In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies

Guidance for Industry

DRAFT GUIDANCE

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For questions regarding this draft document, contact (CDER) Office of Clinical Pharmacology, Guidance and Policy Team at CDER_OCP_GPT@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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Clinical Pharmacology

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TABLE OF CONTENTS

In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies Guidance for Industry[1]

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I. INTRODUCTION

This guidance is intended to help drug developers plan and evaluate studies to determine the drug-drug interaction (DDI) potential of an investigational drug product.[2] It focuses on in vitro experimental approaches to evaluate the interaction potential between investigational drugs that involves metabolizing enzymes and/or transporters. This guidance also discusses how in vitro results can inform future clinical DDI studies. The appendices of this guidance include considerations when choosing in vitro experimental systems, key issues regarding in vitro experimental conditions, and more detailed explanations regarding model-based DDI prediction strategies. See section VIII for a list of terms used in this guidance and their definitions.

If an in vitro assessment suggests that the sponsor should conduct a clinical DDI study, the sponsor should refer to a related guidance addressing the conduct and interpretation of clinical drug interaction studies (draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*).[3] Together, these two guidances describe a systematic, risk-based approach to assessing the DDI potential of investigational drugs and making recommendations to mitigate DDIs and will replace the 2012 draft guidance entitled

[1] This guidance has been prepared by the Office of Clinical Pharmacology, Office of Translational Sciences in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] Only small molecule drugs are covered in this guidance. Interactions involving biologics (therapeutic proteins) are beyond the scope of this guidance.

[3] When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA Drugs guidance Web page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

Drug Interaction Studies – Study Design, Data analysis, Implications for Dosing, and Labeling Recommendations.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Evaluating the DDI potential of an investigational new drug involves: (1) identifying the principal routes of the drug's elimination; (2) estimating the contribution of enzymes and transporters to the drug's disposition; and (3) characterizing the effect of the drug on enzymes and transporters. This evaluation often starts with in vitro experiments to identify potential factors influencing drug disposition to elucidate potential DDI mechanisms and to yield kinetic parameters for use in further studies. Results of in vitro experiments, along with clinical pharmacokinetic (PK) data, provide mechanistic information that can inform the need and proper design of potential future clinical studies. Various modeling approaches can translate in vitro observations into in vivo predictions of potential clinical DDIs. For example, when evaluating the drug as a perpetrator (i.e., an inhibitor or inducer) of a metabolism-mediated DDI, basic models (Einolf 2007; Einolf, Chen, et al. 2014; Vieira, Kirby, et al. 2014), static mechanistic models (Einolf 2007; Fahmi, Hurst, et al. 2009; Einolf, Chen, et al. 2014), or dynamic mechanistic models including physiologically-based pharmacokinetic (PBPK) models (Zhao, Zhang, et al. 2011; Zhao, Rowland, et al. 2012; Jones, Chen, et al. 2015; Wagner, Zhao, et al. 2015; FDA draft guidance for industry *Physiologically Based Pharmacokinetic Analyses — Format and Content*[4]) can guide decisions on when and how to conduct a clinical DDI study. This guidance outlines a general framework for conducting in vitro experiments and interpreting in vitro study results to determine the potential for clinical DDIs.

The recommendations in this guidance are based on current scientific understanding. The recommendations outlined here may be periodically updated as the scientific field of DDIs evolves and matures. Refer to the appendices for general considerations regarding in vitro systems for drug development and regulatory purposes.

III. EVALUATING METABOLISM-MEDIATED DRUG INTERACTIONS

Many drugs undergo metabolism as a major mechanism of bioactivation (e.g., in the case of

[4] When final, this guidance will represent the FDA's current thinking on this topic.

prodrugs) or clearance from the body. Drugs can be metabolized in several organs, including, but not limited to, the liver, kidney, gut wall, and lung. Drug metabolism primarily occurs in the liver and intestine. These organs express a wide variety of drug metabolizing enzymes and are responsible for the biotransformation of many drugs. Hepatic metabolism occurs primarily through the cytochrome P450 (CYP) family of enzymes located in the hepatic endoplasmic reticulum but can also occur through non-CYP enzymes, including Phase II glucuronosyl- and sulfo-transferases. Sponsors should examine the potential for interactions between these metabolizing enzymes and investigational drugs. Although certain clinical PK information is necessary to quantify the potential for interactions between metabolizing enzymes and investigational drugs, sponsors should initiate in vitro metabolic studies before first-in-human studies to inform the need and design of these clinical studies. We recommend that the sponsor conducts the following in vitro studies to evaluate the potential for metabolism-mediated drug interactions.

A. Determining if the Investigational Drug is a Substrate of Metabolizing Enzymes

1. Conducting In Vitro Studies

The sponsor should routinely evaluate CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 using in vitro phenotyping experiments to determine which enzymes metabolize the investigational drug. However, it is possible that the investigational drug undergoes significant in vivo metabolism that is not mediated by these major CYP enzymes. In this event, the investigational drug is probably a substrate for additional enzymes, and the sponsor should determine what additional enzymes contribute to the metabolism of the investigational drug. These additional enzymes include but are not limited to:

- CYP enzymes including CYP2A6, CYP2J2, CYP4F2, and CYP2E1
- Other Phase I enzymes including monoamine oxidase (MAO), flavin monooxygenase (FMO), xanthine oxidase (XO), and alcohol/aldehyde dehydrogenase
- Phase II enzymes including UDP glucuronosyl transferases (UGTs)

2. Data Analysis and Interpretation

The contribution of a specific metabolizing enzyme to an investigational drug's clearance is considered significant if the enzyme is responsible for $\geq$ 25% of the drug's elimination based on the in vitro phenotyping studies and human PK data. Under these circumstances, the sponsor should conduct clinical DDI studies using strong index inhibitors and/or inducers of the enzyme (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study*

Design, Data Analysis, and Clinical Implications[5]). Refer to the appendix, section VII.A.1, for additional considerations regarding data analysis.

B. Determining if the Investigational Drug is an Inhibitor of Metabolizing Enzymes

1. Conducting In Vitro Studies

The sponsor should evaluate an investigational drug's potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 in both a reversible manner (i.e., reversible inhibition) and time-dependent manner (i.e., time-dependent inhibition (TDI)).

2. Data Analysis and Interpretation

For basic models of reversible inhibition, the sponsor should calculate the predicted ratio of the area under the plasma concentration-time curve (AUC) for the victim (substrate) drug in the presence and absence of an inhibitor. This ratio is referred to as R_1 (see the appendix, section VII.C1). For CYP3A, $R_{1,gut}$ should also be calculated as shown in Figure 1.

Figure 1: Equations to Calculate the Predicted Ratio of Victim Drug's AUC in the Presence and Absence of an Inhibitor for Basic Models of Reversible Inhibition

$$R_1 = 1 + (I_{max,u} / K_i)$$

$$R_{1,gut} = 1 + (I_{gut} / K_i)$$

R_1 or **$R_{1,gut}$** is the predicted ratio of the victim drug's AUC in the presence and absence of an inhibitor for basic models of reversible inhibition.
$I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug.*
I_{gut} is the intestinal luminal concentration of the interacting drug calculated as the dose/250 mL.
K_i is the unbound inhibition constant determined in vitro.

Note: I and K_i need to be expressed in the same unit (e.g., in a molar concentration unit).

*Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

For basic models of TDI, the sponsor should calculate the predicted ratio of the victim drug AUC in the presence and absence of an inhibitor (R_2) as described in Figure 2.

[5] When final, this guidance will represent the FDA's current thinking on this topic.

Figure 2: Equations to Calculate the Predicted Ratio of the Victim Drug's AUC in the Presence and Absence of an Inhibitor for Basic Models of TDI (Yang, Liao, et al. 2008; Grimm, Einolf, et al. 2009; Vieira, Kirby, et al. 2014)

$$R_2 = (k_{obs} + k_{deg}) / k_{deg}$$

Where $k_{obs} = (k_{inact} \times 50 \times I_{max,u}) / (K_I + 50 \times I_{max,u})$

R_2 is the predicted ratio of the victim drug's AUC in the presence and absence of an inhibitor for basic models of enzyme TDI.
k_{obs} is the observed (apparent first order) inactivation rate constant of the affected enzyme.
k_{deg} is the apparent first-order degradation rate constant of the affected enzyme.
K_I is the inhibitor concentration causing half-maximal inactivation.
k_{inact} is the maximal inactivation rate constant.
$I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug.*

Note: I and K_I need to be expressed in the same unit (e.g., in a molar concentration unit).

*Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

If $R_1 \geq 1.02$, $R_2 \geq 1.25$ (Vieira, Kirby et al. 2014) or the $R_{1,gut} \geq 11$ (Tachibana, Kato, et al. 2009; Vieira, Kirby, et al. 2014), the sponsor should further investigate the DDI potential by either using mechanistic models (see the appendix, section VII.C) or conducting a clinical DDI study with a sensitive index substrate. If the predicted AUC ratio (AUCR) of a sensitive index substrate in the presence and absence of an investigational drug is ≥ 1.25 based on static mechanistic models or dynamic mechanistic models (e.g., PBPK models) (see appendix, section VII.C), the sponsor should conduct a clinical DDI study using a sensitive index substrate.

When static mechanistic models or PBPK models (see appendix, section VII.C) are used for predicting DDIs caused by enzyme inhibition, the models should include the inhibition mechanism only (i.e., the model should not include concurrent induction predictions for an investigational drug that is hypothesized to be both an inducer and inhibitor) to definitively assess the potential of the investigational drug to inhibit metabolizing enzymes.

C. Determining if the Investigational Drug is an Inducer of Metabolizing Enzymes

1. Conducting In Vitro Studies

The sponsor should evaluate the potential of an investigational drug to induce CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5. Initially, sponsors can conduct experiments to evaluate CYP1A2, CYP2B6, and CYP3A4/5 only. If no induction of CYP3A4/5 enzymes is observed, evaluating the induction potential of CYP2C enzymes is not necessary

because both CYP3A4/5 and CYP2C enzymes are induced via activation of the pregnane X receptor (PXR). If the investigational drug induces CYP3A4/5, however, the sponsor should evaluate the potential of the investigational drug to induce CYP2C.

2. Data Analysis and Interpretation

Several basic methods can assess the potential of an investigational drug to induce metabolizing enzymes (Fahmi, Kish, et al. 2010; Fahmi and Ripp 2010; Einolf, Chen, et al. 2014). Three of them are described in detail below:

- **Fold-change method**: The sponsor can examine the fold-change in CYP enzyme mRNA levels when incubated with the investigational drug by using a cutoff determined from known positive and negative controls to calibrate the system. For example, a ≥ 2-fold increase in mRNA and a response ≥ 20% of the response of the positive control in the presence of an investigational drug are interpreted as a positive finding.

- **Correlation methods**: The sponsor may use correlation methods with predicted positive criteria defined by known positive (e.g., known inducers of the same enzyme) and negative controls as described in Figure 3.

Figure 3: Two Correlation Methods to Assess the Potential of an Investigational Drug to Induce Metabolizing Enzymes (Fahmi and Ripp, 2010)

Correlation Method 1: Calculate a relative induction score (RIS) using $(E_{max} \times I_{max,u}) / (EC_{50} + I_{max,u})$
OR
Correlation Method 2: Calculate $I_{max,u} / EC_{50}$ values

Determine the magnitude of a clinical induction effect (e.g., AUC ratio of index substrate in the presence and absence of inducers) according to a calibration curve of RIS scores or $I_{max,u}/EC_{50}$ for a set of known inducers of the same enzyme.

E_{max} is the maximum induction effect determined in vitro.
EC_{50} is the concentration causing half-maximal effect determined in vitro.
$I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug.*

*Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

- **Basic kinetic model**: To use this method, the sponsor should calculate the ratio of victim drug AUC in the presence and absence of an inducer (R_3) as described in Figure 4.

