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

PRELIMINARY CONCEPT PAPER

For Discussion Purposes Only

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Concept paper for discussion purposes only

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I. INTRODUCTION

This concept paper provides recommendations to sponsors of new drug applications (NDAs) and biologics license applications (BLAs) for therapeutic biologics (hereafter drugs) who intend to perform *in vitro* and *in vivo* drug metabolism and drug-drug interaction studies. The concept paper reflects the Agency's current view that the metabolism of an investigational new drug should be defined during drug development and that its interactions with other drugs should be explored as part of an adequate assessment of its safety and effectiveness. For drug-drug interactions, the approaches considered in the concept paper are offered with the understanding that the relevance of a particular study depends on the characteristics and proposed indication of the drug under development. Furthermore, not every drug-drug interaction is metabolism-based, but may arise from changes in pharmacokinetics caused by absorption, tissue and/or plasma binding, distribution, and excretion interactions. Drug interactions related to transporters are being documented with increasing frequency and are important to consider in drug development. Although less well studied, drug-drug interactions may alter pharmacokinetic/pharmacodynamic (PK/PD) relationships. These important areas are not considered in detail in this concept paper.

Discussion of metabolic and other types of drug-drug interactions is provided in the following CDER guidances, *Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro (1997), In Vivo Drug Metabolism/Drug Interaction Studies — Study Design, Data Analysis, and Recommendations for Dosing and Labeling (1999)* and International Conference on Harmonisation (ICH) *E8 General Considerations for Clinical Trials* (December 1997), *E7 Studies in Support of Special Populations: Geriatrics* (August 1994), and *E3 Structure and Content of Clinical Study Reports* (July 1996), and the Agency guidances *Studying Drugs Likely to be Used in the Elderly* (November 1989) and *Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs* (July 1993).

II. BACKGROUND

A. Metabolism

The desirable and undesirable effects of a drug arising from its concentrations at the sites of action are usually related either to the amount administered (dose) or to the resulting blood concentrations, which are affected by its absorption, distribution, metabolism

and/or excretion. Elimination of a drug or its metabolites occurs either by metabolism, usually by the liver or gut mucosa, or by excretion, usually by the kidneys and liver. In addition, protein therapeutics may be eliminated via a specific interaction with cell surface receptors, followed by internalization and lysosomal degradation within the target cell. Hepatic elimination occurs primarily by the cytochrome P450 family (CYP) of enzymes located in the hepatic endoplasmic reticulum but may also occur by non-P450 enzyme systems, such as N-acetyl and glucuronosyl transferases. Many factors can alter hepatic and intestinal drug metabolism, including the presence or absence of disease and/or concomitant medications. While most of these factors are usually relatively stable over time, concomitant medications can alter metabolism abruptly and are of particular concern. The influence of concomitant medications on hepatic and intestinal metabolism becomes more complicated when a drug, including a prodrug, is metabolized to one or more active metabolites. In this case, the safety and efficacy of the drug/prodrug are determined not only by exposure to the parent drug but by exposure to the active metabolites, which in turn is related to their formation, distribution, and elimination.

B. Drug-Drug Interactions

Many metabolic routes of elimination, including most of those occurring via the P450 family of enzymes, can be inhibited, activated, or induced by concomitant drug treatment. Observed changes arising from metabolic drug-drug interactions can be substantial — an order of magnitude or more decrease or increase in the blood and tissue concentrations of a drug or metabolite — and can include formation of toxic metabolites or increased exposure to a toxic parent compound. These large changes in exposure can alter the safety and efficacy profile of a drug and/or its active metabolites in important ways. This is most obvious and expected for a drug with a narrow therapeutic range (NTR), but is also possible for non-NTR drugs as well (e.g., HMG CoA reductase inhibitors). Depending on the extent and consequence of the interaction, the fact that a drug's metabolism can be significantly inhibited by other drugs and that the drug itself can inhibit the metabolism of other drugs can require important changes in either its dose or the doses of drugs with which it interacts, that is, on its labeled conditions of use. Rarely, metabolic drug-drug interactions may affect the ability of a drug to be safely marketed.

The following general principles underlie the recommendations in this concept paper:

- Adequate assessment of the safety and effectiveness of a drug includes a description of its metabolism and the contribution of metabolism to overall elimination.

- Metabolic drug-drug interaction studies should explore whether an investigational agent is likely to significantly affect the metabolic elimination of drugs already in the marketplace and, conversely, whether drugs in the marketplace are likely to affect the metabolic elimination of the investigational drug.

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Even drugs that are not substantially metabolized can have important effects on the metabolism of concomitant drugs. For this reason, metabolic drug-drug interactions should be explored, even for an investigational compound that is not eliminated significantly by metabolism. Although classical biotransformation studies are not a general requirement for the evaluation of therapeutic biologics (ICH document S6 "Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals"), certain protein therapeutics modify the metabolism of drugs that are metabolized by the P450 enzymes. Type I interferons, for example, inhibit CYP1A2 production at the transcriptional and post-translational levels, inhibiting clearance of theophylline. The increased clinical use of therapeutic proteins may raise concerns regarding the potential for their impacts on drug metabolism. Generally, these interactions cannot be detected by in vitro assessment. Consultation with the FDA is appropriate before initiating metabolic drug-drug interaction studies involving biologics.

- In some cases, metabolic drug-drug interaction studies are not informative until metabolites and prodrugs have been identified and their pharmacological properties described.

- Identifying metabolic differences in patient groups based on genetic polymorphism, or on other readily identifiable factors, such as age, race, and gender, can aid in interpreting results. The extent of interactions may be defined by these variables (e.g., CYP2D6 genotypes). Further, a minor pathway may become important in subjects lacking a particular enzyme and the evaluation of the drug interaction via the minor pathway may be appropriate in these subjects.

- The impact of an investigational or approved interacting drug can be either to inhibit, stimulate, or induce metabolism.

- A specific objective of metabolic drug-drug interaction studies is to determine whether the interaction is sufficiently large to necessitate a dosage adjustment of the drug itself or the drugs it might be used with, or whether the interaction would require additional therapeutic monitoring.

- In some instances, understanding how to adjust dosage in the presence of an interacting drug, or how to avoid interactions, may allow marketing of a drug that would otherwise have been associated with an unacceptable level of toxicity. Sometimes a drug interaction may be used intentionally to increase levels or reduce elimination of another drug (e.g., ritonavir and lopinavir). Rarely, the degree of interaction caused by a drug, or the degree to which other drugs alter its metabolism, may be such that it cannot be marketed safely.

- The blood or plasma concentrations of the parent drug and/or its active

metabolites (systemic exposure) may provide an important link between drug dose (exposure) and desirable and/or undesirable drug effects. For this reason, the development of sensitive and specific assays for a drug and its key metabolites is critical to the study of metabolism and drug-drug interactions.

- For drugs whose presystemic or systemic clearance occurs primarily by metabolism, differences arising from various sources, including administration of another drug, are an important source of inter-individual and intra-individual variability.

- Unlike relatively fixed influences on metabolism, such as hepatic function or genetic characteristics, metabolic drug-drug interactions can lead to abrupt changes in exposure. Depending on the nature of the drugs, these effects could potentially occur when a drug is initially administered, when it has been titrated to a stable dose, or when an interacting drug is discontinued. Interactions can occur after even a single concomitant dose of an inhibitor.

- The effects of an investigational drug on the metabolism of other drugs and the effects of other drugs on an investigational drug's metabolism should be assessed relatively early in drug development so that the clinical implications of interactions can be assessed as fully as possible in later clinical studies.

- Transporter-based interactions have been increasingly documented. Various reported interactions attributed to other mechanisms of interactions, such as protein-displacement or enzyme inhibition may be due in part to the inhibition of transport proteins, such as P-glycoprotein (P-gp), organic anion transporter (OAT), organic anion transport protein (OATP), organic cation transporter (OCT), etc. Examples of transporter-based interactions include the interactions between digoxin and quinidine, fexofenadine and ketoconazole or erythromycin, penicillin and probenecid, dofetilide and cimetidine, paclitaxel and valspodar, etc. Of the various transporters, P-gp is the most well understood and may be appropriate to evaluate during drug development.

