The AAPS Journal, Vol. 15, No. 3, July 2013 (© 2013)
DOI: 10.1208/s12248-013-9470-x

Commentary

Drug–Drug Interaction Studies: Regulatory Guidance and An Industry Perspective

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Received 27 November 2012; accepted 25 February 2013; published online 30 March 2013

Abstract. Recently, the US Food and Drug Administration and European Medicines Agency have issued new guidance for industry on drug interaction studies, which outline comprehensive recommendations on a broad range of *in vitro* and *in vivo* studies to evaluate drug–drug interaction (DDI) potential. This paper aims to provide an overview of these new recommendations and an in-depth scientifically based perspective on issues surrounding some of the recommended approaches in emerging areas, particularly, transporters and complex DDIs. We present a number of theoretical considerations and several case examples to demonstrate complexities in applying (1) the proposed transporter decision trees and associated criteria for studying a broad spectrum of transporters to derive actionable information and (2) the recommended model-based approaches at an early stage of drug development to prospectively predict DDIs involving time-dependent inhibition and mixed inhibition/induction of drug metabolizing enzymes. We hope to convey the need for conducting DDI studies on a case-by-case basis using a holistic scientifically based interrogative approach and to communicate the need for additional research to fill in knowledge gaps in these areas where the science is rapidly evolving to better ensure the safety and efficacy of new therapeutic agents.

KEY WORDS: drug–drug interaction studies; industry perspective; regulatory guidance.

INTRODUCTION

Drug–drug interactions (DDI) can cause profound clinical effects, either by reducing therapeutic efficacy or enhancing toxicity of drugs. DDIs are one of the major causes for drug withdrawal from the market (1). These factors when considered together with an increasing frequency in polypharmacy has prompted regulatory agencies, including the Food and Drug Administration (FDA) and European Medicines Agency (EMA), to publish guidance documents for industry that are designed to help pharmaceutical researchers characterize and better understand DDI potential for a new molecular entity (1,2). While DDI can result in alterations of either drug pharmacokinetics (PK), pharmacodynamics (PD) or both, it is the PK interactions that have been the main focus for both the US and EU DDI guidelines. PK drug interactions, typically characterized by alterations of plasma concentration–time profiles, could be mediated *via* mechanistic changes in processes of absorption, distribution, metabolism, and/or excretion (ADME) of a drug substance (victim) by another compound (perpetrator) when they are given concomitantly. In both the EU and USA, the first DDI guidance was published in 1997 (1,3), and the most recent guidance version has been issued as a draft in 2010 (EMA) or 2012 (USA) (4). Compared to earlier editions, both of the new draft guidance documents contain much more extensive recommendations on *in vitro* and *in vivo* studies. They suggest approaches to evaluate DDIs mediated *via* cytochromes P450 (CYPs) as well as non CYP enzymes, and including those in emerging areas of science such as modeling approaches and transporters. The latest revisions are conceivably based on the considerable scientific progress made over the last two decades towards understanding the role of drug metabolizing enzymes in DDIs, as well as increasing numbers of reports on effects related to transporters. The EMA final version has recently been issued in July 2012, with an effective date of January 2013 (5). The US guidance has yet to be finalized; reviews of public comments from the due date May 2012 are presumably in progress, and therefore it is possible that the recommendations in the current draft may not be part of the final recommendations. Nevertheless, the newer aspects of the draft guidance seems to have already been implemented at the FDA, as Merck Research Laboratories has already received several specific recommendations from FDA reviewers that referenced the latest draft guidance.

Electronic supplementary material The online version of this article (doi:10.1208/s12248-013-9470-x) contains supplementary material, which is available to authorized users.

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The primary objectives of this paper are to (1) provide an overview on the new draft of the US and final EU guidance documents, with respect to their similarities/differences in the recommended DDI studies and associated approaches, and (2) highlight potential complications associated with aspects of the proposed emerging approaches to evaluate transporter-mediated DDIs and application of model-based predictions in early drug development for predicting potential for DDIs arising from time-dependent inhibitors (TDI) and mixed TDI/inducers. We exemplify our concerns with case examples gathered from the literature and our own internal drug development programs. Finally, we put forward an approach from an industry perspective in applying the regulatory guidance to support drug development. It is our intention that this paper would promote a continued scientific dialog on the optimal means for implementing DDI research and be helpful in molding guidance recommendations in the aforementioned evolving areas.

OVERVIEW OF THE LATEST REGULATORY GUIDANCE ON DDI STUDIES: WHAT IS NEW

Both US and EU guidance documents contain many new features and are similar in concepts. The key common updates can be summarized as follows:

- An increased emphasis on transporter-based DDI evaluations, involving both *in vitro* and *in vivo* studies of many different transporters.
- A more detailed guidance on how to incorporate results from *in vitro* enzyme/transporter studies to inform the nature and extent of clinical DDI studies. Application of model-based approaches, and especially physiologically based pharmacokinetic (PBPK), are highly recommended. Results from PBPK analysis carries more weight than other models.
- An expansion of the guidance on DDI evaluations to include metabolites.
- An emphasis on the need to understand and characterize the impact of various enzyme/transporter genotypes and other complex DDIs

Some specifics are described below.

FDA Draft Guidance

Compared to the 2006 Guidance version, many more studies have been added in this new guidance (Table I). With respect to studies on drug metabolizing enzymes, key changes include recommendations on approaches to studying DDIs for substrates of UDP-glucuronosyltransferases (UGTs; Fig. 2 of the draft guidance). Surprisingly, guidance does not mention DDI evaluations for UGT inhibitors. For *in vitro* evaluations of CYPs, there were no significant changes except explicit inclusion of time-dependent inhibition to be studied for all major CYPs. For enzyme induction studies, an evaluation of the change in mRNA levels is now the recommended end point replacing the previous recommendation of using enzyme activity measurement. Other major changes center around recommended model-based approaches and associated criteria in using *in vitro* CYP inhibition and induction results to determine the need for *in vivo* DDI studies. These approaches are recommended for not only CYP inhibitors or inducers, but also compounds demonstrating time-dependent inhibition and those with mixed inhibition and induction effects (Fig. 4 of the draft guidance). The models included are both basic and mechanistic (static and PBPK) in nature. In particular, mechanistic PBPK models have been emphasized and mentioned numerous times in the guidance. Indeed, while the agency recommends a stepwise, model-based evaluation of metabolism-based interactions starting from a basic model for initial assessment, the criteria set for the basic (based on total C_{max}) and mechanistic static models (with "AUCR" values; fold changes in area under plasma concentration–time curve, AUC, of a probe substrate in the presence and absence of a perpetrator, of 0.8–1.25) are so conservative such that PBPK will ultimately be needed. There are also specific recommendations with respect to testing DDI potential due to metabolites, especially those with circulating levels of ≥25% or more of the parent compounds' exposures.

On the transporter front, six additional transporters (organic anion transporting polypeptides OATP1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*), organic cation transporter OCT2 (*SLC22A2*), organic anion transporters OAT1 (*SLC22A6*) and OAT3 (*SLC22A8*), and breast cancer resistance protein BCRP (*ABCG2*)) have been included on the list to be studied, on top of the existing P-glycoprotein (Pgp, *ABCB1*). The guidance on transporters is rather detailed with many decision trees, mirroring what had been proposed by the International Transporter Consortium (ITC) with respect to considerations as to when to conduct *in vitro* and *in vivo* transporter studies, and including a listing of recommended inhibitors and probe substrates (6), but with somewhat more stringent criteria. Specifically, a more stringent cutoff of 25% for the involvement of hepatic elimination or renal secretion is recommended by FDA, to trigger evaluation of drugs as substrates of OATP1B or OCT/OAT, instead of 30% or 50% cutoff, respectively, recommended by the ITC. Additionally, the FDA recommends using total C_{max} (vs unbound C_{max} by ITC) to estimate the value for inhibitor concentration "I_1" for BCRP and Pgp inhibitors, and the cut-off "R" (fold change in exposure of statins, OATP1B substrates) value of 1.25 calculated based on an absorption rate constant ka of 0.1 min^{-1} (vs "R" value of 2 calculated based on the *ka* value of 0.03 min^{-1} by ITC) for OATP1B inhibitors (see additional details in the "Transporter-based DDIs" section below).