Figure 4: An Equation to Calculate the Predicted Ratio of the Victim Drug's AUC in the Presence and Absence of an Inducer for Basic Models of Induction

$$R_3 = 1 / [1 + (d \times E_{max} \times 10 \times I_{max,u}) / (EC_{50} + (10 \times I_{max,u}))]$$

R_3 is the predicted ratio of the victim drug's AUC in the presence and absence of an inducer for basic models of enzyme induction.
d is the scaling factor and is assumed to be 1 unless supported by prior experience with the system used.
E_{max} is the maximum induction effect determined in vitro.
$I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug.*
EC_{50} is the concentration causing half-maximal effect determined in vitro.

*Considering uncertainties in the protein binding measurements, the unbound fraction should be set to 1% if experimentally determined to be <1%.

If any of these methods indicates that the investigational drug has the potential to induce metabolizing enzymes (using specific cutoff values developed by individual laboratories for Methods 1 and 2 or if $R_3 \leq 0.8$), the sponsor should further investigate the enzyme induction potential of the investigational drug by using mechanistic models (see the appendix, section VII.C) or by conducting a clinical DDI study with a sensitive index substrate. If the predicted AUCR of a sensitive index substrate in the presence and absence of an investigational drug is ≤ 0.8 based on static mechanistic models or dynamic mechanistic models (e.g., PBPK models; see appendix, section VII.C), the sponsor should further investigate potential DDIs by conducting a clinical DDI study using a sensitive index substrate.

When static mechanistic models or dynamic mechanistic models (e.g., PBPK models; see appendix, section VII.C) are used for predicting DDIs caused by enzyme induction, the models should include the induction mechanism only (i.e., the model should not include concurrent inhibition predictions for an investigational drug that is hypothesized to be both an inducer and inhibitor) to definitively assess the potential of an investigational drug to induce metabolizing enzymes.

3. Additional Considerations

The AUCR cutoffs of > 0.8 (for induction) and < 1.25 (for inhibition) using mechanistic models are the suggested default values to indicate that the investigational drug has no effect on the levels of metabolizing enzymes.

When evaluating whether an investigational drug is an inhibitor of multiple CYP enzymes, the sponsor can prioritize in vivo DDI evaluations for various CYP enzymes with sensitive index substrates of respective pathways (see the FDA's draft guidance for industry *Clinical Drug*

Interaction Studies — Study Design, Data Analysis, and Clinical Implications[6]) based on rank-ordered R_1, R_2, or the predicted AUCR values, preferably using the in vitro inhibition parameters obtained in the same study.[7] That is, the sponsor may first carry out an in vivo study with a sensitive index substrate of the CYP with the largest R or AUCR value. If this in vivo study shows no interaction, in vivo evaluations of other CYPs with lower potencies (e.g., smaller R or AUCR) are not needed. However, if this in vivo study shows a positive interaction between the drug and the sensitive index CYP substrate, the sponsor should conduct additional in vivo studies for other CYPs, starting with the CYP with the next largest R or AUCR value. Alternatively, the sponsor can use a PBPK model to inform the need for the conduct of additional studies. The sponsor should verify and update any PBPK models to demonstrate that the model can adequately describe the observed findings from the first in vivo study with a sensitive index substrate.

IV. EVALUATING TRANSPORTER-MEDIATED DRUG INTERACTIONS

Membrane transporters can have clinically relevant effects on the pharmacokinetics and pharmacodynamics of a drug in various organs and tissues by controlling its absorption, distribution, and elimination (Giacomini, Huang, et al. 2010; Giacomini and Huang 2013). In contrast to drug metabolizing enzymes that are largely expressed in the liver and small intestines, transporters are expressed in tissues throughout the human body and govern the access of endogenous and exogenous substances to various sites in the body. Transporters, in concert with metabolizing enzymes, can govern a drug's disposition and pharmacological action. Conversely, a drug can also modulate transporter expression or activity, resulting in altered disposition of endogenous (e.g., creatinine, glucose) or exogenous substances.

Several transporters interact with drugs in clinical use (Giacomini, Huang, et al. 2010; Giacomini and Huang 2013), for example:

- P-glycoprotein (P-gp or Multi-drug Resistance 1 (MDR1) protein)
- Breast cancer resistance protein (BCRP)
- Organic anion transporting polypeptide 1B1/1B3 (OATP1B1/OATP1B3)
- Organic anion transporter 1/3 (OAT1/OAT3)
- Multidrug and toxin extrusion (MATE) proteins
- Organic cation transporter 2 (OCT2)

[6] When final, this guidance will represent the FDA's current thinking on this topic.

[7] An orally administered drug may inhibit intestinal metabolic enzymes (e.g., CYP3A) in addition to hepatic enzymes. Therefore, in vivo DDI for CYP3A inhibition needs to be considered if $R_{1,gut}$ is greater than or equal to 11, even if R_1 for CYP3A is not the largest value among the major CYPs evaluated.

The consequence of a drug interaction mediated by transporters may not be apparent if a clinical DDI study only measures systemic drug exposures. However, understanding whether the drug is a substrate or perpetrator (i.e., inhibitor or inducer) of these key transporters can explain some clinical consequences, such as increased toxicity or altered efficacy, that result from altered tissue distribution of a drug that is a substrate of a transporter.

This section focuses on transporters that have clinical evidence suggesting their involvement in drug interactions (Giacomini, Huang, et al. 2010; Brouwer, Keppler, et al. 2013; Giacomini and Huang 2013; Tweedie, Polli, et al. 2013; Zamek-Gliszczynski, Lee, et al. 2013). The sponsor should evaluate the interactions between investigational drugs acting as substrates and/or perpetrators of these transporters as outlined below. The timing of the in vitro evaluation of each transporter may vary depending on the therapeutic indications of the investigational drug. For example, if the intended population is likely to use statins, the sponsor should examine the potential of the investigational drug to interact with OATP1B1/1B3 before clinical studies in patients. If in vitro experiments indicate a low potential for an interaction between the transporter and investigational drug, subjects taking statins may be included in clinical studies to better represent the intended patient population.

A. Determining if the Investigational Drug is a Substrate of the Transporters P-gp and BCRP

P-gp and BCRP are expressed in various tissues including the gastrointestinal tract, liver, kidney, and brain. Thus, both transporters have the potential to impact the oral bioavailability, the tissue distribution, and the hepatic and renal elimination of substrates.

1. Conducting In Vitro Studies

Sponsors should evaluate most investigational drugs in vitro to determine whether they are substrates of P-gp and BCRP using the experimental systems described in the appendix, section VII.B. P-gp and BCRP are not expected to impact the oral bioavailability of highly permeable and highly soluble drugs. In vitro assessment of these drugs as P-gp or BCRP substrates is not suggested unless there are potential safety concerns with the drug distributing into tissues (e.g., the kidney and brain). See the FDA's guidance for industry entitled *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*[8] to determine if the investigational drug can be classified as highly permeable and/or highly soluble (e.g., biopharmaceutics classification system class 1 drugs).

[8]When final, this guidance will represent the FDA's current thinking on this topic.

2. Data Analysis and Interpretation

The following results suggest that an investigational drug is an in vitro P-gp substrate:

- A net flux ratio (or efflux ratio (ER)) of ≥ 2 for an investigational drug in cells that express P-gp (e.g., Caco-2 cells or other cells overexpressing P-gp)[9]

- A flux that is inhibited by at least one known P-gp inhibitor at a concentration at least 10 times its K_i (e.g., the ER decreases to < 50% of the ER in the absence of inhibitor or the flux reduced to unity).

When using Caco-2 cells that express multiple efflux transporters, the sponsor should use more than one P-gp inhibitor to determine the specificity of the efflux. The sponsor may use a net flux ratio cutoff other than 2 or a specific relative ratio to positive controls if prior experience with the cell system justifies these alternative methods.

If in vitro studies indicate that a drug is a P-gp substrate, the sponsor should consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known P-gp inhibitors in the indicated patient population (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[10]).

The sponsor may also use the above procedures to determine whether the drug is a BCRP substrate by using known BCRP inhibitors. If in vitro studies indicate that a drug is a BCRP substrate, the sponsor should consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known BCRP inhibitors in the indicated patient population (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[11]).

B. Determining if the Investigational Drug is a Substrate of the Hepatic Transporters OATP1B1 and OATP1B3

OATP1B1 and OATP1B3 are key uptake transporters expressed on the sinusoidal membrane of hepatocytes and play an important role in the hepatic uptake of various drugs.

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[9] The ER can be calculated as the ratio of the basal to apical (B-A) transport rate to the apical to basal (A-B) transport rate. The net flux ratio can be calculated as the ratio of the ER between cells expressing the transporter of interest to cells not expressing the transporter.

[10] When final, this guidance will represent the FDA's current thinking on this topic.

[11] When final, this guidance will represent the FDA's current thinking on this topic.

1. Conducting In Vitro Studies

If in vitro studies or human/animal absorption, distribution, metabolism, and/or excretion (ADME) data suggest that an investigational drug's hepatic uptake or elimination is significant (i.e., the drug's clearance through hepatic metabolism or biliary secretion is ≥ 25% of the total drug's clearance), or the drug's uptake into the liver is clinically important (e.g., for biotransformation or to exert a pharmacological effect), the sponsor should evaluate the investigational drug in vitro to determine whether it is a substrate for the hepatic uptake transporters OATP1B1 and OATP1B3 (see the appendix, section VII.B).

2. Data Analysis and Interpretation

An investigational drug is considered an in vitro substrate for OATP1B1 or OATP1B3 if: (1) the uptake of the drug in OATP1B1- or OATP1B3-transfected cells is ≥ 2-fold of the drug's uptake in empty vector-transfected cells; and (2) a known inhibitor (e.g., rifampin) can decrease the drug's uptake to ≤ 50% at a concentration at least 10 times that of the K_i or IC_{50}. The sponsor may justify alternative cutoff ratios based on its prior experience with the cell system.

If in vitro studies indicate that a drug is an OATP1B1 or OATP1B3 substrate, the sponsor should consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic index, and likely co-medications that are known OATP1B1 or OATP1B3 inhibitors in the indicated patient populations (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[12]).

C. Determining if the Investigational Drug is a Substrate of the Renal Transporters OAT, OCT, and MATE

OAT1, OAT3, and OCT2 are renal transporters expressed on the basolateral membrane of the renal proximal tubule. MATE1 and MATE2-K are expressed on the brush border membrane. All of the aforementioned renal transporters can play a role in the active renal secretion of investigational drugs.

1. Conducting In Vitro Studies

If the investigational drug's ADME data suggest that active renal secretion is significant for a drug (i.e., active secretion of the parent drug by the kidney is ≥ 25% of the total clearance), the sponsor should evaluate the drug in vitro to determine whether it is a substrate of OAT1/3, OCT2

[12] When final, this guidance will represent the FDA's current thinking on this topic.

and MATE1 and MATE2-K (see appendix, section VII.B). See Figure 5 for the equation to calculate active secretion.

Figure 5: An Equation to Calculate Active Secretion*

Active secretion = $CL_r - (f_{u,p} \times GFR)$

Cl_r is the renal clearance.
$f_{u,p}$ is the unbound fraction in plasma.
GFR is the glomerular filtration rate.

*This equation is valid assuming that there is no re-absorption. The GFR is set as 125 mL/min for subjects with normal renal function if the GFR is not measured.

2. Data Analysis and Interpretation

The investigational drug is an in vitro substrate for the above renal transporters if: (1) the ratio of the investigational drug's uptake in the cells expressing the transporter versus the drug's uptake in control cells (or cells containing an empty vector) is ≥ 2; and (2) a known inhibitor of the transporter decreases the drug's uptake to $\leq 50\%$ at a concentration at least 10 times its K_i or IC_{50}. The sponsor may justify alternative cutoff ratios based on its prior experience with the cell system.

If in vitro studies indicate that a drug is a substrate of one or more of these renal transporters, the sponsor should consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known inhibitors of these renal transporters in the indicated patient populations (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[13]).

D. Determining if the Investigational Drug is an Inhibitor of a Transporter

1. Conducting In Vitro Studies

The sponsor should conduct in vitro studies to evaluate whether an investigational drug is an inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OCT2, MATEs (MATE-1, MATE-2K), OAT1, and OAT3 (see appendix, section VII.B for considerations regarding in vitro systems).

2. Data Analysis and Interpretation

[13] When final, this guidance will represent the FDA's current thinking on this topic.

