Guidance for Industry

Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations

DRAFT GUIDANCE

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

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Clinical Pharmacology

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I. INTRODUCTION

This guidance provides recommendations for sponsors of new drug applications (NDAs) and biologics license applications (BLAs) for therapeutic biologics regulated by CDER regarding *in vitro* and *in vivo* studies of drug metabolism, drug transport, and drug-drug or drug-therapeutic protein interactions. Drug interactions can result when one drug alters the pharmacokinetics of another drug or its metabolites. Drug interactions also can reflect the additive nature of the pharmacodynamic effect of either drug when taken with the other drug. The main focus of this guidance is pharmacokinetic drug interactions. This guidance reflects the Agency's view that the pharmacokinetic interactions between an investigational new drug and other drugs should be defined during drug development, as part of an adequate assessment of the drug's safety and effectiveness. It is important to understand the nature and magnitude of drug-drug interactions (DDI) for several reasons. Concomitant medications, dietary supplements, and some foods, such as grapefruit juice, may alter metabolism and/or drug transport abruptly in individuals who previously had been receiving and tolerating a particular dose of a drug. Such an abrupt alteration in metabolism or transport can change the known safety and efficacy of a drug.

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[1] This guidance has been prepared by the Drug-Drug Interaction Working Group in the Office of Clinical Pharmacology, Office of Translational Sciences, in the Center for Drug Evaluation and Research (CDER), with input from other offices in CDER.

II. SUMMARY OF GUIDANCE

The key recommendations for sponsors to consider when evaluating drug-drug interactions during drug development are listed below. The various sections of this guidance provide more details for each recommendation.

- Interactions between an investigational new drug and other drugs should be defined during drug development, as part of an adequate assessment of the drug's safety and effectiveness. The objective of drug-drug interaction studies is to determine whether potential interactions between the investigational drug and other drugs exist and, if so, whether the potential for such interactions indicates the need for dosage adjustments, additional therapeutic monitoring, a contraindication to concomitant use, or other measures to mitigate risk.

- Development of a drug should include identification of the principal routes of elimination, quantitation of the contribution by enzymes and transporters to drug disposition, and characterization of the mechanism of drug-drug interactions.

- Sponsors who believe a complete evaluation of the potential for drug-drug interactions is not necessary for an investigational drug because of the target population and likely co-administered drugs should contact the Office of Clinical Pharmacology and the clinical division in the Office of New Drugs.

- This guidance and its appendices include numerous decision trees intended to help sponsors determine what types of drug-drug interaction studies may be needed (see Figures 2 through 7 and Appendix Figures A-1 through A-6).

- The study of drug-drug interaction for a new drug generally begins with in vitro studies to determine whether a drug is a substrate, inhibitor, or inducer of metabolizing enzymes. The results of in vitro studies will inform the nature and extent of in vivo studies that may be required to assess potential interactions. Along with clinical pharmacokinetic data, results from in vitro studies may serve as a screening mechanism to rule out the need for additional in vivo studies, or provide a mechanistic basis for proper design of clinical studies using a modeling and simulation approach.

- When testing an investigational drug for the possibility that its metabolism is inhibited or induced (i.e., as a substrate), selection of the interacting drugs should be based on in vitro or in vivo studies identifying the enzyme systems that metabolize the investigational drug. The choice of the interacting drug can then be based on known, important inhibitors and inducers of the pathway under investigation. Strong inhibitors and inducers provide the most sensitive assessment and should generally be tested first (see section V.C).

- If potential drug-drug interactions are identified based on in vitro and/or in vivo studies, sponsors should design further studies or collect information to determine (1) whether

additional studies are needed to better quantify the effect and to examine the effects of weaker inhibitors (early studies usually examine strong inhibitors) on the investigational drugs as substrates and effects of investigational drugs (as inhibitors) on a range of substrates, and (2) whether dosage adjustments or other prescribing modifications (e.g., additional safety monitoring or contraindications) are needed based on the identified interaction(s) to avoid undesired consequences.

- The potential for drug interactions with metabolites of investigational drugs (metabolites present at ≥25% of parent drug AUC) should be considered (see section IV.A.3).

- Metabolic drug-drug interactions should also be explored for investigational drugs that are not eliminated significantly by metabolism because such drugs can inhibit or induce a co-administered drug's metabolic pathway (see section IV.A.1).

- When evaluating a new drug as a potential cytochrome P450 (CYP) enzyme inhibitor, sponsors should consider a stepwise, model-based evaluation of metabolism-based interactions (from basic model for initial assessment to more mechanistic models including physiologically-based pharmacokinetic (PBPK) modeling) (see section IV.A.1). The criteria used for assessing "equivalence" (e.g., predicted AUC ratio of 0.8-1.25 using population-based PBPK models) may be used as an initial cutoff in deciding whether in vivo studies are needed. The criteria discussed in this guidance document are suggested values. We are open to discussion based on sponsors' interpretation.

    - PBPK is a useful tool that can help sponsors (1) better design drug-drug interaction studies, including dedicated trials and population pharmacokinetic studies, and (2) quantitatively predict the magnitude of drug-drug interactions in various clinical situations. PBPK models also may offer useful alternatives to dedicated clinical studies.

    - When submitting PBPK studies to CDER, sponsors should provide details of model assumptions, physiological and biological plausibility, the origin of the parameters, and information on uncertainty and variability.

- The evaluation of CYP enzyme induction should begin with studies of CYP1A2, CYP2B6, and CYP3A in vitro (Figure 4). If the in vitro induction results are positive according to predefined thresholds using basic models, the investigational drug is considered an enzyme inducer and further in vivo evaluation may be warranted. Alternatively, a sponsor can estimate the degree of drug-drug interactions using mechanistic models to determine the need for further in vivo evaluation (see section IV.A.1.b-3).

    - It should be noted that there may be mechanisms of induction that are presently unknown. Therefore, a potential human teratogen needs to be studied in vivo for effects on contraceptive steroids if the drug is intended for use in fertile women, regardless of in vitro induction study results.

- In addition to CYPs, other metabolizing enzymes (e.g., uridine diphosphate (UDP)-glucuronosyl transferases (UGTs)) that may be important for the drugs under evaluation should also be considered (see section IV.A.1).

- A number of transporter-based interactions have been documented in recent years (see Table 1, section III.B.2).

    - All investigational drugs should be evaluated in vitro to determine whether they are a potential substrate of P-glycoprotein (P-gp) or Breast Cancer Resistance Protein (BCRP) (see Figure 6, left panel, section IV.A.2). Investigational drugs should be evaluated in vitro to determine whether they are a substrate of hepatic uptake transporters Organic Anion Transporting Polypeptide 1B1(OATP1B1) or OATP1B3 when their hepatic pathway is significant (see Figure 6, middle panel, section IV.A.2). Similarly, investigational drugs should be evaluated in vitro to determine whether they are a substrate of Organic Aniton Transporter 1 (OAT1) or OAT3 or Organic Cation Transporter 2 (OCT2) when renal active secretion is important (Figure 6, right panel, section IV.A.2).

    - Because there have been clinically significant interactions demonstrated for critical drugs that are known substrates for P-gp (e.g., digoxin), BCRP (e.g., rosuvastatin), OATP1B1/OATP1B3 (e.g., statin drugs), OAT1/OAT3 (e.g., methotrexate, tenofovir) and OCT2 (e.g., metformin), evaluation of investigational drugs as inhibitors for these transporters should be conducted (see section IV.A.2).

    - The need for further in vivo drug interaction studies based on in vitro evaluation will be based on the criteria described in the decision trees in Figures A1-A6 in the Appendix.

- Because of the lack of a validated in vitro system to study transporter induction, the definitive determination of induction potential of an investigational drug on transporters is based on in vivo induction studies. The sponsor should consult with CDER about studying induction of transporters in vivo.

- Human clinical studies to assess drug-drug interactions may include simultaneous administration of a mixture of substrates of multiple CYP enzymes and transporters in one study (i.e., a "cocktail approach") to evaluate a drug's inhibition or induction potential (see section V.C.3). Negative results from a well-conducted cocktail study may eliminate the need for further evaluation of particular CYP enzymes and transporters. However, positive results may indicate that further in vivo evaluation should be conducted.

- The potential for interactions with drug products should be considered for certain classes of therapeutic proteins (TPs) (see Figure 7, section IV.B.2).

- If an investigational TP is a cytokine or cytokine modulator, studies should be conducted to determine the TP's effects on CYP enzymes or transporters. The in vivo evaluations of TPs in targeted patient populations can be conducted with individual substrates for specific CYP enzymes and transporters, or studies can be conducted using a "cocktail approach" (see section V.C.3).

- For TPs that will be used in combination with other drug products (small molecule or TP) as a combination therapy, studies should evaluate the effect of each product on the other. This evaluation is particularly important when the drug used in combination has a narrow therapeutic range.

- When there are known mechanisms or prior experience with certain PK or PD interactions for other similar TPs, appropriate in vitro or in vivo assessments for possible interactions should be conducted.

- Refer to section V for information regarding in vivo drug interaction study design. The section also contains tables on classification of in vivo inhibitors (Table 3) or inducers for CYP enzymes (Table 4), examples of sensitive in vivo CYP substrates and CYP substrates with narrow therapeutic ranges (Table 5), examples of in vivo inhibitors and inducers of selected transporters (Table 6), examples of in vivo substrates of selected transporters (Table 7) and examples of in vivo CYP3A and P-gp inhibitors and their relative potency (Table 8).

  - Simulations (e.g., by population-based PBPK models) can provide valuable insight into optimizing the study design (see section IV.A.1).

  - Detailed information on the dose given and time of administration should be documented for the co-administered drugs. When relevant for the specific drug, the time of food consumption should be documented.

  - Population pharmacokinetic (PopPK) analyses of data obtained from large-scale clinical studies that include sparse or intensive blood sampling can help characterize the clinical impact of known or newly identified interactions and determine recommendations for dosage modifications for the investigational drug as a substrate (section V.B). DDI analyses using a population PK approach should focus on excluding a specific clinically meaningful PK change. Because exposure of co-administered drugs is not monitored in most PopPK studies, the PopPK approach may not be useful to assess the effect of the investigational drugs on other drugs.

- The likelihood of drug interactions in specific populations (e.g., patients with organ impairment, and pediatric and geriatric patients) should be considered on a case-by-case basis. PBPK modeling (if well verified for intended purposes) can be helpful to guide the determination of the need to conduct population-specific studies (see "Populations" in section V.B and "Complex Drug Interactions" section V.C.4).

- Additional study design issues are discussed throughout the guidance (e.g., route of administration (section V.D), dose selection (section V.E), defining endpoints (section V.F), and statistical considerations (section V.G)).

- Labeling recommendations with regard to drug interactions are described in section VI.

    - A forest plot is considered a useful tool for presenting changes in pharmacokinetic exposure measures by various intrinsic and extrinsic factors including drug interactions in the PHARMACOKINETIC subsection of the labeling (see Figure 8, section VI).

    - If the sponsor wishes to include a statement in the labeling that no known drug-drug interaction of clinical significance exists, the sponsor should recommend specific *no effect* boundaries, or clinical equivalence intervals, for a drug-drug interaction and should provide the scientific justification for the recommendations. No effect boundaries represent the interval within which a change in a systemic exposure measure is considered not clinically meaningful. These conclusions can be based on exposure-response or dose-response data.

- Sponsors are encouraged to communicate with the Office of Clinical Pharmacology or the appropriate clinical review divisions within CDER regarding questions about drug interactions, in particular when

    - Using mechanistic or PBPK models for the prediction of drug-drug interactions including evaluation of complex drug-drug interactions
    - Determining the need to evaluate drug interactions with non-CYP enzymes or additional transporters that are not included in the decision trees
    - Determining drug-drug interaction studies involving TPs.

III. BACKGROUND

A. Relevance of Drug Interactions

The desirable and undesirable effects of a drug are related to its concentration at various sites of action, which is usually related to the blood or tissue concentration of the drug. The blood or tissue concentrations resulting from a dose are determined by the drug's absorption, distribution, metabolism, and excretion (ADME). Elimination of a drug or its active metabolites occurs either by metabolism to an inactive metabolite that is excreted, or by direct excretion of the drug or active metabolites. The kidneys and liver are responsible for most drug excretion. Drug interactions related to metabolism and excretion are well-recognized, but effects related to transporters are being documented with increasing frequency and are, therefore, important to consider in drug development. Therapeutic proteins may be eliminated through a specific

interaction with cell surface receptors, followed by internalization and lysosomal degradation within the target cell.

The overall objective of interaction studies for a new drug is to determine:

- whether any interactions are sufficiently large to necessitate a dosage adjustment of the drug itself or of the drugs with which it might be used,
- whether any interactions calls for additional therapeutic monitoring, or
- whether there should be a contraindication to concomitant use when lesser measures cannot mitigate risk.

In some instances, understanding how to adjust a dose or dosage regimen in the presence of an interacting drug, or how to avoid drug-drug interactions, may allow marketing of a drug that would otherwise have an unacceptable level of risk. In a few cases, consequences of an interaction have led to the conclusion that the drug could not be marketed safely. In almost all of these cases, that conclusion was strengthened by the availability of alternative drugs with lower risks for interactions. Several drugs have been withdrawn from the market because of significant drug interactions that led to QT prolongation and Torsades de Pointes (TdP) arrhythmias, after warnings in drug labels did not adequately manage the risk of drug interactions. For example, terfenadine and astemizole, two early nonsedating antihistamines metabolized by CYP3A, were withdrawn after labeling failed to reduce cases of TdP sufficiently, because fexofenadine and loratadine fulfilled the need for nonsedating antihistamines that had no risk of TdP. Cisapride, a CYP3A metabolized drug, was withdrawn because its gastrointestinal benefits were not felt to outweigh its TdP risk. A fourth drug, mibefradil (a calcium channel blocker similar to verapamil and diltiazem) was a strong CYP3A inhibitor and, when used with simvastatin, caused rhabdomyolysis because of markedly increased simvastatin exposure.

B. Drug Interaction Considerations for Small Molecule Drugs

The main focus of this guidance is pharmacokinetic drug interactions. The drug development process should include evaluation of a new drug's potential to affect the metabolism or transport of other drugs and the potential for the new drug's metabolism or transport to be affected by other drugs. Use of in vitro tools to determine whether a drug is a substrate, inhibitor, or inducer of metabolizing enzymes, followed by in vivo interaction studies to assess potential interactions, has become an integral part of drug development and regulatory review. In addition to the evaluation of metabolic drug interactions, the role of transporters in drug interactions should be evaluated. This section will separately discuss drug-drug interactions at the levels of metabolizing enzymes and transporters, and also consider situations when multiple drug-drug interaction mechanisms are present.

1. Metabolism-Based Drug-Drug Interactions

Hepatic metabolism occurs primarily through the cytochrome P450 family (CYP) of enzymes located in the hepatic endoplasmic reticulum, but may also occur through non-CYP enzyme systems, such as glucuronosyl- and sulfo-transferases, which can, in general, inactivate a drug and increase its renal elimination. Some drug metabolizing enzymes are present in the gut wall and other extrahepatic tissues, in addition to the liver.

Many metabolic routes of elimination can be inhibited or induced by concomitant drug treatment. Metabolic drug-drug interactions can cause substantial changes — an order of magnitude or more decrease or increase in the blood and tissue concentrations of a drug or metabolite — and can affect the extent to which toxic or active metabolites are formed. These large changes in exposure can alter the safety and efficacy profile of a drug and its active metabolites, regardless of whether the drug has a narrow therapeutic range (NTR). For example, certain HMG-CoA reductase inhibitors (e.g., lovastatin, simvastatin) that are extensively metabolized by CYP3A can have a 10-fold or more increase in blood levels when their metabolism is inhibited by co-administration with strong CYP3A inhibitors such as mibefradil or ketoconazole, or even moderate inhibitors such as erythromycin. Although the HMG-CoA reductase inhibitors are not NTR drugs, the blood level increases caused by interactions between HMG-CoA reductase inhibitors and CYP3A inhibitors can cause myopathy and in some cases rare and life-threatening rhabdomyolysis.

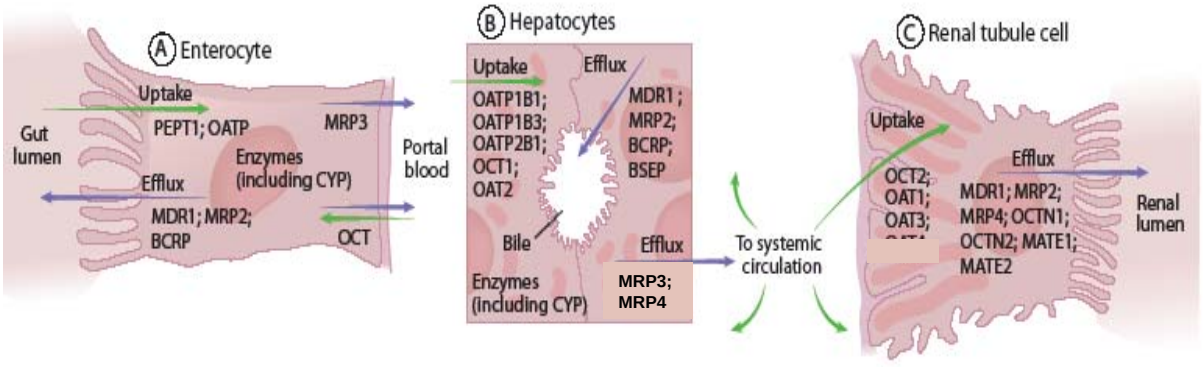
In addition to evaluating a drug as a substrate of an enzyme that another drug may inhibit or induce, it is important to determine whether an investigational drug significantly affects the metabolic elimination of drugs already in the marketplace. Metabolic drug-drug interactions should be explored for investigational drugs that are not eliminated significantly by metabolism because such drugs can inhibit or induce a co-administered drug's metabolism pathway.

Drug-drug interactions can differ among individuals based on genetic variation of a polymorphic enzyme. For example, a strong CYP2D6 inhibitor (e.g., fluoxetine) will increase the plasma levels of a CYP2D6 substrate (e.g., atomoxetine) in subjects who are extensive metabolizers (EM) of CYP2D6, but will have minimal effect in subjects who are poor metabolizers (PM) of CYP2D6, because these individuals have no active enzyme to inhibit. It is noted that CYP2D6 PMs will already have greatly increased levels of atomoxetine if given usual doses. There are also situations where inhibition may have a greater effect in PMs than EMs. If a drug is metabolized by a minor pathway (nonpolymorphic enzyme) and a major pathway (polymorphic enzyme), inhibition of the minor pathway will usually have minimal effect on plasma concentrations in EMs. However, the minor pathway plays a greater role in clearance of the drug in PMs of the major pathway. Thus, inhibition of the minor pathway in PMs of the major pathway can have a significant effect on drug clearance and resulting drug concentrations. Therefore studying the effect of interactions may be recommended in subjects with varied genotypes or phenotypes.

2. Transporter-Based Drug-Drug Interactions

Although less well-recognized than metabolizing enzymes, membrane transporters can have important effects on pharmacokinetics and drug exposure. To date, most identified transporters belong to one of two superfamilies: ATP-Binding Cassette (ABC) and Solute Carrier (SLC). Transporters govern the transport of solutes (e.g., drugs and other xenobiotics) in and out of cells. In contrast to metabolizing enzymes, which are largely concentrated in the liver and intestine, transporters are present with varying abundance in all tissues in the body and play important roles in drug distribution, tissue-specific drug targeting, drug absorption, and elimination. For example, recent research indicates an important role of transporters in the absorption, distribution, and excretion of drugs (see Figure 1 below and Table 1). Transporters can also work in concert with metabolizing enzymes and play a role in drug metabolism.

Figure 1. Illustration of Examples of Efflux and Uptake Transporters in the Gut Wall (A), Liver (B), and Kidneys (C) that May Be Involved in a Drug's Absorption, Distribution, Metabolism, and Excretion.



Abbreviations: MRP: multidrug resistance associated protein; PEPT1, peptide transporter 1; OATP: organic anion transporting polypeptide; OAT: organic anion transporter; OCT: organic cation transporter; BCRP: breast cancer resistance protein; MDR1: multidrug resistance 1(P-glycoprotein (P-gp)); MATE: multidrug and toxic compound extrusion protein (Adapted from Huang S-M, Lesko LJ, and Temple R, "Adverse Drug Reactions and Pharmacokinetic Drug Interactions," Chapter 20, Adverse Drug Reactions and Drug Interactions in Part I (Section 4), *Pharmacology and Therapeutics: Principles to Practice*, Waldman SA and Terzic A, Eds., Elsevier, 2009).

A number of transporter-based interactions have been documented in recent years. Analogous to drug interactions mediated by P450 enzymes, co-administration of a drug that is an inhibitor or an inducer of a drug transporter may affect the pharmacokinetics of a drug that is a substrate for that transporter. It has been shown that various drugs (e.g., quinidine, verapamil, itraconazole) increase plasma levels of digoxin by inhibiting the efflux transporter, P-gp, at the intestinal level. Plasma levels of many HMG-CoA reductase inhibitors, including rosuvastatin, pravastatin, and pitavastatin, are increased by

co-administration of inhibitors of hepatic uptake transporters (e.g., OATP1B1), such as cyclosporine and single dose rifampin. For example, co-administration of cyclosporine increases the area under the plasma concentration-time curve (AUC) of pravastatin, rosuvastatin, and pitavastatin by 10-fold, 7-fold, and 5-fold, respectively. This effect and a number of other transporter interactions are shown in Table 1 below. Because these statins are not significantly metabolized, the interactions appear to result from inhibition of transporters, including OATP1B1. Table 1 also shows a substantial effect of lopinavir/ritonavir on bosentan, which is potentially important because of bosentan's dose-related hepatotoxicity. Probenecid increases plasma concentrations of cidofovir, furosemide, and acyclovir because it inhibits their active renal tubular secretion by the transporters OAT1 and OAT3. Table 1 lists additional clinically relevant transporter-based drug-drug interactions.

