Guidance for Industry

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

DRAFT GUIDANCE

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For questions regarding this draft document contact (CDER) Shiew-Mei Huang, 301-796-1541, or Lei Zhang, 301-796-1635.

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Office of Communications
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Center for Drug Evaluation and Research
Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
Phone: 301-796-3400; Fax: 301-847-8714
druginfo@fda.hhs.gov

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Guidance for Industry[1]

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I.   INTRODUCTION

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

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

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

40
41 **II.   SUMMARY OF GUIDANCE**
42
43   The key recommendations for sponsors to consider when evaluating drug-drug interactions
44   during drug development are listed below. The various sections of this guidance provide more
45   details for each recommendation.
46
47   • Interactions between an investigational new drug and other drugs should be defined during
48   drug development, as part of an adequate assessment of the drug's safety and effectiveness.
49   The objective of drug-drug interaction studies is to determine whether potential interactions
50   between the investigational drug and other drugs exist and, if so, whether the potential for
51   such interactions indicates the need for dosage adjustments, additional therapeutic
52   monitoring, a contraindication to concomitant use, or other measures to mitigate risk.
53
54   • Development of a drug should include identification of the principal routes of elimination,
55   quantitation of the contribution by enzymes and transporters to drug disposition, and
56   characterization of the mechanism of drug-drug interactions.
57
58   • Sponsors who believe a complete evaluation of the potential for drug-drug interactions is not
59   necessary for an investigational drug because of the target population and likely co-
60   administered drugs should contact the Office of Clinical Pharmacology and the clinical
61   division in the Office of New Drugs.
62
63   • This guidance and its appendices include numerous decision trees intended to help sponsors
64   determine what types of drug-drug interaction studies may be needed (see Figures 2 through
65   7 and Appendix Figures A-1 through A-6).
66
67   • The study of drug-drug interaction for a new drug generally begins with in vitro studies to
68   determine whether a drug is a substrate, inhibitor, or inducer of metabolizing enzymes. The
69   results of in vitro studies will inform the nature and extent of in vivo studies that may be
70   required to assess potential interactions.   Along with clinical pharmacokinetic data, results
71   from in vitro studies may serve as a screening mechanism to rule out the need for additional
72   in vivo studies, or provide a mechanistic basis for proper design of clinical studies using a
73   modeling and simulation approach.
74
75   • When testing an investigational drug for the possibility that its metabolism is inhibited or
76   induced (i.e., as a substrate), selection of the interacting drugs should be based on in vitro or
77   in vivo studies identifying the enzyme systems that metabolize the investigational drug. The
78   choice of the interacting drug can then be based on known, important inhibitors and inducers
79   of the pathway under investigation. Strong inhibitors and inducers provide the most sensitive
80   assessment and should generally be tested first (see section V.C).
81
82   • If potential drug-drug interactions are identified based on in vitro and/or in vivo studies,
83   sponsors should design further studies or collect information to determine (1) whether

2

84   additional studies are needed to better quantify the effect and to examine the effects of
85   weaker inhibitors (early studies usually examine strong inhibitors) on the investigational
86   drugs as substrates and effects of investigational drugs (as inhibitors) on a range of
87   substrates, and (2) whether dosage adjustments or other prescribing modifications (e.g.,
88   additional safety monitoring or contraindications) are needed based on the identified
89   interaction(s) to avoid undesired consequences.
90

91   •   The potential for drug interactions with metabolites of investigational drugs (metabolites
92   present at ≥25% of parent drug AUC) should be considered (see section IV.A.3).
93

94   •   Metabolic drug-drug interactions should also be explored for investigational drugs that are
95   not eliminated significantly by metabolism because such drugs can inhibit or induce a co-
96   administered drug's metabolic pathway (see section IV.A.1).
97

98   •   When evaluating a new drug as a potential cytochrome P450 (CYP) enzyme inhibitor,
99   sponsors should consider a stepwise, model-based evaluation of metabolism-based
100   interactions (from basic model for initial assessment to more mechanistic models including
101   physiologically-based pharmacokinetic (PBPK) modeling) (see section IV.A.1). The criteria
102   used for assessing "equivalence" (e.g., predicted AUC ratio of 0.8-1.25 using population-
103   based PBPK models) may be used as an initial cutoff in deciding whether in vivo studies are
104   needed. The criteria discussed in this guidance document are suggested values. We are open
105   to discussion based on sponsors' interpretation.
106

107   -   PBPK is a useful tool that can help sponsors (1) better design drug-drug interaction
108   studies, including dedicated trials and population pharmacokinetic studies, and (2)
109   quantitatively predict the magnitude of drug-drug interactions in various clinical
110   situations. PBPK models also may offer useful alternatives to dedicated clinical
111   studies.
112

113   -   When submitting PBPK studies to CDER, sponsors should provide details of model
114   assumptions, physiological and biological plausibility, the origin of the parameters,
115   and information on uncertainty and variability.
116

117   •   The evaluation of CYP enzyme induction should begin with studies of CYP1A2, CYP2B6,
118   and CYP3A in vitro (Figure 4). If the in vitro induction results are positive according to
119   predefined thresholds using basic models, the investigational drug is considered an enzyme
120   inducer and further in vivo evaluation may be warranted. Alternatively, a sponsor can
121   estimate the degree of drug-drug interactions using mechanistic models to determine the need
122   for further in vivo evaluation (see section IV.A.1.b-3).
123

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

128
129   • In addition to CYPs, other metabolizing enzymes (e.g., uridine diphosphate (UDP)-
130   glucuronosyl transferases (UGTs)) that may be important for the drugs under evaluation
131   should also be considered (see section IV.A.1).
132
133   • A number of transporter-based interactions have been documented in recent years (see Table
134   1, section III.B.2).
135
136   - All investigational drugs should be evaluated in vitro to determine whether they are a
137   potential substrate of P-glycoprotein (P-gp) or Breast Cancer Resistance Protein
138   (BCRP) (see Figure 6, left panel, section IV.A.2). Investigational drugs should be
139   evaluated in vitro to determine whether they are a substrate of hepatic uptake
140   transporters Organic Anion Transporting Polypeptide 1B1(OATP1B1) or OATP1B3
141   when their hepatic pathway is significant (see Figure 6, middle panel, section
142   IV.A.2). Similarly, investigational drugs should be evaluated in vitro to determine
143   whether they are a substrate of Organic Aniton Transporter 1 (OAT1) or OAT3 or
144   Organic Cation Transporter 2 (OCT2) when renal active secretion is important
145   (Figure 6, right panel, section IV.A.2).
146
147   - Because there have been clinically significant interactions demonstrated for critical
148   drugs that are known substrates for P-gp (e.g., digoxin), BCRP (e.g., rosuvastatin),
149   OATP1B1/OATP1B3 (e.g., statin drugs), OAT1/OAT3 (e.g., methotrexate, tenofovir)
150   and OCT2 (e.g., metformin), evaluation of investigational drugs as inhibitors for
151   these transporters should be conducted (see section IV.A.2).
152
153   - The need for further in vivo drug interaction studies based on in vitro evaluation will
154   be based on the criteria described in the decision trees in Figures A1-A6 in the
155   Appendix.
156
157   • Because of the lack of a validated in vitro system to study transporter induction, the
158   definitive determination of induction potential of an investigational drug on transporters is
159   based on in vivo induction studies. The sponsor should consult with CDER about studying
160   induction of transporters in vivo.
161
162   • Human clinical studies to assess drug-drug interactions may include simultaneous
163   administration of a mixture of substrates of multiple CYP enzymes and transporters in one
164   study (i.e., a "cocktail approach") to evaluate a drug's inhibition or induction potential (see
165   section V.C.3). Negative results from a well-conducted cocktail study may eliminate the
166   need for further evaluation of particular CYP enzymes and transporters. However, positive
167   results may indicate that further in vivo evaluation should be conducted.
168
169   • The potential for interactions with drug products should be considered for certain classes of
170   therapeutic proteins (TPs) (see Figure 7, section IV.B.2).
171

172   -   If an investigational TP is a cytokine or cytokine modulator, studies should be
173   conducted to determine the TP's effects on CYP enzymes or transporters. The in
174   vivo evaluations of TPs in targeted patient populations can be conducted with
175   individual substrates for specific CYP enzymes and transporters, or studies can be
176   conducted using a "cocktail approach" (see section V.C.3).
177
178   -   For TPs that will be used in combination with other drug products (small molecule or
179   TP) as a combination therapy, studies should evaluate the effect of each product on
180   the other. This evaluation is particularly important when the drug used in
181   combination has a narrow therapeutic range.
182
183   -   When there are known mechanisms or prior experience with certain PK or PD
184   interactions for other similar TPs, appropriate in vitro or in vivo assessments for
185   possible interactions should be conducted.
186
187   • Refer to section V for information regarding in vivo drug interaction study design. The
188   section also contains tables on classification of in vivo inhibitors (Table 3) or inducers for
189   CYP enzymes (Table 4), examples of sensitive in vivo CYP substrates and CYP substrates
190   with narrow therapeutic ranges (Table 5), examples of in vivo inhibitors and inducers of
191   selected transporters (Table 6), examples of in vivo substrates of selected transporters (Table
192   7) and examples of in vivo CYP3A and P-gp inhibitors and their relative potency (Table 8).
193
194   -   Simulations (e.g., by population-based PBPK models) can provide valuable insight
195   into optimizing the study design (see section IV.A.1).
196
197   -   Detailed information on the dose given and time of administration should be
198   documented for the co-administered drugs. When relevant for the specific drug, the
199   time of food consumption should be documented.
200
201   -   Population pharmacokinetic (PopPK) analyses of data obtained from large-scale
202   clinical studies that include sparse or intensive blood sampling can help characterize
203   the clinical impact of known or newly identified interactions and determine
204   recommendations for dosage modifications for the investigational drug as a substrate
205   (section V.B). DDI analyses using a population PK approach should focus on
206   excluding a specific clinically meaningful PK change. Because exposure of co-
207   administered drugs is not monitored in most PopPK studies, the PopPK approach may
208   not be useful to assess the effect of the investigational drugs on other drugs.
209
210   • The likelihood of drug interactions in specific populations (e.g., patients with organ
211   impairment, and pediatric and geriatric patients) should be considered on a case-by-case
212   basis. PBPK modeling (if well verified for intended purposes) can be helpful to guide the
213   determination of the need to conduct population-specific studies (see "Populations" in
214   section V.B and "Complex Drug Interactions" section V.C.4).
215

216   •   Additional study design issues are discussed throughout the guidance (e.g., route of
217   administration (section V.D), dose selection (section V.E), defining endpoints (section V.F),
218   and statistical considerations (section V.G)).
219
220   •   Labeling recommendations with regard to drug interactions are described in section VI.
221
222   -   A forest plot is considered a useful tool for presenting changes in pharmacokinetic
223   exposure measures by various intrinsic and extrinsic factors including drug
224   interactions in the PHARMACOKINETIC subsection of the labeling (see Figure 8,
225   section VI).
226
227   -   If the sponsor wishes to include a statement in the labeling that no known drug-drug
228   interaction of clinical significance exists, the sponsor should recommend specific *no*
229   *effect* boundaries, or clinical equivalence intervals, for a drug-drug interaction and
230   should provide the scientific justification for the recommendations. No effect
231   boundaries represent the interval within which a change in a systemic exposure
232   measure is considered not clinically meaningful. These conclusions can be based on
233   exposure-response or dose-response data.
234
235   •   Sponsors are encouraged to communicate with the Office of Clinical Pharmacology or the
236   appropriate clinical review divisions within CDER regarding questions about drug
237   interactions, in particular when
238
239   -   Using mechanistic or PBPK models for the prediction of drug-drug interactions
240   including evaluation of complex drug-drug interactions
241   -   Determining the need to evaluate drug interactions with non-CYP enzymes or
242   additional transporters that are not included in the decision trees
243   -   Determining drug-drug interaction studies involving TPs.
244
245
246
247 **III.   BACKGROUND**
248
249 **A.   Relevance of Drug Interactions**
250
251 The desirable and undesirable effects of a drug are related to its concentration at various sites of
252 action, which is usually related to the blood or tissue concentration of the drug. The blood or
253 tissue concentrations resulting from a dose are determined by the drug's absorption, distribution,
254 metabolism, and excretion (ADME). Elimination of a drug or its active metabolites occurs either
255 by metabolism to an inactive metabolite that is excreted, or by direct excretion of the drug or
256 active metabolites. The kidneys and liver are responsible for most drug excretion. Drug
257 interactions related to metabolism and excretion are well-recognized, but effects related to
258 transporters are being documented with increasing frequency and are, therefore, important to
259 consider in drug development. Therapeutic proteins may be eliminated through a specific

260 interaction with cell surface receptors, followed by internalization and lysosomal degradation
261 within the target cell.
262
263 The overall objective of interaction studies for a new drug is to determine:
264
265 • whether any interactions are sufficiently large to necessitate a dosage adjustment of the
266 drug itself or of the drugs with which it might be used,
267 • whether any interactions calls for additional therapeutic monitoring, or
268 • whether there should be a contraindication to concomitant use when lesser measures
269 cannot mitigate risk.
270
271 In some instances, understanding how to adjust a dose or dosage regimen in the presence of an
272 interacting drug, or how to avoid drug-drug interactions, may allow marketing of a drug that
273 would otherwise have an unacceptable level of risk. In a few cases, consequences of an
274 interaction have led to the conclusion that the drug could not be marketed safely. In almost all of
275 these cases, that conclusion was strengthened by the availability of alternative drugs with lower
276 risks for interactions. Several drugs have been withdrawn from the market because of significant
277 drug interactions that led to QT prolongation and Torsades de Pointes (TdP) arrhythmias, after
278 warnings in drug labels did not adequately manage the risk of drug interactions. For example,
279 terfenadine and astemizole, two early nonsedating antihistamines metabolized by CYP3A, were
280 withdrawn after labeling failed to reduce cases of TdP sufficiently, because fexofenadine and
281 loratadine fulfilled the need for nonsedating antihistamines that had no risk of TdP. Cisapride, a
282 CYP3A metabolized drug, was withdrawn because its gastrointestinal benefits were not felt to
283 outweigh its TdP risk. A fourth drug, mibefradil (a calcium channel blocker similar to verapamil
284 and diltiazem) was a strong CYP3A inhibitor and, when used with simvastatin, caused
285 rhabdomyolysis because of markedly increased simvastatin exposure.
286
287
288 **B.** **Drug Interaction Considerations for Small Molecule Drugs**
289
290 The main focus of this guidance is pharmacokinetic drug interactions. The drug development
291 process should include evaluation of a new drug's potential to affect the metabolism or transport
292 of other drugs and the potential for the new drug's metabolism or transport to be affected by
293 other drugs. Use of in vitro tools to determine whether a drug is a substrate, inhibitor, or inducer
294 of metabolizing enzymes, followed by in vivo interaction studies to assess potential interactions,
295 has become an integral part of drug development and regulatory review. In addition to the
296 evaluation of metabolic drug interactions, the role of transporters in drug interactions should be
297 evaluated. This section will separately discuss drug-drug interactions at the levels of
298 metabolizing enzymes and transporters, and also consider situations when multiple drug-drug
299 interaction mechanisms are present.
300
301 *1.* *Metabolism-Based Drug-Drug Interactions*
302

303	Hepatic metabolism occurs primarily through the cytochrome P450 family (CYP) of
304	enzymes located in the hepatic endoplasmic reticulum, but may also occur through non-
305	CYP enzyme systems, such as glucuronosyl- and sulfo-transferases, which can, in
306	general, inactivate a drug and increase its renal elimination. Some drug metabolizing
307	enzymes are present in the gut wall and other extrahepatic tissues, in addition to the liver.
308	
309	Many metabolic routes of elimination can be inhibited or induced by concomitant drug
310	treatment. Metabolic drug-drug interactions can cause substantial changes — an order of
311	magnitude or more decrease or increase in the blood and tissue concentrations of a drug
312	or metabolite — and can affect the extent to which toxic or active metabolites are
313	formed. These large changes in exposure can alter the safety and efficacy profile of a
314	drug and its active metabolites, regardless of whether the drug has a narrow therapeutic
315	range (NTR). For example, certain HMG-CoA reductase inhibitors (e.g., lovastatin,
316	simvastatin) that are extensively metabolized by CYP3A can have a 10-fold or more
317	increase in blood levels when their metabolism is inhibited by co-administration with
318	strong CYP3A inhibitors such as mibefradil or ketoconazole, or even moderate inhibitors
319	such as erythromycin. Although the HMG-CoA reductase inhibitors are not NTR drugs,
320	the blood level increases caused by interactions between HMG-CoA reductase inhibitors
321	and CYP3A inhibitors can cause myopathy and in some cases rare and life-threatening
322	rhabdomyolysis.
323	
324	In addition to evaluating a drug as a substrate of an enzyme that another drug may inhibit
325	or induce, it is important to determine whether an investigational drug significantly
326	affects the metabolic elimination of drugs already in the marketplace. Metabolic drug-
327	drug interactions should be explored for investigational drugs that are not eliminated
328	significantly by metabolism because such drugs can inhibit or induce a co-administered
329	drug's metabolism pathway.
330	
331	Drug-drug interactions can differ among individuals based on genetic variation of a
332	polymorphic enzyme. For example, a strong CYP2D6 inhibitor (e.g., fluoxetine) will
333	increase the plasma levels of a CYP2D6 substrate (e.g., atomoxetine) in subjects who are
334	extensive metabolizers (EM) of CYP2D6, but will have minimal effect in subjects who
335	are poor metabolizers (PM) of CYP2D6, because these individuals have no active
336	enzyme to inhibit. It is noted that CYP2D6 PMs will already have greatly increased
337	levels of atomoxetine if given usual doses. There are also situations where inhibition
338	may have a greater effect in PMs than EMs. If a drug is metabolized by a minor pathway
339	(nonpolymorphic enzyme) and a major pathway (polymorphic enzyme), inhibition of the
340	minor pathway will usually have minimal effect on plasma concentrations in EMs.
341	However, the minor pathway plays a greater role in clearance of the drug in PMs of the
342	major pathway. Thus, inhibition of the minor pathway in PMs of the major pathway can
343	have a significant effect on drug clearance and resulting drug concentrations. Therefore
344	studying the effect of interactions may be recommended in subjects with varied
345	genotypes or phenotypes.
346	
347	*2. Transporter-Based Drug-Drug Interactions*

348
349   Although less well-recognized than metabolizing enzymes, membrane transporters can
350   have important effects on pharmacokinetics and drug exposure. To date, most identified
351   transporters belong to one of two superfamilies: ATP-Binding Cassette (ABC) and
352   Solute Carrier (SLC). Transporters govern the transport of solutes (e.g., drugs and other
353   xenobiotics) in and out of cells. In contrast to metabolizing enzymes, which are largely
354   concentrated in the liver and intestine, transporters are present with varying abundance in
355   all tissues in the body and play important roles in drug distribution, tissue-specific drug
356   targeting, drug absorption, and elimination. For example, recent research indicates an
357   important role of transporters in the absorption, distribution, and excretion of drugs (see
358   Figure 1 below and Table 1). Transporters can also work in concert with metabolizing
359   enzymes and play a role in drug metabolism.
360
361
362   **Figure 1. Illustration of Examples of Efflux and Uptake Transporters in the Gut**
363   **Wall (A), Liver (B), and Kidneys (C) that May Be Involved in a Drug's Absorption,**
364   **Distribution, Metabolism, and Excretion.**
365
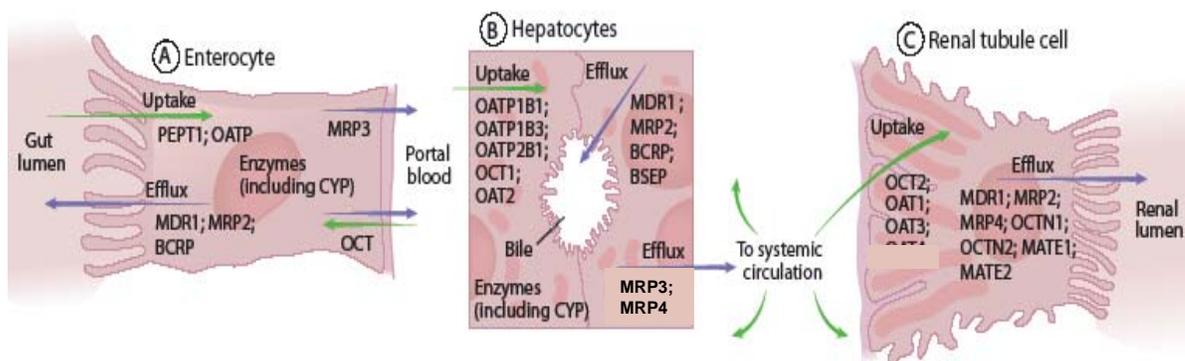
366
367   Abbreviations: MRP: multidrug resistance associated protein; PEPT1, peptide transporter 1; OATP:
368   organic anion transporting polypeptide; OAT: organic anion transporter; OCT: organic cation transporter;
369   BCRP: breast cancer resistance protein; MDR1: multidrug resistance 1(P-glycoprotein (P-gp)); MATE:
370   multidrug and toxic compound extrusion protein (Adapted from Huang S-M, Lesko LJ, and Temple R,
371   "Adverse Drug Reactions and Pharmacokinetic Drug Interactions," Chapter 20, Adverse Drug Reactions
372   and Drug Interactions in Part I (Section 4), *Pharmacology and Therapeutics: Principles to Practice*,
373   Waldman SA and Terzic A, Eds., Elsevier, 2009).
374
375   A number of transporter-based interactions have been documented in recent years.
376   Analogous to drug interactions mediated by P450 enzymes, co-administration of a drug
377   that is an inhibitor or an inducer of a drug transporter may affect the pharmacokinetics of
378   a drug that is a substrate for that transporter. It has been shown that various drugs (e.g.,
379   quinidine, verapamil, itraconazole) increase plasma levels of digoxin by inhibiting the
380   efflux transporter, P-gp, at the intestinal level. Plasma levels of many HMG-CoA
381   reductase inhibitors, including rosuvastatin, pravastatin, and pitavastatin, are increased by