P-gp and BCRP: The sponsor should conduct studies to determine if an investigational drug inhibits the net flux of a known P-gp or BCRP substrate in Caco-2, P-gp- or BCRP-overexpressed cells and determine the drug's inhibition potency (i.e., IC_{50} or K_i). The investigational drug has the potential to inhibit P-gp or BCRP in vivo if the investigational drug is administered orally, and the $I_{gut}/IC_{50} \geq 10$ where I_{gut} = dose of inhibitor/250 mL. To determine the IC_{50}, a unidirectional assay based on the probe substrate can also be considered. This cutoff value is based on a limited dataset (Zhang, Zhang, et al. 2008; Tachibana, Kato, et al. 2009; Agarwal, Arya, et al. 2013; Ellens, Deng, et al. 2013). The sponsor may calibrate its internal in vitro systems with known inhibitors and non-inhibitors and propose a different cutoff value with proper justification (see appendix, section VII.B for detailed recommendations).

If in vitro studies indicate that a drug is a P-gp or BCRP inhibitor, the sponsor should consider whether to conduct an in vivo study based on likely concomitant medications that are known P-gp or BCRP substrates in the indicated patient populations (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[14]*).*

OATP1B1 and OATP1B3: The sponsor should conduct studies to determine the inhibition potency (i.e., IC_{50} or K_i) of the investigational drug on the uptake of a known OATP1B1 or OATP1B3 substrate in cells overexpressing the relevant transporter. Because some known OATP1B1/3 inhibitors demonstrate time-dependent inhibition, the sponsor should determine IC_{50} values following pre-incubation with the investigational drug for a minimum of 30 minutes (Amundsen, Christensen, et al. 2010; Gertz, Cartwright, et al. 2013; Izumi, Nozaki, et al. 2015). The investigational drug has the potential to inhibit OATP1B1/3 in vivo if the R value (as described in Figure 6 below) is ≥ 1.1.

Figure 6: Equation to Calculate the Predicted Ratio of the Victim Drug AUC in the Presence and Absence of the Investigational Drug to Determine the Potential to Inhibit OATP1B1/3*

$$R = 1 + ((f_{u,p} \times I_{in,max})/IC_{50}) \geq 1.1$$

R is the predicted ratio of the victim drug's AUC in the presence and absence of the investigational drug as the inhibitor.
$f_{u,p}$ is the unbound fraction in plasma.
IC_{50} is the half-maximal inhibitory concentration.
$I_{in,max}$ is the estimated maximum plasma inhibitor concentration at the inlet to the liver. It is calculated as:

$$I_{in,max} = (I_{max} + (F_a F_g \times k_a \times Dose))/Q_h/R_B$$

Continued

[14] When final, this guidance will represent the FDA's current thinking on this topic.

Figure 6 continued. Equation to Calculate the Predicted Ratio of the Victim Drug AUC in the Presence and Absence of the Investigational Drug to Determine the Potential to Inhibit OATP1B1/3*

F_a is the fraction absorbed.
F_g is the intestinal availability.
k_a is the absorption rate constant.
Q_h is the hepatic blood flow rate.
R_B is the blood-to-plasma concentration ratio.

*If unknown, $F_aF_g = 1$ and $k_a = 0.1$/min can be used as a worst-case estimate.
Considering uncertainties in the protein binding measurements, the unbound fraction ($f_{u,p}$) should be set to 1% if experimentally determined to be less than 1%.

The cutoff value described in Figure 6 is based on limited published data (Yoshida, Maeda, et al. 2012; Tweedie, Polli, et al. 2013; Vaidyanathan, Yoshida, et al. 2016). Sponsors may calibrate their internal in vitro systems with known inhibitors and non-inhibitors of these transporter systems and propose a specific cutoff value with proper justification.

If in vitro studies indicate that a drug is an OATP1B1 or OATP1B3 inhibitor, the sponsor should consider whether to conduct an in vivo study based on whether the likely concomitant medications used in the indicated patient populations are known OATP1B1or OATP1B3 substrates (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[15]).

OAT, OCT, and MATE: Sponsors should conduct studies to determine the inhibition potency (i.e., IC_{50} or K_i) of the investigational drug on the uptake of a known substrate for renal transporters (i.e., OAT1, OAT3, OCT2, MATE1, and MATE2K) in cells overexpressing these transporters. The investigational drug has the potential to inhibit these transporters in vivo if the $I_{max,u}/IC_{50}$ value is ≥ 0.1 for OAT1/OAT3/OCT2 or the $I_{max,u}/IC_{50}$ value is ≥ 0.02 for MATEs.[16] These cutoff values are based on limited data (Dong, Yang, et al. 2016a; Dong, Yang, et al. 2016b). Sponsors may calibrate their unique in vitro systems with known inhibitors and non-inhibitors of these transporter systems and propose a different cutoff value with proper justification. Creatinine is also a substrate for OCT2, MATEs, and OAT2 (Lepist, Zhang, et al. 2014). Elevated serum creatinine levels in observed in clinical studies could be due to inhibition of these transporters by the investigational drug. Confirmation of the mechanism of an increase in serum creatinine with the investigational drug requires additional evidence such as clinical

[15] When final, this guidance will represent the FDA's current thinking on this topic.

[16] Considering uncertainties in the protein binding measurements, the unbound fraction should be set to 1% if experimentally determined to be less than 1%.

mechanistic studies.

If in vitro studies indicate that a drug is an inhibitor of these renal transporters, the sponsor should consider whether to conduct an in vivo study based on whether the likely concomitant medications used in the indicated patient populations are known substrates of these renal transporters (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[17]).

E. Determining if the Investigational Drug is an Inducer of a Transporter

Certain transporters such as P-gp are induced through mechanisms similar to those for CYP enzymes (e.g., by activation of specific nuclear receptors). Because of these similarities, information from CYP3A induction studies can inform P-gp induction studies (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*[18]). However, in vitro methods to evaluate the induction of P-gp and other transporters are not well established at this time. Therefore, the FDA does not currently recommend in vitro evaluation of investigational drugs as transporter inducers.

V. EVALUATION OF THE DDI POTENTIAL OF METABOLITES

Sponsors should evaluate the DDI potential of an investigational drug's metabolites for their impact on the drug's safety and efficacy using a risk-based assessment that considers safety margins, likely concomitant medications, and therapeutic indications.

A metabolite with significant plasma exposure or pharmacological activities may need to be evaluated for its DDI potential as a substrate or as an inhibitor of metabolizing enzymes (see sections V.A and V.B below). In vitro studies normally use a synthesized or purified metabolite standard or radiolabeled drug. Alternative methods are acceptable if the sponsor can justify that the DDI potential of the metabolites can be adequately assessed (Callegari, Kalgutkar, et al. 2013; Yu and Tweedie 2013; Yu, Balani, et al. 2015).

Published data have shown that some Phase II metabolites can be better substrates (more polar than the parent) or inhibitors of various transporters leading to a higher chance of DDIs than the parent drug (Zamek-Gliszczynski et al, 2014). Therefore the DDI potential of a metabolite as a substrate or a perpetrator of major drug transporters should be assessed on a case-by-case basis. The same principles and strategies mentioned above for parent drug should be applied where

[17] When final, this guidance will represent the FDA's current thinking on this topic.

[18] When final, this guidance will represent the FDA's current thinking on this topic.

applicable.

A. Metabolite as a Substrate

1. Conducting In Vitro Studies

The sponsor should evaluate the potential for a metabolite to cause DDIs by acting as a substrate of metabolizing enzymes if the metabolite: (1) is active (i.e., has the potential to affect safety or efficacy based on in vitro pharmacology and toxicology assessments); and (2) contributes to ≥ 50% of the overall activity, considering both in vitro receptor potency and in vivo exposure.

2. Data Analysis and Interpretation

The sponsor should consider in vivo DDI studies of the metabolite based on in vitro assessments using the same strategies as those for the parent drugs (see section III.A).

B. Metabolite as an Inhibitor

1. Conducting In Vitro Studies

If in vitro assessments suggest that the parent drug inhibits major CYP enzymes and transporters and in vivo DDI studies are warranted, in vitro assessments of metabolites as enzyme or transporter inhibitors may not be needed because the in vivo inhibition potential of metabolites would be evaluated in vivo along with the parent drug, unless clinically relevant exposures of the metabolite cannot be adequately represented in the in vivo DDI study (i.e., the study duration does not allow the metabolite to accumulate). However, if in vitro assessments suggest that the parent drug alone will not inhibit major CYP enzymes or transporters, in vivo DDIs caused by metabolites may still be possible. In this situation, the sponsor should evaluate the in vitro inhibition potential of a metabolite on CYP enzymes taking into account the following factors: (1) the systemic exposure of the metabolite relative to the parent drug; and (2) any structural alerts, such as Quantitative Structure-Activity Relationship (QSAR) for potential time-dependent inhibition (Yu and Tweedie 2013; Yu, Balani, et al. 2015). Additional considerations are discussed in detail below:

- The sponsor should conduct an in vitro inhibition study of the metabolite if a metabolite is less polar than the parent drug and the $AUC_{metabolite} \geq 25\% \times$ the AUC_{parent} (i.e., $AUC_{metabolite} \geq 0.25 \times AUC_{parent}$).

- The sponsor should conduct an in vitro inhibition study of the metabolite if a metabolite is more polar than the parent drug, and the $AUC_{metabolite} \geq 100\% \times AUC_{parent}$ (i.e., $AUC_{metabolite} \geq AUC_{parent}$).

- The sponsor should conduct an in vitro inhibition study of the metabolite on CYP enzymes if a metabolite with a structural alert for TDI has an $AUC_{metabolite} \geq 25\% \times$ the AUC_{parent} and an $AUC_{metabolite} \geq 10\% \times$ the AUC of the total drugs (determined with radioactivity). If there are no radioactivity data but the $AUC_{metabolite} \geq 25\% \times$ the AUC_{parent}, then the sponsor should conduct an in vitro DDI assessment with the metabolite.

2. Data Analysis and Interpretation

Based on the results of in vitro DDI assessments of the metabolite, the sponsor should consider an in vivo DDI study of the metabolite using the same strategies as those for the parent drug (see section III.B).

VI. LABELING RECOMMENDATIONS

Prescription information must include a summary of drug interaction information that is essential for the safe and effective use of the drug product by the health care provider and must be based on data derived from human experience whenever possible.[19] In the absence of clinical information, the sponsor should include in vitro information regarding the characterization of metabolic and transporter pathways as well as PK interactions between the drug and other prescription drugs, over-the-counter drugs, classes of drugs, dietary supplements, and foods or juices (including inhibition, induction, and genetic characteristics) in prescription drug labeling, if clinically significant. In addition, the results of pertinent in vitro studies that establish the absence of an effect must be included.[20] In vitro information that has been superseded by clinical information should not be included in the prescription drug labeling unless it is essential to understanding the clinical results.

This in vitro information should generally be placed under the *12.3 Pharmacokinetics* subsection of the CLINICAL PHARMACOLOGY section. In rare cases, the clinical significance of the in vitro information may require placement in other sections of the prescription drug labeling (e.g., BOXED WARNING, CONTRAINDICATIONS, WARNINGS AND PRECAUTIONS, and/or DRUG INTERACTIONS sections).

See the following FDA guidances for industry for labeling recommendations relevant to drug metabolism and transporter pathways as well as clinical DDIs:

[19] 21 CFR 201.56(a)(3).

[20] 21 CFR 201.57(c)(13)(c)(i)(C).

667 • *Clinical Pharmacology Labeling for Human Prescription Drug and Biological Products*
668 *— Considerations, Content, and Format*[21]
669
670 • *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical*
671 *Implications*[22]
672

[21] This guidance is available on the FDA Drugs guidance Web page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

[22] When final, this guidance will represent the FDA's current thinking on this topic.

