Citation: Clin Transl Sci (2019) 12, 113–121; doi:10.1111/cts.12627

REVIEW

Emerging Role of Organ-on-a-Chip Technologies in Quantitative Clinical Pharmacology Evaluation

Nina Isoherranen[1,2,†], Rajanikanth Madabushi[1,†] and Shiew-Mei Huang[1,*]

The recently enacted Prescription Drug User Fee Act (PDUFA) VI includes in its performance goals "enhancing regulatory science and expediting drug development." The key elements in "enhancing regulatory decision tools to support drug development and review" include "advancing model-informed drug development (MIDD)." This paper describes (i) the US Food and Drug Administration (FDA) Office of Clinical Pharmacology's continuing efforts in developing quantitative clinical pharmacology models (disease, drug, and clinical trial models) to advance MIDD, (ii) how emerging novel tools, such as organ-on-a-chip technologies or microphysiological systems, can provide new insights into physiology and disease mechanisms, biomarker identification and evaluation, and elucidation of mechanisms of adverse drug reactions, and (iii) how the single organ or linked organ microphysiological systems can provide critical system parameters for improved physiologically-based pharmacokinetic and pharmacodynamic evaluations. Continuous public-private partnerships are critical to advance this field and in the application of these new technologies in drug development and regulatory review.

The US Food and Drug Administration Reauthorization Act (FDARA)[1] of 2017 includes the reauthorization of the Prescription Drug User Fee Act (PDUFA) that provides the US Food and Drug Administration (FDA) with the necessary resources to maintain a predictable and efficient review process for human drug and biologic products. The provisions in PDUFA VI[2] continue to include "enhancing regulatory science and expediting drug development" to build on the success of the FDA's regulatory science program. The US FDA Office of Clinical Pharmacology (OCP) has accordingly set the Office goals to enhance drug development, promote regulatory science and innovation, and inform the safe and optimal use of medications. To achieve these goals, the Office strives to play a pivotal role in advancing development of innovative new medicines by applying state-of-the-art regulatory science and clinical pharmacology principles, and to promote therapeutic optimization and individualization through best practices in research, policy development, and drug evaluation throughout the product cycle.[3] In OCP's Good Review Practices,[4] there are four key questions to address in the clinical pharmacology review: (i) To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness? (ii) Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought? (iii) Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors? (iv) Are there clinically relevant food–drug or drug–drug interactions (DDIs), and what is the appropriate management strategy? To address these key clinical pharmacology questions, modeling and simulations play a critical role in the review and analyses,[5] and experimental data are needed from various preclinical and clinical studies to aid in this exercise. **Figure 1** depicts quantitative clinical pharmacology models that are currently being used in drug development and regulatory review.[6,7]

Disease models are developed to quantify disease progression with placebo or drug treatment and need to incorporate relevant clinical end points or biomarkers to inform clinical trial design. Drug models, on the other hand, are central to clinical pharmacology review and describe the time course of plasma/tissue concentrations of drugs and/or their metabolites with various dosing regimens, and the relationships between the exposure (or pharmacokinetics (PKs)) and response (or pharmacodynamics (PDs)) for both desired and undesired effects, and individual patient characteristics. The exposure–response relationships are critical to evaluate the benefit–risk ratio of a specific drug treatment or combination of treatments and to provide optimized dosing regimens for patients with various intrinsic (age, gender, race, genetics, hepatic impairment, renal impairment, etc.) and extrinsic (concomitant medications) factors (**Figure 1**). In addition to disease and drug models, clinical trial models are developed that describe patient adherence and discontinuation and that quantify the patient population covariates important for product safety and efficacy. These trial models are also used to inform the inclusion/exclusion criteria of specific trials that may depend on various factors, such as dosing frequency or convenience related to certain regimens, lack of efficacy, and adverse events.

To enable successful implementation of drug-disease trial models or PK-PD models, we need appropriate biomarkers

†These authors contributed equally to this work.

[1]Office of Clinical Pharmacology (OCP), Office of Translational Sciences, Center for Drug Evaluation and Research, US Food and Drug Administration (FDA), Silver Spring, Maryland, USA; [2]Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, Washington, USA. *Correspondence: Shiew-Mei Huang (Shiewmei.Huang@fda.hhs.gov)

Received: October 23, 2018; accepted: January 26, 2019. doi:10.1111/cts.12627

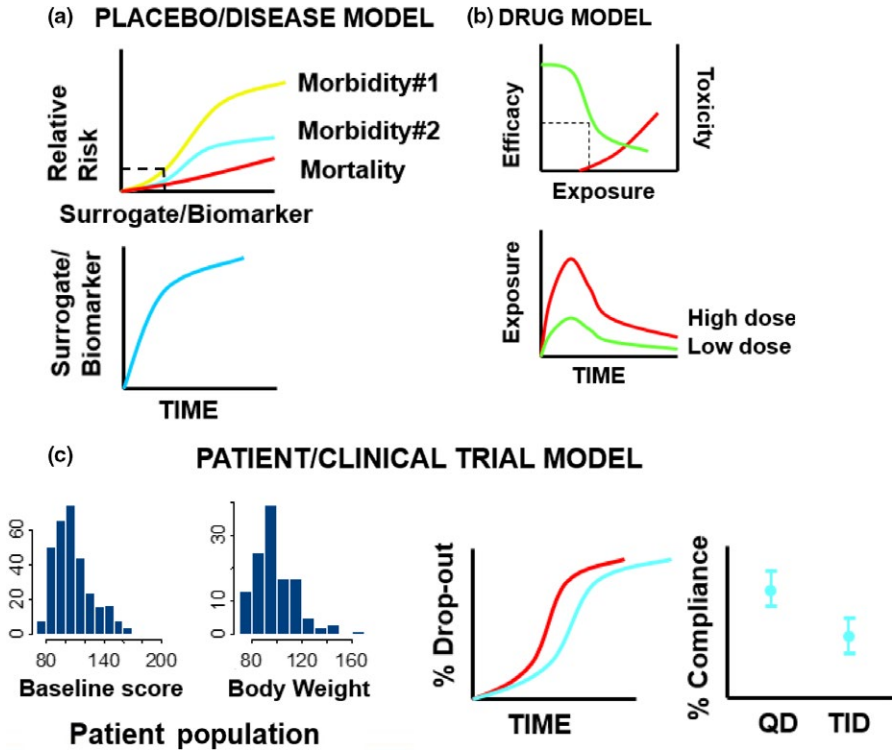

Figure 1 Quantitative clinical pharmacology models.[6,7] (**a**) Disease models that quantify disease progression under placebo based on relevant clinical end points or biomarkers to inform clinical trial design and are typically used at the end of phase 2a or phase 2 to help sponsors design phase 3 trials. (**b**) Drug models that describe the relationship between exposure (or pharmacokinetics) and/or response (or pharmacodynamics) for both desired and undesired effects, and individual patient characteristics. (**c**) Clinical trial models describe the inclusion/exclusion criteria, patient discontinuation and adherence, and attempt to quantify the patient population covariates important for product safety and efficacy. Figure reproduced from public domain.[6]

Table 1 Disease and trial model application: Select cases of disease and trial models in which various end points and biomarkers have been successfully developed to inform study designs for phase 3 pivotal trials, pediatric trials, combination treatment trials, etc.