With respect to *in vivo* DDI studies, one key change involves recommendations to include a third arm in clinical studies to evaluate the time required for activity to return to baseline when evaluating an enzyme inducer or TDI. For compounds with concurrent inhibition/induction (such as rifampin which is a known OATP1B inhibitor and CYP3A inducer), additional study arms may be needed to determine appropriate timing of administration of substrate and duration of interaction effect. Additionally, both UGT1A1 and OATP1B1 have now been included on the recommended list for genotyping or phenotyping studies, expanding the original panel of polymorphic enzymes CYP2C9, 2C19, and 2D6. For the first time, the guidance also includes recommendations to conduct drug interaction studies for therapeutic proteins, with primary focuses on *in vivo* (much less on *in vitro*) clinical studies for cytokines or cytokine modulators. The changes include recommended studies for these protein-based drug products that will be used in combination therapy with other drug products (small molecule or proteins) or those with the potential for PK- or PD-related

Table I. List of New *In Vitro* Studies Recommended by the Latest Regulatory (US and EU) Guidance

	Enzymes	Transporters
Substrate	• UGTs decision tree for substrates with ≥25% contribution of total clearance (US only)	• Pgp[a], BCRP for all NCEs • OATP1B1; 1B3 (≥25% hepatic elimination); OCT2, OAT1; OAT3 (≥25% renal secretion) • BCS class I waived (US only)
Inhibitor	• UGT1A1; UGT2B7 (EU only)	• Pgp[a], BCRP, OATP1B1/3; OCT2; OAT1/3 (all NCEs); BSEP (EU only)
Inducer	• Emphasis on mRNA end point	• *In vitro* studies not recommended; *in vivo* studies maybe required on a case by case basis
Metabolite considerations	• Metabolites with ≥25% of parent AUC (and ≥10% of total for EU)	• Metabolites with ≥25% of parent AUC (and ≥10% of total for EU)

BSEP human bile salt export pump
[a] Not new for US

DDIs based on prior experience/known mechanisms. The recommendation to conduct clinical DDI studies are particularly important if specific labeling language is sought. In an absence of the studies, general labeling language indicating potential for interactions with CYPs or transporters is allowed for cytokines/cytokine modulators.

EMA Guidance

Similarly, there are numerous changes in the latest EMA guidance as compared to the 1997 version (Table I). While the latest EMA guidance is similar in concept to the 2012 draft US guidance, it contains some major differences. Key differences summarized in Table II include overall style, the type of recommended *in vitro* studies, approaches and criteria to help determine whether *in vivo* clinical studies are needed. Notably, the EU, and not the USA, provides guidance on timing as to when studies should be performed (e.g., most of *in vitro* studies before phase II start). In addition, the criteria used for CYP inhibition, especially for the basic model, to trigger *in vivo* clinical studies are quite different between the two agencies. The EU favors the use of unbound plasma concentrations to reflect the concentration of the inhibitor at the site where the enzymes reside, whereas the USA recommends total plasma concentrations be used for the front line predictions, conceivably to force further considerations to the proposed funneling system with mechanistic-based models as the next in line. Such differences in guidance recommendation can pose a difficult challenge to drug developers especially when the same experimental data may give rise to different predictions and interpretations of the potential for drug interactions based upon these different specifications by the authorities. For example, a compound with a very low plasma protein binding value may be considered exhibiting low potential for DDIs not requiring a clinical DDI study based on the US but not the EU guidance, and *vice versa*. Additionally, while the US provides specific recommendations on the use of predictive models based on *in vitro* data for compounds with mixed inhibitory and induction effects on CYPs, the EU specifically states that *in vivo* clinical studies need to be conducted for these compounds, given limited experience in DDI predictions for the mixed

Table II. Key Differences Between US and EU Recommendations

		USA	EU
Styles	• Overall	• Prescriptive	• More conceptual
	• Decision tree	• Many (12 total)	• Limited
	• Table-substrate/inhibitor/inducer	• Laundry list of possible	• Distilled list
	• Study timing	• No guidance (ultimately before NDA filing)	• Most *in vitro* before PhII
Enzymes	• CYP inhibition (basic criteria)	• C_{max} total (10-x factor)	• C_{max}, free (fu≥1%, 50-x factor)
	• CYP Induction	• No mention on RIS	• Include RIS (unbound Cinlet)
	• Mixed inhibitor/inducer	• Mechanistic model	• *In vitro* not recommended
	• Other enzymes- substrate	• UGT decision tree	• No specifics
	• Other enzymes- inhibitor	• None	• Include UGT1A1 and 2B7
Transporters	• Substrate	• BCS class I waiver	• Not mention
	• Inhibitor (OATP1B criteria)	• 10x-total C_{max}; R value	• 25-x unbound Cinlet
Metabolites	• Victim	• 25% of parent AUC	• Active (50% of total activity)
	• Perpetrator	• 25% of parent AUC	• 25% of parent+10% total
Protein binding	• Displacement study	• No guidance	• Highly bound compounds (fu<1%)
Therapeutic protein		• Decision tree for immunomodulators+others	• None (a separate guidance specifies immunomoderator related DDI)

BSEP human bile salt export pump

inhibitors/inducers. Interestingly, unlike the USA, the EU provides no specific guidance on evaluation of UGT substrates, but instead explicitly recommends testing compounds for inhibition of UGT1A1 and 2B7.

Other major differences are in the area of transporters. The EU guidance includes human bile salt export pump (BSEP) as another transporter to be studied, primarily for safety reasons, and provides only a more general guidance on transporter studies *versus* a prescriptive decision tree approach proposed in the US draft guidance. Similar to the criteria used for drug metabolizing enzymes, the EU uses unbound plasma concentration (vs total concentrations by the US) for establishing the transporter criteria (see additional details in the "Transporter-based DDIs" section below). The EU also provides more clarity on the need to conduct *in vitro* and *in vivo* DDI studies related to metabolites. Furthermore, the EU, but not the USA, includes guidance on protein binding displacement. In contrast, the EU guidance focuses only on small molecule drugs; the EU provides more limited guidance on DDIs related to therapeutic proteins, primarily immunomodulators, in a separate guidance document (7).

POTENTIAL COMPLICATIONS ASSOCIATED WITH SOME OF THE RECOMMENDED DDI STUDY ASPECTS

In this section, we present a number of theoretical considerations and specific examples, on issues surrounding some of the proposed recommendations in emerging areas. Specifically, potential complications associated with transporter-based DDIs and model-based approaches for predicting time-dependent effects, including mixed enzyme inhibition–induction, are highlighted and discussed in depth.

Transporter-Based DDIs

In contrast to CYP-mediated DDIs where knowledge gained over the past 20–30 years has established a foundation for more quantitative predictions of drug clearance and potential for DDI, our understanding of the potential for DDI arising from interactions with transporters is still in its infancy. Indeed, the EU guidance, while emphasizing the importance of transporter-based DDI evaluations, only provides a more general recommendation on study approaches, acknowledging that *in vitro*/*in vivo* extrapolation of drug transporter interactions is currently less mature and requires additional experience and continued scientific development. Presented below are four key scientific considerations to help illustrate the complications associated with evaluations of transporter-mediated DDIs in general and pertaining to the regulatory guidance, supportive of our view that given the current stage of knowledge in this field, it is premature to provide and apply a detailed guidance on transporter studies and associated criteria on a broad basis.

Complications Related to Multiplicity of Transporters

Unique to transporters is their multiplicity nature, which poses one of the greatest challenges to our understanding of the role of a specific transporter in drug disposition and its impact on DDIs. Transporters, uptake or efflux, are directional, have broad tissue distribution, and are involved in multiple drug disposition processes. Many transporters have overlapping substrate specificity which causes the functional redundancy. These transporters may work in sequential transport steps, parallel directions, or countercurrent (opposite) directions. For sequential transport activities, the impact of transporter inhibition on drug disposition will depend on whether the transporter is rate limiting to the overall disposition process. In the case of parallel or countercurrent transport processes, the impact of individual transporters will be dependent on their relative contributions to the overall process. Further complicating this, inhibition of one transporter can be compensated for by another such that there are no measurable effects on drug disposition (examples are provided in subsequent sections below; additional examples can be found from references 15 and 27 in Table III). In such cases, extrapolating *in vitro* inhibition studies to *in vivo* PK implications can be misleading. Moreover, if both the perpetrator and the victim interact with multiple transporters, the effects can be unpredictable, dose-dependent, and highly variable, particularly in cases where transporters function in opposite directions. These complexities limit direct extrapolation of *in vitro* results to the *in vivo* situation based simply on whether a compound is a substrate or inhibitor of an individual transporter of interest, and highlight the need for a comprehensive holistic understanding of transporters involved in drug disposition. Considering the large number (over 400) of gene products from the human ABC and SLC transporter gene families (6), comprehensive understanding of the contribution of each individual transporter to drug disposition is not readily achievable with currently available tools.

The complexities introduced by the transporter multiplicity can be further highlighted by several *in vivo* studies. Recent results (16) showed that in comparison to a small impact of Oatp1b2 knockout on pravastatin pharmacokinetics, a more substantial alteration in its PK was observed in Oatp1a/1b knockout mice in which elimination of both Oatp1a and 1b activities resulted in a sevenfold increase in systemic exposure. However, inhibition of activities of multiple transporters with activities counter to each other can also have a compensatory effect such that the net effect on PK cancel each other out. In the case of oral administration of methotrexate (17), individual knockout of Mrp2, Mrp3, and Bcrp resulted in 1.4, 0.8, and 1.7-fold changes in the systemic exposure. Dual knockout of Mrp2 and Bcrp resulted in three-fold increase in methotrexate systemic exposure (measured by AUC). However, additional knockout of Mrp3 reduced the systemic exposure back to levels in wild-type (wt) mice (AUC in the triple knockout mice was 90% of that in wt mice). While the observations can easily be understood based on the orientation of Mrp3 transport relative to Mrp2 and Bcrp efflux, prospective predictions, based on the inhibition of individual transporters is difficult in the absence of a more holistic understanding of the elimination process.