III. GENERAL STRATEGIES

To the extent possible, drug development should follow a sequence where early *in vitro* and *in vivo* investigations can either fully address a question of interest or provide information to guide further studies. Optimally, a sequence of studies should be planned, moving from *in vitro* studies, to early exploratory studies, to later more definitive studies, employing special study designs and methodology where necessary and appropriate. In many cases, negative findings from early *in vitro* and early clinical studies can eliminate the need for later clinical investigations. Early investigations should explore whether a drug is eliminated primarily by excretion or metabolism, with identification of the principal metabolic routes in the latter case.

Using suitable in vitro probes and careful selection of interacting drugs for early *in vivo* studies, the potential for drug-drug interactions can be studied early in the development process, with further study of observed interactions assessed later in the process, as needed. In certain cases and with careful study designs and planning, these early studies may also provide information about dose, concentration, and response relationships in the general population, subpopulations, and individuals, which can be useful in interpreting the consequences of a metabolic drug-drug interaction.

A. *In Vitro* Studies

A complete understanding of the relationship between *in vitro* findings and *in vivo* results of metabolism/drug-drug interaction studies is still emerging. Nonetheless, *in vitro* studies can frequently serve as an adequate screening mechanism to rule out the importance of a metabolic pathway and drug-drug interactions that occur via this pathway so that subsequent *in vivo* testing is unnecessary. This opportunity should be based on appropriately validated experimental methods and rational selection of substrate/interacting drug concentrations. For example, if suitable *in vitro* studies at therapeutic concentrations indicate that CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A enzyme systems do not metabolize an investigational drug, then clinical studies to evaluate the effect of CYP2D6 inhibitors or CYP1A2, CYP2C9, CYP2C19, or CYP3A inhibitors/inducers on the elimination of the investigational drug will not be needed. Similarly, if *in vitro* studies indicate that an investigational drug does not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A metabolism, then corresponding *in vivo* inhibition-based interaction studies of the investigational drug and concomitant medications eliminated by these pathways are not needed.

The CYP2D6 enzyme has not been shown to be inducible. Recent data have shown co-induction of CYP3A and CYP2C/CYP2B enzymes. Therefore, if *in vitro* studies indicate that an investigational drug does not induce CYP1A2 or CYP3A metabolism, then corresponding *in vivo* induction-based interaction studies of the investigational drug and concomitant medications eliminated by CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A may not be needed.

Drug interactions based on CYP2B6 and CYP2C8 are emerging as important interactions. When appropriate, in vitro evaluations based on these enzymes may be conducted. The other CYP enzymes CYP2A6, CYP2E1, are less likely to be involved in clinically important drug interactions, but should be considered when appropriate.

Section VI describes general considerations in the *in vitro* evaluation of CYP-related metabolism and interactions. Appendices A, B, and C provide considerations in the experimental design, data analysis, and data interpretation in drug metabolizing enzyme identification including CYP enzymes (new drug as a substrate), CYP inhibition (new

drug as an inhibitor) and CYP induction (new drug as an inducer), respectively.

B. Specific *In Vivo* Clinical Investigations

Appropriately designed pharmacokinetic studies, usually performed in the early phases of drug development, can provide important information about metabolic routes of elimination, their contribution to overall elimination, and metabolic drug-drug interactions. Together with information from *in vitro* studies, these investigations can be a primary basis of labeling statements and can often help avoid the need for further investigations. Further recommendations about these types of studies appear in section IV of this concept paper.

C. Population Pharmacokinetic Screens

Population pharmacokinetic analyses of data obtained from large-scale clinical studies with sparse or intensive blood sampling can be valuable in characterizing the clinical impact of known or newly identified interactions, and in making recommendations for dosage modifications. The result from such analyses can be informative and sometimes conclusive when the clinical studies are adequately designed to detect significant changes in drug exposure due to drug-drug interactions. Simulations can provide valuable insights into optimizing the study design. It may be possible that population pharmacokinetic analysis could detect unsuspected drug-drug interactions. Population analysis can also provide further evidence of the absence of a drug-drug interaction when this is supported by prior evidence and mechanistic data. However, it is unlikely that population analysis can be used to prove the absence of an interaction that is strongly suggested by information arising from *in vivo* studies specifically designed to assess a drug-drug interaction. To be optimally informative, population pharmacokinetic studies should have carefully designed study procedures and sample collections. A guidance for industry on population pharmacokinetics is available.[1]

IV. DESIGN OF *IN VIVO* DRUG-DRUG INTERACTION STUDIES

If *in vitro* studies and other information suggest a need for *in vivo* metabolic drug-drug interaction studies, the following general issues and approaches should be considered. In the following discussion, the term *substrate* (S) is used to indicate the drug studied to determine if its exposure is changed by another drug, which is termed the *interacting drug* (I). Depending on the study objectives, the substrate and the interacting drug may be the investigational agents or approved products.

[1] CDER/CBER guidance for industry "Population pharmacokinetics", February 1999

A. Study Design

In vivo drug-drug interaction studies generally are designed to compare substrate concentrations with and without the interacting drug. Because a specific study may consider a number of questions and clinical objectives, many study design for studying drug-drug interactions can be considered. A study can use a randomized crossover (e.g., S followed by S+I, S+I followed by S), a one-sequence crossover (e.g., S always followed by S+I or the reverse), or a parallel design (S in one group of subjects and S+I in another). The following possible dosing regimen combinations for a substrate and interacting drug may also be used: single dose/single dose, single dose/multiple dose, multiple dose/single dose, and multiple dose/multiple dose. The selection of one of these or another study design depends on a number of factors for both the substrate and interacting drug, including (1) acute or chronic use of the substrate and/or interacting drug; (2) safety considerations, including whether a drug is likely to be an NTR (narrow therapeutic range) or non-NTR drug; (3) pharmacokinetic and pharmacodynamic characteristics of the substrate and interacting drugs; and (4) the need to assess induction as well as inhibition. The inhibiting/inducing drugs and the substrates should be dosed so that the exposures of both drugs are relevant to their clinical use. The following considerations may be useful:

- Changes in pharmacokinetic parameters may be used to indicate the clinical importance of drug-drug interactions. Interpretation of findings from these studies will be aided by a good understanding of dose/concentration and concentration/response relationships for both desirable and undesirable drug effects in the general population or in specific populations. A guidance[1] for industry published in April 2003 provides considerations in the evaluation of exposure-response relationships. In certain instances, reliance on endpoints other than pharmacokinetic measures/parameters may be useful.

- When both substrate and interacting drug are likely to be given chronically over an extended period of time, administration of the substrate to steady state with collection of blood samples over one or more dosing intervals could be followed by multiple dose co-administration of the interacting drug, again with collection of blood for measurement of both the substrate and the interacting drug (as feasible) over the same intervals. This is an example of a one-sequence crossover design.

- The time at steady state before collection of endpoint observations depends on

[1] CDER/CBER guidance for industry "Exposure-response relationships- study design, data analysis and regulatory applications" April 2003

whether inhibition or induction is to be studied. Inducers can take several days or longer to exert their effects, while inhibitors generally exert their effects more rapidly. For this reason, a more extended period of time after attainment of steady state for the substrate and interacting drug may be necessary if induction is to be assessed.

- When attainment of steady state is important and either the substrate or interacting drugs and/or their metabolites exhibit long half-lives, special approaches may be useful. These include the selection of a one-sequence crossover or a parallel design, rather than a randomized crossover study design.

- When a substrate and/or an interacting drug need to be studied at steady state because the effect of interacting drug is delayed as is the case for inducers and certain inhibitors, documentation that near steady state has been attained for the pertinent drug and metabolites of interest is important. This documentation can be accomplished by sampling over several days prior to the periods when samples are collected. This is important for both metabolites and the parent drug, particularly when the half-life of the metabolite is longer than the parent, and is especially important if both parent drug and metabolites are metabolic inhibitors or inducers.

