3 DDI Risk Assessment and Evaluation in Pharmaceutical Development: Interfacing Drug Metabolism and Clinical Pharmacology

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3.1 SUMMARY

Scientifically guided risk assessment and clinical pharmacologic evaluation of pharmacokinetic (PK) drug–drug interactions (DDI) are key components of contemporary drug development and play an integral role in the overall benefit/risk evaluation of new molecular entities (NMEs). DDI risk assessment and clinical evaluation should consider the potential for the NME to alter the PK of coadministered drugs (NME as the precipitant) and also the potential for coadministered drugs to alter NME PK (NME as the object). Risk assessment begins with *in vitro* and mechanistic studies of the NME's potential to alter drug disposition via inhibition or induction of drug-metabolizing enzymes and transport proteins, and additionally, studies that elucidate

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the clearance mechanisms of the NME itself. This is followed by a mechanistic and quantitative translation of *in vitro* parameters to estimate clinical PK consequences of a potential DDI (i.e., expected increase in object drug exposure). When the risk for DDI cannot be definitively dismissed as low based on translational assessment from the results of *in vitro* studies, controlled clinical DDI studies are designed to evaluate the clinical pharmacologic correlates of the *in vitro* data. Finally, the results of clinical DDI studies will need to be interpreted in the context of knowledge of the therapeutic index of the object drug, gained from exposure–response understanding for both safety and efficacy endpoints. This chapter provides a technical overview and strategic perspectives on each of these aspects of DDI risk assessment and clinical evaluation, with the use of examples that illustrate applications in drug development and the translation of scientific knowledge to prescribing guidance, in the context of currently available regulatory guidance and published scientific opinion.

3.2 INTRODUCTION AND MEDICAL IMPORTANCE

DDI represent an important mechanism of adverse drug reactions making their evaluation a key element of contemporary drug discovery, development, and regulation, given their potential impact as an integral component of clinical therapeutics. DDI profiling and risk forecasting when incorporated into the overall framework of multidimensional optimization can help identify candidates with likely low clinical DDI risk in the discovery stage and subsequently enable development of rational clinical pharmacologic DDI evaluation strategies to manage risk in clinical drug development. Finally, the ultimate goal of nonclinical and clinical DDI studies is to permit integration of DDI knowledge acquired in the development phase into prescribing guidance in a manner that enables optimal postmarketing risk management following marketing authorization.

A clinically significant DDI occurs when the therapeutic or toxic effects of a medication are altered by administration with another drug. DDIs are broadly classified into two categories: (i) PK interactions wherein one drug alters the absorption, distribution, metabolism, or elimination of another coadministered drug resulting in altered blood/target organ levels and potential effects on efficacy and/or safety; and (ii) pharmacodynamic (PD) interactions wherein one drug alters the pharmacologic effect (efficacy and/or safety) of another coadministered drug without affecting its PK. This chapter discusses DDIs resulting from PK mechanisms, with a focus on metabolic interactions and approaches for risk assessment and clinical pharmacologic evaluation of such interactions in contemporary drug development.

The medical importance of DDIs, and, in particular, the need for scientifically guided premarketing risk evaluation can be readily appreciated from the results of a review of drugs withdrawn from the US market from 1998 through 2003 because of their involvement in major clinical DDIs with commonly coadministered agents [1]. A notable example is the calcium channel blocker mibefradil that was used in the medical management of hypertension and angina. Mibefradil, a strong mechanism-based inactivator of cytochrome P450 3A (CYP3A) and an inhibitor of the efflux transporter P-glycoprotein (P-gp) produced dangerous and occasionally fatal interactions with sensitive substrates of CYP3A, such as the calcium channel blocker felodipine. This led to

the United States Prescribing Information (USPI) for mibefradil to be revised to recommend the contraindication of concomitant use of 26 drugs spanning multiple therapeutic areas. Mibefradil was voluntarily withdrawn in June 1998 by the manufacturer within a year following approval because of recognition of these serious interactions produced by this drug and the complexity of prescribing information needed to administer the drug safely. Additionally, considering that other pharmacotherapeutic options without similar serious DDI risks were available for the medical management of the conditions for which the use of mibefradil was indicated, the overall benefit/risk ratio for mibefradil was unfavorable. This example illustrates the importance of early assessment of risk for NMEs to produce DDIs with coadministered agents via effects on their PK (e.g., via metabolic inhibition by the NME). Other notable examples of drug withdrawals due to unacceptable DDI liabilities include the nonsedating antihistamine drugs, terfenadine and astemizole, and the prokinetic agent cisapride that was used for the management of gastroesophageal reflux disease. All three drugs were associated with two common properties. These drugs were inhibitors of the ion channel hERG, which plays a critical role in cardiac repolarization, and were additionally cleared almost exclusively via metabolism by CYP3A such that large and clinically significant increases in their exposure could occur on coadministration of inhibitors of this enzyme. This combination of an unfavorable off-target pharmacologic effect associated with proarrhythmic risk and exquisite sensitivity to metabolic inhibition resulted in major clinically deleterious interactions. The clinical consequence was an increased risk for the fatal cardiac arrhythmia *torsades de pointes* when coadministered with many commonly administered therapeutics, including antibiotics such as erythromycin and consumer products such as grapefruit juice. This set of examples illustrates the importance of an adequate level of premarketing characterization of DDI risks in the context of the NMEs safety profile and therapeutic index.

Although CYP enzyme-based DDIs have received the greatest amount of attention, it should be noted that DDIs at the level of non-CYP enzymes can also be clinically significant. An example of a serious DDI involving inhibition of a non-CYP enzyme that has resulted in fatal outcomes is the case of interactions between the antiviral drug sorivudine and the cytotoxic anticancer agent 5-fluorouracil (5-FU) or 5-FU prodrugs that are metabolized by the enzyme dihydropyrimidine dehydrogenase (DPD). This interaction is explained by mechanism-based inactivation (MBI) of DPD by a metabolite of sorivudine resulting in an essentially irreversible and long-lasting impairment of DPD activity and 5-FU clearance [2–4]. Another example of clinically significant DDIs resulting from inhibition on non-CYP enzymes is the interaction between the xanthine oxidase (XO) inhibitor allopurinol and 6-mercaptopurine (6-MP) or its imidazolyl derivative, azathioprine. Inhibition of XO-mediated metabolism of 6-MP by allopurinol has been shown to result in a fivefold increase in systemic exposure of orally administered 6-MP [5], necessitating a reduction in the dose of 6-MP or azathioprine in patients requiring coadministration with allopurinol [6].

As a consequence of serious medically significant DDI reports, as exemplified by the above discussed examples, risk assessment and clinical management of DDIs has gained importance across the sectors of medical practice, pharmaceutical research and development, and drug regulation. This is especially important considering the increase in pharmacotherapy options for patients and the emergence of polypharmacy, especially in certain therapeutic areas such as oncology, HIV medicine, and in the geriatric population [7]. Finally, the identification of clinically important interactions with food

products such as grapefruit juice, and importantly herbal products, is also a serious issue. This is partly because they are unfortunately often perceived as innocuous substances without risks. These factors have highlighted DDI awareness and magnified its importance as a public health issue [1,8]. In the face of these drivers of awareness and recognition of the importance of DDIs, there have been significant scientific advances over the last two decades, resulting in critical enablers that now permit a rational and mechanistic approach to drug interaction risk assessment and clinical pharmacologic evaluation. These enablers include: (i) biological and chemical reagents and *in vitro* technology platforms to study drug metabolism and DDIs, made possible by molecular cloning of the human drug-metabolizing enzymes and transporters; and (ii) advances in the science of quantitative *in vitro–in vivo* extrapolation (IVIVE) through modeling and simulation to forecast clinical pharmacologic correlates and optimize designs of clinical DDI studies from the results of nonclinical studies. Finally and importantly, advances in quantitative clinical pharmacology have enabled exposure–response understanding of the efficacy and safety profiles of drug candidates during the course of their clinical development. Such exposure–response characterization is necessary for the quantitative and objective estimation of therapeutic index, which is critically important to assess the clinical significance of drug interactions and contribute to prescribing guidance [1].

3.3 FRAMEWORK FOR DDI RISK ASSESSMENT AND EVALUATION IN DRUG DEVELOPMENT

A simple conceptual framework for DDI risk assessment and clinical evaluation in drug development is illustrated in Fig. 3.1. For representative purposes, interactions affecting clearance processes are illustrated in this figure, although this framework is equally applicable for interactions resulting from modulation of drug absorption. As shown in this framework, there are two broad categories of DDIs that require consideration as part of drug development. First, it is important to assess the risk for clinically meaningful alterations in exposure of an NME when it is coadministered with other (marketed) agents that may alter its PK (i.e., NME as the *substrate* drug and the *object* or *victim* of the interaction). A second assessment is of the risk for the NME to produce clinically significant alterations in the PK of concomitant agents, especially those with a narrow therapeutic range (i.e., NME as the *precipitant* or *perpetrator* agent because of its ability to act as a clinically meaningful *inhibitor* or *inducer*). The following sections of this chapter discuss the scientific considerations, experimental methodologies, and strategies employed in answering each of these questions and ultimately integrating the knowledge gained from these assessments into the overall clinical pharmacologic characterization of an NME to inform prescribing guidance. While the focus of this chapter is on metabolic DDIs, it is important to note that transporter-mediated DDIs also represent an important emerging area, and there have been many recent examples of clinically important interactions resulting from modulation of absorption as well as clearance processes via effects of coadministered agents on drug transporter expression and/or activity. A detailed discussion of experimental approaches and strategies for transporter-based DDI risk assessment, and their clinical evaluation is not within the scope of this chapter and the reader is referred to a recent review for current opinion on this topic [9]. Finally, although the focus of this chapter is on DDI risk assessment

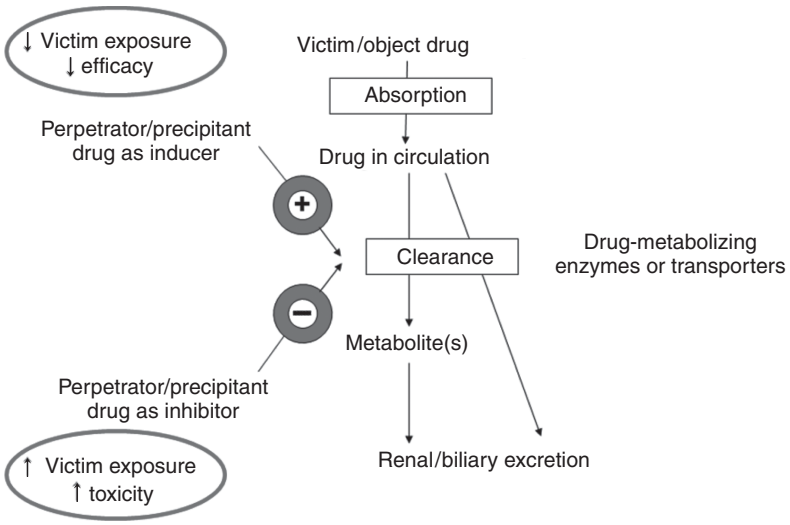


Figure 3.1 Conceptual framework for DDI Risk assessment and evaluation in drug development, considering the NME as both the victim or object drug and as the perpetrator or precipitant drug.

and evaluation for small molecule drugs, it should be noted that the topic of PK DDIs with biotherapeutics (e.g., therapeutic antibodies) is an emerging area of science. There have been many recent reports of DDIs between therapeutic antibodies and small molecules resulting, for instance, from indirect immunomodulatory mechanisms that can alter the expression and activity of drug-metabolizing enzymes. For an overview of this emerging area and current expert opinion, the reader is referred to recent review articles [10–13].

3.4 NME AS PRECIPITANT: DDI RISK ASSESSMENT FOR CYP INHIBITION

The assessment of CYP-inhibitory DDI potential of an NME in drug development is a two-step process: (i) *In vitro* estimation of inhibitory potency; and (ii) Translation of *in vitro* inhibitory potency to project potential clinical pharmacologic correlates (i.e., drug interaction magnitude).

3.4.1 *In vitro* CYP inhibition Studies

The *in vitro* experimental models, methodology, and associated data analytic methods for the assessment of the potential for an NME to produce inhibition of the activity of the major drug-metabolizing human CYP isoforms have been developed and refined over the last two decades and are now well established to permit their routine integration into drug discovery and development [14–19]. The most commonly applied approach involves measuring the effect of increasing concentrations of the NME on the metabolic rate in pooled human liver microsomes, of selective index reactions that are

representative of the activities of individual drug-metabolizing CYP isoforms, to estimate IC_{50} values as measures of inhibitory potency. Clinically important CYP isoforms from the standpoint of human drug metabolism and DDIs include CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5 (referred to as 3A). Examples of commonly utilized index reactions for each of these enzymes have been published [15,16,19]. These studies are typically conducted at a substrate concentration equal to the K_m of the index reaction, such that the apparent K_i can be approximated as $0.5 \times IC_{50}$ under the conservative assumption of competitive inhibition. For enzymes that are inhibited sufficiently to enable IC_{50} estimation, a more complete enzyme-kinetic analysis of inhibitory mechanism for definitive estimation of the apparent K_i can be performed following the IC_{50} analysis. Such an experiment will provide definitive information on the kinetic mechanism of inhibition and the associated inhibition constant [20], thereby minimizing the impact of assuming competitive inhibition in subsequent *IVIVE* of clinical interaction magnitude. Such additional refinement, while useful, especially when the underlying mechanism of inhibition is atypical/complex [21], may not be a critical requirement from a pragmatic standpoint for meeting the overall strategic goal of DDI risk assessment in drug development, as estimates of K_i from IC_{50} appear to be sufficiently informative in assessing the risk for PK DDIs and in guiding development of the optimal clinical DDI evaluation strategy [22,23].

An important consideration in the design of *in vitro* CYP inhibition DDI studies is the use of experimental conditions to minimize the extent of nonspecific microsomal binding of the NME. Originally identified as an important factor that can influence estimation of *in vitro* K_m values and thereby confound the accuracy of *in vitro–in vivo* scaling of drug clearance [24–27], nonspecific microsomal binding has been established as a source of overestimation of IC_{50}/K_i estimates (i.e., underestimation of inhibitory potency) in CYP inhibition studies [20,28–30]. With the use of sensitive LC/MS/MS bioanalytical methods, it is possible to keep the human liver microsomal protein concentrations to <0.2 mg/mL [19,22] such that the extent of microsomal binding would likely be minimized for most NMEs [e.g., microsomal unbound fraction ($f_{u,mic}$) ≥ 0.8]. If this is not feasible or when dealing with NMEs that are expected to be meaningfully bound even at low microsomal concentrations based on their physicochemical properties (e.g., highly lipophilic bases) or other available data, it would be important to account for this phenomenon and apply necessary corrections for microsomal unbound fraction in the calculation of the unbound inhibitory potency (e.g., $K_{i,u} = f_{u,mic} \times$ observed K_i). Of note, the importance of considering microsomal binding in the calculation of K_i values for basic drugs is recognized in the European Medicines Agency (EMA) draft guideline [31]. This is best done via *in vitro* measurement of the unbound fraction of the NME at the microsomal concentration employed in the CYP inhibition studies [20,28,29]. Alternatively, initial estimates of $f_{u,mic}$ can be calculated from physicochemical properties of the NME using *in silico* models, which have undergone considerable refinement with improvement in their predictive accuracy in recent years [32–34] in relation to the first described model [35]. For highly lipophilic drugs (e.g., $\log P/D > 3$), uncertainty in estimating $f_{u,mic}$ is still high enough that it is advisable to directly measure this parameter for such NMEs [33].

Pooled human liver microsomes represent the most commonly used and generally preferred *in vitro* system for the conduct of CYP inhibition studies. However, it should be noted that CYP inhibition studies can also be performed using human hepatocytes, and there are certain situations where such evaluations may be helpful,

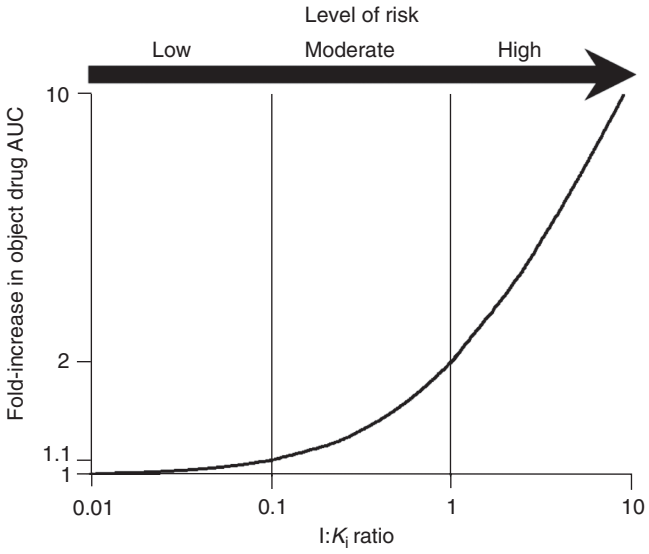
as is discussed later in the section on "mechanism-based inactivation" (Section 3.5). Specifically, when extensive non-CYP-mediated and/or nonmicrosomal metabolism of the NME is expected or known, use of hepatocytes for estimating inhibitory potency for CYP inhibition by the NME may need to be considered as it is possible that there could be *in vitro* system-dependent differences in outcomes, with the outcomes in hepatocytes rather than human liver microsomes being more representative of the clinical outcomes [36].

3.4.2 DDI Risk Assessment from *in vitro* CYP Inhibition Data

Following estimation of inhibitory potency (K_i or IC_{50}), the next step is to put these *in vitro* data in context of the exposures/PK properties of the NME at the clinical dose(s) to forecast the level of risk for DDI with substrates of the enzyme being inhibited, to enable development of risk management plans in later phases of clinical drug development and to guide the strategy/design of clinical DDI studies. Ideally, the objective of IVIVE is to permit prediction of the magnitude of increase in exposure of a coadministered object drug and where possible, a more specific prediction of the nature of the altered plasma concentration–time profile and associated population variability. Substantial progress has been made in the IVIVE of CYP-inhibitory DDI over the last decade, with some recently published examples of successful retrospective predictions from large databases [20,22,37,38]. However, uncertainty still remains in certain key parameters that are critical to the prediction of DDI magnitude (e.g., PK metric that is best suited to serve as a surrogate of enzyme-available inhibitor concentration), as will be reviewed later. Therefore, the quantitative prediction of CYP-inhibitory DDI in drug development remains an emerging area of science without a clear consensus on the best approach that balances scientific rigor and an adequately conservative predictive performance. The approach currently recommended by the FDA Draft DDI Guidance [39], consistent with leading scientific opinion on the topic [14], involves a simple and empirical, yet useful approach intended to provide a conservative categorization of the level of risk based on the [I]:K_i ratio, where [I] is the total systemic maximum plasma concentration of the NME achieved following administration at the highest clinical dose/frequency (e.g., steady-state C_{max}). The underlying scientific rationale for this risk level classification approach is based on the hyperbolic relationship (Fig. 3.2) between [I]:K_i and fold increase in AUC of an orally administered substrate drug whose clearance is entirely mediated 100% via metabolism by the enzyme that is inhibited by the NME—assumptions that intentionally err on the conservative side to project the maximum possible magnitude of a DDI (Eq. 3.1).

$$\frac{AUC_{inhibited}}{AUC_{control}} = \frac{CL_{int,control}}{CL_{int,inhibited}} = 1 + \frac{[I]}{K_i} \tag{3.1}$$

The [I]:K_i ratio cut-offs of <0.1 (corresponding projected maximum increase in AUC of <1.1-fold) and >1 (corresponding projected maximum increase in AUC of greater than twofold) are to be used for qualitative risk level classification as "low" and "high," respectively, and are not intended to serve as quantitative predictions of DDI magnitude. The application of this classification is simply to eliminate the need for unnecessary follow-up clinical DDI studies for enzymes that are inhibited with an inferred low DDI risk, and to sequentially prioritize the conduct of clinical DDI studies



$[I]/K_i$	Prediction
$[I]/K_i > 1$	Likely
$1 > [I]/K_i > 0.1$	Possible
$0.1 > [I]/K_i$	Remote

Figure 3.2 Relationship between the I:K_i ratio for CYP inhibition by an NME and the associated level of DDI risk assessed based on the maximum expected fold increase in systemic exposure of an orally administered object drug whose oral clearance is mediated solely by hepatic metabolism by the inhibited enzyme.

for enzymes that are inhibited with decreasing levels of DDI risk, as will be discussed later. Consider, for example, the anticancer drug everolimus, an mTOR (mammalian target of rapamycin) inhibitor, indicated for the treatment of advanced renal cell carcinoma. Everolimus inhibits CYP2D6 activity *in vitro*; however, the clinically observed mean steady-state C_{max} at the recommended 10 mg daily dose is more than 12-fold below the CYP2D6 inhibitory K_i, based on which the everolimus USPI concludes that an effect of everolimus on the metabolism of CYP2D6 substrates is unlikely [40]. This example illustrates how *in vitro* data, when clearly indicative of low DDI risk, can inform prescribing guidance without the need for unnecessary clinical DDI studies.

