1521-009X/47/10/1206–1221$35.00
Drug Metabolism and Disposition

https://doi.org/10.1124/dmd.119.087270
Drug Metab Dispos 47:1206–1221, October 2019

Perspectives from the Innovation and Quality Consortium Induction Working Group on Factors Impacting Clinical Drug-Drug Interactions Resulting from Induction: Focus on Cytochrome 3A Substrates

Diane Ramsden, Conrad Fung, Niresh Hariparsad, Jane R. Kenny, Michael Mohutsky, Neil J. Parrott, Sarah Robertson, and Donald J. Tweedie

Alnylam Pharmaceuticals, Cambridge, Massachusetts (D.R.); Vertex Pharmaceuticals, Boston, Massachusetts (C.F., N.H., S.R.); Genentech, South San Francisco, California (J.R.K.); Eli Lilly and Company, Indianapolis, Indiana (M.M.); Roche Innovation Center, Basel, Switzerland (N.J.P.); and Merck & Co., Inc., Kenilworth, New Jersey (D.T.)

Received March 22, 2019; accepted August 6, 2019

ABSTRACT

A recent publication from the Innovation and Quality Consortium Induction Working Group collated a large clinical data set with the goal of evaluating the accuracy of drug-drug interaction (DDI) prediction from in vitro data. Somewhat surprisingly, comparison across studies of the mean- or median-reported area under the curve ratio showed appreciable variability in the magnitude of outcome. This commentary explores the possible drivers of this range of outcomes observed in clinical induction studies. While recommendations on clinical study design are not being proposed, some key observations were informative during the aggregate analysis of clinical data. Although DDI data are often presented using median data, individual data would enable evaluation of how differences in study design, baseline expression, and the number of subjects contribute. Since variability in perpetrator pharmacokinetics (PK) could impact the overall DDI interpretation, should this be routinely captured? Maximal induction was typically observed after 5–7 days of dosing. Thus, when the half-life of the inducer is less than 30 hours, are there benefits to a more standardized study design? A large proportion of CYP3A4 inducers were also CYP3A4 inhibitors and/or inactivators based on in vitro data. In these cases, using CYP3A selective substrates has limitations. More intensive monitoring of changes in area under the curve over time is warranted. With selective CYP3A substrates, the net effect was often inhibition, whereas less selective substrates could discern induction through mechanisms not susceptible to inhibition. The latter included oral contraceptives, which raise concerns of reduced efficacy following induction. Alternative approaches for modeling induction, such as applying biomarkers and physiologically based pharmacokinetic modeling (PBPK), are also considered.

SIGNIFICANCE STATEMENT

The goal of this commentary is to stimulate discussion on whether there are opportunities to optimize clinical drug-drug interaction study design. The overall aim is to reduce, understand and contextualize the variability observed in the magnitude of induction across reported clinical studies. A large clinical CYP3A induction dataset was collected and further analyzed to identify trends and gaps. Reporting individual victim PK data, characterizing perpetrator PK and including additional PK assessments for mixed-mechanism perpetrators may provide insights into how these factors impact differences observed in clinical outcomes. The potential utility of biomarkers and PBPK modeling are discussed in considering future directions.

Introduction

As part of an overall assessment of current practices and recommendations in regulatory drug-drug interaction (DDI) guidelines, the Induction Working Group (IWG) of the International Consortium of Innovation and Quality in Pharmaceutical Development (IQ) collated an extensive data set of in vitro and clinical DDI induction data. The most commonly reported induction was with cytochrome P450 (P450) 3A, which became the focus of the analysis. This endeavor highlighted a large degree of variability in derived in vitro induction parameters (Kenny et al., 2018), as well as variability in the observed clinical DDI across multiple studies. Clinical DDI data were collected for numerous compounds for which in vitro induction data were available (Kenny et al., 2018) to support evaluation of in vitro to in vivo extrapolation (IVIVE). Since most clinical studies did not report individual subject data, the mean or median reported area under the curve ratio (AUCR) values were collected for comparison across studies. A limitation of this approach, as discussed below, is that the mean or median reported AUCR will be dependent on the number of subjects studied and sample size differences will contribute to the apparent variability observed across studies. The median of the reported AUCR values was determined, and

https://doi.org/10.1124/dmd.119.087270.
This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: AUC, area under the curve; AUCR, area under the curve ratio; CAR, constitutive androstane receptor; C_{ave}, average concentration; C_{max}, maximum concentration; DDI, drug-drug interaction; EE, ethinylestradiol; EMA, European Medicines Agency; E_{max}, maximum fold increase (or induction) minus baseline of 1-fold; FDA, Food and Drug Administration; F_G, fraction escaping gut metabolism; F_H, fraction escaping liver unchanged; f_m, fraction of metabolism; f_mP450, fraction metabolized by cytochrome P450; HV, healthy volunteer; IQ, innovation and quality consortium; IVIVC, in vitro in vivo correlation; IVIVE, in vitro to in vivo extrapolation; IWG, Induction Working Group; LNG, levonorgestrel; OC, oral contraceptive; P450, cytochrome P450; PBPK, physiologically based pharmacokinetic modeling; PK, pharmacokinetics; PXR, pregnane-X receptor.

no weighting of data based on sample size was applied. In addition, it is important to distinguish between, on the one hand, the systematic sources of variation associated with deterministic factors and, on the other, the random variation (cross-study, intersubject, intrasubject, etc.) customarily associated with experimentation.

The clinical data were refined based on the dose level (e.g., rifampicin dose = 600 mg daily) and a minimum duration of dosing (>5 days), which included different probe drugs as long as they were metabolized in part by CYP3A. Importantly, within this refined clinical dataset, there remained a high degree of variability (Fig. 1), which shows the spread of clinical AUCR and was also discussed in Kenny et al. (2018). In the case of strong CYP3A inducers, rifampicin, phenytoin, and carbamazepine, the clinical effect ranged from strong induction (AUCR < 0.2) to no effect or even weak inhibition (rifampicin and phenytoin). Similarly, in vitro inducers of CYP3A, which are also reversible or time-dependent in vitro inhibitors of CYP3A, such as nelfinavir, nevirapine, rosiglitazone, ritonavir, and saquinavir, showed a range of clinical response from strong induction to strong inhibition.

There are multiple factors which can potentially contribute to the magnitude of clinical response, such as the dose and exposure of the inducer, the subject population, the substrate drug (victim), route of substrate administration (impacted by hepatic ± intestinal contribution), whether the inducer is also an inhibitor or inactivator (and hence timing of substrate administration could be important), and the duration of treatment with inducer. However, even in the case of rifampicin, the magnitude of difference in reported AUCR is still ~10-fold across studies with oral midazolam and a 600-mg dose of rifampicin administered daily for >5 days. What else could be driving this range of responses? Is it possible or even practical to standardize the design of clinical DDI studies or further optimize conditions to reduce variability between studies? Can differences in clinical outcomes be better understood or controlled through orthogonal analysis, for example, applying biomarker data indicative of induction, by PBPK modeling or some other approach, or could the variability determined by these endpoints just confuse the issue? Importantly, do inconsistencies in outcomes impact the conclusions being derived from these studies; for example, is the compound a mild, moderate, or potent inducer, and will these conclusions extrapolate to other drugs defined within the same potency class? Some variability in the in vitro data across companies is to be expected since protocols, reagents, donors, and analysis (e.g., methods and instrumentation) differ between companies. In an effort to present data as it will be generated in real life, the IWG deliberately did not control for different methods used by different investigators, but rather provided examples that illustrate ranges of cross-study and intrastudy variation that would be encountered when comparing their results with published data. Even when conditions and materials are controlled, the size of the data set can impact the perceived variability within a laboratory, as well as between companies. Small in vitro sample sizes are inherently less representative of the full population and can result in mean and standard deviation values that differ from those of the overall population. A resampling exercise in which subsamples of 5, 10, 15, or 20 subjects were randomly selected from a collated set of in vitro donor data (Kenny et al., 2018; Fig. 1), demonstrated how the ranges of sample mean and standard deviation values are narrower when larger data sets are obtained (data not shown). It is not clear to what extent the sample size is the sole contributor to the overall in vitro variability, as any analysis will also be complicated by other contributing factors. This commentary attempts to address potential causes of variability observed in clinical DDI data for CYP3A inducers and to identify opportunities for better characterization of induction to minimize variability, with an eventual goal of optimizing the design of clinical DDI studies. Toward this goal, although formal recommendations are not being made, we hope that highlighting certain aspects of induction studies within the this commentary will add to the recommendations from the IQ IWG (Hariparsad et al., 2017; Kenny et al., 2018) and stimulate a dialogue.

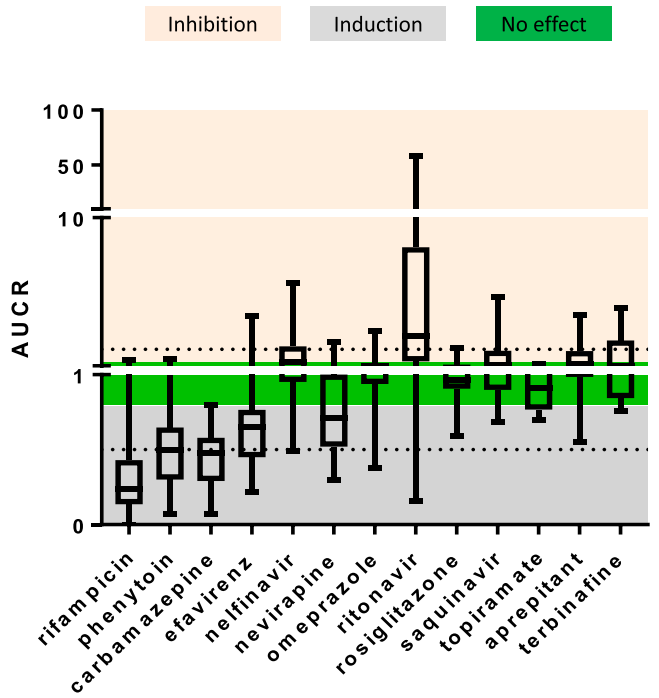

Fig. 1. Box and whisker plots showing the 25th to 75th percentiles within the box; the center line designates median, and the whiskers extend to the minimum and maximum reported AUCR values. Data are presented using a linear *y*-axis. Clinical AUCR values were collected from the University of Washington drug interaction database and are the reported mean or median change observed after administration with in vitro inducers for ≥5 days. Substrates collected include any with some metabolism through CYP3A. Number of studies collected varies across inducers. Raw data values are contained within Kenny et al. (2018). In general, a range of response was observed across the in vitro inducers and was dependent on multiple factors.

Are clinical DDI studies designed with consideration of the subject number needed to account for the variability in victim PK observed, and are these studies powered to establish a meaningful difference? The DDI potential of a P450 inducer is generally concluded on the basis of results from just one or two clinical DDI studies, conducted by the sponsor company. A key conclusion from Kenny et al. (2018) was that simple models could be used to assess clinical risk despite both the expected range of responses between individuals and the less expected range within responses observed in the clinical data and especially in the in vitro data. In that analysis, quantitative predictions that fell within 2-fold or within bioequivalence limits (0.8–1.25) were improved across 63% of the prediction methods when the median in vitro parameters were used in the prediction models and comparison was made to the lowest clinical AUCR, indicative of the most potent induction, rather than the median clinical AUCR, which was determined using data from all substrates. In many cases, the lowest AUCR was observed using a substrate that was not as selective toward CYP3A and was also metabolized by coregulated enzymes (Supplemental Table 2, Kenny et al., 2018). While the contribution of CYP3A to the overall metabolism of the substrate contributed to some of the variability in the clinical response, high variability was still observed between studies with selective CYP3A substrates (Table 1). Here we ask, to what extent does overall PK of the substrate (victim), including the contribution of metabolism to total clearance, fraction of metabolism (f_m) through other induced enzymes, the fraction escaping gut metabolism (F_G), the contribution of transporters to substrate disposition, and the inducer

TABLE 1

Maximum over minimum point estimates within each study for mean clinical change with all CYP3A substrates compared with sensitive CYP3A substrates

Inducer	Max AUCR/Min AUCR	
	All Substrates	CYP3A Sensitive
Aprepitant	7.5	3.3
Bosentan	2.2	2.0
Efavirenz	18	4.0
Lersivirine	2.2	1.8
Nelfinavir	12	5.5
Omeprazole	8.2	3.4
Phenytoin	20	5.5
Rifampicin	277	213
Ritonavir	307	49.1
Saquinavir	7.9	6.8
Terbinafine	5.8	2.8

AUCR, area under the curve ratio.
Adapted from Kenny et al. (2018), with permission.

(perpetrator, often not captured during the study) contribute to the variability in clinical outcomes? Consideration will also be extended as to whether the perpetrator is an inhibitor of the induced enzyme, coregulated enzymes, and/or transporters.

Contributors to the Overall Variability Observed in Clinical Studies

Differences in Levels of CYP3A. Variability in the expression and function of CYP3A, both interindividual and intraindividual (changes over time), has been well described (Thummel et al., 1994; Paine et al., 1997; Lin et al., 2001). This is clinically relevant because it can lead to variability in PK, PD, toxicity, and DDI and needs to be taken into account for deriving predictions. There is also the potential for gut extraction to contribute to observed differences in clinical outcomes, which is discussed in further detail below. Intrinsic (genetic, physiologic) and extrinsic (environmental, diet) factors both contribute. Midazolam clearance (CYP3A-mediated) varies by 5- to 11-fold (Floyd et al., 2003; He et al., 2006), with some studies reporting greater than a 20-fold range (Lin et al., 2001; Zhu et al., 2003). There are published examples of higher clearance of midazolam in South Asian and Japanese subjects compared with Caucasians and Europeans (Kato et al., 2010; van Dyk et al., 2018). A recent study (van Dyk et al., 2018) showed that the baseline midazolam AUC was 38% higher in Caucasians than in South Asians. Measurable differences in the magnitude of inhibition and induction DDI were also observed between Caucasians and South Asians. Women have exhibited up to 26% higher clearance for CYP3A substrates compared with men, which was more pronounced with intravenous midazolam (Greenblatt and von Moltke, 2008; Hu and Zhao, 2010). Gorski et al. (2003) also reported a large difference in response to rifampicin, with men showing a higher induction of oral midazolam clearance than women (Gorski et al., 2003). A recent review aimed at evaluating whether evidence for sex differences in DDI exist concluded that sex differences in DDI appear to be limited (Naidoo and Chetty, 2019). However, the number of clinical studies evaluating DDI potential in females was small (five), and comparisons of sex effects in DDI studies require further study given the sparsity of clinical trials where both sexes are included (7.7%). Studies have indicated that the exposure of oxycodone, which is primarily metabolized by CYP3A, can be dependent on age, with 2-fold greater mean exposure in elderly than in young adults (Liukas et al., 2008). Differences can also exist in the PK for CYP3A substrates between healthy volunteers (HVs) and patients (Yang et al., 2003; Nebert et al., 2013), which can lead to differences in DDI outcomes. As an example, when the effect of rifampicin on saquinavir was evaluated in HV and in patients with HIV, the magnitude of change was far greater in HV subjects compared with patients (70.4% vs. 35.9% decrease in AUC, respectively) (Grub et al., 2001).

