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Clinical Probes and Endogenous Biomarkers as Substrates for Transporter DDI

Evaluation: Perspectives from the International Transporter Consortium

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Abbreviation List:

ADME, Absorption, distribution, metabolism, and excretion; AUC, area under the concentration-time curve; AUCR, ratio of AUC; BCRP, breast cancer resistance protein; 6 β HC, 6 β -hydroxycortisol; CL, clearance; CYP, cytochrome P450; CB, conjugated bilirubin; CP, coproporphyrin; C_{max}, maximum concentration; C_{max}R, ratio of C_{max}; CL_r, renal clearance; CL_rR,

71 ratio of CL_r ; DDI, drug-drug interaction; DE, dabigatran etexilate; GCDCA-S,
72 glycochenodeoxycholate-3-O-sulfate; GWAS, genome wide association study; HDA,
73 hexadecanedioate; IVIVE, *in vitro* to *in vivo* extrapolation; ITC, International Transporter
74 Consortium; IC_{50} , half maximal inhibitory concentration; K_m , Michaelis constant; K_i , inhibition
75 rate constant; MDR, multidrug resistance; MRP, multidrug resistance protein; MATE, multidrug
76 and toxin extrusion protein; NME, new molecular entity; NMN, N1-methylnicotinamide; NTCP,
77 sodium taurocholate co-transporting polypeptide; OATP, organic anion transporting polypeptide;
78 OAT, organic anion transporter; OCT, organic cation transporter; P-gp, P-glycoprotein; PBPK,
79 physiologically-based pharmacokinetic; PK, pharmacokinetics; PPB, plasma protein binding; $t_{1/2}$,
80 half-life; THTR, thiamine transporter; TDA, tetradecanedioate; UCB, unconjugated bilirubin.

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Abstract (150/150)

Drug transporters can govern the absorption, distribution, metabolism, and excretion of substrate drugs and endogenous substances. Investigations to examine their potential impact to pharmacokinetic drug-drug interactions (DDIs) are an integral part of the risk assessment in drug development. To evaluate a new molecular entity as a potential perpetrator of transporters, use of well characterized and/or clinically relevant probe substrates with good selectivity and sensitivity are critical for robust clinical DDI assessment that could inform DDI management strategy in the product labeling. The availability of endogenous biomarkers to monitor transporter-mediated DDIs in early phases of clinical investigations would greatly benefit downstream clinical plans. This article reviews the state-of-the-art in transporter clinical probe drugs and emerging biomarkers including current challenges and limitations, delineates methods and workflows to identify and validate novel endogenous biomarkers to support clinical DDI evaluations, and proposes how these probe drugs or biomarkers could be used in drug development.

Drug transporters can modulate the absorption, distribution, metabolism, and excretion (ADME) of substrate drugs and endogenous substances, ultimately determining their exposure in systemic circulation and tissues.¹ Transporter substrate or modulator (inhibitor or inducer) drugs can become clinical victims or perpetrators of DDIs, respectively, when the transporter in question is a substantial contributor to the pharmacokinetics (PK) of the victim drug and can be inhibited or induced in the clinical setting. For example, lapatinib, a P-glycoprotein (P-gp) inhibitor, increased digoxin exposure by 2.8-fold (TYKERB labeling at Drugs@FDA), whereas tipranavir/ritonavir, a P-gp inducer, decreased saquinavir/ritonavir exposure by 76% (APTIVUS labeling at Drugs@FDA). Understanding DDIs is an integral part of risk assessment in drug development considering the common practice of concomitant use of multiple medications.¹⁻³

Previous International Transporter Consortium (ITC) whitepapers and regulatory guidelines have proposed an integrated approach (*in vitro*, *in vivo*, *in silico*) to assess DDI potential and to inform safe drug use.¹⁻¹⁰ Multiple factors contribute to the decision whether a clinical DDI study may be necessary, and if so, its prioritization during drug development, and subsequent interpretation of clinical DDI data to inform the product labeling. From the perspective of a new molecular entity (NME) as a transporter inhibitor, prediction of clinical DDI potential involves comparison of *in vitro* transporter inhibitory potency to various transporters that govern the victim drug's intestinal, liver portal, systemic and tissue exposure. In this regard, *in vitro* transporter inhibition assays have been routinely used to determine whether an NME is an inhibitor of clinically-relevant transporters, which serve as a trigger for follow up clinical DDI studies.^{9, 10} However, this approach may result in false positive or false negative predictions due to the limitations of *in vitro* transporter assays and/or gaps in *in vitro* to *in vivo* extrapolation (IVIVE).^{7, 11} For instance, the assumptions are often made that the transporter-mediated pathway accounts for 100% of drug elimination, however, the contribution of transporter(s) to overall elimination of drugs is often not known or cannot be accurately measured.

Endogenous substances can exhibit transporter-mediated disposition, and their concentrations may be altered when transporter activity is modulated.^{12, 13, 14} These

endogenous substances have the potential to serve as biomarkers to study transporter function *in vivo* in humans. Transporter biomarkers offer the potential for evaluating NMEs as transporter inhibitors in early clinical studies, and with appropriate validation, may obviate the need for dedicated clinical DDI studies. For the purposes of drug development planning, it would be invaluable to corroborate or refute inhibition of transporters predicted from *in vitro* data using biomarkers, particularly in situations where *in vitro* prediction methods have high false positive rates. For clinical DDI studies evaluating an NME as an inhibitor, use of selective and sensitive probe substrate drugs is a key to determine clinical risk for transporter inhibition, provide a mechanistic insight, extrapolate the results to other unstudied drugs, and inform the drug labeling to provide recommendations based on the DDI results. However, this has been challenging by the multiplicity of transporter substrates and inhibitors, the presence of multiple drug binding sites in many drug transporters, and complex interplay with drug metabolizing enzymes^{4-6, 10}. Therefore, probe drugs currently used for clinical DDI studies are largely based on the likelihood of co-administration, without comprehensive understanding and evaluation of their ADME properties/*in vitro* transporter profiles, which limits extrapolation of the results to other drugs. Validated clinical probes are critical for robust clinical DDI assessment that ultimately drives product labeling to inform concomitant drug use. **Table 1** outlines key features of an ideal probe drug or a biomarker which can be used *in vivo* to assess transporter perpetrator DDIs. Notably, very few such probe substrate drugs or biomarkers have been identified to date.

This article reviews the state-of-the-art of transporter clinical probe drugs and emerging biomarkers, provides recommendations on use of relevant transporter probe substrates for DDI evaluation, and some potential transporter endogenous biomarkers for further characterization and validation, as well as current challenges and limitations. Furthermore, the article practically delineates methods and workflows to identify and validate novel endogenous biomarkers to support clinical DDI evaluation, and proposes their potential utility and application in drug development.

STATE-OF-THE ART IN CLINICAL PROBE DRUGS AND POTENTIAL ENDOGENOUS BIOMARKERS FOR CLINICAL TRANSPORTER DDI ASSESSMENT

Clinical DDI assessment for an NME as a transporter inhibitor is typically conducted by administering an accepted probe substrate with the NME. In this section, we have conducted a critical and comprehensive analysis of probe substrate drugs commonly used for the transporters recommended by the ITC and regulatory agencies for clinical DDI evaluation based on their ADME properties, *in vitro* transporter profiles, and *in vitro* and clinical DDI data.^{1, 4-6, 9, 10}

Figure 1 illustrates recommended probe substrate drugs with a relatively high selectivity (based on relative contribution of transporter-mediated pathways to overall elimination of drugs) and sensitivity (based on clinical DDI studies with known potent transporter inhibitors and/or pharmacogenomic studies in subjects carrying non- or reduced-function alleles)^{15, 16} with detailed ADME properties, *in vitro* transporter profile, and examples of clinical DDIs summarized in **Table 2**. These probe substrate drugs in general meet the following criteria:

- *In vitro* transporter profiles are relatively well characterized, and show higher transport activity and selectivity for the transporter of interest
 - Clinical data show significant contribution of the transporter of interest in the overall elimination as indicated by
 - the ratio of area under the plasma concentration-time profile (AUCR) ≥ 2
 - and/or the ratio of plasma clearance (CL) (or renal clearance (CL_r) for renal transporters) $CL_R \text{ or } CL_{rR} \leq 0.5$
- Either in the presence of a potent transporter inhibitor or in subjects with reduced function caused by a transporter polymorphism
- Are commercially available and have relatively rich clinical DDI data

In this section, potential utility and limitations of probe substrate drugs summarized in **Figure 1** are discussed, together with recent examples of clinical probe drug cocktails used to simultaneously explore DDIs involving multiple drug transporters and metabolizing enzymes.¹⁷⁻

²⁰

Several endogenous substrates have been identified as potential endogenous biomarkers to evaluate inhibition of several hepatic and renal transporters in humans.^{12, 13, 14} The endogenous substrates with initial clinical DDI evaluation are highlighted in **Figure 1**. Recent progress in identifying and evaluating these potential biomarkers are discussed below and some key preclinical and clinical data are summarized in **Table 3** and **Table S1**.

Liver transporters: Organic Anion Transporting Polypeptide 1B1/1B3 (OATP1B) and Organic Cation Transporter 1 (OCT1)

OATP1B1/1B3

- **Probe Drugs**

Pitavastatin, rosuvastatin, and atorvastatin are widely used as clinical probe drugs for the evaluation of OATP1B-mediated DDIs, either following a clinically-relevant oral dose or a microdose as part of a drug cocktail^{18, 21, 22} (**Figure 1, Table 2**). Although considered as OATP1B substrates, the aforementioned drugs as well as other proposed “probes” have been shown to be substrates for other transporters *in vitro*, such as OATP2B1, sodium-taurocholate co-transporting polypeptide (NTCP), breast cancer resistance protein (BCRP), P-glycoprotein (P-gp), organic anion transporter 3 (OAT3), and multidrug resistance-associated protein 4 (MRP4) and MRP3.^{21, 23, 24}

Pitavastatin is considered as the most sensitive OATP1B1 probe,²² supported by >80% fraction transported via OATP1B1, which has been estimated from *in vitro*, human ADME, *SLCO1B1* (OATP1B1) c. 521T>C pharmacogenomic data and DDI data with intravenous (i.v.) vs.

orally-administered rifampin, a potent inhibitor for OATP1B. Pharmacogenomic data highlight similar sensitivity to *SLCO1B1* for simvastatin acid,^{15, 16, 25} but its complex PK (lactone-acid interconversion) and OATP1B-CYP3A interplay may limit its general use as a selective OATP1B clinical probe. In the case of rosuvastatin, the alteration of the activity of multiple non-OATP1B transporters expressed either in the intestine (BCRP and OATP2B1), liver (NTCP and OATP2B1), or kidney (OAT3) may be the driver behind the observed change in its PK following the oral administration of an inhibitor that can affect these transporters. Like rosuvastatin, atorvastatin presents a suitable OATP1B probe drug because of supporting pharmacogenomics data,²⁶ clinical DDI data with rifampin and mechanistic insight regarding OATP1B as the rate-determining step driving its hepatic disposition.²⁷ Nonetheless, the potential effect of inhibiting intestinal CYP3A4 and BCRP/P-gp efflux should also be considered when interpreting the observed changes in its systemic exposure.

- Endogenous Substrates (Potential Biomarker)

To date, the endogenous substrates of OATP1B include coproporphyrins (CPs), bile acids (BAs), sulfated and glucuronidated BAs, fatty acid dicarboxylates, bilirubin, glucuronidated bilirubin, thyroid hormones and their metabolites, steroids, and sulfated and glucuronidated steroids. Among them CPI, CPIII, glycochenodeoxycholate-3-O-sulfate (GCDCA-S), hexadecanedioate (HDA), tetradecanedioate (TDA), conjugated and unconjugated bilirubin (CB and UCB) present as potential biomarkers for clinical evaluation of OATP1B-mediated DDIs, but each has limitations^{12, 13 14} (**Figure 1, Table 3**). In most cases, evidence for their utility as OATP1B biomarkers is based on animal data and careful profiling of plasma and urine of human *SLCO1B* genotyped subjects. For example, patients with Rotor's syndrome lack expression of OATP1B1 and OATP1B3 are characterized by increased urinary excretion of CPI and higher bilirubin in the blood due to reduced secretion into the bile.²⁸ Likewise, pharmacogenomic studies indicate that individuals genotyped *SLCO1B1**15 and c.521T>C show increased exposure of UCB, CB and some BAs.²⁹⁻³¹ Compelling clinical evidence supporting HDA, TDA, GCDCA-S and bilirubin as potential endogenous biomarkers for OATP1B1 has come from metabolomic and genome-wide association studies (GWAS), which show a significant association of *SLCO1B1*

521T>C with the plasma levels of these endogenous substances.³²⁻³⁴ The clinical data are consistent with the results obtained in *Oatp1a/1b*^{-/-} mice, as they exhibit higher plasma CPI, CPIII, UCB, CB and BA concentrations^{28, 35, 36} (**Table S1**). In addition, *in vitro* studies using transporter-transfected cells and hepatocytes with an OATP1B inhibitor (e.g., rifampin) confirm that CPI, CPIII, GCDCA-S, HDA, TDA, CB and UCB are substrates for OATP1B1 (**Table 3**). These biomarkers are also substrates for OATP1B3 with the exception of HDA and TDA. In addition, they are also transported by other hepatic and renal transporters such as MRP2 and MRP3 (CPI, CPIII and CB), OATP2B1 (CPIII), OAT1 (HDA and TDA), OAT3 (GCDCA-S, HDA, and TDA), and NTCP (GCDCA-S) (**Table 3**), which may limit their selectivity.

Concerns regarding transporter selectivity aside, the above mentioned endogenous biomarkers are capable of recapitulating some clinical OATP1B DDIs. For example, a single oral dose of rifampin (600 mg) has been shown to markedly increased the AUC of GCDCA-S (10-20-fold).⁴¹ This increase was significantly greater than the changes in plasma simeprevir, bosentan, and repaglinide exposures in the same study (7.2-, 3.2- and 1.9-fold, respectively).³⁴ Results of recent studies have also shown that changes in CPI plasma exposure correlate with presumably different levels of OATP1B inhibition imposed by OATP1B inhibitors that showed different *in vivo* inhibition (e.g., simeprevir, JNJ-A, and GDC-0810) with known OATP1B substrates.³⁷ To date, however, reported OATP1B clinical DDI studies with concomitant monitoring of both clinical probe drugs and endogenous biomarker(s) are still limited in number.³⁸⁻⁴¹ (**Table 3**).

In summary, CPI, CPIII, GCDCA-S, HDA, TDA, CB and UCB have demonstrated initial promise as clinical OATP1B biomarkers, in which, CPI appears to be the most promising biomarker with higher selectivity and sensitivity. Further characterization and validation is needed to determine their predictive value for OATP1B-related DDIs. Until other confounding factors, including overlapping substrate selectivity for transporters and enzymes, disease, diet, and circadian rhythm, are fully understood, a single OATP1B biomarker may not be sufficient for effective overall evaluation of OATP1B inhibition risk and the use of multiple biomarkers may be warranted.