Disease	Objective	Application	Reference
Non-small cell lung cancer	Quantify tumor size and survival relationship to guide future drug development decisions	The model was used to successfully predict the failure of an ongoing phase 3 trial	9,10
Alzheimer's disease	Quantify disease progression and dropout pattern under placebo	The disease model and dropout model were incorporated into CAMD's drug development tool for qualification to facilitate the development of disease-modifying treatment	11
Pediatric pulmonary arterial hypertension	Quantify hemodynamics and 6MWD relationship to establish new efficacy end point	The outcome was used to change the primary efficacy end point in an ongoing pediatric trial	12
Attention deficit hyperactivity disorder	Quantify disease progression and dropout pattern under placebo or active drugs	The models were applied in clinical trial simulation to design a new pediatric phase 3 trial (dose selection, trial duration justification, and patient population selection)	13
Parkinson's disease	Derive end points to discern disease-modifying and symptomatic effects	Disease and dropout models were applied to design a delayed start phase 3 trial	14
Obesity	Quantify clinical progression and dropout pattern under placebo	The disease model and dropout model were incorporated in clinical trial design	15
Bipolar disorder	Quantify bipolar disorder progression and dropout pattern under placebo	The disease model and dropout model were incorporated in clinical trial design	16
HIV/HCV	Quantify HIV/HCV disease progression under drugs with various mechanism of actions	The models were applied to design clinical trials for combination therapies (dose selection, trial duration justification, and patient population selection)	17

6MWD, 6-Minute Walk Distance; CAMD, Coalition Against Major Diseases; HCV, hepatitis C virus.

that provide insights into mechanistic underpinnings of disease progression and drug effects. The PK-PD models (**Figure 1**), depending on the purpose or use, have ranged from simple, linear models, such as those describing drug exposure–QT prolongation relationship, to more complex, mechanistic models, such as physiologically-based

pharmacokinetic (PBPK) and quantitative systems pharmacology models.[8] **Table 1** lists cases of disease and trial models in which various end points and biomarkers have been successfully developed to inform study designs for phase 3 pivotal trials, pediatric trials, combination treatment trials, etc. There is a continued need in end points/biomarker development to facilitate the development of novel and biosimilar therapeutic proteins[18] and products for rare diseases.[19] The following sections provide insight into the current and future potential use of microphysiological systems (e.g., organ-on-a-chip) in these areas.

USE OF MICROPHYSIOLOGICAL SYSTEMS (ORGAN-ON-A-CHIP) FOR MECHANISTIC DISEASE, PHARMACOLOGY, AND TOXICOLOGY ASSESSMENT

As indicated earlier, development and application of mathematical modeling that leverage our understanding of physiology, pathology, and pharmacology to inform drug development has long been appreciated. With the advent of new technologies and innovations in computation, our ability to integrate mathematical models to gain better understanding of disease, drug effects, and inform clinical trial design, and eventually clinical practice, has improved.[20] All these advances bode well for realizing the potential of quantitative systems pharmacology approaches as well as quantitative disease-drug trial models.[7,21,22] However, the success of these computational approaches depends on the availability of critical and reliable experimental data that can inform such models. There is a clear need to develop a better understanding of the physiology, disease and its progression, and identification of mechanistic and PD biomarkers that are translatable to outcomes of interest. Better tools are also needed to anticipate and predict drug toxicity. As shown in **Figure 2**, microphysiological systems can be viewed as an innovative technology that has the potential to enhance the role of quantitative clinical pharmacology in advancing drug discovery and development.[23,24] To realize the full potential of these emerging technologies, collaboration across various stakeholders in this multidisciplinary setting is essential. Several public-private partnerships in the development and application of various microphysiological systems to evaluate the efficacy, safety, and toxicity of therapies have already been established.[25-28]

Physiology and disease

Microphysiological systems represent advancement over conventional technologies by leveraging the recent advances in microfluidics, microfabrication, and cell biology to better mimic and control the physiological microenvironments. As such, they are more suitable for investigating complex organ-level physiology, tissue architecture, and functions than traditional 2-D cell culture models. This is exemplified by the human breathing lung-on-a-chip, which was used to characterize complex physiological responses, such as activation of endothelial cells, increased expression of adhesion molecules, adhesion, and transmigration of neutrophils across tissue layers and engulfing of bacteria in response to an inflammatory insult by placing bacteria in the alveolar compartment.[29]

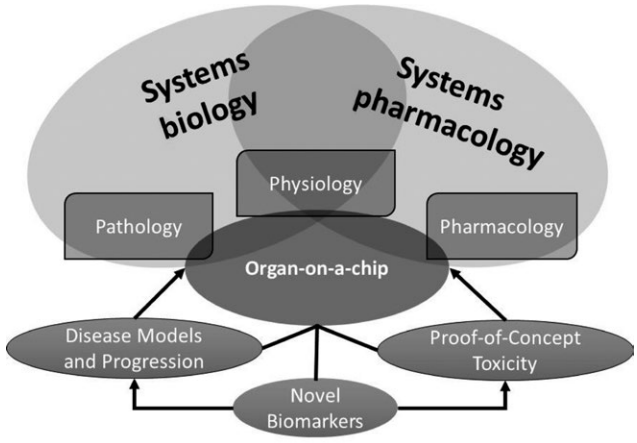

Figure 2 Potential role of microphysiological systems to inform quantitative clinical pharmacology models. Better understanding of physiology, pathology, and pharmacology is critical for developing systems biology and systems pharmacology models. Microphysiological systems can be viewed as an innovative technology that has the potential to enhance the understanding of physiology, pathology, and pharmacology. Specific applications of the microphysiological systems in the areas of biomarker development; demonstrating proof-of-concept, elucidating the mechanism of drug toxicity, and characterizing the complex physiologic changes that occur in disease states can provide the necessary information to advance the role of quantitative clinical pharmacology models in drug development.

