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## **Emerging transporters of clinical importance: an update from the International Transporter Consortium**

Hillgren, K M; Keppler, D; Zur, A A; Giacomini, K M; Stieger, B; Cass, C E; Zhang, L

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# Clinical Pharmacology & Therapeutics

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Kathleen M. Hillgren, Dietrich Keppler, Arik Zur, Kathleen M. Giacomini, Bruno Stieger, Carol E. Cass, and Lei Zhang

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## Emerging Transporters of Clinical Importance: An Update from the International Transporter Consortium

Kathleen M. Hillgren<sup>1\*</sup>, Dietrich Keppler<sup>2\*</sup>, Arik Zur<sup>3</sup>, Kathleen M. Giacomini<sup>4</sup>, Bruno Stieger<sup>5</sup>,  
Carol E. Cass<sup>6</sup>, and Lei Zhang<sup>7\*</sup>

(on behalf of the International Transporter Consortium)

<sup>1</sup> Drug Disposition, Lilly Research Laboratories, Indianapolis, IN 46285, USA; Email: [k\\_hillgren@lilly.com](mailto:k_hillgren@lilly.com); Tel: 1-317-433-6678

<sup>2</sup> German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany; Email: [d.keppler@dkfz.de](mailto:d.keppler@dkfz.de); Tel: 49-6221-422400

<sup>3</sup> Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, USA; Email: [Arik.Zur@ucsf.edu](mailto:Arik.Zur@ucsf.edu); Tel: 415-476-1936

<sup>4</sup> Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, USA; Email: [Kathy.Giacomini@ucsf.edu](mailto:Kathy.Giacomini@ucsf.edu); Tel: 415-476-1936

<sup>5</sup> University Hospital, Department of Clinical Pharmacology and Toxicology, 8091 Zurich, Switzerland; Email: [bstieger@kpt.uzh.ch](mailto:bstieger@kpt.uzh.ch); Tel: 41-44-634-3169

<sup>6</sup> Department of Oncology, University of Alberta, Cross Cancer Institute, 11560 University Ave, Edmonton, AB, T6G2H7, Canada; Email: [carol.cass@ualberta.ca](mailto:carol.cass@ualberta.ca); Tel: 780-436-4911

<sup>7</sup> Office of Clinical Pharmacology, Office of Translational Sciences, CDER, FDA, 10903 New Hampshire Ave, Building 51, Room 3196, Silver Spring, MD, USA; Email: [leik.zhang@fda.hhs.gov](mailto:leik.zhang@fda.hhs.gov); Tel: 301-797-1635

\* Corresponding authors

\*Kathleen M. Hillgren, Ph.D.  
Department of Drug Disposition  
Lilly Research Laboratories  
Indianapolis, IN 46285, USA  
Tel: 317-433-6678  
Email: [k\\_hillgren@lilly.com](mailto:k_hillgren@lilly.com)

\*Prof. Dr. Dietrich Keppler  
German Cancer Research Center (DKFZ)  
Im Neuenheimer Feld 280  
D-69120 Heidelberg, Germany  
Tel: 49-6221-422400  
Fax: 49-6221-422402  
Email: [d.keppler@dkfz.de](mailto:d.keppler@dkfz.de)

\*Lei Zhang, Ph.D.

Office of Clinical Pharmacology  
Office of Translational Sciences  
CDER, FDA  
10903 New Hampshire Ave  
Building 51, Room 3196  
Silver Spring, MD 20993-0002, USA  
Tel: 301-797-1635  
Email: [leik.zhang@fda.hhs.gov](mailto:leik.zhang@fda.hhs.gov)

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**Abstract (150 words)**

The International Transporter Consortium (ITC) has described recently seven transporters of particular relevance for drug development (Giacomini et al., Nat. Rev. Drug Discov. 9: 215-236, 2010). Based on the second ITC transporter workshop in 2012, we have identified additional transporters of emerging importance in pharmacokinetics, interference of drugs with transport of endogenous compounds and drug-drug interactions in humans. The multidrug and toxin extrusion proteins (MATEs, gene symbol *SLC47A*) mediate excretion of organic cations into bile and urine. MATEs are important in renal drug-drug interactions. Multidrug resistance proteins (MRPs or ABCCs) are drug and conjugate efflux pumps, and impaired activity of MRP2 results in conjugated hyperbilirubinemia. The bile salt export pump (BSEP or ABCB11) prevents accumulation of toxic bile salt concentrations in hepatocytes, and BSEP inhibition or deficiency may cause cholestasis and liver injury. Additionally, examples are presented on the roles of nucleoside and peptide transporters in drug targeting and disposition.

**INTRODUCTION**

Transporters are membrane bound proteins that control access of endogenous substances and xenobiotics (drugs) to various sites of the human body (1, 2). In contrast to drug metabolizing enzymes, which are largely concentrated in the liver and intestine, transporters are present in all tissues in the body and play important roles in drug absorption, distribution, tissue-specific drug targeting, and elimination, thus influencing drug pharmacokinetics (PK) and pharmacodynamics (PD). Transporters can also work in concert with metabolizing enzymes in affecting a drug's PK and PD. Similar to metabolizing enzymes, transporters have substrate

binding sites that are saturable and can be inhibited (3). Moreover, some transporters are inducible, or can change their localization by endocytic retrieval and exocytic insertion (3).

The human genome project has identified more than 400 transporters that belong to one of two superfamilies: ATP-binding Cassette (ABC) or Solute Carrier (SLC). Changes in transporter expression or activity either via genetic factors or drug-drug interactions (DDIs) can contribute to variability in drug exposure and response. The advancement of molecular cloning of cDNAs encoding various transporters and *in vitro* cell expression systems to study interactions of drugs with transporters has enabled researchers to examine the underlying mechanisms of DDIs mediated by transporters. This improved understanding has provided the foundation to predict *in vivo* DDIs based on *in vitro* assays. For example, it was found that many statin drugs are substrates of the organic anion transporting polypeptide (OATP1B1, gene symbol *SLCO1B1*); their interactions with cyclosporine (a broad transporter and enzyme inhibitor) are therefore “anticipated”. In addition, recent findings that many HIV protease inhibitors are OATP1B1/OATP1B3 inhibitors are critical in the design of clinical DDI studies to manage a myriad of potential drug interactions between HIV protease inhibitors and other concomitantly administered drugs, including statins (4).

In 2007, the International Transporter Consortium (ITC), which includes members from academia, industry and the Food and Drug Administration (FDA), was formed with the goal of determining transporters that are of emerging importance in clinical DDIs, establishing standards for *in vitro* evaluation of transporter-based interactions that may reduce the need for *in vivo* studies, and achieving, where possible, a consensus on current knowledge of transporters in drug

development (5). The ITC organized an FDA critical path initiative-funded transporter workshop in October 2008 and authored a transporter whitepaper that was published in Nature Reviews Drug Discovery in March of 2010 (2). The publication shared experiences, stimulated further discussion, and provided strategic directions in the following scientific areas: key transporters with clinical implications, *in vitro* methodologies, and decision trees for key transporters as to when to conduct *in vitro* and *in vivo* DDI evaluations (2).