VII. APPENDICES

A. Evaluating Metabolism-Based Drug Interactions In Vitro

Various hepatic in vitro systems can be used to evaluate the drug interaction potential of an investigational drug, including:

(1) Subcellular human liver tissue fractions such as reconstituted microsomal systems, supernatants after 9000 g centrifugation of liver homogenate (S9), and cytosol (adding appropriate co-factors as necessary)

(2) Recombinant CYP enzymes in various expression systems that can identify the production of individual drug metabolites and the involvement of certain classes of enzymes

(3) Human liver tissues, including freshly prepared hepatocytes and cryopreserved hepatocytes that preserve enzyme architecture and contain the full complement of Phase I and Phase II drug metabolizing enzymes

Although the main focus of this guidance is on CYP and hepatic metabolism, sponsors should consider non-CYP, enzyme-based metabolism (e.g., Phase II enzymes) and metabolism occurring in extra-hepatic tissues when relevant for their investigational drugs.

1. Determining if the Investigational Drug is an Enzyme Substrate

Drug metabolizing enzyme identification studies, often referred to as reaction phenotyping studies, are a set of in vitro experiments that identify the specific enzymes responsible for the metabolism of a drug. Along with other information (e.g., in vivo pharmacokinetics, enzyme polymorphism or DDI data), in vitro phenotyping data are often used to quantify disposition pathways of an investigational drug.

a. Conducting metabolic pathway identification experiments

Metabolic pathway identification experiments identify the number and structures of metabolites produced by a drug and whether the metabolic pathways are parallel or sequential. These experiments use intact human liver systems (e.g., human hepatocytes), human liver microsomes, or recombinant enzyme systems. Data obtained from metabolic pathway identification experiments help to determine whether and how to conduct a reaction phenotyping study.

b. Identifying the enzymes that metabolize an investigational drug

The sponsor should conduct in vitro experiments to identify specific metabolizing enzymes that are involved in the metabolism of an investigational drug, preferably before first-in-human studies. There are two widely used methods for identifying the individual CYP enzymes

responsible for a drug's metabolism: (1) the first method uses chemicals, drugs, or antibodies as specific enzyme inhibitors in pooled (e.g., a pool of more than 10 donors) human liver microsomes and (2) the second method uses individual human recombinant CYP enzymes. The sponsor should consider the following recommendations when performing reaction phenotyping experiments:

- The sponsor should use both methods to identify the specific enzymes responsible for a drug's metabolism.

- When using individual recombinant CYP enzymes, the sponsor should consider the difference in the amount of CYP contents between recombinant CYP enzyme systems and the human liver.

- The in vitro system for these studies should: (1) be robust and reproducible; and (2) include the necessary selective in vitro probe substrate as a positive control to prove the activity of each enzyme. A list of probe substrates can be found on the FDA's Web site on Drug Development and Drug Interactions.[23]

- Whenever possible, the sponsor should conduct all experiments with drug concentrations deemed appropriate by kinetic experiments and under initial rate conditions (linearity of metabolite production rates with respect to time and enzyme concentrations). The sponsor should conduct an adequate number of replicates (e.g., three or more replicates per drug concentration) in a single study.

- When conducting an in vitro study to examine the contribution of individual CYP enzymes to the overall metabolism of an investigational drug, the measurement of parent drug depletion is preferred over the measurement of metabolite formation, unless all of the major metabolites have been identified and quantified in metabolite formation experiments.

- When conducting in vitro studies to examine the contribution of individual CYP enzymes to the formation of a specific metabolite, the sponsor should measure the formation rate of the metabolite.

- The sponsor should develop validated and reproducible analytical methods to measure levels of the parent drug and each metabolite.

[23] A list of probe substrates: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1.

- The use of a radiolabeled drug substrate is advantageous because samples can be analyzed using liquid chromatography coupled with a radioactivity detector or a mass spectrometer to identify and quantify drug-related species.

- The sponsor should separately evaluate individual isomers of racemic drugs when it is important to understand the different disposition characteristics of each isomer (e.g., when two isomers have different pharmacological activities).

- Most chemical inhibitors are not specific for an individual CYP enzyme. The sponsor should verify the selectivity and potency of inhibitors in the same experimental conditions using probe substrates for each CYP enzyme. Commonly used in vitro CYP enzyme inhibitors can be found on the FDA's Web site on Drug Development and Drug Interactions.[24]

- The sponsor should test the inhibitory effect of an antibody to a CYP enzyme at sufficiently low and high concentrations to establish a titration curve and ensure the maximal inhibition of a particular pathway (ideally resulting in greater than 80 percent inhibition). The sponsor should verify the effect of an antibody using probe substrates of each CYP isoform and with the same experimental conditions.

2. Determining if the Investigational Drug is an Enzyme Inhibitor or Inducer

a. Conducting in vitro enzyme inhibition studies

The potential of an investigational drug to inhibit CYP enzymes is usually investigated in human liver tissue systems using probe substrates to determine the inhibition mechanisms (e.g., reversible or time-dependent inhibition) and inhibition potencies (e.g., K_i for reversible inhibition, and K_I and k_{inact} for time-dependent inhibition). The in vitro systems used for these studies include human liver microsomes, microsomes obtained from recombinant CYP-expression systems, or hepatocytes (Bjornsson, Callaghan, et al. 2003).

Kinetic data from in vitro inhibition studies of an investigational drug can be used in quantitative models to predict the investigational drug's effects on the pharmacokinetics of other drugs in humans. These analyses inform the decision on whether to conduct an in vivo DDI study using sensitive enzyme index substrates (see section III.B.2).

The sponsor should consider the following recommendations when designing an in vitro CYP

[24] Examples of in vitro selective inhibitors for P450-mediated metabolism: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1-2

inhibition study:

- A probe substrate should be selective (e.g., predominantly metabolized by a single enzyme in pooled human liver microsomes or recombinant CYPs) and have simple metabolic schemes (ideally, the drug does not undergo sequential metabolism). Commonly used in vitro probe substrates and their marker reactions can be found on the FDA Web site on Drug Development and Drug Interactions.[25]

- The sponsor should use a validated and reproducible analytical assay to measure the formation of a probe substrate's metabolite.

- The in vitro system of choice for enzyme inhibition should be robust and reproducible and include the appropriate strong probe inhibitors as positive controls (see the FDA's Web site on Drug Development and Drug Interactions).[26] Kinetic constants (K_i, IC_{50}, K_I, and/or k_{inact}) of the probe inhibitors should be comparable to literature-reported values. In vitro systems may be pooled human liver microsomes (e.g., pooled from more than 10 donors), pooled cryopreserved hepatocytes (e.g., pooled from more than 10 donors), or individual microsomes expressing recombinant CYP enzymes. To obtain inhibition parameters, the sponsor may consider primary hepatocytes enriched with human plasma as an in vitro system that represents physiological conditions (Lu, Miwa, et al. 2007; Mao, Mohutsky, et al. 2012).

- When used as an inhibitor, the investigational drug concentrations should generally be as high as possible to maximize the inhibition effect. However, the drug concentration should not exceed the drug's solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cell models.

- The sponsor should test four to six different concentrations of the investigational drug with the probe substrate. The sponsor should first conduct experiments with a high concentration of test drug to study its inhibition potential on a particular enzyme (e.g., 50 times the unbound C_{max}, or 0.1 times the dose/250 mL). If the initial high concentration

[25] Examples of in vitro marker reactions for P450-mediated metabolism and in vitro selective inhibitors for P450-mediated metabolism: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1

[26] Examples of in vitro selective inhibitors for P450-mediated metabolism: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1-2

of the test drug is able to inhibit a particular enzyme, the sponsor should test lower drug concentrations to calculate the drug's IC_{50} or K_i value.

- Typical experiments to determine the IC_{50} value of a drug involve incubating the substrate at a concentration at or below its K_m to more closely relate the inhibitor's IC_{50} to its K_i. For K_i determinations, the sponsor should vary both the substrate and inhibitor concentrations to cover ranges above and below the substrate's K_m and the inhibitor's K_i.

- Microsomal protein concentrations are usually less than 1 mg/mL. The sponsor should correct for nonspecific binding during the incubation if this binding is expected to influence the analysis of kinetic data. Nonspecific binding can be measured experimentally (e.g., using equilibrium dialysis or ultrafiltration) (Hallifax and Houston 2006) or predicted using in silico methods (Gertz, Kilford, et al. 2008).

- Because buffer strength, type, and pH can all significantly affect the determination of V_{max} and K_m, the sponsor should use standardized assay conditions.

- In general, the sponsor should avoid any significant depletion of the substrate or inhibitor. However, when substrates have a low K_m, it may be difficult to avoid substrate depletion at low substrate concentrations. In these circumstances, the sponsor should consider substrate depletion when determining inhibition kinetics.

- The sponsor should choose an incubation time and an enzyme amount that result in linear formation of the metabolite (at an initial rate of the metabolite formation).

- The sponsor should use any organic solvents at low concentrations (<1% (volume/volume) and preferably < 0.5%) because some solvents can inhibit or induce enzymes. The experiment should include a no-solvent control and a solvent (vehicle) control.

- The sponsor should determine inhibition kinetics according to appropriate mechanisms (e.g., competitive, noncompetitive, or TDI).

- The sponsor should routinely study TDI in standard in vitro screening protocols by pre-incubating the investigational drug (e.g., for at least 30 min) before adding any substrate. Any significant time-dependent and co-factor-dependent (e.g., NADPH for CYPs) loss of initial product formation may indicate TDI. In these circumstances, the sponsor should conduct definitive in vitro studies to obtain TDI parameters (i.e., k_{inact} and K_I) (Grimm, Einolf, et al. 2009).

b. Evaluating enzyme induction in vitro

The sponsor can investigate the potential of an investigational drug to act as an inducer of CYP enzymes in plateable, cryopreserved or freshly isolated, human hepatocytes. Other, alternative in vitro systems such as immortalized hepatic cell lines may be used and are acceptable methods to determine the CYP induction potential of investigational drugs. Cell receptor assays may be used, but the results from these studies are considered supportive, rather than definitive, in nature. The sponsor should justify both why any alternative in vitro system is appropriate for the purpose of the study as well as the method to interpret the data. Acceptable study endpoints include mRNA levels and/or enzyme activity levels using a probe substrate (Fahmi and Ripp 2010; Einolf, Chen, et al. 2014). A major challenge of measuring only the enzyme's activity is that the induction may be masked in the presence of concomitant inhibition. Transcriptional analysis through the measurement of mRNA levels may address this challenge. Regardless of which in vitro system and endpoint are chosen, the sponsor should validate the system to show that all major CYP enzymes are functional and inducible with positive controls.

When using in vitro systems to study enzyme induction, the sponsor should consider the following recommendations:

- The sponsor should evaluate the ability of an investigational drug to induce the major CYPs, including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5.

- The sponsor should individually evaluate CYP1A2, CYP2B6, and CYP3A4/5 first because they are induced via different nuclear receptors.

- Activation of a nuclear receptor, PXR, may lead to co-induction of CYP3A4/5 and CYP2C enzymes. Thus, a negative in vitro result for CYP3A4/5 induction eliminates the need for additional in vitro or in vivo induction studies for CYP3A4/5 and CYP2C enzymes. If in vitro CYP3A4/5 induction results are positive, the sponsor should evaluate the ability of the investigational drug to induce CYP2C8, CYP2C9, and CYP2C19 either in vitro or in vivo.

- The in vitro system of choice to evaluate enzyme induction should be robust and reproducible and include appropriate clinical inducers and/or non-inducers as positive and negative controls (see the FDA's Web site on Drug Development and Drug Interactions).[27] When applicable, the sponsor should conduct pilot experiments to establish a test system (e.g., a particular lot of cryopreserved human hepatocytes) for

[27] For more information, see: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm.

routine studies of CYP induction (Fahmi, Kish et al. 2010; Fahmi and Ripp 2010; Einolf, Chen et al. 2014).

- The sponsor should investigate drug concentrations that reflect the expected or observed human plasma drug concentrations or intestinal drug concentrations (for CYP3A4/5). Drug concentrations should span the range of therapeutic exposures. If the drug solubility permits, this range of drug concentrations should include at least one drug concentration that is an order of magnitude greater than the maximum unbound steady-state plasma drug concentration in vivo. The sponsor should conduct three replicate experiments per drug concentration. The sponsor should measure the concentration of unbound test drug to help predict the magnitude of clinical DDIs.

- The sponsor should use hepatocyte preparations from at least three donors. If the result from at least one donor's hepatocytes exceeds the predefined threshold, the sponsor should consider the drug an inducer in vitro and conduct a follow-up evaluation.

