Drug–Drug Interaction Studies

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Contents

Introduction 2

General DDI Considerations Inclusive Comparison of the DDI Guidelines EMA/FDA/PMDA 2

Nonclinical Assessment of DDI by In Vitro Investigations: Determination if a Drug Is a Victim or Perpetrator of a Potential DDI 4
Metabolism-Based DDIs 4
Transporter-Based DDIs 6

General Strategies for the Planning and Conduct of DDI Trials 6

Practical Considerations for DDI Trials 9
Study Design 9
Study Population 10
Choice of Substrate and Interacting Drugs 11
Complex Drug Interactions 14
Route of Administration 16
Dose Selection 16
Endpoints 16
Statistical Considerations, Clinical Relevance, and Sample Size 17
Cocktail Approaches 17
Pharmacogenomic Considerations 18
DDI as Part of Pop PK in Phase II and Phase III Trials 18

DDI Considerations for NBEs 19

References and Further Reading 20

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Abbreviations

ABC	ATP-binding cassette
AhR	Aryl hydrocarbon receptor
AUC	Area under the plasma concentration-time curve
BCRP	Breast cancer resistance protein
BSEP	Bile salt export pump
CAR	Constitutive androstane receptor
CYP	Cytochrome P450
FMO	Flavin monooxygenase
MAO	Monoamine oxidase
MATE	Multidrug and toxin extrusion
MRP	Multidrug resistance-associated protein
NTR	Narrow therapeutic range
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
PBPK	Physiologically-based pharmacokinetic
PD	Pharmacodynamics
P-gp	P- glycoprotein
PK	Pharmacokinetics
PXR	Pregnane X receptor
SLC	Solute carrier
TDI	Time dependent inhibition
UGT	Uridine diphosphate (UDP)-glucuronosyl transferase
XO	Xanthine oxidase

Introduction

Drug drug interaction (DDI) can result when one drug alters the pharmacokinetics of another drug or its metabolites. The assessment of pharmacokinetic DDIs during clinical development is a part of the general clinical pharmacology and safety assessment of a new investigational compound. Market withdrawals of drugs were frequently caused by DDIs which underlines the importance of addressing these issues during drug development. This is also reflected by the latest DDI (DDI) guidelines from European Medicines Agency EMA (2012), Food and Drug Administration (FDA) (2012), and Pharmaceuticals and Medical Devices Agency (2014). The details of all aspects which have to be considered in the design of DDI studies are outlined in the respective guidelines from EMA (2012), FDA (2012), and PMDA (2014). This section is aiming to give a summary of the respective considerations of these guidelines for the design of DDI studies and also contains many aspects of the respective guidelines including the most relevant decision trees and tables.

This chapter:

- Describes how an evaluation of DDI is performed from in vitro to in vivo studies within clinical development
- Reflects recent recommendations by authorities as regards the design, conduct, and reporting of DDI studies
- Presents the requirement to assess the clinical significance of DDIs

General DDI Considerations Inclusive Comparison of the DDI Guidelines EMA/FDA/PMDA

The main focus of this chapter is on pharmacokinetic DDIs. The understanding of the nature and magnitude of DDI is important for several reasons. Concomitant medications, dietary supplements, and some foods, such as grapefruit juice, may alter metabolism and/or drug transport abruptly in individuals who previously had been receiving and tolerating a particular dose of a drug. A respective alteration in metabolism or transport can change the known safety and efficacy of a drug. In a few cases, consequences of an interaction have led to the conclusion that the drug could not be marketed safely. Several drugs have been withdrawn from the market because of significant DDIs that led to, e.g., QT prolongation and Torsades de Pointes (TdP) arrhythmias, after warnings in drug labels did not adequately manage the risk of DDIs.

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

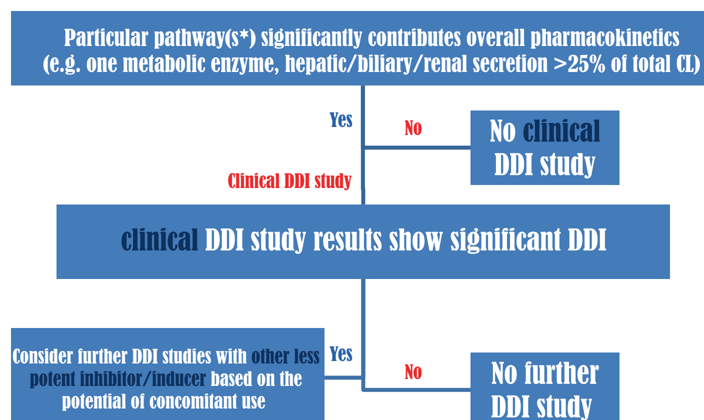

Fig. 1 General DDI decision tree for a drug as a victim for a DDI (*FDA requests the investigation on multiple enzymes)

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

Therefore, the development of an investigational drug should include identification of the principal routes of elimination, quantitation of the contribution by enzymes and transporters to drug disposition, and characterization of the mechanism of DDIs.

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

Human clinical studies to assess DDIs are either designed for a dedicated administration of a perpetrator and a victim drug or may contain simultaneous administration of a mixture of substrates of multiple CYP enzymes and transporters in one study (i.e., a "cocktail approach") to evaluate a drug's inhibition or induction potential.

All three DDI Guidelines (EMA-CHMP 2012; US-FDA 2012; PMDA. Pharmaceuticals & Medical Device Agency-Japan 2014) are displaying general decision trees as to when a drug has to be considered for a DDI trial, when the drug is a **victim** for an enzyme or drug transporter (Fig. 1)

Additionally also decision trees can be found in all three DDI Guidelines (EMA-CHMP 2012; US-FDA 2012; PMDA. Pharmaceuticals & Medical Device Agency-Japan 2014) which display as to when a drug has to be considered for a DDI trial, when the drug is acting as a **perpetrator** for an enzyme or drug transporter DDI (Fig. 2).

Additionally, complex DDIs, which can occur in specific populations (e.g., patients with organ impairment, and pediatric and geriatric patients), should be considered on a case-by-case basis. Moreover also PK modeling approaches (if well verified for intended purposes) can be helpful to guide the determination of the need to conduct specific DDI studies or even to avoid respective DDI studies in special cases.