382   co-administration of inhibitors of hepatic uptake transporters (e.g., OATP1B1), such as
383   cyclosporine and single dose rifampin. For example, co-administration of cyclosporine
384   increases the area under the plasma concentration-time curve (AUC) of pravastatin,
385   rosuvastatin, and pitavastatin by 10-fold, 7-fold, and 5-fold, respectively. This effect and
386   a number of other transporter interactions are shown in Table 1 below. Because these
387   statins are not significantly metabolized, the interactions appear to result from inhibition
388   of transporters, including OATP1B1. Table 1 also shows a substantial effect of
389   lopinavir/ritonavir on bosentan, which is potentially important because of bosentan's
390   dose-related hepatotoxicity. Probenecid increases plasma concentrations of cidofovir,
391   furosemide, and acyclovir because it inhibits their active renal tubular secretion by the
392   transporters OAT1 and OAT3. Table 1 lists additional clinically relevant transporter-
393   based drug-drug interactions.
394
395   Transporters can affect the safety profile of a drug by affecting the concentration of a
396   drug or its metabolites in various tissues. An example of transporter-mediated effects on
397   drug toxicity involves the drug cidofovir. This antiviral drug causes nephrotoxicity;
398   however, when administered with probenecid, an inhibitor of organic anion transport in
399   the kidney, the uptake of cidofovir into the renal tubular cell is blocked and
400   nephrotoxicity is reduced. Another example involves simvastatin, polymorphism of
401   OATP1B1 was found to correlate with the prevalence of myopathy in patients receiving
402   simvastatin. Transporter-based drug interactions and the potential effect of drug
403   transporters on safety make it important to determine whether transporters affect the
404   absorption and disposition of an investigational drug and whether the investigational drug
405   can affect the absorption and disposition of other drugs through an effect on transporters.
406
407   *3.*   *Multiple Drug-Drug Interaction Mechanisms*
408
409   The above sections separately discuss drug-drug interactions related to effects on
410   enzymes and transporters, but drug interactions for a specific drug may occur based on a
411   combination of mechanisms. Such "complex drug interaction" scenarios include, but are
412   not limited to:
413
414   • Concurrent inhibition and induction of one enzyme or concurrent inhibition of
415   enzyme and transporter by a drug
416   • Increased inhibition of drug elimination by the use of more than one inhibitor of the
417   same enzyme that metabolizes the drug
418   • Increased inhibition of drug elimination by use of inhibitors of more than one enzyme
419   that metabolizes the drug
420   • Inhibition by a drug and its metabolite or metabolites, both of which inhibit the
421   enzyme that metabolizes the substrate drug
422   • Inhibition of an enzyme other than the genetic polymorphic enzyme in poor
423   metabolizers taking substrate that is metabolized by both enzymes
424   • Use of enzyme/transporter inhibitors in subjects with varying degrees of impairment
425   of drug eliminating organs (e.g., liver or kidney)

426
427   When there are multiple factors that affect clearance and multiple mechanisms of drug–
428   drug interactions, the prediction of in vivo interactions from results of in vitro assessment
429   is challenging. Modeling and simulations accounting for multiple mechanisms can be
430   helpful in the design of clinical studies to inform the potential for drug interaction or
431   prediction of the extent of interactions (see section V.C.4).
432

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

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

434

435
436 [a] Abbreviations: BCRP, breast cancer resistance protein; P-gp, p-glycoprotein; MDR, multidrug resistance: LST, liver-specific transporters; OATP, organic anion transporting
437 polypeptide; OCT, organic cation transporter; OAT, organic anion transporter
438 [b] Implicated transporter refers to the likely transporter; however, because the studies are in vivo, it is not possible to assign definitively specific transporters to these interactions.
439 [c] Minimum predose plasma level (C_{trough}) data from Day 4 (48-fold), Day 10 (5-fold) after co-administration.
440 [d] Interaction could be partly mediated by OATP1B1.
441 [e] Interaction could be partly mediated by BCRP.
442 [f] Interaction could be partly mediated by OAT1.
443

444 **C.   Drug Interaction Considerations for Therapeutic Proteins**
445
446   Therapeutic proteins (TPs) typically do not undergo metabolism or transport as their
447   clearance pathway, therefore the potential is limited for small molecule drugs (termed
448   "drug" in this document) to affect TPs through metabolism or transport pathways.
449   However, a drug may affect the clearance of TPs through the drug's effect on
450   immunogenicity (e.g., methotrexate reduces the clearance of infliximab, possibly due to
451   methotrexate's effect on the antibodies formed against infliximab). In addition, TPs that
452   are cytokines or cytokine modulators may modify the metabolism of drugs that are
453   substrates for P450 enzymes through their effects on the regulation pathways of P450
454   enzymes. For example, cytokines such as IL-6 can produce concentration-dependent
455   inhibition on various CYP isoforms at the transcription level or by alteration of CYP
456   enzyme stability in patients with infection or inflammation, and increase the plasma
457   concentrations of specific CYP substrate drugs. In contrast, cytokine modulators such as
458   tocilizumab (anti-IL-6 receptor antibody) may reverse the apparent "inhibition" effect of
459   the cytokines on CYP substrates, resulting in a "normalization" of CYP activities.
460
461   General points to be considered for evaluation of TP-drug interactions are discussed in
462   section IV.B.2.
463
464
465   **IV.   GENERAL STRATEGIES**
466
467   Development of a drug should include identification of the principal routes of
468   elimination, quantitation of the contribution by enzymes and transporters to drug
469   disposition, and characterization of the mechanism of drug-drug interactions. The
470   quantitative assessment of drug-drug interaction potential for an investigational drug
471   employs a variety of models including basic models, mechanistic static models, and more
472   comprehensive dynamic models (e.g., physiologically-based pharmacokinetic (PBPK)
473   models). Appropriately designed pharmacokinetic studies, usually performed in the early
474   phases of drug development, can provide important information about metabolic and
475   excretory routes of elimination, their contribution to overall elimination, and metabolic or
476   transporter-mediated drug-drug interactions. Together with information from in vitro
477   studies, these in vivo investigations can be used for PBPK model construction and
478   refinement. Quantitative assessment of the findings from these studies helps address key
479   regulatory questions regarding whether, when, and how to conduct further clinical drug-
480   drug interaction studies. In many cases, negative findings from early in vitro and clinical
481   studies eliminate the need for later clinical investigations of drug-drug interaction
482   potential. If potential drug-drug interactions are identified based on in vitro and/or in
483   vivo studies, sponsors should design further studies or collect information to determine
484   (1) whether additional studies are needed to better quantify the effect and to examine the
485   effects of weaker inhibitors (early studies usually examine strong inhibitors) on the
486   investigational drugs as substrates and effects of investigational drugs (as inhibitors) on a
487   range of substrates, and (2) whether dosage adjustments or other prescribing

488 modifications (e.g., additional safety monitoring or contraindications) are needed based
489 on the identified interaction(s) to avoid undesired consequences. Further
490 recommendations about the types of in vivo studies that should be conducted in certain
491 circumstances appear in section V of this guidance.
492
493 Drug interaction information is used along with information about exposure-response
494 relationships in the general population and specific populations, to help predict the
495 clinical consequences of drug-drug interactions.
496
497 **A.   In Vitro Studies**
498
499 Findings from in vitro metabolism, transport, and drug interaction studies are valuable in
500 quantitatively assessing the drug-drug interaction potential of an investigational drug.
501 Along with clinical pharmacokinetic data, results from in vitro studies can serve as a
502 screening mechanism to rule out the need for additional in vivo studies, or provide a
503 mechanistic basis for proper design of clinical studies using a modeling and simulation
504 approach. Considerations critical for conducting in vitro studies include, but are not
505 limited to, appropriately validated experimental methods, choice of test systems, and
506 rational selection of substrate/interacting drug and their concentrations.
507
508 *1.   In Vitro Metabolism Studies*
509
510 Figure 2 below shows a decision tree that describes when in vivo metabolism-
511 based interaction studies are indicated, based on in vitro metabolism, in vitro
512 drug-drug interaction, and/or other appropriate pharmacokinetic data.

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

514

Conduct In Vitro Metabolism and Drug-Drug Interaction Studies in Human Tissues[a]
- Phase I enzymes: CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A, others
- Phase II enzymes: UGTs (see Figure 3)

Is investigational drug a substrate of an enzyme responsible for ≥25% of its systemic clearance[b]?

Is investigational drug an interacting drug of an enzyme? (see Figure 4) [c]

No

Yes or inconclusive

Yes

No

Is investigational drug a substrate of multiple metabolizing enzymes together responsible for ≥25% of its systemic clearance[b]?

Conduct in vivo studies with strong inhibitor(s)/inducer(s) or if appropriate, compare PK in different genotypes

Conduct in vivo studies with most sensitive/specific substrate(s)

Label as such based on in vitro data

No

Yes

Label as such based on in vitro and in vivo disposition data[e]

Evaluate potential of complex drug-drug interaction[d]

Presence of significant interaction?

Presence of significant interaction?

Yes

No

Yes

No

Conduct in vivo studies with other less strong inhibitors/inducers selected based on likely co-administration or if appropriate, apply mechanistic modeling (see Figure 4)[e]

No further studies needed → General label based on in vitro and in vivo data[e]

Conduct in vivo studies with other substrates selected based on likely co-administration and/or narrow therapeutic range or if appropriate, apply mechanistic modeling (see Figure 4)

No further studies needed → General label based on in vitro and in vivo data

Dosage adjustment needed?

Dosage adjustment needed?

Yes   **No**

Yes   **No**

515
516

16

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

518   [b] Results from in vitro enzyme phenotyping experiments, human pharmacokinetic studies such as an intravenous study, a mass-balance study, and

519   pharmacokinetic studies in which renal/biliary clearances are determined can be evaluated together to determine the percent contribution of enzyme to

520   overall in vivo drug elimination in humans.

521   [c] See Figure 4 for calculation of R values and cutoff values. Sponsor may conduct an in vivo cocktail study in humans (Reference: *Clinical Pharmacology*

522   *and Therapeutics*, 81: 298-304, 2007). See section V.C.3.

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

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

525
526   *a.   Drug Metabolizing Enzyme Identification — the Investigational Drug as*
527   *an Enzyme Substrate*
528
529   The metabolic profile of the investigational drug should be characterized from in
530   vitro studies. The in vitro systems include human liver tissues such as liver
531   microsomes, microsomes expressing recombinant enzymes, or freshly isolated or
532   cryopreserved human hepatocytes. Generally, decisions on the need for in vivo
533   drug interaction studies with enzyme inhibitors/inducers are based on the
534   quantitative measurement of the contribution of the enzyme to the overall
535   systemic clearance of the substrate. We consider metabolism to be a significant
536   pathway when it constitutes 25% or more of the drug's overall elimination. When
537   the contribution is ≥25% or unknown, in vivo studies using appropriate
538   inhibitor(s)/inducer(s) are warranted. The sequence of these in vivo studies
539   should start with a strong inhibitor/inducer. If the results from the study with
540   strong inhibitors/inducers indicate positive interactions, the impact of a less
541   strong inhibitor/inducer should be evaluated. The subsequent evaluations with a
542   less strong inhibitor/inducer can be conducted through a clinical study.
543   Alternatively, it may be possible to conduct the evaluation through PBPK
544   modeling (see section IV.A.1.b-3 below related to model building and strategies
545   to evaluate drug-drug interaction using PBPK). The choice of in vivo enzyme
546   inhibitors/inducers can be found in section V.
547
548   Minor elimination pathways mediated by a drug metabolizing enzyme may
549   require further investigation under certain conditions. The contribution of these
550   enzymes may become significant in specific populations (e.g., in subjects with
551   renal impairment when the substrate drug is significantly eliminated through renal
552   excretion, in poor metabolizers when substrate drug is predominantly metabolized
553   by the polymorphic enzymes, or in subjects taking a strong inducer of the enzyme
554   of minor pathway). The likelihood of metabolism-based drug interactions in
555   these populations should be considered on a case-by-case basis (also see
556   "Populations" in section V.B and "Complex Drug Interactions" section V.C.4).
557

558
559 *Phase I Metabolizing Enzymes*
560
561 Routine assessment to identify the following CYP enzymes for potential
562 metabolism-mediated interactions is recommended: CYP1A2, CYP2B6,
563 CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. If an investigational drug
564 is a substrate in vitro for a particular CYP, in vivo interaction studies with a
565 strong inhibitor and inducer for that CYP (refer to the later sections about
566 classification of CYP inhibitors and inducers) are recommended to determine the
567 extent of changes in the investigational drug's pharmacokinetics. Negative
568 results alleviate the need for further in vivo studies with less strong inhibitors or
569 inducers, if the study is well designed and appropriate.
570
571 If a drug is not metabolized by the major CYPs (listed above), the drug's
572 likelihood of being a substrate for other CYP enzymes (e.g., CYP2A6, CYP2J2,
573 CYP4F2, CYP2E1) or non-CYP Phase I enzymes should be considered. Non-
574 CYP Phase I enzymes (enzymes involved in oxidation, reduction, hydrolysis,
575 cyclization, and decyclization reactions) that are involved in drug metabolism
576 include monoamine oxidase (MAO), flavin monooxygenase (FMO), xanthine
577 oxidase (XO), and alcohol/aldehyde dehydrogenase. The potential for an
578 investigational drug to be a substrate for these enzymes can be studied on a case-
579 by-case basis (e.g., based on prior knowledge of the drug class).
580
581 *Phase II Metabolizing Enzymes*
582
583 Phase II enzymes (enzymes that are involved in conjugation reactions —
584 conjugation involving, for example, glucuronic acid, sulfonates, glutathione, or
585 amino acids) have historically attracted less attention than CYP enzymes in drug
586 interaction evaluations, most likely because of the lack of tools to study them and
587 a lower incidence of observed adverse drug-drug interactions.
588
589 Recently, there has been an increased interest in drug-drug interactions involving
590 UGTs (UDP glucuronosyl transferases), enzymes responsible for the
591 biotransformation of many drugs. For example, UGT1A1, 1A3, 1A4, 1A6, 1A9,
592 2B7, and 2B15 have been shown to play important roles in drug metabolism.
593 However, determination of the contribution of each UGT isoform to the overall
594 elimination is not as straightforward as that for CYP enzymes because of the
595 absence of data on the abundance of these isoforms in drug eliminating organs,
596 and the lack of specific inhibitors. For example, atazanavir has been used as a
597 UGT1A1 inhibitor; however, it also inhibits CYP3A. Therefore, an investigation
598 of a UGT-based drug-drug interaction may follow the decision tree outlined in
599 Figure 3. If glucuronidation is a predominant pathway of drug elimination, in
600 vitro studies (see Figure 3 below) to determine whether the drug is a substrate of
601 UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, or 2B15 are recommended. These in vitro

602   studies can be conducted using recombinant human UGTs, many of which are
603   available from commercial sources. Results from these studies inform future in
604   vivo drug interaction studies. In certain cases, comparative PK data in subjects
605   with various UGT genotypes may be used to identify important UGT pathways
606   and estimate the possible extent of interactions (e.g., comparison of PK in
607   subjects who are poor metabolizers versus those who are extensive metabolizers).
608   For example, UGT1A1 polymorphic variants affect the level of SN38, an active
609   metabolite of irinotecan, which has both safety and efficacy implications. The
610   clinical significance of interactions mediated by UGTs depends on the extent of
611   interaction and the therapeutic range of the substrate drug.
612
613   **Figure 3. Evaluation of Investigational Drugs as UGT Substrates**
614
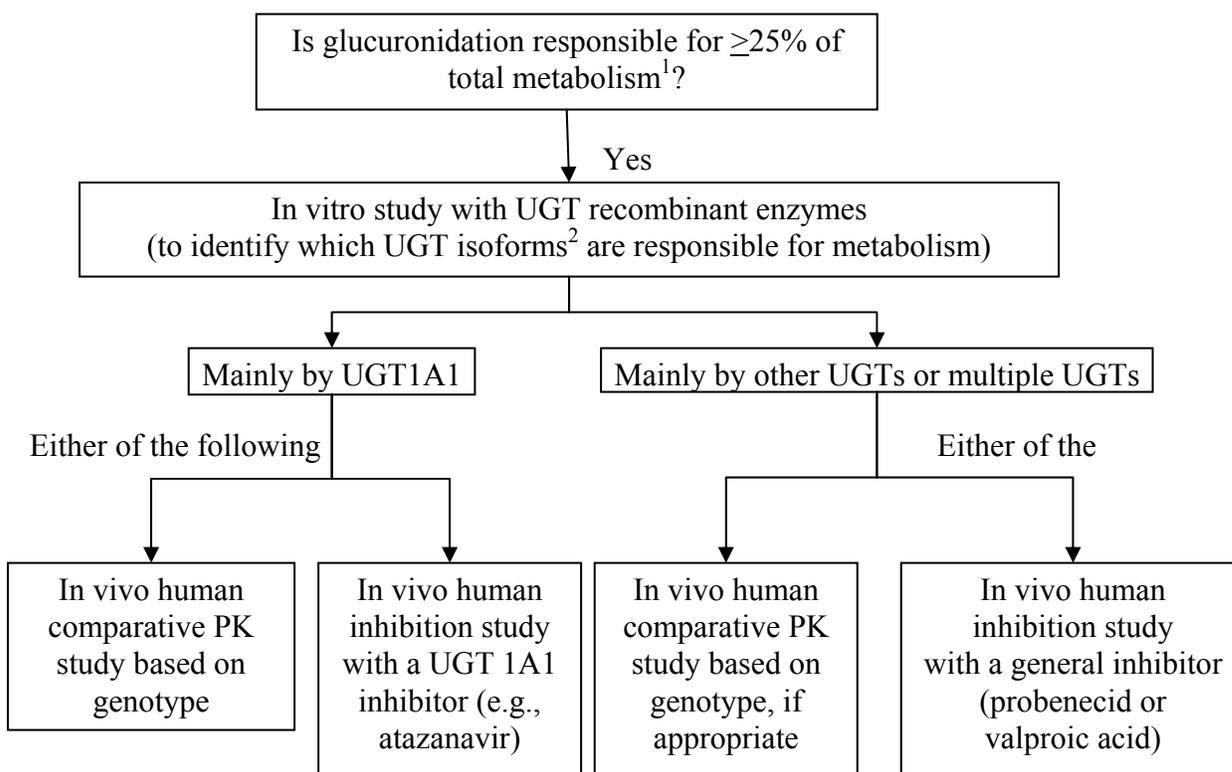
615
616   [1] In an in vitro system capable of informing contribution by UGT and non-UGT enzymes (e.g., hepatocytes
617   or microsomes supplemented with appropriate co-factors).
618   [2] Main UGTs recommended to be studied: UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15.
619
620
621