In a study in healthy male Chinese subjects (Yin et al., 2004), the interindividual variation in the urinary 6β-hydroxycortisol/cortisol ratio was reported to be 30-fold, whereas the intraindividual variability was only 30%. The authors concluded that genetics contributed approximately 90% to interindividual variability, which is in accord with other studies (Ozdemir et al., 2000). Intraindividual variability of 5%–20% in CYP3A activity has been reported, measured by intravenous doses of alfentanil or midazolam (Kashuba et al., 1998; Kharasch et al., 1999). Diurnal variations in midazolam clearance in healthy volunteers, due to variation in enzyme activity and absorption rate, have also been observed with higher clearance in the evening compared to morning (Klotz and Ziegler, 1982; van Rongen et al., 2015). Since DDI studies likely synchronize to either evening or morning dosing, this may not significantly impact the magnitude of DDI observed within subjects in the same study, although differences in design could, in theory, contribute to the variability observed across studies in aggregate analyses. CYP3A5 genotype, particularly substrate and inhibitor overlap with CYP3A4, and differential regulation, are additional complicating factors (Pearson et al., 2007; Lolodi et al., 2017). While individuals carrying the lower activity CYP3A4*22 allele require lower statin doses (Wang et al., 2011) and have been proposed (along with variants of CYP3A5 and PXR) to contribute to higher tacrolimus levels (Pallet et al., 2015), the low incidence of this allelic variant probably does not contribute significantly to the overall variability of CYP3A activities in vivo. Genetic influence in twin studies has been shown to account for 66%–88% of interindividual variability (Klein and Zanger, 2013), with cytochrome P450 reductase and peroxisome proliferator-activated receptor α identified as potential contributors. Levels can also be impacted by vitamin D (Wang et al., 2013) and liver- enriched transcription factors, FoxA2 and PXR (Thirumaran et al., 2012). The accommodating active site of CYP3A4, including allosteric interactions with broad substrate and inhibitor interactions modulating activity, may add to this complexity (Shou et al., 2001; Atkins, 2005; Davydov and Halpert, 2008). α-Napthoflavone is an example of an activator of CYP3A activity in vitro (Domanski et al., 1998) and carbamazepine activity is increased by progesterone (Denisov et al., 2015). Whether these effects observed in vitro translate to in vivo changes remains controversial, and as such it is unclear whether modulation of carbamazepine activity, as a CYP3A, substrate could impact its effective concentration as an inducer.

Is there a way to account and correct for some aspects within a clinical DDI study that possibly contribute to variable outcomes such that extrapolation to other substrates and/or inducers would be more predictable? For example, could the addition of an orthogonal measure of CYP3A activity, such as a biomarker, provide additional insights into the magnitude of response or would that additional measurement of changes in CYP3A activity simply exhibit its own independent variability and complicate rather than deconvolute? There have been a number of endogenous and exogenous markers of CYP3A activity evaluated over the years, such as the erythromycin breath test (Watkins et al., 1989), 6β-hydroxycortisol (and the ratio to cortisol) (Ged et al., 1989), 4β-hydroxycholesterol (Mao et al., 2017), quinine (Wanwimolruk et al., 2002), and more recently ω or ω-1 hydroxylated medium chain acylcarnitines (Kim et al., 2018). All have had mixed success and limitations. The utility of erythromycin was limited by its selectivity and specificity as a substrate and an inhibitor of other contributing enzymes and transporters [e.g., inhibition of P-glycoprotein (P-gp)] (Schwarz et al., 2000; Eberl et al., 2007). The overlap in selectivity

of CYP3A and P-gp is well documented, and the dual effect of P-gp–enhancing CYP3A intestinal first pass metabolism can add to the variability in first- pass metabolism (Wacher et al., 1995). The promise of these approaches should continue to stimulate research.

Selectivity of Substrate toward CYP3A4. As described by Kenny et al. (2018), clinical induction data were collected for all the in vitro inducers and substrates (victim drugs), including those substrates where CYP3A contributed to any extent toward the metabolism (as defined by in vivo or in vitro data). One limitation of including all substrates, regardless of how extensively they are metabolized by CYP3A, is that the magnitude of DDI effect for an inducer is dependent on the relative contribution of CYP3A, or other inducible enzymes, to the overall metabolism of the substrate (i.e., fraction metabolized, or f_m). All regulatory agencies are aligned on the recommendation to use oral midazolam to investigate CYP3A induction clinically. Midazolam has a high CYP3A fraction metabolism (f_{m3A4} = 0.93), with equal contribution of liver and gut (F_G = 0.51) (Fahmi et al., 2008; Gertz et al., 2010). These relative contributions are reflected in the differences in the magnitude of induction observed for intravenous and oral midazolam, indicating that an important contribution to net outcome is at the enterocyte level (see additional discussion below; Fig. 2). The Food and Drug Administration (FDA) DDI guidance acknowledges that there are many sensitive CYP3A substrates, other than midazolam, that can be used to evaluate potential CYP3A induction clinically (Food and Drug Administration FDA, 2017). An advantage of midazolam is its short half-life; as such, a full AUC can be gathered within 24 hours of dosing. For substrates with longer half-lives and for which collection periods extend beyond 24 hours, consideration should be given to continued dosing of the inducer over the collection period. A list of potential sensitive substrates is maintained on the FDA's Web site for Drug Development and Drug Interactions https://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm. The FDA also points out that some substrates, such as omeprazole and repaglinide, while indicated as substrates for other CYPs, namely CYP2C19 and CYP2C8, respectively, also undergo metabolism by CYP3A.

Steady-state data are available for the same inducer across multiple substrates (Table 2). The clinical induction response was similar across substrates for rufinamide, terbinafine, bosentan, and nevirapine (Table 2). Larger differences between substrates were observed for inducers with competing mechanisms of DDI (induction vs. inhibition or inactivation),

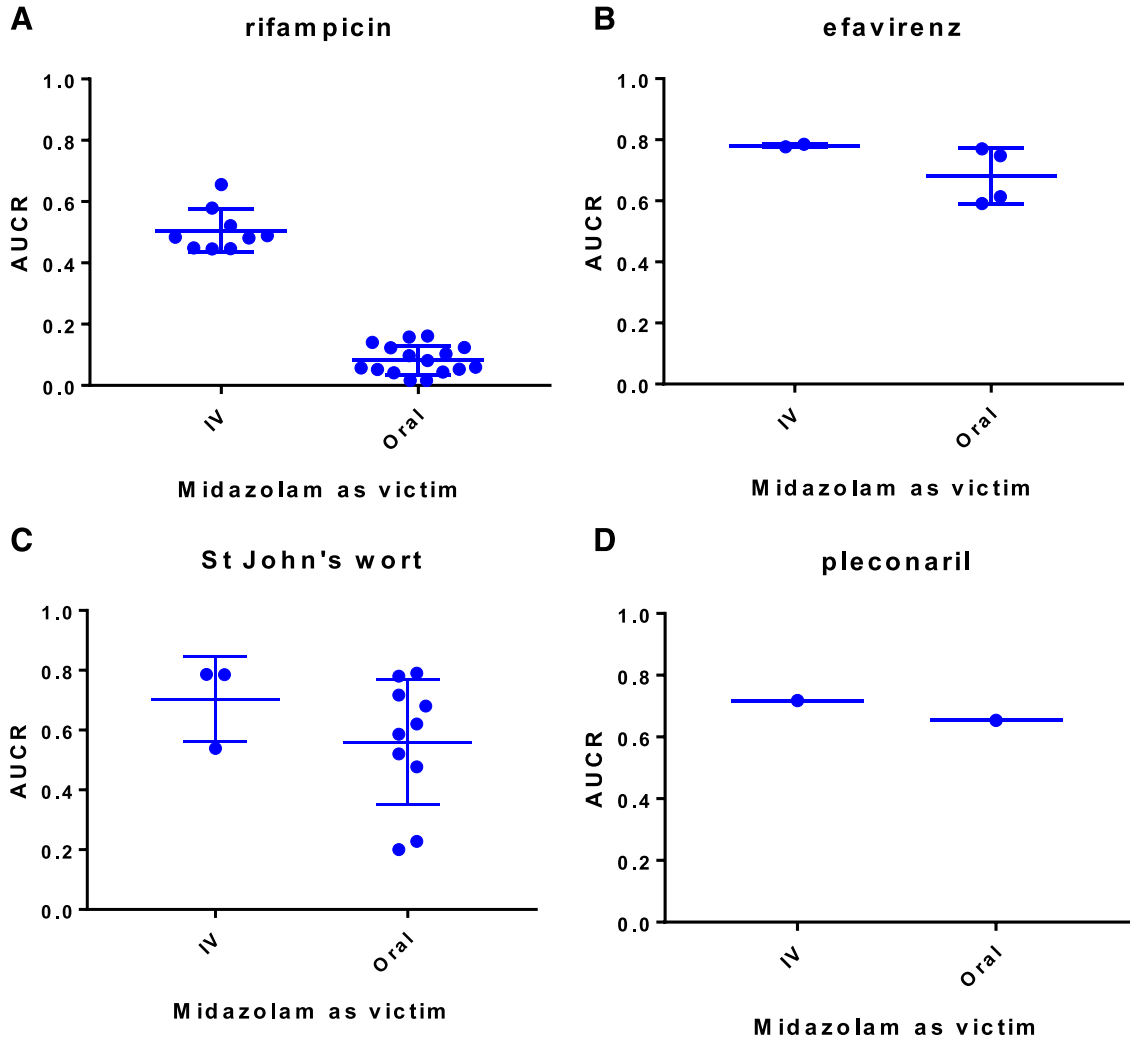

Fig. 2. Clinical induction data collected from the University of Washington drug interaction database for inducers (Panel A, rifampicin, B), efavirenz, C), St John's wort and D, pleconaril) where both intravenous and oral midazolam changes were evaluated, although not necessarily within the same study. The reported AUCR reflects the mean or median study data and does not account for differences in number of subjects between studies. The center blue line represented the mean of the collected data, and the error bars represent the standard deviation. The trend is for a greater inductive response with oral midazolam, suggesting an important role of enterocyte-expressed enzyme in first-pass metabolism and fraction escaping gut metabolism upon induction.

TABLE 2

Effect of inducer and substrate on area under the curve ratio (AUCR)

Inducer	AUCR of Object at Inducer Steady State																				
	mid	vcz	trz	smv	alf	alp	amp	art	atz	atv	Caf	CsA	dtg	EE	etr	fex	ima	ind	itz	lop	met
efavirenz	0.591	0.217	—	0.396	0.393	—	0.626	0.445	—	0.586	—	—	0.43	—	0.694	0.774	—	0.754	—	0.49	0.43
phenytoin	—	0.28	—	—	—	—	—	—	—	0.461	—	0.529	—	0.51	—	—	—	—	0.07	0.7	0.479
omeprazole	—	1.41	—	—	—	—	0.96	—	0.554	—	1.13	—	1.00	—	1.41	—	0.971	0.533	0.849	0.921	—
ritonavir	26.4	0.16	21.1	5.57	12.0	2.48	3.22		25.9	—	0.245	—	—	0.613	—	1.39	0.967	5.5	—	—	—
rifampicin	0.016	—	0.05	0.09	0.052	0.117	0.183	—	0.240	0.197	0.629	0.323	0.46	0.345	0.135	0.346	0.261	—	0.237	0.323	0.232
rufinamide	—	—	0.633	—	—	—	—	—	—	—	—	—	—	0.774	—	—	—	—	—	—	—
terbinafine	0.755	—	0.811	—	0.84	—	—	—	—	—	1.30	0.861	—	—	—	—	—	—	—	—	—
bosentan	—	—	—	0.656	—	—	—	—	—	—	—	—	—	0.69	—	—	—	—	—	—	—
carbamazepine	—	—	—	0.255	—	—	—	—	—	—	—	—	—	0.58	—	—	—	—	—	—	—
saquinavir	5.18	—	—	—	—	—	0.684	—	1.17	—	—	—	—	—	—	—	—	—	—	0.931	—
nevirapine	—	—	—	—	—	—	0.78	0.32	—	—	—	-	0.81	0.678	—	—	—	0.675	0.377	—	0.512

alf, alfentanil; alp, alprazolam; amp, amprenavir; art, artemether; atv, atorvastatin; atz, atazanavir; caf, caffeine; CsA, cyclosporine A; dtg, dolutegravir; EE, ethinyl estradiol; etr, etravirine; fex, fexofenadine; ima, imatinib; ind, indinavir; itz, itraconazole; lop, lopinavir; met, methadone; mid, midazolam; smv, simvastatin; trz, triazolam; vcz, voriconazole; —, no data.

such as efavirenz, phenytoin, ritonavir, nelfinavir, and saquinavir. When both the induction and inhibition mechanisms contribute to the observed DDI outcome the f_mCYP3A versus coregulated proteins can drive the direction of DDI. A large data set was available for rifampicin, which enabled comparisons between substrates and the magnitude of DDI (Table 2). Rifampicin is defined as a strong inducer (AUCR <0.2) (Food and Drug Administration FDA, 2017), and the most pronounced induction occurred with oral midazolam (AUCR = 0.016). Strong induction of a similar magnitude was observed for the sensitive substrates, triazolam, simvastatin, and alfentanil (AUCR 0.05–0.09). Strong induction was also observed with rifampicin for the moderately sensitive substrates alprazolam and atorvastatin, as well as amprenavir and etravirine, which are not recommended substrates (Food and Drug Administration FDA, 2017). Moderate induction by rifampicin (AUCR 0.2–0.5) was observed with substrates not considered as sensitive toward CYP3A (Table 2), likely due to the limited role of CYP3A or other inducible enzymes toward their metabolism and/or a lesser role of metabolic clearance versus renal clearance toward overall elimination. Importantly, there was no correlation when comparing midazolam AUCR with the AUCR observed for substrates that are less selective toward CYP3A, including atazanavir, caffeine, ethinylestradiol, fexofenadine, lopinavir, and methadone. In contrast, sensitive substrates, such as triazolam, simvastatin, alfentanil, and amprenavir, were positively correlated with midazolam (Fig. 3).

