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Review Article

Drug Transporters: Regulatory Guidance from ICH, USFDA, EMA and PMDA

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ABSTRACT

Drug transporters play a critical role in disposition of xenobiotics, and altered transport function could lead to adverse effects. Given their importance in drug-drug interactions, regulatory agencies recommend testing transporter inhibitory potential for all investigational drugs. Based on route of elimination and target organ, agencies also recommend evaluating if investigational drugs are substrates for major transporters. This review compares guidance from United States (USFDA), European Union (EMA), Japanese (PMDA) and International Council for Harmonization (ICH) pertinent to drug transporters.

Keywords: Drug-drug interaction; Food and Drug Administration (FAD); ICH M12; EMA; PMDA; Guidance

ABBREVATIONS

ICH: International Council for Harmonization; FAD: Food and Drug Administration; DDI: Direct Dial Inward; MDR1: Multidrug Resistance; BCRP: Breast cancer resistance protein Pregnane; PXR: X receptor; MPP: Massively Parallel Processing; CAR: Corrective Action Request; OATP1B1: Organic Anion Transporting Polypeptide 1B1; BCR: Bulbocavernosus Reflex; MATE1: Multidrug and Toxin Extrusion Protein-1; TEA: Transient Epileptic Amnesia; NSAID: Non-Steroidal Anti-inflammatory Drugs

INTRODUCTION

Drug transporters are membrane proteins that aid in the exchange of chemicals across biological membranes. Along with multitude of endogenous chemicals, for instance, creatinine, bile acids, steroid hormones, drug molecules are also substrates for transporters. Given the ubiquitous expression of transporters across different organ systems, they play critical role in absorption, distribution, and excretion of many drugs [1]. For the substrate drugs, efficacy and adverse events can be contingent on their interaction with relevant drug transporter. Because of their critical importance in drug disposition, regulatory agencies around the world recommend studies to evaluate the inhibitory and substrate potential of new drug entities pertinent to major drug transporters.

International Council for Harmonization (ICH) released a draft version of their drug-drug interaction (DDI) guidance in August 2022, wherein they provided detailed instructions on drug transporter *in vitro* assays, probe substrates and inhibitors, assay systems, data interpretation and clinical follow up [2]. Prior to that, in January 2020, US FDA released a similarly detailed two DDI related guidance documents [3,4]. European Medical Agency (EMA) provided their recommendations for the DDI studies in a guidance released in June 2012 [5]. In 2019, Pharmaceutical and Medical Device Agency (PMDA) of Japan released the official English translation of their drug interaction study guidance [6].

This review focuses on summarizing the *in vitro* and clinical guidance from the four agencies mentioned above- ICH, USFDA, EMA and PMDA. There are several similarities in terms of suggestions from all the agencies, as we have presented those in the form of tables for each major transporter. Transporters are an evolving area of science. Over the past two decades, our understanding of drug transporters has improved significantly. This is reflected in the details of the regulatory suggestions in recent years. ICH M12 and USFDA 2020 guidance are the most detailed and specific and have used more direct recommendations compared to EMA and PMDA guidance. All the major transporters that are mentioned in these guidelines are described in this review. Also, for some other transporters, where detailed guidance is not provided, but are mentioned in the guidelines, we have included those towards the end of the review. Moreover, in the future directions, we reflect on

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recent progress in biomarkers of the transport function, and their anticipated inclusion in the future DDI guidelines from agencies across the world.

MDR1/BCRP

MDR1, also known as P-glycoprotein (P-gp), is an efflux transporter (~170 kDa) encoded by ATP binding cassette subfamily B member 1 (ABCB1). BCRP (Breast cancer resistant protein) is a 75 kDa protein that is encoded by ABC sub-family G member 2 (ABCG2). MDR1 and BCRP are highly expressed in several tissues and organs, including Blood-Brain-Barrier (BBB), intestinal epithelial cells, and canalicular cells in hepatocytes, kidney cells, and placenta [7-9]. The main function of MDR1 and BCRP is to restrict the distribution of its substrates into organs, particularly with respect to CNS exposure, and eliminate its substrates from excretory organs by mediating biliary, renal, and gut secretions. MDR1 is the most extensively studied and well-understood transport system among many other transporters. Although it is less well studied compared to MDR1, BCRP is generally co-expressed with MDR1 and shares many substrates, inhibitors, and inducers. All guidance (FDA, EMA, PMDA, and ICH) recommend testing of MDR1 and BCRP interactions *in vitro* as a minimum requirement.

