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In Vitro Drug  
Interaction Studies —  
Cytochrome P450  
Enzyme- and  
Transporter-Mediated  
Drug Interactions  
Guidance for Industry

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

January 2020  
Clinical Pharmacology

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# In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry

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**U.S. Department of Health and Human Services  
Food and Drug Administration  
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# **In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry<sup>1</sup>**

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

## **I. INTRODUCTION**

This final guidance is intended to help drug developers plan and evaluate studies to determine the drug-drug interaction (DDI) potential of an investigational drug product.<sup>2</sup> The final guidance focuses on in vitro approaches to evaluate the interaction potential between investigational drugs with cytochrome P450 enzymes (CYPs) and transporters as well as 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. Note that at this time, the in vitro methods to evaluate the induction of P-gp and other transporters are not well established; therefore, recommendations for the in vitro evaluation of investigational drugs as transporter inducers are not provided.

If an in vitro assessment suggests that the sponsor should conduct a clinical DDI study, the sponsor should refer to the January 2020 final FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*.<sup>3</sup> Together, these two final guidances describe a systematic, risk-based approach to assessing the DDI potential of investigational drugs and making recommendations to mitigate DDIs.

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

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<sup>1</sup> 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.

<sup>2</sup> Only small molecule drugs are covered in this guidance. Interactions involving biologics (therapeutic proteins) are beyond the scope of this guidance.

<sup>3</sup> We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/RegulatoryInformation/Guidances/default.htm>.

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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 for and proper design of potential future clinical studies.

Various modeling approaches can help translate in vitro observations into in vivo predictions of potential clinical DDIs. For example, when evaluating the drug as a perpetrator 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; September 2018 FDA guidance for industry *Physiologically Based Pharmacokinetic Analyses — Format and Content*) can help 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 to evaluate DDIs 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 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; however, 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 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 by initiating in vitro metabolic studies before first-in-human studies to inform the need for and design of clinical PK studies. We recommend that the sponsor conducts the following in vitro studies to evaluate the potential for metabolism-mediated drug interactions.

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### **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 CYP3A using in vitro phenotyping experiments to determine which enzymes metabolize the investigational drug. If the investigational drug is not found to undergo significant in vivo metabolism by these major CYP enzymes, the sponsor should then 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 aldehyde oxidase (AO), carboxylesterase (CES), monoamine oxidase (MAO), flavin monooxygenase (FMO), xanthine oxidase (XO), and alcohol/aldehyde dehydrogenase (ADH/ALDH)
- Phase II enzymes including UDP glucuronosyl transferases (UGTs) and sulfotransferases (SULTs)

#### *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 January 2020 FDA final guidance for industry entitled *Clinical Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*).

### **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 CYP3A 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, the sponsor should calculate the ratio of intrinsic clearance values of a probe substrate for an enzymatic pathway in the absence and in the presence of the interacting drug. This ratio is referred to as  $R_1$  for reversible inhibition. For CYP3A,  $R_{1,\text{gut}}$  should also be calculated as shown in Figure 1.

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**Figure 1: Equations to Calculate the R value for Basic Models of Reversible Inhibition (Vieira, Kirby, et al. 2014)**

$$R_1 = 1 + (I_{\max,u} / K_{i,u})$$

$$R_{1,\text{gut}} = 1 + (I_{\text{gut}} / K_{i,u})$$

$I_{\max,u}$  is the maximal unbound plasma concentration of the interacting drug at steady state.\*

$I_{\text{gut}}$  is the intestinal luminal concentration of the interacting drug calculated as the dose/250 mL.

$K_{i,u}$  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  $R_2$  as described in Figure 2.

**Figure 2: Equations to Calculate the R value for Basic Models of TDI (Yang, Liao, et al. 2008; Grimm, Einolf, et al. 2009; Vieira, Kirby, et al. 2014)**

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

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

$k_{\text{obs}}$  is the observed (apparent first order) inactivation rate of the affected enzyme.

$k_{\text{deg}}$  is the apparent first-order degradation rate constant of the affected enzyme.

$K_{I,u}$  is the unbound inhibitor concentration causing half-maximal inactivation.

$k_{\text{inact}}$  is the maximal inactivation rate constant.