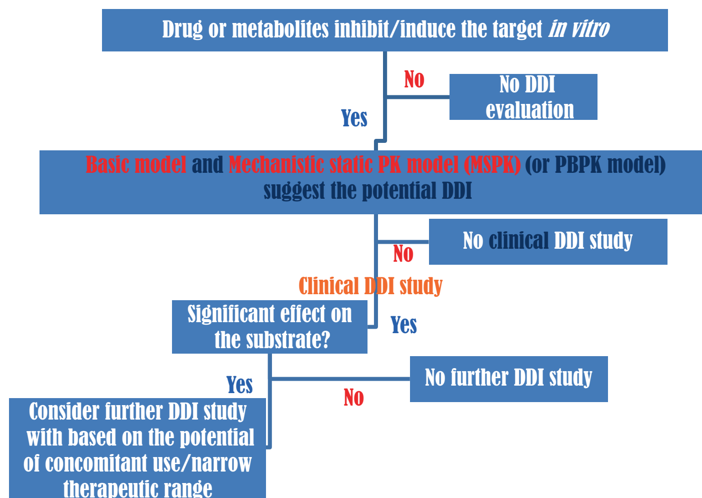

Fig. 2 General DDI decision tree for a drug acting as a perpetrator for a DDI

Nonclinical Assessment of DDI by In Vitro Investigations: Determination if a Drug Is a Victim or Perpetrator of a Potential DDI

The drug development process should include evaluation of a new drug's potential to affect the metabolism or transport of other drugs and the potential for the new drug's metabolism or transport to be affected by other drugs. Use of in vitro tools to determine whether a drug is a substrate, inhibitor, or inducer of metabolizing enzymes or drug transporters, followed by in vivo interaction studies to assess potential interactions, has become an integral part of drug development and regulatory review. These results will be the basis for the determination of a clinical DDI study is needed or not. Authorities may consider in vitro data sufficient to exclude DDI liabilities if there is a clear indication from parameters outlined in regulatory documents (e.g., $[I]/K_i$ for CYP-based interactions in the absence of liver partitioning) of little or no DDI potential. These negative in vitro data can obviate the need for further in vivo clinical activities considering the pathways that were excluded from being clinically meaningful.

This section will separately discuss in vitro investigations at the levels of metabolizing enzymes and transporters. Also general considerations for situations when complex or multiple DDI mechanisms will be presented will be briefly described.

Metabolism-Based DDIs

Hepatic metabolism occurs primarily through the cytochrome P450 family (CYP) of enzymes located in the hepatic endoplasmic reticulum, but may also occur through non-CYP enzyme systems, such as glucuronosyl- and sulfo-transferases, which can, in general, inactivate a drug and increase its renal elimination. Some drug metabolizing enzymes are present in the gut wall and other extrahepatic tissues, in addition to the liver. Many metabolic routes of elimination can be inhibited or induced by concomitant drug treatment. Metabolic DDIs can cause substantial changes (an order of magnitude or more decrease or increase in the blood and tissue concentrations of a drug or metabolite) and can also significantly affect the extent to which toxic or active metabolites are formed. These large changes in exposure can alter the safety and efficacy profile of a drug and its active metabolites, regardless of whether the drug has a narrow therapeutic range (NTR).

Nonclinical in vitro experiments provide data on DDI potentially mediated by CYP enzymes (or others like (Uridyl diphosphate (UDP))-glucuronosyltransferases) using systems such as human liver microsomes, expressed human recombinant enzymes, and hepatocytes. These data are used in order to determine the substrate specificity of an NCE for a specific metabolic enzyme, the inhibitory potential (half maximal inhibitory concentration (IC_{50}), inhibition

Table 1 Targets to be examined: whether the drug is a **substrate** or not

	FDA	EMA	PMDA
Metabolic enzymes			
CYPs [recommendation]	CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A [2A5, 2 J12,4F2, 2E1]	CYP1A2, 2B5, 2C8, 2C9, 2C19, 2D6, 3A	CYP1A2, 2B5, 2C8, 2C9, 2C19, 2D6, 3A [2A5, 2 J12,4F2, 2E1]
UGTs	UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15[b]	Not specified	UGT1A1, 1A3, 1A4, 1A5, 1A9, 2B7, 2B15[b]
Others [recommendation]	MAO, FMO, XO, ALDH, ADH	Not specified	MAO, FMO, XO, AO, ALDH, ADH
Transporters			
Gut and systemic	P-gp, BCRP	Not specified	P-gp, BCRP
Hepatic ($CL_H > 25\%$ of CL_{tot})		CATP1B1/1B3[a]	
Renal ($CL_{R,\ secretion} > 25\%$ of CL_{tot})	OCT2, OAT1/3	Not specified	OCT2, OAT1/3, MATE1/2-K

[a]In case the autoradiography (ARG) in animal shows significant accumulation in the liver (PMDA)
[b]Require the identification of UGT subtype
[c]In case the drug is not metabolized by major CYPs

Table 2 Targets to be examined: whether a drug is an **inhibitor** or not

	FDA	EMA	PMDA
Metabolic enzymes			
CYPs	CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A	CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A*	CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A*
UGTs	UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15	UGT1A1, 2B7	UGT1A1, 2B7
Transporters			
Gut and systemic	P-gp, BCRP		
Hepatic ($CL_H > 25\%$ of CL_{tot}) [recommendation]	OA1P1B1/1B3 [MRPs, BSEP]	OATP1B1/1B3, [BSEP, OCT1]	OATP1B1/1B3, [MRP2, BSEP, OCT1]
Renal ($CL_{R,\ secretion} > 25\%$ of CL_{tot}) [recommendation]	OC12, OAT1/3 [MRPs, MA1E1/2-K]	OCT2,OAT1/3 [MATE1/2-K]	OCT2,OAT1/3, MATE1/ 2-K, [MRP2, 4]

*For CYP3A4, investigation with multiple substrate with different binding site are required

constant (K_i) for competitive inhibitors, and rate of enzyme inactivation (k_{inact}) for mechanism-based inhibitors) or induction in case of enzyme inducers (rate of metabolism). The evaluation of CYP enzyme induction may begin with studies of CYP1A2, CYP2B6, and CYP3A in vitro. If the in vitro induction results are positive according to predefined thresholds using basic models, the investigational drug is considered an enzyme inducer and further in vivo evaluation may be warranted

An overview as regards which metabolic enzymes to be examined based on FDA, EMA, and PMDA guideline, e.g., whether a drug is a substrate, inhibitor, or inducer or not is given in Tables 1, 2, and 3.