The approach to DDI risk assessment using the [I]:K_i ratio alone (with [I] defined as the clinically observed systemic C_{max} of the NME), while simple and pragmatically straightforward, is not without limitations. The assumptions underlying this simple classification system have been addressed in multiple quantitative enrichments to the framework that have built in considerations of the appropriate metric of [I], route of administration of the victim drug, and potential for extrahepatic metabolism. Finally, if the goal is to make quantitative predictions of DDI magnitude that extend beyond risk

level categorization, it is important to consider the fraction of victim drug clearance that is mediated by metabolism via the inhibited enzyme ($f_{m(CYPi)}$). Each of these aspects is reviewed briefly in the following subsections.

3.4.3 Considerations in Defining [I]

A key consideration in DDI risk assessment from *in vitro* enzyme inhibitory potency is the selection of the best PK surrogate of the enzyme-available inhibitor concentration in the liver. The selection of an appropriate surrogate for [I] has been an extensively debated topic for nearly two decades, with multiple reports of variable predictive success resulting from use of various surrogates—some more scientifically valid and appealing than others. Considering that this continues to be an emerging area of science without global regulatory consensus guidelines available at the time of writing of this chapter, this chapter does not provide any definitive recommendations on the preferred approach to estimate [I] for DDI predictions in drug development but will only discuss the relative merits and limitations of the available approaches. For metabolism by liver microsomal enzymes, enzyme-available inhibitor concentration in the hepatocyte should in principle equal the unbound concentration in hepatic cytosol around the enzyme, which is unfortunately impossible to directly measure in humans. Pharmacologic principles and specifically the unbound drug hypothesis would suggest that the unbound plasma concentration of the inhibitor would be expected to be in equilibrium with the unbound concentration within the hepatocyte (in the absence of active hepatic uptake), suggesting that it may be the most appropriate surrogate for [I]. However, multiple reports have consistently demonstrated that the use of unbound systemic exposure (either steady-state average concentration over the dosing interval or steady-state peak concentration) results in substantial underpredictions of DDI magnitude, with unacceptably high false negative prediction rates, such that major clinically important DDIs would be missed. Multiple reasons can potentially explain the poor performance of unbound systemic exposure as a surrogate for [I]. For orally dosed inhibitors that undergo first-pass hepatic extraction, significant portal/systemic concentration gradients can be expected. Therefore, the unbound hepatic inlet concentration has been proposed as a suitable surrogate for [I] based on the recognition that use of systemic unbound concentrations can underestimate the transiently high concentrations that will be "seen" by the drug-metabolizing enzymes in the liver following an oral dose of the NME, and therefore result in underprediction of DDI magnitude. This can be readily calculated using Equation 3.2 where I_{max} is the systemic maximum concentration of the inhibitor, f_u is its plasma free fraction, f_a is its fraction absorbed, k_a is its absorption rate constant, and Q_h is hepatic blood flow [41,42].

$$I_{inlet,max,u} = f_u \times \left(I_{max} + \frac{f_a \cdot dose \cdot k_a}{Q_h} \right) \tag{3.2}$$

Another potential reason for underprediction of DDI magnitude with the use of unbound systemic concentrations is the active hepatic uptake of certain inhibitors that can result in unbound hepatocellular concentrations in excess of the corresponding unbound systemic concentrations. Considering these complexities and uncertainties, and in the interest of having a conservative, yet simple approach to DDI risk assessment from *in vitro* CYP-inhibitory potency data, two distinct approaches are currently

advocated by the FDA and the EMA draft DDI guidance documents, respectively. The regulatory draft guidance document from FDA conservatively recommends the use of total systemic C_{max} of the CYP-inhibitory NME for calculating the [I]:K_i ratio, with a 10-fold safety factor as discussed earlier (i.e., risk for DDI cannot be excluded unless [I]:K_i ratio is <0.1) [39]. The EMA draft guideline that was recently released for comment considers plasma protein binding and recommends unbound systemic C_{max} as the surrogate of [I] but accordingly recommends the use of higher safety factors to account for factors such as portal/systemic concentration gradients and uncertainties in K_i estimation, and thereby minimize false negative risk assessments. A 50-fold safety factor is recommended (i.e., risk for DDI cannot be excluded unless [I]:K_i ratio is $\leq$1/50), with a higher safety factor of 250-fold (i.e., risk for DDI cannot be excluded unless [I]:K_i ratio is $\leq$1/250) for drugs with >99% plasma protein binding [31]. In addition to consideration of the systemic unbound exposure of the inhibitor, the EMA draft guideline also recommends consideration of intestinal exposure when the enzyme being inhibited is known to have pronounced intestinal expression (e.g., CYP3A). Two measures of intestinal exposure are recommended to be calculated: a maximum luminal concentration calculated as the maximum dose taken at one occasion divided by 250 mL and a predicted maximum concentration in the enterocyte using Equation 3.3 [43], where Q_{ent} is the enterocytic blood flow (~248 mL/min):

$$I_{gut} = \frac{f_a \times \text{Dose} \times k_a}{Q_{ent}} \tag{3.3}$$

Safety factors of 10-fold (for the calculated maximum luminal concentration) and 50-fold (for the calculated concentration in the enterocyte) are recommended to be jointly applied (in addition to the previously discussed approach to risk assessment based on the systemic unbound C_{max}) for translational assessment of clinical DDI risk from *in vitro* CYP-inhibitory potency data [31]. The performance characteristics of these suggested safety factors remain to be investigated.

3.4.4 Quantitative Predictions of DDI Magnitude: Victim Drug-Specific Considerations

An important factor that determines the magnitude of a DDI is not only the potency of inhibition of the drug-metabolizing enzyme by the NME and the NME's exposure at clinical dose(s) but also the fraction of total clearance of the object drug that is mediated by metabolism via the specific inhibited enzyme (Eq. 3.4; Fig. 3.3), denoted variably by f_m, $f_{m(CYP)}$, $f_{m(CYPi)}$, or the contribution ratio (CR) in previous investigations of CYP-based DDIs [22,23,44–48].

$$\frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{(1 - f_{m(CYPi)}) + \frac{f_{m(CYPi)}}{\left(1+\frac{I}{K_i}\right)}} \tag{3.4}$$

The approach to DDI risk assessment based solely on I:K_i ratio (Fig. 3.2) conservatively assumes that $f_{m(CYPi)}$ of the object drug is unity, although this is seldom the case even for highly selective probe substrates of the major human CYP enzymes, with estimated values of $f_{m(CYP1A2)}$ of ~0.95 for caffeine, $f_{m(CYP2C9)}$ of ~0.91 for *S*-warfarin, $f_{m(CYP2C19)}$ of ~0.87 for omeprazole, $f_{m(CYP2D6)}$ of ~0.9 for desipramine,

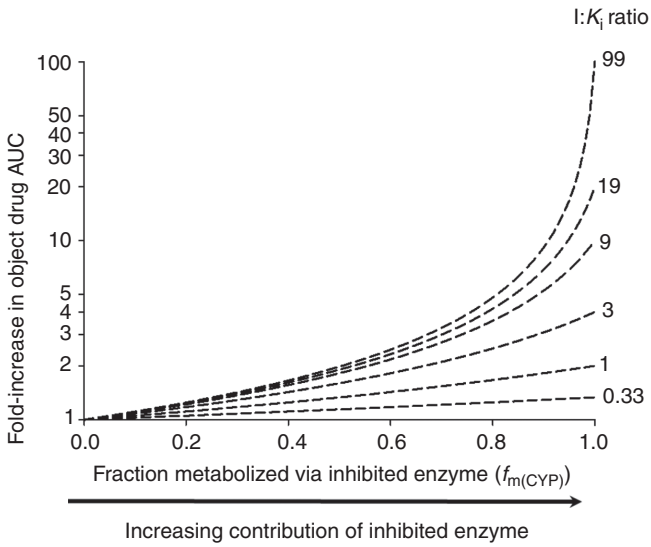


Figure 3.3 Fraction of total clearance of the object drug that is mediated by metabolism via the specific inhibited enzyme ($f_{m(CYPi)}$) and the precipitant's I:K_i ratio as determinants of clinical DDI magnitude.

and $f_{m(CYP3A)}$ of ~0.93 for midazolam, as reviewed previously [49]. Considering the nonlinear relationship between $f_{m(CYPi)}$ of the object drug and predicted interaction magnitude for a given I:K_i ratio (Fig. 3.3), sensitivity of the IVIVE of DDI to the assumption of $f_{m(CYPi)}$ of unity can be quite large and result in a substantial overprediction of DDI magnitude, even when dealing with a highly selective substrate with $f_{m(CYPi)} \geq 0.9$. Accurately defining $f_{m(CYPi)}$ of the object drug with a high level of precision (e.g., 0.85 vs 0.95) is not straightforward. One approach involves the derivation of $f_{m(CYPi)}$ from the results of clinical DDI studies with a strong and selective inhibitor (e.g., ketoconazole for CYP3A), and has been utilized for estimating $f_{m(CYP3A)}$ of midazolam and other CYP3A substrates [22,48]. It is important to note that the assumption of complete and specific inhibition of the target enzyme by a strong inhibitor is inherent to such an approach. The adequacy of this assumption of complete inhibition of the target enzyme depends on the potency of the inhibitor in relation to time course of inhibitor concentrations achieved in the DDI study and the mechanism of inhibition (reversible vs mechanism based). Another approach that is probably scientifically most appealing (provided all necessary data are available to enable its application) involves a calculation of $f_{m(CYPi)}$ from various sources of data, including a quantitative knowledge of the clearance mechanisms of the object drug (e.g., as gained from the results of a radiolabeled mass balance study with adequate quantitative tracking of the chemical fate of the object drug in excreta) and quantitative *in vitro* reaction phenotyping of its biotransformation pathways. This approach has been used successfully in estimating $f_{m(CYP1A2)}$ of theophylline and clozapine [22,49]. With this approach, it is important to note that although the identification of major versus minor contributing enzymes can be expected to be consistent between the complementary approaches of *in vitro* reaction phenotyping, quantitatively equivalent estimates of relative contributions of each enzyme are not always obtained, introducing uncertainty in the derived $f_{m(CYPi)}$.

Finally, the most straightforward approach is in the setting where the target enzyme is polymorphically expressed and clinical PK data are available in extensive metabolizer (EM) and poor metabolizer (PM) populations. In such cases, $f_{m(CYPi)}$ can be readily estimated as one minus the inverse of the ratio of exposure of the object drug following oral dosing in PM versus EM subjects. Such an approach has been used successfully in estimating the $f_{m(CYPi)}$ of commonly used probe substrates of CYP2C9, CYP2C19, and CYP2D6 [22,45,49]. While the certainty in estimation of $f_{m(CYPi)}$ can be considered to be high in these cases when EM/PM PK data are available, the same is not true when dealing with the other methods of estimating this input parameter, where the associated uncertainty is relatively greater. The topic of defining $f_{m(CYPi)}$ is discussed further in a later section in the context of risk assessment for DDI with the NME as the object drug, but the principles are equally applicable to defining this critical parameter of the object drug to enable quantitative predictions of the magnitude of CYP-inhibitory DDI produced by an NME as the precipitant.

3.4.5 Quantitative Predictions of DDI Magnitude: NME-Specific Considerations

When using Equation 3.4 to make quantitative predictions of DDI magnitude, a key question as discussed earlier for qualitative (categorical) risk assessment is the selection of PK surrogate for [I] of the NME inhibitor. Various measures of [I] (e.g., total systemic C_{max}, unbound systemic C_{max}, total maximum hepatic inlet concentration, and unbound maximum hepatic inlet concentration) have been investigated in prospective and retrospective exercises of IVIVE of CYP-inhibitory DDI without general consensus on the surrogate for [I] associated with best predictive performance. In a large retrospective analysis based on a primary database of *in vitro* CYP-inhibitory potencies by Obach and colleagues [22], use of unbound maximum hepatic inlet concentration (Eq. 3.2) provided the best predictive accuracy relative to other metrics of [I], including total systemic C_{max}, unbound systemic C_{max}, and total maximum hepatic inlet concentration. In contrast to these observations, use of the unbound maximum hepatic inlet concentration of the inhibitor resulted in an overall underprediction of DDI magnitude in another retrospective analysis by Brown and colleagues [20], also based on a primary database of *in vitro* CYP-inhibitory potencies. This latter study found total systemic average concentration to be associated with the best predictive accuracy (91% of predictions within twofold of the observed DDI magnitude), which is unfortunately at odds with expectations from the unbound drug hypothesis and is therefore harder to explain from a mechanistic standpoint. The case of inhibition of CYP2C8 by the leukotriene receptor antagonist montelukast represents an example that supports the importance of considering plasma protein binding of the inhibitor during IVIVE of DDI magnitude. Montelukast has been identified to be a potent CYP2C8 inhibitor with a K_i of ~9 nM [30]. Total systemic plasma concentrations of montelukast at the 10 mg/day therapeutic dosing regimen (steady-state average concentration of ~0.4 μM; steady-state C_{max} of ~0.9 μM) are in far excess of the *in vitro* K_i for CYP2C8 inhibition, such that nearly complete inhibition of CYP2C8 should be anticipated. However, clinical DDI studies with CYP2C8 substrates such as repaglinide [50] and pioglitazone [51] have consistently demonstrated that montelukast does not alter the PK of these agents, confirming the lack of CYP2C8 inhibition by montelukast *in vivo*. Montelukast is highly plasma protein bound (>99%) and the estimated unbound hepatic inlet C_{max} calculated using Equation 3.2 is <11 nM, translating to a <2.2-fold DDI magnitude even if

100% of clearance of the substrate drug is via CYP2C8-mediated metabolism [30]. As plasma protein binding of montelukast is not precisely known and exact estimates of $f_{m(CYP2C8)}$ of rosiglitazone, pioglitazone, and repaglinide are difficult to obtain considering involvement of nonmetabolic OATP1B1 transporter-mediated clearance of these agents, it is difficult to more precisely predict the quantitative extent of DDIs with montelukast. Nevertheless, it is clear that predictions that do not consider the plasma protein binding of montelukast would substantially overpredict the risk for clinical DDIs with CYP2C8 substrates.

For CYP3A substrates administered orally, the quantitative prediction of DDI magnitude following administration of a CYP3A inhibitor should consider inhibition of intestinal metabolism in addition to hepatic metabolism of the object drug, due to the established expression of CYP3A in the small intestine and clinically important contribution to the clearance of many (but not all) substrates. The previously discussed IVIVE analysis by Obach and colleagues [22], accounted for inhibition of intestinal first-pass metabolism for CYP3A inhibitory DDIs, based on inclusion of the fold increase in the intestinal bioavailability as a multiplicative term together with the hepatic component. This is implemented using Equation 3.5 [52], where F_G is the baseline value of intestinal bioavailability of the object drug, I_g is the estimated intestinal concentration of the inhibitor (calculated using Eq. 3.3).

$$\frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{F_G + \frac{(1-F_G)}{\left(1+\frac{I_g}{K_i}\right)}} \times \frac{1}{(1 - f_{m(CYPi)}) + \frac{f_{m(CYPi)}}{\left(1+\frac{I}{K_i}\right)}} \tag{3.5}$$

The contribution of CYP3A inhibition in the intestine to the overall magnitude of a DDI clearly depends on the baseline F_G of the object drug. For highly potent inhibitors, the $I_g : K_i$ ratio may be large enough such that the first term in Equation 3.5 can be approximated as the inverse of baseline F_G, as has been done in the above discussed analysis by Brown and colleagues [20] for the CYP3A-inhibitory azole antifungal agents. For a detailed review of the assumptions and considerations associated with IVIVE of intestinal inhibition in the overall prediction of DDI magnitude, the reader is referred to a recent comprehensive review on this topic [53].

3.5 NME AS PRECIPITANT: MECHANISM-BASED CYP INACTIVATION DDI

Many clinically important PK DDIs result from impairment of metabolic clearance via MBI of CYP enzymes. This can be readily appreciated from an examination of the strong and moderate inhibitors of the major human drug-metabolizing enzyme CYP3A identified in the 2006 FDA draft guidance on drug interaction studies [39]. Fourteen out of 19 identified clinically significant CYP3A inhibitors are either established or putative mechanism-based inactivators of the enzyme, ranging from the natural product grapefruit juice to prescription drugs spanning several therapeutic classes, including antiretroviral agents (e.g., ritonavir, saquinavir), antibiotics widely used in general practice (e.g., clarithromycin, erythromycin), the calcium channel blockers diltiazem and verapamil, and the antidepressant agent nefazodone.

3.5.1 *In vitro* Diagnosis of MBI

The *in vitro* CYP inhibition studies and conceptual framework to IVIVE of DDI magnitude discussed in the previous section are only applicable to the setting of reversible CYP inhibition and are not appropriate when the investigational agent is a mechanism-based inactivator of a drug-metabolizing enzyme. Reversible CYP inhibition is only concentration dependent (at a given substrate concentration), whereas MBI is characterized by both concentration *and* time-dependent inactivation of the enzyme, secondary to metabolic activation of the inactivator (often described as a suicide inhibitory mechanism). An efficient approach to diagnosis of MBI by an NME is a test of enhancement of its apparent inhibitory activity following preincubation with human liver microsomes (or specific recombinant CYP isoforms) and NADPH before incubation with the isoform-selective index substrate. If the test compound is a mechanism-based inactivator, the above described preincubation process will result in a leftward shift in the IC_{50} curve (relative to a control experiment conducted alongside with the cofactor NADPH omitted in the preincubation period) and a correspondingly smaller estimate of the IC_{50} [54,55]. For simple reversible inhibitors, the IC_{50} curves will be expected to be essentially indistinguishable when interpreted in context of experimental variability, or may actually demonstrate an apparently greater IC_{50} following preincubation, as a consequence of metabolic consumption of the reversible inhibitor during the preincubation. If the above described diagnostic test is positive indicating time-dependent inhibition, further DDI risk assessment requires a full *in vitro* kinetic analysis to estimate the parameters characterizing MBI by the NME. An abbreviated empirical IVIVE approach using the "shifted IC_{50}" estimated following preincubation of the inactivator with human liver microsomes and NADPH, in the context of the reversible inhibition model (i.e., using one-half of the "shifted IC_{50}" in place of K_i in Eq. 3.5) has been explored [55,56]. The "shifted IC_{50}" is inversely related to the $k_{inact} : K_I$ ratio, which is a measure of the inactivator's intrinsic efficiency [57,58]. However, the performance of quantitative DDI predictions using the "shifted IC_{50}" is suboptimal, and it is therefore recommended that the appropriate mechanistic model for IVIVE of MBI based on *in vitro* parameters derived from a full enzyme-kinetic characterization of inactivation be utilized for definitive quantitative predictions and clinical DDI risk assessment [56].