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

---

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

661 components make the PBPK model readily capable of investigating drug-drug
662 interactions in the presence of multiple intrinsic and/or extrinsic factors (section
663 V.C.4). These features make PBPK a useful option for sponsors to (1) better
664 design drug-drug interaction studies, including dedicated trials and population
665 pharmacokinetic studies, and (2) quantitatively predict the magnitude of drug-
666 drug interactions in various clinical situations, including the existence of multiple
667 patient factors such as renal impairment and/or genetic deficiency in certain
668 metabolizing enzymes. Regardless of which prediction model is used, the
669 sponsors should provide details of model assumptions, physiological and
670 biological plausibility, the origin of the parameters, and information on
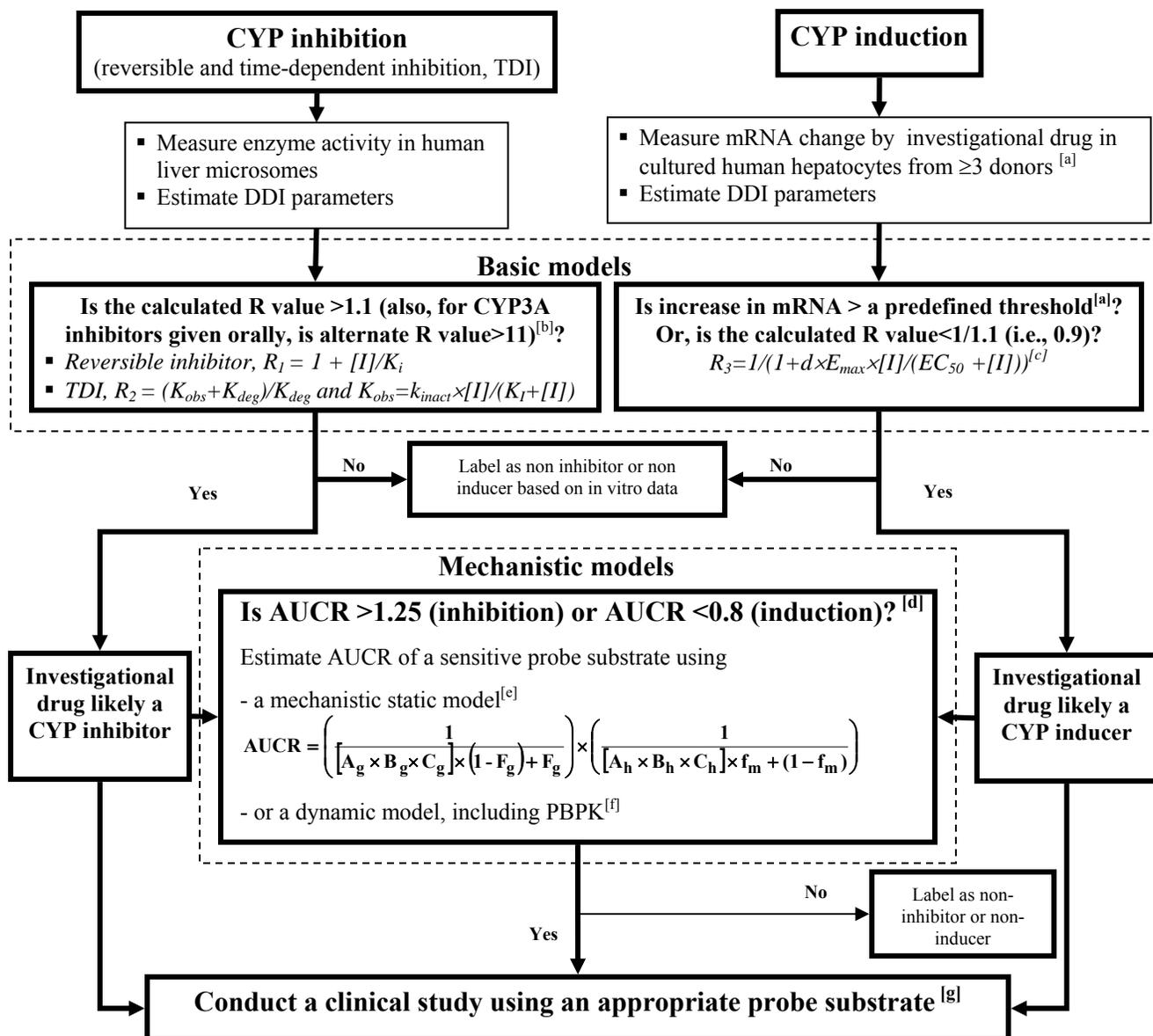671 uncertainty and variability.
672
673 The sections that follow include details on enzyme inhibitor and inducer,
674 respectively.

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

CYP inhibition
(reversible and time-dependent inhibition, TDI)

CYP induction

- Measure enzyme activity in human liver microsomes
- Estimate DDI parameters

- Measure mRNA change by investigational drug in cultured human hepatocytes from ≥3 donors [a]
- Estimate DDI parameters

Basic models

Is the calculated R value >1.1 (also, for CYP3A inhibitors given orally, is alternate R value>11)[b]?
- *Reversible inhibitor, $R_1 = 1 + [I]/K_i$*
- *TDI, $R_2 = (K_{obs}+K_{deg})/K_{deg}$ and $K_{obs}=k_{inact}\times[I]/(K_I+[I])$*

Is increase in mRNA > a predefined threshold[a]? Or, is the calculated R value<1/1.1 (i.e., 0.9)?
$R_3=1/(1+d\times E_{max}\times[I]/(EC_{50} +[I]))^{[c]}$

No → Label as non inhibitor or non inducer based on in vitro data ← **No**

Yes **Yes**

Investigational drug likely a CYP inhibitor

Mechanistic models

Is AUCR >1.25 (inhibition) or AUCR <0.8 (induction)? [d]

Estimate AUCR of a sensitive probe substrate using

- a mechanistic static model[e]

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

- or a dynamic model, including PBPK[f]

Investigational drug likely a CYP inducer

No → Label as non-inhibitor or non-inducer

Yes

Conduct a clinical study using an appropriate probe substrate [g]

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

689 $k_{inact} \times [I]/(K_I + [I])$; and R is the ratio of intrinsic clearance by metabolizing enzyme in the absence and in the presence
690 of inhibitor.

691

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

695

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

701

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

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

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

713

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

723

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

725
726   *b-1.   Investigational drug as an enzyme inhibitor using basic models*
727

728   The potential of an investigational drug to inhibit CYP enzymes is usually investigated in
729   vitro using human liver tissues such as human liver microsomes or cDNA-expressed
730   microsomes to determine the inhibition mechanisms (e.g., reversible or time-dependent
731   inhibition) and inhibition potency (e.g., K_i).
732

733   The R value is dependent on the in vitro inhibition parameters and the maximum inhibitor
734   concentration [I] that can be achieved in vivo with the highest dose. Although several
735   algorithms to calculate [I] have been proposed, selection of [I] should justify maximum
736   exposure of interacting drug at different tissues (Footnote [b] of Figure 4). The use of a
737   cutoff R value of 1.1 where [I] represents maximum total (free and bound) system
738   concentration of the inhibitor is based on an earlier FDA recommendation for reversible
739   inhibition (Huang et al. 2007). Note an orally administered drug may inhibit CYPs that
740   have a high expression in the intestine (e.g., CYP3A). Under such circumstances, I_{gut}
741   (defined as molar dose/250 mL) may represent the maximum inhibitor concentrations
742   better than the systemic concentrations. An alternate R value ($R=1+I_{gut}/K_i$) of 11 should
743   be used as a conservative criteria to avoid false negatives. This basic static model has two
744   major uses. First, it eliminates unnecessary clinical studies when the R value is below the
745   threshold of 11 (for orally administered drugs that may inhibit CYP3A) or 1.1. Second, it
746   allows rank ordering of inhibition potential across different CYP enzymes (Figure 2) for
747   the same drug so that in vivo drug-drug interaction evaluations can be prioritized. For
748   example, an in vivo study with a sensitive substrate of the CYP with the largest R may be
749   carried out first. If the in vivo study shows no interaction, in vivo evaluation of other
750   CYPs with smaller R will not be needed. However, there are exceptions to this approach.
751   For example, if a metabolite present at $\geq$25% of the parent drug AUC inhibits CYP
752   enzymes in vitro, an R value for the metabolite should be calculated based on metabolite
753   exposure and its inhibition potency (e.g., K_i) for the CYPs. The rank order of the
754   metabolite R values should be considered when determining what in vivo studies need to
755   be conducted.
756

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

769   longer lasting after discontinuation of the inhibitor than in a situation when the inhibitory
770   interaction is reversible. For example, the maximum inhibition of CYP3A in humans by
771   erythromycin administered 200 mg three times a day appeared to occur after 4 days of
772   dosing (the AUC values of oral midazolam, a probe substrate of CYP3A, increased 2.3-,
773   3.4-, and 3.4- fold, respectively, on days 2, 4, and 7) (Okudaira et al. 2007). Therefore,
774   TDI should be studied in standard in vitro screening protocols by pre-incubating the drug
775   (a potential inhibitor) before the addition of a substrate. Any time-dependent loss of
776   initial product formation rate may indicate time-dependent inhibition, and definitive in
777   vitro studies to obtain TDI parameters (i.e., k_{inact} and K_I where k_{inact} and K_I are maximal
778   inactivation rate constant and apparent inactivation constant, respectively) are
779   recommended. Details of this tiered approach were proposed by the PhRMA Drug
780   Metabolism Technical Group (Grimm et al. 2009). However, prediction of TDI in vivo
781   from in vitro inactivation parameters remains challenging because of the complexity of the
782   mechanism as compared to reversible inhibition. Generally, TDI is evaluated under the
783   condition when the affected enzyme level reaches a new steady state in the presence of the
784   inhibitor, and the inhibitor does not affect de novo synthesis of the enzyme. In contrast to
785   reversible inhibition, the R value (Figures 4) for time-dependent inhibition is dependent on
786   the rate constant for enzyme degradation, in addition to inhibitor exposure level and the
787   TDI parameters (k_{inact} and K_I). Furthermore, the degradation kinetics for each CYP has
788   not been unambiguously determined (Yang et al. 2008). If in vitro results suggest a TDI
789   potential (e.g., R>1.1), an in vivo study is recommended. Alternatively, the sponsor can
790   estimate the degree of drug-drug interactions using mechanistic models (see Figures 4,
791   and section IV.A.1.b-3)

792
793

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

795

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

804

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

813   (Fahmi, Kish, et al. 2010).
814   • Vehicle control, positive control (usually a known strong inducer), and negative
815   control (usually a known non-inducer) should be included in the experiment.
816   Concentrations of the positive control inducers can be found in Table 2.
817
818   Studies indicate that activation of the nuclear receptor, Pregnane X receptor (PXR), results
819   in co-induction of CYP3A and CYP2C. Thus, a negative in vitro result for CYP3A
820   induction eliminates the need for additional in vitro or in vivo induction studies for
821   CYP3A and CYP2C enzymes. If CYP3A induction results are positive, then induction of
822   CYP2C should be studied either in vitro or in vivo. Because CYP1A2 and CYP2B6 may
823   be induced by different nuclear receptors (e.g., aryl hydrocarbon receptor (AhR), or
824   constitutive androstane receptor (CAR)), they may not be co-induced with CYP3A.
825   Therefore, the potential for induction of CYP1A2 and CYP2B6 should be evaluated
826   regardless of the CYP3A result.
827
828   Initially, CYP1A2, CYP2B6, and CYP3A should be evaluated in vitro (Figure 4). If the
829   in vitro induction results are positive according to predefined thresholds using basic
830   models, the investigational drug is considered an enzyme inducer and therefore further in
831   vivo evaluation may be warranted. Alternatively, a sponsor can estimate the degree of
832   drug-drug interactions using mechanistic models (see Figures 4, and section IV.A.1.b-3)
833   to determine the need for further in vivo evaluation.
834
835   **Table 2**. **In Vitro CYP Inducers**

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

836   *Note that this is not an exhaustive list. For an updated list, see the following link
837   http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling
838   /ucm080499.htm.
839
840

841

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

844

845

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

848

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

870

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

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

878
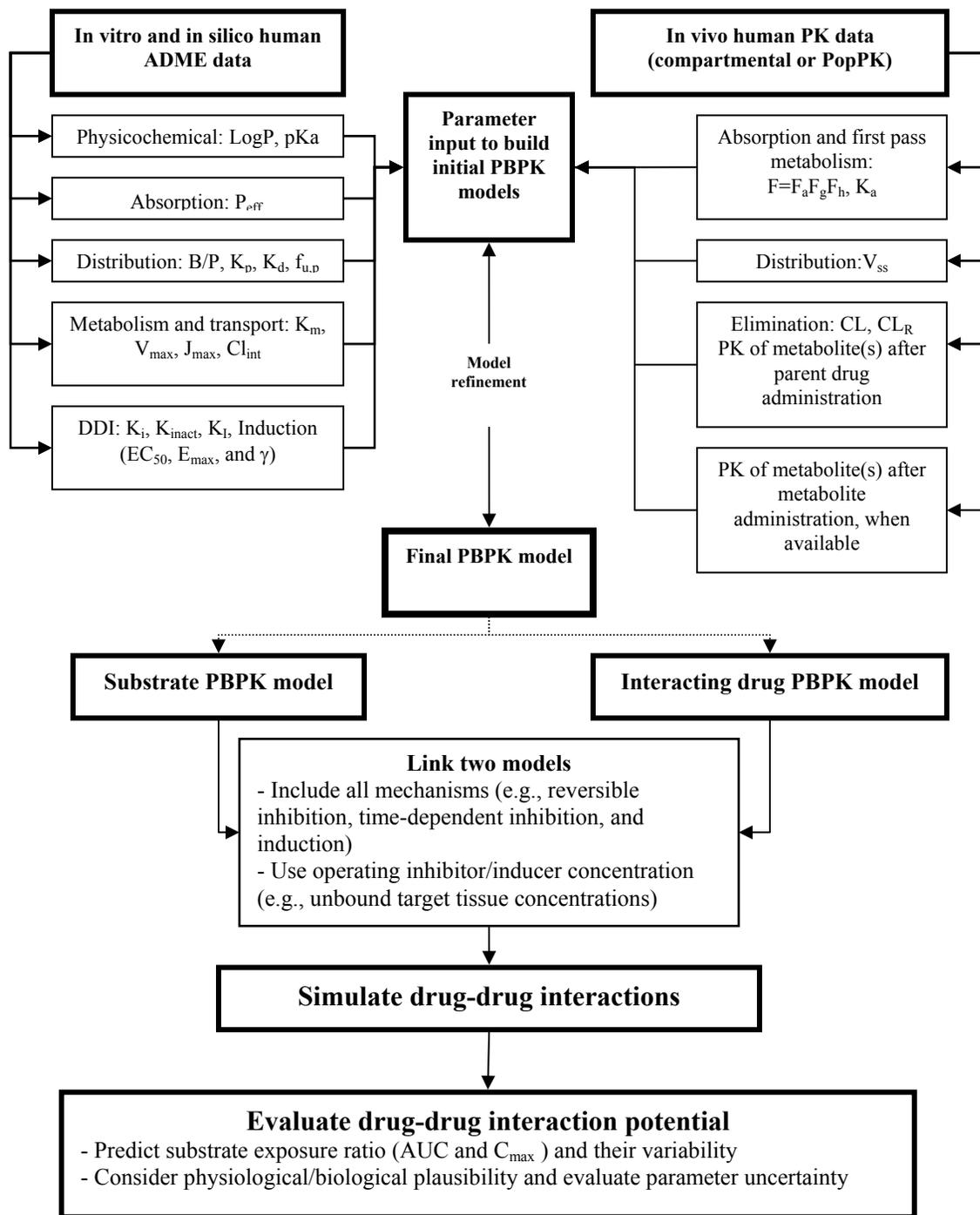
879   Abbreviations: ADME, absorption, distribution, metabolism and excretion; AUC, area under the plasma
880   concentration versus time curve; B/P, blood to plasma ratio; C_{max}, maximum concentration; CL, clearance; CL_r,
881   renal clearance; DDI, drug-drug interactions; EC_{50} or IC_{50}, concentration causing half maximal effect or inhibition;
882   E_{max} or I_{max}, maximum effect or inhibition; F, bioavailability; F_a, fraction absorbed; F_g, bioavailability in the gut; F_h,
883   bioavailability in the liver; $f_{u,p}$, unbound fraction in plasma; γ, Hill coefficient; J_{max}, maximum rate of transporter-
884   mediated efflux/uptake; K_a, first-order absorption rate constant; K_d, dissociation constant of drug-protein complex;
885   K_i, reversible inhibition constant, concentration causing half maximal inhibition; K_I, apparent inactivation constant,
886   concentration causing half maximum inactivation; k_{inact}, apparent maximum inactivation rate constant; K_m,
887   Michaelis-Menten constant, substrate concentration causing half maximal reaction or transport; K_p, tissue-to-plasma
888   partition coefficient; LogP, Logarithm of the octanol-water partition coefficient; P_{eff}, jejunum permeability; PK,
889   pharmacokinetics; PopPK, population pharmacokinetics; V, volume of distribution; V_{max}, maximum rate of
890   metabolite formation.
891
892
893   *2.   In Vitro Transporter Studies*
894
895   *a.   The Investigational Drug as a Substrate for Transporters*
896
897   Both P-gp and BCRP are expressed in the gastrointestinal tract, liver, and kidney, and
898   have a role in limiting oral bioavailability. Therefore, all investigational drugs should be
899   evaluated in vitro to determine whether they are a potential substrate of P-gp or BCRP
900   (See Figure 6, left panel).
901
902   A bidirectional assay in Caco-2 cells or overexpressed cell lines is a preferred method for
903   in vitro evaluation. If the results are positive, an in vivo evaluation in humans is
904   recommended (see Figure A1 in the Appendix for a decision tree on when an in vivo
905   human study is recommended based on the in vitro data).
906
907   For drugs that are highly permeable and highly soluble, the intestinal absorption is not a
908   rate-limiting step, and, therefore, it may be appropriate to exempt such drugs from the in
909   vivo evaluation with a P-gp or BCRP inhibitor. (For further discussion regarding the
910   defining a drug as highly soluble and high permeable (e.g., biopharmaceutical
911   classification class (BCS) 1 drugs), see the Guidance for Industry on *Waiver of In Vivo*
912   *Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage*
913   *Forms Based on a Biopharmaceutics Classification System*,
914   http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm0
915   70246).
916
917   Investigational drugs should be evaluated in vitro to determine whether they are a
918   substrate of hepatic uptake transporters OATP1B1/OATP1B3 when their hepatic
919   pathway is significant (e.g., clearance through hepatic or biliary secretion is more than or
920   equal to 25% of the total clearance)[3] (Figure 6, middle panel). Similarly, investigational
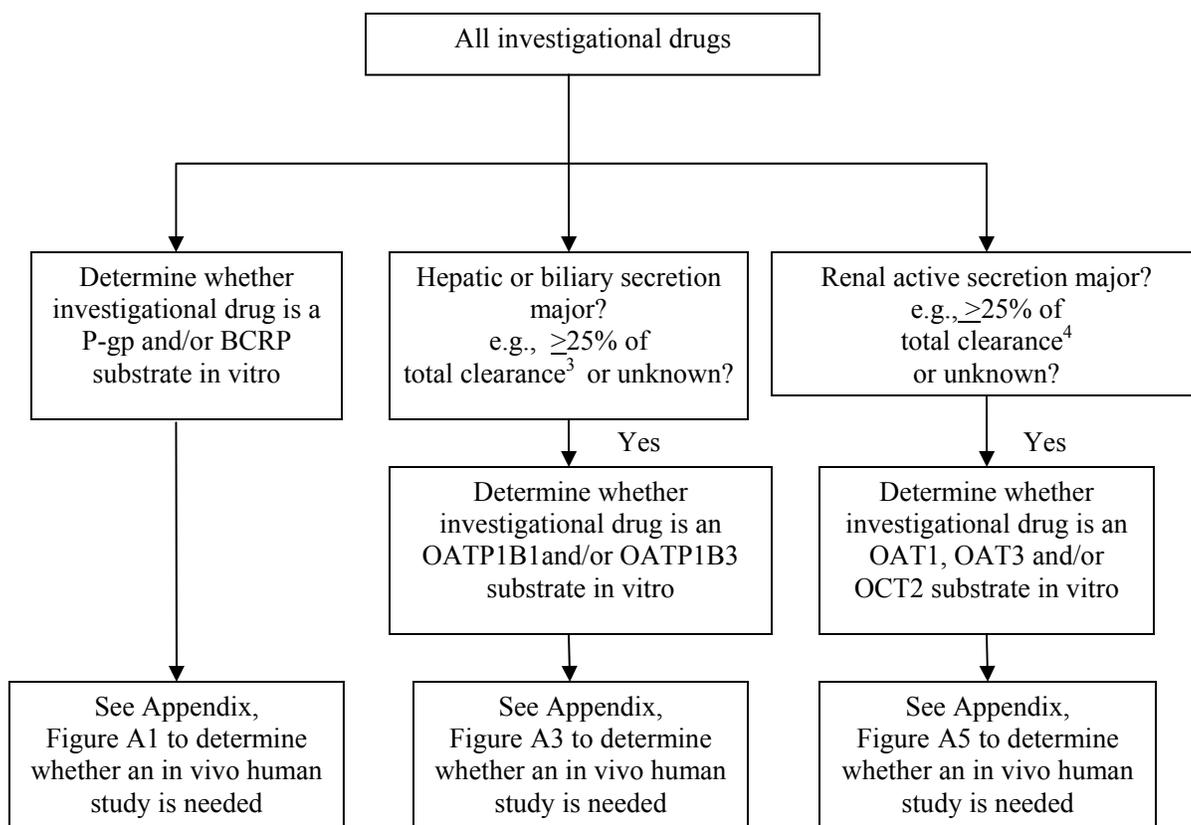921   drugs should be evaluated in vitro to determine whether they are a substrate of OAT1/3