Transporters can affect the safety profile of a drug by affecting the concentration of a drug or its metabolites in various tissues. An example of transporter-mediated effects on drug toxicity involves the drug cidofovir. This antiviral drug causes nephrotoxicity; however, when administered with probenecid, an inhibitor of organic anion transport in the kidney, the uptake of cidofovir into the renal tubular cell is blocked and nephrotoxicity is reduced. Another example involves simvastatin, polymorphism of OATP1B1 was found to correlate with the prevalence of myopathy in patients receiving simvastatin. Transporter-based drug interactions and the potential effect of drug transporters on safety make it important to determine whether transporters affect the absorption and disposition of an investigational drug and whether the investigational drug can affect the absorption and disposition of other drugs through an effect on transporters.

3. Multiple Drug-Drug Interaction Mechanisms

The above sections separately discuss drug-drug interactions related to effects on enzymes and transporters, but drug interactions for a specific drug may occur based on a combination of mechanisms. Such "complex drug interaction" scenarios include, but are not limited to:

- Concurrent inhibition and induction of one enzyme or concurrent inhibition of enzyme and transporter by a drug
- Increased inhibition of drug elimination by the use of more than one inhibitor of the same enzyme that metabolizes the drug
- Increased inhibition of drug elimination by use of inhibitors of more than one enzyme that metabolizes the drug
- Inhibition by a drug and its metabolite or metabolites, both of which inhibit the enzyme that metabolizes the substrate drug
- Inhibition of an enzyme other than the genetic polymorphic enzyme in poor metabolizers taking substrate that is metabolized by both enzymes
- Use of enzyme/transporter inhibitors in subjects with varying degrees of impairment of drug eliminating organs (e.g., liver or kidney)

When there are multiple factors that affect clearance and multiple mechanisms of drug–drug interactions, the prediction of in vivo interactions from results of in vitro assessment is challenging. Modeling and simulations accounting for multiple mechanisms can be helpful in the design of clinical studies to inform the potential for drug interaction or prediction of the extent of interactions (see section V.C.4).

433 **Table 1. Selected Transporter[a]-Mediated Clinical Significant Drug-Drug Interactions**

Gene	Aliases[a]	Tissue	Function	Interacting Drug	Substrate (Affected Drug)	Changes in Substrate Plasma AUC (AUC ratios)
ABC Transporters of clinical importance in the absorption, disposition, and excretion of drugs						
ABCB1	P-gp, MDR1	Intestinal enterocyte, kidney proximal tubule, hepatocyte (canalicular), brain endothelia	Efflux	Dronedarone Quinidine Ranolazine Tipranavir/Ritonavir	Digoxin Digoxin Digoxin Loperamide	2.6-fold 1.7-fold 1.6-fold 0.5-fold
				Tipranavir/Ritonavir	Saquinavir/Ritonavir	0.2-fold
ABCG2	BCRP	Intestinal enterocyte, hepatocyte (canalicular), kidney proximal tubule, brain endothelia, placenta, stem cells, mammary gland (lactating)	Efflux	GF120918	Topotecan	2.4-fold
SLC Transporters of clinical importance in the disposition and excretion of drugs						
SLCO1B1	OATP1B1 OATP-C OATP2 LST-1	Hepatocyte (sinusoidal)	Uptake	Lopinavir/ritonavir Cyclosporine Rifampin (single dose)	Bosentan Pravastatin Glyburide	5-48 fold[c] 9.9-fold 2.3-fold
SLCO1B3	OATP1B3, OATP-8	Hepatocyte (sinusoidal)	Uptake	Cyclosporine Cyclosporine Lopinavir/ritonavir	Rosuvastatin[d, e] Pitavastatin[d] Rosuvastatin[d]	7.1- fold[d] 4.6-fold 2.1-fold
SLC22A2	OCT2	Kidney proximal tubule	Uptake	Cimetidine Cimetidine Cimetidine	Dofetilide Pindolol Metformin	1.5-fold 1.5-fold 1.4-fold
SLC22A6	OAT1	Kidney proximal tubule, placenta	Uptake	Probenecid Probenecid Probenecid	Cephradine Cidofovir Acyclovir	3.6-fold 1.5-fold 1.4-fold
SLC22A8	OAT3	Kidney proximal tubule, choroid plexus, brain endothelia	Uptake	Probenecid	Furosemide[f]	2.9-fold

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436 [a] Abbreviations: BCRP, breast cancer resistance protein; P-gp, p-glycoprotein; MDR, multidrug resistance: LST, liver-specific transporters; OATP, organic anion transporting
437 polypeptide; OCT, organic cation transporter; OAT, organic anion transporter
438 [b] Implicated transporter refers to the likely transporter; however, because the studies are in vivo, it is not possible to assign definitively specific transporters to these interactions.
439 [c] Minimum predose plasma level (C_{trough}) data from Day 4 (48-fold), Day 10 (5-fold) after co-administration.
440 [d] Interaction could be partly mediated by OATP1B1.
441 [e] Interaction could be partly mediated by BCRP.
442 [f] Interaction could be partly mediated by OAT1.
443

C. Drug Interaction Considerations for Therapeutic Proteins

Therapeutic proteins (TPs) typically do not undergo metabolism or transport as their clearance pathway, therefore the potential is limited for small molecule drugs (termed "drug" in this document) to affect TPs through metabolism or transport pathways. However, a drug may affect the clearance of TPs through the drug's effect on immunogenicity (e.g., methotrexate reduces the clearance of infliximab, possibly due to methotrexate's effect on the antibodies formed against infliximab). In addition, TPs that are cytokines or cytokine modulators may modify the metabolism of drugs that are substrates for P450 enzymes through their effects on the regulation pathways of P450 enzymes. For example, cytokines such as IL-6 can produce concentration-dependent inhibition on various CYP isoforms at the transcription level or by alteration of CYP enzyme stability in patients with infection or inflammation, and increase the plasma concentrations of specific CYP substrate drugs. In contrast, cytokine modulators such as tocilizumab (anti-IL-6 receptor antibody) may reverse the apparent "inhibition" effect of the cytokines on CYP substrates, resulting in a "normalization" of CYP activities.

General points to be considered for evaluation of TP-drug interactions are discussed in section IV.B.2.

IV. GENERAL STRATEGIES

Development of a drug should include identification of the principal routes of elimination, quantitation of the contribution by enzymes and transporters to drug disposition, and characterization of the mechanism of drug-drug interactions. The quantitative assessment of drug-drug interaction potential for an investigational drug employs a variety of models including basic models, mechanistic static models, and more comprehensive dynamic models (e.g., physiologically-based pharmacokinetic (PBPK) models). Appropriately designed pharmacokinetic studies, usually performed in the early phases of drug development, can provide important information about metabolic and excretory routes of elimination, their contribution to overall elimination, and metabolic or transporter-mediated drug-drug interactions. Together with information from in vitro studies, these in vivo investigations can be used for PBPK model construction and refinement. Quantitative assessment of the findings from these studies helps address key regulatory questions regarding whether, when, and how to conduct further clinical drug-drug interaction studies. In many cases, negative findings from early in vitro and clinical studies eliminate the need for later clinical investigations of drug-drug interaction potential. If potential drug-drug interactions are identified based on in vitro and/or in vivo studies, sponsors should design further studies or collect information to determine (1) whether additional studies are needed to better quantify the effect and to examine the effects of weaker inhibitors (early studies usually examine strong inhibitors) on the investigational drugs as substrates and effects of investigational drugs (as inhibitors) on a range of substrates, and (2) whether dosage adjustments or other prescribing

modifications (e.g., additional safety monitoring or contraindications) are needed based on the identified interaction(s) to avoid undesired consequences. Further recommendations about the types of in vivo studies that should be conducted in certain circumstances appear in section V of this guidance.

Drug interaction information is used along with information about exposure-response relationships in the general population and specific populations, to help predict the clinical consequences of drug-drug interactions.

A. In Vitro Studies

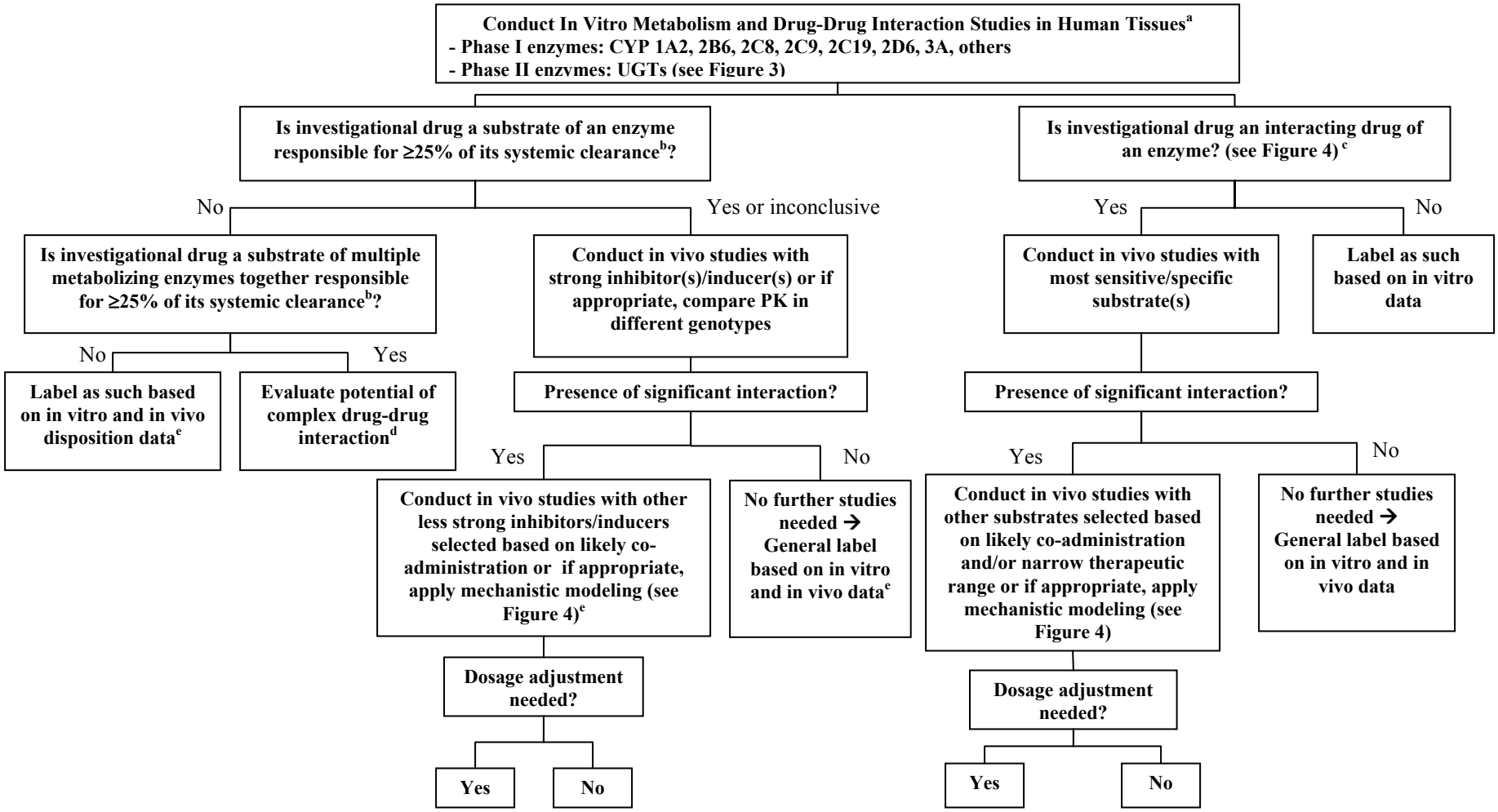
Findings from in vitro metabolism, transport, and drug interaction studies are valuable in quantitatively assessing the drug-drug interaction potential of an investigational drug. Along with clinical pharmacokinetic data, results from in vitro studies can serve as a screening mechanism to rule out the need for additional in vivo studies, or provide a mechanistic basis for proper design of clinical studies using a modeling and simulation approach. Considerations critical for conducting in vitro studies include, but are not limited to, appropriately validated experimental methods, choice of test systems, and rational selection of substrate/interacting drug and their concentrations.

1. In Vitro Metabolism Studies

Figure 2 below shows a decision tree that describes when in vivo metabolism-based interaction studies are indicated, based on in vitro metabolism, in vitro drug-drug interaction, and/or other appropriate pharmacokinetic data.

513 **Figure 2. Metabolism-Based Drug-Drug Interaction Studies — Decision Tree**

514



515
516

[a] Other Phase I enzymes (CYP and non-CYP) are discussed in section IV.A.1.a.

[b] Results from in vitro enzyme phenotyping experiments, human pharmacokinetic studies such as an intravenous study, a mass-balance study, and pharmacokinetic studies in which renal/biliary clearances are determined can be evaluated together to determine the percent contribution of enzyme to overall in vivo drug elimination in humans.

[c] See Figure 4 for calculation of R values and cutoff values. Sponsor may conduct an in vivo cocktail study in humans (Reference: *Clinical Pharmacology and Therapeutics*, 81: 298-304, 2007). See section V.C.3.

[d] See section V.C.4 for evaluation of complex drug interactions.

[e] Additional population pharmacokinetic analysis may assist the overall evaluation of the investigational new drug as a substrate.

a. Drug Metabolizing Enzyme Identification — the Investigational Drug as an Enzyme Substrate

The metabolic profile of the investigational drug should be characterized from in vitro studies. The in vitro systems include human liver tissues such as liver microsomes, microsomes expressing recombinant enzymes, or freshly isolated or cryopreserved human hepatocytes. Generally, decisions on the need for in vivo drug interaction studies with enzyme inhibitors/inducers are based on the quantitative measurement of the contribution of the enzyme to the overall systemic clearance of the substrate. We consider metabolism to be a significant pathway when it constitutes 25% or more of the drug's overall elimination. When the contribution is ≥25% or unknown, in vivo studies using appropriate inhibitor(s)/inducer(s) are warranted. The sequence of these in vivo studies should start with a strong inhibitor/inducer. If the results from the study with strong inhibitors/inducers indicate positive interactions, the impact of a less strong inhibitor/inducer should be evaluated. The subsequent evaluations with a less strong inhibitor/inducer can be conducted through a clinical study. Alternatively, it may be possible to conduct the evaluation through PBPK modeling (see section IV.A.1.b-3 below related to model building and strategies to evaluate drug-drug interaction using PBPK). The choice of in vivo enzyme inhibitors/inducers can be found in section V.

Minor elimination pathways mediated by a drug metabolizing enzyme may require further investigation under certain conditions. The contribution of these enzymes may become significant in specific populations (e.g., in subjects with renal impairment when the substrate drug is significantly eliminated through renal excretion, in poor metabolizers when substrate drug is predominantly metabolized by the polymorphic enzymes, or in subjects taking a strong inducer of the enzyme of minor pathway). The likelihood of metabolism-based drug interactions in these populations should be considered on a case-by-case basis (also see "Populations" in section V.B and "Complex Drug Interactions" section V.C.4).

Phase I Metabolizing Enzymes

Routine assessment to identify the following CYP enzymes for potential metabolism-mediated interactions is recommended: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. If an investigational drug is a substrate in vitro for a particular CYP, in vivo interaction studies with a strong inhibitor and inducer for that CYP (refer to the later sections about classification of CYP inhibitors and inducers) are recommended to determine the extent of changes in the investigational drug's pharmacokinetics. Negative results alleviate the need for further in vivo studies with less strong inhibitors or inducers, if the study is well designed and appropriate.

If a drug is not metabolized by the major CYPs (listed above), the drug's likelihood of being a substrate for other CYP enzymes (e.g., CYP2A6, CYP2J2, CYP4F2, CYP2E1) or non-CYP Phase I enzymes should be considered. Non-CYP Phase I enzymes (enzymes involved in oxidation, reduction, hydrolysis, cyclization, and decyclization reactions) that are involved in drug metabolism include monoamine oxidase (MAO), flavin monooxygenase (FMO), xanthine oxidase (XO), and alcohol/aldehyde dehydrogenase. The potential for an investigational drug to be a substrate for these enzymes can be studied on a case-by-case basis (e.g., based on prior knowledge of the drug class).

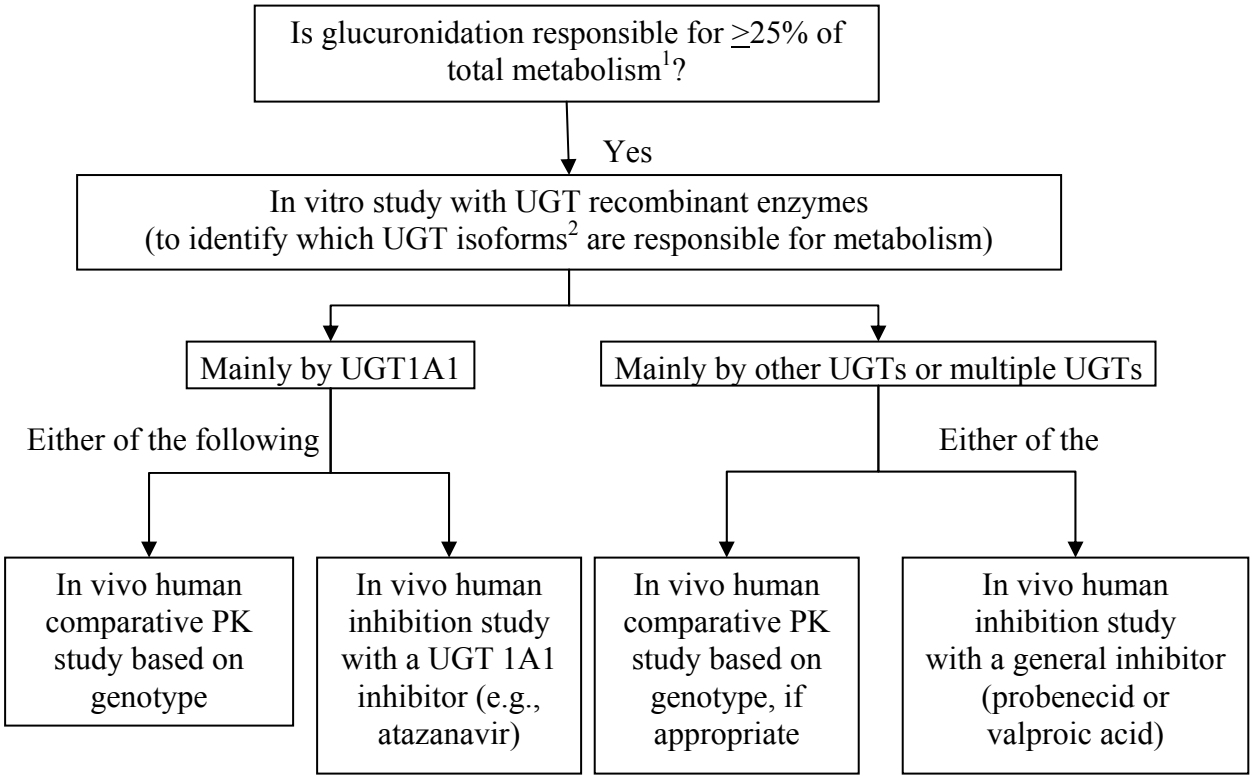
Phase II Metabolizing Enzymes

Phase II enzymes (enzymes that are involved in conjugation reactions — conjugation involving, for example, glucuronic acid, sulfonates, glutathione, or amino acids) have historically attracted less attention than CYP enzymes in drug interaction evaluations, most likely because of the lack of tools to study them and a lower incidence of observed adverse drug-drug interactions.

Recently, there has been an increased interest in drug-drug interactions involving UGTs (UDP glucuronosyl transferases), enzymes responsible for the biotransformation of many drugs. For example, UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15 have been shown to play important roles in drug metabolism. However, determination of the contribution of each UGT isoform to the overall elimination is not as straightforward as that for CYP enzymes because of the absence of data on the abundance of these isoforms in drug eliminating organs, and the lack of specific inhibitors. For example, atazanavir has been used as a UGT1A1 inhibitor; however, it also inhibits CYP3A. Therefore, an investigation of a UGT-based drug-drug interaction may follow the decision tree outlined in Figure 3. If glucuronidation is a predominant pathway of drug elimination, in vitro studies (see Figure 3 below) to determine whether the drug is a substrate of UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, or 2B15 are recommended. These in vitro

studies can be conducted using recombinant human UGTs, many of which are available from commercial sources. Results from these studies inform future in vivo drug interaction studies. In certain cases, comparative PK data in subjects with various UGT genotypes may be used to identify important UGT pathways and estimate the possible extent of interactions (e.g., comparison of PK in subjects who are poor metabolizers versus those who are extensive metabolizers). For example, UGT1A1 polymorphic variants affect the level of SN38, an active metabolite of irinotecan, which has both safety and efficacy implications. The clinical significance of interactions mediated by UGTs depends on the extent of interaction and the therapeutic range of the substrate drug.