OCT1

- Probe Drugs

Metformin is a well-known substrate of OCT1. It is also a substrate for OCT2 and multidrug and toxin extrusion protein MATE1 and MATE2K. Metformin may not be a suitable clinical OCT1 probe drug if only changes in PK is monitored,⁴² because OCT1 modulation does not impact metformin systemic PK, and it only impacts metformin hepatic distribution and pharmacodynamics (PD) response.^{42, 43} Currently, OCT1 probe substrate drugs have only been validated clinically in pharmacogenetic studies by comparing individuals expressing inactive or reduced function of OCT1 vs. wildtype controls.^{15, 42, 44} Clinical OCT1 probes identified to date include sumatriptan,⁴⁵ ondansetron, and tropisetron.⁴⁶ Overall, sumatriptan and tropisetron are promising probe substrates of OCT1 because they exhibit a 2.2-fold and 4.9-fold increase in AUC, respectively, in subjects with a polymorphism resulting in reduced OCT1 activity vs. controls.^{45, 46} Of the two, sumatriptan is most promising as a drug probe for OCT1 because evidence is available from a prospective study in healthy subjects⁴⁵ (**Table 2**). Additional DDI studies with OCT1 inhibitors are warranted to better characterize their clinical utility as probe drugs for OCT1.

Fenoterol is also a promising clinical OCT1 probe with both notable systemic PK and PD effects of OCT1 modulation, but it is practically problematic, because it is not approved in the US.⁴⁷ The O-desmethyl metabolite of tramadol^{48, 49} is a sensitive OCT1 probe with both notable PK and PD effects during OCT1 modulation. However, metabolites are generally not preferred probes in the drug development setting due to additional data interpretation complexity associated with metabolite formation that may be influenced by NME and population variability (e.g., pharmacogenetic factors). Morphine⁵⁰ may be a useful OCT1 probe, but clinical studies conducted to-date were inconsistent, and morphine cannot presently be recommended as an OCT1 probe until these findings are clarified (e.g., oral administration of the prodrug versus parenteral administration of morphine directly). Besides OCT1, morphine is a substrate for UGTs and P-gp. Moreover, morphine is a controlled substance and DDI studies with controlled substances are not practical (or safe).

- Endogenous Substrates (Potential Biomarker)

Thiamine is a substrate of OCT1⁵¹ (**Table S1**), however, clinical evidence supporting thiamine as an endogenous OCT1 probe is lacking. Preclinical studies using *Oct1*^{-/-} mice showed that the concentrations of thiamine and its metabolites in the knockout mouse liver following i.v. administration were reduced by more than 50% compared to wild-type animals, whereas plasma levels were increased 30-40%, confirming the role of Oct1 in thiamine hepatic disposition *in vivo*. However, thiamine is not selective for OCT1 because it is also a substrate for OCT2, MATE1, MATE2K, thiamine transporter 1 and 2 (THTR1 and THTR2).^{52, 53} Notably, up to 9% of Caucasians exhibit an OCT1 deficient phenotype, which has not been associated with markedly elevated systemic thiamine concentrations possibly due to variability associated with dietary intake. This may make thiamine an impractical clinical OCT1 biomarker.

Recently, acylcarnitines, intermediate metabolites of mitochondrial oxidation, was identified as endogenous substrates of OCT1 through GWAS, targeted metabolomics, transporter transfected cells, and Oct1 liver specific knockout mice.⁵⁴ These studies have illustrated the role of OCT1 in the efflux of acylcarnitines from the liver to the circulation. Additional clinical DDI studies, however, are needed to assess the suitability of acylcarnitine as a potential endogenous biomarker of OCT1. It is worth noting that the GWAS showed that serum acylcarnitine levels are also significantly associated with organic cation/carnitine transporter 1 (OCTN1; *SLC22A4*), UGT1A1 and carnitine palmitoyltransferase 1 (CPT1) genes⁵⁵ and acylcarnitine is also an *in vitro* substrate of OCTN2, which may limit the selectivity of acylcarnitine as a biomarker for OCT1.⁵⁵

Kidney Transporters: Organic Anion Transporters OAT1/3 and Organic Cation Transporters OCT2 and MATE1/2K

OAT1/3

- Probe Drugs

Despite overlapping substrate selectivity between OAT1 and OAT3, adefovir is a relatively selective OAT1 probe, because its transport by OAT3 is minimal⁵⁶ (**Figure 1, Table 2**). Adefovir is also transported by the apically localized renal efflux transporter MRP4.⁵⁷ However, plasma and renal clearance of adefovir was only slightly decreased in *Mrp4*^{-/-} mice, suggesting that change in Mrp4 activity may not have significant impact on its plasma exposure.⁵⁷ Probenecid, a potent inhibitor of OATs, caused a 2.1-fold increase in adefovir plasma AUC (**Table 2**), suggesting that adefovir may be used as a probe drug for OAT1. In contrast, benzylpenicillin (also named as Penicillin G) (**Figure 1, Table 2**) is a more selective OAT3 substrate compared to OAT1.^{56, 58} Probenecid increased plasma AUC of benzylpenicillin by 3.3-fold⁵⁶ (**Table 2**), suggesting that it may serve as an OAT3 probe substrate. As discussed in drug probe cocktail section below, furosemide, a dual substrate of OAT1 and OAT3, could be a useful probe to study the inhibition to both OAT1 and OAT3.¹⁷

- Endogenous Substrates (Potential Biomarker)

Taurine is an endogenous OAT1 substrate, whereas 6 β -hydroxycortisol (6 β HC) and GCDCA-S are substrates of OAT3; they may therefore potentially serve as endogenous biomarkers for assessing DDIs with these transporters^{12, 13} (**Figure 1, Table 3**). In a clinical study, probenecid exhibited dose-dependent reduction of CL_r of taurine and GCDCA-S without altering their plasma AUC. Moreover, the CL_r values for either compound correlated strongly with benzylpenicillin (an OAT3 probe drug) and 6 β HC, suggesting that either compound could be a useful biomarker for OAT1 or OAT3.⁵⁹ Unlike 6 β HC, the CL_r of taurine and GCDCA-S was below GFR, indicating potential reabsorption from the urine in addition to tubular secretion. The selectivity of GCDCA-S as an endogenous OAT3 probe is limited since probenecid can inhibit apically-localized OAT4 and MRP2, which may be involved in the reabsorption and urinary excretion of GCDCA-S, respectively. Additionally, GCDCA-S is also known as an endogenous substrate of hepatic OATP1B⁶⁰ (**Table 3**) and, as such, may confound its selectivity for OAT3. 6 β HC is formed by hepatic CYP3A4 and excreted into urine. It is also known as a biomarker for induction of CYP3A4 and any change in CYP3A4 activity may therefore influence the sensitivity and selectivity of 6 β HC as an OAT3 probe.⁶¹ Besides OAT3, 6 β HC is a substrate for MATE1 and

MATE2K.⁶² Probenecid significantly increased plasma AUC, but reduced CL_r of 6βHC, whereas pyrimethamine (a MATEs inhibitor) did not change systemic 6βHC AUC or CL_r significantly.⁶² Neither probenecid nor pyrimethamine affected CYP3A4 and 11β-hydroxysteroid dehydrogenase 2 activity responsible for the formation of 6βHC and conversion of 6βHC to 6β-hydroxycortisone, respectively.⁶² Recently, pyridoxic acid and homovanillic acid have been identified as endogenous substrates of OAT1/3 using metabolomic analysis and DDI studies in cynomolgus monkeys.⁶³ Additional studies are needed to validate their potential utility as OAT1/3 endogenous biomarkers in human.

OCT2, MATE1/2K

- Probe Drugs

Metformin is a well-known and broadly used probe drug to study inhibition of the renal uptake transporter OCT2, and efflux transporters MATE1/2K.⁶⁴ For example, MATE1/2K inhibitor pyrimethamine significantly changed metformin plasma AUC and CL_r by the inhibition of active renal secretion^{65,66} (**Figure 1, Table 2**). However, distribution of metformin to the liver, its primary site of action, but not clearance, is mediated by OCT1.⁶⁷ Recent studies suggest that metformin is also a substrate of OCT3, which is a key to its skeletal muscle distribution (another site of action). The absorption and distribution of metformin is a complex and poorly understood process that involves at least several transporters, including THTR-2 and OCT1/3.⁶⁴ Lack of selectivity of most transporter inhibitors complicates further the interpretation of metformin renal DDI data. For instance, famotidine (a selective MATE1 inhibitor) at a high dose (200-800 mg/day) resulted in an increase, rather than the expected decrease in metformin CL_r.⁶⁸ Famotidine co-administration also caused a transient enhancement of the anti-hyperglycemic effects of metformin, likely due to enhanced hepatic distribution through MATE1 inhibition.⁶⁸ Due to the unique complexity of metformin mechanistic disposition, a separate commentary in this issue of CPT is dedicated to clinical DDI study design consideration for metformin.⁴³

- Endogenous Substrates (Potential Biomarker)

N1-methylnicotinamide (NMN) is an endogenous substrate of OCT2, MATE1/ 2K.⁶⁹ Pyrimethamine almost completely inhibited its CL_r via active secretion without altering its plasma exposure.⁶⁹ Trimethoprim, another OCT2/MATEs inhibitor, also reduced NMN CL_r, which correlated with the change in metformin exposure in the same healthy subjects.⁷⁰ Recent studies reported increased NMN CL_r in pregnant women which was positively correlated with increase in metformin CL_r.⁷¹ These clinical observations suggest the potential use of NMN as an endogenous probe for DDIs involving OCT2/MATEs (**Figure 1, Table 3**). However, it is worth noting that renal elimination of NMN also involves active tubular reabsorption.⁷²

Creatinine is commonly used as a biomarker for renal function. Increased serum creatinine and reduced creatinine CL_r could be a result of transient inhibition of renal transporters involved in its active secretion (OCT2, OAT2, MATE1/2K), without altering GFR⁷³ (**Figure 1, Table 3**). However, creatinine levels are influenced by many factors such as weight, gender, age, muscle metabolism, and diet. A retrospective analysis of the change of creatinine level and DDI data with OCT2/MATEs inhibition suggests that creatinine is not a sensitive biomarker for OCT2/MATEs inhibition, despite the fact that elevation of serum creatinine is frequently associated with inhibition of OCT2/MATEs.⁷³ Therefore, incorporation of creatinine with other biomarkers, such as NMN may be needed to evaluate the risk for OCT2/MATEs inhibition.

Gut Transporters: P-gp and BCRP

P-gp

- Probe Drugs

Digoxin, dabigatran etexilate (DE), and fexofenadine are recommended by regulatory agencies as clinical probe drugs for P-gp inhibition (**Figure 1, Table 2**). However, there are a

number of limitations regarding the suitability of digoxin as a P-gp probe including the involvement of additional transporters and/or the low sensitivity of the DDI signal largely due to high oral bioavailability (60-80%). Despite these limitations, clinical DDIs with digoxin have been most extensively evaluated because digoxin exhibits a narrow therapeutic index and is commonly used in the clinical setting. The impact of NMEs on digoxin PK should therefore be carefully monitored as a safety precaution.⁷⁴ However, DDI data obtained with digoxin cannot be readily extrapolated to other P-gp substrate drugs.⁷⁵ The limitation of fexofenadine as a P-gp probe is the involvement of OATPs (OATP2B1, OATP1B1/1B3) and OAT3 in its absorption, hepatic and renal elimination.^{76, 77} Fexofenadine therefore can be selected as a P-gp probe only when OATPs /OAT3 inhibition is not involved in the DDIs. DE, a prodrug of dabigatran, is a P-gp substrate, whereas its active moiety dabigatran is not.⁷⁸ Thus, DE could be a more selective P-gp probe to study P-gp inhibition in the gut, as P-gp-mediated efflux is restricted to limiting intestinal absorption of DE. However, PK variability, conversion of DE to dabigatran via carboxylesterases, and potential saturation of P-gp in the gut at therapeutic dose may impact its sensitivity and selectivity.⁷⁹ Given their relatively low bioavailability (**Table 2**), DE and fexofenadine may be more appropriate P-gp probes in comparison to digoxin when evaluating intestinal P-gp inhibition.

- Endogenous Substrates (Potential Biomarker)

To date, no endogenous probe for P-gp has been identified.¹³

BCRP

- Probe Drugs

It is challenging to identify clinical BCRP probes due to the multiplicity of substrates and inhibitors, as well as potential BCRP interactions both in the gut and liver. Following a comprehensive review of *in vitro*, clinical DDI, and pharmacogenomic data, sulfasalazine has been proposed as an *in vivo* probe for evaluating intestinal BCRP inhibition, intravenous

rosuvastatin for evaluating hepatic BCRP inhibition, and oral rosuvastatin for both intestinal and hepatic BCRP inhibition.²⁴ Sulfasalazine has limited intestinal absorption due to its low permeability, low solubility, and BCRP-mediated intestinal efflux (**Figure 1, Table 2**). Plasma AUC of sulfasalazine in wild-type mice increased by 8-fold, following oral dosing of curcumin, a potent BCRP inhibitor, whereas no change was observed in *Bcrp*^{-/-} mice.⁸⁰ In human, curcumin increased sulfasalazine AUC by 1.8- to 3.2-fold⁸⁰ (**Table 2**), suggesting that BCRP plays a significant role in limiting the intestinal absorption of sulfasalazine. Of note, sulfasalazine is an MRP2 and P-gp substrate as well, which may limit its intestinal absorption but to a much lower extent than BCRP based on the observations from transporter knockout mice. Furthermore, sulfasalazine is also transported by OATP2B1 in the gut, which is most likely saturated at sulfasalazine's therapeutic dose, and therefore, may not have significant impact on its intestinal absorption.⁸⁰

As discussed above, rosuvastatin is a substrate for multiple uptake and efflux transporters, including BCRP. A polymorphism in *ABCG2* (c.421C>A) results in a significant increase of rosuvastatin plasma AUC^{15, 16} (**Table S2**), supporting its use as a BCRP probe. As inhibition of BCRP may alter both the intestinal absorption and hepatobiliary excretion of rosuvastatin, it can be a useful probe to assess inhibitory effects on both gut and hepatic BCRP.²⁴ Differentiation between the contribution of intestinal and hepatic BCRP is possible only if the appropriate study design is used, as discussed in study design section.

- Endogenous Substrates (Potential Biomarker)

To date, no endogenous probe for BCRP has been identified.¹³

Transporter Probe Drug Cocktails

Since many perpetrator drugs inhibit multiple transporters, concurrent administration of a cocktail containing multiple probe drugs at therapeutic or microdose could be an efficient and valuable tool. The cocktail approach has been successfully applied in drug development and

recognized by regulatory agencies to assess DDIs with CYP enzymes.⁸¹ However, the evaluation and use of dedicated drug cocktails to study transporter-related DDIs is still limited.^{20,82, 83} In 2011, Maeda, et al²⁷ designed a clinical study to identify the rate-determining process in hepatic elimination of atorvastatin using a microdose cocktail of atorvastatin along with probe substrates for OATP1B (pravastatin) and CYP3A (midazolam). Recently, two transporter probe drug cocktails have been proposed for studying DDIs involving transporters and their interplay with CYP3A. A transporter probe cocktail containing a conventional dose of digoxin (0.25 mg, P-gp), rosuvastatin (10 mg, OATP1B, and BCRP), furosemide (5 mg, OAT1/3), and metformin (500 mg, OCT2, MATE1/2K) has been evaluated *in vitro*¹⁷ and in clinical PK studies^{84 18}. In general, the PK of each probe substrate administered in a cocktail was comparable to when dosed individually, except for a small decrease in C_{max} of furosemide and a moderate increase in rosuvastatin C_{max} (38.6%) and AUC (43.4%). A reduction of the dose of metformin (50 mg or 10 mg) and furosemide (1 mg) is further recommended to minimize potential interactions between these probes.⁸⁴ Recently, in a new trial where the doses of metformin and furosemide as putative perpetrators were reduced to 10 mg and 1 mg, respectively, their DDI effects on rosuvastatin were shown to be eliminated.⁸⁵ Another microdose cocktail has recently been proposed and validated to study DDIs with OATP1B, P-gp, BCRP, and their interplay with CYP3A.¹⁹ This five-component cocktail contained midazolam (10 µg, CYP3A), DE (375 µg, P-gp), pitavastatin (10 µg, OATP1B), rosuvastatin (25 µg, OATP1B, BCRP) and atorvastatin (50 µg, OATP1B, BCRP, P-gp, and CYP3A). Except for DE, which showed a ~2-fold higher magnitude of DDIs with P-gp inhibitors versus a conventional dose, likely caused by saturation of gut P-gp, PK and the magnitude of DDIs with other microdosed probe drugs were generally comparable to those at their respective conventional doses. Probe drug cocktails allow the simultaneous assessment of DDI risk for different transporters/enzymes and thus can provide a mechanistic understanding of complex DDIs involving multiple transporters and enzymes.