The difference between the minimum and maximum induction observed across all substrates after 600 mg of rifampicin treatment was 277×, whereas the difference when considering only oral midazolam as the probe substrate was 10×. The impact of sample size (as mentioned previously) could also contribute to these ranges. Some substrate-dependent outcomes contributed to the observed variability, highlighting that f_m is an important driver to the magnitude of induction observed. For instance, rifampicin treatment results in a more pronounced change in zolpidem exposure (AUCR = 0.29) compared with theophylline (AUCR = 0.73). The f_{m3A4} is ~0.4 for zolpidem but much lower for theophylline (0.05–0.1) (Gillum et al., 1996; Villikka et al., 1997). The magnitude of effect on theophylline is also complicated by the relative contribution of CYP1A2 in its metabolism and by induction of CYP1A2 by rifampicin (Rae et al., 2001; Backman et al., 2006; Chen and Raymond, 2006). This is just one example that helps to illustrate the complexity of assessing in vivo P450 induction, which can be dependent on many factors.

Contribution of Intestinal Metabolism. Paine et al. (1997) noted interindividual differences in intestinal levels of CYP3A, the regiospecificity in content of CYP3A (jejunum > duodenum > ileum), differences in the relative levels of P450 reductase affecting CYP3A activity, and a lack of correlation of intestinal levels of CYP3A to hepatic levels within individuals, all of which could complicate an assessment of the relative contribution of intestine versus liver for metabolism of CYP3A substrates. The fraction of the substrate escaping intestinal (F_G) and hepatic (F_H) first-pass metabolism is also an important driver to the magnitude of AUCR observed on induction. F_G can vary significantly across sensitive substrates; for example, simvastatin F_G is ~0.1 while alprazolam is ~1 (Gertz et al., 2010). By dosing a CYP3A substrate both intravenously and orally, it might be possible to separate the induction of liver alone from the combined enterocyte and liver induction and in turn may help evolve prediction methods. Differential exposure to an orally administered inducer by the intestine and liver obviously can contribute to the relative extent of induction in these tissues. Different half-lives for CYP3A have also been used for modeling the impact of mechanism-based inactivation using in vitro kinetic parameters (Obach et al., 2007). Although static models used to assess the induction liability of compounds are informed by the

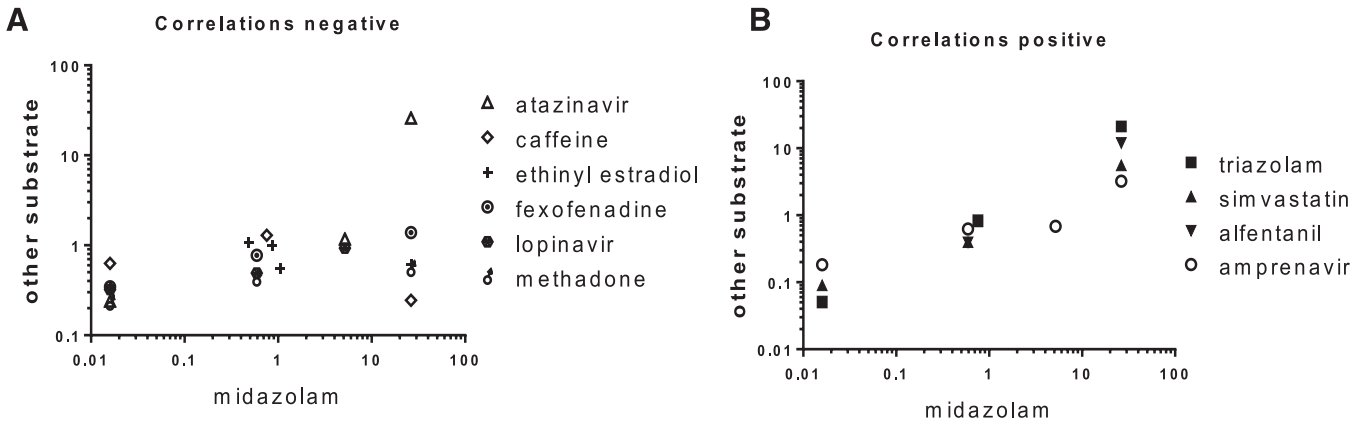

Fig. 3. Pearson correlation analysis, with two-tailed *P* value, and significance of 0.05 conducted using GraphPad Prism version 7 (GraphPad Software San Diego, CA). Correlation analysis of the AUCR observed for inducers with oral midazolam compared with individual other substrates. (A) Shows where correlations were not observed and includes the substrates, atazanavir (correlated using one-tailed), caffeine, ethinyl estradiol, fexofenadine, lopinavir, and methadone. (B) Includes other sensitive CYP3A substrates that show a positive correlation with oral midazolam AUCR.

maximal concentrations in the plasma ($I_{max,u}$) and the gut, a recent publication (Chang et al., 2017) has suggested that CYP3A4 induction by rifampicin in human hepatocyte culture is driven by overall exposure rather than by maximum exposure. Using various target-engagement study designs, the group showed that AUC or C_{ave}, rather than C_{max}, could most closely recover the observed changes in enzyme levels. Clinical studies to determine the influence of AUC versus C_{max} or comparative studies where the inducer is dosed both orally and intravenously have not been conducted. In several studies, probe substrates were administered both intravenously and orally after oral administration of inducer (Fig. 2; Table 3), which allows quantification of the difference in induction between intestine and liver.

The influence of rifampicin on the CYP3A4 substrate alfentanil, a moderate clearance drug, showed that the fraction escaping the liver unchanged (F_H), measured after an intravenous dose of alfentanil, decreased from 0.74 in the control situation to 0.29 with rifampicin treatment (Kharasch et al., 2011). These investigators demonstrated that after oral administration of alfentanil, F_G decreased from 0.68 to 0.19 with rifampicin administration, highlighting a similar change in intestinal and hepatic induction. Consistent with the observations made with rifampicin, administration of a weak inducer, armodafinil, resulted in an AUC ratio of 0.83 after intravenous midazolam and 0.68 after oral midazolam administration (Darwish et al., 2008). A comparison of the impact of administration of an inducer on liver (intravenous) versus intestinal extraction (oral) was made for eight CYP3A substrates. These substrates ranged from low to high extraction (Table 3). The equations described in Kharasch et al. (2011) were used to estimate F_G and EH, hepatic extraction from the reported or derived CL_{iv} values. An average Q_p of 17 ml/kg was applied. In general, the ratio of induced to noninduced was similar between liver and intestine, suggesting equal hepatic and enterocyte induction. There were notable exceptions, including cases where hepatic induction was greater (pleconaril + midazolam; rifampicin + methadone and verapamil) and where gut induction appeared greater (rifampicin + quinidine; phenobarbital + verapamil). It is plausible that interaction with transporters such as P-gp may lead to these observations (quinidine and verapamil are substrates, whereas rifampicin is an inhibitor). The use of PBPK modeling may offer an approach to interrogate the mechanisms behind these observations.

Although both rifampicin and armodafinil have shown that relative extent of induction can be similar between liver and intestine, this does not appear to be the case with all inducers. For example, efavirenz, a moderate inducer of CYP3A and CYP2B6, induces hepatic but not intestinal CYP3A (Mouly et al., 2002). It is not known whether the lack of intestinal induction of CYP3A is because there is no increase in enzyme levels or that the assay to measure changes in intestinal enzyme levels (western blotting) lacks sensitivity. The complexity added by efavirenz induction being mediated through CAR and PXR could also confound interpretation of outcome since the expression of CAR compared with PXR may be different between tissues. A study of intravenous and oral midazolam with efavirenz dosed to steady state has not been reported. Data with single dose efavirenz are available (Mikus et al., 2017).

Gorski et al. (2003) reported on this comparison and concluded that, for a given subject, the extent of induction was high in either the liver or intestine but not in both. They also noted that, in general, the lower the baseline oral clearance, the greater the change in oral clearance with rifampicin induction. Care should be taken when comparing the effect of the inducer on the liver and intestine since the overall influence of the inducer on the AUC ratio from the liver can be limited when clearance approaches hepatic blood flow, whereas the change in intestinal extraction will not be subject to this limitation. These authors specifically commented that midazolam is a moderate extraction ratio drug; after rifampicin treatment, the hepatic extraction ratio was 0.6, which the authors concluded still allowed for increases in intrinsic clearance to be detected. Additionally, (Fromm et al., 1996) have demonstrated that rifampicin does not alter hepatic blood flow and as such should not be of concern.

Although further studies are necessary to examine more fully the relative induction of the intestine versus the liver, the clinical evidence presented here suggests that induction of intestine does indeed play an important role in the changes of probe substrates, such as midazolam and alfentanil. The similar effects on the liver and gut with a strong, moderate, or weak inducer suggests similar processes control the intestinal and hepatic enzyme changes. Additional work is needed to derive further evidence of whether AUC/C_{ave} or C_{max} are better predictors for the overall effect. Currently, induction potential is evaluated in vitro using hepatocytes, and the derived induction parameters are then used as a surrogate for induction in enterocytes. This approach likely has limitations, and more work is needed to fully understand those deficiencies.

Dependence of Inducer Dose Level on Magnitude of Response. Review of regulatory submissions in 2013 and 2014 revealed that while 30% of submitted drugs were positive for in vitro induction, only a small fraction (<5%) resulted in an in vivo induction signal (Yu et al., 2014, 2016). The outlook from submissions during 2015 was slightly different and may reflect adoption of the DDI guidance recommendations (Yu et al., 2017). Of 33 approved NDAs, 27 were assessed for in vitro

TABLE 3

Evaluation of EH vs. EG for eight CYP3A substrates with varying hepatic extraction

Object	Precipitant	Dose (mg)/Duration (Days)	Reported F_G	Extraction Ratio/Ranking	Calculated		Induced/Baseline		Interpretation
					F_G	EH	EH	EG	
	rifampicin	5/5 or 6	0.51	0.3–0.5 (intermediate to high)	0.44	0.36	1.2	0.97	Similar
		10/5 or 6					1.3	0.98	Similar
		25/5 or 6					1.6	1.1	Similar
midazolam		75/5 or 6					1.7	1.3	Similar
		600/5			0.47	0.26	2.0	1.9	Similar
	armodafinil	100–250/28			0.46	0.39	1.2	1.1	Similar
	pleconaril	400 (tid)/6			0.59	0.52	1.4	0.13	>Hepatic
cyclosporine	rifampicin	600/11	0.44	0.22 (intermediate)	0.27	0.02	1.4	1.2	Similar
methadone	rifampicin	600/10	0.78	0.09 (low)	0.69	0.10	2.8	1.2	>Hepatic
	rifampicin	600/4	0.60	0.14 (low)	0.61	0.29	2.5	2.0	Similar
alfentanil	rifampicin	600/5			0.62	0.26	2.6	2.0	Similar
	efavirenz	600/20			0.68	0.30	1.9	1.6	Similar
nifedipine	rifampicin	600/7	0.40	0.64 (high)	0.75	0.52	1.7	2.8	Similar
quinidine	rifampicin	600/7	0.90	0.4 (high)	1.0	0.24	3.7	59	>Gut
tacrolimus	rifampicin	600/18	0.14	low to intermediate	0.11	NC	1.5	1.1	Similar
verapamil	rifampicin	600/13 and 15	0.65	high	1.0	0.86	1.1	0.2	>Hepatic
	phenobarbital	100/21			0.77	0.50	1.9	5.1	>Gut

EG = gastrointestinal extractionF_g, fraction escaping gut metabolism.

induction potential. Eight showed positive CYP3A induction, and one showed in vitro CYP3A downregulation. Of the seven tested for clinical induction, using a sensitive CYP3A substrate, three were positive, representing a shift upward compared with previous years (43%). Like the observations made in Kenny et al. (2018), most of the in vitro inducers also showed inhibition. Since the magnitude of induction is dependent on the concentration evaluated, consideration of the dose level and whether E_{max} has been achieved is critical to clinical study design and deriving comparisons across inducers. Importantly, the evaluation of DDI is typically limited to the highest labeled dose. Most of the clinical induction data collected by the IQ IWG did not contain the same compound and substrate pair across different dose levels; however, dose-response data are available for rifampicin with midazolam and alfentanil, as well as data for more than one dose level of several other inducers (avasimibe, bosentan, eslicarbazepine, rifabutin, ritonavir, brivaracetam, oxcarbazepine, and lersivirine) (Fig. 4). Except for rifabutin and oxcarbazepine, the magnitude of induction tended to increase with the increase in inducer dose level. It is plausible that the lowest dose level tested for rifabutin and oxcarbazepine may have already achieved E_{max}. As expected, the slopes of the induction dose response curves differed across compounds, likely owing to differences in potency across compounds, as can be observed in the kinetic parameters determined during in vitro induction assays.

Contribution of Inducer PK; Rifampicin as a Prototypical Inducer. There was a pronounced difference in the AUCR observed across victim drugs when coadministered with rifampicin (perpetrator) (Table 1). How do differences in rifampicin PK and autoinduction time course impact the observed induction responses? In many of the published clinical DDI studies, the perpetrator PK was not assessed. This highlights a missed opportunity to characterize pharmacokinetic-pharmacodynamic relationships for enzyme inducers to better understand the variability in response and to improve predictive translational modeling efforts.