- The sponsor should demonstrate that the experimental approach is capable of identifying the absence and presence of the investigational drug's induction potential and avoids false negative predictions with the selected system and endpoints.

- Incubation of an investigational drug usually lasts for 48-72 hours to allow complete induction to occur. Incubations include a daily addition of the investigational drug, and the medium containing the drug is changed regularly. The optimal time course for incubation should allow the sponsor to detect enzyme induction without causing cell toxicity. The sponsor should justify the rationale for shorter incubation durations.

- Data on the actual concentration of drug in the system are important for extrapolating in vitro results to in vivo scenarios. The sponsor should measure concentrations of the parent drug in the medium at several time points during the last day of the incubation, unless loss of the parent drug due to in vitro drug metabolism, degradation, or lysosomal trapping is negligible, or if loss of the parent drug was quantified in the system before the induction assay and compensated for through the amount of drug added or the intervals between medium changes.

B. Evaluating Transporter-Mediated Drug Interactions In Vitro

In vitro transporter assays can determine whether an investigational drug is a substrate or inhibitor of a particular transporter. Coupled with appropriate in vitro-to-in vivo extrapolation methods (see section IV), these assays can determine if the sponsor should conduct an in vivo drug interaction study. Currently, in vitro methods to evaluate transporter induction are not well understood.

1. General Considerations When Using In Vitro Experimental Systems to Evaluate Transporter-Mediated Drug Interactions

a. Selecting an in vitro test system

The sponsor should choose an in vitro test system that is suitable for a specific transporter, such as a membrane vesicle system, a polarized cell-based bidirectional assay for efflux transporters, or a cell-based assay for uptake transporters. Selecting the in vitro model may depend on the purpose of the study and the questions to be addressed. Table 1 summarizes examples of in vitro systems to investigate potential transporter-mediated drug interactions with an investigational drug as either a substrate or an inhibitor of a specific transporter.

Table 1. Examples of In Vitro Systems to Investigate Transporter-Mediated Drug Interactions

Transporter	In Vitro Systems
ABC Transporters	
BCRP, P-gp	Caco-2 cells, commercial or in-house membrane vesicles, knock-out/down cells, transfected cells (MDCK, LLC-PK_1, etc.)
Solute Carrier (SLC) Transporters	
OATPs	Hepatocytes, transfected cells (CHO, HEK293, MDCK, etc.)
OATs, OCTs	Transfected cells (CHO, HEK293, MDCK, etc.)
MATEs*	Commercial or in-house membrane vesicles, transfected cells (CHO, HEK293, MDCK)

CHO: Chinese hamster ovary cell
HEK293: human embryonic kidney 293 cell
LLC-PK1: Lewis-lung cancer porcine kidney 1 cell
MDCK: Madin-Darby canine kidney cell

*The function of MATEs depends on the driving force from oppositely directed proton gradient; therefore, the appropriate pH of MATE assay system should be employed.

Details regarding each in vitro test system to investigate transporter-mediated drug interactions are described below:

- Membrane vesicles:
  - In vitro systems using inside-out membrane vesicles evaluate whether an investigational drug is a substrate or inhibitor of P-gp or BCRP but may fail to identify highly permeable drugs as substrates.
  - Assays using membrane vesicles should directly measure the adenosine triphosphate (ATP)-dependent, transporter-mediated uptake of drugs.
- Bi-directional transport assays with cell-based systems:

- Bidirectional assays evaluate whether an investigational drug is a substrate or inhibitor of efflux transporters such as P-gp or BCRP.

- Cell monolayers grow on semi-porous filters in a device with apical (AP) and basolateral (BL) chambers.

- The sponsor should add the test drug to either the AP or BL side of the cell monolayer and measure the amount of the drug permeating through the cell monolayers in the receiver chamber over time.

- The sponsor should calculate the apparent permeability (P_{app}) of the drug in both the AP→BL (absorption) and BL→AP (efflux) directions and calculate an efflux ratio from the ratio of BL→AP to AP→BL P_{app} values for the substrate.

- When using transfected cell lines, the sponsor should compare the efflux ratios of the transfected cell line to the parental or empty vector-transfected cell line.

- Uptake assays with cell-based systems:

  - Uptake assays evaluate whether an investigational drug is a substrate or inhibitor of SLC transporters such as OCT, OAT, OATP and MATE.

  - When transfected cell lines are used, the sponsor should compare the drug uptake in the transfected cell line to the parental or empty vector-transfected cell line.

  - The sponsor may use human hepatocytes or hepatic cell lines in suspension, plated, or sandwich-cultured assays.

b. Determining in vitro test conditions

The sponsor should validate the model system and experimental conditions, including culture and transport assay conditions, within the same laboratory. The sponsor should include appropriate positive and negative controls in the test study to ensure the validity of the study's results. The sponsor should consider the following recommendations during assay development and validation:

- The sponsor should develop and optimize transport assays to ensure consistent transporter expression (e.g., mRNA expression, protein expression) and transporter function (e.g., uptake, efflux).

- The sponsor should verify the functionality of the assay by conducting studies with known positive and negative controls (see the FDA's Web site on Drug Development and Drug Interactions[28]).

- The sponsor should characterize the following conditions whenever applicable: the source of the membrane vesicles or cells, the cell culture conditions (e.g., cell passage number, seeding density, monolayer age), the probe substrate/inhibitor concentrations, the incubation time, the buffer/pH conditions, the sampling interval, and the methods for calculating parameters such as the IC_{50}, K_i, and K_m.

- The sponsor should use any organic solvents at low concentrations (< 1% volume/volume and preferably < 0.5%) because some solvents can affect cell integrity or transporter function. The experiment should include a no-solvent control and a solvent (vehicle) control.

- For both substrate and inhibitor studies, the sponsor should attempt to assess the impact of the following factors:

    - The stability of the test drug for the duration of study
    - The effect of nonspecific binding of the test drug to cells/apparatus
    - The test drug's solubility limits
    - The effect of prefiltration
    - The effect of adding serum protein to the media
    - The effect of other experimental steps involved in transport studies

- The sponsor should conduct transport studies under linear transport rate conditions.

- The sponsor should establish laboratory acceptance criteria for study results (e.g., monolayer integrity, passive permeability, efflux or uptake of probe substrates, K_m for a probe substrate, IC_{50} for probe inhibitor). The K_m value of a probe substrate or the IC_{50} value of a probe substrate or inhibitor should be comparable to literature-reported values.

- The substrate (which may be the test drug) should be readily measured with no interference from the assay matrix.

2. Determining if the Investigational Drug is a Transporter Substrate

[28] For more information, see: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm.

When using in vitro systems to study whether an investigational drug is a substrate of transporters, the sponsor should consider the following factors:

- The sponsor should evaluate multiple concentrations of the test drug to cover the range of clinically relevant concentrations.

- Several factors may limit test drug concentrations in the in vitro assays, including aqueous solubility, nonspecific binding to the culture vessel, and cytotoxicity.

- The sponsor should evaluate the recovery (mass balance), stability, and/or nonspecific binding of the test drug.

- If the in vitro system expresses multiple transporters, the sponsor should conduct additional experiments to confirm the findings with two or more known potent inhibitors.

3. Determining if the Investigational Drug is a Transporter Inhibitor

When using in vitro systems to study whether an investigational drug is an inhibitor of transporters, the following should be considered:

- Test-drug concentrations should generally be as high as possible to maximize the inhibition effect. However, the drug concentration should not exceed the drug's solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cells.

- The sponsor should evaluate approximately four to six concentrations of the test drug with the probe substrate. The sponsor should start with a high concentration of the test drug, at least an order of magnitude higher than the drug's clinically relevant concentration. Because transporters are expressed in different locations in tissues, the sponsor should consider different clinically relevant concentrations (e.g., the unbound C_{max} for renal uptake transporters, unbound maximum hepatic inlet concentration for hepatic uptake transporters (see Figure 6), or dose/250 mL for apical intestinal transporters). If the test drug demonstrates inhibitory activity, the sponsor should test additional concentrations to calculate IC_{50} or K_i values. The sponsor can then compare these values to clinical plasma or intestinal concentrations to predict the potential for DDIs.

- Experiments should include a probe substrate concentration range that results in linear transport of the substrate. The probe substrate concentration should be at or below its K_m for the transporter.

- The sponsor should consider a pre-incubation step with the test drug (for a minimum of 30 minutes) for OATP1B1 and OATP1B3 inhibition to evaluate whether TDI could result

in a lower IC_{50} of the test drug. For example, recent data show that cyclosporine and its metabolite AM1 are time-dependent OATP1B inhibitors (Amundsen, Christensen et al. 2010; Gertz, Cartwright et al. 2013; Izumi, Nozaki et al. 2015).

- Inhibition can be substrate dependent; therefore, the sponsor should determine the inhibition constant of the test drug with a probe substrate that may also be used in later clinical studies. Alternatively, the sponsor may use a probe substrate that usually generates a lower IC_{50} for known inhibitors to avoid underestimating the interaction potential of the investigational drug.

- The sponsor could use positive and negative controls to calibrate their internal in vitro systems to generate cutoff values to inform potential future clinical DDI studies.

C. Using Model-Based Predictions to Determine a Drug's Potential to Cause DDIs

Mathematical models can evaluate the results of in vitro and in vivo DDI studies to determine whether, when, and how to conduct further clinical DDI studies in drug development. In many cases, negative findings from early in vitro and clinical studies, in conjunction with model-based predictions, can eliminate the need for additional clinical investigations of an investigational drug's DDI potential.

Mathematical models that integrate in vitro findings and are verified with clinical PK data can play an important role in predicting the DDI potential of an investigational drug under various scenarios. There are several models to consider when evaluating the drug as a perpetrator of a metabolism-based DDI. *Basic models* generally serve simple purposes, such as the identification of low levels of inhibition or induction of metabolizing enzymes by an investigational drug. *Static mechanistic models* can account for the disposition characteristics of both the perpetrator and the index substrate drugs (Fahmi, Hurst, et al. 2009). *Dynamic mechanistic models*, including PBPK models that integrate system-dependent parameters (e.g., based on human physiology) and drug-dependent parameters (Zhao, Zhang, et al. 2011) and their time course of changes, can support decisions on when and how to conduct a clinical DDI study. Furthermore, these models can quantitatively predict the magnitude of DDI in various clinical situations, such as in patients with renal impairment or patients with genetic deficiencies in certain metabolizing enzymes.

1. General Considerations When Using Predictive Models to Evaluate Enzyme-Based DDIs

a. Basic models to predict the effect of a drug as an enzyme modulator

Evaluating a drug as a potential enzyme inhibitor or inducer begins with the use of a basic model, which includes the following components:

- The R value is the ratio of intrinsic clearance values of an index substrate for an enzymatic pathway in the absence and presence of a potential modulator (perpetrator). Assuming changes in intrinsic clearance are proportional to those in total clearance, the R value can be used to represent the AUC ratio of a victim drug in the presence and absence of a potential modulator (perpetrator). This guidance uses R_1 (including $R_{1,g}$), R_2, and R_3 for reversible inhibition, time-dependent inhibition, and induction, respectively. The R value is calculated using the concentration of the interacting drug available at the enzyme site (defined as [I]) and the appropriate kinetic parameters for each basic model (see section III)).

- [I] represents the concentration of the interacting drug (potential inhibitor or inducer) available at the enzyme site. The basic models described in this guidance use the maximal unbound plasma concentration of the interacting drug for [I]. For CYP3A at the gut, [I] is I_{gut}, which is calculated as dose/250 mL for the basic model.

- Kinetic parameters are estimates for each basic model (reversible inhibition, time-dependent inhibition, and induction).

The sponsor should compare the calculated R values to the predefined cutoff criteria to determine whether it is possible to rule out the potential for a DDI. If the basic model does not rule out the potential for a DDI, the sponsor should further evaluate the DDI potential of the investigational drug by conducting additional modeling analyses, using static mechanistic models or PBPK models (see below) or by conducting an in vivo DDI study.

b. Using static mechanistic models to predict the effect of a drug as an enzyme modulator

Static mechanistic models incorporate more detailed drug disposition and drug interaction mechanisms for both interacting and substrate drugs (Fahmi, Hurst, et al. 2009). The following equation can be used to calculate the overall effect (inhibition or induction) of the investigational drug on substrate drugs (represented as the AUCR) (see Figure 7).