The ITC whitepaper identified seven transporters as having compelling clinical evidence of involvement in clinical DDIs (Figure 1). These were: P-gp (MDR1, gene symbol *ABCB1*), breast cancer resistance protein (BCRP, *ABCG2*), OATP1B1, OATP1B3 (gene symbols *SLCO1B1* and *SLCO1B3*), organic cation transporter 2 (OCT2, *SLC22A2*) and organic anion transporters 1 and 3 (OAT1/OAT3, *SLC22A6/SLC22A7*) (2). The whitepaper recommended that these transporters should be studied *in vitro* to determine the potential of clinical DDIs and proposed decision trees to determine if clinical studies should be conducted to evaluate the propensity for clinically relevant DDIs.

The FDA's 2012 draft drug interaction guidance (6) included recommendations on when to evaluate transporter-based DDIs. The European Medicines Agency (EMA) also included recommendations on transporters in their recently published drug interaction guidelines (7).

In addition to contributing to DDIs, transporters may contribute to drug tissue toxicity or serve as treatment targets. In March 2012, the ITC organized a second transporter workshop that included discussions of emerging transporters involved in DDIs, efficacy, and drug induced

toxicity, including multidrug and toxin extrusion transporters (MATEs, gene symbol *SLC47A*), multidrug resistance proteins (MRPs, ABCCs, gene symbols *ABCCs*) and the bile salt export pump (BSEP, ABCB11, gene symbol *ABCB11*) (Figure 1) (8). In addition, clinically important examples involving nucleoside transporters and peptide transporters were also presented (8).

The goal of this whitepaper is to discuss the research and clinical observations available for emerging transporters of clinical importance. The clinical relevance and importance of these transporters and recommendations on how to study them during drug development and what criteria may be used to assess their role in DDIs and toxicity are discussed.

## **The emerging transporters MATE1 and MATE2K**

### ***General description***

The multidrug and toxin extrusion proteins (MATEs) include three major functional solute carriers: MATE1 (*SLC47A1*), MATE2 (*SLC47A2*) and the splice variant MATE2K. The three functional isoforms are expressed abundantly in the apical membrane of the renal proximal tubule and play roles in the secretion of cations and zwitterions into urine (1, 9, 10). They function as cation/H<sup>+</sup> antiporters in tandem with organic cation transporters (*e.g.*, OCT2) localized at the basolateral membrane of proximal tubule cells. In addition, MATE1 is highly expressed on the canalicular membrane of hepatocytes where it appears to cooperate with OCT1 in mediating biliary excretion. The PK, efficacy, safety and/or tissue levels of a substrate of MATEs may be altered by changes in transporter function and/or level of expression caused by genetic polymorphisms or DDIs. For more detail, see recent reviews that describe molecular



characteristics, expression and function of MATEs (9, 10). Current evidence is strongest for MATE1 and MATE2K and accordingly this consortium update focuses on them.

### ***Structure***

The human *SLC47A1* (MATE1) and *SLC47A2* (MATE2) genes are located in tandem on chromosome 17p11.2 and encode membrane proteins of 570 and 602 amino acids, respectively (11). MATE2K, which lacks part of exon 7 due to alternative splicing, is a 566 amino acid protein. Various structural studies suggest that human MATE1 topology includes a 13 transmembrane helix with an extracellular COOH terminus (12). Homology models of human MATEs based on the available bacterial X-ray structure (*i.e.*, NorM) are emerging but have not yet been used effectively in structure based screens of large drug databases (13). Site directed mutagenesis studies of human MATEs have identified several structural motifs with importance for transport activity (a detailed list of residues can be found in the supplementary material).

### ***Genetic variants***

Polymorphisms found in the *SLC47A1* and *SLC47A2* genes include both regulatory single nucleotide polymorphisms (rSNPs) and non-synonymous and synonymous SNPs in the coding region (cSNPs) (9). Seven rSNPs in MATE1/2 have been characterized in reporter assays, and fifteen cSNPs have been characterized in transporter assays (14). A detailed list of the reported polymorphisms in the two genes is summarized in Supplementary Table 1. The clinical significance of some of these SNPs has been linked to clinical effects in metformin treated subjects. Separate studies have shown that the MATE1 intronic variants rs2289669 and

rs8065082 have significant effects on metformin response. Similarly a common promoter region variant in MATE2K, rs12943590 (-130G>A), was associated with reduced metformin response (15).

### ***Methodology for evaluating function***

Under physiological conditions, proximal tubule cells express MATE1 and MATE2K in the brush border membrane, where sodium/proton exchangers maintain a proton gradient in which the extracellular fluids of the tubule lumen are slightly more acidic than the cytosol. For *in vitro* studies, cell lines expressing MATE1 and MATE2 or MATE2K are recommended. Currently, there is little information on differences in substrate or inhibitor specificity between MATE2 and MATE2K, therefore, cell lines expressing MATE2K are generally used as models of both MATE2 isoforms. Several *in vitro* assays have been designed to mimic physiologic parameters with epithelial cell lines and used to test MATE1 or MATE2K function. Human Embryonic Kidney cells (HEK), Chinese Hamster Ovary cells (CHO) or Madin-Darby Canine Kidney cells (MDCK) are the most commonly used cell lines and are transfected with a MATE recombinant ortholog or vector control and used in adherent culture. Ammonia pre-pulse is commonly used to acidify the cytosol and reverse transport direction. Then a substrate is applied and taken into the cell against the flux of protons from the cytosol. Although these assays measure substrate influx, MATEs exhibit reversible transport, and the assays can be easily used to identify MATE ligands. Variations to the experimental setting have been reported and include extracellular pH changes to avoid preloading of cells, and use of polarized cells and double transfected cells (MATE1/2K and OCT2) for measurement of transport through a monolayer of differentiated cells (16). These *in vitro* assays are instrumental when assessing whether a new

molecular entity (NME) is a MATE substrate or inhibitor (17). Mouse is the preferred animal model to study *Mate1*, but not *Mate2* because mice do not express either *Mate2* or *Mate2K* in their kidneys (18). Mice have two functional genomic variants for *Slc47a1* (i.e., *mSlc471a* and *mSlc471b*). (9, 10). *mMate1* knockout mice have been instrumental in elucidating the systemic distribution and clearance of endogenous compounds and xenobiotics *in vivo*. The physiologic and pharmacological relevance of *mMate1* has been demonstrated for metformin, cephalexin, cisplatin, paraquat and other molecules, and usually correlates well with the human ortholog. However, differences in ligand affinity between orthologs as well as general limitations of interpretation of rodent PK data (*e.g.*, Cyp450 activity) need to be considered (Supplementary Table 2).

### ***Substrate and inhibitor selectivity***

To date, over 900 compounds have been tested as substrates and inhibitors of MATEs, and several structure activity relationship models have been proposed. Typical substrates for MATEs, which overlap with OCT substrates, are hydrophilic, low molecular weight organic cations such as metformin and 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>). However, in contrast to OCTs, MATEs appear to transport a wider range of chemical structures including anionic compounds (*e.g.*, acyclovir, gancyclovir and estrone sulfate) and zwitterions (*e.g.*, cephalexine and cephradine). Supplementary Table 2 lists various ligands reported in the literature and their relative selectivities for MATEs or OCTs. For transepithelial flux, it is thought that MATEs partner with OCTs for cationic substrates and with organic anion transporters (OATs) for zwitterionic and anionic substrates. The substrate specificities of MATE1 and MATE2K are highly similar, but not identical, as is underscored by the MATE2K preferred substrate,

oxaliplatin, which interacts poorly with MATE1. Physicochemical properties of inhibitors of MATE1 and MATE2K include a positive charge at pH 7.4, a high LogP value and a large molecular weight (16, 17). Selectivity for MATEs over OCT2 is exhibited by cimetidine and pyrimethamine, both of which can interact with MATEs at clinically relevant concentrations.