Rifampicin induces multiple drug-metabolizing enzymes in vitro (Rae et al., 2001) by binding to the pregnane X receptor (PXR) and to a more limited extent through crosstalk with the constitutive androstane receptor (CAR) (Chen and Raymond, 2006). Affected enzymes include multiple P450s (CYP1A, CYP2A6, CYP2B6, CYP2C, CYP3A), uridine 5′-diphospho-glucuronosyltransferases (UGT) (UGT1A1, UGT2B7), glutathione S-transferases, flavin-containing monooxygenases, and P-gp (Rae et al., 2001). The PK of rifampicin is highly variable (10-fold interindividual difference) and can be impacted by disease state (Wilkins et al., 2008; (Milán Segovia et al., 2013); Seng et al., 2015; Stott et al., 2018). Rifampicin also demonstrates greater-than-dose-proportional increases in exposure due to extensive and saturable first-pass metabolism (Ruslami et al., 2007), as well as time-dependent PK as a result of autoinduction of metabolism, leading to increased clearance with repeat dosing (Acocella, 1978; Loos et al., 1985). Variability in rifampicin PK has been linked to polymorphisms of OATP1B1 (Kwara et al., 2014) and to SNPs in serine esterase arylacetamide deacetylase, which mediates its metabolism (Nakajima et al., 2011; Shimizu et al., 2012). Indeed, the variability in rifampicin PK has been hypothesized to play a role in the observed variability in clinical induction (Almond et al., 2016).

Possible Role of Transporters. There is broad overlap of substrates between CYP3A and P-gp (Wacher et al., 1995). In addition, a greater appreciation of the role of an increasing array of drug transporters (Tweedie et al., 2013) in the disposition of drugs adds to the complexity of data interpretation and contributes to the variability in DDI response across subjects. Differences in the induction parameters between in vitro systems was postulated to be due to expression of uptake transporters (Sun et al., 2017). Differences in OATP expression and function across subjects could have a profound effect on the magnitude of induction when the inducer is a substrate for uptake transport. The same is likely true for drugs that act as substrates or inhibitors of efflux transporters since they can modulate the intracellular concentration of the inducer. Although it has been postulated that P-gp expression can affect the magnitude of induction through alteration of intracellular levels, data in the literature confirming this association have not been reproducible (Lamba et al., 2010; Klein et al., 2012). Rifampicin is also a potent inhibitor of OATP1B1 and can impact the PK of OATP substrates (Vavricka et al., 2002; Kalliokoski and Niemi, 2009), as exemplified in studies showing that the dosing time for repaglinide, relative to rifampicin treatment, impacted the magnitude of exposure change. Quantitative modeling of the net outcome of the complex interaction between rifampicin and repaglinide demonstrated that inhibition of OATP1B1 can partially explain this result (Varma et al., 2013a,b). In another example, lenvatinib, which is netabolized primarily by CYP3A (~80% of P450-dependent), is also a substrate and a weak

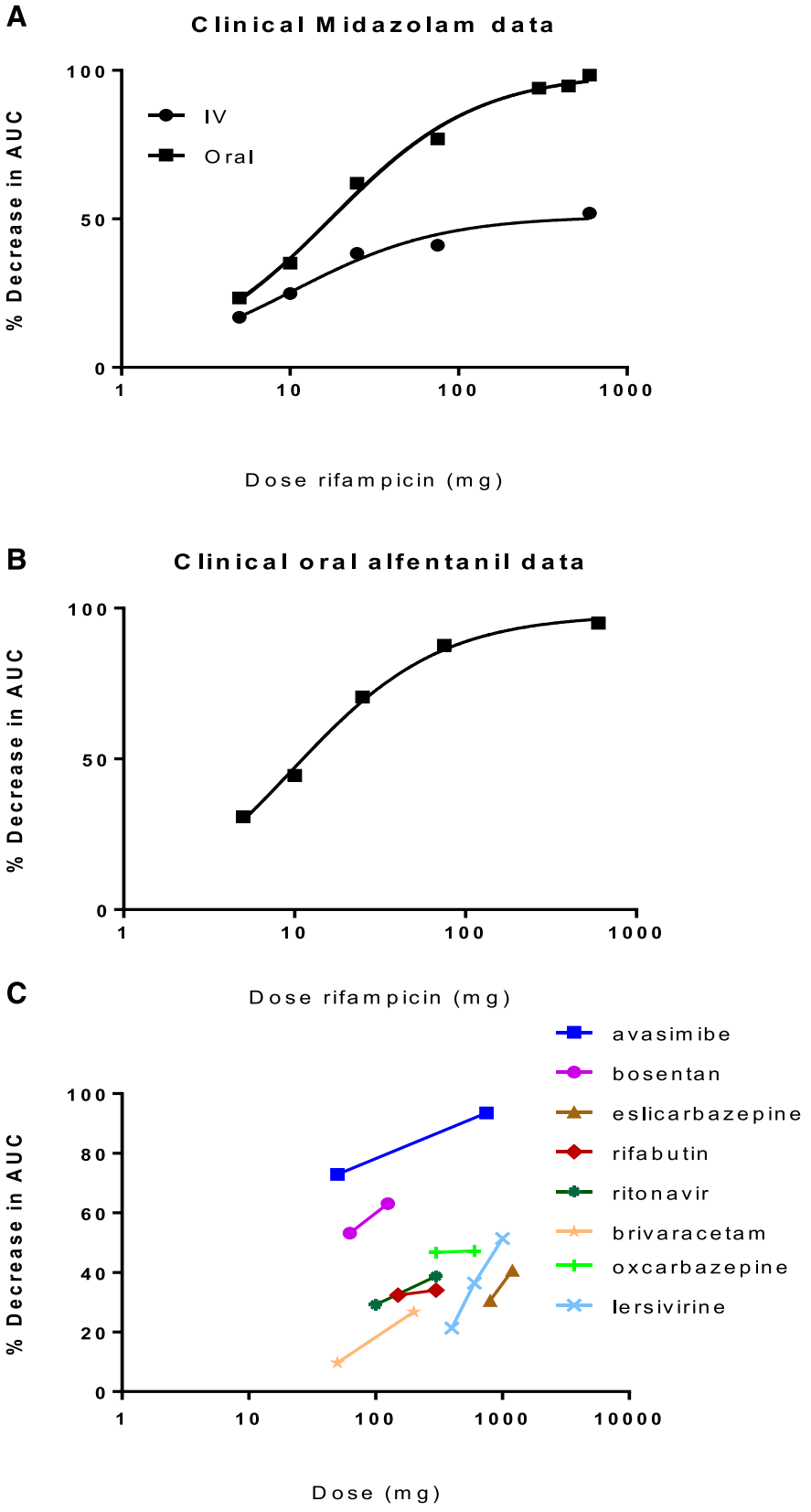

Fig. 4. Data collected from University of Washington drug interaction database and raw data values are contained within Kenny et al. (2018). (A) Shows the effect of increasing dose level of rifampicin on oral and intravenous midazolam exposure. When midazolam is dosed orally, the magnitude of induction (% decrease in AUC) is larger than when midazolam is dosed intravenously. (B) Shows the effect of increasing dose level of rifampicin on oral alfentanil exposure. As expected, the increase in rifampicin dose increases the % decrease in AUC. (C) Shows other inducers where multiple dose levels were investigated using the same substrate drug. In most cases the magnitude of induction increases with increasing dose level. The slope of effect is different across inducers, as is the magnitude of response.

inhibitor of P-gp. The AUCR of lenvatinib increased to 1.3-fold with a concomitant single dose of rifampicin, whereas with multiple doses of rifampicin, the AUCR was slightly reduced to 0.83-fold (Shumaker et al., 2014). Lenvatinib is also an inhibitor of multiple other transporters, including BSEP, OAT1, OAT3, OATP1B1, OCT1, and OCT2 (NDA 206947). These examples highlight an important role of transporters to net clinical outcomes. Delineating the role of transporters can be challenging given the substrate overlap between transporters and

enzymes. Recently, biomarkers such as coproporphyrin I have shown promise in reflecting OATP activity and as such may help in understanding DDI due to OATP inhibition (Barnett et al., 2018, 2019). Since induction can cause increases in multiple enzymes and transporters involved, this, along with other transporter/enzyme interactions, should be considered in the clinical study design. Appropriate clinical design, in combination with mechanistic modeling, can help to tease out relative roles and aid in building better characterized and more comprehensive PBPK models for predicting effects with other substrates.

Effect of Perpetrator Dosing Duration. Induction often occurs by an increase in the rate of enzyme synthesis through activation of transcription described by the following equation: $Amt\ Enzyme_{ss} = \frac{Synthesis\ rate}{K_{deg}}$. Considering constant inducer concentrations, the time to steady state is controlled by the degradation half-life of the affected enzyme when the half-life of the drug is less than the degradation half-life of the enzyme. The reported half-life values of CYP3A4 are variable (Yang et al., 2008), but multiple recent reports have coalesced on a half-life of around 30 hours (Ramsden et al., 2015; Takahashi et al., 2017; Chan et al., 2018). Interestingly, the European Medicines Agency (EMA) originally took a more conservative stance and recommended the use of 80 hours (https://www.ema.europa.eu/documents/other/overview-comments-received-guideline-investigation-drug-interactions_en.pdf), which would imply that 17 days are needed to achieve 97% of steady-state values. Most inducers collected for the IVIVE evaluation by the IQ IWG had reported half-lives of less than 30 hours. Exceptions were clobazam, efavirenz, ezetimibe, nevirapine, phenobarbital, terbinafine, and teriflunomide. Clinical induction studies collated for IVIVE evaluation by the IWG showed cases where induction of CYP3A activity was observed by day 3 and maximized by day 4 or 5. Regulatory agencies recommend that the perpetrator dosing interval be designed to achieve steady state of the inducer and enzyme. As highlighted in this article, when the half-life of the inducer is less than 30 hours, the time to reach steady state of CYP3A activity will be driven by the half-life of the induced enzyme. Monitoring of 6 β-hydroxycortisol urinary excretion showed that phenytoin treatment resulted in measurable and statistically significant induction by day 4, with induction apparent within 48 hours of phenytoin administration (Fleishaker et al., 1995). A similar observation was made when using morning spot urinary 6β-hydroxycortisol/cortisol after rifampicin induction; at day 4 or 5, induction was similar compared with day 14 or longer (Tran et al., 1999). These effects were also observable with moderate and weak inducers (Fig. 5). Taken together, the data indicate that a 5- or 7-day dosing regimen, for compounds with a half-life $<$30 hours, likely will be sufficient to achieve maximal induction and appears to support the value of 30 hours versus 80 hours for the half-life of CYP3A. A recent publication used a verified PBPK model and available rifampicin/midazolam clinical DDI studies to make recommendations on time-course of induction (Kapetas et al., 2019). This analysis indicated that hepatic induction of CYP3A4 appears to take longer than intestinal induction ($>$5 days) and therefore recommend that rifampicin be dosed for at least 10 days. They also discuss the potential for earlier time points to contribute to the observed variability. This finding contrasts with the analysis conducted here, which also includes data from weak and moderate inducers and highlights the need for further exploration. Clearly, when measuring the induction of other P450 isoforms, their half-life needs to be taken into consideration if longer than for CYP3A4 (e.g., CYP2D6, 51 hours) (Venkatakrishnan and Obach, 2005) and CYP1A2 (51 hours) (Diaz et al., 1990). As with CYP3A4, care should be taken when applying the longer experimentally derived k_{deg} values as these may also be dependent on the method used.

Clinical Examples of Inhibition and Induction and Impact on Study Design. An important finding from the IWG data collection efforts was that a large proportion (~61%) of in vitro CYP3A inducers also demonstrated in vitro inhibition (Kenny et al., 2018), a complicating factor in the design and interpretation of DDI studies. Understanding the time dependency of changes in PK attributable to inhibition and induction, as well as the magnitude of change at steady state, is important, as exemplified by aprepitant. When administered daily for 4 days, aprepitant results in weak inhibition (AUCR = 1.25) as measured by intravenous midazolam, whereas the same dose results in mild induction after 8 days (AUCR = 0.81) (Shadle et al., 2004). The PK of aprepitant is also time-dependent, as it has been reported to undergo both autoinhibition (day 7/day 1) and autoinduction (day 56/day 1) (Prueksaritanont et al., 2013).

Significant efforts have been focused on trying to predict mixed-mechanism DDIs with static and PBPK modeling (Prueksaritanont et al., 2013, Almond et al., 2016, Gu et al., 2018). Although promising, these efforts have highlighted a need for better understanding of the translation of in vitro data to clinical DDI results, particularly when multiple pathways and modes of interaction are present. An important aspect that bears consideration is that, in the case of induction, other proteins can be upregulated by the same nuclear receptor pathway (Urquhart et al., 2007), whereas inhibition typically affects specific single enzymes. A clinical example is that of ritonavir and voriconazole, where the effect on voriconazole exposure is dependent on the duration of ritonavir dosing. After 2 days of ritonavir administration, moderate clinical inhibition of voriconazole was apparent and dependent on CYP2C19 genotype (1.5- to 9.1-fold) (Mikus et al., 2006), whereas after 20 days, the result was strong clinical induction (0.16 AUCR) (Liu et al., 2007). Voriconazole is metabolized extensively by CYP2C9, CYP2C19, UGTs, and, to a lesser extent, by CYP3A. As CYP2C9, CYP2C19, and UGT1A1 are coregulated with CYP3A, but not likely inhibited by ritonavir at clinical concentrations (Zhang et al., 2005; Englund et al., 2014), it is possible that these enzymes play a larger role in the metabolism of voriconazole after inhibition of CYP3A, resulting in an overall strong induction effect with longer duration of ritonavir dosing.