Figure 7: Equation to Calculate AUCR of the Substrate Drugs (AUC plus investigational drug/AUC minus investigational drug)

$$AUCR = \left(\frac{1}{\left[A_g \times B_g \times C_g\right] \times \left(1 - F_g\right) + F_g} \right) \times \left(\frac{1}{\left[A_h \times B_h \times C_h\right] \times f_m + (1 - f_m)} \right)$$

A is the effect of reversible inhibitions.
B is the effect of TDI.
C is the effect of induction.
F_g is the fraction available after intestinal metabolism.
f_m is the fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction.
Subscripts 'h' denote liver.
Subscripts 'g' denote gut.

Each value can be estimated with the following equations:

	Gut	Liver
Reversible inhibition	$A_g = \frac{1}{1 + \frac{[I]_g}{K_i}}$	$A_h = \frac{1}{1 + \frac{[I]_h}{K_i}}$
Time-dependent inhibition	$B_g = \frac{k_{deg,g}}{k_{deg,g} + \frac{[I]_g \times k_{inact}}{[I]_g + K_I}}$	$B_h = \frac{k_{deg,h}}{k_{deg,h} + \frac{[I]_h \times k_{inact}}{[I]_h + K_I}}$
Induction	$C_g = 1 + \frac{d \bullet E_{max} \bullet [I]_g}{[I]_g + EC_{50}}$	$C_h = 1 + \frac{d \bullet E_{max} \bullet [I]_h}{[I]_h + EC_{50}}$

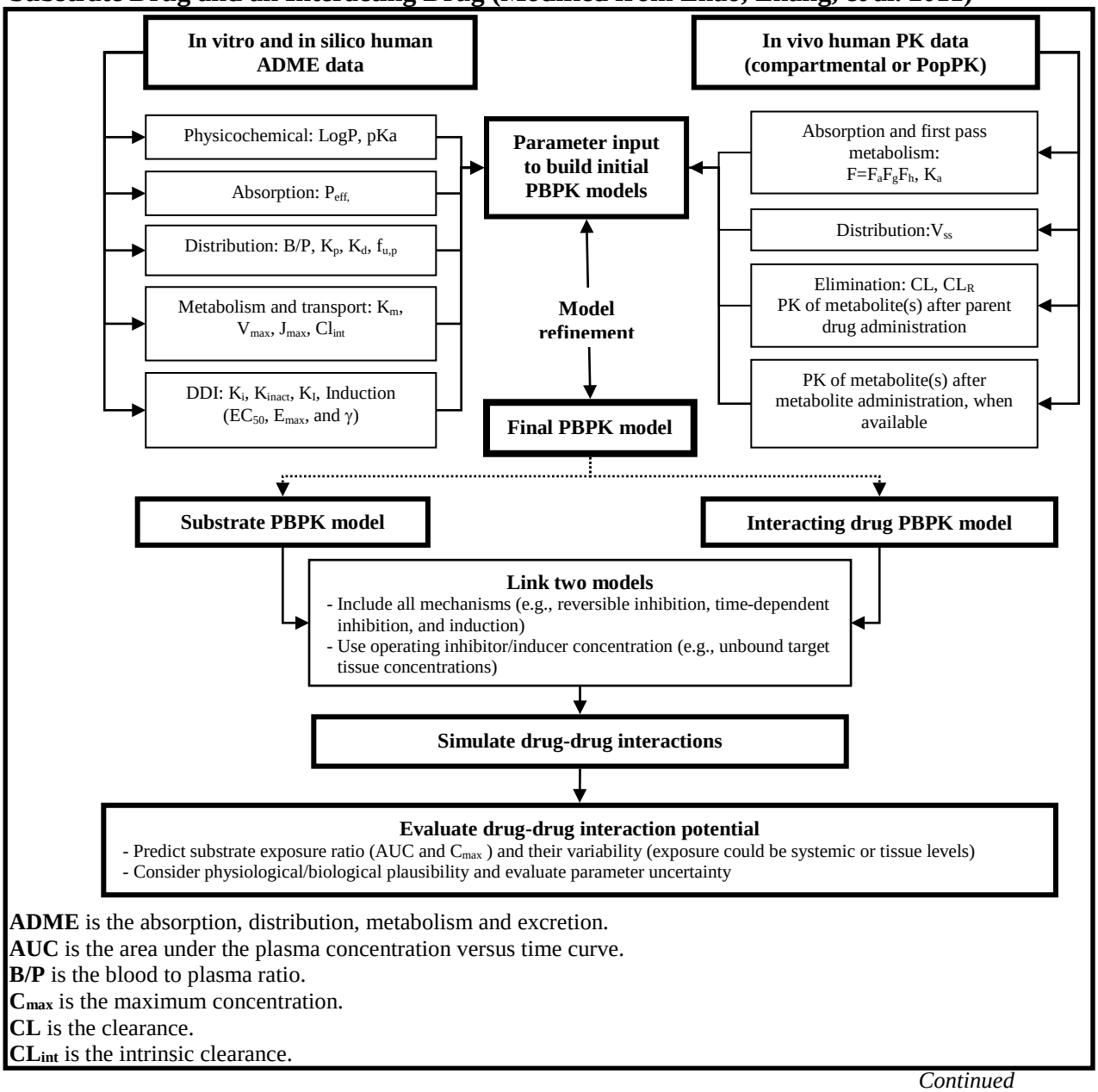
$[I]_h$ = $f_{u,p} \times (C_{max} + F_a \times k_a \times Dose/Q_h/R_B)$ (Ito, Iwatsubo, et al. 1998)
$[I]_g$ = $F_a \times k_a \times Dose/Q_{en}$ (Rostami-Hodjegan and Tucker 2004)
$f_{u,p}$ is the unbound fraction in plasma. When it is difficult to measure accurately due to high protein binding (i.e., $f_{u,p}$ <0.01) in plasma, a value of 0.01 should be used for $f_{u,p}$.
C_{max} is the maximal total (free and bound) inhibitor concentration in the plasma at steady state.
F_a is the fraction absorbed after oral administration; a value of 1 should be used when the data are not available.
k_a is the first order absorption rate constant in vivo; a value of 0.1 min^{-1} (Ito, Iwatsubo, et al. 1998) can be used when the data are not available.
Q_{en} is the blood flow through enterocytes (e.g., 18 L/hr/70 kg (Yang, Jamei, et al. 2007a)).
Q_h is the hepatic blood flow (e.g., 97 L/hr/70 kg (Yang, Jamei, et al. 2007b)).
R_B is the blood-to-plasma concentration ratio.

One should separately use inhibition mechanisms (A and B only) to predict a drug's enzyme inhibition potential (i.e., assuming C is equal to 1), and use induction mechanisms (C only) to predict a drug's enzyme induction potential (i.e., assuming A and B are equal to 1).

c. Using PBPK models to predict enzyme-based DDIs

PBPK models can predict the DDI potential of an investigational drug as an enzyme substrate or an enzyme perpetrator. Figure 8 shows a general PBPK model-based framework to predict the DDI potential for the purposes of DDI study planning in clinical development.

Figure 8. A PBPK Model-Based Framework to Explore the DDI Potential Between a Substrate Drug and an Interacting Drug (Modified from Zhao, Zhang, et al. 2011)*



ADME is the absorption, distribution, metabolism and excretion.
AUC is the area under the plasma concentration versus time curve.
B/P is the blood to plasma ratio.
C_{max} is the maximum concentration.
CL is the clearance.
CL_{int} is the intrinsic clearance.

Continued

Figure 8 continued. A PBPK Model-Based Framework to Explore the DDI Potential Between a Substrate Drug and an Interacting Drug (Modified from Zhao, Zhang, et al. 2011)*

CL_R is the renal clearance.
DDI is a drug-drug interaction.
EC_{50} is the concentration causing half maximal effect.
E_{max} is the maximum effect.
F is the bioavailability.
F_a is the fraction absorbed.
F_g is the bioavailability in the gut.
F_h is the bioavailability in the liver.
$f_{u,p}$ is the unbound fraction in plasma.
γ is the Hill coefficient.
IC_{50} the concentration causing half maximal inhibition.
I_{max} is the maximum effect or inhibition.
J_{max} is the maximum rate of transporter-mediated efflux/uptake.
K_a is the first-order absorption rate constant.
K_d is the dissociation constant of a drug-protein complex.
K_i is the reversible inhibition constant, concentration causing half maximal inhibition
K_I is the apparent inactivation constant, concentration causing half maximum inactivation
k_{inact} is the apparent maximum inactivation rate constant.
K_m is the Michaelis-Menten constant, substrate concentration causing half maximal reaction or transport
K_p is the tissue to plasma partition coefficient.
LogP is the logarithm of the octanol-water partition coefficient.
MOA is the mechanism of action.
PD is the pharmacodynamics of a drug
P_{eff} is the jejunum permeability.
PK is pharmacokinetics of a drug.
PopPK is population pharmacokinetics.
V is the volume of distribution.
V_{max} is the maximum rate of metabolite formation.

*Note: PBPK models for both substrate and interacting drug (inhibitor or inducer) should be constructed separately using in vitro and in vivo disposition parameters and be verified before they are linked through appropriate mechanisms to predict the degree of DDI.

- When using PBPK modeling, the sponsor should provide comprehensive justifications on any model assumptions, the physiological and biochemical plausibility of the model, variability, and uncertainty measures. Submissions using advanced models like PBPK models should include a description of the structural model, the sources and justifications for both system- and drug-dependent parameters, the types of error models, all model outputs, the data analysis, and an adequate sensitivity analysis (see the FDA's guidance for industry *Physiologically Based Pharmacokinetic Analyses — Format and Content*[29]).

[29] When final, this guidance will represent the FDA's current thinking on this topic.

When using predefined models (structural and error) from commercially available software, the sponsor should specify the software version and list any deviations from the predefined models (Zhao, Rowland, et al. 2012).

- When using PBPK modeling to predict the DDI potential of the investigational drug as an *enzyme substrate*, the sponsor should address the following questions (Vieira, Kim, et al. 2014; Wagner, Pan, et al. 2015; Wagner, Pan, et al. 2016):

    - Can the base PBPK model of the investigational substrate describe the available clinical PK data using different dosing regimens (e.g., a dose proportionality study) and dosing routes (e.g., intravenous or oral)?

    - Are elimination pathways quantitatively assigned in the substrate's model according to available in vitro and in vivo data?

    - Are index perpetrator models verified with regard to their modulating effect on enzyme activity in humans?

    - Are there sensitivity analyses for parameters exhibiting a high level of uncertainty?

    - If complex metabolic and transport mechanisms are expected, do the substrate and modulator models include the major disposition and interaction mechanism and are they verified in a step-wise manner? (see also 2.b below for transporters)

  The sponsor may use PBPK models to predict the effects of enzyme modulators on the PK of an investigational substrate if the models can describe the available data on DDIs for a strong enzyme inhibitor or inducer (Wagner, Zhao, et al. 2015; Wagner, Pan, et al. 2015; Wagner, Pan, et al. 2016).

- When using PBPK modeling to predict the drug interaction potential of an investigational drug as an *enzyme perpetrator*, the sponsor should address the following questions (Vieira, Zhao, et al. 2012; Wagner, Pan, et al. 2015; Wagner, Pan, et al. 2016):

    - Can the base PBPK model of the investigational perpetrator describe the available clinical PK data using different dosing regimens (e.g., a dose proportionality study) and dosing routes (e.g., intravenous or oral)?

    - Are index substrate models verified with regard to the effect of altered enzyme activity on its PK in humans?

    - Were inhibition and induction mechanisms separately considered to ensure a conservative prediction of in vivo enzyme inhibition or induction?

- Did the simulation use the highest clinical dose of the investigational perpetrator?

- Are there sensitivity analyses for parameters exhibiting high levels of uncertainty?