---

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

922   and OCT2 when their renal active secretion is important (active secretion by kidney is
923   more than or equal to 25% of total clearance)[4] (Figure 6, right panel).
924

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

```
                          ┌─────────────────────────┐
                          │  All investigational    │
                          │        drugs            │
                          └─────────────────────────┘
```

Determine whether investigational drug is a P-gp and/or BCRP substrate in vitro	Hepatic or biliary secretion major? e.g., $\geq$25% of total clearance[3] or unknown?	Renal active secretion major? e.g., $\geq$25% of total clearance[4] or unknown?
	Yes	Yes
	Determine whether investigational drug is an OATP1B1and/or OATP1B3 substrate in vitro	Determine whether investigational drug is an OAT1, OAT3 and/or OCT2 substrate in vitro
See Appendix, Figure A1 to determine whether an in vivo human study is needed	See Appendix, Figure A3 to determine whether an in vivo human study is needed	See Appendix, Figure A5 to determine whether an in vivo human study is needed

927
928
929   Other transporters (e.g., MRP (multidrug resistance-associated protein)) may need to be
930   studied based on knowledge of other drugs in the same therapeutic class as the
931   investigational new drug. Information for the other drugs may include observed drug-
932   drug interactions that are attributed to these other transporters. New information in the
933   literature may raise questions about additional transporters.
934

935

936   *b.   The Investigational Drug as an Inhibitor of Transporters*

937

---

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

938   Because many drugs may be used concomitantly with digoxin (a P-gp substrate) and
939   statins (BCRP and OATP1B1/1B3 substrates), evaluation of investigational drugs as
940   inhibitors of P-gp, BCRP, and OATP1B1/OATP1B3 should be considered. An
941   investigational drug also should be evaluated to determine whether it inhibits OCT2,
942   OAT1, and OAT3, because there have been clinically significant interactions
943   demonstrated for critical drugs that are known OCT substrates (e.g., metformin) or OAT
944   substrates (e.g., methotrexate, tenofovir, zidovudine). The need for further in vivo drug
945   interaction studies will be based on the criteria described in the decision trees in Figures
946   A2, A4, and A6 in the Appendix.
947
948   The decision as to whether the investigational drug should be evaluated as an inhibitor
949   for other transporters will be based on the therapeutic class, where unexpected drug-drug
950   interactions may have been observed and attributed to these other transporters, and when
951   new information becomes available in the literature.
952
953   *c.*   *The Investigational Drug as an Inducer of Transporters*
954
955   Transporters can be induced by mechanisms similar to those for CYP enzymes (e.g., by
956   activation of specific nuclear receptors). The expression levels of some transporters are
957   regulated in coordination with metabolizing enzymes, and they share common nuclear
958   factors. For example, a large number of drugs and dietary supplements (e.g., rifampin,
959   St. John's wort) concomitantly induce the expression of CYP3A and MDR1 (P-gp),
960   MRP2, MRP3, MRP4, and OATP1A2.
961
962   However, methods for in vitro evaluation for transporter induction are not well
963   understood. Cell lines are being used for in vitro P-gp induction including human colon
964   adenocarcinoma cell line LS 180/WT, and its adriamycin-resistant (LS 180/AD 50) or
965   vinblastine-resistant (LS 180/V) sublines. Further development is needed to validate the
966   utility of the in vitro assays to determine the need for an in vivo induction study. Until a
967   well-accepted system is developed, activation of nuclear receptor assays may be used as
968   an initial evaluation of the induction potential of an investigational drug on transporters.
969
970   The definitive determination of induction potential is based on in vivo induction studies.
971   The sponsor should consult with FDA about studying induction of transporters in vivo.
972
973   *3.*   *Considerations of the Metabolites of Investigational Drugs*
974
975   Metabolites formed in vivo may reach significant exposure (e.g., $\geq 25\%$ of the parent
976   drug) and elicit pharmacological and/or toxicological effects. Therefore, the same
977   considerations on further metabolism, transport, and drug interaction studies described
978   above should be considered for relevant metabolites of the investigational drugs. The
979   decision on which metabolite(s) should be investigated depends on multiple factors,
980   including the knowledge in pharmacological/toxicological activities (from in vitro human
981   cell line data and/or in vivo animal data) and the knowledge in metabolites' disposition

982 kinetics. For example, metabolites deemed insignificant after a single dose of the parent
983 drug may accumulate to appreciable exposure after multiple dosing if they have long
984 half-lives. Unexpected high exposure of metabolites may be attained in subjects with
985 decreased function of organs responsible for their elimination and in the event of drug
986 interactions affecting the disposition of the parent drug. Appropriate assays for
987 metabolites should be in place to monitor the metabolite levels along with the parent.
988
989 Given the complexity of the kinetics and interaction mechanisms of formed metabolites,
990 modeling and simulation integrating knowledge of drug disposition kinetics of both
991 parent investigational drug and metabolites may provide a useful tool to facilitate the
992 evaluation of drug interaction potential of metabolites (see earlier sections).
993
994 **B.**   **In Vivo Studies**
995
996 *1.*   *In Vivo Drug-Drug Interactions*
997
998 For detailed discussion on recommendations of in vivo human drug-drug interaction
999 studies, please refer to section V below
1000
1001 *2.*   *In Vivo Drug-Therapeutic Protein (TP) Interactions*
1002
1003 Drug-TP interactions have been observed and information about these interactions is
1004 included in labeling. Figure 7 lists the types of studies that have been conducted during
1005 drug development to evaluate TP and small molecule drug interactions. The following
1006 are general considerations:
1007
1008 • If an investigational TP is a cytokine or cytokine modulator, studies should be
1009   conducted to determine the TP's effects on CYP enzymes or transporters (Huang et
1010   al. 2010, Le Vee M et al. 2009). In vitro or animal studies have limited value in the
1011   qualitative and quantitative projection of clinical interactions because translation of in
1012   vitro to in vivo and animal to human results to date has been inconsistent,
1013   necessitating in vivo drug interaction studies. The in vivo evaluations of TPs in
1014   targeted patient populations can be conducted with individual substrates for specific
1015   CYP enzymes and transporters, or studies can be conducted using a "cocktail
1016   approach" (see section V.C).
1017
1018 • For TPs that will be used in combination with other drug products (small molecule or
1019   TP) as a combination therapy, studies should evaluate the effect of each product on
1020   the other. The studies should assess effects on pharmacokinetics (PK) and, when
1021   appropriate, pharmacodynamics (PD) of either drug. This evaluation is particularly
1022   important when the drug used in combination has a narrow therapeutic range (e.g.,
1023   chemotherapeutic agents).
1024
1025 • When there are known mechanisms or prior experience with certain PK or PD

1026   interactions, appropriate in vitro or in vivo assessments for possible interactions
1027   should be conducted. Some interactions between drugs and TPs are based on
1028   mechanisms other than CYP or transporter modulation. For example, methotrexate's
1029   immunosuppressive effect may alter the clearance of concomitantly administered TPs
1030   through the reduction of antibodies formed against TP. Other examples include
1031   heparin's effect on palifermin (increased exposure) and paclitaxel's effect on
1032   etanercept (increased exposure).
1033
1034

1035   **Figure 7. Summary of The Types of Studies That Have Been Used During Drug**
1036 **Development to Evaluate Therapeutic Protein (TP)–Small-Molecule Drug (D) Interactions.**
1037   **This includes an evaluation of the effect of TP on D (TP→D) and the effect of D on TP**
1038 **(D→TP). The broken lines suggest the limited use of in vitro studies for informing in vivo**
1039   **study design or labeling. CYP, cytochrome P450. (Modified from Huang et al. 2010)**
1040
1041
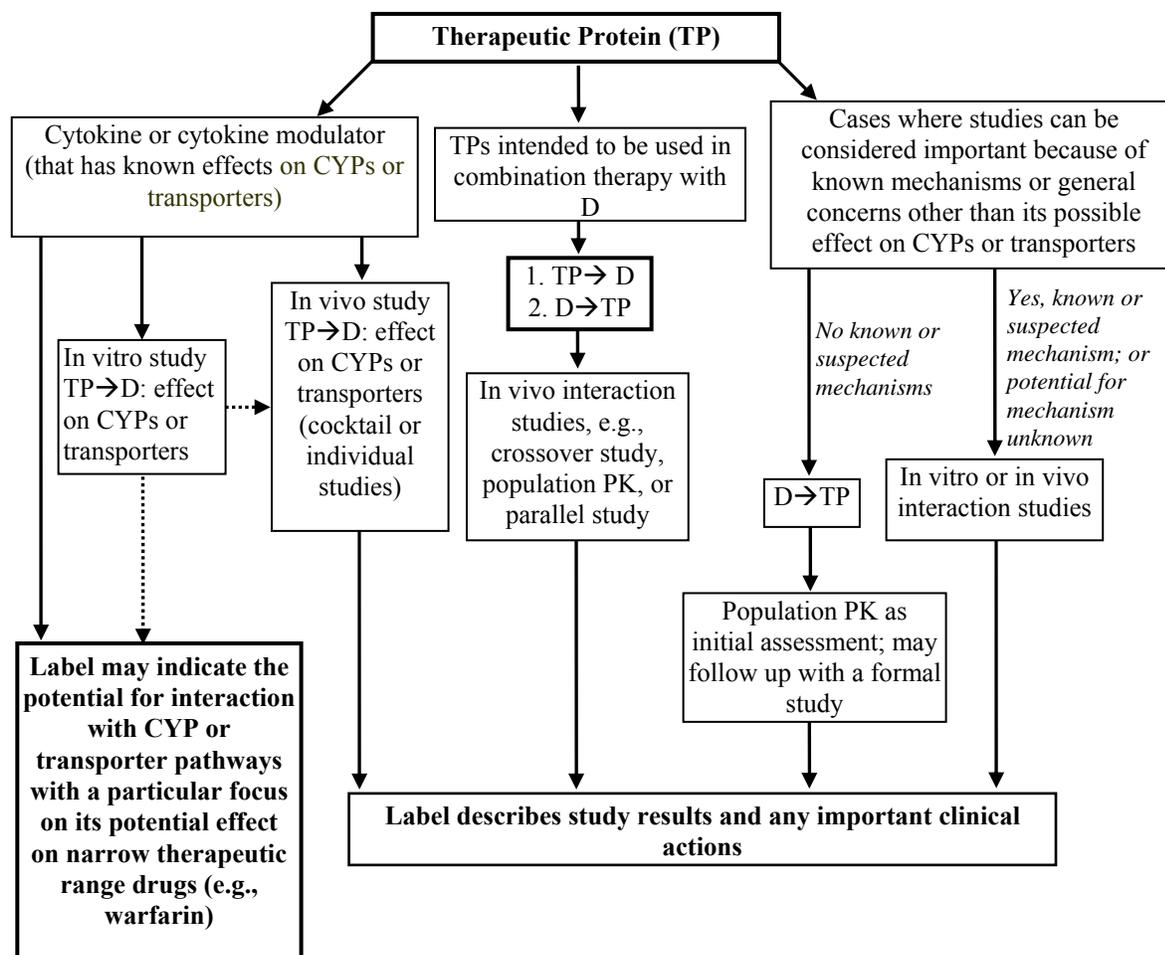
1042
1043
1044

1045
1046

1047 **C.   Using a Population Pharmacokinetic Approach to Assess Drug-Drug Interactions**
1048
1049   Population pharmacokinetic (PopPK) analyses of data obtained from large-scale clinical studies
1050   that include sparse or intensive blood sampling can help characterize the clinical impact of
1051   known or newly identified interactions and determine recommendations for dosage
1052   modifications for the investigational drug as a substrate. The results of such analyses can be
1053   informative and sometimes conclusive when the clinical studies are adequately designed to
1054   detect significant changes in drug exposure due to drug-drug interactions. PopPK evaluations
1055   may also detect unsuspected drug-drug interactions, a particularly important possibility given the
1056   complexity of the potential interactions (see section V.C.4), not all of which are likely to have
1057   been anticipated and studied. PopPK evaluations can also provide further evidence of the
1058   absence of a drug-drug interaction, when supported by prior evidence and mechanistic data. It is
1059   unlikely, however, that population analysis will persuasively show the absence of an interaction
1060   that is suggested by information from in vivo studies specifically designed to assess a drug-drug
1061   interaction. To be optimally informative, PopPK studies should have carefully designed study
1062   procedures and sample collection protocols. Simulations (e.g., by population-based PBPK
1063   models) can provide valuable insight into optimizing the study design (see section IV.A above).
1064   Detailed information on the dose given and time of administration should be documented for the
1065   co-administered drugs. When relevant for the specific drug, the time of food consumption
1066   should be documented. Population analyses should focus on excluding a specific clinically
1067   meaningful PK change. A guidance for industry on *Population Pharmacokinetics* is available at
1068   ([http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.](http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm)
1069   [htm](http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.htm)). Because exposure of co-administered drugs is not monitored in most PopPK studies, the
1070   PopPK approach may not be useful to assess the effect of the investigational drugs on other
1071   drugs.
1072
1073
1074   **V.   DESIGN OF IN VIVO DRUG-DRUG INTERACTION STUDIES**
1075
1076   If in vitro studies and other information suggest that in vivo drug-drug interaction studies would
1077   be helpful (e.g., based on the decision trees in Figures 2-7), the following general issues and
1078   approaches should be considered. In the following discussion, the term *substrate* (S) is used to
1079   indicate the drug studied to determine whether its exposure is changed by another drug. The
1080   other drug is termed the *interacting drug* (I).
1081
1082   **A.   Study Design**
1083
1084   In vivo drug-drug interaction studies generally are designed to compare substrate concentrations
1085   with and without the interacting drug. Because a specific study can address a number of
1086   questions and clinical objectives, many study designs for investigating drug-drug interactions
1087   can be considered. In general, crossover designs in which the same subjects receive substrate
1088   with and without the interacting drug are more efficient. A study can use a randomized
1089   crossover (e.g., S followed by S+I, S+I followed by S), one-sequence crossover (e.g., S followed
1090   by S+I), or a parallel (S in one group of subjects and S+I in another group) design, and there may

36

1091 be reason to have another period when the I is removed to assess effect duration. The following
1092 possible dosing regimen combinations for a substrate and interacting drug can also be used:
1093 single dose/single dose, single dose/multiple dose, multiple dose/single dose, and multiple
1094 dose/multiple dose. Additional factors include consideration of the sequence of administration
1095 and the time interval between dosing of substrate and inhibitor/inducer.
1096
1097 The selection of a study design depends on a number of factors for both the substrate and
1098 interacting drug, including (1) whether the substrate and/or interacting drug is used acutely or
1099 chronically; (2) safety considerations, including whether a substrate is a narrow therapeutic
1100 range (NTR)[5] or non-NTR drug; (3) pharmacokinetic and pharmacodynamic characteristics of
1101 the substrate and interacting drugs; (4) whether there is a desire to assess induction as well as
1102 inhibition; (5) whether the inhibition is delayed; and (6) whether there is a need to assess
1103 persistence of inhibition or induction after withdrawal of the interacting drug. The interacting
1104 drugs and the substrates should be dosed so that the exposures of both drugs are relevant to their
1105 clinical use, including the highest doses likely to be used in clinical practice, and plasma levels
1106 of both drugs should be obtained to show this. Simulations can help select an appropriate study
1107 design (see section IV.A). The following considerations may be useful:
1108
1109 - When attainment of steady state is important, and either the substrate or interacting
1110   drug or their metabolites have long half-lives, one or both periods of a crossover
1111   study should be long, but several other approaches can be considered, depending on
1112   pharmacokinetic characteristics of the drug and metabolites. For example, if the
1113   substrate has a long half-life, a loading dose could be used to reach steady state
1114   concentrations earlier in a one-sequence crossover followed by an S+I period long
1115   enough to allow I to reach steady state (here too, using a loading dose could shorten
1116   that period).
1117
1118 - When it is important that a substrate and/or an interacting drug be studied at steady
1119   state for a long duration because the effect of an interacting drug is delayed, as is the
1120   case for inducers and TDIs, documentation that near steady state has been attained for
1121   the pertinent substrate drug and metabolites as well as the interacting drug is critical,
1122   and both S and I should be present long enough to allow the full effect to be seen.
1123   This documentation can be accomplished by sampling over several days prior to the
1124   periods when test samples are collected. This information is important for
1125   metabolites and the parent drug, particularly when the half-life of the metabolite is
1126   longer than the parent. It is also important when the interacting drug and metabolites
1127   are both metabolic inhibitors (or inducers). Finally, it is critical to evaluate the time it
1128   takes for the enzyme activities to return to normal when induction or TDI is involved
1129   so that a third crossover period in which the interacting drug (I) is removed will
1130   generally be recommended.
1131

---

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

1132 • Studies can usually be open label (unblinded), unless pharmacodynamic endpoints
1133   (e.g., adverse events that are subject to bias) are critical to the assessment of the
1134   interaction.
1135

1136 • For a rapidly reversible inhibitor, administration of the interacting drug either just
1137   before or simultaneously with the substrate on the test day might increase sensitivity
1138   by ensuring maximum exposure to the two drugs together. For a mechanism-based
1139   inhibitor (a drug that requires metabolism before it can inactivate the enzyme; an
1140   example is erythromycin), administration of the inhibitor prior to the administration
1141   of the substrate drug can maximize the effect. If the absorption of an interacting drug
1142   may be affected by other factors (e.g., the gastric pH), it may be appropriate to
1143   control the variables or confirm the absorption through plasma level measurements of
1144   the interacting drug.
1145

1146 • Timing of administration may be critical in situations of concurrent inhibition and
1147   induction. For example, if the investigational drug is a substrate for both enzymes
1148   and OATP, and rifampin is used as an enzyme inducer, the simultaneous
1149   administration of the drug with rifampin (an OATP inhibitor) may underestimate
1150   enzyme induction, so delayed administration of the substrate is recommended. The
1151   optimal delayed time should be determined. In addition, it is critical to evaluate the
1152   duration of the interaction effect after the interacting drug has been removed.
1153

1154 • When the effects of two drugs on one another are of interest, the potential for
1155   interactions can be evaluated in a single study or two separate studies. Some design
1156   options are randomized three-period crossover, parallel group, and one-sequence
1157   crossover.
1158

1159 • To avoid variable study results because of uncontrolled use of dietary/nutritional
1160   supplements, tobacco, alcohol, juices, or other foods that may affect various
1161   metabolizing enzymes and transporters during in vivo studies, it is important to
1162   exclude, when appropriate, subjects who used prescription or over-the-counter
1163   medications, dietary/nutritional supplements, tobacco, or alcohol within 1 week prior
1164   to enrollment. In addition, investigators should explain to subjects that for at least 1
1165   week prior to the start of the study until its conclusion, they should not eat any food
1166   or drink/beverage containing *alcohol, grapefruit or grapefruit juice, apple or orange*
1167   *juice, vegetables from the mustard green family* (e.g., kale, broccoli, watercress,
1168   collard greens, kohlrabi, brussels sprouts, mustard), and *charbroiled meats.* In some
1169   instances, it is advisable to confine subjects to a study unit for the week prior to
1170   study.
1171

1172 • Because interactions might differ in subgroups of different pharmacogenetic
1173   genotypes, genotyping for the enzymes and transporters involved in the interaction
1174   should be carried out when appropriate.
1175

1176

1177 **B.   Study Population**

1178

1179   In most situations, clinical drug-drug interaction studies can be performed using healthy
1180   volunteers, and findings in healthy volunteers will predict findings in the patient population for
1181   which the drug is intended. Safety considerations, however, may preclude the use of healthy
1182   subjects in studies of certain drugs. In addition, there are circumstances in which subjects drawn
1183   from the intended patient population offer advantages, including the opportunity to study
1184   pharmacodynamic endpoints not present in or relevant to healthy subjects.