MECHANISTIC CONSIDERATIONS AND CHALLENGES OF USING PROBE DRUGS AND ENDOGENOUS BIOMARKERS TO ASSESS TRANSPORTER INHIBITION

This section describes mechanistic considerations and challenges associated with the use of exogenously administered probe drugs and endogenous biomarkers to study transporter inhibition. Some of the challenges, such as the lack of selectivity towards individual transporters, are common for biomarkers and probe drugs. However, there are also unique challenges. For example, for many endogenous biomarkers, the kinetic determinants of their systemic exposure are poorly understood or defined. These limitations need to be considered when designing clinical studies or extrapolating observed DDIs to new combinations of drugs.

Probe Drugs

There are multiple factors that need to be considered prior to qualifying a given drug as a useful “probe” for examining the *in vivo* activity of a transporter of interest, e.g., absence of additional mechanisms (i.e., selectivity) or the availability of candidate probe drugs in a form that can be safely and ethically administered to humans in a feasible manner (**Table 1**). The paucity of probe drugs possessing sufficient selectivity places a major limitation on the ability to demonstrate the importance of a given transporter (**Table 2**). As highlighted, in certain instances testing candidate probe substrates and inhibitors in preclinical species may provide valuable information to guide future clinical investigations. Ultimately, the validation of such probes requires multiple rounds of investigation to identify the appropriate study design and the relatively specific substrate/inhibitor sets that can be utilized clinically.

Overlapping Substrate Specificities of Multiple Transporters and Metabolic Enzymes

Because most transporter probes are substrates of multiple transporters and/or enzymes, there are substantial challenges in selecting a specific probe. As discussed earlier, rosuvastatin is a substrate of BCRP and several other transporters (e.g., OATP1B1, -1B3, -2B1,

NTCP, and OAT3). If a perpetrator drug is an inhibitor of these transporters, observed DDIs may represent a worst-case scenario for rosuvastatin DDIs, but not inhibition by BCRP alone. In such case, a rosuvastatin clinical DDIs need to be interpreted in conjunction with *in vitro* and other clinical DDI studies with single or multiple transporter probes of interest or endogenous biomarkers. One promising approach to address the overlapping selectivity of transporters and enzymes, and to maximize clinical study design is to use a transporter and enzyme probe drug cocktail, as discussed in the previous section.

In Vivo Quantitation of Transporter Activities

Probe drugs may be used to quantitatively evaluate the effect of an NME on transporter activity in various organs. However, the nature of the biological matrices that can be easily collected (e.g., blood, urine, feces and saliva) often imposes a major limitation on the practical use of probe drugs in such clinical investigations. The use of stable- or radioisotope-labeled micro-tracers administered intravenously offers the opportunity to evaluate the effect of the perpetrator drugs on the bioavailability and total body clearance of probe drugs. However, limited ability to assess drug concentration-time profiles in tissues imposes limitations on understanding some of transporter DDIs where changes in systemic exposure do not necessarily reflect events at the tissue level.^{24, 68, 86} In such situations, measurement of the tissue concentration-time profiles by non-invasive approaches, such as positron emission tomography (PET), is important for quantifying transporter activities.⁸⁷ The integrated efforts of combining *in vitro*, imaging and modelling approaches can improve our predictability of tissue concentrations and DDIs.^{88, 89}

Other Challenges and Considerations

Taking into consideration of substrate-dependent inhibition *in vitro*,⁷ translation of clinical DDI data obtained using one probe to other substrate drugs remains uncertain. *In vitro* studies may be able to bridge the gap assuming the substrate dependence is relevant *in vivo*. In

addition, translation of the data from healthy subjects to specific populations, such as the subjects with hepatic or renal impairment also remains challenging due to limited information on the change of transporter activity in these populations.^{90, 91}

Endogenous Biomarkers

Mechanistic Understanding of the Formation, Disposition, and Elimination of Biomarkers

One of the major challenges in translating response of endogenous biomarkers to the inhibition of drug transporters is the poor understanding of the mechanisms involved in their formation, disposition, and/or elimination. Using OATP1B biomarkers as an example, **Figure 2** summarizes key elements that need to be considered when interpreting biomarker data. Factors potentially impacting the interpretation of renal transporter biomarker data are shown in **Figure S1**. They can be broadly categorized into three elements as described below: selectivity, specificity, sensitivity, and intrinsic or dietary factors. Current knowledge on some emerging biomarker candidates is summarized in **Table 3**, but a considerable amount of relevant information is still missing.

Selectivity and specificity of biomarkers for assessing transporter inhibition needs to be carefully considered, as highlighted in the case of probe drugs, but to date there has been limited clinical validation of the specificity and selectivity of potential biomarkers. Sensitivity of the endogenous biomarker is another key determinant of their utility in assessing transporter function, especially if the endogenous biomarker data are to be used to support the lack of an interaction between an NME and a transporter. Additionally, understanding of both intra- and inter-subject variability of biomarker levels is needed to evaluate the utility of biomarker in capturing weak or moderate transporter inhibitors/DDIs.

Some potential endogenous biomarkers, such as bile acids or bilirubin, are associated with either the development of diseases or organ injury; therefore, they are not ideal biomarkers for DDI assessment (**Table 1**). Additionally, some biomarkers may be derived from

dietary constituents or metabolites, and in that case the potential effects of food should be carefully ruled out. Effect of perpetrator drugs on the biosynthesis of endogenous biomarkers also needs to be considered, as illustrated recently for CPI and creatinine.^{92, 93, 94}

Genetic polymorphisms of drug transporters can shed a light on the mechanistic understanding of endogenous biomarker disposition. One salient example is the altered disposition and elimination of CPI and CPIII due to genetic alterations that affect MRP2 (*ABCC2*) and OATP1B1 (*SLCO1B1*) transport activities.¹² In that case, the observed changes in plasma or urine concentrations of these biomarkers may provide quantitative information on the contribution of individual transporters to a given clearance pathway. It is anticipated that the incorporation of such quantitative measures can be particularly useful in verifying physiologically-based pharmacokinetic (PBPK) models to describe the PK of endogenous biomarkers.

Kinetic Consideration for Interpreting Biomarker Data

One major difference between endogenous biomarkers and probe drugs is that the baseline concentrations of the endogenous biomarkers are determined by the rate of synthesis (as opposed to dose in the case of clinical probe) (R_{in}), along with the rate of degradation/elimination (R_{out}). When an endogenous biomarker follows first-order elimination, the concentration of the biomarker (C) at any given time can be described as:

$$dC/dt = R_{in} - CL * C \text{ (Equation 1)}$$

Where CL represents the clearance of the biomarker. Thus, determination of R_{in} and CL values is the key for quantitative, model-based analysis of biomarker kinetic profiles. When R_{in} and CL are stable over time (i.e., no diurnal fluctuation), the steady-state concentration (C_{ss}) is described as:

$$C_{ss} = R_{in} / CL \text{ (Equation 2)}$$

Unlike the probe drugs for which we can control the administered dose, it is challenging to directly estimate R_{in} . This has a significant implication on a model-based analysis of biomarker profile and subsequent quantitative interpretation of DDI observations. One strategy is to utilize isotope tracers of endogenous biomarker⁹⁵ to determine C_{ss} and CL of a biomarker, and then infer R_{in} based on Equation 2. To obtain CL, kinetic profiles after i.v. administration of the biomarker are the most informative. Another approach is to administer isotopic precursor to determine synthesis rate. Alternatively, clinical data for an endogenous biomarker in the presence and absence of a transporter inhibitor can be modeled simultaneously to estimate the rate of biomarker synthesis and elimination clearances, assuming that the inhibitor only affects transporter-mediated CL, with no effect on R_{in} .⁹² Currently such information is limited to only a few transporter biomarkers⁹⁶ and more research is needed to fill the gap to better characterize the kinetic behaviors of biomarkers.

Selection of descriptive PK parameters for biomarkers requires careful considerations. The simplest case is a direct evaluation of the changes in pathway-specific clearance, e.g., CL_r , when examining effects on renal transporters. Amount of biomarker excreted into urine over a particular time (A_e) can be expressed as follows:

$$A_e = CL_r * AUC \text{ (Equation 3)}$$

Therefore, measurement of biomarker AUC and A_e in a given time interval allows direct calculation of the effect of test drugs on renal clearance of the biomarker.

The systemic level of a biomarker could be used for the evaluation of transporter function in situations where the direct measurement is not possible (e.g., modulation in biliary excretion). In such cases, the time course of the biomarker after administration of a perpetrator drug is critical to allow correct interpretation of the DDI data. This time course will be influenced by the half-life ($t_{1/2}$) of not only the biomarker (which may be synthesis, absorption, or elimination rate-limited), but also the PK of the transporter inhibitor. For example, if an inhibitor has a long $t_{1/2}$, its effect on the transporter will be sustained after it reaches its maximum concentration, and the biomarker concentrations will approach new steady-state in

606 accordance with the $t_{1/2}$ of the perpetrator drug. In this situation, AUC would be an appropriate
607 metric to interpret changes in transporter function. **Figure 3** and **Supplementary Materials**
608 show various simulations under different scenarios to illustrate these points, and the
609 recommendations on the interpretation of systemic exposure of biomarkers.

611 *Other Considerations*

612 Similar to the clinical probe drugs, there is a potential substrate-dependency in the *in*
613 *vitro* transporter IC₅₀ or K_i values with either different biomarkers and/or biomarkers to
614 substrate drugs. Therefore, translation of DDI data using one biomarker to predict DDI risk with
615 NMEs needs to be fully evaluated assuming the relevance of substrate-dependent phenomenon
616 *in vivo*. The importance of investigating more than one biomarker is also applicable to the
617 evaluation of inhibitor effect on other transporters. Fully validated bioanalytical methods for
618 reliable and reproducible measurements of biomarkers are critical (**Table 1**).

621 **IDENTIFICATION AND VALIDATION OF ENDOGENOUS BIOMARKERS FOR TRANSPORTER DDI** 622 **EVALUATION**

623 The identification of novel, sensitive endogenous biomarkers to assess transporter
624 inhibition in a clinical setting follows a series of sequential steps. Initially, a candidate biomarker
625 can be identified from a broad scope of potential data sources and subsequently narrowed
626 down through steps of hit identification, lead characterization and eventually clinical validation
627 as detailed in the workflow in **Figure 4**.

629 *In Vitro* and Metabolomics Approaches

The process of identification may include a combination of *in vitro* and *in vivo* approaches to select a candidate endogenous molecule. For example, metabolomics approaches combined with *in vitro* assays can be used to examine the uptake of endogenous molecules into cells individually expressing transporters of interest following incubation with biological matrices. The utility of these candidate molecules can then be further challenged in systems of increasing complexity such as knockout rodent models, non-human primates and humans following the administration of a potent and relatively selective transport inhibitor. Finally, the candidate biomarker may be examined in larger populations to verify that variability in its plasma concentrations correlate with known genetic polymorphisms affecting the activity of the transporter of interest. However, there are only a few transporters with clinically meaningful genetic polymorphisms, which may limit this approach.^{15, 16} **Table S3** provides details and examples on each experimental system. Each system will generate matching test and control samples that will be quantified using metabolomics and enable inferential comparison of dozens of endogenous and exogenous metabolites in parallel and generate candidates. The selected molecules should exhibit robust and consistent responses in all available test systems before they can be considered as biomarkers for the transporter being investigated.

Clinical Studies to Characterize and Validate Novel Endogenous Biomarkers to Study Transporter Inhibition

The identification of an endogenous biomarker in a clinical setting is aimed at investigating the sensitivity selectivity, and specificity of candidates identified using the steps outlined above. Factors affecting the baseline concentration of a candidate biomarker such as diet, age, exercise, diurnal variation, and disease state may all confound the ability to observe meaningful changes in the biomarker level upon inhibition of an implicated transporter. Similarly, the participation of additional unidentified transporters in the systemic clearance of the candidate biomarker may also pose an additional challenge. Finally, an understanding of the synthetic and catabolic pathways of the candidate biomarker is necessary to ensure that the

test inhibitor does not acutely nor chronically interfere with either pathway. Mechanistic models that combine rates of synthesis, degradation and transport can be developed as described in previous sections to further verify the practicality of the candidate biomarker and perform power calculations to guide the design of clinical DDI studies, as illustrated recently for CPI.⁹²

Further validation of a candidate biomarker can be done by examining the effect of specific transport inhibitors on the biomarker exposure, preferably in a well-controlled cross-over study in subjects that have consented to genomic analysis of the suspected transporter that is known to be polymorphic. Inclusion of a validated probe drug in the study is recommended to enable parallel examination of observed effects. The number of individuals participating in a given trial and the length of treatment should be determined following the appropriate statistical and kinetic analysis of the candidate biomarker PK and extent of contribution of the implicated transporter to the overall systemic clearance. Moreover, confirmatory studies should utilize optimized analytical methods and procedures capable of detecting low levels of the biomarker in the relevant biological matrix. Study design considerations are described in detail in the next section. The results of the clinical study should be used to compare the magnitude of changes in biomarker exposure relative to the changes measured for the transporter probe drug for each of the tested inhibitors.

CLINICAL DDI STUDY DESIGN FOR EVALUATION OF TRANSPORTER INHIBITION WITH CLINICAL PROBE DRUGS OR ENDOGENOUS BIOMARKERS

Probe Drugs

Due to the complexity of transporter-mediated drug disposition and DDIs, a rational clinical DDI study design is critical to establish whether an NME can act as an *in vivo* inhibitor of drug transporter(s). Regulatory DDI guidance documents have provided some

recommendations on the design of clinical DDI studies, which in general, can be applied to transporter DDIs.⁴⁻⁶ Additional practical considerations for designing clinical DDI studies using the probe drugs or cocktails highlighted in previous section are discussed below.