For a new drug that has complex DDI, it is important to understand the potential for the DDI outcome to change between day 1 and steady state. To evaluate inducers with mixed DDI mechanisms, clinical DDI studies should be designed to ensure maximal inhibition (i.e., day 1, assuming competitive inhibition with no accumulation) and induction effects (i.e., steady state). This approach could provide information enabling physicians to modify dosing of narrow therapeutic co-medications where a DDI effect could change from largely inhibition to a combination of inhibition and induction. A clinical study design that includes additional PK sampling to evaluate victim drug PK, after single and repeat dosing of the perpetrator, would be helpful. As indicated with the ritonavir example, induction typically takes longer to become evident compared with competitive inhibition, which can be observed immediately. The extent of inhibition will depend on the concentration of the perpetrator and possible accumulation with repeat dosing. For enzyme inactivation, the extent of inhibition will depend on the concentration of the inactivator and its inactivation parameters (Obach et al., 2007; Venkatakrishnan and Obach, 2007; Venkatakrishnan et al., 2007). Theoretically, a situation could arise where the net effect of inactivation and induction is AUCR = 1, and in that case, it is challenging to distinguish between similar extents of inactivation and induction leading to no net effect and the case where no inactivation or induction occurs at all. Since induction will likely impact other proteins, using solely CYP3A as an indicator of full induction would not be beneficial in this case. As such, a clinical study design determining victim PK on several days may be warranted. In addition, a cocktail of probe drugs could be administered to more fully characterize the possible effect on other drug-metabolizing enzymes and transporters (Stopfer et al., 2016);

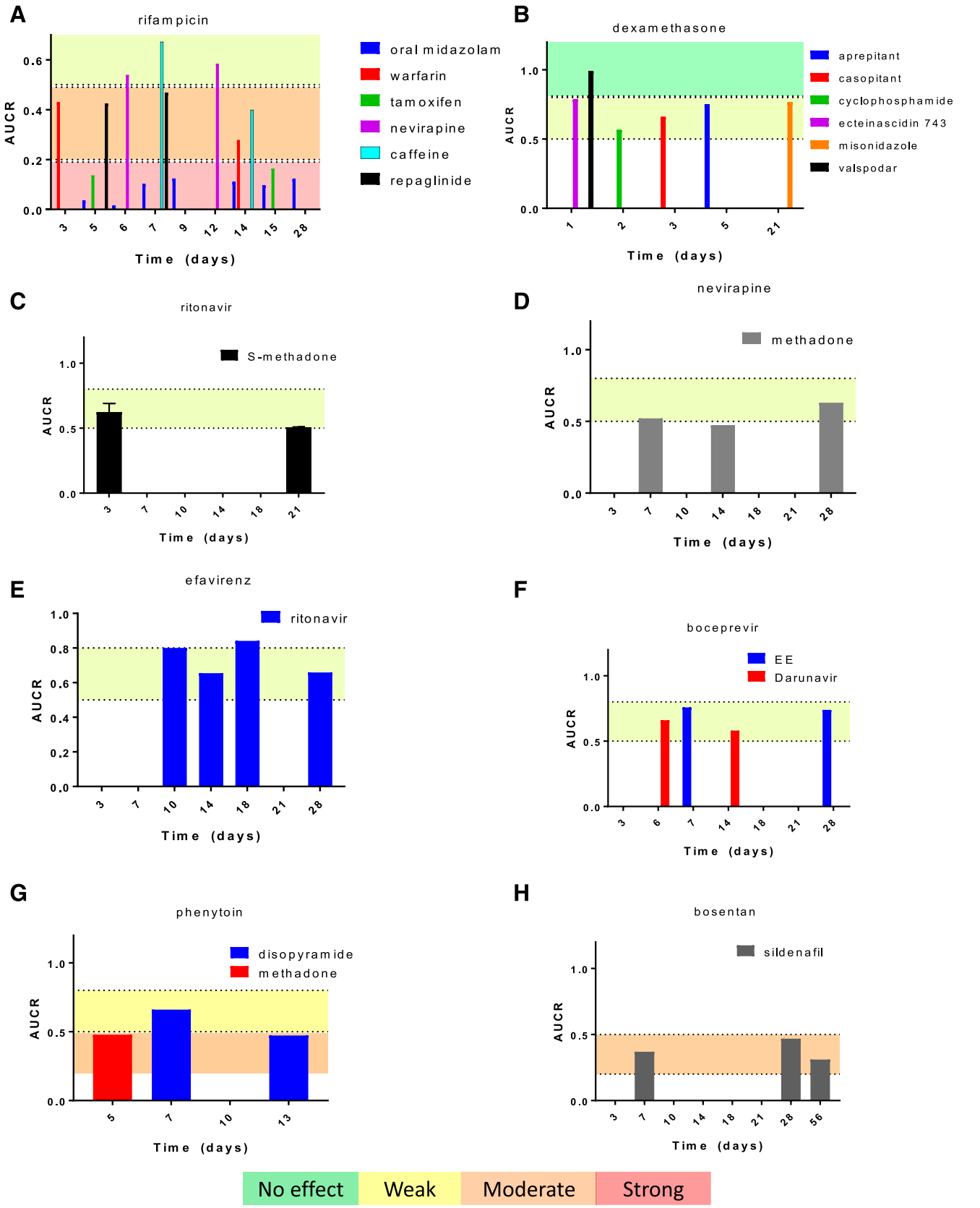

Fig. 5. Comparison of the magnitude of induction observed across different duration of dosing using the same clinical inducer and substrate pairs. (A) Shows that the time course of maximal rifampicin induction is similar across a range of substrates with varying selectivity toward CYP3A. With the exception of caffeine, all substrates confirm maximal induction by day 5 or 7. The impact of dosing duration on the magnitude of response for weak (dexamethasone (B), ritonavir (C), nevirapine (D), efavirenz (E), and boceprevir (F)) inducers using the same substrate is consistent with the rifampicin data. These data demonstrate that 5–7 days is sufficient to reach steady state, even for weak inducers. Similarly, phenytoin (G) and bosentan (H), which are moderate inducers, result in maximal induction in the same time frame as does the strong inducer rifampicin.

Prueksaritanont et al., 2017). Many DDI studies do not evaluate perpetrator concentrations, often extrapolating from other studies. In most cases, it may be important to characterize perpetrator PK to help in developing models for extrapolating drug interaction, which could reduce the number of clinical DDI studies and allow for labeling recommendations.

The potential for complex DDI with transporters also needs to be considered during study design. Although rifampicin is generally regarded as a prototypical inducer that is absent of competing mechanisms (e.g., inhibition), it is now known to be a potent inhibitor of OATPs (Karlgren et al., 2012) and was determined to be a reversible inhibitor of CYP3A (Kajosaari et al., 2005). This is relevant to study design where, in the case of repaglinide, a substrate of OATP1B1 and CYP3A (Kajosaari et al., 2005; Yoshikado et al., 2017), there was a marked difference in AUCR when repaglinide was dosed simultaneously with rifampicin (48.1% decrease) versus 24 hours after rifampicin (79.6% decrease) (Bidstrup et al., 2004). Thus, for substrates of OATPs, when evaluating rifampicin induction, staggered dosing is recommended. Similarly, when also assessing direct inhibitors of CYP3A, the magnitude of induction by rifampicin may also be underestimated if they are coadministered. Furthermore, consideration of the need for staggered dosing should be made on a case by case basis for medications intended for concomitant administration.

Extrapolating Induction across Substrates, Oral Contraceptives. The impact of inducers on the exposure of oral contraceptives (OCs) is a special consideration during drug development. Predicting the impact of CYP3A inducers on ethinyl estradiol (EE) and progestin [e.g., levonorgestrel (LNG), norethindrone, dosperinone] exposure is difficult because of the role of multiple enzymes in their metabolism, variability in PK, and other factors. There is also uncertainty around the impact on exposure as it relates to loss of efficacy (i.e., the PKPD relationship for OCs), with a dichotomous effect on ovulation rather than a graded response as with other drugs. The combined significance of therapeutic failure with the high prevalence of OC use has meant that DDI studies with OCs may be conducted as part of drug development to provide information in the product label, even for drugs characterized as having low risk of CYP3A induction. The role of CYP3A in the overall metabolism of EE and progestins was reviewed (Zhang et al., 2018). These findings demonstrated that OCs were only minimally sensitive to strong CYP3A inhibitors, which is likely due to the fact that multiple enzymes, including CYP2C9, 2C19, 3A, UGTs, and sulfotransferases, contribute to their metabolism. Since inducers of CYP3A can also induce other enzymes involved in OC metabolism, it is important to understand the relative role of enzymes responsible for metabolism in weighing the need for conducting a clinical DDI trial for an OC potential inducer.

Given the large number of clinical induction studies, the IWG collected with EE or progestins (LNG and norethindrone), evaluation of induction of OC compared with other sensitive substrates of CYP3A, such as oral midazolam, triazolam, tipranavir and others, was possible (Fig. 6). Clinical data for both a CYP3A-sensitive substrate and OC were available for 15 of the 35 in vitro inducers included in the original IWG analysis (Kenny et al., 2018). To build on this data set for this analysis, clinical data were collected for additional inducers. In total, 23 perpetrator drugs had clinical studies with both an OC or progestin and a sensitive CYP3A substrate. Six compounds had only OC data (felbamate, festerodine, mavoglurant, rosiglitazone, telaprevir, and topiramate) and 14 with only sensitive CYP3A substrate data. Whereas several clinical studies had the same perpetrator and both EE or a progestin and a sensitive CYP3A substrate, the data set for EE and oral midazolam was not large enough to draw any conclusions on extrapolation of substrate effects given that the AUCR values for midazolam and EE did not show a correlation (Fig. 3).

In general, all clinically relevant inducers could be identified based on DDI results with an OC, although in the case of some moderate and strong inducers, the magnitude of effect was lower with the OC compared with a sensitive CYP3A substrate (Fig. 6). In the case of weak inducers (Fig. 6B), three of the six showed similar magnitude between substrates, while two showed greater induction with the sensitive CYP3A substrate. Oxcarbazepine showed greater induction of EE and LNG (Fig. 6B). Bosentan, efavirenz (Fig. 6C), and carbamazepine (Fig. 6D) resulted in similar AUCR for EE or LNG compared with the sensitive CYP3A substrate simvastatin. EE identified ritonavir, aprepitant, perampanel, and brivaracetam (Fig. 6E) as mild inducers, whereas other sensitive substrates showed net inhibition or no effect. Etravirine, flibanserin, rifaximin, and teriflunomide showed no effect with OC, while sensitive CYP3A substrates (Fig. 6A) also showed no effect or mild inhibition. Based on clinical data, ethinyl estradiol is both an in vitro time-dependent inhibitor of CYP3A and an apparent inducer (Rodrigues and Lu, 2004; Chang et al., 2009; Zimmerlin et al., 2011), complicating the interpretation of data. This analysis confirms that evaluation of EE or progestins in DDI studies can appropriately identify CYP3A inducers, but the magnitude of effect on other substrates, including sensitive CYP3A substrates, may be difficult to extrapolate based on the results. Conducting clinical DDI studies with OCs may add additional value for understanding induction risk, particularly when the inducer is also a time-dependent inhibitor of CYP3A.

Use of PBPK to Help Understand Variability and Predict Complex DDIs. Physiologically based pharmacokinetic (PBPK) modeling is an integral part of drug discovery and development (Jones et al., 2015) and is increasingly a part of submissions to regulatory agencies (Wagner et al., 2015). Regulators are developing guidelines around its utility (https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm531207.pdf; https://www.ema.europa.eu/documents/scientific-guideline/guideline-qualification-reporting-physiologically-based-pharmacokinetic-pbpk-modelling-simulation_en.pdf), and industry is responding with recommendations on validating and reporting PBPK data (Shebley et al., 2018). Literature examples have provided evidence that PBPK modeling may better predict complex DDI, aid in clinical trial design, and enable predictions in lieu of dedicated clinical trials (Sager et al., 2015; Einolf et al., 2017; Asaumi et al., 2018; Gupta et al., 2018; Shebley et al., 2018). Despite these significant advances, there are still areas where confidence in PBPK predictions from in vitro data are insufficient to achieve regulatory acceptance. For example, although PBPK model building may still be possible when transporters play a major role (Prueksaritanont et al., 2013; Yu et al., 2017) it often requires deriving key model parameters based on observed clinical data since in vitro data do not directly translate, thus limiting this approach as a way to replace clinical studies. Induction is another area where confidence in PBPK modeling needs to be strengthened. FDA and EMA appear to have different perspectives on the utility of PBPK for induction. The FDA have accepted PBPK models of induction in NDA submissions to support drug labeling. Some recent examples include PBPK modeling that supported dosing recommendations for rifampin with cobimetinib and panobinostat and for efavirenz with cobimetinib and sonidegib (Yoshida et al., 2017). However, the FDA has also indicated that there is room for improvement in PBPK models of rifampicin and that, in general, more research is needed to update inducer drug models (Hsueh et al., 2018). Whereas the FDA believes that PBPK prediction of induction may be sufficient to support dosing recommendations, the EMA has been more reluctant and has requested more validation data (Shepard et al., 2015; Zhao, 2017).

