clearance model. *Clin Pharmacol Ther* **100**:413–418.

Qiu X, Zhang H, and Lai Y (2014) Quantitative targeted proteomics for membrane transporter proteins: method and application. *AAPS J* **16**:714–726.

Rahman MF, Raj R, and Govindarajan R (2018) Identification of structural and molecular features involved in the transport of 3′-deoxy-nucleoside analogs by human equilibrative nucleoside transporter 3. *Drug Metab Dispos* **46**:600–609.

Ryan J, Morgan RE, Chen Y, Volak L, Dunn RT, and Dunn KW (2018) Intravital multiphoton microscopy with fluorescent bile salts in rats as an in vivo biomarker for hepatobiliary transport inhibition. *Drug Metab Dispos* **46**:704–718.

Ryan JC, Dunn KW, and Decker BS (2014) Effects of chronic kidney disease on liver transport: quantitative intravital microscopy of fluorescein transport in the rat liver. *Am J Physiol Regul Integr Comp Physiol* **307**:R1488–R1492.

Sager JE, Yu J, Ragueneau-Majlessi I, and Isoherranen N (2015) Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. *Drug Metab Dispos* **43**:1823–1837.

Sato M, Toshimoto K, Tomaru A, Yoshikado T, Tanaka Y, Hisaka A, Lee W, and Sugiyama Y (2018) Physiologically based pharmacokinetic modeling of bosentan identifies the saturable hepatic uptake as a major contributor to its nonlinear pharmacokinetics. *Drug Metab Dispos* **46**:740–748.

Shen H, Yang Z, Mintier G, Han YH, Chen C, Balimane P, Jemal M, Zhao W, Zhang R, Kallipatti S, et al. (2013) Cynomolgus monkey as a potential model to assess drug interactions involving hepatic organic anion transporting polypeptides: in vitro, in vivo, and in vitro-to-in vivo extrapolation. *J Pharmacol Exp Ther* **344**:673–685.

Tachikawa M, Sumiyoshiya Y, Saigusa D, Sasaki K, Watanabe M, Uchida Y, and Terasaki T (2018) Liver zonation index of drug transporter and metabolizing enzyme protein expressions in mouse liver acinus. *Drug Metab Dispos* **46**:610–618.

Tahara H, Shono M, Kusuhara H, Kinoshita H, Fuse E, Takadate A, Otagiri M, and Sugiyama Y (2005) Molecular cloning and functional analyses of OAT1 and OAT3 from cynomolgus monkey kidney. *Pharm Res* **22**:647–660.

Takano J, Maeda K, Kusuhara H, and Sugiyama Y (2018) Organic anion transporting polypeptide 1a4 is responsible for the hepatic uptake of cardiac glycosides in mice. *Drug Metab Dispos* **46**:652–657.

Takechi T, Hirota T, Sakai T, Maeda N, Kobayashi D, and Ieiri I (2018) Inter-individual differences in the expression of ABC and SLC family transporters in human skin: DNA methylation regulates transcriptional activity of the human ABCC3 (MRP3) gene. *Drug Metab Dispos* **46**:628–635.

Tweedie D, Polli JW, Berglund EG, Huang SM, Zhang L, Poirier A, Chu X, and Feng B; International Transporter Consortium (2013) Transporter studies in drug development: experience to date and follow-up on decision trees from the International Transporter Consortium. *Clin Pharmacol Ther* **94**:113–125.

Varma MV and El-Kattan AF (2016) Transporter-enzyme interplay: deconvoluting effects of hepatic transporters and enzymes on drug disposition using static and dynamic mechanistic models. *J Clin Pharmacol* **56** (Suppl 7):S99–S109.

Varma MV, Steyn SJ, Allerton C, and El-Kattan AF (2015) Predicting clearance mechanism in drug discovery: extended clearance classification system (ECCS). *Pharm Res* **32**:3785–3802.

Vildhede A, Nguyen C, Erickson BK, Kunz RC, Jones R, Kimoto E, Bourbonais F, Rodrigues AD, and Varma MV (2018) Comparison of proteomic quantification approaches for hepatic drug transporters: multiplexed global quantitation correlates with targeted proteomic quantitation. *Drug Metab Dispos* **46**:692–696.

Weber C, Schmitt R, Birnboeck H, Hopfgartner G, van Marle SP, Peeters PA, Jonkman JH, and Jones CR (1996) Pharmacokinetics and pharmacodynamics of the endothelin-receptor antagonist bosentan in healthy human subjects. *Clin Pharmacol Ther* **60**:124–137.

Xiang Z, Li W, Wang L, Yi J, Chen K, and Hong M (2018) Identification of a NFκB inhibition site on the proximal promoter region of human organic anion transporting polypeptide 1A2 coding gene *SLCO1A2*. *Drug Metab Dispos* **46**:643–651.

Yasunaga M, Takemura M, Fujita K, Yabuuchi H, and Wada M (2008) Molecular cloning and functional characterization of cynomolgus monkey multidrug resistance-associated protein 2 (MRP2). *Eur J Pharm Sci* **35**:326–334.

Zhuang X and Lu C (2016) PBPK modeling and simulation in drug research and development. *Acta Pharm Sin B* **6**:430–440.

Address correspondence to: Dr. Qingcheng Mao, Department of Pharmaceutics, University of Washington, Box 357610, H272F Health Science Building, Seattle, WA 98195. E-mail: qmao@uw.edu; or Dr. Yurong Lai, Gilead Sciences, Inc., Foster City, CA 94404. E-mail: Yurong.Lai@gilead.com; or Dr. Joanne Wang, Department of Pharmaceutics, University of Washington, Box 357610, H272J Health Science Building, Seattle, WA 98195. E-mail: jowang@uw.edu