Platforms that characterize complex physiology can be leveraged to understand the changes that occur in disease states. Such an application is illustrated by the work done using a neurovascular unit blood–brain barrier (BBB) microfluidic device.[30] This system comprises vascular perfusion channels, barrier membrane, brain compartment, and brain perfusion channels, and was derived using primary human brain-derived microvascular endothelial cells, pericytes, astrocytes, and human induced pluripotent stem cell-(iPSC)-derived human cortical neurons along with co-differentiating astrocytes. Using the system, the authors were able to observe time-dependent changes in the BBB integrity and function, including partial recovery and cytokine activation in response to inflammatory stimulus. By integrating metabolomics and pathway identification analysis, the metabolic signature and pathways associated with the inflammatory stimulation could be mapped. Another example is integration of the human small airway-on-a-chip with a smoke machine and microrespirator to study smoke-induced pathophysiology. By lining the device with living human bronchiolar epithelium from normal and patients with chronic obstructive pulmonary disease, comparative biological responses were obtained that led to the identification of ciliary micropathologies, chronic obstructive pulmonary disease–specific molecular signatures, and epithelial responses to smoke.[31]

Peer-reviewed literature is emerging with examples of disease models using human microphysiological systems (or organs-on-chips).[32–34] When combined with patient-derived or genetically engineered iPSCs to induce disease-causing mutations, the pathophysiology of rare and ultrarare disease subsets can be elucidated. An example of this is the

work done to elucidate the pathophysiology underlying the cardiomyopathy of Barth syndrome. Using Barth syndrome iPSC-derived cardiomyocytes, the metabolic, structural, and functional abnormalities associated with mutations in the gene encoding tafazzin were defined. The authors further demonstrated with Cas9-mediated genome editing that mutation in the gene encoding tafazzin was sufficient to cause the disease phenotype.[35] The use of patient-derived cells opens the potential for personalized disease modeling as well as the ability to model the longitudinal progression of the disease both at an individual as well as at the population level.

Pharmacology

In the context of drug discovery and development, models that can realistically replicate physiology and disease states are useful in providing reliable inputs for developing systems biology and disease models and have the potential to be transformative. Several current and future applications can be envisioned. Microphysiological systems may provide a reliable means to reproduce the pharmacology and clinically relevant downstream responses of drug treatment. This is particularly useful for lead development and optimization.[36] Further, microphysiological system platforms that probe higher-order functionality can be very useful for the demonstration of proof-of-concept for new molecular entities early in drug discovery and development. As a proof-of-concept for such an approach, Berdichevsky *et al.*[37] demonstrated the inhibition of spontaneous electrical excitation of the brain slice treated with a glutamate receptor antagonist, using a microdevice that enables culture of brain slices in separate media formulations while retaining *in vivo* neural network connections and electrophysiological behavior.

In vitro models that can reliably demonstrate proof-of-concept are of particular utility in development of drugs for rare diseases for which appropriate animal models are sparse. Such models can be used to characterize the time course of drug effects on the organ-level structure and function and can potentially be used to evaluate the relationship to clinical outcomes of interest. By functionally integrating multiple organs, a more realistic estimate of the clinical response can be projected.[38] Such an approach combined with patient-derived iPSC has the potential to capture the variability in response stemming from the role of different critical organs that either process the drug of interest (PK) or that are the target of the drug effect in the organ of interest (PD).

Toxicity

The most common application reported for a microphysiological system is to either predict or characterize the mechanism of on-target and off-target drug toxicity in humans.[34,39–42] An example of such application is the characterization of hepatotoxicity of several hepatotoxic drugs using human, 3-D, microfluidic, four-cell, sequentially layered, self-assembly liver model.[32] The system reproduced the acute toxicity (1–2 days) seen with a high concentration of troglitazone as well as the gradual and delayed (28 days) toxicity produced by a lower concentration of troglitazone. In addition, the system could demonstrate the immune-mediated toxicity with trovafloxacin. Such data could inform predictive mathematical models of liver injury. Further, functionally integrating organs provides an opportunity to model the secondary toxic effects of metabolites. This was demonstrated by the functional integration of a microfluidic liver–kidney model to demonstrate the nephrotoxic responses to the hepatic metabolite of ifosfamide.[43]

USE OF MICROPHYSIOLOGICAL SYSTEMS TO SUPPORT PBPK MODELING AND PREDICTIONS OF DRUG DISPOSITION AND DRUG–DRUG INTERACTIONS

Drug models to address how individual intrinsic and extrinsic factors affect the PK and PD of drugs are routinely developed. For example, to address potential and severity of DDIs, various *in vitro*, *in vivo*, and *in silico* models are used.[44–47] PBPK models have been increasingly developed and used to address DDI in regulatory submissions[5,48] and published literature[49] (**Figure 3**).

The key parameters required to build a reliable PBPK model for a drug include, in addition to a good system model, determination of the absorption, distribution, metabolism, and excretion (ADME) characteristics for the drug: absorption kinetics (rate and extent), distribution parameters (organ partitioning, and perfusion vs. permeability limitations), metabolism (in drug eliminating organs, such as the liver), and excretion (by the kidneys and into the bile). Microphysiological systems hold unique promise in providing a refined tool to establish these parameters that form the foundation of PBPK model development and DDI predictions. In the following section, the potential of different organ-on-a-chip approaches in providing critical ADME values for PBPK model development is reviewed. An overall schematic of a basic PBPK model and how microphysiological systems may fit into populating the drug properties in such models are shown in **Figure 4**.

Absorption

At present, the absorption characteristics of many drugs are not well defined, and modeling the extent and rate of absorption is often confounded by low confidence in parameter estimates for drug absorption. This is largely due to the lack of data on absolute bioavailability due to absence of PK information after intravenous administration of the drug of interest, and the confounding effects of distribution kinetics in modeling rate of absorption. Current static cell models to evaluate intestinal absorption, such as Caco-2 cells, are predominantly useful for determining the passive permeability values for a compound of interest and for identifying potential efflux and uptake transporters contributing to the absorption kinetics of the drug. However, predicting the ultimate rate and extent of absorption *in vivo* in humans from *in vitro* systems remains a considerable challenge due to the complex physiology of the human intestine and the processes involved in drug absorption from the gastrointestinal tract.