Subjects

Transporter DDI studies are generally conducted in healthy subjects, rather than patients, unless there are safety concerns. Extrapolation of DDI results from healthy subjects to a specific and/or diseased population requires prior knowledge on alternation of transporter expression and/or activity in addition to the changes of relevant physiological parameters. Limited knowledge is currently available in this emerging area to allow direct translation of clinical DDIs to these specific populations⁹¹. Carriers of certain genetic polymorphisms (e.g., *SLCO1B1* c.521T>C and *ABCG2* c.421C>A) will have a diminished response to transport inhibitors as previously described.^{15, 16, 89} In the context of this article, these variants are specifically relevant for pitavastatin for OATP1B1 (*SLCO1B1*) and rosuvastatin for BCRP (*ABCG2*).^{24, 97} Thus, it is recommended to genotype subjects for *SLCO1B1* and *ABCG2* prior to enrollment or at least collect such information after the trial to help data analysis and interpretation¹⁵, e.g., in case outliers are identified. Currently, genetic information may not be available in cocktail DDI studies that are conducted as a nested arm in a large clinical trial (e.g., secondary DDI endpoint in a phase I trial). However, if the DDI under investigation involves OATP1B1 and/or BCRP inhibition, the inclusion of the genetic analysis in study protocols would be helpful to interpret DDI results.

Dose and Dose Regimen

In general, the doses of probe and perpetrator drugs should be clinically relevant to appropriately characterize the DDIs. Based on this consideration, the maximum approved dose and shortest dosing interval should be used for transporter inhibitors. For probe drugs,

potential saturation of drug transporters at a given dose (e.g., intestinal P-gp) needs to be considered, as it may reduce the sensitivity of the measurements. Cocktail probes (at either microdose or therapeutic dose) need to be validated with both PK and clinical DDI studies to confirm the translation of the data to clinical DDIs and the lack of interaction between different probes. In some cases, the magnitude of DDIs at microdose level can be higher than those observed at therapeutic dose due to the saturation of transporters at therapeutic doses (especially relevant in the intestine).¹⁹ In such cases, microdose data could be considered a worst-case scenario for transporter DDIs and observed DDIs may not be directly translated to therapeutic dose for dosing adjustment.¹⁹ NMEs with complex inhibition/induction liabilities may require single-dose assessment of the inhibitory effects, and combined induction/inhibition effects following chronic administration (e.g., rifampin, an inhibitor of OATP1B and inducer of CYP3A/P-gp⁹⁸).

As with all DDI studies, the sequence of administration and the time interval between dosing of probe drug and perpetrator can impact the outcome. For example, when using the prodrug DE as an *in vivo* probe for intestinal P-gp, oral dosing of the P-gp inhibitor should precede DE administration to ensure maximum exposure considering rapid hydrolysis of DE to the non-P-gp substrate dabigatran.⁹⁹

In most cases, both perpetrator and probe drugs are orally administered which may reflect the clinical setting. However, use of both i.v. and oral dosing of probe drugs or perpetrators may provide additional mechanistic insight into the relative contributions of intestinal vs. hepatic transporters to the observed DDIs. For example, oral rosuvastatin is recommended to evaluate the DDIs resulting from the inhibition of both intestinal and hepatic BCRP, whereas i.v. rosuvastatin can be used to evaluate the contribution of hepatic BCRP.²⁴ Similarly, i.v. and oral rifampin have been used as a perpetrator to understand the selectivity of pitavastatin (OATP1B) and rosuvastatin (BCRP/OATP1B) as DDI probe drugs for hepatic and intestinal transport activity, respectively.²²

737 Study Endpoints

738 Changes in systemic exposure of probe drugs (AUC and $C_{\max}$ ratio with and without
739 inhibitor) are generally used to describe the extent of transporter DDIs. Besides these
740 parameters, CL_r of probe drugs should also be measured over the PK sampling period when
741 studying renal transporter DDIs. In such cases, urinary pH should also be measured to
742 understand potential variability of reabsorption for probes with PK_a values within urinary pH
743 range. PD endpoints are generally not included in DDI studies. However, metformin may
744 represent an example that including PD endpoints could help to interpret clinical DDI data and
745 enable rational dose adjustment.^{42, 43}

746

747 Endogenous Biomarkers

748 The design of a clinical study to explore changes in the level of a validated endogenous
749 biomarker after administration of an NME will benefit from a clear understanding of biomarker
750 clearance(s). Characterization of such kinetic parameters may require monitoring of the
751 disposition of an endogenous biomarker using radiolabeled or stable isotopes. The availability
752 of quantitative data on disposition mechanisms (metabolic and renal clearance, *in vitro*
753 transporter kinetics) and rates of synthesis allows the subsequent development of biomarker
754 PBPK models. Such models can be used to simulate the changes in exposure due to the
755 inhibition of transporter(s) and aid in the design of clinical investigations aimed at maximizing
756 the sensitivity of a given biomarker. In all cases, a crossover study design is preferred given the
757 expected variability in the exposure, rate of elimination, and rate of synthesis in humans. The
758 study design should take into consideration the appropriate time frame to collect the matched-
759 samples based on PBPK simulations conducted in the absence or presence of the test inhibitor.

760 The use of a standard cut-off value for the change in biomarker exposure may not be
761 possible with all candidate transporters due to variability in the magnitude of interactions.
762 Instead, a systematic increase in the exposure of the biomarker that corresponds to an increase
763 in the dose of the test inhibitor is recommended as a positive indication of an interaction with

the transporter of interest. Ideally, bioanalysis of the biomarker concentration in the biological matrices (e.g., urine, plasma, saliva) can be added onto typical first-in-human investigations aimed at verifying the safety and tolerability of NME that have been identified as transport inhibitors *in vitro* or in preclinical species. Such studies will provide a comprehensive dataset to verify the dose-dependent change in the exposure of the biomarker for the transporter of interest up to supra-therapeutic dose levels.

Altered transport activity due to genetic polymorphisms may complicate the ability to detect exposure changes of a drug probe and can affect biomarker concentrations at baseline. Therefore, understanding of this covariate on the systemic exposure of the biomarker and associated variability is important. Moreover, the extent of change above baseline during the course of treatment is likely to be lower in subjects with a reduced function allele than in wild type subjects. Prior knowledge on the genetic makeup of subjects participating in the trial would be beneficial for biomarkers of transporters with clinically relevant polymorphism, e.g., *SLCO1B1*, *ABCG2*, and *SLC22A1* (OCT1), to explain inter-individual variability.

To address multiple sources of inter-individual variability in biomarker baseline concentrations, a cross-over study design in a sufficiently large number of test subjects (based on power calculations) is required to demonstrate statistical significance. Ideally, the biomarker concentration would be monitored at the beginning of the study period and again after 5-7 days to allow for subject acclimation under the clinical trial conditions. Moreover, synchronization of study procedures ensures that potential diurnal fluctuations in the biomarker level in individuals are not drastically affected in the subjects undergoing the study. Subjects would subsequently receive the test inhibitor preferably following an 8-10 hours fast until steady-state exposure is reached. Test inhibitor should be administered in a consistent manner to all test subjects (e.g., oral administration followed by the consumption of 250 mL of water) and subjects would consume other meals in a standardized fashion to rule out any potential food effect on biomarker and inhibitor PK. In each period of the study, blood samples (or other matrices) will be collected post-dose, the number of samples and time of collection should closely match the PK of the test inhibitor to ensure capturing changes in the biomarker

level when the inhibitor is at $C_{\max}$ and C_{trough} levels. Additional treatment cycles where the dose of the inhibitor is escalated should be done in a consistent manner and additional sets of blood samples are collected at steady-state levels. Changes in the biomarker exposure that match the inhibitor dose would be assessed. Ideally changes in the biomarker exposure in individual subjects should correlate with the inhibitor dose as an indication that the examined biomarker is suitable to address the inhibition of the transport activity.

RECOMMENDATION AND PERSPECTIVES OF APPLYING PROBE DRUGS AND ENDOGENOUS BIOMARKERS TO STUDY TRANSPORTER INHIBITION

The utility of probe drugs and endogenous biomarkers to assess inhibition potential of NMEs on drug transporters are summarized in **Figure 5**. Monitoring plasma and/or urine exposure of endogenous biomarkers for various transporters could be incorporated in early human PK studies, such as phase I dose escalation studies. Most of the biomarkers reported thus far are not fully validated for their sensitivity, selectivity and specificity as endogenous probes of transporters of interest. Therefore, at present time (**Figure 5A**), *in vivo* biomarker data can only be used for designing DDI assessment strategy or for prioritizing the clinical DDI studies with probe substrate drugs (as individual or in a cocktail) or with the most relevant concomitant medications. The data need to be interpreted with the understanding that positive data may imply a potential DDI, but may not be specific for a particular transporter and may potentially be confounded by inhibition on the synthesis of the biomarker. Furthermore, lack of interaction between the NME and the biomarker does not ensure one did not occur and additional targeted evaluation of particular DDIs of concern using probe substrate drugs would be warranted.

Once selectivity and sensitivity of an endogenous biomarker have been validated (**Figure 5B**) in humans, it can be used for early assessment of transporter-based DDIs in single and multiple ascending dose phase I studies. We envisage that the use of a sensitive and selective

819 biomarker would potentially obviate the need to administer a probe substrate drug in a
820 dedicated DDI study, avoiding unnecessary drug exposure to participants and to reduce cost.
821 The *in vivo* biomarker data would also be useful to develop and optimize mechanistic PBPK
822 models for predicting unstudied scenarios for dosing recommendation or for informing
823 additional clinical studies. *In vivo* biomarker data together with clinical DDI results obtained
824 from individual and/or cocktail probe drugs and PBPK modeling can also provide a mechanistic
825 insight to predict complex DDIs involving inhibition of multiple transporters and enzymes. Such
826 an integrated approach will ensure understanding of the full dose response with respect to
827 transporter inhibition/induction liability of an NME as a perpetrator, while minimizing DDI
828 studies conducted in response to false positive predictions using *in vitro* data. It is worth noting
829 that continuing efforts to identify and validate new and existing biomarkers are critically
830 needed. Another targeted effort is to identify probe substrate drugs that are suitable for
831 cassette DDI assessment and exhibit high sensitivity to evaluate transporter inhibition, but with
832 minimal potential for mutual interactions between the components of the cocktail. Pre-
833 competitive efforts and dedicated consortia may be valuable in coordinating targeted efforts
834 and accelerating the incorporation of biomarkers and probe drug cocktails in drug
835 development.

836 In summary, multiple endogenous molecules whose exposure is modulated due to
837 interactions with drug transporters have been identified to support the clinical development of
838 safe and effective therapeutics. Ideally, suitable biomarkers would enable gauging the risk of
839 interactions with clinically relevant transporters. In reality, examples of promising candidate
840 biomarkers are currently only being identified and studied for interactions with several hepatic
841 (OATP1B) and renal transporters (OCT2/MATE1/2K, OAT1/3), but there is a considerable
842 increase in published information. There has been a clear absence of candidate biomarkers to
843 examine P-gp and BCRP inhibition, which is likely due to lack or limited involvement of gut P-gp,
844 and BCRP on *in vivo* disposition of endogenous substrates and the difficulty associated with
845 estimating the extent of individual contribution of gut, hepatic and renal P-gp and BCRP to the
846 systemic clearance of biomarkers. Continued measurement of candidate biomarkers in clinical
847 studies where transporter interactions are anticipated needs to become more widespread. This

is only possible if suitable language is included in early clinical study protocols to ensure adequate consent from the human subjects. This will ultimately enhance the confidence in the suitability of measuring changes in biomarker concentrations as an indicator of potential clinical transporter DDIs from pharmaceutical and regulatory perspectives.

DISCLAIMER

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FIGURE LEGENDS

Figure 1. Recommended probe drugs for clinical DDI studies to evaluate inhibitory effects of an NME on major drug transporters expressed in gut, liver and kidney and potential endogenous biomarkers for several hepatic and renal transporters.

The criteria for inclusion of probe drugs in this figure are discussed in the STATE-OF-THE ART IN CLINICAL PROBE DRUGS AND POTENTIAL ENDOGENOUS BIOMARKERS FOR CLINICAL TRANSPORTER DDI ASSESSMENT section. The endogenous substrates with clinical DDI evaluation of potent transporter inhibitor(s) have been included into this figure as potential endogenous biomarkers. Digoxin, dabigatran etexilate (DE), and fexofenadine are probe drugs for multidrug resistance protein MDR1 P-glycoprotein (P-gp, *ABCB1*), and sulfasalazine and rosuvastatin for breast cancer resistance protein (BCRP, *ABCG2*). Pitavastatin, atorvastatin, and rosuvastatin are probe drugs for the organic anion transporting polypeptides OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*); Coproporphyrin I (CPI) and CPIII, glycochenodeoxycholate-3-O-sulfate (GCDCA-S), conjugated and unconjugated bilirubin (CB and UCB) are potential endogenous biomarkers for OATP1B1 and -1B3. Hexadecanedioate (HDA) and tetradecanedioate (TDA) are potential biomarkers for OATP1B1 (they are not substrates for OATP1B3). Sumatriptan is a probe drug for organic cation transporter 1 (OCT1; *SLC22A1*). Metformin is a probe drug for organic cation transporter 2 (OCT2, *SLC22A2*) and multidrug and toxin extrusion protein MATE1 (*SLC47A1*) and MATE2K (*SLC47A2*); N1-methylnicotinamide (NMN) and creatinine are potential endogenous biomarkers of OCT2/MATE1/2K. Adefovir is a probe drug for organic anion transporter 1 (OAT1, *SLC22A6*); taurine is a potential endogenous biomarker for OAT1. Benzylpenicillin is a probe drugs of organic anion transporter 3 (OAT3; *SLC22A8*); 6 β -hydroxycortisol (6 β HC) and GCDCA-S are potential endogenous biomarkers of OAT3.

^a: Potential endogenous biomarkers with relatively higher selectivity and/or sensitivity for clinical evaluation based on current literature data.

^b: Substrates of OATP1B1, but not -1B3.

Green colored circles: uptake transporters; Purple colored circles: efflux transporters.

Figure 2. Mechanistic challenges when developing transporter endogenous biomarker models: OATP1B1/1B3 biomarkers as an example.

Figure 3. Comparison of endogenous biomarkers and probe drugs with different $t_{1/2}$ (A) and recommended method for interpreting systemic exposure changes in biomarkers with competitive inhibition of transporters (B-C).

A: Grey lines represent simulated inhibitor concentration and % of inhibition of the elimination pathway of a probe drug and biomarker. Red, green, and blue lines represent simulated concentration-time profiles of probe drugs and biomarkers in the absence (up to day 6) and presence (on and after day 7) of inhibitors, with colors represent different terminal $t_{1/2}$. B-C: See **Supplementary Materials** for details. $*C_{max}$ may be influenced by change in other parameters, such as distribution volume, and may not always represent change in clearance.

Figure 4: Workflow for the identification, characterization, and validation of transporter endogenous biomarkers.

^a. Recombinant system may be a cellular or a vesicular system that expresses high levels of the target transporter. Transport in the overexpressing system is compared with one in a control system with non-/ or low transporter function.

^b. One of the two data types should be a mammalian *in vivo* model with genetically reduced transport function.

^c. Testing of multiple inhibitors or doses may be required to provide a range of inhibition potency. If a selective inhibitor is not available, multiple inhibitors with differential overlapping selectivity may be required.

^d. Possible regulatory review process. For example, FDA guidance for drug development tools.¹⁰⁰

919

920 **Figure 5. Examples of current (A) and future (B) considerations regarding the potential utility**
921 **of endogenous biomarkers (pre-validated (A) and validated(B)) and probe drugs to assess**
922 **transporter-related inhibition in drug development.**

923 ^a. For transporters without identified biomarkers (e.g., gut transporters), consider to conduct
924 clinical DDI with single or cocktail drug probe(s) at clinically relevant perpetrator dose.

