ABSTRACT
Understanding the extended clearance concept and establishing a physiologically based pharmacokinetic (PBPK) model are crucial for investigating the impact of changes in transporter and metabolizing enzyme abundance/functions on drug pharmacokinetics in blood and tissues. This mini-review provides an overview of the extended clearance concept and a PBPK model that includes transporter-mediated uptake processes in the liver. In general, complete in vitro and in vivo extrapolation (IVIVE) poses challenges due to missing factors that bridge the gap between in vitro and in vivo systems. By considering key in vitro parameters, we can capture in vivo pharmacokinetics, a strategy known as the top-down or middle-out approach. We present the latest progress, theory, and practice of the Cluster Gauss-Newton method, which is used for middle-out analyses. As examples of poor IVIVE, we discuss “albumin-mediated hepatic uptake” and “time-dependent inhibition” of OATP1Bs. The hepatic uptake of highly plasma-bound drugs is more efficient than what can be accounted for by their unbound concentration alone. This phenomenon is referred to as “albumin-mediated” hepatic uptake. IVIVE was improved by measuring hepatic uptake clearance in vitro in the presence of physiologic albumin concentrations. Lastly, we demonstrate the application of Cluster Gauss-Newton method-based analysis to the target-mediated drug disposition of bosentan. Incorporating saturable target binding and OATP1B-mediated hepatic uptake into the PBPK model enables the consideration of nonlinear kinetics across a wide dose range and the prediction of receptor occupancy over time.

SIGNIFICANCE STATEMENT
There have been multiple instances where researchers’ endeavors to unravel the underlying mechanism of poor in vitro-in vivo extrapolation have led to the discovery of previously undisclosed truths. These include 1) albumin-mediated hepatic uptake, 2) the target-mediated drug disposition in small molecules, and 3) the existence of a trans-inhibition mechanism by inhibitors for OATP1B-mediated hepatic uptake of drugs. Consequently, poor in vitro-in vivo extrapolation and the subsequent inquisitiveness of scientists may serve as a pivotal gateway to uncover hidden mechanisms.

Introduction
For the past 45 years, I have been utilizing the physiologically based pharmacokinetic (PBPK) model to predict in vivo kinetics, including metabolic clearance, tissue uptake and excretion clearance, and drug-drug interactions, based on in vitro metabolism, transport, and binding experiments using cells and organelles (Iwatsubo et al., 1997; Kusuhara and Sugiyama, 2009; Shitara et al., 2013). The pharmacokinetics (PK) of drugs in vivo can be mathematically described by models incorporating parameters related to biochemical interactions between enzymes and...
In this mini-review, we provide an overview of recent research conducted at the University of Tokyo, RIKEN, and Josai International University. Over the past 20 years, our primary focus has been on addressing the challenge of predicting hepatic clearance in humans through in vitro experiments, specifically employing the approach of in vitro-in vivo extrapolation (IVIVE). This mini-review not only emphasizes our own contributions but also encompasses the work of other researchers in the field. The theoretical framework and methodology used in our research are grounded in PBPK modeling and the extended clearance concept. Moreover, we introduce a novel methodology called the Cluster-Gauss-Newton method (CGNM). Through this review, we aim to provide insights into the current state-of-the-art advancements in drug discovery based on TP recognition is expected to enable the development of drugs with ideal PK properties that maintain drug efficacy while minimizing tissue/cell transfer associated with adverse drug reactions. There is a growing interest in TP research from the standpoint of drug development. Transporters, similar to drug-metabolizing enzymes, exhibit diverse characteristics, including multiplicity, genetic polymorphism, organ specificity, inducible expression, and broad substrate recognition (Giacomini et al., 2010; Giacomini and Sugiyama, 2023). In drug development, determining the factors governing a drug’s PK properties in a clinical setting is crucial. Biopharmaceutics drug disposition classiﬁcation system was proposed by Benet and his colleagues (Wu and Benet 2005; Benet et al. 2011). TP effects in the intestine and the liver are not clinically relevant for biopharmaceutics drug disposition classification system class 1 drugs but potentially can have a high impact for class 2 (efﬂux in the gut and efﬂux and uptake in the liver) and class 3 (uptake and efﬂux in both gut and liver) drugs.