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

- For a rapidly reversible inhibitor, administration of the interacting drug either just before or simultaneously with the substrate on the test day might be the appropriate design to increase sensitivity. For a mechanism-based inhibitor, it may be important to administer the inhibitor prior to (e.g., 1 hour) the administration of the substrate drug to maximize the effect. If the absorption of an interacting drug (e.g., an inhibitor or an inducer) may be affected by other factors (e.g., the gastric pH), it may be appropriate to control the variables and confirm the absorption via plasma level measurements of the interacting drug.

- If the drug interaction effects are to be assessed for both agents in a combination regimen, the assessment can be done in two separate studies. If the pharmacokinetic and pharmacodynamic characteristics of the drugs make it feasible, the dual assessment can be done in a single study. Some design options are randomized three-period crossover, parallel group, and one-sequence crossover.

X In order to avoid variable study results due to uncontrolled use of dietary supplements, juices or other foods that may affect various metabolizing enzymes and transporters during *in vivo* studies, it is important to exclude their use when

appropriate. Examples of statements in a study protocol include "Participants will be excluded for the following reasons: ….. use of prescription or over-the-counter medications, *including herbal products,* or alcohol within two weeks prior to enrollment", "For at least two weeks prior to the start of the study until its conclusion, volunteers will not be allowed to eat any food or drink any beverage containing *alcohol, grapefruit or grapefruit juice, apple or orange juice, vegetables from the mustard green family* (e.g., kale, broccoli, watercress, collard greens, kohlrabi, Brussels sprouts, mustard) and *charbroiled meats."*

X If not precluded by considerations of safety or tolerability due to adverse effects, it may be appropriate to estimate the systemic concentrations of a drug and/or its metabolites when there is maximum inhibition of its clearance pathway. For example, there may be a need to evaluate the drug's QT/QTc prolonging potential at substantially higher concentrations than those anticipated following the therapeutic doses[2]. In these instances, higher systemic concentrations may be achieved by administration of supra-therapeutic doses or by maximum inhibition of a drug's clearance pathway. If the drug is mainly metabolized by one single enzyme, high exposure can be achieved by the use of an inhibitor of this major metabolic pathway. In certain situations when there may be multiple metabolic pathways or multiple clearance pathways (metabolism and renal excretion), the studies may be conducted with the administration of multiple inhibitors or under multiple impaired conditions. [3] For example, for a drug that is mainly metabolized by CYP3A, the QT evaluation can be conducted with a strong CYP3A inhibitor. Studies of QT prolonging effect of telithromycin with co-administration of ketoconazole illustrate this. When a drug is a substrate for both CYP2D6 and CYP3A, a study involving co-administration of ketoconazole or ritonavir (for CYP3A inhibition) in poor metabolizers of CYP2D6 may be appropriate. For a drug that is both metabolized by CYP3A and excreted via the kidney, it may be appropriate to conduct a study when ketoconazole or ritonavir is co-administered with the investigational drug in patients with renal-impairment. For safety concerns, lower doses of the investigational drug may be appropriate for the initial evaluation to estimate the fold-increase in the systemic exposure. However, prior to the investigation using multiple inhibitors or multiple impaired conditions, the effect of individual inhibition should have been characterized and the combined effects deemed significant based on simulations.

B. Study Population

[2] ICH E14 step 2 document, "The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-antiarrhythmic Drugs"

[3] Comment is requested on the use of multiple inhibitors or multiple impaired conditions to achieve maximum inhibition of the investigational drug's clearance pathway.

Clinical drug-drug interaction studies may generally be performed using healthy volunteers or volunteers drawn from the general population, on the assumption that findings in this population should predict findings in the patient population for which the drug is intended. Safety considerations, however, may preclude the use of healthy subjects. In certain circumstances, subjects drawn from the general population and/or patients for whom the investigational drug is intended offer certain advantages, including the opportunity to study pharmacodynamic endpoints not present in healthy subjects and reduced reliance on extrapolation of findings from healthy subjects. In either patient or healthy/general population subject studies, performance of phenotype or genotype determinations to identify genetically determined metabolic polymorphisms is often important in evaluating effects on enzymes with polymorphisms, notably CYP2D6, CYP2C19, and CYP2C9 - the CYP enzymes considered as known valid metabolic biomarkers.[4] The extent of drug interactions (inhibition or induction) may be different depending on the subjects' genotype for the specific enzyme being evaluated. Similarly, drug interaction via a minor pathway may become important for subjects lacking the major enzyme that contribute to the metabolism of the drug in the general population.

C. Choice of Substrate and Interacting Drugs

1. Investigational Drug as an Inhibitor or an Inducer of CYP Enzymes

In contrast to earlier approaches that focused mainly on a specific group of approved drugs (digoxin, hydrochlorothiazide) where co-administration was likely or the clinical consequences of an interaction were of concern, improved understanding of the metabolic basis of drug-drug interactions enables more general approaches to and conclusions from specific drug-drug interaction studies. In studying an investigational drug as the interacting drug, the choice of substrates (approved drugs) for initial in vivo studies depends on the P450 enzymes affected by the interacting drug. In testing inhibition, the substrate selected should generally be one whose pharmacokinetics is markedly altered by co-administration of known specific inhibitors of the enzyme systems (i.e., a very sensitive substrate should be chosen) to assess the impact of the interacting investigational drug. Examples of substrates include, but are not limited to, (1) midazolam for CYP3A; (2) theophylline for CYP1A2; (3) S-warfarin for CYP2C9; (4) omeprazole for CYP2C19; and (5) desipramine for CYP2D6. Additional examples of substrates, along with inhibitors and inducers of specific CYP enzymes are listed in Table 1. If the initial study is positive for inhibition or induction, further studies of other substrates may be useful, representing a range of substrates based on the likelihood of co-administration.

Table 1. Examples of *in vivo* substrate, inhibitor and inducer for specific CYP enzymes

[4] Draft guidance for industry "voluntary pharmacogenomic data submission", November 2003

have been recommended for study (oral administration) [1,2]

CYP	Substrate	Inhibitor	Inducer
1A2	theophylline, caffeine	fluvoxamine	smoking[3]
2B6	efavirenz		rifampin
2C8	repaglinide, rosiglitazone	gemfibrozil	rifampin
2C9	warfarin, tolbutamide	fluconazole, amiodarone (use of PM subjects) [4]	rifampin
2C19	omeprazol, esoprazol, lansoprazol, pantoprasol	omeprazole, fluvoxamine, moclobemide (use of PM subjects) [4]	rifampin
2D6	desipramine, dextromethorphan, atomoxetine	paroxetine, quinidine, (use of PM subjects) [4]	None identified
2E1	chlorzoxazone	disulfirum	ethanol
3A4/ 3A5	midazolam, buspirone, felodipine, simvastatin, lovastatin, eletriptan, sildenafil, simvastatin, triazolam	atanazavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, voriconazole	rifampin, carbamazepine

[1] substrates for any particular CYP enzyme listed in this table are those with plasma AUC values increased by 2-fold or higher when co-administered with inhibitors of that CYP enzyme; for CYP3A, only those with plasma ACU increased by 5-fold or higher are listed. Inhibitors listed are those that increase plasma AUC values of substrates for that CYP enzyme by 2-fold or higher. For CYP3A inhibitors, only those increase AUC of CYP3A substrates by 5-fold or more are listed. Inducers listed are those that decrease plasma AUC values of substrates for that CYP enzyme by 30% or higher.

[2] note that this is not an extensive list; for an updated list, see URL???

[3] a clinical study may be conducted in smokers as compared to non-smokers (in lieu of an interaction study with an inducer), when appropriate

[4] a clinical study may be conducted in poor metabolizers (PM) as compared to extensive metabolizers (EM) for the specific CYP enzyme (in lieu of an interaction study with an inhibitor), when appropriate.