### ***Clinical significance and drug development recommendations***

Two lines of evidence suggest that MATEs are important determinants of both PK and PD in humans. First, as noted above, studies of genetic polymorphisms in both *SLC47A1* and *SLC47A2* suggest that these transporters play important roles in the renal elimination and pharmacologic effects of metformin. In some cases measurement of PD endpoints seem more sensitive for DDI identification than apparent changes in plasma concentrations. Second, DDI studies with selective inhibitors of MATEs such as cimetidine and pyrimethamine (*e.g.*, >10-fold more potent in comparison to OCT2) have demonstrated substantial effects of MATE inhibitors on renal drug clearance and drug concentrations in plasma (AUC and  $C_{\max}$ ) (Table 1). Previous studies had suggested that cimetidine causes DDIs by inhibiting OCT2; however, in light of the *in vitro* studies showing that cimetidine interacts more potently with MATEs than with OCT2, these DDIs are now believed to be mediated through inhibition of MATE1 and/or MATE2K.

In view of the strong *in vitro* and *in vivo* evidence that MATEs are sites of renal DDIs, the ITC recommends that clinical decision trees for evaluating the interactions of MATEs with NMEs be adopted and included along with OCT2 and OAT1/OAT3 (Figures 2 and 3). The

first decision tree, is targeted to NMEs for which renal secretion is an important route of drug elimination. For such compounds, *in vitro* uptake studies in MATE transfected cells are recommended. If the NME is found to be a substrate of MATEs (or OCT2), a clinical study is recommended to be considered with cimetidine or pyrimethamine as inhibitors. When evaluating the potential of a NME to cause a DDI due to inhibition of MATEs, the decision tree in Figure 3, which is based on previously published ITC recommended cutoffs, suggests an empirical approach (4). That is, therapeutic maximum unbound concentrations of the NME that are greater than or equal to 1/10th of its *in vitro* IC<sub>50</sub> or K<sub>i</sub> value suggest a DDI potential with drugs that are substrates for MATEs or OCT2. For most clinical studies of MATE substrates or inhibitors, creatinine clearance, which is often used as a measure of filtration clearance, may be replaced by other methods to evaluate filtration clearance since creatinine is a substrate of MATEs (19, 20).

## **Multidrug Resistance Proteins (MRPs, ABCCs): The drug and conjugate efflux pumps**

### **MRP2, MRP3, and MRP4**

#### ***General description***

Among the nine multidrug resistance proteins (MRPs) encoded in the human genome, MRP2 (*ABCC2*), MRP3 (*ABCC3*), and MRP4 (*ABCC4*) have received particular attention because of their roles in the disposition of drugs and conjugates (21). MRPs are unidirectional, ATP-dependent efflux pumps in the plasma membrane of human cells and many other organisms (22). Human MRP2, formerly also termed canalicular multispecific organic anion transporter (cMOAT), was the second member of the MRP subfamily to be identified by molecular cloning of its cDNA, and is localized to the apical membrane of hepatocytes, intestinal epithelia, and proximal tubules of the kidney (23). Accordingly, it is essential for hepatobiliary and renal

elimination of many anionic substrates, including drugs and conjugates such as bilirubin glucuronides. Conjugated hyperbilirubinemia in patients suggests MRP2 dysfunction. MRP2 of intestinal epithelia pumps its substrates into the intestinal lumen. In hepatocytes, MRP3 and MRP4 are localized to the basolateral (sinusoidal) membrane and mediate efflux of substrates, particularly glucuronides and glutathione conjugates, into sinusoidal blood. Thus, they play a compensatory role under conditions of cholestasis and when MRP2 function is impaired. MRP4 has a number of additional localizations important for drug elimination and action: it is localized to the apical (luminal) membrane of kidney proximal tubules and epithelia of the blood-brain barrier (24) and to membranes of blood platelets (25).

### ***Structure and function***

Human MRP2 is an integral plasma membrane glycoprotein composed of 1,545 amino acids. The total number of amino acids for MRP3 and MRP4 is 1,527 and 1,325, respectively, and the amino acid identities of MRP2 compared to MRP3 and MRP4 are 48% and 39%, respectively (22, 23). All MRPs contain two ATP-binding domains. The *ABCC* genes encoding the MRP transport proteins considered here are located on different chromosomes, namely 10q24 for MRP2, 17q22 for MRP3, and 13q32 for MRP4 (22, 23). At present, MRP2 orthologs have been cloned from more than 20 organisms, ranging from cellular slime molds, plants, bony fishes, and birds to many mammalian species, including human, rat, mouse, dog, rabbit, and monkey. Thus, MRP2 is a phylogenetically ancient efflux pump involved in detoxification. Genetic variants in mammals leading to an inactive MRP2 transporter are usually well compensated by alternative efflux pathways, including basolateral hepatocellular MRP3 and apical MRP4 in kidney proximal tubule cells.

The members of the MRP subfamily exhibit an overlap in substrate selectivity, but differ in cell and tissue distribution and in domain-specific cellular localization (21). MRP2 is the only member that is localized exclusively to the apical membrane domain of polarized cells. Specific antibodies serve to localize the MRP2 protein in the apical domain of various polarized cell types, including hepatocytes, kidney proximal tubule cells, human small intestine, colon, gall bladder, segments of bronchi, and placenta. MRP2 protein is absent or below current detection limits in several other normal human cell types and tissues, including endothelial cells of the blood-brain barrier.

Typical substrates for which both human MRP2 and rat Mrp2 have high affinities include many glutathione conjugates of drugs and endogenous compounds, notably the arachidonic acid-derived glutathione conjugate termed leukotriene C<sub>4</sub>, as well as glucuronic acid conjugates, including bilirubin monoglucuronide and bilirubin bisglucuronide (23). Synthetic substrates include bromosulfophthalein, cholecystokinin octapeptide (CCK-8), cholyl-L-lysyl-fluorescein, fluo-3, carboxy-2',7'-dichlorofluorescein (CDF), and methotrexate. Major differences in the substrate specificities of recombinant human and rat MRP2/Mrp2 have not yet been detected (23).

Important substrates for human MRP3 include glucuronic acid conjugates of drugs and endogenous substances (26), particularly bilirubin monoglucuronide and bilirubin bisglucuronide (21). MRP3 plays a clinically important role in jaundice with conjugated hyperbilirubinemia. It is the interaction between canalicular MRP2 and sinusoidal (basolateral) MRP3 with efflux of

bilirubin glucuronides from hepatocytes into blood that explains the conjugated hyperbilirubinemia in cholestasis and under conditions of impaired function of MRP2. Subsequently, bilirubin glucuronides can be eliminated via the kidneys into urine. In intestinal epithelia, basolateral MRP3 may also function in transport of glucuronides, formed intracellularly, into blood. Detailed studies in knock-out mice lacking *Mrp3* support the role of this basolateral efflux pump in the transport of bilirubin glucuronides from cells into blood (27).

The substrate specificity of MRP4 is relatively broad compared to other MRP subfamily members (21, 24, 28). This is due in part to the broadening of the substrate spectrum in the presence of GSH, which is normally present in living cells at millimolar concentrations, as exemplified by the MRP4-mediated ATP-dependent transport of bile acids (28) and leukotriene B<sub>4</sub>, which occur only in the presence of GSH. Additional substrates of MRP4 include leukotriene C<sub>4</sub>, prostanoids, cyclic nucleotides, ADP, urate, dehydroepiandrosterone 3-sulfate, and the synthetic nucleoside phosphonate analog 9-(2-phosphonylmethoxyethyl) adenine (PMEA) (24, 26).