Figure 3. Evaluation of Investigational Drugs as UGT Substrates



[1] In an in vitro system capable of informing contribution by UGT and non-UGT enzymes (e.g., hepatocytes or microsomes supplemented with appropriate co-factors).

[2] Main UGTs recommended to be studied: UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15.

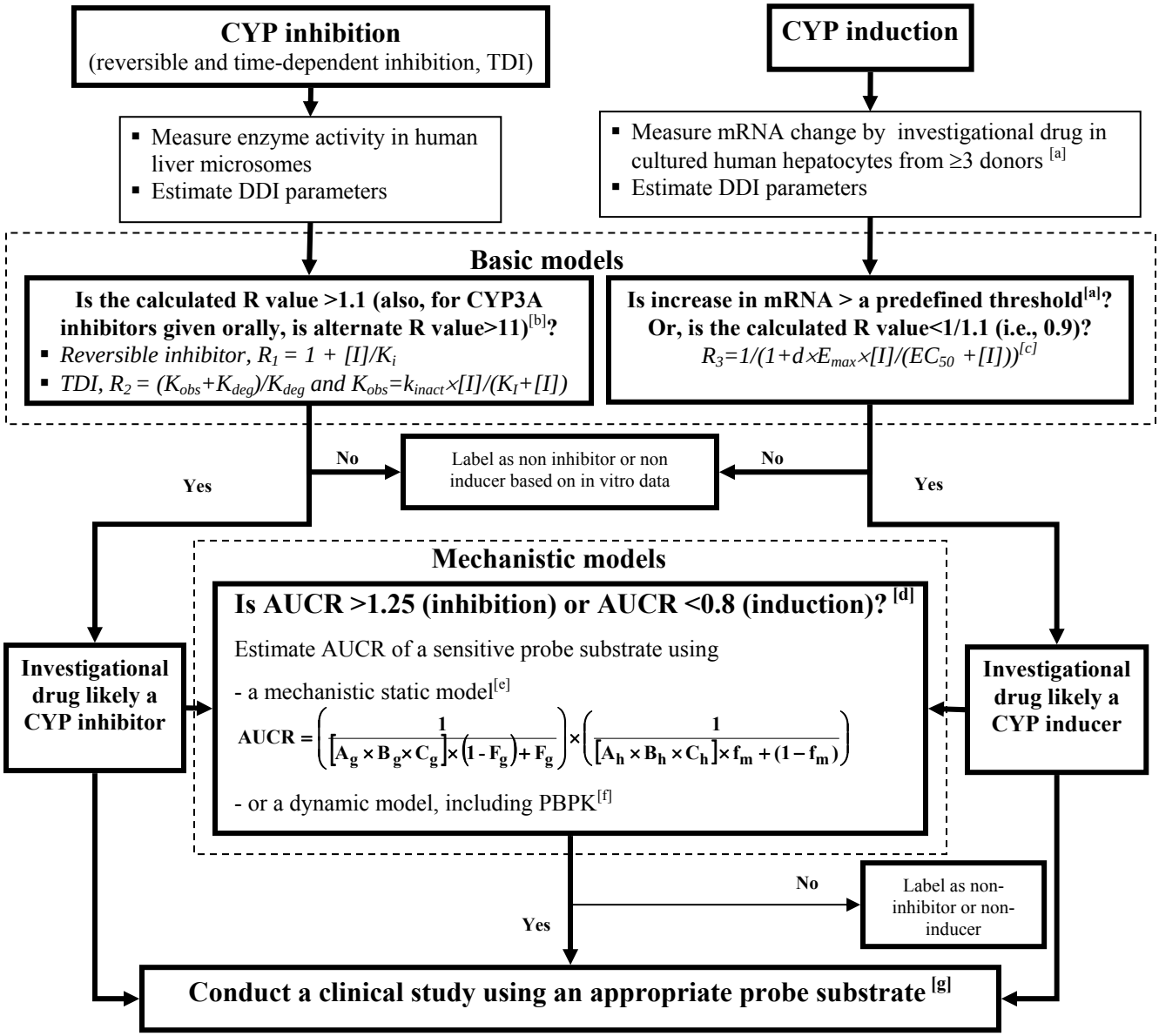
622
623 *b. Evaluation of Investigational Drug as an Enzyme Inhibitor or Inducer*
624
625 The decision to conduct an in vivo drug-drug interaction study for an
626 investigational drug as an enzyme inhibitor and/or inducer should be based on
627 quantitative analysis of both in vitro and clinical pharmacokinetic data. Such
628 analysis is accomplished by a variety of algorithms and models including basic
629 models, mechanistic static models, and more comprehensive dynamic models
630 (e.g., physiologically-based pharmacokinetic (PBPK) models, see Figure 4).
631
632 Basic models have been predominantly used because they are simple and
633 practical. These models are conservative, but in some cases they eliminate the
634 need for later clinical investigations of drug-drug interaction potential. For
635 example, the cut-off value to decide whether further in vivo investigation of a
636 drug as an inhibitor or an inducer is needed is generally calculated based on the
637 ratio of intrinsic clearance values of a probe substrate for an enzymatic pathway
638 in the absence and in the presence of the interacting drug (i.e., the R value[2]).
639 Based on the estimation of an R value,[2] a decision can be made about whether an
640 in vivo drug-drug interaction study is needed. Alternatively, in vitro data can be
641 incorporated into mechanistic models to further investigate drug-drug interaction
642 potential and determine the need to conduct a clinical drug-drug interaction study.
643
644 Mechanistic static models incorporate more detailed drug disposition and drug
645 interaction mechanisms for both interacting and substrate drugs (Fahmi et al.
646 2009). For example, these models integrate parameters such as bioavailability (in
647 gut and liver) and fractional metabolism data (e.g., "f_m" by a certain CYP
648 enzyme) for substrate drugs and parameters related to all interaction mechanisms
649 (inhibition and induction) for interacting drugs.
650
651 A PBPK model integrates system-dependent parameters (e.g., based on human
652 physiology) and drug-dependent parameters, which can be continuously refined.
653 When appropriately constructed, the PBPK model offers clear advantages over
654 static models. First, the PBPK model reflects the dynamics of drug-drug
655 interactions, allowing the investigation of the effect of an interacting drug on the
656 entire pharmacokinetic profile of the substrate. Second, the PBPK model can be
657 used to evaluate concurrent mechanisms of drug-drug interactions, including the
658 effect of inhibitory metabolites. Third, the emerging population-based PBPK
659 models provide greater insight into the causes of uncertainty and variability when
660 evaluating drug-drug interactions. Additionally, the inherent system-dependent

[2] Ratio of estimated intrinsic clearance values in the absence and presence of an inhibitor or an inducer. For a drug that is a reversible inhibitor, $R=1+[I]/K_i$. K_i is the unbound inhibition constant determined in vitro. Sometimes inhibitor concentration causing 50% inhibition (IC_{50}) is determined, and K_i can be calculated as $IC_{50}/2$ by assuming competitive inhibition. See Figure 4 for discussion of [I] values.

components make the PBPK model readily capable of investigating drug-drug interactions in the presence of multiple intrinsic and/or extrinsic factors (section V.C.4). These features make PBPK a useful option for sponsors to (1) better design drug-drug interaction studies, including dedicated trials and population pharmacokinetic studies, and (2) quantitatively predict the magnitude of drug-drug interactions in various clinical situations, including the existence of multiple patient factors such as renal impairment and/or genetic deficiency in certain metabolizing enzymes. Regardless of which prediction model is used, the sponsors should provide details of model assumptions, physiological and biological plausibility, the origin of the parameters, and information on uncertainty and variability.

The sections that follow include details on enzyme inhibitor and inducer, respectively.

675 **Figure 4. General Scheme of Model-Based Prediction: The Investigational Drug (and**
676 **Metabolite Present at ≥25% of Parent Drug AUC) as an Interacting Drug of CYP Enzymes**



677
678 [a] An in vitro induction system may be established in cultured human hepatocytes from ≥3 donors. Use sufficient
679 numbers of clinical inducers and non-inducers to determine a cutoff value (e.g., as described in Fahmi, Kish et al,
680 2010). Note that these cutoff values may vary among different laboratories because of the variability among
681 hepatocyte lots.
682
683 [b] Equations are as described in Bjornsson et al. 2003. [I] can be estimated by the maximal total (free and bound)
684 systemic inhibitor concentration in plasma and the cutoff for R is 1.1. In addition, for CYP3A inhibitors that are
685 dosed orally, [I] should also be estimated by $[I]=I_{gut}$=Molar Dose/250 mL and the cutoff for this alternate R is 11
686 (Zhang et al. 2008). K_{deg} is the apparent first order degradation rate constant of the affected enzyme; K_i is the
687 unbound reversible inhibition constant determined in vitro; k_{inact} and K_I are maximal inactivation rate constant and
688 apparent inactivation constant, respectively; K_{obs} is the apparent inactivation rate constant and K_{obs}=

$k_{inact}\times[I]/(K_I+[I])$; and R is the ratio of intrinsic clearance by metabolizing enzyme in the absence and in the presence of inhibitor.

[c] Equation is described in Fahmi et al. 2009. EC_{50} is the concentration causing half maximal effect; E_{max} is the maximum induction effect; and [I] is maximal total (free and bound) systemic inducer concentration in plasma; d is a scaling factor that is assumed as 1 for the basic model.

[d] These are suggested values according to the lower and upper limit of equivalence range. However, we are open to discussion based on sponsors' interpretation. If the calculated AUCR using a mechanistic static model is outside the equivalence range, the sponsor has the option to use a dynamic model (e.g., a PBPK model) supported by available clinical pharmacokinetic data to calculate AUCR and determine whether or not there is a need to conduct clinical drug-drug interaction studies.

[e] A mechanistic static model (or a "net effect model") is modified from that reported by Fahmi et al. 2009.

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

Where F_g is the fraction available after intestinal metabolism; f_m is the fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction; subscripts "h" and "g" denote liver and gut, respectively; $[I]_h=f_{u,b}\times([I]_{max,b}+F_a\times K_a\times Dose/Q_h)$ (Ito et al. 2002); $[I]_g = F_a\times K_a\times Dose/Q_{en}$ (Rostami-Hodjegan and Tucker 2004). In these equations, $f_{u,b}$ is the unbound fraction in blood, when it is difficult to measure due to high protein binding in plasma, a value of 0.01 should be used for fu,b; $[I]_{max,b}$ is the maximal total (free and bound) inhibitor concentration in the blood at steady state; Fa is the fraction absorbed after oral administration, a value of 1 should be used when the data is not available; K_a, is the first order absorption rate constant in vivo and a value of 0.1 min^{-1} (Ito et al. 1998) can be used when the data is not available; and Q_{en} and $Q_{h,}$ are blood flow through enterocytes (e.g., 18 L/hr/70 kg, Yang et al. 2007 (a)) and hepatic blood flow (e.g., 97 L/hr/70 kg, Yang et al. 2007 (b)), respectively.

[f] Dynamic models, including physiologically-based pharmacokinetic (PBPK) models, can be developed using both in vitro drug disposition data (e.g., protein/tissue binding, metabolism, transport, and drug-drug interaction) and physicochemical properties. The model should be refined when human pharmacokinetic data become available. The model can then be used to evaluate the drug-drug interaction potential with a sensitive substrate of the CYP enzymes of interest (Rostami-Hodjegan and Tucker 2007). The model of the substrate needs to be developed and drug interaction mechanisms should be appropriately defined by linking the models of the substrate and the interacting drug (see section IV.A.1.b-3 and Figure 5 for more details). If a metabolite is involved in a drug-drug interaction, a model for the metabolite can be established and linked to the parent drug to evaluate its inhibition/induction potential.

[g] See Table 5 (section V.C below) and Zhang et al. 2010.

b-1. Investigational drug as an enzyme inhibitor using basic models

The potential of an investigational drug to inhibit CYP enzymes is usually investigated in vitro using human liver tissues such as human liver microsomes or cDNA-expressed microsomes to determine the inhibition mechanisms (e.g., reversible or time-dependent inhibition) and inhibition potency (e.g., K_i).

The R value is dependent on the in vitro inhibition parameters and the maximum inhibitor concentration [I] that can be achieved in vivo with the highest dose. Although several algorithms to calculate [I] have been proposed, selection of [I] should justify maximum exposure of interacting drug at different tissues (Footnote [b] of Figure 4). The use of a cutoff R value of 1.1 where [I] represents maximum total (free and bound) system concentration of the inhibitor is based on an earlier FDA recommendation for reversible inhibition (Huang et al. 2007). Note an orally administered drug may inhibit CYPs that have a high expression in the intestine (e.g., CYP3A). Under such circumstances, I_{gut} (defined as molar dose/250 mL) may represent the maximum inhibitor concentrations better than the systemic concentrations. An alternate R value ($R=1+I_{gut}/K_i$) of 11 should be used as a conservative criteria to avoid false negatives. This basic static model has two major uses. First, it eliminates unnecessary clinical studies when the R value is below the threshold of 11 (for orally administered drugs that may inhibit CYP3A) or 1.1. Second, it allows rank ordering of inhibition potential across different CYP enzymes (Figure 2) for the same drug so that in vivo drug-drug interaction evaluations can be prioritized. For example, an in vivo study with a sensitive substrate of the CYP with the largest R may be carried out first. If the in vivo study shows no interaction, in vivo evaluation of other CYPs with smaller R will not be needed. However, there are exceptions to this approach. For example, if a metabolite present at ≥25% of the parent drug AUC inhibits CYP enzymes in vitro, an R value for the metabolite should be calculated based on metabolite exposure and its inhibition potency (e.g., K_i) for the CYPs. The rank order of the metabolite R values should be considered when determining what in vivo studies need to be conducted.

Most inhibitory drug interactions with CYP enzymes are reversible, but in some cases the inhibitory effect increases over time and is not promptly reversible. This effect is due to irreversible covalent binding or quasi-irreversible noncovalent tight binding of a chemically reactive intermediate to the enzyme that catalyzes its formation. This class of inhibitory drug interactions is called time-dependent inhibition (TDI). Examples of TDI of CYP3A include the HIV protease inhibitors ritonavir and saquinavir, the macrolide antibiotics erythromycin and clarithromycin, and the calcium channel blockers verapamil and diltiazem. In the case of diltiazem, both parent drug diltiazem and its primary metabolite, N-desmethyldiltiazem, are time-dependent CYP3A inhibitors. An example of TDI of CYP2D6 is paroxetine, which significantly inhibits the metabolism of desipramine, tamoxifen, dextromethorphan, and bufuralol. When TDI is the mode of inhibition, the inhibitory interaction will generally be greater over time following multiple dosing and be

longer lasting after discontinuation of the inhibitor than in a situation when the inhibitory interaction is reversible. For example, the maximum inhibition of CYP3A in humans by erythromycin administered 200 mg three times a day appeared to occur after 4 days of dosing (the AUC values of oral midazolam, a probe substrate of CYP3A, increased 2.3-, 3.4-, and 3.4- fold, respectively, on days 2, 4, and 7) (Okudaira et al. 2007). Therefore, TDI should be studied in standard in vitro screening protocols by pre-incubating the drug (a potential inhibitor) before the addition of a substrate. Any time-dependent loss of initial product formation rate may indicate time-dependent inhibition, and definitive in vitro studies to obtain TDI parameters (i.e., k_{inact} and K_I where k_{inact} and K_I are maximal inactivation rate constant and apparent inactivation constant, respectively) are recommended. Details of this tiered approach were proposed by the PhRMA Drug Metabolism Technical Group (Grimm et al. 2009). However, prediction of TDI in vivo from in vitro inactivation parameters remains challenging because of the complexity of the mechanism as compared to reversible inhibition. Generally, TDI is evaluated under the condition when the affected enzyme level reaches a new steady state in the presence of the inhibitor, and the inhibitor does not affect de novo synthesis of the enzyme. In contrast to reversible inhibition, the R value (Figures 4) for time-dependent inhibition is dependent on the rate constant for enzyme degradation, in addition to inhibitor exposure level and the TDI parameters (k_{inact} and K_I). Furthermore, the degradation kinetics for each CYP has not been unambiguously determined (Yang et al. 2008). If in vitro results suggest a TDI potential (e.g., R>1.1), an in vivo study is recommended. Alternatively, the sponsor can estimate the degree of drug-drug interactions using mechanistic models (see Figures 4, and section IV.A.1.b-3)

b-2. The investigational drug as an enzyme inducer using a basic model

Several algorithms and quantitation approaches have been proposed for studying enzyme induction using in vitro data (Shou et al. 2008; Almond et al. 2009; Fahmi et al. 2009; Fahmi, Kish, et al. 2010; Fahmi and Ripp 2010). Human hepatocytes continue to be the system of choice for evaluating enzyme induction in vitro. Although freshly isolated human hepatocytes have been the gold standard, advancement in cryopreservation technology has made the cryopreserved hepatocytes available for routine use. When determining enzyme induction potential of an investigational drug using cultured human hepatocytes, the following are considered critical:

- To account for inter-individual variability, hepatocyte preparations from at least three (3) donors are recommended. If the result in hepatocytes from at least one donor exceed the predefined threshold (see Figure 4, R value estimated using a basic model), the drug is considered an inducer and a follow-up evaluation is needed (e.g., see Figure 4, estimate AUCR using a mechanistic model or conduct a clinical study).
- Performance of these hepatocyte preparations in identifying enzyme induction potential of a sufficient number of clinical inducers should be demonstrated.
- The changes in the mRNA level of the target gene should be used as an endpoint

(Fahmi, Kish, et al. 2010).

- Vehicle control, positive control (usually a known strong inducer), and negative control (usually a known non-inducer) should be included in the experiment. Concentrations of the positive control inducers can be found in Table 2.

Studies indicate that activation of the nuclear receptor, Pregnane X receptor (PXR), results in co-induction of CYP3A and CYP2C. Thus, a negative in vitro result for CYP3A induction eliminates the need for additional in vitro or in vivo induction studies for CYP3A and CYP2C enzymes. If CYP3A induction results are positive, then induction of CYP2C should be studied either in vitro or in vivo. Because CYP1A2 and CYP2B6 may be induced by different nuclear receptors (e.g., aryl hydrocarbon receptor (AhR), or constitutive androstane receptor (CAR)), they may not be co-induced with CYP3A. Therefore, the potential for induction of CYP1A2 and CYP2B6 should be evaluated regardless of the CYP3A result.

Initially, CYP1A2, CYP2B6, and CYP3A should be evaluated in vitro (Figure 4). If the in vitro induction results are positive according to predefined thresholds using basic models, the investigational drug is considered an enzyme inducer and therefore further in vivo evaluation may be warranted. Alternatively, a sponsor can estimate the degree of drug-drug interactions using mechanistic models (see Figures 4, and section IV.A.1.b-3) to determine the need for further in vivo evaluation.

Table 2. In Vitro CYP Inducers

CYP	In Vitro Inducer * as Positive Controls	Recommended Concentration (μM) of the Positive Controls	Reported Fold Induction In Enzyme Activities
1A2	omeprazole lansoprazole	25-100 10	14-24 10
2B6	phenobarbital	500-1000	5-10
2C8	rifampin	10	2-4
2C9	rifampin	10	4
2C19	rifampin	10	20
2D6	none identified		
3A4	rifampin	10-50	4-31

*Note that this is not an exhaustive list. For an updated list, see the following link http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.