All four guidance recommend using *in vitro* system as the first step to determine if an investigational drug is a substrate or inhibitor for MDR1 and BCRP. The recommendations for selecting *in vitro* systems are similar among guidelines. Caco-2 (from Cancer coli, "colon cancer") or over-expressing cell lines can be used for such tests. However, ICH and EMA both emphasized that the *in vitro* experiments need to be performed under well-controlled conditions. For example, sufficient total recovery of the drugs to be demonstrated (e.g., 80%). When determining the permeability of test articles, Caco-2 cells can be used, and well-validated high and low permeable controls should be included. In addition, EMA recommended using two separate systems to test for P-gp due to the high inter-laboratory variability in the inhibition parameter estimation while FDA suggests that the study results are justified as long as the test system is validated.

Since the *in vitro* methods to evaluate the induction of transporters are not well established, FDA did not provide recommendations for *in vitro* evaluation of investigational drugs as transporter inducers. PMDA only reminded sponsors to pay attention to investigational drug tissue distribution which may lead to the induction of efflux transporters in specific tissues. However, both ICH and EMA suggested an *in vivo* investigation if a PXR and/or CAR mediated induction is observed.

The interpretation for *in vitro* data among different guidelines for determining if the investigational drug is the substrate of MDR1 or BCRP are similar as shown in Table 1. However, the calculation and interpretation for inhibitors varies. FDA and ICH accept the calculation using IC_{50} or K_i while EMA mentioned K_i only and PMDA recommends IC_{50}. PMDA also suggests ER should be used as an index for IC_{50} calculation. The cutoff value is similar across four guidelines. But the threshold to determine if a metabolite or a parenterally administered drug inhibits P-gp or BCRP is different. ICH suggested a more conservative value (K_i or $IC_{50} > 50 \times C_{max,u}$) compared to FDA ($K_i$ or $IC_{50} > 10 \times C_{max,u}$).

Table 1: Comparison of the regulatory guidance for efflux transporter MDR1 and BCRP

Guidance	ICH	USFDA	EMA	PMDA
When to conduct substrate assay	All drugs	All drugs	All investigational drugs. For metabolite, if active secretion is the major elimination pathway (estimated contribution to *in vivo* pharmacological effect ≥ 50% of total effect), EMA recommends identifying transporters involved.	All drugs
Drug is a MDR1/BCRP substrate if	ER or net ER ≥ 2 and ER or net ER can be inhibited by more than 50% by a known inhibitor of the transporter	ER or net ER ≥ 2 and ER or net ER can be inhibited by more than 50% by a known inhibitor of the transporter	ER or net ER ≥ 2 and ER or net ER can be inhibited by more than 50% by a known inhibitor of the transporter. In addition, EMA suggested that ER>2 indicates the involvement of active uptake transporters.	ER or net ER ≥ 2 and ER or net ER can be inhibited by more than 50% by a known inhibitor of the transporter
When to conduct inhibition assay	All drugs	All drugs	All drugs	All drugs
Drug is an MDR1/BCRP inhibitor if	K_i or $IC_{50} < 0.1 \times$ (Dose/250 mL) for orally administered drugs; Ki or $IC_{50} > 50 \times C_{max,u}$ for parenterally administered drugs and metabolite formed post-absorption	K_i or $IC_{50} < 0.1 \times$ (Dose/250 mL) for orally administered drugs; K_i or $IC_{50} > 10 \times C_{max,u}$ for parenterally administered drugs and metabolite formed post-absorption	$K_i < 0.1 \times$ (Dose/250 mL) for intestinally expressed transporters	$[I]/IC_{50} \geq 10$ ([I] should be set based on the expected maximum concentration) maximum single dose of

If *in vitro* studies show the investigational drug is the substrate of P-gp or BCRP, FDA and PMDA suggested the need for a clinical DDI trial can be determined based on drug's putative site of action, route of elimination, likely concomitant drugs, and safety considerations. ICH had similar suggestions and added more, including the consideration for drug's passive permeability, route of administration, and *in vivo* absorption and elimination. Other than EMA, the other three guidelines all recommended a clinical DDI to determine if an investigational drug is the substrate for P-gp or BCRP when intestinal absorption is limited or biliary excretion/active renal secretion is a major elimination pathway.

On the other hand, if *in vitro* studies show the investigational drug is the inhibitor of P-gp or BCRP, FDA, ICH, and PMDA all suggested sponsor to consider a clinical DDI study based on likely concomitant drugs and safety considerations. The preference for substrate drug is given to the drug whose pharmacokinetic profile is markedly altered by co-administration of known inhibitors of P-gp or BCRP and is also a likely concomitant drug.