$I_{\max,u}$  is the maximal unbound plasma concentration of the interacting drug at steady state.\*

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

When static mechanistic models or dynamic models (see appendix, section VII.C.1) 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

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investigational drug that is hypothesized to be both an inducer and inhibitor) to 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. Initially, sponsors can conduct experiments to evaluate CYP1A2, CYP2B6, and CYP3A4 only. If no induction of CYP3A4 enzymes is observed, evaluating the induction potential of CYP2C enzymes is not necessary because both CYP3A4 and CYP2C enzymes are induced via activation of the pregnane X receptor (PXR). If the investigational drug induces CYP3A4 and the results suggest that a clinical study is warranted, the sponsor should evaluate the potential of the investigational drug to induce CYP2C. However, a negative in vivo study with a CYP3A sensitive substrate can be used to rule out induction potential of an investigational drug on CYP2C enzymes, as long as the potential of CYP3A inhibition by the drug and its metabolite(s) can be excluded.

#### *2. Data Analysis and Interpretation*

The induction results should be evaluated separately for each donor. If the result from at least one donor exceeds the pre-defined threshold, the sponsor should consider the drug to have induction potential and conduct a follow-up evaluation. 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; Kenny, Ramsden, et al. 2018). Three are described in detail below:

**1. 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 drug is interpreted as an inducer if: (1) it increased mRNA expression of a CYP enzyme in a concentration-dependent manner; and (2) the fold change of CYP mRNA expression relative to the vehicle control is  $\geq 2$ -fold at the expected hepatic concentrations of the drug. Expected drug concentrations in the liver can be calculated by assuming a certain fold of  $I_{\max,u}$  (e.g., 30-fold of mean unbound maximal steady-state plasma concentration at therapeutic dose). Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% ( $f_{u,p} = 0.01$ ) if experimentally determined to be  $< 1\%$  when calculating  $I_{\max,u}$ .

However, the induction potential should not be ruled out for an investigational drug that increases CYP enzyme mRNA less than 2-fold the of vehicle control, if the increase is more than 20% of the response of the positive control. Further evaluation is recommended when there is an inconclusive finding.



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To calculate the percent of the response to the positive control, the following equation should be used:

$$\% \text{ of positive control} = (\text{mRNA fold increase of test drug treated cells} - 1) \times 100 / (\text{mRNA fold increase of positive control} - 1)$$

**2. Correlation methods:** The sponsor may use correlation methods as described in Figure 3 to predict the magnitude of a clinical induction effect (e.g., AUC ratio of index substrate in the presence and absence of inducers) of an investigational drug according to a calibration curve of relative induction scores (RIS) or  $I_{\max,u}/EC_{50}$  for a set of known inducers of the same enzyme. If the predicted magnitude is more than a predefined cut-off (e.g., AUC ratio  $\leq 0.8$ ), a drug is considered have induction potential in vivo.

The calibration can be established once for one batch of hepatocytes and does not need to be determined for each experiment. Sometimes,  $E_{\max}$  or  $EC_{50}$  cannot be estimated due to an incomplete in vitro induction profile (e.g., limited by solubility or cytotoxicity of tested drug). An alternative correlation approach may be used if the method is validated.

**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  
 $E_{\max}$  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 at steady state.\*  
\*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%.

**3. Basic kinetic model:** To use this method, the sponsor should calculate the R value ( $R_3$ ) as described in Figure 4 and compare to a predefined cut-off determined from a set of inducers and non-inducers. For example, a  $R_3$  value  $\leq 0.8$  may indicate that the investigational drug has induction potential in vivo.

**Figure 4: An Equation to Calculate the R value for Basic Models of Induction (Kenny, Ramsden, et al. 2018)**

$R_3 = 1 / [1 + d \times ((E_{\max} \times 10 \times I_{\max,u}) / (EC_{50} + 10 \times I_{\max,u}))]$   
 $R_3$  is the predicted ratio of intrinsic clearance values of a probe substrate for an enzymatic pathway in the absence and presence of an inducer.  
 $d$  is the scaling factor and is assumed to be 1. A different value can be used if supported by prior experience with the system used (Vermet, Raoust, et al. 2016).  
 $E_{\max}$  is the maximum induction effect determined in vitro.  
 $I_{\max,u}$  is the maximal unbound plasma concentration of the interacting drug at steady state.\*  
 $EC_{50}$  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%.

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If these methods indicate that the investigational drug has the potential to induce metabolizing enzymes (using specific cutoff values mentioned above or developed by individual laboratories for these methods), the sponsor should further investigate the enzyme induction potential of the investigational drug by using mechanistic models (see appendix, section VII.C.1) 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  $\leq 0.8$  based on static mechanistic models or dynamic mechanistic models (e.g., PBPK models; see appendix, section VII.C.1), 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 (see appendix, section VII.C.1) 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 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 January 2020 FDA guidance for industry *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*) 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.<sup>4</sup> 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 mechanistic dynamic model to inform the need for additional studies. The sponsor should verify and update dynamic models to demonstrate that the model can adequately describe the observed findings from the first in vivo study with a sensitive index substrate. In the presence of inhibitory metabolites of an investigational drug, their contribution and rank order of metabolite  $R$  values should also be considered when determining what in vivo studies should be conducted.