3.5.2 *In vitro* Kinetic Characterization of MBI

The two primary kinetic parameters of relevance to IVIVE of MBI DDI are a capacity term reflecting the maximum rate of loss of enzymatic activity that the inactivator is capable of producing (k_{inact}), and a potency term (K_I) reflecting the concentration of the inactivator at which half-maximal inactivation rate ($0.5 \times k_{inact}$) is achieved. The inactivator at varying concentrations ([I]) is preincubated with the source of enzyme (human liver microsomes or recombinant CYP preparation) and NADPH for varying durations of time (t) before dilution into a secondary metabolic incubation (activity assay) containing NADPH and an index substrate of the target CYP isoform at concentrations in excess of its K_m. Dilution following the preincubation and use of saturating substrate concentrations in the activity assay help minimize any surmountable reversible inhibition, which may otherwise potentially confound estimation of the true kinetic parameters of CYP inactivation. The time course of loss of CYP activity over the duration of the preincubation is analyzed to estimate the apparent first-order

inactivation rate constant λ at each concentration of inactivator.

$$\text{Activity} = \text{Activity}_{t=0} \cdot e^{-\lambda t} \tag{3.6}$$

The parameters k_{inact} and K_{I} characterizing the hyperbolic relationship between λ and [I] are subsequently estimated using either a nonlinear regression approach or the less preferred approach involving linear transformations of the data (e.g. Kitz–Wilson analysis).

$$\lambda = \frac{k_{\text{inact}} \cdot [\text{I}]}{[\text{I}] + K_{\text{I}}} \tag{3.7}$$

The design of *in vitro* inactivation kinetic studies and subsequent data analysis should be performed in a manner that minimizes bias and increases precision of the estimated parameters [59]. For a detailed review of best practices for the experimental designs and data analytical approaches to *in vitro* studies of CYP inactivation, the reader is referred to a recent review that provides an expert opinion on the topic from a PhRMA perspective [60].

A complete elimination of bias in parameter estimates may be difficult to achieve using conventional experimental and data analytical approaches for inactivation kinetic characterization, as illustrated via theoretical simulations of the fidelity of such traditional approaches [61]. For example, metabolic depletion of the inactivator during the preincubation (as a consequence of which, nominal concentrations may overestimate the actual concentrations during the preincubation) as well as possible inactivation of the enzyme during the metabolic incubation with probe substrate (i.e., inadequate "quenching" of inactivation by dilution when the metabolic incubations with the probe substrate are conducted for relatively long durations) can introduce bias in k_{inact} and K_{I} estimates. These considerations emphasize the importance of using the lowest required microsomal concentration during the preincubation and the shortest possible duration of incubation for the metabolic activity measurement. Other features of the experimental protocol (e.g., dilution factor employed between the preincubation and incubation steps) can additionally influence the estimated inactivation kinetic parameters as illustrated in a series of studies on CYP2D6 inactivation by 3,4-methylenedioxymethamphetamine [62,63]. To address these issues, Yang and colleagues [64] have proposed a mechanistically based experimental protocol to eliminate the bias introduced in the estimation of inactivation kinetic parameters with the above described conventional approach. This approach involves three separate experiments to assess the metabolism of the inactivator, its ability to cause competitive inhibition, and its ability to cause time-dependent inhibition, followed by a simultaneous analysis of all resulting data to permit a model-based extraction of MBI kinetic parameters [64]. A variation of this approach referred to as *progress curve analysis* has been described and applied to estimate and distinguish the potencies of reversible inhibition and MBI of the CYP1A2 inactivators resveratrol, furafylline, and dihydralazine within the same experiment [65]. The complete time courses of substrate turnover were characterized in the absence of the inactivator and in the presence of varying concentrations of the inactivator. The reversible K_{i} was estimated based on the concentration–effect relationship for inhibition of initial velocity of substrate metabolism and the inactivation kinetic parameters (K_{I} and k_{inact}) were estimated based on kinetic analysis of the entire progress curve of substrate turnover

followed by characterization of the concentration–effect relationship for inactivation using the time-averaged inactivator concentration over the duration of the incubation [65]. Additional research is required to fully evaluate the performance characteristics of these novel promising approaches across CYP isoforms and their application for MBI DDI risk assessment.

3.5.3 Considerations in *In vitro* System Selection for MBI Kinetic Studies

In vitro studies of MBI can be performed using recombinantly expressed CYP enzyme isoforms, human liver microsomes, or hepatocytes. It should be noted that MBI kinetic parameters estimated using certain recombinantly expressed CYP preparations may not be reflective of those estimated using native human liver microsomes [66]. Therefore, caution should be exercised in their use for DDI risk assessment, as has been demonstrated for CYP3A inhibitory DDIs produced by macrolide antibiotics [67]. A recent study suggested a better predictive performance of IVIVE based on *in vitro* inactivation kinetic parameters determined in primary human hepatocytes compared to human liver microsomes [68]. Nevertheless, when viewed in the context of multiple reports that suggest reasonably successful classification of the level of DDI risk using time-dependent inhibition studies in human liver microsomes, and the relative convenience and opportunity for "standardization" of this experimental system (e.g., use of pooled human liver microsomes to represent the "population average" distribution of enzymes), the most commonly utilized system for studies of MBI for DDI risk assessment is human liver microsomes. Consistently, human liver microsomes represent the recommended *in vitro* system for estimation of kinetic parameters of inactivation in the expert opinion from the PhRMA [60].

It should, however, be noted that if substantial non-CYP- and/or nonmicrosomal-mediated metabolism of the NME is expected and if such metabolism modifies the inactivation effects of the NME, data from human hepatocytes rather than human liver microsomes may be more translatable to the clinical setting. This has been demonstrated recently for gemfibrozil, bupropion, and ezetimibe [36]. Gemfibrozil produces clinically relevant inhibition of CYP2C8 through MBI of the enzyme by its acyl glucuronide. Bupropion produces clinically relevant inhibition of CYP2D6 through metabolism-dependent inhibition of the enzyme by its reduced metabolites *erythro*- and *threo*-hydrobupropion. As hepatocytes represent a more complete biotransformation system compared to human liver microsomes, potent inhibition of CYP2C8 and CYP2D6 by gemfibrozil and bupropion, respectively, are readily observed following preincubation of hepatocytes with these drugs but are not observed using human liver microsomes as the *in vitro* system, where direct evaluation of the metabolites is necessary to observe potent enzyme inhibition [36]. The reverse is true in the case of ezetimibe, where potent MBI of CYP3A4 is observed in human liver microsomes, whereas this is not the case when using hepatocytes as the *in vitro* system, because of turnover of ezetimibe by direct glucuronidation, which occurs *in vivo*, resulting in the lack of clinically relevant CYP3A inhibition [36].

Considering the relatively large microsomal concentrations generally used in the preincubation step in *in vitro* kinetic studies of MBI, nonspecific microsomal binding can be significant, introducing bias in K_I estimates, jeopardizing their applicability in subsequent IVIVE of DDI magnitude. Therefore, correction of apparent K_I estimates for microsomal binding can be important in getting unbiased estimates of inactivator

potency for highly lipophilic basic inactivators that can be substantially bound to microsomal phospholipids. For example, failure to consider nonspecific microsomal binding of paroxetine results in a greater than threefold underprediction of the magnitude of interactions with CYP2D6 substrates [69].

3.5.4 DDI Risk Assessment and IVIVE for MBI

The theoretical framework that guides IVIVE of DDI magnitude resulting from CYP inactivation was first described by Hall and colleagues in 2000 [70]. As with IVIVE of reversible inhibition, the first step in IVIVE of MBI is to estimate the effect of the inactivator on intrinsic clearance of metabolism via the inactivated enzyme. Considering that steady-state levels of CYP enzymes *in vivo* are maintained by a balance of zero-order production (k_{syn}) and first-order degradation (rate constant, k_{deg}), the perturbation of this system by an inactivator (i.e., introduction of an additional mechanism of loss of active enzyme characterized by the apparent first-order rate constant λ) without an effect on enzyme synthesis rate would be expected to result in the following derivation of the expression for fold reduction in intrinsic clearance:

$$\frac{CL_{int,control}}{CL_{int,I}} = \frac{E_{ss}}{E_{ss,I}} = \frac{k_{syn}/k_{deg}}{k_{syn}/k_{deg} + \lambda} = \frac{k_{deg} + \lambda}{k_{deg}} = \frac{k_{deg} + \frac{[I]\cdot k_{inact}}{[I]+K_I}}{k_{deg}} \tag{3.8}$$

The model described above provides the fundamental basis of IVIVE of MBI DDI and is shown in Fig. 3.4. The 2010 EMA draft guideline on DDIs recommends use of the derived expression in Equation 3.8 to predict the effect of NMEs that display time-dependent inhibition *in vitro* on intrinsic clearance of the enzyme being inactivated. If $\geq$ 30% inhibition is predicted using this equation (with the estimates of [I] and safety factors recommended in the guideline as discussed previously for reversible inhibition), *in vivo* inhibition may not be excluded and a clinical DDI study is recommended [31].

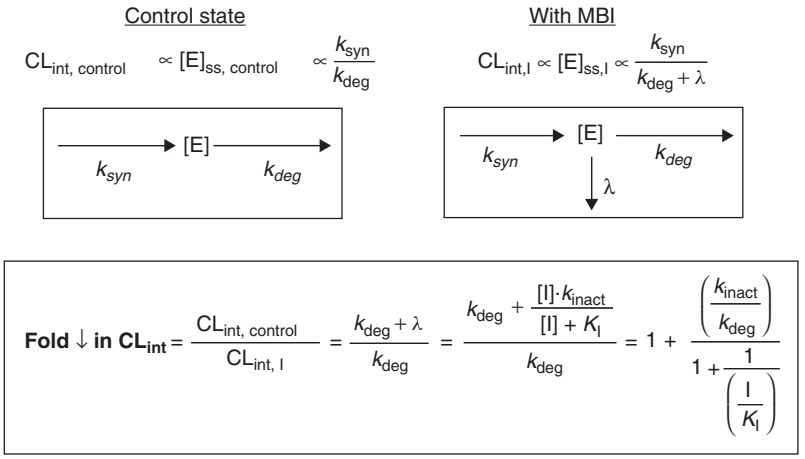


Figure 3.4 Schematic illustration of the enzyme-kinetic mechanistic model underlying mechanism-based inactivation.

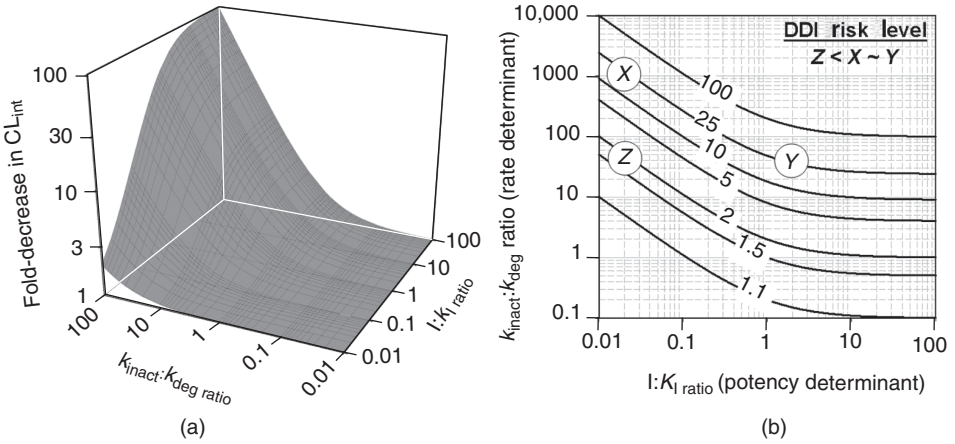


Figure 3.5 (a) MBI surface plot showing the fold reduction in intrinsic clearance as a function of I:K_I and $k_{inact} : k_{deg}$. (b) Contour map positioning as a tool for DDI risk assessment via MBI. See text for details.

Rearranging the terms in the derived expression in Equation 3.8, a modified form of this expression can be obtained and is shown in Equation 3.9 [71].

$$\frac{CL_{int,control}}{CL_{int,I}} = 1 + \frac{\left(\frac{k_{inact}}{k_{deg}}\right)}{1 + \frac{1}{\left(\frac{I}{K_I}\right)}} \tag{3.9}$$

It follows from Equation 3.9 that the fold decrease in intrinsic clearance of metabolism via the enzyme being inactivated is a complex nonlinear function of I:K_I and $k_{inact} : k_{deg}$ (Fig. 3.5a) and that various combinations of I:K_I and $k_{inact} : k_{deg}$ can result in identical fold reductions in intrinsic clearance. MBI interaction contour maps can thus be generated to serve as a visual aid to guide risk assessment for DDI from *in vitro* inactivation kinetics, initial estimates of k_{deg}, and of enzyme-available inactivator concentration *in vivo*. Each contour is a slice of the surface at a given magnitude of fold decrease in intrinsic clearance of the enzyme, and tracks combinations of I:K_I and $k_{inact} : k_{deg}$ producing identical fold reductions in intrinsic clearance (Fig. 3.5b). Therefore, rank ordering of the level of DDI risk associated with individual compounds within or across chemical series can be readily done based on the interaction contour on which they fall (or are closest to), which may not be readily apparent based on inspection of the four independent input parameters (k_{inact}, K_I, k_{deg}, [I]). This is illustrated in Fig. 3.5b where three hypothetical compounds characterized as inactivators (*X* with I:K_I of 0.02 and $k_{inact} : k_{deg}$ of 1000; *Y* with I:K_I of 2 and $k_{inact} : k_{deg}$ of 40; and *Z* with I:K_I of 0.02 and $k_{inact} : k_{deg}$ of 40) are compared on a MBI contour map. Despite vastly different values of I:K_I and $k_{inact} : k_{deg}$, the level of DDI risk associated with *X* and *Y* can be inferred to be comparable because they both fall close to the same contour (tracking an ~25-fold reduction in intrinsic clearance), albeit at different positions as dictated by their relative potencies and rates of inactivation. In contrast, compound *Z* represents a significant improvement over *X* or *Y* with respect to risk for MBI DDI, as is evident from its position on a contour

tracking a less than twofold reduction in intrinsic clearance. An important lesson learned from examination of compound X is that despite a very small I:K_I ratio of 0.02 (which would typically be considered to be associated with negligible risk and a remote possibility of a clinically meaningful interaction if the inhibition mechanism were reversible), the resulting fold reduction in intrinsic clearance is predicted to be very large (~20-fold), as a consequence of very rapid inactivation that is three orders of magnitude faster than the rate of degradation of the inactivated CYP isoform. This emphasizes the importance of not only potency considerations but also rate considerations when making inferences regarding the level of DDI risk associated with MBI. Making comparisons of K_I against clinical exposures of the inactivator is inappropriate for risk assessment unless considered in context of the $k_{inact} : k_{deg}$ ratio. Examples include CYP2D6 inactivation by paroxetine, CYP2C19 inactivation by ticlopidine, and CYP3A4/5 inactivation by erythromycin, verapamil, and diltiazem, which are all associated with clinically significant DDIs despite the corresponding I:K_I ratios being below 0.1 [55,71].

For the quantitative prediction of DDI magnitude with CYP inactivators, the fold decrease in intrinsic clearance of the inactivated enzyme (Eq. 3.9) takes the place of the "$1 + (I/K_i)$" term in Equation 3.5, resulting in the following equation (where $k_{deg(intestine)}$ and $k_{deg(liver)}$ are the first-order turnover rate constants for the intestinal and hepatic enzyme):

$$\frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{F_G + \dfrac{(1-F_G)}{\left(1+\dfrac{\left(\frac{k_{inact}}{k_{deg(intestine)}}\right)}{1+\frac{1}{\left(\frac{I_g}{K_I}\right)}}\right)}} \times \frac{1}{(1-f_{m(CYPi)}) + \dfrac{f_{m(CYPi)}}{\left(1+\dfrac{\left(\frac{k_{inact}}{k_{deg(liver)}}\right)}{1+\frac{1}{\left(\frac{I}{K_I}\right)}}\right)}} \quad (3.10)$$

Use of this equation has resulted in reasonable predictions of the magnitude of DDIs produced by CYP inactivators based on retrospective analyses from primary *in vitro* databases that suggest that unbound systemic exposure appears to be the best surrogate for hepatic [I], with the use of Equation 3.3 for estimation of intestinal [I] [55,56].

3.5.5 CYP Turnover Half-Life: A key parameter for IVIVE of MBI DDI

It is readily apparent on inspection of Equations 3.8–3.10 that a key drug-independent physiological parameter that is necessary to translate *in vitro* data on CYP inactivation to predict clinical pharmacologic consequences is the degradation/turnover rate constant (k_{deg}) of the enzyme *in vivo*. While relatively good estimates of *in vivo* k_{deg} are available for certain human CYP enzymes (e.g., hepatic CYPs 1A2, 2C8, 2D6, 2E1, and intestinal CYP3A), the same is not true for other enzymes where *in vivo* estimates are either lacking (e.g., CYP2C9, CYP2C19) or are available with a high degree of uncertainty reflected by a wide range of estimates across literature reports that have estimated this parameter using complementary methods and data analytic approaches with varying associated assumptions (e.g., hepatic CYP3A4). A detailed review of the methods used to estimate k_{deg}, their merits and limitations, and a critical analysis of available data in this regard is outside the scope of this chapter and the reader is referred to a recent detailed overview [72]. For CYP1A2, a half-life of 39 h is estimated

based on time course of deinduction on smoking cessation [73]. For CYP2D6, a half-life of 51 h has been derived by noncompartmental deconvolution of the steady-state serum half-life of paroxetine from the time course of recovery of CYP2D6 activity following cessation of treatment with the inactivator [69,74]. In the case of CYP2E1, the time course of both deinduction (during the withdrawal phase in alcoholics) and recovery following disulfiram inactivation have been characterized and both estimates yield comparable CYP2E1 turnover half-lives of 60 h [75] and 51 h [76], respectively. A recent report estimated the half-life of hepatic CYP2C8 to be 22 h based on analysis of the time course of recovery of repaglinide oral clearance following inactivation by gemfibrozil [77]. The turnover half-life of intestinal CYP3A (likely reflective of the kinetics of enterocyte turnover in the intestinal epithelium) has been estimated as 23 h based on the time course of recovery following inactivation by grapefruit juice ingestion [78]. For hepatic CYP3A, a wide range of estimates (24–140 h) is available based on analysis of clinical data of the time course of induction or deinduction, or of recovery from inactivation using CYP3A substrates of varying specificity and different methods of data analysis ranging from nonlinear regression of time course of induction/deinduction [72,79] to more sophisticated analyses using mechanistic population PK models describing autoinduction [80]. CYP3A turnover half-lives of 28 h [81,82], 36 h [37,55,71,83], 48 h [68], and 72 h [56,84] have been used in previous analyses of IVIVE of MBI DDI. Considering the uncertainty in half-life of certain human CYP enzymes and hepatic CYP3A, in particular, a useful approach to IVIVE of CYP3A MBI is to select a certain reasonable initial estimate (within the 24–72 h range, for instance) and then examine the sensitivity of the predicted drug interaction magnitudes around this value. The key question is whether the prediction for a given inactivator using the initial estimate of k_{deg} falls on the dynamic range of the sensitivity curve or on a relatively flat portion of the curve, as this would translate to a relatively low versus high confidence in prediction, respectively. The previously discussed MBI contour mapping approach can be useful in visually interpreting the results of analyses of sensitivity of the outcome of IVIVE of MBI DDI to uncertainty in k_{deg} when reliable estimates of this parameter are not available [49].

3.6 NME AS PRECIPITANT: CYP INDUCTION DDI

Induction of drug-metabolizing enzymes is an important mechanism of clinically significant DDIs. Increases in enzyme content secondary to either increased expression or protein stabilization by an NME can result in an increase in intrinsic clearance of metabolism by the induced enzyme, leading to decreased systemic exposure of coadministered drugs that are substrates for the enzyme that is induced. Although this typically manifests itself as a reduction in therapeutic efficacy (e.g., loss of oral contraceptive efficacy due to decreased exposure by enzyme-inducing antiepileptic drugs) and increased dosage requirements (e.g., higher cyclosporine A dose requirement in patients taking St. John's wort) in the setting of induction, increases in metabolism can also result in an alteration of the safety profile when dealing with drugs with active and/or toxic metabolites. Unlike reversible inhibition, induction DDIs are time dependent in their onset and offset, complicating their clinical management both after initiation of treatment with an inducer and in the deinduction period following cessation of treatment with the inducer.