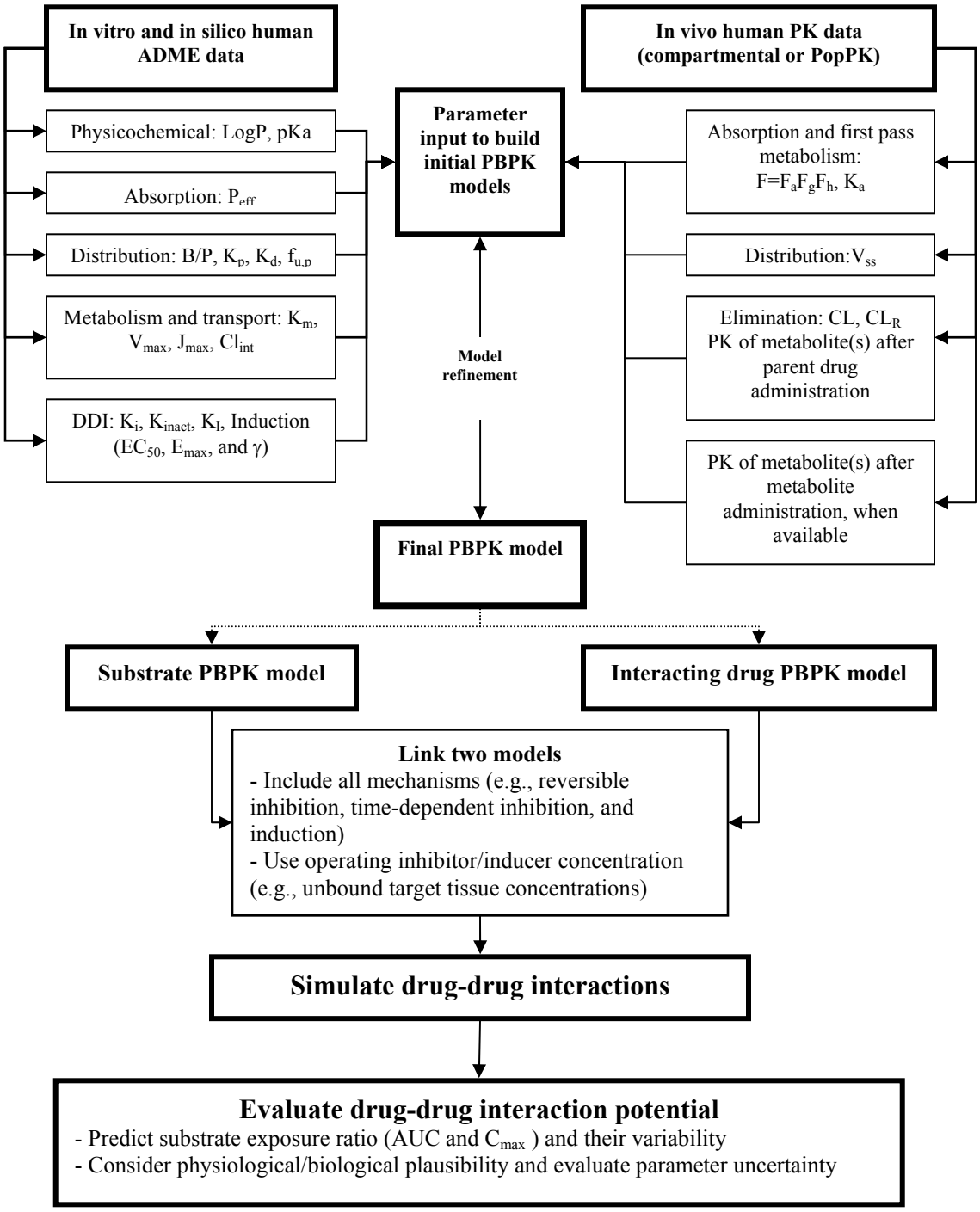
At present, data generated from other in vitro systems are considered complementary and may be reviewed along with data generated with cultured hepatocyte systems.

b-3. The investigational drug as enzyme inhibitor and/or inducer using mechanistic models

Figure 4 includes a framework for assessing drug-drug interactions using more mechanistic models, including PBPK models. Algorithms of enzyme inhibition and enzyme induction, described according to basic models in above sections (b-1 and b-2), can be incorporated into these mechanistic models. As mentioned earlier, PBPK models offer useful alternatives to dedicated clinical studies. This alternative is especially important when the sponsor would like to support the absence of meaningful clinical drug-drug interactions with an investigational drug that showed interaction potential according to a basic model. At present, the field of predicting drug-drug interactions by PBPK models is still developing and the best practices are being defined. Hence, sponsors should provide comprehensive justifications on model assumptions, physiological and biochemical plausibility, variability, and uncertainty measures. The submission containing the use of such advanced models should include a description of the structural model, source and justifications for both system- and drug-dependent parameters, type of error models, model output, data analysis, and adequate sensitivity analyses. If predefined models (structural and error) from commercially available software are employed, versions and deviations from the predefined models should be specified. Sponsors are encouraged to communicate with the FDA regarding the use of these models for the prediction of drug-drug interactions. The criteria used for assessing "equivalence" (e.g., predicted AUC ratio of 0.8-1.25 using population-based PBPK models) may be used as an initial cutoff in deciding whether in vivo studies are needed. However, these are suggested values. We are open to discussion based on sponsors' interpretation.

Figure 5 shows a general scheme that uses a PBPK model to predict the degree of drug-drug interactions. PBPK models for both substrate and interacting drug (inhibitor or inducer) should be constructed separately using in vitro and in vivo disposition parameters, before they are linked through appropriate mechanisms to predict the degree of drug-drug interaction.

Figure 5. Using a PBPK Model to Explore Drug-Drug Interaction Potential Between a Substrate Drug and an Interacting Drug (Modified from Zhao et al. 2011).



Abbreviations: ADME, absorption, distribution, metabolism and excretion; AUC, area under the plasma concentration versus time curve; B/P, blood to plasma ratio; C_{max}, maximum concentration; CL, clearance; CL_r, renal clearance; DDI, drug-drug interactions; EC_{50} or IC_{50}, concentration causing half maximal effect or inhibition; E_{max} or I_{max}, maximum effect or inhibition; F, bioavailability; F_a, fraction absorbed; F_g, bioavailability in the gut; F_h, bioavailability in the liver; $f_{u,p}$, unbound fraction in plasma; γ, Hill coefficient; J_{max}, maximum rate of transporter-mediated efflux/uptake; K_a, first-order absorption rate constant; K_d, dissociation constant of drug-protein complex; K_i, reversible inhibition constant, concentration causing half maximal inhibition; K_I, apparent inactivation constant, concentration causing half maximum inactivation; k_{inact}, apparent maximum inactivation rate constant; K_m, Michaelis-Menten constant, substrate concentration causing half maximal reaction or transport; K_p, tissue-to-plasma partition coefficient; LogP, Logarithm of the octanol-water partition coefficient; P_{eff}, jejunum permeability; PK, pharmacokinetics; PopPK, population pharmacokinetics; V, volume of distribution; V_{max}, maximum rate of metabolite formation.

2. In Vitro Transporter Studies

a. The Investigational Drug as a Substrate for Transporters

Both P-gp and BCRP are expressed in the gastrointestinal tract, liver, and kidney, and have a role in limiting oral bioavailability. Therefore, all investigational drugs should be evaluated in vitro to determine whether they are a potential substrate of P-gp or BCRP (See Figure 6, left panel).

A bidirectional assay in Caco-2 cells or overexpressed cell lines is a preferred method for in vitro evaluation. If the results are positive, an in vivo evaluation in humans is recommended (see Figure A1 in the Appendix for a decision tree on when an in vivo human study is recommended based on the in vitro data).

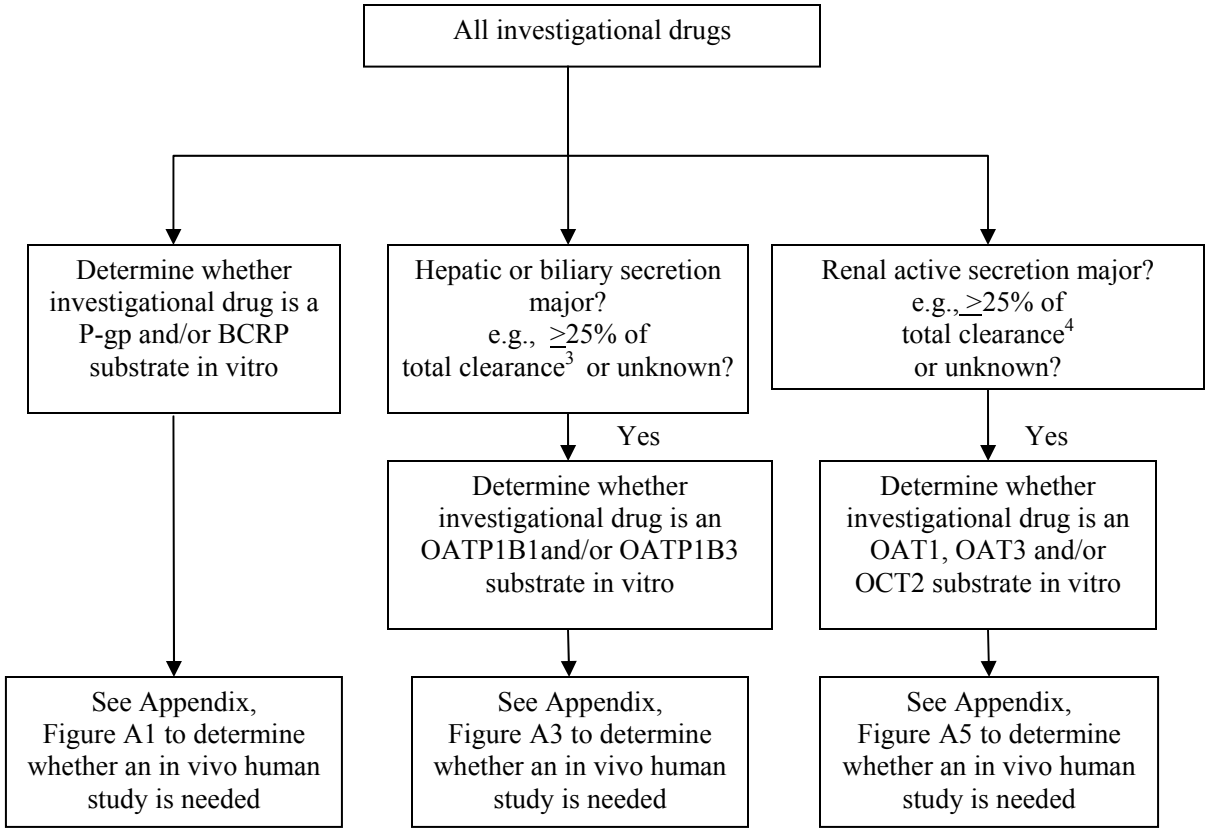
For drugs that are highly permeable and highly soluble, the intestinal absorption is not a rate-limiting step, and, therefore, it may be appropriate to exempt such drugs from the in vivo evaluation with a P-gp or BCRP inhibitor. (For further discussion regarding the defining a drug as highly soluble and high permeable (e.g., biopharmaceutical classification class (BCS) 1 drugs), see the Guidance for Industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*, http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070246).

Investigational drugs should be evaluated in vitro to determine whether they are a substrate of hepatic uptake transporters OATP1B1/OATP1B3 when their hepatic pathway is significant (e.g., clearance through hepatic or biliary secretion is more than or equal to 25% of the total clearance)[3] (Figure 6, middle panel). Similarly, investigational drugs should be evaluated in vitro to determine whether they are a substrate of OAT1/3

[3] Biliary secretion can be estimated from preclinical data, in vitro heptocyte uptake data or radiolabeled ADME data, and nonrenal clearance data.

and OCT2 when their renal active secretion is important (active secretion by kidney is more than or equal to 25% of total clearance)[4] (Figure 6, right panel).

Figure 6. Evaluation of Investigational Drugs as Substrates for P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2 Transporters.



Other transporters (e.g., MRP (multidrug resistance-associated protein)) may need to be studied based on knowledge of other drugs in the same therapeutic class as the investigational new drug. Information for the other drugs may include observed drug-drug interactions that are attributed to these other transporters. New information in the literature may raise questions about additional transporters.

b. The Investigational Drug as an Inhibitor of Transporters

[4] Percent (%) active renal secretion was estimated from $(CL_r – fu*GFR)/CL_{Total}$; fu is the unbound fraction in plasma.

Because many drugs may be used concomitantly with digoxin (a P-gp substrate) and statins (BCRP and OATP1B1/1B3 substrates), evaluation of investigational drugs as inhibitors of P-gp, BCRP, and OATP1B1/OATP1B3 should be considered. An investigational drug also should be evaluated to determine whether it inhibits OCT2, OAT1, and OAT3, because there have been clinically significant interactions demonstrated for critical drugs that are known OCT substrates (e.g., metformin) or OAT substrates (e.g., methotrexate, tenofovir, zidovudine). The need for further in vivo drug interaction studies will be based on the criteria described in the decision trees in Figures A2, A4, and A6 in the Appendix.

The decision as to whether the investigational drug should be evaluated as an inhibitor for other transporters will be based on the therapeutic class, where unexpected drug-drug interactions may have been observed and attributed to these other transporters, and when new information becomes available in the literature.

c. The Investigational Drug as an Inducer of Transporters

Transporters can be induced by mechanisms similar to those for CYP enzymes (e.g., by activation of specific nuclear receptors). The expression levels of some transporters are regulated in coordination with metabolizing enzymes, and they share common nuclear factors. For example, a large number of drugs and dietary supplements (e.g., rifampin, St. John's wort) concomitantly induce the expression of CYP3A and MDR1 (P-gp), MRP2, MRP3, MRP4, and OATP1A2.

However, methods for in vitro evaluation for transporter induction are not well understood. Cell lines are being used for in vitro P-gp induction including human colon adenocarcinoma cell line LS 180/WT, and its adriamycin-resistant (LS 180/AD 50) or vinblastine-resistant (LS 180/V) sublines. Further development is needed to validate the utility of the in vitro assays to determine the need for an in vivo induction study. Until a well-accepted system is developed, activation of nuclear receptor assays may be used as an initial evaluation of the induction potential of an investigational drug on transporters.

The definitive determination of induction potential is based on in vivo induction studies. The sponsor should consult with FDA about studying induction of transporters in vivo.

3. Considerations of the Metabolites of Investigational Drugs

Metabolites formed in vivo may reach significant exposure (e.g., ≥ 25% of the parent drug) and elicit pharmacological and/or toxicological effects. Therefore, the same considerations on further metabolism, transport, and drug interaction studies described above should be considered for relevant metabolites of the investigational drugs. The decision on which metabolite(s) should be investigated depends on multiple factors, including the knowledge in pharmacological/toxicological activities (from in vitro human cell line data and/or in vivo animal data) and the knowledge in metabolites' disposition

kinetics. For example, metabolites deemed insignificant after a single dose of the parent drug may accumulate to appreciable exposure after multiple dosing if they have long half-lives. Unexpected high exposure of metabolites may be attained in subjects with decreased function of organs responsible for their elimination and in the event of drug interactions affecting the disposition of the parent drug. Appropriate assays for metabolites should be in place to monitor the metabolite levels along with the parent.

Given the complexity of the kinetics and interaction mechanisms of formed metabolites, modeling and simulation integrating knowledge of drug disposition kinetics of both parent investigational drug and metabolites may provide a useful tool to facilitate the evaluation of drug interaction potential of metabolites (see earlier sections).

B. In Vivo Studies

1. In Vivo Drug-Drug Interactions

For detailed discussion on recommendations of in vivo human drug-drug interaction studies, please refer to section V below

2. In Vivo Drug-Therapeutic Protein (TP) Interactions

Drug-TP interactions have been observed and information about these interactions is included in labeling. Figure 7 lists the types of studies that have been conducted during drug development to evaluate TP and small molecule drug interactions. The following are general considerations:

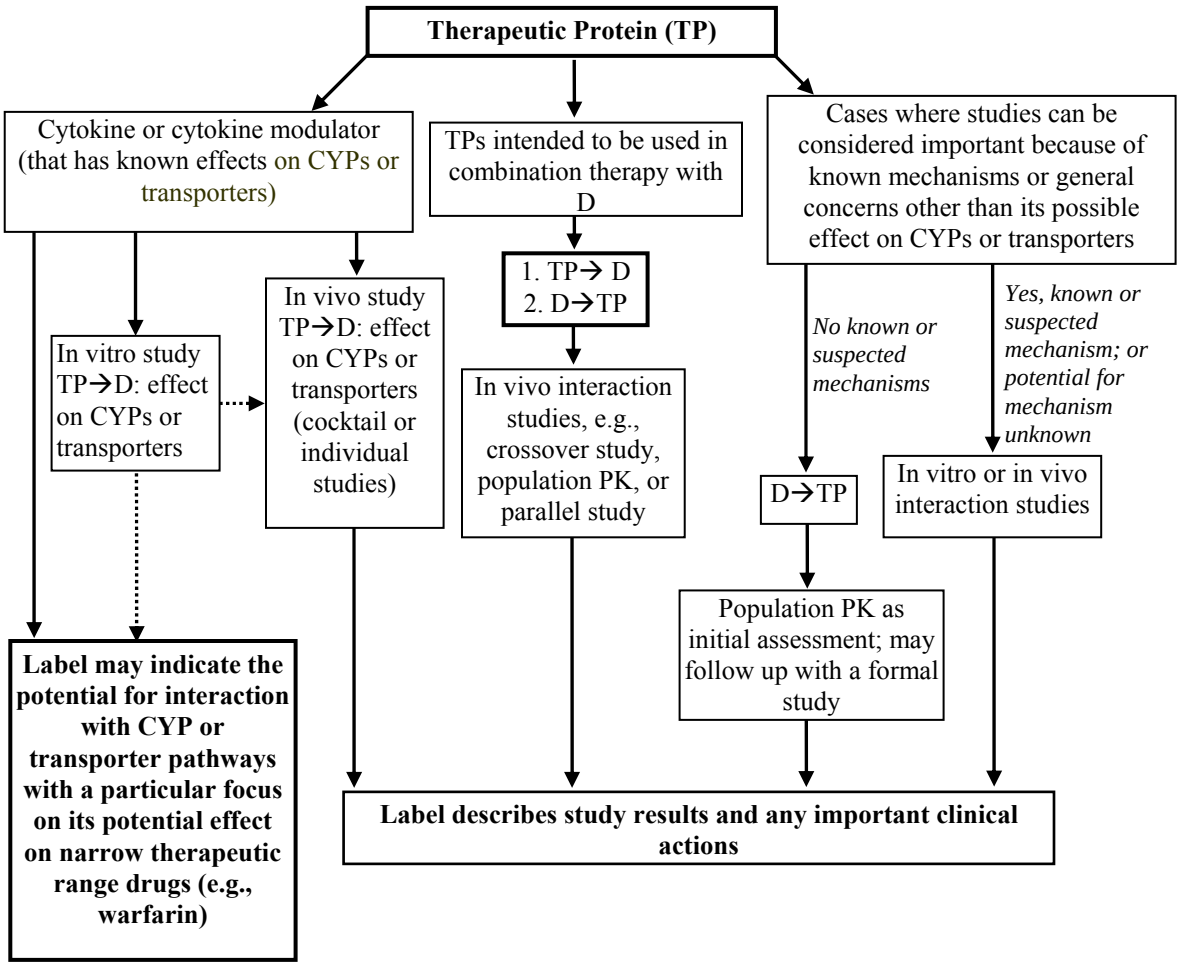
- If an investigational TP is a cytokine or cytokine modulator, studies should be conducted to determine the TP's effects on CYP enzymes or transporters (Huang et al. 2010, Le Vee M et al. 2009). In vitro or animal studies have limited value in the qualitative and quantitative projection of clinical interactions because translation of in vitro to in vivo and animal to human results to date has been inconsistent, necessitating in vivo drug interaction studies. The in vivo evaluations of TPs in targeted patient populations can be conducted with individual substrates for specific CYP enzymes and transporters, or studies can be conducted using a "cocktail approach" (see section V.C).

- For TPs that will be used in combination with other drug products (small molecule or TP) as a combination therapy, studies should evaluate the effect of each product on the other. The studies should assess effects on pharmacokinetics (PK) and, when appropriate, pharmacodynamics (PD) of either drug. This evaluation is particularly important when the drug used in combination has a narrow therapeutic range (e.g., chemotherapeutic agents).

- When there are known mechanisms or prior experience with certain PK or PD

interactions, appropriate in vitro or in vivo assessments for possible interactions should be conducted. Some interactions between drugs and TPs are based on mechanisms other than CYP or transporter modulation. For example, methotrexate's immunosuppressive effect may alter the clearance of concomitantly administered TPs through the reduction of antibodies formed against TP. Other examples include heparin's effect on palifermin (increased exposure) and paclitaxel's effect on etanercept (increased exposure).

Figure 7. Summary of The Types of Studies That Have Been Used During Drug Development to Evaluate Therapeutic Protein (TP)–Small-Molecule Drug (D) Interactions. This includes an evaluation of the effect of TP on D (TP→D) and the effect of D on TP (D→TP). The broken lines suggest the limited use of in vitro studies for informing in vivo study design or labeling. CYP, cytochrome P450. (Modified from Huang et al. 2010)



C. Using a Population Pharmacokinetic Approach to Assess Drug-Drug Interactions

Population pharmacokinetic (PopPK) analyses of data obtained from large-scale clinical studies that include sparse or intensive blood sampling can help characterize the clinical impact of known or newly identified interactions and determine recommendations for dosage modifications for the investigational drug as a substrate. The results of such analyses can be informative and sometimes conclusive when the clinical studies are adequately designed to detect significant changes in drug exposure due to drug-drug interactions. PopPK evaluations may also detect unsuspected drug-drug interactions, a particularly important possibility given the complexity of the potential interactions (see section V.C.4), not all of which are likely to have been anticipated and studied. PopPK evaluations can also provide further evidence of the absence of a drug-drug interaction, when supported by prior evidence and mechanistic data. It is unlikely, however, that population analysis will persuasively show the absence of an interaction that is suggested by information from in vivo studies specifically designed to assess a drug-drug interaction. To be optimally informative, PopPK studies should have carefully designed study procedures and sample collection protocols. Simulations (e.g., by population-based PBPK models) can provide valuable insight into optimizing the study design (see section IV.A above). Detailed information on the dose given and time of administration should be documented for the co-administered drugs. When relevant for the specific drug, the time of food consumption should be documented. Population analyses should focus on excluding a specific clinically meaningful PK change. A guidance for industry on *Population Pharmacokinetics* is available at (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm). Because exposure of co-administered drugs is not monitored in most PopPK studies, the PopPK approach may not be useful to assess the effect of the investigational drugs on other drugs.

V. DESIGN OF IN VIVO DRUG-DRUG INTERACTION STUDIES

If in vitro studies and other information suggest that in vivo drug-drug interaction studies would be helpful (e.g., based on the decision trees in Figures 2-7), the following general issues and approaches should be considered. In the following discussion, the term *substrate* (S) is used to indicate the drug studied to determine whether its exposure is changed by another drug. The other drug is termed the *interacting drug* (I).