If the initial study is negative with the most sensitive substrates, it can be presumed that less sensitive substrates will also be unaffected.

CYP3A inhibitors may be classified based on their *in vivo* fold-change in the plasma AUC of oral midazolam or other CYP3A substrate, when given concomitantly. For example, if an investigational drug increases the AUC of oral midazolam or other CYP3A substrates by 5-fold or more ($\geq$5-fold), it may be labeled as "strong" CYP3A inhibitor. If an investigational drug, when given at the highest dose and shortest dosing interval, increases the AUC of oral midazolam or other sensitive CYP3A substrates by between 2- and 5 fold ($\geq$ 2- and <5-fold) when given, it may be labeled as "moderate" CYP3A inhibitor. When an investigational drug is determined to be a "strong" or "moderate" inhibitor of

CYP3A", its interaction with "sensitive CYP3A substrates" or "CYP3A substrates with narrow therapeutic range" (see Table 2 in section V for a list) may be described in various sections of the labeling, as appropriate.

When an *in vitro* evaluation cannot rule out that an investigational drug is an inducer of CYP3A (section VI), *in vivo* evaluation may be conducted using the most sensitive substrate (e.g., oral midazolam). When midazolam has been co-administered following administration of multiple doses of the investigational drug, as may have been conducted as part of an *in vivo* inhibition evaluation, and the results are negative, it can be concluded that the investigational drug is not an inducer of CYP3A (in addition to the conclusion that it is not an inhibitor of CYP3A). *In vivo* induction evaluation has often been conducted with oral contraceptives. However, as they are not the most sensitive substrates, negative data may not exclude the possibility that the investigational drug may be an inducer of CYP3A.

2. Investigational Drug as Substrate of CYP Enzymes

In testing an investigational drug for the possibility that its metabolism is inhibited or induced (i.e., as a substrate), selection of the interacting drugs should be based on *in vitro* or other metabolism studies identifying the enzyme systems that metabolize the drug. The choice of interacting drug should then be based on known, important inhibitors of the pathway under investigation. For example, if the investigational drug is shown to be metabolized by CYP3A and the contribution of this enzyme to the overall elimination of this drug is substantial, the choice of inhibitor and inducer could be ketoconazole and rifampin, respectively, because of the substantial effects of these interacting drugs on CYP3A metabolism (i.e., they are the most sensitive in identifying an effect of interest). If the study results are negative, then absence of a clinically important drug-drug interaction for the metabolic pathway could be claimed. If the clinical study of the strong, specific inhibitor/inducer is positive and the sponsor wishes to claim lack of an interaction between the test drug and other less potent specific inhibitors or inducers, or give advice on dosage adjustment, further clinical studies would generally be recommended (see Table 1 for a list of CYP inhibitors and inducers and Table 3, section V for additional 3A inhibitors). If a drug is metabolized by CYP3A and its plasma AUC was increased by 5-fold or higher by CYP3A inhibitors, it is considered a "sensitive substrate" of CYP3A. The labeling may indicate that it is a sensitive CYP3A substrate and its use with strong or moderate inhibitors may be cautioned based on the drug's exposure- response relationship (see section V for labeling implications). Certain approved drugs are not optimal selections as the interacting drug. For example, cimetidine is not considered an optimal choice to represent drugs inhibiting a given pathway because its inhibition affects multiple metabolic pathways as well as certain drug

transporters.

3. Investigational Drug as Substrate or Inhibitor of P-gp Transporter

In testing an investigational drug for the possibility that its disposition may be inhibited or induced (i.e., as a substrate of P-gp), selection of the interacting drugs may be based on whether the investigational drug is also a CYP3A substrate. If it is also a substrate of CYP3A, it may be appropriate to use a dual inhibitor of both CYP3A and P-gp, such as ritonavir. If the investigational drug is not a substrate of CYP3A, it may be appropriate to use a strong inhibitor of P-gp, such as cyclosporine or verapamil.

In testing an investigational drug for the possibility that it may be an inhibitor of P-gp, selection of digoxin or other known substrates of P-gp may be appropriate.

D. Route of Administration

The route of administration chosen for a metabolic drug-drug interaction study is important. For an investigational agent used as either an interacting drug or substrate, the route of administration should generally be the one planned for in product labeling. When multiple routes are being developed, the necessity for doing metabolic drug-drug interaction studies by all routes should be based on the expected mechanism of interaction and the similarity of corresponding concentration-time profiles for parent and metabolites. If only oral dosage forms will be marketed, studies with an intravenous formulation would not usually be needed, although information from oral and intravenous dosings may be useful in discerning the relative contributions of alterations in absorption and/or presystemic clearance to the overall effect observed for a drug interaction. Sometimes certain routes of administration can reduce the utility of information from a study. For example, an intravenous study may not reveal an interaction for substrate drugs where intestinal CYP3A activity markedly alters bioavailability. For an approved agent used either as a substrate or interacting drug, the route of administration will depend on available marketed formulations, which in most instances will be oral.

E. Dose Selection

For both a substrate (investigational drug or approved drug) and interacting drug (investigational drug or approved drug), testing should maximize the possibility of finding an interaction. For this reason, the maximum planned or approved dose and shortest dosing interval of the interacting drug (as inhibitors or inducers) should be used. For example, when using ketoconazole as an inhibitor of CYP3A, dosing at 400 mg QD for multiple days would be preferable to dosing at lower doses. When using rifampin as an inducer, dosing at 600 mg QD for multiple days would be preferable to dosing at lower

doses. Doses smaller than those to be used clinically may be needed for substrates on safety grounds and may be more sensitive to the effect of the interacting drug.

F. Endpoints

1. Pharmacokinetic Endpoints

The following measures and parameters are recommended for assessment of the substrate: (1) exposure measures such as AUC, Cmax, time to Cmax (Tmax), and others as appropriate; and (2) pharmacokinetic parameters such as clearance, volumes of distribution, and half-lives. In some cases, these measures may be of interest for the inhibitor or inducer as well, notably where the study is assessing possible interactions between both study drugs. Additional measures may help in steady state studies (e.g., trough concentration (Cmin)) to demonstrate that dosing strategies were adequate to achieve near steady state before and during the interaction. In certain instances, an understanding of the relationship between dose, blood concentrations, and response may lead to a special interest in certain pharmacokinetic measures and/or parameters. For example, if a clinical outcome is most closely related to peak concentration (e.g., tachycardia with sympathomimetics), Cmax or another early exposure measure might be most appropriate. Conversely, if the clinical outcome is related more to extent of absorption, AUC would be preferred. The frequency of sampling should be adequate to allow accurate determination of the relevant measures and/or parameters for the parent and metabolites. For the substrate, whether the investigational drug or approved drug, determination of the pharmacokinetics of important active metabolites is important. This concept paper focuses on metabolic drug-drug interactions, however, protein binding determinations are considered necessary to distinguish between induction or stimulation of metabolism and displacement from protein-binding site. The latter is not considered to be a source of clinically important drug interactions because unbound drug concentrations are unaffected.

2. Pharmacodynamic Endpoints

Pharmacokinetic measures are usually sufficient for metabolic drug-drug interaction studies, although pharmacodynamic measures can sometimes provide additional useful information. Pharmacodynamic measures may be needed when a pharmacokinetic/pharmacodynamic relationship for the substrate endpoints of interest is not established or when pharmacodynamic changes do not result solely from pharmacokinetic interactions (e.g, additive cardiovascular effect of quinidine and tricyclic antidepressants). When an approved drug is studied as a substrate, the pharmacodynamic impact of a given change in blood level (Cmax, AUC) caused by an investigational interaction should be known from other interaction

studies about the approved drug, with the possible exception of older drugs.

G. Sample Size and Statistical Considerations

For both investigational drugs and approved drugs, when used as substrates and/or interacting drugs in drug-drug interaction studies, the desired goal of the analysis is to determine the clinical significance of any increase or decrease in exposure to the substrate in the presence of the interacting drug. Assuming unchanged PK/PD relationships, changes may be evaluated by comparing pharmacokinetic measures of systemic exposure that are most relevant to an understanding of the relationship between dose (exposure) and therapeutic outcome.