3.6.1 Molecular Mechanisms of Induction and *In vitro* Assay Platforms

The major mechanism of induction of drug clearance is via increased expression of drug-metabolizing enzymes and transport proteins by the inducer via increased transcription. Therefore, risk assessment for induction DDIs requires the use of whole cell systems with intact transcriptional and translational machinery as opposed to inhibition DDI studies that can be readily performed in human liver microsomes. The major molecular mechanisms of induction DDI involve binding to and activation of receptors that are involved in the regulation of transcription of genes encoding drug-metabolizing enzymes and/or transporters. Although many such receptors and transcriptional mechanisms have been identified as regulators of gene expression, the three receptors that mediate gene transcription that is induced in the majority of clinically relevant induction DDIs include (i) the aromatic hydrocarbon (Ah) receptor, (ii) the constitutive androstane receptor (CAR), and (iii) the pregnane X receptor (PXR). For a detailed overview of the molecular mechanisms of enzyme induction, the reader is referred to recent review articles [85–87].

The *in vitro* system that is currently considered as reliable for evaluating the potential for an NME to produce induction of drug-metabolizing enzymes is cultured human hepatocytes, either as primary cultures of fresh human hepatocytes or as attachable cryopreserved hepatocytes, as reflected in the recently published perspective of the PhRMA [88]. It is recommended for *in vitro* induction studies in human hepatocytes that the potential for an NME to produce induction of CYPs 1A2, 2B6, and 3A4 be evaluated, as the genes encoding these enzymes are considered to be representative sensitive targets that respond to induction via the AhR, CAR, and PXR, respectively. Significant overlap and cross talk between the CAR and PXR systems is well established, with coinduction of CYPs 2B6 and 3A4 by prototypic inducers such as rifampin. Accordingly, it was originally considered to be sufficient to test NMEs for potential for induction of CYPs 1A2 and 3A4, to enable risk assessment for induction DDIs that may result from increased expression of AhR and CAR/PXR target genes, respectively, as reflected in the recommendations in the 2006 FDA Draft Guidance [39]. However, more recent studies have indicated that although human PXR regulates both CYP3A4 and CYP2B6 with less selectivity, human CAR exhibits marked selectivity for CYP2B6 over CYP3A4 such that selective CAR activators can induce CYP2B6 substantially without appreciable levels of CYP3A4 induction [89]. Additionally, some inducers of CYP2B6 and CYP3A4 (e.g., carbamazepine, nevirapine, efavirenz) produce their effects primarily via CAR rather than PXR activation [90]. On the basis of these advances in our understanding of the molecular mechanisms of induction, it is now generally accepted that a comprehensive and definitive *in vitro* evaluation of risk for an NME to produce DDIs via CYP/transporter induction should include analysis of the potential for induction of CYPs 1A2, 2B6, and 3A4 in human hepatocytes [88], or in an appropriately qualified cell line that maintains inducible regulation via the AhR, CAR, and PXR mechanisms as also noted in the 2010 EMA Draft DDI guideline [31].

Although recognized as the gold standard *in vitro* system for induction DDI risk assessment, limitations do exist in the availability of high quality human hepatocytes as reproducibly performing biological reagents for *in vitro* induction assays. While interindividual variability in responsiveness still requires consideration, the challenges related to supply limitations and the intermittent availability of fresh human hepatocytes have been substantially offset by the availability of cryopreserved human hepatocytes

and establishment of experimental approaches that enable their reliable use as models for induction studies [91]. In an effort to qualify biological reagents for *in vitro* induction studies with reproducible performance characteristics that model the performance of high quality human hepatocytes, hepatocyte-derived cell lines have received considerable attention as surrogate models of human hepatocytes for *in vitro* induction studies.

Successful use of the immortalized hepatocyte cell line Fa2N-4 has been described in the *in vitro* assessment of enzyme induction for PXR target genes (e.g., CYP3A4, MDR1, CYP2C9) and AhR target genes (e.g., CYP1A2) [92], with promising *in vitro–in vivo* correlations to enable induction DDI predictions [93]. However, since this immortalized hepatocyte cell line does not express CAR, induction of CYP2B6 is not observed in response to CAR-selective inducers, and induction of CYP3A4 in Fa2N-4 cells following treatment with CAR-selective inducers is either blunted or absent when compared to cryopreserved human hepatocytes [94,95]. Additionally, the level of expression of several hepatic transporters in Fa2N-4 cells is substantially lower than in human hepatocytes. Specifically, expression of the uptake transporters OATP1B1 and OATP1B3 is substantially reduced, as a result of which the apparent potency for rifampin induction of CYP3A4 in Fa2N-4 cells is 10-fold lesser than that observed in cryopreserved human hepatocytes [94], consistent with rifampin being a substrate for OATP-mediated hepatic uptake, which regulates its intracellular concentrations and apparent induction potency [96]. Therefore, Fa2N-4 cells may represent a good surrogate for primary human hepatocytes for evaluating the potential for an NME to produce AhR- or PXR-mediated induction, but not CAR-mediated induction, limiting their utility as a definitive *in vitro* model for CYP induction studies in a drug development setting [88].

The differentiated hepatoma cell line HepaRG appears to be a promising surrogate for human hepatocytes, based on recent studies that have demonstrated expression, activity, and inducibility of CYPs 1A2, 2B6, and 3A4 [97,98], consistent with expression of AhR, CAR, and PXR under optimally cultured conditions [99]. Unlike Fa2N-4 cells, where CYP2B6 induction is not observed following treatment with CAR-selective inducers such as phenytoin or phenobarbital due to lack of CAR expression [94], induction of CYP2B6 by these inducers has been observed in HepaRG cells [98]. Additionally, an excellent correlation between parameters characterizing the maximum magnitude, potency, and efficiency of inducers (E_{max}, EC_{50}, and E_{max}:EC_{50} ratio, respectively) in primary human hepatocytes versus HepaRG cells has also been described [100]. The experience gained from the above discussed examples of studies on the Fa2N-4 and HepaRG cell lines suggest that a comprehensive qualification of induction of CYPs 1A2, 2B6, and 3A4 using appropriate positive controls that measure regulation of all three pathways (AhR-, CAR-, and PXR-mediated induction) is critical to the use of any such cell line as a surrogate in place of primary human hepatocytes for *in vitro* induction studies.

Assays that measure binding to nuclear receptors and those that measure transactivation of reporter gene expression can be useful in the drug discovery setting due to their relatively higher throughput compared to definitive hepatocyte-based induction assays. Additionally, nuclear receptor transactivation assays can be useful in elucidating the molecular mechanism of the observed induction in hepatocytes (e.g., does an NME produce induction primarily via PXR or CAR activation?). However, since the mechanisms of induction are complex and involve multiple nuclear receptors with cross talk between the mechanisms, these assays alone are not considered sufficient in

excluding the potential for enzyme induction by an NME in drug development settings to support clinical DDI risk assessment [88].

3.6.2 Endpoints in *In vitro* Induction Studies

The general approach to *in vitro* induction studies in human hepatocytes (or adequately qualified cell lines with appropriate scientific justification of suitability, as discussed earlier) involves treatment with the NME for two to three days, with daily replacement of the culture medium containing the NME. Usually, a range of NME concentrations including the therapeutic systemic concentrations and including up to an order of magnitude higher concentration are evaluated to assess concentration response and the studies are conducted in hepatocytes from at least three different donor livers. At the end of the incubation period, the endpoints typically measured include (i) activity of CYPs 1A2, 2B6, and 3A4 *in situ* or in microsomes prepared from the hepatocytes using isoform-selective index reactions; and/or (ii) mRNA expression of CYPs 1A2, 2B6, and 3A4 using techniques such as reverse transcription-polymerase chain reaction [88]. Many CYP3A4 inducers are additionally time-dependent inhibitors of the enzyme's activity, making it important to include mRNA measurements in addition to enzyme activity assessments to aid appropriate mechanistic interpretation of the results, translation of relevance to the clinical setting, and appropriate clinical DDI study design, as reflected in the PhRMA perspective [88]. In fact, the EMA draft guideline mandates the inclusion of mRNA measurement in *in vitro* induction studies for the interpretation of study results if inhibition of the studied enzyme may not be excluded at the concentrations used or if a downregulation is suspected based on activity measurements [31]. A classical example of a drug producing complex simultaneous inhibition/induction effects is the HIV protease inhibitor ritonavir, with *in vitro* induction studies showing increases in mRNA expression of CYP3A4 due to PXR-mediated induction and decreased CYP3A4 activity due to time-dependent inactivation of the induced enzyme [101]. The clinical pharmacologic picture is consequently characterized by complex dose- and time-dependent interactions that are additionally dependent on the PK properties of the substrate drug. For example, in the case of the CYP3A substrate alprazolam, the net effect was a clinically significant level of inhibition of oral clearance (~2.5-fold increase in AUC) following short-term low dose treatment with four doses of 200 mg ritonavir administered BID, considered to be representative of a dosage schedule that may be used to initiate treatment with ritonavir [102]. In contrast, mild and clinically insignificant level of induction of alprazolam oral clearance (12% decrease in AUC) was observed as the net effect following a 10-day treatment with usual therapeutic dose of 500-mg BID of ritonavir as reflected in the ritonavir USPI [103]. Depending on the substrate drug's PK properties, the outcome of multiple-dose treatment with therapeutic doses of ritonavir can be variable, with substantial impairment of oral clearance (i.e., net inhibition rather than induction) observed even in the setting of a week-long treatment with high dose (500-mg BID) ritonavir for substrates such as sildenafil [104]. These consequences are obviously not straightforward to predict from the *in vitro* results alone, making it important to measure both mRNA and activity-based endpoints in the *in vitro* induction studies especially when there is a potential for concurrent inhibition. It is important to recognize that the use of CYP3A4 activity and/or mRNA measurement as an endpoint in *in vitro* induction studies is to serve as a sensitive marker of PXR-mediated induction and its associated pleiotropic

phenotype. For instance, if CYP3A4 induction is not observed in an *in vitro* induction study in human hepatocytes following treatment with an NME at concentrations up to 10 times the mean systemic C_{max} at clinically relevant doses, one can not only conclude that the risk for the NME to produce DDIs via CYP3A induction is low but also that the risk for it to produce DDIs via induction of other coinduced PXR targets such as CYPs 2C8, 2C9, 2C19, and MDR1 P-gp is also low as per the 2006 FDA Draft guidance [39]. With an NME that has properties such as ritonavir (i.e., PXR-mediated inducer and mechanism-based inactivator of CYP3A), if activity data alone were measured and used in risk assessment, it could result in a false negative risk assessment for induction DDIs. The conclusion that the risk for induction DDI is low may still be generally valid for potential interactions with CYP3A substrates since the net effect of inhibition may likely predominate and the time-dependent inhibition studies would lead to a clinical DDI evaluation of the effect of the NME on the PK of a sensitive CYP3A substrate to assess clinical relevance. However, a bigger impact is on risk assessment for induction of non-CYP3A targets of PXR, where a low risk for induction DDI with substrates of such enzymes or transporters (e.g., oral contraceptives, digoxin, warfarin) may be erroneously concluded in the absence of mRNA measurements that would be required for observing induction of gene expression, which would not be reflected by CYP3A4 activity measurements alone. Therefore, it is critical that the conclusion of lack of CYP induction be unbiased by factors such as concurrent inhibition or inactivation that could jeopardize the ability to pick up induction potential of an NME.

A recent analysis of CYP3A4 inducers evaluated the performance characteristics of mRNA versus enzyme activity as endpoints in cryopreserved human hepatocyte induction studies with respect to their sensitivity and specificity in the identification of the risk for induction DDIs and inferred that measurement of CYP3A4 mRNA levels represents the most sensitive endpoint for detecting induction in human hepatocytes [105]. Across inducers, the maximum fold increase in CYP3A4 mRNA expression observed in cryopreserved human hepatocytes was generally greater than the corresponding maximum observed fold increase in CYP3A4 catalytic activity [105], consistent with similar findings in a previous study using fresh human hepatocytes [100]. This is explained, at least in part, by concurrent inhibition of CYP3A4 by the inducer. Interestingly, when using mRNA expression of CYP3A4 as the endpoint, even with CAR-selective inducers such as phenytoin and phenobarbital, the fold increase in mRNA of CYP3A4 was consistently higher than the fold increase in mRNA of the other PXR/CAR-regulated drug-metabolizing enzymes examined (CYPs 2B6, 2C9, 2C19, 3A5), leading to the conclusion that CYP3A4 mRNA measurement may suffice as a single endpoint for evaluating risk for induction via the PXR and/or CAR pathways [105].

3.6.3 DDI Risk Assessment from *In vitro* CYP Induction Data

The translation of the results of *in vitro* induction studies performed in human hepatocytes (or adequately qualified cell lines that are responsive to PXR-, CAR-, and AhR-mediated induction, as discussed earlier) to clinical PK DDI risk assessment is an emerging area of science. Empirical cut-offs for fold increase in mRNA or activity over vehicle control and for the percentage of the observed induction by a strong inducer positive control (e.g., rifampin for CYP3A) tested alongside in the same experimental system have been used for classifying *in vitro* positives versus negatives for induction DDI risk assessment. For instance, observation of <40% of the positive

control's effect with use of a strong prototypic inducer as the positive control (e.g., rifampin for CYP3A) has been suggested as sufficient for classifying an NME as an *in vitro* negative for induction, eliminating the need for further clinical evaluation of DDI risk [14]. These approaches unfortunately lack a mechanistic basis and in the absence of a formal statistical optimization of the cut-off values for the specific experimental system, they should be used with caution. A recent study evaluated the performance characteristics of various preselected cut-offs for mRNA and activity endpoints in the categorization of risk for clinically relevant CYP3A induction DDIs using *in vitro* CYP3A4 induction data on 20 clinically significant CYP3A inducers and 15 noninducers in cryopreserved human hepatocytes [105]. This retrospective analysis concluded that a cut-off of fourfold increase in mRNA expression provided 98% sensitivity while maintaining a specificity of ~70% and importantly also demonstrated that the previously suggested cut-off of "40% of the positive control's effect" was associated with an unacceptably low sensitivity (i.e., high false negative rate) [105]. A similar observation questioning the conservativeness of the "40% of positive control" cut-off has been made in a previous study in primary human hepatocytes [106]. Opportunities exist for statistical refinement/optimization of empirical cut-offs using approaches such as receiver operator characteristics analyses that have been used with success in optimizing cut-offs for $I:IC_{50}$ ratios for assessment of risk for digoxin DDIs via P-gp inhibition [107].

3.6.4 Quantitative Predictions of Induction DDI Magnitude

For a detailed overview of approaches to quantitative IVIVE of induction DDIs, the reader is referred to a recent review article [108]. The first step in this process is to characterize the concentration–effect relationship for the induction observed *in vitro* (either for the mRNA increase and/or increase in enzyme activity), which is typically accomplished using a typical E_{max} model. IVIVE is then performed assuming that the fold increase in enzyme expression or activity estimated from the *in vitro* concentration–effect relationship at the *in vivo*–relevant concentration of the inducer directly translates to fold increase in intrinsic clearance of metabolism via the induced enzyme *in vivo* [109]. For an orally administered object drug without appreciable intestinal extraction (or under the assumption of lack of meaningful effect of the inducer on its intestinal extraction ratio), the following equation mechanistically allows the translation of the estimated *in vitro* concentration–effect relationship to a predicted interaction magnitude and is essentially similar in form to Equation 3.4 that was presented previously for inhibition DDI:

$$\frac{AUC_{induced}}{AUC_{control}} = \frac{1}{(1 - f_{m(CYPi)}) + \left(f_{m(CYPi)} \cdot \left(1 + \frac{E_{max}[Ind]}{EC_{50}+[Ind]}\right)\right)} \tag{3.11}$$

With enzyme activity as the endpoint in the *in vitro* induction assay in fresh human hepatocytes, use of unbound systemic steady-state maximum concentration of the inducer to estimate [Ind] and use of unbound EC_{50} of the inducer *in vitro* (calculated from the *in vitro* induction EC_{50} and the measured unbound fraction of the inducer in hepatocytes), Equation 3.11 has been successfully used to predict the magnitude of CYP3A induction DDIs [106]. Interestingly, when mRNA expression rather than enzyme activity was used as the *in vitro* endpoint, the performance of

IVIVE was poorer, with a trend for overprediction of the magnitude of clinical DDIs, explained by a higher E_{max} for mRNA expression compared to enzyme activity as the *in vitro* endpoint. This is not surprising considering that the fold increase in mRNA (a measure of the extent of induction) may not necessarily quantitatively equal the fold increase in enzyme content (which is governed by the efficiency of mRNA translation to protein), which ultimately determines enzyme activity. Nevertheless, this does not necessarily mean that enzyme activity rather than mRNA is a better endpoint for concentration–effect analysis of *in vitro* induction data to enable IVIVE. As discussed earlier, since certain CYP3A inducers also produce some level of inhibition of the enzyme's activity either via reversible or time-dependent mechanisms, an advantage of the mRNA endpoint is that it is a "pure" reflection of the induction effect unbiased by effects of the inducer on enzyme activity, the IVIVE of which should ideally be performed using approaches previously discussed for inhibition DDIs. If, however, mRNA is used as the endpoint, since a one-to-one correspondence between fold increase in mRNA and fold increase in enzyme content/activity cannot be assumed, there is a need for introducing an empirical scalar (multiplicative adjustment factor on E_{max} in Equation 3.11) that can be derived using a calibration approach using positive controls within the same experimental system, as has been described by Fahmi and colleagues [83]. In their model, Fahmi and colleagues additionally consider induction of intestinal metabolism, as well as concurrent reversible inhibition and time-dependent inhibition, taken together in the extrapolation of net *in vivo* DDI magnitude using a more general form of Equation 3.10 where the composite multiplicative effect of the inhibition, inactivation, and induction of intrinsic clearance of the affected enzyme is considered [83].

In addition to the above discussed mechanistic/semimechanistic IVIVE approaches to the prediction of induction DDIs, empirical *in vitro–in vivo* correlation approaches have also been described. These approaches involve establishment of a descriptive correlation relating the *in vitro* induction data and clinical exposures of the inducer to the observed clinical DDI magnitude for a database of prototypic inducers using a fit-for-purpose empirical mathematical model. Such a model is developed from a database of *in vitro* induction parameters, human exposure information, and clinical DDI outcomes. It is intended to provide a quantitative knowledge management framework that can aid forecasting of the clinical DDI magnitude associated with an NME from *in vitro* induction results and human exposure of the NME at clinically relevant doses (or predicted human exposures, if applied before entry into humans). With the immortalized cell line Fa2N-4 as the *in vitro* system, Ripp and colleagues described application of a relative induction score (RIS) approach for predicting the magnitude of clinical DDIs of inducers with midazolam (sensitive CYP3A substrate) and ethinyl estradiol (substrate for CYP3A as well as other PXR-inducible enzymes) [93]. The parameters E_{max} and EC_{50} describing the concentration–effect relationship for the fold increase in CYP3A4 mRNA *in vitro* were estimated for each inducer. The RIS for each inducer in the database was then calculated by substituting the unbound clinical C_{max} of the inducer for the concentration term into the estimated *in vitro* concentration–effect relationship for CYP3A4 induction. The RIS therefore is simply a calculated fold increase in CYP3A4 mRNA at the unbound C_{max} of the inducer based on the concentration–effect relationship characterized for the inducer *in vitro*. The relationships between the RIS and percent decrease in object drug (midazolam or ethinyl estradiol) AUC were fitted to empirical sigmoid E_{max} models. These models provide quantitative frameworks to guide prediction of clinical DDI magnitude with

midazolam and ethinyl estradiol from *in vitro* induction parameters (EC_{50} and E_{max}) and the NME's clinical PK data [93]. Although first described using Fa2N-4 cells as the *in vitro* model, the RIS approach has been subsequently qualified as applicable using cryopreserved human hepatocytes as well [110]. Using HepaRG cells as the *in vitro* model, Kanebratt and Andersson described an *in vitro–in vivo* correlation relating the ratio of total systemic exposure (AUC) of the inducer to the concentration of the inducer that produced a twofold increase in mRNA expression of CYP3A4 *in vitro* (F_2) as the predictor variable and the clinical DDI magnitude expressed as percent decrease in AUC of a selective and sensitive CYP3A substrate as the dependent variable. Similar to the RIS-based approach, the relationship between the AUC/F_2 ratio and percent decrease in object drug AUC was fitted to a hyperbolic E_{max} model to provide a quantitative framework to guide prediction of clinical DDI magnitude from *in vitro* induction data (F_2) and clinical exposures (AUC) of an NME [98].