A. Study Design

In vivo drug-drug interaction studies generally are designed to compare substrate concentrations with and without the interacting drug. Because a specific study can address a number of questions and clinical objectives, many study designs for investigating drug-drug interactions can be considered. In general, crossover designs in which the same subjects receive substrate with and without the interacting drug are more efficient. A study can use a randomized crossover (e.g., S followed by S+I, S+I followed by S), one-sequence crossover (e.g., S followed by S+I), or a parallel (S in one group of subjects and S+I in another group) design, and there may

be reason to have another period when the I is removed to assess effect duration. The following possible dosing regimen combinations for a substrate and interacting drug can also be used: single dose/single dose, single dose/multiple dose, multiple dose/single dose, and multiple dose/multiple dose. Additional factors include consideration of the sequence of administration and the time interval between dosing of substrate and inhibitor/inducer.

The selection of a study design depends on a number of factors for both the substrate and interacting drug, including (1) whether the substrate and/or interacting drug is used acutely or chronically; (2) safety considerations, including whether a substrate is a narrow therapeutic range (NTR)[5] or non-NTR drug; (3) pharmacokinetic and pharmacodynamic characteristics of the substrate and interacting drugs; (4) whether there is a desire to assess induction as well as inhibition; (5) whether the inhibition is delayed; and (6) whether there is a need to assess persistence of inhibition or induction after withdrawal of the interacting drug. The interacting drugs and the substrates should be dosed so that the exposures of both drugs are relevant to their clinical use, including the highest doses likely to be used in clinical practice, and plasma levels of both drugs should be obtained to show this. Simulations can help select an appropriate study design (see section IV.A). The following considerations may be useful:

- When attainment of steady state is important, and either the substrate or interacting drug or their metabolites have long half-lives, one or both periods of a crossover study should be long, but several other approaches can be considered, depending on pharmacokinetic characteristics of the drug and metabolites. For example, if the substrate has a long half-life, a loading dose could be used to reach steady state concentrations earlier in a one-sequence crossover followed by an S+I period long enough to allow I to reach steady state (here too, using a loading dose could shorten that period).

- When it is important that a substrate and/or an interacting drug be studied at steady state for a long duration because the effect of an interacting drug is delayed, as is the case for inducers and TDIs, documentation that near steady state has been attained for the pertinent substrate drug and metabolites as well as the interacting drug is critical, and both S and I should be present long enough to allow the full effect to be seen. This documentation can be accomplished by sampling over several days prior to the periods when test samples are collected. This information is important for metabolites and the parent drug, particularly when the half-life of the metabolite is longer than the parent. It is also important when the interacting drug and metabolites are both metabolic inhibitors (or inducers). Finally, it is critical to evaluate the time it takes for the enzyme activities to return to normal when induction or TDI is involved so that a third crossover period in which the interacting drug (I) is removed will generally be recommended.

[5] NTR drugs are defined as those drugs for which there is little separation between therapeutic and toxic doses or the associated blood or plasma concentrations (i.e., exposures) (see page 40).

- Studies can usually be open label (unblinded), unless pharmacodynamic endpoints (e.g., adverse events that are subject to bias) are critical to the assessment of the interaction.

- For a rapidly reversible inhibitor, administration of the interacting drug either just before or simultaneously with the substrate on the test day might increase sensitivity by ensuring maximum exposure to the two drugs together. For a mechanism-based inhibitor (a drug that requires metabolism before it can inactivate the enzyme; an example is erythromycin), administration of the inhibitor prior to the administration of the substrate drug can maximize the effect. If the absorption of an interacting drug may be affected by other factors (e.g., the gastric pH), it may be appropriate to control the variables or confirm the absorption through plasma level measurements of the interacting drug.

- Timing of administration may be critical in situations of concurrent inhibition and induction. For example, if the investigational drug is a substrate for both enzymes and OATP, and rifampin is used as an enzyme inducer, the simultaneous administration of the drug with rifampin (an OATP inhibitor) may underestimate enzyme induction, so delayed administration of the substrate is recommended. The optimal delayed time should be determined. In addition, it is critical to evaluate the duration of the interaction effect after the interacting drug has been removed.

- When the effects of two drugs on one another are of interest, the potential for interactions can be evaluated in a single study or two separate studies. Some design options are randomized three-period crossover, parallel group, and one-sequence crossover.

- To avoid variable study results because of uncontrolled use of dietary/nutritional supplements, tobacco, alcohol, juices, or other foods that may affect various metabolizing enzymes and transporters during in vivo studies, it is important to exclude, when appropriate, subjects who used prescription or over-the-counter medications, dietary/nutritional supplements, tobacco, or alcohol within 1 week prior to enrollment. In addition, investigators should explain to subjects that for at least 1 week prior to the start of the study until its conclusion, they should not eat any food or drink/beverage containing *alcohol, grapefruit or grapefruit juice, apple or orange juice, vegetables from the mustard green family* (e.g., kale, broccoli, watercress, collard greens, kohlrabi, brussels sprouts, mustard), and *charbroiled meats.* In some instances, it is advisable to confine subjects to a study unit for the week prior to study.

- Because interactions might differ in subgroups of different pharmacogenetic genotypes, genotyping for the enzymes and transporters involved in the interaction should be carried out when appropriate.

B. Study Population

In most situations, clinical drug-drug interaction studies can be performed using healthy volunteers, and findings in healthy volunteers will predict findings in the patient population for which the drug is intended. Safety considerations, however, may preclude the use of healthy subjects in studies of certain drugs. In addition, there are circumstances in which subjects drawn from the intended patient population offer advantages, including the opportunity to study pharmacodynamic endpoints not present in or relevant to healthy subjects.

The extent of drug interactions (inhibition or induction) may be different depending on the subjects' genotype for the specific enzyme or transporter being evaluated. For example, subjects lacking the major polymorphic clearance pathway will show reduced total metabolism or transport. However, alternative pathways can become quantitatively more important in these subjects. In such cases, the alternative pathways should be understood and studied appropriately. Thus, phenotype or genotype determinations to identify genetically determined metabolic or transporter polymorphisms are important when evaluating effects on enzymes or transporters with polymorphisms, such as CYP2D6, CYP2C19, CYP2C9, UGT1A1, and OATP1B1 (SLCO1B1). In addition, it is valuable to specify the need for stratifying the population based on genotype while conducting the DDI studies. Another alternative is to consider powering the study for the genotype status that is likely to have the highest potential for interaction.

C. Choice of Substrate and Interacting Drugs

1. CYP-Mediated Interactions

a. The Investigational Drug as a Substrate of CYP Enzymes — Effect of Other Drugs on Investigational Drugs

When testing an investigational drug for the possibility that its metabolism is inhibited or induced (i.e., as a substrate), selection of the interacting drugs should be based on in vitro or in vivo studies identifying the enzyme systems that metabolize the investigational drug. The choice of the interacting drug can then be based on known, important inhibitors and inducers of the pathway under investigation. Strong inhibitors and inducers provide the most sensitive assessment and should generally be tested first. Consider, for example, an investigational drug metabolized by CYP3A with the contribution of this enzyme to the overall elimination of this drug that is either substantial (≥ 25% of the clearance pathway) or unknown. In this case, the inhibitor and inducer can be ketoconazole and rifampin, a strong inhibitor and a strong inducer, respectively. Other strong inhibitors or inducers are acceptable. If the study results are negative, then absence of a clinically important drug-drug interaction for the metabolic pathway is demonstrated. If the clinical study of the strong inhibitor or inducer is positive, the

sponsor would generally evaluate effects through in vivo studies or mechanistic modeling of other less potent specific inhibitors or inducers, and develop labeling advice on dosage adjustment (the classification of CYP inhibitors and inducers is discussed in the next section; see Table 3 for a list of CYP inhibitors and Table 4 for CYP inducers). If the investigational drug is metabolized by CYP3A and its plasma AUC is increased 5-fold or higher by strong CYP3A inhibitors, it is considered a *sensitive substrate* of CYP3A. The labeling would indicate that the drug is a "sensitive CYP3A substrate" and that its use with strong or moderate inhibitors may call for caution, depending on the drug's exposure-response relationship. If the investigational drug is metabolized by CYP3A and its exposure-response relationship indicates that a two-fold increase in the exposure levels by the concomitant use of CYP3A inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes), it is considered a "CYP3A substrate with narrow therapeutic range" (Table 5) (see section VI for more labeling recommendations).

1235

1236 **Table 3. Classification of In Vivo Inhibitors of CYP Enzymes[(1)]**

CYP Enzymes	**Strong Inhibitors[(2)]** ≥ 5-fold increase in AUC or > 80% decrease in CL	**Moderate inhibitors[(3)]** ≥ 2 but < 5-fold increase in AUC or 50-80% decrease in CL	**Weak inhibitors[(4)]** ≥ 1.25 but < 2-fold increase in AUC or 20-50% decrease in CL
CYP1A2	Ciprofloxacin, enoxacin, fluvoxamine	Methoxsalen, mexiletine, oral contraceptives, phenylpropanolamine, thiabendazole, vemurafenib, zileuton	Acyclovir, allopurinol, caffeine, cimetidine, Daidzein,[(5)], disulfiram, Echinacea,[(5)] famotidine, norfloxacin, propafenone, propranolol, terbinafine, ticlopidine, verapamil
CYP2B6			Clopidogrel, ticlopidine prasugrel
CYP2C8	Gemfibrozil[(6)]		Fluvoxamine, ketoconazole, trimethoprim
CYP2C9		Amiodarone, fluconazole, miconazole, oxandrolone	Capecitabine, cotrimoxazole, etravirine, fluvastatin, fluvoxamine, metronidazole, sulfinpyrazone, tigecycline, voriconazole, zafirlukast
CYP2C19	Fluconazole,[(7)] fluvoxamine,[(8)] ticlopidine[(9)]	Esomeprazole, fluoxetine, moclobemide, omeprazole, voriconazole	Allicin (garlic derivative), armodafinil, carbamazepine, cimetidine, etravirine, human growth hormone (rhGH), felbamate, ketoconazole, oral contraceptives[(10)]
CYP3A	Boceprevir, clarithromycin, conivaptan, grapefruit juice,[(11)] indinavir, itraconazole,	Amprenavir, aprepitant, atazanavir, ciprofloxacin, crizotinib, darunavir/ritonavir, diltiazem, erythromycin, fluconazole,	Alprazolam, amiodarone, amlodipine, atorvastatin, bicalutamide, cilostazol, cimetidine, cyclosporine, fluoxetine, fluvoxamine, ginkgo,[(5)]

	ketoconazole, lopinavir/ritonavir, mibefradil, (12) nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, voriconazole	fosamprenavir, grapefruit juice,(11) imatinib, verapamil	goldenseal,(5) isoniazid, lapatinib, nilotinib, oral contraceptives, pazopanib, ranitidine, ranolazine, tipranavir/ritonavir, ticagrelor, zileuton
CYP2D6	Bupropion, fluoxetine, paroxetine, quinidine	Cinacalcet, duloxetine, terbinafine	Amiodarone, celecoxib, clobazam, cimetidine, desvenlafaxine, diltiazem, diphenhydramine, Echinacea,(5) escitalopram, febuxostat, gefitinib, hydralazine, hydroxychloroquine, imatinib, methadone, oral contraceptives, pazopanib, propafenone, ranitidine, ritonavir, sertraline, telithromycin, verapamil, vemurafenib

(1) Please note the following: This is not an exhaustive list. For an updated list, see the following link http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.

(2) A strong inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a substrate for that CYP by equal or more than 5-fold.

(3) A moderate inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 5-fold but equal to or more than 2-fold.

(4) A weak inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 2-fold but equal to or more than 5-fold.

(5) Herbal product.

(6) Gemfibrozil also inhibits OATP1B1.

(7) Fluconazole is listed as a strong CYP2C19 inhibitor based on the AUC ratio of omeprazole, which is also metabolized by CYP3A; fluconazole is a moderate CYP3A inhibitor.

(8) Fluvoxamine strongly inhibits CYP1A2 and CYP2C19, but also inhibits CYP2C8/2C9 and CYP3A;

(9) Ticlopidine strongly inhibits CYP2C19, but also inhibits CYP3A, CYP2B6, and CYP1A2.

(10) Effect seems to be due to CYP2C19 inhibition by ethinyl estradiol.

(11) The effect of grapefruit juice varies widely among brands and is concentration-, dose-, and preparation-dependent. Studies have shown that it can be classified as a "strong CYP3A inhibitor" when a certain preparation was used (e.g., high dose, double strength) or as a "moderate CYP3A inhibitor" when

another preparation was used (e.g., low dose, single strength).
(12) Withdrawn from the United States market.

Table 4. Classification of In Vivo Inducers of CYP Enzymes[(1)]

CYP Enzymes	**Strong Inducers** ≥ 80% decrease in AUC	**Moderate Inducers** 50-80% decrease in AUC	**Weak Inducers** 20-50% decrease in AUC
CYP1A2		Montelukast, phenytoin, smokers versus non-smokers[(2)]	Moricizine, omeprazole, phenobarbital,
CYP2B6		Efavirenz, rifampin	Nevirapine
CYP2C8		Rifampin	
CYP2C9		Carbamazepine, rifampin	Aprepitant, bosentan, phenobarbital, St. John's wort[(3,4)]
CYP2C19		Rifampin	Artemisinin
CYP3A	Avasimibe,[(5)] carbamazepine, phenytoin, rifampin, St. John's wort[(3)]	Bosentan, efavirenz, etravirine, modafinil, nafcillin	Amprenavir, aprepitant, armodafinil, clobazamechinacea,[(4)] pioglitazone, prednisone, rufinamide, vemurafenib
CYP2D6	None known	None known	None known

(1) Please note the following: This is not an exhaustive list. For an updated list, see the following link: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.
(2) For a drug that is a substrate of CYP1A2, the evaluation of the effect of induction of CYP1A2 can be carried out by comparative PK studies in smokers vs. non-smokers.
(3) The effect of St. John's wort varies widely and is preparation-dependent.
(4) Herbal product.
(5) Not a marketed drug.

Table 5. Examples[1] of Sensitive In Vivo CYP Substrates and CYP Substrates with Narrow Therapeutic Range

CYP Enzymes	Sensitive substrates[2]	Substrates with narrow therapeutic range[3]
CYP1A2	Alosetron, caffeine, duloxetine, melatonin, ramelteon, tacrine, tizanidine	Theophylline, tizanidine
CYP2B6 [4]	Bupropion, efavirenz	
CYP2C8	Repaglinide[5]	Paclitaxel
CYP2C9	Celecoxib	Warfarin, phenytoin
CYP2C19	Clobazam, lansoprazole, omeprazole, S-mephenytoin	S-mephenytoin
CYP3A[6]	Alfentanil, aprepitant, budesonide, buspirone, conivaptan, darifenacin, darunavir, dasatinib, dronedarone, eletriptan, eplerenone, everolimus, felodipine, indinavir, fluticasone, lopinavir, lovastatin, lurasidone, maraviroc, midazolam, nisoldipine, quetiapine, saquinavir, sildenafil, simvastatin, sirolimus, tolvaptan, tipranavir, triazolam, ticagrelor, vardenafil	Alfentanil, astemizole,[7] cisapride,[7] cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, terfenadine[7]
CYP2D6	Atomoxetine, desipramine, dextromethorphan, metoprolol, nebivolol, perphenazine, tolterodine, venlafaxine	Thioridazine, pimozide

(1) Note that this is not an exhaustive list. For an updated list, see the following link: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.

(2) *Sensitive CYP substrates* refers to drugs whose plasma AUC values have been shown to increase 5-fold or higher when co-administered with a known CYP inhibitor or AUC ratio in poor metabolizers vs. extensive metabolizers is greater than 5-fold.

(3) CYP *substrates with narrow therapeutic range* refers to drugs whose exposure-response relationship indicates that small increases in their exposure levels by the concomitant use of CYP inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes).

(4) The AUC of these substrates were not increased by 5-fold or more with a CYP2B6 inhibitor, but they represent the most sensitive substrates studied with available inhibitors evaluated to date.

(5) Repaglinide is also a substrate for OATP1B1, and it is only suitable as a CYP2C8 substrate if the inhibition of OATP1B1 by the investigational drug has been ruled out.

(6) Because a number of CYP3A substrates (e.g., darunavir, maraviroc) are also substrates of P-gp, the observed increase in exposure could be due to inhibition of both CYP3A and P-gp.

(7) Withdrawn from the United States market.

NTR drugs are defined as those drugs for which there is little separation between therapeutic and toxic doses or the associated blood or plasma concentrations (i.e., exposures). In general, the toxicity in question is serious toxicity, not symptomatic reversible toxicity (most drugs have adverse effects of various kinds within the therapeutic range).

Classic examples of NTR drugs include:

- Warfarin, where a modest increase from the titrated (by international normalized ratio, INR) concentration can cause major bleeding.
- Drugs with concentration-related QT effects (cisapride, astemizole, dofetilide), where a previously tolerated dose could become toxic with a doubling of serum concentration.
- Most cytotoxic oncologic drugs.
- Aminoglycoside antibiotics.

Although there is no well-established rule, drugs for which a doubling of serum concentration would cause serious toxicity can be considered NTR. Note, however, that even reasonably well-tolerated drugs can become toxic if blood levels are greatly increased (e.g., by CYP450 inhibition). For example, lovastatin and simvastatin, used over a substantial dose range, can cause myopathy leading to rare and life-threatening rhabdomyolysis if taken with a strong CYP3A inhibitor (such as mibefradil, now removed from the U.S. market), which can cause a large-fold increase in blood levels.

If an orally administered drug is a substrate of CYP3A and has low oral bioavailability because of extensive presystemic extraction by enteric CYP3A, grapefruit juice may have a significant effect on its systemic exposure. Use of the drug with grapefruit juice may call for caution, depending on the drug's exposure-response relationship (see section VI for labeling recommendations).

If a drug is a substrate of CYP3A or P-gp and co-administration with St. John's wort, an inducer of this enzyme and transporter, can decrease the systemic exposure and effectiveness, St. John's wort will be listed in the labeling along with other known inducers, such as rifampin, rifapentin, phenytoin, carbamazepine, or phenobarbital, as possibly decreasing plasma levels of the drug.

If a drug is metabolized by a polymorphic enzyme (such as CYP2D6, CYP2C9, CYP2C19, or UGT1A1), the comparison of pharmacokinetic parameters of this drug in poor metabolizers and extensive metabolizers may substitute for an interaction study for that particular pathway, as the PK in the poor metabolizers will indicate the effect of a strong inhibitor. When the study suggests the presence of a significant interaction with

strong inhibitors or in poor metabolizers, further evaluation, including mechanistic modeling with weaker inhibitors or intermediate metabolizers, may be recommended.

b. The Investigational Drug as an Inhibitor or an Inducer of CYP Enzymes — Effect of Investigational Drugs on Other Drugs

When studying an investigational drug as the interacting drug, the choice of substrates (approved drugs) for initial in vivo studies depends on the P450 enzymes affected by the interacting drug. When testing inhibition, the substrate selected should generally be one whose pharmacokinetics are markedly altered by the co-administration of known specific inhibitors of the enzyme systems (sensitive substrates) to see the largest impact of the interacting investigational drug. Examples of such substrates include (1) midazolam for CYP3A; (2) theophylline for CYP1A2; (3) bupropion for CYP2B6; (4) repaglinide for CYP2C8; (5) warfarin for CYP2C9 (with the evaluation of S-warfarin); (6) omeprazole for CYP2C19; and (7) desipramine for CYP2D6 (see Table 5 above for additional substrates). If the initial study determines that an investigational drug either inhibits or induces metabolism of sensitive substrates, further studies using other substrates, representing a range of therapeutic classes, based on the likelihood of co-administration, may be useful. If the initial study with the most sensitive substrates is negative, it can be presumed that less sensitive substrates also will be unaffected. It should be noted that several of the substrates recommended for drug interaction studies are not specific because they are substrates for more than one CYP enzyme or may be substrates for transporters. While a given substrate may not be metabolized by a single enzyme (e.g., dextromethorphan elimination is carried out primarily by CYP2D6 but other enzymes also contribute in a minor way), its use in an interaction study is appropriate if the inhibitor (the investigational drug) to be evaluated is selective for the CYP enzyme of interest.

If an investigational drug is a CYP inhibitor, it may be classified as a strong, moderate, or weak inhibitor based on its effect on a sensitive CYP substrate. For example, CYP3A inhibitors can be classified based on the magnitude of the change in plasma AUC of oral midazolam or other CYP3A substrates that are similar in characteristics (e.g., fm (% clearance contributed by CYP3A), half-life, not subject to transporter effect) as midazolam, when the substrate is given concomitantly with the inhibitor (see Table 3 above). If the investigational drug increases the AUC of oral midazolam or other CYP3A substrates by 5-fold or higher ($\geq$ 5-fold), it can be considered a *strong* CYP3A inhibitor. If the investigational drug, when given at its highest dose and shortest dosing interval (to maximize exposure and inhibitory effect), increases the AUC of oral midazolam or other sensitive CYP3A substrates by between 2- and 5-fold ($\geq$ 2- and <5-fold), it can be considered a *moderate* CYP3A inhibitor. If the investigational drug, when given at the highest dose and shortest dosing interval, increases the AUC of oral midazolam or other sensitive CYP3A substrates by between 1.25- and 2-fold ($\geq$ 1.25- and < 2-fold), it can be considered a *weak* CYP3A inhibitor. When the investigational drug is determined to be an inhibitor of CYP3A, its interaction with CYP3A substrates should be described in

various sections of the labeling, as appropriate (see section VI, Labeling Recommendations).

