# M12 Drug Interaction Studies

## Guidance for Industry

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FOREWORD

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has the mission of achieving greater regulatory harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed, registered, and maintained in the most resource-efficient manner.  By harmonizing the regulatory expectations in regions around the world, ICH guidelines have substantially reduced duplicative clinical studies, prevented unnecessary animal studies, standardized safety reporting and marketing application submissions, and contributed to many other improvements in the quality of global drug development and manufacturing and the products available to patients.

ICH is a consensus-driven process that involves technical experts from regulatory authorities and industry parties in detailed technical and science-based harmonization work that results in the development of ICH guidelines.  The commitment to consistent adoption of these consensus-based guidelines by regulators around the globe is critical to realizing the benefits of safe, effective, and high-quality medicines for patients as well as for industry.  As a Founding Regulatory Member of ICH, the Food and Drug Administration (FDA) plays a major role in the development of each of the ICH guidelines, which FDA then adopts and issues as guidance to industry.

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## I.    INTRODUCTION (1)[2]

### A.    Objective (1.1)

This guidance provides recommendations to promote a consistent approach in designing, conducting, and interpreting enzyme- or transporter-mediated in vitro and clinical drug-drug interaction (DDI) studies during the development of a therapeutic product. A consistent approach will reduce uncertainty for the pharmaceutical industry to meet the requirements of multiple regulatory agencies and lead to more efficient utilization of resources. In addition, this approach will lead to the effective and safe treatment for patients who take multiple medications.

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

### B.    Background (1.2)

In clinical practice, patients are often prescribed more than one drug, which can result in a DDI. Some patients, in particular fragile older patients or patients with serious or multiple health issues, can be prescribed a large number of different drugs (i.e., polypharmacy). The occurrence of DDIs is a common clinical problem that can increase the risk of adverse effects, sometimes leading to hospital admissions. Alternatively, some DDIs can reduce or enhance efficacy of the treatment. Hence, it is important to consider an investigational drug's potential to interact with other drugs.

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[1] This guidance was developed within the Expert Working Group (*Multidisciplinary*) of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. This document has been endorsed by the ICH Assembly at *Step 4* of the ICH process May 2024. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the ICH regions.

[2] The numbers in parentheses reflect the organizational breakdown of the document endorsed by the ICH Assembly at Step 4 of the ICH process May 2024.

Regional guidances for investigations of DDIs have been available for decades and have undergone several updates as scientific progress has been made. In general, the proposed approach to the evaluation of interaction potential of investigational drugs has been similar between regions, but despite harmonization initiatives, some differences have remained. This ICH guidance aims to harmonize recommendations for in vitro and clinical evaluation of DDIs.

This guidance provides general recommendations on how to evaluate the DDI potential of an investigational drug. It is recognized that the DDI evaluation is generally tailored based on the specific drug, intended patient population, and therapeutic context. Alternative approaches may be acceptable if properly justified. The focus of the guidance is the development of new drugs, but if new scientific information regarding the potential for DDIs is obtained after drug approval, additional DDI evaluation should be considered.

## C.      Scope (1.3)

The scope of the guidance is limited to pharmacokinetic interactions, with a focus on metabolic enzyme- and transporter-mediated interactions. These aspects in general apply to the development of small chemical molecules. DDI evaluation of biologics is covered briefly, with focus on monoclonal antibodies and antibody-drug conjugates. Recommendations are provided on how to investigate interactions mediated by inhibition or induction of enzymes or transporters, both in vitro and in vivo (the terms *clinical* and *in vivo* are used interchangeably in this document), and on how to translate the results to appropriate treatment recommendations. The guidance also includes recommendations on how to address metabolite-mediated interactions. The guidance also covers use of model-based data evaluations and DDI predictions.

The development and emergence of other modalities such as oligonucleotides, small interfering ribose nucleic acids, and peptides is acknowledged. However, these modalities are out of scope for this guidance. Where applicable, regional guidances should be considered.

Other types of pharmacokinetic interactions, such as impact on absorption (e.g., gastric pH change, gastric motility change, formation of chelation or complexation), food effects, or protein binding displacement, are not part of this document and may be covered by regional guidances. Similarly, DDIs that are a result of pharmacodynamic interactions are beyond the scope of this guidance.

## D.      General Principles (1.4)

The potential for an investigational drug to cause DDIs should be investigated in a stepwise manner during drug development. The potential for an investigational drug to cause pharmacokinetic interactions both as an object (effect of other drugs on the investigational drug) and as a precipitant (effect of the investigational drug on concomitant drugs) should be evaluated. All aspects mentioned below are further expanded and discussed later in the document. Note that historically, some regions have used the term *victim* instead of *object* and the term *perpetrator* instead of *precipitant*. Because objects of DDIs are often substrates of

enzymes and/or transporters, the term *substrate* in this guidance refers to drugs that may be objects of DDIs.

Evaluating the potential of an investigational drug as an object of a metabolic enzyme- or transporter-mediated DDI involves identification of the principal routes of the drug's elimination. For drugs whose major elimination routes are not urinary excretion of unchanged parent drug or that are not biologics eliminated through unspecific catabolism, the keystone of the identification of principal elimination routes is a well-performed clinical mass balance study. In some instances, (e.g., if a large part of the dose is found as unchanged parent drug in feces), an absolute bioavailability study can also be a useful complement to aid interpretation of principal elimination. Using data from the mass balance study, the quantitative contributions of the different elimination pathways should be estimated based on the amount of dose excreted as primary and secondary metabolites along specific routes. For quantitatively important elimination pathways, in vitro and clinical studies should be used to identify the main enzymes and/or transporter proteins involved in these pathways. The ability to predict interactions affecting the investigational drug is dependent on the identification of these proteins.

Evaluating the DDI potential of an investigational drug as a precipitant involves characterizing the effect of the drug on enzymes and transporters. This evaluation often starts with in vitro experiments to elucidate potential DDI mechanisms. Identification of DDI risks should then be followed by clinical DDI studies based on mechanistic knowledge, and the results should be translated to appropriate clinical management recommendations for drugs as a precipitant of DDIs.

The results of DDI evaluations inform the protocols for clinical studies in patients regarding the use of concomitant drugs. Information about the interaction potential should be gained as early in drug development as practically possible to ensure safety and avoid unnecessary restrictions of concomitant medications and/or exclusion of patients who require the concomitant medications in clinical studies. The timing of the different nonclinical and clinical studies is dependent on the context and type of product; some general recommendations are given below. Predictive modeling (see Appendix D (section 7.5)) can also assist evaluation of the DDI potential.

- In vitro data on the investigational drug as a substrate of metabolic enzymes is generally recommended to be obtained before starting the clinical phase in patients to evaluate metabolic stability and identify the potential main metabolic pathway(s) and enzyme(s) that metabolize the investigational drug (reaction phenotyping studies). If in vitro studies suggest the possibility of a clinically significant interaction with inhibitors or inducers of a metabolic enzyme, additional actions (e.g., clinical DDI studies) may be needed before studies in patients. Until appropriate additional actions are taken, a conservative strategy, such as excluding patients on certain concomitant drugs that are inhibitors and/or inducers, may be needed.

- Absorption, distribution, metabolism, and/or excretion (ADME) properties determine whether in vitro data of the investigational drug as a substrate for transport proteins should be collected. If a drug has limited absorption or is expected to undergo significant active hepatic uptake, biliary excretion, or active renal secretion as unchanged drug, the

relevant transporter(s) should be identified in vitro before initiating clinical studies in patients to avoid restrictions on concomitant medications in protocols.

- In vitro data on the effects of the investigational drug as a precipitant on the major cytochrome P450 (CYP) enzymes and transporters is generally recommended to be available before initiating larger studies in subjects.

- The results of the mass balance study are generally recommended to be available before starting phase 3. Based on results of the mass balance study and in vitro studies, clinical studies with strong index enzyme inhibitors and inducers should be considered to confirm and quantify the main metabolism pathways and define the risk for clinically significant DDIs.

- The pharmacokinetic DDI potential of metabolites with significant plasma exposure or pharmacological activity (see section II.C (2.3)) is recommended to be considered similarly as for the parent drug, but these investigations can generally be completed later in development when more knowledge about the exposure and activity of metabolites is available.

## II. IN VITRO EVALUATION (2)

### A. Evaluation of Metabolism-Mediated Interactions (2.1)

In vitro studies are important first steps to identify risks for a drug to be an object or precipitant of DDIs related to inhibition or induction of drug metabolizing enzymes.

### 1. *Drug as a Substrate of Metabolizing Enzymes (2.1.1)*

Typically, an in vitro screening to identify the main enzymes responsible for the metabolism of a new drug is performed early in drug development. If oxidative metabolism is important, the identification of catalyzing enzymes usually starts by determining whether the investigational drug is an in vitro substrate for the most common CYP enzymes involved in drug metabolism: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A (CYP3A4 and CYP3A5) using in vitro phenotyping experiments. If the drug is not found to undergo metabolism by these major CYP enzymes, other enzymes can be investigated. These additional enzymes can include, but are not limited to:

- Other CYP enzymes, including CYP2A6, CYP2E1, CYP2J2, and CYP4F2, and other phase 1 enzymes, including alcohol/aldehyde dehydrogenase, aldehyde oxidase, carboxylesterase, flavin monooxygenase, monoamine oxidase, and xanthine oxidase.

- Phase 2 enzymes: the most frequently evaluated phase 2 enzymes, Uridine 5'-diphospho-glucuronosyltransferases (UGTs), are responsible for glucuronide conjugation of drugs and metabolites. Potential UGTs to be investigated in vitro include UGT1A1, 1A3, 1A4, 1A6, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, and 2B17.

- Other phase 2 enzymes, including glutathione S-transferases, N-acetyltransferases, sulfotransferases.

Details on the experimental setup for in vitro studies to identify enzymes catalyzing the main elimination pathways are given in Appendix B sections 1 and 2 (7.3.1 and 7.3.2).

In vitro phenotyping, metabolic profiling, and mass balance studies are generally used to identify and quantify the various elimination pathways of a drug. An enzyme that is estimated to contribute to greater than or equal to 25 percent of total elimination generally needs additional clinical characterization to quantify the risk of interaction with the investigational drug as a substrate. The characterization is often done by performing clinical DDI studies using a strong index inhibitor of the enzyme when available (refer to section III.B.3.a (3.2.3.1)). For some enzymes, pharmacogenetic studies may substitute for clinical DDI studies with a strong index inhibitor (refer to section IV.A (4.1)). A clinical study with a strong inducer is also generally needed to fully characterize the risk of DDI because inducers can upregulate expression of multiple enzymes and some transporters (except CYP2D6, which is generally considered not inducible by drugs) (refer to section III.B.3.a (3.2.3.1)).

### 2. *Drug as an Inhibitor of CYP Enzymes (2.1.2)*

An investigational drug's potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A in both a reversible manner and time-dependent inhibition (TDI) manner should be evaluated.

### a. Reversible inhibition (2.1.2.1)

In the reversible inhibition experiments, a $K_i$ (inhibition constant) is usually determined experimentally or estimated based on half-maximal inhibitory concentration ($IC_{50}$) using unbound concentrations (refer to Appendix B section 3 (7.3.3)). If the initial experiments testing a sufficiently high concentration of the investigational drug already indicate that the unbound inhibition constant ($K_{i,u}$) will be markedly higher than the cutoffs given (see below), the risk for clinical inhibition can normally be excluded without further data.

The risk for reversible enzyme inhibition can be excluded based on in vitro data (*basic method*) if:

$$K_{i,u} > 50 \times C_{max,u} \ (\text{i.e.,} \ \frac{C_{max,u}}{K_{i,u}} < 0.02)$$

$K_{i,u}$ is the unbound inhibition constant.
$C_{max,u}$ is the unbound $C_{max}$ (maximum plasma concentration) at the highest recommended dose at steady state.

For the purposes of calculating the unbound measure of concentration, the measured $f_{u,p}$ (fraction unbound in plasma) can be used for all drugs, including highly protein bound drugs (i.e., greater than 99 percent protein binding), if the accuracy and precision of the protein binding measurement has been demonstrated. Such a demonstration should include validation data of the protein binding assay including bioanalytical method and appropriate positive controls (i.e.,

drugs with high binding to relevant plasma proteins). If the reliability of $f_{u,p}$ measurements less than 1 percent cannot be demonstrated, a default value of 1 percent unbound fraction in plasma (i.e., $f_{u,p} = 0.01$) should be used (see Appendix A (section 7.2) for details). This consideration for $f_{u,p}$ applies in other contexts where basic method, mechanistic static, and dynamic models (often referred to as physiologically based pharmacokinetic (PBPK) modeling) can be used to interpret the in vitro results of enzyme and transporter inhibition/induction experiments.

For orally administered drugs that are inhibitors of CYP3A, the risk of intestinal CYP3A inhibition can be excluded if:

$$K_{i,u} > 0.1 \times \frac{maximum\ clinical\ dose}{250\ mL} \left(i.e., \frac{Dose/250\ mL}{K_{i,u}} < 10\right)$$

$K_{i,u}$ = unbound inhibition constant; mL = milliliter.

If risk for clinical inhibition cannot be excluded using this basic method, mechanistic static and/or PBPK models can be used to interpret the in vitro experiment results (refer to Appendix D (section 7.5)). If in vitro data and modeling do not exclude the risk for clinically relevant inhibition, a clinical DDI study with a sensitive index substrate should be conducted.

If a clinical study using a substrate for an enzyme that was inhibited in vitro by an investigational drug with a low $K_{i,u}$ shows lack of inhibition, then the risk for clinical inhibition can be excluded for other enzymes having a larger $K_{i,u}$. Such an inference should be made only for the enzymes that are expressed at the same site and for which the inhibition potencies are determined in the same human liver microsome (HLM)/hepatocyte batch (rank order approach) (1). Of note, an orally administered drug can inhibit intestinal metabolic enzymes (e.g., CYP3A) in addition to hepatic enzymes. In such situations, the risk for inhibition of CYP3A in the gastrointestinal (GI) tract should be considered even if systemic inhibition of CYP3A can be excluded using the rank order approach based on a negative clinical study on another CYP enzyme. In the presence of inhibitory metabolites of an investigational drug, their contribution should also be considered when using rank order approach to determine if clinical studies should be conducted.

b.     TDI (2.1.2.2)

If an in vitro assay (described in Appendix B section 3 (7.3.3)) indicates an increased enzyme inhibition potential with drug preincubation, the inhibitory kinetic parameters ($K_{I,u}$ and $k_{inact}$) will be determined and the following equation can be used as the basic method to evaluate the risk for TDI (2). The risk for clinical inhibition can be excluded based on in vitro data if:

$$\frac{(k_{obs} + k_{deg})}{k_{deg}} < 1.25$$

$$\text{where } k_{obs} = \frac{(k_{inact} \times 5 \times C_{max,u})}{(K_{I,u} + 5 \times C_{max,u})}$$

$k_{obs}$ is the apparent first-order inactivation rate constant of the affected enzyme.
$k_{deg}$ is the apparent first-order degradation rate constant of the affected enzyme (refer to Table 6).
$K_{I,u}$ is the unbound inhibitor concentration causing half-maximal inactivation.
$k_{inact}$ is the maximal inactivation rate constant.

$C_{max,u}$ is the maximal unbound plasma concentration of the inhibitor drug at steady state. The $f_{u,p}$ (fraction unbound in plasma) should be set to 1 percent if the reliability of $f_{u,p}$ measurements less than 1 percent cannot be demonstrated (also refer to section II.A.2.a (2.1.2.1)).
Note: $C_{max,u}$ and $K_{I,u}$ should be expressed in the same unit (e.g., in a molar concentration unit).

If the above ratio is greater than or equal to 1.25, mechanistic static and/or PBPK models can be used to interpret the in vitro experiment results (refer to Appendix D (section 7.5)). If in vitro data and modeling do not exclude the risk for clinical inhibition, a clinical DDI study with a sensitive index substrate should be conducted. The rank order approach, mentioned above for reversible inhibitors, does not apply to TDIs.

### 3. *Drug as an Inhibitor of UGTs (2.1.3)*

It is recognized that a drug which is not a substrate of an enzyme can still be an inhibitor. However, considering the generally limited magnitude of UGT inhibition-mediated DDIs (3), a routine evaluation of investigational drugs to inhibit UGTs may not be warranted. If direct glucuronidation is the major elimination pathway of an investigational drug, it is recommended to study in vitro whether the drug can inhibit UGTs. Potential UGTs to be studied include UGT1A1, UGT1A4, UGT1A9, UGT2B7, and UGT2B15. The evaluation is usually performed using recombinant UGTs or HLM with relatively selective substrates (refer to Table 8, in Appendix E section 2.a (7.6.2.1) for an illustrative list of substrates) (4). When an investigational drug will be commonly administered with a drug that is mainly metabolized by direct glucuronidation, it is recommended to evaluate in vitro the potential inhibitory effect of the investigational drug on the UGT isoform(s) responsible for the elimination of the other drug.

Due to limited availability of data from clinical DDI studies that evaluate inhibition of UGT isoenzymes, cutoffs for determining DDI risk using basic models like those for CYP enzymes have not been established. This is an area of ongoing research and, in the interim, sponsors can consider the same criterion that is applied to CYPs (i.e., $C_{max,u}/K_{i,u}$ less than 0.02 ($C_{max,u}$ is the unbound $C_{max}$ (maximum plasma concentration) at the highest recommended dose at steady state.)) or propose an alternative with justification.

### 4. *Drug as an Inducer of CYP Enzymes (2.1.4)*

An investigational drug's potential to induce enzymes via activation of nuclear receptors pregnane X receptor (PXR), constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AhR), and if relevant other drug regulation pathways should be evaluated. For technical advice on the experiments, refer to Appendix B section 4 (7.3.4).

To assess the DDI liability of a drug as an inducer, studies should be performed in human hepatocytes from at least three individual donors, and the extent of enzyme induction should be measured at the mRNA level. The enzymes CYP3A4, CYP2B6, and CYP1A2 should be included as markers of induction mediated via PXR/CAR (CYP3A4, CYP2B6) and AhR (CYP1A2). Enzymes CYP3A4 and CYP2C8, CYP2C9, and CYP2C19 are induced via activation of the PXR. CYP2C8, CYP2C9, and CYP2C19 are generally less inducible compared to CYP3A4. Therefore, the potential of the investigational drug to induce CYP2C8, CYP2C9, and CYP2C19 should be evaluated in vitro and/or in vivo when it has been shown to be an inducer of

CYP3A4 in a clinical study. A negative result for induction in a clinical study with a sensitive CYP3A4 substrate can be used to rule out an investigational drug's potential to induce CYP2C8, CYP2C9, and CYP2C19 enzymes provided that the potential of CYP3A4 inhibition by the drug and its major metabolite(s) can be excluded. When in vitro induction of CYP2C19 is evaluated, the mRNA responses to inducers are often limited, and thus the activity should be measured using a probe substrate to evaluate the CYP2C19 induction potential of the investigational drug (see Appendix B section 4 (7.3.4)).

As described below, there are several methods that can be used to interpret mRNA data from in vitro induction experiments and to assess the in vivo potential of a drug to induce enzymes. It is recommended to first use the basic qualitative method (mRNA fold-change). If the basic method indicates induction potential, the evaluation can continue using more quantitative approaches (e.g., correlation methods), provided it is possible to study a wide range of concentrations of the investigational drug to determine induction parameters (e.g., maximum induction effect ($E_{max}$) and $EC_{50}$ (concentration causing half-maximal effect)). For the more quantitative approaches, one qualified batch of hepatocytes is sufficient. The basic method only uses in vitro data from the investigational drug, whereas correlation methods compare the induction response of the drug to that of multiple established clinical inducers of the enzyme of interest.

In addition, mechanistic static or PBPK models can potentially be used (refer to Appendix B (section 7.5)). If a risk for induction cannot be excluded based on in vitro data and modeling, clinical studies with sensitive substrates of the enzymes of interest should be conducted.

> a.    Basic *mRNA fold-change* method (2.1.4.1)

The induction results should be evaluated separately for each donor. The levels of mRNA should be compared to the control (vehicle) incubations, and a fold-change over the vehicle control should be calculated. In vivo induction potential cannot be excluded if the drug in hepatocytes from at least one donor meets both of the following criteria, and further evaluation of the induction potential should be conducted:

- Increases mRNA expression of a CYP enzyme in a concentration-dependent manner; and

- The fold-change of CYP mRNA expression is greater than or equal to 2-fold at concentrations less than or equal to $50 \times C_{max,u}$.