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<sup>4</sup> An orally administered drug may inhibit intestinal metabolic enzymes (e.g., CYP3A) in addition to hepatic enzymes. Therefore, in vivo DDI for CYP3A inhibition should be considered if  $R_{1,\text{gut}}$  is greater than or equal to 11, even if  $R_1$  for CYP3A is not the largest value among the major CYPs evaluated.

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Concurrent prediction of inhibition and induction using mechanistic static models or dynamic models (see appendix, section VII.C.1) can be considered for predicting the net effect of an investigational drug that is hypothesized to be both an inhibitor and an inducer of metabolizing enzymes. However, there is a concern with concurrent predictions, as over-prediction of inhibition may mask the induction effect leading to a false negative prediction of the overall effect (Einolf, Chen, et al. 2014). If the induction potential is over-predicted, it will mask the inhibition effect.

In vitro induction studies may also detect enzyme down-regulation. However, research in this area is presently very limited, and the mechanisms behind these effects are unclear. If concentration-dependent down-regulation is observed in vitro and is not attributable to cytotoxicity, additional in vitro or in vivo studies may be needed to understand the potential clinical consequences (Hariparsad, Ramsden, et al. 2017).

#### **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. In concert with metabolizing enzymes, transporters 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 (MATE1/MATE2-K)
- Organic cation transporter 2 (OCT2)

Understanding whether the drug is a substrate or inhibitor 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 inhibitors 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

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intended population is likely to use statins, the sponsor should examine the potential of the investigational drug to interact with OATP1B1/1B3 before initiation of 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 generally 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 2017 FDA 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* to determine if the investigational drug can be classified as highly permeable and/or highly soluble (e.g., biopharmaceutics classification system class 1 drugs).

#### *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  $\geq 2$  for an investigational drug in cells that express P-gp (e.g., Caco-2 cells or transfected cells overexpressing P-gp)<sup>5</sup>
- A flux that is inhibited by at least one known P-gp inhibitor at a concentration at least 10 times its  $K_i$  or  $IC_{50}$  (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.

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<sup>5</sup> 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.

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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 January 2020 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*).

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 January 2020 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*).

### **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.

#### *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  $\geq 25\%$  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). Other factors to be considered include the drug's physiological properties, e.g., low passive membrane permeability, high hepatic concentrations relative to other tissues, organic anion/charged at physiological pH, which support the importance of active uptake of the drug into liver.

#### *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  $\geq 2$ -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  $\leq 50\%$  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 2019 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*).

### **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 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  $\geq 25\%$  of the systemic clearance), the sponsor should evaluate the drug in vitro to determine whether it is a substrate of OAT1/3, OCT2, 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\***

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

$\text{CL}_r$  is the renal clearance.

$f_{u,p}$  is the unbound fraction in plasma.

$\text{GFR}$  is the glomerular filtration rate.

\*This equation is valid assuming that there is no re-absorption (e.g., no active re-absorption and passive re-absorption is equal to passive secretion). 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  $\geq 2$ ; 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  $\text{IC}_{50}$ . 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 January 2020 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*).

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

## *Contains Nonbinding Recommendations*

### *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 (MATE1, MATE2-K), OAT1, and OAT3 (see appendix, section VII.B for considerations regarding in vitro systems).

### *2. Data Analysis and Interpretation*

**P-gp and BCRP**: The sponsor should conduct studies to determine if an investigational drug inhibits the efflux ratio or net flux of a known P-gp or BCRP substrate in Caco-2, P-gp- or BCRP-overexpressed cells or inhibits uptake of substrate when membrane vesicles are used, 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  $I_{gut}/IC_{50}$  or  $K_i \geq 10$  where  $I_{gut}$  = dose of inhibitor/250 mL. If a metabolite of the drug is an inhibitor or the investigational drug is administered by parenteral route, in vivo inhibition of P-gp or BCRP may occur if the  $I_1/IC_{50}$  or  $K_i \geq 0.1$ , where  $I_1$  is the  $C_{max}$  of the metabolite or the inhibitor drug. These cutoff values are 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.

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 January 2020 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*).

**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. Time-dependent inhibition has been demonstrated for a few OATP1B1/3 inhibitors (Amundsen, Christensen, et al. 2010; Gertz, Cartwright, et al. 2013; Izumi, Nozaki, et al. 2015; Pahwa, Alam, et al. 2017). Sponsors may consider adding a pre-incubation step as part of assay validation when determining  $IC_{50}$  values for an investigational drug. The investigational drug has the potential to inhibit OATP1B1/3 in vivo if the R value (as described in Figure 6 below) is  $\geq 1.1$ .