When an in vitro evaluation does not rule out the possibility that an investigational drug is an inducer of CYP3A (see section IV.A), an in vivo evaluation can be conducted using the most sensitive substrate (e.g., oral midazolam, see Table 5 above). When midazolam, the most sensitive substrate, is co-administered orally following the administration of multiple doses of the investigational drug, and there is no interaction, it can be concluded that the investigational drug is not an inducer of CYP3A (in addition to the conclusion that it is not an inhibitor of CYP3A). A caveat to this interpretation is that if the investigational drug is both an inducer and inhibitor of CYP3A, such as ritonavir, the net effect at any time it is introduced may vary. In this case, the net effect of the drug on CYP3A function may be time-dependent.

In vivo induction evaluations have often been conducted using oral contraceptives as the substrate. However, oral contraceptives are not the most sensitive substrates for CYP3A, so a negative result does not exclude the possibility that the investigational drug is an inducer of CYP3A. Some compounds listed in Table 5 as sensitive substrates for the other enzymes can also be used as substrates with the investigational drug as an inducer. For example, omeprazole and repaglinide are CYP2C19 and CYP2C8 substrates, respectively, but they are also metabolized by CYP3A. If omeprazole is used as a substrate to study CYP2C19 induction, measurement of its metabolites (CYP2C19-mediated hydroxy-omeprazole and CYP3A4-mediated omeprazole sulfone) will be recommended for the interpretation of the study results.

2. Transporter-Mediated Interactions

Similar to CYP enzymes, transporters may be inhibited or induced. Inhibition of transporters by interacting drugs can lead to altered exposure of other drugs that are substrates of transporters. Therefore, the potential for an investigational drug as a substrate, inhibitor, or inducer for transporters should be evaluated during drug development.

Clinically significant P-gp-mediated drug interactions, mostly related to digoxin, have been reported (Table 1). With the availability of genetic tools, our understanding of roles of other transporters in drugs' ADME, and transporter-based interactions has improved. A recent genome-wide association study showed that OATP1B1 polymorphism was associated with increased incidence of myopathy in patients taking 80 mg of simvastatin daily (Link et al. 2008). Cyclosporine increases some statin drugs' exposure 5- to 10-fold, which appeared to be mediated by inhibition of OATP and possibly BCRP (Table 1). These data indicate that significant interactions between drugs can occur at the transporter level.

In this guidance, BCRP, OATP, OATs, and OCTs are considered important transporters in addition to P-gp (International Transporter Consortium 2010) and should be routinely evaluated. Refer to Figure 6 for a possible decision tree that could be used to guide the decision of when to study these transporters in vitro during drug development. Additional decision trees to determine when to evaluate drug interactions in vivo are presented in the Appendix (Figures A1-6).

Because the field of transporter pharmacology is rapidly evolving, other transporters (e.g., multidrug resistance-associated proteins (MRPs), multidrug and toxin extrusion (MATE) transporters, and bile salt export pump (BSEP) transporters) should be considered when appropriate.

a. The Investigational Drug as a Substrate of Transporters — the Effect of Other Drugs on an Investigational Drug

When testing an investigational drug for the possibility that its transport is inhibited or induced (i.e., as a substrate), selection of the interacting drugs should be based on in vitro or in vivo studies identifying the transporters that are involved in the absorption and disposition of the investigational drug (e.g., absorption and efflux in the gastrointestinal tract, uptake and secretion in the liver, and the secretion and re-absorption in the kidney). The choice of the interacting drug should be based on known, important inhibitors of the pathway under investigation. Strong inhibitors provide the most sensitive assessment and should generally be tested first. As there is overlapping selectivity in substrate and inhibitor among transporters, negative results from a study using a broad inhibitor may rule out the possibility for drug interaction mediated by multiple pathways. For example, it may be appropriate to use an inhibitor of many transporters (e.g., cyclosporine, which inhibits P-gp, OATP, and BCRP) to study its effect on a drug that may be a substrate for these transporters. A negative result rules out the involvement of these transporters in the drug's disposition. However, if the result is positive, it will be difficult to determine the relative contribution of each transporter to the disposition of the substrate drug. In contrast, if the goal of the study is to determine the role of a specific pathway in the PK of a substrate drug, then a selective and potent inhibitor for that transporter should be used. Table 6 lists examples of inhibitors and inducers of selected transporters.

As an alternative, comparative PK of an investigational drug in subjects with different genotypes of specific transporters (e.g., OATP1B1 c.521 T vs C) can be evaluated to determine the importance of a specific transporter in the clearance pathway for the drug. On the other hand, polymorphism data on P-gp is controversial and may not be used to determine the role of P-gp in the disposition of investigational drugs that are substrates of P-gp.

Table 6. Examples of In Vivo Inhibitors and Inducers of Selected Transporters[1]

Transporter	Gene	Inhibitor[2]	Inducer[3]
P-gp	*ABCB1*	Amiodarone, azithromycin,[4] captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin,[5] felodipine, itraconazole, ketoconazole,[4] lopinavir and ritonavir, quercetin,[4] quinidine, ranolazine, ticagrelor, verapamil	Avasimibe, [6] carbamazepine,[7] phenytoin, rifampin, St John's wort,[8] tipranavir/ritonavir
BCRP	*ABCG2*	Cyclosporine, elacridar (GF120918), eltrombopag, gefitinib	Not known
OATP1B1	*SLCO1B1*	Atazanavir,[10] cyclosporine, eltrombopag, gemfibrozil, lopinavir, [10] rifampin,[9] ritonavir, [11] saquinavir, [10] tipranavir[10]	Not known
OATP1B3	*SLCO1B3*	Atazanavir, [10] cyclosporine, lopinavir, [10] rifampin, [9] ritonavir,[11] saquinavir[10]	Not known
OCT2	*SLC22A2*	Cimetidine, quinidine	Not known
OAT1	*SLC22A6*	Probenecid	Not known
OAT3	*SLC22A8*	Probenecid cimetidine, diclofenac	Not known

(1) Please note this is not an exhaustive list. For an updated list, see the following link http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.
(2) Inhibitors listed for P-gp are those that showed >25% increase in digoxin AUC or otherwise indicated if substrate is other than digoxin.
(3) Inducers listed for P-gp are those that showed >20% decrease in digoxin AUC or otherwise indicated if substrate is other than digoxin.
(4) Inhibitors listed are those that showed >25% increase in fexofenadine AUC.
(5) Inhibitors listed are those that showed >25% increase in talinolol AUC.
(6) Not a marketed drug.
(7) Inducers listed are those that showed >20% decrease in fexofenadine AUC.
(8) Herbal product.
(9) Given as a single dose.
(10) In vitro inhibitors for OATP. Separation of the in vivo inhibition effect from ritonavir is difficult because this drug is usually co-administered with ritonavir.
(11) The in vivo inhibition effect of ritonavir cannot be easily estimated because it is usually co-administered with other HIV protease inhibitors that are inhibitors for OATP as well.

b. The Investigational Drug as an Inhibitor or an Inducer of Transporters — Effect of the Investigational Drugs on Other Drugs

When studying an investigational drug as the interacting drug, the choice of substrates (approved drugs in the United States) for initial in vivo studies depends on the transport pathway that may be affected by the interacting drug. In general, when testing inhibition, the substrate selected should be one whose pharmacokinetics are markedly altered by co-administration of known specific inhibitors of the transporter pathway to see the largest impact of the interacting investigational drug. The choice of substrates can also be determined by the therapeutic area of the investigational drug and the probable co-administered drugs that are known substrates for transporters. Table 7 lists selected examples of substrates for P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, and OAT3. However, because many drugs are substrates of multiple transporters or enzymes, specific substrates for each transporter are not available. For example, rosuvastatin is a substrate for BCRP, OATP1B1, and OATP1B3; lapatinib is a substrate for both P-gp and BCRP. The observed clinical interactions may be a result of inhibition of multiple pathways if the investigational drug is also an inhibitor for the same multiple pathways.

Table 7. Examples of In Vivo Substrates for Selected Transporters(1)

Transporter	Gene	Substrate
P-gp	*ABCB1*	Aliskiren, ambrisentan, colchicine, dabigatran etexilate, digoxin, everolimus, fexofenadine, imatinib, lapatinib, maraviroc, nilotinib, posaconazole, ranolazine, saxagliptin, sirolimus, sitagliptin, talinolol, tolvaptan, topotecan
BCRP	*ABCG2*	Methotrexate, mitoxantrone, imatinib, irrinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan
OATP1B1	*SLCO1B1*	Atrasentan, atorvastatin, bosentan, ezetimibe, fluvastatin, glyburide, SN-38 (active metabolite of irinotecan), rosuvastatin, simvastatin acid, pitavastatin, pravastatin, repaglinide, rifampin, valsartan, olmesartan
OATP1B3	*SLCO1B3*	Atorvastatin, rosuvastatin, pitavastatin, telmisartan,(2) valsartan, olmesartan
OCT2	*SLC22A2*	Amantadine, amiloride, cimetidine, dopamine, famotidine, memantine, metformin, pindolol, procainamide, ranitidine, varenicline, oxaliplatin
OAT1	*SLC22A6*	Adefovir, captopril, furosemide, lamivudine, methotrexate, oseltamivir, tenofovir, zalcitabine, zidovudine
OAT3	*SLC22A8*	Acyclovir, bumetanide, ciprofloxacin, famotidine, furosemide, methotrexate, zidovudine, oseltamivir acid, (the active metabolite of oseltamivir), penicillin G, pravastatin, rosuvastatin, sitagliptin

(1) Please note this is not an exhaustive list. For an updated list, see the following link http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.

(2) Selective for OATP1B3.

Because of the lack of a validated in vitro system to study transporter induction, the definitive determination of induction potential of an investigator on transporters is based on in vivo induction studies. The sponsor should consult with FDA about studying induction of transporters in vivo. For example, because of similarities in the mechanisms of CYP3A and P-gp induction, information from the testing of CYP3A inducibility can inform decisions about P-gp. If an investigational drug is found not to induce CYP3A in vitro, no further tests of CYP3A and P-gp induction in vivo are necessary. If a study of the investigational drug's effect on CYP3A activity in vivo is indicated from a positive in vitro screen, but the drug is shown not to induce CYP3A in vivo, then no further test of P-gp induction in vivo is necessary. However, if the in vivo CYP3A induction test is positive, then an additional study of the investigational drug's effect on a P-gp probe substrate is recommended. If the drug is also an inhibitor for P-gp, then the induction study can be conducted with the inhibitor study using a multiple-dose design.

3. Cocktail Approach

Simultaneous administration of a mixture of substrates of multiple CYP enzymes and transporters in one study (i.e., a "cocktail approach") in human volunteers is another way to evaluate a drug's inhibition or induction potential, provided that the study is designed properly and the following factors are present: (1) the substrates are specific for individual CYP enzymes or transporters; (2) there are no interactions among these substrates; and (3) the study is conducted in a sufficient number of subjects (see section V.G). Negative results from a well-conducted cocktail study can eliminate the need for further evaluation of particular CYP enzymes. However, positive results can indicate that further in vivo evaluation should be conducted to provide quantitative exposure changes (such as AUC, C_{max}), if the initial evaluation only assessed the changes in the urinary parent to metabolite ratios. The data generated from a cocktail study can supplement data from other in vitro and in vivo studies in assessing a drug's potential to inhibit or induce CYP enzymes and transporters.

4. Complex Drug Interactions

a. Multiple CYP Inhibitors

There may be situations when an evaluation of the effect of multiple CYP inhibitors on the drug can be informative. For example, it may be appropriate to conduct an interaction study with more than one inhibitor simultaneously if all of the following conditions are met: (1) the drug exhibits blood concentration-dependent important safety concerns; (2) multiple CYP enzymes are responsible for the metabolic clearance of the drug; (3) the predicted residual or non-inhibitable drug clearance is low. Under these conditions, the effect of multiple CYP-selective inhibitors on the investigational drug's blood AUC may be much greater than when the inhibitors are given individually with the drug, and more than the product of changes in AUC observed with each individual inhibitor. The magnitude of the combined effect will depend on the residual fractional clearance (the smaller the fraction, the greater the concern) and the relative fractional clearances of the inhibited pathways. Modeling and simulation approaches can help project the magnitude of the effect based on single pair drug interaction studies.

If results from a study with a single inhibitor have already triggered a major safety concern (i.e., a contraindication), multiple inhibitor studies are unlikely to add value.

b. Enzyme/Transporter Interplay

There is an overlap in enzyme and transporter specificity. For example, there is considerable overlap between CYP3A and P-gp inhibitors and inducers. Itraconazole inhibits CYP3A and P-gp and rifampin induces CYP3A and P-gp. However, dual inhibitors for CYP3A and P-gp do not necessarily have similar inhibition potency on

CYP3A and P-gp (Table 8). For example, the strong CYP3A inhibitor voriconazole does not cause a large increase in exposure of a P-gp substrate, such as digoxin or fexofenadine. In addition, some potent P-gp inhibitors such as amiodarone and quinidine (causing ≥ 1.5-fold change in digoxin or fexofenadine AUC) are weak CYP3A inhibitors. The differential inhibition effects on CYP3A and P-gp should be considered when inhibitors are selected for study of interactions with an investigational drug that is a CYP3A, P-gp, or dual CYP3A and P-gp substrate (Zhang et al. 2009). To assess the worst case scenario for a dual CYP3A and P-gp substrate, inhibition should be studied using an inhibitor that shows strong inhibition for both P-gp and CYP3A, such as itraconazole. However, under this condition, if the result is positive, specific attribution of an AUC change to P-gp or CYP3A4 may not be possible. For labeling purposes, evaluation either through in vivo interaction studies or mechanistic modeling with less strong inhibitors for either pathways or inhibitors for one particular pathway only may be recommended. If the goal is to determine the specific contribution of CYP3A or P-gp on the AUC change, then a strong inhibitor for CYP3A only or a potent inhibitor for P-gp only should be selected to discern the effect of CYP3A vs. P-gp. Table 8 lists examples of CYP3A and P-gp inhibitors and their relative potency.

Table 8. Examples of In Vivo CYP3A and P-gp Inhibitors and Their Relative Potency

	P-gp Inhibitor	Non-P-gp Inhibitor
Strong CYP3A Inhibitor	Itraconazole, lopinavir/ritonavir, telaprevir, clarithromycin, ritonavir,* ketoconazole,* indinavir/ritonavir,*conivaptan	Voriconazole
Moderate CYP3A Inhibitor	Verapamil, erythromycin,* diltiazem, dronedarone	None identified
Weak CYP3A Inhibitor	Lapatinib, quinidine, ranolazine, amiodarone, felodipine, azithromycin*	Cimetidine

* Data derived with fexofenadine; all other data were derived with digoxin.

Notes:

(1) The University of Washington Drug Interaction Database was used to search the data that defined the in vivo potency of various inhibitors for CYP3A (midazolam was searched as a substrate) and P-gp (digoxin or fexofenadine was searched as a substrate).

(2) P-gp inhibitors or non-P-gp inhibitors are defined as those drugs that increase the AUC of digoxin or fexofenadine by ≥ 1.25-fold or <1.25-fold, respectively. (The asterisk indicates data derived with fexofenadine; all other data were derived with digoxin.)

(3) Strong, moderate, or weak CYP3A inhibitors are defined as those drugs that increase the AUC of oral midazolam or other CYP3A substrates ≥5-fold, 2-5-fold, and 1.25-2-fold, respectively.

In addition to the possibility that a drug is an inhibitor or inducer of multiple enzymes/transporters, a drug can be an inhibitor of one enzyme/transporter and inducer of another enzyme/transporter. For example, ritonavir is an inhibitor of CYP3A and an inducer of UGT; tipranavir is an inhibitor of CYP3A and an inducer of P-gp. Rifampin, an established inducer of multiple CYP enzymes and transporters, was recently found to be an inhibitor of the uptake transporter OATP1B1 and may inhibit the uptake of an investigational drug that is a substrate of OATP1B1. Accordingly, if a drug is a CYP enzyme substrate and an OATP1B1 substrate, an induction study with rifampin should be designed and interpreted carefully. The net steady state effect may vary depending on the relative size of the individual effect on transporter and enzyme activities. Timing of administration may become critical in situations when both enzymes and transporters can be affected. These overlapping selectivities contribute to complex drug interactions and make the prediction of in vivo outcome based on in vitro evaluation challenging or impossible (Zhang et al. 2009).

The implications of simultaneous inhibition of a dominant CYP enzyme(s) and an uptake or efflux transporter that controls the availability of the drug to CYP enzymes can be just as profound as that of multiple CYP inhibition. For example, the large effect of co-administration of itraconazole and gemfibrozil on the systemic exposure (AUC) of repaglinide may be attributed to collective inhibitory effects on both the enzyme (CYP2C8) and transporters (OATP1B1) by intraconazole and gemfibrozil and their respective metabolites.

c. Effect of Organ Impairment

Another type of complex drug interaction is the co-administration of substrate and enzyme/transporter inhibitor in subjects with organ impairment. For example, if a substrate drug is eliminated through both hepatic metabolism and renal secretion/filtration, the use of an enzyme inhibitor in subjects with renal impairment may cause a more than projected increase in exposure of substrate drug based on individual effect alone.

Unfortunately, current knowledge does not permit the presentation of specific guidance for studying some of these complex drug interaction scenarios because dedicated in vivo studies in humans may not be feasible or may raise ethical and practical considerations. Modeling and simulation approaches integrating prior in vitro and in vivo ADME and drug interaction data may be useful for evaluating complex drug interactions. For example, results from dedicated single pair drug interaction studies and separate pharmacokinetic evaluation in subjects with organ impairment may provide useful information to strengthen the model for the evaluation of complex drug interactions.

d. Pediatrics and Geriatrics

Age-related changes in physiological processes governing drug disposition and drug

effect have been investigated. In some cases, disproportional alterations in binding proteins, drug metabolizing enzymes and/or transporters, and renal filtration/secretion caused by developmental changes have been known to result in different drug disposition characteristics in pediatric and geriatric populations. However, dedicated drug interaction studies in these populations may not be feasible. Simulations using system biology approaches such as PBPK models (see section IV.A) may be helpful to predict drug interaction potential when the model can be constructed based on sufficient in vitro and clinical pharmacology and drug interaction data and incorporates development changes. Population pharmacokinetic approaches with sparse sampling can be used if properly designed (section IV.C).

e. Genetics

When a drug-drug interaction study uses a probe drug (e.g., omeprazole for CYP2C19) to evaluate the impact of the investigational drug on a polymorphic enzyme, individuals who have no functional enzyme activity would not be appropriate study subjects. Drug interaction studies that evaluate enzymes or transporters with known polymorphisms should include collection of genotype or phenotype information to allow appropriate interpretation of the study results. In some instances, an evaluation of the extent of drug interactions in subjects with various genotypes may be helpful (refer to the FDA guidance for industry on *Clinical Pharmacogenomics: Premarketing Evaluation in Early Phase Clinical Studies,* http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm).

D. Route of Administration

The route of administration chosen for a metabolic drug-drug interaction study is important. For an investigational agent, the route of administration generally should be the one planned for clinical use. When multiple routes are being developed, the need for metabolic drug-drug interaction studies by each route depends on the expected mechanisms of interaction and the similarity of corresponding concentration-time profiles for parent drug and metabolites. If only oral dosage forms will be marketed, studies with an intravenous formulation are not usually recommended, although information from oral and intravenous dosing may be useful in discerning the relative contributions of alterations in absorption and/or presystemic clearance to the overall effect observed for a drug interaction. Sometimes certain routes of administration can reduce the utility of information from a study. For example, intravenous administration of a substrate drug may not reveal an interaction for substrate drugs where intestinal CYP3A activity markedly alters bioavailability.

E. Dose Selection

The doses of the substrate and interacting drug used in studies should maximize the possibility of demonstrating an interaction. For this reason, the maximum planned or approved dose and

shortest dosing interval of the interacting drug (as inhibitors or inducers) should be used. For example, when using ketoconazole as an inhibitor of CYP3A, the decision whether to dose at 400 mg QD or 200 mg BID for multiple days can be determined based on the pharmacokinetic characteristics (e.g., the half-life) of the substrate drug (Zhao et al. 2009). When using rifampin as an inducer, dosing at 600 mg QD for multiple days would be preferable to lower doses. When there are safety concerns, doses lower than those used clinically may be recommended for substrates. In such instances, any limitations of the sensitivity of the study to detect the drug-drug interaction due to the use of lower doses should be discussed by the sponsor in the protocol and study report.

F. Endpoints

Changes in pharmacokinetic parameters generally are used to assess the clinical importance of drug-drug interactions. Interpretation of findings (i.e., deciding whether a given effect is clinically important) depends on a good understanding of dose/concentration and concentration/response relationships for both desirable and undesirable drug effects in the general population or in specific populations. The FDA guidance for industry on *Exposure-Response Relationships — Study Design, Data Analysis, and Regulatory Applications* (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm) provides considerations in the evaluation of exposure-response relationships. In certain instances, reliance on pharmacodynamic endpoints in addition to pharmacokinetic measures and/or parameters may be useful. Examples include INR measurement (e.g., when studying warfarin interactions) or QT interval measurements.