Role of Transporters in Pharmacokinetics

In living organisms, a diverse array of TPs are expressed in various tissues, playing a crucial role in facilitating the active uptake of drugs and endogenous substances, as well as their active excretion/efﬂux. Understanding drug discovery based on TP recognition is expected to enable the development of drugs with ideal PK properties that maintain drug efficacy while minimizing tissue/cell transfer associated with adverse drug reactions. There is a growing interest in TP research from the standpoint of drug development. Transporters, similar to drug-metabolizing enzymes, exhibit diverse characteristics, including multiplicity, genetic polymorphism, organ specificity, inducible expression, and broad substrate recognition (Giacomini et al., 2010; Giacomini and Sugiyama, 2023). In drug development, determining the factors governing a drug’s PK properties in a clinical setting is crucial. Biopharmaceutics drug disposition classification system was proposed by Benet and his colleagues (Wu and Benet 2005; Benet et al. 2011). TP effects in the intestine and the liver are not clinically relevant for biopharmaceutics drug disposition classification system class 1 drugs but potentially can have a high impact for class 2 (efﬂux in the gut and efﬂux and uptake in the liver) and class 3 (uptake and efﬂux in both gut and liver) drugs.

Incorporating new technologies and evaluation systems at an early stage of development can optimize PK properties, leading to efficient drug development. Thus, it is important to establish evaluation methods for quantifying the contribution ratio of TPs in each tissue and extrapolation methods from in vitro to in vivo. These approaches enable the utilization of gene expression systems in drug development, evaluation of drug-drug interactions, analysis of interindividual variation resulting from genetic polymorphisms (Rostami-Hodjegan, 2012; Yee et al., 2018), and examination of drug PK in special populations, such as those with hepatic/renal failure, aged patients, pediatrics, and during pregnancy (Howard et al., 2018). By enhancing our understanding of TPs and their impact on PK, we can advance drug discovery and improve patient care.

The Role of PBPK Modeling in Drug Approval Applications

In recent years, regulatory agencies, including the U.S. Food and Drug Administration, have increasingly relied on PK predictions using PBPK models to inform decision-making processes related to clinical trials and dosing strategies. This shift reflects the understanding that conducting exhaustive clinical trials encompassing all possible drug combinations and patient backgrounds is impractical, and regulatory requirements should not hinder the progress of drug development (Zhao et al., 2011). By accumulating data on alterations in the quantity and quality of various metabolic enzymes, TPs, and drug target proteins associated with physiologic and pathologic conditions, drug development endeavors to generate medications that minimize drug-drug interactions, exhibit reduced susceptibility to interindividual variations (including genetic variability), and possess an expanded therapeutic range (Zhao et al., 2011; Maeda and Sugiyama, 2013; Cheung et al., 2019). The utilization of PBPK models facilitates informed decision-making and enhances the efficiency of drug approval processes (Rostami-Hodjegan, 2012; Jamei, 2016).

Recent advancements in mathematical modeling have revolutionized the analysis of extensive clinical PK data accumulated over time. These models facilitate the integration of PK data with in vitro metabolism and transport data, enabling the quantification of their interrelationships. In the context of drug-drug interactions (DDIs), the contribution of relevant enzymes or TPs to the overall clearance of the victim drug, as well as the strength of inhibition (1/Ki) exerted by the inhibitor on the enzyme or TP, can be predicted using clinical reports. Notably, the withdrawal of

![Drug Interaction Diagram](image-url)