925

926 ^b. If drugs can potentially inhibit multiple transporters, consider design a drug probe cocktail
927 studies using *in vitro* and clinically validated probes. If no significant DDIs observed, dedicated
928 clinical DDI studies with single drug probe at conventional doses may not be needed; If
929 significant DDIs observed with selected transporters, dedicated clinical DDI studies with drug
930 probes at clinically relevant exposure (both perpetrator and probes) are needed.

References:

1. International Transporter, C., *et al.* Membrane transporters in drug development. *Nat Rev Drug Discov.* **9**, 215-236 (2010).
2. Tweedie, D., *et al.* 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 (2013).
3. Lee, S. C., Arya, V., Yang, X., Volpe, D. A., & Zhang, L. Evaluation of transporters in drug development: Current status and contemporary issues. *Adv Drug Deliv Rev.* **116**, 100-118 (2017).
4. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry. October 2017.
5. European Medicines Agency. Guideline on the investigation of drug interactions. London, UK: EMA; 2013.
6. Ministry of Health, Labour and Welfare, Japan. Guideline of drug interaction studies for drug development and appropriate provision of information. Tokyo, Japan: Ministry of Health; 2014.
7. Zamek-Gliszczynski, M. J., *et al.* ITC recommendations for transporter kinetic parameter estimation and translational modeling of transport-mediated PK and DDIs in humans. *Clin Pharmacol Ther.* **94**, 64-79 (2013).
8. Rekić, D., *et al.* Clinical Drug-Drug Interaction Evaluations to Inform Drug Use and Enable Drug Access. *J Pharm Sci.* **106**, 2214-2218 (2017).
9. Yoshida, K., *et al.* *In vitro-In vivo* Extrapolation of Metabolism- and Transporter-Mediated Drug-Drug Interactions-Overview of Basic Prediction Methods. *J Pharm Sci.* **106**, 2209-2213 (2017).
10. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). *In vitro* Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies Guidance for Industry. October 2017.
11. Brouwer, K. L., *et al.* *In vitro* methods to support transporter evaluation in drug discovery and development. *Clin Pharmacol Ther.* **94**, 95-112 (2013).
12. Chu, X., Chan, G. H., & Evers, R. Identification of Endogenous Biomarkers to Predict the Propensity of Drug Candidates to Cause Hepatic or Renal Transporter-Mediated Drug-Drug Interactions. *J Pharm Sci.* **106**, 2357-2367 (2017).
13. Rodrigues, A. D., Taskar, K. S., Kusuhara, H., & Sugiyama, Y. Endogenous Probes for Drug Transporters: Balancing Vision With Reality. *Clin Pharmacol Ther.* (2017).
14. Muller, F., Sharma, A., König, J., & Fromm, M. F. Biomarkers for In Vivo Assessment of Transporter Function. *Pharmacol Rev.* **70**, 246-277 (2018).
15. Yee, S. W., *et al.* Influence of Transporter Polymorphisms on Drug Disposition and Response: A Perspective from the International Transporter Consortium. *Clin Pharmacol Ther.* (2018).
16. Giacomini, K. M., *et al.* International Transporter Consortium commentary on clinically important transporter polymorphisms. *Clin Pharmacol Ther.* **94**, 23-26 (2013).
17. Ebner, T., Ishiguro, N., & Taub, M. E. The Use of Transporter Probe Drug Cocktails for the Assessment of Transporter-Based Drug-Drug Interactions in a Clinical Setting-Proposal of a Four Component Transporter Cocktail. *J Pharm Sci.* **104**, 3220-3228 (2015).
18. Stopfer, P., *et al.* Pharmacokinetic Evaluation of a Drug Transporter Cocktail Consisting of Digoxin, Furosemide, Metformin, and Rosuvastatin. *Clin Pharmacol Ther.* **100**, 259-267 (2016).
19. Prueksaritanont, T., *et al.* Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A. *Clin Pharmacol Ther.* **101**, 519-530 (2017).

20. Zhang, L.& Sparreboom, A. Predicting transporter-mediated drug interactions: Commentary on: "Pharmacokinetic evaluation of a drug transporter cocktail consisting of digoxin, furosemide, metformin and rosuvastatin" and "Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A". *Clin Pharmacol Ther.* **101**, 447-449 (2017).
21. Momper, J. D., Tsunoda, S. M.,&Ma, J. D. Evaluation of Proposed *In vivo* Probe Substrates and Inhibitors for Phenotyping Transporter Activity in Humans. *J Clin Pharmacol.* **56 Suppl 7**, S82-98 (2016).
22. Prueksaritanont, T., *et al.* Pitavastatin is a more sensitive and selective organic anion-transporting polypeptide 1B clinical probe than rosuvastatin. *Br J Clin Pharmacol.* **78**, 587-598 (2014).
23. Elsby, R., Hilgendorf, C.,&Fenner, K. Understanding the critical disposition pathways of statins to assess drug-drug interaction risk during drug development: it's not just about OATP1B1. *Clin Pharmacol Ther.* **92**, 584-598 (2012).
24. Lee, C. A., *et al.* Breast cancer resistance protein (ABCG2) in clinical pharmacokinetics and drug interactions: practical recommendations for clinical victim and perpetrator drug-drug interaction study design. *Drug Metab Dispos.* **43**, 490-509 (2015).
25. Pasanen, M. K., Neuvonen, M., Neuvonen, P. J.,&Niemi, M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. *Pharmacogenet Genomics.* **16**, 873-879 (2006).
26. Pasanen, M. K., Fredrikson, H., Neuvonen, P. J.,&Niemi, M. Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther.* **82**, 726-733 (2007).
27. Maeda, K., *et al.* Identification of the rate-determining process in the hepatic clearance of atorvastatin in a clinical cassette microdosing study. *Clin Pharmacol Ther.* **90**, 575-581 (2011).
28. van de Steeg, E., *et al.* Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. *J Clin Invest.* **122**, 519-528 (2012).
29. Zhang, W., *et al.* OATP1B1 polymorphism is a major determinant of serum bilirubin level but not associated with rifampicin-mediated bilirubin elevation. *Clin Exp Pharmacol Physiol.* **34**, 1240-1244 (2007).
30. van der Deure, W. M., *et al.* Organic anion transporter 1B1: an important factor in hepatic thyroid hormone and estrogen transport and metabolism. *Endocrinology.* **149**, 4695-4701 (2008).
31. Xiang, X., *et al.* Effect of SLCO1B1 polymorphism on the plasma concentrations of bile acids and bile acid synthesis marker in humans. *Pharmacogenet Genomics.* **19**, 447-457 (2009).
32. Johnson, A. D., *et al.* Genome-wide association meta-analysis for total serum bilirubin levels. *Human Molecular Genetics.* **18**, 2700-2710 (2009).
33. Yee, S. W., *et al.* Metabolomic and Genome-wide Association Studies Reveal Potential Endogenous Biomarkers for OATP1B1. *Clin Pharmacol Ther.* **100**, 524-536 (2016).
34. Takehara, I., *et al.* Investigation of Glycochenodeoxycholate Sulfate and Chenodeoxycholate Glucuronide as Surrogate Endogenous Probes for Drug Interaction Studies of OATP1B1 and OATP1B3 in Healthy Japanese Volunteers. *Pharm Res.* **34**, 1601-1614 (2017).
35. van de Steeg, E., *et al.* Organic anion transporting polypeptide 1a/1b-knockout mice provide insights into hepatic handling of bilirubin, bile acids, and drugs. *J Clin Invest.* **120**, 2942-2952 (2010).
36. Shen, H., *et al.* Coproporphyrins I and III as Functional Markers of OATP1B Activity: *In vitro* and *In vivo* Evaluation in Preclinical Species. *J Pharmacol Exp Ther.* **357**, 382-393 (2016).
37. Kunze, A., Ediage, E. N., Dillen, L., Monshouwer, M.,&Snoeys, J. Clinical Investigation of Coproporphyrins as Sensitive Biomarkers to Predict Mild to Strong OATP1B-Mediated Drug-Drug Interactions. *Clin Pharmacokinet.* (2018).

38. Lai, Y., *et al.* Coproporphyrins in Plasma and Urine Can Be Appropriate Clinical Biomarkers to Recapitulate Drug-Drug Interactions Mediated by Organic Anion Transporting Polypeptide Inhibition. *J Pharmacol Exp Ther.* **358**, 397-404 (2016).
39. Liu, L., *et al.* A Phase 1, Open-Label, Study to Evaluate the Effect of GDC-0810 on the Pharmacokinetics of Pravastatin in Healthy Female Subjects of Non-Child Bearing Potential. *Clin Pharmacol Ther.* **101**, S75 (2017).
40. Shen, H., *et al.* Comparative Evaluation of Plasma Bile Acids, Dehydroepiandrosterone Sulfate, Hexadecanedioate, and Tetradecanedioate with Coproporphyrins I and III as Markers of OATP Inhibition in Healthy Subjects. *Drug Metab Dispos.* **45**, 908-919 (2017).
41. Takehara, I., *et al.* Comparative Study of the Dose-Dependence of OATP1B Inhibition by Rifampicin Using Probe Drugs and Endogenous Substrates in Healthy Volunteers. *Pharm Res.* **35**, 138 (2018).
42. Zamek-Gliszczynski, M. J., Giacomini, K. M., & Zhang, L. Emerging Clinical Importance of Hepatic Organic Cation Transporter 1 (OCT1) in Drug Pharmacokinetics, Dynamics, Pharmacogenetic Variability, and Drug Interactions. *Clin Pharmacol Ther.* (2017).
43. Zamek-Gliszczynski, M. J., *et al.* ITC Commentary on Metformin Clinical Drug-Drug Interaction Study Design That Enables an Efficacy- and Safety-Based Dose Adjustment Decision. *Clin Pharmacol Ther.* (2018).
44. Zamek-Gliszczynski, M. J. Transporters in Drug Development: 2018 ITC Recommendations for Emerging Transporters of Clinical Importance. *Clin Pharmacol Ther (this issue)*.
45. Matthaei, J., *et al.* OCT1 mediates hepatic uptake of sumatriptan and loss-of-function OCT1 polymorphisms affect sumatriptan pharmacokinetics. *Clin Pharmacol Ther.* **99**, 633-641 (2016).
46. Tzvetkov, M. V., Saadatmand, A. R., Bokelmann, K., Meineke, I., Kaiser, R., & Brockmoller, J. Effects of OCT1 polymorphisms on the cellular uptake, plasma concentrations and efficacy of the 5-HT(3) antagonists tropisetron and ondansetron. *Pharmacogenomics J.* **12**, 22-29 (2012).
47. Tzvetkov, M. V., *et al.* Increased Systemic Exposure and Stronger Cardiovascular and Metabolic Adverse Reactions to Fenoterol in Individuals with Heritable OCT1 Deficiency. *Clin Pharmacol Ther.* (2017).
48. Tzvetkov, M. V., Saadatmand, A. R., Lotsch, J., Tegeder, I., Stingl, J. C., & Brockmoller, J. Genetically polymorphic OCT1: another piece in the puzzle of the variable pharmacokinetics and pharmacodynamics of the opioidergic drug tramadol. *Clin Pharmacol Ther.* **90**, 143-150 (2011).
49. Stamer, U. M., Musshoff, F., Stuber, F., Brockmoller, J., Steffens, M., & Tzvetkov, M. V. Loss-of-function polymorphisms in the organic cation transporter OCT1 are associated with reduced postoperative tramadol consumption. *Pain.* **157**, 2467-2475 (2016).
50. Tzvetkov, M. V. OCT1 pharmacogenetics in pain management: is a clinical application within reach? *Pharmacogenomics.* **18**, 1515-1523 (2017).
51. Chen, L., *et al.* OCT1 is a high-capacity thiamine transporter that regulates hepatic steatosis and is a target of metformin. *Proc Natl Acad Sci U S A.* **111**, 9983-9988 (2014).
52. Ashokkumar, B., Vaziri, N. D., & Said, H. M. Thiamin uptake by the human-derived renal epithelial (HEK-293) cells: cellular and molecular mechanisms. *American Journal of Physiology: Renal Physiology.* **291**, F796-805 (2006).
53. Kato, K., *et al.* Investigation of endogenous compounds for assessing the drug interactions in the urinary excretion involving multidrug and toxin extrusion proteins. *Pharm Res.* **31**, 136-147 (2014).
54. Kim, H. I., *et al.* Fine Mapping and Functional Analysis Reveal a Role of SLC22A1 in Acylcarnitine Transport. *American Journal of Human Genetics.* **101**, 489-502 (2017).
55. Suhre, K., *et al.* Human metabolic individuality in biomedical and pharmaceutical research. *Nature.* **477**, 54-60 (2011).

56. Maeda, K., *et al.* Inhibitory effects of p-aminohippurate and probenecid on the renal clearance of adefovir and benzylpenicillin as probe drugs for organic anion transporter (OAT) 1 and OAT3 in humans. *Eur J Pharm Sci.* **59**, 94-103 (2014).
57. Imaoka, T., Kusuhaara, H., Adachi, M., Schuetz, J. D., Takeuchi, K., & Sugiyama, Y. Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. *Mol Pharmacol.* **71**, 619-627 (2007).
58. Mathialagan, S., Piotrowski, M. A., Tess, D. A., Feng, B., Litchfield, J., & Varma, M. V. Quantitative Prediction of Human Renal Clearance and Drug-Drug Interactions of Organic Anion Transporter Substrates Using *In vitro* Transport Data: A Relative Activity Factor Approach. *Drug Metab Dispos.* **45**, 409-417 (2017).
59. Tsuruya, Y., *et al.* Investigation of Endogenous Compounds Applicable to Drug-Drug Interaction Studies Involving the Renal Organic Anion Transporters, OAT1 and OAT3, in Humans. *Drug Metab Dispos.* **44**, 1925-1933 (2016).
60. Jadavji, N. M., Emmerson, J. T., MacFarlane, A. J., Willmore, W. G., & Smith, P. D. B-vitamin and choline supplementation increases neuroplasticity and recovery after stroke. *Neurobiology of Disease.* **103**, 89-100 (2017).
61. Peng, C. C., Templeton, I., Thummel, K. E., Davis, C., Kunze, K. L., & Isoherranen, N. Evaluation of 6beta-hydroxycortisol, 6beta-hydroxycortisone, and a combination of the two as endogenous probes for inhibition of CYP3A4 *in vivo*. *Clin Pharmacol Ther.* **89**, 888-895 (2011).
62. Imamura, Y., *et al.* 6beta-Hydroxycortisol is an endogenous probe for evaluation of drug-drug interactions involving a multispecific renal organic anion transporter, OAT3/SLC22A8, in healthy subjects. *Drug Metab Dispos.* **42**, 685-694 (2014).
63. Shen, H., *et al.* Discovery and Validation of Pyridoxic Acid and Homovanillic Acid as Novel Endogenous Plasma Biomarkers of Organic Anion Transporter (OAT) 1 and OAT3 in Cynomolgus Monkeys. *Drug Metab Dispos.* **46**, 178-188 (2018).
64. Liang, X. & Giacomini, K. M. Transporters Involved in Metformin Pharmacokinetics and Treatment Response. *J Pharm Sci.* **106**, 2245-2250 (2017).
65. Oh, J., *et al.* Inhibition of the multidrug and toxin extrusion (MATE) transporter by pyrimethamine increases the plasma concentration of metformin but does not increase antihyperglycaemic activity in humans. *Diabetes Obes Metab.* **18**, 104-108 (2016).
66. Kusuhaara, H., *et al.* Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at oral microdose and at therapeutic dose in healthy subjects. *Clin Pharmacol Ther.* **89**, 837-844 (2011).
67. Gong, L., Goswami, S., Giacomini, K. M., Altman, R. B., & Klein, T. E. Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet Genomics.* **22**, 820-827 (2012).
68. Hibma, J. E., *et al.* The Effect of Famotidine, a MATE1-Selective Inhibitor, on the Pharmacokinetics and Pharmacodynamics of Metformin. *Clin Pharmacokinet.* **55**, 711-721 (2016).
69. Ito, S., *et al.* N-methylnicotinamide is an endogenous probe for evaluation of drug-drug interactions involving multidrug and toxin extrusions (MATE1 and MATE2-K). *Clin Pharmacol Ther.* **92**, 635-641 (2012).
70. Muller, F., *et al.* N(1)-methylnicotinamide as an endogenous probe for drug interactions by renal cation transporters: studies on the metformin-trimethoprim interaction. *Eur J Clin Pharmacol.* **71**, 85-94 (2015).
71. Bergagnini-Kolev, M. C., Hebert, M. F., Easterling, T. R., & Lin, Y. S. Pregnancy Increases the Renal Secretion of N(1)-methylnicotinamide, an Endogenous Probe for Renal Cation Transporters, in Patients Prescribed Metformin. *Drug Metab Dispos.* **45**, 325-329 (2017).