Results of drug-drug interaction studies should be reported as 90% confidence intervals about the geometric mean ratio of the observed pharmacokinetic measures with (S+I) and without the interacting drug (S).[3] Confidence intervals provide an estimate of the distribution of the observed systemic exposure measure ratio of S+I versus S alone and convey a probability of the magnitude of the interaction. In contrast, tests of significance are not appropriate because small, consistent systemic exposure differences can be statistically significant ($p < 0.05$) but not clinically relevant.

When a drug-drug interaction is clearly present (e.g., comparisons indicate twofold or greater increments in systemic exposure measures for S+I) the sponsor should be able to provide specific recommendations regarding the clinical significance of the interaction based on what is known about the dose-response and/or PK/PD relationship for either the investigational agent or the approved drugs used in the study. This information should form the basis for reporting study results and for making recommendations in the package insert with respect to either the dose, dosing regimen adjustments, precautions, warnings, or contraindications of either the investigational drug or the approved drug. FDA recognizes that dose-response and/or PK/PD information may sometimes be incomplete or unavailable, especially for an approved drug used as S.

Second, the sponsor may wish to make specific claims in the package insert that no drug-drug interaction is expected. In these instances, the sponsor should be able to recommend specific *no effect* boundaries, or clinical equivalence intervals, for a drug-drug interaction. No effect boundaries define the interval within which a change in a systemic exposure measure is considered not clinically meaningful. There are two approaches to define no effect boundaries.

Approach 1: No effect boundaries can be based on population (group) average dose

[3] Schuirmann, D.J., "A Comparison of the Two One-Sided Tests Procedure and the Power Approach for Assessing the Bioequivalence of Average Bioavailability," *J. Pharmacokin. and Biopharm.*, 15:657-80, 1987.

and/or concentration-response relationships, PK/PD models, and other available information for the substrate drug. If the 90% confidence interval for the systemic exposure measurement in the drug-drug interaction study falls completely within the *no effect* boundaries, the sponsor may conclude that no clinically significant drug-drug interaction was present.

Approach 2: In the absence of *no effect* boundaries defined in (1) above, a sponsor may use a default *no effect* boundary of 80-125% for both the investigational drug and the approved drugs used in the study. When the 90% confidence intervals for systemic exposure ratios fall entirely within the equivalence range of 80-125%, standard Agency practice is to conclude that no clinically significant differences are present.

The selection of the number of subjects for a given drug-drug interaction study will depend on how small an effect is clinically important to detect, or rule out, the inter- and intrasubject variability in pharmacokinetic measurements, and possibly other factors or sources of variability not well recognized. In addition, the number of subjects will depend on how the results of the drug-drug interaction study will be used, as described above.

This concept paper should not be interpreted by sponsors as generally recommending the inclusion of some number of subjects in a drug-drug interaction study such that the 90% confidence interval for the ratio of pharmacokinetic measurements falls entirely within the no effect boundaries of 80-125%. This approach, however, could be deemed appropriate by a sponsor, after considering the expected outcome of a drug-drug interaction study, the anticipated magnitude of variability in pharmacokinetic measurements, and the desired label claim that no clinically significant drug-drug interaction was present.

V. LABELING IMPLICATIONS

All relevant information on the metabolic pathways and metabolites and pharmacokinetic interaction should be included in the PHARMACOKINETICS subsection of the CLINICAL PHARMACOLOGY section of the labeling. The clinical consequences of metabolism and interactions should be placed in DRUG INTERACTIONS, WARNINGS AND PRECAUTIONS, BOXED WARNINGS, CONTRAINDICATIONS, or DOSAGE AND ADMINISTRATION sections, as appropriate. Such information related to clinical consequences should not be included in detail in more than one consequences related section, but rather referenced from one section to other sections as needed. When the metabolic pathway or interaction data resulted in recommendations for dosage adjustments, contraindications, warnings (e.g., co-administration should be avoided), that were included in the BOXED WARNINGS, CONTRAINDICATIONS, WARNINGS AND PRECAUTIONS, or DOSAGE AND ADMINISTRATION sections, these

recommendations should also be included in the corresponding "HIGHLIGHTS" section of the labeling with appropriate referencing of other labeling sections. Refer to the guidance for industry "*Labeling for Human Prescription Drug and Biological Products – Implementing the New Content and Format Requirements"* and *"Clinical Pharmacology and Drug Interaction Labeling"* for more information on presenting drug interaction information in labeling.

The following general principles affect labeling for specific metabolism or drug interaction data.

- In certain cases, information based on clinical studies not using the labeled drug under investigation can be described with an explanation that similar results may be expected for the labeled drug. For example, if a drug has been determined to be a strong inhibitor of CYP3A, it does not need to be tested with all CYP3A substrates to warn about an interaction with "sensitive CYP3A substrates" and "CYP3A substrates with narrow therapeutic range". Table 2 lists examples of "sensitive CYP3A substrates" and "CYP3A substrates with narrow therapeutic range".

Table 2. Examples[(1)] of sensitive CYP3A substrates or CYP3A substrates with narrow therapeutic range

Sensitive CYP3A substrates[(2)]	CYP3A Substrates with Narrow therapeutic range[(3)]
budesonide, buspirone, eletriptan, felodipine, imatinab, lovastatin, midazolam, saquinavir, sildenafil, simvastatin, triazolam	Alfentanil, astemizole(a), cisapride(a), cyclosporine, diergotamine, ergotamine, fentanyl, irinotecan, pimozide, quinidine, sirolimus, tacrolimus, terfenadine(a)

[(1)] note that this is not an extensive list; for an updated list, see URL???
[(2)] "sensitive CYP3A substrates" refer to drugs whose plasma AUC values are increased 5-fold or more when co-administered with CYP3A inhibitors
[(3)] "CYP3A substrates with narrow therapeutic range" refer to drugs whose exposure-response data are such that increases in their exposure levels by the concomitant use of CYP3A inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes); (a) not available in US

- If a drug has been determined to be a sensitive CYP3A substrate or a CYP3A substrate with a narrow therapeutic range, it does not need to be tested with all strong or moderate inhibitors of CYP3A to warn about an interaction with "strong" or "moderate" CYP3A inhibitors. Table 3 lists examples of "strong CYP3A inhibitors" and "moderate CYP3A inhibitors". Similarly, if a drug has been determined to be a sensitive CYP3A substrate or a CYP3A substrate with a narrow therapeutic range, it does not need to be tested with all CYP3A inducers to warn about an interaction with CYP3A inducers. Examples of CYP3A inducers include *rifampin, rifabutin, rifapentin, dexamethasone, phenytoin, carbamazepine, phenobarbital and St. John's Wort.*

Table 3. Classification of CYP3A inhibitors[(1)]

Strong CYP3A inhibitors	Moderate CYP3A inhibitors
atanazavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, voriconazole	Amprenavir, aprepitant, diltiazem, erythromycin, fluconazole, fosaprenavir, grapefruit juice(a), verapamil

[(1)] please note the following:
- A "strong inhibitor" is one that caused a ≥ 5-fold increase in the plasma AUC values of CYP3A substrates (not limited to midazolam) in clinical evaluations
- A "moderate inhibitor" is one that caused a ≥ 2- but < 5-fold increase in the AUC values of sensitive CYP3A substrates when the inhibitor was given at the highest approved dose and the shortest dosing interval in clinical evaluations
- Note that this is not an extensive list; for an updated list, see URL???

(a) the effect of grapefruit juice varies widely

VI. Appendices- In vitro drug metabolism studies

Appendix A. Drug metabolism enzyme identification

Drug metabolizing enzyme identification studies, often referred to as reaction phenotyping studies, are a set of experiments that identify the specific enzymes responsible for metabolism of a drug. Oxidative and hydrolytic reactions involve cytochrome P450 (CYP) and non-CYP enzymes. For many drugs, transferase reactions are preceded by oxidation or hydrolysis of the drug. However, direct transferase reactions may represent a major metabolic pathway for compounds containing polar functional groups.