3.7 NME AS PRECIPITANT: CLINICAL PHARMACOLOGIC EVALUATION

The key goal of the *in vitro* DDI risk assessment program evaluating the effects of an NME on the activity and expression of CYP enzymes is to guide development of a scientifically appropriate clinical DDI evaluation strategy. Importantly, the *in vitro* data are utilized to eliminate the need for unnecessary DDI studies in cases where it can be concluded that there is a remote possibility of a DDI via CYP inhibition or induction based on the CYP-inhibitory potency or concentration response for CYP induction viewed in the context of clinical exposures of the NME. For instance, there are many recent examples of approved drugs across therapeutic areas where an I:K_i ratio of <0.1 (Fig. 3.2) has been used to conclude that clinical DDIs via inhibition of the particular CYP isoform are unlikely without the need for specific clinical DDI studies. When the *in vitro* data cannot be used to exclude the risk for a potential DDI, prioritization of clinical DDI studies is necessary such that the conduct of clinical DDI studies that are more likely to result in clinically meaningful interactions is planned ahead of the conduct of less critical DDI studies that may result in smaller magnitude interactions.

For CYP-inhibitory DDIs, developing the strategy for clinical pharmacologic evaluation of DDIs is performed using the rank-order approach [22,23], a central assumption of which is that the rank order of potencies of inhibition of multiple CYP isoforms by an NME will be preserved *in vivo* such that the rank order of clinical DDI magnitude with isoform-selective probe substrates would be similar to the rank order of *in vitro* potencies versus each of those enzymes. The use of this approach in guiding clinical DDI strategy is illustrated schematically in Fig. 3.6. Consider, for example, a situation where an NME produces concentration-dependent *in vitro* inhibition of multiple CYP isoforms (*A* through *E*), with *A* being the most potently inhibited enzyme (lowest IC_{50}) followed by *B*, *C*, *D*, and *E* in that order. In such a situation, since *A* is the most potently inhibited enzyme, the clinical pharmacology plan should typically consider a DDI study with a probe substrate for enzyme *A* as the first clinical DDI study. If the results of this DDI study indicate the absence of an interaction, it would not be necessary to conduct DDI studies with probe substrates for enzymes *B–E* since they are less-potently inhibited *in vitro* and the resulting clinical DDI magnitude would

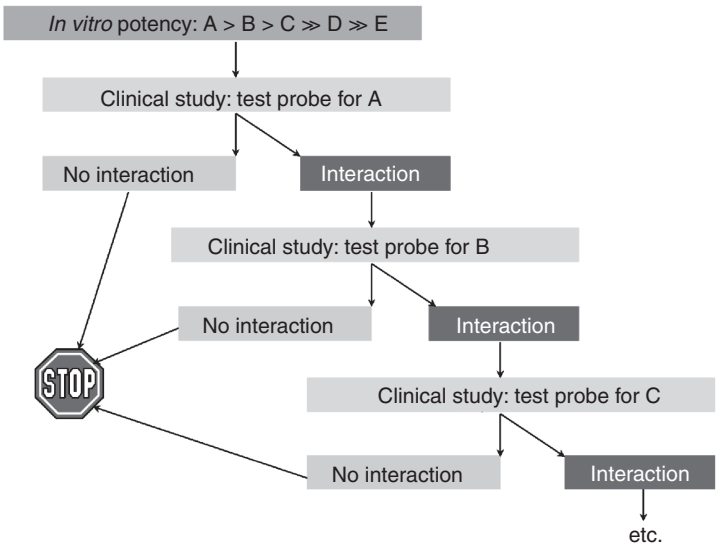


Figure 3.6 Rank-order approach for developing clinical DDI evaluation strategy based on *in vitro* CYP inhibition potency data.

therefore be expected to be lesser than that observed on inhibition of enzyme *A*. If, however, the clinical DDI study evaluating the effect of the NME on a selective probe substrate for enzyme *A* reveals a clinically meaningful interaction, the next DDI study to be considered is with a probe substrate for enzyme *B* (the enzyme with the second lowest IC_{50}), with the need for a DDI study with a probe substrate of enzyme *C* evaluated based on the results of the DDI study with the substrate of enzyme *B*. This iterative process is repeated until the lack of a clinical DDI is demonstrated, at which point it can be concluded that no additional DDI studies are necessary. This is analogous to a bridging approach where the first DDI study demonstrating the lack of an interaction serves as the link between the *in vitro* and clinical settings, enabling extrapolation of the *in vitro* data for less-potently inhibited enzymes to the conclusion that clinical DDIs via inhibition of these enzymes is unlikely. The fidelity of the rank-order approach has been retrospectively examined by Obach and colleagues [22,23] from a primary *in vitro* database of CYP isoform-specific inhibitory potency data for 21 drugs for which the results of clinical DDI studies with selective substrates of at least 3 CYP isoforms were available in the published literature. A twofold clinical DDI magnitude (i.e., ratio of exposure of the probe substrate in the presence of the CYP inhibitor in reference to that observed in the absence of the CYP inhibitor) was used to categorize an interaction as positive for purposes of this exercise. This analysis indicated that in 18 out of the 21 cases examined, retrospective application of the above described iterative rank-order approach would have been successful, supporting the performance of this strategy to define the clinical DDI strategy in a drug development setting.

There is precedence for the use of the rank-order approach to inform regulatory decision making. For example, *in vitro* CYP inhibition studies indicated that temsirolimus is an inhibitor of both CYP2D6 (I:K_i ratio of 0.38) and CYP3A4 (I:K_i ratio

of 0.19) [111]. A clinical DDI study was performed to evaluate the effect of temsirolimus at the clinically recommended dose of 25 mg on the PK of the CYP2D6 substrate desipramine. This study concluded the lack of an effect of temsirolimus at the recommended clinical dose on CYP2D6 activity in humans based on 90% confidence intervals for the ratio of geometric least square means of desipramine AUC and C_{max} (when administered with temsirolimus in reference to when administered alone) being contained within the 80–125% range [112]. The Clinical Pharmacology Review of the temsirolimus NDA concluded that based on the I:K_i ratios from *in vitro* studies, if temsirolimus did not produce clinically meaningful inhibition of CYP2D6, it can be extrapolated that temsirolimus would not inhibit CYP3A *in vivo* [111]. Accordingly, the USPI for temsirolimus indicates that no clinically significant effect is anticipated when temsirolimus is coadministered with agents that are metabolized by CYP2D6 or CYP3A4 [113]. Another example illustrating the application of the rank-order approach is for the Bcr-Abl kinase inhibitor nilotinib. Nilotinib was an *in vitro* inhibitor of multiple human liver microsomal CYP isoforms including CYPs 2C9, 2C8, 3A, 2D6, and 2C19 in decreasing order of potency, with corresponding I:K_i ratios of 32.6, 18.2, 9.6, 2.9, and 1.1, respectively, calculated using the mean steady-state C_{max} of nilotinib at the recommended dose of 400 mg BID as the measure of [I] [114]. As the clinical DDI implications of these *in vitro* observations were not fully elucidated at the time of approval of this anticancer agent, follow-up clinical DDI evaluation was required as a phase 4 postapproval commitment. On the basis of the observed rank order of CYP-inhibitory potency indicating inhibition of CYP2C9 with the highest potency, it was recommended in the Clinical Pharmacology Review of the nilotinib NDA that the sponsor conduct a DDI study evaluating the effect of multiple-dose administration of nilotinib on the PK of a sensitive CYP2C9 substrate (e.g., *S*-warfarin). Further, it was indicated that if a significant interaction was demonstrated, additional DDI studies evaluating the effects of multiple-dose administration of nilotinib on the PK of a sensitive CYP2C8 substrate and/or a sensitive CYP3A substrate may be needed [114], suggesting application of the rank-order approach in defining the appropriate clinical DDI evaluation strategy based on the results of *in vitro* CYP inhibition studies.

It is important to note that the rank-order approach is not without assumptions: one, is that the factors that determine quantitative translation of the *in vitro* K_i to the magnitude of the clinical DDI are similar across all CYP isoforms. However, this may not be the case when comparing across enzymes that may be expressed in the small intestine and liver (e.g., CYP3A) and those that are mainly expressed in the liver (e.g., other CYP isoforms). This is because inhibition of intestinal presystemic extraction of the probe drug can contribute to the overall magnitude of the observed interaction via CYP3A inhibition in addition to the impact of inhibition of hepatic metabolism, depending on the baseline F_G of the probe (Eq. 3.5). Consider, for example, an NME that inhibits CYP1A2 and CYP3A4, with a marginally greater potency of inhibition of CYP1A2 versus CYP3A4. If a DDI study with a sensitive CYP1A2 substrate (e.g., caffeine) demonstrates lack of an interaction, the possibility of *in vivo* inhibition of CYP3A may still be difficult to completely exclude for orally administered CYP3A substrates subject to substantial intestinal extraction (e.g., midazolam, simvastatin, buspirone). This is because the rank order of I:K_i ratio using systemic C_{max} of the NME as the estimate of [I] may not directly translate to the rank order of *in vivo* DDI magnitude due to the impact of higher intestinal concentrations of the inhibitor that may contribute to a greater effect on the PK of an orally administered CYP3A substrate

than would be expected based on considerations of systemic exposure alone. It is of interest that the 2010 EMA DDI Draft Guideline specifically addresses this caveat of the rank-order approach and does not permit extrapolation of the lack of inhibition *in vivo* for orally administered CYP3A substrates based on a lower potency of CYP3A inhibition and lack of an *in vivo* interaction with a probe substrate of a more potently inhibited enzyme [31]. Despite this caveat and the recommendation to avoid extrapolation to CYP3A for orally administered substrates, the rank-order approach, nevertheless, provides a useful framework to streamline clinical development planning by guiding clinical DDI strategy, prioritizing the conduct of clinical DDI studies and eliminating the need for unnecessary studies.

3.7.1 Probe Substrate Drug Selection in Clinical DDI Studies

A critical decision in clinical DDI study design for the evaluation of the CYP-inhibitory or inducing effect of an NME is selection of the probe substrate of the affected enzyme. Ideally, the probe drug should be a sensitive and selective substrate of the enzyme whose inhibition or induction by the NME is being investigated *in vivo*. A probe drug can be considered a sensitive substrate of a CYP enzyme if its exposure has been shown to increase greater than or equal to fivefold by a known inhibitor of that enzyme [39]. The reason it is important to select a sensitive probe substrate is because if lack of interaction is demonstrated with a sensitive substrate, it can be presumed that less sensitive substrates will also be unaffected, eliminating the need for conducting additional DDI studies to evaluate the potential clinical relevance of DDIs with less sensitive (but perhaps more clinically relevant) coadministered agents that are metabolized by the same enzyme as the probe drug. Consider, for instance, the drugs theophylline and caffeine. Both methylxanthine derivatives are substrates of CYP1A2, with a major portion of their clearance mediated via oxidative metabolism by this enzyme. It is estimated that ~95% of *in vivo* clearance of caffeine is via CYP1A2-mediated metabolism, whereas the relative contribution of CYP1A2 to theophylline clearance is lower, with an estimated contribution of ~80% as inferred from the results of human radiolabeled mass balance/metabolism and *in vitro* reaction phenotyping studies [49]. As indicated by Equation 3.4, the maximum possible magnitude of a DDI resulting from inhibition of CYP1A2 with caffeine as the substrate ($f_{m(CYP1A2)}$ of ~0.95) would be ~20-fold, whereas this ceiling for maximum possible magnitude of a CYP1A2-inhibitory DDI with theophylline ($f_{m(CYP1A2)}$ of ~0.8) would be calculated to be ~5-fold. Accordingly, DDI studies with the strong CYP1A2 inhibitor fluvoxamine have revealed a 5- to 12-fold decrease in caffeine oral clearance on coadministration with fluvoxamine [115,116], while the effects of fluvoxamine on theophylline AUC are more modest, characterized by an ~3-fold increase in AUC [117,118]. Although theophylline has a narrow therapeutic range and the consequences of inhibition of its clearance by an NME would be greater from a medical standpoint compared to the implications of impairment of caffeine clearance, the above discussed scientific considerations justify the use of caffeine rather than theophylline as a sensitive CYP1A2 probe substrate in the design of clinical DDI studies aimed at evaluating an NME as a potential inhibitor of this enzyme. If a caffeine DDI study establishes the lack of an interaction, it can be presumed that the exposure of theophylline will not be affected by the NME due to the greater sensitivity of caffeine compared to theophylline as a CYP1A2 substrate *in vivo* and conduct of a

theophylline DDI study would be unnecessary. In contrast, if the exposure of caffeine is increased meaningfully (e.g., greater than or equal to twofold), it cannot be presumed that the PK of theophylline will be unaffected. If the NME is being developed in a therapeutic area or patient population where coadministration with theophylline is likely, it may be necessary from a medical standpoint to conduct a DDI study with theophylline to quantify the magnitude of the specific interaction and provide prescribing guidance.

When dealing with CYP3A, due to the expression of the enzyme both in the liver and in the small intestine, the sensitivity of a probe substrate of this enzyme depends not only on the contribution of CYP3A to the overall hepatic metabolism of the probe but also on the extent of contribution of intestinal CYP3A to its presystemic extraction following oral administration (Eq. 3.5). Therefore, two critical parameters: $f_{m(CYP3A)}$ and F_G determine the sensitivity of substrates of CYP3A. Consider, for example, three substrates of CYP3A: midazolam, alprazolam, and buspirone. The rank order of sensitivity of these three drugs to CYP3A-inhibitory DDIs is as follows: buspirone > midazolam > alprazolam. This is readily apparent on examination of Fig. 3.7, where the effects of various CYP3A inhibitors (ketoconazole, itraconazole, grapefruit juice, nefazodone, erythromycin, diltiazem, verapamil, and ritonavir) on midazolam AUC on the X-axis is compared against their corresponding effects on buspirone (circle symbols) or alprazolam (square symbols) AUC on the Y-axis. This analysis indicates that alprazolam is generally less sensitive to CYP3A inhibition than midazolam (square symbols are below the line of identity) but that buspirone is generally more sensitive than midazolam (circle symbols are above the line of identity). These observations are explained by the PK properties of these object drugs, with differences in sensitivity of buspirone and midazolam being explained by buspirone having a smaller F_G than midazolam (0.21 versus 0.5) and differences in the sensitivity of alprazolam and midazolam being explained by differences in $f_{m(CYP3A)}$ (alprazolam: 0.74, midazolam: 0.93) as well as F_G (alprazolam: 0.99, midazolam: 0.5). Clearly, CYP3A is a major contributor to alprazolam clearance *in vivo* such that coadministration with strong inhibitors such as ketoconazole can result in clinically significant increases in alprazolam exposure of approximately fourfold increase in AUC [119] with such coadministration not recommended in clinical practice [120]. However, alprazolam is not a sufficiently sensitive CYP3A substrate and is therefore not a suitable probe for evaluating the potential CYP3A inhibitory effect of an NME in a clinical DDI study. In contrast, both midazolam and buspirone represent sensitive probe substrates of CYP3A for use in clinical DDI studies as coadministration with CYP3A inhibitors (e.g., ketoconazole, ritonavir, itraconazole) has resulted in fivefold or greater magnitudes of DDI with these substrates (Fig. 3.7).

Examples of selective probe substrates for the major human drug-metabolizing CYP isoforms include caffeine and tizanidine for CYP1A2; omeprazole for CYP2C19; desipramine for CYP2D6; and midazolam, triazolam, buspirone, simvastatin, and felodipine for CYP3A [39]. For some CYP isoforms, established sensitive probe substrates are lacking and in these cases, representative reasonably selective substrates will need to be used. In some cases, although the clinically dosed substrate drug is a racemic mixture, the PK of specific enantiomers will need to be characterized using chiral bioanalytical methods due to stereoselective metabolism of individual enantiomers by specific enzymes. For example, although racemic warfarin is administered as the probe substrate for evaluation of CYP2C9 inhibitory effect of an NME, the PK of *S*-warfarin will need to be characterized as only this enantiomer is

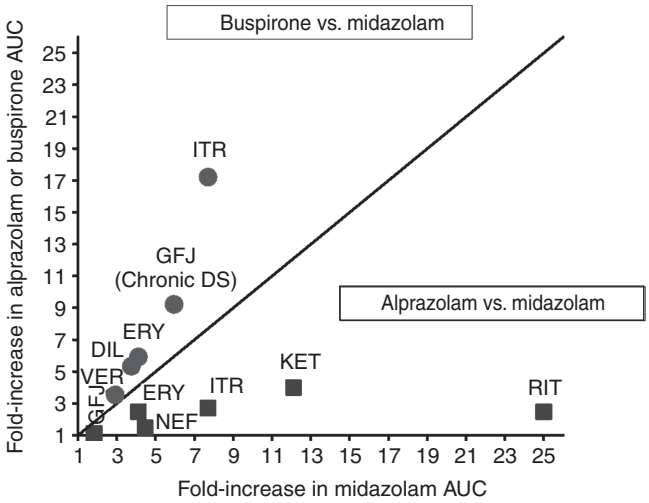


Figure 3.7 Illustration of differential substrate sensitivity to metabolic inhibitory DDIs, based on examination of the effects of different CYP3A inhibitors on the AUCs of three representative CYP3A substrate object drugs midazolam, alprazolam, and buspirone. The squares indicate the relationship between the effects of these inhibitors on alprazolam AUC (Y-axis) and their corresponding effects on midazolam AUC (X-axis). The circles indicate the relationship between the effects of these inhibitors on buspirone AUC (Y-axis) and their corresponding effects on midazolam AUC (X-axis). The line represents the line of identity ($X = Y$). The CYP3A inhibitors included are diltiazem (DIL), erythromycin (ERY), single-strength grapefruit juice (GFJ), chronic double-strength grapefruit juice (GFJ, chronic DS), itraconazole (ITR), ketoconazole (KET), nefazodone (NEF), and ritonavir (RIT).

selectively metabolized by CYP2C9 [31]. Finally, in some cases, specific pathways of metabolism of the probe substrate (as opposed to total clearance) are known to be selectively catalyzed by the enzyme of interest because of the existence of additional metabolic pathways catalyzed by other enzymes. In these cases, a metabolite/parent exposure ratio rather than parent drug AUC is used as the endpoint for DDI studies. For example, hydroxylation of the antidepressant bupropion is a specific index of CYP2B6 activity, as supported by *in vitro* metabolism studies of this pathway in human liver microsomes and using recombinantly expressed human CYP isoforms [121]. Consistently, use of the hydroxybupropion/bupropion exposure ratio as an endpoint in clinical DDI studies is supported by its ability to identify the CYP2B6 inhibitory potential of the antiplatelet agents, ticlopidine and clopidogrel, that produced 90% and 70% decrease, respectively, in the hydroxybupropion/bupropion AUC ratio [122].

3.8 NME AS OBJECT OF DDI

The preceding sections of this chapter have provided an overview of the approaches used for assessment of the risk for DDIs produced by the NME as the precipitant drug and their clinical pharmacologic evaluation, either as a potential inhibitor or inducer of the mechanisms of disposition of coadministered agents. The scientific considerations

underlying risk assessment for DDIs resulting from modulation of disposition of the NME and strategies for their clinical pharmacologic evaluation in drug development will now be discussed using representative examples.