An added important barrier to this comprehensive view is the fact that new information on transporters continues to rapidly evolve. In this regard, recent publications (18,19) suggested that multidrug and toxin extrusion proteins MATE1 and MATE2-K may be more important renal transporters than OCT2, one of the renal transporters recommended to be studied by both the ITC and regulatory agencies. Cimetidine, currently recommended by the draft FDA guidance as an *in vivo* inhibitor for OCT2, is a more potent

Table III. Pharmacokinetics and Tissue Levels of Drugs in Wild-Type Animals and Transporter Knock-Out (ko) Rodents

		% Bioavailability		Fold change of plasma or tissue levels (ko/wt)				
Transporter	Drug	ko	wt	Plasma	Liver	Kidney	Brain	Reference
Mdr-1a/b	Topotecan			0.7	0.8		1.5	(20)
	Digoxin			2.5	2.4	2.3	38.5	(21)
	Quinidine			3.7	4.3	2.5	29.2	(21)
	Digoxin			2.9	2.5	2.6	27.2	(21)
	Taxol			1.1				(21)
Bcrp	Methotrexate	15.6	14.0	1.7[a]				(17)
				1.6	1.72			(17)
	Topotecan			2.4	2.1		1.6	(20)
	Digoxin	33	37	0.6				(13)
	Nitrofurantoin			1.3				(13)
		94	83	1.5				(13)
	Sulfasalazine			1.8				(13)
		18.9	1.1	33.1				(13)
	Compound A			3.0				(13)
		4.4	1.1	9.8				(13)
Bcrp/Mdr-1a/b	Dasatinib			1.4			10.0	(23)
	Erlotinib			1.0				(24)
		60	40	1.5				(24)
Oatp1b2	Rifampin			1.7	0.4			(25)
	Rifampicin			1.9	0.4			(26)
	Rifamycin SV			6.3	1.8			(26)
	Cerivastatin			1.0	0.9			(26)
	Lovastatin acid			1.4	1.0			(26)
	Pravastatin			0.4	1.3			(26)
	Pravastatin			1.8	0.6			(26)
	Simvastatin acid			0.9	0.6			(26)
Mrp2	Methotrexate	9.8	14.0	1.4				(17)
				2.0				(17)
Mrp3	Methotrexate	13.1	14.0	0.8				(17)
				0.8		0.5		(17)
Oct2	Tetraethylammonium			1.1	0.9	0.7	1.0	(27)
Oct1	Tetraethylammonium			0.5	0.1	1.4	1.3	(15)
Oct1	Methyl-4-phenylpyridinium			1.3	0.3		1.0	(15)
Oct1	Metaiodobenzylguanidine			0.9	0.2		0.9	(15)
Oct1	Cimetidine			1.0	0.8		0.8	(15)
Oct1	Choline			0.9	1.1		0.9	(15)

All studies were performed in mice with the exception of Huang *et al.* (13) which were performed in rats
[a] Tissue levels at 2 h post-oral dose

inhibitor of MATE1 and MATE2-K. Similarly, metformin, the recommended *in vivo* OCT2 probe, is not as sensitive a probe for OCT2 as OCT1 and MATE1/MATE2-K, which also contribute to its hepatic and renal disposition. It is worth mentioning that this rapidly evolving science could make many prescriptive aspects related to transporter-based DDI described in the draft US guidance outdated soon after it is finalized. Additionally, the cimetidine case study illustrates the importance of demonstrating whether the transporter of interest is rate limiting to the overall disposition, which unfortunately cannot simply be obtained from an *in vitro* determination of a transporter substrate as commonly practiced. Consistent with this, a single dose of the widely accepted clinically potent OATP1B inhibitor rifampin had significant impact on the pharmacokinetics of atorvastatin and glyburide, but not warfarin, in spite of the fact that all three compounds have been shown *in vitro* to be a substrate of OATP1B and susceptible to inhibition by rifampin (28–30).

Complications Due to Lack of Specific Inhibitors and Probes for Transporters

Another important obstacle to our understanding of the role of transporters in drug disposition and DDIs is the lack of specific inhibitors/probes for transporters, including those on the recommended list in the current US draft guidance. In this regard, the findings of the ITC (6) indicate methotrexate as a "selected substrate" for five transporters while cyclosporine is listed as a "selected inhibitor" for seven transporters. The lack of specific probes is largely a result of the multiplicity of transporters, which in theory can be addressed to some extent if specific inhibitors were to be available. Without specific inhibitors/probes, result interpretations with respect to the transporter roles and impact on DDIs can be ambiguous, and there are severe limitations in extrapolating the results from *in vitro* to *in vivo* and from one DDI study with one transporter substrate/inhibitor to another. This particular issue is fundamental to an ongoing debate with

respect to how important transporter-based DDIs are and to general concerns regarding how to derive meaningful and actionable information based on the recommended *in vivo* clinical DDI studies using a single probe or inhibitor as described in the current draft FDA guidance.

Much of what is known today about the transporter involvement in drug disposition is extrapolated from *in vitro* studies using cells expressing endogenous transporters (e.g., Caco-2 cells) or transfected with specific transporters. Many of these cell lines unfortunately also express significant background levels of other transporters, requiring use of specific inhibitors to aid in proper identification of the role a given transporter may play. However, given the deficiency of specific inhibitors, interpretation of *in vitro* transport results in some cases may be ambiguous and misleading. Similarly, the lack of selective inhibitors poses a significant issue for determination of the *in vivo* impact of specific transporters on exposure. Even with relatively specific inhibitors, interpretation of *in vivo* studies aimed at determining the impact of specific transporters is not straightforward. Early studies showed increased exposure (3.8-fold) of imatinib when administered with the Pgp inhibitor valspodar or the dual Pgp/Bcrp inhibitor elacridar in wt mice (31). These results were interpreted to support Pgp and Bcrp in limiting imatinib exposure. However in a study by Oostendorp et al. (32), coadministration with elacridar or pantoprazole resulted in a threefold higher systemic exposure and reduced clearance of imatinib in both wt and Mdr1a/1b/Bcrp triple knockout mice. Elacridar was specifically designed to be, and is considered as, a Pgp/Bcrp inhibitor. Imatinib is a Pgp/Bcrp dual substrate which is extensively metabolized by CYPs (CYP3A, 1A1/2, 1B1, 2C8/9, and 2D6) (33). Given that the knockout mice are deficient in both Pgp and Bcrp activity, the changes in imatinib PK were ascribed to unanticipated inhibition of other elimination mechanisms potentially mediated by Oatps or Octs. These results highlight the lack of specificity of even the best characterized transporter inhibitors and are particularly concerning since unanticipated effects on other drug elimination processes may compromise the cause–effect relationship needed for meaningful extrapolation of *in vitro* inhibition data to avoid DDIs.

More concerning is that clinical relevance of drug transporters in DDIs has been mainly derived based on studies with nonselective inhibitors and substrates. Cyclosporine (CsA) and statins, commonly used as model inhibitors and substrates, respectively, in clinical studies as well as in the draft FDA guidance have broad interactions with transporters and metabolism enzymes. Clinical DDIs reported with CsA (as perpetrator) and organic anion transporting polypeptide (OATP) substrates were commonly cited to underscore the *in vivo* impact of OATP inhibition. It is important to point out that as a known immunosuppressant, clinical DDI studies with CsA have been mostly performed in transplant patients who were on multiple drugs, and the clinical DDI data were often compared to historic pharmacokinetic data in healthy volunteers resulting in generally high DDI magnitude (Electronic Supplementary Material (ESM) Table S1). As such, the results of these comparisons may not accurately reflect interaction potential based simply upon a mechanism of OATP inhibition. Only 4 out of 18 studies were conducted in healthy subjects in an open-label, randomized, cross-over or one-sequence design (ESM Table S1). The results from these healthy volunteer studies showed disparate effects of CsA on the fold change of plasma AUC of atorvastatin (15.3-fold; 34), pitavastain (4.5-fold, NDA #022363), bosentan (1.9-fold; 35), and repaglinide (2.5-fold; 36). *In vitro*, OATP-mediated uptake has been shown as a major contributor for hepatic uptake of these compounds (37,38). Nonetheless, differences in the magnitude of the CsA impact *in vivo* among the four drugs likely reflect the effects on multiple transporters (e.g., OATP1B1, -1B3, Pgp, BCRP) and drug metabolizing enzymes (CYP3A). Furthermore, inhibition of transporters and enzymes by CsA may occur either in the liver or gut. Thus, the wide-ranging magnitude of DDI changes among these four drugs is a complex reflection of differences in the anatomical location and involvement of multiple enzymes and transporters involved in the elimination of the drugs that are affected by CsA (39). It is also important to note that CsA is known to have nephrotoxic and other undesirable effects (40), which might be additional contributing factors to alterations in plasma exposure of victim drugs, independent of its effect on drug transporters or enzymes. These complications dampen the notion put forth in the US draft guidance that CsA is a broad inhibitor of transporters and CYPs and therefore the results from clinical DDI studies with CsA could serve as the worst case scenario for substrates of transporters and enzymes. In this regard, rifampin may serve as a better alternative OATP1B1, -1B3 inhibitor, provided that it is given as a single dose and preferably intravenously to reduce potential complications due to its inhibitory effects on Pgp and MRP2 at the intestinal level (28). Nevertheless, more research is needed to confirm whether a single dose rifampin is indeed an *in vivo* selective inhibitor of hepatic OATP1B, with minimal effects on other yet to be identified transporters.