Inhibitors of high selectivity are not yet available for MRP2, MRP3, or MRP4. Useful nonselective inhibitors, however, include several quinoline derivatives, developed as leukotriene D<sub>4</sub> receptor antagonists such as MK-571. MK-571 was originally identified as a potent inhibitor of MRP1-mediated transport and is now widely used as a nonspecific *in vitro* inhibitor of the transport of many organic anions mediated by MRP1, MRP2, MRP3, and MRP4 (21). However, it should be noted that, in addition to the action of MK-571 on MRP subfamily members, this anionic quinoline derivative is a potent inhibitor of the hepatocellular uptake transporters



OATP1B1, OATP1B3, and OATP2B1 (29). Cyclosporin A acts as a good but nonselective inhibitor of MRP2, in addition to its more potent inhibition of MDR1, BSEP (30), and the uptake transporters OATP1B1 and OATP1B3 (29). Corresponding inhibitory actions on uptake as well as efflux have been described for rifampicin and rifamycin SV. Thus, for studies *in vivo* and with intact cells, the lack of selectivity with respect to MRP subfamily members as well as the action of the inhibitors on uptake transporters must be considered.

### ***Genetic variants***

A large number of genetic variants in the *ABCC2* gene encoding MRP2 have been identified in various ethnic groups, but only some of these variants lead to loss of the MRP2 protein or to loss of functionally active MRP2 in apical plasma membranes (23). A complete loss of functionally active MRP2 in the hepatocyte canalicular membrane has been recognized as the molecular basis of Dubin-Johnson syndrome (31), which is associated with mild, predominantly conjugated hyperbilirubinemia, and upregulation of basolateral MRP3 in human hepatocytes and Mrp3 in Mrp2-deficient rats (21). Under this condition, MRP3/Mrp3 compensate for the impairment of MRP2/Mrp2 function by efflux into blood of conjugated bilirubin and other endogenous and xenobiotic anionic substrates of MRP2 and MRP3.

Multiple genetic polymorphisms exist in the *ABCC3* gene (32). However, so far no major PK consequences have been documented. The *ABCC4* gene is highly polymorphic, and large variability of *ABCC4*/MRP4 mRNA (38-fold) and protein (45-fold) expression was found in human liver, and significant upregulation was observed in livers of patients with cholestasis (33). A common single-nucleotide polymorphism (rs3765534; >18% in the Japanese population),

which reduces MRP4 function, seems to be associated with thiopurine-induced hematopoietic toxicity (34).

### ***Methodology for evaluating function***

The exact substrate specificity of the ATP-dependent efflux pumps of the MRP subfamily, MRP2, MRP3, and MRP4, may be defined in inside-out-oriented membrane vesicles for which substrate concentrations can be adjusted and metabolism or complex formation, as it may occur in intact cells and *in vivo*, can be controlled. Moreover, Table 2 presents some recommended probe substrates and inhibitors. It should be noted that many MRP substrates are only generated intracellularly in Phase II reactions forming the respective glutathione, sulfate, and glucuronide conjugates, as illustrated by *S*-(2,4-dinitrophenyl)glutathione and glucuronosyl bilirubin.

An extensive analysis of the prediction and identification of *in vitro* drug interactions with human MRP2 was published by Pedersen *et al.* (35) using inside-out-oriented membrane vesicles from Sf9 cells and estradiol-17 $\beta$ -D-glucuronide as the substrate. This study with a large set of structurally diverse drugs and drug-like compounds identified many inhibitors, noninhibitors, and even stimulators of MRP2-mediated transport (35). The stimulators exhibited similar charge distributions as MRP2 substrates, with at least one negative charge at physiologic pH. As pointed out above, such a study gains additional physiological relevance when performed in the presence and absence of GSH which is present in living cells at millimolar concentrations. Moreover, for drugs and drug candidates that undergo predominantly hepatobiliary elimination, it is essential to determine if glucuronate or glutathione conjugates are formed intracellularly and represent inhibitors or substrates of MRP2, MRP3, or MRP4.

For a NME, it is important to examine MRP2 inhibition if drug-induced conjugated hyperbilirubinemia is observed in patients or in preclinical species. To date no classical PK DDI has been attributed to MRP2 and there is no evidence that MRP3 or MRP4 should be examined or that there is a likelihood of DDIs or toxicity due to being a substrate of MRP2. It is plausible, but not proven, that hepatotoxicity may result, however, from compounds or metabolites that potentially inhibit MRP2 as well as MRP3 and MRP4 and thus cannot exit from hepatocytes via alternative pathways.

### **The bile salt export pump (BSEP) and its role in hepatotoxicity**

#### ***General Description***

In addition to elimination of drugs and xenobiotics, bile formation is another key function of the liver. Bile has two major physiologic functions: it provides a vehicle for excretion of poorly water soluble substances (*e.g.*, cholesterol) and is essential for digestion and absorption of fat and fat soluble vitamins in the intestine. Bile is mainly composed of small ions, organic anions, lipids (*e.g.*, phosphatidylcholine) and bile salts (36). Bile salts are amphipathic molecules, have detergent properties and are highly concentrated in bile (36) where they form mixed micelles with phospholipids, which act as carriers for poorly water soluble substances (36). Due to their detergent properties, bile salts can be highly toxic to hepatocytes if they accumulate intracellularly (37).

Bile salts are taken up from the sinusoidal blood plasma by several transporters into hepatocytes (38), where they mix with newly synthesized bile salts. Bile salts are secreted by the

BSEP into canaliculi, which constitute the starting points of the biliary tree (39, 40). The BSEP mediates the rate-limiting step of bile salt transport across hepatocytes for which there is not a backup system in the canalicular membrane. Consequently, any impairment of BSEP function can potentially lead to accumulation of cytotoxic bile salts in hepatocytes.

### ***Function***

BSEP has a narrow substrate specificity and transports conjugated monovalent bile salts in addition to taurolithocholyl 3-sulfate (39, 41). Data on transport of unconjugated bile acids such as cholic acid are conflicting (40). For taurocholate,  $K_m$  values ranging from 5 to 22  $\mu\text{M}$  have been reported by different groups in different expression systems (40) and other bile salts have  $K_m$  values in this range (40). The intrinsic clearances for human, rat and mouse BSEP have the same rank order (40, 42), indicating similar transport properties of BSEPs from these three species. In addition to bile salts, pravastatin has been reported to be a BSEP substrate, albeit with low affinity (40), and indirect evidence from sandwich-cultured hepatocytes suggests that the antifungal micafungin may be transported by BSEP (43). Taking together the evidence obtained so far, it seems unlikely that BSEP plays a major role in drug export from hepatocytes into bile and consequently in drug disposition.

### ***Genetic variants***

Much has been learned about the physiologic role of BSEP from patients with inherited liver diseases due to impaired BSEP function. *ABCB11* encoding BSEP was identified by positional cloning in families with progressive familial intrahepatic cholestasis (PFIC) type 2 (44). Four mutations identified in this study predict premature truncation of BSEP, while the

other mutations are missense mutations. Such patients develop progressive cholestatic liver disease with low biliary bile salt concentrations (45). Basolateral efflux via MRP4 and the organic solute transporter OSTalpha/OSTbeta is not sufficient to compensate for genetic variants resulting in the absence of BSEP protein, clearly demonstrating the absence of a functional backup system in the canalicular membrane for secretion of bile salts. Some mutations in *ABCB11* lead to less severe forms of cholestatic liver disease called benign recurrent intrahepatic cholestasis type 2 (BRIC2). The clinical spectrum of *ABCB11* mutations covering BRIC2 to PFIC2 is also known as BSEP deficiency syndrome (45).