2. General Considerations When Using Predictive Models to Evaluate Transporter-Mediated DDIs

a. Using basic models to predict the effect of a drug as a transporter inhibitor

Evaluating a drug as a potential transporter inhibitor begins with the use of a basic model, applying the same concepts as for metabolizing enzymes (see section IV.B). The predictions and predefined cutoff criteria from this basic model approach determine the need to further evaluate transporter inhibition in vivo. Generally, the sponsor should assume reversible inhibition and use the IC_{50} as a practical substitute for the K_i. The basic models described in this document use the gut luminal concentration, the maximal unbound plasma concentration, and/or the estimated maximal unbound concentration at the hepatic inlet of the interacting drug for [I]. The sponsor should compare the calculated R or $[I]/IC_{50}$ values to predefined cutoff criteria to determine whether it is possible to rule out the potential for a DDI. If the basic model does not rule out the potential for DDIs, the sponsor should further evaluate the DDI potential of the investigational drug.

b. Using PBPK models to predict transporter-based DDIs

PBPK models can include ADME processes mediated by transporters as well as passive diffusion and metabolism. Compared to CYP enzymes, the predictive performance of PBPK modeling for transporter-based DDIs has not been established (Wagner, Zhao, et al. 2015). This is largely due to knowledge gaps in transporter biology and limited experience in determining and modeling the kinetics of transporters (Pan, Hsu, et al, 2016). Recent applications of PBPK models to evaluate the interplay between transporters and enzymes suggest that a model of an investigational drug as a substrate of multiple transporters and enzymes is only adequate for confident predictions of untested DDI scenarios after the model has been verified with clinical data from a wide range of DDI or pharmacogenetic studies for the applicable enzyme or transporter (Varma, Lai, et al. 2012; Gertz, Cartwright, et al. 2013; Varma, Lai, et al. 2013; Varma, Lin, et al. 2013; Jamei, Bajot, et al. 2014; Varma, Scialis, et al. 2014; Snoeys, Beumont, et al. 2015). For drugs that are potential transporter inhibitors, the sponsor should establish and verify models for transporter substrates (Gertz, Tsamandouras, et al. 2014; Tsamandouras, Dickinson, et al. 2015; Snoeys, Beumont, et al. 2015).

VIII. ABBREVIATIONS AND ACRONYMS

ADME: absorption, distribution, metabolism, and/or excretion
AP: apical
ATP: adenosine triphosphate
AUC: area under the plasma concentration-time curve
$AUC_{metabolite}$: area under the plasma concentration-time curve of metabolite
AUC_{parent}: area under the plasma concentration-time curve of parent drug
AUCR: area under the plasma concentration-time curve ratio
BL: basolateral
B/P: blood to plasma ratio
BCRP: breast cancer resistance protein
CHO: Chinese hamster ovary cell
Cl_{int}: intrinsic clearance
CL_r: renal clearance
C_{max}: total maximal concentration in plasma
CYP: cytochrome P450
d: scaling factor
DDI: drug-drug interaction
EC_{50}: concentration causing half maximal effect determined in vitro
E_{max}: maximum induction effect determined in vitro
ER: efflux ratio
F_a: fraction absorbed
F_g: intestinal availability
f_m: fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction.
FMO: flavin monooxygenase
$f_{u,p}$: unbound fraction in plasma
GFR: glomerular filtration rate
HEK293: human embryonic kidney 293 cell
[I]: concentration of the interacting drug
IC_{50}: half-maximal inhibitory concentration
I_{gut}: intestinal luminal concentration estimated as dose/250 mL
$I_{in,max}$: estimated maximum plasma inhibitor concentration at the inlet to the liver
$I_{max,u}$: maximal unbound plasma concentration of the interacting drug
J_{max}: maximal flux rate
k_a: absorption rate constant
k_d: dissociation constant
k_p: partition coefficient
k_{deg}: apparent first-order degradation rate constant of the affected enzyme
K_i: inhibition constant
K_I: inhibitor concentration causing half-maximal inactivation
k_{inact}: maximal inactivation rate constant

K_m: Michaelis-Menton constant
k_{obs}: observed (apparent first order) inactivation rate constant of the affected enzyme
LLC-PK1: Lewis-lung cancer porcine kidney 1 cell
LogP: octanol-water partition coefficient
MAO: monoamine oxidase
MATE: multidrug and toxin extrusion
MDCK: Madin-Darby canine kidney cell
MDR1: multi-drug resistance 1 protein
NADPH: nicotinamide adenine dinucleotide phosphate (reduced form)
OAT: organic anion transporter
OATP: organic anion transporting polypeptide
OCT: organic cation transporter
P_{app}: apparent permeability
PBPK: physiologically-based pharmacokinetic
PD: pharmacodynamics
P-gp: P-glycoprotein
PK: pharmacokinetic
pKa: negative logarithm of the ionization constant (Ka) of an acid, a measure of the strength of an acid
PXR: pregnane X receptor
Q_{en}: blood flow through enterocytes
Q_h: hepatic blood flow rate
R: ratio of victim AUC in the presence and absence of perpetrators (inhibitors or inducers), predicted with basic models
R_B: blood to plasma ratio
S9: supernatants after 9000 g centrifugation
SCH: sandwich cultured hepatocytes
SLC: solute carrier
TDI: time-dependent inhibition
UGT: uridine diphosphate (UDP)-glucuronosyl transferase
V_{max}: maximal rate
V_{ss}: steady-state volume of distribution
XO: xanthine oxidase

IX. REFERENCES

Agarwal, S, V Arya, and L Zhang, 2013, Review of P-gp Inhibition Data in Recently Approved New Drug Applications: Utility of the Proposed [I(1)]/IC(50) and [I(2)]/IC(50) Criteria in the P-gp Decision Tree, J Clin Pharmacol, 53(2):228-233.

Amundsen, R, H Christensen, B Zabihyan, and A Asberg, 2010, Cyclosporine A, But Not Tacrolimus, Shows Relevant Inhibition of Organic Anion-Transporting Protein 1B1-Mediated Transport of Atorvastatin, Drug Metab Dispos, 38(9):1499-1504.

Bjornsson, TD, JT Callaghan, HJ Einolf, V Fischer, L Gan, S Grimm, J Kao, SP King, G Miwa, L Ni, G Kumar, J McLeod, RS Obach, S Roberts, A Roe, A Shah, F Snikeris, JT Sullivan, D Tweedie, JM Vega, J Walsh, SA Wrighton, and R Pharmaceutical, G Manufacturers of America, Drug Metabolism/Clinical Pharmacology Technical Working, FDACfD Evaluation and Research, 2003, The Conduct of In Vitro and In Vivo Drug-Drug Interaction Studies: A Pharmaceutical Research and Manufacturers of America (PhRMA) Perspective, Drug Metab Dispos, 31(7):815-832.

Brouwer, KL, D Keppler, KA Hoffmaster, DA Bow, Y Cheng, Y Lai, JE Palm, B Stieger, R Evers, and C International Transporter, 2013, In Vitro Methods to Support Transporter Evaluation in Drug Discovery and Development, Clin Pharmacol Ther, 94(1):95-112.

Callegari, E, AS Kalgutkar, L Leung, RS Obach, DR Plowchalk, and S Tse, 2013, Drug Metabolites as Cytochrome p450 Inhibitors: A Retrospective Analysis and Proposed Algorithm for Evaluation of the Pharmacokinetic Interaction Potential of Metabolites in Drug Discovery and Development, Drug Metab Dispos, 41(12):2047-2055.

Dong Z, X Yang, V Arya, and L Zhang, 2016a, Comparing Various In Vitro Prediction Criteria to Assess the Potential of a New Molecular Entity (NME) to Inhibit Organic Anion Transporter 1 and 3 (OAT1 and 3), Clin Pharmacol Ther, 99(S1):S94.

Dong Z, X Yang, V Arya, and L Zhang, 2016b, Comparing Various In Vitro Prediction Criteria to Assess the Potential of a New Molecular Entity (NME) to Inhibit OCT2 and MATE Transporters In Vivo, Clin Pharmacol Ther, 99(S1):S94.

Einolf, HJ, 2007, Comparison of Different Approaches to Predict Metabolic Drug-Drug Interactions, Xenobiotica, 37(10-11):1257-1294.

Einolf, HJ, L Chen, OA Fahmi, CR Gibson, RS Obach, M Shebley, J Silva, MW Sinz, JD Unadkat, L Zhang and P Zhao, 2014, Evaluation of Various Static and Dynamic Modeling Methods to Predict Clinical CYP3A Induction Using In Vitro CYP3A4 mRNA Induction Data, Clin Pharmacol Ther, 95(2):179-188.

Ellens, H, S Deng, J Coleman, J Bentz, ME Taub, I Ragueneau-Majlessi, SP Chung, K Heredi-Szabo, S Neuhoff, J Palm, P Balimane, L Zhang, M Jamei, I Hanna, M O'Connor, D Bednarczyk, M Forsgard, X Chu, C Funk, A Guo, KM Hillgren, L Li, AY Pak, ES Perloff, G Rajaraman, L Salphati, JS Taur, D Weitz, HM Wortelboer, CQ Xia, G Xiao, T Yamagata, and CA Lee, 2013, Application of Receiver Operating Characteristic Analysis to Refine the Prediction of Potential Digoxin Drug Interactions, Drug Metab Dispos, 41(7):1367-1374.

Fahmi, OA, S Hurst, D Plowchalk, J Cook, F Guo, K Youdim, M Dickins, A Phipps, A Darekar, R Hyland, and RS Obach, 2009, Comparison of Different Algorithms for Predicting Clinical Drug-Drug Interactions, Based on the Use of CYP3A4 In Vitro Data: Predictions of Compounds as Precipitants of Interaction, Drug Metab Dispos, 37(8):1658-1666.

Fahmi, OA, M Kish, S Boldt, and RS Obach, 2010, Cytochrome P450 3A4 mRNA is a More Reliable Marker than CYP3A4 Activity for Detecting Pregnane X Receptor-Activated Induction of Drug-Metabolizing Enzymes, Drug Metab Dispos, 38(9):1605-1611.

Fahmi, OA and SL Ripp, 2010, Evaluation of Models for Predicting Drug-Drug Interactions Due to Induction, Expert Opin Drug Metab Toxicol, 6(11):1399-1416.

Gertz, M, CM Cartwright, MJ Hobbs, KE Kenworthy, M Rowland, JB Houston, and A Galetin, 2013, Cyclosporine Inhibition of Hepatic and Intestinal CYP3A4, Uptake and Efflux Transporters: Application of PBPK Modeling in the Assessment of Drug-Drug Interaction Potential, Pharm Res, 30(3):761-780.

Gertz, M, N Tsamandouras, C Säll, JB Houston, and A Galetin, 2014, Reduced Physiologically Based Pharmacokinetic Model of Repaglinide: Impact of OATPB1 and CYP2C8 Genotype and Source of In Vitro Data on the Prediction of Drug-Drug Interaction Risk, Pharm Res, 31(9):2367-2382.

Gertz, M, PJ Kilford, JB Houston, and A Galetin, 2008, Drug Lipophilicity and Microsomal Protein Concentration as Determinants in the Prediction of the Fraction Unbound in Microsomal Incubations, Drug Metab Dispos, 36(3):535-542.

Giacomini, KM and SM Huang, 2013, Transporters in Drug Development and Clinical Pharmacology, Clin Pharmacol Ther, 94(1):3-9.

Giacomini, KM, SM Huang, DJ Tweedie, LZ Benet, KL Brouwer, X Chu, A Dahlin, R Evers, V Fischer, KM Hillgren, KA Hoffmaster, T Ishikawa, D Keppler, RB Kim, CA Lee, M Niemi, JW Polli, Y Sugiyama, PW Swaan, JA Ware, SH Wright, SW Yee, MJ Zamek-Gliszczynski, and L Zhang, 2010, Membrane Transporters in Drug Development, Nat Rev Drug Discov, 9(3):215-236.

Grimm, SW, HJ Einolf, SD Hall, K He, HK Lim, KH Ling, C Lu, AA Nomeir, E Seibert, KW

Skordos, GR Tonn, R Van Horn, RW Wang, YN Wong, TJ Yang, and RS Obach, 2009, The Conduct of In Vitro Studies to Address Time-Dependent Inhibition of Drug-Metabolizing Enzymes: A Perspective of the Pharmaceutical Research and Manufacturers of America, Drug Metab Dispos, 37(7):1355-1370.