Current 2-D *in vitro* models suffer from the lack of flow on either side of the cell system, generating an artificially

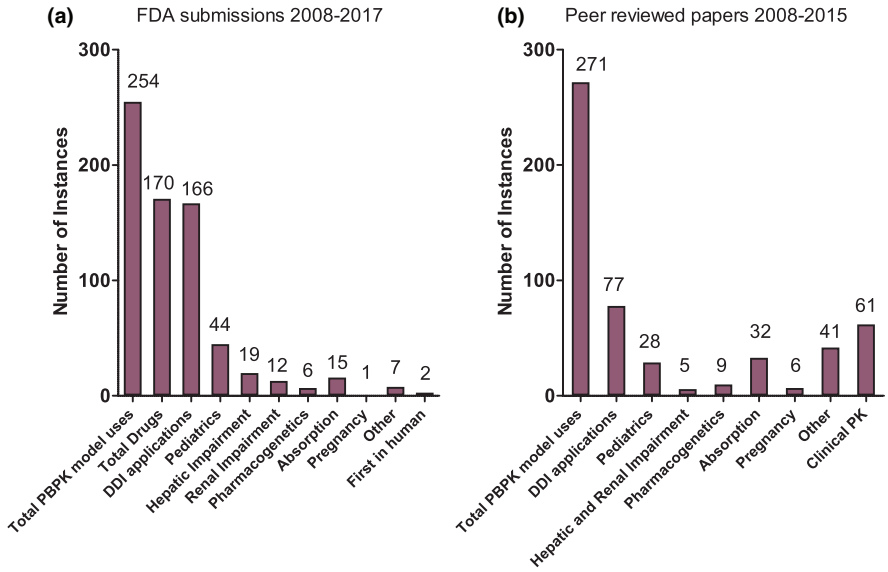

Figure 3 Physiologically-based pharmacokinetic (PBPK) model use (**a**) in regulatory submissions to the US Food and Drug Administration (FDA) and (**b**) in peer reviewed literature. The figure shows the numbers of drugs and specific PBPK model applications used in **a** and the numbers of individual papers and the numbers of specific applications reported in **b**. For the FDA submissions, some PBPK models were used for multiple applications and, hence, the total numbers of applications cannot be directly compared with the number of drug submissions. The data in **a** are adapted from Grimstein *et al.*[5,46] and personal communication with Yaning Wang. The data in **b** are adapted from Sager *et al.*[47] PK, pharmacokinetic.

static system to define drug absorption that lacks any dynamic concentration gradients and flow dynamics observed in the human gastrointestinal tract. The microphysiological systems offer great promise in regenerating the microenvironment of the human gut, including the mechanical, absorptive (passive and active), and flow characteristics. For example, the gut-on-a-chip system[50] that encompasses Caco-2 cells with epithelial cells layered between two microfluidic channels incorporates fluid flow on both sides of the Caco-2 cells, which mimic the shear stress and dynamics of the human intestine. This system incorporates spontaneous formation of microvilli and a polarized epithelium that seems to mimic the human intestine better than static (2-D) Caco-2 models. A weakness of this model, among other intestinal microphysiological systems, is that it uses Caco-2 cells, which are known to lack expression of critical intestinal metabolic enzymes, such as cytochrome P450 (CYP)3A4, that decrease drug bioavailability. Therefore, evaluation of intestinal first pass metabolism seems limited using this system. Nevertheless, this system was shown to have excellent epithelial integrity based on transepithelial electrical resistance measurements, and the data suggest that the system has great potential in refining predictive parameters for the absorption characteristics of drugs. The proof-of-concept of determining intestinal permeability has been demonstrated by Gao *et al.*[51] who used a microfluidic device with Caco-2 cells to evaluate the intestinal permeability of curcumin. This study also included an evaluation of the effect of different flow rates on permeability measurements. Using the microfluidic system, the authors determined the low passive permeability of curcumin and measured an efflux ratio of 0.68. More recently, a similar system, including Caco-2 cells and microfluidic flow, was used to evaluate permeability of caffeine and atenolol together with irinotecan.[52] Surprisingly, the permeability values obtained from the microphysiological system with Caco-2 cells resulted in consistently higher apparent permeability values than those obtained from conventional 2-D Caco-2 transwell studies. This may be due to the spontaneous formation of microvilli in the microphysiological system, which increases the effective surface area or other differences in the monolayer formed, or the presence of the unstirred water layer in the two systems. Yet this difference illustrates the need for more comprehensive studies of permeability values in microphysiological systems that can be used to establish how these values quantitatively scale to *in vivo* human PK modeling, and to populate PBPK models that simulate drug absorption.

An interesting aspect of some of the human gut microphysiological systems is that they allow coculture of human gut microbiome with the epithelial cells.[50] Although no applications of this coculture system to drug metabolism and absorption have yet been published, one can envision that, in the future, this system will allow studies to extrapolate the contribution of gut microbiome to the absorption characteristics and enterohepatic recycling of xenobiotics.

Distribution

Current methods of modeling distribution of drugs into specific organs rely mainly on few mathematical models of predicting tissue partitioning (K_p),[53,54] on extrapolating distribution characteristics from preclinical species via body-size-based interspecies scaling (allometric scaling), and on applying limited imaging data to distribution modeling. The distribution kinetics of many drugs are not