Likewise, many clinically relevant DDIs mediated *via* drug transporters have been reported with nonspecific substrates, and the scarcity of selective inhibitors presents a major barrier in determining a relative contribution of an individual transporter to overall uptake or secretion processes in a given tissue, critical information linking to DDI potential. Rosuvastatin, recommended by the FDA draft guidance as a probe substrate for both OATP1B1 and BCRP in the clinic, is generally believed not to undergo significant metabolism (41), and instead its elimination and disposition mechanism is largely driven by transporters including OATP1B (42) and BCRP (43). Interestingly, the magnitude of DDIs between rosuvastatin and several known OATP inhibitors was at most twofold (fold changes of AUC was 1.6-, 1.8-, 2.1-, and 1.4-fold for eltrombopag, gemfibrozil, lopinavir/ritonavir, and tipranavir/ritonavir, respectively) (11,44–46). The only exception is with CsA, with the fold change in AUC of 7.1-fold, but this was observed in heart transplant patients when compared with historical data in healthy controls (ESM Table S1). A similarly modest change in rosuvastatin exposure (fold change in AUC and C_{max} was 1.2 and 2.9, respectively) has also been recently observed in a clinical DDI study with one of our internal candidates (compound B in Table IV) when given at 300 mg twice daily (BID) for 10 days with a single 5-mg oral dose of rosuvastatin in healthy subjects. This compound was classified as a relatively potent *in vitro* inhibitor of OATP1B1 (IC_{50}= 0.3 μM, pitavastain as a probe substrate), OATP1B3 (IC_{50}= 0.3 μM; bromosulfophthalein as a probe substrate) and BCRP (IC_{50} ~13 μM; methotrexate as a probe substrate). The modest changes reported thus far raise a question as to whether rosuvastatin disposition is limited by these transporters and

therefore its suitability for being a recommended probe substrate. In this regard, the current FDA transporter decision trees assume that a single transporter-mediated uptake/efflux accounts for 100% of the elimination of a probe substrate or victim drug, which is not the case for rosuvastatin and most, if not all, clinically used drugs. It is also noteworthy that rosuvastatin undergoes renal elimination to some extent (~28% of total clearance) in humans (47), and that the notion that rosuvastatin is eliminated mainly unchanged should be interpreted with caution. Rosuvastatin is capable of undergoing glucuronidation and lactonization similar to other statins (47), and rosuvastatin lactone has been observed in excreta and in plasma in humans (48–50), raising the possibility of an additional complicating factor resulting from reversible metabolism. It would be of high interest to conduct a clinical DDI study using an intravenous single dose of rifampin to better assess the *in vivo* relevance of OATP impact on rosuvastatin pharmacokinetics while avoiding a potential complication due to gut Pgp-mediated DDI (rosuvastatin has also been reported as a Pgp substrate (39)). Additionally, since pitavastatin possess similarities to rosuvastatin with respect to its interactions with transporters OATP1B1, -1B3, BCRP, Pgp and MRP2, and UGT enzymes, but with much less dependency on renal elimination pathway (39), we are currently conducting a clinical DDI study to determine the relative impact of a single dose rifampin on the PK of pitavastatin *versus* that of rosuvastatin. The outcome of our ongoing clinical DDI study should provide valuable information regarding the clinical relevance of OATP1B and help determine whether pitavastatin, a currently employed *in vitro* probe, may also be used as an *in vivo* probe for OATP1B.

Impact of Individual Transporters Alone on DDI is Usually Small

Thus far, the magnitude of DDIs reported in clinical studies with inhibitors or substrates of transporters other than OATP1B (see above), and particularly for the renal transporters OCTs and OATs, is generally less than threefold (6). In principle, gene knockout animals represent the most extreme case for DDI in which complete inhibition of transporter activity is achievable. Although loss of one transporter may result in a compensatory increased gene expression for complementary transporters and metabolism enzymes, gene profiling results in Mrp2 (51) and Oatp1b2 (26) do not show a clear gene expression pattern that would compensate for the transporter knockout (e.g., in case of Mrp2 knockout mice, loss of Mrp2 activity, and increase of Mrp4 activity in liver would both contribute to increased systemic exposure). Results to date in transporter knockout animals showed changes in systemic exposure generally within twofold in knockout animals in the absence of transporters commonly cited as important in drug disposition, including Oatp1 (Table III). The only exception is with Bcrp knockout and sulfasalazine where a much more dramatic impact on oral exposure (33-fold increase) was observed in the knockout rats (13). Interestingly, this was not duplicated in humans, where ABCG2 421 C>A SNP led to only a 2- to 3.5-fold increase in oral exposure of sulfasalazine (13 and references therein). Consistent with this notion, clinical data in subjects with genetic mutations of transporters reported thus far with OATP1B1 (*SLCO1B1*) and BCRP (*ABCG2*) and other transporters also showed less than or equal to threefold change in systemic exposure of substrate compounds. In a series of clinical studies with the same group of subjects, the plasma AUC of simvastatin acid, atorvastatin, pravastatin, rosuvastatin, and fluvastatin in subjects with the SLCO1B1 c.521CC genotype was 3.2-, 2.4-, 1.9-, 1.6-, and 1.2-fold greater than in those with the SLCO1B1 c.521TT genotype, respectively (52–54). The ABCG2 c.421C>A (rs2231142) SNP also affects the PK of several statins, including rosuvastatin (2.0- and 2.4-fold greater in rosuvastatin AUC in the c.421AA homozygote compared with c.421CA or c.421CC genotypes, respectively), and atorvastatin, fluvastatin, and simvastatin lactone (increased exposure by about 1.7-, 1.7-, and 2.1-fold, respectively, in subjects with the c. 421AA genotype compared with c. 421CC reference genotype) (55,56). As a comparison, changes in the exposure reported in CYP knockout animals or human subjects with polymorphic enzymes are much higher often >20-fold (57–59). The relatively modest impact of genetic variation in drug transporters, when compared to transporter DDI studies, in which the majority of perpetrators, such as CsA, are inhibitors for multiple transporters/enzymes further supports that the magnitude of DDIs reported with OATP inhibitors is likely overestimated, due to multiple process/mechanisms involved *in vivo*.

The relatively small magnitude of transporter-mediated DDIs or transporter polymorphisms prompts a larger question of whether routine studies on many drug transporters, as recommended by the recent guidance, are justifiable. Given the relatively small magnitude of DDI reportedly associated with the renal transporters OCT and OAT, together with no clear evidence thus far related to the impact of their gene mutations on drug exposures, it would be more reasonable to limit *in vitro* and *in vivo* studies of transporters only to compounds that have, or to those likely to be prescribed with drugs with, narrow therapeutic index. It is worth noting that although the impacts of transporters on systemic exposure are relatively modest, their effects on drug distribution or tissue exposure could theoretically be more dramatic. Thus far, this has been documented for mouse Mdr1 in which Mdr1 activity in the blood–brain barrier minimized CNS exposure to Pgp substrates. Knock-out of Mdr1 can lead to >10-fold increase in CNS exposure (Table III), while having little if any impact on systemic exposure (21,60). In general, high systemic levels of Pgp inhibitors are needed to significantly alter brain exposure, and this has been shown not to be achievable with therapeutic doses of commonly known transporter inhibitors, including the most potent inhibitor CsA (61). Although it could be argued that for some compounds changes in distribution can have adverse effects, routine transporter substrate/inhibition studies are unlikely to provide prospective predictions of distribution and altered pharmacology. In such cases, more focused studies aligned with preclinical safety studies to address whether changes in tissue exposure correspond with observed toxicity.

Transporter Data are Dependent on In Vitro Model Systems and Methodology Used

Recent work by a Pgp IC_{50} working group consisting of 22 participating pharmaceutical and contract research laboratories and one academic institution showed substantial inter-laboratory variability of Pgp IC_{50} values (18- to 796-

Table IV. *In Vitro* Evaluation of Compounds as a Potential Perpetrator for OATP1B1-Mediated DDIs