72. Weber, W., Toussaint, S., Looby, M., Nitz, M., & Kewitz, H. System analysis in multiple dose kinetics: evidence for saturable tubular reabsorption of the organic cation N1-methylnicotinamide in humans. *Journal of Pharmacokinetics and Biopharmaceutics*. **19**, 553-574 (1991).
73. Chu, X., Bleasby, K., Chan, G. H., Nunes, I., & Evers, R. The Complexities of Interpreting Reversible Elevated Serum Creatinine Levels in Drug Development: Does a Correlation with Inhibition of Renal Transporters Exist? *Drug Metab Dispos*. **44**, 1498-1509 (2016).
74. Nader, A. M. & Foster, D. R. Suitability of digoxin as a P-glycoprotein probe: implications of other transporters on sensitivity and specificity. *J Clin Pharmacol*. **54**, 3-13 (2014).
75. Lee, C. A., Kalvass, J. C., Galetin, A., & Zamek-Gliszczynski, M. J. ITC commentary on the prediction of digoxin clinical drug-drug interactions from *in vitro* transporter assays. *Clin Pharmacol Ther*. **96**, 298-301 (2014).
76. Cvetkovic, M., Leake, B., Fromm, M. F., Wilkinson, G. R., & Kim, R. B. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos*. **27**, 866-871 (1999).
77. Shirasaka, Y., *et al.* Concentration-dependent effect of naringin on intestinal absorption of beta(1)-adrenoceptor antagonist talinolol mediated by p-glycoprotein and organic anion transporting polypeptide (Oatp). *Pharm Res*. **26**, 560-567 (2009).
78. Hartter, S., *et al.* Decrease in the oral bioavailability of dabigatran etexilate after co-medication with rifampicin. *Br J Clin Pharmacol*. **74**, 490-500 (2012).
79. Chu, X., *et al.* Dabigatran Etexilate and Digoxin: a Critical Comparison as Clinical Probes for P-gp Inhibition Evaluation. *Clin Pharmacol Ther* (this issue).
80. Kusuvara, H., *et al.* Pharmacokinetic interaction study of sulphasalazine in healthy subjects and the impact of curcumin as an *in vivo* inhibitor of BCRP. *Br J Pharmacol*. **166**, 1793-1803 (2012).
81. Zhou, H., Tong, Z., & McLeod, J. F. "Cocktail" approaches and strategies in drug development: valuable tool or flawed science? *J Clin Pharmacol*. **44**, 120-134 (2004).
82. Lenuzza, N., *et al.* Safety and pharmacokinetics of the CIME combination of drugs and their metabolites after a single oral dosing in healthy volunteers. *Eur J Drug Metab Pharmacokinet*. **41**, 125-138 (2016).
83. Kashiwara, Y., *et al.* Small-Dosing Clinical Study: Pharmacokinetic, Pharmacogenomic (SLCO2B1 and ABCG2), and Interaction (Atorvastatin and Grapefruit Juice) Profiles of 5 Probes for OATP2B1 and BCRP. *J Pharm Sci*. **106**, 2688-2694 (2017).
84. Stopfer, P., *et al.* Effects of Metformin and Furosemide on Rosuvastatin Pharmacokinetics in Healthy Volunteers: Implications for Their Use as Probe Drugs in a Transporter Cocktail. *Eur J Drug Metab Pharmacokinet*. (2017).
85. Stopfer, P., *et al.* Optimization of a drug transporter probe cocktail: potential screening tool for transporter-mediated drug-drug interactions. *Br J Clin Pharmacol*. (2018).
86. Watanabe, T., Kusuvara, H., Maeda, K., Shitara, Y., & Sugiyama, Y. Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. *J Pharmacol Exp Ther*. **328**, 652-662 (2009).
87. Langer, O. Use of PET Imaging to Evaluate Transporter-Mediated Drug-Drug Interactions. *J Clin Pharmacol*. **56 Suppl 7**, S143-156 (2016).
88. Guo, & *et al.* Advancing Predictions of Tissue and Intracellular Drug Concentrations Using *In vitro*, Imaging and PBPK Modeling Approaches. *Clin Pharmacol Ther* (this issue).
89. Chu, X., *et al.* Intracellular drug concentrations and transporters: measurement, modeling, and implications for the liver. *Clin Pharmacol Ther*. **94**, 126-141 (2013).
90. Miners, J. O., Yang, X., Knights, K. M., & Zhang, L. The Role of the Kidney in Drug Elimination: Transport, Metabolism, and the Impact of Kidney Disease on Drug Clearance. *Clin Pharmacol Ther*. **102**, 436-449 (2017).

91. Evers, R., *et al.* Disease-Associated Changes in Drug Transporters May Impact the Pharmacokinetics and/or Toxicity of Drugs: A White Paper from the International Transporter Consortium. *Clin Pharmacol Ther.* (2018).
92. Barnett, S., *et al.* Gaining Mechanistic Insight Into Coproporphyrin I as Endogenous Biomarker for OATP1B-Mediated Drug-Drug Interactions Using Population Pharmacokinetic Modeling and Simulation. *Clin Pharmacol Ther.* (2017).
93. Agarwal, R., Hynson, J. E., Hecht, T. J., Light, R. P., & Sinha, A. D. Short-term vitamin D receptor activation increases serum creatinine due to increased production with no effect on the glomerular filtration rate. *Kidney Int.* **80**, 1073-1079 (2011).
94. Weir, M. R. Short-term effects of vitamin D receptor activation on serum creatinine, creatinine generation, and glomerular filtration. *Kidney Int.* **80**, 1016-1017 (2011).
95. Wolfe, R. R. & Chinkes, D. L. *Isotope tracers in metabolic research: principles and practice of kinetic analysis* (John Wiley & Sons: 2005).
96. Koskelo, P. & Kekki, M. Multicompartment analysis of ¹⁴C-labelled coproporphyrin and uroporphyrin kinetics in human beings. *Annals of Clinical Research.* **8 Suppl 17**, 198-202 (1976).
97. Tsamandouras, N., *et al.* Identification of the effect of multiple polymorphisms on the pharmacokinetics of simvastatin and simvastatin acid using a population-modeling approach. *Clin Pharmacol Ther.* **96**, 90-100 (2014).
98. Reitman, M. L., *et al.* Rifampin's acute inhibitory and chronic inductive drug interactions: experimental and model-based approaches to drug-drug interaction trial design. *Clin Pharmacol Ther.* **89**, 234-242 (2011).
99. Hartter, S., Sennewald, R., Nehmiz, G., & Reilly, P. Oral bioavailability of dabigatran etexilate (Pradaxa((R))) after co-medication with verapamil in healthy subjects. *Br J Clin Pharmacol.* **75**, 1053-1062 (2013).
100. Guidance for Industry and FDA Staff on the Qualification Process for Drug Development Tools, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) January 2014.
101. Chen, Y., *et al.* Effect of a single-dose rifampin on the pharmacokinetics of pitavastatin in healthy volunteers. *Eur J Clin Pharmacol.* **69**, 1933-1938 (2013).
102. Kim, S. J., *et al.* Clarification of the Mechanism of Clopidogrel-Mediated Drug-Drug Interaction in a Clinical Cassette Small-dose Study and Its Prediction Based on *In vitro* Information. *Drug Metab Dispos.* **44**, 1622-1632 (2016).
103. Wu, H. F., *et al.* Rosuvastatin Pharmacokinetics in Asian and White Subjects Wild Type for Both OATP1B1 and BCRP Under Control and Inhibited Conditions. *J Pharm Sci.* **106**, 2751-2757 (2017).
104. Lau, Y. Y., Huang, Y., Frassetto, L., & Benet, L. Z. effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. *Clin Pharmacol Ther.* **81**, 194-204 (2007).
105. He, Y. J., *et al.* Rifampicin alters atorvastatin plasma concentration on the basis of SLCO1B1 521T>C polymorphism. *Clinica Chimica Acta.* **405**, 49-52 (2009).
106. Cheng, Z., *et al.* Hydrophilic anti-migraine triptans are substrates for OATP1A2, a transporter expressed at human blood-brain barrier. *Xenobiotica.* **42**, 880-890 (2012).
107. Tfelt-Hansen, P. & Hougaard, A. Sumatriptan: a review of its pharmacokinetics, pharmacodynamics and efficacy in the acute treatment of migraine. *Expert Opin Drug Metab Toxicol.* **9**, 91-103 (2013).
108. Prescribing Information of IMITREX.
109. Jalava, K. M., Partanen, J., & Neuvonen, P. J. Itraconazole decreases renal clearance of digoxin. *Ther Drug Monit.* **19**, 609-613 (1997).
110. Vermeer, L. M., Isringhausen, C. D., Ogilvie, B. W., & Buckley, D. B. Evaluation of Ketoconazole and Its Alternative Clinical CYP3A4/5 Inhibitors as Inhibitors of Drug Transporters: The *In vitro* Effects

of Ketoconazole, Ritonavir, Clarithromycin, and Itraconazole on 13 Clinically-Relevant Drug Transporters. *Drug Metab Dispos.* **44**, 453-459 (2016).

- **Supplementary Section 1**
- **Table S1.**
- **Table S2.**
- **Table S3.**
- **Supplementary Materials**
- **Figure S1.**
- **Figure S2.**

Table 1. Ideal features of transported probe drugs or biomarkers for the evaluation of clinical DDIs.

- **Transport.** Active transport and transport kinetics of the clinical probe or biomarker are demonstrated using suitable *in vitro* assays. Active transport significantly contributes to the *in vitro* and *in vivo* clearance
- **Selectivity and Specificity.** The contribution of the transporter of interest to the overall clearance of the candidate clinical probe or biomarker is clearly established kinetically. Transport represents the rate-limiting step to overall systemic clearance despite the presence of downstream disposition pathways (e.g., metabolism).
- **Sensitivity.** Active transport significantly contributes to the *in vitro* or *in vivo* clearance and can be demonstrated clinically from known genetic polymorphisms or DDI studies that affect the transport activity. The extent of change in exposure due to inhibition correlates with the potency of co-administered inhibitors and can be measured with high sensitivity and accuracy using robust validated bioanalytical methods.
- ***In vivo* PK.** Detailed characterization of the PK parameters including various disposition (and formation) pathways. The PK profile should provide sufficient flexibility to allow demonstration of changes in exposure preferably following common early clinical study protocols
- **Additional desirable features**
 - a. **Predictability with modeling.** The magnitude of change in PK profiles is predictable using PBPK models.
 - b. **Regulatory acceptance.** The clinical probe and biomarker are accepted by regulatory agencies in support of *in vitro* data generation, IVIVE exercises, definitive clinical DDI assessment, and product labeling.
 - c. **Administration to subjects.** Clinical drug probe can be administered using multiple routes and in combination with other agents as a cocktail. Changes in clinical drug PK corresponds to those of candidate biomarkers that utilize the same transport mechanism
 - d. Candidate biomarker concentrations are not significantly altered due to physiological or pathophysiological changes when compared to those when transport activity is impaired
 - e. Low likelihood of interference with rates of synthesis or catabolism of candidate biomarkers due to transporter inhibition

Table 2: Examples of probe drugs for OATP1B, OCT1, P-gp, BCRP, OAT1, OAT3, OCT2, MATE1, and MATE2K for clinical DDI evaluation.

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K _m) ^a	Key human ADME property and C _{max} and C _{maxu} at therapeutic dose ^b	Clinical DDI with known inhibitors			References
			Inhibitor (dose regimen), C _{max} , C _{maxu} , I ₂ , and/or I _{inmaxu}	PK Change (inhibitor/control)	Inhibitor selectivity (IC ₅₀ or K _i) ^a	
OATP1B1/OATP1B3						
Pitavastatin (HMG-CoA reductase inhibitor)	<ul style="list-style-type: none">• OATP1B1: 0.81-5.53 μM• OATP1B3: 2.6-3.25μM• OATP2B1: 1.17-25 μM• OATP1A2: substrate• NTCP: 37.3-38.2 μM• P-gp: 83.7 μM• BCRP: 1.2-5.73 μM• MRP3: 448 μM• MRP4: substrate	<ul style="list-style-type: none">• F: 51%• PPB: 99.5-99.6%• V_d: 148L• Metabolism: CYP2C9 (marginally), CYP2C8 (lesser), and UGT1A3 and UGT2B7 (formed major metabolites in human plasma)• Elimination: renal (15%); feces (79%)• t_{1/2}: 12 hrs• C_{max}: 29.6 nM; C_{maxu}: 0.13 nM (2mg oral SD)	Rifampin (iv or po 600mg SD) PO: <ul style="list-style-type: none">• C_{max}: 12.2-29.8 μM• C_{maxu}: 3.04-7.38 μM• I₂: 2916 μM• I_{inmaxu}: 15.2-19.5 μM IV: <ul style="list-style-type: none">• C_{max}: 24.3 μM• C_{maxu}: 6.08 μM	IV Rifampin: <ul style="list-style-type: none">• AUCR: 7.55• C_{max}R: 6.00 PO Rifampin: <ul style="list-style-type: none">• AUCR: 4.22-6.4• C_{max}R: 4.08-9.2	<ul style="list-style-type: none">• OATP1B1: 0.28-120 μM• OATP1B3: 0.22-190 μM• OATP2B1: 0.48-91 μM• OATP1A2: 51 μM• OAT1: 47.8-212 μM• OAT3: 30.2-33 μM• BCRP: 14-56 μM• P-gp: 4.3-175 μM• BSEP: 10.5-204 μM• MDR3: 139 μM• MRP1: 32 μM• MRP2: 7.9-83 μM• MRP3: 97 μM• MRP4: 88 μM• NTCP: 277 μM	22, 101, 102