It can be inferred from patients with benign BSEP deficiency syndrome that in symptom-free intervals the residual BSEP transport capacity is sufficient for a seemingly normal liver function. Consequently, inhibition of BSEP activity by endogenous or exogenous substances below (a yet unknown) threshold function can lead to cholestasis. Cyclosporine is a drug leading to acquired cholestasis and consequently to drug induced liver injury (DILI) in some patients (41). Using rat Bsep as a model, competitive inhibition of Bsep by drugs known to be associated with drug-induced cholestasis was demonstrated (46). This finding was later extended to human BSEP (42, 47). To date, a large variety of drugs and xenobiotics have been reported to interfere with BSEP function *in vitro*, the information for which is summarized in (40, 41, 48, 49).

Drug-induced cholestasis is a rare but often serious side effect of drugs (50). Consequently, investigations for identification of susceptibility factors are ongoing, but have so far proved difficult (50). With respect to factors affecting BSEP, information is scarce. The most common polymorphism in *ABCB11* is c.1331T>C (p.V444A) and is ethnicity dependent with a

frequency around 60% in Caucasian populations (51). The c.1331C variant leads to lower BSEP protein levels (52) and is overrepresented in patients with drug induced cholestasis as well as in patients with intrahepatic cholestasis of pregnancy (50, 51). As the two BSEP variants have indistinguishable kinetic properties and both variants are almost equally abundant in some populations, the role of this polymorphism as a susceptibility factor for drug- induced cholestasis is, as yet, unknown.

### ***Methodology for evaluating function***

Bile salts are negatively charged and can barely cross membranes. Therefore, studies with isolated membrane vesicles are the best technical approach to investigate transport mediated by BSEP. ATP-dependent taurocholate transport into canalicular plasma membrane vesicles is electrogenic (40). Inhibition studies with NMEs and BSEP are preferentially done at taurocholate concentrations of 5 to 10  $\mu\text{M}$  in the presence of a permeant anion (for compensation of charge movement). The suggested taurocholate concentration is in the range of the  $K_m$  value of BSEP (40) and allows an estimation of  $K_i$  values from  $\text{IC}_{50}$  values. If a NME inhibits BSEP with a low  $K_i$  value, additional experiments may be warranted. One additional test system for a NME is human hepatocytes cultured in a sandwich configuration (53). This system offers the advantage that it is metabolically competent and to some extent includes the potential impact of metabolites on BSEP. In addition, experiments in a preclinical species, *e.g.* rats, may be warranted. If the NME is also an inhibitor of rat Bsep, application of the NME in a repeat dose study for several weeks to animals at a dose leading to at least the expected human serum concentrations will be helpful. It is recommended that this test includes sampling of serum on a daily basis followed by measurements of bile salt concentration, alkaline phosphatase, and transaminases. A course of

serum parameters showing a steady increase of bile salts, even if before elevation of transaminases, points to a likely Bsep interaction. At the end of the experiment, bile flow may be determined in control and treated animals, as any reduction of bile flow is a very sensitive indicator for cholestasis. If a NME showing a Bsep interaction in rodents is given to humans, serum bile salt levels as well as serum markers may be monitored from the very beginning. For humans, the revised standards for drug-induced liver injury may serve as a guideline (54).

### ***Clinical significance and drug development recommendations***

The decision whether or not to investigate a NME for BSEP interaction may depend on various factors. As BSEP is an unlikely player in disposition of NMEs, concerns may focus on potential DILI related to BSEP. Here, inhibition of BSEP will lead to intracellular accumulation of bile salts followed by cytotoxic events.

Currently, it is impossible to define a value for a BSEP inhibition constant that will realistically predict significant BSEP-mediated DILI. The reasons are: 1) While a trend between low  $IC_{50}$  values of BSEP inhibition and DILI was described (48, 49), no correlation between  $C_{max, unbound}$  with the potency of BSEP inhibition and with DILI was observed (48), showing that the uptake mechanism of drugs into hepatocytes is a major contributor to intracellular drug concentrations. At present, the exact transport mechanism and in particular the energetics of the OATPs, which are the major drug uptake systems into hepatocytes, are poorly understood. It should also be noted that cyclosporin A is a very potent BSEP inhibitor although it is widely used in solid organ transplantation despite the occurrence of cholestatic episodes. In addition, there is large interindividual variability of BSEP protein levels in normal human liver

contributing to adverse actions (52). 2) Many drugs form an array of metabolites, some of which may be even more potent BSEP inhibitors than the parent drug and act synergistically. Examples are troglitazone and bosentan (41). The bosentan interaction is discussed in its EU label. 3) Some drugs may in addition to interference with BSEP also impair mitochondrial function (*e.g.*, troglitazone (41)) forming reactive intermediates. Such a situation may lead to a synergism in toxicity, as elevated intracellular bile salts may aggravate mitochondrial toxicity of a metabolite (41). And 4) There are BSEP inhibitors that require parallel transport activity of BSEP and MRP2 in the same membrane. This was first demonstrated for estradiol 17 $\beta$ -glucuronide (46) and later for drug metabolites (50). Drugs have been identified that are BSEP inhibitors but are not associated with DILI. In conclusion, prospective BSEP testing cannot be endorsed at this moment without a strategy to assess clinical relevance of such inhibition, but *in vitro* characterization of BSEP-drug interactions are certainly warranted after the appearance of cholestatic issues in clinical trials or safety studies. However, the EMA DDI guideline states that investigating BSEP inhibitory potential should be considered. If inhibition is indicated, adequate biochemical monitoring including serum bile salts is recommended during drug development.

### **Other Transporters of Clinical Importance**

The transporters described above and in the previous ITC white paper have the potential to interact with a broad range of drugs. However, it is important to keep in mind that for certain classes of drugs other transport mechanisms may be important. Two such examples are given below: Human equilibrative nucleoside transporter 1 (hENT1) which is important in the delivery



of certain drugs to tumors and the human intestinal Peptide Transporter which is important in the oral delivery of many peptide-like drugs.

## **Human equilibrative nucleoside transporter 1 (hENT1)**

### ***General description***

Nucleosides have diverse roles in many processes, including cellular metabolism, signaling and proliferation. Many nucleoside analogs are used clinically for treatment of cancer and viral diseases. Since most nucleosides are hydrophilic, their cellular uptake and release is dependent on the activity of membrane transport proteins. Two evolutionarily unrelated protein families are responsible for nucleoside transport in humans: the Solute Carrier (SLC)28 and SLC29 families of nucleoside transporters (NTs), whose members are known, respectively, as the human (h) concentrative and equilibrative NTs (hCNTs; hENTs)(for recent reviews see (55-57)). Three isoforms in the hCNT family and four in the hENT family have been identified and functionally characterized by molecular cloning and expression of their cDNAs. These isoforms, the genes encoding them and their chromosomal locations are: hCNT1, *SLC28A1*, 15q25.3; hCNT2, *SLC28A2*, 15q15; hCNT3, *SLC28A3*, 9q22.2; hENT1, *SLC29A1*, 6p21.1; hENT2, *SLC29A2*, 11q13; hENT3, *SLC29A3*, 10q22.1; hENT4, *SLC29A4*, 7p22.1. The focus in this whitepaper will be on hENT family members and their roles in delivery of drugs to tumors. Current understanding of the distribution of hENTs in intestine, kidney, liver and brain (57) is summarized in Figure 1 .