## Contains Nonbinding Recommendations

**Figure 6: Equation to Calculate the R Value of the Investigational Drug to Determine the Potential to Inhibit OATP1B1/3\***

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

$f_{u,p}$  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:

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

$F_a$  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,  $F_a = 1$ ,  $F_g = 1$  and  $k_a = 0.1/\text{min}$  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 OATP1B1 or OATP1B3 substrates (see the January 2020 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter--Mediated Drug Interactions*).

**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 MATE2-K) 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  $\geq 0.1$ .<sup>6</sup> 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 observed in clinical studies could be due to inhibition of these transporters by the investigational drug (Chu, Bleasby, et al. 2016; Mathialagan, Rodrigues, et al. 2017; Arya, Yang, et al. 2014). Confirmation of the mechanism of an increase in serum creatinine with the investigational drug requires additional evidence such as clinical mechanistic studies.

<sup>6</sup> Considering uncertainties in the protein binding measurements, the unbound fraction should be set to 1% if experimentally determined to be less than 1%.



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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 January 2020 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions*).

### **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 January 2020 FDA guidance for industry entitled *Clinical Drug Interaction Studies — Cytochrome P450 Enzymes and Transporters-Mediated Drug Interactions*). At this time, the in vitro methods to evaluate the induction of P-gp and other transporters are not well established, therefore recommendations for in vitro evaluation of investigational drugs as transporter inducers are not provided.

## **V. EVALUATING 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 a perpetrator of metabolizing enzymes (see sections below). In vitro studies normally use a synthesized or purified metabolite standard. 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). If basic models suggest that the metabolite(s) may have in vivo DDI liability and a static or dynamic mechanistic modeling approach (e.g., PBPK) is used for DDI assessment of a drug, metabolite(s) should be incorporated in these models.

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 the parent drug should be applied where applicable.

### **A. Metabolite as a Substrate**

#### *1. Conducting In Vitro Studies*

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The risk of a clinically relevant DDI through altered formation or elimination of metabolites should be investigated if changes in metabolite exposure levels may result in clinically meaningful alteration of efficacy or safety in vivo. The risk of a DDI when the metabolite acts as a substrate should be evaluated for a pharmacologically active metabolite that contributes to  $\geq 50\%$  of the overall activity. Both the in vitro receptor potency and the in vivo unbound systemic exposure (expressed in molar unit) of a metabolite relative to the parent drug need to be taken into consideration when evaluating the contribution of the metabolite to efficacy. If plasma protein binding of the parent drug and the metabolite is high, it is preferred to determine their protein binding in the same study to reduce inter-study variability. If available, data related to target tissue distribution of parent drug and the metabolite may need to be considered when evaluating the contribution of metabolite to in vivo efficacy.

### *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 systemic exposure (in molar unit) and polarity (e.g., measured or predicted LogP, the elution order on the chromatogram of reverse phase-high performance liquid chromatography) of the metabolite relative to the parent drug. The sponsor should conduct an in vitro CYP enzyme inhibition study if: (1) the metabolite is less polar than the parent drug and the  $AUC_{\text{metabolite}} \geq 25\%$  of  $AUC_{\text{parent}}$ ; or (2) the metabolite is more polar than the parent drug and the  $AUC_{\text{metabolite}} \geq AUC_{\text{parent}}$ . A lower cut-off value for the metabolite-to-parent AUC ratio may also be considered for metabolites with structural alerts for potential mechanism-based inhibition (Orr, Ripp, et al. 2012; Yu and Tweedie 2013; Yu, Balani, et al. 2015).

### *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 except that  $R_{1,\text{gut}}$  may not be applicable (see section III.B).

## **VI. LABELING RECOMMENDATIONS**

The Prescribing 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.<sup>7</sup> 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 the Prescribing Information, if clinically significant. In addition, the results of pertinent in vitro studies that establish the absence of an effect must be included.<sup>8</sup> In vitro information that has been superseded by clinical information should not be included in the Prescribing Information unless it is essential to understanding the clinical results.

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 Prescribing Information (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:

- *Clinical Pharmacology Labeling for Human Prescription Drug and Biological Products — Considerations, Content, and Format* (December 2016)
- *Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions* (January 2020)

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<sup>7</sup> 21 CFR 201.56(a)(3).

<sup>8</sup> 21 CFR 201.57(c)(13)(c)(i)(C).