1. Pharmacokinetic Endpoints

Substrate PK exposure measures such as AUC, C_{max}, time to C_{max} (T_{max}), and others as appropriate should be obtained in every study. Calculation of pharmacokinetic parameters such as clearance, volumes of distribution, and half-lives may help in the interpretation of the results of the trial. In some cases, obtaining these measures for the inhibitor or inducer may be of interest as well, notably where the study is intended to assess possible changes in the disposition of both study drugs. Additional measures may help in steady state studies (e.g., trough concentration) to demonstrate that dosing strategies were adequate to achieve near steady state before and during the interaction. In certain instances, an understanding of the relationship between dose, blood concentrations, and response may lead to a special interest in certain pharmacokinetic measures and/or parameters. For example, if a clinical outcome is most closely related to peak concentration (e.g., tachycardia with sympathomimetics), C_{max} or an early exposure measure may be most appropriate for evaluation. Conversely, if the clinical outcome is related more to extent of absorption, AUC would be preferred. The frequency of sampling should be adequate to allow accurate determination of the relevant measures and/or parameters for the parent molecule and metabolites. For the substrate, whether the investigational drug or the approved drug, determination of the pharmacokinetics of relevant metabolites is important. Also, measurement of these metabolites may be useful

to differentiate the effect of inhibitor/inducer on pathways mediated by different CYP enzymes.

2. Pharmacodynamic Endpoints

Pharmacokinetic measures are usually sufficient for drug-drug interaction studies, although pharmacodynamic measures can sometimes provide additional useful information, especially for therapeutic proteins. Pharmacodynamic measures may be indicated when a pharmacokinetic/pharmacodynamic relationship for the substrate endpoints of interest is not established or when pharmacodynamic changes do not result solely from pharmacokinetic interactions (e.g., additive effect of quinidine and tricyclic antidepressants on QT interval). In most cases, when an approved drug is studied as a substrate, the pharmacodynamic impact of a given change in blood level (C_{max}, AUC) caused by an investigational interacting drug should be known from other data. If a PK/PD study is needed, it generally should include a larger population of subjects/patients than the typical PK study (e.g., a study of QT interval effects or platelet aggregation effects).

G. Statistical Considerations and Sample Size

The goal of the interaction study is to determine whether there is any increase or decrease in exposure to the substrate in the presence of the interacting drug. If there is, its implications should be assessed by an understanding of PK/PD relations both for C_{max} and AUC.

Results of drug-drug interaction studies should be reported as 90% confidence intervals about the geometric mean ratio of the observed pharmacokinetic measures with (S+I) and without the interacting drug (S alone). Confidence intervals provide an estimate of the distribution of the observed systemic exposure measure ratio of (S+I) versus (S alone) and convey a probability of the magnitude of the interaction. In contrast, tests of significance are not appropriate because small, consistent systemic exposure differences can be statistically significant ($p < 0.05$), but not clinically relevant.

When a drug-drug interaction of potential importance is clearly present, the sponsor should provide specific recommendations regarding the clinical significance of the interaction based on what is known about the dose-response and/or PK/PD relationship for the substrate drug used in the study. This information can form the basis for reporting study results and for making recommendations in the labeling. FDA recognizes that dose-response and/or PK/PD information can sometimes be incomplete or unavailable, especially for an older approved drug used as a substrate.

If the sponsor wishes to include a statement in the labeling that no known drug-drug interaction of clinical significance exists, the sponsor should recommend specific *no effect* boundaries, or clinical equivalence intervals, for a drug-drug interaction and should provide the scientific justification for the recommendations. No effect boundaries represent the interval within which

a change in a systemic exposure measure is considered not clinically meaningful. These conclusions can be based on dose-response data (e.g., if doses of x and 2x are known not to have different effectiveness or toxic effects) or on PK/PD modeling (a known flat concentration-response relationship).

There are two approaches to defining no effect boundaries:

Approach 1: No effect boundaries can be based on the population (group) average dose-related and/or individual concentration-response relationships derived from PK/PD models, and other available information for the substrate drug to define a degree of difference caused by the interaction that is of no clinical consequence. If the 90% confidence interval for the systemic exposure measurement change in the drug-drug interaction study falls completely within these no effect boundaries, the sponsor can conclude that no clinically significant drug-drug interaction is present.

Approach 2: In the absence of no effect boundaries defined in Approach 1, a sponsor can use a default no effect boundary of 80-125% for both the investigational drug and the approved drugs used in the study. When the 90% confidence intervals for systemic exposure ratios fall entirely within the equivalence range of 80-125%, standard Agency practice is to conclude that no clinically significant differences are present. This is, however, a very conservative standard and a substantial number of subjects (sample size) would need to be studied to meet it.

The selection of the number of subjects for a given drug-drug interaction study will depend on how small an effect is clinically important to detect or rule out the inter- and intra-subject variability in pharmacokinetic measurements, and possibly other factors or sources of variability not well recognized.

VI. LABELING RECOMMENDATIONS

Drug interaction information is generally included in the DRUG INTERACTIONS and CLINICAL PHARMACOLOGY sections of labeling and presents information that is essential for prescribers to appropriately use the drug. When drug interaction information has important implications for the safe and effective use of the drug, it will often be included in varying levels of detail in other sections of the labeling, such as DOSAGE AND ADMINISTRATION, CONTRAINDICATIONS or WARNINGS AND PRECAUTIONS. The labeling should include clinically relevant information about metabolic and transport pathways, metabolites, pharmacokinetic or pharmacodynamic interactions, and clinical implications of pharmacokinetic or pharmacodynamic interactions or genetic polymorphisms of drug metabolizing enzymes and transporters, if applicable. The description of clinical implications should include dose adjustments or monitoring recommendations, when relevant. General content recommendations for the appropriate labeling sections are provided below.

Drug interaction information in the labeling may not always result from a dedicated drug

interaction study. In certain cases, information can be extrapolated from one drug interaction study with a set of drugs to another set of drugs, with an explanation that similar results are expected. For example:

- An investigational drug that is a strong inhibitor or a strong inducer of CYP3A does not need to be tested with all CYP3A substrates to warn about an interaction with sensitive CYP3A substrates and CYP3A substrates with a narrow therapeutic range. A study involving a single sensitive substrate with the investigational drug would lead to labeling language about the use of the investigational drug with all sensitive and NTR substrates of the affected enzyme.

- A drug that is a sensitive CYP3A substrate or a CYP3A substrate with a narrow therapeutic range does not need to be tested with all strong or moderate inhibitors or inducers of CYP3A to warn about an interaction with CYP3A inhibitors or inducers. The labeling can include such a warning in the absence of a study if its metabolism is predominantly by the CYP3A route.

A. Drug Interactions Section of Labeling

The DRUG INTERACTIONS section includes a description of the clinical implications of clinically significant interactions with other drugs (including prescription and over-the-counter drugs), classes of drugs, dietary supplements, and foods and practical instructions for preventing or managing them. Recommendations for dose adjustments of co-administered drugs are included in this section. This section also includes practical guidance on known interference with laboratory tests. Interactions mentioned in DOSAGE AND ADMINISTRATION, CONTRAINDICATIONS, or WARNINGS AND PRECAUTIONS must be discussed in more detail in the DRUG INTERACTIONS section (21 CFR 201.57(c)(8)(i)). The need for dose adjustments of co-administered drugs is summarized in this section and presented in more detail in DOSAGE AND ADMINISTRATION. Drug interaction findings with negative results (i.e., no interaction was found) should generally not appear in this section unless this information is clinically relevant for the prescriber (e.g., if two drugs are commonly used together or if a drug does not have the same interaction as other drugs in the same class). This section may also include a brief summary of potential mechanisms of drug interactions. (e.g., "Drug X is a strong CYP3A inhibitor and may increase concentrations of CYP3A substrates." or "Drug X does not inhibit or induce CYPs 1A2, 2C9, or 2C19."). This section does not include details of drug interaction studies, but instead cross-references the information in the CLINICAL PHARMACOLOGY section.

Drug interactions that have the most clinical relevance (e.g., result in serious or otherwise clinically significant outcomes) should be listed first. Because the number of drug interactions and complexity of the information in this setting varies, we recommend using the most appropriate format to enhance communication of the information. For example, for drugs with extensive drug interaction information, a table may be the most effective format to convey the information. The table can list, when applicable, the co-administered drugs, potential or known

interactions (information regarding the increase or decrease in concentrations of drug, co-administered drug, or relevant metabolites), and clinical comments (clinical concern, dose adjustments, or advice regarding monitoring). When appropriate, the use of numbered subsections or subheadings within a subsection are recommended to organize the information (e.g., "Effect of Drug X on other drugs," "Effect of other drugs on Drug X," or subheadings for specific drugs or drug classes). Because this section may include information about both known and predicted drug interactions, it may be helpful to describe the data source for the information (e.g., indicate when the information is based on a specific drug interaction study and when it is based on a known mechanism, including simulation results, without a study).

B. Clinical Pharmacology Section of Labeling

Information in the PHARMACOKINETICS subsection (12.3 Pharmacokinetics) of the CLINICAL PHARMACOLOGY section is generally organized under descriptive subheadings (e.g., absorption, distribution, metabolism, excretion, pharmacokinetics in specific populations, and drug interactions). The PHARMACOKINETICS subsection should include descriptive information related to mechanisms of drug interactions, and details of the relevant drug interaction study results. The text should cross-reference other sections of the labeling that describe clinical management instructions, dose adjustments, or major safety concerns related to drug interactions (e.g., WARNINGS AND PRECAUTIONS or CONTRAINDICATIONS).

If the drug is a metabolizing enzyme or transporter substrate, such information should be included in PHARMACOKINETICS under "Metabolism," the text should describe the metabolic pathway(s), relevant metabolites formed, specific drug metabolizing enzymes, and whether there is genetic variation in the drug metabolizing enzymes. If the drug is metabolized by an enzyme subject to genetic variability, the information should be included under "Metabolism" and cross-referenced to the fuller discussion under a PHARMACOGENOMICS subsection of the CLINICAL PHARMACOLOGY section.

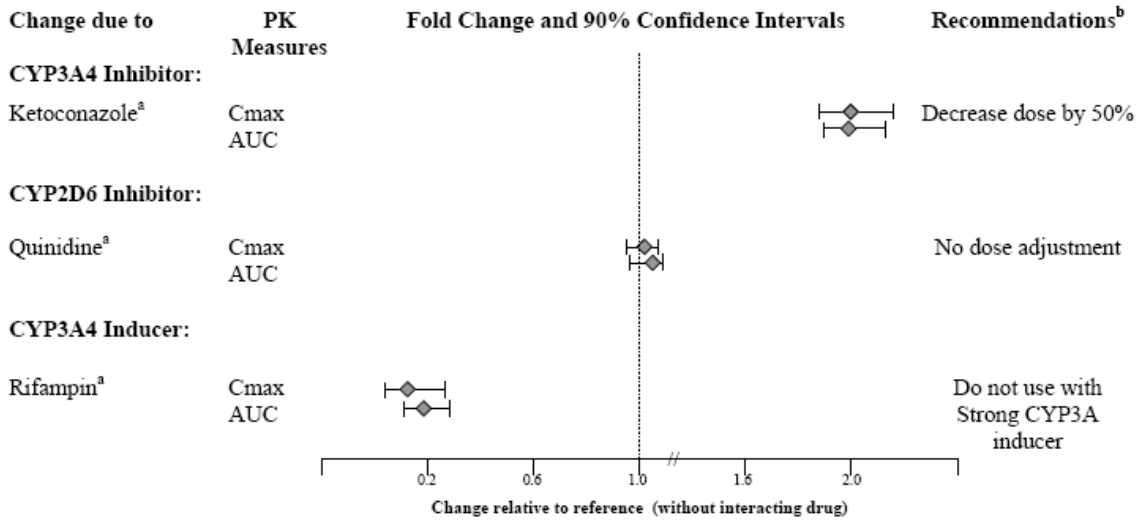
Information under the "Drug Interactions" subheading includes a more detailed description of the potential mechanisms of drug interactions than the description in the DRUG INTERACTIONS section of labeling. The data source for the conclusions (e.g., known CYP3A inhibitor based on in vitro and in vivo studies) should be briefly described.

Under "Drug Interactions" study results may be presented in a forest plot (described below), a table, or as text, depending on the number of studies and level of detail needed for clarity. The information should include only those study features that are essential to understand the results. In most cases it is not necessary to include study design, number of subjects, or population (e.g., healthy volunteers or patients) studied. The most relevant study design feature is likely the dose and duration for each drug; when relevant, the information should be included. The results should be presented as the change in relevant pharmacokinetic exposure measures (e.g., AUC and C_{max} and where appropriate C_{min}, T_{max}). It is important to indicate the variability of the interaction. Results should generally be presented as geometric mean change and the 90% confidence interval around the geometric mean change. For example, a 48% percent increase in

AUC could be expressed as ↑48% (90%CI: ↑24%, ↑76%) or as a ratio or fold change, where the 48% percent increase would be expressed as 1.48 (90% CI: 1.24, 1.76).

In the PHARMACOKINETICS subsection, a forest plot is a useful tool for presenting changes in pharmacokinetic exposure measures caused by various intrinsic and extrinsic factors such as drug interactions, hepatic impairment, and renal impairment (see Figure 8 below). The forest plot should display the fold-change in key pharmacokinetic measures such as geometric mean AUC and geometric mean C_{max} along with the 90% confidence intervals. Such graphs should clearly state the reference arm (or identify it in text accompanying the figure) and can include the doses of studied drugs, if relevant. Separate plots can display the effect of others on the labeled drug, effects of the drug on other drugs, and the effects of impaired hepatic or renal function.

Figure 8. The Effect of Various CYP Inhibitors on a Hypothetical Drug's PK as Displayed as 90% Confidence Interval of Geometric Mean AUC and C_{max} Ratios.



[a] For illustration purpose only. Assuming the interacting drugs affect CYPs only
[b] Recommendation will be drug dependent

C. Other Labeling Sections

As stated above, when drug interaction information has important implications for the safe and effective use of the drug, the information may be distributed among several other labeling sections (e.g., DOSAGE AND ADMINISTRATION, CONTRAINDICATIONS, WARNINGS AND PRECAUTIONS, or PATIENT COUNSELING INFORMATION), with a cross-reference to the DRUG INTERACTIONS or CLINICAL PHARMACOLOGY sections for more detailed information.

- DOSAGE AND ADMINISTRATION — This section includes information about drug interaction information that has important implications for a drug's dosing regimen (e.g., dosage adjustments, timing of dose relative to dosing of another drug).

- CONTRAINDICATIONS — This section describes when other drugs should not be co-administered with the drug because the risk outweighs any potential benefit.

- WARNINGS AND PRECAUTIONS — This section includes a brief discussion of any known or predicted drug interactions with serious or otherwise clinically significant outcomes.

- PATIENT COUNSELING INFORMATION — This section includes information necessary for patients to use the drug safely and effectively, such as avoiding drinking grapefruit juice.

For more specific recommendations on labeling content for these sections of labeling, refer to the following guidances for industry: *Warnings and Precautions, Contraindications, and Boxed Warning Sections of Labeling for Prescription Drug and Biological Products – Content and Format,* and *Dosage and Administration Section of Labeling for Human Prescription Drug and Biological Products – Content and Format.* These guidances and other labeling guidances are available at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065010.htm.

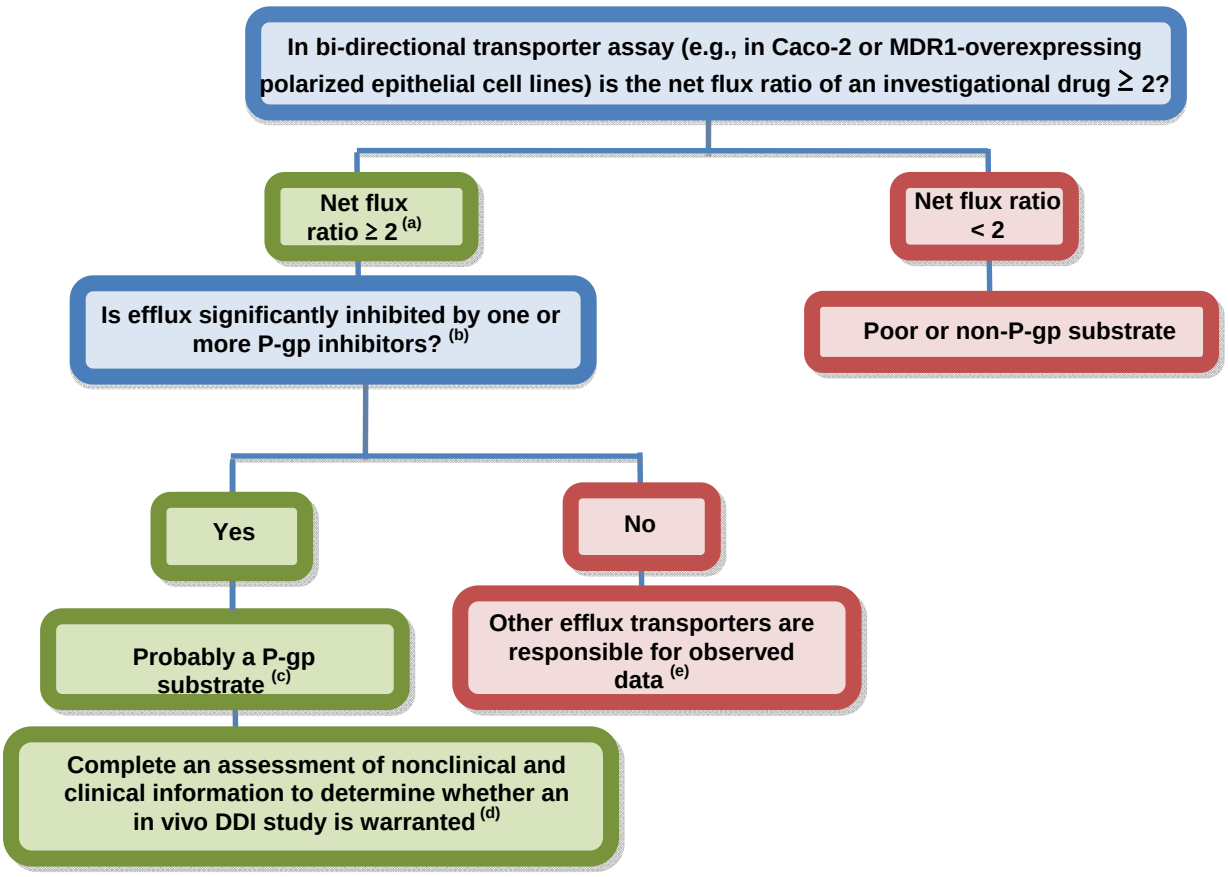
List of Figures in the Appendix

APPENDIX

Models for Determining When In Vivo Transporter-Mediated Drug Interaction Studies Are Needed

P-gp and BCRP:

Figure A1. Decision tree to determine whether an investigational drug is a substrate for P-gp and when an in vivo clinical study is needed. A similar model can be applied to a BCRP substrate — refer to IV.A.2.a, Figure 6 (Modified From Figures in Giacomini et al. 2010).



(a) An acceptable system produces net flux ratios of probe substrates similar to the literature values. A net flux ratio ≥ 2 for the investigational drug is a positive signal for further evaluation. A net flux ratio "cutoff" higher than 2 or a relative ratio to positive controls may be used to avoid false positives if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.

(b) Reduction of the flux ratio significantly (> 50%) or to unity.

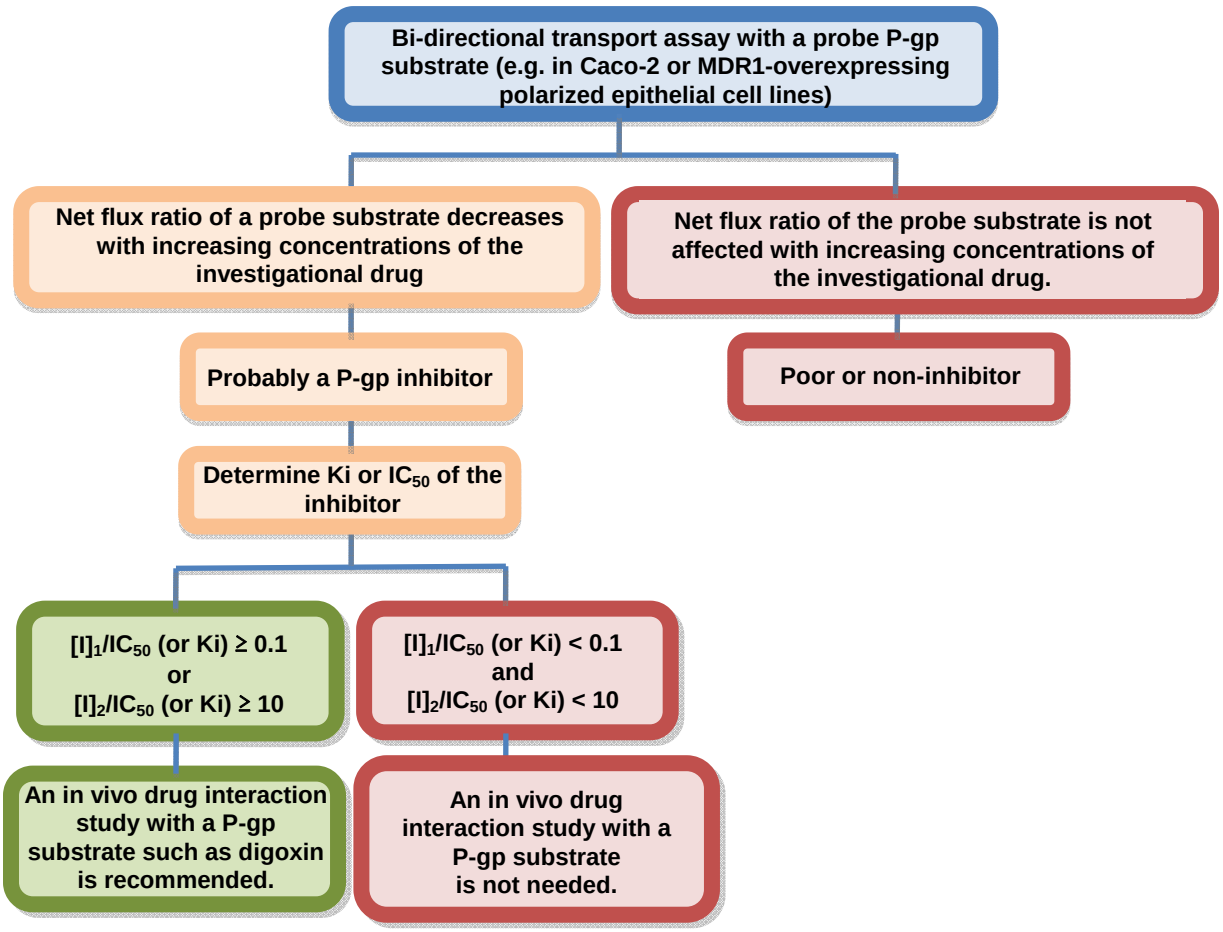
(c) Additional data are needed to establish clinical relevance of the in vitro data. In particular, the relative contribution of the transporter-mediated pathway to the overall clearance of the drug is the primary determinant of whether an inhibitor will have a major effect on the disposition of the investigational new drug.