For drugs with potential to induce transporters, FDA and PMDA didn't give clear statement while FDA recommends the sponsor to consult FDA if an investigational study is needed to evaluate whether an investigational drug is an inducer of transporters. Since P-gp is co-regulated with Cytochrome *P450 3A4* (*CYP3A*), ICH recommended sponsors to consider whether to conduct clinical DDI studies to evaluate the potential effect of a drug on other transporters regulated through the same pathways as *CYP3A* (e.g., PXR and/or CAR). EMA suggested an interaction study with the transporter inducer that if these transporter inducers are marketed within the European. EMA also recommended an *in vivo* study if transporter-enzyme interplay (e.g., P-gp and *CYP3A*) is observed.

OATP1B1/1B3

Organic anion transporting polypeptide (OATP) 1B1 and 1B3 are expressed primarily on sinusoidal/basolateral membrane of the hepatocytes [10]. They play role in hepatic uptake of substrate drugs and endogenous molecules, which are primarily organic anions. Endogenous substrates for OATP1B1 and 1B3 include bile acids, bilirubin, coproporphyrins I and III, prostaglandin E2 and some peptides [11]. Cholesterol lowering drugs including atorvastatin, rosuvastatin, fluvastatin as well as bosentan, repaglinide, and methotrexate are some examples of the marketed drugs that are substrates for OATP transporters [12].

Inhibition of transport function for OATP1B1/1B3 can result in systemic accumulation of the substrate drugs leading to adverse events or increased DDI risk. Notably, accumulation of statins could lead to enhanced risk of rhabdomyolysis. Lau et al conducted a clinical investigation of healthy volunteers co-administered with atorvastatin and rifampin, a potent inhibitor of OATP1B1/1B3 [13]. Rifampin co-administration increased Area Under the Curve (AUC) for atorvastatin, and its major metabolites 4 to 6 times compared to single administration. Shitara reviewed the clinical relevance of the OATP1B1/1B3 inhibition and also described the effects of genetic polymorphism on the pharmacokinetics of the statins [10].

As detailed in, the guidance across agencies has similarities for substrate assays for OATP1B1/1B3 (Table 2). If the hepatic metabolism or biliary excretion contributes to 25% or more clearance of investigational drug, then sponsors are recommended to determine if that drug is a substrate for OATP1B1/1B3. In addition to these criteria, USFDA also mentioned the physicochemical characteristics of the molecule to be taken into account. For instance, active uptake of drugs is important if they possess low passive membrane permeability, high hepatic concentrations relative to other tissues, or organic anion/charged at physiological pH. For substrate assay, data interpretation is not specified in the EMA guidance, however PMDA clarified apparent update by human hepatocytes and its inhibition by known OATP inhibitors observed, or uptake observed in OATP expressing cells compared to control cells. USFDA and ICH further specified that investigational drug is a substrate of OATP1B1/1B3 if ≥ 2-fold uptake of the drug in OATP expressing cells than control cells and uptake in OATP expressing cells can be inhibited by more than 50% by a known OATP inhibitor.

Table 2: Comparison of the regulatory guidance for hepatic uptake transporters OATP1B1/1B3.

Guidance	ICH	USFDA	EMA	PMDA
When to conduct substrate assay	Consider if hepatic metabolism or biliary excretion accounts for ≥ 25% of elimination of a drug or if the pharmacological target of a drug is in the liver	Same as ICH and additionally, drug's physiological properties, e.g., low passive membrane permeability, high hepatic concentrations relative to other tissues, organic anion/charged at physiological pH, which support the importance of active uptake of the drug into liver	Consider if hepatic metabolism or biliary excretion accounts for ≥ 25% of elimination of a drug	Consider if hepatic metabolism or biliary excretion accounts for ≥ 25% of elimination of a drug
Drug is a OATP substrate if	≥ 2-fold uptake of the drug in OATP expressing cells than control cells and uptake in OATP expressing cells can be inhibited by more than 50% by a known OATP inhibitor	≥ 2-fold uptake of the drug in OATP expressing cells than control cells and uptake in OATP expressing cells can be inhibited by more than 50% by a known OATP inhibitor at a concentration at least 10 times that of the Ki or IC_{50}	General recommendation: use transporter expressing cell line, and specific known inhibitor to confirm the data. The concentration range of the drug should be relevant to site of transport.	Apparent update by human hepatocytes and its inhibition by known OATP inhibitors observed, or uptake observed in OATP expressing cells compared to control cells
When to conduct inhibition assay	All drugs	All drugs	All drugs	All drugs
Drug is an OATP inhibitor if	K_i or $IC_{50} > 10 \times C_{max,inlet,u}$ (i.e., $C_{max,inlet,u}/K_i$ or $IC_{50} < 0.1$)	$R=1+((f_{u,p} \times I_{in,max})/IC_{50}) \geq 1.1$	$K_i < 25 \times ([I]_{u,inlet,max})$	$1+((_{fu,p} \times {}_{Iin,max})/K_i) \geq 1.1$