Perpetrator (dose, regimen)	IC_{50} (μM)[a] OATP1B1	C_{max} (μM)	C_{max}/IC_{50} (FDA)[b] (≥0.1)	*R* value (ITC)[c] (≥2)	*R* value (FDA)[b] (≥1.25)	$I_{in,\ max,\ u}/IC_{50}$ (EMA)[d] (≥0.04)	Victim (clinical DDIs)	Fold changes of victim AUC	Fold changes of victim C_{max}	References for clinical DDI data
Boceprevir(800mg TID)	18±2.4	3.0	0.17	1.5	2.6	1.6	Atorvastatin Pravastatin	2.3 1.6	2.7 1.25	(8)
Compound A (500mg)	0.4±0.09	5.5	12.5	1.5	2.4	1.4	Simvastatin	1.9[e]	2.2[e]	Data on file
Compound A (500mg)[f]	0.4±0.09	5.2	11.8	1.5	2.4	1.4	Simvastatin	2.8[e]	2.4[e]	Data on file
Compound B (300mg BID)	0.3±0.01	5.4	18.1	1.8	2.8	1.8	Rosuvastatin (5mg SD)	1.2	2.9	Data on file
Compound C (150mg QD, low fat food)	>50	2.0	<0.04	<1.0	<1.0	<0.004	Simvastatin (40mg SD)	1.34	1.26	Data on file
Compound C (100mg QD, high fat food)	>50	3.4	<0.07	<1.0	<1.0	<0.003	Atorvastatin (10mg SD)	1.07	1.17	Data on file
CsA (100mg)	0.3±0.1	0.6	2.0	1.8	3.1	2.1	Pitavastatin	4.5	NA	(9)
Rifampin (600mg)	1.4±0.2	7.9	5.81	2.8	5.6	4.6	Atorvastatin	8.5	14.9	(10)
Lopinavir (400mg)	0.4±0.1	20	55.6	2.4	3.6	2.6	Rosuvastatin	2.1	4.7	(11)
Telaprevir (750mg TID)	3.4±0.1	2.5	0.74	3.9	9.95	8.95	Atorvastatin	7.9	10.6	(12)
Amprenavir (600mg)[g]	10.0±1.6	12	1.2	1.4	1.91	0.91	Rosuvastatin	1.08 (P=0.86)	1.45 (P=0.76)	(48)
Ritonavir[h] (100mg BID)	0.8±0.2	1.73	2.06	1.1	1.26	0.26	Rosuvastatin	1.08 (P=0.86)	1.45 (P=0.76)	(48)
Gemfibrozil (600mg BID)	89.5±17.5	100	1.12	1.1	1.09	0.09	Pitavastatin Atorvastatin	1.25 1.24	NA 1.17	(NDA# 022363, 2009) (14)

[a] In-house data obtained using pitavastatin (0.1 μM) as a probe substrate and OATP1B1 transfected MDCKII cells
[b] FDA Guidance criteria (4)
[c] ITC criteria (6)
[d] EMA guidance criteria (5)
[e] Measured simvastatin acid
[f] Simvastatin administered 12 h later
[g] The prodrug fosamprenavir was used in the study
[h] Co-administered with fosamprenavir
NA-Data are not available

fold difference) obtained using various *in vitro* assay systems (62). Such large differences have been ascribed to interlaboratory differences in the behavior of Pgp expression systems, assay protocols, and the methods used to calculate IC_{50} values. Similar *in vitro* system-dependent IC_{50} values have also been observed with boceprevir; minimal inhibition (<10% decrease in net transport) of Pgp-mediated digoxin transport was observed using MDCKII-MDR1 cells, while studies in Caco-2 cells indicated that boceprevir as an inhibitor of Pgp-mediated transport of digoxin, with IC_{50}= 25 μM (63). The reason for the discrepancy between these two assay systems is unclear, but it may suggest that boceprevir affects other uptake or efflux transporter expressed in Caco-2 cells which are involved in the transport of digoxin.

Substrate-dependent inhibition of OATP transporters has also been reported, with different IC_{50} or K_i values with different substrates, suggesting that OATPs have multiple binding sites (64,65). Consistent with this, our recent studies (63) using estrone sulfate and estropipate, potent prototypical inhibitors for OATP1B1 (66), also yielded different IC_{50} values for the uptake of pitavastatin (IC_{50}, 0.6 and 0.8 μM, respectively) as compared to that of atorvastatin (IC_{50}>50, >10 μM, respectively). Further complicating this is the fact that currently we have little understanding of the kinetics of drug–transporter interactions. The current assumption of a simple competitive inhibition is commonly used to explain DDIs related to transporters. However, recently emerging information suggests that this may not be the case. For example, CsA has been shown to exhibit a long lasting inhibition on hepatic uptake of bromosulfophthalein, a probe substrate for OATPs, in rats (67), and on OATP1B1 and -1B3 mediated uptake *in vitro* (68,69). Conceivably, this may be a contributing factor, among several others, to the unusually high magnitude of DDIs associated with CsA.

The complexities associated with assay systems and methodologies highlight the need to develop better tools and approaches to appropriately model *in vitro* transporter data for IC_{50} determination. Indeed, this is an area of focus by the ITC (62). Furthermore, assay methodology, including clinically relevant probes or inhibitors, must be harmonized in order for a single cutoff criterion as currently suggested in both the EU and US guidance documents to provide meaningful information indicative of DDI potential. It is also worth noting that the current FDA criteria based on an R value of 1.25 has been shown to yield an unacceptably high false-positive (100%) shown for OATP1B (70). This is concerning considering that using the proposed criterion *in vivo* clinical DDI will need to be conducted regardless of the *in vitro* outcome. In this regard, our internal data based on IC_{50} values for limited set of OATP1B inhibitors from a single *in vitro* assay system (OATP1B1 transfected MDCKII cells with pitavastatin as a probe substrate) also showed many false positives using the criteria proposed by both agencies, either based on a ratio between inhibitor concentrations (total for the US or free for the EU) and IC_{50}, or R value (US only), and many false negatives with the ITC criteria R value of 2 (Table IV), supportive of continued research in this area, including searches for a more suitable *in vivo* probe, to aid in a more rationale-based criteria setting.

Model-Based Predictions of DDIs

Recently, there have been an increasing number of publications on mechanistic modeling, including PBPK to predict DDI mediated *via* enzyme and/or transporter inhibition, induction or both. Understandably, given the knowledge gained with CYPs over the last few decades, many successful quantitative predictions have been reported for the DDI magnitude in humans due particularly to either inhibition or induction of CYPs, although not without some false predictions (71–73). Thus far, most of the reports on more complex DDIs, including time-dependent inhibition and mixed inhibition and induction, and enzyme-transporter interplays, are retrospective (74–76), and successes in prospective predictions have not been well established. Indeed, the EMA guidance explicitly excludes the use of mechanistic modeling for quantitative prediction of the interaction resulting from *both* induction and inhibition (reversible or mechanism-based), citing limited experience as a reason. These modeling approaches, while theoretically sound, are dependent heavily on quality inputs and fundamental knowledge of the system, as well as comprehensive information about the compound itself. While much is known about the former as compared to transporters, the latter will not be easily obtained in the early stages of drug development and will usually require further support or validation with additional clinical experiences. In this section, we present arguments supported with case examples to highlight complications associated with modeling approaches for prediction of TDI and particularly mixed TDI and induction as described in the regulatory guidance. We hope to convey that more research is needed even for CYP3A, the most studied enzyme, as well as other enzymes, before the recommended model-based approach and associated criteria can be reliably applied for these complex DDIs.

Predictions Using the Mechanistic Static Model for TDIs and Mixed Inhibitors/Inducers

The so-called "mechanistic static model" or "net effect" described in the US FDA guidance to assess investigational drugs as inhibitors (reversible inhibitors or TDIs), inducers and mixed inhibitors and inducers (Fig. 4 of the US draft guidance) is primarily based on a model described by Fahmi *et al.* (77). The proposed model relies on using a single maximum inhibitor concentration at both the gut (I_{gut}—total drug concentration in the gut) and hepatic (I_h—estimated maximum unbound liver concentration) levels. In the original work (77), I_h was based on free systemic C_{max} for the inactivation and induction portions and free portal C_{max} for the reversible inhibition portion of the model. It remains unclear why different surrogates for the perpetrator concentration (free systemic *versus* portal C_{max}) were required to optimize the model performance. The FDA is more conservative in this regard recommending the use of free portal C_{max} for I_h for all inhibitor concentrations in the model. Based on the database totaling 30 drugs, Fahmi *et al.* (77) showed that while the predictions were within twofold of the observed values for DDI mediated *via* CYP inhibition (reversible or mechanism-based) or CYP3A4 induction alone, the success rate was low for compounds anticipated to be both inhibitors and inducers. Of the five compounds tested that belong to this class, only one compound was predicted well with the mechanistic static model; all other predictions were at least

two- to three-fold off (either false positive or negatives) the observed values (77). It is important to point out that a twofold cut off is commonly used as a criterion judging the success rate of prediction models, and that the success rate was still low even with a twofold cutoff for predicting mixed inhibition induction. The criteria proposed for an initial cutoff in the latest US guidance is much more conservative, with predicted relative AUC values of 80–125%. Not surprisingly, a recent poster at the 2012 ASCPT meeting (78) shows up to a 90% false-positive rate, with the higher end associated with TDI or compounds with mixed inhibition and induction of CYP3A (n=12).

Similarly, we have experienced overpredictions with the mechanistic static model based either on the free portal or systemic C_{max} for all compounds (n=9) in our database that qualified as TDIs and mixed inhibitors/inducers of CYP3A. In fact, up to 10-fold and three- to five-fold overpredictions were observed with the recommended free portal C_{max} (calculated based on the recommended default absorption rate constant value of 0.1 min^{-1}) and systemic C_{max} (clinically observed C_{max} at steady-state, adjusted for free fraction), respectively (Table V). In both cases, the predictions gave no false negatives (Table V). In this analysis, an enzyme turnover rate (k_{deg}) for CYP3A of 0.03 h^{-1} was used; this k_{deg} value has recently been shown to yield more accurate predictions for time dependent inhibition of diltiazem and other TDIs on midazolam (74,79). Although limited, our data suggest that the results from the mechanistic static model may provide a more reasonable estimate for DDI risk if clinically observed unbound C_{max}, instead of the agency's recommended unbound portal C_{max} is used. It is noteworthy that the default ka value of 0.1 min^{-1} was partly responsible for the difference observed between the two approaches, and that the previously recommended ka value of 0.03 min^{-1} by Ito *et al.* (80) would narrow, although not eliminate, the difference (data not shown), and therefore may be a better value of choice. Additionally, although the results thus far support what appears to be the main intent from the regulatory perspective of using the static model for ruling out the need to do clinical DDI studies, from a drug industry perspective this perceived benefit is minimized by the high likelihood that the predictions will suggest that essentially no clinical studies can be excluded, regardless of *in vitro* study outcomes.