An efficient approach is to determine the metabolic profile (identify metabolites that are formed and their quantitative importance) of a drug and estimate the relative contribution of CYP enzymes to clearance before initiating studies to identify specific CYP enzymes that metabolize the drug. Identification of CYP enzymes is warranted if CYP enzymes contribute > 25% of a drug's total clearance. The identification of drug metabolizing CYP enzymes in vitro helps predict the potential for in vivo drug-drug interactions and the impact of polymorphic enzyme activity on drug disposition and the formation of toxic or active metabolites. There are few documented cases of clinically significant drug-drug interactions related to non-CYP enzymes, but the identification of drug metabolizing enzymes in this class (i.e., glucuronosyltransferases, sulfotransferases, and N-acetyl transferases) is encouraged. Although classical biotransformation studies are not a general requirement for the evaluation of therapeutic biologics, certain protein therapeutics modify the metabolism of drugs that are metabolized by CYP enzymes. Given their unique nature, consultation with FDA is appropriate before initiating drug-drug interaction studies involving biologics.

1. Metabolic pathway identification experiments (Determination of metabolic profile)

a) Rationale and Goals- Data obtained from drug metabolic pathway identification experiments in vitro help determine whether experiments to identify drug metabolizing enzymes are warranted, and guide the appropriate design of any such experiments. The metabolic pathway identification experiments should identify the number and classes of metabolites produced by a drug and whether the metabolic pathways are parallel or sequential.

b) Tissue selection for metabolic pathway identification experiments

Freshly isolated hepatocytes are the preferred tissue for conducting metabolic pathway identification experiments. Hepatocytes provide cellular integrity with respect to enzyme architecture and contain the full complement of drug metabolizing enzymes. Alternative tissues include cyropreserved hepatocytes and freshly isolated liver slices. However, these tissues provide qualitative rather than quantitative information.

Subcellular liver tissue fractions or recombinant enzymes can be used in combination with the tissues mentioned above to identify the individual drug metabolites produced and classes of enzyme involved, but the methods do not provide quantitative information of fraction metabolized by a specific enzyme or pathway. Subcellular liver tissue fractions include microsomes, S9, and cytosol; appropriate co-factors are necessary.

c) Design of metabolic pathway identification experiments

The preferred first approach to metabolic pathway identification is to incubate the drug with hepatocytes or liver slices, followed by chromatographic analysis of the incubation medium by HPLC-MS/MS. This type of experiment leads to the direct identification of metabolites formed by oxidative, hydrolytic and transferase reactions and provides information concerning parallel vs. sequential pathways. An alternate approach is to analyze the incubation medium by HPLC using UV, fluorescent, or radiochemical detection.

In view of the known multiplicity and overlapping substrate specificity of drug metabolizing enzymes and the possibility of either parallel or sequential metabolic pathways, experiments should include several drug concentrations and incubation times. Expected steady-state in vivo plasma drug concentrations serve as a guide for the range of drug concentrations used for these experiments.

d) As indicated in the PhRMA position paper on drug-drug interactions (Bjornsson TD, et al, 2003), the methods listed in Table 1 can be used to identify CYP and non-CYP oxidative pathways responsible for the observed metabolites.

Table 1. Methods to identify pathways involved in the oxidative biotransformation of a drug

In vitro System	**Condition**	**Tests**
microsomes	+/- NADPH	CYP, FMO vs other oxidases
microsomes, hepatocytes	+/- 1-aminobenzotriazole	broad specificity CYP inactivator
Microsomes	45^{o}C pretreatment	inactivates FMO
S-9	+/- pargyline	broad MAO inactivator
S-9, cytosol	+/- menadione, allopurinol	Mo-CO (oxidase) inhibitors

2. Studies designed to identify drug metabolizing CYP enzymes

If human in vivo data or metabolic pathway identification studies conducted in vitro indicate CYP enzymes contribute >25% of a drug's clearance, studies to identify drug metabolizing CYP enzymes in vitro are recommended. This recommendation includes cases in which oxidative metabolism is followed by transferase reactions, because a drug-drug interaction that inhibits oxidation of the parent compound can result in elevated levels of the parent compound**.**

a) General experimental methods for identifying drug metabolizing CYP enzymes

There are four well characterized methods for identifying the individual CYP enzymes responsible for a drug's metabolism. The respective methods use 1) specific chemical inhibitors; 2) individual human recombinant CYP enzymes, 3) antibodies as specific enzyme inhibitors; 4) a bank human liver microsomes characterized for CYP activity that were prepared from individual donor livers. At least two of the methods should be performed to identify the specific enzyme(s) responsible for a drug's metabolism.

Either pooled human liver microsomes or microsomes prepared from individual liver donors may be used for the methods a.1 and a.3. For correlation analysis (a.4), a bank of characterized microsomes from individual donor livers is required.

Experiments to identify the CYP enzymes responsible for a drug's metabolism should be conducted, whenever possible, with pharmacologically relevant concentrations of drugs. It is recommended that enzyme identification experiments be conducted under initial rate conditions (linearity of metabolite production rates with respect to time and enzyme concentrations). In some cases the experiments may be conducted under nonlinear conditions due to analytical sensitivity; results of these experiments should be interpreted with caution. Thus, reliable analytical methods should be developed to quantitate each metabolite produced by individual CYP enzymes selected for identification. For racemic drugs, individual isomers should be evaluated separately.

b) Considerations regarding the use of **specific chemical inhibitors** to identify drug metabolizing CYP enzymes

Most chemical inhibitors are not absolutely specific for an individual CYP enzyme, but a valuable attribute of chemical inhibitors is their commercial availability. Although not all inclusive, the chemical inhibitors listed in Table 2 can be used to identify individual CYP enzymes responsible for a drug's metabolism and determine the relative contribution of an individual CYP enzyme.

Table 2: Chemical inhibitors for in vitro experiments

CYP	Inhibitor [1] Preferred	Ki (µM)	Inhibitor [1] Acceptable	Ki (µM)
1A2	furafylline [2]	0.6-0.73	α-naphthoflavone	0.01
2A6	tranylcypromine methoxsalen [2]	0.02-0.2 0.01-0.2	pilocarpine tryptamine	4 1.7 [3]
2B6			3-isopropenyl-3-methyl diamantane [4] 2-isopropenyl-2-methyl adamantane [4] sertraline phencyclidine triethylenethiophosphoramide (thiotepa) clopidogrel ticlopidine	2.2 5.3 3.2 [5] 10 4.8 0.5 0.2
2C8	quercetin	1.1	trimethoprim gemfibrozil rosiglitazone pioglitazone	32 69-75 5.6 1.7
2C9	sulfaphenazole	0.3	fluconazole fluvoxamine Fluoxetine	7 6.4-19 18-41
2C19			ticlopidine nootkatone	1.2 0.5
2D6	quinidine	0.027-0.4		
2E1			diethyldithiocarbamate clomethiazole diallyldisulfide	9.8-34 12 150
3A4/5	ketoconazole itraconazole	0.0037- 0.18 0.27, 2.3	troleandomycin verapamil	17 10, 24

(1) Substrates used for inhibition studies include: **CYP1A2**, phenacetin-o-deethylation, theophylline-N-demethylation; **CYP2A6**, coumarin-7-hydroxylation; **CYP2B6**, 7-pentoxyresorufin-O-dependentylation, bupropion hydroxylation, 7-ethoxy-4-(trifluoromethyl)-coumarin O-deethylation, S-mephenytoin-N-demethylation; Bupropion-hydroxylation; **CYP2C8**, taxol 6-alpha-hydroxylation; **CYP2C9**, tolbutamide 4-methylhydroxylation, S-warfarin-7-hydroxylation, phenytoin 4-hydroxylation; **2CYP2C19**, (S)-mephenytoin 4-hydroxylation **CYP2D6**, dextramethorphan O-demethylation, desbrisoquine hyddroxylase; **CYP2E1**, chlorzoxazone 6-hydroxylation, aniline 4-hydroxylase; **CYP3A4/5**, testosterone-6ß-hydroxylation, midazolam-1-hydroxylation; cyclosporine hydroxylase; nefedipine dehydrogenation.