**Fig. 1.** Drug interaction between cerivastatin and gemfibrozil. In 2001, a significant number of patients died from rhabdomyolysis after taking cerivastatin, leading to its withdrawal from the market. Further investigations revealed that some of the patients who died were also using gemfibrozil, suggesting a potential drug interaction. Subsequent studies demonstrated that gemfibrozil’s metabolite, the glucuronide conjugate, acts as a potent inhibitor of CYP2C8, the primary metabolic enzyme of cerivastatin, as well as inhibiting OATP1B1, a liver uptake transporter (Shitara et al., 2013). This case highlighted the importance of considering not only metabolizing enzymes but also transporters as targets of drug interactions.
cerivastatin from the market due to rhabdomyolysis highlighted the significance of simultaneous inhibition of metabolic enzymes and TPs (Shitara et al., 2013; Iwaki et al., 2019) (Fig. 1). Subsequent studies have further elucidated the substantial interactions resulting from concurrent inhibition of TPs and metabolic enzymes in clinical practice (Yao et al., 2018) (Fig. 2). Theoretical frameworks have been established to predict instances of simultaneous dual inhibition (Fig. 2) (Ueda et al., 2001; Yao et al., 2018; Iwaki et al., 2019). These clinical events have driven the development of methodologies to predict DDIs and interindividual variability arising from genetic polymorphisms, primarily based on in vitro studies (Ueda et al., 2001; Asaumi et al., 2018; Yao et al., 2018; Taskar et al., 2020; Chu et al., 2022). Regulatory authorities have started incorporating these predicted outcomes into drug package inserts, even in the absence of direct clinical evidence (Kuemmel et al., 2020; Musuamba et al., 2021). The integration of mathematical modeling in PK analyses enhances our understanding of drug interactions and variability, facilitating informed decision-making in clinical practice.

We here want to compare the application of PBPK modeling with the extended clearance concept (Gillette and Pang, 1977; Shitara et al., 2005; Zhao et al., 2012; Shitara et al., 2013; Fujino et al., 2018; Liang and Lai, 2021). In pharmacology, clearance refers to the rate at which a drug is eliminated from the body. The clearance concept was originally proposed by two groups (Rowland et al., 1973; Wilkinson and Shand, 1975; Pang and Rowland, 1977). The clearance concept quantitatively revealed how clearance can be influenced by various factors such as liver and kidney function, enzyme activity, membrane permeability, and blood flow to the organs involved in drug elimination. PBPK modeling employs ordinary differential equations to describe the mass balance of a drug in all organs, including the blood compartment. By numerically solving these equations, drug concentration profiles in the blood and organs can be described. Consequently, calculations such as the area under the concentration-time curve (AUC) in the blood and in organs can be performed. PBPK models are also capable of handling nonlinear kinetics. In contrast, the clearance concept involves integrating the ordinary differential equations from zero to infinity to determine how the AUC in blood and organs can be represented by specific parameters. This method requires analytical integration and may pose challenges when applied to cases involving nonlinear kinetics. Within the realm of clearance concepts, the extended clearance concept (ECC) quantitatively describes the influence of biomembrane permeability by considering the transmembrane permeation process in elimination organs such as the liver and kidney (Gillette and Pang, 1977; Shitara et al., 2005, 2013). In contrast, the traditional clearance concept often assumes rapid equilibrium in tissue distribution of drugs. Considering these distinctions, PBPK modeling is particularly suitable for describing the time profiles of drug concentrations in the blood and tissues following drug administration, even in the presence of nonlinear kinetics. If the goal is to describe AUC or average concentration in a linear condition, ECC can be employed to achieve this objective.

**Prediction of Changes in Hepatic Clearance with Simultaneous Inhibition of Serial Clearance Pathway**

In cases where parallel clearance pathways are involved, the DDI guidance provides methods to predict hepatic clearance by considering the in vitro Ki and unbound inhibitor concentration for each pathway, while accounting for their respective contributions (fraction metabolized value in the case of metabolism) (Maeda and Sugiyama, 2013; Kuemmel et al., 2020; Musuamba et al., 2021). However, the prediction of...
simultaneous inhibition of serial pathways, such as uptake/metabolism or uptake/biliary excretion in the liver [e.g., organic anion transporting polypeptides (OATPs)/cytochrome P450s or OATPs/MRP2], remains a challenge. To achieve accurate predictions, it is crucial to identify the rate-determining process among elementary processes, including uptake, basolateral efflux, biliary excretion, and metabolism, that significantly contribute to hepatic clearance (Fig. 2). The theoretical development and experimental proof of this methodology based on the ECC was already published in 2001 (Ueda et al., 2001). However, estimating the intracellular unbound inhibitor concentration poses a major obstacle in applying this methodology, particularly when predicting intracellular enzyme and efflux TP-mediated inhibitions and inductions (Ueda et al., 2001; Asaumi et al., 2018; Yao et al., 2018).