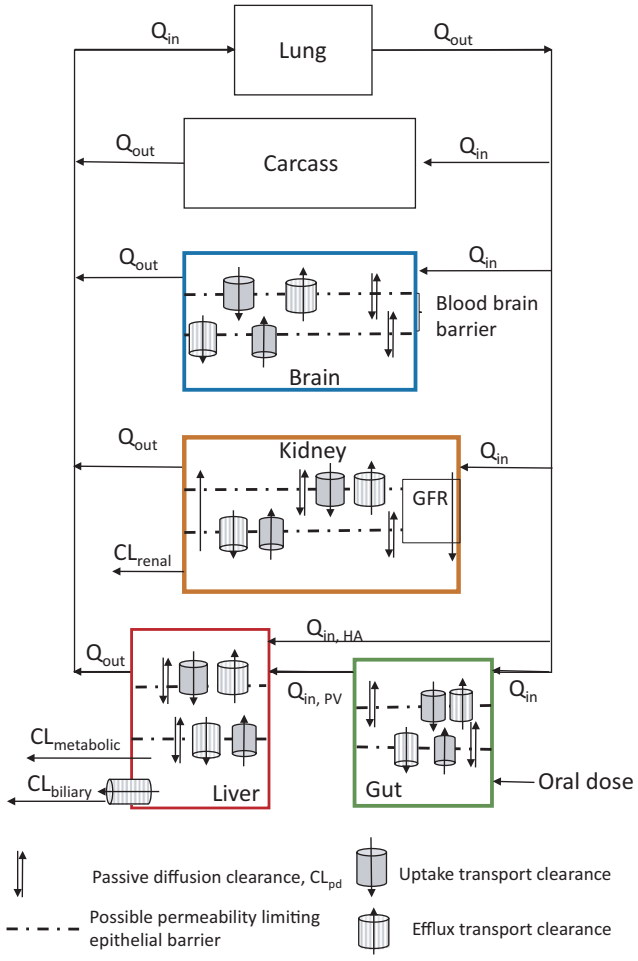

Figure 4 Overall structure of a simple physiologically-based pharmacokinetic (PBPK) model and the incorporation of data from microphysiological systems into the model. The potential role of microphysiological systems in informing drug PBPK model parameters are indicated by colored boxes. CL, clearance; GFR, glomerular filtration rate; HA, hepatic artery; PV, portal vein; Q, blood flow rate.

well-defined due to the lack of good experimental data of rates and extents of distribution after i.v. dosing. The microphysiological systems that incorporate flow characteristics offer a unique opportunity to evaluate drug distribution into specific organs in a preclinical setting and to support PBPK model development. Specifically, microphysiological systems that combine fluid flows and include protein binding with organ models can provide rates for how quickly drugs distribute to given organs prior to steady-state conditions and to what extent drugs partition to those organs under equilibrium conditions. Although no system has been applied to predicting drug distribution kinetics yet, if such application was shown to be successful, this could considerably improve current PBPK models. On the other hand, microphysiological systems that combine multiple organs also provide exciting opportunities for predicting drugs and their metabolites distribution. As an example of this, it was predicted using a liver–brain connected chip system that trimethylamine N-oxide, a metabolite of trimethylamine formed in the liver, would pass the BBB and be detected in the brain.[55] It was subsequently confirmed that trimethylamine N-oxide crosses the BBB. This example demonstrates the opportunity to gather important information of the pharmacological and toxicological role of metabolites in an *in vitro* system that has not been previously possible. This example also suggests that microphysiological systems that allow study of drug distribution across tight barriers, such as the BBB or blood–testis barrier, could, in the future, be used to characterize permeability-rate-limited drug distribution, an area that is very challenging in current PBPK modeling (**Figure 4**). However, the question remains on how to scale the kinetic values obtained in microphysiological systems to a whole organ, and proof-of-concept studies in this area are needed. Similarly, further research is needed to explore the possibilities to study transporter contribution to distribution kinetics, and DDIs that may affect distribution kinetics.

Metabolism

At present, well-established methods, such as human liver microsomes and 2-D cultured human hepatocytes, are used to predict metabolic clearance in humans and to populate the clearance parameters in PBPK models. In general, the value of these 2-D systems in predicting human metabolic drug clearance is well recognized. However, these systems typically allow evaluation of metabolic processes solely in the hepatocytes and do not provide information on how other liver cell types may influence drug clearance. In addition, it is well recognized that these systems do not provide good information of transporter-metabolism interplay that could be kinetically extrapolated to PBPK models. Microfluidic liver models or liver-on-a-chip may offer key advantages in this regard, especially when hepatic zonation is observed. Indeed, a microphysiological liver model that includes the four main liver cell types (hepatocytes, Kupffer cells, stellate cells, and endothelial cells) in a ratio mimicking the human liver has been reported.[32] Relevant to ADME characterization and PBPK modeling, this system was shown to be viable for 28 days and have metabolic functions similar to the human liver as measured by metabolism of diclofenac, phenolphthalein, ethoxyresorufin, and testosterone metabolism. In contrast, some of the other liver chip models that have been explored up to date have suffered from the fact that they have used HepG2 cells, which do not have the complement of metabolic enzymes and metabolic capacity that is commonly observed in an adult human liver.

In the context of human clearance predictions and PBPK modeling, liver microphysiological systems that incorporate human hepatocytes have been shown to maintain the expression and activity of main drug metabolizing enzymes, and the data generated from these systems have been used to predict human *in vivo* metabolic clearance.[56,57] Although the quantitative predictive value of liver chips for estimating *in vivo* clearance needs further validation, these methods clearly show that microphysiological systems that incorporate hepatocyte culture with flow offer a promising system to predict both the *in vivo* metabolic clearance of drugs and

the interindividual variability of drug clearance. For example, the data collected for lidocaine clearance in a liver microphysiological system were successfully used to populate a PBPK model of lidocaine disposition *in vivo*.[57] The liver chip systems may also provide great value in exploring sequential metabolic processes that occur in the liver and between liver cell types, as it has been shown that hepatocytes cultured under flow undergo zonation similar to human liver *in vivo*.[58]

The full potential of liver chips can perhaps be realized via the use of these systems in combination with other organs, as metabolism in the liver is typically considered the culprit in forming metabolites that may then cause toxicities in other organs. For evaluation of such organ-to-organ interplay, a liver–kidney combined microphysiological system has been used to demonstrate how metabolites of aristolochic acid cause kidney toxicity.[59] In that study, the nitroreduction of aristolochic acid in the liver chip followed by sulfate conjugation and active uptake transport by organic anion transporter 1 into tubular cells in the kidney chip was demonstrated. This example illustrates the unique opportunity to evaluate complex metabolism-transport interplay between organs using linked microphysiological tissue models. In another recent study, the PK of diclofenac was characterized in such "physiome-on-a-chip" system.[60]

Excretion

At present, there are no good static systems to predict renal clearance due to the complex physiology of the kidney and the generation of concentration gradients and pH gradients in the kidneys during passive reabsorption of drugs and water from tubular lumen. Passive permeability measures from Caco-2 or Manine-Darby canine kidney (MDCK) cells are generally used to estimate and predict reabsorption clearance in the kidneys and can be used to populate PBPK models.[61,62] This approach has many weaknesses, and, therefore, better *in vitro* models that closely mimic the processes observed in the human kidneys are needed to improve the validity of kidney PBPK models and predictions of renal clearance and renal transporter contribution to renal clearance. The kidney-on-a-chip system that incorporates two fluid flow compartments that mimic the blood and tubular lumen in the kidneys separated by the layer of tubular cells may be the best experimental model to generate appropriate *in vitro* values for predicting and modeling renal clearance.[63,64] However, as of now, no studies have applied the kidney chip to human renal clearance modeling and predictions. In addition, the anatomic complexity of the kidneys and how the complexity impacts function is a significant and ongoing challenge to recapitulate in a microphysiological system. A "tubule" on a chip is not representative of a "kidney" on a chip.