When the response of the positive control is less than 6-fold increase in mRNA, the induction potential cannot be ruled out for an investigational drug that increases CYP enzyme mRNA less than 2-fold of the vehicle control but more than 20 percent of the response of the positive control. Further evaluation is recommended when there is an inconclusive finding (see Appendix B section 4 (7.3.4) and the International Council for Harmonisation (ICH) guidance for industry *M12 Drug Interaction Studies: Questions and Answers* (August 2024) (ICH M12 Q&A)).[3]

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[3] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

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

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

        b.     Correlation methods (2.1.4.2)

Correlation methods compare the induction effect of the investigational drug to that of established in vivo inducers of the enzyme of interest (5). The magnitude of an in vivo induction effect (e.g., area under the curve (AUC) ratio of sensitive substrate in the presence and absence of inducers) of an investigational drug is predicted based on a calibration curve of relative induction scores (RIS, see equation below) or $C_{max,u}/EC_{50,u}$ (unbound concentration causing half the maximal effect) versus the in vivo induction effect for a set of known inducers of the same enzyme (also refer to Appendix B, section 4 (7.3.4)). If the predicted AUC ratio greater than 0.8, the analysis can be used to exclude the risk for in vivo induction.

$$RIS = \frac{E_{max} \ \times C_{max,u}}{EC_{50,u} + C_{max,u}}$$

$EC_{50,u}$ is the unbound concentration causing half the maximal effect.
$E_{max}$ is the maximum induction effect.
$C_{max,u}$ is the unbound maximum plasma concentration of a drug at steady state. The $f_{u,p}$ (fraction unbound in plasma) should be set to 1 percent if the reliability of $f_{u,p}$ measurements less than 1 percent cannot be demonstrated (also refer to section II.A.2.a (2.1.2.1)).

Sometimes, $E_{max}$ or $EC_{50,u}$ cannot be estimated due to an incomplete in vitro induction profile (e.g., limited by solubility or cytotoxicity of tested drug). An alternative correlation approach can be used if the method is validated (6).

        c.     Basic kinetic model (2.1.4.3)

Mechanistic models have been proposed to predict the sum of different interaction processes (reversible inhibition, TDI, induction) systemically as well as in the GI tract. This approach is further discussed in Appendix B (section 7.5).

A limited version of this approach is described as below. If R (predicted AUC ratio of a sensitive enzyme substrate with and without an inducer) greater than 0.8, the analysis can be used to exclude the risk for in vivo induction.

$$R = \frac{1}{1 + d \ \times \ \dfrac{(E_{max} \ \times 10 \ \times C_{max,u})}{\left(EC_{50,u} + 10 \ \times C_{max,u}\right)}}$$

R is predicted AUC ratio of a sensitive enzyme substrate with and without an inducer.
$C_{max,u}$ is the unbound maximum plasma concentration in plasma. The $f_{u,p}$ (fraction unbound in plasma) should be set to 1 percent if the reliability of is 0.01, $f_{u,p}$ measurements less than 1 percent cannot be demonstrated (also refer to section II.A.2.a (2.1.2.1)).

d is the scaling factor (7). If the scaling factor has not been determined in a calibrated hepatocyte batch (see section 7.3.4), d=1 should be used.

If the above methods indicate that the investigational drug has the potential to induce metabolizing enzymes (using specific cutoff values mentioned above or developed by individual laboratories for these methods), the enzyme induction potential of the investigational drug should be further investigated by conducting a clinical DDI study with a sensitive index substrate or using mechanistic models (refer to Appendix D (section 7.5)).

        d.      Additional considerations related to induction (2.1.4.4)

Currently, in vitro methods to evaluate UGT induction are not well established. If an investigational drug has been observed to be an inducer of CYP enzymes via activation of nuclear receptors such as PXR or CAR, it is likely that UGTs regulated through these receptors will be induced. Refer to section III.B.4.c (3.2.4.3), which describes conducting clinical DDI studies mediated by induction of UGT for more considerations.

In vitro induction studies can also detect enzyme downregulation. However, research in this area is presently very limited, and the mechanisms behind these effects are unclear. If concentration-dependent downregulation (less than 50 percent of the mRNA control response) is observed in vitro and is not attributable to cytotoxicity, additional in vitro or clinical studies can be considered to understand the potential clinical consequences.

## B.      Evaluation of Transporter-Mediated Interactions (2.2)

### *1.      Drug as a Substrate of Transporters (2.2.1)*

P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are efflux transporters expressed in the GI tract and can affect the oral bioavailability of drugs. Thus, the possibility of being a substrate of P-gp and/or BCRP is often evaluated in vitro for investigational drugs given orally. Because P-gp and BCRP are also expressed in the liver (P-gp, BCRP) and kidneys (P-gp), an in vitro study should be considered for a drug if biliary excretion or active renal secretion is a major elimination pathway of the drug. In addition, evaluating P-gp- and BCRP-mediated transport may be useful in the assessment of the drug's brain penetration.

Organic anion transporting polypeptide (OATP)1B1 and OATP1B3 are important hepatic uptake transporters. Examination of whether an investigational drug is a substrate for OATP1B1 and 1B3 should be considered if hepatic metabolism or biliary excretion accounts for greater than or equal to 25 percent of elimination of a drug or if the pharmacological target of a drug is in the liver.

Renal uptake transporters (organic anion transporter (OAT)1, OAT3, and organic cation transporter (OCT)2) and renal efflux transporters (multidrug and toxin extrusion protein (MATE)1 and MATE2-K) are often involved in active renal secretion of drugs. In vitro studies to evaluate a drug as a substrate of these transporters should be considered if the drug clearance by renal active secretion is greater than or equal to 25 percent of its systemic clearance. Assuming there is no reabsorption (e.g., passive reabsorption is equal to passive secretion, and

there is no active reabsorption), active secretion clearance can be calculated as $(CLr - (f_{u,p} \times GFR))$, where GFR is glomerular filtration rate and CLr is renal clearance. If pharmacokinetic data following intravenous administration are not available, systemic clearance may be computed by multiplying apparent total clearance by estimated bioavailability.

In addition to the above-mentioned transporters, in vitro evaluation of a drug as substrate of additional transporters may be considered on a case-by-case basis as new information emerges and our understanding evolves. For example, multidrug resistance-associated protein 2 is also an efflux transporter in similar locations as P-gp and BCRP; OATP2B1 is an uptake transporter present in the liver and intestine and is involved in absorption of certain drugs; and OCT1 is a hepatic transporter mediating the uptake of some drugs into the liver. The decision to evaluate additional transporters can take into consideration the site of action, passive permeability, and knowledge about absorption and elimination pathways of a drug.

> a.    Data analysis and interpretation (2.2.1.1)

When examining the possibility that an investigational drug is a substrate of transporters, in vitro studies should be performed using experimental systems with the transporter activity confirmed using probe substrates and inhibitors (refer to Tables 10 and 11, in Appendix E section 3 (7.6.3) for some examples). Further details about considerations when performing in vitro studies are described in Appendix C sections 1 and 2 (7.4.1 and 7.4.2).

For uptake studies, if there is significant uptake of a tested drug in transporter-expressed cells relative to the vehicle control-transfected cells (e.g., greater than or equal to 2-fold of controls), and the uptake in transporter-expressed cells can be inhibited by more than 50 percent by a known inhibitor of the transporter, the tested drug can be considered a substrate of the transporter examined.

For bidirectional transport studies, if there is significant directional transport of a tested drug in transporter-expressed cells relative to untransfected or parental cells (e.g., net efflux ratio greater than or equal to 2) or Caco-2 cells (e.g., efflux ratio greater than or equal to 2), and the efflux ratio can be inhibited by more than 50 percent by a known inhibitor of the transporter, the tested drug can be considered as a substrate of the transporter examined.

A cutoff other than 2 or a specific relative ratio to positive controls can be used if prior experience with the cell system used justifies these alternative methods. Sponsors can also propose criteria for vesicle assays based on prior experience and internal data.

If in vitro studies indicate that a drug is a substrate of a transporter, clinical studies should be considered. Refer to section III.B.5.a (3.2.5.1) for more details.

> 2.    *Drug as an Inhibitor of Transporters (2.2.2)*

Studies should be conducted to evaluate whether an investigational drug is an inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K. Sponsors can consider evaluating the inhibition potential of a drug on other transporters as necessary. The

decision to evaluate additional transporters can take into consideration if commonly coadministered drugs are substrates for these transporters. In vitro studies should be performed using an experimental system whose transport activity is confirmed using probe substrates and inhibitors (see Appendix E section 3 (7.6.3) for more details). Considerations about how in vitro studies should be conducted are described in Appendix C sections 1 and 3 (7.4.1 and 7.4.3).

The risk for transporter inhibition by an investigational drug in humans can be excluded based on in vitro data using the basic methods and the criteria outlined in Table 1. The contribution of drug metabolites to transporter inhibition should also be considered (see section II.C.2 (2.3.2)).

**Table 1. Recommended Ratio and Cutoff Value for Drug as Inhibitor of Transporters**

| | |
|---|---|
| P-gp or BCRP | $IC_{50,u}{}^{*} > 0.1 \times$ (Dose/250 mL) (i.e., (Dose/250 mL)/ $IC_{50,u} < 10$) for orally administered drugs |
| OATP1B1 or OATP1B3 | $IC_{50,u} > 10 \times C_{max, inlet,u}{}^{\#}$ (i.e., $C_{max,inlet,u}$ / $IC_{50,u} < 0.1$) |
| OAT1, OAT3, OCT2 | $IC_{50,u} > 10 \times C_{max,u}$ (i.e., $C_{max,u}$/ $IC_{50,u} < 0.1$) |
| MATE1/MATE2-K | $IC_{50,u} > 50 \times C_{max,u}$ (i.e., $C_{max,u}$/ $IC_{50,u} < 0.02$) |

P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP = organic anion transporting polypeptide; OAT = organic anion transporter; MATE = multidrug and toxin extrusion protein; $IC_{50,u}$ = unbound half-maximal inhibitory concentration; mL = milliliter; $K_{i,u}$ = unbound inhibition constant; $K_m$ = Michaelis-Menton constant; fu,p = fraction unbound in plasma.

$C_{max,u}$ is unbound maximal plasma concentration of an inhibitor at steady state after therapeutic dose.
\* The $K_{i,u}$ of an inhibitor approaches $IC_{50,u}$ when substrate concentration is much less than $K_m$ assuming competitive inhibition (8).
\# $C_{max,inlet,u}$ is estimated unbound maximum plasma concentration of an inhibitor at liver inlet. $C_{max,inlet,u}$=fu,p x ($C_{max}$ + (Fa×Fg×ka×Dose)/Qh/$R_B$) (36). If unknown, Fa= 1, Fg = 1 and k= 0.1/minute can be used as a worst-case estimate. The $f_{u,p}$ should be set to 1 percent if the reliability of $f_{u,p}$ measurements less than 1 percent cannot be demonstrated (also refer to section II.A.2.a (2.1.2.1)).

The recommended ratio and cutoff value for P-gp or BCRP is for orally administered drugs. If the investigational drug is administered parenterally or if it is a metabolite formed post-absorption that inhibits P-gp or BCRP, $IC_{50,u} > 50 \times C_{max,u}$ (i.e., $C_{max,u}$/ $IC_{50,u} < 0.02$) can be used.

The cutoff values in Table 1 were determined based on in vitro-to-in vivo extrapolation analyses mainly using $IC_{50}$ and are based on limited published data. Other cutoff values can be proposed if justified based on in vitro-to-in vivo extrapolation and a calibration of the specific in vitro systems with known inhibitors and noninhibitors of these transporter systems.

If the above analysis indicates that a drug inhibits a transporter, a clinical study should be considered based on whether the likely concomitant medications used in the indicated patient populations are known substrates of the inhibited transporter and the safety profiles of those substrates. Alternatively, the inhibition potential of a drug can be evaluated using mechanistic static models, PBPK modeling, or endogenous biomarkers. These approaches should be supported by submission of evidence supporting validity of the methods.

3. *Drug as an Inducer of Transporters (2.2.3)*

Currently, in vitro methods to evaluate transporter induction are not well established. If an investigational drug has been observed to be an inducer of CYP enzymes via activation of nuclear receptors such as PXR or CAR, it is likely that transporters regulated through these receptors will be induced, such as P-gp. Refer to section III.B.5.c (3.2.5.3), which describes conducting clinical DDI studies mediated by transporters, for more considerations.

## C. DDI Potential of Metabolites (2.3)

Assessing the DDI liability of an investigational drug's metabolites often starts with in vitro experiments and generally uses the same strategies as those for parent drugs. The need to evaluate the DDI potential of metabolites is based on pharmacologic activity or plasma exposure, as described below.

1. *Metabolite as a Substrate (2.3.1)*

The risk of DDIs through altered formation or elimination of a metabolite should be investigated if available nonclinical or clinical data indicate that a change in metabolite exposure can result in clinically meaningful alteration of efficacy or safety of a drug (*target* as well as *off-target* effects). The enzymes responsible for formation and elimination of a metabolite should be identified in vitro if the metabolite contributes to an in vivo target effect to a similar or greater extent than the parent drug. The contribution of the metabolite to efficacy should be estimated by taking into account unbound metabolite and parent drug exposures (e.g., AUC expressed in molar units) in humans, pharmacological potency (e.g., receptor binding affinity, enzyme inhibitory potency), and if available, data related to target tissue distribution. Identification of the enzyme(s) responsible for formation and elimination of the active metabolite should be studied in the same manner as the identification of the enzymes involved in the elimination of the parent drug. If the plasma protein binding of the metabolite is high, the measured $f_{u,p}$ can be used if the accuracy and precision of protein binding measurement has been demonstrated (see Appendix A (section 7.2) for details). Similarly, if a metabolite is suspected to cause substantial adverse effects based on available nonclinical or clinical information, the enzymes involved in the formation and elimination of that metabolite should be identified. Similar to metabolic phenotyping for parent drugs, the characterization of enzymes involved in metabolite formation and metabolism should also start with major CYP enzymes and can examine other enzymes when appropriate.

The general principles described above can also be applied to characterization of a metabolite as a substrate of major transporters, with consideration of the relevance of transporter-mediated distribution or elimination in the disposition of a metabolite.

Whether a sponsor should conduct a clinical DDI study with an inhibitor or inducer of an enzyme or a transporter depends on the estimated fraction of formation or elimination of a metabolite mediated by an enzyme or transporter, how much the metabolite contributes to the clinical effect, the exposure-response relationship of the metabolite if known, and likely concomitant medications that affect the enzyme or transporter.

> 2.    *Metabolite as an Inhibitor (2.3.2)*

If in vitro assessments suggest that the parent drug alone does not inhibit major CYP enzymes/transporters or is not expected to inhibit enzymes/transporters clinically, DDI liability due to metabolites as inhibitors can still exist. As a pragmatic rule, it is recommended to investigate the CYP enzyme and transporter inhibitory potential of metabolites that have total $AUC_{metabolite}$ greater than or equal to 25 percent of $AUC_{parent}$ and also account for at least 10 percent of drug-related material in circulation (i.e., considered as a major metabolite, often determined based on radioactivity data).

If in vitro assessments suggest that the parent drug inhibits major CYP enzymes and transporters and clinical DDI studies are planned, in vitro assessments of metabolites as inhibitor of those enzymes or transporters may not be needed. The inhibition potential of metabolites would be implicitly reflected in the clinical DDI study along with the parent drug, unless clinically relevant exposures of the metabolite cannot be adequately represented in the clinical DDI study (i.e., the study duration does not allow the metabolite to accumulate). It is noted that in vitro assessments of metabolites can become useful in interpreting the results of DDI studies.

Based on the results of in vitro DDI assessments of a metabolite, the determination of whether to conduct a clinical DDI study follows the same approaches as those for the parent drug. Usually, metabolites are irrelevant for the evaluation of intestinal CYP or transporter inhibition except when formed substantially in the gut or intestinal cells. If basic methods suggest that the metabolite(s) could have in vivo DDI liability, and a mechanistic static or PBPK model is then used to evaluate the DDI risk of a drug, metabolite(s) should be incorporated in those models.

> 3.    *Metabolite as an Inducer (2.3.3)*

Although metabolites can induce CYP enzymes, the in vitro evaluation of the parent drug as a potential inducer could also reflect induction by metabolites because metabolites can be generated during incubation of the parent drug with hepatocytes. However, when the drug is a prodrug or when a metabolite is mainly formed extra-hepatically, in vitro evaluation of a metabolite's induction potential on CYP enzymes is recommended if the metabolite is a major metabolite and has $AUC_{metabolite}/AUC_{parent}$ greater than or equal to 25 percent and accounts for at least 10 percent of drug-related material in circulation. Based on the results of in vitro assessments of the metabolite, the determination of whether to conduct a clinical DDI study follows the same approach as for the parent drug.

## III.    CLINICAL EVALUATION (3)

### A.    Types of Clinical DDI Studies (Terminology) (3.1)

There are different study types that can be conducted to determine the presence or absence of a clinical DDI and the magnitude of the DDI if one exists. The study types described in this section

are not mutually exclusive. The specific goal of a study should be considered when determining the type of study to conduct.

Regulatory decision-making generally relies upon prospective studies specifically designed to evaluate the potential for DDIs. Retrospective evaluation of drug concentrations from studies not designed to evaluate DDIs rarely includes sufficient accuracy and precision to provide an adequate assessment. DDIs identified or ruled out using a retrospective analysis may need to be confirmed using a prospective evaluation.

In some situations, predictive modeling approaches (mechanistic static or PBPK) can be used to translate in vitro results to the clinical setting, without a clinical DDI study. The scenarios and best practice considerations are described in Appendix D (section 7.5).

### 1. *Stand-Alone and Nested DDI Studies (3.1.1)*

A stand-alone DDI study is a clinical study with the primary objective of determining the presence or absence of a clinical DDI and the magnitude of the DDI. Alternatively, a nested DDI study evaluates DDIs as part of larger studies in subjects (e.g., phase 2/3) for which DDI evaluation is not the primary objective. The nested DDI evaluation is prospectively planned and appropriately designed (refer to section III.B.2 (3.2.2) for more details).

### 2. *DDI Studies With Index Precipitants and Index Substrates (3.1.2)*

Precipitants (inhibitors or inducers) and objects (substrates) with well-understood and predictable pharmacokinetic and DDI properties with regard to level of inhibition, induction, or metabolic pathway are known as *index drugs*. The most common purpose of studies conducted with these drugs is to estimate the greatest magnitude of interaction for the studied pathway. For drugs that are evaluated as objects of a DDI, the greatest magnitude of interaction generally results from concomitant administration of a strong index inhibitor or inducer of the drug's metabolic pathway(s). For drugs evaluated as precipitants of DDIs, the greatest magnitude of interaction generally results from concomitant administration of the drug with a sensitive index substrate.

A distinctive feature of index studies is that the results usually can be extrapolated to other drug combinations. Thus, after conducting a study with an index inhibitor, one can assume that other inhibitors of equal strength for that metabolic pathway will generally have a similar DDI effect size. Additionally, if one concludes that the change in drug exposure following a concomitant strong index inhibitor is not clinically relevant, the same can be concluded for all other inhibitors for that particular metabolic pathway without additional studies. Results from DDI studies with index precipitants or substrates are also used to help design DDI studies with commonly used concomitant medications in the investigational drug's target population.

A list of index drugs (either as substrates, inhibitors, or inducers of CYPs) is presented in Appendix F, section 1 (7.7.1).

Index substrates or precipitants have not been identified for transporters and several metabolic pathways (e.g., CYP2B6, UGTs). The lack of index substrates or precipitants is mainly due to

selectivity issues. However, information similar to that provided by studies with index precipitants or substrates (i.e., the likelihood of a DDI due to a specific pathway) is often important for these pathways. Although index substrates and precipitants have not been identified, Appendix F sections 2 and 3 (7.7.2 and 7.7.3) list examples of drugs that can be useful for DDI studies because they provide informative results and explain the limitations of the drugs. However, extrapolating the results of these studies can be more difficult and complex than extrapolating results from studies with index drugs.

     *3.      DDI Studies With Expected Concomitant Drugs (3.1.3)*

In addition to DDI studies with index drugs, as described above, it can be informative to conduct studies that investigate DDIs between the investigated drug and drugs likely to be administered to the target population. These studies can also be considered when a drug is used as an add-on to other therapies or as part of a fixed-dose combination. When choosing drugs to evaluate in these studies, sponsors should consider the mechanistic understanding of the potential for DDIs (based on in vitro studies and clinical studies with index drugs) and the relative frequency of coadministration. Another situation where DDI evaluation is often based on the likelihood of coadministration is for transporter-mediated pathways and several metabolic pathways (UGTs; CYP2B6) because of a general lack of index substrates or precipitants for these pathways.

DDI studies with expected concomitant drugs can provide information about specific dose adjustments for the investigational drug and/or concomitant drug or inform the need for monitoring for adverse effects or reduced efficacy. However, although these studies are often informative to patients and medical professionals, the results may be difficult to extrapolate to other drugs.

     *4.      Cocktail Approach (3.1.4)*

A cocktail study includes the simultaneous administration of substrates of multiple enzymes and/or transporters to study subjects. A cocktail approach can simultaneously evaluate a drug's inhibition or induction potential for multiple enzymes and transporters if the study is properly designed and conducted (refer to section III.B.6 (3.2.6) for additional details).

     *5.      Biomarker Approach (3.1.5)*

An emerging approach in the evaluation of DDI risk assessment is the deployment of endogenous biomarkers that are substrates for drug metabolism and transport. This approach is enabled by the measurement of endogenous biomarkers in plasma and/or urine before and after administration of the investigational drug. The biomarker-based approach may provide an early indication of DDI potential of a drug as a precipitant via specific pathway by monitoring endogenous biomarkers in clinical studies (refer to section III.B.7 (3.2.7) for additional details).

**B.      Study Planning and Considerations for Clinical DDI Studies (3.2)**

The objective of most DDI studies is to determine the ratio of a measure of substrate drug exposure (e.g., AUC ratio) in the presence and absence of a precipitant drug. The following

considerations are important when designing prospective clinical DDI studies to unambiguously determine this ratio.