Because of the intensive number of input parameters and blinded nature of the data collected, PBPK modeling was out of scope for the work done by the IWG. However, although the recent IWG paper

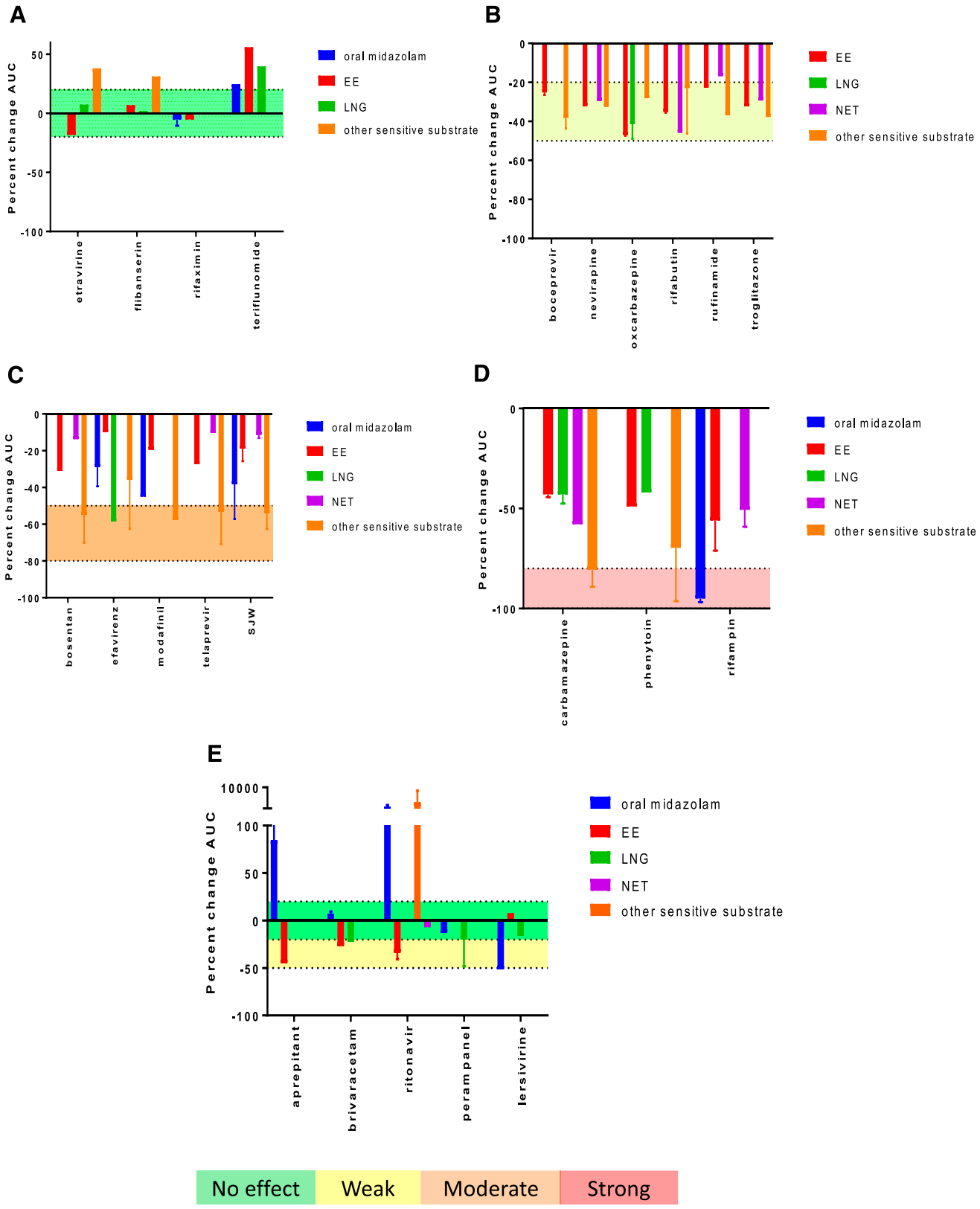

Fig. 6. Comparison of oral midazolam, a sensitive CYP3A substrate or oral contraceptives (EE, LNG, or NET) AUCR values across a range of induction categories. (A) Shows in vitro inducers that do not show induction (percent change in AUC is not > -20). (B) Shows weak inducers (percent change in AUC between -20 and -50). (C) Shows moderate inducers (percent change in AUC between -50 and -80). (D) Shows potent inducers (percent change in AUC > -80). (E) Shows inducers that demonstrated a differential effect depending on the substrate being used (from inhibition to weak induction).

(Kenny et al., 2018) concluded that induction risk assessment is possible using basic models described in the regulatory guidance, the extent of overprediction and false-positive rate point to the need for better quantitative prediction.

Concluding Remarks and Future Directions. An unexpected outcome of the clinical data collection carried out by the IWG was the large range of AUCR values observed for CYP3A inducers across studies. Analysis of the clinical data revealed that many factors could contribute to the observed variability, including the selectivity of the substrate and the dose level of the inducer. Does the overlap in substrate selectivity for CYP3A4 substrates with other drug-metabolizing enzymes and transporters complicate interpretation of clinical outcomes? Would administration of a cocktail of substrates with differential selectivity to CYP3A4 and other proteins (at doses providing systemic concentrations below their respective K_m values) provide data that, with an appropriate deconvolution of contribution from different enzymes and transporters, tease out the role of just CYP3A4? Alternatively, administration of a microdose (Prueksaritanont et al., 2017) of drug combinations, or even a single drug, might circumvent interactions with other proteins, particularly as clinically meaningful inhibitors. It is important to consider whether the perpetrator drug is also an inhibitor of enzymes or transporters involved in the metabolism and disposition of a substrate. If so, it would be beneficial to evaluate PK at multiple time points and/or consider dose staggering. Analysis of trends from the CYP3A clinical induction data also indicated that 5–7 days of dosing may suffice to achieve maximal effects when the inducer half-life is shorter than 30 hours. However, a recent publication by Kapetas et al. (2019), using simulations derived from a verified PBPK model, indicated that while induction of intestinal CYP3A reaches steady-state by day 5, this time course resulted in significant under-prediction of hepatic induction. Thus, analysis before day 10 could result in incorrect assignment of relative extraction between intestine and liver and may contribute to the variability of outcome observed. These conflicting reports clearly highlight the value in continuing to advance the understanding of induction-mediated DDI as emphasized here. Another important aspect to consider is whether the variability observed from in vitro induction parameters (Kenny et al., 2018) is reflective of intrasubject variability. This would require investigating the clinical induction response in the same subjects over repeated clinical studies. In addition, although there is a difference in the magnitude of change between sensitive CYP3A substrates and OC for most inducers, all clinically relevant inducers were identified as such in OC DDI studies, and CYP3A dual inhibitor/inducers resulted in clinical induction of OC. In cases where the perpetrator is determined from in vitro data to be both an inducer and inhibitor of CYP3A, the induction potential of other coregulated enzymes and transporters may not be appropriately characterized by only using an index or sensitive CYP3A substrate. In these cases, evaluation of marker substrates for coregulated proteins or consideration of potential loss of efficacy for important comedicants should be made and perhaps evaluated clinically.

Biomarkers are considered a favorable means for monitoring induction as additional dosing is not required, and analysis can be conducted on plasma samples already being taken to assess drug levels. Additionally, urinary excretion of a biomarker provides a noninvasive sample collection. Indeed, the draft FDA guidance on clinical drug interaction study designs (Food and Drug Administration FDA, 2017) highlights how biomarker data can provide useful information on the drug's effect on a metabolic pathway but do not recommend biomarkers for index studies because of the lack of clear and consistent ability to extrapolate to other substrates. A recent example of complex DDI PBPK modeling for midostaurin, which, along with its metabolites, are substrates, reversible and time-dependent inhibitors, and inducers of CYP3A4, relied on 4β-hydroxycholesterol data to increase confidence in DDI predictions (Gu et al., 2018). Although additional examples are needed, this highlights how biomarker data have the potential to help bridge gaps and build confidence during PBPK modeling efforts. Evaluation of 4β-hydroxycholesterol has been included as standard during early clinical phase 1 studies to identify in vivo induction earlier on and in the rare cases when in vitro assays are not able to predict induction (Jones et al., 2017). Although there are no universally accepted biomarkers of CYP3A activity, continued efforts are encouraged to identify endogenous biomarker(s) that could be used to dissociate contributors of the overall variability in DDI response and/or provide correction factors to reduce overall variability. Since static models do not take into consideration the fluctuation of inducer concentration throughout the dosing period and, as such, possible changes in response during the day, PBPK modeling should be further evaluated as a valuable tool for predicting induction. Overall, a better understanding of the temporal aspects contributing to an inductive effect would be helpful, such as duration that a concentration needs to be maintained above an effective value.

Since multiple parameters should be considered when designing a clinical study to evaluate induction, it is not possible to be prescriptive in study design. If the drug of interest is a potential perpetrator of induction, consideration should be given to the half-life of the inducer, any time-dependent PK, and the potential for mixed inhibition/induction or transporter effects. If the drug of interest is a potential victim and the half-life of the drug is less than the degradation half-life of the protein of interest (e.g., CYP3A), serious consideration should be given to adopting a study design that controls for this aspect (e.g., duration of dosing, dose, number of subjects). Monitoring the levels of the perpetrator (even for standard inducers such as rifampicin) will inform on the relative contribution of the inducer PK to the overall variability and provide vital information for future PBPK models. Is there an acceptable path forward to get a better handle on some of the contributors to variability? As outlined earlier, larger data sets may help to define more accurately the ranges of effects in vitro. Further analysis would require generation of larger data sets, but this is not feasible for any one company in a practical drug development paradigm. Would companies be willing to share data from positive controls in their studies so that cumulative data would inform on the contribution of data size to the variability in response? The IWG discussed whether recommending a cutoff value for maximum fold induction for a positive control inducer such as rifampicin in vitro could reduce overall variability in mRNA and enzyme activity outcomes. Such a recommendation was not made as there is no agreement on what that top value should be. Larger data sets may also inform on this aspect. Do outlier values diminish the credibility of in vitro responses, and should these values be excluded? Further insights into these questions are needed.

The recommendations from the IWG (Hariparsad et al., 2017; Kenny et al., 2018) are intended to optimize in vitro induction studies as a contributor to overall improved IVIVE. Additional insights into the duration of in vitro induction studies and recommendations on in vitro induction data analysis by the IWG are imminent (S. Wong, personal communication). Future challenges will include improved predictions of mild and moderate inducers, better deconvolution of contributions by intestinal versus hepatic enzymes and transporters, as well as identifying alternative mechanisms of induction and understanding our ability to confidently extrapolate from in vitro to clinical outcomes for these mechanisms. For example, do current sandwich-cultured hepatocytes preserve these mechanisms? Certainly, there are many examples of successful prediction of induction from in vitro data that support the value of these approaches, and we need to ensure that we maintain that success as new challenges unfold.

Acknowledgments

This commentary was developed with the support of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ, www.iqconsortium.org). IQ is a not-for-profit organization of pharmaceutical and biotechnology companies with a mission of advancing science and technology to augment the capability of member companies to develop transformational solutions that benefit patients, regulators, and the broader research and development community. We thank the IQ IWG for valuable discussions and manuscript review, the IQ Translational ADME and Clinical Pharmacology Leadership Groups for manuscript review, the IQ member companies for contributing data, Dr. Scott Obach (Pfizer), Dr. Christopher Gibson (Merck), Dr. Michael Sinz (Bristol Myers Squibb) for valuable feedback on the manuscript draft, and Sophie Mukadam (Genentech) for assistance in literature data mining.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Ramsden, Fung, Hariparsad, Kenny, Mohutsky, Parrott, Robertson, Tweedie.

References

Acocella G (1978) Clinical pharmacokinetics of rifampicin. *Clin Pharmacokinet* **3**:108–127.

Almond LM, Mukadam S, Gardner I, Okialda K, Wong S, Hatley O, Tay S, Rowland-Yeo K, Jamei M, Rostami-Hodjegan A, et al. (2016) Prediction of drug-drug interactions arising from CYP3A induction using a physiologically based dynamic model. *Drug Metab Dispos* **44**: 821–832.

Asaumi R, Toshimoto K, Tobe Y, Hashizume K, Nunoya KI, Imawaka H, Lee W, and Sugiyama Y (2018) Comprehensive PBPK model of rifampicin for quantitative prediction of complex drug-drug interactions: CYP3a/2C9 induction and OATP inhibition effects. *CPT Pharmacometrics Syst Pharmacol* **7**:186–196.

Atkins WM (2005) Non-Michaelis-Menten kinetics in cytochrome P450-catalyzed reactions. *Annu Rev Pharmacol Toxicol* **45**:291–310.

Backman JT, Granfors MT, and Neuvonen PJ (2006) Rifampicin is only a weak inducer of CYP1A2-mediated presystemic and systemic metabolism: studies with tizanidine and caffeine. *Eur J Clin Pharmacol* **62**:451–461.

Barnett S, Ogungbenro K, Menochet K, Shen H, Humphreys GW, and Galetin A (2019) Comprehensive evaluation of the utility of 20 endogenous molecules as biomarkers of OATP1B inhibition compared with rosuvastatin and coproporphyrin I. *J Pharmacol Exp Ther* **368**: 125–135.

Barnett S, Ogungbenro K, Ménochet K, Shen H, Lai Y, Humphreys WG, and Galetin A (2018) Gaining mechanistic insight into coproporphyrin I as endogenous biomarker for OATP1B-mediated drug-drug interactions using population pharmacokinetic modeling and simulation. *Clin Pharmacol Ther* **104**:564–574.

Bidstrup TB, Stilling N, Damkier P, Scharling B, Thomsen MS, and Brøsen K (2004) Rifampicin seems to act as both an inducer and an inhibitor of the metabolism of repaglinide. *Eur J Clin Pharmacol* **60**:109–114.

Chan CYS, Roberts O, Rajoli RKR, Liptrott NJ, Siccardi M, Almond L, and Owen A (2018) Derivation of CYP3A4 and CYP2B6 degradation rate constants in primary human hepatocytes: a siRNA-silencing-based approach. *Drug Metab Pharmacokinet* **33**:179–187.

Chang C, Yang X, Fahmi OA, Riccardi KA, Di L, and Obach RS (2017) An exposure-response analysis based on rifampin suggests CYP3A4 induction is driven by AUC: an in vitro investigation. *Xenobiotica* **47**:673–681.

Chang SY, Chen C, Yang Z, and Rodrigues AD (2009) Further assessment of 17alpha-ethinyl estradiol as an inhibitor of different human cytochrome P450 forms in vitro. *Drug Metab Dispos* **37**:1667–1675.

Chen J and Raymond K (2006) Roles of rifampicin in drug-drug interactions: underlying molecular mechanisms involving the nuclear pregnane X receptor. *Ann Clin Microbiol Antimicrob* **5**:3.

Darwish M, Kirby M, Robertson P Jr, and Hellriegel ET (2008) Interaction profile of armodafinil with medications metabolized by cytochrome P450 enzymes 1A2, 3A4 and 2C19 in healthy subjects. *Clin Pharmacokinet* **47**:61–74.

Davydov DR and Halpert JR (2008) Allosteric P450 mechanisms: multiple binding sites, multiple conformers or both? *Expert Opin Drug Metab Toxicol* **4**:1523–1535.

Denisov IG, Grinkova YV, Baylon JL, Tajkhorshid E, and Sligar SG (2015) Mechanism of drug-drug interactions mediated by human cytochrome P450 CYP3A4 monomer. *Biochemistry* **54**: 2227–2239.

Diaz D, Fabre I, Daujat M, Saint Aubert B, Bories P, Michel H, and Maurel P (1990) Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P450. *Gastroenterology* **99**: 737–747.