3.8.1 Determinants of DDI involving the NME as the Object Drug

The cornerstone for assessment of risk for PK DDIs for an NME as the object drug is a quantitative understanding of the relative contributions of each mechanism to total human clearance at the level of its specific molecular determinants, that is, specific enzyme or transporter isoforms, denoted variably by f_m, $f_{m(CYP)}$, $f_{m(CYPi)}$, or the CR in previous investigations of CYP-based DDIs [22,44–48,123,124], as is evident from the previously discussed Equations 3.4 and 3.11. Derivation of the $f_{m(CYPi)}$ for an NME to define the contribution of a specific enzyme to its overall clearance is a two-step process. The first step involves an estimation of the *in vivo* contribution of specific biotransformation pathways to total drug clearance in humans, and the second step involves an estimation of the relative contribution of specific enzyme isoforms to each of these *in vivo* biotransformation pathways using *in vitro* reaction phenotyping approaches. The preferred approach for the first step of this two-step process is a radiolabeled mass balance study that includes identification and quantification of metabolites recovered in excreta (urine and feces) to enable construction of the overall human biotransformation scheme for the NME and estimate relative contributions of each primary biotransformation pathway to the overall clearance of the drug [125]. The second step involves an *in vitro* analysis of the principal biotransformation pathways identified in the first step, using an appropriate *in vitro* system that models human biotransformation of the NME, to define the relative contribution of each molecular determinant (e.g., CYP isoform) to the clearance of the NME via that biotransformation pathway. The results from these two distinct steps will need to be integrated to derive the $f_{m(CYPi)}$ for each contributing enzyme isoform to enable DDI risk assessment for the NME as the object drug. For example, if two primary biotransformation pathways A and B are identified for an NME based on analysis of excretory metabolites in a radiolabeled mass balance study, with relative contributions of 30% and 60% to total clearance, and if reaction phenotyping studies on pathways A and B indicate 30% and 50% contributions of CYP3A, respectively, it can be inferred that ~40% of the total clearance of the NME is expected to occur via CYP3A-mediated metabolism (calculated as the sum of 30% of 30% for pathway A and of 50% of 60% for pathway B). Similarly, if the reaction phenotyping studies indicate that the contributions of CYP2D6 to pathways A and B are 50% and 10%, respectively, it can be inferred that ~20% of the total clearance of the NME is expected to occur via CYP2D6-mediated metabolism.

3.8.2 Strategy for Clinical DDI Evaluation

On the basis of current regulatory guidance [31,39], clinical DDI studies evaluating the effects of strong inhibitors/inducers of a participating enzyme are not necessary if the relative contribution of the enzyme to overall clearance is <25%. Accordingly, in the above discussed scenario, the clinical pharmacology plan for such an NME should typically include DDI studies with a strong CYP3A inhibitor (e.g., ketoconazole) and strong CYP3A inducer (e.g., rifampin), whereas studies evaluating the effect of a strong

CYP2D6 inhibitor (e.g., paroxetine) or of the impact of the CYP2D6 polymorphism (e.g., EM vs. PM study) would not be required, as a complete inhibition of CYP2D6 or lack of activity of this enzyme would not be expected to result in a clinically meaningful exposure alteration (contribution <25% would translate to a <1.33-fold increase in exposure on complete inhibition of the enzyme, based on Equation 3.4).

If more than one enzyme is predicted to contribute meaningfully (>25%) to the overall clearance of an NME, DDI studies with strong inhibitors and inducers of each of these pathways should be typically planned. However, a step-wise approach can be used starting with the DDI study evaluating the effect of a strong inhibitor of the highest contributor enzyme followed by conduct of DDI studies with strong inhibitors of the less important contributors (analogous to the rank-order approach described earlier for CYP inhibition studies for the NME as the inhibitor). For each of the contributing enzymes, if a strong inhibitor produces a large and clinically meaningful increase in NME exposure, the need for additional DDI studies with less potent (e.g., moderate) inhibitors of that enzyme will need to be considered to guide DDI risk management and inform prescribing guidance. An example that illustrates this is for the CYP3A substrate everolimus, where the results of DDI studies with strong and moderate CYP3A inhibitors have informed prescribing guidance [126]. The value of such characterization in informing prescribing guidance is discussed later. If a contributing enzyme is polymorphically expressed, with existence of PM genotypes that are deficient in enzyme activity, a PK study evaluating the effect of the polymorphism on the NME's PK (e.g., in EMs vs PMs) can serve the purpose of a DDI study with a strong inhibitor and an additional strong inhibitor DDI study would not be necessary. However, it is important to note that patients with PM genotypes for the major molecular determinant of clearance of the NME may still be at risk of DDIs resulting from inhibition of the alternative "minor" clearance mechanism(s). Although these alternative clearance pathways may be considered minor contributor(s) to total clearance in EMs, they will be important contributor(s) in PMs. For example, consider a drug that is cleared primarily via metabolism by the polymorphic enzyme CYP2D6 (f_i(%)80%), with the remainder of its clearance occurring via metabolism by CYP1A2. Although CYP2D6 EMs will not be at risk of DDIs via inhibition of CYP1A2, it is important to note that clinically relevant increases in exposure to the NME can still be produced by CYP1A2 inhibitors (e.g., fluvoxamine) in the CYP2D6 PM subpopulation. As will be discussed later, population-based simulations integrating *in vitro* quantitative metabolism phenotyping data together with the available clinical PK characteristics of the NME in EM and PM subpopulations represent a powerful approach to DDI risk forecasting in such special populations and can additionally inform the need for and design of DDI studies in such special subpopulations.

3.8.3 Risk Assessment Strategies in Early Clinical Development

Although the above described two-step process that requires data from a human radiolabeled mass balance study is the gold standard approach for definitive DDI risk assessment and development of the clinical DDI strategy for the NME as the object drug, it is not always practically feasible in early clinical development planning to complete a radiolabeled mass balance study ahead of initiation of clinical development in patient populations (i.e., ahead of phase 2 proof-of-concept clinical studies). Nevertheless, early assessment of DDI risk is necessary to enable appropriate risk management

strategies in clinical studies in patient populations. Some key questions that will need to be answered include: (i) Are certain DDIs expected to be either large in magnitude or clinically meaningful (e.g., with strong inhibitors of an enzyme that is expected to be a major contributor to clearance of the NME), to require exclusion of specific categories of concomitant medications? (ii) Is the expected magnitude and clinical significance of certain DDIs small enough to permit inclusion of patients on certain concomitant medications (e.g., patients on weak or moderate inhibitors of a contributing enzyme) provided adequate safety monitoring is included? (iii) Is there advice that can be provided on alternate concomitant medications within a particular therapeutic category that may be best suited from the standpoint of DDI risk minimization (e.g., sertraline or citalopram in place of paroxetine or bupropion as a selective serotonin reuptake inhibitor antidepressant for an NME that is expected to be metabolized by CYP2D6; azithromycin or amoxicillin as potential antibiotics in place of clarithromycin for an NME that is expected to be metabolized by CYP3A). In oncology drug development, DDI risk assessment and the development of risk management strategies will need to begin sooner than in many other therapeutic areas considering that phase 1 first-in-human studies are commonly done in patients with advanced cancer that are often on multiple concomitant medications, especially when dealing with cytotoxic NMEs that cannot be studied in healthy volunteers [127]. Therefore, from a practical standpoint, alternate methods for an earlier provisional estimation of $f_{m(CYPi)}$ are often necessary before initiation of phase 2 clinical studies, and in some cases, possibly even ahead of first-in-human studies if drug development is to initiate in patient populations. *In vitro* metabolite profiling and identification studies using radiolabeled substrate and human biomaterials (e.g., human liver microsomes, hepatocytes, S9 fractions) are generally successful in identifying the primary biotransformation pathways of drugs in humans [128]. To enable identification of the primary biotransformation pathways in humans with reasonable confidence in the absence of data from a human mass balance study, a bridging approach may be used that leverages *in vitro* and *in vivo* metabolism data from preclinical species using radiolabeled NME together with *in vitro* metabolite profiling using human biomaterials. The observation of *in vitro* to *in vivo* correspondence in the biotransformation pathways of the NME in preclinical species (e.g., rat, dog) can increase confidence in use of *in vitro* metabolite profiling studies using human biomaterials (e.g., human liver microsomes, human hepatocytes) to estimate the contributions of individual metabolic pathways (e.g., oxidative vs conjugative) to overall human clearance and identify pathways of importance. These data can then be used to design *in vitro* quantitative reaction phenotyping studies of the principal biotransformation pathways to estimate the relative contributions of individual enzyme isoforms to the overall clearance of the NME.

3.8.4 Quantitative Reaction Phenotyping of NME Metabolism

Irrespective of the approach used to defining the contribution of specific biotransformation pathways to NME clearance (*in vivo* radiolabeled mass balance study in humans as the gold standard vs a provisional estimate using radiolabeled *in vitro* metabolite profiling studies with human biomaterials), the second step of the two-step process of estimating $f_{m(CYPi)}$ involves quantitative reaction phenotyping of the principal biotransformation pathways. *In vitro* drug metabolism studies have reached an impressive level of sophistication to permit derivation of a quantitative phenotype

(i.e., description of the percent contributions of each enzyme to total metabolism *in vitro*) of CYP-mediated biotransformation of drug candidates. This is possible using the complementary approaches of selective chemical and/or antibody-mediated inhibition, scaling from kinetic studies on recombinantly expressed individual CYP isoforms, and correlation analyses of metabolic rates of the NME against the content/activity of individual CYP isoforms in a panel of human liver microsomes. A detailed overview of reaction phenotyping strategies and experimental approaches is not within scope of this chapter, and the reader is referred to review articles on the subject [14,129–134].

Although the *in vitro* prediction of *in vivo* glucuronidation-mediated clearance and reaction phenotyping of conjugative metabolism is an emerging area of science, significant progress has been made in recent years with availability of recombinantly expressed uridine diphosphate glucuronosyltransferase (UGT) isoforms, characterization of isoform-selective substrates/index reactions, and limited examples of isoform-selective inhibitors [135]. For example, optimization of experimental conditions for successful quantitative phenotyping of the relative contributions of parallel pathways of direct glucuronidation versus oxidative CYP-mediated metabolism to the overall metabolism of drugs has been recently described [136]. Once the relative contribution of glucuronidation to total hepatic metabolism is defined using such an approach, the relative contribution of specific molecular determinants can be further assessed with the use of recombinantly expressed UGT isoforms, with the use of selective UGT inhibitors where available, and with studies using genotyped liver microsomal samples for UGTs displaying polymorphic expression. A recent example of such an application is the use of kinetic studies on human liver microsomes with functional UGT2B17 (*1/*1 genotype) versus UGT2B17-deficient liver microsomes (*2/*2 genotype) coupled with kinetic studies of recombinantly expressed UGT isoforms to reveal an important contribution of UGT2B17 to the metabolism of the histone deacetylase inhibitor anticancer drug vorinostat [137]. Application of a two-stage approach involving (i) estimation of the relative contribution of glucuronidation versus CYP-mediated metabolism to total metabolic clearance and (ii) reaction phenotyping of the glucuronidation and oxidation components, should therefore permit derivation of initial estimates of the contribution of each molecular determinant (i.e., individual UGT and CYP isoforms) to the overall hepatic clearance of an NME.

3.8.5 Considerations of Pharmacokinetic Properties of the NME in Risk Assessment

Factors that need to be considered in translational assessment of the level of DDI risk include the route of administration and the expected hepatic extraction ratio. For intravenously administered high clearance drugs, clearance is largely dependent on hepatic blood flow, and the PK of such drugs will therefore be relatively less sensitive to alterations in hepatic metabolic intrinsic clearance by coadministered inhibitors or inducers of metabolic enzymes. In contrast, intravenously administered low clearance drugs whose clearance is largely dependent on hepatic intrinsic clearance are relatively less sensitive to changes in hepatic blood flow and will be more sensitive to DDIs via inhibition of hepatic metabolism [138]. Unlike intravenously administered drugs, the systemic exposures of orally administered drugs that are primarily cleared via the hepatic route will be equally sensitive to DDI via inhibition of hepatic metabolism irrespective of their baseline extraction ratio. However, the specific

nature of the PK interaction (i.e., change in shape of the oral dose PK profile due to a DDI) will be different, with the half-life being altered (reflecting alteration of systemic clearance) without an appreciable change in peak plasma concentrations for low clearance drugs and the peak concentrations being principally affected (reflecting an alteration of bioavailability) without an appreciable alteration of half-life for high clearance drugs. Additionally, when CYP3A is identified as a contributor to the human liver microsomal metabolism of an NME, intestinal first-pass extraction of the NME can occur following oral administration, inhibition of which can increase DDI magnitude beyond what would be predicted solely from the *in vitro* liver microsomal metabolic phenotype. Considering the above discussed multiple factors (route of administration, extraction ratio, intestinal extraction, etc.) that influence translation of $f_{m(CYPi)}$ to the projected maximum DDI magnitude, physiologically based IVIVE permits integration of all available data on the NME and inhibitor of interest, to predict clinical DDI magnitude [124,139], as will be discussed later.

3.8.6 Considerations of Transporter Contribution to NME Disposition

Uncertainty in the quantitative translation of the drug-metabolizing enzyme phenotype derived from *in vitro* studies can be expected to be higher when the NME is also a substrate for nonmetabolic transporter-based clearance mechanisms. The Biopharmaceutics Drug Disposition Classification System (BDDCS) provides a useful framework to guide assessment of the importance of intestinal and hepatic transport as well as enzyme-transporter interplay to the NME's disposition and sensitivity to DDIs, resulting from metabolic and/or transporter inhibition [9,140]. Additionally, knowledge of the BDDCS class of the NME can help guide expectations regarding the level of confidence in IVIVE of metabolic DDI risk based solely on the NME's metabolic phenotype derived from *in vitro* metabolism studies. For instance, the confidence in predictions from *in vitro* metabolism data alone may be relatively high for BDDCS class 1 (i.e., highly soluble and highly permeable/extensively metabolized) NMEs. This is because transporter effects are expected to be minimal in the gut and liver, and it should therefore suffice to consider the impact of intestinal and hepatic drug-metabolizing enzyme inhibition for class 1 NMEs. In contrast, considerations of the contributions of intestinal efflux and hepatic uptake and efflux, as well as enzyme-transporter interplay are particularly important for BDDCS class 2 (i.e., poorly soluble and highly permeable/extensively metabolized) NMEs [141]. Importantly, for BDDCS class 2 NMEs that are substrates of both intestinal drug-metabolizing enzymes (e.g., CYP3A, intestinal UGT isoforms) and efflux transporters (e.g., P-gp), large clinically relevant DDIs can result from coadministration with dual inhibitors of the metabolizing enzyme and efflux transporter (e.g., ritonavir, a potent inhibitor of CYP3A and P-gp). Predicting the clinical correlates of the dynamic interplay of metabolism and transport for BDDCS class 2 drugs requires careful consideration of expectations at each anatomic site of the interaction (e.g., intestinal vs hepatic extraction), given the inverse orientation of P-gp and CYP3A in these organs [142]. Notable progress has been made in the IVIVE and phenotyping of hepatic uptake and efflux clearance through the use of cryopreserved hepatocytes, heterologously expressing cellular models of coupled vectorial transport, and vesicular models for *in vitro* kinetic studies of drug uptake by transporters such as OATP1B1 and OATP1B3 and efflux by transporters such as P-gp, BCRP, and MRP2.

Application of a relative activity factor-based scaling approach has enabled quantification of the contributions of individual organic anion transporting polypeptide (OATP) isoforms to overall hepatic uptake clearance [143–146], with simulation of clinical PK correlates made possible through use of physiologically based PK (PB-PK) models [147–149]. Despite these emerging scientific developments, approaches for estimating the clinical PK correlates of inhibiting individual molecular mechanisms of clearance for drugs cleared via mixed mechanisms (metabolism and transport) remain to be characterized from a standpoint of translational validity. Of note, the examples illustrating the application of the aforementioned methods for transporter identification and phenotyping have been primarily focused on nonmetabolized drugs (primarily BDDCS class 3 drugs) and additional evaluations are necessary to qualify more general applications of the framework for metabolized compounds that may additionally be substrates of active efflux and/or uptake transporters.

3.8.7 Risk Assessment for DDIs with Inducers

An important difference between susceptibility of an NME as an object drug to induction versus inhibition DDI is that when an enzyme is a minor contributor (e.g., $f_{m(CYPi)} < 25\%$) to total clearance of an NME, while the magnitude of an inhibitory DDI will be relatively small even on essentially complete inhibition of the enzyme (<1.33-fold), the magnitude of an interaction resulting from strong induction of that enzyme can reach clinically significant magnitudes [123]. Therefore, the relative contribution of inducible enzymes will need to be very small to conclude that the risk for induction DDI is low (e.g., resulting in a <25% decrease in NME exposure). For example, it has been shown that even if the contribution of CYP3A to the overall clearance of an object drug is only 25%, the enzyme-inducing anticonvulsants phenytoin and carbamazepine can be expected to produce an ~40–60% decrease in exposures of the object drug and the strong CYP3A inducer rifampin can be expected to produce an ~65% decrease in the object drug exposure, due to the high intrinsic efficiency of these agents as CYP3A inducers [123].

Additionally, it is important to recognize that induction of multiple drug-metabolizing enzymes and transporters occurs in a pleiotropic manner via common upstream nuclear receptors that regulate the expression of a battery of drug-metabolizing enzyme encoding genes. Therefore, even though the relative contribution of CYP3A per se may be well below 25%, if other PXR-inducible enzymes such as CYPs 2C9 and 2C19 or drug transporters such as P-gp contribute additionally to the overall clearance of the NME, the possibility of clinically significant decreases in exposure following administration of strong PXR agonist inducers such as rifampin cannot be excluded. A recent example illustrating this concept is the interaction between the strong CYP3A inducer rifampin and the antibiotic linezolid, which is not directly metabolized by CYP enzymes, not a substrate of P-gp, and is primarily metabolized by a nonenzymatic chemical oxidation mechanism. In a clinical DDI study, rifampin produced an ~30% reduction in linezolid exposure, with *in silico* simulations demonstrating that an interaction of such magnitude can be explained by a CYP3A contribution to linezolid clearance of <10% [150]. Therefore, when determining the need for a clinical DDI study for an NME with a strong inducer (e.g., rifampin), it may be important to consider such studies even if the contribution of inducible clearance pathways is <25%, because of the possibility that the contribution

of the inducible clearance mechanism can be large enough in the induced state to meaningfully increase the overall clearance of the NME and result in clinically relevant decreases in systemic exposure.

3.9 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING AND SIMULATION OF DDIs

The approaches discussed thus far, while useful for initial risk assessment and quantitative predictions of expected interaction magnitude, nevertheless, represent a simplification of the problem of IVIVE of DDIs, as they assume a single constant value of [I], substrate concentrations below the K_m, and do not consider the time-varying concentration of the inhibitor or inducer, or the object drug in the context of typical clinical dosing regimens. These aspects can only be incorporated when the theoretical frameworks for IVIVE of CYP inhibition or induction DDI discussed in the previous sections of this chapter are implemented in the context of a PB-PK model. Use of such a model-based approach allows prediction of not only the fold increase in exposure (AUC) of the object drug but also simulation of the expected PK profile following administration of the metabolic inhibitor or inducer, enabling forecasting of effects on parameters such as peak plasma concentration and half-life. With the availability of automated software to enable such simulations from *in vitro* data the application of full PB-PK model-based methods in drug discovery and development is increasing. The increased use of such model-based approaches is exemplified by many recent publications highlighting the utility of these approaches in the prediction of DDI magnitude [37,38,68,124,139,151,152]. This framework allows simultaneous modeling of object and precipitant drug PK including complexities such as nonlinear PK characteristics, metabolite kinetics and interactions resulting from effects of metabolites in addition to the parent perpetrator agent, and can additionally permit incorporation of multiple mechanisms of interactions (e.g., reversible inhibition, MBI, induction) when dealing with mixed mechanism precipitants with complex properties [38,81,82,153]. In addition to enabling simulation of the typical effect of the metabolic inhibitor on the full PK profile of the object drug, tools such as the Simcyp® population simulator allow forecasting of the associated intersubject variability of the DDI in the population because variability in demographic (e.g., body weight), physiological (e.g. liver blood flow), biochemical (e.g., abundance of individual CYP isoforms in the liver, serum albumin concentration), and genetic (e.g., population frequency of CYP2D6 EM vs PM genotypes) parameters are all considered together with drug-specific *in vitro* input parameters (e.g., CYP inhibition K_i, K_m for metabolism of the object drug by individual CYP isoforms) in forecasting outcomes of the DDI in the population [43,154,155]. The use of PB-PK model-based predictions of the outcome of DDIs can be particularly important in guiding the design of clinical DDI studies ahead of conducting them. Simulation studies can be particularly valuable in estimating the required duration of washout between periods of crossover DDI studies as well as guiding the minimum necessary duration of dosing of the precipitant drug to elicit the maximum possible interaction magnitude, both in context of the investigational agent as the object and as the precipitant. A recent example of such an application is in the evaluation of the impact of dose, dosing duration, and dosing frequency of ketoconazole as a strong CYP3A inhibitor on the magnitude of DDI with CYP3A substrates of varying PK characteristics to develop

general recommendations on the optimal dosing regimen of ketoconazole for use as a strong CYP3A inhibitor in clinical DDI studies based on the object drug's characteristics [156]. These approaches can be particularly useful in evaluating whether the magnitude of the interaction can be offset by altering the timing of administration of the object drug and the metabolic inhibitor [157], enabling scientifically guided risk management strategies in later-phase clinical trials, where appropriate, based on the PK properties and clinical dosing regimens of the object and precipitant drugs.