1. *Study Design (3.2.1)*

    a. Study population and number of subjects (3.2.1.1)

Most clinical DDI studies can be conducted using healthy subjects, under the assumption that findings in healthy subjects translate to findings in the intended patient population. However, safety considerations can prevent the use of healthy subjects in studies of certain drugs. For some drugs, use of the intended patient population in DDI studies can allow for evaluation of pharmacodynamic endpoints that cannot be studied in healthy subjects, in addition to pharmacokinetic endpoints.

The number of subjects included in a DDI study should be sufficient to provide a reliable estimate of the magnitude and variability of the interaction (see ICH M12 Q&A).

    b. Dose (3.2.1.2)

For studies intended to identify the interaction of greatest magnitude, the doses of the precipitant drug used in DDI studies should maximize the possibility of identifying a DDI. Thus, the maximum dose and the shortest dosing interval of the precipitant under the recommended clinical use should generally be evaluated.

If the substrate drug has dose-proportional pharmacokinetics, sponsors can study any dose in the range where exposure to the drug increases in a dose-proportional manner. If the substrate drug does not have dose-proportional pharmacokinetics, the therapeutic dose most likely to demonstrate the largest magnitude DDI should be used. When there are safety concerns, lower doses of the substrate drug, including doses lower than therapeutic doses, can be used.

For studies with anticipated concomitant drugs when a clinically significant DDI is anticipated based on prior in vitro or clinical DDI information, it can be informative to build a dose adjustment of the substrate drug into the study to allow identification of doses that can be administered together in clinical practice. In such a scenario, a clinically relevant dose of the precipitant should be used.

    c. Single or multiple doses (3.2.1.3)

The precipitant drug is often administered in a multiple-dose regimen in DDI studies. However, sponsors can evaluate single-dose administration of a precipitant if the interaction potential is only relevant during absorption (e.g., inhibition of intestinal P-gp or BCRP).

In addition, DDI studies can evaluate single-dose administration of a precipitant if the exposure of precipitant following a single dose is representative of exposure at steady state and if the precipitant is not a potential inducer or time-dependent inhibitor. The single dose can be the therapeutic dose or a higher dose, depending on accumulation. Safety of a higher dose would

need to be understood before conducting the study. When studied with a substrate with a long half-life, it may be necessary to administer a precipitant multiple times to cover the full time course of the substrate exposure. The duration of the treatment with the precipitant should be long enough to cover at least 90 percent of the plasma concentration-time curve of the substrate, considering that its half-life may be longer in the presence of an inhibitor drug. However, if the substrate has a very long terminal half-life that does not allow dosing with the precipitant to cover the full plasma concentration-time curve, population pharmacokinetic analysis or PBPK analysis can be used to bridge the clinical DDI study results to the maximum effect on the exposure of the substrate.

If a metabolite of the precipitant has a long half-life or has demonstrated TDI of the enzyme being evaluated in the DDI study, the duration of the treatment with the parent drug should be sufficient for steady state of enzyme inhibition due to parent drug and metabolite to be reached.

Inducers should be administered as multiple doses to ensure the maximal induction of a specific pathway. The duration should consider the time to reach steady state of the inducer, the turnover time of the affected enzyme or transporter, and the half-life of substrate drug. Typical pretreatment durations range from 7 to 14 days.

When there are multiple mechanisms of interactions for a specific precipitant, single-dose administration can be appropriate in certain situations (e.g., evaluation of rifampin as an inhibitor of OATP1B1), while multiple-dose administration can be appropriate in other situations (e.g., evaluation of rifampin as a CYP3A inducer).

If the substrate demonstrates time-dependent pharmacokinetics (clearance changes over time), multiple-dose administration of the substrate and a precipitant should be evaluated. If the substrate does not demonstrate time-dependent pharmacokinetics, the substrate can be administered as single doses, and the observed magnitude increase in exposure can be extrapolated to steady-state conditions.

> d.      Route of administration and formulations (3.2.1.4)

The route of administration of the investigational drug evaluated in DDI studies should generally be the one planned for routine clinical use. When multiple routes of administration are developed for clinical use, the route of drug administration for DDI studies should be selected based on the expected mechanisms of the DDIs and the similarity of the concentration-time profiles for the parent drug and metabolites after different routes of administration.

Formulation-related differences in DDIs are also reported to occur (9,10). The possibility of formulation differences in interaction potential should be considered when extrapolating interaction results between formulations. In general, DDI potential can be extrapolated between formulations by comparing their rate and extent of absorption.

e.      Parallel versus crossover studies (3.2.1.5)

Crossover studies (one-sequence or randomized) are preferred over parallel study designs to reduce variability. Duration of the washout period should be based on the pharmacokinetics of the substrate and the precipitant, the anticipated impact on the substrate's half-life, and the duration necessary for enzyme activity to return to baseline or for potential pharmacodynamic effects to return to pretreatment levels (if pharmacodynamic effects are also assessed). In some situations, additional periods can be informative. The situations include evaluation of the time it takes for enzyme activity to return to normal following removal of an inducer or time-dependent inhibitor, evaluation of two drugs that may affect each other (each drug alone and in combination), or evaluation of the effects of acute and chronic treatment of a drug.

Parallel, two-arm studies can be appropriate when a crossover study design is not feasible, such as when one of the drugs (or major active metabolite, if applicable) has a long half-life. Typically, parallel-design studies call for larger sample sizes than crossover studies, and subjects should be matched for intrinsic factors likely to affect pharmacokinetics.

f.      Timing of drug administration (3.2.1.6)

In most DDI studies, the precipitant and substrate drugs can be administered at the same time. However, the timing of administration of the precipitant is critical if it is both an inhibitor and an inducer. In such scenarios, the timing of administration of the precipitant and the pharmacokinetic sampling times should consider the objective of the DDI study. For an index study, to ensure the maximum induction effect is identified, staggered administration of the precipitant and substrate drugs is recommended to prevent inhibition from masking induction. The anticipated clinical dosing scenario should be used if the objective of the study is evaluation of concomitant medications.

If a large part of an interaction occurs during absorption or first pass, staggered dosing schedules can be studied (clinical study or PBPK) to understand whether such a method is a viable mitigation strategy for the DDI.

When evaluating the interaction between drugs that require different food conditions for optimal absorption, the timing of drug administration should be adjusted to maximize the potential to detect an interaction (i.e., index studies) and/or to reflect the clinically relevant conditions (i.e., DDI study with potential concomitant drugs).

g.      Comedications and other extrinsic factors affecting DDIs (3.2.1.7)

To reduce variability in the magnitude of DDIs, use of the following should be excluded to the extent possible during DDI studies: other medications, dietary/nutritional supplements, herbal supplements, tobacco, smoking, alcohol, foods, and fruit juices that may affect the expression or function of enzymes and transporters. The exclusion should begin for a sufficient time before subjects enter the study and continue for the duration of the study.

h.       Sample and data collection (3.2.1.8)

Sampling times for pharmacokinetic assessment should be sufficient to characterize the AUC extrapolated to infinity ($AUC_{0-inf}$) (for single-dose studies) or the AUC for one dosing interval following multiple doses, generally at steady state ($AUC_{0-tau}$) (for multiple-dose studies) and $C_{max}$ of the substrate drug administered alone and under conditions of the anticipated interaction. Data on additional pharmacokinetic parameters should be collected based on the pharmacokinetic or pharmacological relevance for the proposed indication (e.g., the minimum concentration ($C_{min}$), partial AUC). The sampling times for single-dose studies should be planned so that the mean difference between the AUC from time zero to time of last quantifiable observation (t) ($AUC_{0-t}$) and the $AUC_{0-inf}$ is less than 20 percent, accounting for potential longer elimination half-life due to the DDI. Samples collected should contain the moieties needed to interpret study results; in most cases, the moiety needed to interpret results will be the parent drug. Metabolite concentrations should be determined if they provide information about the effect of a DDI on safety or efficacy or if the data inform the mechanism of the drug interaction. For example, when a clinical DDI study evaluates drugs that may interact via multiple pathways, measuring metabolites may help determine the enzymes and/or transporters responsible for the interaction.

In addition to collecting samples for pharmacokinetic assessment of the object drug, optional collection of a few sparse samples for the inhibitor or inducer can help ensure the plasma concentrations of the inhibitor or inducer are within the expected range. In addition, urine samples can be collected for understanding DDIs involving renal transporters,

When in vitro data provide a plausible DDI mechanism that cannot be evaluated with systemic drug exposure, collection and analysis of pharmacodynamic data can be informative. One possible scenario where this could occur is when transporter inhibition alters access of the drug to specific organs or tissues. In such scenarios, clinical consequences, such as altered efficacy or increased toxicity resulting from altered tissue distribution of a substrate drug, can be measured as pharmacodynamic endpoints, and in vitro evidence of a drug's interaction potential can support data interpretation.

2.       *Specific Considerations for Nested DDI Studies (3.2.2)*

Nested DDI studies are clinical DDI investigations that are part of other studies (e.g., phase 2/3) in which the assessment of DDI is not the primary objective. However, these studies are designed prospectively to investigate DDIs as an exploratory or secondary objective. Nested DDI studies are usually used to evaluate the drug as an object of interactions with concomitant drugs and sometimes can also be used to assess the drug as a precipitant. 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 DDIs. An advantage of nested DDI studies is the fact that they are conducted in a patient population and may more closely represent the anticipated clinical setting. However, nested DDI studies can also be challenging because they call for careful attention to study design and data collection. In some cases, PBPK modeling can assist the design of nested DDI studies (refer to Appendix D section 2 (7.5.2)).

A nested DDI study can evaluate the effect of concomitant drugs that are used for the full duration of the clinical study or those that are added in response to the subject's condition during the study. Concomitant drugs to be evaluated should be prespecified. The drugs are typically selected because there is a mechanistic reason to anticipate an interaction. Relevance in the patient population is also a consideration. The study design can specify individual drugs or a grouping, based on mechanism (e.g., strong CYP3A inhibitors). However, if a grouping is evaluated it is important to consider the potential for differences in the effect of different drugs in the group and the effect of the potential variability on data analysis and translation of the findings (11).

Simulations can be used to determine the appropriate number of pharmacokinetic samples and to assist in the selection of sampling times. A power analysis can also be performed to estimate the minimum effect size that is likely to be detected with acceptable precision in a study using a given number of subjects on a concomitant drug.

Collection of the following data is critical to ensure interpretable results: timing of drug administration (investigational drug and concomitant drug), drug dose, timing relative to food (when relevant), other concomitant drugs, and pharmacokinetic sampling date and time (actual, not scheduled). It is also important to document the start date and discontinuation date of the concomitant drug relative to when an interaction will be observed, particularly when the concomitant drug is an inducer or time-dependent inhibitor.

A nested DDI study is typically evaluated using population pharmacokinetic analysis, which should be performed according to well-established scientific practice using a model that is robust and fit-for-purpose. The sample collection plan for population pharmacokinetic analysis for the DDI assessment should be established before conducting the study. In general, the standard analysis approach is a binary evaluation that includes the concomitant drug as a categorical covariate. Sponsors should consider whether their selected analysis methods will provide the desired level of precision in DDI evaluation. Regardless of analysis method, all assumptions should be stated.

In some instances, unplanned analyses of potential DDIs in phase 2/3 studies are conducted to explain clinical study results, such as safety or efficacy issues in a group of patients, or to screen for potential DDIs not anticipated at the time the studies were designed. If the data collected meet the criteria described in this section, except for preplanned DDI assessment, it can be possible to draw conclusions about the presence or absence of an interaction. In situations where the data do not permit an accurate assessment of a DDI, a further assessment of the DDI potential should be conducted.

       3.       *Considerations for CYP-Mediated Interactions (3.2.3)*

            a.      Investigational drug as a substrate for CYP enzymes (3.2.3.1)

When evaluating the investigational drug as a substrate, the first clinical DDI studies should, in general, determine the effects of a strong index inhibitor and a strong index inducer on the investigational drug. Moderate index inhibitors or inducers can be used if strong index inhibitors

or inducers are not available for a particular enzyme. Some of these inhibitors and inducers can also affect other metabolism and/or transporter pathways; thus, when selecting index inhibitors and inducers for prospective DDI studies, all metabolic and transport pathways of the investigational drug should be considered. Studies with other strong inhibitors and inducers of CYP enzymes can also be appropriate, considering the criteria listed in Appendix F section 1 (7.7.1). If the investigational drug is a substrate for multiple enzymes and/or transporters, measuring metabolites can, in some cases, help interpret study results and interaction mechanisms.

If a DDI study with a strong index inhibitor or inducer indicates no DDI is present, additional clinical studies with other inhibitors or inducers of the same enzyme are not needed. However, because a negative DDI study may reveal that the enzyme proposed to be the major metabolizing enzyme based on in vitro data is not contributing to the elimination of the drug, this may instead indicate that further investigations of alternative pathways should be considered.

If a DDI study with strong index inhibitors or inducers indicates that there is a clinically relevant interaction, evaluating the impact of moderate inhibitors or inducers can be useful to gain a full understanding of the investigational drug's DDI potential. The evaluated moderate inhibitors and inducers may be anticipated concomitant medications in the intended patient population. The effect of the additional inhibitors and inducers can be evaluated in a clinical interaction study, or in some cases, modeling approaches can provide additional information (refer to Appendix D (section 7.5)). If it is anticipated that coadministration with strong inducers or inhibitors should be avoided, a DDI study with a moderate inducer or inhibitor may be preferable as the initial study.

If the investigational drug is subject to significant metabolism by a genetically polymorphic enzyme for which a well-defined, poor metabolizer phenotype exists that results in nonfunctional enzyme activity, a comparison of the pharmacokinetic parameters of the drug in individuals with the poor metabolizer phenotype versus those with a normal metabolizer phenotype can substitute for an interaction study for that particular pathway (refer to section IV.A (4.1)).

> b.  Investigational drug as an inhibitor or an inducer of CYP enzymes (3.2.3.2)

When studying an investigational drug as a potential inhibitor or inducer of a CYP enzyme, the sensitive index substrate selected for the initial clinical studies should be sensitive to changes in activity or amount of the CYP enzyme being evaluated (refer to Appendix F section 1 (7.7.1)). Because some substrates are not specific for one CYP enzyme and sometimes are also substrates of transporters, the most appropriate substrate should be selected considering the inhibitor/inducer characteristics of the investigational drug, based on available in vitro and clinical data. Other CYP enzyme substrates can also be appropriate. If the substrate drug is metabolized by more than one enzyme, measuring metabolites sometimes can help with interpretation of study results.

If the initial study with a sensitive index substrate is negative, studies with less sensitive substrates of the enzyme are not needed. If an initial study determines that an investigational

drug either inhibits or induces the metabolism of sensitive index substrates, further evaluations using other substrates (e.g., relevant comedications) can be useful. The magnitude of the effect of the investigational drug on the sensitive index substrate and the potential for concomitant use with other drugs that are substrates of the same enzyme should be considered.

If the investigational drug is both an inducer and an inhibitor of an enzyme, the net effect of the drug on enzyme function may be time dependent. The timing of pharmacokinetic endpoints should permit an understanding of the changes in effects over time, when relevant. To achieve this understanding, the pharmacokinetics of the substrate drug should be evaluated at early and late time points during the administration of the investigational drug in the test period. The observed effect of reversible inhibition may be more pronounced in the beginning of the treatment, and the induction may be most pronounced after ending the treatment.

### 4. *Considerations for Evaluation of UGT-Mediated Interactions (3.2.4)*

#### a. Investigational drug as a substrate of UGTs (3.2.4.1)

Based on limited literature evidence, the magnitude of DDIs mediated through inhibition of UGTs (reflected by AUC ratio of a substrate in the presence of an inhibitor compared to no inhibitor) is typically less than the magnitude observed with CYP inhibition (3). For an investigational drug that is mainly eliminated by direct glucuronidation, clinical DDI studies with UGT inhibitors should be conducted on a case-by-case basis, considering the safety profile of the drug and the likelihood of its concomitant use with inhibitors of that UGT isoform (refer to Table 16, in Appendix F section 2 (7.7.2) for some examples of UGT inhibitors). Some UGT substrates are also substrates of other enzymes or transporters, and the interaction with a UGT inhibitor may involve other mechanisms when the UGT inhibitor also affects those enzymes or transporters. Thus, it may be valuable to also measure the glucuronide conjugate concentrations in addition to the UGT substrate itself. The change of glucuronide metabolite relative to the parent drug may provide insight into the underlying mechanism of interaction. In addition, some glucuronide metabolites are active or reactive and may significantly contribute to efficacy or safety of a drug. In such cases, the concentrations of glucuronide conjugates should be measured in addition to parent drug concentrations.

Genetic variation in certain UGT enzymes (for example, UGT1A1, UGT2B7, UGT2B10, UGT2B15, and UGT2B17) has been reported to contribute to variation in the pharmacokinetics of drugs metabolized by UGTs. In certain cases, comparative pharmacokinetic data in subjects with various UGT genotypes can be used to identify the importance of the UGT pathway(s) in the elimination of a drug and to estimate the extent of DDI with inhibitors of UGT.

In addition, UGTs can be induced, for example, by certain PXR agonists (e.g., moderate or strong CYP3A inducers). The impact of inducers on an investigational drug that is mainly metabolized by UGTs should also be considered and evaluated depending on the likelihood of its concomitant use with UGT inducers and the dose/exposure-efficacy relationship of the investigational drug.

       b.      Investigational drug as an inhibitor of UGTs (3.2.4.2)

As indicated in section II.A.3 (2.1.3), considering the generally limited magnitude of UGT inhibition-mediated DDIs, a routine evaluation of UGT inhibition by an investigational drug may not be warranted. Following the in vitro assessment described in section II.A.3 (2.1.3), a decision on whether to perform a clinical DDI study to evaluate the effect of a drug as a UGT inhibitor should also take into consideration the likelihood of the drug's concomitant use with known substrates of the UGT isoform (refer to Table 15, in Appendix F section 2 (7.7.2) for examples) and the safety profiles of those substrates.

       c.      Investigational drug as an inducer of UGTs (3.2.4.3)

There is limited understanding about gene expression of UGTs. However, limited clinical DDI studies indicate certain UGTs may be induced by agonists of PXR and/or CAR, which also regulate CYP3A4 expression. UGTs are less inducible than CYP3A4. Thus, for a drug found to induce CYP3A4 in vitro and further evaluated with a clinical DDI study, the effect of the drug on CYP3A4 substrates may inform its potential induction effect on UGTs. In such a situation, if a drug reduces the AUC of a sensitive substrate of CYP3A by greater than or equal to 50 percent, a clinical DDI study should be considered taking into account the following: the magnitude of exposure change of the CYP3A substrate, the likelihood of concomitant use of the investigational drug with UGT substrates, whether there are other enzymes/transporters involved in the pharmacokinetics of UGT substrates that can also be regulated by PXR/CAR agonists, and the dose or exposure-efficacy relationship of those UGT substrates. It is noted that some CYP3A4 inducers have their induction effect overridden by their inhibition effect on CYP3A. Thus, while those drugs inhibit CYP3A4 in clinical studies, they may exhibit induction effects on UGTs.

      5.      *Considerations for Evaluation of Transporter-Mediated Interactions (3.2.5)*

       a.      Investigational drug as a substrate of transporters (3.2.5.1)

If in vitro studies indicate that the investigational drug is a transporter substrate, sponsors should determine whether to conduct clinical DDI studies based on the drug's passive permeability, route of administration, absorption and elimination, putative site of action, safety profile, dose or exposure-response (efficacy and safety) relationship, and likely concomitant drugs that are known inhibitors or inducers of the transporters. The information in Table 2 helps guide when a clinical DDI study should be considered for investigational drugs that are transporter substrates in vitro:

**Table 2. Considerations for Clinical Evaluation of Drug as Substrate of Transporters**

| Transporters | When a Clinical DDI Study Should Be Considered |
|---|---|
| P-gp and BCRP | When intestinal absorption is limited, or biliary excretion/active renal secretion is a major elimination pathway. |
| OATP1B1 and OATP1B3 | When hepatic (metabolic/biliary) elimination is a significant clearance pathway ($\geq$25%) for the investigational drug or the action site of the drug is in liver, and the drug's properties support the importance of active uptake of the drug into the liver. |
| OAT1 and OAT3, OCT2, MATE1, and MATE2-K | When the investigational drug undergoes significant active renal secretion (i.e., accounting for $\geq$ 25% of systemic clearance) |

DDI = drug-drug interaction; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP = organic anion transporting polypeptide; OAT = organic anion transporter; MATE = multidrug and toxin extrusion protein.

When evaluating an investigational drug as a substrate in transporter-mediated DDIs, the selected precipitant drug should be a known inhibitor of the transporter under investigation. Because of a general lack of index precipitants for transporter-mediated pathways, the choice of transporter precipitant is generally based on the likelihood of concomitant use (e.g., to obtain clinically relevant DDI information that can inform labeling regarding the management of a DDI). Some examples are provided in Table 19 in Appendix F section 3.b (7.7.3.2).