(2) Furafylline and methoxsalen are mechanism-based inhibitors and should be preincubated before adding substrate.

(3) cDNA expressing microsomes from human lymphoblast cells.

(4) Supersomes, microsomal isolated from insect cells transfected with baculovirus containing CYP2B6.

(5) IC_{50} values

The effectiveness of competitive inhibitors is dependent on concentrations of the drug and inhibitor. Experiments designed to identify and to quantitate the relative importance of individual CYP enzymes mediating a drug's metabolism should use drug concentrations $=K_m$. The experiments should include the inhibitor at concentrations that ensure selectivity and adequate potency. It is also acceptable to use a range of inhibitor concentrations.

Noncompetitive and mechanism-based inhibitors are not dependent on the drug (substrate) concentration. When using a mechanism-based inhibitor, it is necessary to pre-incubate the inhibitor for 30 minutes.

For additional information concerning inhibition experiments see Inhibition Section.

c) Considerations regarding the use of **recombinant enzymes** to identify drug metabolizing CYP enzymes

When a drug is metabolized by only one recombinant human CYP enzyme, interpretation of the results is relatively straightforward. However, if more than one recombinant CYP enzyme is involved, measurement of enzyme activity alone does not provide information concerning the relative importance of the individual pathways.

Recombinant CYP enzymes are not present in their native environment and are often over expressed. Accessory proteins (NADPH-CYP reductase and cytochrome b5) or membrane lipid composition may differ from native microsomes. Several approaches have been reported to quantitatively scale metabolic activity obtained using recombinant CYP enzymes to activities expected in the human liver microsomes; however, these methods have not been validated and their results are suspect.

d) Considerations regarding the use of **specific antibodies** to identify drug metabolizing CYP enzymes

The inhibitory effect of an inhibitory antibody should be tested at sufficiently low and high concentrations to establish the titration curve. If only one CYP enzyme is involved in the drug's metabolism, > 80% inhibition is expected in a set of pooled or individual microsomes. If the extent of inhibition is low, it is difficult to determine whether the partial inhibition is due to the involvement of other CYPs in metabolism of the drug or the antibody has poor potency.

e) Considerations regarding the use of **correlation analyses** to identify drug metabolizing CYP enzymes

This approach relies on statistical analyses to establish a correlation between the production rate of an individual metabolite and activities determined for each CYP enzyme in a set of

871 microsomes prepared from individual donor livers.
872
873 The set of characterized microsomes should include microsomes prepared from at least 10
874 individual donor livers. The variation in metabolic activity for each CYP enzyme should be
875 sufficient between individual donor livers to ensure adequate statistical power. Enzyme
876 activities in the set of microsomes used for correlation studies should be determined using
877 appropriate probe substrates and experimental conditions.
878
879 Results are suspect when a single outlying point dictates the correlation coefficient. If the
880 regression line does not pass through or near the origin, it may indicate that multiple CYP
881 enzymes are involved or reflect a set of microsomes that are inherently insensitive.
882
883

Appendix B. Evaluation of CYP inhibition

A drug that inhibits a specific drug-metabolizing enzyme can decrease the metabolic clearance of a co-administered drug that is a substrate of the inhibited pathway. A consequence of decreased metabolic clearance is elevated blood concentrations of the coadministered drug, which may cause adverse effects or enhanced therapeutic effects. Also, the inhibited metabolic pathway could lead to decreased formation of an active compound, resulting in decreased efficacy.

1. Probe substrates

In vitro experiments that are conducted to determine whether a drug inhibits a specific CYP enzyme involve incubation of the drug with probe substrates for the CYP enzymes.

There are two scientific criteria for selection of a probe substrate - the substrate should be selective (predominantly metabolized by a single enzyme in pooled human liver microsomes or recombinant P450s) and should have a simple metabolic scheme (ideally no sequential metabolism). There are also some practical criteria- commercial availability of substrate and metabolite(s); assays that are sensitive, rapid, and simple; and a reasonable incubation time.

Preferred substrates listed in Table 3 meet a majority of the criteria listed above. Acceptable substrates meet some of the criteria, and are considered acceptable by the scientific community.

Table 3. Preferred and acceptable chemical substrates for in vitro experiments

CYP	Substrate Preferred	Km (μM)	Substrate Acceptable	Km (μM)
1A2	phenacetin-O-deethylation	1.7-152	7-Ethoxyresorufin-O-deethylation Theophylline-N-demethylation Caffeine-3-N-demethylation Tacrine 1-hydroxylation	0.18-0.21 280-1230 220-1565 2.8, 16
2A6	coumarin-7-hydroxylation nicotine C-oxidation	0.30-2.3 13-162		
2B6	Efavirenz hydroxylase	17-23	Propofol hydroxylation S-mephenytoin-N-demethylation Bupropion-hydroxylation	3.7-94 1910 67-168
2C8	Taxol 6-hydroxylation	5.4-19	Amodiaquine N-deethylation Rosiglitazone para-hydroxylation	2.4, 4.3-7.7
2C9	tolbutamide methyl-hydroxylation S-warfarin 7-hydroxylation diclofenac 4'-hydroxylation	67-838 1.5-4.5 3.4-52	Flurbiprofen 4'-hydroxylation Phenytoin-4-hydroxylation	6-42 11.5-117
2C19	S-mephenytoin 4'-hydroxylation	13-35	Omeprazole 5-hydroxylation Fluoxetine O-dealkylation	17-26 3.7-104
2D6	(±)-bufuralol 1'-hydroxylation dextromethorphan O-demethylation	9-15 0.44-8.5	Debrisoquine 4-hydroxylation	5.6
2E1	chlorzoxazone 6-hydroxylation	39-157	p-nitrophenol 3-hydroxylation Lauric acid 11-hydroxylation Aniline 4-hydroxylation	3.3 130 6.3-24
3A4/5*	midazolam 1-hydroxylation testosterone 6β-hydroxylation	1-14 52-94	Erythromycin N-demethylation Dextromethorphan N-demethylation Triazolam 4-hydroxylation Terfenadine C-hydroxylation Nifedipine oxidation	33 – 88 133-710 234 15 5.1- 47

*Recommend use of 2 structurally unrelated CYP3A4/5 substrates for evaluation of in vitro CYP3A inhibition. If the drug inhibits at least one CYP3A substrate in vitro, then in vivo evaluation is warranted.

2. Design considerations for in vitro CYP inhibition studies

a. Typical kinetic experiments for determining IC50 or Ki involve incubating varying concentrations of substrate and inhibitor with fixed amounts of enzyme for a constant period of time. The substrate and inhibitor concentrations used should cover the range above and below the Km and Ki, respectively.

b. Microsomal protein concentration usually ranges from 0.01 to 0.5 mg/ml.

c. Because buffer strength, type, and pH can all significantly affect Vmax and Km, standardized assay conditions are recommended.

d. Preferably no more than 10% substrate or inhibitor depletion should occur. However, with low Km substrates, it may be difficult to avoid >10% substrate depletion at low substrate concentrations.

e. The relationship between time and amount of product formed should be linear.

f. The relationship between amount of enzyme and product formation should be linear.

g. Any solvents should be used at low concentrations (≤1% (v/v) and preferably <0.1%). Some of the solvents inhibit or induce enzymes. The experiment may include a no-solvent control and a solvent control.

h. Use of an active control (known inhibitor) is optional

3. Determining whether an NME is a reversible inhibitor

Theoretically, significant enzyme inhibition occurs when the concentration of the inhibitor present at the active site is comparable to or in excess of the Ki. In theory, the degree of interaction (R, expressed as percent change in AUC) can be estimated by the following equation: R = 1+ [I]/Ki, where [I] is the concentration of inhibitor exposed to the active site of the enzyme and Ki is the inhibition constant.