Traditionally, it has been assumed that intracellular and extracellular concentrations of unbound drugs are equal in the field of PK. However, emerging evidence has highlighted the involvement of active TPs in drug uptake and efflux processes, challenging this assumption (Giacomini et al., 2010; Giacomini and Sugiyama, 2023). It is now recognized that intracellular and extracellular concentrations of drugs may not be equal. To evaluate active and passive uptake clearance separately under linear conditions, the initial rate of drug uptake into hepatocytes at various drug concentrations can be measured, enabling the determination of active uptake clearance (Vmax/Km) and passive uptake clearance (PSinf,dif) (Yabe et al., 2011). Estimating tissue-to-plasma unbound concentration ratio (Kpuu values) has been proposed by assuming that passive transport clearance is the same for both uptake (PSinf,dif) and efflux (PSeff,dif). However, this assumption is not valid for charged compounds. Notably, hepatocytes possess an inside negative membrane potential of approximately ~40 mV, leading to PSinf,dif < PSeff,dif for anions and PSinf,dif > PSeff,dif for cations. To address this, we have proposed a methodology for estimating hepatocyte-to-medium unbound concentration ratio (Kpuu values) that considers the membrane potential (Yoshikado et al., 2017). Since then, various advancements have been made in the measurement of Kpuu values (Guo et al., 2018), recommending the assessment of Kp values in hepatocytes in the presence of albumin/plasma and the estimation of in vivo Kpuu through intracellular and extracellular binding measurements (Riccardi et al., 2017; Di et al., 2021).

Revisiting the Free Hypothesis for Improved In Vitro-In Vivo Extrapolation: Significance of Measuring Unbound Uptake Clearance in the Presence of Physiologic Albumin Concentration

In pharmacology, the “free hypothesis” refers to the assumption that the unbound (free) concentration of a drug in the bloodstream is the pharmacologically active form. According to this hypothesis, only the unbound fraction of a drug is available for distribution to tissues, metabolism, and elimination processes. While many studies support these hypotheses, there have been challenges to their validity dating back 35 to 40 years (Forker and Luxon, 1981; Weisiger et al., 1981; Tsao et al., 1986). However, limitations in demonstrating these hypotheses in human PK studies hindered further investigation. Recently, this research area has regained attention, particularly in IVIVE studies of hepatic clearance using anionic drugs. It has been observed that the predicted hepatic clearance of highly protein-bound compounds is underestimated based on the free hypothesis, and this underestimation can be improved by measuring hepatic uptake in vitro in the presence of physiologic albumin concentrations (Fig. 3) (Poulin et al., 2012; Miyachi et al., 2018; Bowman et al., 2019; Kim et al., 2019; Miyachi et al., 2022). This phenomenon is explained by considering a model in which a binding site on the hepatocyte surface interacts with albumin, facilitating the dissociation of the free drug from albumin on the cell surface and subsequent uptake into cells (Fig. 4) (Miyachi et al., 2018; Kim et al., 2019).
2019). This model is referred to as the albumin-mediated facilitated dissociation model. Saturable binding of albumin to the hepatocyte surface occurs with Kd values ranging from 25 to 160 μM (Weisiger et al., 1981; Tsao et al., 1986; Miyauuchi et al., 2018; Kim et al., 2019; Miyauuchi et al., 2022). Other research groups have also attempted to improve the accuracy of IVIVE for highly plasma-protein-bound drugs by measuring hepatic clearance in the presence of plasma or physiologic concentrations of albumin, utilizing mechanism-based models (Poulin et al., 2012; Poulin and Haddad, 2018; Miyauuchi et al., 2022). Bi et al. (Bi et al., 2021) employed 19 OATP1B compounds to determine unbound hepatic uptake clearance in the absence of plasma, based on our proposed albumin-mediated facilitated dissociation model. This relationship is well explained by the facilitated dissociation model, but not by other free hypothesis adjusted models (Bi et al., 2021). However, a recent study by Yin et al. (Yin et al., 2022) raises questions regarding the phenomenon of albumin-mediated hepatic uptake, suggesting it may be an artifact stemming from the nonspecific binding of the albumin-drug complex to the cell surface. Further discussions and investigations are needed to address this concern.