## **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 human 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 elimination 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

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specific enzyme inhibitors in human liver microsomes or hepatocytes (e.g., a pool of more than 10 donors); 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 human recombinant CYP enzymes, the sponsor should consider the difference in the amount and enzyme activity of CYPs between the recombinant CYP enzyme systems and the human liver (Venkatakrisnan, von Moltke, et al. 2000; Chen, Liu, et al. 2011).
- The in vitro system for these studies should: (1) be robust and reproducible; and (2) be characterized with in vitro probe substrate 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.<sup>9</sup>
- Whenever possible, the sponsor should conduct all experiments with drug concentrations deemed appropriate by kinetic experiments, relevant to clinical setting, 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, there are two widely used methods: measurement of parent drug depletion; and measurement of metabolite formation. For the latter method, it is desirable that 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.
- The use of a radiolabeled drug substrate is advantageous because samples can be analyzed using liquid chromatography coupled with a radioactivity detector and a mass spectrometer to identify and quantify drug-related species.

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<sup>9</sup> A list of probe substrates:

<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1>.

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- 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.<sup>10</sup>
- 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 (TDI)) and inhibition potencies (e.g.,  $K_i$  for reversible inhibition, and  $K_i$  and  $k_{inact}$  for TDI). 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 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).

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<sup>10</sup> Examples of in vitro selective inhibitors for P450-mediated metabolism:  
<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1-2>

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Commonly used in vitro probe substrates and their marker reactions can be found on the FDA Web site on Drug Development and Drug Interactions.<sup>11</sup>

- 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).<sup>12</sup> Kinetic constants ( $K_i$ ,  $IC_{50}$ ,  $K_I$ , and/or  $k_{inact}$ ) of the probe inhibitors should be comparable to the range of 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).
- 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). However, the drug concentration should not exceed the drug's solubility limits or cause deleterious effects in cell models (e.g., cytotoxicity). If the initial high concentration 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. The sponsor should test at least four different concentrations of the investigational drug with the probe substrate.
- 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

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<sup>11</sup> 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>

<sup>12</sup> Examples of in vitro selective inhibitors for P450-mediated metabolism:  
<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1-2>

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2006) or predicted using *in silico* methods. It is recommended to experimentally determine nonspecific binding for highly lipophilic drugs (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 activate enzymes. The experiment should include a solvent (vehicle) control, and when necessary, also a no-solvent control.
- The sponsor can determine inhibition kinetics according to appropriate mechanisms (e.g., reversible inhibition or TDI). For a reversible inhibitor, half of the measured  $IC_{50}$  can be used as an estimate of  $K_i$ , if the substrate concentration used is equal to its  $K_m$  for the enzyme (Haput, Kazmi, et al. 2015).
- 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 why any alternative *in vitro* system is appropriate for 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



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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.
- The sponsor should individually evaluate CYP1A2, CYP2B6, and CYP3A4 first because they are induced via different nuclear receptors.
- Activation of a nuclear receptor, PXR, may lead to co-induction of CYP3A4 and CYP2C enzymes. Thus, a negative in vitro result for CYP3A4 induction eliminates the need for additional in vitro or in vivo induction studies for CYP3A4 and CYP2C enzymes. If in vitro CYP3A4 induction results are positive and suggest a clinical DDI study is warranted, then 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 inducers and non-inducers as positive and negative controls (see the FDA's Web site on Drug Development and Drug Interactions).<sup>13</sup> Once the system is validated, a non-inducer (as negative control) can be included as optional in the test study to evaluate an investigational drug. When applicable, the sponsor should conduct pilot experiments to establish a test system (e.g., a particular lot of cryopreserved human hepatocytes) for routine studies of CYP induction (Fahmi, Kish et al. 2010; Fahmi and Ripp 2010; Einolf, Chen et al. 2014).
- Drug concentrations investigated should span the range of therapeutic exposures. If the drug solubility permits, this range of drug concentrations should include at least one 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. If the drug is highly bound to human plasma protein, and the medium contains serum (or proteins, e.g., bovine serum albumin), or if the drug may have significant non-specific binding, sponsors are encouraged to measure the concentration of unbound test drug in the medium of incubation. Correction for binding may be needed when interpreting the data, to help predict the magnitude of a clinical DDI (Sun, Chothe, et al. 2017; Chang, Yang, et al. 2017).

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<sup>13</sup> For more information, see:

<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm>.

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- When primary human hepatocytes are used, the sponsor should use 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 can identify 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 times.
- Actual concentrations of the drug in the system are important for extrapolating in vitro results to in vivo scenarios. Sponsors are encouraged to measure concentrations of the parent drug in the medium, preferably at several time points during the last day of the incubation (Sun, Chothe, et al. 2017; Chang, Yang, et al. 2017).