Hallifax, D and JB Houston, 2006, Binding of Drugs to Hepatic Microsomes: Comment and Assessment of Current Prediction Methodology With Recommendation for Improvement, Drug Metab Dispos, 34(4):724-726; author reply 727.

Ito, K, T Iwatsubo, S Kanamitsu, K Ueda, H Suzuki, and Y Sugiyama, 1998, Prediction of Pharmacokinetic Alterations Caused by Drug-Drug Interactions: Metabolic Interaction in the Liver, Pharmacol Rev, 50(3):387-412.

Izumi, S, Y Nozaki, K Maeda, T Komori, O Takenaka, H Kusuhara, and Y Sugiyama, 2015, Investigation of the Impact of Substrate Selection on In Vitro Organic Anion Transporting Polypeptide 1B1 Inhibition Profiles for the Prediction of Drug-Drug Interactions, Drug Metab Dispos, 43(2):235-247.

Jamei, M, F Bajot, S Neuhoff, Z Barter, J Yang, A Rostami-Hodjegan, and K Rowland-Yeo, 2014, A Mechanistic Framework for In Vitro-In Vivo Extrapolation of Liver Membrane Transporters: Prediction of Drug-Drug Interaction Between Rosuvastatin and Cyclosporine, Clin Pharmacokinet, 53(1):73-87.

Jones, H, Y Chen, C Gibson, T Heimbach, N Parrott, S Peters, J Snoeys, V Upreti, M Zheng, and S Hall, 2015, Physiologically Based Pharmacokinetic Modeling in Drug Discovery and Development: A Pharmaceutical Industry Perspective, Clin Pharmacol Ther, 97(3):247-262.

Lepist, EI, X Zhang, J Hao, J Huang, A Kosaka, G Birkus, BP Murray, R Bannister, T Cihlar, Y Huang, and AS Ray, 2014, Contribution of the Organic Anion Transporter OAT2 to the Renal Active Tubular Secretion of Creatinine and Mechanism for Serum Creatinine Elevations Caused by Cobicistat, Kidney Int, 86(2):350-357.

Lu, C, GT Miwa, SR Prakash, LS Gan, and SK Balani, 2007, A Novel Model for the Prediction of Drug-Drug Interactions in Humans Based on In Vitro Cytochrome P450 Phenotypic Data, Drug Metab Dispos, 35(1):79-85.

Mao, J, MA Mohutsky, JP Harrelson, SA Wrighton, and SD Hall, 2012, Predictions of Cytochrome P450-Mediated Drug-Drug Interactions Using Cryopreserved Human Hepatocytes: Comparison of Plasma and Protein-Free Media Incubation Conditions, Drug Metab Dispos, 40(4):706-716.

Pan Y, V Hsu, M Grimstein, L Zhang, V Arya, V Sinha, JA Grillo, and P Zhao, 2016, The Application of Physiologically Based Pharmacokinetic Modeling to Predict the Role of Drug Transporters: Scientific and Regulatory Perspectives, J Clin Pharmacol, 56:S122-31.

Rostami-Hodjegan, A and G Tucker, 2004, In Silico Simulations to Assess the In Vivo Consequences of In Vitro Metabolic Drug-Drug Interactions, Drug Discov Today Technol, 1(4):441-448.

Snoeys, J, M Beumont, M Monshouwer, and S Ouwerkerk-Mahadevan, 2015, Mechanistic Understanding of the Nonlinear Pharmacokinetics and Intersubject Variability of Simeprevir: A PBPK-Guided Drug Development Approach, Clin Pharmacol Ther, 99(2):224-234.

Tachibana, T, M Kato, T Watanabe, T Mitsui, and Y Sugiyama, 2009, Method for Predicting the Risk of Drug-Drug Interactions Involving Inhibition of Intestinal CYP3A4 and P-Glycoprotein, Xenobiotica, 39(6):430-443.

Tweedie, D, JW Polli, EG Berglund, SM Huang, L Zhang, A Poirier, X Chu, B Feng, and C International Transporter, 2013, Transporter Studies in Drug Development: Experience to Date and Follow-Up on Decision Trees from the International Transporter Consortium, Clin Pharmacol Ther, 94(1):113-125.

Tsamandouras, N, G Dickinson, Y Guo, S Hall, A Rostami-Hodjegan, A Galetin, and L Aarons, 2015, Development and Application of a Mechanistic Pharmacokinetic Model for Simvastatin and Its Active Metabolite Simvastatin Acid Using an Integrated Population PBPK Approach, Pharm Res, 32(6):1864-1883.

Vaidyanathan, J, K Yoshida, V Arya, and L Zhang, 2016. Comparing Various In Vitro Prediction Criteria to Assess the Potential of a New Molecular Entity (NME) to Inhibit Organic Anion Transporting Polypeptide 1B1 (OATP1B1) In Vivo, J Clin Pharm, 56 Suppl 7:S59-72.

Varma, MV, Y Lai, B Feng, J Litchfield, TC Goosen, and A Bergman, 2012, Physiologically Based Modeling of Pravastatin Transporter-Mediated Hepatobiliary Disposition and Drug-Drug Interactions, Pharm Res, 29(10):2860-2873.

Varma, MV, Y Lai, E Kimoto, TC Goosen, AF El-Kattan, and V Kumar, 2013, Mechanistic Modeling to Predict the Transporter- and Enzyme-Mediated Drug-Drug Interactions of Repaglinide, Pharm Res, 30(4):1188-1199.

Varma, MV, J Lin, YA Bi, CJ Rotter, OA Fahmi, JL Lam, AF El-Kattan, TC Goosen, and Y Lai, 2013, Quantitative Prediction of Repaglinide-Rifampicin Complex Drug Interactions Using Dynamic and Static Mechanistic Models: Delineating Differential CYP3A4 Induction and OATP1B1 Inhibition Potential of Rifampicin, Drug Metab Dispos, 41(5):966-974.

Varma, MV, RJ Scialis, J Lin, YA Bi, CJ Rotter, TC Goosen, and X Yang, 2014, Mechanism-Based Pharmacokinetic Modeling to Evaluate Transporter-Enzyme Interplay in Drug Interactions and Pharmacogenetics of Glyburide, AAPS J, 16(4):736-748.

Vieira, ML, B Kirby, I Ragueneau-Majlessi, A Galetin, JY Chien, HJ Einolf, OA Fahmi, V Fischer, A Fretland, K Grime, SD Hall, R Higgs, D Plowchalk, R Riley, E Seibert, K Skordos, J Snoeys, K Venkatakrishnan, T Waterhouse, RS Obach, EG Berglund, L Zhang, P Zhao, KS Reynolds, and SM Huang, 2014, Evaluation of Various Static In Vitro-In Vivo Extrapolation Models for Risk Assessment of the CYP3A Inhibition Potential of an Investigational Drug, Clin Pharmacol Ther, 95(2):189-198.

Vieira MD, MJ Kim, S Apparaju , V Sinha , I Zineh, SM Huang, and P Zhao, 2014, PBPK model describes the effects of comedication and genetic polymorphism on systemic exposure of drugs that undergo multiple clearance pathways, Clin Pharmacol Ther, 95(5):550-557.

Vieira, ML, P Zhao, EG Berglund, KS Reynolds, L Zhang, LJ Lesko, and SM Huang, 2012, Predicting Drug Interaction Potential With a Physiologically Based Pharmacokinetic Model: A Case Study of Telithromycin, a Time-Dependent CYP3A Inhibitor, Clin Pharmacol Ther, 91(4):700-708.

Wagner, C, P Zhao, Y Pan, V Hsu, J Grillo, S Huang, and V Sinha, 2015, Application of Physiologically Based Pharmacokinetic (PBPK) Modeling to Support Dose Selection: Report of an FDA Public Workshop on PBPK, Clin Pharm Ther: Pharmacometrics & Systems Pharmacology, 4:226-230.

Wagner, C, Y Pan, V Hsu, JA Grillo, L Zhang, KS Reynolds, V Sinha, and P Zhao, 2015, Predicting the Effect of Cytochrome P450 Inhibitors on Substrate Drugs: Analysis of Physiologically Based Pharmacokinetic Modeling Submissions to the US Food and Drug Administration, Clin Pharmacokinet, 54(1):117-127.

Wagner C, Y Pan, V Hsu , V Sinha, and P Zhao, 2016, Predicting the Effect of CYP3A Inducers on the Pharmacokinetics of Substrate Drugs Using Physiologically Based Pharmacokinetic (PBPK) Modeling: An Analysis of PBPK Submissions to the US FDA, Clin Pharmacokinet, 55(4):475-83.

Yang, J, M Jamei, KR Yeo, A Rostami-Hodjegan, and GT Tucker, 2007a, Misuse of the Well-Stirred Model of Hepatic Drug Clearance, Drug Metab Dispos, 35(3):501-502.

Yang, J, M Jamei, KR Yeo, GT Tucker, and A Rostami-Hodjegan, 2007b, Prediction of Intestinal First-Pass Drug Metabolism, Curr Drug Metab, 8(7):676-684.

Yang, J, M Liao, M Shao, M Jamei, KR Yeo, GT Tucker, and A Rostami-Hodjegan, 2008, Cytochrome P450 Turnover: Regulation of Sythesis and Degradation, Methods for Determining

Rates, and Implications for the Prediction of Drug Interactions, Curr Drug Metab, 9(5):384-394.

Yoshida, K, K Maeda, and Y Sugiyama, 2012, Transporter-Mediated Drug-Drug Interactions Involving OATP Substrates: Predictions Based on In Vitro Inhibition Studies, Clin Pharmacol Ther, 91(6):1053-1064.

Yu, H, SK Balani, W Chen, D Cui, L He, WG Humphreys, J Mao, WG Lai, AJ Lee, HK Lim, C MacLauchlin, C Prakash, S Surapaneni, S Tse, A Upthagrove, RL Walsky, B Wen, and Z Zeng, 2015, Contribution of Metabolites to P450 Inhibition-Based Drug-Drug Interactions: Scholarship from the Drug Metabolism Leadership Group of the Innovation and Quality Consortium Metabolite Group, Drug Metab Dispos, 43(4):620-630.

Yu, H and D Tweedie, 2013, A Perspective on the Contribution of Metabolites to Drug-Drug Interaction Potential: The Need to Consider Both Circulating Levels and Inhibition Potency, Drug Metab Dispos, 41(3):536-540.

Zamek-Gliszczynski, MJ, CA Lee, A Poirier, J Bentz, X Chu, H Ellens, T Ishikawa, M Jamei, JC Kalvass, S Nagar, KS Pang, K Korzekwa, PW Swaan, ME Taub, P Zhao, A Galetin, and C International Transporter, 2013, ITC Recommendations for Transporter Kinetic Parameter Estimation and Translational Modeling of Transport-Mediated PK and DDIs in Humans, Clin Pharmacol Ther 94(1):64-79.

Zamek-Gliszczynski, MG, X Chu, JW Polli, MF Paine, and A Galetin, 2014, Understanding the Transport Properties of Metabolites: Case Studies and Considerations for Drug Development, Drug Metab Dispos, 42(4):650-654.

Zhang, L, YD Zhang, JM Strong, KS Reynolds, and SM Huang, 2008, A Regulatory Viewpoint on Transporter-Based Drug Interactions, Xenobiotica, 38(7-8):709-724.

Zhao, P, M Rowland, and SM Huang, 2012, Best Practice in the Use of Physiologically Based Pharmacokinetic Modeling and Simulation to Address Clinical Pharmacology Regulatory Questions, Clin Pharmacol Ther, 92(1):17-20.

Zhao, P, L Zhang, JA Grillo, Q Liu, JM Bullock, YJ Moon, P Song, SS Brar, R Madabushi, TC Wu, BP Booth, NA Rahman, KS Reynolds, E Gil Berglund, LJ Lesko, and SM Huang, 2011, Applications of Physiologically Based Pharmacokinetic (PBPK) Modeling and Simulation During Regulatory Review, Clin Pharmacol Ther, 89(2):259-267.