Although the [I]/Ki ratio is used to predict the likelihood of inhibitory drug interactions, there are factors that affect selection of the relevant [I] and Ki. Factors that affect [I] include uncertainty regarding the concentration that best represents concentration at the enzyme binding site and uncertainty regarding the impact of first-pass exposure. Factors that affect Ki include substrate specificity, binding to components of incubation system, substrate and inhibitor depletion.

Current recommended approach-
Due to the concerns listed above, the use of [I]/Ki to predict the potential for inhibitory drug interactions needs to be further evaluated. Thus, we use a conservative approach to determine the likelihood of an in vivo interaction, based on in vitro data. Calculate [I]/Ki, where [I] represents the mean steady-state Cmax value for total drug (bound plus unbound) following administration of the highest proposed clinical dose. As the ratio increases, the likelihood of an interaction increases. If the ratio is <0.02, the likelihood of an interaction is remote, and an in vivo metabolism-based drug-drug interaction study is not needed. Quantitative predictions of the magnitude of an in vivo interaction, based on in vitro data, are not possible at this time. Although quantitative predictions of in vivo drug-drug interactions from in vitro studies are not possible, rank order across the different CYP enzymes for the same drug may help prioritize in vivo drug-drug interaction evaluations.

4. Determining whether an NME is a mechanism based inhibitor

Time-dependent inhibition should be examined in standard in vitro screening protocols, because the phenomenon cannot be predicted with complete confidence from chemical structure. A 30 minute pre-incubation of a potential inhibitor, prior to addition of substrate, is recommended. Any time-dependent and concentration-dependent loss of initial product formation rate indicates

mechanism based inhibition. For compounds containing amines, metabolic intermediate complex formation can be followed spectroscopically. Detection of time-dependent inhibition kinetics in vitro should be followed up with in vivo studies in humans (or possibly in a human hepatocyte study).

Appendix C. Evaluation of CYP induction

A drug that induces a drug-metabolizing enzyme can increase the rate of metabolic clearance of a co-administered drug that is a substrate of the induced pathway. A potential consequence of this type of drug-drug interaction is sub-therapeutic blood concentrations. Alternatively, the induced metabolic pathway could lead to increased formation of an active compound resulting in an adverse event.

1. Chemical inducers as a positive control

If one is evaluating the potential for a drug to induce a specific CYP enzyme, the experiment should include an acceptable enzyme inducer as a control such as those listed in Table 4. The use of a positive control helps quantify enzyme catalytic activity. The positive controls should be potent inducers (> 2 fold increase in enzyme activity of probe substrate at inducer concentrations < 500 µM). The selection of test drug probes is discussed in Section A.

Table 4. chemical inducers for in vitro experiment[1]

CYP	Inducer [1] -Preferred	Inducer Concentrations (µM)	Fold Induction	Inducer [1] -Acceptable	Inducer Concentrations (µM)	Fold Induction
1A2	omeprazole ß-naphthoflavone[2] 3-methylcholanthrene	25-100 33-50 1,2	14-24 4-23 6-26	lansoprazole	10	10
2A6	dexamethasone	50	9.4	pyrazole	1000	7.7
2B6	phenobarbital	500-1000	5-10	phenytoin	50	5-10
2C8	rifampin	10	2-4	phenobarbital	500	2-3
2C9	rifampin	10	3.7	phenobarbital	100	2.6
2C19	rifampin	10	20			
2D6	none identified					
2E1	none identified					
3A4	rifampin[3]	10-50	4-31	phenobarbital[3] phenytoin rifapentine troglitazone taxol dexamethasone[4]	100-2000 50 50 10-75 4 33-250	3-31 12.5 9.3 7 5.2 2.9- 6.9

(1) Except for the cases noted below, the following test substrates were used: CYP1A2, 7-ethoxyresorufin; CYP 2A6, coumarin; CYP2C9, tolbutamide, CYP2C19, S-mephenytoin; CYP3A4, testosterone.
(2) CYP1A2: 1 of 4 references for β-naphthoflavone used phenacetin
(3) CYP3A4: 2 of 13 references for rifampin and 1 of 3 references for phenobarbital used midazolam
(4) CYP3A4: 1 of the 4 references for dexamethasone used nifedipine

2. Design of drug induction studies in vitro

Presently, the most reliable method to study a drug's induction potential is to quantify the enzyme activity of primary hepatocyte cultures following treatments including the potential

inducer drug, a probe inducer drug (positive control, see Table 4), and non treated hepatocytes (negative control), respectively. Either freshly isolated hepatocyte cultures or cryopreserved hepatocytes that can be thawed and cultured are acceptable for these studies.

a) Test drug concentrations should be utilized based on the expected human plasma drug concentrations. At least three concentrations spanning the therapeutic range should be studied, including at least one concentration that is an order of magnitude greater than the average expected plasma drug concentration. If this information is not available, concentrations ranging over at least two orders of magnitude should be studied.

b) Following treatment of hepatocytes for 3-4 days, the resulting enzyme activities should be determined using appropriate CYP-specific probe drugs (see Table 3). Either whole cell monolayers or isolated microsomes can be utilized to monitor drug-induced enzyme changes, however, the former tissue is the simplest and most direct method,

c) When conducting experiments to determine enzyme activity, the experimental conditions listed in section B.2 are relevant.

d) Based on inter-individual differences in induction potential, experiments should be conducted with hepatocytes prepared from at least three individual donor livers.

3. Endpoints for subsequent prediction of enzyme induction

When analyzing the results of experiments to determine enzyme activity, the following issues are relevant.

a) The simplest and most frequently used endpoints to identify enzyme induction are the fold induction activity:

fold induction = (activity of test drug treated cells) / (activity of negative control)

or percent of positive control activity:

% positive control = (activity of test drug treated cells x 100) / (activity of positive control)

b) An alternative endpoint is the use of an EC_{50} (effective concentration at which 50% maximal induction occurs) value, which represents a potency index that can be used to compare the potency of different compounds.

c) A drug that produces a > 2 fold increase in probe drug enzyme activity or the fold-change that is more than 40% of the positive control can be considered as an enzyme inducer in vitro

and in vivo evaluation is warranted.

4. Other methods proposed for identifying enzyme induction in vitro

Although the most reliable method for quantifying a drug's induction potential involves measurement of enzyme activities after incubation of the drug in primary cultures of human hepatocytes, other methods are being evaluated. Several of these methods are described briefly below.

a) Western immunoblotting or immunoprecipitation probed with specific polyclonal antibodies. Relative quantification of specific P450 enzyme protein requires that the electrophoretic system clearly resolve the individual enzymes and/or the primary antibodies be specific for the enzyme quantified. Enzyme antibody preparations are highly variable.

b) Measurement of mRNA levels using reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR can quantify mRNA expression for a specific CYP enzyme but is not necessarily informative of enzyme activities. The measurement of mRNA levels are helpful when both enzyme inhibition and induction are operative.

c) Receptor gene assays for receptors mediating induction of P450 enzymes. Cell receptors mediating CYP1A, CYP2B and CYP3A induction have been identified. Higher throughput AhR (aromatic hydrocarbon receptor) and PXR (pregnane X receptor) binding assays and cell-based reporter gene assays have been developed and utilized to screen for compounds that have CYP1A and CYP3A induction potential. However, correlation of receptor binding and activation with in vivo CYP enzyme induction requires additional validation.

d) Enzyme activity in immortal cell lines. Differential expression of the individual CYP450 enzymes and corresponding regulatory factors (e.g., nuclear receptors and associated cofactors) over time in culture suggests that this model system is not reflective of in vivo profiles. Although negative results from this method cannot rule out an induction effect, positive results can indicate a need for further clinical evaluation.

VII. References:

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