DDI studies with transporter inhibitors can be used to understand the underlying mechanisms of DDIs or to determine the anticipated largest magnitude DDI. If in vitro studies indicate a drug is a substrate of multiple transporters, a clinical study can be conducted with a broad inhibitor of multiple transporters to determine the anticipated largest magnitude DDI. For example, cyclosporine, which inhibits intestinal P-gp and BCRP and hepatic OATPs, can be used as the inhibitor in a DDI study. Negative results from this kind of study may rule out the need to further evaluate the drug as a substrate for any of the individual transporters. If the study result is positive, additional studies with more selective inhibitors of specific transporter pathways can be conducted to determine the impact of inhibition of each transporter on the disposition of the substrate drug. The same paradigm can apply to an investigational drug that is a substrate for both transporters and metabolic enzymes (e.g., CYP3A and P-gp).

If the goal of the study is to determine the role of a specific pathway in the pharmacokinetics of a substrate drug and resulting DDIs due to that pathway, then a more selective inhibitor should be used. Use of these inhibitors in clinical studies can provide a mechanistic understanding of transporter-mediated DDIs. Some transporters, including OATP1B1 and BCRP, are encoded by genetically polymorphic genes (SLCO1B1 and ABCG2, respectively) for which phenotypes with reduced functionality exist. Similar to drugs that are substrates of CYPs encoded by polymorphic genes, the relative contribution of a specific transporter to the disposition of the investigational drug can be evaluated in subjects with different transporter genotypes, if a nonfunctional phenotype exists (refer to section IV.A (4.1)).

Examples of transporter inhibitors are listed in Appendix F section 3.b (7.7.3.2). Many of them not only inhibit the specified transporters but also can inhibit other transporters and/or CYP

enzymes. Thus, extrapolation of results from transporter inhibition studies to other drugs can be challenging. Interpretation of the study results should consider the knowledge of transport and metabolic pathways for the investigational drug.

> b.  Investigational drug as an inhibitor of transporters (3.2.5.2)

If in vitro studies indicate that the investigational drug is a transporter inhibitor, the determination of whether to conduct a clinical DDI study should be based on likely concomitant drugs and safety considerations. When studying the investigational drug's potential to act as an inhibitor drug for a transporter, a substrate drug whose pharmacokinetic profile is markedly altered by coadministration of known inhibitors of that transporter and is also a likely concomitant drug is preferred. Some examples of transporter substrates that can be used in DDI studies are listed in Appendix F section 3.a (7.7.3.1). Because many drugs are substrates of multiple transporters and/or enzymes, the observed clinical interactions can be a result of the modulation of multiple pathways if the investigational drug is also an inhibitor or inducer for those pathways. Extrapolation of results from these studies to other drugs can thus be challenging. The choice of substrates can be determined by the therapeutic area of the investigational drug and the likely concomitant drugs that are known substrates of the transporters.

In some cases, an alteration in drug transport may not be fully reflected by changes in plasma concentrations of parent drug alone. Therefore, measurement of metabolite or pharmacodynamic markers to reflect altered distribution to the organs expressing the transporter may be considered to help interpret the potential for an interaction.

Recent literature reports indicate potential utility of endogenous substrates for some drug transporters (see section III.B.7.a (3.2.7.1)). Evaluating the change in exposure of the endogenous substrate when the investigational drug is administered may provide information regarding the drug's potential as a transporter inhibitor.

> c.  Investigational drug as an inducer of transporters (3.2.5.3)

Since P-gp is coregulated with CYP3A, for example by agonists of PXR and/or CAR, but is less inducible than CYP3A (12,13), if an investigational drug reduces the AUC of a sensitive substrate of CYP3A by 50 percent or more (i.e., being a moderate or strong inducer), a further clinical study to evaluate potential induction effect of the drug on P-gp substrates should be considered. This case-by-case consideration should take into account the following factors: the magnitude of CYP3A substrate AUC change by the investigational drug, the likelihood of concomitant use of the drug with P-gp substrates, whether there are other enzymes/transporters involved in the pharmacokinetics of P-gp substrates that can also be regulated by PXR and/or CAR agonists, and the dose or exposure-efficacy relationship of P-gp substrates. It is noted that some CYP3A4 inducers have their induction effect overridden by their inhibition effect on CYP3A. Thus, while those drugs inhibit CYP3A4 in clinical studies, they may exhibit induction effects on P-gp. Sponsors should also consider whether to conduct clinical DDI studies to evaluate the potential effect of a drug on other transporters regulated through the same pathways as CYP3A.

6. *Cocktail Studies — Considerations for CYP or Transporter Cocktail Studies (3.2.6)*

A cocktail approach can simultaneously evaluate a drug's inhibition or induction potential for multiple CYPs and transporters if the study is properly designed. Results from a well-conducted cocktail study that includes all elements of a prospective DDI study can be interpreted the same way as results from any other well-conducted DDI study (see sections III.B.1.a through h (3.2.1.1. through 3.2.1.8)). The criteria for the selection of cocktail drugs are: (a) the substrates are specific for individual CYP enzymes or transporters; and (b) there are no interactions among the substrates. If these criteria are not met, the lack of specificity or the interaction among substrates should be understood and incorporated into the interpretation of the study results. It should be noted that findings obtained with a microdose of a substrate cannot always be extrapolated to a therapeutic dose of that substrate.

7. *Considerations for Biomarker Approach (3.2.7)*

An alternative approach to assess an investigational drug's potential as a precipitant is evaluating the change in exposure of a well-characterized endogenous substrate. Sufficient analytical validation should be conducted to ensure an adequate level of quality and consistency to allow for reliable interpretation of the results. Reported examples of biomarkers include, but are not limited to, plasma coproporphyrin I (hepatic OATP1B1/3), plasma and urine $N^1$-methylnicotinamide and $N^1$-methyladenosine (renal OCT2, MATE1, MATE2K), plasma pyridoxic acid (renal OAT1/3), and plasma 4β-hydroxycholesterol/cholesterol ratio and urine 6β-hydroxycortisol/cortisol ratio for CYP3A (14,15,16,17). It should be noted that not all endogenous biomarkers are validated and characterized in terms of their performance characteristics such as sensitivity, selectivity, specificity, dynamic range, correlation with pharmacokinetic parameters of the probe drugs, and variability (due to factors such as diet, age, exercise, diurnal variation, and disease state) (18,19). Availability of such data can enable sponsor-regulator dialogue regarding the prioritization of, need for, and design of endogenous biomarker-based DDI evaluation.

a. Investigational drug as an inhibitor of hepatic OATP1B (3.2.7.1)

As an example, recent literature reports support the utility of plasma coproporphyrin I (CPI) for evaluation of hepatic OATP1B inhibition potential. Monitoring of plasma CPI can be incorporated in early human healthy volunteer pharmacokinetic studies, such as phase 1 single- or multiple-dose escalation studies. Plasma CPI measured before the administration of the investigational drug (predose) represents baseline concentration and baseline $AUC_{0-t}$ (baseline $AUC_{0-t}$ = baseline CPI x t). Serial samples for CPI following the administration of the investigational drug will allow the characterization of $C_{max}$ and AUC of CPI. The metrics of interest are the ratio of CPI $C_{max}$ and $AUC_{0-t}$ after administration of an investigational drug to a baseline assessment. If the ratio is less than 1.25, this indicates a low likelihood of a clinical DDI via OATP1B inhibition (20).

## IV.    OTHER TOPICS (4)

### A.    Pharmacogenetics (4.1)

Pharmacogenetic variations in genes encoding drug metabolizing enzymes or drug transporters can affect the pharmacokinetics of a drug, increase interindividual variability in drug exposure, affect safety or efficacy, and alter the magnitude of DDIs. Important pharmacogenes include those that encode phase 1 (e.g., CYP2C9, CYP2C19, CYP2D6) and phase 2 (e.g., NAT2, UGT1A1) drug metabolizing enzymes as well as genes that encode drug transporters (e.g., BCRP, OATP1B1). Polymorphisms in metabolizing enzymes can lead to increased, normal, decreased, or absent enzyme activity resulting in ultra-rapid (UM), normal or extensive (NM or EM, hereafter referred to as NM), intermediate (IM), and poor (PM) metabolizers, respectively. Polymorphisms in drug transporters can increase or decrease transport of a drug across membranes. These drug metabolizing enzyme and transporter polymorphisms can affect the systemic or tissue concentrations of a drug and/or its metabolite(s).

The scope of this section is limited to the evaluation of the impact of pharmacogenetics on DDIs and on DDI evaluation. While the considerations described below use metabolizing enzymes as examples, the concept can also be applicable to transporters with polymorphisms.

If an investigational drug is a substrate/inhibitor for a polymorphic enzyme and a DDI study with an index inhibitor/substrate is conducted to evaluate pharmacokinetic changes, it is recommended to prospectively characterize the subjects' genotypes. Exclusion of PMs is recommended, to allow characterization of the greatest magnitude of interaction. If PMs are not excluded, the DDI effect should be evaluated separately in subjects with different phenotypes (e.g., PM, IM, NM), as relevant.

If an investigational drug is subject to metabolism by an enzyme with a well-defined PM phenotype (for example, CYP2D6, CYP2C19), exposure in PM is expected to be similar to the effect of a strong inhibitor of that pathway. A comparison of the pharmacokinetic parameters of the drug in individuals with the PM phenotype with those with an NM phenotype can substitute for a DDI study of that pathway with a strong inhibitor. Similarly, the exposures in subjects with a polymorphic PM phenotype could be estimated using the results of a clinical DDI study with a strong inhibitor. If there is a significant difference in exposure between individuals with the PM and NM phenotypes, further studies to evaluate the DDI potential with moderate inhibitors or inducers of the specific enzyme should be considered.

When an enzyme encoded by a polymorphic gene is one of two major elimination routes of an investigational drug, the interaction effects of inhibiting the other enzymes is expected to vary in different phenotypes of the polymorphic enzyme. In a DDI study evaluating the impact of inhibitors of the other enzyme, prospective genotyping and enrichment of subjects with absent or decreased function of the polymorphic gene besides NM subjects can help assess the interaction effects in the various phenotypes. Because the DDI magnitude may become large in PMs or IMs of the polymorphic enzyme when combined with an inhibitor of a parallel pathway, depending on the safety profile of the drug, different doses should be considered in those subjects. PBPK

modeling can be useful to supplement such studies or to extrapolate the interaction effects in different genotypes (refer to Appendix D section 2 (7.5.2)).

A retrospective pharmacogenetic analysis can help elucidate reasons for a high variability in a DDI study. When study enrollment is not based on the genotype of a polymorphic metabolizing enzyme or transporter, a retrospective analysis of the metabolizing enzyme or transporter of interest can help to characterize differences in the magnitude of the DDI across genotype groups and explain why some subjects have unanticipated increases or decreases in drug concentrations.

Guidance on DNA sample collection for prospective and retrospective pharmacogenetic analysis can be found elsewhere. The frequency of certain pharmacogenetic variations can vary across populations. Therefore, when performing pharmacogenetic analysis, an individual's race/ethnicity should be considered. In addition, regional regulations on sampling and analyzing human-derived materials need to be followed.

## B.     Therapeutic Protein DDIs (4.2)

In general, the risk of pharmacokinetic DDIs is lower for therapeutic proteins compared to small molecules. The in vitro assays that are applicable for small molecules are generally not applicable to proteins.

When evaluating the potential for a DDI between therapeutic proteins and small molecules or between multiple therapeutic proteins, the mechanisms of a potential DDI should be considered, taking into account the pharmacology and clearance of the therapeutic proteins as well as any coadministered medications in the patient population.

DDI risk for emerging modalities such as oligonucleotides, small interfering ribonucleic acids, modified ribose nucleic acids, and peptides are out of scope for this guidance.

### 1.     *Proinflammatory Cytokine-Related Mechanism (4.2.1)*

Certain therapeutic proteins may exert an indirect effect on expression of CYP enzymes and thus affect the pharmacokinetics of small molecules. Therapeutic proteins that are proinflammatory cytokines (e.g., peginterferon) or that can increase cytokine levels can downregulate the expression of CYP enzymes, thereby decreasing the metabolism of drugs that are CYP substrates and increasing their exposure levels. The increase in cytokine levels as a result of drug treatment can be transient or persistent; a sponsor should consider this increase when determining whether to conduct a DDI study as well as the design of that study.

Conversely, therapeutic proteins that reduce the elevated cytokine levels (e.g., inhibitors of tumor necrosis factor) can relieve the CYP downregulation from an inflammatory environment (e.g., rheumatoid arthritis), thereby increasing CYP expression and activity and reducing exposure for CYP substrates.

If the investigational drug is a cytokine or a cytokine modifier, sponsors should consider whether to perform a clinical DDI study to evaluate the effects of the investigational therapeutic protein

on sensitive substrates for CYP enzymes. Known drug effects on metabolism in disease states with similar or higher inflammatory burden, differences in exposure levels of sensitive CYP substrates in healthy subjects versus patients in the indicated population, and the magnitude of the drug effect on cytokine levels should be considered when determining whether to conduct a clinical study. In some cases, a DDI study in the relevant indicated population should be conducted to further inform instructions for use of the drug. Important design aspects include the disease type and severity in the included patients and the dose and treatment time of the precipitant drug.

> 2. *Antibody-Drug Conjugates (4.2.2)*

For antibody-drug conjugates (ADCs), the small molecule drug component conjugated to the antibody component can be released in unconjugated form. Therefore, the DDI potential of both the antibody and the small molecule drug component should be considered. In general, for the small molecule component, the potential to inhibit or induce enzymes and transporters should be addressed in a similar manner as described for small molecules in this guidance. In many cases, however, the systemic concentration of small molecule drug component might be too low to act as a precipitant clinically.

It is important to understand the formation, distribution, and elimination kinetics of the small molecule and to assess the systemic exposure of the small molecule drug component of the ADC. It might be necessary to evaluate the small molecule component (administered as an ADC) as a substrate drug, in particular if increased levels of free drug may be associated with safety concerns. Understanding the exposure-response relationship of the components of the ADC is important in determining whether to conduct DDI studies and their significance.

## V. REPORTING AND INTERPRETING CLINICAL DDI STUDY RESULTS (5)

A DDI study report should include and justify the study design and data analysis method based on what is known about the mechanism of the DDI and the pharmacokinetic properties of the precipitant and substrate drugs. Data analysis of pharmacokinetic parameters (and pharmacodynamic parameters, when relevant) should include all subjects enrolled in the study who have evaluable pharmacokinetic (and/or pharmacodynamic) data. If a subject is dropped from the study or has incomplete plasma concentration sampling during a treatment period, the possibility that the observation is due to an interaction should be considered. When indicated, the interaction effect should be presented with and without the individuals proposed for exclusion and a short description of each dropout should be provided.

### A. Pharmacokinetic Data Analysis (5.1)

> 1. *Noncompartmental Analysis (5.1.1)*

The following exposure measures should be determined for each subject: $AUC_{0\text{-inf}}$, $AUC_{0\text{-t}}$, the percent extrapolated from $AUC_{0\text{-t}}$ to $AUC_{0\text{-inf}}$, $C_{max}$, and time to $C_{max}$ ($T_{max}$). For multiple-dose studies, $C_{max}$, $C_{min}$, and $AUC_{TAU}$ (area under plasma concentration-time curve over dosing

interval) at steady state should also be reported. Additional parameters can help to interpret the pharmacokinetic results: clearance (CL or CL/F), half-life, and volume of distribution. Parameters for metabolites, when measured, should also be presented. Noncompartmental analysis can be used to evaluate stand-alone DDI studies conducted to evaluate the investigational drug as an object or precipitant.

### 2. *Population Pharmacokinetic Analysis (5.1.2)*

Pharmacokinetic data collected in nested DDI studies should typically be evaluated using population pharmacokinetic methods. DDIs should be evaluated using all plausible structural elements of the pharmacokinetic model (e.g., CL or CL/F, relative bioavailability, rate of absorption). Population pharmacokinetic analyses should derive pharmacokinetic parameters appropriate for the study design and pharmacokinetic properties of the drug, such as AUC and $C_{max}$. For multiple-dose studies, $C_{max}$, $C_{min}$, and $AUC_{0-tau}$ at steady state should be reported.

## B. Reporting DDI Results (5.2)

Typical pharmacokinetic endpoints for DDI studies should include changes in drug exposure parameters for the substrate drug, such as AUC, $C_{max}$, and, when applicable, $C_{min}$ (see section V.A.1 (5.1.1)). Pharmacokinetic results of DDI studies should be reported as the geometric mean ratio of the observed pharmacokinetic exposure measures with and without the precipitant drug and the associated 90 percent confidence interval. Measures of the observed variability of the interaction, such as the range of AUC or $C_{max}$ ratios for individuals in a crossover study, should be reported.

There are multiple methods of displaying the data, and sponsors are encouraged to select the most appropriate methods based on the data and the situation. Data can be displayed graphically, for example, by using forest plots. A comparison of the individual pharmacokinetic parameters with and without concomitant medication can also be presented graphically (e.g., as spaghetti plots or individual ratio plots). The proportion of individuals for whom exposure extends beyond the no-effect boundary (see section V.C.1 (5.3.1)) can also be presented.

If pharmacodynamic endpoints are also assessed in the DDI study, the results should be reported and summarized.

## C. Interpreting DDI Study Results (5.3)

### 1. *Determination of No-Effect Boundaries (5.3.1)*

The results of a DDI study should be interpreted based on the no-effect boundaries for the substrate drug. No effect-boundaries represent the interval within which a change in systemic exposure measure is considered not significant enough to warrant clinical action (e.g., avoiding coadministration, dose or schedule adjustment, additional therapeutic monitoring).

It is preferable for no-effect boundaries to be developed based on exposure-response relationships derived from clinical study results, as well as other relevant information for the

substrate drug (e.g., safety data, the maximum-tolerated dose). A good understanding of exposure-response relationships for desirable and undesirable drug effects, as well as knowledge of the variability of exposures in the indicated population, facilitates data interpretation. In the absence of a defined exposure-response relationship, the totality of evidence should be considered when determining the clinical impact of a DDI. Sometimes, a 90 percent confidence interval of 80 percent to 125 percent is proposed as a default no-effect boundary. This is in general an acceptable approach but is considered overly conservative for most drugs, where small changes in exposure are unlikely to be of clinical consequence.

In general, the point estimate for the ratio between the exposure of the substrate with and without the precipitant can be used to describe the magnitude of the interaction and to determine whether interventions such as dose adjustments should be considered. Sponsors should also consider the variability of the interaction. The number of subjects included in the study should be sufficient to provide a reliable estimate of the magnitude and variability of the interaction (see ICH M12 Q&A).

> 2.     *Investigational Drug as a Precipitant of DDIs: Classification System (5.3.2)*

The classification system assists in the extrapolation of DDI study results to drugs that have not been evaluated in a clinical DDI study.

If an investigational drug is a CYP inhibitor, it can be classified as a strong, moderate, or weak inhibitor based on its effect on a sensitive index CYP substrate. The convention is to categorize CYP inhibition in the following way:

- A strong inhibitor increases the AUC of a sensitive index CYP substrate greater than or equal to 5-fold.

- A moderate inhibitor increases the AUC of a sensitive index CYP substrate greater than or equal to 2-fold to less than 5-fold.

- A weak inhibitor increases the AUC of a sensitive index CYP substrate greater than or equal to 1.25-fold to less than 2-fold.

If an investigational drug is a CYP inducer, it can be classified as a strong, moderate, or weak inducer based on its effect on a sensitive index CYP substrate. The convention is to categorize CYP induction in the following way:

- A strong inducer decreases the AUC of a sensitive index CYP substrate by greater than or equal to 80 percent.

- A moderate inducer decreases the AUC of a sensitive index CYP substrate by greater than or equal to 50 percent to less than 80 percent.

- A weak inducer decreases the AUC of a sensitive index CYP substrate by greater than or equal to 20 percent to less than 50 percent.

These categories generally describe the effect of the investigational drug when given at the highest clinical dose and the shortest dosing interval within its therapeutic dose range/dosing regimen. It is noted that the effects of some inhibitors or inducers are dose dependent.

Although CYP inhibitor and inducer classifications are typically based on DDI studies with sensitive index substrates, if the metabolic properties of a different sensitive substrate are well understood, it can be possible to classify the investigational drug based on a study with the alternative substrate.

Currently, there are no classification systems for transporters or non-CYP enzymes. The interacting mechanisms may involve other transporters and/or enzymes, making it challenging to classify inhibitors using the same criteria as those for CYP enzymes. In addition, the magnitude of DDIs mediated by many of the transporters or non-CYP enzymes (e.g., UGTs) has a more limited range.

   3.      *Extrapolating Study Results (5.3.3)*

Clinical evaluation of all possible combinations of drugs is not feasible. When possible, results from DDI studies should be extrapolated to other drugs and clinical situations. Results from DDI studies with index drugs generally represent the largest magnitude interaction by a specific mechanism and can be used to predict the magnitude of other interactions by the same mechanism. The classification system for CYP inhibitors and inducers assists the extrapolation. For example, if there is no effect on the exposure of an investigational drug when coadministered with a strong CYP3A index inhibitor, then one can generally assume that there is no effect when other strong, moderate, or weak CYP3A4 inhibitors are coadministered with the investigational drug. If administration of a strong CYP2D6 index inhibitor results in a significant increase in exposure of the investigational drug, these results can be directly extrapolated to other strong CYP2D6 inhibitors. In some cases, extrapolation of positive findings to moderate and weak inhibitors can be possible using mechanistic modeling (see Appendix D (section 7.5)).