#### **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.

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

<b>Transporter</b>	<b>In Vitro Systems</b>
<i>ABC Transporters</i>	
BCRP, P-gp	Caco-2 cells, commercial or in-house membrane vesicles, knock-out/down cells, transfected cells (MDCK, LLC-PK <sub>1</sub> , etc.)
<i>Solute Carrier (SLC) Transporters</i>	
OATP1B1/3	Hepatocytes, transfected cells (CHO, HEK293, MDCK, etc.)
OAT1/3, OCT2	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: Lilly Laboratory 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 or highly non-specific binding drugs as substrates.
  - P-gp and BCRP assays using membrane vesicles should directly measure the adenosine triphosphate (ATP)-dependent, transporter-mediated uptake of drugs with control vesicles for comparisons.
- 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 should be grown 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.

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- 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 with appropriate control conditions to account for endogenous transporter activity and non-specific binding. One approach is to compare the efflux ratios from 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 OCTs, OATs, OATPs and MATEs.
    - 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 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 function (e.g., uptake, efflux) with control experiments (e.g., positive controls for substrates/inhibitors, non-transfected control cells).
- 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<sup>14</sup>).
- The sponsor should characterize the following conditions whenever applicable: the source of the membrane vesicles or cells, cell culture conditions (e.g., cell passage number, seeding density, monolayer age), probe substrate/inhibitor concentrations,

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<sup>14</sup> For more information, see:

<http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm>.

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incubation time, buffer/pH conditions, sampling interval, and 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 solvent (vehicle) control, and when necessary, also a no-solvent control.
- For both substrate and inhibitor studies, the sponsor should demonstrate sufficient total recovery of the drugs. If the total recovery falls below a pre-specified boundary set by the laboratories, the nature and extent of the effects leading to a decrease of recovery should be investigated and considered when evaluating the potential DDI risk of a test drug. 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 adding serum or proteins to the media
- 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*

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 concentrations of the test drug in 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.
- 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.

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### 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:

- 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. However, the drug concentration should not exceed the drug's solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cells. 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 or the unbound maximum hepatic inlet concentration for hepatic uptake transporters (see Figure 6)). For apical intestinal transporters, the tested drug concentration should cover  $0.1 \times \text{dose}/250 \text{ mL}$ . If the test drug demonstrates inhibitory activity, the sponsor should test additional concentrations to calculate  $IC_{50}$  or  $K_i$  values. The sponsor should evaluate at least four concentrations of the test drug with the probe substrate. The sponsor can then compare  $IC_{50}$  or  $K_i$  values to clinical plasma or estimated 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 may consider a pre-incubation step with the test drug for OATP1B1 and OATP1B3 inhibition to evaluate whether this results 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 may 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 or clinical studies, in conjunction with model-based

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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 probe 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, i.e.,  $R_1$ ,  $R_{1,\text{gut}}$  (only for CYP3A), and  $R_2$  (only for TDI) for inhibition effect;  $R_3$ , fold of change, and correlation methods for induction.

The sponsor should compare the calculated R values or fold-change to the recommended cutoff criteria to determine whether it is possible to rule out the potential for a DDI. Sponsors may calibrate their internal in vitro systems with known perpetrators and non-perpetrators of an enzyme and propose specific cutoff values with proper justification. 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 of the investigational drug on substrate drugs (represented as the AUCR) (see Figure 7).

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

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

The equation assumes that the drug has negligible extrahepatic clearance.

**A** is the effect of reversible inhibitions.

**B** is the effect of TDI.

**C** is the effect of induction.

**F<sub>g</sub>** is the fraction available after intestinal metabolism.

**f<sub>m</sub>** is the fraction of hepatic 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	$C_g = 1 + \frac{d \cdot E_{max} \cdot [I]_g}{[I]_g + EC_{50}}$	$C_h = 1 + \frac{d \cdot E_{max} \cdot [I]_h}{[I]_h + EC_{50}}$

$[I]_h = f_{u,p} \times (C_{max} + (F_a \times F_g \times k_a \times Dose)/Q_h/R_B)$  (Ito, Iwatsubo, et al. 1998)

$[I]_g = F_a \times k_a \times Dose / Q_{en}$  (Rostami-Hodjegan and Tucker 2004)

**f<sub>u,p</sub>** 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<sub>max</sub>** is the maximal total (free and bound) inhibitor concentration in the plasma at steady state.

**F<sub>a</sub>** is the fraction absorbed after oral administration; a value of 1 should be used when the data are not available.