Among members of the hENT family, the properties of hENT1 and hENT2 are well established (56). Both translocate nucleosides bidirectionally down their concentration gradients,

have broad substrate selectivities, and are widely distributed among tissues. They are functionally distinguished by differences in their sensitivities to transport inhibitors. hENT1 is potently inhibited by nitrobenzylmercaptapurine ribonucleoside (NBMPR) and its structural analogs with  $K_i$  values in the nM range whereas hENT2 is relatively insensitive with  $K_i$  values in the  $\mu$ M range. Because NBMPR and its structural analogs are highly specific for hENT1, they have been used extensively as molecular probes for identification and isolation of hENT1 in cells and tissues. hENT1 and hENT2 are integral membrane proteins with 11 transmembrane helices and regions important for inhibitor and substrate interactions have been identified for both proteins (57).

hENT1 and hENT2 both transport a wide range of purine and pyrimidine nucleosides, and hENT1 has higher apparent affinities for its substrates than hENT2. Both also have the capacity to transport nucleobases (hENT2 > hENT1), although the physiological importance of these activities is unknown (56, 57). In general, nucleoside analog drugs are poorer substrates for hENT1 and hENT2 than their physiological counterparts. Among clinical anticancer nucleosides, fludarabine, cladribine, clofarabine, 5-fluorouridine, 2'-deoxy-5-fluorouridine, azacytidine and decitabine are substrates of both, whereas gemcitabine and cytarabine are preferred by hENT1. Among clinical antiviral nucleosides, hENT2 appears to be more important than hENT1 in cellular uptake of zidovudine, zalcitabine and didanosine.

### ***Transport assays***

Because most human cell types contain multiple hNT types, the most reliable functional studies of hNTs are those conducted with (i) recombinant proteins produced individually in

otherwise NT-deficient cells, primarily the yeast *Saccharomyces cerevisiae* (engineered to lack NT activity), oocytes of *Xenopus laevis* (lack endogenous NT activity) and human cell lines (mutants that lack NT activity) and (ii) native proteins in cells that either produce a single transporter type (*e.g.*, hENT1-containing human erythrocytes) or that can be manipulated pharmacologically by treatment with selective NT inhibitors such that only a single transporter type is functional (*e.g.*, treatment of hENT1/2-containing cells with NBMPR). For methodological details see (58, 59). Studies of radioisotope fluxes can be undertaken with these systems to identify substrates and inhibitors, which for some hNT and cell combinations is difficult because of rapid equilibration of intra and extracellular nucleosides.

#### ***Clinical implications: hENT1 in anticancer therapies with nucleoside drugs***

Although the potential role of hENT1 in the clinical activity of nucleoside drugs has been recognized for decades (reviewed in (60)), evidence of its importance has only recently been obtained. The development of monoclonal antibodies against hENT1 (61) provided the tools for analysis of the distribution of hENT1 in human tissues, including immunohistochemistry studies of human cancer samples, which revealed marked variations in its abundance in a variety of cancers, giving rise to the hypothesis that low levels of hENT1 in target tumors predicts resistance to nucleoside drugs. In a retrospective study of pancreatic adenocarcinoma samples from deceased patients who received gemcitabine mono-chemotherapy for palliation, patients whose tumor cells all exhibited hENT1 immunostaining had longer survival times than those whose tumors contained cells that lacked hENT1 immunostaining (13 vs 4 months), indicating a relationship between hENT1 abundance and response to gemcitabine (62). A subsequent study of hENT1 abundance and gemcitabine therapy in a larger group of pancreatic adenocarcinoma

patients yielded similar findings (63). It has been proposed that resistance to nucleoside drugs because of low hNT abundance in target tumor cells can be circumvented by the development of nucleoside drugs that enter cells by other routes (*e.g.*, passive diffusion). Since pharmacologic resistance to anticancer nucleoside drugs is likely if other drugs administered simultaneously inhibit cellular uptake, NMEs with nucleoside-like structures may be assessed for their potential to inhibit hENT1 if they are likely to be administered with ENT substrates.

### Peptide transport

Another class of transporter that will handle selected targeted drugs is the Peptide Transporter Family (gene symbol *SLC15*) (64). There are four transporters in the *SLC15* family (*SLC15A1-4*). The proteins encoded and their chromosomal location are PEPT1 (*SLC15A1*, *13q33-q34*), PEPT2 (*SLC15A2*, *3q13.3-q21*), PHT1 (*SLC15A4*, *12q24.32*) and PHT2 (*SLC15A3*, *11q12.2*). PHT1 and 2 transport histidine and peptides and have not been shown clinically to play a major role in the disposition of drugs. PEPT1 and PEPT2 are the most studied transporters in the family and are key in the disposition of peptide-like drugs (*e.g.*, beta lactam antibiotics, ACE and renin inhibitors) and in peptide prodrugs (valacyclovir)(65). PEPT1 is apically located in the intestine and to a lesser extent in the kidney. PEPT1 is responsible for the uptake of di- and tri- peptides from the intestinal lumen into the blood and in the kidney for the scavenging of peptides from the urine back into the renal proximal tubule cells. PEPT2 is located in the kidney, but is also found at the blood cerebrospinal fluid barrier and the lung. PEPT2 is the primary mechanism in the proximal tubule cells for the reabsorption of di- and tri-

peptides from the urine. In the brain, PEPT2 is used to clear di- and tri-peptides from the cerebrospinal fluid (CSF) back into the blood and in the lung PEPT2 absorbs peptides.

Because of PEPT1's location and absorptive function in the intestine, it is a useful mechanism to increase the bioavailability of small, hydrophilic, charged molecules (64). It has been utilized to increase the bioavailability of amino acid- and nucleoside-like drugs. It is also known to transport some antibiotics. It transports molecules via a proton dependent mechanism taking advantage of the acidic micro-environment of the intestine to co-transport a substrate along with a proton(65). PEPT1 is considered a low affinity high capacity transporter. The affinity ( $K_m$ ) for natural substrates is in the high micromolar range and for drugs such as beta lactam antibiotics the  $K_m$  values are in the low millimolar range. However, since it has high capacity and is expressed along the length of the gut, it efficiently scavenges its substrates' uptake into intestinal enterocytes where they are eventually shunted into the blood.

The genetics of PEPT1 have been studied *in vitro* and the most frequent coding region SNP (S117N) does not lead to functional change *in vitro* (66). Two SNPs that do lead to functional changes *in vitro* (P586L and F28Y) have very low frequency in all populations studied to date (65). To date no clinical consequences for any PEPT1 variants have been found.

Functional studies for PEPT1 have been carried out in a number of heterologous expression systems. The transporter is easily expressed transiently in mammalian cell lines such as HEK, COS or HeLa or it can also be stably expressed (64). Many laboratories have also used oocyte expression to study peptide transport. PEPT1 is also expressed in intestinal cell lines

such as CACO-2. However, the expression level is low and requires 14 to 21 days in culture for maximal expression. The role of PEPT1 in absorption can also be studied in knockout mice (67). Although the knock-out strains have been characterized and used to study probe substrates, they are not currently commercially available.