Table 3 Targets to be examined: whether the drug is an **inducer** or not

	FDA	BVIA	PMDA
Metabolic enzymes			
Enzyme (transcriptional factor)	CYP3A4/5 (PXR),[if positive, CYP2C8, 2C9, 2C19]CYP1A2 (AhR),CYP2B6 (CAR)	CYP3A4/5 (PXR), CYP1A2 (AhR), CYP2B6 (CAR)	CYP3A4/5 (PXR),[if positive, CYP2C9 e t al] CYP1A2 (AhR), CYP2B6 (CAR)
Transporter			
	"Methods for in vitro evaluation are not well understood" "Should consult with FDA about studying induction in vivo"	P-gp (in case PXR and/or CAR mediated induction observed)	Not mentioned

Transporter-Based DDIs

Although less well-recognized than metabolizing enzymes, membrane transporters can have important effects on pharmacokinetics and drug exposure. To date, most identified transporters belong to one of two superfamilies: ATP-binding cassette (ABC) and solute carrier (SLC). Transporters govern the transport of solutes (e.g., drugs and other xenobiotics) in and out of cells. In contrast to metabolizing enzymes, which are largely concentrated in the liver and intestine, transporters are present with varying abundance in all tissues in the body and play important roles in drug distribution, tissue-specific drug targeting, drug absorption, and elimination. Transporters can also work in concert with metabolizing enzymes (see also ▶ "Complex DDI Interactions")

A number of transporter-based interactions have been documented in recent years. Analogous to drug interactions mediated by P450 enzymes, coadministration of a drug that is an inhibitor or an inducer of a drug transporter may affect the pharmacokinetics of a drug that is a substrate for that transporter. Transporters can affect the safety profile of a drug by affecting the concentration of a drug or its metabolites in various tissues. Transporter-based drug interactions and the potential effect of drug transporters on safety make it important to determine whether transporters affect the absorption and disposition of an investigational drug and whether the investigational drug can affect the absorption and disposition of other drugs through an effect on transporters.

The effect of a compound on drug transporter function will be investigated, e.g., in bidirectional transport experiments in tissue cultures. Results of these experiments show whether the investigated compound is a drug transporter substrate (by determining the net transport rate, efflux ratio, or Michaelis constant (Km)) or an inhibitor of the transporter (by IC_{50} or K_i values). Because of the lack of a validated in vitro system to study transporter induction, the definitive determination of induction potential of an investigational drug on transporters is based on in vivo induction studies.

An overview as regards which drugs transporters to be examined based on FDA, EMA and PMDA guideline, e.g., whether a drug is a substrate, inhibitor, or inducer or not is given in Tables 1, 2, and 3.

General Strategies for the Planning and Conduct of DDI Trials

The evaluation of a DDI potential for a compound is at first based on all in vitro data collected for a compound to whether the drug is a substrate, inhibitor, or inducer or a metabolic enzyme or drug transporter in relation to the (expected) in vivo plasma concentrations (e.g., maximum plasma concentrations). The respective cut offs, which have to be considered, are given based on a "basic model" for reversible, time-dependent inhibition and also induction. Respective overviews as regards specific recommendations (e.g., use of unbound or total drug concentrations) and cut off values (as to whether a clinical DDI trial needs to

Table 4 Basic model: for reversible inhibition (R = 1 + [I]/Ki or IC50)

	FDA	EMA	PMDA
Metabolic enzyme: Reversible inhibition			
Systemic	[I] = total C_{max} $R > 1.1$	[I] = unbound C_{max} $R \geq 1.02$	[I] = total C_{max} $R > 1.1$
Gut	$[I]_G$ = Dose/250 mL, R > (≥) 11		
Transporter: Reversible inhibition			
Systemic			
P-gp, BCRP	[I] = total C_{max} $R \geq 1.1$	[I] = unbound C_{max} $R \geq 1.02$	[I] = total C_{max} $R \geq 1.1$
CAT1,3, OCT2 **MATE1, 2-K(PMDA)**	[I] = unbound C_{max} $R \geq 1.1$	[I] = unbound C_{max} $R \geq 1.02$	[I] = unbound C_{max} $R \geq 1.25$
CA7P1B1,3	[I] = total C_{max} $R \geq 1.1$ and [I] = unbound $C_{max,\ inlet}$[a] $R \geq 1.25$	[I] = unbound $C_{max,\ inlet}$[a] $R \geq 1.04$	[I] = unbound $C_{max,\ inlet}$[a] $R \geq 1.25$
Gut			
P-gp, BCRP	$[I]_G$ = Dose/250 mL, R > (≥) 11		

[a]$C_{max,inlet}$ is calculated as $f_{u,b}$ x ($[I]_{max,b}$ + F_a x F_g x k_a x Dose/Q_h)

Table 5 Basic model: time-dependent inhibition (TDI)

	FDA	EMA	PMDA
Time dependent inhibition (TDI)			
Systemic	[I] = total C_{max} $R > 1.1$	[I] = unbound C_{max} $R \geq 1.25$	[I] = total C_{max} $R > 1.1$
Gut	$[I]_G$ = Dose/250 mL $R > 11$	$[I]_G$ = Dose/250 mL $R \geq 1.25$	$[I]_G$ = Dose/250 mL $R > 11$

Table 6 Basic model: induction [R = 1 + Emax × [I]/(EC50 + [I])]

	FDA	EMA	PMDA
Metabolic enzyme induction			
mRNA change	> predefined threshold	>2-fold (concentration dependent increase) or 20% increase of the increase in positive control[a]	
Cut off value	[I] = total C_{max} $R < 0.9$	[I] = unbound C_{max} R: Not defined	[I] = total C_{max} $R < 0.9$
Transporter induction			
	Not mentioned		

[a]It is acceptable to use the enzyme activity as a measure, in case that the inhibition of enzyme can be clearly denied (PMDA)

be performed) of the guidelines from different authorities are given in Tables 4, 5, and 6. Mechansitic static models and/or more comprehensive dynamic models (e.g., physiologically based PK (PBPK) models) may be used additionally, and specific recommendations can be found in the guidelines from FDA, EMA, and PMDA. It should be noted that currently only the FDA provides a dedicated flowchart how to explore DDI potential with PBPK models. The recommended cases to use PBPK (EMA/FDA) are to predict DDI's worst case scenarios (additive "multiple DDI mechanisms" combined with, e.g., organ impairment), dose-dependent DDIs, the effect of a less potent inhibitor, or the impact of a DDI in subpopulations.

The initial approach to assessing clinical DDIs is the evaluation of underlying mechanisms with

Table 7 Overview as regards inhibitor, inducer, and substrate lists provided by authorities

	FDA	EMA	PMDA
Metabolic enzyme (in vitro/in vivo)			
Inhibitor	×/◎	○/◎	○/◎
Inducer	◎/◎	×/×	○/◎
Substrate	×/○	○/○	○/◎
Transporter (in vitro/in vivo)			
Inhibitor	×/○	×/×	◎/○
Inducer	×/○	×/×	◎/○
Substrate	×/○	×/×	◎/○

◎: listed with intensity for each drug (i.e., classification [weak, moderate, or strong] or Ki value), ○: listed, ×: not listed

probe compounds. Examples of appropriate probe compounds that are considered to be specific and representative for a defined metabolic pathway or drug transporter are defined in current regulatory guidelines (e.g., (1,2,3)). A brief overview, which inhibitors, inducers, and substrates are specified in the respective guidelines, can be found in Table 7.