Predictions Using Mechanistic Dynamic Models for TDIs and Mixed Inhibitors/Inducers

The high incidence for overprediction by the static model is not surprising given the fact that this model only captures the maximum plasma concentration of the perpetrator. In theory, the next-in-line dynamic PBPK modeling approach recommended by both the agencies, which accounts for temporal change in the perpetrator plasma concentrations, should improve the prediction, provided that the model incorporates all necessary

Table V. *In Vitro* Evaluations and Model-Based Predictions of Compounds as a Potential Perpetrator for CYP3A-Mediated DDIs

	Input parameters of perpetrator								Midazolam exposure changes (AUCR)[a]		
Perpetrator	Dose (mg) regimen	Observed $C_{max,ss}$ (μM)	Plasma free fraction	Reversible inhibition IC_{50} (μM)	Time-dependent inhibition KI (μM)	Time-dependent inhibition kinact (hr^{-1})	Induction EC50 (μM)	Induction E_{max} (fold)	Predicted Net Effect[b]	Predicted PBPK[c]	Observed
Compound 1	80 QD	1.6	0.005	4	12	8.4	14	11	4.9 (2.0)	1.3	1.5
Compound 2	20 QD	0.54	0.025	100	17	7	1.3	15	1.9 (1.6)	1.3	0.82
Compound 3	200 BID	8.0	0.030	7.9	3.6	1.7	0.5	15.5	2.8 (1.4)	2.8	0.8
Aprepitant	160 QD	3.0	0.003	0.84	2.23	6.0	0.275	4.05	3.0 (2.7)	2.8	1.6
Compound 4	150 QD	3.0	0.030	28	9.5	6.6	–	–	17.0 (4.7)	3.9	1.4
Compound 5	500 QD	5.8	0.041	75	2.3	1.4	–	–	17.0 (7.2)	3.8	3.8
Compound 6	600 QD	2.4	0.016	19	42	8.4	–	–	7.3 (2.2)	N/A	1.8
Boceprevir	800 TID	3.3	0.300	7.7	6.1	7.2	–	–	24.6 (18.5)	6.8	5.3
Teleprevir	750 TID	4.5	0.300	1.2	0.5	4.5	–	–	24.9 (23.7)	10.2	9.0

All *in vitro* data were generated in-house. Clinical data are internal data, except for Boceprevir and Teleprevir are from references (81) and (82), respectively

[a] All predictions for AUCR were based on k_{deg}=0.03 h^{-1}. Additional information is included in the ESM S2

[b] Predicted net effect values were based on free hepatic portal concentrations, while values in parentheses were obtained using free plasma C_{max}

[c] PBPK was simulated using SimCYP with clinical PK data of perpetrators. Additional information used in the simulation is included in the ESM S2

and adequately characterized system and drug parameters. Indeed, a general trend of improvement was observed with a PBPK model using the same *in vitro* dataset (Table V; see also ESM S2) and a k_{deg} value as for the static model, but with clinically observed plasma concentration-time profiles for the perpetrators at steady-state (Table V). Notably, by using the clinically observed perpetrator concentrations at steady-state, our PBPK model results represent the best case scenario by reducing uncertainty in extrapolating the inhibitor concentrations using translational bottom-up approaches. Despite this, a 2- to 3.5-fold over prediction was observed for compound 3 (mixed inhibitor/inducer) and compound 4 (a TDI), suggesting some limitations of the current model.

The model limitations are also supported by a recent industry/FDA/academia collaboration that was recently completed evaluating current static and time-based models for induction DDI (C. Gibson, personal communication). Two perpetrators included in the study were pioglitazone and troglitazone, each of which has been shown *in vitro* and *in vivo* to be inducers but also have clearly observable time-dependant inhibition of CYP3A *in vitro* in a human liver microsomal model. A summary of the PBPK model parameters for pioglitazone and troglitazone and model validation using existing clinical data are shown in ESM S3. After incorporation of all the observed *in vitro* induction and time-dependent inhibition parameters for both perpetrators (ESM S3), they were both simulated to be net inhibitors rather than inducers, at steady-state. With the k_{deg} value of 0.03 h^{-1}, the modeled GMR (95% CI) for the pioglitazone/midazolam DDI was 1.87 (1.78–1.97) *versus* the observed GMR of 0.74, and the corresponding value for the troglitazone/simvastatin DDI was 6.47 (6.10–6.86) *versus* the observed interaction of 0.59 (83). Interestingly, these predicted results are the best-case outcome, given that the prediction became worse using a k_{deg} value of either 0.019 h^{-1} (predicted GMR=2.1 and 7.0 for pioglitazone/simvastatin and troglitazone/simvastatin, respectively) or 0.008 h^{-1} (predicted GMR=2.8 and 8.8 for pioglitazone/simvastatin and troglitazone/simvastatin, respectively); both k_{deg} values have also been commonly employed for CYP3A in many reportedly successful DDI predictions resulting from CYP3A inhibition (74,79,84). Furthermore, correction of the Ki and KI values of pioglitazone and troglitazone using the measured nonspecific binding (0.11 and 0.07, respectively—data not shown) caused the prediction to be even worse (i.e., predicted a greater magnitude of net inhibition). These poor predictions, with respect to both the magnitude and the direction of DDI (predicted net inhibition vs observed net induction) were made in spite of using clinically observed perpetrator concentrations at steady-state of both troglitazone and pioglitazone. Such examples strongly suggest deficiencies in the current model approaches and limiting their use in predicting DDI liability of compounds with mixed time-dependent inhibition and induction.

Complications Associated with Using a Single Value of CYP3A4 Degradation Rate (k_{deg}) to Describe Both Induction and Inhibition

While the k_{deg} value of 0.03 h^{-1} has provided reasonable predictions for the magnitude of DDIs for many CYP3A-mediated TDIs, including several of our compounds, our experience using the PBPK models to study the time course of return to baseline for midazolam PK following 28 days of rifampin administration to healthy volunteers suggest a CYP3A4 k_{deg} of 0.008 h^{-1} is required to describe the time course of the "de-induction" (85). For presentation in this manuscript, the aforementioned studies were re-evaluated using the same version of simCYP (v11.1) and were modeled using both k_{deg} values (0.03 h^{-1} and 0.008 h^{-1}) for comparison to the clinical observations. Additionally, the reversible and time-dependent inactivation parameters of CYP3A4 by diltiazem were modified to Ki=36.1 μM, KI=2.97 μM and kinact=4.2 h^{-1}, consistent to those previously published (79). All other model parameters for midazolam, rifampin and diltiazem were used as their default values in simCYP v11.1. As shown in Fig. 1a, the CYP3A k_{deg} value of 0.03 h^{-1} reported by Friedman *et al.* to best describe the time-course of CYP3A inhibition by diltiazem performed relatively poorly compared to using a k_{deg} value of

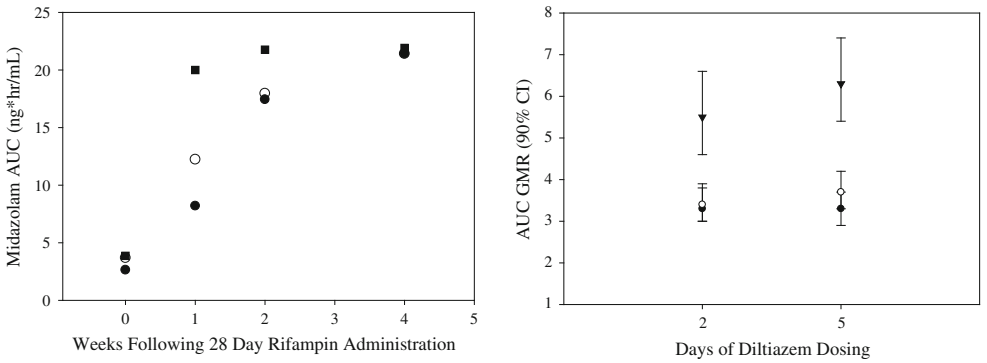

Fig. 1. a Plot of midazolam AUC following 28 day once daily (QD) oral administration of 600 mg of Rifampin showing the washout of the induction effect in midazolam PK modeled using to different values of k_{deg} (0.03 and 0.008 h^{-1}). Observed midazolam exposure shown in *filled circles*, modeled midazolam exposure using CYP3A4 k_{deg}=0.03 h^{-1} shown in *filled squares*, modeled midazolam exposure using CYP3A4 k_{deg}=0.008 h^{-1} shown in *open circles*. Observed data extracted from Reitman *et al.* (85). See additional details in ESM S3. **b** Plot of observed and simulated drug interaction, expressed as geometric mean AUC ratio (GMR) of 240 mg QD diltiazem (sustained released, SR) for 5 days on midazolam using two different values of the CYP3A4 k_{deg} (0.03 and 0.008 h^{-1}). Observed GMR with 90% CI shown in closed circles, simulated GMR (90% CI) using k_{deg}=0.03 h^{-1} shown in *open circles*, simulated GMR (90% CI) using k_{deg}=0.008 h^{-1} shown in *closed triangles*. See additional details in ESM S3