1185

1186   The extent of drug interactions (inhibition or induction) may be different depending on the
1187   subjects' genotype for the specific enzyme or transporter being evaluated. For example, subjects
1188   lacking the major polymorphic clearance pathway will show reduced total metabolism or
1189   transport. However, alternative pathways can become quantitatively more important in these
1190   subjects. In such cases, the alternative pathways should be understood and studied
1191   appropriately. Thus, phenotype or genotype determinations to identify genetically determined
1192   metabolic or transporter polymorphisms are important when evaluating effects on enzymes or
1193   transporters with polymorphisms, such as CYP2D6, CYP2C19, CYP2C9, UGT1A1, and
1194   OATP1B1 (SLCO1B1). In addition, it is valuable to specify the need for stratifying the
1195   population based on genotype while conducting the DDI studies. Another alternative is to
1196   consider powering the study for the genotype status that is likely to have the highest potential for
1197   interaction.

1198

1199

1200   **C.   Choice of Substrate and Interacting Drugs**

1201

1202   *1.   CYP-Mediated Interactions*

1203

1204   *a.   The Investigational Drug as a Substrate of CYP Enzymes — Effect of Other*
1205   *Drugs on Investigational Drugs*

1206

1207   When testing an investigational drug for the possibility that its metabolism is inhibited or
1208   induced (i.e., as a substrate), selection of the interacting drugs should be based on in vitro
1209   or in vivo studies identifying the enzyme systems that metabolize the investigational
1210   drug. The choice of the interacting drug can then be based on known, important
1211   inhibitors and inducers of the pathway under investigation. Strong inhibitors and
1212   inducers provide the most sensitive assessment and should generally be tested first.
1213   Consider, for example, an investigational drug metabolized by CYP3A with the
1214   contribution of this enzyme to the overall elimination of this drug that is either substantial
1215   ($\geq 25\%$ of the clearance pathway) or unknown. In this case, the inhibitor and inducer can
1216   be ketoconazole and rifampin, a strong inhibitor and a strong inducer, respectively.
1217   Other strong inhibitors or inducers are acceptable. If the study results are negative, then
1218   absence of a clinically important drug-drug interaction for the metabolic pathway is
1219   demonstrated. If the clinical study of the strong inhibitor or inducer is positive, the

1220   sponsor would generally evaluate effects through in vivo studies or mechanistic modeling
1221   of other less potent specific inhibitors or inducers, and develop labeling advice on dosage
1222   adjustment (the classification of CYP inhibitors and inducers is discussed in the next
1223   section; see Table 3 for a list of CYP inhibitors and Table 4 for CYP inducers). If the
1224   investigational drug is metabolized by CYP3A and its plasma AUC is increased 5-fold or
1225   higher by strong CYP3A inhibitors, it is considered a *sensitive substrate* of CYP3A. The
1226   labeling would indicate that the drug is a "sensitive CYP3A substrate" and that its use
1227   with strong or moderate inhibitors may call for caution, depending on the drug's
1228   exposure-response relationship. If the investigational drug is metabolized by CYP3A and
1229   its exposure-response relationship indicates that a two-fold increase in the exposure
1230   levels by the concomitant use of CYP3A inhibitors may lead to serious safety concerns
1231   (e.g., Torsades de Pointes), it is considered a "CYP3A substrate with narrow therapeutic
1232   range" (Table 5) (see section VI for more labeling recommendations).
1233
1234

1235

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

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

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

1237

(1) Please note the following: This is not an exhaustive list. For an updated list, see the following link http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm.
(2) A strong inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a substrate for that CYP by equal or more than 5-fold.
(3) A moderate inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 5-fold but equal to or more than 2-fold.
(4) A weak inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 2-fold but equal to or more than 5-fold.
(5) Herbal product.
(6) Gemfibrozil also inhibits OATP1B1.
(7) Fluconazole is listed as a strong CYP2C19 inhibitor based on the AUC ratio of omeprazole, which is also metabolized by CYP3A; fluconazole is a moderate CYP3A inhibitor.
(8) Fluvoxamine strongly inhibits CYP1A2 and CYP2C19, but also inhibits CYP2C8/2C9 and CYP3A;
(9) Ticlopidine strongly inhibits CYP2C19, but also inhibits CYP3A, CYP2B6, and CYP1A2.
(10) Effect seems to be due to CYP2C19 inhibition by ethinyl estradiol.
(11) The effect of grapefruit juice varies widely among brands and is concentration-, dose-, and preparation-dependent. Studies have shown that it can be classified as a "strong CYP3A inhibitor" when a certain preparation was used (e.g., high dose, double strength) or as a "moderate CYP3A inhibitor" when

1257   another preparation was used (e.g., low dose, single strength).
1258   (12) Withdrawn from the United States market.
1259

1260   **Table 4. Classification of In Vivo Inducers of CYP Enzymes[1]**

1261

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

1262
1263   (1) Please note the following: This is not an exhaustive list. For an updated list, see the following link:
1264   http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabelin
1265   g/ucm080499.htm.
1266   (2) For a drug that is a substrate of CYP1A2, the evaluation of the effect of induction of CYP1A2 can be
1267   carried out by comparative PK studies in smokers vs. non-smokers.
1268   (3) The effect of St. John's wort varies widely and is preparation-dependent.
1269   (4) Herbal product.
1270   (5) Not a marketed drug.

1271 **Table 5. Examples[1] of Sensitive In Vivo CYP Substrates and CYP Substrates with**
1272 **Narrow Therapeutic Range**

1273

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

1274
1275 (1) Note that this is not an exhaustive list. For an updated list, see the following link:
1276 http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabelin
1277 g/ucm080499.htm.
1278 (2) *Sensitive CYP substrates* refers to drugs whose plasma AUC values have been shown to increase 5-fold
1279 or higher when co-administered with a known CYP inhibitor or AUC ratio in poor metabolizers vs.
1280 extensive metabolizers is greater than 5-fold.
1281 (3) CYP *substrates with narrow therapeutic range* refers to drugs whose exposure-response relationship
1282 indicates that small increases in their exposure levels by the concomitant use of CYP inhibitors may lead to
1283 serious safety concerns (e.g., Torsades de Pointes).
1284 (4) The AUC of these substrates were not increased by 5-fold or more with a CYP2B6 inhibitor, but they
1285 represent the most sensitive substrates studied with available inhibitors evaluated to date.
1286 (5) Repaglinide is also a substrate for OATP1B1, and it is only suitable as a CYP2C8 substrate if the
1287 inhibition of OATP1B1 by the investigational drug has been ruled out.
1288 (6) Because a number of CYP3A substrates (e.g., darunavir, maraviroc) are also substrates of P-gp, the
1289 observed increase in exposure could be due to inhibition of both CYP3A and P-gp.
1290   (7) Withdrawn from the United States market.

1291

1292 NTR drugs are defined as those drugs for which there is little separation between
1293 therapeutic and toxic doses or the associated blood or plasma concentrations (i.e.,
1294 exposures). In general, the toxicity in question is serious toxicity, not symptomatic
1295 reversible toxicity (most drugs have adverse effects of various kinds within the
1296 therapeutic range).

1297 Classic examples of NTR drugs include:

1298 • Warfarin, where a modest increase from the titrated (by international normalized
1299   ratio, INR) concentration can cause major bleeding.

1300 • Drugs with concentration-related QT effects (cisapride, astemizole, dofetilide), where
1301   a previously tolerated dose could become toxic with a doubling of serum
1302   concentration.

1303 • Most cytotoxic oncologic drugs.

1304 • Aminoglycoside antibiotics.

1305

1306 Although there is no well-established rule, drugs for which a doubling of serum
1307 concentration would cause serious toxicity can be considered NTR. Note, however, that
1308 even reasonably well-tolerated drugs can become toxic if blood levels are greatly
1309 increased (e.g., by CYP450 inhibition). For example, lovastatin and simvastatin, used
1310 over a substantial dose range, can cause myopathy leading to rare and life-threatening
1311 rhabdomyolysis if taken with a strong CYP3A inhibitor (such as mibefradil, now
1312 removed from the U.S. market), which can cause a large-fold increase in blood levels.

1313

1314 If an orally administered drug is a substrate of CYP3A and has low oral bioavailability
1315 because of extensive presystemic extraction by enteric CYP3A, grapefruit juice may have
1316 a significant effect on its systemic exposure. Use of the drug with grapefruit juice may
1317 call for caution, depending on the drug's exposure-response relationship (see section VI
1318 for labeling recommendations).

1319

1320 If a drug is a substrate of CYP3A or P-gp and co-administration with St. John's wort, an
1321 inducer of this enzyme and transporter, can decrease the systemic exposure and
1322 effectiveness, St. John's wort will be listed in the labeling along with other known
1323 inducers, such as rifampin, rifapentin, phenytoin, carbamazepine, or phenobarbital, as
1324 possibly decreasing plasma levels of the drug.

1325

1326 If a drug is metabolized by a polymorphic enzyme (such as CYP2D6, CYP2C9,
1327 CYP2C19, or UGT1A1), the comparison of pharmacokinetic parameters of this drug in
1328 poor metabolizers and extensive metabolizers may substitute for an interaction study for
1329 that particular pathway, as the PK in the poor metabolizers will indicate the effect of a
1330 strong inhibitor. When the study suggests the presence of a significant interaction with

1331   strong inhibitors or in poor metabolizers, further evaluation, including mechanistic
1332   modeling with weaker inhibitors or intermediate metabolizers, may be recommended.
1333
1334   b.   *The Investigational Drug as an Inhibitor or an Inducer of CYP Enzymes — Effect*
1335   *of Investigational Drugs on Other Drugs*
1336
1337   When studying an investigational drug as the interacting drug, the choice of substrates
1338   (approved drugs) for initial in vivo studies depends on the P450 enzymes affected by the
1339   interacting drug. When testing inhibition, the substrate selected should generally be one
1340   whose pharmacokinetics are markedly altered by the co-administration of known specific
1341   inhibitors of the enzyme systems (sensitive substrates) to see the largest impact of the
1342   interacting investigational drug. Examples of such substrates include (1) midazolam for
1343   CYP3A; (2) theophylline for CYP1A2; (3) bupropion for CYP2B6; (4) repaglinide for
1344   CYP2C8; (5) warfarin for CYP2C9 (with the evaluation of S-warfarin); (6) omeprazole
1345   for CYP2C19; and (7) desipramine for CYP2D6 (see Table 5 above for additional
1346   substrates). If the initial study determines that an investigational drug either inhibits or
1347   induces metabolism of sensitive substrates, further studies using other substrates,
1348   representing a range of therapeutic classes, based on the likelihood of co-administration,
1349   may be useful. If the initial study with the most sensitive substrates is negative, it can be
1350   presumed that less sensitive substrates also will be unaffected. It should be noted that
1351   several of the substrates recommended for drug interaction studies are not specific
1352   because they are substrates for more than one CYP enzyme or may be substrates for
1353   transporters. While a given substrate may not be metabolized by a single enzyme (e.g.,
1354   dextromethorphan elimination is carried out primarily by CYP2D6 but other enzymes
1355   also contribute in a minor way), its use in an interaction study is appropriate if the
1356   inhibitor (the investigational drug) to be evaluated is selective for the CYP enzyme of
1357   interest.
1358
1359   If an investigational drug is a CYP inhibitor, it may be classified as a strong, moderate, or
1360   weak inhibitor based on its effect on a sensitive CYP substrate. For example, CYP3A
1361   inhibitors can be classified based on the magnitude of the change in plasma AUC of oral
1362   midazolam or other CYP3A substrates that are similar in characteristics (e.g., fm (%
1363   clearance contributed by CYP3A), half-life, not subject to transporter effect) as
1364   midazolam, when the substrate is given concomitantly with the inhibitor (see Table 3
1365   above). If the investigational drug increases the AUC of oral midazolam or other CYP3A
1366   substrates by 5-fold or higher ($\geq$ 5-fold), it can be considered a *strong* CYP3A inhibitor.
1367   If the investigational drug, when given at its highest dose and shortest dosing interval (to
1368   maximize exposure and inhibitory effect), increases the AUC of oral midazolam or other
1369   sensitive CYP3A substrates by between 2- and 5-fold ($\geq$ 2- and <5-fold), it can be
1370   considered a *moderate* CYP3A inhibitor. If the investigational drug, when given at the
1371   highest dose and shortest dosing interval, increases the AUC of oral midazolam or other
1372   sensitive CYP3A substrates by between 1.25- and 2-fold ($\geq$ 1.25- and < 2-fold), it can be
1373   considered a *weak* CYP3A inhibitor. When the investigational drug is determined to be
1374   an inhibitor of CYP3A, its interaction with CYP3A substrates should be described in

1375 various sections of the labeling, as appropriate (see section VI, Labeling
1376 Recommendations).
1377
1378 When an in vitro evaluation does not rule out the possibility that an investigational drug
1379 is an inducer of CYP3A (see section IV.A), an in vivo evaluation can be conducted using
1380 the most sensitive substrate (e.g., oral midazolam, see Table 5 above). When midazolam,
1381 the most sensitive substrate, is co-administered orally following the administration of
1382 multiple doses of the investigational drug, and there is no interaction, it can be concluded
1383 that the investigational drug is not an inducer of CYP3A (in addition to the conclusion
1384 that it is not an inhibitor of CYP3A). A caveat to this interpretation is that if the
1385 investigational drug is both an inducer and inhibitor of CYP3A, such as ritonavir, the net
1386 effect at any time it is introduced may vary. In this case, the net effect of the drug on
1387 CYP3A function may be time-dependent.
1388
1389 In vivo induction evaluations have often been conducted using oral contraceptives as the
1390 substrate. However, oral contraceptives are not the most sensitive substrates for CYP3A,
1391 so a negative result does not exclude the possibility that the investigational drug is an
1392 inducer of CYP3A. Some compounds listed in Table 5 as sensitive substrates for the
1393 other enzymes can also be used as substrates with the investigational drug as an inducer.
1394 For example, omeprazole and repaglinide are CYP2C19 and CYP2C8 substrates,
1395 respectively, but they are also metabolized by CYP3A. If omeprazole is used as a
1396 substrate to study CYP2C19 induction, measurement of its metabolites (CYP2C19-
1397 mediated hydroxy-omeprazole and CYP3A4-mediated omeprazole sulfone) will be
1398 recommended for the interpretation of the study results.
1399
1400
1401 *2.   Transporter-Mediated Interactions*
1402
1403 Similar to CYP enzymes, transporters may be inhibited or induced. Inhibition of
1404 transporters by interacting drugs can lead to altered exposure of other drugs that are
1405 substrates of transporters. Therefore, the potential for an investigational drug as a
1406 substrate, inhibitor, or inducer for transporters should be evaluated during drug
1407 development.
1408
1409 Clinically significant P-gp-mediated drug interactions, mostly related to digoxin, have
1410 been reported (Table 1). With the availability of genetic tools, our understanding of roles
1411 of other transporters in drugs' ADME, and transporter-based interactions has improved.
1412 A recent genome-wide association study showed that OATP1B1 polymorphism was
1413 associated with increased incidence of myopathy in patients taking 80 mg of simvastatin
1414 daily (Link et al. 2008). Cyclosporine increases some statin drugs' exposure 5- to 10-
1415 fold, which appeared to be mediated by inhibition of OATP and possibly BCRP (Table
1416 1). These data indicate that significant interactions between drugs can occur at the
1417 transporter level.
1418

1419   In this guidance, BCRP, OATP, OATs, and OCTs are considered important transporters
1420   in addition to P-gp (International Transporter Consortium 2010) and should be routinely
1421   evaluated. Refer to Figure 6 for a possible decision tree that could be used to guide the
1422   decision of when to study these transporters in vitro during drug development.
1423   Additional decision trees to determine when to evaluate drug interactions in vivo are
1424   presented in the Appendix (Figures A1-6).
1425
1426   Because the field of transporter pharmacology is rapidly evolving, other transporters
1427   (e.g., multidrug resistance-associated proteins (MRPs), multidrug and toxin extrusion
1428   (MATE) transporters, and bile salt export pump (BSEP) transporters) should be
1429   considered when appropriate.
1430
1431
1432   *a.*   *The Investigational Drug as a Substrate of Transporters — the Effect of Other*
1433   *Drugs on an Investigational Drug*
1434
1435   When testing an investigational drug for the possibility that its transport is inhibited or
1436   induced (i.e., as a substrate), selection of the interacting drugs should be based on in vitro
1437   or in vivo studies identifying the transporters that are involved in the absorption and
1438   disposition of the investigational drug (e.g., absorption and efflux in the gastrointestinal
1439   tract, uptake and secretion in the liver, and the secretion and re-absorption in the kidney).
1440   The choice of the interacting drug should be based on known, important inhibitors of the
1441   pathway under investigation. Strong inhibitors provide the most sensitive assessment and
1442   should generally be tested first. As there is overlapping selectivity in substrate and
1443   inhibitor among transporters, negative results from a study using a broad inhibitor may
1444   rule out the possibility for drug interaction mediated by multiple pathways. For example,
1445   it may be appropriate to use an inhibitor of many transporters (e.g., cyclosporine, which
1446   inhibits P-gp, OATP, and BCRP) to study its effect on a drug that may be a substrate for
1447   these transporters. A negative result rules out the involvement of these transporters in the
1448   drug's disposition. However, if the result is positive, it will be difficult to determine the
1449   relative contribution of each transporter to the disposition of the substrate drug.
1450   In contrast, if the goal of the study is to determine the role of a specific pathway in the
1451   PK of a substrate drug, then a selective and potent inhibitor for that transporter should be
1452   used. Table 6 lists examples of inhibitors and inducers of selected transporters.
1453
1454   As an alternative, comparative PK of an investigational drug in subjects with different
1455   genotypes of specific transporters (e.g., OATP1B1 c.521 T vs C) can be evaluated to
1456   determine the importance of a specific transporter in the clearance pathway for the drug.
1457   On the other hand, polymorphism data on P-gp is controversial and may not be used to
1458   determine the role of P-gp in the disposition of investigational drugs that are substrates of
1459   P-gp.
1460

1461   **Table 6**. **Examples of In Vivo Inhibitors and Inducers of Selected Transporters[1]**

1462

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

1463   (1) Please note this is not an exhaustive list. For an updated list, see the following link
1464   http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabelin
1465   g/ucm080499.htm.
1466   (2) Inhibitors listed for P-gp are those that showed >25% increase in digoxin AUC or otherwise indicated
1467   if substrate is other than digoxin.
1468   (3) Inducers listed for P-gp are those that showed >20% decrease in digoxin AUC or otherwise indicated
1469   if substrate is other than digoxin.
1470   (4) Inhibitors listed are those that showed >25% increase in fexofenadine AUC.
1471   (5) Inhibitors listed are those that showed >25% increase in talinolol AUC.
1472   (6) Not a marketed drug.
1473   (7) Inducers listed are those that showed >20% decrease in fexofenadine AUC.
1474   (8) Herbal product.
1475   (9) Given as a single dose.
1476   (10) In vitro inhibitors for OATP. Separation of the in vivo inhibition effect from ritonavir is difficult
1477   because this drug is usually co-administered with ritonavir.
1478   (11) The in vivo inhibition effect of ritonavir cannot be easily estimated because it is usually co-
1479   administered with other HIV protease inhibitors that are inhibitors for OATP as well.
1480

1481   *b.*   *The Investigational Drug as an Inhibitor or an Inducer of Transporters — Effect*
1482   *of the Investigational Drugs on Other Drugs*
1483
1484   When studying an investigational drug as the interacting drug, the choice of substrates
1485   (approved drugs in the United States) for initial in vivo studies depends on the transport
1486   pathway that may be affected by the interacting drug. In general, when testing inhibition,
1487   the substrate selected should be one whose pharmacokinetics are markedly altered by co-
1488   administration of known specific inhibitors of the transporter pathway to see the largest
1489   impact of the interacting investigational drug. The choice of substrates can also be
1490   determined by the therapeutic area of the investigational drug and the probable co-
1491   administered drugs that are known substrates for transporters. Table 7 lists selected
1492   examples of substrates for P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, and
1493   OAT3. However, because many drugs are substrates of multiple transporters or enzymes,
1494   specific substrates for each transporter are not available. For example, rosuvastatin is a
1495   substrate for BCRP, OATP1B1, and OATP1B3; lapatinib is a substrate for both P-gp and
1496   BCRP. The observed clinical interactions may be a result of inhibition of multiple
1497   pathways if the investigational drug is also an inhibitor for the same multiple pathways.