If a clinical relevant DDI cannot be excluded through screening with a probe compound, further clinical DDI evaluations may become necessary (see Figs. 1 and 2). The demonstration of a relevant DDI in a study with a probe compound can result in the need of further studies with concomitant medications for the NME (Figs. 1 and 2) to determine:

1. Whether additional studies are needed to better quantify the effect and to examine the effects of weaker inhibitors (early studies usually examine strong inhibitors) on the investigational drugs as substrates and effects of investigational drugs (as inhibitors) on a range of substrates.
2. Whether dosage adjustments or other prescribing modifications (e.g., additional safety monitoring or contraindications) are needed based on the identified interaction(s) to avoid undesired consequences. Drug interaction information is used along with information about exposure-response relationships in the general population and specific populations, to help predict the clinical consequences of DDIs.

Should in vitro data show that a drugis an inhibitor or inducer of enzymes, it may be recommended to conduct a DDI study as early as possible in clinical development in order to exclude any possible liability of this interaction. For substrates of specific drug metabolizing or drug transporter pathways, the timing of DDI in vivo clinical studies depends on the safety range and on the frequency of co-medications that would act as inhibitor on the compound especially during Phase II or III.

Independently of underlying mechanisms for potential DDIs, it has to be evaluated whether compounds with narrow therapeutic windows (relevant co-medications) in the targeted therapeutic area need to be evaluated in addition to the before mentioned studies which use probe drugs. It should be noted that DDI studies with specific co-medications might be necessary in order to have a specific label of no clinically relevant DDI.

In general, the described principles of nonclinical and clinical assessment of DDI are also valid for oncological NCEs. However, studies in healthy volunteers might not be possible due to low tolerability of the compound. Furthermore, study design options might be limited due to a reduced clinical state of the patients. In general, all "combination trials" of standard chemotherapy together with a NME should be designed to investigate possible DDIs between the different compounds in light of this document (approaches to be discussed on a case-by-case basis).

Practical Considerations for DDI Trials

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

Study Design

In vivo DDI studies generally are designed to compare substrate concentrations with and without the interacting drug. Because a specific study can address a number of questions and clinical objectives, many study designs for investigating DDI can be considered. In general, crossover designs in which the same subjects receive substrate with and without the interacting drug are more efficient. A study can use a randomized crossover (e.g., Substrate (S) followed by S+ Inhibitor (I), S + I followed by S), one-sequence crossover (e.g., S followed by S + I), or a parallel (S in one group of subjects and S + I in another group) design, and there may be reasons to have another period when the I is removed to assess effect duration. The following possible dosing regimen combinations for a substrate and interacting drug can also be used: single dose/single dose, single dose/multiple dose, multiple dose/single dose, and multiple dose/multiple dose. Additional factors include consideration of the sequence of administration and the time interval between dosing of substrate and inhibitor/inducer. The selection of a study design depends on a number of factors for both the substrate and interacting drug, including:

1. Whether the substrate and/or interacting drug is used acutely or chronically
2. Safety considerations, including whether a substrate is a NTR drug (NTR drugs are defined as those drugs for which there is little separation between therapeutic and toxic doses or the associated blood or plasma concentrations) or non-NTR drug
3. Pharmacokinetic and pharmacodynamic characteristics of the substrate and interacting drugs
4. Whether there is a desire to assess induction as well as inhibition
5. Whether the inhibition is delayed
6. Whether there is a need to assess persistence of inhibition or induction after withdrawal of the interacting drug

The interacting drugs and the substrates should be dosed so that the exposures of both drugs are relevant to their clinical use, including the highest doses likely to be used in clinical practice, and plasma levels of both drugs should be obtained to show this. The following considerations may be useful:

- When attainment of steady state is important (especially for the drug being the perpetrator drug), and either the substrate or interacting drug or their metabolites have long half-lives, one or both periods of a crossover study should be long, but several other approaches can be considered, depending on pharmacokinetic characteristics of the drug and metabolites. For example, if the substrate has a long half-life, a loading dose could be used to reach steady-state concentrations earlier in a one-sequence crossover followed by an S + I period long enough to allow I to reach steady state (here too, using a loading dose could shorten that period).
- When it is important that a substrate and/or an interacting drug be studied at steady state for a long duration because the effect of an interacting drug is delayed, as is the case for inducers and time-dependent imhibition (TDI), documentation that near steady state has been attained for the pertinent substrate drug and metabolites as well as the interacting drug is critical, and both S and I should be present long enough to allow the full effect to be seen. This documentation can be accomplished by

sampling over several days prior to the periods when test samples are collected. This information is important for metabolites and the parent drug, particularly when the half-life of the metabolite is longer than the parent. It is also important when the interacting drug and metabolites both are metabolic inhibitors (or inducers). Finally, it is critical to evaluate the time it takes for the enzyme activities to return to normal when induction or TDI is involved so that a third crossover period in which the interacting drug (I) is removed will generally be recommended.

- Studies can usually be open label (unblinded), unless pharmacodynamic endpoints (e.g., adverse events that are subject to bias) are critical to the assessment of the interaction.
- For a rapidly reversible inhibitor, administration of the interacting drug either just before or simultaneously with the substrate on the test day might increase sensitivity by ensuring maximum exposure to the two drugs together. For a mechanism-based inhibitor (a drug that requires metabolism before it can inactivate the enzyme; an example is erythromycin), administration of the inhibitor prior to the administration of the substrate drug can maximize the effect. If the absorption of an interacting drug may be affected by other factors (e.g., the gastric pH), it may be appropriate to control the variables or confirm the absorption through plasma level measurements of the interacting drug.
- Timing of administration may be critical in situations of concurrent inhibition and induction. For example, if the investigational drug is a substrate for both enzymes and OATP, and rifampin is used as an enzyme inducer, the simultaneous administration of the drug with rifampin (an OATP inhibitor) may underestimate enzyme induction, so delayed administration of the substrate is recommended. The optimal delayed time should be determined. In addition, it is critical to evaluate the duration of the interaction effect after the interacting drug has been removed.
- When the effects of two drugs on one another are of interest, the potential for interactions can be evaluated in a single study or two separate studies. Some design options are randomized three-period crossover, parallel group, and one-sequence crossover.
- To avoid variable study results because of uncontrolled use of dietary/nutritional supplements, tobacco, alcohol, juices, or other foods that may affect various metabolizing enzymes and transporters during in vivo studies, it is important to exclude, when appropriate, subjects who used prescription or over-the-counter medications, dietary/nutritional supplements, tobacco, or alcohol within 1 week prior to enrollment. In addition, investigators should explain to subjects that for at least 1 week prior to the start of the study until its conclusion.
- Because interactions might differ in subgroups of different pharmacogenetic genotypes, genotyping for the enzymes and transporters involved in the interaction should be carried out when appropriate.
- Detailed information on the dose given and time of administration should be documented for the coadministered drugs.