The Mechanism Through Which In Vivo Drug-Drug Interactions Cannot Be Accurately Predicted Using Ki Values Obtained from In Vitro Experiments

As the analysis of DDIs has accumulated, it has become evident that in vitro parameters, such as Ki values, often do not accurately reflect the in vivo situation. This discrepancy raises the question of why this occurs. For instance, when studying the inhibition of OATP1B by cyclosporin A, it was observed that preincubating OATP1B-expressing cells or hepatocytes with cyclosporin A for 30 to 60 minutes resulted in Ki values more than 10-fold lower than those measured without preincubation (Shitara and Sugiyama, 2017; Tătărai et al., 2019; Izumi et al., 2023). Although the Ki values obtained with preincubation are closer to in vivo Ki values compared with those obtained from in vitro preincubation experiments may be quantitatively explained (Shitara and Sugiyama, 2017).

In the case of mechanism-based inhibition of drug-metabolizing enzymes, clinical trials have shown significant variation in the degree of DDI when the timing of inhibitor administration and substrate drug is shifted. This phenomenon has been successfully captured through PBPK modeling that incorporates the mechanism-based inhibition mechanism (Honkalammi et al., 2011; Kim et al., 2017; Varma et al., 2019). Incorporating the trans-inhibition mechanism of inhibitors described here into the PBPK model holds the potential to enhance IVIVE with improved predictability (Shitara and Sugiyama, 2017).

The current quantitative prediction of DDIs based on in vitro Ki values remains inadequate, as previously mentioned. However, there have been significant advancements in the development of successful methods for predicting the magnitude of DDIs associated with OATP1B using endogenous biomarkers like coproporphyrin-I (Chu et al., 2018; Rodrigues et al., 2018; Barnett et al., 2019; Mochizuki et al., 2022; Yoshikado et al., 2022). These methods have even demonstrated their efficacy in predicting changes in PK among special populations (Lin et al., 2023). It is important to note that the aforementioned approach becomes feasible only during the clinical phase of a project. In recent studies, researchers have employed a “middle-out” method in PBPK modeling approach in preclinical models, including monkeys, to bridge the gap in IVIVE and the application of scaling factors for PK predictions in the early stages of drug discovery (Gu et al., 2020).

Advancing the Middle-Out Approach for PBPK Modeling Methodology Based on Cluster Gauss-Newton Method

The CGNM algorithm, developed by Aoki et al. (Aoki et al., 2022), offers a solution for optimizing parameters in PBPK models (Fig. 5). PBPK models face challenges where some parameters may not be identifiable from available data, and initial parameter estimates for optimization methods may not be readily available. In our research group, we have employed CGNM to investigate nonlinear PK and DDIs using PBPK models (Koyama et al., 2021; Mochizuki et al., 2022; Yoshikado et al., 2022). We have found that CGNM simplifies the process of fitting PBPK models to available data, enabling a top-down approach to derive in vivo parameters even for complex PBPK models by matching the model with clinical PK data. However, we have observed a discrepancy...
between the mathematically optimal parameter combinations obtained through CGNM and the knowledge derived from in vitro experiments. Identifying the cause of this discrepancy poses a significant challenge. It is likely that the simplifying assumptions made during model development contribute to this bias. As it is impractical to include all drug absorption, distribution, metabolism, and excretion and physiologic mechanisms, our model may not fully capture the complexity of PK. Consequently, the parameter combinations that appear mathematically optimal may be biased due to the omission of relevant mechanisms during model building, raising concerns about their biologic accuracy. In contrast, the conventional bottom-up approach of IVIVE often requires the use of scaling factors to align predictions with in vivo PK observations. This discrepancy arises due to various factors, including variations in measured values from in vitro experimental systems under different conditions and the inability of these systems to fully replicate physiologic processes. For example, extrapolating in vitro hepatic uptake of drugs with high plasma albumin binding to in vivo scenarios based on the free hypothesis, as discussed earlier (Miyachi et al., 2022), may lead to inconsistencies. Overall, the development of PBPK models using the CGNM approach presents a promising advancement in overcoming parameter optimization challenges. Addressing the discrepancies between mathematically optimal parameter combinations and knowledge derived from in vitro experiments remains a complex task, emphasizing the need for careful consideration of model assumptions and the limitations of in vitro systems.