**F<sub>g</sub>** is the fraction available after intestinal metabolism; a value of 1 should be used when the data are not available.

**k<sub>a</sub>** is the first order absorption rate constant in vivo; a value of 0.1 min<sup>-1</sup> (Ito, Iwatsubo, et al. 1998) can be used when the data are not available.

**Q<sub>en</sub>** is the blood flow through enterocytes (e.g., 18L/hr/70 kg (Yang, Jamei, et al. 2007a)).

**Q<sub>h</sub>** is the hepatic blood flow (e.g., 97 L/hr/70 kg (Yang, Jamei, et al. 2007b)).

**R<sub>B</sub>** is the blood-to-plasma concentration ratio.

**d** is the scaling factor and is assumed to be 1. A different value can be used if supported by prior experience with the system used (Einolf, Chen, et al. 2014; Vermet, Raouf, et al. 2016).

In addition to the combination of inhibition and induction, 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



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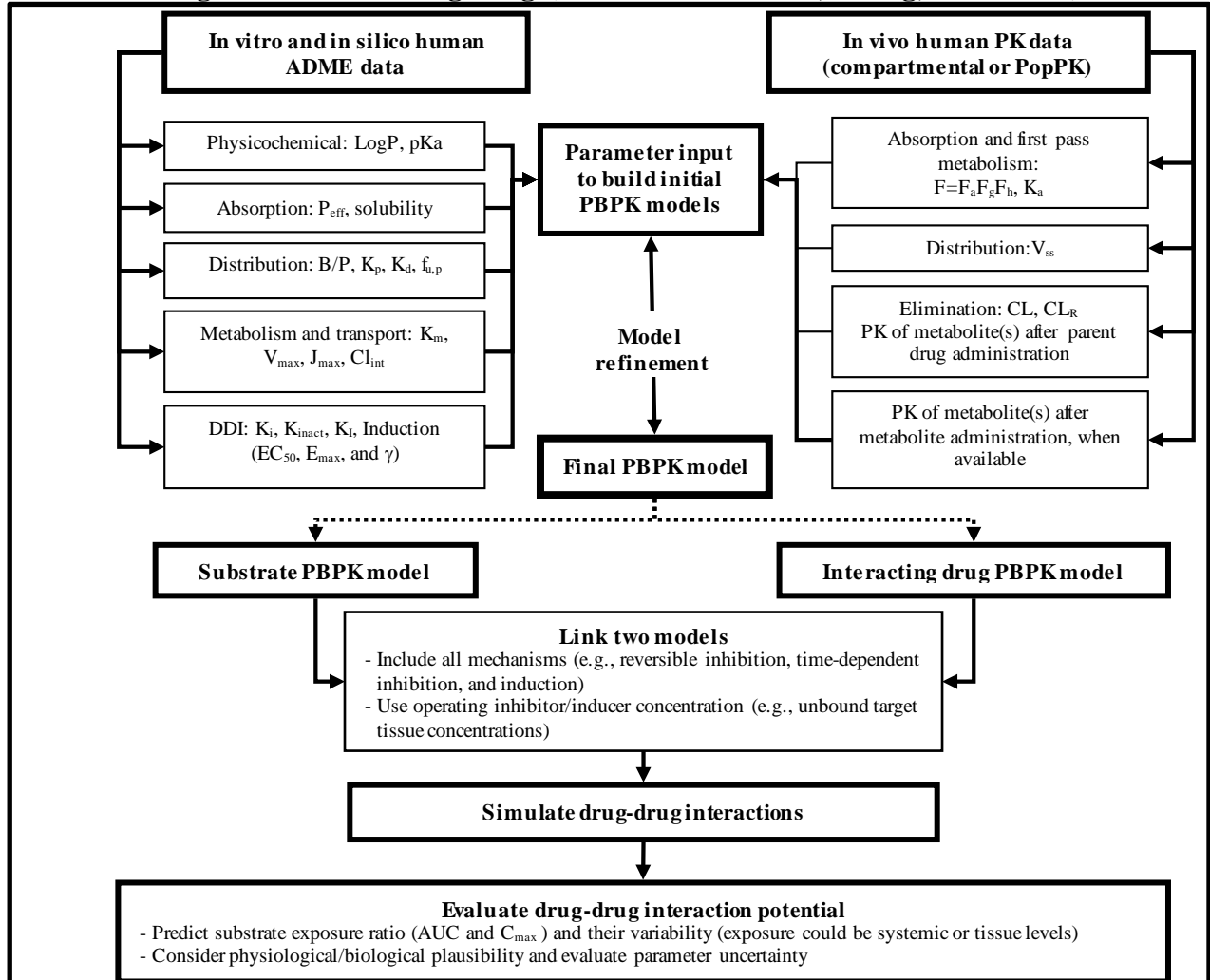
potential (i.e., assuming A and B are equal to 1). It should be noted that concurrent prediction may lead to a false negative prediction if the inhibition potential is over-predicted, thus masking the induction effect. If the induction potential is over-predicted, it will mask the inhibition effect.