Because of the lack of specific transporter substrates and inhibitors and the possible interplay with metabolism, it is generally challenging to extrapolate results from DDI studies evaluating transporter-mediated DDIs or transporter-metabolism interactions from one drug to other drugs. However, if the ADME properties of the investigational drug and potential concomitant drugs are well understood, it is possible to extrapolate to transporter-mediated interactions with other concomitant drugs.

      a.      Extrapolating complex scenarios (5.3.3.1)

Most DDI studies evaluate the interaction between two drugs and consider the effect on single transporters or enzymes. However, DDIs for a specific drug may result from a combination of mechanisms, patients may receive more than two potentially interacting drugs, and the magnitude of an interaction may be different in different populations. Some of the resulting *complex DDI scenarios* are listed below:

- Concurrent inhibition of an enzyme and a transporter by a drug or drugs;

- Concurrent inhibition of multiple transporters by a drug or drugs;

- Concurrent inhibition and induction of a drug's metabolic pathways, involving one or more enzymes;

- Increased inhibition of drug elimination by use of inhibitors of more than one enzyme that metabolizes the drug;

- Inhibition of an enzyme other than the genetic polymorphic enzyme in poor metabolizers taking a substrate that is metabolized by both enzymes;

- Effect of enzyme/transporter inhibitors in subjects with varying degrees of impairment of drug eliminating organs (e.g., liver, kidney); and

- The two drugs affect one another's pharmacokinetics (both act as precipitant and object).

When there are multiple factors that affect the absorption and disposition of an investigational drug as well as multiple mechanisms of DDIs, sponsors should consider evaluating the effect of the combination of mechanisms and/or individual factors on drug exposure to facilitate risk assessment and provide recommendations. The complex scenarios can be evaluated by integrating knowledge from the relevant in vitro and clinical studies as well as endogenous biomarker data. Predictive modeling can sometimes be used to determine whether a clinical study would be informative or to inform the design of clinical studies.


## VI.  RISK ASSESSMENT AND MANAGEMENT (6)

Risk assessment should inform the use of DDI management strategies (i.e., DDI prevention and risk minimization strategies). A DDI is clinically relevant if concomitant use of the drugs leads to safety, effectiveness, or tolerability concerns greater than those present when the drugs are administered alone.

In general, DDI management strategies should result in drug concentrations of the substrate drug falling within the no-effect boundaries. The risk assessment and development of risk minimization strategies should consider the following factors:

- The exposure-response relationships for safety and efficacy

- The variability of the observed DDI data, if available

- The expected duration of concomitant drug use (e.g., acute, short-term, or chronic use of one or both drugs)

- The anticipated timing of the introduction of the concomitant medication;

- The mechanism of the DDI (e.g., reversible or TDI, induction, combined inhibition and induction)

- The availability of monitoring parameters (e.g., therapeutic drug monitoring, laboratory tests)

- The ability to interrupt the investigational drug or concomitant interacting medication and the availability of other therapeutic options for either drug

- The clinical importance of the relevant adverse outcome relative to the clinical benefit of the drugs

After considering the items above, DDI management strategies can include the following: (note that there may be regional regulatory differences in how recommendations are worded)

- Contraindicating or avoiding concomitant use

- Temporarily discontinuing one of the interacting drugs

- Modifying the dosing regimen of one of the drugs

- Staggering drug administration (e.g., administer the investigational drug at a different time than a concomitant drug)

- Implementing specific monitoring strategies (e.g., therapeutic drug monitoring, laboratory testing)

- Replacing one of the interacting drugs with a drug not expected to interact

## APPENDICES (7)
## GLOSSARY (7.1)

**ADC:** antibody-drug conjugate

**ADME:** absorption, distribution, metabolism, and/or excretion

**AUC:** area under the concentration versus time curve

**AUC$_{0-inf}$:** AUC extrapolated to infinity

**AUC$_{0-t}$:** AUC from time zero to time of last quantifiable observation (t)

**AUC$_{0-tau}$:** AUC for one dosing interval following multiple doses, generally at steady state

**AUCR:** ratio of AUCs of the substrate (object) drug in the presence and absence of a precipitant drug

**BCRP:** breast cancer resistance protein

**CAR:** constitutive androstane receptor

**C$_{max}$:** maximum concentration of the drug after dosing

**C$_{max,u}$:** unbound C$_{max}$

**C$_{max,inlet,u}$:** estimated unbound C$_{max}$ of an inhibitor at liver inlet

**C$_{min}$:** minimum concentration during one dosing interval at steady state

**CYP:** cytochrome P450

**DDI:** drug-drug interaction

**EC$_{50}$:** concentration causing half-maximal effect

**E$_{max}$:** maximum induction effect

**f$_m$:** fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction.

**f$_{u,p}$:** unbound fraction in plasma

**HLM:** human liver microsome

**IC$_{50}$:** half-maximal inhibitory concentration

**IC$_{50,u}$:** unbound IC$_{50}$

**Index precipitant:** a drug recommended for use in a stand-alone clinical DDI study because it has a well-established potency and selectivity profile that causes a defined degree of inhibition or induction of a given elimination pathway when administered with a sensitive and specific substrate of that pathway

**Index substrate:** a drug recommended for use in a stand-alone clinical DDI study as substrate because it has a well-established sensitivity and specificity profile that demonstrates a defined degree of change in exposures when administered with a strong inhibitor or inducer for that specific elimination pathway

**Investigational drug:** a medicinal product or a drug under development that is investigated as to its potential to act as an affecting drug or an affected drug

**k$_{deg}$:** apparent first-order degradation rate constant of the affected enzyme

**K$_i$:** inhibition constant

**K$_I$:** inhibitor concentration causing half-maximal inactivation

**k$_{inact}$:** maximal inactivation rate constant

**K$_m$:** Michaelis-Menton constant

**K$_{obs}$:** apparent first-order inactivation rate constant of the affected enzyme

**MATE:** multidrug and toxin extrusion

**MRP:** multidrug resistance-associated protein

**NADPH:** nicotinamide adenine dinucleotide phosphate (reduced form)

**Nested DDI study:** a DDI investigation that is part of a clinical study (e.g., phase 2/3) in which the assessment of DDI is not the primary objective

**No-effect boundaries:** interval within which a change in a systemic exposure measure is considered not significant enough to warrant clinical action (e.g., dose or schedule adjustment, additional therapeutic monitoring, avoid use)

**OAT:** organic anion transporter

**OATP:** organic anion transporting polypeptide

**Object:** a substrate of enzyme or transporter

**OCT:** organic cation transporter

**pAUC:** partial AUC; area under the concentration versus time curve between two specific time points

**PBPK:** physiologically based pharmacokinetic

**P-gp:** P-glycoprotein

**Precipitant:** a drug that can induce or inhibit an enzyme or a transporter

**Probe substrate:** a drug used in in vitro studies that measure individuals' enzyme inhibition or induction properties of an investigational drug. The probe substrate should be selective, or the formation of a specific metabolite should be selective for the evaluated enzyme.

**PXR:** pregnane X receptor

**Stand-alone DDI study:** a clinical DDI study with the primary objective of determining the presence or absence of a clinical DDI and the magnitude of the DDI

**Studies with index precipitants and index substrates:** clinical DDI studies conducted with index precipitants or index substrates that aim to investigate the greatest magnitude of interaction with the investigational drug for the studied pathway and which results usually can be extrapolated to other drug combinations

**TDI:** time-dependent inhibition

**$T_{max}$:** time to $C_{max}$

**$t_{1/2}$:** elimination half-life

**UGT:** uridine diphosphate (UDP)-glucuronosyl transferase

## APPENDIX A: PROTEIN BINDING (7.2)

A key parameter of interest in predicting the risk of drug-drug interactions (DDIs) for an investigational drug as a precipitant is plasma protein binding. Historically, considering uncertainties in protein binding measurements for highly bound drugs, regulatory agencies have recommended the $f_{u,p}$ (fraction unbound in plasma) be set at 0.01 (i.e., 1 percent). This conservative approach was adopted to protect against false negative DDI predictions. Recent advances have resulted in the development of methodologies that allow accurate and precise measurement of protein binding for highly protein bound drugs. When choosing a protein binding assay, it is important to ensure the suitability of a given assay methodology for the investigational drug. The next step is to demonstrate the protein binding assay is precise and accurate. Such a demonstration should include validation data of the protein binding assay qualified with appropriate positive controls (i.e., range of compounds with high binding to relevant plasma proteins). The bioanalytical methods used in these experiments should have appropriate precision and accuracy at the required sensitivity range (i.e., calibration standards and quality controls at 15 percent; lower limit of quantification at 20 percent). Protein binding assays that have demonstrated these features can be used to determine the protein binding of an investigational drug (21). When novel and emerging protein binding assay methodologies are being established, the assay method should be qualified against an orthogonal method that is previously established and accepted for a range of highly bound compounds (22).

Of note, the protein binding experiments for the investigational drug should always include a positive control for which the assay was previously qualified to ensure in-study performance. The positive control should be demonstrated to be within 3-fold of the mean $f_{u,p}$ value that was previously reported with the qualification of the protein binding assay method.

**APPENDIX B: IN VITRO EVALUATION OF METABOLISM-BASED DDIs (7.3)**

### 1. In Vitro Systems (7.3.1)

Various hepatic in vitro systems can be used to evaluate the risk for enzyme-mediated interactions for an investigational drug, including:

- Subcellular human liver tissue fractions such as microsomal systems (human liver microsomes (HLMs), containing cytochrome P450 (CYP) and uridine diphosphate (UDP)-glucuronosyl transferase (UGT) enzymes); supernatants after 9000 g centrifugation of liver homogenate (S9, containing microsomal as well as cytosolic enzymes such as sulfotransferases, glutathione transferases, aldehyde dehydrogenase, aldehyde oxidase, and alcohol dehydrogenase), and cytosol (adding cofactors as appropriate). For HLM, a pool of at least 10 donors is suggested.

- Recombinant, such as human CYP and UGT enzymes. These systems usually express only one single enzyme.

- Human liver tissues, including freshly prepared or cryopreserved hepatocytes that preserve enzyme architecture and contain the full complement of phase 1 and 2 drug metabolizing enzymes. For phenotyping and inhibition experiments, hepatocytes pooled from at least 5 to 10 donors is suggested, whereas for induction experiments usually hepatocytes from at least 3 individual donors should be used.

The in vitro systems used should be robust and reproducible.

Microsomal protein concentrations should be minimized, and standardized assay conditions (e.g., buffer strength, pH) should be used. An incubation time and an enzyme amount that result in linear formation of the metabolite (at an initial rate of the metabolite formation) are recommended.

For phenotyping experiments, the system should be characterized with in vitro probe substrates to prove the activity of each enzyme. In general, a probe substrate should be selective (e.g., predominantly metabolized by a single enzyme), or a specific metabolite of a probe substrate is primarily formed by a single enzyme. A list of examples of probe substrates with their marker reactions can be found in Table 4, in Appendix E section 1.a (7.6.1.1). For studies of time-dependent inhibition or induction, appropriate inhibitors or inducers should be included as positive controls (refer to Appendix E section 1 (7.6.1) for more details).

For enzyme inhibition studies, if the investigational drug is metabolized by the enzymes present in the incubation, the probe substrate should, if possible, have a markedly faster metabolism rate than the investigational drug to minimize the influence of investigational drug metabolism (decreasing concentrations) on the estimation of inhibitory parameters.

Robust analytical methods should be used to quantify an investigational drug and its relevant metabolite(s) in phenotyping experiments as well as probe substrates and/or their relevant

metabolites in inhibition and induction experiments (when enzyme activities are measured). Good laboratory practice standard is not required, but a full description of the analytical methods employed should be provided.

It is recognized that obtaining high drug concentrations in the in vitro studies of enzyme inhibition or induction may not be possible in some circumstances due to poor aqueous solubility or cell toxicity. If limited by solubility, cosolvents can be used to reach the highest concentration possible. Any organic solvents should be used at low concentrations (less than 1 percent volume/volume and preferably less than 0.5 percent) because some solvents can inhibit or activate enzymes. The experiment should include a solvent (vehicle) control and, when appropriate (e.g., when uncommon solvents are used), also a no-solvent control to evaluate potential effect of solvent on enzyme reaction. There is at present much uncertainty regarding how to interpret in vitro inhibition and induction data when sufficiently high concentrations cannot be tested; absence of a clinical drug-drug interaction (DDI) can be further justified by using other methods to calculate inhibition and induction parameters (6) and by considering pharmacokinetic characteristics of the compound, such as solubility limited absorption, dose and time dependency, etc. Otherwise, clinical studies are recommended to test the DDI potential of these compounds.

Limited drug stability or nonspecific binding in the incubations (e.g., with apparatus, microsomes, or hepatocytes) can also create experimental challenges in in vitro studies of enzyme inhibition or induction. Actual unbound concentrations of the drug in the in vitro system (e.g., incubation medium) should in general be used for extrapolating in vitro results to clinical scenarios. Nonspecific binding can be measured experimentally (e.g., using equilibrium dialysis) or predicted using *in silico* methods. For highly lipophilic drugs, it is preferred to experimentally determine nonspecific binding (23).

For induction experiments, sponsors are encouraged to measure concentrations of the parent drug in the medium on the last day of incubation with hepatocytes and nonspecific binding should also be considered. When measured concentrations in medium are less than 80 percent of concentrations due to non-metabolism/transporter related confounders, sponsors should discuss the potential impact of the discrepancy on data interpretation with regulatory authorities (24,25).

## 2. Investigational Drug as an Enzyme Substrate: Reaction Phenotyping (7.3.2)

Drug metabolizing enzyme identification studies, often referred to as reaction phenotyping studies, identify the specific enzymes contributing to the main elimination pathways of a drug. Along with other information (e.g., clinical pharmacokinetics, mass-balance study, pharmacogenetic data, available DDI data), in vitro phenotyping data are often used to identify and quantify elimination pathways of an investigational drug.

Although the main focus of this guidance is on hepatic CYP involved metabolism, in order to identify the metabolic pathways for the individual investigational drug, non-CYP enzyme-based metabolism and metabolism occurring in extra-hepatic tissues should also be considered for certain drugs.

        *a.*       *Metabolic Pathway Identification (7.3.2.1)*

Metabolic pathway identification experiments should be performed early in drug development to identify the number and structures of metabolites formed when a drug is metabolized and to investigate whether the metabolic pathways are parallel or sequential. These experiments use HLM, intact human liver systems (e.g., hepatocytes), or recombinant enzyme systems. Data obtained from metabolic pathway identification experiments help to determine whether and how to conduct a reaction phenotyping study.

        *b.*       *Metabolic Enzyme Identification (7.3.2.2)*

Reaction phenotyping can be done in HLM or hepatocytes using selective enzyme inhibitors and/or in human recombinant enzymes. When using individual human recombinant enzymes, the difference in the amount and enzyme activity of CYPs between the recombinant CYP enzyme systems and the human liver should be considered. Whenever possible, all experiments should be conducted with drug concentrations relevant to the clinical setting, and under initial rate conditions (e.g., linearity of metabolite production rates with respect to time and enzyme concentrations).

The contribution of individual enzymes to the overall metabolism of an investigational drug can be examined by measurement of parent drug depletion or measurement of metabolite formation. For the latter method, all the major metabolites should have been identified and quantified in metabolite formation experiments. The use of a radiolabeled drug substrate is advantageous because samples can be analyzed using liquid chromatography coupled with a radioactivity detector and a mass spectrometer to identify and quantify drug-related species. Evaluation of individual isomers of racemic drugs is recommended when it is important to understand the different disposition characteristics of each isomer (e.g., when two isomers have different pharmacological activities).

Some chemical inhibitors are not specific for an individual CYP enzyme. The selectivity and potency of inhibitors should be verified in the same experimental conditions using probe substrates for each CYP enzyme (see Appendix E section 1.a (7.6.1.1) for more details). If specific antibodies are used instead of inhibitors, the inhibitory effect of an antibody to a CYP enzyme should be tested at sufficiently low and high concentrations to establish a titration curve and ensure the maximal inhibition of a particular pathway (ideally resulting in greater than 80 percent inhibition). The effect of an antibody should be verified using probe substrates of each CYP isoform in the same experimental conditions.

For UGT enzymes, in vitro studies are most commonly performed with HLM or recombinant UGT enzymes as the enzyme source. When HLMs are used as an enzyme source, either addition of alamethicin or sonication is necessary for activating HLM, and add BSA to prevent inhibition by long-chain fatty acid (4). Determination of the contribution of each UGT isoform to the overall elimination is sometimes challenging due to lack of selective inhibitors, variability of results depending on experiment conditions, and instability of glucuronide metabolite in biologic matrices from a mass balance study.

### 3. Investigational Drug as an Enzyme Inhibitor (7.3.3)

The potential of an investigational drug to inhibit CYP enzymes is usually investigated using selective probe substrates to determine the type of inhibition (e.g., reversible inhibition or time-dependent inhibition (TDI)) and measure of inhibition potency (e.g., unbound inhibition constant ($K_{i,u}$) for reversible inhibition; $K_{I,u}$ (unbound inhibitor concentration causing half-maximal inactivation) and the maximal inactivation rate constant ($k_{inact}$) for TDI). The in vitro systems used for these studies include pooled HLM, microsomes obtained from recombinant CYP-expression systems, or pooled human hepatocytes.

For reversible inhibition, experiments with a high concentration of test drug can be performed first to study its inhibition potential on a particular enzyme (e.g., 50 x $C_{max,u}$ (unbound maximum concentration of the drug after dosing) or 0.1 x Dose/250 milliliters, refer to section II.A.2.a (2.1.2.1)). If clinical interaction cannot be excluded at the high concentration, lower drug concentrations should be tested to estimate the drug's unbound half-maximal inhibitory concentration ($IC_{50,u}$) or $K_{i,u}$ value; it is recommended to examine at least four different concentrations of the investigational drug. To determine the $K_{i,u}$ for inhibition, varying concentrations of both the inhibitor and substrate, to cover ranges above and below the substrate's Michaelis-Menton constant ($K_m$), should be tested. For competitive inhibition or uncompetitive inhibition, $IC_{50,u}/2$ can be used as an estimate for $K_{i,u}$ if the substrate concentration in the incubation is the same as its $K_m$ value (26). If the substrate concentration is much less than the $K_m$ value, then the $IC_{50,u}$ value will approximate the $K_{i,u}$ value for a competitive inhibitor. More accurate estimation of the $K_{i,u}$ value can be derived from the $IC_{50,u}$ value using the Cheng-Prusoff equation (8). For noncompetitive inhibition, $K_{i,u}$ value is equal to $IC_{50,u}$ regardless of substrate concentration used (27). Thus, $IC_{50,u}/2$ can still be used as a conservative estimate.

Evaluation of in vitro TDI potential of an investigational drug consists of two steps. The first step is a screening step to identify potential for TDI of CYP enzymes, and the second step measures the inhibition potency. There are various assays to identify TDI of CYP enzymes. For example, TDI can be detected by assessing a difference in half-maximal inhibitory concentration ($IC_{50}$) curves generated with and without a preincubation with nicotinamide adenine dinucleotide phosphate (NADPH) (i.e., $IC_{50}$ shift), decreases in enzyme activity (measurement of the pseudo first-order rate constant ($k_{obs}$)), or percent activity loss with the inactivator over time. In the $IC_{50}$ shift assay, pooled HLM should typically be preincubated for 30 minutes with the investigational drug with or without NADPH. A left shift of the $IC_{50}$ curve (e.g., greater than or equal to 1.5-fold or 2-fold) from the samples preincubated with NADPH compared to those without, suggests a potential for enzyme inactivation by the investigational drug. A known TDI should be included as positive control, which should provide a response that is within an established historical range to demonstrate sensitivity of the experimental system; otherwise, the assay should be repeated. Dilution and nondilution methods can be used for the $IC_{50}$ shift assay. Dilution assays may be more sensitive for screening purposes because inactivation of the enzymes occurs at higher concentrations of the investigational drugs whereas non-dilution method may be useful in instances of poor solubility of the drug or metabolically labile compounds.

To rule out a TDI, the decreases in CYP enzyme activity with time can also be evaluated at a single concentration of the investigational drug (e.g., $k_{obs}$, percent activity loss). A reduction in

CYP enzyme activity greater than a predefined threshold for the assay (e.g., of greater than 20 percent reduction in activity, a $k_{obs}$ value of greater than 0.01 min$^{-1}$) can be used to define a positive result.

When a drug is identified as a TDI with initial assessment as described above, definitive in vitro studies should be performed to obtain TDI parameters (i.e., $k_{inact}$ and $K_{I,u}$) in pooled HLM for DDI predictions. Human hepatocytes and rhCYP can also be considered for TDI assessment.

### 4. Investigational Drug as an Enzyme Inducer (7.3.4)

The potential of an investigational drug to act as an inducer of CYP enzymes is normally investigated in plateable, cryopreserved or freshly isolated, human hepatocytes. Alternative in vitro systems such as immortalized hepatic cell lines and cell receptor assays can be used, but the results from these studies are generally considered supportive rather than definitive in nature. If an alternative in vitro system is used as the main method, the sponsor should provide a justification supporting the appropriateness of the in vitro system as well as data interpretation.