Study Population

In most situations, clinical DDI studies can be performed using healthy volunteers, and findings in healthy volunteers will predict findings in the patient population for which the drug is intended. Safety considerations, however, may preclude the use of healthy subjects in studies of certain drugs. In addition, there are circumstances in which subjects drawn from the intended patient population offer advantages, including the opportunity to study pharmacodynamic endpoints not present in or relevant to healthy subjects. The extent of drug interactions (inhibition or induction) may be different depending on the subjects' genotype for the specific enzyme or transporter being evaluated. For example, subjects lacking the major polymorphic clearance pathway will show reduced total metabolism or transport. However, alternative pathways can become quantitatively more

important in these subjects. In such cases, the alternative pathways should be understood and studied appropriately. Thus, phenotype or genotype determinations to identify genetically determined metabolic or transporter polymorphisms are important when evaluating effects on enzymes or transporters with polymorphisms, such as CYP2D6, CYP2C19, CYP2C9, UGT1A1, and OATP1B1 (SLCO1B1). In addition, it is valuable to specify the need for stratifying the population based on genotype while conducting the DDI studies. Another alternative is to consider powering the study for the genotype status that is likely to have the highest potential for interaction.

Choice of Substrate and Interacting Drugs

CYP-Mediated Interactions

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

When testing an investigational drug for the possibility that its metabolism is inhibited or induced (i.e., being the victim drug of a DDI as a substrate), selection of the interacting drug can then be based on known, important inhibitors and inducers of the pathway under investigation. Strong inhibitors and inducers provide the most sensitive assessment and should generally be tested first. Consider, for example, an investigational drug metabolized by CYP3A with the contribution of this enzyme to the overall elimination of this drug that is either substantial ($\geq$ 25% of the clearance pathway) or unknown. In this case, the inhibitor and inducer can be itraconazole and rifampin, a strong inhibitor and a strong inducer, respectively. Respective strong inhibitors or inducers should be looked after in the respective sections of the guidelines from FDA, EMA, and PMDA or in the most current literature. If the study results are negative, then absence of a clinically important DDI for the metabolic pathway is demonstrated. If the clinical study of the strong inhibitor or inducer is positive, effects through in vivo studies of other less potent specific inhibitors or inducers may be needed to be evaluated. If the investigational drug is metabolized by CYP3A and its plasma AUC (Area under the plasma concentration time curve) is increased fivefold or higher by strong CYP3A inhibitors, it is considered a *sensitive substrate* of CYP3A. The labeling would indicate that the drug is a "sensitive CYP3A substrate" and that its use with strong or moderate inhibitors may call for caution, depending on the drug's exposure-response relationship.

For further information as regards the labeling of respective DDI effects, please look at the respective section of the DDI guidelines from EMA, FDA, and PMDA.

If a drug is metabolized by a polymorphic enzyme (such as CYP2D6, CYP2C9, 1327 CYP2C19, or UGT1A1), the comparison of pharmacokinetic parameters of this drug in poor metabolizers and extensive metabolizers may substitute for an interaction study for that particular pathway, as the PK in the poor metabolizers will indicate the effect of a strong inhibitor. When the study suggests the presence of a significant interaction with strong inhibitors or in poor metabolizers, further clinical DDI evaluation, e.g., with weaker inhibitors or intermediate metabolizers, may be recommended (additionally also mechansitic modeling approaches may be used supporting respective investigantions).

The Investigational Drug as an Inhibitor or an Inducer of CYP Enzymes: Effect of Investigational Drugs on Other Drugs

When studying an investigational drug as the interacting drug (being the perpetrator drug), the choice of substrates (approved drugs) for initial in vivo studies depends on the P450 enzymes affected by the interacting drug. When testing inhibition, the substrate selected should generally be one whose pharmacokinetics are markedly altered by the coadministration of known specific inhibitors of the enzyme systems (sensitive substrates) to see the largest impact of the interacting investigational drug. Examples of such substrates include (refer also to the respective section in the EMA, FDA, and PMDA DDI guidelines and most recent literature):

1. Midazolam for CYP3A
2. Theophylline for CYP1A2
3. Bupropion for CYP2B6
4. Repaglinide for CYP2C8
5. Warfarin for CYP2C9 (with the evaluation of S-warfarin)
6. Omeprazole for CYP2C19
7. Desipramine for CYP2D6

If the initial study determines that an investigational drug either inhibits or induces metabolism of sensitive substrates, further studies using other substrates, representing a range of therapeutic classes, based on the likelihood of coadministration, may be useful. If the initial study with the most sensitive substrates is negative, it can be presumed that less sensitive substrates also will be unaffected. It should be noted that several of the substrates recommended for drug interaction studies are not specific because they are substrates for more than one CYP enzyme or may be substrates for drug transporters. While a given substrate may not be metabolized by a single enzyme (e.g., dextromethorphan elimination is carried out primarily by CYP2D6 but other enzymes also contribute in a minor way), its use in an interaction study is appropriate if the inhibitor (the investigational drug) to be evaluated is selective for the CYP enzyme of interest. If an investigational drug is a CYP inhibitor, it may be classified as a strong, moderate, or weak inhibitor based on its effect on a sensitive CYP substrate. For example, CYP3A inhibitors can be classified based on the magnitude of the change in plasma AUC of oral midazolam or other CYP3A substrates that are similar in characteristics (e.g., fm (%clearance contributed by CYP3A), half-life, not subject to transporter effect) as midazolam, when the substrate is given concomitantly with the inhibitor. If the investigational drug increases the AUC of oral midazolam or other CYP3A substrate by fivefold or higher ($\geq$ fivefold), it can be considered a *strong* CYP3A inhibitor. If the investigational drug, when given at its highest dose, increases the AUC of oral midazolam or other sensitive CYP3A substrates by between two- and fivefold ($\geq$ two- and $<$fivefold), it can be considered a *moderate* CYP3A inhibitor. If the investigational drug, when given at the highest dose and shortest dosing interval, increases the AUC of oral midazolam or other sensitive CYP3A substrates by between 1.25- and 2-fold ($\geq$1.25- and $<$2-fold), it can be considered a *weak* CYP3A inhibitor.