### ***Clinical significance and drug development recommendations***

PEPT1 works well as a mechanism for oral delivery of small charged amino acid, peptide or nucleoside like drugs (65). The transporter appears not to be easily saturated and can deliver relatively large doses of drugs. For example, the cephalosporin antibiotics are given at gram doses. Review of labels for drugs (*e.g.*, cephalosporins, valacyclovir, enalapril) that utilize peptide transporters for absorption shows little concern for interactions during absorption as there are no consistent food interactions or interactions with other drugs transported by PEPT1. It is of note that PEPT1 appears to be upregulated in inflammatory bowel disease leading to expression of PEPT1 in the colon which is believed to be part of the proinflammatory response (68). Thus, although PEPT1 is normally thought of as a delivery mechanism, it may also be a pharmacological target for inflammatory bowel disease. There have also been studies as to its role in various cancers as a possible delivery mechanism (64, 65).

### **SUMMARY AND FUTURE PERSPECTIVES**

Membrane transporters of emerging importance in pharmacokinetics, drug-drug interactions, and interference of drugs with the transport of endogenous compounds are discussed in this paper. We focus here on human transporters involved in the uptake and efflux

of drugs that were not considered in detail in our previous ITC whitepaper on membrane transporters (2).

The multidrug and toxin extrusion proteins (MATEs) were discovered only in 2005 (11) and include MATE1, MATE2 and the splice variant MATE2K. They are expressed abundantly in the apical membrane of the renal proximal tubule and play a role in the secretion of cations and zwitterions into the urine. MATE1 is also highly expressed in the canalicular membrane of hepatocytes where it contributes to biliary excretion of cationic compounds. Many clinically used drugs, including metformin, cimetidine, and acyclovir, were identified as substrates for MATEs. There is compelling *in vitro* and *in vivo* evidence that MATEs are major sites of renal drug-drug interactions. ITC recommends that NMEs be evaluated as potential substrates or inhibitors for MATEs along with OCT2 and OAT1/OAT3, and decision trees for evaluating the interactions of MATEs with NMEs are presented. These decision trees will need to be tested in the clinic and revised if necessary as more data becomes available.

Within the subfamily of multidrug resistance proteins (MRPs, ABCCs), the ATP-driven efflux pumps MRP2, MRP3, and MRP4 have received particular attention because of their role in the disposition of anionic drugs and anionic conjugates. Human MRP2 is localized to the apical membrane of hepatocytes, intestinal epithelia, and proximal tubules of the kidney. Accordingly, it is essential for the hepatobiliary and renal elimination of many anionic substrates, including drugs and conjugates such as bilirubin glucuronides. In hepatocytes, MRP3 and MRP4 are localized to the basolateral (sinusoidal) membrane and mediate efflux of substrates, particularly glucuronides and glutathione conjugates, into sinusoidal blood and play a

compensatory role in cholestasis. Human MRP4 has a number of localizations important for drug elimination and action, including its presence in the apical (luminal) membrane of the kidney proximal tubule, the epithelium of the blood-brain barrier and membranes of the blood platelet. Drug-induced conjugated hyperbilirubinemia, in patients or in preclinical species, points to an inhibition of MRP2. Thus, under many conditions, conjugated hyperbilirubinemia may necessitate *in vitro* analyses using, for instance, inside-out-oriented membrane vesicles to elucidate the interaction of a compound with MRP2.

The bile salt export pump (BSEP) is exclusively localized in the hepatocyte canalicular membrane and prevents accumulation of toxic concentrations of bile salts in hepatocytes by unidirectional ATP-dependent transport into the biliary tree. BSEP has no functional backup and impairment of BSEP function may lead to liver disease as evidenced by increased plasma bile salt concentrations and liver enzymes. NMEs leading to cholestasis with increased plasma bile salt concentrations in preclinical species or in humans may be examined for their inhibitory potential using inside-out membrane vesicles containing recombinant human BSEP in ATP-dependent transport assays with taurocholate (cholytaurine) as a probe substrate. Future work is needed to identify susceptibility factors and/or genes leading to drug-induced cholestasis due to BSEP impairment. Such information may be obtained from retrospective analysis of clinical data, but will subsequently need to be verified in prospective multicenter trials.

Other transporters have shown importance in drug delivery. In this whitepaper, we presented two examples, NTs and PEPTs.



NTs (*e.g.*, hENT1) are important for the uptake of nucleosides and their analogs into cells and are widely distributed among cells and tissues in humans. Many nucleoside analogs are used clinically for treatment of cancer and viral diseases. Recent data showed a correlation between abundance of hENT1 and survival of pancreatic cancer patients treated with gemcitabine.

Peptide transporters, as exemplified by PEPT1 in the apical membrane of small intestine, are not only important for nutritional absorption of di- and tri-peptides but also for delivery of amino acid-like or dipeptide-like drugs and prodrugs. Drug substrates include many  $\beta$ -lactam antibiotics and prodrugs such as valaciclovir. Transporter assays (*e.g.*, using transfected PEPT1-expressing HeLa cells) serve not only in studies on the substrate properties of new chemical entities but also in the *in vitro* analysis of drug-drug interactions.

Consistent methods for studying transporters are still under development. However, recommendations and a detailed discussion of determination of kinetic parameters of transporter substrates are given in an accompanying article in this issue (69). In brief, transport studies with transfected cells are recommended for uptake transport systems while transport studies with inside-out vesicles isolated from transfected cells are recommended for efflux transporters. Kinetic parameters have to be determined using conditions of initial uptake rates. For inhibition experiments  $K_i$  values obtained from Dixon plots are preferred over  $IC_{50}$  values.

In summary, NMEs that are actively renally secreted are recommended for investigation as a MATE1 or MATE2K substrates in addition to being investigated as OAT1, OAT3 or OCT2

substrates according to Figure 2. It is recommended that the potential of all NMEs to inhibit MATE1 and MATE2k be determined *in vitro*. Figure 3 may be followed to determine if a clinical interaction study is necessary. The inhibition of both BSEP and MRPs has the potential to cause cholestasis and if signs of cholestasis are seen, retrospective analysis of inhibition of the BSEP and MRPs can help determine the mechanism of toxicity. Because there are no known cut-offs for the inhibition potential ( $IC_{50}$  or  $K_i$  values) of these transporters that indicate increased risk of hepatotoxicity, screening of their inhibition on a routine basis is not yet warranted. Three other transporters that were highlighted in this review, hENT1, hENT2 and PEPT1, although examples of transporters that do not need to be routinely screened, are also known to distribute specific type of drugs and thus are important determinants for efficacy and distribution. The hENTs transport nucleoside drugs and PEPT1 transports a variety of drugs with peptide-like properties.

**Disclaimer:**

The opinions expressed in this paper reflect the views of the authors and should not be construed to represent FDA's views or policies.

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## Figure Legends

**Figure-1: Proposed expansion of transporters for evaluation during drug development by ITC.**

Proposed expansion of transporters for evaluation during drug development by ITC.

Transporters were highlighted on the basis of evidence of clinical drug interactions and relevance to toxicity or efficacy, as well as availability of in vitro assays, substrates and inhibitors.

Transporters recommended for evaluation in the 2012 FDA Draft Drug Interaction Guidance are marked with red circles. MATEs (green circles) are being proposed for prospective investigation in drug development as discussed at the ITC Second Workshop. In addition MRP2 and BSEP inhibition are recommended for retrospective studies based on preclinical and clinical observations (yellow circles.) Examples given in this paper (ENTs and PEPTs) are in light blue circles. Modified from ref. 8 by Sook Wah Yee, University of California, San Francisco.