When dealing with the investigational agent as the object drug, physiologically based modeling and simulation can be particularly useful in projecting the impact of DDIs in special patient populations (e.g., patients with renal impairment or genetically distinct subpopulations such as CYP2D6 PMs), where the relative contributions of individual clearance mechanisms to the overall clearance of the investigational agent can be different from those in the general patient population. Consider, for example, a drug that is metabolized by CYP2C19 (major contributor) and CYP3A4 (minor pathway with $<20\%$ contributor to total clearance). The effects of CYP3A4 inhibitors can be expected to be minimal and not of clinical significance in patients with functional CYP2C19, whereas this cannot be expected for CYP2C19 PMs where loss of functional activity of CYP2C19 can result in this drug essentially behaving as a "pure" CYP3A substrate, with potentially major risks for clinically important DDIs with strong CYP3A inhibitors. Conducting a DDI study in PMs of CYP2C19 can be operationally challenging, considering the low frequency of the PM genotypes in Western populations. The challenges can be even worse if the investigational agent is a cytotoxic antineoplastic agent that cannot be dosed in healthy volunteers, which would then necessitate the conduct of such a DDI study in a genetically defined special population of cancer patients. Simulations of the expected effect of strong inhibitors (e.g., ketoconazole) on the PK of such a drug in CYP2C19 PMs, using *in vitro* reaction phenotyping data and available clinical PK data in EM versus PM subjects as prior knowledge can represent a powerful approach to forecasting the level of DDI risk in such special populations. Whether such simulations can be solely used in lieu of a dedicated DDI study will, of course, depend on the specific case under consideration and factors such as the population frequency of the special subpopulation, predicted magnitude of the interaction, sensitivity of the predictions to uncertainties in input parameters or key assumptions, as well as the clinical impact of the predicted interaction based on exposure–response understanding of safety of the drug in question. Nevertheless, having the results of such simulations in a drug development setting can guide determination of the timing and design of such special population DDI studies and facilitate scientific discussions with regulatory agencies on the proposed clinical DDI risk assessment and evaluation strategy. Another setting where PB-PK model-based simulations can be helpful is in the forecasting of level of DDI risk via a new route of administration to guide product labeling. An example of such an application is the use of a model-based approach to predicting the magnitude of effect of the strong CYP3A inhibitor ritonavir on the PK of intravenously administered sildenafil, with qualification of the model based on its ability to adequately predict the interaction between oral sildenafil and ritonavir [158]. This analysis provided support for the conclusion that the magnitude of the effect of CYP3A inhibitors on intravenous sildenafil can be expected to be lesser than for oral sildenafil, resulting in the following text in the Clinical Pharmacology section of the USPI: "*Predictions based on a pharmacokinetic model suggest that drug–drug interactions with CYP3A inhibitors will be less than those observed after oral sildenafil administration*" [159].

3.10 DESIGN, ANALYSIS AND INTERPRETATION OF CLINICAL DDI STUDY RESULTS

When designing a clinical DDI study between two drugs, factors requiring consideration include the PK properties of the object and precipitant drugs, the mechanism of the expected interaction (e.g., reversible inhibition, time-dependent inhibition, induction) and wherever possible, the expected magnitude and time course of the interaction (e.g., as predicted from the *in vitro* data and available understanding of the PK properties of the object and precipitant drugs). When evaluating the effect of an NME as an inhibitor or inducer on the PK of a selective probe substrate of an enzyme, the most commonly employed approach is to estimate the effect of multiple-dose administration of the NME on the PK of a single dose of the probe substrate. The NME is typically administered at the highest dose and administration frequency of clinical relevance to estimate the maximum possible magnitude of the interaction. The NME should be dosed until PK steady-state conditions are achieved before administration of the probe substrate. Dosing of the NME should be continued through the period of PK characterization of the probe substrate to maintain the condition of metabolic inhibition or induction. When evaluating the effect of a probe inhibitor or inducer on the PK of an NME, the most commonly applied design involves evaluating the effect of steady-state administration of the selected inhibitor or inducer on the single-dose PK of the NME, provided the multiple-dose PK of the NME are predictable from its single-dose PK profile (i.e., there are no time-dependent nonlinearities in the PK of the object NME).

3.10.1 Common Clinical DDI Study Designs

Many different designs are used for clinical DDI studies [160]. Examples of commonly employed DDI study designs are shown in Fig. 3.8 and will be discussed here. A widely utilized and robust DDI study design is the randomized crossover design (Fig. 3.8a). In this design, subjects are randomized to one of two sequences: a or b. Subjects randomized to sequence a are administered a single test dose of the object drug for PK characterization in period 1 followed by administration of the object drug for PK characterization during multiple-dose administration of the precipitant (at PK steady state of the precipitant) in period 2 to enable assessment of the effect of the precipitant on the object's PK. Dosing of the precipitant drug is continued through the period of PK assessment of the object drug. In sequence b, the order of treatments is reversed such that the single-dose PK of the object drug are evaluated in the setting of steady-state precipitant coadministration in period 1, followed by evaluation of the single-dose PK of the object drug in the absence of the precipitant in period 2. Periods 1 and 2 should be separated by an adequately long washout period that permits complete washout of both the object drug and the precipitant and ensures recalibration to baseline conditions of metabolic activity at the start of the second period. When the precipitant is a reversible CYP inhibitor, the duration of washout can be generally determined easily as five half-lives of the precipitant (and of any known CYP-inhibitory circulating metabolites). In contrast, when the precipitant is a mechanism-based inhibitor or an inducer, the washout period will typically need to be longer than anticipated from PK considerations alone, as the time course of recovery from CYP inactivation or of deinduction, as discussed previously, are dependent not only on the half-life of the precipitant drug but also importantly governed by the biological turnover half-life of

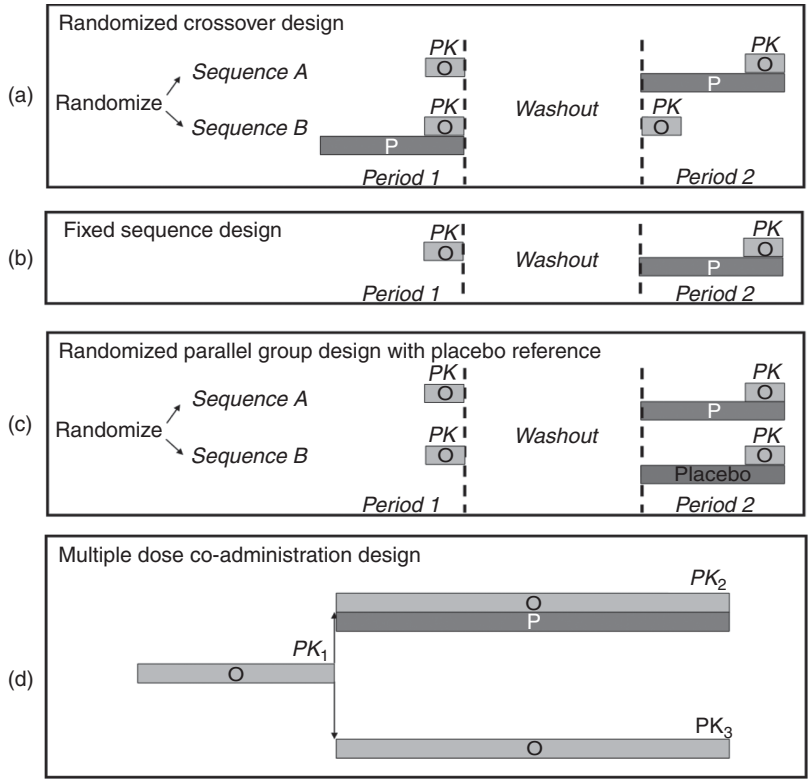


Figure 3.8 Schematic illustration of clinical DDI study designs (O, object drug; P, precipitant drug; PK, pharmacokinetic sampling).

the enzyme that is subject to inactivation or induction. The duration of the washout phase should be adequately long for scientific robustness of the study design. However, in the interest of operational feasibility, the washout period should not be longer than necessary, and optimization is essential. This can be done using physiologically based PK modeling and simulation of DDIs. In some cases, a randomized crossover study design may not be feasible because of a very long half-life of one or both drugs that would make it impractical to incorporate a sufficiently long washout phase between the study periods. In these cases, a fixed-sequence study design (also sometimes referred to as a *one-way crossover design*) is often used where the PK characterization of the object drug alone precedes the PK characterization of the object drug administered with the precipitant (Fig. 3.8b). The duration of the washout period with such a design needs to consider only the half-life of the object drug.

The robustness of the randomized crossover study design over the fixed-sequence design stems from the randomization to one of two sequences such that any potential confounding impact of period and/or sequence effects can be minimized. The risk of a sequence/period effect on PK endpoints is generally low (provided washout is adequate and there is no carryover between periods), given the objective nature of these measurements. Therefore, in most cases, a fixed-sequence study design will be appropriate. In fact, the fixed-sequence design was the most commonly utilized study

design (70% of all studies evaluated) for DDI studies based on an analysis of New Drug Application submissions to the US FDA during the period from December 1995 to November 1996 [160]. However, if the DDI study includes measurement of subjective PD endpoints (e.g., CNS depressant effects using a visual analog scale) in addition to PK endpoints, the randomized crossover design is recommended to minimize potential subjective influences on the study conclusions. This may be applicable, for instance, in DDI studies aimed at evaluating the effect of MDR1 P-gp inhibition on the PK of an NME that is known to be a P-gp substrate. In addition to increasing systemic drug exposure by an increase in the extent of oral absorption (via inhibition of intestinal efflux transport) and/or a decrease in renal and/or biliary clearance, it is possible that P-gp inhibition at the blood–brain barrier may additionally increase CNS drug availability beyond the extent that would be expected based on increases in systemic drug exposure alone. There is currently little clinical evidence to support the clinical significance of such DDIs that may increase human CNS drug distribution via inhibition of P-gp at the blood–brain barrier, although some examples have been reported in experimental clinical pharmacology studies [161,162]. There is little rationale to advocate direct assessment of CNS drug distribution via invasive approaches such as sampling of cerebrospinal fluid in DDI studies evaluating the effects of a P-gp inhibitor (e.g., quinidine) on the PK of a P-gp substrate NME. Nevertheless, if the NME is a P-gp substrate with potential for target or off-target CNS PD effects, it may be useful to include CNS PD and/or safety evaluations in addition to systemic PK measurements in DDI studies evaluating the effect of a P-gp inhibitor. Considering the subjective nature of such endpoints, it would be important to utilize a randomized crossover design instead of a fixed-sequence design in such DDI studies, to permit unbiased evaluation of the PD and/or CNS safety data of special interest. If a randomized crossover design is not feasible (e.g., due to a long half-life of the precipitant drug) and if the potential bias introduced by a fixed-sequence design is to be avoided (e.g., if subjective PD endpoints are being measured), an alternate design that may be used is the randomized parallel group design with a placebo reference (Fig. 3.8c). In this design, subjects are randomized into one of two groups. One group of subjects undergo PK characterization of the object drug alone in period 1 followed by PK characterization of the object drug in the setting of coadministration with the precipitant drug in period 2, and a second group of subjects undergo PK characterization of the object drug alone in period 1 and of the object drug following blinded administration of a placebo in period 2. Such a design provides the ability to separate out the effect of a potential DDI from any confounding period effects.

When evaluating the effect of a probe inhibitor or inducer on the PK of an NME as the object drug, if the PK of the object NME are characterized by time-dependent nonlinear kinetics (e.g., autoinduction or autoinhibition of clearance) and if understanding the effect of enzyme inhibition or induction on the PK of the NME following multiple-dose administration are of primary clinical relevance, the DDI study may need to be designed as a steady-state coadministration study because conclusions from a single-dose DDI study may not be translatable to the clinically relevant setting of multiple-dose administration. As an example, the exposure of single-dose imatinib is increased by the strong CYP3A inhibitor ketoconazole by 40% in a healthy volunteer DDI study [163]. However, a subsequent DDI study in cancer patients in the clinically relevant setting of multiple-dose administration of imatinib failed to reveal an effect of short-term ritonavir (a CYP3A inhibitor that is comparably potent as ketoconazole, with

short-term administration selected to avoid its CYP3A induction effects) on the steady-state exposure of imatinib [164]. Although the mechanism underlying this difference is not entirely clear, it is possible that it may be explained by CYP3A inhibition by imatinib [165] that may translate to an autoinhibition of the CYP3A-mediated component of its oral clearance, thereby resulting in a potentially decreased contribution of CYP3A to steady-state imatinib oral clearance compared to the corresponding contribution following single-dose administration. When designing a multiple-dose coadministration DDI study, the steady-state PK of the NME would need to be characterized following multiple-dose coadministration with the probe inhibitor or inducer and compared to the steady-state PK of the NME administered alone. The multiple-dose coadministration of the probe inhibitor or inducer will need to be performed for a sufficiently long period until attainment and characterization of the new PK steady state of the NME under maximally inhibited or induced conditions of its metabolism. As discussed earlier, optimization of study duration can be achieved using physiologic model-based IVIVEs and comparison of the performance of competing study designs via simulations. One approach to a multiple-dose coadministration DDI study is to utilize a randomized crossover design (similar to that shown in Fig. 3.8b, except that multiple dosing of the NME object drug would be employed instead of single-dose administration). Such a design can be potentially onerous to execute as the study duration will be quite long (especially for drugs with long half-lives), as it would be comprised by the time taken for achievement of PK steady state of the NME alone, the time taken for achievement of PK steady state of the NME in the setting of coadministration of the precipitant drug, and the interperiod washout, which would need to be long enough to ensure return to baseline metabolic conditions and ensure complete washout of both the drugs. Therefore, when multiple-dose coadministration is required for a steady-state DDI evaluation, the use of a fixed-sequence design may be preferred as a simpler alternative (similar to that shown in Fig. 3.8a, except that multiple dosing of the NME object drug would be employed instead of single-dose administration). Even in the setting of a fixed-sequence design, if the half-life of the object drug is long, the total study duration for a steady-state DDI study can be excessive and potentially impractical. In such cases, alternate study designs can be considered, which eliminate the interperiod washout phase. An example of such an alternate design for a multiple-dose coadministration DDI study is shown in Fig. 3.8d. In this design, multiple-dose administration of the object NME is initiated in all subjects and continued until PK steady state is approximately achieved, at which point a PK characterization over the steady-state dosing interval is performed (PK_1). Following completion of this PK assessment of the NME alone, one-half of the subjects continue multiple-dose administration of the object NME alone and another half of the subjects continue multiple-dose administration of the object NME, with coadministration of the probe inhibitor or inducer. The assignment of subjects to the treatment of NME alone versus NME plus probe inhibitor or inducer can be based on randomization that is performed at entry into the study. Both groups of subjects continue their respective multiple-dose study treatments for a prespecified duration that is designed to be adequate to allow achievement of the new PK steady state of the NME when coadministered with the probe inhibitor or inducer. At this point, all subjects undergo PK characterization of the object NME again (PK_2 representing the assessments in subjects coadministered the NME with the probe precipitant; PK_3 representing the assessments in subjects that continued administration of the NME alone). Statistical analysis of the PK_2/PK_1 ratios of systemic exposures

in reference to the PK_3/PK_1 ratios of systemic exposures of the object NME should permit estimation of the magnitude of the interaction.

3.10.2 Statistical Considerations in Design and Analysis of Clinical DDI Studies

A key consideration in the design of clinical PK DDI studies is the determination of an appropriate sample size to enable an informative analysis of the resulting data to guide interpretation of the clinical significance of any observed interaction. DDI studies are generally designed with a sufficient number of subjects that can permit estimation of the magnitude of the interaction with a reasonable level of precision, based on prior knowledge of the variability in PK of the object drug. The operational metric of variability is the within-subject variance in the object drug's PK endpoints (e.g., AUC and C_{max}) for designs where each subject serves as his/her own control and undergoes PK characterization of the object drug alone as well as in the setting of coadministration of the precipitant drug. For less commonly utilized parallel group DDI study designs, the operational metric of variability is the total variance in PK endpoints thereby translating to a larger sample size. The level of precision desired in the estimate of interaction magnitude depends on the broader strategic objective of the DDI study. If the objective is to claim lack of an interaction in proposed labeling and if the exposure–Response relationships for efficacy and safety of the object drug have not been adequately understood, the study would need to be sized to include a sufficient number of subjects for the 90% confidence intervals for the ratio of geometric mean AUC and C_{max} (object plus precipitant in reference to object alone) to be contained within the 80–125% range (default bioequivalence range). However, if the intent is to estimate the magnitude of the interaction with a level of precision that is sufficiently high to allow decisions to be made regarding the potential clinical significance of the interaction to inform risk management strategies and prescribing guidance, sizing the study to show bioequivalence may not be necessary. When dealing with the NME as the object drug, the desired level of precision in estimating the magnitude of the effect of a probe inhibitor or inducer should be ideally based on no-effect boundaries derived from quantitative clinical pharmacologic understanding of the therapeutic index of the object drug [1]. When robust exposure–response relationships for the therapeutic and adverse effects of the NME are available, such understanding provides a powerful framework to prospectively define clinically relevant no-effect boundaries (as opposed to defaulting to the standard bioequivalence criteria) and to interpret the results of the DDI study to guide next steps. PK data from a DDI study are typically analyzed using noncompartmental methods to calculate individual values of PK parameters (e.g., AUC and C_{max}) of the object drug when administered alone and when administered with the precipitant drug. Statistical analysis of PK parameters is generally done using a mixed effects analysis of variance on log-transformed AUC and C_{max}, with estimation of the ratio of geometric mean AUC and C_{max} (object plus precipitant in reference to object alone) and associated 90% confidence intervals. This approach of estimation of interaction magnitude is preferred over hypothesis testing and use of p-values for the assessment of the statistical significance of a DDI, as has been illustrated using representative examples from a review of analyses of DDI studies in New Drug Application submissions to the US FDA during the period from December 1995 to November 1996 [160]. As a hypothetical example, consider the following two scenarios: Study A yielding an estimated ratio of geometric mean AUCs of 89%,

with a 90% confidence interval ranging from 85% to 95% and a p-value of <0.05; and Study B yielding an estimated ratio of geometric mean AUCs of 120%, with a 90% confidence interval ranging from 60% to 220% and a p-value $\gg 0.1$. Although Study A revealed a "statistically significant" interaction based on a p-value of <0.05, this is simply a function of the low within-patient variability in the object drug's exposure resulting in the ability of the study to demonstrate statistical significance in the small observed decrease in object drug exposure (by 11% on average). However, the 90% confidence interval for the ratio of geometric mean AUC is contained within the 80–125% range, and the observed mean decrease in exposure of 11% would be of no clinical relevance in most cases. Therefore, the conclusion of statistical significance based on a p-value alone is not particularly meaningful or informative to guide next steps in terms of whether the observed interaction requires risk management via dosage increases and/or other precautionary guidance to prescribers. In fact, no further clinical action would typically be necessary for DDIs of this magnitude, and this inference can be reached based on the point estimate of interaction magnitude and the associated 90% confidence interval as a measure of precision in its estimation. In contrast, although the p-value for Study B was $\gg 0.1$, a conclusion of the lack of interaction based solely on the "lack of statistical significance" is not appropriate. Unfortunately, the results of Study B cannot be considered as adequately informative to guide next steps unless exposure–response considerations can be used to justify a wide therapeutic range of the object drug such that decreases in exposure by up to 40% or increases in exposure as large as 2.2-fold would not be of clinical relevance. The poor precision in the estimated interaction magnitude in Study B is likely due to high within-patient variability in the object drug's exposure. Therefore, although the point estimate of interaction magnitude (1.2-fold increase in AUC) is relatively low, the large uncertainty in its estimation precludes definitive conclusions regarding whether or not there is a clinically meaningful DDI. Again, the p-value is of no value in reaching this conclusion, and it would be inappropriate to use it to suggest the lack of a meaningful interaction. The ability to show statistical significance or the failure to demonstrate statistical significance should not drive interpretation of clinical DDI study results and the use of a confidence interval approach (90% confidence interval about the geometric mean ratio of the AUC and C_{max} with and without the interacting drug) as opposed to testing for statistical significance is considered as appropriate in the analysis and interpretation of DDI study results. This is reflected in expert opinion articles [166] as well as the Draft FDA and EMA DDI guidance documents [31,39].