1498

1499 **Table 7. Examples of In Vivo Substrates for Selected Transporters[1]**

1500

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

1501 (1) Please note this is not an exhaustive list. For an updated list, see the following link
1502 http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabelin
1503 g/ucm080499.htm.
1504 (2) Selective for OATP1B3.

1505

1506 Because of the lack of a validated in vitro system to study transporter induction, the
1507 definitive determination of induction potential of an investigator on transporters is based
1508 on in vivo induction studies. The sponsor should consult with FDA about studying
1509 induction of transporters in vivo. For example, because of similarities in the mechanisms
1510 of CYP3A and P-gp induction, information from the testing of CYP3A inducibility can
1511 inform decisions about P-gp. If an investigational drug is found not to induce CYP3A in
1512 vitro, no further tests of CYP3A and P-gp induction in vivo are necessary. If a study of
1513 the investigational drug's effect on CYP3A activity in vivo is indicated from a positive in
1514 vitro screen, but the drug is shown not to induce CYP3A in vivo, then no further test of
1515 P-gp induction in vivo is necessary. However, if the in vivo CYP3A induction test is
1516 positive, then an additional study of the investigational drug's effect on a P-gp probe
1517 substrate is recommended. If the drug is also an inhibitor for P-gp, then the induction
1518 study can be conducted with the inhibitor study using a multiple-dose design.

1519

1520   *3.   Cocktail Approach*
1521
1522   Simultaneous administration of a mixture of substrates of multiple CYP enzymes and
1523   transporters in one study (i.e., a "cocktail approach") in human volunteers is another way
1524   to evaluate a drug's inhibition or induction potential, provided that the study is designed
1525   properly and the following factors are present: (1) the substrates are specific for
1526   individual CYP enzymes or transporters; (2) there are no interactions among these
1527   substrates; and (3) the study is conducted in a sufficient number of subjects (see section
1528   V.G). Negative results from a well-conducted cocktail study can eliminate the need for
1529   further evaluation of particular CYP enzymes. However, positive results can indicate
1530   that further in vivo evaluation should be conducted to provide quantitative exposure
1531   changes (such as AUC, C_{max}), if the initial evaluation only assessed the changes in the
1532   urinary parent to metabolite ratios. The data generated from a cocktail study can
1533   supplement data from other in vitro and in vivo studies in assessing a drug's potential to
1534   inhibit or induce CYP enzymes and transporters.
1535
1536
1537   *4.   Complex Drug Interactions*
1538
1539   *a.   Multiple CYP Inhibitors*
1540
1541   There may be situations when an evaluation of the effect of multiple CYP inhibitors on
1542   the drug can be informative. For example, it may be appropriate to conduct an
1543   interaction study with more than one inhibitor simultaneously if all of the following
1544   conditions are met: (1) the drug exhibits blood concentration-dependent important safety
1545   concerns; (2) multiple CYP enzymes are responsible for the metabolic clearance of the
1546   drug; (3) the predicted residual or non-inhibitable drug clearance is low. Under these
1547   conditions, the effect of multiple CYP-selective inhibitors on the investigational drug's
1548   blood AUC may be much greater than when the inhibitors are given individually with the
1549   drug, and more than the product of changes in AUC observed with each individual
1550   inhibitor. The magnitude of the combined effect will depend on the residual fractional
1551   clearance (the smaller the fraction, the greater the concern) and the relative fractional
1552   clearances of the inhibited pathways. Modeling and simulation approaches can help
1553   project the magnitude of the effect based on single pair drug interaction studies.
1554
1555   If results from a study with a single inhibitor have already triggered a major safety
1556   concern (i.e., a contraindication), multiple inhibitor studies are unlikely to add value.
1557
1558   *b.   Enzyme/Transporter Interplay*
1559
1560   There is an overlap in enzyme and transporter specificity. For example, there is
1561   considerable overlap between CYP3A and P-gp inhibitors and inducers. Itraconazole
1562   inhibits CYP3A and P-gp and rifampin induces CYP3A and P-gp. However, dual
1563   inhibitors for CYP3A and P-gp do not necessarily have similar inhibition potency on

1564   CYP3A and P-gp (Table 8). For example, the strong CYP3A inhibitor voriconazole does
1565   not cause a large increase in exposure of a P-gp substrate, such as digoxin or
1566   fexofenadine. In addition, some potent P-gp inhibitors such as amiodarone and quinidine
1567   (causing ≥ 1.5-fold change in digoxin or fexofenadine AUC) are weak CYP3A inhibitors.
1568   The differential inhibition effects on CYP3A and P-gp should be considered when
1569   inhibitors are selected for study of interactions with an investigational drug that is a
1570   CYP3A, P-gp, or dual CYP3A and P-gp substrate (Zhang et al. 2009). To assess the
1571   worst case scenario for a dual CYP3A and P-gp substrate, inhibition should be studied
1572   using an inhibitor that shows strong inhibition for both P-gp and CYP3A, such as
1573   itraconazole. However, under this condition, if the result is positive, specific attribution
1574   of an AUC change to P-gp or CYP3A4 may not be possible. For labeling purposes,
1575   evaluation either through in vivo interaction studies or mechanistic modeling with less
1576   strong inhibitors for either pathways or inhibitors for one particular pathway only may be
1577   recommended. If the goal is to determine the specific contribution of CYP3A or P-gp on
1578   the AUC change, then a strong inhibitor for CYP3A only or a potent inhibitor for P-gp
1579   only should be selected to discern the effect of CYP3A vs. P-gp.   Table 8 lists examples
1580   of CYP3A and P-gp inhibitors and their relative potency.

1581

1582   **Table 8. Examples of In Vivo CYP3A and P-gp Inhibitors and Their Relative Potency**

1583

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

1584   *   Data derived with fexofenadine; all other data were derived with digoxin.

1585

1586   Notes:
1587   (1) The University of Washington Drug Interaction Database was used to search the data that defined the in
1588   vivo potency of various inhibitors for CYP3A (midazolam was searched as a substrate) and P-gp (digoxin
1589   or fexofenadine was searched as a substrate).
1590   (2) P-gp inhibitors or non-P-gp inhibitors are defined as those drugs that increase the AUC of digoxin or
1591   fexofenadine by ≥ 1.25-fold or <1.25-fold, respectively. (The asterisk indicates data derived with
1592   fexofenadine; all other data were derived with digoxin.)
1593   (3) Strong, moderate, or weak CYP3A inhibitors are defined as those drugs that increase the AUC of oral
1594   midazolam or other CYP3A substrates ≥5-fold, 2-5-fold, and 1.25-2-fold, respectively.

1595

1596	In addition to the possibility that a drug is an inhibitor or inducer of multiple
1597	enzymes/transporters, a drug can be an inhibitor of one enzyme/transporter and inducer
1598	of another enzyme/transporter. For example, ritonavir is an inhibitor of CYP3A and an
1599	inducer of UGT; tipranavir is an inhibitor of CYP3A and an inducer of P-gp. Rifampin,
1600	an established inducer of multiple CYP enzymes and transporters, was recently found to
1601	be an inhibitor of the uptake transporter OATP1B1 and may inhibit the uptake of an
1602	investigational drug that is a substrate of OATP1B1. Accordingly, if a drug is a CYP
1603	enzyme substrate and an OATP1B1 substrate, an induction study with rifampin should be
1604	designed and interpreted carefully. The net steady state effect may vary depending on
1605	the relative size of the individual effect on transporter and enzyme activities. Timing of
1606	administration may become critical in situations when both enzymes and transporters can
1607	be affected. These overlapping selectivities contribute to complex drug interactions and
1608	make the prediction of in vivo outcome based on in vitro evaluation challenging or
1609	impossible (Zhang et al. 2009).
1610	
1611	The implications of simultaneous inhibition of a dominant CYP enzyme(s) and an uptake
1612	or efflux transporter that controls the availability of the drug to CYP enzymes can be just
1613	as profound as that of multiple CYP inhibition. For example, the large effect of co-
1614	administration of itraconazole and gemfibrozil on the systemic exposure (AUC) of
1615	repaglinide may be attributed to collective inhibitory effects on both the enzyme
1616	(CYP2C8) and transporters (OATP1B1) by intraconazole and gemfibrozil and their
1617	respective metabolites.
1618	
1619	*c. Effect of Organ Impairment*
1620	
1621	Another type of complex drug interaction is the co-administration of substrate and
1622	enzyme/transporter inhibitor in subjects with organ impairment. For example, if a
1623	substrate drug is eliminated through both hepatic metabolism and renal
1624	secretion/filtration, the use of an enzyme inhibitor in subjects with renal impairment may
1625	cause a more than projected increase in exposure of substrate drug based on individual
1626	effect alone.
1627	
1628	Unfortunately, current knowledge does not permit the presentation of specific guidance
1629	for studying some of these complex drug interaction scenarios because dedicated in vivo
1630	studies in humans may not be feasible or may raise ethical and practical considerations.
1631	Modeling and simulation approaches integrating prior in vitro and in vivo ADME and
1632	drug interaction data may be useful for evaluating complex drug interactions. For
1633	example, results from dedicated single pair drug interaction studies and separate
1634	pharmacokinetic evaluation in subjects with organ impairment may provide useful
1635	information to strengthen the model for the evaluation of complex drug interactions.
1636	
1637	*d. Pediatrics and Geriatrics*
1638	
1639	Age-related changes in physiological processes governing drug disposition and drug

1640   effect have been investigated. In some cases, disproportional alterations in binding
1641   proteins, drug metabolizing enzymes and/or transporters, and renal filtration/secretion
1642   caused by developmental changes have been known to result in different drug disposition
1643   characteristics in pediatric and geriatric populations. However, dedicated drug
1644   interaction studies in these populations may not be feasible. Simulations using system
1645   biology approaches such as PBPK models (see section IV.A) may be helpful to predict
1646   drug interaction potential when the model can be constructed based on sufficient in vitro
1647   and clinical pharmacology and drug interaction data and incorporates development
1648   changes. Population pharmacokinetic approaches with sparse sampling can be used if
1649   properly designed (section IV.C).

1650

1651   *e.*   *Genetics*

1652

1653   When a drug-drug interaction study uses a probe drug (e.g., omeprazole for CYP2C19) to
1654   evaluate the impact of the investigational drug on a polymorphic enzyme, individuals
1655   who have no functional enzyme activity would not be appropriate study subjects. Drug
1656   interaction studies that evaluate enzymes or transporters with known polymorphisms
1657   should include collection of genotype or phenotype information to allow appropriate
1658   interpretation of the study results. In some instances, an evaluation of the extent of drug
1659   interactions in subjects with various genotypes may be helpful (refer to the FDA
1660   guidance for industry on *Clinical Pharmacogenomics: Premarketing Evaluation in Early*
1661   *Phase Clinical Studies,*
1662   http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm0
1663   64982.htm).

1664

1665 **D.**   **Route of Administration**

1666

1667 The route of administration chosen for a metabolic drug-drug interaction study is important. For
1668 an investigational agent, the route of administration generally should be the one planned for
1669 clinical use. When multiple routes are being developed, the need for metabolic drug-drug
1670 interaction studies by each route depends on the expected mechanisms of interaction and the
1671 similarity of corresponding concentration-time profiles for parent drug and metabolites. If only
1672 oral dosage forms will be marketed, studies with an intravenous formulation are not usually
1673 recommended, although information from oral and intravenous dosing may be useful in
1674 discerning the relative contributions of alterations in absorption and/or presystemic clearance to
1675 the overall effect observed for a drug interaction. Sometimes certain routes of administration
1676 can reduce the utility of information from a study. For example, intravenous administration of a
1677 substrate drug may not reveal an interaction for substrate drugs where intestinal CYP3A activity
1678 markedly alters bioavailability.

1679

1680 **E.**   **Dose Selection**

1681

1682 The doses of the substrate and interacting drug used in studies should maximize the possibility of
1683 demonstrating an interaction. For this reason, the maximum planned or approved dose and

1684   shortest dosing interval of the interacting drug (as inhibitors or inducers) should be used. For
1685   example, when using ketoconazole as an inhibitor of CYP3A, the decision whether to dose at
1686   400 mg QD or 200 mg BID for multiple days can be determined based on the pharmacokinetic
1687   characteristics (e.g., the half-life) of the substrate drug (Zhao et al. 2009). When using rifampin
1688   as an inducer, dosing at 600 mg QD for multiple days would be preferable to lower doses. When
1689   there are safety concerns, doses lower than those used clinically may be recommended for
1690   substrates. In such instances, any limitations of the sensitivity of the study to detect the drug-
1691   drug interaction due to the use of lower doses should be discussed by the sponsor in the protocol
1692   and study report.

1693
1694   **F.**   **Endpoints**

1695
1696   Changes in pharmacokinetic parameters generally are used to assess the clinical importance of
1697   drug-drug interactions. Interpretation of findings (i.e., deciding whether a given effect is
1698   clinically important) depends on a good understanding of dose/concentration and
1699   concentration/response relationships for both desirable and undesirable drug effects in the
1700   general population or in specific populations. The FDA guidance for industry on *Exposure-*
1701   *Response Relationships — Study Design, Data Analysis, and Regulatory Applications*
1702   (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064982.
1703   htm) provides considerations in the evaluation of exposure-response relationships. In certain
1704   instances, reliance on pharmacodynamic endpoints in addition to pharmacokinetic measures
1705   and/or parameters may be useful. Examples include INR measurement (e.g., when studying
1706   warfarin interactions) or QT interval measurements.

1707
1708   *1.*   *Pharmacokinetic Endpoints*

1709
1710   Substrate PK exposure measures such as AUC, C_{max}, time to C_{max} (T_{max}), and others as
1711   appropriate should be obtained in every study. Calculation of pharmacokinetic
1712   parameters such as clearance, volumes of distribution, and half-lives may help in the
1713   interpretation of the results of the trial. In some cases, obtaining these measures for the
1714   inhibitor or inducer may be of interest as well, notably where the study is intended to
1715   assess possible changes in the disposition of both study drugs. Additional measures may
1716   help in steady state studies (e.g., trough concentration) to demonstrate that dosing
1717   strategies were adequate to achieve near steady state before and during the interaction. In
1718   certain instances, an understanding of the relationship between dose, blood
1719   concentrations, and response may lead to a special interest in certain pharmacokinetic
1720   measures and/or parameters. For example, if a clinical outcome is most closely related to
1721   peak concentration (e.g., tachycardia with sympathomimetics), C_{max} or an early exposure
1722   measure may be most appropriate for evaluation. Conversely, if the clinical outcome is
1723   related more to extent of absorption, AUC would be preferred. The frequency of
1724   sampling should be adequate to allow accurate determination of the relevant measures
1725   and/or parameters for the parent molecule and metabolites. For the substrate, whether the
1726   investigational drug or the approved drug, determination of the pharmacokinetics of
1727   relevant metabolites is important. Also, measurement of these metabolites may be useful

1728   to differentiate the effect of inhibitor/inducer on pathways mediated by different CYP
1729   enzymes.
1730
1731   *2.   Pharmacodynamic Endpoints*
1732
1733   Pharmacokinetic measures are usually sufficient for drug-drug interaction studies,
1734   although pharmacodynamic measures can sometimes provide additional useful
1735   information, especially for therapeutic proteins. Pharmacodynamic measures may be
1736   indicated when a pharmacokinetic/pharmacodynamic relationship for the substrate
1737   endpoints of interest is not established or when pharmacodynamic changes do not result
1738   solely from pharmacokinetic interactions (e.g., additive effect of quinidine and tricyclic
1739   antidepressants on QT interval). In most cases, when an approved drug is studied as a
1740   substrate, the pharmacodynamic impact of a given change in blood level (C_{max}, AUC)
1741   caused by an investigational interacting drug should be known from other data. If a
1742   PK/PD study is needed, it generally should include a larger population of
1743   subjects/patients than the typical PK study (e.g., a study of QT interval effects or platelet
1744   aggregation effects).
1745
1746   **G.   Statistical Considerations and Sample Size**
1747
1748   The goal of the interaction study is to determine whether there is any increase or decrease in
1749   exposure to the substrate in the presence of the interacting drug. If there is, its implications
1750   should be assessed by an understanding of PK/PD relations both for C_{max} and AUC.
1751
1752   Results of drug-drug interaction studies should be reported as 90% confidence intervals about
1753   the geometric mean ratio of the observed pharmacokinetic measures with (S+I) and without the
1754   interacting drug (S alone). Confidence intervals provide an estimate of the distribution of the
1755   observed systemic exposure measure ratio of (S+I) versus (S alone) and convey a probability of
1756   the magnitude of the interaction. In contrast, tests of significance are not appropriate because
1757   small, consistent systemic exposure differences can be statistically significant ($p < 0.05$), but not
1758   clinically relevant.
1759
1760   When a drug-drug interaction of potential importance is clearly present, the sponsor should
1761   provide specific recommendations regarding the clinical significance of the interaction based on
1762   what is known about the dose-response and/or PK/PD relationship for the substrate drug used in
1763   the study. This information can form the basis for reporting study results and for making
1764   recommendations in the labeling. FDA recognizes that dose-response and/or PK/PD information
1765   can sometimes be incomplete or unavailable, especially for an older approved drug used as a
1766   substrate.
1767
1768   If the sponsor wishes to include a statement in the labeling that no known drug-drug interaction
1769   of clinical significance exists, the sponsor should recommend specific *no effect* boundaries, or
1770   clinical equivalence intervals, for a drug-drug interaction and should provide the scientific
1771   justification for the recommendations. No effect boundaries represent the interval within which

1772   a change in a systemic exposure measure is considered not clinically meaningful. These
1773   conclusions can be based on dose-response data (e.g., if doses of x and 2x are known not to have
1774   different effectiveness or toxic effects) or on PK/PD modeling (a known flat concentration-
1775   response relationship).
1776
1777   There are two approaches to defining no effect boundaries:
1778
1779   *Approach 1:* No effect boundaries can be based on the population (group) average dose-related
1780   and/or individual concentration-response relationships derived from PK/PD models, and other
1781   available information for the substrate drug to define a degree of difference caused by the
1782   interaction that is of no clinical consequence. If the 90% confidence interval for the systemic
1783   exposure measurement change in the drug-drug interaction study falls completely within these
1784   no effect boundaries, the sponsor can conclude that no clinically significant drug-drug
1785   interaction is present.
1786
1787   *Approach 2:* In the absence of no effect boundaries defined in Approach 1, a sponsor can use a
1788   default no effect boundary of 80-125% for both the investigational drug and the approved drugs
1789   used in the study. When the 90% confidence intervals for systemic exposure ratios fall entirely
1790   within the equivalence range of 80-125%, standard Agency practice is to conclude that no
1791   clinically significant differences are present. This is, however, a very conservative standard and
1792   a substantial number of subjects (sample size) would need to be studied to meet it.
1793
1794   The selection of the number of subjects for a given drug-drug interaction study will depend on
1795   how small an effect is clinically important to detect or rule out the inter- and intra-subject
1796   variability in pharmacokinetic measurements, and possibly other factors or sources of variability
1797   not well recognized.
1798
1799
1800   **VI.   LABELING RECOMMENDATIONS**
1801
1802   Drug interaction information is generally included in the DRUG INTERACTIONS and
1803   CLINICAL PHARMACOLOGY sections of labeling and presents information that is essential
1804   for prescribers to appropriately use the drug. When drug interaction information has important
1805   implications for the safe and effective use of the drug, it will often be included in varying levels
1806   of detail in other sections of the labeling, such as DOSAGE AND ADMINISTRATION,
1807   CONTRAINDICATIONS or WARNINGS AND PRECAUTIONS. The labeling should include
1808   clinically relevant information about metabolic and transport pathways, metabolites,
1809   pharmacokinetic or pharmacodynamic interactions, and clinical implications of pharmacokinetic
1810   or pharmacodynamic interactions or genetic polymorphisms of drug metabolizing enzymes and
1811   transporters, if applicable. The description of clinical implications should include dose
1812   adjustments or monitoring recommendations, when relevant. General content recommendations
1813   for the appropriate labeling sections are provided below.
1814
1815   Drug interaction information in the labeling may not always result from a dedicated drug