It is recommended to measure the extent of enzyme induction at the mRNA level. Enzyme activity can also be measured, but measuring only the enzyme activity is usually not recommended because the induction could be masked when the investigational drug is also an inhibitor. However, when in vitro induction of CYP2C19 is evaluated, enzyme activity should be measured, since its mRNA change is often limited even in response to positive control (28).

Regardless of which in vitro system and endpoint are chosen, the system should be qualified to show that all major CYP enzymes are functional and inducible with positive controls. The response of positive controls (measured as mRNA fold change) is normally at least a 6-fold increase for CYP1A2, CYP2B6, and CYP3A4, which is considered indicative of satisfactory sensitivity of hepatocyte lots (refer to section II.A.4.a (2.1.4.1)) (29). In vitro induction of CYP2C8, CYP2C9, and CYP2C19 can also be measured, but their mRNA change is often limited even in response to the positive control (e.g., rifampicin). Therefore, a 6-fold increase of the positive control for these enzymes is not mandatory as a guarantee of sensitivity. Conclusive interpretation of the results is often challenging because of the limited fold of increase in mRNA of these enzymes to positive controls.

Duration of incubation of an investigational drug is usually 48 to 72 hours to allow complete induction to occur. Justification should be provided for shorter incubation times (28). Incubations normally include a daily addition of the investigational drug, and the medium containing the drug should be changed regularly. More frequent addition of a drug can be considered if its stability is low. The optimal time course for incubation should allow detection of enzyme induction without causing cell toxicity. If cytotoxicity occurs, reduced incubation durations can be used if adequate sensitivity of the assay can be demonstrated.

Quality and viability of the cultured hepatocytes should be verified and documented by cell morphology and biochemistry tests. A suitable viability assessment is normally performed before and at the end of the incubation period to certify that cell toxicity is not influencing the induction

response. If toxicity/loss of viability is observed, influence on the study results should be discussed in the study report, and clinical studies may be considered.

If hepatocytes from a donor (a) do not respond satisfactorily to the positive induction controls, (b) demonstrate viability less than 80 percent before addition of the investigational drug, or (c) demonstrate viability less than 70 percent at the end of the incubation compared to solvent (vehicle) control, the cells should be replaced by hepatocytes from a new donor.

To rule out that the investigational drug is an in vitro inducer, an induction study including three donor hepatocytes should be run with at least three replicates of three to five different concentrations of the test investigational drug, encompassing $50 \times C_{max,u}$. The basic mRNA fold-change method can be used to evaluate clinical induction potential based upon the criteria described earlier (refer to section II.A.4.a (2.1.4.1)).

When there is an induction signal, five to eight concentrations are recommended for an accurate determination of maximum induction effect ($E_{max}$) and unbound concentration causing half the maximal effect ($EC_{50,u}$). The sponsor can further use the correlation method or mechanistic static models to predict the magnitude of a clinical induction effect of an investigational drug. These methods utilize full concentration-response curves for induction, to estimate $E_{max}$ and $EC_{50,u}$ of the investigational drug. In addition, to use these methods, a batch of hepatocytes should be "calibrated" (5). For the correlation method, a large set of inducers ($n$ greater than or equal to 8) covering the full clinical induction potency range and including at least two weak inducers are recommended for calibration. $E_{max}$ and $EC_{50,u}$ are determined for all inducers, and a correlation is established between a certain matrix (incorporating $E_{max}$ and/or $EC_{50,u}$ and clinical concentrations of inducers) and clinical change in the area under the concentration versus time curve (AUC) of a sensitive substrate of a specific enzyme (e.g., midazolam for CYP3A) for each inducer. For the mechanistic method, an empirical calibration factor, $d$ factor to enable in vitro-to-in vivo induction scaling, should be determined for a hepatocyte batch. The $d$ factor can be estimated by correlating the predicted and observed induction effects (i.e., AUC ratio of a sensitive substrate of a particular enzyme) of a set of known inducers and performing a linear regression to identify a $d$ value that can minimize the prediction error (7). If the $d$ factor is not estimated, it should be set as a default value of 1.

For the correlation or mechanistic static methods, sponsors can use only one hepatocyte donor. The calibration can be established once for that batch of hepatocytes rather than multiple times for each experiment with investigational drugs. When performing the in vitro study evaluating the induction potential of an investigational drug, criteria for acceptable assay variability should be established. To confirm performance and sensitivity of the experimental system, at least two of the inducers (weak and strong) of the calibration set should be included as controls. The responses of the controls should fall within the defined assay variability in order to utilize the calibration set of that hepatocyte batch. If this method is used, both the calibration data set/calibration report and the data on the investigational drug should be submitted.

**APPENDIX C: IN VITRO EVALUATION OF TRANSPORTER-BASED DDIs (7.4)**

### 1.    In Vitro Systems (7.4.1)

Various in vitro transporter assays can be used to evaluate the risk for transporter-mediated interactions of an investigational drug. Selecting the in vitro model can depend on the purpose of the study and the questions to be addressed. Available in vitro systems include:

- Membrane vesicles

  In vitro systems using inside-out membrane vesicles from cells transfected with a transporter can be used to evaluate whether an investigational drug is a substrate or inhibitor of efflux transporters such as P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP) but may fail to identify highly permeable drugs or highly nonspecific binding drugs as substrates.

  P-gp and BCRP assays using membrane vesicles should directly measure the adenosine triphosphate-dependent, transporter-mediated uptake of drugs. For studies evaluating a drug as substrate of transporters, control groups should be included, for example, adenosine monophosphate (AMP)-treatment or nontransfected vesicles.

- Bidirectional transport assays with cell-based systems

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

  The permeability of the substrate should be investigated in both directions and evaluated under conditions for which the transport rate is linear. The apparent permeability ($P_{app}$) of the drug in both the AP→BL (absorption: apical to basolateral) and BL→AP (efflux: basolateral to apical) directions can be calculated, as well as an efflux ratio (ER) of BL→AP to AP→BL.

$$ER = \frac{P_{app,BL-AP}}{P_{app,AP-BL}}$$

  When using transfected cell lines, ERs of the transfected cell line should be compared with appropriate control conditions to account for endogenous transporter activity and nonspecific binding. One approach is to compare the ERs from transfected cell line to the parental or empty vector-transfected cell line.

$$Net\ ER = \frac{ER_{transfected}}{ER_{parental}}$$

  The integrity of monolayer membrane should be measured before and after experiments by examining whether transepithelial/transendothelial electrical resistance values or permeability of paracellular markers fall within the predefined acceptance range.

- Uptake assays with cell-based systems

  Uptake assays can be used to evaluate whether an investigational drug is a substrate or inhibitor of solute carrier transporters such as organic cation transporters (OCTs), organic anion transporters (OATs), organic anion transporting polypeptides (OATPs) and multidrug and toxin extrusions (MATEs) but can also be used to investigate efflux transporters.

  When transfected cell lines are used to evaluate whether a drug is a substrate of a transporter, the drug uptake in the transfected cell line should be compared to the parental or empty vector-transfected cell line, or a comparison of the uptake with or without an inhibitor of the transporter should be performed. When assessing a drug as an inhibitor of a transporter, evaluation of the uptake of a known probe substrate using transporter-transfected cell lines alone can be sufficient. Besides transfected cell lines, human hepatocytes or hepatic cell lines in suspension or plated can be used.

The model system and experimental conditions should be validated, including culture and transport assay conditions. Transport studies should be performed under linear transport rate conditions (probe substrate concentration used is usually below its Michaelis-Menton constant ($K_m$) for the transporter). Appropriate positive controls should be included in the test study to ensure the validity of the study's results. The assays should be optimized to ensure consistent transporter function (e.g., uptake, efflux) with control experiments (e.g., positive and negative controls for substrates/inhibitors (refer to Tables 10 and 11, in Appendix E section 3 (7.6.3) for some examples), nontransfected control cells). The following conditions should be considered whenever applicable: the source of the membrane vesicles or cells, cell culture conditions (e.g., cell passage number, seeding density, monolayer age), probe substrate/inhibitor concentrations, incubation time, buffer/pH conditions, sampling interval, and methods for estimating parameters such as the half-maximal inhibitory concentration ($IC_{50}$), inhibition constant ($K_i$), and $K_m$. In addition, adding serum or plasma proteins to the media can also affect transport activity.

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

The substrate should be readily measured with no interference from the assay matrix.

Any organic solvents should only be used at low concentrations (less than 1 percent volume/volume and preferably less than 0.5 percent) because some solvents can affect cell integrity or transporter function. The experiment should include a solvent (vehicle) control and, when appropriate (e.g., uncommon solvents are used), also a no-solvent control to evaluate potential effect of solvent on transporter activity.

Several factors may cause actual drug concentrations in the in vitro assays to deviate from nominal concentrations, including poor aqueous solubility, nonspecific binding, and instability.

Sponsors are encouraged to measure drug concentrations in the medium. Correction for binding or stability or solubility issues should be considered when interpreting the data.

## 2. Investigational Drug as a Transporter Substrate (7.4.2)

The concentration range of an investigational drug should be relevant to the site of transport and should be based on the expected clinical concentration range. For transporters expressed in multiple organs (e.g., P-gp, BCRP), the sponsors should provide justification for the choice of concentrations, taking into consideration the sites where the transporter is likely to play a role for drug disposition. When a range of drug concentrations is relevant, it is important to ensure that low concentrations are included because high concentrations may saturate transporters that are still active at lower drug concentrations.

If the in vitro system expresses multiple transporters (e.g., Caco-2 cells, hepatocytes), the sponsor should conduct additional experiments to confirm the findings with two or more known potent inhibitors, including the ones that are relatively specific for individual transporters.

If active transport is concluded, the passive permeability in the absence of transporters is one of the factors that could be taken into account to estimate the clinical importance of the transporter. For intestinal transporters, the role of these transporters may be limited if the permeability in the absence of transporters is high (greater than or equal to the permeability constant of the highly permeable control drug). In this case, the effect of active drug transport may be negligible as compared to the passive, concentration-gradient driven absorption of the drug. To estimate the permeability of a drug in the absence of transporters, for bidirectional assays (e.g., Caco-2 cells) the permeability constant can, for example, be determined at concentrations high enough to completely saturate the transporters (assessed as an ER ratio of 0.5 to 2). If this approach is used, it should be established that the cell monolayer is unaffected. Alternatively, the permeability of a drug can be measured in the presence of a broad inhibitor of transporters. The investigation should include a well-validated, high and low permeable reference substance (for example, metoprolol and mannitol; refer to the International Council for Harmonisation guidance for industry *M9 Biopharmaceutics Classification System-Based Biowaivers* (May 2021)[1] for more details).

## 3. Investigational Drug as a Transporter Inhibitor (7.4.3)

Normally the investigation of transporter inhibition starts with testing a high concentration of the test drug, for example, $10 \times C_{max,u}$ (unbound maximum concentration of the drug after dosing) for OAT1/3 and OCT2, $50 \times C_{max,u}$ for MATEs, $10 \times$ liver inlet $C_{max,u}$ for OATP1B1/3, and $0.1 \times$ the highest therapeutic dose/250 milliliters for orally administered P-gp or BCRP inhibitors. The drug concentration should, however, not exceed the drug's solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cells. There is at present much uncertainty regarding how to extrapolate in vitro results to in vivo when sufficiently high concentrations cannot be tested. Thus, the general recommendation is to test the drug-drug interaction (DDI) potential of these compounds in vivo, unless in vitro results are sufficiently justified.

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[1] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

If the test drug demonstrates inhibitory activity at the recommended cutoff concentration, the sponsor should test additional concentrations to estimate $IC_{50}$ or $K_i$ values. The sponsor should evaluate at least four concentrations of the investigational drug with the probe substrate. The sponsor can then compare $IC_{50}$ or $K_i$ values to clinical plasma or estimated intestinal concentrations of a drug to predict the potential for DDIs (refer to section II.B.2 (2.2.2)).

For some transporters (e.g., OATP1B1, OATP1B3) and experimental systems, it can be relevant to determine $IC_{50}$ or $K_i$ following preincubation with the investigational drug because some inhibitors demonstrated more inhibition potency after preincubation (30,31,32). This is an area of emerging information, and sponsors are encouraged to follow current literature for information on transporters of interest and relevant experimental protocols.

**APPENDIX D: PREDICTIVE MODELING (7.5)**

This appendix (section) describes how mechanistic modeling approaches can be used to (1) characterize the potential for drug-drug interactions (DDIs), (2) indicate whether a dedicated clinical DDI study should be conducted, and (3) support clinical recommendations in the absence of a clinical DDI study. The modeling approaches discussed are static mechanistic models and dynamic mechanistic models (also known as physiologically based pharmacokinetic (PBPK) models).

Various mathematical and mechanistic modeling approaches in conjunction with findings from in vitro and early clinical studies can help translate in vitro observations into predictions of potential clinical DDIs.

Section II (2) of this guidance describes the evaluation of in vitro metabolism and transporter studies to determine whether further evaluation of a drug as an object or precipitant of cytochrome P450 (CYP) enzyme- or transporter-mediated interactions should be conducted. If those assessments indicate further evaluations should be conducted, they may be conducted using mechanistic static models or PBPK models, as described in Appendix D sections 1 and 2 (7.5.1 and 7.5.2). For some drug development programs, multiple approaches for assessing DDI risk may be feasible.

Depending on the results of the mechanistic static or PBPK modeling, follow-up clinical DDI studies could be needed.

The use of appropriate in vitro experimental conditions is critical to any model used for a quantitative prediction.

1. **Using Mechanistic Static Models for DDI Predictions (7.5.1)**

A mechanistic static model incorporates detailed drug disposition and drug interaction mechanisms for both interacting and substrate drugs (2,33,34). The model includes the effect of reversible and time-dependent enzyme inhibition, as well as enzyme induction. Thus, the model can estimate the effect of several interaction processes. The overall effect of the precipitant drug on the substrate drug is represented as AUCR (ratio of the area under the concentration versus time curve (AUC) of the substrate drug in the presence and absence of the precipitant drug) and is given by the equation below. Input parameters should be sufficiently supported by data and/or scientific literature.

   a. *Evaluation of an Investigational Drug as a DDI Precipitant of CYP-Mediated DDIs (7.5.1.1)*

For a drug that is both an inhibitor and an inducer of an enzyme, in addition to the combination of inhibition and induction, a drug's inhibition potential alone (A and B only, assuming C is equal to 1 in the equation below), and induction potential alone (C only, assuming A and B are equal to 1 in the equation below) should be conducted. Concurrent prediction can lead to a false

negative prediction if the inhibition potential is overpredicted, thus masking the induction effect (35). If the induction potential is overpredicted, it will mask the inhibition effect.

> b. *Evaluation of Investigational Drug as an Object of CYP-Mediated DDIs (7.5.1.2)*

In principle, mechanistic static models can be used to predict DDI effects with a less potent precipitants after the model has been confirmed with index precipitants.

**Equation to Calculate AUCR of the Substrate Drugs (AUC Plus Investigational Drug/AUC Minus Investigational Drug)**

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

AUC = area under the concentration versus time curve; AUCR = ratio of the AUC of the substrate drug in the presence and absence of the precipitant drug.
The equation assumes that the drug has negligible extrahepatic clearance.
A is the effect of reversible inhibitions.
B is the effect of time-dependent inhibition.
C is the effect of induction.
$F_g$ is the fraction available after intestinal metabolism.
$f_m$ is the fraction of hepatic clearance of the substrate mediated by the cytochrome P450 enzyme that is subject to inhibition/induction.
Subscripts $h$ denote liver.
Subscripts $g$ denote gut.

**Table 3. Equations to Calculate AUCR of the Substrate Drug for Reversible and Time-Dependent Inhibition**

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

AUCR = ratio of the area under the concentration versus time curve of the substrate drug in the presence and absence of the precipitant drug; $E_{max}$ = maximum induction effect.
Each value can be estimated with the following equations:
$[I]_h = f_{u,p} \times (C_{max} + (Fa \times Fg \times ka \times Dose)/Qh/R_B)$ (36).
$[I]_g = Fa \times ka \times Dose/Qen$ (35).
$f_{u,p}$ is the unbound fraction in plasma. The $f_{u,p}$ should be set to 1 percent if the reliability of $f_{u,p}$ measurements less than 1 percent cannot be demonstrated (also refer to section II.A.2.a (2.1.2.1)). Since the potential impact of $f_{u,p}$ on the prediction of drug-drug interaction is high, sensitivity analyses for $f_{u,p}$ should be provided for highly protein bound drugs.
$C_{max}$ is the maximal total (free and bound) inhibitor concentration in the plasma at steady state.
Fa is the fraction absorbed after oral administration; a value of 1 should be used when the data are not available.

Fg is the fraction available after intestinal metabolism; a value of 1 should be used when the data are not available.
ka is the first-order absorption rate constant in vivo; a value of 0.1 min-1 (36) can be used as a worst-case estimate when the data are not available.
Qen is the blood flow through enterocytes (e.g., 18 liters (L)/hour (hr)/70 kilograms (kg)) (37).
Qh is the hepatic blood flow (e.g., 97 L/hr/70 kg) (38).
$R_B$ is the blood-to-plasma concentration ratio.
d is a scaling factor determined in a calibrated hepatocyte batch based on positive control inducers (7,33). If not determined, it is assumed to be 1. A different value can be used if supported by prior experience with the system used.

Reports of modeling exercises and results should provide support for input parameters based on data and/or scientific literature.

If the model estimates AUCR between 0.80 to 1.25, the risk of a clinically relevant interaction is low, and additional evaluations of the drug as a precipitant for the studied enzyme are not needed. If AUCR is outside 0.80 to 1.25, further evaluation should be conducted to quantify the effect. Alternatively, sponsors should provide sufficient justification(s) if no further assessments are planned.

Mechanistic static models are currently used to determine whether the potential for a DDI can be ruled out. This use, along with the current equations used for drug concentrations in the gut and liver (above), can be overly conservative and thus result in false positive results. However, when more relevant drug concentrations in gut and liver are considered, mechanistic static models can provide quantitative estimates of DDIs (2,34).

For quantitative estimation of an interaction, reports of results should include justifications for both system- and drug-dependent parameters and sensitivity analyses to cover for uncertainties in model parameters, when relevant.

### c. *Evaluation of the Potential for Transporter-Mediated DDIs (7.5.1.3)*

Although there are fewer examples, the mechanistic static models can be used to evaluate transporter-mediated DDIs, when the fraction of substrate drug transported at tissues relevant to the transporter of interest (fT) is derived from clinical data. For evaluating a drug as an inhibitor, the fT of a substrate should be confirmed with clinical DDI studies with other inhibitors. The potential applications and considerations listed for PBPK modeling (refer to Appendix D section 2.b (7.5.2.2)) are generally also relevant for mechanistic static modeling.

### 2. **Using PBPK Models to Predict Enzyme or Transporter-Based DDIs (7.5.2)**

PBPK models can assist in the evaluation of the DDI potential of an investigational drug and/or a metabolite as an object or precipitant of enzyme- or transporter-mediated interactions. When PBPK modeling is used to support drug development and regulatory decisions, it is important to justify any model assumptions, the physiological and biochemical plausibility of the model, variability, and uncertainty measures. PBPK analysis reports should include a description of the context of use for the model, model structure and development plan, the sources and justifications for both system- and drug-dependent parameters, and an adequate sensitivity

analysis plan. When using predefined models (structural and error), the software version and any deviations from predefined models should be described.

In general, broad recommendations for PBPK model verification and validation and the reporting of the results are beyond the scope of this guidance (refer to regional guidances where available). Instead, this guidance describes the utility of PBPK modeling for the evaluation of DDIs, with the understanding that models should be demonstrated as fit-for-purpose. Specific best practice considerations for use of PBPK modeling for the evaluation of DDIs are also described below.

> a.   *Potential Applications of PBPK to the Evaluation of CYP-Mediated DDIs (7.5.2.1)*

Related to evaluation of CYP-mediated DDIs, PBPK models can help select key DDI studies for a development program and inform the study design for clinical DDI studies. They can also be used to explain pharmacokinetic observations, such as observed pharmacokinetic differences that are due to genetic polymorphism.

When evaluating a drug as a potential object of CYP-mediated DDIs, PBPK models can be used to predict DDI effects with a less potent precipitant after the model has been confirmed with index precipitants. They can also predict clinically relevant DDI scenarios, such as the effect following multiple dose administration of the object drug if only single dose administration is evaluated in a clinical DDI study.

When evaluating a drug as a potential precipitant of CYP-mediated DDIs, PBPK models can be used to support the lack of clinical DDI potential and to predict DDI effects under different dosing regimens after the model has been confirmed with a sensitive index substrate.

> i.   Modeling considerations — PBPK for evaluation of CYP interactions for drugs as substrates (7.5.2.1.1)

Sponsors should consider the following when using PBPK modeling to predict the DDI potential of the investigational drug (including clinically relevant metabolite(s)) as a CYP enzyme substrate:

- The base PBPK model of the investigational drug should describe the available clinical pharmacokinetic data using different dosing regimens (e.g., a dose proportionality study, repeated dosing) and dosing routes (e.g., intravenous, oral).

- The major metabolic and other elimination pathways should be quantitatively assigned in all relevant tissues in the investigational drug's model according to available in vitro and clinical data.