When an in vitro evaluation does not rule out the possibility that an investigational drug is an inducer of CYP3A, an in vivo evaluation can be conducted using the most sensitive substrate (e.g., oral midazolam). When midazolam, is coadministered orally following the administration of multiple doses of the investigational drug, and there is no interaction, it can be concluded that the investigational drug is not an inducer of CYP3A (in addition to the conclusion that it is not an inhibitor of CYP3A). A caveat to this interpretation is that if the investigational drug is both an inducer and inhibitor of CYP3A, such as ritonavir, the net effect at any time it is introduced may vary. In this case, the net effect of the drug on CYP3A function may be time dependent. In vivo induction evaluations have often been conducted using oral contraceptives as the substrate. However, oral contraceptives are not the most sensitive substrates for CYP3A, so a negative result does not exclude the possibility that the investigational drug is an inducer of CYP3A. Some compounds listed as sensitive substrates for the other enzymes can also be used as substrates with the investigational drug as an inducer. For example, omeprazole and repaglinide are CYP2C19 and CYP2C8 substrates, respectively, but they are also metabolized by CYP3A. If omeprazole is used as a substrate to study CYP2C19 induction, measurement of its metabolites (CYP2C191397-mediated hydroxy-omeprazole and CYP3A4-mediated omeprazole sulfone) will be recommended for the interpretation of the study results.

Transporter-Mediated Interactions

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

transporters should be evaluated during drug development.

In the most recent guidances from EMA, FDA, and PMDA, BCRP, OATP, OATs, and OCTs are considered important transporters (see also Tables 1, 2, and 3) in addition to P-gp and should be routinely evaluated. Because the field of transporter pharmacology is rapidly evolving, other transporters (e.g., multidrug resistance-associated proteins (MRPs), multidrug and toxin extrusion (MATE) transporters, and bile salt export pump (BSEP) transporters) should be considered when appropriate.

The Investigational Drug as a Substrate of Transporters – The Effect of Other Drugs on an Investigational Drug

When testing an investigational drug for the possibility that its transport is inhibited or induced (i. e., as a substrate), selection of the interacting drugs should be based on in vitro or in vivo studies identifying the transporters that are involved in the absorption and disposition of the investigational drug (e.g., absorption and efflux in the gastrointestinal tract, uptake and secretion in the liver, and the secretion and reabsorption in the kidney). The choice of the interacting drug should be based on known, important inhibitors of the pathway under investigation. Strong inhibitors provide the most sensitive assessment and should generally be tested first. As there is overlapping selectivity in substrate and inhibitor among transporters, negative results from a study using a broad inhibitor may rule out the possibility for drug interaction mediated by multiple pathways. For example, it may be appropriate to use an inhibitor of many transporters (e.g., cyclosporine, which inhibits P-gp, OATP, and BCRP) to study its effect on a drug that may be a substrate for these transporters. A negative result rules out the involvement of these transporters in the drug's disposition. However, if the result is positive, it will be difficult to determine the relative contribution of each transporter to the disposition of the substrate drug. In contrast, if the goal of the study is to determine the role of a specific pathway in the PK of a substrate drug, then a selective and potent inhibitor for that transporter should be used. As an alternative, comparative PK of an investigational drug in subjects with different genotypes of specific transporters can be evaluated to determine the importance of a specific transporter in the clearance pathway for the drug. On the other hand, polymorphism data on P-gp is controversial and may not be used to determine the role of P-gp in the disposition of investigational drugs that are substrates of P-gp.

The Investigational Drug as an Inhibitor or an Inducer of Transporters – Effect of the Investigational Drugs on Other Drugs

When studying an investigational drug as the interacting drug, the choice of substrates for initial in vivo studies depends on the transport pathway that may be affected by the interacting drug. In general, when testing inhibition, the substrate selected should be one whose pharmacokinetics are markedly altered by coadministration of known specific inhibitors of the transporter pathway to see the largest impact of the interacting investigational drug. The choice of substrates can also be determined by the therapeutic area of the investigational drug and the probable coadministered drugs that are known substrates for transporters (respective lists of selected substrates for transporters can be found in the respective guidelines of the EMA, FDA and PMDA guideline and most recent literature). However, because many drugs are substrates of multiple transporters or enzymes, specific substrates for each transporter are not available. The observed clinical interactions may be a result of inhibition of multiple pathways if the investigational drug is also an inhibitor for the same multiple pathways.

Because of the lack of a validated in vitro system to study transporter induction, the definitive determination of induction potential of an investigator on transporters is based on in vivo induction studies. For example, because of similarities in the mechanisms of CYP3A and P-gp induction, information from the testing of CYP3A inducibility can inform decisions about P-gp. If an

investigational drug is found not to induce CYP3A in vitro, no further tests of CYP3A and P-gp induction in vivo are necessary. If a study of the investigational drug's effect on CYP3A activity in vivo is indicated from a positive in vitro screen, but the drug is shown not to induce CYP3A in vivo, then no further test of P-gp induction in vivo is necessary. However, if the in vivo CYP3A induction test is positive, then an additional study of the investigational drug's effect on a P-gp probe substrate is recommended. If the drug is also an inhibitor for P-gp, then the induction study can be conducted with the inhibitor study using a multiple-dose design.

EMA, FDA, and PMDA DDI guidelines contain valuable information regarding the classification of in vivo inhibitors or inducers for CYP enzymes, examples of sensitive in vivo CYP substrates and CYP substrates with narrow therapeutic ranges, examples of in vivo inhibitors and inducers of selected transporters, examples of in vivo substrates of selected transporters and examples of in vivo CYP3A and P-gp inhibitors and their relative potency.

Complex Drug Interactions

The above sections separately discussed DDIs related to effects on enzymes and transporters, but drug interactions for a specific drug may occur based on a combination of mechanism and have to be taken in consideration, when a clinical DDI trial needs to be designed.