What is in the future for microphysiological systems and PBPK modeling?

The current state-of-the-art for gut chips is the use of Caco-2 cells. The next steps for the field may involve the use of cryopreserved human enterocytes[65] in the microphysiological systems that would allow simultaneous assessment of the metabolic capacity and permeability in the intestine. If such systems could provide reliable predictive information of bioavailability (F_a and F_g) as well as rate of absorption, the values generated would be of great advantage to PBPK modeling efforts. It is likely that intestinal chip systems in the future will allow evaluation of DDIs involving intestinal uptake and efflux transporters in the presence of flow. Such data would provide critical improvements to parameter inputs for PBPK modeling.

The microphysiological systems involving human liver cells, such as hepatocytes, have the great advantage of living for relatively long periods in culture, which is of importance in preclinical assessment of DDI potential. A major issue with current hepatocyte systems is their short lifetime in culture (72–96 hours), which typically limits studies of enzyme activity under steady-state conditions because of the apparent long half-lives of metabolic enzymes. As such, most induction evaluations are currently based on mRNA changes. Microphysiological systems that allow studies over several weeks hold great promise in generating predictive values of induction, downregulation, and time-dependent/irreversible inhibition of CYP enzymes. Long-term cultures would presumably allow assessment of homeostatic processes that may contribute to interplay between regulation of metabolic enzyme expression and direct modulation of their activity. In addition, long-term microphysiological systems will enable pulse-chase types of studies that may finally provide the CYP enzyme half-life estimates that are critical for PBPK model development.

Recent advances in human transporter biology have greatly improved our understanding of transporters' role in drug PK and PD.[66] Although transporter involvement in DDIs, drug disposition, and drug effects has been increasingly recognized, critical issues remain that need to be addressed to comprehensively evaluate DDI mechanisms, including metabolic enzyme-transporter interplay.[67,68] In this area, microphysiological systems hold great promise to provide a novel platform to assess not only transported function and activity but also the impact of transporters on tissue drug accumulation.

CONCLUSIONS

Contemporary clinical pharmacology reviews of new drug submissions critically address key questions related to the safe and effective use of new drugs in individual patients with varying intrinsic and extrinsic factors. Appropriate applications of the state-of-the-art *in vitro*, *in vivo*, and *in silico* models (including the disease-drug trial models) are critical in drug development and regulatory review. With advances in the microphysiological system (organ-on-a-chip) technology, we will likely see more integration with model-informed drug development approaches for better understanding of disease, predicting drug effects, designing clinical trials, and, ultimately, better individualized patient treatment.

Acknowledgments. The authors thank Yaning Wang for providing **Figure 1** and **Table 1**. The authors thank Paul C. Brown, Anuradha Ramamoorthy, Alexandre Ribeiro, David Strauss, Yaning Wang, and Issam Zineh for helpful discussions and careful review of this manuscript.

Funding. This research was supported by the US Food and Drug Administration's (FDA's) Medical Countermeasures Initiative. Nina Isoherranen was supported in part by an appointment to the Research Participation Program at the Center for Drug Evaluation and Research, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and the FDA, and by the National Center for Advancing Translational Sciences and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health under Award Number UG3TR002158. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflict of Interest. The authors declared no competing interests for this work. As an Associate Editor for *Clinical and Translational Science*, Nina Isoherranen was not involved in the review or decision process for this paper.

Disclaimer. The contents of this article reflect the views of the authors and should not be construed to represent the FDA's views or policies. No official support or endorsement by the FDA is intended or should be inferred. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the FDA.