3.10.3 Assessment of Clinical Relevance of DDIs

A key strategic objective of clinical DDI studies performed using probe substrates or inhibitors is to utilize the results to evaluate the clinical relevance of potential interactions of the NME with other object drugs with similar clearance mechanisms or precipitant drugs with similar CYP or transporter inhibitory or inductive properties. As discussed previously, use of a sensitive substrate of the target CYP isoform (when investigating the NME as a potential precipitant) or a strong inhibitor or inducer of the contributing CYP isoform (when investigating the NME as the object drug) in the probe DDI study are important to enable assessment of relevance of the interaction with similarly or less sensitive substrates, or with similarly or less potent inhibitors or inducers. This is important because the conduct of each and every possible DDI study

is neither practical nor warranted unless a specific drug combination is of substantial clinical relevance in the target patient population such that specific investigation of a particular interaction will yield medically important information to guide prescribing. For example, consider an NME that is an inhibitor of a drug-metabolizing enzyme, as confirmed in a clinical DDI study evaluating its effects on the PK of a selective and sensitive substrate of the inhibited enzyme. If this NME is expected to be frequently coadministered in clinical practice with a specific object drug with a narrow therapeutic range that is metabolized by the inhibited enzyme, an exact recommended dose reduction or a precise dose titration plan for the object drug of interest may be desirable. This may not be possible to derive based solely on the results of the DDI study conducted with a probe substrate. In such a situation, it can be useful to perform an additional DDI study with the specific object drug of relevance to the intended setting of clinical use of the NME, with the primary objective of contributing to specific prescribing guidance in patients requiring such coadministration.

Some key questions that often need to be considered as part of evaluating the implications of the findings from a clinical DDI study and informing prescribing guidance include:

1. Can the drugs be safely coadministered through appropriate risk management (e.g., safety monitoring, dose reduction) or should use be contraindicated?
2. If the interaction is clinically meaningful, is a dosage adjustment necessary or is it sufficient to advise cautious coadministration with appropriate clinical safety monitoring?
3. If the NME is the object drug, are additional DDI studies needed with other clinically relevant and/or less potent inhibitors or inducers to inform prescribing guidance?
4. If the NME is the precipitant drug, are additional DDI studies needed with other clinically relevant and/or less sensitive substrates of the affected enzyme to inform prescribing guidance?

Modeling and simulation can be particularly helpful in answering these questions with quantitative rigor. The answers to questions 1 and 2 are best developed based on the observed DDI magnitude viewed in context of the clinical safety profile of the drug, exposure–response relationships for efficacy endpoints, and exposure–response relationships for adverse effects. If recommendation of a dose modification of the NME object drug is considered appropriate, integration of DDI study results into population PK/PD models for safety and efficacy of the NME can permit simulation of the performance characteristics of the proposed dose-modification guidelines, an approach analogous to that described for developing dosing recommendations for special populations of patients with impaired renal or hepatic function [167,168]. Consider, for instance, a scenario where the results of a clinical DDI study of an orally administered NME with a strong inhibitor of its metabolism indicate a substantial exposure increase such that a dose reduction of the NME would be necessary to allow coadministration with strong inhibitors. The results of the DDI study can be used to calculate the reduced dose of the NME based on the fold increase in NME exposure estimated in the clinical DDI study. However, in many such instances, only a limited number of dose strengths of the NME may be available and delivery of the calculated reduced dose

may require a unit dose strength that is not available. In such situations, modeling and simulation can represent a valuable approach to test the performance characteristics of alternate dose reduction guidelines that may potentially be supported by the available dose strengths of the NME without having to manufacture and qualify biopharmaceutic performance of lower dose strengths. The key question is whether the proposed dose reduction guideline can be expected to result in a population distribution of NME exposures that would not compromise the benefit/risk balance in patients receiving the strong enzyme inhibitor, which can be addressed using simulations that integrate the DDI study results into the results of population PK/PD models for safety and efficacy endpoints.

Questions 3 and 4 can be addressed using PB-PK modeling and simulation leveraging the available *in vitro* data on both clinically studied and unstudied interactions together with the PK data from the completed clinical DDI studies to simulate the expected outcomes from unstudied interactions. Sensitivity analyses evaluating the impact of unknown or inaccessible model parameters can be particularly helpful in assessing the level of uncertainty in predictions to guide the decision of whether additional clinical DDI studies are of added value.

3.11 EXAMPLES ILLUSTRATING TRANSLATION OF DDI KNOWLEDGE TO PRESCRIBING GUIDANCE

The interpretation of clinical significance of DDIs and the use of clinical DDI study results to guide product labeling, both for the NME as the object drug and for the NME as the precipitant drug, can be better appreciated using some representative examples. If a DDI is characterized by a large clinically significant exposure increase or decrease, and/or a large intersubject variability in interaction magnitude, recommendation of coadministration of the interacting drugs through use of dose modification and/or clinical safety monitoring guidelines may sometimes be difficult. This is because it may be questionable whether a positive benefit/risk balance is preserved when indicating use of the drug in patients receiving such major interacting concomitant medications. This is particularly the case when the object drug has a narrow therapeutic range such that serious unacceptable toxicities can be expected at higher exposures that may be observed as a consequence of DDIs. In such cases, the combination may be contraindicated or alternatively it may be recommended that such concomitant use be avoided. However, if the interaction magnitude is not excessive, it may be possible to utilize the results of controlled clinical DDI studies to provide pharmacokinetically derived dose-modification recommendations in prescribing guidance. These concepts are illustrated using three representative examples (tizanidine, everolimus, and darunavir/ritonavir) that demonstrate the translation of knowledge gained from DDI investigations to the development of risk management guidelines in prescribing guidance.

Example 1: Tizanidine. The centrally acting α_2-adrenergic agonist tizanidine indicated for the management of spasticity is an ultrasensitive substrate of CYP1A2. When administered with the strong CYP1A2 inhibitor fluvoxamine, a 33-fold mean increase in tizanidine AUC is observed, with a 12-fold mean increase in C_{max}, and a nearly 3-fold prolongation of half-life [169]. Importantly, these PK changes were accompanied by serious clinical consequences of hypotension

(characterized by a mean systolic blood pressure of ~80 mm Hg when coadministered with fluvoxamine) and marked drowsiness and psychomotor impairment reflecting exaggerated PD secondary to the large increase in tizanidine exposure. Additionally, the variability in interaction magnitude was also significant with the 33-fold mean increase in AUC comprised of a range of 14- to 103-fold across individual subjects. In another DDI study that evaluated the effect of another strong CYP1A2 inhibitor ciprofloxacin on tizanidine's clinical pharmacology, a 10-fold mean increase in AUC was observed with clinically significant potentiation of its hypotensive and sedative effects [170]. Considering the large magnitude of these exposure increases and their associated serious clinical consequences that can jeopardize patient safety, it is clear that coadministration of tizanidine with fluvoxamine or ciprofloxacin would not preserve a positive benefit/risk balance for patients. With 2 mg being the lowest available dose strength and 8–12 mg three times a day representing the highest recommended dose of tizanidine, the challenges associated with recommending a dose reduction that would permit safe use of tizanidine in patients requiring fluvoxamine or ciprofloxacin treatment become immediately apparent. Accordingly, the USPI for tizanidine contraindicates its use in patients receiving the strong CYP1A2 inhibitors, fluvoxamine or ciprofloxacin [171]. Additionally, based on *in vitro* studies of the enzymology of tizanidine metabolism that have identified CYP1A2 as the principal determinant of its biotransformation [172] and knowledge of CYP1A2 inhibitory drugs, an extension of DDI risk assessment to other CYP1A2 inhibitors is also possible. Accordingly, the USPI also includes the following statement warning prescribers regarding potential interactions that may be expected with other CYP1A2 inhibitors: "*Because of potential drug interactions, concomitant use of tizanidine with other CYP1A2 inhibitors, such as zileuton, other fluoroquinolones, antiarrhythmics (amiodarone, mexiletine, propafenone, and verapamil), cimetidine, famotidine, oral contraceptives, acyclovir, and ticlopidine should ordinarily be avoided. If their use is clinically necessary, they should be used with caution.*" [171]. This example illustrates the extension of DDI risk assessment to other precipitant drugs based on understanding of the underlying mechanisms and molecular determinants of established interactions.

Example 2: Everolimus. As another example, consider the anticancer drug everolimus, a substrate of CYP3A4 and P-gp. Ketoconazole (strong CYP3A inhibitor and P-gp inhibitor) produced a 15-fold increase in everolimus AUC, and erythromycin and verapamil (moderate CYP3A inhibitors and inhibitors of P-gp) produced 4.4- and 3.5-fold increases in everolimus AUC, respectively [126]. Considering the relatively large magnitude of the observed interaction with ketoconazole, the everolimus USPI recommends that concomitant use of strong CYP3A4 or P-gp inhibitors be avoided [40]. If patients require coadministration with a moderate CYP3A4 inhibitor, the everolimus USPI recommends use with caution at a reduced dose of 2.5 mg/day (one-fourth of the usual recommended dose of 10 mg/day), with a dose increase to 5 mg/day, which may be considered based on patient tolerance [40]. Finally, in a DDI study with the strong CYP3A inducer rifampin, a 64% reduction of everolimus AUC was observed [173]. Accordingly, the USPI recommends that concomitant use of strong CYP3A inducers be avoided and that if such combined use cannot be avoided, a dose increase from 10 to 20 mg/day may be considered

in 5 mg increments [40]. This example illustrates how adequately designed clinical DDI studies can inform the development of pharmacokinetically derived dose-modification guidelines for use in clinical practice when coupled with adequate safety monitoring.

Example 3: Ritonavir-Boosted Darunavir. The examples discussed above represent relatively simple illustrations of the concepts of translating the results of clinical DDI studies to guide prescribing information. There are many such examples across multiple therapeutic areas. Of note, particularly complex dose-modification guidelines are recommended for some antiretroviral agents where clinical use is indicated in the setting of multiagent combination therapy with other antiretroviral agents that have complex inhibitory or inducing effects on CYP enzymes as well as drug transporters. As an example, consider the prescribing information for ritonavir-boosted darunavir that exemplifies the translation to prescribing guidance, of knowledge collectively gained from *in vitro* drug metabolism and DDI studies, understanding of human disposition mechanisms of darunavir as well as coadministered agents, and the results of clinical DDI studies with probe substrates and inhibitors/inducers as well as commonly coadministered drugs. Darunavir is a CYP3A substrate and ritonavir coadministration boosts darunavir exposure by 11-fold due to the strong CYP3A inhibitory effect of ritonavir [174], representing a beneficial and necessary DDI in the context of its clinical use. In fact, to achieve sufficient exposures for antiretroviral therapy, darunavir should only be used with 100-mg BID ritonavir [175]. However, the state of nearly complete deficiency in CYP3A activity produced by regular use of ritonavir in patients receiving treatment with ritonavir-boosted darunavir results in clinically important DDI implications that complicate coadministration of other therapeutic agents that are substrates of CYP3A. In particular, serious safety concerns can be expected when the coadministered agent (i.e., object drug) is highly dependent on CYP3A-mediated metabolism for its clearance and additionally has a narrow therapeutic range. Accordingly, the USPI for darunavir provides a list of such drugs classified by therapeutic class, whose coadministration with darunavir/ritonavir is contraindicated (e.g., alfuzosin, dihydroergotamine, ergonovine, ergotamine, methylergonovine, cisapride, pimozide, oral midazolam, oral triazolam, lovastatin, simvastatin, and sildenafil for treatment of pulmonary arterial hypertension) together with rationale for the contraindication and the potential clinical consequences of coadministration based on knowledge of the safety profile of these object drugs [175]. For other object drugs that are substrates of CYP3A or CYP2D6 (since ritonavir is also an inhibitor of CYP2D6), the USPI offers cautionary guidance and dosage recommendations, where appropriate, based on knowledge of the therapeutic range and safety profile of the object drug and sensitivity to CYP3A or CYP2D6 inhibitory DDIs. Examples of such drugs include but are not limited to CYP3A substrates such as atorvastatin, cyclosporine, and tacrolimus, and CYP2D6 substrates such as risperidone, thioridazine, and metoprolol [175]. Additionally, because of pleiotropic induction of drug-metabolizing enzymes by ritonavir via PXR-mediated mechanisms, exposures of object drugs such as ethinyl estradiol (substrate of CYP3A and phase 2 metabolizing enzymes including UGTs and sulfotransferases) and *S*-warfarin (CYP2C9 substrate) were decreased by 44% and 21%, respectively, in DDI studies performed with oral contraceptives and warfarin, respectively. These findings translate, respectively, to

recommendations for alternate methods of nonhormonal contraception in patients treated with darunavir/ritonavir and monitoring of the international normalized ratio in patients receiving warfarin in combination with darunavir/ritonavir therapy [175]. Finally, with respect to DDI risk assessment for darunavir as the object drug, the USPI contraindicates coadministration with rifampin and St. John's wort based on knowledge of the clinically significant CYP3A inducing effects of these agents that may translate to a decrease in darunavir exposure, which may lead to loss of therapeutic effect and development of resistance [175].

3.12 CONCLUDING REMARKS

DDIs represent an important source of variability in PK that can impact drug response, including both the desired therapeutic effects and the unintended adverse effects. This chapter has provided an overview of the scientific principles, experimental approaches and clinical pharmacologic strategies applied in the investigation of PK DDI characteristics of investigational small molecule drugs during their development, and the application of knowledge gained from such evaluations to contribute to prescribing guidance. This chapter focussed on metabolic DDIs, although it should be noted that the general principles discussed here are also applicable to PK DDIs via nonmetabolic mechanisms (e.g., transporter-mediated DDIs; absorption DDIs via physicochemical or physiologic mechanisms), the investigation of which are equally important. DDI risk assessment and evaluation in drug development needs to consider the complementary scenarios of the NME as a victim or object and as a perpetrator or precipitant. A conceptual framework that illustrates the various considerations around DDI risk assessment and evaluation is provided in Fig. 3.9. The central goal of clinical pharmacologic understanding in drug development is to enable definition of the right dose of the right drug to the right patient populations. To this effect, it is important in drug development to characterize the impact of the various intrinsic and extrinsic sources of PK and PD variability that can influence the therapeutic as well as adverse effects of the drug, thereby altering the overall benefit–risk balance. This is illustrated on the scheme shown on the left panel of Fig. 3.9. DDIs represent an important source of variability, but their effects and clinical relevance will need to be interpreted in the context of other factors that may influence PK (genetic variation in drug-metabolizing enzymes or transporters, effects of food, age, body size, eliminating organ function, etc.) and PDs (PD DDIs, genetic variation in drug target proteins, altered sensitivity due to age or comorbid disease, etc.). Forest plots represent a powerful graphical framework to provide a concise, yet comprehensive, visual illustration of the comparative effects of various intrinsic and extrinsic factors (including the effects of co-administered agents) on the PK exposure parameters of a drug, as applied in recent USPI examples [176]. When utilized in a drug development setting and to guide development of proposed product labeling, such visual frameworks can permit an integrated assessment of the relative magnitude of various DDIs for the NME as the object drug, also in relation to other sources of PK variability including other extrinsic factors as well as intrinsic factors. The right panel of Fig. 3.9 provides a pyramid view of the integrated approach to DDI risk assessment and evaluation in drug development that was focussed on in this chapter. The base of the pyramid reflects the scientific considerations and approaches

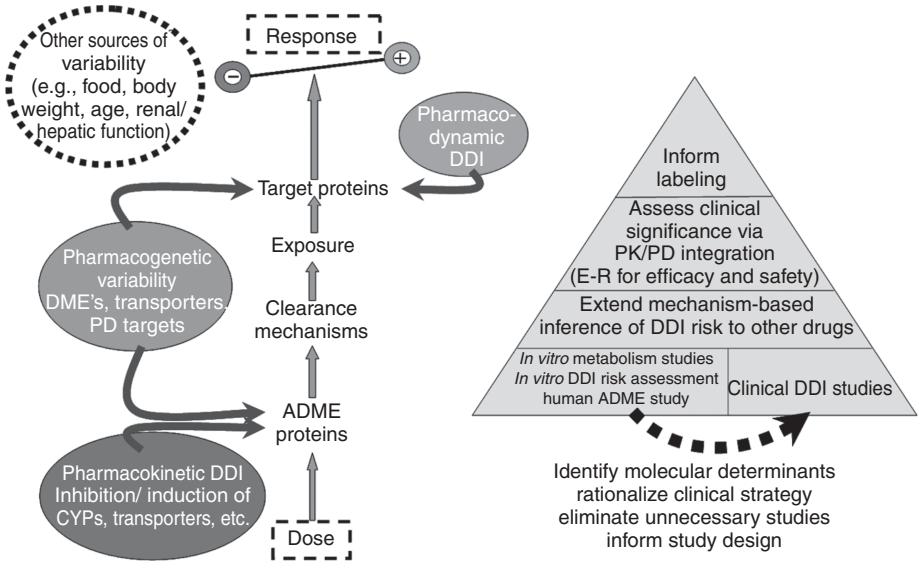


Figure 3.9 Integrated approach to strategic integration of DDI risk assessment and evaluation in contemporary drug development.

(experimental and clinical pharmacologic) employed in DDI risk assessment and evaluation that provide the data that serve as the foundation for translation to clinical significance. Of note, knowledge gained from mechanistic *in vitro* drug metabolism and DDI studies and understanding of clearance mechanisms of the object drug, represents critical prior information to enable DDI risk assessment and evaluation of the need for further clinical DDI evaluation. Importantly, understanding of the enzymology of metabolism and disposition of the NME and its effects on the activity and expression of drug-metabolizing enzymes and transporters *in vitro* together with quantitative IVIVE can eliminate the need for unnecessary clinical DDI studies and streamline the clinical DDI evaluation program. Such understanding can permit forecasting of DDI risk with unstudied objects (for NME as precipitant) and unstudied precipitants (for NME as object) based on knowledge of the clearance mechanisms of coadministered agents and knowledge of the effects of the coadministered agents on the clearance mechanisms of the NME, respectively. The quantitative translation of results from *in vitro* DDI studies to predictions of clinical pharmacologic correlates represents an emerging area of research, and this chapter has provided an overview of some of these approaches based on published expert opinion and emerging regulatory guidance (US and EU) in this area. Ultimately, the clinical relevance of PK changes resulting from DDIs will need to be assessed in the context of exposure–response understanding for the efficacy and safety characteristics of the object drug to contribute to product labeling. As is readily apparent from the above discussed integrated approach, DDI risk assessment and evaluation is a highly interdisciplinary science. Strong partnerships and collaborations across drug metabolism scientists, clinical pharmacologists, physicians, pharmacometricians, statisticians, pharmacogeneticists, and regulatory scientists engaged in the cross-functional mission of contemporary drug development are therefore critical to success.

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