1816 interaction study. In certain cases, information can be extrapolated from one drug interaction
1817 study with a set of drugs to another set of drugs, with an explanation that similar results are
1818 expected. For example:
1819

1820 • An investigational drug that is a strong inhibitor or a strong inducer of CYP3A does
1821 not need to be tested with all CYP3A substrates to warn about an interaction with
1822 sensitive CYP3A substrates and CYP3A substrates with a narrow therapeutic range.
1823 A study involving a single sensitive substrate with the investigational drug would
1824 lead to labeling language about the use of the investigational drug with all sensitive
1825 and NTR substrates of the affected enzyme.
1826

1827 • A drug that is a sensitive CYP3A substrate or a CYP3A substrate with a narrow
1828 therapeutic range does not need to be tested with all strong or moderate inhibitors or
1829 inducers of CYP3A to warn about an interaction with CYP3A inhibitors or inducers.
1830 The labeling can include such a warning in the absence of a study if its metabolism is
1831 predominantly by the CYP3A route.
1832

1833 **A.   Drug Interactions Section of Labeling**
1834

1835 The DRUG INTERACTIONS section includes a description of the clinical implications of
1836 clinically significant interactions with other drugs (including prescription and over-the-counter
1837 drugs), classes of drugs, dietary supplements, and foods and practical instructions for preventing
1838 or managing them. Recommendations for dose adjustments of co-administered drugs are
1839 included in this section. This section also includes practical guidance on known interference
1840 with laboratory tests. Interactions mentioned in DOSAGE AND ADMINISTRATION,
1841 CONTRAINDICATIONS, or WARNINGS AND PRECAUTIONS must be discussed in more
1842 detail in the DRUG INTERACTIONS section (21 CFR 201.57(c)(8)(i)). The need for dose
1843 adjustments of co-administered drugs is summarized in this section and presented in more detail
1844 in DOSAGE AND ADMINISTRATION. Drug interaction findings with negative results (i.e.,
1845 no interaction was found) should generally not appear in this section unless this information is
1846 clinically relevant for the prescriber (e.g., if two drugs are commonly used together or if a drug
1847 does not have the same interaction as other drugs in the same class). This section may also
1848 include a brief summary of potential mechanisms of drug interactions. (e.g., "Drug X is a strong
1849 CYP3A inhibitor and may increase concentrations of CYP3A substrates." or "Drug X does not
1850 inhibit or induce CYPs 1A2, 2C9, or 2C19."). This section does not include details of drug
1851 interaction studies, but instead cross-references the information in the CLINICAL
1852 PHARMACOLOGY section.
1853

1854 Drug interactions that have the most clinical relevance (e.g., result in serious or otherwise
1855 clinically significant outcomes) should be listed first. Because the number of drug interactions
1856 and complexity of the information in this setting varies, we recommend using the most
1857 appropriate format to enhance communication of the information. For example, for drugs with
1858 extensive drug interaction information, a table may be the most effective format to convey the
1859 information. The table can list, when applicable, the co-administered drugs, potential or known

1860   interactions (information regarding the increase or decrease in concentrations of drug, co-
1861   administered drug, or relevant metabolites), and clinical comments (clinical concern, dose
1862   adjustments, or advice regarding monitoring). When appropriate, the use of numbered
1863   subsections or subheadings within a subsection are recommended to organize the information
1864   (e.g., "Effect of Drug X on other drugs," "Effect of other drugs on Drug X," or subheadings for
1865   specific drugs or drug classes). Because this section may include information about both known
1866   and predicted drug interactions, it may be helpful to describe the data source for the information
1867   (e.g., indicate when the information is based on a specific drug interaction study and when it is
1868   based on a known mechanism, including simulation results, without a study).
1869
1870   **B.   Clinical Pharmacology Section of Labeling**
1871
1872   Information in the PHARMACOKINETICS subsection (12.3 Pharmacokinetics) of the
1873   CLINICAL PHARMACOLOGY section is generally organized under descriptive subheadings
1874   (e.g., absorption, distribution, metabolism, excretion, pharmacokinetics in specific populations,
1875   and drug interactions). The PHARMACOKINETICS subsection should include descriptive
1876   information related to mechanisms of drug interactions, and details of the relevant drug
1877   interaction study results. The text should cross-reference other sections of the labeling that
1878   describe clinical management instructions, dose adjustments, or major safety concerns related to
1879   drug interactions (e.g., WARNINGS AND PRECAUTIONS or CONTRAINDICATIONS).
1880
1881   If the drug is a metabolizing enzyme or transporter substrate, such information should be
1882   included in PHARMACOKINETICS under "Metabolism," the text should describe the
1883   metabolic pathway(s), relevant metabolites formed, specific drug metabolizing enzymes, and
1884   whether there is genetic variation in the drug metabolizing enzymes. If the drug is metabolized
1885   by an enzyme subject to genetic variability, the information should be included under
1886   "Metabolism" and cross-referenced to the fuller discussion under a PHARMACOGENOMICS
1887   subsection of the CLINICAL PHARMACOLOGY section.
1888
1889   Information under the "Drug Interactions" subheading includes a more detailed description of
1890   the potential mechanisms of drug interactions than the description in the DRUG
1891   INTERACTIONS section of labeling. The data source for the conclusions (e.g., known CYP3A
1892   inhibitor based on in vitro and in vivo studies) should be briefly described.
1893
1894   Under "Drug Interactions" study results may be presented in a forest plot (described below), a
1895   table, or as text, depending on the number of studies and level of detail needed for clarity. The
1896   information should include only those study features that are essential to understand the results.
1897   In most cases it is not necessary to include study design, number of subjects, or population (e.g.,
1898   healthy volunteers or patients) studied. The most relevant study design feature is likely the dose
1899   and duration for each drug; when relevant, the information should be included. The results
1900   should be presented as the change in relevant pharmacokinetic exposure measures (e.g., AUC
1901   and C_{max} and where appropriate C_{min}, T_{max}). It is important to indicate the variability of the
1902   interaction. Results should generally be presented as geometric mean change and the 90%
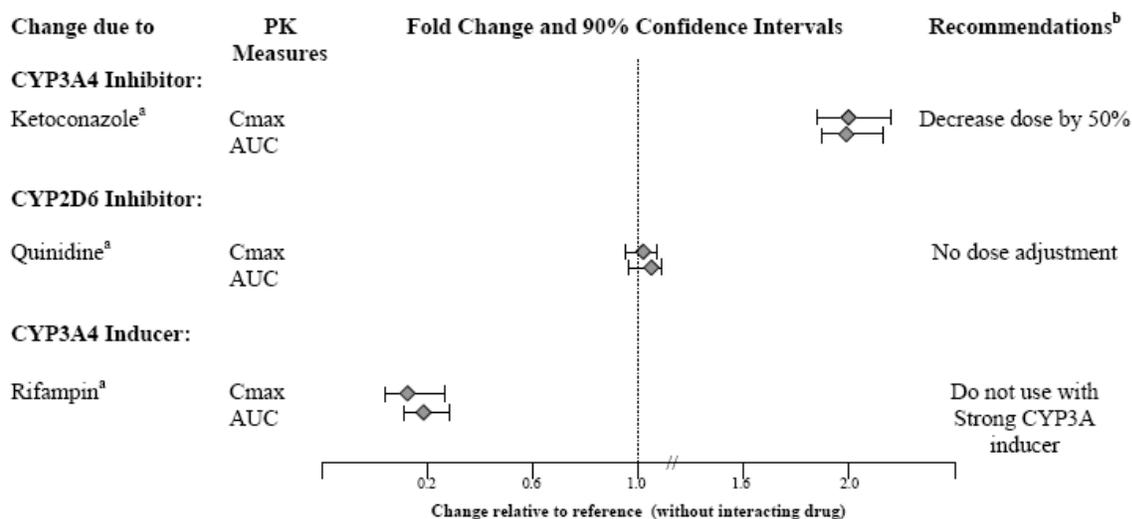1903   confidence interval around the geometric mean change. For example, a 48% percent increase in

1904 AUC could be expressed as ↑48% (90%CI: ↑24%, ↑76%) or as a ratio or fold change, where the
1905 48% percent increase would be expressed as 1.48 (90% CI: 1.24, 1.76).
1906
1907 In the PHARMACOKINETICS subsection, a forest plot is a useful tool for presenting changes in
1908 pharmacokinetic exposure measures caused by various intrinsic and extrinsic factors such as
1909 drug interactions, hepatic impairment, and renal impairment (see Figure 8 below). The forest
1910 plot should display the fold-change in key pharmacokinetic measures such as geometric mean
1911 AUC and geometric mean C_{max} along with the 90% confidence intervals. Such graphs should
1912 clearly state the reference arm (or identify it in text accompanying the figure) and can include the
1913 doses of studied drugs, if relevant. Separate plots can display the effect of others on the labeled
1914 drug, effects of the drug on other drugs, and the effects of impaired hepatic or renal function.
1915

1916 **Figure 8. The Effect of Various CYP Inhibitors on a Hypothetical Drug's PK as Displayed**
1917 **as 90% Confidence Interval of Geometric Mean AUC and C_{max} Ratios.**

1918

Change due to	PK Measures	Fold Change and 90% Confidence Intervals	Recommendations[b]
CYP3A4 Inhibitor:			
Ketoconazole[a]	Cmax		Decrease dose by 50%
	AUC		
CYP2D6 Inhibitor:			
Quinidine[a]	Cmax		No dose adjustment
	AUC		
CYP3A4 Inducer:			
Rifampin[a]	Cmax		Do not use with Strong CYP3A inducer
	AUC		

Change relative to reference (without interacting drug)

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

1919
1920 **C.   Other Labeling Sections**
1921
1922 As stated above, when drug interaction information has important implications for the safe and
1923 effective use of the drug, the information may be distributed among several other labeling
1924 sections (e.g., DOSAGE AND ADMINISTRATION, CONTRAINDICATIONS, WARNINGS
1925 AND PRECAUTIONS, or PATIENT COUNSELING INFORMATION), with a cross-reference
1926 to the DRUG INTERACTIONS or CLINICAL PHARMACOLOGY sections for more detailed
1927 information.
1928
1929   • DOSAGE AND ADMINISTRATION — This section includes information about
1930   drug interaction information that has important implications for a drug's dosing
1931   regimen (e.g., dosage adjustments, timing of dose relative to dosing of another drug).
1932
1933   • CONTRAINDICATIONS — This section describes when other drugs should not be
1934   co-administered with the drug because the risk outweighs any potential benefit.
1935
1936   • WARNINGS AND PRECAUTIONS — This section includes a brief discussion of
1937   any known or predicted drug interactions with serious or otherwise clinically
1938   significant outcomes.
1939
1940   • PATIENT COUNSELING INFORMATION — This section includes information
1941   necessary for patients to use the drug safely and effectively, such as avoiding
1942   drinking grapefruit juice.
1943
1944 For more specific recommendations on labeling content for these sections of labeling, refer to the
1945 following guidances for industry: *Warnings and Precautions, Contraindications, and Boxed*
1946 *Warning Sections of Labeling for Prescription Drug and Biological Products – Content and*
1947 *Format,* and *Dosage and Administration Section of Labeling for Human Prescription Drug and*
1948 *Biological Products – Content and Format.* These guidances and other labeling guidances are
1949 available at
1950 http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065010.htm.
1951

1952
1953   **List of Figures in the Appendix**
1954

1969 **APPENDIX**
1970
1971 **Models for Determining When In Vivo Transporter-Mediated Drug**
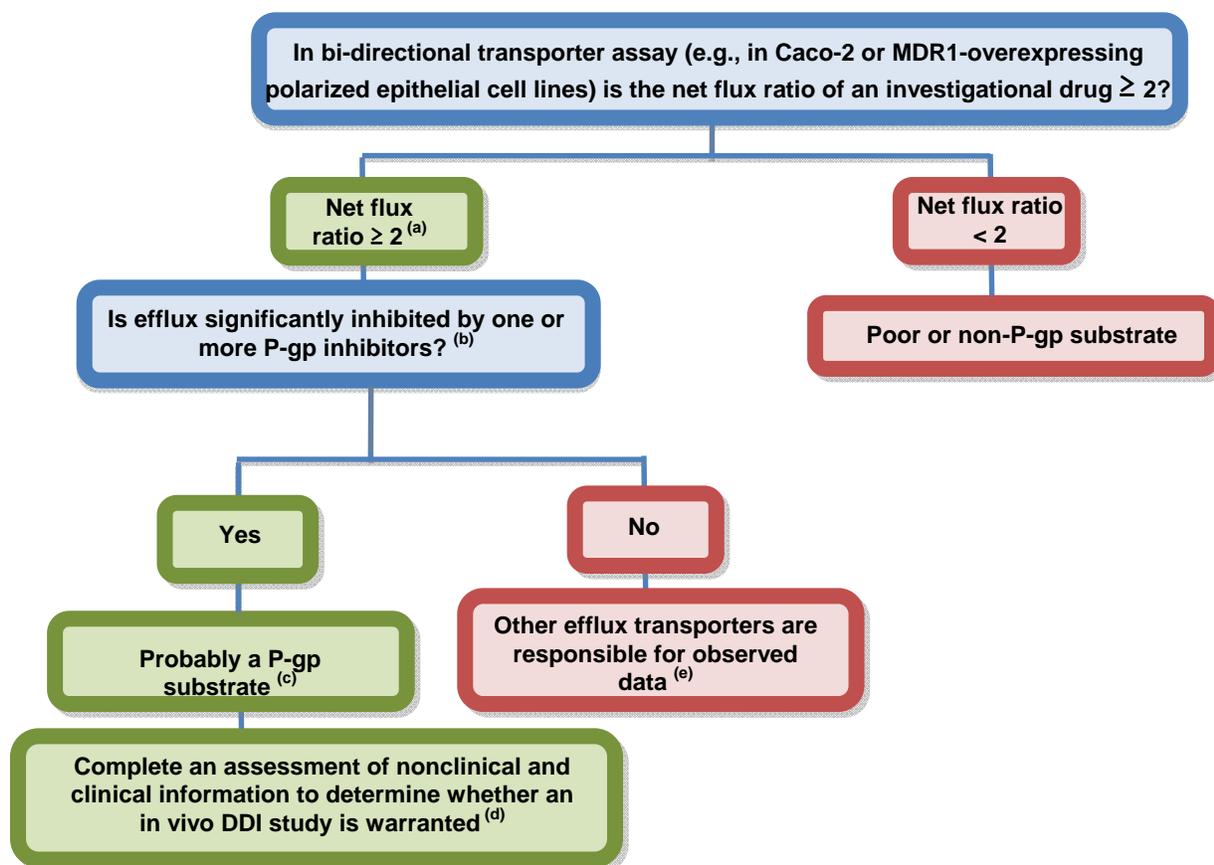1972 **Interaction Studies Are Needed**
1973
1974 *P-gp and BCRP:*
1975
1976 **Figure A1. Decision tree to determine whether an investigational drug is a substrate for P-**
1977 **gp and when an in vivo clinical study is needed. A similar model can be applied to a BCRP**
1978 **substrate — refer to IV.A.2.a, Figure 6 (Modified From Figures in Giacomini et al. 2010).**
1979

1980
1981
1982 [a] An acceptable system produces net flux ratios of probe substrates similar to the literature values. A net flux ratio
1983 ≥ 2 for the investigational drug is a positive signal for further evaluation. A net flux ratio "cutoff" higher than 2 or a
1984 relative ratio to positive controls may be used to avoid false positives if a ratio of 2 is deemed non-discriminative as
1985 supported by prior experience with the cell system used.
1986 [b] Reduction of the flux ratio significantly (> 50%) or to unity.
1987 [c] Additional data are needed to establish clinical relevance of the in vitro data. In particular, the relative
1988 contribution of the transporter-mediated pathway to the overall clearance of the drug is the primary determinant of
1989 whether an inhibitor will have a major effect on the disposition of the investigational new drug.
1990 [d] Selection of inhibitors could be based on likelihood of co-administration and/or its inhibition potency on P-gp.

1991   Strong P-gp inhibitors (e.g., itraconazole, verapmil) provide the most sensitive assessment and should generally be
1992   tested first. If the drug is also a substrate for CYP3A, then inhibitors for both CYP3A and P-gp should be selected
1993   (Table 8).
1994   [e] Based on existing knowledge of the compound class, further studies may be warranted to determine which efflux
1995   transporters are involved. Determining whether the drug is a BCRP substrate may be explored. A similar decision
1996   model may be used for a BCRP substrate; however, clinical studies would differ.
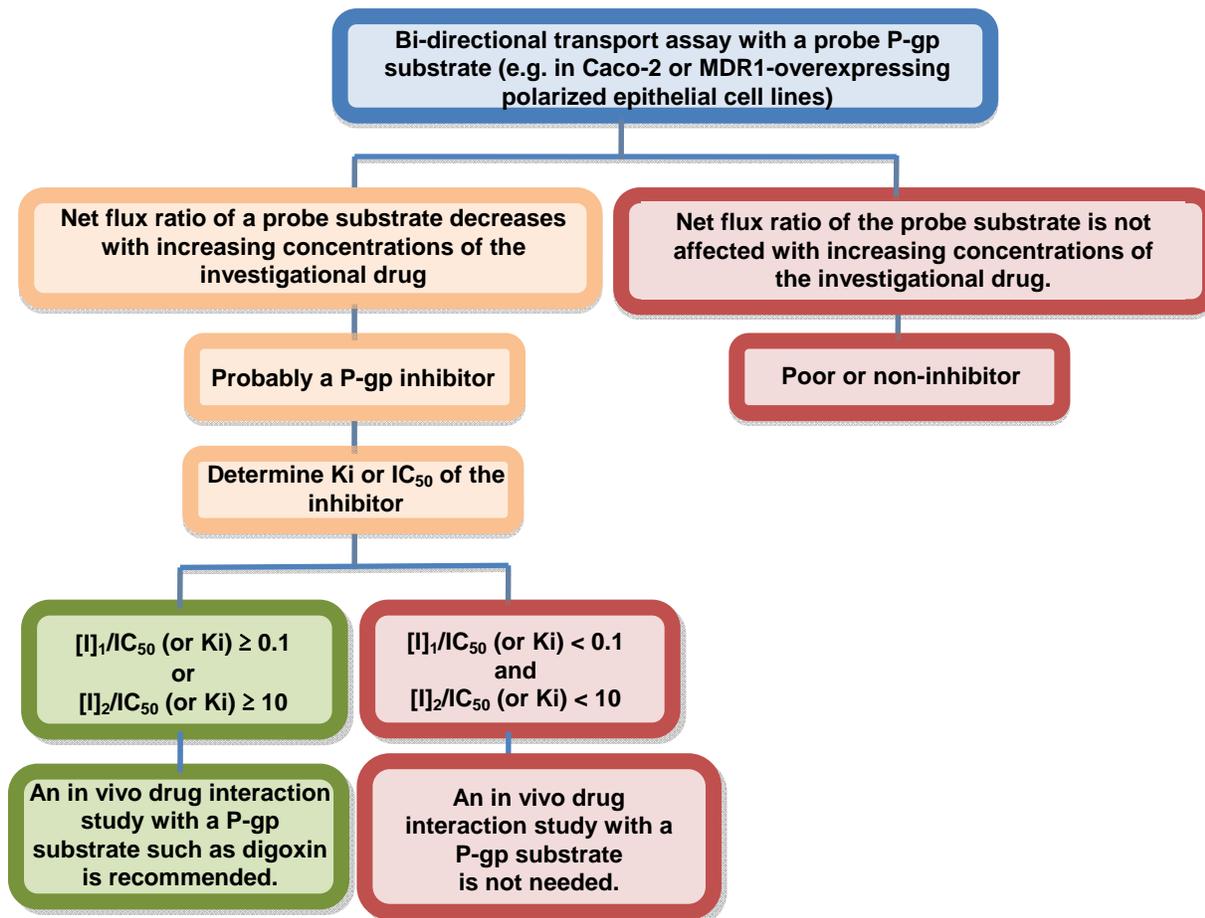
1997