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K_m) ^a	Key human ADME property and C_{max} and C_{maxu}	Clinical DDI with known inhibitors			References
Rosuvastatin (HMG-CoA reductase inhibitor)	<ul style="list-style-type: none"> • OATP1B1: 0.8-19.87 μM • OATP1B3: 9.8-16.5 μM • OATP2B1: 2.4-26.1 μM • OATP1A2: 2.6-60.2 μM • OAT3: 7.4 μM • BCRP: 2.02-10.8 μM • NTCP: 26.2-97 μM • P-gp: substrate • MRP4: substrate 	<ul style="list-style-type: none"> • F: 20% • PPB: 88% • V_d: 134L • Metabolism: not extensively metabolized (~10% as metabolite). CYP2C9 (major) • Elimination: renal (28%); feces (72%) • $t_{1/2}$: 19 hrs • C_{max}: 4.6 nM; C_{maxu} 0.55nM (10 mg oral QD for 10 days) 	Rifampin (iv or po 600mg SD) PO: <ul style="list-style-type: none"> • C_{max}: 12.2-30.6 μM • C_{maxu}: 3.04-7.65 μM • I_2: 2916 μM • I_{inmaxu}: 15.2-19.8 μM IV: <ul style="list-style-type: none"> • C_{max}: 24.3 μM • C_{maxu}: 6.08 μM 	IV Rifampin: <ul style="list-style-type: none"> • AUCR: 3.21-3.37 • $C_{max}R$: 5.51-8.55 PO Rifampin: <ul style="list-style-type: none"> • AUCR: 4.37-4.8 • $C_{max}R$: 9.93-13.2 		19, 22, 38, 103
Atorvastatin (HMG-CoA reductase inhibitor)	<ul style="list-style-type: none"> • OATP1B1: 0.62-18.8 μM • OATP1B3: 0.73 μM • OATP2B1: 2.84 μM • NTCP: 40.3-185 μM • P-gp: 110 μM • BCRP: substrate 	<ul style="list-style-type: none"> • F: 14% • PPB: $\geq 98\%$ • V_d: 381L • Metabolism: extensively metabolized. CYP3A4 (major) • Elimination: primarily into bile (both parent and metabolites); renal (<2%); $t_{1/2}$: 19.5 hrs • C_{max}: 12.3 nM; 	Rifampin (iv or po 600mg SD) <ul style="list-style-type: none"> • C_{max}: 9.35-22.6 μM • C_{maxu}: 1.41-5.65 μM • I_2: 2916 μM • I_{inmaxu}: 8.70-17.8 μM IV: <ul style="list-style-type: none"> • C_{max}: 17.1 μM • C_{maxu}: 4.28 μM 	IV Rifampin: <ul style="list-style-type: none"> • AUCR: 7.82 • $C_{max}R$: 10.5 PO Rifampin: <ul style="list-style-type: none"> • AUCR: 8.51-12 • $C_{max}R$: 14.9-21.1 		19, 27, 104, 105

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K_m) ^a	Key human ADME property and C_{max} and C_{min} at	Clinical DDI with known inhibitors			References
		C_{max} : 0.25 nM (20 mg oral QD for 14 days)				
OCT1						
Sumatriptan (5- hydroxytryptamine receptor agonist for migraine treatment)	<ul style="list-style-type: none"> • OCT1: 55.4 μM • OATP1A2: 94.5 μM 	<ul style="list-style-type: none"> • F: 15% • PPB: 14-21% • V_d: 2.4 L/kg • Metabolism: extensively metabolized by monoamine oxidase type A (MAOA) • Elimination: primarily in urine 60% as the major metabolite IAA (3% unchanged); fecal (~40%); • $t_{1/2}$: 2.5 hrs 	<p>No clinical DDI studies are available with OCT1 inhibitors</p> <p>PGx study compared OCT1*1-*2 (active) to OCT1*3-*6 (deficient)</p>	<ul style="list-style-type: none"> • AUCR: 2.2 (higher in subjects with two deficient alleles relative to one or more active alleles) • $C_{max}R$: 1.8 (higher in subjects with two deficient alleles relative to one or more active alleles) 	NA	45, 106-108

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K _m) ^a	Key human ADME property and C _{max} and C _{min} ^{at}	Clinical DDI with known inhibitors			References
P-gp						
Digoxin (Cardiac/ digitalis glycosides)	• P-gp: 73-220 μM • OATP4C1: 7.8 μM	• F: 60-80% • PPB: 25% • V _d : 3.12 L/kg • Metabolism: minimal (16% of dose by non- CYP metabolism) • Elimination: renal (50-70% as unchanged drug, after IV dose) • t _{1/2} : 39 hrs • C _{max} : 0.97-2.1 nM ; C _{maxu} : 0.73-2.58 nM (0.25mg oral SD)	Itraconazole (oral 200 mg QD x 5 days) • C _{max} : 746.8 nM; • C _{maxu} : 1.49 nM • I ₂ : 1133.7 μM	• AUCR: 1.68 • C _{max} R: 1.34	• P-gp: 0.05-9.5 μM • BCRP: 1.9-10 μM • OATP2B1: 2.5 μM • OCT1: 0.74 μM • MRP3: 2.1 μM • BSEP: 1.8-3 μM	109, 110, g
			Verapamil (oral 80 mg TID x 2-4 weeks or 120 mg TID x 2 weeks) • C _{avg} : 73-132 nM • C _{avg,u} : 7.3-13.2 nM • I ₂ : 704-1056 μM	• AUCR: 1.5 • C _{max} R: 1.44- 1.77 • CL _{renal} R: 1.04- 1.13 • CL _{biliary} R: 0.54 •	• P-gp: 0.1-234 μM • BCRP: 3.65 μM • OATP1A2: 2.6 μM • OATP1B1: 14.8-84.3 μM • OATP1B3: 87.3-89.5 μM • OATP4C1: 110 μM • OCT1: 0.09-59 μM • OCT2: 7.4-91.9 μM • MATE1: 27.5-41.9 μM • MATE2K: 32.1-37.9 μM • MDR3: 7 μM • MRP3: 26.3 μM • BSEP: 20.5 μM	g
Fexofenadine (Histamine H1- receptor antagonist)	• P-gp: 25.9-150 μM • OATP1B1: 61.6 μM • OATP1B3: 108 μM • OATP2B1: 0.14	• F: NR • PPB: 60-70% • Metabolism: minimal (5% of dose by hepatic metabolism) • Elimination:	Itraconazole (oral 50/100/200 mg SD, or 200 mg QD x 6 days, or 100 mg BID x 5 days) • C _{max} : 25.5-483.8 nM • C _{maxu} : 0.05-0.97 nM	• AUCR: 2.09- 2.53 • C _{max} R: 1.78- 2.69 • CL _r R: NS	See above	g

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K_m) ^a	Key human ADME property and C_{max} and $C_{max,u}$	Clinical DDI with known inhibitors			References
	μM • OAT3: 70.2 μM • BSEP: substrate • MATE1: substrate • MRP3: substrate	renal (11%); feces (80%) • $t_{1/2}$: 14 hrs • C_{max} : 531 nM; $C_{max,u}$: 159-212 nM (oral 60mg BID)	• I_2 : 283-1134 μM • $I_{inmax, u}$: 9.5-38.8 nM			
			Verapamil (oral 80 mg TID x 6 days, 240 mg QD x 1 day) • C_{max} : S- verapamil: 46.2 nM; R- verapamil: 154 nM (240mg QD) • $C_{max,u}$: S- verapamil: 4.62 nM; R-verapamil 15.4 nM) (240 mg QD) • I_2 : 703.9-2112 μM • $I_{inmax,u}$: 3.52 μM	• AUCR: 1.8- 2.52 • $C_{max}R$: 1.5- 2.92 • CL_{tR} : NS	See above	^g
Dabigatran etexilate (DE) (Anticoagulant)	P-gp: 1 μM (predicted)	Dabigatran ^c : • F: 3-7% • PPB: 35% • V_d : 50-70 L • Metabolism: carboxylesterase catalyzed hydrolysis to dabigatran, and,	Itraconazole (oral 200 mg QD x 5 days) • C_{max} : 1.9 μM • $C_{max,u}$: 0.068 μM • I_2 : 1133.7 μM • $I_{inmax,u}$: 0.75 μM	• AUCR ^d : 6.92 • $C_{max}R^d$: 6.42	See above	^{19, g}

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K_m) ^a	Key human ADME property and C_{max} and C_{maxu} then form acyl glucuronides by conjugation	Clinical DDI with known inhibitors			References
			Verapamil (oral 120 mg IR SD or oral 240 mg ER SD)	<ul style="list-style-type: none"> AUCR: 1.71-2.43 $C_{max}R$: 1.91-2.79 	See above	
		<ul style="list-style-type: none"> Elimination: (after IV dose): renal (85%); (after PO dose): urine (7%) and feces (86%) $t_{1/2}$: 12-17 hrs C_{max}: 174 nM; C_{maxu}: 113 nM (100mg DE oral SD) C_{max}: 341 nM; C_{maxu}: 222 nM (200mg DE oral SD) 	<ul style="list-style-type: none"> C_{max}: 205.5 -275 nM C_{maxu}: 20.5 -27.5 nM I_2: 1056 -2112 μM 			99
BCRP						
Rosuvastatin (HMG Co-A reductase inhibitor)	See above	See above	Fostamatinib ^e (oral 100 mg BID, x 9 days) <ul style="list-style-type: none"> C_{max} (active moiety R 406) : 1.6 μM C_{maxu} (active moiety R 406): 0.029 μM I_2 (fostamatinib): 691 μM I_{inmaxu} (R406): 	<ul style="list-style-type: none"> AUCR: 1.96 $C_{max}R$: 1.88 	Fostamatinib: <ul style="list-style-type: none"> BCRP: 0.05 μM P-gp: 3.2 μM active moiety R406: <ul style="list-style-type: none"> BCRP: 0.031 μM OATP1B1: >10 μM OAT3: NI 	^g

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K_m) ^a	Key human ADME property and C_{max} and C_{min}	Clinical DDI with known inhibitors			References
			0.289 μ M			
			Rifampin (oral 600mg SD IV and PO) PO: <ul style="list-style-type: none"> C_{max}: 12.2 μM C_{maxu}: 3.04 μM I_2: 2916 μM I_{inmaxu}: 15.2 μM IV: <ul style="list-style-type: none"> C_{max}: 24.3 μM C_{maxu}: 6.08 μM 	PO Rifampin: <ul style="list-style-type: none"> AUCR: 4.37 C_{maxR}: 9.93 IV Rifampin: <ul style="list-style-type: none"> AUCR: 3.30 C_{maxR}: 5.51 	See above	²²

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K _m) ^a	Key human ADME property and C _{max} and C _{max,u}	Clinical DDI with known inhibitors			References
Sulfasalazine (Salicylate- sulfonamide)	<ul style="list-style-type: none">• BCRP: 0.7 μM• OATP2B1: 1.73 μM• MRP2: substrate• P-gp: substrate	<ul style="list-style-type: none">• F: 3-12%• PPB: >99.3%• V_d: 0.11 L/kg• Metabolism: metabolized by intestinal bacteria to 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP) in the intestine, and in liver to these moieties by some extent.• Elimination: Absorbed SP and 5-ASA and their metabolites are primarily eliminated into urine; CL_r: 37% of CL.• t_{1/2}: 7.6 hrs• C_{max}: 37.6-79 μM; C_{max,u}: 0.26-0.55 μM (3-4 g oral SD)	Curcumin (oral 2 g, SD) C _{avg} : < 0.5 ng/mL (lower limit of quantification)	Sulfasalazine at therapeutic dose (2g) <ul style="list-style-type: none">• AUCR: 3.23• C_{max}R: 3.72 Sulfasalazine at microdose (100 μg) <ul style="list-style-type: none">• AUCR: 1.83C_{max}R: 1.96	<ul style="list-style-type: none">• BCRP: 0.62-32 μM• MRP1: 16.1 μM• OATP1B1: 33.7μM• OATP1B3: 3.81 μM• P-gp: 5.8-50.5 μM	80

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K _m) ^a	Key human ADME property and C _{max} and C _{max,u}	Clinical DDI with known inhibitors			References
OAT1/OAT3						
Adefovir ^f (Antivirals)	<ul style="list-style-type: none">• OAT1: 11.4-30 μM• MRP4: substrate	<ul style="list-style-type: none">• F: 59 %.• PPB: ≤ 4 %• V_{dss}: 352-392ml/kg• Metabolism: adefovir dipivoxil is rapidly converted to adefovir• Elimination: renal (45% in 24 hrs as adefovir); active secretion and glomerular filtration• t_{1/2}: 7.48 hrs• C_{max}: 0.067 μM; C_{max,u}: 0.065 μM (10 mg adefovir dipivoxil QD)	Probenecid (oral, 750mg, SD) <ul style="list-style-type: none">• C_{max}: 189 μM• C_{max,u}: 18.9 μM	<ul style="list-style-type: none">• AUCR: 2.09• GFR ratio: 0.97• CL_{rR}: 0.44	<ul style="list-style-type: none">• OAT1: 3.6-27.4 μM• OAT3: 0.76-29.8 μM• MRP2: 35.7-2300 μM• MRP4: 8-77% inhibition at 100 μM• OAT2: 393-853 μM• OAT4: 15.5-134 μM• OATP1B1: 39.8-740 μM• OATP1B3: 130 μM• URAT1: 13.2-50 μM	^{56, g}

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K_m) ^a	Key human ADME property and C_{max} and C_{maxu}	Clinical DDI with known inhibitors			References
Furosemide (Diuretics)	<ul style="list-style-type: none"> • OAT1: 38.9 μM • OAT3: 21.5 μM • MRP4: substrate • MRP2: substrate • BCRP: substrate • OATP1B1: substrate • OATP1B3: substrate 	<ul style="list-style-type: none"> • F: 71% • PPB: 98.6% • V_d: 0.13 L/kg • Metabolism: glucuronidation, 12% of oral dose • Elimination: renal (65%) • $t_{1/2}$: 1.3 hrs • C_{max}: 5.14 μM; C_{maxu}: 0.07 μM (40mg oral SD) 	Probenecid (oral, 1g, SD)	<ul style="list-style-type: none"> • AUCR: 2.68 • CL_{mr}R: 0.41 • CL_rR: 0.34 	See above	17, g
Benzylpenicillin (Penicillin G) (antibiotics)	<ul style="list-style-type: none"> • OAT3: 13.9-66 μM • OAT1: 884 μM, or not a substrate • OATP1B1: substrate • OATP1B3: substrate • 	<ul style="list-style-type: none"> • F%: 15-30% (fasting) • PPB: 45-68% • V_d: 0.53-0.67 L/kg • Metabolism: 16-30% intramuscular dose is metabolized to penicilloic acid • Elimination: renal • $t_{1/2}$: 0.4-0.6 hr • C_{max} and C_{maxu}, NR 	Probenecid (oral, 1500mg, SD)	<ul style="list-style-type: none"> • AUCR: 3.27 • CL_rR: 0.22 	See Above	56, g