To address the inconsistencies between top-down and bottom-up approaches, the middle-out approach, which combines both approaches, has gained popularity. The middle-out approach aims to obtain PBPK models in drug development to showcase the computational efficiency and robustness of CGNM compared with the standard Levenberg-Marquardt method, as well as state-of-the-art multistart and derivative-free methods (Aoki et al., 2022).

**Cluster Gauss-Newton Method (CGNM)**

- Requires only setting wide ranges for initial values of parameters.
- Obtains multiple sets of optimized parameters.
- Can estimate many unknown parameters.

**Conventional method (e.g. Levenberg–Marquardt method)**

- Requires appropriate initial value for parameters.
- Obtains only a single set of optimized parameters.
- Requires derivatives (Jacobian).
- Has to start with different initial parameters.

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**Fig. 5.** Cluster Gauss-Newton method. CGNM is an algorithm designed to find multiple approximate minimizers for nonlinear least squares problems, with applications to parameter estimation in pharmacokinetic models. This figure demonstrates the use of PBPK models in drug development to showcase the computational efficiency and robustness of CGNM compared with the standard Levenberg-Marquardt method, as well as state-of-the-art multistart and derivative-free methods (Aoki et al., 2022).

**Fig. 6.** Middle-out approach in CGNM. In the middle-out approach using CGNM, the minimization process goes beyond minimizing the SSR and also includes minimizing the SSP, such as Km values obtained in vitro and those estimated from blood concentration-time profiles. This approach aims to refine the parameter estimation using CGNM (Yoshikado et al., 2022). SSR, sum of squares of residuals; SSP, sum of squares of differences between parameters.
model fits that are consistent with both in vitro and in vivo data. One possible strategy is to fit the PBPK model to a combined dataset of in vivo and in vitro data. This can be mathematically formulated, as illustrated in Fig. 6, and can be viewed as setting a prior in Bayesian statistics (Cole et al., 2014) or as a form of regularization in the frequentist sense (Bishop, 1995). However, due to the inherent differences between in vitro and in vivo data, simply pooling the data may not be sufficient. It may be crucial to assign appropriate weights to the data, which introduces subjectivity into the analysis. Our objective is to establish a general strategy or guideline for conducting middle-out approach analyses. We aim to achieve this by applying the proposed approach to various clinical and in vitro experimental data sets, as well as different PBPK models (Yoshikado et al., 2022) (see Fig. 6). Importantly, it should be emphasized that in top-down and middle-out analyses, the mathematically optimal solution (minimum sum of squared residuals) may not always be biologically or pharmacokinetically valid. In some cases, a solution with a slightly higher sum of squared residuals may be deemed more biologically plausible. By investigating the middle-out approach and its application to diverse datasets and models, our research aims to provide valuable insights and establish guidelines for effectively integrating in vitro and in vivo data in PBPK modeling. This will contribute to improved accuracy and reliability in optimizing PBPK models for various applications.

Target-Mediated Drug Disposition Analysis Using the PBPK Model

In this section, we demonstrate the application of CGNM to analyze the nonlinear PK of a small molecule drug exhibiting target-mediated drug disposition (TMDD). TMDD refers to the phenomenon where drug binding to a molecular target influences the drug's disposition, resulting in dose- and time-dependent PK profiles (An, 2017; Lee et al., 2023). TMDD was first described by Levy in 1994 using warfarin as an example (Levy, 1994). While TMDD has been extensively studied in the context of biologics, such as antibodies, its role in small molecule drugs is also important and warrants quantitative prediction (Dua et al., 2015). To achieve this, we focus on analyzing specific examples and assessing the contribution of saturable binding to molecular targets in

Saturation of hepatic uptake alone does not explain the time profile in blood concentrations of low doses of bosentan.