All four agencies recommend evaluation of the drugs as inhibitors of the OATP1B1/1B3 for all investigational drugs. For data interpretation, it's essential to consider the inlet maximum unbound concentration (Cu,in,max or Iu,in,max) since that's the concentration available when drugs interact with OATP transporters. The data interpretation criteria for USFDA, ICH and PMDA are the same, however it differs for EMA. Additionally, USFDA and ICH also mention use of preincubation with drugs while conducting inhibition assays for OATP transporters, which is derived from published evidence [14].

No agency makes any mention of endogenous biomarkers for OATP1B transport function.

OAT1, OAT3, OCT2, MATE1, MATE2K

Organic Anion Transporters (OAT) and Organic Cation Transporters (OCT) are expressed in renal proximal tubular epithelium. OAT1 and 3 are anion exchanging antiporters, whereas OCT2 primarily transports cations. These three transporters aid in uptake of endogenous chemicals and drugs from blood to renal tubular epithelium. OCT2 works in concert with MATE1/2K for elimination of compounds in the urine [15].

Multidrug and Toxin Extrusion (MATE) pumps are bidirectional proton antiporters that function based on pH gradient across the membrane. MATEs play an important role in renal and biliary excretion of metformin. Human MATE1 and MATE2K are localized on renal proximal tubular brush border membranes [16]. Besides kidney, other organs with high expression of MATE1 include adrenal gland, liver, and skeletal muscle. On the contrary, MATE2K is exclusively expressed in kidneys. MATE1 and MATE2K have similar substrate specificity for the majority of compounds, however certain substrates distinctly demonstrate uniqueness of each transporter. For instance, cationic compounds including cimetidine, metformin, guanidine, Tetraethylammonium (TEA), MPP, topotecan are substrates for both MATE1 and MATE2K. However, zwitterionic compounds cephalexin and cephradine are substrates for MATE1, but not MATE2K. Anionic compounds estrone sulfate, acyclovir and ganciclovir are transported by both MATE1 and MATE2K [17]. Given their localization within the renal proximal cells, MATE transporters work in concert with OCT1 and OCT2. Organic cation transporters uptake the substrates from blood side, and then MATEs efflux it into the luminal side to be excreted in urine. MDCK cells were double transfected with OCT1/MATE1 or OCT2/MATE1 in order to study the combined effect of uptake and efflux transporters in renal elimination of substrate compound TEA [18]. Compared to control cells, the double transfected cells showed greater basolateral to apical transport of TEA, and this transport declined significantly in presence of 1-methyl-4-phenylpyridinium, which could be attributed to competitive inhibition.

OAT1 and 3 move chemicals from blood to renal tubular cells against the concentration gradient, but without use of ATP energy. Instead, drug transport by OAT is accompanied by endogenous anion transport from renal cell to the blood along the concentration gradient. The dicarboxylates are in turn exchanged by sodium dicarboxylate co-transporter, which relies on sodium gradient created by sodium potassium ATPase [19]. Endogenous substrates for OATs include uric acid, prostaglandin E2 and cyclic nucleotides [20].

Several marketed drugs are substrates for OAT1 and OAT3, including antivirals, beta lactam antibiotics, Non-Steroidal Anti-inflammatory Drugs (NSAID) and diuretics [21]. Methotrexate co-administration with NSAID ketoprofen has been reported to cause severe clinical adverse events [22]. Given that methotrexate is a substrate for OAT1 and OAT3, its potent inhibition by ketoprofen illustrates the mechanistic link for the observed DDI [23].

Endogenous substrates for OCT2 include creatinine, acetylcholine, monoamine neurotransmitters, and bile acids. Anti-diabetic drug Metformin is a well-known substrate for OCT2. Inhibition of OCT2 and MATE transporters in kidney by cimetidine, and subsequent metformin toxicity are well documented [24].