- The uncertainty of the PBPK model parameters should be assessed using sensitivity analysis.

- The index precipitant models should describe the available clinical pharmacokinetic data using different dosing regimens (e.g., a dose proportionality study) and, as appropriate, different dosing routes (e.g., intravenous, oral).

- The acceptability of index precipitant models should be independently confirmed with regard to their modulating effect on the pharmacokinetics of sensitive enzyme substrates in humans.

- If complex metabolic and transport mechanisms are expected, the substrate and precipitant models should include the relevant disposition and interaction mechanisms and should be identifiable and deemed fit-for-purpose.

> ii.  Modeling considerations — PBPK for evaluation of CYP interactions for drugs as precipitants (7.5.2.1.2)

Sponsors should consider the following when using PBPK modeling to predict the drug interaction potential of an investigational drug (including clinically relevant metabolite(s)) as a CYP enzyme precipitant:

- The base PBPK model of the investigational precipitant (and its metabolites, when relevant) should describe the available clinical pharmacokinetic data using different dosing regimens (e.g., a dose proportionality study, repeated dosing) and, as appropriate, dosing routes (e.g., intravenous, oral).

- The DDI parameters should be assigned in the precipitant's model according to available in vitro and clinical data such as clinical DDI study or studies.

- For precipitants that exhibit both inhibition and induction, the inhibition and induction mechanisms should be separately considered, in addition to the combination of inhibition and induction, to ensure a conservative prediction of clinical enzyme inhibition or induction. In most cases, the clinically relevant effect of interest is the combined effect.

- The sensitive index substrate models should describe the available clinical pharmacokinetic data using different dosing regimens (e.g., a dose proportionality study) and, as appropriate, different dosing routes (e.g., intravenous, oral).

- The sensitive index substrate models should be independently confirmed with regard to the effect of a strong index precipitant-mediated altered enzyme activity on its pharmacokinetics in humans.

- The simulation should include the highest clinical dose and shortest dosing interval of the investigational precipitant. The pharmacokinetics and modulating effect of the highest dose should be confirmed before use in the simulation.

- Sensitivity analyses should be conducted for parameters exhibiting high levels of uncertainty. For example, because the potential impact of $f_{u,p}$ (fraction unbound in

plasma) on the prediction of DDI is high, sensitivity analyses for $f_{u,p}$ is expected for highly protein bound drugs.

b.    *Potential Applications of PBPK to the Evaluation of Transporter-Mediated DDIs (7.5.2.2)*

Related to evaluation of transporter-mediated DDIs, PBPK models can be used to support the initial study design for clinical DDI studies when a DDI liability is identified.

When evaluating a drug as a potential object of transporter-mediated DDIs, PBPK models can be used to explain pharmacokinetic observations, such as pharmacokinetic differences that are due to genetic polymorphism (e.g., OATP1B1 (OATP (organic anion transporting polypeptide))). PBPK models can also be used to explore involvement of specific transporters in a drug's absorption, distribution, metabolism, and/or excretion and DDI liability.

When evaluating a drug as a potential inhibitor of transporter-mediated DDIs, PBPK models may support negative DDI prediction when the drug is an in vitro inhibitor for a basolateral uptake transporter (e.g., OAT1/3 (OAT (organic anion transporter))). They can also be used to evaluate the effect of an investigational drug on the pharmacokinetics of a transporter substrate with a well-characterized pathway.

i.    Modeling considerations — drug as a transporter substrate (7.5.2.2.1)

In general, quantitatively confirming the model regarding the involvement of the specific transporter in the relevant organ(s) is challenging. Comprehensive model exploration and/or clinical studies should be conducted for quantitative model confirmation.

ii.    Modeling considerations — drug as a transporter inhibitor (7.5.2.2.2)

In general, when using PBPK models to evaluate a drug as a transporter inhibitor, the substrate model should be confirmed for the relevant transporter(s). Further, the analysis report should include a sensitivity analysis addressing uncertainties related to the precipitant's inhibition constant and the concentration that is relevant for the DDI.

**APPENDIX E: LIST OF DRUGS THAT CAN BE USED IN IN VITRO STUDIES (7.6)**

1. **CYP Enzymes (7.6.1)**

    a. *CYP Enzyme Substrates for In Vitro Studies (7.6.1.1)*

Probe substrates are used to measure inhibitor/inducer properties of a drug candidate on individual cytochrome P450 (CYP) enzymes (see Table 4 for examples of substrates; the list is not exhaustive, and sponsors can use other substrates/metabolites with appropriate justification). The substrates should be selective, or the formation of a specific metabolite is selective for a CYP enzyme. Concentration of the substrate should be at or below its Michaelis-Menton constant for the reaction.

**Table 4. Examples of Probe Substrates for CYP Enzymes (In Vitro Studies)**

| CYP Enzyme | Probe substrate | Marker reaction |
|---|---|---|
| CYP1A2 | Phenacetin<br>7-Ethoxyresorufin | Phenacetin O-deethylation<br>7-Ethoxyresorufin-O-deethylation |
| CYP2B6 | Bupropion<br>Efavirenz | Bupropion hydroxylation<br>Efavirenz hydroxylation |
| CYP2C8 | Paclitaxel<br>Amodiaquine | Paclitaxel 6α-hydroxylation<br>Amodiaquine N-deethylation |
| CYP2C9 | S-warfarin<br>Diclofenac | S-warfarin 7-hydroxylation<br>Diclofenac 4'-hydroxylation |
| CYP2C19 | S-Mephenytoin | S-Mephenytoin 4'-hydroxylation |
| CYP2D6 | Bufuralol<br>Dextromethorphan | Bufuralol 1'-hydroxylation<br>Dextromethorphan O-demethylation |
| CYP3A<br>(recommend using two structurally different substrates) | Midazolam<br>Testosterone | Midazolam 1'-hydroxylation<br>Testosterone 6β-hydroxylation |

CYP = cytochrome P450.

    b. *CYP Enzymes Inhibitors/Inducers for In Vitro Studies (7.6.1.2)*

The enzyme inhibitors and inducers are used to phenotype individual CYP enzymes involved in the drug candidate metabolism in vitro. In general, the inhibitors should be selective at the concentration used. The following tables are provided to help sponsors design in vitro studies and to evaluate the interaction potential (Tables 5 through 7). These tables are not exhaustive, and sponsors can use other inhibitors/inducers with appropriate justification.

**Table 5. Examples of Inhibitors for CYP Enzymes (In Vitro Studies)**

| CYP Enzyme | Inhibitor |
|---|---|
| CYP1A2 | α-Naphthoflavone, Furafylline* |
| CYP2B6 | Clopidogrel*, Ticlopidine*, Thiotepa* |
| CYP2C8 | Gemfibrozil glucuronide*, Montelukast, Phenelzine* |
| CYP2C9 | Sulfaphenazole, Tienilic acid* |
| CYP2C19 | Loratadine, Ticlopidine* |
| CYP2D6 | Paroxetine*, Quinidine |
| CYP3A | Azamulin*, Itraconazole, Ketoconazole, Troleandomycin* |

CYP = cytochrome P450.
* Designated as time-dependent inhibitor. When used, those inhibitors should be preincubated with the experimental system.

**Table 6. Representative Values of the Turnover Rate Constant ($K_{deg}$) and Half-Life ($t_{1/2}$) of Major CYP Enzymes to Aid in the Assessment of Time-Dependent Inhibition**

| Enzymes (hepatic) | t1/2 (hr) | Kdeg (/min) |
|---|---|---|
| CYP1A2 | 38 | 0.00030 |
| CYP2B6 | 32 | 0.00036 |
| CYP2C8 | 22 | 0.00053 |
| CYP2C9 | 104 | 0.00011 |
| CYP2C19 | 26 | 0.00044 |
| CYP2D6 | 51 | 0.00023 |
| CYP3A4 | 36 | 0.00032 |
| CYP3A4 (intestinal) | 24 | 0.00048 |

CYP = cytochrome P450; hr = hour; min = minute.

**Table 7. Examples of Inducers for CYP Enzymes (In Vitro Studies)**

| CYP Enzyme | Inducer |
|---|---|
| CYP1A2 | Omeprazole |
| CYP2B6 | Phenobarbital |
| CYP2C8 | Rifampicin |
| CYP2C9 | Rifampicin |
| CYP2C19 | Rifampicin |
| CYP3A4 | Rifampicin |

CYP = cytochrome P450.

2. **UGTs (Uridine Diphosphate (UDP)-Glucuronosyl Transferases) (7.6.2)**

a. *UGT Substrates for In Vitro Studies (7.6.2.1)*

The list provided in Table 8 is not exhaustive, and sponsors can use other substrates with appropriate justification.

**Table 8. Examples of Substrates for UGTs (In Vitro Studies)**

| UGT enzyme | Substrate |
|---|---|
| UGT1A1 | β-Estradiol, PF-06409577 |
| UGT1A3 | Telmisartan |
| UGT1A4 | Trifluoperazine, 1'-Hydroxymidazolam |
| UGT1A6 | Deferiprone, 5-Hydroxytryptophol, Serotonin |
| UGT1A9 | Mycophenolic acid, Propofol |
| UGT2B7 | Morphine, Zidovudine |
| UGT2B10 | Cotinine, RO5263397 |
| UGT2B15 | S-Oxazepam |
| UGT2B17 | Testosterone |

UGT = uridine diphosphate (UDP)-glucuronosyl transferase.

b. *UGT Inhibitors for In Vitro Studies (7.6.2.2)*

There is a lack of relatively selective inhibitors for UGT1A3, UGT1A6, UGT2B7, and UGT2B15. In the absence of selective inhibitors, a combination of methods including use of recombinant UGT isoform, human liver microsome expressing polymorphic variants of UGT isoform (where appropriate), the relative activity factor or relative expression factor approach, and activity correlation approach can be employed. Comparative studies with multiple inhibitors can also help assess the involvement of a particular isoform. When using individual recombinant enzyme preparations, the difference in the amount and enzyme activity of UGTs between the recombinant enzyme systems and the human liver should be considered.

The list provided in Table 9 is not exhaustive, and sponsors can use other inhibitors with appropriate justification.

**Table 9. Examples of Inhibitors for UGTs (In Vitro Studies)**

| UGT enzyme | Inhibitor |
|---|---|
| UGT1A1 | Nilotinib, Regorafenib |
| UGT1A3 | - |
| UGT1A4 | Hecogenin |
| UGT1A6 | - |
| UGT1A9 | Magnolol, Niflumic acid |

| UGT enzyme | Inhibitor |
|---|---|
| UGT2B7 | 16α- and 16β-Phenyllongifolol*, fluconazole** |
| UGT2B10 | Desloratadine |
| UGT2B15 | - |
| UGT2B17 | Imatinib |

UGT = uridine diphosphate (UDP)-glucuronosyl transferase.
*16α- and 16β-Phenyllongifolol also inhibit UGT2B4. Their effects on UGT2B10 remains unknown.
** Fluconazole also inhibits UGT2B10 and UGT2B17.

### 3.    Transporters (7.6.3)

Some substrates are not specific for an individual transporter. When an experimental system expressing multiple transporters is used, a more specific substrate is preferred. The following tables provide examples of transporter substrate and inhibitors for in vitro studies (Tables 10 and 11).

**Table 10. Examples of Substrates for Transporters (In Vitro Studies)**

| Transporter | Substrate |
|---|---|
| P-gp | Digoxin, *N*-methyl-quinidine (NMQ), Quinidine, Vinblastine |
| BCRP | Estrone-3-sulfate, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), Prazosin, Rosuvastatin, Sulfasalazine |
| OATP1B1, OATP1B3 | Cholecystokinin octapeptide (CCK-8, selective for OATP1B3), Estradiol-17β-glucuronide, Pitavastatin, Pravastatin, Rosuvastatin |
| OAT1 | Adefovir, Cidofovir, *p*-aminohippurate (PAH), Tenofovir |
| OAT3 | Benzylpenicillin, Estrone-3-sulfate, Methotrexate |
| MATE1, MATE2-K | Creatinine, Metformin, 1-methyl-4-phenylpyridinium (MPP+), Tetraethylammonium (TEA) |
| OCT2 | Creatinine, Metformin, Tetraethylammonium (TEA) |

P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP = organic anion transporting polypeptide; OAT = organic anion transporter; MATE = multidrug and toxin extrusion protein; OCT = organic cation transporter.

**Table 11. Examples of Inhibitors for Transporters (In Vitro Studies)**

| Transporter | Inhibitor |
|---|---|
| P-gp | GF120918 (dual P-gp/BCRP inhibitor), Verapamil, Valspodar (PSC833), Zosuquidar (LY335979) |
| BCRP | Fumitremorgin C, GF120918 (dual P-gp/BCRP inhibitor), Ko143, Novobiocin |
| OATP1B1, OATP1B3 | Bromosulfophthalein (BSP), Cyclosporine, Rifampin, Rifamycin SV |
| OAT1, OAT3 | Benzylpenicillin*, Probenecid |
| MATE1, MATE2-K | Cimetidine, Pyrimethamine, Quinidine |

| Transporter | Inhibitor |
|---|---|
| OCT2 | Cimetidine, Clonidine, Pyrimethamine, Verapamil |

P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP = organic anion transporting polypeptide; OAT = organic anion transporter; MATE = multidrug and toxin extrusion protein; OCT = organic cation transporter.
* Relatively selective inhibitor for OAT3.

**APPENDIX F: LIST OF DRUGS THAT CAN BE USED IN CLINICAL STUDIES (7.7)**

1. **Cytochrome P450 (CYPs) Enzymes (7.7.1)**

    a. *CYP Enzyme Substrates for Clinical Studies (7.7.1.1)*

Ideally, drugs selections should be based on sensitivity, specificity, safety profiles, and reported clinical drug-drug interaction (DDI) studies with inhibitors, as well as an absence of studies that indicate the drug does not meet the criteria described below:

- Index substrates predictably exhibit exposure increase due to inhibition of a given metabolic pathway and results are available from prospective clinical DDI studies. These drugs can be safely administered with potential inhibitors, sometimes with a dose reduction.

- Sensitive index substrates are index drugs that demonstrate an increase in area under the concentration versus time curve (AUC) of greater than or equal to 5-fold with strong index inhibitors of a given metabolic pathway in clinical DDI studies.

- Moderately sensitive substrates are drugs that demonstrate an increase in AUC of greater than or equal to 2-fold to less than 5-fold with strong index inhibitors of a given metabolic pathway in clinical DDI studies.

Sponsors are encouraged to consider the unique characteristics of each drug when designing DDI studies. For example, a drug could be a substrate for multiple CYPs or a CYP plus a transporter. In such a case, the selection of an index drug for a study should take into consideration the knowledge about the potential precipitant (enzymes and/or transporters, which it could inhibit). The drugs listed in Table 12 below have been identified as appropriate index substrates for clinical DDI studies. Other drugs can be proposed, considering the criteria above.

**Table 12. Examples of Index Substrates for CYP Enzymes (Clinical Studies)**

| CYP Enzyme | Sensitive index substrate (unless otherwise noted) | Comments |
|---|---|---|
| CYP1A2 | Caffeine | |
| CYP2B6 | Bupropion | Bupropion is metabolized by CYP2B6 and non-CYP enzymes. Thus, by itself is not a sensitive substrate. Hydroxybupropion should also be measured because it is primarily formed by CYP2B6. Hydroxybupropion concentration changes should be considered when determining clinical significance because it is the major active moiety. |

| CYP Enzyme | Sensitive index substrate (unless otherwise noted) | Comments |
|---|---|---|
| CYP2C8 | Repaglinide | Also metabolized by CYP3A though to a lesser extent. Transported by OATP1B1. |
| CYP2C9 | S-warfarin, Flurbiprofen, Tolbutamide | Moderately sensitive substrates |
| CYP2C19 | Omeprazole | Also metabolized by CYP3A though to a lesser extent. Measurement of metabolite concentrations should be considered when there are multiple interacting mechanisms involved. |
| CYP2D6 | Desipramine, Dextromethorphan, Nebivolol | Dextromethorphan is also metabolized by CYP3A to a lesser extent. |
| CYP3A | Midazolam, Triazolam | |

CYP = cytochrome P450; OATP = organic anion transporting polypeptide.

> b.      *CYP Enzyme Inhibitors for Clinical Studies (7.7.1.2)*

Index inhibitors predictably inhibit metabolism via a given pathway, and results are available from prospective clinical DDI studies. Strong and moderate inhibitors are drugs that increase the AUC of sensitive index substrates of a given metabolic pathway greater than or equal to 5-fold and greater than or equal to 2-fold to less than 5-fold, respectively.

Ideally, index inhibitors should be selected based on potency and selectivity of inhibition, safety profiles, and availability of reported clinical DDI studies with different clinical substrates, as well as an absence of studies that indicate the drug does not meet the criteria described above.

Sponsors are encouraged to consider the unique characteristics of each drug when designing DDI studies. For example, a drug could inhibit multiple CYPs or a CYP plus a transporter. Sponsors should select an index inhibitor for a study based on knowledge about the potential CYPs and transporters involved with the substrate's disposition.

The drugs listed in Table 13 below have been identified as appropriate index inhibitors for clinical DDI studies. Other drugs can be proposed, considering the criteria described above.

**Table 13. Examples of Index Inhibitors for CYP Enzymes (Clinical Studies)**

| CYP Enzyme | Strong index inhibitors | Comments |
|---|---|---|
| CYP1A2 | Fluvoxamine | Also, strong inhibitor of CYP2C19; weak inhibitor of CYP2C9, CYP2D6, and CYP3A. |
| CYP2B6 | *No strong index inhibitors are available for CYP2B6* | Ticlopidine can be used as a CYP2B6 inhibitor. It decreases hydroxybupropion formation by more than 80%. Ticlopidine is also a strong inhibitor of CYP2C19. |
| CYP2C8 | Gemfibrozil | Also, inhibits OATP1B1 and OAT3. |

| CYP Enzyme | Strong index inhibitors | Comments |
|---|---|---|
| CYP2C9 | Fluconazole (moderate inhibitor) | Also, strong inhibitor of CYP2C19; moderate inhibitor CYP3A. |
| CYP2C19 | Fluvoxamine Fluconazole | Fluvoxamine: Also, strong inhibitor of CYP1A2; weak inhibitor of CYP2C9, CYP2D6, and CYP3A. Fluconazole: Also, moderate inhibitor of CYP2C9 and CYP3A. |
| CYP2D6 | Fluoxetine Paroxetine | Fluoxetine: Also, strong inhibitor of CYP 2C19. |
| CYP3A | Clarithromycin Itraconazole | Clarithromycin and itraconazole both inhibit P-gp. Itraconazole also inhibits BCRP. |

CYP = cytochrome P450; OATP = organic anion transporting polypeptide; OAT = organic anion transporter; P-gp = P-glycoprotein; BCRP = breast cancer resistance protein.

> c. *CYP Enzyme Inducers for Clinical Studies (7.7.1.3)*

Inducers in Table 14 below were selected based on potency of induction, safety profiles, and availability of clinical DDI studies with different clinical substrates. Due to the mechanisms of induction, inducers usually regulate the expression of multiple enzymes and transporters.

Strong and moderate inducers decrease the AUC of sensitive index substrates of a given metabolic pathway by greater than or equal to 80 percent and greater than or equal to 50 percent to less than 80 percent, respectively.

**Table 14. Examples of Inducers for CYP Enzymes (Clinical Studies) — the List Is Not Exhaustive and Other Inducers Can Be Used**

| CYP Enzyme | Strong inducers | Moderate inducers |
|---|---|---|
| CYP1A2* | | Phenytoin, Rifampin, Cigarette smoking |
| CYP2B6 | Carbamazepine | Rifampin, Efavirenz |
| CYP2C8 | | Rifampin |
| CYP2C9 | | Rifampin |
| CYP2C19 | Rifampin | |
| CYP3A | Carbamazepine, Phenytoin, Rifampin | Efavirenz |

CYP = cytochrome P450.
*CYP1A2: Phenytoin, rifampin, and cigarette smoking are weak-to-moderate inducers based on limited number of clinical drug-drug interaction studies conducted with caffeine, tizanidine, and theophylline.

> **2. UGTs (Uridine Diphosphate (UDP)-Glucuronosyl Transferases) (7.7.2)**

UGT substrates and inhibitors/inducers that are useful for clinical DDI studies are listed below (Tables 15 through 17). UGT substrates, inhibitors, and inducers are less established (see section III.B.4 (3.2.4)). Other substrates/inhibitors/inducers can be used with appropriate justifications.