Domanski TL, Liu J, Harlow GR, and Halpert JR (1998) Analysis of four residues within substrate recognition site 4 of human cytochrome P450 3A4: role in steroid hydroxylase activity and alpha-naphthoflavone stimulation. *Arch Biochem Biophys* **350**:223–232.

Eberl S, Renner B, Neubert A, Reisig M, Bachmakov I, König J, Dörje F, Mürdter TE, Ackermann A, Dormann H, et al. (2007) Role of p-glycoprotein inhibition for drug interactions: evidence from in vitro and pharmacoepidemiological studies. *Clin Pharmacokinet* **46**:1039–1049.

Einolf HJ, Lin W, Won CS, Wang L, Gu H, Chun DY, He H, and Mangold JB (2017) Physiologically based pharmacokinetic model predictions of panobinostat (LBH589) as a victim and perpetrator of drug-drug interactions. *Drug Metab Dispos* **45**:1304–1316.

Englund G, Lundquist P, Skogastierna C, Johansson J, Hoogstraate J, Afzelius L, Andersson TB, and Projean D (2014) Cytochrome p450 inhibitory properties of common efflux transporter inhibitors. *Drug Metab Dispos* **42**:441–447.

Fahmi OA, Maurer TS, Kish M, Cardenas E, Boldt S, and Nettleton D (2008) A combined model for predicting CYP3A4 clinical net drug-drug interaction based on CYP3A4 inhibition, inactivation, and induction determined in vitro. *Drug Metab Dispos* **36**:1698–1708.

Fleishaker JC, Pearson LK, and Peters GR (1995) Phenytoin causes a rapid increase in 6 beta-hydroxycortisol urinary excretion in humans--a putative measure of CYP3A induction. *J Pharm Sci* **84**:292–294.

Floyd MD, Gervasini G, Masica AL, Mayo G, George AL Jr, Bhat K, Kim RB, and Wilkinson GR (2003) Genotype-phenotype associations for common CYP3A4 and CYP3A5 variants in the basal and induced metabolism of midazolam in European- and African-American men and women. *Pharmacogenetics* **13**:595–606.

Food and Drug Administration (FDA) (2017) Clinical drug interaction studies- study design, data analysis, and clinical implications guidance for industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD. https://www.fda.gov/media/108130/download

Fromm MF, Busse D, Kroemer HK, and Eichelbaum M (1996) Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. *Hepatology* **24** (4):796–801, doi: 10.1002/hep.510240407 8855178.

Ged C, Rouillon JM, Pichard L, Combalbert J, Bressot N, Bories P, Michel H, Beaune P, and Maurel P (1989) The increase in urinary excretion of 6 beta-hydroxycortisol as a marker of human hepatic cytochrome P450IIIA induction. *Br J Clin Pharmacol* **28**:373–387.

Gertz M, Harrison A, Houston JB, and Galetin A (2010) Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos* **38**:1147–1158.

Gillum JG, Israel DS, Scott RB, Climo MW, and Polk RE (1996) Effect of combination therapy with ciprofloxacin and clarithromycin on theophylline pharmacokinetics in healthy volunteers. *Antimicrob Agents Chemother* **40**:1715–1716.

Gorski JC, Vannaprasaht S, Hamman MA, Ambrosius WT, Bruce MA, Haehner-Daniels B, and Hall SD (2003) The effect of age, sex, and rifampin administration on intestinal and hepatic cytochrome P450 3A activity. *Clin Pharmacol Ther* **74**:275–287.

Greenblatt DJ and von Moltke LL (2008) Gender has a small but statistically significant effect on clearance of CYP3A substrate drugs. *J Clin Pharmacol* **48**:1350–1355.

Grub S, Bryson H, Goggin T, Lüdin E, and Jorga K (2001) The interaction of saquinavir (soft gelatin capsule) with ketoconazole, erythromycin and rifampicin: comparison of the effect in healthy volunteers and in HIV-infected patients. *Eur J Clin Pharmacol* **57**:115–121.

Gu H, Dutreix C, Rebello S, Ouatas T, Wang L, Chun DY, Einolf HJ, and He H (2018) Simultaneous physiologically based pharmacokinetic (PBPK) modeling of parent and active metabolites to investigate complex CYP3A4 drug-drug interaction potential: a case example of midostaurin. *Drug Metab Dispos* **46**:109–121.

Gupta N, Hanley MJ, Venkatakrishnan K, Bessudo A, Rasco DW, Sharma S, O'Neil BH, Wang B, Liu G, Ke A, et al. (2018) Effects of strong CYP3A inhibition and induction on the pharmacokinetics of ixazomib, an oral proteasome inhibitor: results of drug-drug interaction studies in patients with advanced solid tumors or lymphoma and a physiologically based pharmacokinetic analysis. *J Clin Pharmacol* **58**:180–192.

Hariparsad N, Ramsden D, Palamanda J, Dekeyser JG, Fahmi OA, Kenny JR, Einolf H, Mohutsky M, Pardon M, Siu YA, et al. (2017) Considerations from the IQ induction working group in response to drug-drug interaction guidance from regulatory agencies: focus on downregulation, CYP2C induction, and CYP2B6 positive control. *Drug Metab Dispos* **45**:1049–1059.

He P, Court MH, Greenblatt DJ, and von Moltke LL (2006) Factors influencing midazolam hydroxylation activity in human liver microsomes. *Drug Metab Dispos* **34**:1198–1207.

Hsueh CH, Hsu V, Pan Y, and Zhao P (2018) Predictive performance of physiologically-based pharmacokinetic models in predicting drug-drug interactions involving enzyme modulation. *Clin Pharmacokinet* **57**:1337–1346.

Hu ZY and Zhao YS (2010) Sex-dependent differences in cytochrome P450 3A activity as assessed by midazolam disposition in humans: a meta-analysis. *Drug Metab Dispos* **38**:817–823.

Jones BC, Rollison H, Johansson S, Kanebratt KP, Lambert C, Vishwanathan K, and Andersson TB (2017) Managing the risk of CYP3A induction in drug development: a strategic approach. *Drug Metab Dispos* **45**:35–41.

Jones HM, Chen Y, Gibson C, Heimbach T, Parrott N, Peters SA, Snoeys J, Upreti VV, Zheng M, and Hall SD (2015) Physiologically based pharmacokinetic modeling in drug discovery and development: a pharmaceutical industry perspective. *Clin Pharmacol Ther* **97**:247–262.

Kajosaari LI, Laitila J, Neuvonen PJ, and Backman JT (2005) Metabolism of repaglinide by CYP2C8 and CYP3A4 in vitro: effect of fibrates and rifampicin. *Basic Clin Pharmacol Toxicol* **97**:249–256.

Kalliokoski A and Niemi M (2009) Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* **158**:693–705.

Kapetas AJ, Sorich MJ, Rodrigues AD, and Rowland A (2019) Guidance for rifampin and midazolam dosing protocols to study intestinal and hepatic cytochrome P450 (CYP) 3A4 induction and de-induction. *AAPS J* **21**:78.

Karlgren M, Vildhede A, Norinder U, Wisniewski JR, Kimoto E, Lai Y, Haglund U, and Artursson P (2012) Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): influence of protein expression on drug-drug interactions. *J Med Chem* **55**:4740–4763.

Kashuba AD, Bertino JS Jr, Rocci ML Jr, Kulawy RW, Beck DJ, and Nafziger AN (1998) Quantification of 3-month intraindividual variability and the influence of sex and menstrual cycle phase on CYP3A activity as measured by phenotyping with intravenous midazolam. *Clin Pharmacol Ther* **64**:269–277.

Kato M, Chiba K, Ito T, Koue T, and Sugiyama Y (2010) Prediction of interindividual variability in pharmacokinetics for CYP3A4 substrates in humans. *Drug Metab Pharmacokinet* **25**:367–378.

Kenny JR, Ramsden D, Buckley DB, Dallas S, Fung C, Mohutsky M, Einolf HJ, Chen L, Dekeyser JG, Fitzgerald M, et al. (2018) Considerations from the innovation and quality induction working group in response to drug-drug interaction guidances from regulatory agencies: focus on CYP3A4 mRNA in vitro response thresholds, variability, and clinical relevance. *Drug Metab Dispos* **46**:1285–1303.

Kharasch ED, Jubert C, Senn T, Bowdle TA, and Thummel KE (1999) Intraindividual variability in male hepatic CYP3A4 activity assessed by alfentanil and midazolam clearance. *J Clin Pharmacol* **39**:664–669.

Kharasch ED, Vangveravong S, Buck N, London A, Kim T, Blood J, and Mach RH (2011) Concurrent assessment of hepatic and intestinal cytochrome P450 3A activities using deuterated alfentanil. *Clin Pharmacol Ther* **89**:562–570.

Kim B, Lee J, Shin KH, Lee S, Yu KS, Jang IJ, and Cho JY (2018) Identification of ω- or (ω-1)-hydroxylated medium-chain acylcarnitines as novel urinary biomarkers for CYP3A activity. *Clin Pharmacol Ther* **103**:879–887.

Klein K, Thomas M, Winter S, Nussler AK, Niemi M, Schwab M, and Zanger UM (2012) PPARA: a novel genetic determinant of CYP3A4 in vitro and in vivo. *Clin Pharmacol Ther* **91**:1044–1052.

Klein K and Zanger UM (2013) Pharmacogenomics of cytochrome P450 3A4: recent progress toward the "missing heritability" problem. *Front Genet* **4**:12.

Klotz U and Ziegler G (1982) Physiologic and temporal variation in hepatic elimination of midazolam. *Clin Pharmacol Ther* **32**:107–112.

Kwara A, Cao L, Yang H, Poethke P, Kurpewski J, Tashima KT, Mahjoub BD, Court MH, and Peloquin CA (2014) Factors associated with variability in rifampin plasma pharmacokinetics and the relationship between rifampin concentrations and induction of efavirenz clearance. *Pharmacotherapy* **34**:265–271.

Lamba V, Panetta JC, Strom S, and Schuetz EG (2010) Genetic predictors of interindividual variability in hepatic CYP3A4 expression. *J Pharmacol Exp Ther* **332**:1088–1099.

Lin YS, Lockwood GF, Graham MA, Brian WR, Loi CM, Dobrinska MR, Shen DD, Watkins PB, Wilkinson GR, Kharasch ED, et al. (2001) In-vivo phenotyping for CYP3A by a single-point determination of midazolam plasma concentration. *Pharmacogenetics* **11**:781–791.

Liu P, Foster G, Gandelman K, LaBadie RR, Allison MJ, Gutierrez MJ, and Sharma A (2007) Steady-state pharmacokinetic and safety profiles of voriconazole and ritonavir in healthy male subjects. *Antimicrob Agents Chemother* **51**:3617–3626.

Liukas A, Kuusniemi K, Aantaa R, Virolainen P, Neuvonen M, Neuvonen PJ, and Olkkola KT (2008) Plasma concentrations of oral oxycodone are greatly increased in the elderly. *Clin Pharmacol Ther* **84**:462–467.

Lolodi O, Wang YM, Wright WC, and Chen T (2017) Differential regulation of CYP3A4 and CYP3A5 and its implication in drug discovery. *Curr Drug Metab* **18**:1095–1105.

Loos U, Musch E, Jensen JC, Mikus G, Schwabe HK, and Eichelbaum M (1985) Pharmacokinetics of oral and intravenous rifampicin during chronic administration. *Klin Wochenschr* **63**: 1205–1211.

Mao J, Martin I, McLeod J, Nolan G, van Horn R, Vourvahis M, and Lin YS (2017) Perspective: 4β-hydroxycholesterol as an emerging endogenous biomarker of hepatic CYP3A. *Drug Metab Rev* **49**:18–34.

Mikus G, Heinrich T, Bödigheimer J, Röder C, Matthee AK, Weiss J, Burhenne J, and Haefeli WE (2017) Semisimultaneous midazolam administration to evaluate the time course of CYP3A activation by a single oral dose of efavirenz. *J Clin Pharmacol* **57**:899–905.

Mikus G, Schöwel V, Drzewinska M, Rengelshausen J, Ding R, Riedel KD, Burhenne J, Weiss J, Thomsen T, and Haefeli WE (2006) Potent cytochrome P450 2C19 genotype-related interaction between voriconazole and the cytochrome P450 3A4 inhibitor ritonavir. *Clin Pharmacol Ther* **80**:126–135.

Milán Segovia RC, Domínguez Ramírez AM, Jung Cook H, Magaña Aquino M, Vigna Pérez M, Brundage RC, and Romano Moreno S (2013) Population pharmacokinetics of rifampicin in Mexican patients with tuberculosis. *J Clin Pharm Ther* **38**:56–61.

Mouly S, Lown KS, Kornhauser D, Joseph JL, Fiske WD, Benedek IH, and Watkins PB (2002) Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. *Clin Pharmacol Ther* **72**:1–9.

Naidoo P and Chetty M (2019) Progress in the Consideration of Possible Sex Differences in Drug Interaction Studies. *Curr Drug Metab* **20** (2):114–123, doi: 10.2174/1389200220666181128160813 30488793.

Nakajima A, Fukami T, Kobayashi Y, Watanabe A, Nakajima M, and Yokoi T (2011) Human arylacetamide deacetylase is responsible for deacetylation of rifamycins: rifampicin, rifabutin, and rifapentine. *Biochem Pharmacol* **82**:1747–1756.

Nebert DW, Wikvall K, and Miller WL (2013) Human cytochromes P450 in health and disease. *Philos Trans R Soc Lond B Biol Sci* **368**:20120431.

Obach RS, Walsky RL, and Venkatakrishnan K (2007) Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. *Drug Metab Dispos* **35**: 246–255.

Ozdemir V, Kalow W, Tang BK, Paterson AD, Walker SE, Endrenyi L, and Kashuba AD (2000) Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. *Pharmacogenetics* **10**:373–388.

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD, and Thummel KE (1997) Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* **283**:1552–1562.

Pallet N, Jannot AS, El Bahri M, Etienne I, Buchler M, de Ligny BH, Choukroun G, Colosio C, Thierry A, Vigneau C, et al. (2015) Kidney transplant recipients carrying the CYP3A4*22 allelic variant have reduced tacrolimus clearance and often reach supratherapeutic tacrolimus concentrations. *Am J Transplant* **15**:800–805.