Verapamil and cimetidine combination was used to prevent cisplatin-induced nephrotoxicity in testicular cancer patients, possibly indicating role of OCT2 and MATE in renal cisplatin transport [25]. HER2-positive breast cancer treatment agent Tucatinib inhibits OCT2, MATE1 and MATE2K *in vitro* with IC_{50} values of 14.7, 0.34 and 0.135 μM, respectively. Metformin and tucatinib co-administration study was performed in 18 healthy volunteers to study the clinical interaction. Tucatinib increased the metformin exposure by 1.4-fold, but the maximum plasma concentration remained unchanged. Tucatinib also decreased creatinine clearance by 23%, but the renal function markers including GFR remained unchanged [26].

Compares the regulatory guidance for OAT1, OAT3, OCT2, MATE1 and MATE2K (Table 3). All agencies recommend that sponsors should determine if investigational drug is a substrate for OAT1, OAT3, OCT2, MATE1 and MATE2K if it's 25% or more of its clearance can be attributed to kidneys. ICH further adds that these transporters should also be evaluated if renal toxicity is a concern for drugs. For data interpretation of substrate assay, EMA guidance does not specify numerical criteria, but rather provides general recommendation of using transfected cell lines and known inhibitors of these transporters. ICH, USFDA and PMDA suggest that drug is a substrate for OAT, OCT, MATE transporters if its transport is greater than or equal to 2-fold in expressing cells compared to control cells, and if this transport is inhibited at least 50% by known inhibitors. All four agencies recommend inhibitory potential check for OAT, OCT, MATE transporters for all investigational drugs. There are minor numerical differences between the data interpretation criteria for inhibition assay, however the parameters used in the calculation remain the same across the agencies.

USFDA mentioned monitoring endogenous creatinine levels as a possible biomarker for OCT2 and MATE transporters. Although details of the numerical criteria are not specified, this is the first mention of the endogenous biomarkers in the *in vitro* DDI guidance for renal transporters.

ENDOGENOUS BIOMARKERS FOR TRANSPORTER FUNCTION

Transporter isoforms differ significantly between the species. For instance, human OATP1B1 and 1B3 refer to rodent OATP1B2. Along with differences in nomenclature, some transporters also demonstrate functional differences between species. Estrone-3-sulfate and progesterone sulfate are transported by human OATP2B1, but not rodent Oatp2b1 [27]. For P-glycoprotein, rodent brain capillaries expression levels are about 3-times higher compared to human brain capillaries [28]. Indinavir, which is a known P-glycoprotein substrate, showed differences in the extent

of transport in human and rodent transfected cells [29]. These and many other examples illustrate that rodent studies may not be a true reflection of transporter function in humans. The regulatory agencies suggest use of *in vitro* models, which include cell lines transfected with human transporters or human primary hepatocytes for studying the investigational drugs. Based on *in vitro* data, formulae are used to deduce *in vivo* transporter DDI risk. Model-based approaches are also suggested for *in vitro* to *in vivo* extrapolation of the DDI risk, however, animal studies are not recommended.

In recent years, several publications have illustrated endogenous chemicals as biomarkers of transport function *in vivo* [30]. For OATP1B function, coproporphyrins (CP) were proposed as sensitive biomarkers with clinical evidence [31]. For BCRP function, Riboflavin as a biomarker was proposed and process of biomarker validation was described [32].

Besides creatinine as a potential biomarker for OCT2 and MATE transporters, regulatory guidelines do not mention any other biomarkers. However, given the ongoing research and increasing evidence, future DDI guidance may include monitoring of biomarkers as additional evidence to transport function.

Table 3: Comparison of the regulatory guidance for transporters OAT1, OAT3, OCT2 and MATE1/2K

Guidance	ICH	USFDA	EMA	PMDA
When to conduct substrate assay	If drug undergoes significant active renal secretion (i.e., accounting for ≥ 25% of systemic clearance) or there are concerns about renal toxicity	Active secretion of the drug by the kidney is ≥ 25% of the systemic clearance	Same as USFDA	Same as USFDA
Drug is a MATE/ OAT/OCT substrate if	The ratio of the investigational drug's uptake in the cells expressing the transporter versus the drug's uptake in control cells (or cells containing an empty vector) is ≥ 2; and a known inhibitor of the transporter decreases the drug's uptake to ≤ 50%	The ratio of the investigational drug's uptake in the cells expressing the transporter versus the drug's uptake in control cells (or cells containing an empty vector) is ≥ 2; and a known inhibitor of the transporter decreases the drug's uptake to ≤ 50% at a concentration at least 10 times its Ki or IC_{50}	General recommendation: use transporter expressing cell line, and specific known inhibitor to confirm the data. The concentration range of the drug should be relevant to site of transport.	Same as ICH
When to conduct inhibition assay	All drugs	All drugs	All drugs	All drugs
Drug is a MATE/ OAT/OCT inhibitor if	$C_{max,u}$/ K_i or IC_{50} ≥ 0.1 for OAT1, OAT3 and OCT2 $C_{max,u}$/ Ki or IC_{50} ≥ 0.02 for MATE1/2K	$I_{max,u}/IC_{50}$ ≥ 0.1	K_i<50 × $C_{u,max}$ (Which is same as $C_{max,u}$/ IC_{50} ≥ 0.02)	1+($C_{u,max}/K_i$) ≥ 1.1 for OAT1, OAT3 and OCT2 1+($C_{u,max}/K_i$) ≥ 1.02 for MATE1/2K