**Table 15. Examples of Substrates for UGTs (Clinical Studies)**

| UGT enzyme | Substrates | Comments |
|---|---|---|
| UGT1A1 | Bictegravir | Also metabolized by CYP3A |
| | Cabotegravir | Also glucuronidated by UGT1A9 |
| | Dolutegravir | Also metabolized by CYP3A and glucuronidated by UGT1A3 and UGT1A9 |
| | SN-38 | Active metabolite of irinotecan. Also glucuronidated by UGT1A9 |
| UGT1A4 | Lamotrigine | Also glucuronidated by UGT2B7 |
| | Pexidartinib | Also metabolized by CYP3A |
| UGT1A9 | Dapagliflozin | Also metabolized by CYPs |
| | Ertugliflozin | Also glucuronidated by UGT2B4 and UGT2B7 |
| | Sotagliflozin | Also metabolized by CYP3A |
| UGT2B7 | Indomethacin | Indomethacin and naproxen form acyl glucuronides that are unstable. Since there is possibility that the acyl glucuronides are transported by OAT1/3, alternative options may be considered if co-administered with an OAT1/3 inhibitor |
| | S-Naproxen | |
| | Zidovudine | Also glucuronidated by UGT2B4 |
| UGT2B15 | Lorazepam | S-lorazepam and S-oxazepam are glucuronidated by UGT2B15. R-enantiomers are also glucuronidated by other UGT2B isoforms and UGT1A9 |
| | Oxazepam | |

UGT = uridine diphosphate (UDP)-glucuronosyl transferase; CYP = cytochrome P450; OAT = organic anion transporter.

**Table 16. Examples of Inhibitors for UGTs (Clinical Studies)**

| UGT enzyme | Inhibitors | Comments |
|---|---|---|
| UGT1A1 | Atazanavir | Also, an inhibitor of CYP3A |
| UGT1A4 | Probenecid | Also, an inhibitor of OAT1 and OAT3 |
| UGT1A9 | Mefenamic Acid | Also, an inhibitor of UGT2B7 |
| UGT2B7 | Probenecid | Also, an inhibitor of OAT1 and OAT3 |
| UGT2B15 | Probenecid | Also, an inhibitor of OAT1 and OAT3 |

UGT = uridine diphosphate (UDP)-glucuronosyl transferase; CYP = cytochrome P450; OAT = organic anion transporter.

**Table 17. Examples of Inducers for UGT (Clinical Studies)**

| UGT enzyme | Inducers |
|---|---|
| UGT1A1 | Carbamazepine, Efavirenz, Phenobarbital, Rifampin, Saint-John's-wort, Tipranavir combined with ritonavir |
| UGT1A4 | Carbamazepine, Lopinavir combined with ritonavir, Phenobarbital, Phenytoin, Rifampin |
| UGT1A9 | Rifampin |

| UGT enzyme | Inducers |
|---|---|
| UGT2B7 | Rifampin |
| UGT2B15 | Rifampin, Phenytoin |

UGT = uridine diphosphate (UDP)-glucuronosyl transferase.

### 3. Transporters (7.7.3)

#### a. *Transporter Substrates for Clinical Studies (7.7.3.1)*

Transporter substrates that are useful for clinical DDI studies are listed in Table 18 below. Many of them are substrates of multiple transporters and/or enzymes. Thus, the extrapolation of results from these studies to other drugs can be challenging and as indicated earlier (see section III.B.5 (3.2.5)), index substrates are not available for transporters. Interpretation of the study results should take into consideration the knowledge of the transporter inhibition properties for the investigational drug as well as its effect on metabolic enzymes. It is most useful to select a transporter substrate that is likely to be administered in the intended patient population for the investigational drug.

The listed substrates exhibit markedly altered pharmacokinetic profiles following coadministration of known inhibitors of the transporter, meeting the criteria below. In addition, they are generally safe for use in clinical DDI studies.

Criteria

The criteria below were used to select recommended transporter substrates for use in DDI studies to characterize a drug's transporter inhibition properties. Results from studies conducted with clinically relevant doses were used for selection of drugs. When possible, drugs most relevant for global drug development programs were selected.

- P-glycoprotein (P-gp): (1) AUC fold-increase greater than or equal to 2 with itraconazole, quinidine, or verapamil coadministration, (2) in vitro transport by P-gp expression systems, and (3) not extensively metabolized in vivo.

- Breast cancer resistance protein (BCRP): (1) AUC fold-increase greater than or equal to 2 with pharmacogenetic alteration of ABCG2 (421C>A) and (2) in vitro transport by BCRP expression systems.

- OATP1B1/OATP1B3 (organic anion transporting polypeptide(OATP)): (1) AUC fold-increase greater than or equal to 2 with rifampin (single dose) or cyclosporine coadministration, or pharmacogenetic alteration of SLCO1B1 (521T>C) and (2) in vitro transport by OATP1B1 or OATP1B3 expression systems.

- OAT1/OAT3 (organic anion transporter (OAT)): (1) AUC fold-increase greater than or equal to 2 with probenecid coadministration, (2) fraction excreted into urine as an unchanged drug greater than or equal to 0.5, and (3) in vitro transport by OAT1 and/or OAT3 expression systems.

- OCT2/MATEs (organic cation transporter (OCT)/multidrug and toxin extrusion protein (MATE)): (1) AUC fold-increase greater than or equal to 2 with dolutegravir or pyrimethamine; (2) fraction excreted into urine as an unchanged drug greater than or equal to 0.5, and (3) in vitro transport by OCT2 and/or MATEs expression system.

Note: The list is not exhaustive, and sponsors can use substrates that are not listed in the table if the drug's transport properties are well understood and similar to the criteria above**.**

**Table 18. Examples of Substrates for Transporters (Clinical Studies)**

| Transporter | Substrates | Comments* |
|---|---|---|
| P-gp | Dabigatran etexilate Digoxin*** Fexofenadine | Dabigatran etexilate** – only affected by intestinal P-gp. Fexofenadine – also substrate for OATP1B1, 1B3 and 2B1. |
| BCRP | Rosuvastatin Sulfasalazine | Rosuvastatin – also a substrate for OATP1B1, 1B3, 2B1, and OAT3. Sulfasalazine – only affected by intestinal BCRP. |
| OATP1B1, OATP1B3 | Atorvastatin Bosentan Pitavastatin Pravastatin Rosuvastatin Simvastatin acid | Atorvastatin – also a substrate of BCRP, P-gp, and CYP3A. Pravastatin – also a substrate of MRP2 and OAT3. Rosuvastatin – also a substrate for BCRP, OAT3, and OATP2B1. Simvastatin – also a substrate of CYP3A. |
| OAT1 OAT3 | Adefovir Baricitinib Cefaclor Furosemide Oseltamivir carboxylate Penicillin G | Adefovir**** – Higher contribution of OAT1 than OAT3. Baricitinib, cefaclor and Penicillin G – Higher contribution of OAT3 than OAT1. Furosemide – dual substrate of OAT1/OAT3 is also a substrate of BCRP, OATP2B1, and UGT. |
| MATE1, MATE2-K, OCT2 | Metformin | |

P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP = organic anion transporting polypeptide; OAT = organic anion transporter; MATE = multidrug and toxin extrusion protein; OCT = organic cation transporter; MRP = multidrug resistance-associated protein 2; CYP = cytochrome P450;UGT = uridine 5'-diphospho-glucuronosyltransferases.
* Due to the evolving nature of the understanding, some of the drugs listed in the table could be substrates of other transporters that are not listed here.
** Dabigatran etexilate is a prodrug and converted by carboxylesterase (CES) to dabigatran, which is the measured moiety (dabigatran is not a substrate of P-gp). Thus, for correct interpretation of clinical DDI results, preassessment of the inhibitory effects of an investigational drug on CES activity should be considered.
*** For P-gp, renal inhibition can be determined using renal clearance of digoxin.
**** Adefovir dipivoxil is a prodrug and converted by CES to adefovir, which is a substrate of OAT1 and OAT3. Adefovir is the measured moiety in the drug-drug interaction (DDI) study. Thus, for correct interpretation of clinical

DDI results, preassessment of the inhibitory effect of an investigational drug on CES activity should be considered. Adefovir dipivoxil is a substrate of P-gp.

> b. *Transporter Inhibitors for Clinical Studies (7.7.3.2)*

Transporter inhibitors that are useful for clinical DDI studies are listed in Table 19 below. Many of them not only inhibit the specified transporters but also inhibit some other transporters and/or CYP enzymes. Thus, extrapolation of results from these studies to other drugs can be challenging as indicated earlier (see section III.B.5 (3.2.5)), index inhibitors are not available for transporters. Interpretation of the study results should take into consideration the knowledge of transport and metabolic/elimination pathways for the investigational drug. It is most useful to select a transporter inhibitor that is likely to be administered in the intended patient population for the investigational drug.

The listed inhibitors lead to markedly altered pharmacokinetic profiles of known substrates of the transporter following coadministration, meeting the criteria below. In addition, they are generally safe for use in clinical DDI studies.

Criteria

The criteria below were used to select recommended transporter inhibitors for use in DDI studies to characterize a drug's properties as a transporter substrate. Results from studies conducted with clinically relevant doses were used for selection of drugs. When possible, drugs most relevant for global drug development programs were selected.

- P-gp: (1) AUC fold-increase of digoxin, dabigatran, or fexofenadine greater than or equal to 2 with coadministration and (2) in vitro inhibitor.

- BCRP: (1) AUC fold-increase of rosuvastatin greater than or equal to 2 or close to 2-fold with coadministration and (2) in vitro inhibitor.

- OATP1B1/OATP1B3: (1) AUC fold-increase greater than or equal to 2 for at least one of the clinical substrates with coadministration and (2) in vitro inhibitor.

- OAT1/OAT3: (1) AUC fold-increase greater than or equal to 2 for at least one of the clinical substrates with coadministration and (2) in vitro inhibitor.

- OCT2/MATE: (1) AUC fold-increase of metformin greater than or equal to 2 with coadministration and (2) in vitro inhibitor.

Note: The list is not exhaustive, and sponsors can use inhibitors that are not listed in the table if the drug's transporter inhibition properties are well understood and similar to the criteria above.

**Table 19. Examples of Inhibitors for Transporters (Clinical Studies)**

| Transporter | Inhibitor | Comments |
|---|---|---|
| P-gp | Itraconazole Quinidine Verapamil | Itraconazole – also inhibits BCRP and CYP3A Verapamil – also inhibits CYP3A |
| BCRP | Cyclosporine Darolutamide Fostamatinib | Cyclosporine – also inhibits CYP3A, MRP2, OATP1B1, OATP1B3, and P-gp. Fostamatinib – also inhibits P-gp |
| OATP1B1, OATP1B3 | Rifampin (single dose) Cyclosporine | Rifampin – also inhibits P-gp Cyclosporine – also inhibits CYP3A, MRP2, P-gp and BCRP |
| OAT1, OAT3 | Probenecid | Probenecid – also inhibits OATP1B1. |
| MATE1, MATE2-K, OCT2 | Dolutegravir Pyrimethamine | Dolutegravir – in general a more potent inhibitor for OCT2 than for MATEs Pyrimethamine – a relatively specific inhibitor of MATEs. |

P-gp = P-glycoprotein; BCRP = breast cancer resistance protein; OATP = organic anion transporting polypeptide; OAT = organic anion transporter; MATE = multidrug and toxin extrusion protein; OCT = organic cation transporter; CYP = cytochrome P450; MRP =  multidrug resistance-associated protein.

## REFERENCES (8)

1. Rodrigues AD, 2007, Prioritization of Clinical Drug Interaction Studies Using In Vitro Cytochrome P450 Data: Proposed Refinement and Expansion of the "Rank Order" Approach, Drug Metab Lett, 1(1):31–35.

2. Tseng, E, Eng H, Lin J, Cerny MA, Tess DA, Goosen TC, et al., 2021, Static and Dynamic Projections of Drug-Drug Interactions Caused by Cytochrome P450 3A Time-Dependent Inhibitors Measured in Human Liver Microsomes and Hepatocytes, Drug Metab Dispos, 49(10):947–960.

3. Miners JO, Polasek TM, Hulin JA, Rowland A, and Meech R, 2023, Drug-Drug Interactions That Alter the Exposure of Glucuronidated Drugs: Scope, UDP-Glucuronosyltransferase (UGT) Enzyme Selectivity, Mechanisms (Inhibition and Induction), and Clinical Significance, Pharmacol Ther, 248:108459.

4. Miners JO, Rowland A, Novak JJ, Lapham K, and Goosen TC, 2021, Evidence-Based Strategies for the Characterization of Human Drug and Chemical Glucuronidation In Vitro and UDP-Glucuronosyltransferase Reaction Phenotyping, Pharmacol Ther, 218:107689.

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

6. Zhang JG, Ho T, Callendrello AL, Clark RJ, Santone EA, Kinsman S, et al., 2014, Evaluation of Calibration Curve-Based Approaches to Predict Clinical Inducers and Noninducers of CYP3A4 With Plated Human Hepatocytes, Drug Metab Dispos, 42(9):1379–1391.

7. Vermet H, Raoust N, Ngo R, Esserméant L, Klieber S, Fabre G, et al., 2016, Evaluation of Normalization Methods to Predict CYP3A4 Induction in Six Fully Characterized Cryopreserved Human Hepatocyte Preparations and HepaRG Cells, Drug Metab Dispos, 44(1):50–60.

8. Cheng Y and Prusoff WH, 1973, Relationship Between the Inhibition Constant ($K_1$) and the Concentration of Inhibitor Which Causes 50 Per Cent Inhibition ($I_{50}$) of an Enzymatic Reaction, Biochem Pharmacol, 22(23):3099–3108.

9. Durk MR, Jones NS, Liu J, Nagapudi K, Mao C, Plise EG, et al., 2020, Understanding the Effect of Hydroxypropyl-β-Cyclodextrin on Fenebrutinib Absorption in an Itraconazole-Fenebrutinib Drug-Drug Interaction Study, Clin Pharmacol Ther, 108(6):1224–1232.

10. Huppertz A, Ott C, Bruckner T, Foerster KI, Burhenne J, Weiss J, et al., 2019, Prolonged-Release Tacrolimus Is Less Susceptible to Interaction With the Strong CYP3A Inhibitor Voriconazole in Healthy Volunteers, Clin Pharmacol Ther, 106(6):1290–1298.

11. Bonate PL, Ahamadi M, Budha N, de la Peña A, Earp JC, Hong Y, et al., 2016, Methods and Strategies for Assessing Uncontrolled Drug-Drug Interactions in Population Pharmacokinetic

Analyses: Results From the International Society of Pharmacometrics (ISOP) Working Group, J Pharmacokinet Pharmacodyn, 43(2):123–135.

12. Elmeliegy M, Vourvahis M, Guo C, and Wang DD, 2020, Effect of P-Glycoprotein (P-gp) Inducers on Exposure of P-gp Substrates: Review of Clinical Drug-Drug Interaction Studies, Clin Pharmacokinet, 59(6):699–714.

13. Zamek-Gliszczynski MJ, Patel M, Yang X, Lutz JD, Chu X, Brouwer KLR, et al., 2021, Intestinal P-gp and Putative Hepatic OATP1B Induction: International Transporter Consortium Perspective on Drug Development Implications, Clin Pharmacol Ther, 109(1):55–64.

14. Chu X, Liao M, Shen H, Yoshida K, Zur AA, Arya V, et al., 2018, Clinical Probes and Endogenous Biomarkers as Substrates for Transporter Drug-Drug Interaction Evaluation: Perspectives From the International Transporter Consortium, Clin Pharmacol Ther, 104(5):836–864.

15. Willemin ME, Van Der Made TK, Pijpers I, Dillen L, Kunze A, Jonkers S, et al., 2021, Clinical Investigation on Endogenous Biomarkers to Predict Strong OAT-Mediated Drug-Drug Interactions, Clin Pharmacokinet, 60(9):1187–1199.

16. Mao J, Martin I, McLeod J, Nolan G, van Horn R, Vourvahis M, et al., 2017, Perspective: 4β-Hydroxycholesterol as an Emerging Endogenous Biomarker of Hepatic CYP3A, Drug Metab Rev, 49(1):18–34.

17. Galteau MM and Shamsa F, 2003, Urinary 6β-Hydroxycortisol: A Validated Test for Evaluating Drug Induction or Drug Inhibition Mediated Through CYP3A in Humans and in Animals, Eur J Clin Pharmacol, 59(10): 713–733.

18. Gass RJ, Gal J, Fogle PW, Detmar-Hanna D, and Gerber JG, 1998, Neither Dapsone Hydroxylation nor Cortisol 6β-Hydroxylation Detects the Inhibition of CYP3A4 by HIV-1 Protease Inhibitors, Eur J Clin Pharmacol, 54(9–10):741–747.

19. Rodrigues AD, Taskar KS, Kusuhara H, and Sugiyama Y, 2018, Endogenous Probes for Drug Transporters: Balancing Vision With Reality, Clin Pharmacol Ther, 103(3), 434–448.

20. Kikuchi R, Chothe PP, Chu X, Huth F, Ishida K, Ishiguro N, et al., 2023, Utilization of OATP1B Biomarker Coproporphyrin-I to Guide Drug–Drug Interaction Risk Assessment: Evaluation by the Pharmaceutical Industry, Clin Pharmacol Ther, 114(6):1170–1183.

21. Di L, Breen C, Chambers R, Eckley ST, Fricke R, Ghosh A, et al., 2017, Industry Perspective on Contemporary Protein-Binding Methodologies: Considerations for Regulatory Drug-Drug Interaction and Related Guidelines on Highly Bound Drugs, J Pharm Sci, 106(12):3442–3452.

22. Ryu S, Riccardi K, Patel R, Zueva L, Burchett W, and Di L, 2019, Applying Two Orthogonal Methods to Assess Accuracy of Plasma Protein Binding Measurements for Highly Bound Compounds, J Pharm Sci, 108(11):3745–3749.

23. Winiwarter S, Chang G, Desai P, Menzel K, Faller B, Arimoto R, et al., 2019, Prediction of Fraction Unbound in Microsomal and Hepatocyte Incubations: A Comparison of Methods Across Industry Datasets, Mol Pharm, 16(9):4077–4085.

24. Sun Y, Chothe PP, Sager JE, Tsao H, Moore A, Laitinen L, et al., 2017, Quantitative Prediction of CYP3A4 Induction: Impact of Measured, Free, and Intracellular Perpetrator Concentrations From Human Hepatocyte Induction Studies on Drug-Drug Interaction Predictions, Drug Metab Dispos, 45(6):692–705.

25. Chang C, Yang X, Fahmi OA, Riccardi KA, Di L, and Obach RS, 2017, An Exposure-Response Analysis Based on Rifampin Suggests CYP3A4 Induction Is Driven by AUC: an In Vitro Investigation, Xenobiotica, 47(8):673–681.

26. Haupt LJ, Kazmi F, Ogilvie BW, Buckley DB, Smith BD, Leatherman S, et al., 2015, The Reliability of Estimating $K_i$ Values for Direct, Reversible Inhibition of Cytochrome P450 Enzymes From Corresponding $IC_{50}$ Values: A Retrospective Analysis of 343 Experiments, Drug Metab Dispos, 43(11):1744–1750.

27. Cer RZ, Mudunuri U, Stephens R, and Lebeda FJ, 2009, $IC_{50}$-to-$K_i$: A Web-Based Tool for Converting IC50 to Ki Values for Inhibitors of Enzyme Activity and Ligand Binding, Nucleic Acids Res, 37(Web Server issue):W441–W445.

28. Wong SG, Ramsden D, Dallas S, Fung C, Einolf HJ, Palamanda J, et al., 2021, Considerations From the Innovation and Quality Induction Working Group in Response to Drug-Drug Interaction Guidance From Regulatory Agencies: Guidelines on Model Fitting and Recommendations on Time Course for In Vitro Cytochrome P450 Induction Studies Including Impact on Drug Interaction Risk Assessment, Drug Metab Dispos, 49(1):94–110.

29. Chu V, Einolf HJ, Evers R, Kumar G, Moore D, Ripp S, et al., 2009, In Vitro and In Vivo Induction of Cytochrome P450: A Survey of the Current Practices and Recommendations: A Pharmaceutical Research and Manufacturers of America Perspective, Drug Metab Dispos, 37(7):1339–1354.

30. Tátrai P, Schweigler P, Poller B, Domange N, de Wilde R, Hanna I, et al., 2019, A Systematic In Vitro Investigation of the Inhibitor Preincubation Effect on Multiple Classes of Clinically Relevant Transporters, Drug Metab Dispos, 47(7):768–778.

31. Farasyn T, Pahwa S, Xu C, and Yue W, 2021, Pre-incubation With OATP1B1 and OATP1B3 Inhibitors Potentiates Inhibitory Effects in Physiologically Relevant Sandwich-Cultured Primary Human Hepatocytes, Eur J Pharm Sci, 165:105951.

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

33. Fahmi OA, Maurer TS, Kish M, Cardenas E, Boldt S, and Nettleton D, 2008, A Combined Model for Predicting CYP3A4 Clinical Net Drug-Drug Interaction Based on CYP3A4 Inhibition, Inactivation, and Induction Determined In Vitro, Drug Metab Dispos, 36(8):1698–1708.

34. Ramsden D, Perloff ES, Whitcher-Johnstone A, Ho T, Patel R, Kozminski KD, et al., 2022, Predictive In Vitro-In Vivo Extrapolation for Time Dependent Inhibition of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 Using Pooled Human Hepatocytes, Human Liver Microsomes, and a Simple Mechanistic Static Model, Drug Metab Dispos, 50(2):114–127.

35. Einolf HJ, Chen L, Fahmi OA, Gibson CR, Obach RS, Shebley M, et al., 2014, Evaluation of Various Static and Dynamic Modeling Methods to Predict Clinical CYP3A Induction Using In Vitro CYP3A4 mRNA Induction Data, Clin Pharmacol Ther, 95(2):179–188.

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

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

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