Fig. 7. (A) TMDD-PBPK model of bosentan (without considering TMDD). A PBPK model is developed to incorporate saturation mechanisms for target binding, as well as other pharmacokinetic process OATP1B-mediated hepatic uptake. (B) The model parameters are optimized by fitting the model to published data showcasing nonlinear pharmacokinetic profiles over a wide dose range (Koyama et al., 2021). First, when the model does not consider TMDD, it fails to adequately explain the plasma concentration-time profiles at the lowest dose (10 mg i.v.) as shown by a red arrow (Sato et al., 2018).
comparison with other saturation mechanisms in PK, such as saturation of metabolism or transport in the liver or intestinal tract.

As a model case, we present the analysis of bosentan, a small molecule drug displaying TMDD (Koyama et al., 2021). A PBPK model was developed to incorporate saturation mechanisms for target binding and other pharmacokinetic processes, including hepatic uptake saturation (Koyama et al., 2021; Lee et al., 2023). The parameters of the PBPK model were optimized using CGNM (Aoki et al., 2022), fitting the model to published data that exhibited nonlinear PK profiles across a wide dose range. Initially, we analyzed a model without molecular target binding and found that it failed to explain the plasma concentration-time profiles at the lowest dose (10 mg i.v.) (Fig. 7) (Sato et al., 2018). Consequently, CGNM was employed to optimize 10 parameters, including molecular target binding parameters (Kd, koff, Bmax) (Koyama et al., 2021). In the case of bosentan, where the molecular target is expressed on various tissue endothelial cell membranes, the PBPK model incorporated saturable binding parameters (Kd, koff, Bmax) to the molecular target compartment directly connected to the circulating blood compartment (Koyama et al., 2021) (Fig. 7). The parameters of the PBPK model were optimized by fitting the blood PK profiles reported after intravenous and oral administration of bosentan across a wide range of doses (Koyama et al., 2021). The CGNM-based analysis generated multiple optimized parameter sets, which were subsequently used to simulate blood PK and in vivo molecular target occupancy profiles (Fig. 8). Mathematical and statistical analyses were further performed to evaluate the impact of dose selection on parameter estimation for bosentan (Koyama et al., 2021) and warfarin (Lee et al., 2023). The optimized parameter set successfully described the reported blood PK profile of bosentan (Fig. 8).

When considering the findings from the TMDD analysis of warfarin presented in this special issue (Lee et al., 2023), along with the results obtained for bosentan, we observe that for drugs interacting with molecular targets of high affinity and specificity, incorporating saturating molecular target binding enables the prediction of in vivo molecular target occupancy profiles using only dose-dependent drug concentration-time profiles across a wide dose range (Koyama et al., 2021; Lee et al., 2023). Further analyses indicate the potential for more precise prediction of the time profile of molecular target occupancy, particularly if microdosing is employed as the initial dose in the dose-escalation process during phase I clinical trials (Burt et al., 2020; Koyama et al., 2021; Lee et al., 2023). By conducting additional validation studies on other small molecule drugs that exhibit TMDD, our aim is to compile and present the characteristics of drugs for which molecular target occupancy can be reliably predicted in phase I clinical trials. If successful, this approach has the potential to revolutionize the drug development process, offering significant advancements in our ability to predict and optimize the therapeutic effects of novel drug candidates.

**Future Prospects**

Numerous instances have been reported where the conventional IVIVE approach, which involves simply scaling kinetic parameters from in vitro experiments using physiologic factors, fails to quantitatively predict in vivo phenomena (Sato et al., 2018; Kim et al., 2019; Koyama et al., 2021). The reasons behind these discrepancies are multifaceted, including variations in measurements within in vitro experimental systems under different conditions and the challenge of faithfully replicating
complex physiologic systems in vitro. As a result, the middle-out approach, which considers a range of in vitro measurements, is gaining prominence over the pure top-down approach that solely seeks parameters to explain clinical data. In this regard, the middle-out approach can leverage the CGNM algorithm for its implementation.

It is crucial to emphasize that poor IVIVE outcomes should not be seen as failures but rather as opportunities to uncover hidden truths, fueled by the curiosity of scientists. Such challenges drive researchers to explore novel methodologies and approaches, ultimately leading to a deeper understanding of the complex relationship between in vitro and in vivo systems.

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Data Availability

The authors declare that all the data supporting the findings of this study are available within the paper.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Sugiyama, Aoki.

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