OTHER TRANSPORTERS: BSEP, OCT1, MRP2, AND OATP2B1

Bile salt export pump (BSEP, gene symbol *ABCB11*) is a unidirectional, ATP-dependent efflux transporter that is almost exclusively expressed in the liver and responsible for eliminating bile salts from the hepatocytes into bile canaliculi [33]. Bile salt secretion is required for cholesterol removal. Elevated bile acid resulting from BSEP inhibition has been found to be toxic and can lead to cholestasis and Drug-Induced Liver Injury (DILI) [34]. Drugs such as bosentan, troglitazone, and CI-1034 have been found to inhibit BSEP and result in clinical hepatotoxicity [35]. Although FDA 2020 guideline didn't mention BSEP, due to the positive correlation between BSEP and liver toxicity, ICH, EMA, and PDMA all recommend the investigation to determine if the drug can inhibit BSEP. EMA also recommends biochemical monitoring including serum bile salts if *in vitro* studies indicate BSEP inhibition.

MRP2 (gene symbol *ABCC2*), also known as canalicular multi-specific organic anion transporter, is another transporter that is highly expressed in liver where it facilitates the elimination of bilirubin glucuronides and positively charged drugs and conjugates into the bile [36]. MRP2 also plays an important role in oral bioavailability of drugs and their phase II metabolites [37]. MRP2 is also known to be expressed in tumor cells and tissues thus associated with multidrug resistance [38]. Several drugs, including antibiotics (e.g., ampicillin, azithromycin, cefodizime, etc.), anticancer drugs (e.g., Cisplatin, Doxorubicin, Epirubicin, etc.), HIV drugs (e.g., Adevovir, Cidofovir, Indinavir, etc.) are found to be the substrates or inhibitors of MRP2 [39]. Currently, except for ICH and PMDA recommend the sponsor to test for MRP2 inhibition on a case-by-case basis, there is no specific recommendation for MRP2 in FDA or EMA guidance.

OCT1 is another additional transporter that ICH, EMA, and PMDA all recommend being considered for inhibition. OCT1 (SLC22A1) is an uptake transporter that is primarily expressed on the sinusoidal membrane of hepatocytes. It is also found to be expressed abundantly in kidney and gall bladder [40]. OCT1 is reported to possess broad substrate specificity [41]. It plays an important role in cationic drugs and endogenous compounds' distribution and hepatic clearance by the conjunction with MATE1 to facilitate the biliary elimination of its substrates [42]. Several drugs with different indications have been clinically demonstrated to be the substrates and/or inhibitors of OCT1 [40].

OATP2B1 is a newcomer mentioned only in ICH which recommends the sponsor consider testing for OATP2B1 on a case-by-case basis. It is an intestinal and hepatic drug uptake transporter encoded by SLCO2B1 with broad substrate specificity [43]. Among OATPs family, OATP1B1/1B3 are the well-studied transporters. There is little clinical information on OATP2B1 due to the mask effects that intestinal and hepatic DDIs have been mechanistically attributed primarily to OATP1B1 and secondarily to OATP1B3 [44]. Until recently OATP2B1 has been identified as the primary intestinal isoform that can mediate intestinal absorption of its substrate [43]. In liver, OATP2B1 has been demonstrated to have similar expression level as that of OATP1B3 [45]. Eley et al conducted the clinical DDI trial and found the coadministration of Asunaprevir (ASV) with the OATP inhibitor rifampin significantly increased ASV plasma level (15-fold increase in ASV AUCinf). Later the *in vitro* study demonstrated that the ASV transport was mediated by OATP1B1 and OATP2B1, but not by OATP1B3 [46].