0.008 h^{-1}, for describing the time-course of the washout of rifampin's inductive effect on midazolam exposure. Interestingly, although the temporal aspects of the rifampin DDI were not well captured using a CYP3A4 k_{deg} of 0.03 h^{-1}, the magnitude of the observed steady-state interaction on midazolam was reasonably modeled using either value (0.03 or 0.008 h^{-1}). The diltiazem/midazolam DDI study published by Friedman *et al.* (79) was evaluated similarly to test the sensitivity to the CYP3A4 k_{deg} (Fig. 1b). In this case, the model using a CYP3A4 k_{deg} value of 0.03 h^{-1} was able to simulate both the temporal aspects and the magnitude of the observed DDI. As a comparison, a k_{deg} of 0.008 h^{-1} resulted in an overestimation of the magnitude of the observed DDI as well as not capturing the temporal aspects. Although it is possible that interstudy variability may be the cause for the apparent difference in the CYP3A4 k_{deg} value needed to describe the observed interactions mentioned above, based on our experience it can be reasonably concluded that in the current simCYP dynamic model of DDI there is not one value for the hepatic CYP3A4 k_{deg} that will account for both magnitude and temporal aspects of time-dependant inhibition and induction. These examples highlight the complexities in prospectively predicting DDIs and suggest a need for further model and/or parameter refinement.

Other Complications in Predicting DDIs due to TDIs and Mixed Inhibition/Induction

In this section, we present three retrospective case examples to further illustrate complicating factors associated with the PBPK model-based predictions for compounds with mixed inhibition/induction (n=2), and with only time-dependent inhibition on CYP3A (n=1). Unlike the earlier examples, where clinically observed perpetrator concentrations at steady-state were used to predict their effects on midazolam exposure, these cases were based on PBPK models that incorporated observed single dose and multiple dose clinical phase I perpetrator PK data, along with other relevant model parameters (CYP reaction phenotyping data and inhibition/induction parameters, physicochemical properties, etc.), to simulate perpetrator concentrations at steady-state, and predict subsequent effects on midazolam exposure (details provided in ESM S4). Although requiring a more extensive data set, these models are considered more mechanistic, leveraging the model to incorporate changes in clearance for the perpetrator drug as a result of its auto-inhibition/induction potential after multiple dosing. This is an especially important aspect of the model considering that the majority of compounds are substrates of CYP3A, including most of the compounds listed in Table V. We conclude from these and the aforementioned case examples that there is a significant knowledge gap to enable reliable DDI predictions for compounds of this nature (i.e., auto-induction and/or auto-inhibition), and that clinical DDI studies maybe unavoidable until more knowledge, on both the CYP system, including the synthesis and degradation rates, and the drug properties, is gained to help refine and validate the model.

Compound 3-Midazolam DDI. As indicated earlier, the PBPK model based on clinically observed PK of compound 3 and its *in vitro* CYP3A inhibition and induction properties, significantly over predicted the *in vivo* impact resulting from the chronic dosing of compound 3, with midazolam exposure reflecting its net inhibitory effect on CYP3A (Table V). Preclinical *in vitro* studies suggest that compound 3 may both inhibit and induce of CYP3A. Based on a comprehensive set of *in vitro* and *in vivo* studies, including ADME studies using a radiolabeled tracer, compound 3 is expected to be eliminated by metabolism in humans, primarily by CYP3A4 with fraction metabolized by CYP3A (fm_{CYP3A}) of ~0.8. When the parameters describing these attributes were incorporated into the PBPK model, an overprediction of compound 3 exposure was observed following bid dosing for 28 days (Table VI), indicating again the net inhibitory effect on CYP3A. This prediction was inconsistent with the observed multiple dose clinical PK profile of this compound, which suggested no net change in its clearance over this dosing period (i.e., the accumulation ratio of ~1.5 is expected based on its day 1 PK; ESM S4). Although the over-prediction of exposure at steady-state was minimized when the fm_{CYP3A} was arbitrarily changed to ~0.4, the prediction of its effect on midazolam exposure was not improved (Table VI). It is worth noting that in these simulations, the k_{deg} value of 0.03 h^{-1} was used and the results were better than those obtained with other k_{deg} values, including 0.019 h^{-1} (Table VI). Additionally, the model provided a reasonable prediction for midazolam exposure as a result of reversible inhibition after a single dose (day1) of compound 3 (Table VI), suggesting that the limitation of the current PBPK models is primarily on a more complex time-dependent DDI aspect, involving simultaneous induction and inhibition.

Aprepitant-Midazolam DDI. Aprepitant is another drug which has been shown *in vitro* to be a reversible (84) and time-dependent inhibitor of CYP3A4 as well as inducer of CYP3A4 (Table V). Furthermore, the apparent effects of autoinhibition with subsequent autoinduction are apparent in the multiple dose clinical PK profile of the compound (Aprepitant, Summary of Clinical Pharmacology Studies, Rahway (NJ), Merck, 2004, data on file). Increasing AUC is observed between days1 and 7 of aprepitant dosing (160 mg QD), followed by a decreasing trend in exposure on day28 and back to baseline on day56 of chronic dosing (Fig. 2). A minimal accumulation would have been anticipated based simply on the day1 PK profile with a linear kinetic assumption (i.e., no changes in aprepitant clearance due to auto inhibition or induction over the entire dosing period; the aprepitant PK would remain relatively constant over the 56-day dosing period). While our PBPK model (ESM S4) reasonably captured the increased exposure of aprepitant on day7 relative to day1 as a net inhibitory effect on CYP3A activity, it was unable to simulate the decrease in aprepitant exposure on days 28 and 56, an apparent steady-state after chronic dosing (Fig. 2, Table VI). The model predicted a net auto-inhibitory effect through steady-state after 28 days of drug dosing although it would appear that auto-induction had occurred. The inability to accurately simulate the exposure of aprepitant on day56 was accompanied by a poor simulation of the observed midazolam DDI on day56, regardless of which k_{deg} value (0.03 h^{-1} (Table VI) or 0.008 h^{-1} (data not shown)). Interestingly, on day7, when the simulated aprepitant exposure was consistent with observed clinical exposure, the midazolam AUC ratio was accurately predicted with a hepatic CYP3A4 k_{deg} value of 0.019 h^{-1} applied to the model. The k_{deg} value 0.03 h^{-1} resulted in underprediction of aprepitant exposure on day7, and likewise underprediction of the observed midazolam DDI (Table VI). This sensitivity of the PBPK model to the hepatic CYP3A4

Table VI. PBPK Model Predictions of Multiple Dose Pharmacokinetics and Drug-Drug Interaction with Oral Midazolam (2mg)

			Perpetrator					Midazolam	
				C_{max} (μM)		AUC (μM·h)		AUC GMR (90% CI)	
Perpetrator	Dosing day	k_{deg} (h^{-1})	$f_{m,3A4}$	Simulated	Observed	Simulated	Observed	Simulated	Observed
Compound 3 (200 mg BID)	1	0.03	0.4	4.4	5.5	38.6	46.0	1.3 (1.3, 1.3)	1.1 (0.8, 1.3)
			0.8	4.3		37.4		1.3 (1.3, 1.3)	
	28	0.03	0.4	8.4	8.0	77.5	69.8	2.8 (2.5, 3.1)	0.8 (0.6, 1.0)
			0.8	11.2		109.9		3.1 (2.8, 3.5)	
		0.019	0.8	13.7		139.4		4.3 (3.7, 4.9)	
Aprepitant (160 mg QD)	1	0.03	0.9	2.4	2.3	40.5	36.7	–	–
		0.019		2.4		42.3			
	7	0.03	0.9	5.6	7.5	110.6	120.9	3.4 (2.9, 3.9)	4.4 (3.1, 6.3)
		0.019		6.9		140.3		4.8 (4.1, 5.6)	
	56	0.03	0.9	6.0	3.0	119.2	47.9	3.5 (3.0, 4.0)	1.6 (1.1, 2.3)
		0.019		7.5		154.0		4.9 (4.2, 5.8)	
Compound 4 (150 mg QD)	1	0.03	0.9	6.1	7.2	46.6	39.6	–	–
				6.0[a]		*41.0*[a]			
	10	0.03	0.9	14.1	9.2	201.5	51	6.5 (5.5, 7.6)	1.4 (1.3, 1.6)
				12.1[a]		*151.0*[a]		*4.9 (4.2, 5.7)*[a]	

[a] TDI kinetics from hepatocytes; additional details can be found in ESM S4

parameter and its uncertainty, observable across several disparate examples, suggest that it may be difficult, even in the most straightforward cases, to expect the level of precision required to perform within traditional bioequivalence bounds suggested as cutoffs in the draft US guidance.