#### c. Using PBPK models to predict enzyme-based DDIs

PBPK models can predict the DDI potential of an investigational drug and/or a metabolite 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.

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**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.
- $CL_R$**  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.

*Continued*

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### 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)\*

<p><math>f_{u,p}</math> is the unbound fraction in plasma.</p> <p><math>\gamma</math> is the Hill coefficient.</p> <p><math>IC_{50}</math> the concentration causing half maximal inhibition.</p> <p><math>I_{max}</math> is the maximum effect or inhibition.</p> <p><math>J_{max}</math> is the maximum rate of transporter-mediated efflux/uptake.</p> <p><math>K_a</math> is the first-order absorption rate constant.</p> <p><math>K_d</math> is the dissociation constant of a drug-protein complex.</p> <p><math>K_i</math> is the reversible inhibition constant, concentration causing half maximal inhibition</p> <p><math>K_I</math> is the apparent inactivation constant, concentration causing half maximum inactivation</p> <p><math>k_{inact}</math> is the apparent maximum inactivation rate constant.</p> <p><math>K_m</math> is the Michaelis-Menten constant, substrate concentration causing half maximal reaction or transport</p> <p><math>K_p</math> is the tissue to plasma partition coefficient.</p> <p><math>LogP</math> is the logarithm of the octanol-water partition coefficient.</p> <p><b>MOA</b> is the mechanism of action.</p> <p><b>PD</b> is the pharmacodynamics of a drug</p> <p><math>P_{eff}</math> is the jejunum permeability.</p> <p><b>PK</b> is pharmacokinetics of a drug.</p> <p><b>PopPK</b> is population pharmacokinetics.</p> <p><b>V</b> is the volume of distribution.</p> <p><math>V_{max}</math> is the maximum rate of metabolite formation.</p> <p>*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.</p>
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- 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 2018 FDA guidance for industry *Physiologically Based Pharmacokinetic Analyses — Format and Content*). 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)?

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- 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 mechanisms and are they verified? (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?
  - In addition to the combination of inhibition and induction, 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

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and predefined cutoff criteria from this basic model approach determine the need to further evaluate transporter inhibition *in vivo*. Generally, the sponsor could 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).

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### **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<sub>metabolite</sub>: area under the plasma concentration-time curve of metabolite  
AUC<sub>parent</sub>: 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<sub>int</sub>: intrinsic clearance  
CL<sub>r</sub>: renal clearance  
C<sub>max</sub>: total maximal concentration in plasma  
CYP: cytochrome P450  
d: scaling factor  
DDI: drug-drug interaction  
EC<sub>50</sub>: concentration causing half maximal effect determined in vitro  
E<sub>max</sub>: maximum induction effect determined in vitro  
ER: efflux ratio  
F<sub>a</sub>: fraction absorbed  
F<sub>g</sub>: intestinal availability  
f<sub>m</sub>: fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction.  
FMO: flavin monooxygenase  
f<sub>u,p</sub>: unbound fraction in plasma  
GFR: glomerular filtration rate  
HEK293: human embryonic kidney 293 cell  
[I]: concentration of the interacting drug  
IC<sub>50</sub>: half-maximal inhibitory concentration  
I<sub>gut</sub>: intestinal luminal concentration estimated as dose/250 mL  
I<sub>in,max</sub>: estimated maximum plasma inhibitor concentration at the inlet to the liver  
I<sub>max,u</sub>: maximal unbound plasma concentration of the interacting drug at steady state  
J<sub>max</sub>: maximal flux rate  
k<sub>a</sub>: absorption rate constant  
k<sub>d</sub>: dissociation constant  
k<sub>p</sub>: partition coefficient  
k<sub>deg</sub>: apparent first-order degradation rate constant of the affected enzyme  
K<sub>i</sub>: inhibition constant  
K<sub>I</sub>: inhibitor concentration causing half-maximal inactivation  
k<sub>inact</sub>: maximal inactivation rate constant  
K<sub>m</sub>: Michaelis-Menton constant  
k<sub>obs</sub>: observed (apparent first order) inactivation rate of the affected enzyme

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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 ( $K_a$ ) 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

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