CONCLUSION

We reviewed all the major regulatory agencies for their transporter DDI guidance. It's apparent that the most recent ICH as well as USFDA, PMDA have more specific and detailed criteria for transporter evaluation. This is reflective of the evolution in our understanding of the drug transporters arena as a scientific community. As more knowledge and evidence of MATE transporters became available, USFDA included those in their recent guidance as transporters of clinical significance. On similar lines, mention of the preincubation condition for OATP transporter assays is another instance of published evidence enhancing our understanding of these transporters, and regulatory agencies agreeing to the evidence. EMA last released their *in vitro* DDI guidance in 2012 and it is reasonable to assume that it may be updated over next couple years to include several additions as in ICH M12 and USFDA 2020 guidance.

REFERENCES

1. Brouwer KL, Evers R, Hayden E, Hu S, Li CY, Meyer zu, et al. Regulation of drug transport proteins-from mechanisms to clinical impact: A white paper on behalf of the international transporter consortium. Clin Pharmacol Ther. 2022;112:461-484.
2. ICH. ICH Guideline M12 on drug interaction studies. 2022.
3. USFDA. *In Vitro* Drug Interaction Studies-Cytochrome P450 Enzyme and Transporter-Mediated Drug Interactions Guidance for Industry. 2020.
4. USFDA. Clinical Drug Interaction Studies-Cytochrome P450 Enzyme and Transporter-Mediated Drug Interactions-Guidance for Industry. 2020.
5. EMA. Guideline on the investigation of drug interactions. 2012.
6. PMDA. Guideline on drug interaction for drug development and appropriate provision of information. 2018.
7. Borst P, Schinkel AH. P-glycoprotein ABCB1: A major player in drug handling by mammals. J Clin Invest. 2013;123:4131-4133.
8. Cascorbi I. P-glycoprotein: Tissue distribution, substrates, and functional consequences of genetic variations. Handb Exp Pharmacol. 2011:261-283.
9. Nakanishi T, Ross DD. Breast cancer resistance protein (BCRP/ABCG2): Its role in multidrug resistance and regulation of its gene expression. Chin J Cancer. 2012;31:73.
10. Shitara Y. Clinical importance of OATP1B1 and OATP1B3 in drug-drug interactions. Drug Metab Pharmacokinet. 2011;26:220-227.
11. Kullak-Ublick GA, Ismair MG, Stieger B, Landmann L, Huber R, Pizzagalli F, et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. Gastroenterology. 2001;120:525-533.
12. Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, et al. A novel human hepatic Organic Anion Transporting Polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. J Biol Chem. 1999;274:37161-37168.
13. Lau YY, Huang Y, Frassetto L, Benet LZ. Effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. Clin Pharmacol Ther. 2007;81:194-204.

14. Tátrai P, Schweigler P, Poller B, Domange N, de Wilde R, Hanna I, et al. A Systematic *In Vitro* Investigation of the Inhibitor Preincubation Effect on Multiple Classes of Clinically Relevant Transporters. Drug Metab Dispos. 2019;47:768-778.

15. Zamek-Gliszczynski MJ, Chu X, Cook JA, Custodio JM, Galetin A, Giacomini KM, 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;104:781-784.

16. Masuda S, Terada T, Yonezawa A. Identification and functional characterization of a new human kidney-specific H+/organic cation antiporter, kidney-specific multidrug and toxin extrusion 2. J Am Soc Nephrol. 2006;17:2127-2135.

17. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui KI, et al. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. Biochem Pharmacol. 2007;74:359-371.

18. Sato T, Masuda S, Yonezawa A, Tanihara Y, Katsura T, Inui KI, et al. Transcellular transport of organic cations in double-transfected MDCK cells expressing human organic cation transporters hOCT1/hMATE1 and hOCT2/hMATE1. Biochem Pharmacol. 2008;76:894-903.

19. Roth M, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: The organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Br J Pharmacol. 2012;165:1260-1287.

20. Vávra J, Mančíková A, Pavelcová K, Hasíková L, Bohatá J, Stibůrková B. Functional characterization of rare variants in OAT1/SLC22A6 and OAT3/SLC22A8 urate transporters identified in a gout and hyperuricemia cohort. Cells. 2022;11(7):1063.

21. Nigam SK, Bush KT, Martovetsky G, Ahn SY, Liu HC, Richard E, et al. The organic anion transporter (OAT) family: a systems biology perspective. Physiol Rev. 2015;95:83-123.

22. Thyss A, Kubar J, Milano G, Namer M, Schneider M. Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. Lancet. 1986;1:256-258.

23. Iwaki M, Shimada H, Irino Y, Take M, Egashira S. Inhibition of methotrexate uptake *via* organic anion transporters OAT1 and OAT3 by glucuronides of nonsteroidal anti-inflammatory drugs. Biol Pharm Bull. 2017;40:926-931.