Pearson JT, Wahlstrom JL, Dickmann LJ, Kumar S, Halpert JR, Wienkers LC, Foti RS, and Rock DA (2007) Differential time-dependent inactivation of P450 3A4 and P450 3A5 by raloxifene: a key role for C239 in quenching reactive intermediates. *Chem Res Toxicol* **20**:1778–1786.

Prueksaritanont T, Chu X, Gibson C, Cui D, Yee KL, Ballard J, Cabalu T, and Hochman J (2013) Drug-drug interaction studies: regulatory guidance and an industry perspective. *AAPS J* **15**: 629–645.

Prueksaritanont T, Tatosian DA, Chu X, Railkar R, Evers R, Chavez-Eng C, Lutz R, Zeng W, Yabut J, Chan GH, et al. (2017) Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A. *Clin Pharmacol Ther* **101**:519–530.

Rae JM, Johnson MD, Lippman ME, and Flockhart DA (2001) Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. *J Pharmacol Exp Ther* **299**:849–857.

Ramsden D, Zhou J, and Tweedie DJ (2015) Determination of a degradation constant for CYP3A4 by direct suppression of mRNA in a novel human hepatocyte model, hepatoPac. *Drug Metab Dispos* **43**:1307–1315.

Rodrigues AD and Lu P (2004) Is 17alpha-ethinyl estradiol an inhibitor of cytochrome P450 2C19? *Drug Metab Dispos* **32**:364–365.

Ruslami R, Nijland HM, Alisjahbana B, Parwati I, van Crevel R, and Aarnoutse RE (2007) Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. *Antimicrob Agents Chemother* **51**:2546–2551.

Sager JE, Yu J, Ragueneau-Majlessi I, and Isoherranen N (2015) Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. *Drug Metab Dispos* **43**:1823–1837.

Schwarz UI, Gramatté T, Krappweis J, Oertel R, and Kirch W (2000) P-glycoprotein inhibitor erythromycin increases oral bioavailability of talinolol in humans. *Int J Clin Pharmacol Ther* **38**:161–167.

Seng KY, Hee KH, Soon GH, Chew N, Khoo SH, and Lee LS (2015) Population pharmacokinetics of rifampicin and 25-deacetyl-rifampicin in healthy Asian adults. *J Antimicrob Chemother* **70**:3298–3306.

Shadle CR, Lee Y, Majumdar AK, Petty KJ, Gargano C, Bradstreet TE, Evans JK, and Blum RA (2004) Evaluation of potential inductive effects of aprepitant on cytochrome P450 3A4 and 2C9 activity. *J Clin Pharmacol* **44**:215–223.

Shebley M, Sandhu P, Emami Riedmaier A, Jamei M, Narayanan R, Patel A, Peters SA, Reddy VP, Zheng M, de Zwart L, et al. (2018) Physiologically based pharmacokinetic model qualification and reporting procedures for regulatory submissions: a consortium perspective. *Clin Pharmacol Ther* **104**:88–110.

Shepard T, Scott G, Cole S, Nordmark A, and Bouzom F (2015) Physiologically Based Models in Regulatory Submissions: Output From the ABPI/MHRA Forum on Physiologically Based Modeling and Simulation. *CPT Pharmacometrics Syst Pharmacol* **4** (4):221–225, doi: 10.1002/psp4.30 26225245.

Shimizu M, Fukami T, Kobayashi Y, Takamiya M, Aoki Y, Nakajima M, and Yokoi T (2012) A novel polymorphic allele of human arylacetamide deacetylase leads to decreased enzyme activity. *Drug Metab Dispos* **40**:1183–1190.

Shou M, Dai R, Cui D, Korzekwa KR, Baillie TA, and Rushmore TH (2001) A kinetic model for the metabolic interaction of two substrates at the active site of cytochrome P450 3A4. *J Biol Chem* **276**:2256–2262.

Shumaker RC, Aluri J, Fan J, Martinez G, Thompson GA, and Ren M (2014) Effect of rifampicin on the pharmacokinetics of lenvatinib in healthy adults. *Clin Drug Investig* **34**:651–659.

Stopfer P, Giessmann T, Hohl K, Sharma A, Ishiguro N, Taub ME, Zimdahl-Gelling H, Gansser D, Wein M, Ebner T, et al. (2016) Pharmacokinetic Evaluation of a Drug Transporter Cocktail Consisting of Digoxin, Furosemide, Metformin, and Rosuvastatin. *Clin Pharmacol Ther* **100** (3): 259–267, doi: 10.1002/cpt.406 27256812.

Stott KE, Pertinez H, Sturkenboom MGG, Boeree MJ, Aarnoutse R, Ramachandran G, Requena-Méndez A, Peloquin C, Koegelenberg CFN, Alffenaar JWC, et al. (2018) Pharmacokinetics of rifampicin in adult TB patients and healthy volunteers: a systematic review and meta-analysis. *J Antimicrob Chemother* **73**:2305–2313.

Sun Y, Chothe PP, Sager JE, Tsao H, Moore A, Laitinen L, and Hariparsad N (2017) Quantitative prediction of CYP3A4 induction: impact of measured, free, and intracellular perpetrator concentrations from human hepatocyte induction studies on drug-drug interaction predictions. *Drug Metab Dispos* **45**:692–705.

Takahashi RH, Shahidi-Latham SK, Wong S, and Chang JH (2017) Applying stable Isotope labeled amino acids in micropatterned hepatocyte coculture to directly determine the degradation rate constant for CYP3A4. *Drug Metab Dispos* **45**:581–585.

Thirumaran RK, Lamba JK, Kim RB, Urquhart BL, Gregor JC, Chande N, Fan Y, Qi A, Cheng C, Thummel KE, et al. (2012) Intestinal CYP3A4 and midazolam disposition in vivo associate with VDR polymorphisms and show seasonal variation. *Biochem Pharmacol* **84**:104–112.

Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Bacchi CE, Marsh CL, McVicar JP, Barr DM, Perkins JD, et al. (1994) Use of midazolam as a human cytochrome P450 3A probe: II. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation. *J Pharmacol Exp Ther* **271**:557–566.

Tran JQ, Kovacs SJ, McIntosh TS, Davis HM, and Martin DE (1999) Morning spot and 24-hour urinary 6 beta-hydroxycortisol to cortisol ratios: intraindividual variability and correlation under basal conditions and conditions of CYP 3A4 induction. *J Clin Pharmacol* **39**:487–494.

Tweedie D, Polli JW, Berglund EG, Huang SM, Zhang L, Poirier A, Chu X, and Feng B; International Transporter Consortium (2013) Transporter studies in drug development: experience to date and follow-up on decision trees from the International Transporter Consortium. *Clin Pharmacol Ther* **94**:113–125.

Urquhart BL, Tirona RG, and Kim RB (2007) Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. *J Clin Pharmacol* **47**:566–578.

van Dyk M, Marshall JC, Sorich MJ, Wood LS, and Rowland A (2018) Assessment of inter-racial variability in CYP3A4 activity and inducibility among healthy adult males of Caucasian and South Asian ancestries. *Eur J Clin Pharmacol* **74**:913–920.

van Rongen A, Kervezee L, Brill M, van Meir H, den Hartigh J, Guchelaar HJ, Meijer JH, Burggraaf J, and van Oosterhout F (2015) Population pharmacokinetic model characterizing 24-hour variation in the pharmacokinetics of oral and intravenous midazolam in healthy volunteers. *CPT Pharmacometrics Syst Pharmacol* **4**:454–464.

Varma MV, Lai Y, Kimoto E, Goosen TC, El-Kattan AF, and Kumar V (2013a) Mechanistic modeling to predict the transporter- and enzyme-mediated drug-drug interactions of repaglinide. *Pharm Res* **30**:1188–1199.

Varma MV, Lin J, Bi YA, Rotter CJ, Fahmi OA, Lam JL, El-Kattan AF, Goosen TC, and Lai Y (2013b) Quantitative prediction of repaglinide-rifampicin complex drug interactions using dynamic and static mechanistic models: delineating differential CYP3A4 induction and OATP1B1 inhibition potential of rifampicin. *Drug Metab Dispos* **41**:966–974.

Vavricka SR, Van Montfoort J, Ha HR, Meier PJ, and Fattinger K (2002) Interactions of rifamycin SV and rifampicin with organic anion uptake systems of human liver. *Hepatology* **36**:164–172.

Venkatakrishnan K and Obach RS (2005) In vitro-in vivo extrapolation of CYP2D6 inactivation by paroxetine: prediction of nonstationary pharmacokinetics and drug interaction magnitude. *Drug Metab Dispos* **33**:845–852.

Venkatakrishnan K and Obach RS (2007) Drug-drug interactions via mechanism-based cytochrome P450 inactivation: points to consider for risk assessment from in vitro data and clinical pharmacologic evaluation. *Curr Drug Metab* **8**:449–462.

Venkatakrishnan K, Obach RS, and Rostami-Hodjegan A (2007) Mechanism-based inactivation of human cytochrome P450 enzymes: strategies for diagnosis and drug-drug interaction risk assessment. *Xenobiotica* **37**:1225–1256.

Villikka K, Kivistö KT, Luurila H, and Neuvonen PJ (1997) Rifampin reduces plasma concentrations and effects of zolpidem. *Clin Pharmacol Ther* **62**:629–634.

Wacher VJ, Wu CY, and Benet LZ (1995) Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinog* **13**:129–134.

Wagner C, Zhao P, Pan Y, Hsu V, Grillo J, Huang SM, and Sinha V (2015) Application of physiologically based pharmacokinetic (PBPK) modeling to support dose selection: report of an FDA Public Workshop on PBPK. *CPT Pharmacometrics Syst Pharmacol* **4**:226–230.

Wang D, Guo Y, Wrighton SA, Cooke GE, and Sadee W (2011) Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenomic J* **11**:274–286.

Wang Z, Schuetz EG, Xu Y, and Thummel KE (2013) Interplay between vitamin D and the drug metabolizing enzyme CYP3A4. *J Steroid Biochem Mol Biol* **136**:54–58.

Wanwimolruk S, Paine MF, Pusek SN, and Watkins PB (2002) Is quinine a suitable probe to assess the hepatic drug-metabolizing enzyme CYP3A4? *Br J Clin Pharmacol* **54**:643–651.
Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, and Guzelian PS (1989) Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450. Studies in rats and patients. *J Clin Invest* **83**:688–697.
Wilkins JJ, Savic RM, Karlsson MO, Langdon G, McIlleron H, Pillai G, Smith PJ, and Simonsson US (2008) Population pharmacokinetics of rifampin in pulmonary tuberculosis patients, including a semi-mechanistic model to describe variable absorption. *Antimicrob Agents Chemother* **52**:2138–2148.
Yang J, Liao M, Shou M, Jamei M, Yeo KR, Tucker GT, and Rostami-Hodjegan A (2008) Cytochrome p450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. *Curr Drug Metab* **9**:384–394.
Yang LQ, Li SJ, Cao YF, Man XB, Yu WF, Wang HY, and Wu MC (2003) Different alterations of cytochrome P450 3A4 isoform and its gene expression in livers of patients with chronic liver diseases. *World J Gastroenterol* **9**:359–363.
Yin OQ, Shi X, Tomlinson B, and Chow MS (2004) Interindividual and intraindividual variability of the urinary 6beta-hydroxycortisol/cortisol ratio in Chinese subjects: implications of its use for evaluating CYP3A activity. *J Clin Pharmacol* **44**:1412–1417.
Yoshida K, Budha N, and Jin JY (2017) Impact of physiologically based pharmacokinetic models on regulatory reviews and product labels: frequent utilization in the field of oncology. *Clin Pharmacol Ther* **101**:597–602.
Yoshikado T, Maeda K, Furihata S, Terashima H, Nakayama T, Ishigame K, Tsunemoto K, Kusuhara H, Furihata KI, and Sugiyama Y (2017) A clinical cassette dosing study for evaluating the contribution of hepatic OATPs and CYP3A to drug-drug interactions. *Pharm Res* **34**:1570–1583.
Yu J, Ritchie TK, Mulgaonkar A, and Ragueneau-Majlessi I (2014) Drug disposition and drug-drug interaction data in 2013 FDA new drug applications: a systematic review. *Drug Metab Dispos* **42**:1991–2001.
Yu J, Ritchie TK, Zhou Z, and Ragueneau-Majlessi I (2016) Key findings from preclinical and clinical drug interaction studies presented in new drug and biological license applications approved by the Food and Drug Administration in 2014. *Drug Metab Dispos* **44**:83–101.
Yu J, Zhou Z, Owens KH, Ritchie TK, and Ragueneau-Majlessi I (2017) What can be learned from recent new drug applications? A systematic review of drug interaction data for drugs approved by the US FDA in 2015. *Drug Metab Dispos* **45**:86–108.
Zhang D, Chando TJ, Everett DW, Patten CJ, Dehal SS, and Humphreys WG (2005) In vitro inhibition of UDP glucuronosyltransferases by atazanavir and other HIV protease inhibitors and the relationship of this property to in vivo bilirubin glucuronidation. *Drug Metab Dispos* **33**:1729–1739.
Zhang N, Shon J, Kim MJ, Yu C, Zhang L, Huang SM, Lee L, Tran D, and Li L (2018) Role of CYP3A in oral contraceptives clearance. *Clin Transl Sci* **11**:251–260.
Zhao P (2017) Report from the EMA workshop on qualification and reporting of physiologically based pharmacokinetic (PBPK) modeling and simulation. *CPT Pharmacometrics Syst Pharmacol* **6** (2):71–72, doi: 10.1002/psp4.12166 28035755.
Zhu B, Liu ZQ, Chen GL, Chen XP, Ou-Yang DS, Wang LS, Huang SL, Tan ZR, and Zhou HH (2003) The distribution and gender difference of CYP3A activity in Chinese subjects. *Br J Clin Pharmacol* **55**:264–269.
Zimmerlin A, Trunzer M, and Faller B (2011) CYP3A time-dependent inhibition risk assessment validated with 400 reference drugs. *Drug Metab Dispos* **39**:1039–1046.

Address correspondence to: Diane Ramsden, Drug Metabolism and Pharmacokinetics, early Development, Alnylam Pharmaceuticals, 300 Third St, Cambridge, MA 02142. E-mail: dramsden@alnylam.com