Probe drugs (therapeutic class)	<i>In vitro</i> transport profile/kinetic parameters (K_m) ^a	Key human ADME property and C_{max} and C_{maxu}	Clinical DDI with known inhibitors			References
OCT2/MATE1/2K						
Metformin (biguanide, glucose lowering agent)	<ul style="list-style-type: none"> • OCT2: 235-3356 μM • OCT1: 83.6-6360 μM • MATE1: 94.7-4565 μM • MATE2K: 473-2986 μM • • OCT3: 1020-3810 μM • PMAT: 1320 μM 	<ul style="list-style-type: none"> • F: 52% • PPB: negligible • V_d: 1.12L/kg • Metabolism: negligible • Elimination: renal (~90% of the absorbed drug within the first 24 hrs) • $t_{1/2}$: 1.74 hrs • C_{max}: 9.7 μM; C_{maxu}: 9.7 μM (0.5g oral SD) 	Cimetidine (oral, 400mg, bid)	<ul style="list-style-type: none"> • AUCR: 1.46-1.54 • CL_{rR}: 0.55-0.72 	<ul style="list-style-type: none"> • OCT2: 2.51-1650 μM • MATE1: 0.093-16.3 μM • MATE2K: 2.1-46.6 μM • OCT1: 1.4-1010 μM • OCT3: 9.8-111 μM • OAT3: 0.07-215 μM • OAT2: 22-102.3 μM 	^g
			Pyrimethamine (oral, 50mg, SD)	<ul style="list-style-type: none"> • AUCR: 1.35-2.68 • CL_{rR}: 0.28-0.65 	<ul style="list-style-type: none"> • MATE1: 0.04-0.92 μM • MATE2K: 0.046-1.5 μM • OCT2: 0.93-160 μM • OCT1: 1.8-8.5 μM • OAT3: 32 μM • P-gp: 37 μM 	^{65, 66}
			Dolutegravir (oral, 50mg QD and BID)	<ul style="list-style-type: none"> • AUCR: 1.79-2.45 • 	<ul style="list-style-type: none"> • OCT2: 0.066-8.25 μM • MATE1: 4.67-6.34 μM • MATE2K: 9.3-49.3 μM; 	^g

ADME, Absorption, distribution, metabolism, and excretion; AUC, area under the concentration-time curve; AUCR, ratio of AUC; BCRP, breast cancer resistance protein; CYP, cytochrome P450; C_{max} , maximum concentration in plasma; $C_{max}R$, ratio of C_{max} ; C_{avg} , average plasma concentration; CL_r , renal clearance; CL_rR , ratio of CL_r ; $CL_{nr}R$, ratio of non-renal clearance; CL/F , apparent clearance after oral dose; DE, dabigatran etexilate; ER, extended released; F, bioavailability; IC_{50} , half maximal inhibitory concentration; IR, immediate released; K_m , Michaelis constant; K_i , inhibition rate constant; MDR, multidrug resistance; MRP, multidrug resistance protein; MATE, multidrug and toxin extrusion protein; NTCP, sodium taurocholate co-transporting polypeptide; NA, not applicable; NI, not inhibited; NR, not reported; NS, not significant; OATP, organic anion transporting polypeptide; OAT, organic anion transporter; OCT, organic cation transporter; P-gp, P-glycoprotein; PK, pharmacokinetics; PPB, plasma protein binding; $t_{1/2}$, half-life; THTR, thiamine transporter; V_d , volume distribution; V_{dss} , volume distribution at the steady state.

I_2 is gut concentration and calculated as $\text{dose}/250 \text{ mL}$ and I_{inmaxu} is unbound maximal inlet concentration and is calculated as $f_{u,p} * (I_{max} + (FaFg \times ka \times \text{Dose}))/Qh/RB$ where $f_{u,p}$ is unbound fraction in plasma, Fa is the fraction absorbed, Fg is the intestinal availability, ka is the absorption rate constant, Qh is the hepatic blood flow rate and RB is the blood-to-plasma concentration ratio.

^a: Data are obtained from the University of Washington DDI database (<https://www.druginteractioninfo.org>).

^b: PK parameters are obtained from the University of Washington DDI database (<https://www.druginteractioninfo.org>) unless specified in the references; other data of ADME properties are obtained from drug labels

^c: Plasma exposure of dabigatran was measured

^d: DE was administered in microdose (375 μg)

^e: Fostamatinib is a prodrug (R788) that is rapidly and completely metabolized by dephosphorylation in the enterocytes of the intestine to the active systemic metabolite R406.

^f: Administered with adefovir dipivoxil, a diester prodrug of adefovir.

^g: Refer to the University of Washington Drug Interaction and Transport Database (www.druginteractioninfo.org).

Table 3: Examples of potential clinical endogenous biomarkers of OATP1B, OAT1, OAT3, OCT2/MATE1, 2K for DDI evaluation.

Endogenous probe	<i>In vitro</i> transport kinetics/selectivity (K _m)	Formation and elimination mechanisms	Change in patients with genetically altered transporter function	Clinical DDI with known inhibitors		Reference	Comments
				Inhibitor (Dose and administration route) ¹	PK Change (inhibitor/control)		
OATP1B1/OATP1B3							
Coproporphyrin I (CPI) and CPIII	<ul style="list-style-type: none">• OATP1B1: 0.13 (CPI) and 0.22 μM (CPIII)• OATP1B3: 3.95 (CPI) and 1.55 μM (CPIII)• MRP2: 7.7 μM (CPI)• MRP3: substrate (CPI/III)	<ul style="list-style-type: none">• Formation: metabolically stable byproducts of heme biosynthesis• Elimination: biliary and renal excretions	<ul style="list-style-type: none">• Rotor syndrome (deficient in OATP1B): decreased biliary excretion and increased urinary excretion• Dubin-Johnson syndrome (deficient in MRP2): increased urinary excretion	Rifampin (oral 300 and 600 mg SD)	Rifampin 300mg: <ul style="list-style-type: none">• CPI AUCR: 3.0 Rifampin 600 mg: <ul style="list-style-type: none">• CPI AUCR: 4.0-4.6,• CPI C_{max}R: 5.4-6.4• CPIII AUCR: 3.3-3.4• CPIII C_{max}R:3.3	^{38, 40, 41, b}	<ul style="list-style-type: none">• Biliary clearance of CPI is estimated to contribute ≥ 85% CPI total clearance⁹¹
				GDC-0810 (oral 600 mg QD)	<ul style="list-style-type: none">• CPI C_{3hr}R: 1.56• CPIII C_{3hr}R: 1.52	³⁹	<ul style="list-style-type: none">• GDC-0810 increased pravastatin AUCR of 1.4
				JNJ-A (oral 600 mg BID for 8 days)	<ul style="list-style-type: none">• CPI AUCR: 1.4• CPI C_{max}R:1.8• CPIII AUCR: 1.1• CPIII C_{max}R: 1.2	³⁷	<ul style="list-style-type: none">• JNJ-A increased pitavastatin AUCR of 1.5
Glycochenodeoxycholate-3-O-sulfate (GCDCA-S)	<ul style="list-style-type: none">• OATP1B1: 9.95 μM• OATP1B3: 5.23	<ul style="list-style-type: none">• Formation: bile acid sulfation likely by SULT2A1		Rifampin (oral 300 and 600 mg SD)	Rifampin 300mg: <ul style="list-style-type: none">• AUCR: 4.3• Rifampin 600mg:	^{34, 41}	<ul style="list-style-type: none">• Metabolomics and GWAS studies

Endogenous probe	<i>In vitro</i> transport kinetics/selectivity	Formation and elimination mechanisms	Change in patients with genetically altered	Clinical DDI with known inhibitors		Reference	Comments
	μM <ul style="list-style-type: none"> OAT3: 64.3 μM NTCP: substrate 	<ul style="list-style-type: none"> Elimination: biliary excretion, renal excretions, and enterohepatic recirculation 			<ul style="list-style-type: none"> AUCR: 10-20.3 		suggested that GCDCA-S was significantly associated with genetic variants in OATP1B1
Hexadecanedioate (HDA) and tetradecanedioate (TDA)	<ul style="list-style-type: none"> OATP1B1: substrate (uptake ratios ^a 2.2 and 3.4) OAT1: substrates (uptake ratios ^a 2.0 and 4.1) OAT3: substrates (uptake ratios ^a 5.0 and 8.0) 	<ul style="list-style-type: none"> Formation: CYP4A11 is involved in HDA and TDA synthesis and/or metabolism 	<ul style="list-style-type: none"> HDA and TDA are significantly associated with genetic variants in OATP1B1 	Rifampin(oral 600 mg SD)	<ul style="list-style-type: none"> AUCR: 2.2 and 3.2 	^{33, 40}	
Unconjugated and conjugated bilirubin (UCB and CB)	<ul style="list-style-type: none"> OATP1B1: 0.008-0.16 μM (UCB) and 0.1-0.28 μM OATP1B3: 0.039 and 0.50 μM 	<ul style="list-style-type: none"> Formation: bilirubin glucuronidation by UGT1A1 Elimination: biliary excretion and enterohepatic recirculation 	<ul style="list-style-type: none"> Rotor syndrome (deficient in OATP1B): decreased biliary excretion SLCO1B1*15/*15 genotype (decreased OATP1B1 activity): increased plasma UCB and CB levels 	Rifampin (oral 300 and 600 mg SD)	Rifampin 300mg <ul style="list-style-type: none"> AUCR: 1.6 (total bilirubin), 2.3 (CB) Rifampin 600 mg <ul style="list-style-type: none"> AUCR: 1.94 (UCB); 0.85-3.5 (CB); 1.32-2.09 (total bilirubin) 	^{19, 29, 40, 41, b}	
OAT1/OAT3							
Taurine	<ul style="list-style-type: none"> OAT1: 379 μM 	<ul style="list-style-type: none"> Formation: derived from food or produced from cysteine by cysteine dioxygenase and cysteine sulfonate Elimination: renal 		Probenecid (oral 500, 750, and 1500 mg SD)	<ul style="list-style-type: none"> AUCR: 0.97, 0.98, and 1.02, respectively CL_rR: 0.55, 0.40 and 0.29, respectively 	⁵⁹	<ul style="list-style-type: none"> Selective substrate to OAT1 over OAT3

Endogenous probe	<i>In vitro</i> transport kinetics/selectivity	Formation and elimination mechanisms	Change in patients with genetically altered	Clinical DDI with known inhibitors		Reference	Comments
		excretion					
6 β -hydroxyl cortisol (6 β HC)	<ul style="list-style-type: none"> • OAT3: substrate • MATE1: substrate 	<ul style="list-style-type: none"> • Formation: produced from cortisol (an endogenous substrate of OAT3) by hepatic CYP3A4 • Elimination: renal excretion 		Probenecid (oral 750 mg SD)	<ul style="list-style-type: none"> • AUCR: 1.57-1.76 • CL_rR: 0.58-0.63 	^b	<ul style="list-style-type: none"> • Amount (endogenous production of 6βHC) is also influenced by CYP3A activity
Glycochenodeoxycholate-3-O-sulfate (GCDCA-S)	<ul style="list-style-type: none"> • OATP1B1: 9.95 μM • OATP1B3: 5.23 μM • OAT3: 64.3 μM • NTCP: substrate 	<ul style="list-style-type: none"> • Formation: bile acid sulfation likely by SULT2A1 • Elimination: biliary excretion, renal excretions, and enterohepatic recirculation 		Probenecid (oral 500, 750, and 1500 mg SD)	<ul style="list-style-type: none"> • AUCR: 1.06, 1.00, and 1.37, respectively • CL_rR: 0.38, 0.21 and 0.11, respectively 	⁵⁹	
OCT2/MATEs							
Creatinine	<ul style="list-style-type: none"> • OCT2: 1.86-18.8 mM • MATE1: 10.2 mM • MATE2K: 21.6 mM • OAT2: 795-986 μM 	<ul style="list-style-type: none"> • Formation: produced mainly by muscle metabolism • Elimination: primarily filtered through the kidney through the glomeruli; 10–40% is 		Cimetidine (oral 400 mg QD)	<ul style="list-style-type: none"> • AUCR: 1.13-1.26 • CL_rR: 0.80 	^{66, b}	

Endogenous probe	<i>In vitro</i> transport kinetics/selectivity	Formation and elimination mechanisms	Change in patients with genetically altered	Clinical DDI with known inhibitors		Reference	Comments
		actively secreted					
				Pyrimethamine (oral 50-100 mg SD)	<ul style="list-style-type: none"> • AUCR: 1.18-1.26 • CL_rR: 0.73-0.75 	^{66, b}	
				Dolutegravir (oral 50 mg QD or BID for 4 days)	<ul style="list-style-type: none"> • AUCR: 1.09-1.17 • CL_rR: 0.86-0.90 	^b	
N1-methylnicotinamide (NMN)	<ul style="list-style-type: none"> • OCT2: 318 μM • MATE1: 301 μM • MATE2K: 422 μM 	<ul style="list-style-type: none"> • Formation: a nicotinamide metabolite produced by N-methyltransferase Elimination: renal excretions and metabolism 		Pyrimethamine (oral 50 mg SD)	<ul style="list-style-type: none"> • AUCR: 0.84 • CL_rR: 0.30 	⁶⁹	
				Trimethoprim (oral 200 mg BID for 5 days)	<ul style="list-style-type: none"> • AUCR: 1.00 • CL_rR: 0.73 	⁷⁰	

AUCR, ratio of AUC; CL_rR , ratio of CL_r; BID, twice a day; C_{max}R, ratio of C_{max}; K_m , Michaelis constant; QD, once a day; SD, single dose.

^a: Uptake ratio is calculated by dividing uptake activity measured in the transporter cells by that in control cells.

^b: Refer to the University of Washington Drug Interaction and Transport Database (www.druginteractioninfo.org).

A. Comparison of endogenous biomarkers and probe drugs with different $t_{1/2}$

B. Short $t_{1/2}$ of both biomarker and inhibitor

	With diurnal change	No diurnal change
Single- or multiple-dose	Model-based analysis	Model-based analysis (or C_{max}^*)

C. Long $t_{1/2}$ of either biomarker or inhibitor

	With diurnal change	No diurnal change
Multiple-dose	AUC or model-based analysis	AUC, C_{max}^* , or model-based analysis
Single-dose	Model-based analysis; may not capture full inhibition	

Data Source

NHP DDI

Clinical GWAS/PGX

Recombinant *in vitro* systems^a

Rodent KO model

Clinical disease model

Clinical DDI

Quantitation Metabolomics or targeted assay

Identification

Identify candidate biomarkers with suitable sensitivity and/or specificity:

Change from baseline is robust with statistical significance and reproducible in two or more data types^b

Characterization

Clinical Selectivity towards a specific transporter:

Compare the effects of model transport inhibitors and genetic polymorphism on the PK and elimination of candidate biomarkers

In vitro specificity of candidate biomarkers for a transport process:

Characterize the transport, formation, and elimination kinetics of the candidate biomarker and evaluate the effect of inhibitors on these processes

Clinical sensitivity:

Evaluate the magnitude of change from baseline in biological matrices at multiple time points in a sufficiently powered sample size to address physiological variability

Mechanistic clinical deconstruction:

Develop mechanistic PK models to optimize clinical utility. If needed, measure clinically candidate biomarker formation or elimination by administering labeled compound or parallel measurement of precursors

Validation

Optimize analytical procedures and methods

Design a dedicated prospective randomized crossover clinical DDI study with a known probe drug substrate, an inhibitor (multiple^c), and quantitation of the selected candidate biomarker(s)

Use suitable dosing regimen and collect appropriate matrices over a suitable time frame pre- and post-dosing

Compare magnitude of PK changes relative to probe drug substrate(s)

+

Determine the optimal time frame and corresponding sensitivity based on PK changes of probe drug(s)

+

Compare PK changes across a panel of inhibitors to determine the selectivity of the candidate biomarker and the dynamic range

PK changes demonstrate a robust trend with increasing inhibitor concentrations that mirrors those observed with probe drug(s)

Application

Incorporate in clinical programs in “first-in-human” or in DDI investigations in combination with other probe drugs. Qualify the biomarker for the regulatory intended use^d

A. Pre-validated endogenous biomarkers and probe drugs

B. Validated endogenous biomarkers and probe drugs