24. Wagner DJ, Hu T, Wang J. Polyspecific organic cation transporters and their impact on drug intracellular levels and pharmacodynamics. Pharmacol Res. 2016;111:237-246.

25. Th. Sleijfer D, Offerman JJ, Mulder NH, Verweij M, Van Der Hem GK, Koops HS, et al. The protective potential of the combination of verapamil and cimetidine on cisplatin-induced nephrotoxicity in man. Cancer. 1987; 60: 2823-2828.

26. Topletz-Erickson AR, Lee AJ, Mayor JG, Rustia EL, Abdulrasool LI, Wise AL, et al. Tucatinib inhibits renal transporters OCT2 and MATE without impacting renal function in healthy subjects. J Clin Pharmacol. 2021;61:461-471.

27. Hussner J, Foletti A, Seibert I, Fuchs A, Schuler E, Malagnino V, et al. Differences in transport function of the human and rat orthologue of the Organic Anion Transporting Polypeptide 2B1 (OATP2B1). Drug Metab Pharmacokinet. 2021;41:100418.

28. Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, et al. Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. J Neurochem. 2011;117:333-345.

29. Yamazaki M, Neway WE, Ohe T, Chen IW, Rowe JF. *In vitro* substrate identification studies for p-glycoprotein-mediated transport: Species difference and predictability of *in vivo* results. J Pharmacol Exp Ther. 2001;296:723-735.

30. Arya V, Reynolds KS, Yang X. Using endogenous biomarkers to derisk assessment of transporter-mediated drug-drug interactions: A scientific perspective. J Clin Pharmacol. 2022;62:1501-1506.

31. Kunze A, Ediage EN, 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;57:1559-1570.

32. Zhang Y, Shipkova PA, Warrack BM, Nelson DM, Wang L, Huo R, et al. Metabolomic profiling and drug interaction characterization reveal riboflavin as a breast cancer resistance protein-specific endogenous biomarker that demonstrates prediction of transporter activity *in vivo*. Drug Metab Dispos. 2023; 51: 851-861.

33. Kubitz R, Dröge C, Stindt J, Weissenberger K, Häussinger D. The bile salt export pump (BSEP) in health and disease. Clin Res Hepatol Gastroenterol. 2012;36:536-553.

34. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. World J Gastroenterol. 2009;15:1677.

35. Sahi J, Sinz MW, Campbell S, Mireles R, Zheng X, Rose KA, et al. Metabolism and transporter-mediated drug–drug interactions of the endothelin-A receptor antagonist CI-1034. Randomized Controlled Trial. 2006;159:156-168.

36. Myint K, Li Y, Paxton J, McKeage M. Multidrug resistance-associated protein 2 (MRP2) mediated transport of oxaliplatin-derived platinum in membrane vesicles. PloS one. 2015;10:e0130727.

37. Nies AT, Keppler D. The apical conjugate efflux pump ABCC2 (MRP2). Pflugers Arch. 2007;453:643-659.

38. Jemnitz K, Heredi-Szabo K, Janossy J, Ioja E, Vereczkey L, Krajcsi P. ABCC2/Abcc2: A multispecific transporter with dominant excretory functions. Drug metabolism reviews. 2010;42:402-436.

39. Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, et al. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade. Cancer Letters. 2016;370:153-164.

40. Zhou S, Zeng S, Shu Y. Drug-drug interactions at organic cation transporter 1. Front Pharmacol. 2021;12:628705.

41. Brosseau N, Ramotar D. The human organic cation transporter OCT1 and its role as a target for drug responses. Drug Metab Rev. 2019;51:389-407.

42. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH, et al. Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response. Pharmacogenetics and genomics. 2010;20:38-44.

43. Unger MS, Mudunuru J, Schwab M, Hopf C, Drewes G, Nies AT, et al. Clinically relevant OATP2B1 inhibitors in marketed drug space. Mol Pharm. 2020;17:488-498.

44. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, et al. Membrane transporters in drug development. Nat Rev Drug Discov. 2010;9:215-236.

45. Prasad B, Gaedigk A, Vrana M, Gaedigk R, Leeder JS, Salphati L, et al. Ontogeny of hepatic drug transporters as quantified by LC-MS/MS proteomics. Clin Pharmacol Ther. 2016;100:362-370.

46. Eley T, Han YH, Huang SP, He B, Li W, Bedford W, et al. Organic anion transporting polypeptide-mediated transport of, and inhibition by, asunaprevir, an inhibitor of hepatitis C virus NS3 protease. Clin Pharmacol Ther. 2015;97:159-166.