Such "complex drug interaction" scenarios include, but are not limited to:

- Concurrent inhibition and induction of one enzyme or concurrent inhibition of enzyme and transporter by a drug
- Increased inhibition of drug elimination by the use of more than one inhibitor of the same enzyme that metabolizes the drug
- Increased inhibition of drug elimination by use of inhibitors of more than one enzyme that metabolizes the drug
- Inhibition by a drug and its metabolite or metabolites, both of which inhibit the enzyme that metabolizes the substrate drug
- Inhibition of an enzyme other than the genetic polymorphic enzyme in poor metabolizers taking substrate that is metabolized by both enzymes

Multiple CYP Inhibitors

There may be situations when an evaluation of the effect of multiple CYP inhibitors on the drug can be informative. For example, it may be appropriate to conduct a DDI study with more than one inhibitor simultaneously if all of the following conditions are met:

1. The drug exhibits blood concentration-dependent important safety concerns.
2. Multiple CYP enzymes are responsible for the metabolic clearance of the drug.
3. The predicted residual or noninhibitable drug clearance is low.

Under these conditions, the effect of multiple CYP-selective inhibitors on the investigational drug's blood AUC may be much greater than when the inhibitors are given individually with the drug, and more than the product of changes in AUC observed with each individual inhibitor. The magnitude of the combined effect will depend on the residual fractional clearance (the smaller the fraction, the greater the concern) and the relative fractional clearances of the inhibited pathways. Modeling and simulation approaches can help to project the magnitude of the effect based on single pair drug interaction studies. If results from a study with a single inhibitor have already triggered a major safety concern (i.e., a contraindication), multiple inhibitor studies are unlikely to add value.

Enzyme/Transporter Interplay

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

necessarily have similar inhibition potency on CYP3A and P-gp. To assess the worst case scenario for a dual CYP3A and P-gp substrate, inhibition should be studied using an inhibitor that shows strong inhibition for both P-gp and CYP3A, e.g., such as itraconazole. However, under this condition, if the result is positive, specific attribution of an AUC change to P-gp or CYP3A4 may not be possible. If the goal is to determine the specific contribution of CYP3A or P-gp on the AUC change, then a strong inhibitor for CYP3A only or a potent inhibitor for P-gp only should be selected to discern the effect of CYP3A versus P-gp.

In addition to the possibility that a drug is an inhibitor or inducer of multiple enzymes/transporters, a drug can be an inhibitor of one enzyme/transporter and inducer of another enzyme/transporter. For example, rifampin, an established inducer of multiple CYP enzymes and transporters, was recently found to be an inhibitor of the uptake transporter OATP1B1 and may inhibit the uptake of an investigational drug that is a substrate of OATP1B1. Accordingly, if a drug is a CYP enzyme substrate and an OATP1B1 substrate, an induction study with rifampin should be designed and interpreted carefully. The net steady-state effect may vary depending on the relative size of the individual effect on transporter and enzyme activities. Timing of administration may become critical in situations when both enzymes and transporters can be affected. These overlapping selectivities contribute to complex drug interactions and make the prediction of in vivo outcome based on in vitro evaluation challenging or impossible (Zhang et al. 2009a, b). The implications of simultaneous inhibition of a dominant CYP enzyme(s) and an uptake or efflux transporter that controls the availability of the drug to CYP enzymes can be just as profound as that of multiple CYP inhibition. For example, the large effect of coadministration of itraconazole and gemfibrozil on the systemic exposure (AUC) of repaglinide may be attributed to collective inhibitory effects on both the enzyme (CYP2C8) and transporters (OATP1B1) by itraconazole and gemfibrozil and their respective metabolites.

Effect of Organ Impairment

Another type of complex drug interaction is the coadministration of substrate and enzyme/transporter inhibitor in subjects with organ impairment. For example, if a substrate drug is eliminated through both hepatic metabolism and renal secretion/filtration, the use of an enzyme inhibitor in subjects with renal impairment may cause a more than projected increase in exposure of substrate drug based on individual effect alone. Unfortunately, current knowledge does not permit the presentation of specific guidance for studying some of these complex drug interaction scenarios because dedicated in vivo studies in humans may not be feasible or may raise ethical and practical considerations. Modeling and simulation approaches integrating prior in vitro and in vivo ADME and drug interaction data may be useful for evaluating complex drug interactions. For example, results from dedicated single pair drug interaction studies and separate pharmacokinetic evaluation in subjects with organ impairment may provide useful information to strengthen the model for the evaluation of complex drug interactions.

Pediatrics and Geriatrics

Age-related changes in physiological processes governing drug disposition and drug effect have been investigated. In some cases, disproportional alterations in binding proteins, drug metabolizing enzymes and/or transporters, and renal filtration/secretion caused by developmental changes have been known to result in different drug disposition characteristics in pediatric and geriatric populations. However, dedicated drug interaction studies in these populations may not be feasible. Simulations using system biology approaches such as PBPK models may be helpful to predict drug interaction potential when the model can be constructed based on sufficient in vitro and clinical pharmacology and drug interaction data and incorporates development changes.

Route of Administration

The route of administration chosen for a metabolic DDI study is important. For an investigational agent, the route of administration generally should be the one planned for clinical use. When multiple routes are being developed, the need for metabolic DDI studies by each route depends on the expected mechanisms of interaction and the similarity of corresponding concentration-time profiles for parent drug and metabolites. Sometimes certain routes of administration can reduce the utility of information from a study. For example, intravenous administration of a substrate drug may not reveal an interaction for substrate drugs where intestinal CYP3A activity markedly alters bioavailability

Dose Selection

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

Endpoints

Changes in pharmacokinetic parameters generally are used to assess the clinical importance of DDIs. Interpretation of findings (i.e., deciding whether a given effect is clinically important) depends on a good understanding of dose/concentration and concentration/response relationships for both desirable and undesirable drug effects in the general population or in specific populations. In certain instances, reliance on pharmacodynamic endpoints in addition to pharmacokinetic measures and/or parameters may be useful. Examples include INR measurement (e.g., when studying warfarin interactions) or QT interval measurements.

Pharmacokinetic Endpoints

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

Statistical Considerations, Clinical Relevance, and Sample Size

The goal of a DDI study is to determine whether there is any increase or decrease in exposure to the substrate in the presence of the interacting drug. If there is, its implications should be assessed by an understanding of PK/PD relations both for C_{max} and AUC. Results of DDI studies should be reported as 90% confidence intervals about the geometric mean ratio of the observed pharmacokinetic measures with (S + I) and without the interacting drug (S alone). Confidence intervals provide an estimate of the distribution of the observed systemic exposure measure ratio of (S + I) versus (S alone) and convey a probability of the magnitude of the interaction. In contrast, tests of significance are not appropriate because small, consistent systemic exposure differences can be statistically significant ($p < 0.05$), but not clinically relevant.