(d) Selection of inhibitors could be based on likelihood of co-administration and/or its inhibition potency on P-gp.

Strong P-gp inhibitors (e.g., itraconazole, verapmil) provide the most sensitive assessment and should generally be tested first. If the drug is also a substrate for CYP3A, then inhibitors for both CYP3A and P-gp should be selected (Table 8).

[(e)] Based on existing knowledge of the compound class, further studies may be warranted to determine which efflux transporters are involved. Determining whether the drug is a BCRP substrate may be explored. A similar decision model may be used for a BCRP substrate; however, clinical studies would differ.

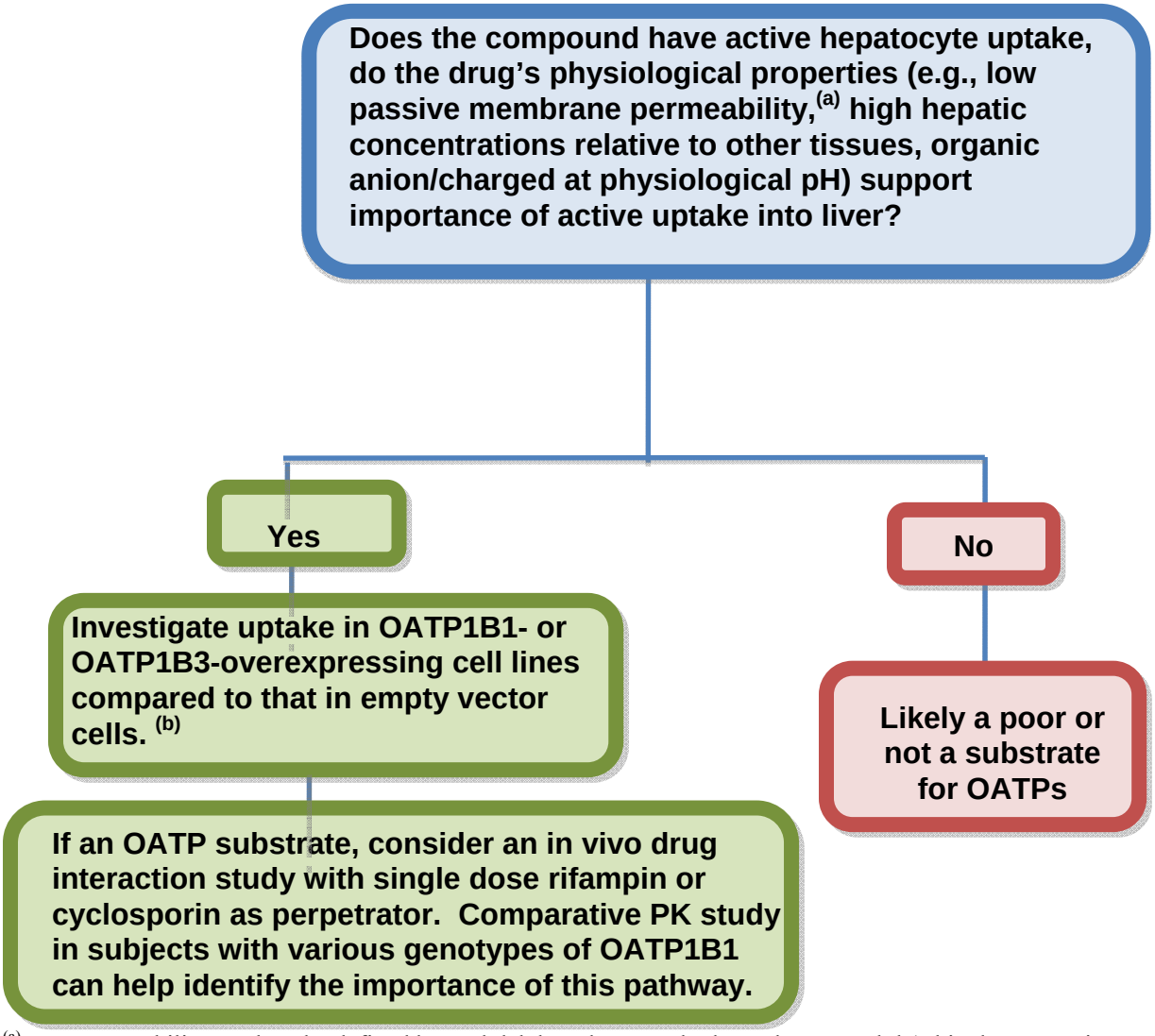
Figure A2. Decision tree to determine whether an investigational drug is an inhibitor of P-gp and when an in vivo clinical study is needed. A similar model can be applied to a BCRP inhibitor) — refer to IV.A.2.b (Modified From Figures in Giacomini et al. 2010)



$[I]_1$ represents the mean steady-state total (free and bound) C_{max} following administration of the highest proposed clinical dose. $[I]_2$= Dose of inhibitor (in mol)/250 mL (if IC_{50} is in a molar unit). For IC_{50} determination, a unidirectional assay (e.g., B to A) based on the probe substrate can also be considered.

OATP1B1 and OATP1B3 (Liver uptake transporters):

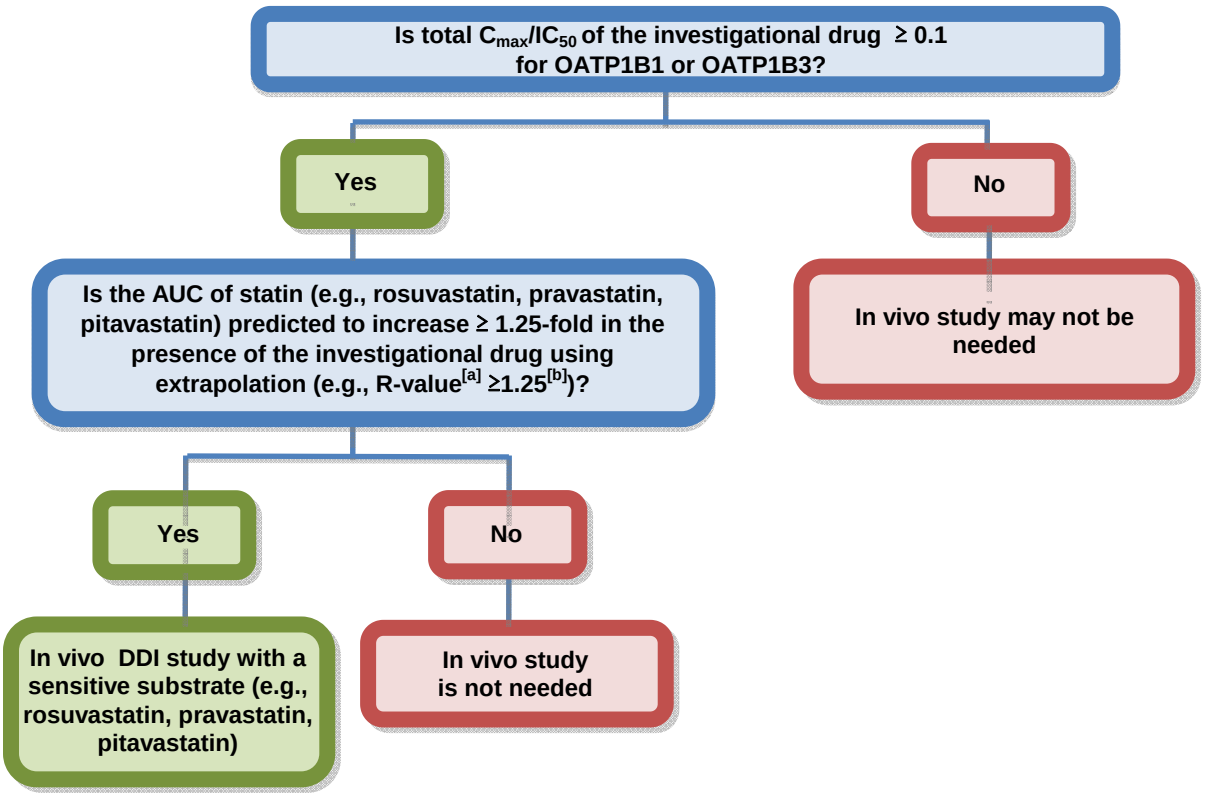
Figure A3. Decision tree to determine whether an investigational drug is a substrate for OATP1B1 or OATP1B3 and when an in vivo clinical study is needed— refer to IV.A.2.a, Figure 6 (Modified From Figures in Giancomini et al. 2010)



(a) Low permeability needs to be defined by each lab based on standards, such as atenolol (a biopharmaceutics classification system (BCS) reference drug). A general guide would be that 10^{-6} cm/sec (10 nm/sec) or lower is classified as "low" permeability.

(b) The following criteria suggest the investigational drug is a substrate of OATP1B1 or OATP1B3: Uptake in OATP1B1- or OATP1B3-transfected cells greater than 2-fold of that in empty vector transfected cells and is inhibitable (e.g, >50% reduction to unity) by a known inhibitor (e.g., rifampin) at a concentration at least 10 times of its K_i. Michaelis–Menten studies may be conducted in the transfected cells to determine the kinetic parameters of the investigational drug. A positive control should be included. In an acceptable cell system, the positive control should show a ≥ 2 fold increase in uptake compared to vector-transfected cells. An uptake ratio (transporter transfected vs. empty vector transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.

Figure A4. Decision tree to determine whether an investigational drug is an inhibitor of OATP1B1 or OATP1B3 and when an in vivo clinical study is needed — refer to IV.A.2.b (Modified From Figures in Giancomini et al. 2010)

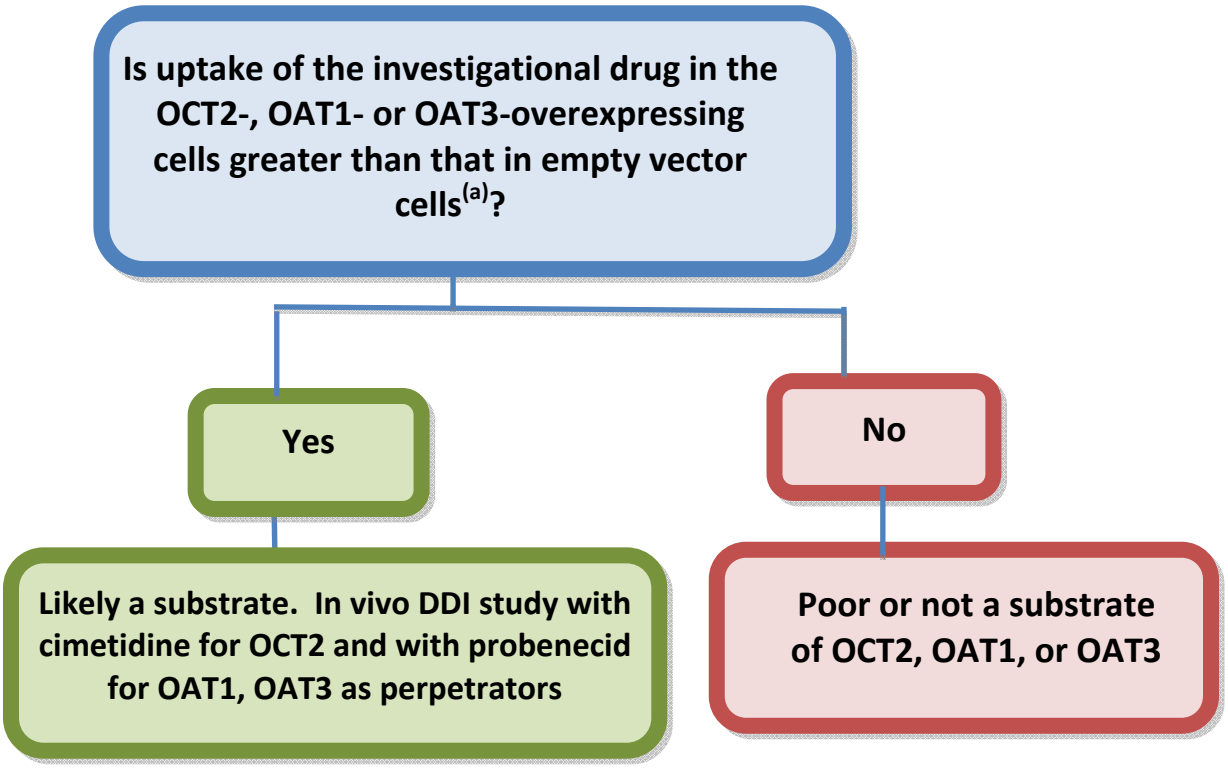


[a] R-value = 1+ (fu x $I_{in,max}/IC_{50}$), where, $I_{in,max}$ is the estimated maximum inhibitor concentration at the inlet to the liver and is equal to: C_{max} + (k_a x Dose x F_a F_g/Qh). C_{max} is the maximum systemic plasma concentration of inhibitor; Dose is the inhibitor dose; F_aF_g is the fraction of the dose of inhibitor which is absorbed; k_a is the absorption rate constant of the inhibitor and Qh is the estimated hepatic blood flow (e.g., 1500 mL/min). If Fa Fg values and ka values are unknown, use 1 and 0.1 min^{-1} (Ito et al. 1998) for FaFg and ka, respectively because the use of theoretically maximum value can avoid false-negative prediction. For drugs whose fu values are less than 0.01 or fu cannot be accurately determined due to high protein-binding, then assume fu = 0.01, to err on the conservative side to avoid false negative predictions.

[b] These are the suggested values according to the upper limit of equivalence range. We are open to discussion based on sponsors' interpretation.

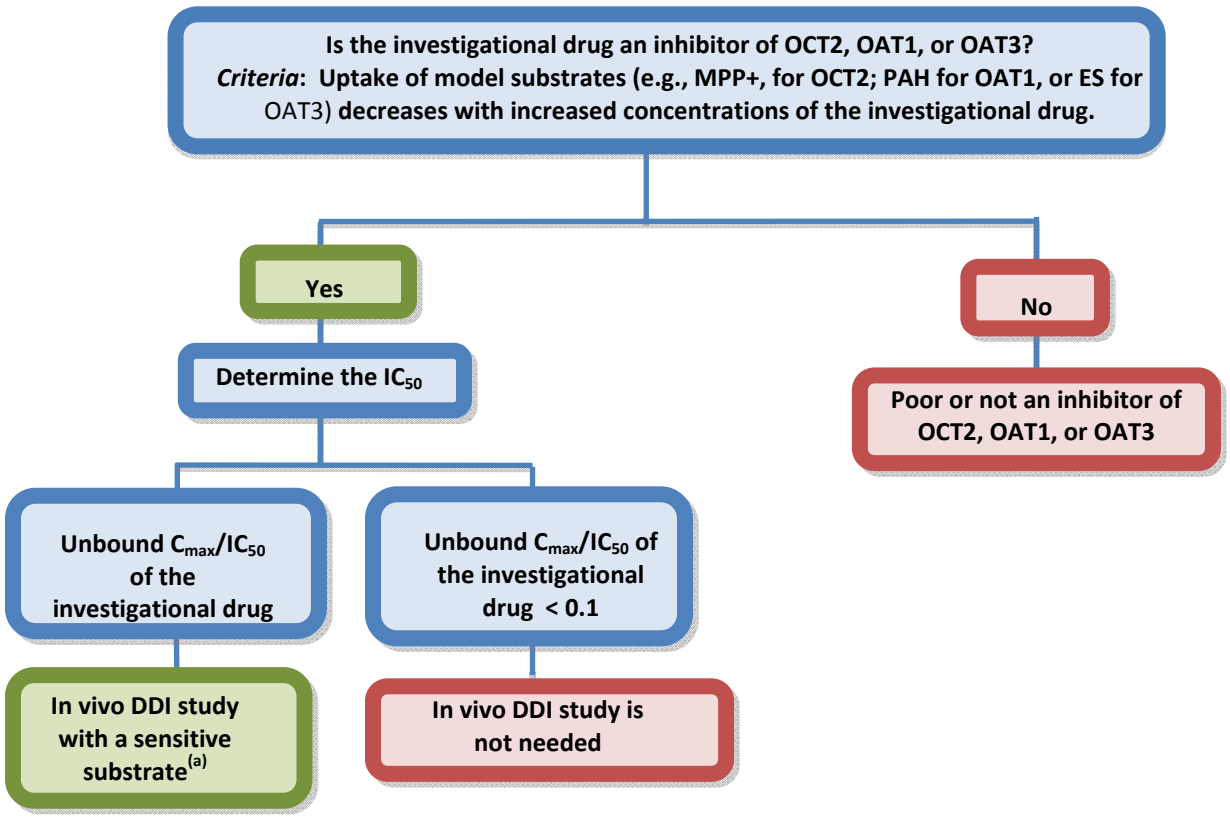
OCT2, OAT1, and OAT3 (renal transporters):

Figure A5. Decision tree to determine whether an investigational drug is a substrate for OCT2, OAT1, or OAT3 and when an in vivo clinical study is needed — refer to IV.A.2.a, Figure 6 (Modified From Figures in Giancomini et al. 2010)



[(a)] The ratio of the investigational drug uptake in the cells expressing the transporter versus the control (or empty vector) cells should be greater than 2. It is important that uptake into the transfected cells be significantly greater than background in a control cell line and be inhibited by a known inhibitor of the transporter. Michaelis–Menten studies may be conducted in the transfected cells to determine the kinetic parameters of the investigational drug. A positive control should be included. In an acceptable cell system, the positive control should show a ≥ 2 fold increase in uptake compared to vector-transfected cells. An uptake ratio (transporter transfected vs. empty vector transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.

Figure A6. Decision tree to determine whether an investigational drug is an inhibitor of OCT2, OAT1, or OAT3 and when an in vivo clinical study is needed — refer to IV.A.2.b (Modified From Figures in Giancomini et al. 2010)



MPP^+, 1-methyl-4-phenylpyridinium; PAH, *para*-aminohippuric acid; ES, estrone-3-sulfate.

[(a)] For the investigational drug that is an OCT2 inhibitor, metformin may be used as the substrate for the clinical drug interaction study.

For investigational drugs that are OAT1 or OAT3 inhibitors, multiple OAT1 or OAT3 substrates could be used in clinical DDI studies, including zidovudine, acyclovir, ciprofloxacin, tenofovir, or methotrexate.

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ABBREVIATIONS

ABC: ATP-binding cassette
ADME: absorption, distribution, metabolism, and/or excretion;
AhR: aryl hydrocarbon receptor
AUC: area under the plasma concentration-time curve
BCRP: breast cancer resistance protein
BCS: biopharmaceutical classification class
BLA: biologics license appliaction
BSEP: bile salt export pump
CAR: constitutive androstane receptor
CCB: calcium channel blocker
CYP: Cytochrome P450
EM: extensive metabolizers
FMO: flavin monooxygenase
INR: international normalized ratio
LST: liver specific transporter
MAO: monoamine oxidase
MATE: multidrug and toxin extrusion
MRP: multidrug resistance-associated protein
NDA: new drug application
NTCP: sodium/taurocholate cotransporting polypeptide
NTR: narrow therapeutic range
OAT: organic anion transporter
OATP: organic anion transporting polypeptide
OCT: organic cation transporter
PBPK: physiologically-based pharmacokinetic
PD: pharmacodynamics
P-gp: P- glycoprotein
PK: pharmacokinetics
PM: poor metabolizers
PXR: pregnane X receptor
SLC: solute carrier
TDI: time dependent inhibition
TdP: torsade de pointes
TP: therapeutic protein
UGT: uridine diphosphate (UDP)-glucuronosyl transferase
XO: xanthine oxidase