1. US Food and Drug Administration Reauthorization Act (FDARA). FDA Reauthorization Act of 2017 (FDARA). <https://www.fda.gov/RegulatoryInformation/LawsEnforcedbyFDA/SignificantAmendmentstotheFDCAct/FDARA/default.htm> (2017).
2. Prescription Drug User Fee Act (PDUFA VI). PDUFA Reauthorization Performance Goals and Procedures Fiscal Years 2018 Through 2022. <https://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM511438.pdf>.
3. Grillo, J.A. & Huang, S.M. Perspectives in regulatory science: translational and clinical pharmacology. *Drug Discov. Today Technol.* **21–22**, 67–73 (2016).
4. US Food and Drug Administration Office of Clinical Pharmacology. OCP review: (OCP Good Review Practices, September 2016). <https://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ManualofPoliciesProcedures/UCM073007.pdf>.
5. Huang, S.-M., Abernethy, D.R., Wang, Y., Zhao, P. & Zineh, I. The utility of modeling and simulation in drug development and regulatory review. *J. Pharm. Sci.* **102**, 2912–2923 (2013).
6. Wang, Y. Modeling and Simulation at CDER. Presented at the FDA Modeling and Simulation Working Group Annual Meeting, July 9, 2018. [for internal info: CDER Modeling and simulation.] <http://inside.fda.gov:9003/CDER/OfficeofTranslationalSciences/CDERModelingSimulation/ucm328008.htm>.
7. Gobburu, J.V. & Lesko, L.J. Quantitative disease, drug and trial models. *Ann. Rev. Pharmacol. Toxicol.* **49**, 291–301 (2009).
8. Wang, Y. & Huang, S.M. Commentary on fit-for-purpose models for regulatory applications. *J. Pharm. Sci.* **108**, 18–20 (2019).
9. Yaning Wang presentation July 2018 oncology workshop.
10. Wang, Y. *et al.* Elucidation of relationship between tumor size and survival in non-small-cell lung cancer patients can aid early decision making in clinical drug development. *Clin. Pharmacol. Ther.* **86**, 167–174 (2009).
11. William-Faltaos, D., Chen, Y., Wang, Y., Gobburu, J. & Zhu, H. Quantification of disease progression and dropout for Alzheimer's disease. *Int. J. Clin. Pharmacol. Ther.* **51**, 120–131 (2013).
12. US Food and Drug Administration (FDA). FDA cardiovascular and renal drugs advisory committee briefing document, July 29, 2010.
13. Knebel, W., Rogers, J., Polhamus, D., Ermer, J. & Gastonguay, M.R. Modeling and simulation of the exposure-response and dropout pattern of guanfacine extended-release in pediatric patients with ADHD. *J. Pharmacokinet. Pharmacodyn.* **42**, 45–65 (2015).
14. Bhattaram, V.A., Siddiqui, O., Kapcala, L.P. & Gobburu, J.V. Endpoints and analyses to discern disease-modifying drug effects in early Parkinson's disease. *AAPS J.* **11**, 456–464 (2009).
15. Clinical pharmacology advisory committee meeting, 2006
16. Sun, W., Laughren, T.P., Zhu, H., Hochhaus, G. & Wang, Y. Development of a placebo effect model combined with a dropout model for bipolar disorder. *J. Pharmacokinet. Pharmacodyn.* **40**, 359–368 (2008).
17. Wang, Y. *et al.* Leveraging prior quantitative knowledge to guide drug development decisions and regulatory science recommendations. Impact of FDA pharmacometrics during 2004–2006. *J. Clin. Pharmacol.* **48**, 146–156 (2008).
18. Wang, Y.-M. *et al.* Role of modeling and simulation in development of novel and biosimilar therapeutic proteins. *J. Pharm. Sci.* **108**, 73–77 (2019).
19. Schuck, R. *et al.* Considerations for developing targeted therapies in low-frequency molecular subsets of a disease. *Clin. Pharmacol. Ther.* **104**, 282–289 (2018).
20. US Food and Drug Administration (FDA). FDA's comprehensive effort to advance new innovations: Initiatives to modernize for innovation. <https://blogs.fda.gov/fdavoice/index.php/2018/08/fdas-comprehensive-effort-to-advance-new-innovations-initiatives-to-modernize-for-innovation/>.
21. van der, Graaf, P.H. & Benson , N. Systems pharmacology: bridging systems biology and pharmacokinetics-pharmacodynamics (PKPD) in drug discovery and development. *Pharm. Res.* **28**, 1460–1464 (2011).
22. Sorger, P.K. *et al.* Quantitative and systems pharmacology in the post-genomic era: new approaches to discovering drugs and understanding therapeutic mechanisms. An NIH White Paper, QSP Workshop Group R. Ward, Editor, 2011.
23. Fabre, K.M., Livingston, C. & Tagle, D.A. Organs-on-chips (microphysiological systems): tools to expedite efficacy and toxicity testing in human tissue. *Exp. Biol. Med. (Maywood)* **239**, 1073–1077 (2014).
24. Cirit, M. & Stokes, C.L. Maximizing the impact of microphysiological systems with in vitro-in vivo translation. *Lab Chip* **18**, 1831–1837 (2018).
25. US Food and Drug Administration (FDA). The FDA CFSAN and Emulate "collaborative agreement to use organs-on-chips technology as a toxicology testing platform for understanding how products affect human health and safety", April 11, 2017. <https://emulatebio.com/press/fda-collab-agreement-emulate/>. Accessed October 15, 2018.
26. Smirnova, L. *et al.* 3S - Systematic, systemic, and systems biology and toxicology. *ALTEX* **35**, 139–162 (2018).
27. US Food and Drug Administration (FDA). FDA collaborative agreenet with CN Bio Innovations to use Organs-on-Chips to improve drug development and evaluation. <https://cn-bio.com/fda-signs-collaborative-agreement-cn-bio-innovations-use-organs-chips-improve-drug-development-evaluation/>.
28. US Food and Drug Administration (FDA). The FDA OCP-DARPA program, as described in Huang, S-M, "Role for Organ-on-a-chip in drug development: a clinical pharmacology perspective" and Ribeiro A, "Application of Organ-on-a-Chip for Mechanistic Assessment", September 22, 2018, Bethesda, MD.
29. Huh, D.D. A human breathing lung-on-a-chip. *Ann. Am. Thorac. Soc.* **12** (suppl. 1), S42–S44 (2015).
30. Brown, A.J. *et al.* Metabolic consequences of inflammatory disruption of the blood-brain barrier in an organ-on-chip model of the human neurovascular unit. *J. Neuroinflammation* **13**, 306 (2016).
31. Kambex, H.B. *et al.* Matched-comparative modeling of normal and diseased human airway responses using a microengineered breathing lung chip. *Cell Syst.* **3**, 456–466 (2016).
32. Vernetti, L.A. *et al.* A human liver microphysiology platform for investigating physiology, drug safety, and disease models. *Exp. Biol. Med. (Maywood)* **241**, 101–114 (2016).
33. Lee, J. & Kim, S. Kidney-on-a-chip: a new technology for predicting drug efficacy, interactions, and drug-induced nephrotoxicity. *Curr. Drug Metab.* **19**, 577–583 (2018).
34. Blutt, S.E. *et al.* Gastrointestinal microphysiologic systems. *Exp. Biol. Med. (Maywood)* **242**, 1633–1642 (2017).
35. Wang, G. *et al.* Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. *Nat. Med.* **20**, 616–623 (2014).
36. Aref, A.R. *et al.* Screening therapeutic EMT blocking agents in a three-dimensional microenvironment. *Integr. Biol. (Camb).* **5**, 381–389 (2013).
37. Berdichevsky, Y., Staley, K.J. & Yarmush, M.L. Building and manipulating neural pathways with microfluidics. *Lab Chip* **10**, 999–1004 (2010).
38. Sung, J.H. *et al.* Using PBPK guided "body-on-a-chip" systems to predict mammalian response to drug and chemical exposure. *Exp. Biol. Med. (Maywood)* **239**, 1225–1239 (2014).
39. Snouber, L.C. *et al.* Metabolomics-on-a-chip of hepatotoxicity induced by anticancer drug flutamide and its active metabolite hydroxyflutamide using HepG2/C3a microfluidic biochips. *Toxicol. Sci.* **132**, 8–20 (2013).
40. Jang, K.J. & Suh, K.Y. A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells. *Lab Chip* **10**, 36–42 (2010).
41. Mahler, G.J. *et al.* Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity. *Biotechnol. Bioeng.* **104**, 193–205 (2009).
42. Mathur, A. *et al.* Human iPSC-based cardiac microphysiological system for drug screening applications. *Sci. Rep.* **5**, 8883 (2015).
43. Choucha-Snouber, L. *et al.* Investigation of ifosfamide nephrotoxicity induced in a liver–kidney co-culture biochip. *Biotechnol. Bioeng.* **110**, 597–608 (2013).
44. US Department of Health and Human Services, US Food and Drug Administration, Center for Drug Evaluation and Research (CDER) Draft guidance/guidance for industry. In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies

Guidance for Industry. <https://wwwfdagov/downloads/Drugs/Guidances/UCM581965pdf>. When finalized, the guidance will be available at the following link: <https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm> (2017).
45. US Department of Health and Human Services, US Food and Drug Administration, Center for Drug Evaluation and Research (CDER) Draft guidance/guidance for industry. Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry. <https://wwwfdagov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362pdf>. When finalized, the guidance will be available at the following link: <https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm06> (2017).
46. Dinko, R. *et al.* Clinical drug-drug interaction evaluations to inform drug use and enable drug access. *J. Pharm. Sci.* **106**, 2214–2218 (2017).
47. Yoshida, K. *et al.* In vitro–in vivo extrapolation of metabolism- and transporter-mediated drug–drug interactions – overview of basic prediction methods. *J. Pharm. Sci.* **106**, 2209–2213 (2017).
48. Grimstein, M. *et al.* Physiologically-based pharmacokinetic (PBPK) modeling in regulatory science: an update from the US Food and Drug Administration's Office of Clinical Pharmacology. *J. Pharm. Sci.* 2019 (revision submitted).
49. Sager, J.E., Yu, J., Ragueneau-Majlessi, I. & Isoherranen, N. Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. *Drug Metab. Dispos.* **43**, 1823–1837 (2015).
50. Kim, H.J., Huh, D., Hamilton, G. & Ingber, D.E. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* **12**, 2165–2174 (2012).
51. Gao, D., Liu, H., Lin, J.M., Wang, Y. & Jiang, Y. Characterization of drug permeability in Caco-2 monolayers by mass spectrometry on a membrane-based microfluidic device. *Lab Chip* **13**, 978–985 (2013).
52. Pocock, K. *et al.* Intestine-on-a-chip microfluidic model for efficient in vitro screening of oral chemotherapeutic uptake. *ACS Biomater. Sci. Eng.* **3**, 951–959 (2017).
53. Rodgers, T. & Rowland, M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *J. Pharm. Sci.* **95**, 1238–1257 (2006).
54. Poulin, P. & Theil, F.P. Development of a novel method for predicting human volume of distribution at steady-state of basic drugs and comparative assessment with existing methods. *J. Pharm. Sci.* **98**, 4941–4961 (2009).
55. Vernetti, L. *et al.* Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. *Sci. Rep.* **7**, 42296 (2017).
56. Novik, E., Maguire, T.J., Chao, P., Cheng, K.C. & Yarmush, M.L. A microfluidic hepatic coculture platform for cell-based drug metabolism studies. *Biochem. Pharmacol.* **79**, 1036–1044 (2010).
57. Tsamandouras, N, Kostrzewski, T., Stokes, C.L., Griffith, L.G., Hughes, D.J. & Cirit, M. Quantitative assessment of population variability in hepatic drug metabolism using a perfused three-dimensional human liver microphysiological system. *J. Pharmacol. Exp. Ther.* **360**, 95–105 (2017).
58. Kang, Y.B.A., Eo, J., Mert, S., Yarmush, M.L. & Usta, O.B. Metabolic patterning on a chip: towards in vitro liver zonation of primary rat and human hepatocytes. *Sci. Rep.* **8**, 8951 (2018).
59. Chang, S.Y. *et al.* Human liver-kidney model elucidates the mechanisms of aristolochic acid nephrotoxicity. *JCI Insight.* **2**(22), e95978 (2017). https://doi.org/10.1172/jci.insight.95978
60. Edington, C.D. *et al.* Interconnected microphysiological systems for quantitative biology and pharmacology studies. *Sci. Rep.* **8**, 4530 (2018).
61. Huang, W. & Isoherranen, N. Development of a dynamic physiologically based mechanistic kidney model to predict renal clearance. *CPT Pharmacometrics Syst. Pharmacol.* **7**, 593–602 (2018).
62. Scotcher, D., Jones, C., Rostami-Hodjegan, A. & Galetin, A. Novel minimal physiologically-based model for the prediction of passive tubular reabsorption and renal excretion clearance. *Eur. J. Pharm. Sci.* **94**, 59–71 (2016).
63. Kelly, E.J. *et al.* Innovations in preclinical biology: ex vivo engineering of a human kidney tissue microperfusion system. *Stem Cell Res. Ther.* **4** (suppl. 1), S17 (2013).
64. Weber, E.J. *et al.* Development of a microphysiological model of human kidney proximal tubule function. *Kidney Int.* **90**, 627–637 (2016).
65. Ho, M.D., Ring, N., Amaral, K., Doshi, U. & Li, A.P. Human enterocytes as an in vitro model for the evaluation of intestinal drug metabolism: characterization of drug-metabolizing enzyme activities of cryopreserved human enterocytes from twenty-four donors. *Drug Metab. Dispos.* **45**, 686–691 (2017).
66. Giacomini, K.M., Galetin, A. & Huang, S.-M. The International Transporter Consortium: summarizing advances in the role of transporters in drug development. *Clin. Pharm. Ther.* **104**, 766–771 (2018).
67. Guo, Y. *et al.* Advancing predictions of tissue and intracellular drug concentrations using in vitro, imaging and physiologically based pharmacokinetic modeling approaches. *Clin. Pharmacol. Ther.* **104**, 865–889 (2018).
68. Zhang, L., Huang, S.M., Reynolds, K., Madabushi, R. & Zineh, I. Transporters in drug development: scientific and regulatory considerations. *Clin. Pharmacol. Ther.* **104**, 793–796 (2018).

Published 2019. This article is a U.S. Government work and is in the public domain in the USA. *Clinical and Translational Science* published by Wiley Periodicals, Inc. on behalf of the American Society for Clinical Pharmacology and Therapeutics. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.