When a DDI of potential importance is clearly present, specific recommendations should be provided regarding the clinical significance of the interaction based on what is known about the dose-response and/or PK/PD relationship for the substrate drug used in the study. This information can form the basis for reporting study results and for making recommendations in the labeling. It should be recognized that dose-response and/or PK/PD information can sometimes be incomplete or unavailable, especially for an older approved drug used as a substrate. If the sponsor wishes to include a statement in the labeling that no known DDI of clinical significance exists, the sponsor should recommend specific *no effect* boundaries, or clinical equivalence intervals, for a DDI and should provide the scientific justification for the recommendations. No effect boundaries represent the interval within which a change in a systemic exposure measure is considered not clinically meaningful. These conclusions can be based on dose-response data or on PK/PD modeling.

There are two approaches to defining no effect boundaries:

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

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

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

Cocktail Approaches

Simultaneous administration of a mixture of substrates of multiple CYP enzymes and transporters in one study (i.e., a “cocktail approach”) in human volunteers is another way to evaluate a drug’s inhibition or induction potential, provided that the study is designed properly and the following factors are present:

1. The substrates are specific for individual CYP enzymes or transporters.
2. There are no interactions among these substrates.

3. The study is conducted in a sufficient number of subjects.

Negative results from a well-conducted cocktail study can eliminate the need for further evaluation of particular CYP enzymes. However, positive results can indicate that further in vivo evaluation should be conducted to provide quantitative exposure changes (such as AUC, C_{max}), if the initial evaluation only assessed the changes in the urinary parent to metabolite ratios. The data generated from a cocktail study can supplement data from other in vitro and in vivo studies in assessing a drug's potential to inhibit or induce CYP enzymes and transporters

Negative results from a well-conducted cocktail study may eliminate the need for further evaluation of particular CYP enzymes and transporters. However, positive results may indicate that further in vivo evaluation should be conducted

Pharmacogenomic Considerations

When a DDI study uses a probe drug (e.g., omeprazole for CYP2C19) to evaluate the impact of the investigational drug on a polymorphic enzyme, individuals who have no functional enzyme activity would not be appropriate study subjects. Drug interaction studies that evaluate enzymes or transporters with known polymorphisms should include collection of genotype or phenotype information to allow appropriate interpretation of the study results. In some instances, an evaluation of the extent of drug interactions in subjects with various genotypes may be helpful. Moreover, DDIs can differ among individuals based on genetic variation of a polymorphic enzyme. For example, a strong CYP2D6 inhibitor (e.g., fluoxetine) will increase the plasma levels of a CYP2D6 substrate (e.g., atomoxetine) in subjects who are extensive metabolizers (EM) of CYP2D6, but will have minimal effect in subjects who are poor metabolizers (PM) of CYP2D6, because these individuals have no active enzyme to inhibit. It is noted that CYP2D6 PMs will already have greatly increased levels of atomoxetine if given usual doses. There are also situations where inhibition may have a greater effect in PMs than EMs. If a drug is metabolized by a minor pathway (nonpolymorphic enzyme) and a major pathway (polymorphic enzyme), inhibition of the minor pathway will usually have minimal effect on plasma concentrations in EMs. However, the minor pathway plays a greater role in clearance of the drug in PMs of the major pathway. Thus, inhibition of the minor pathway in PMs of the major pathway can have a significant effect on drug clearance and resulting drug concentrations. Therefore, studying the effect of interactions may be recommended in subjects with varied genotypes or phenotypes

DDI as Part of Pop PK in Phase II and Phase III Trials

Population pharmacokinetic (PopPK) analyses of data obtained from large-scale clinical studies that include sparse or intensive blood sampling can help characterize the clinical impact of known or newly identified interactions and determine recommendations for dosage modifications for the investigational drug as a substrate. The results of such analyses can be informative and sometimes conclusive when the clinical studies are adequately designed to detect significant changes in drug exposure due to DDIs. PopPK evaluations may also detect unsuspected DDIs, a particularly important possibility given the complexity of the potential interactions, not all of which are likely to have been anticipated and studied. PopPK evaluations can also provide further evidence of the absence of a DDI, when supported by prior evidence and mechanistic data. It is unlikely, however, that population analysis will persuasively show the absence of an interaction that is suggested by information from in vivo studies specifically designed to assess a DDI. To be optimally informative, PopPK studies should have carefully designed study procedures and sample collection protocols. Simulations (e.g., by population-based PBPK models) can provide valuable insight into optimizing the study design. Detailed information on the dose given and time of

administration should be documented for the coadministered drugs. When relevant for the specific drug, the time of food consumption should be documented. Population analyses should focus on excluding a specific clinically meaningful PK change. Because exposure of coadministered drugs is not monitored in most PopPK studies, the PopPK approach may not be useful to assess the effect of the investigational drugs on other drugs.

DDI Considerations for NBEs

A comparison of the most recent DDI guidelines from EMA, FDA, and PMDA, only the guideline FDA provides a flowchart how to evaluate DDI for biologics (see respective part of the FDA DDI guideline). The respective EMA guideline does not mention about DDI of biologics. Whereas the FDA and PMDA recommend to conduct clinical DDI study, in case the biologics are cytokine or cytokine modulator. Additionally, FDA and PMDA recommend to examine PK/PD, in case the biologics is used as combination therapy with other agents. Below, some more specific recommendations for DDI evaluations with NBEs are given based on the FDA DDI guideline:

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

Drug-TP interactions have been observed and information about these interactions is included in labeling and in the following some general considerations are given:

- If an investigational TP is a cytokine or cytokine modulator, studies should be conducted to determine the TP's effects on CYP enzymes or transporters (Huang et al. 2010; Le Vee et al. 2009). In vitro or animal studies have limited value in the qualitative and quantitative projection of clinical interactions because translation of in vitro to in vivo and animal to human results to date has been inconsistent, necessitating in vivo drug interaction studies. The in vivo evaluations of TPs in targeted patient populations can be conducted with individual substrates for specific CYP enzymes and transporters, or studies can be conducted using a "cocktail approach."
- For TPs that will be used in combination with other drug products (small molecule or TP) as a combination therapy, studies should evaluate the effect of each product on the other. The studies should assess effects on pharmacokinetics (PK) and, when appropriate, pharmacodynamics (PD) of either drug. This evaluation is particularly important when the drug used in combination has a NTR (e.g., chemotherapeutic agents).
- When there are known mechanisms or prior experience with certain PK or PD interactions, appropriate in vitro or in vivo assessments for possible interactions should be conducted. Some interactions between drugs and TPs are based on mechanisms other than CYP or transporter modulation. For example, methotrexate's immunosuppressive effect may alter the clearance of concomitantly administered TPs through the reduction of antibodies formed against TP.

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