It is worth noting that the model was unable to predict correctly, with regard to the directional effect (predicted net inhibition vs observed net induction) on the perpetrator exposure, and that in spite of apparent autoinduction on its own metabolism after 56-day dosing, aprepitant caused an inhibitory effect on midazolam exposure (Table VI). The reason for this is unclear and could not be due simply to inaccurate determination of fm_{CYP3A} for aprepitant as changing its fm_{CYP3A} from 0.9 to 0.7 did not improve the prediction (data not shown). Additionally, a clinical DDI study conducted with ketoconazole, a potent CYP3A inhibitor, caused fivefold increase in aprepitant (85), consistent with CYP3A being the major metabolizing enzyme for aprepitant. The observed temporal changes in the aprepitant exposure (increase on day7 then decrease on day28), but not of midazolam, suggest a possibility for changes over time in the relative contribution of CYP3A to overall metabolism of aprepitant that was not replicated with midazolam. Although the underlying mechanism remains to be investigated, this hypothesis is consistent with the fact that aprepitant is also metabolized, albeit to a very limited extent by CYP2C *in vitro*, is not a potent inhibitor of CYP2C (86), and that an inducer of CYP3A can also induce CYP2C (87,88). This example highlights a knowledge gap requiring more

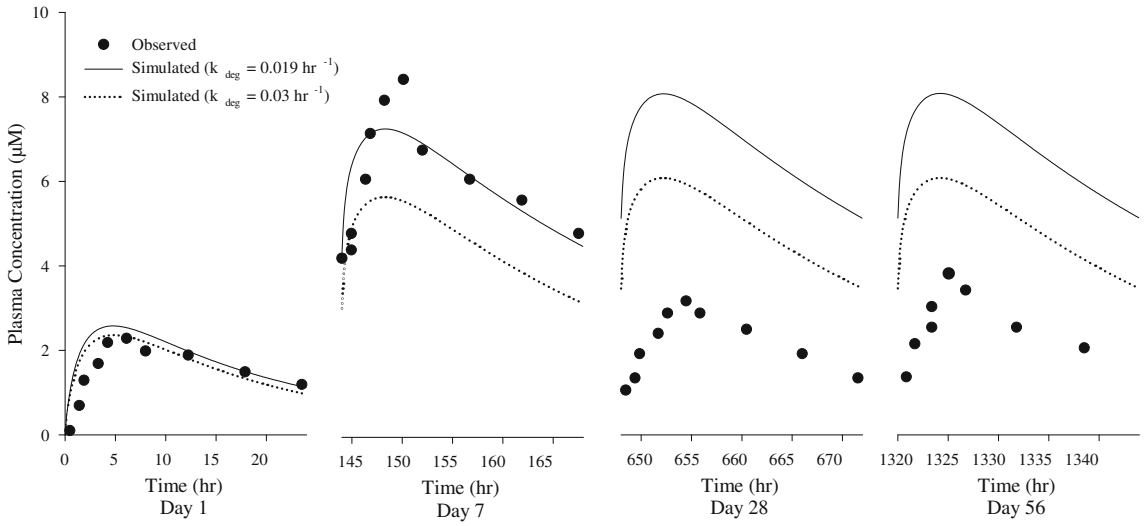

Fig. 2. Plasma concentration–time profiles of aprepitant (observed vs simulated) following aprepitant oral administration at 160 mg QD for 56 days. Observed data were obtained from aprepitant summary of clinical pharmacology studies, Rahway (NJ), Merck (data on file). Simulated data were based on k_{deg} values of 0.019 and 0.03 h^{-1} (see additional details in ESM S4)

targeted studies to better understand the underlying mechanisms for the observed unpredictable time-dependent outcome, which could be incorporated into a more refined PBPK model that better captures all potential contributing factors.

Compound 4-Midazolam DDI. Unlike the above two cases, compound 4, is a substrate and TDI of CYP3A but is unresponsive in human hepatocyte cultures for mRNA CYP3A4 induction suggesting it is not an inducer. Using a similar PBPK modeling approach as described above (ESM S4), about a threefold over prediction of the accumulation ratio of its own exposure was simulated following 10 days of once daily dosing (Table VI). This overprediction is suggestive of autoinhibition, which was not observed clinically; the observed PK on day10 was consistent with no change in clearance from day1. The overpredicted accumulation was reduced, but not eliminated even with fm_{CYP3A} set to 0.5 (data not shown); this fm_{CYP3A} value was thought to be unlikely as it is in contrast with the *in vitro* reaction phenotyping studies with human liver microsomes and clearance mechanisms in animals, which suggested an fm_{CYP3A} of ~0.9. While the approximately fivefold overprediction in midazolam interaction is not unexpected given the overprediction in compound 4 steady state exposure, it was surprising that there was still a 2.8-fold overprediction of the midazolam interaction even when the clinically observed steady state PK profile of compound 4 was well-matched by specifying a time-independent apparent clearance based on that observed *in vivo* (Table V). Thus, the model over predicted the clinical consequence of the *in vitro* time-dependent inhibition of CYP3A-mediated metabolism of both the perpetrator compound 4 and the victim midazolam. Several experiments were conducted to investigate potential causes for these significant over predictions, including investigating reversibility of the inhibition. Such mechanistic understanding is required for modeling TDI since both current static and PBPK approaches to model DDI for compounds are based on an assumption that there is a time-dependent irreversible loss of enzyme activity. The inhibitory effect of compound 4 was confirmed to be irreversible (data not shown), ruling out this as a major contributing factor to the poor modeled result. Results from studies in a human hepatocyte model, which is a more complete system and has been shown to yield *in vitro* inhibition parameters more predictive of an *in vivo* system for some compounds (89,90), showed that the inhibitory potency (kinact/KI ratio) was only marginally reduced (less than twofold), and the reduction had minimal effect on improving the simulation of PK and DDI of compound 4 (Table VI). In addition, the predicted results became slightly worse when simulations were conducted using the k_{deg} value of 0.019 h^{-1}, instead of 0.03 h^{-1} (data not shown), suggesting that the overprediction is not due to the assumed k_{deg} value. The disconnect between *in vitro* TDI and the *in vivo* effect for compound 4 is not entirely clear at the current time, although similar observations have been published to highlight the complexity in obtaining accurate *in vitro* TDI parameters and in DDI prediction for compounds behaving as TDIs *in vitro* (91,92). This provides further support to the notion that prospective PK and DDI modeling of time-dependent effects using PBPK is complex with the current knowledge of the system, even with availability of human PK data.

CONCLUSIONS

The goal of regulatory guidance is to ensure safe and effective use of approved medicines. However, given the complications highlighted above and considering that the majority of drug candidates will fail after a first in human trials (>90%) or after a proof of concept (POC) study (>60%) (93), the recommendations and associated criteria proposed in the DDI guidance, if not appropriately applied during drug development, may result in increased unnecessary studies which add to the already costly process of drug discovery and development (22). This is particularly the case for the transporter related studies, where focus should be primarily on the more established transporters Pgp and OATP1B1. Furthermore, we believe that these transporter-related DDIs should be evaluated on a case-by-case basis using approaches supported by current scientific knowledge in the field and with a holistic view considering the overall properties of a drug candidate including the safety profile of the candidate and/or concomitant drugs. For the majority of compounds, we question the need to conduct *in vitro* studies on a routine basis or *in vivo* clinical DDI studies using the currently recommended probe substrates/inhibitors in the US draft DDI guideline, especially on drug transporters for which the role and impact on drug disposition are presently less well established relative to Pgp and OATP1B1.

Considering the complexities associated with predictions of time- and concentration-dependent effects, clinical DDI studies of TDIs and mixed inhibitors/inducers may be inevitable, and should be done after the clinical dose and efficacious exposure have been determined (i.e., after POC). Given the current state-of-the-art, the recommended mechanistic model-based approaches (including PBPK) based solely on *in vitro* results (bottom-up approach) are not recommended for prospective DDI prediction of these compounds. Instead, the dynamic PBPK models should be applied after availability of phase I human PK data. The PBPK-based simulations which accurately capture the steady-state human PK profiles may help flag potential DDI issues in early development and together with other considerations (e.g., anticipated safety margin, commonly prescribed medications in targeted clinical trial populations) can be used to determine potential risks and aid in prioritization/planning of a clinical DDI strategy, but should not serve as an automatic trigger for a clinical DDI study. When applicable, the regulatory criteria should be used to rule out the need for conducting clinical DDIs, although given the conservative nature of the guidance, this maybe a rare occasion. With at least one properly designed clinical DDI study from which the results can be used to validate a PBPK model and its assumptions, the model may then be used to explore potential outcomes of various "untested" clinical situations (i.e., "what ifs"), and potentially in some cases, the predicted results could be used to justify waiving certain clinical DDI studies. This important role of PBPK will be increasingly realized during later stages of drug development when much more information, including a mechanistic understanding of a compound from targeted issue-driven studies would have already been gained to help parameterize and validate the model.

Overall, the new guidance documents contain many helpful recommendations for assessing a new drug candidate for its DDI potential mediated *via* drug transporters and drug

metabolizing enzymes. However, there are significant knowledge gaps necessitating continued research in the aforementioned emerging areas to aid in developing and refining the recommendations before implementation.

ACKNOWLEDGMENTS

We would like to thank Drs. Ying-Hong Wang, Jairam Palamanda, Prajakti Kothare, Raymond Evers, and David Cutler for their help and valuable inputs, and Drs. Jane Croft Harrelson, Nancy Agrawal, Richard Morrison, and James Yergey for their critical review of the manuscript.

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