1998   **Figure A2. Decision tree to determine whether an investigational drug is an inhibitor of P-**
1999   **gp and when an in vivo clinical study is needed. A similar model can be applied to a BCRP**
2000   **inhibitor) — refer to IV.A.2.b (Modified From Figures in Giacomini et al. 2010)**
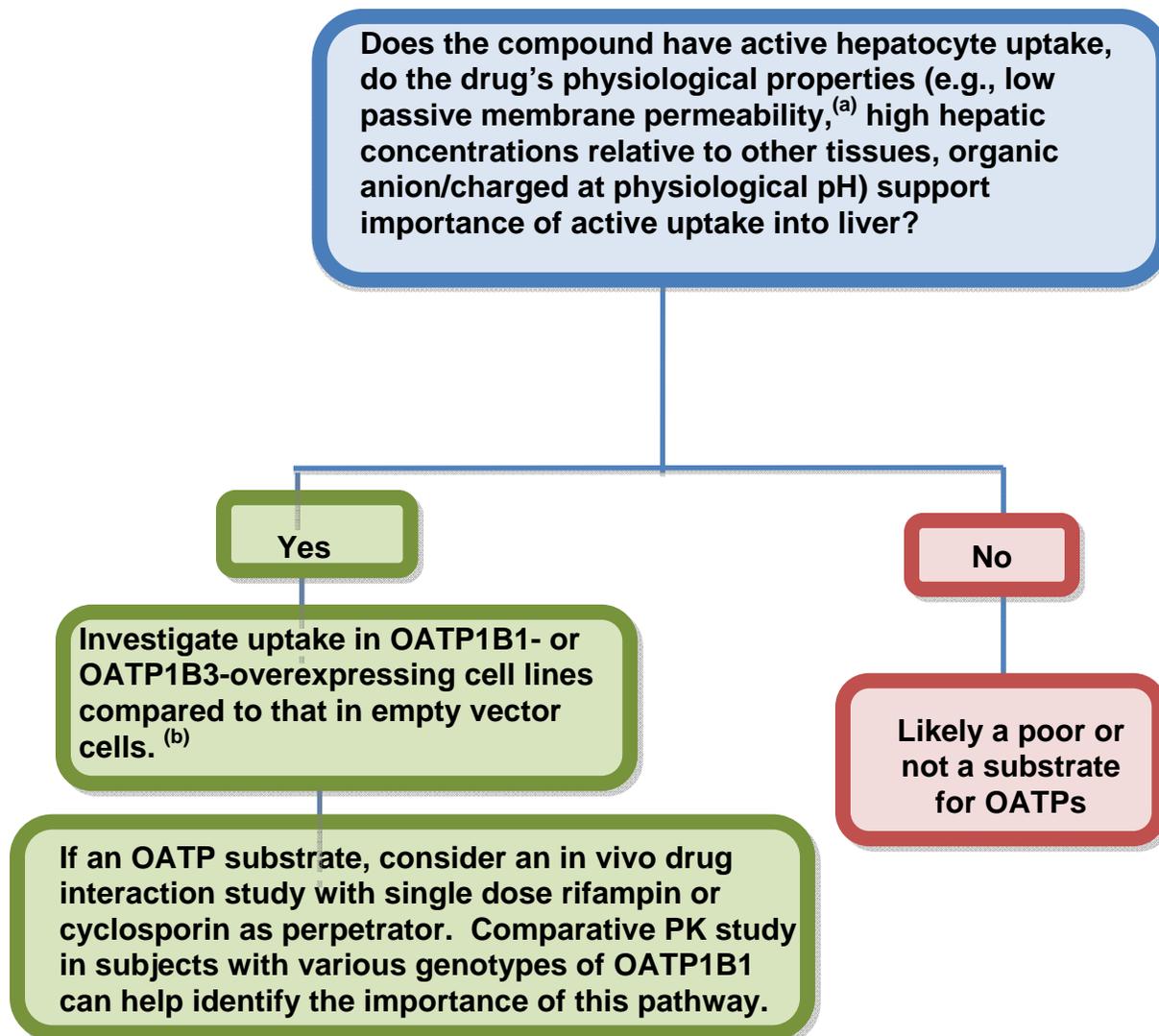
2001

2002
2003
2004 $[I]_1$ represents the mean steady-state total (free and bound) C_{max} following administration of the highest proposed
2005   clinical dose. $[I]_2$= Dose of inhibitor (in mol)/250 mL (if IC_{50} is in a molar unit). For IC_{50} determination, a
2006   unidirectional assay (e.g., B to A) based on the probe substrate can also be considered.
2007
2008

2009   *OATP1B1 and OATP1B3 (Liver uptake transporters):*
2010

2011   **Figure A3. Decision tree to determine whether an investigational drug is a substrate for**
2012   **OATP1B1 or OATP1B3 and when an in vivo clinical study is needed— refer to IV.A.2.a,**
2013   **Figure 6 (Modified From Figures in Giancomini et al. 2010)**

Does the compound have active hepatocyte uptake, do the drug's physiological properties (e.g., low passive membrane permeability,[a] high hepatic concentrations relative to other tissues, organic anion/charged at physiological pH) support importance of active uptake into liver?

Yes

No

Investigate uptake in OATP1B1- or OATP1B3-overexpressing cell lines compared to that in empty vector cells. [b]

Likely a poor or not a substrate for OATPs

If an OATP substrate, consider an in vivo drug interaction study with single dose rifampin or cyclosporin as perpetrator. Comparative PK study in subjects with various genotypes of OATP1B1 can help identify the importance of this pathway.

2014
2015   [a] Low permeability needs to be defined by each lab based on standards, such as atenolol (a biopharmaceutics
2016   classification system (BCS) reference drug). A general guide would be that 10^{-6} cm/sec (10 nm/sec) or lower is
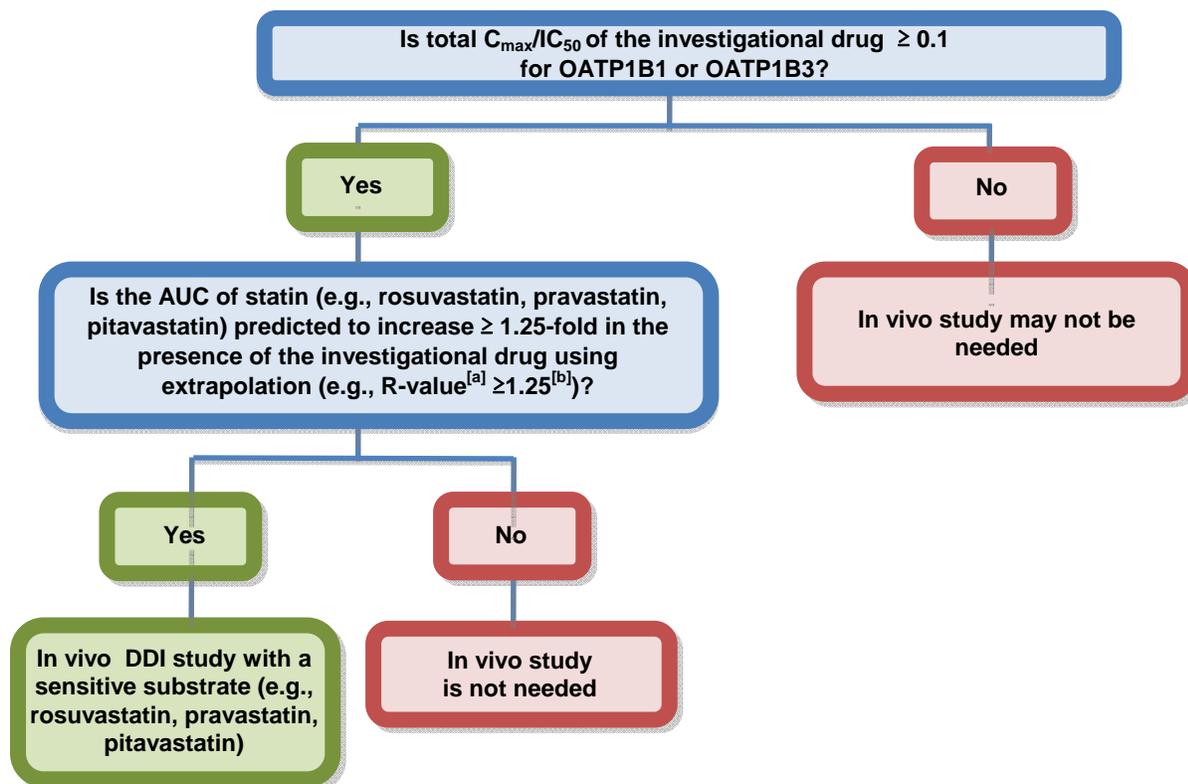2017   classified as "low" permeability.
2018   [b] The following criteria suggest the investigational drug is a substrate of OATP1B1 or OATP1B3: Uptake in
2019   OATP1B1- or OATP1B3-transfected cells greater than 2-fold of that in empty vector transfected cells and is
2020   inhibitable (e.g, >50% reduction to unity) by a known inhibitor (e.g., rifampin) at a concentration at least 10 times
2021   of its K_i. Michaelis–Menten studies may be conducted in the transfected cells to determine the kinetic parameters of
2022   the investigational drug. A positive control should be included. In an acceptable cell system, the positive control
2023   should show a ≥ 2 fold increase in uptake compared to vector-transfected cells. An uptake ratio (transporter
2024   transfected vs. empty vector transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative
2025   as supported by prior experience with the cell system used.

2026

2027 **Figure A4. Decision tree to determine whether an investigational drug is an inhibitor of**
2028 **OATP1B1 or OATP1B3 and when an in vivo clinical study is needed — refer to IV.A.2.b**
2029 **(Modified From Figures in Giancomini et al. 2010)**

2030

Is total C_{max}/IC_{50} of the investigational drug ≥ 0.1 for OATP1B1 or OATP1B3?

- **Yes**
  - **Is the AUC of statin (e.g., rosuvastatin, pravastatin, pitavastatin) predicted to increase ≥ 1.25-fold in the presence of the investigational drug using extrapolation (e.g., R-value[a] ≥1.25[b])?**
    - **Yes**
      - **In vivo DDI study with a sensitive substrate (e.g., rosuvastatin, pravastatin, pitavastatin)**
    - **No**
      - **In vivo study is not needed**
- **No**
  - **In vivo study may not be needed**

2031
2032
2033 [a] R-value = 1+ (fu x $I_{in,max}/IC_{50}$), where, $I_{in,max}$ is the estimated maximum inhibitor concentration at the inlet to the
2034 liver and is equal to: $C_{max} + (k_a$ x Dose x $F_a F_g/Qh)$. C_{max} is the maximum systemic plasma concentration of
2035 inhibitor; Dose is the inhibitor dose; F_aF_g is the fraction of the dose of inhibitor which is absorbed; k_a is the
2036 absorption rate constant of the inhibitor and Qh is the estimated hepatic blood flow (e.g., 1500 mL/min). If Fa Fg
2037 values and ka values are unknown, use 1 and 0.1 min^{-1} (Ito et al. 1998) for FaFg and ka, respectively because the
2038 use of theoretically maximum value can avoid false-negative prediction. For drugs whose fu values are less than
2039 0.01 or fu cannot be accurately determined due to high protein-binding, then assume fu = 0.01, to err on the
2040 conservative side to avoid false negative predictions.
2041
2042 [b]These are the suggested values according to the upper limit of equivalence range. We are open to discussion
2043 based on sponsors' interpretation.
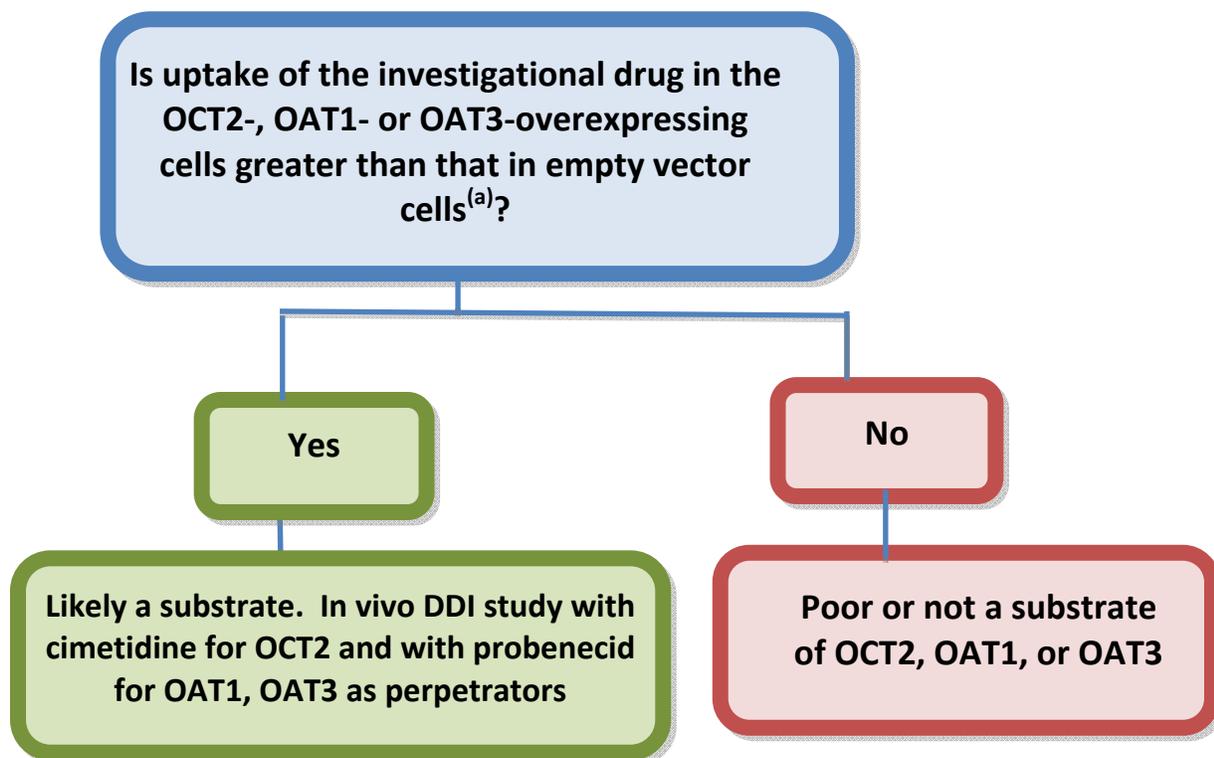
2044   *OCT2, OAT1, and OAT3 (renal transporters):*

2045

2046   **Figure A5. Decision tree to determine whether an investigational drug is a substrate for**
2047   **OCT2, OAT1, or OAT3 and when an in vivo clinical study is needed — refer to IV.A.2.a,**
2048   **Figure 6 (Modified From Figures in Giancomini et al. 2010)**

2049

> **Is uptake of the investigational drug in the OCT2-, OAT1- or OAT3-overexpressing cells greater than that in empty vector cells[a]?**
>
> **Yes** → **Likely a substrate. In vivo DDI study with cimetidine for OCT2 and with probenecid for OAT1, OAT3 as perpetrators**
>
> **No** → **Poor or not a substrate of OCT2, OAT1, or OAT3**

2050
2051
2052   [a] The ratio of the investigational drug uptake in the cells expressing the transporter versus the control (or empty
2053   vector) cells should be greater than 2. It is important that uptake into the transfected cells be significantly greater
2054   than background in a control cell line and be inhibited by a known inhibitor of the transporter. Michaelis–Menten
2055   studies may be conducted in the transfected cells to determine the kinetic parameters of the investigational drug. A
2056   positive control should be included. In an acceptable cell system, the positive control should show a ≥ 2 fold
2057   increase in uptake compared to vector-transfected cells. An uptake ratio (transporter transfected vs. empty vector
2058   transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative as supported by prior
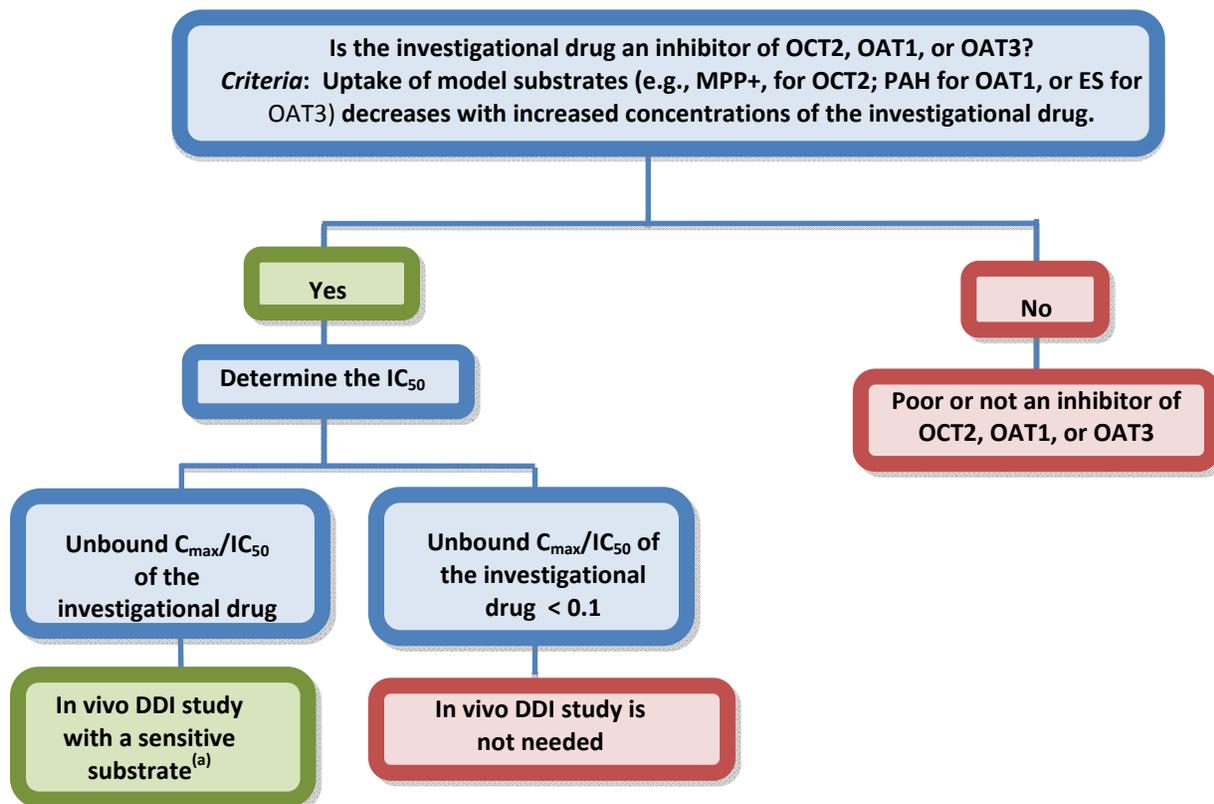2059   experience with the cell system used.
2060
2061

2062

2063 **Figure A6. Decision tree to determine whether an investigational drug is an inhibitor of**
2064 **OCT2, OAT1, or OAT3 and when an in vivo clinical study is needed — refer to IV.A.2.b**
2065 **(Modified From Figures in Giancomini et al. 2010)**

2066

2067
2068
2069
2070 MPP^{+}, 1-methyl-4-phenylpyridinium; PAH, *para*-aminohippuric acid; ES, estrone-3-sulfate.
2071
2072 [a] For the investigational drug that is an OCT2 inhibitor, metformin may be used as the substrate for the clinical
2073 drug interaction study.
2074
2075 For investigational drugs that are OAT1 or OAT3 inhibitors, multiple OAT1 or OAT3 substrates could be used in
2076 clinical DDI studies, including zidovudine, acyclovir, ciprofloxacin, tenofovir, or methotrexate.
2077
2078

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2230
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2233   **ABBREVIATIONS**

2234

ABC: ATP-binding cassette

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

AhR: aryl hydrocarbon receptor

AUC: area under the plasma concentration-time curve

BCRP: breast cancer resistance protein

BCS: biopharmaceutical classification class

BLA: biologics license appliaction

BSEP: bile salt export pump

CAR: constitutive androstane receptor

CCB: calcium channel blocker

CYP: Cytochrome P450

EM: extensive metabolizers

FMO: flavin monooxygenase

INR: international normalized ratio

LST: liver specific transporter

MAO: monoamine oxidase

MATE: multidrug and toxin extrusion

MRP: multidrug resistance-associated protein

NDA: new drug application

NTCP: sodium/taurocholate cotransporting polypeptide

NTR: narrow therapeutic range

OAT: organic anion transporter

OATP: organic anion transporting polypeptide

OCT: organic cation transporter

PBPK: physiologically-based pharmacokinetic

PD: pharmacodynamics

P-gp: P- glycoprotein

PK: pharmacokinetics

PM: poor metabolizers

PXR: pregnane X receptor

SLC: solute carrier

TDI: time dependent inhibition

TdP: torsade de pointes

TP: therapeutic protein

UGT: uridine diphosphate (UDP)-glucuronosyl transferase

XO: xanthine oxidase

2235