**Figure 2: Decision tree for renally cleared substrates proposed by ITC**

a. For simplicity organic anion transporters, OAT1 and OAT3 are not included; however, if secretory clearance is an important route of elimination, NMEs should also be studied as substrates of OAT1 and OAT3.

**a-**Percent (%) active renal secretion was estimated from  $(CL_r - fu * GFR) / CL_{Total}$ ; fu is the unbound fraction in plasma (6).

**b-**The ratio of the investigational drug uptake in the cells expressing the transporter versus the control (or empty vector) cells should be greater than 2. It is important that uptake into the transfected cells be significantly greater than background in a control cell line and be inhibited by a known inhibitor of the transporter. Michaelis–Menten studies may be conducted in the transfected cells to determine the kinetic parameters of the investigational drug. A positive control should be included. In an acceptable cell system, the positive control should show a  $\geq 2$  fold increase in uptake compared to vector-transfected cells. An uptake ratio (transporter transfected vs. empty vector transfected cells) other than 2 may be used if a ratio of 2 is deemed non-discriminative as supported by prior experience with the cell system used.

**c-**If the NME interacts with OCT2 and MATE1/2K, suggested inhibitors to use for a clinical DDI study are pyrimethamine or cimetidine, both of which are clinically validated. If the NME is a substrate of OAT1 or OAT3 and MATE1/2K, probenecid should be considered for use as an inhibitor.

**Figure-3: Decision tree for renal transporter inhibitors proposed by ITC**

**a-**First step might be uptake of model substrate decreases with increasing concentration of NME, then full  $IC_{50}$  curve (modified from Ref 6).

**b-**For NME that is a MATE1, MATE2K or OCT2 inhibitor, metformin may be used as the substrate for the clinical drug interaction study.

**Table 1: Selected putative MATE-mediated clinical drug-drug interactions**

Interacting drug	Affected drug	Clinical effect on affected drug	Ref.
Cimetidine (400-800mg, PO)	Fexofenadine	39% decrease in CLr of fexofenadine	(70)
	Metformin	50% decrease in CLr of metformin and 27% increase in AUC	(71)
	Dofetilide	33% decrease in CLr of dofetilide, 48% and 29% increase in AUC and Cmax respectively	(72)
	Chepalexin	33% decrease in CLr of cephexin	(73)
	Procainamide	44% decrease in CLr of procainamide and 35% increase in AUC	(74)
Chepalexin (500mg, PO)	Metformin	14% decrease in CLr of metformin, 24% and 34% increase in AUC and Cmax respectively	(75)
Pyrimethamine (50mg, PO)	Metformin	35% decrease in CLr of metformin, 42% and 39% increase in Cmax and AUC respectively	(20)
	Creatinine	20% decrease in CLr of creatinine	(20)
<p>Legend: Percent change refers to the difference in the presence or absence of interacting drug normalized to the value in the absence of interacting drug.</p>			

**Table 2. Recommended In Vitro Probe Substrates and Inhibitors for Human MRPs and BSEP ((69) and references therein).**

<b>Transporter</b>	<b>Substrates</b>	<b>Inhibitors</b>
ABCC2 (MRP2)	[ <sup>3</sup> H]-leukotriene C4 [ <sup>3</sup> H]-estradiol 17β-glucuronide carboxy-dichlorofluorescein	MK-571 benzbromarone
ABCC3 (MRP3)	[ <sup>3</sup> H]-estradiol 17β-glucuronide, [ <sup>3</sup> H]-methotrexate carboxy-dichlorofluorescein	Bromosulfophthalein MK-571
ABCC4 (MRP4)	[ <sup>3</sup> H]-leukotriene C4 [ <sup>3</sup> H]-dehydroepiandrosterone 3-sulfate (DHEAS) [ <sup>3</sup> H]-folate	MK-571
ABCB11 (BSEP)	[ <sup>3</sup> H]-taurocholate (cholytaurine)	cyclosporin A PSC-833

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Figure 1

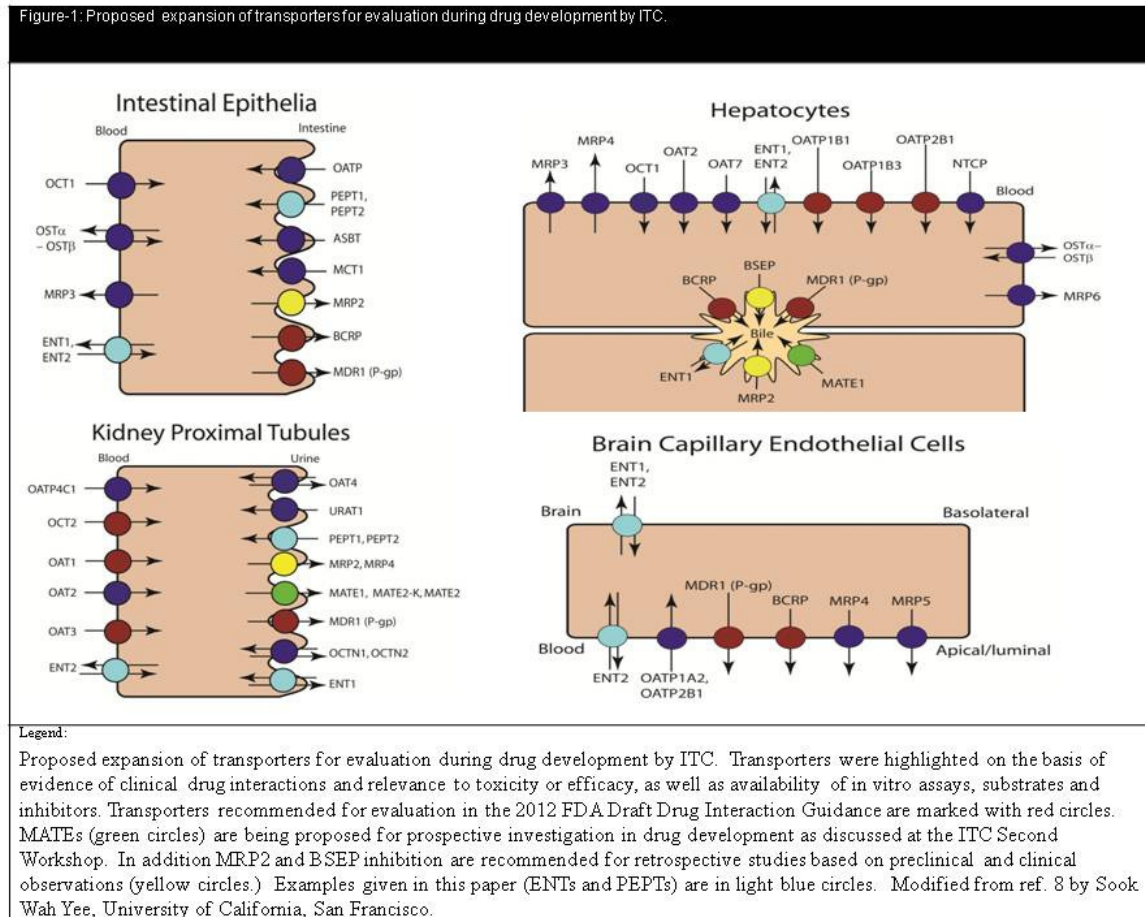


Figure 2

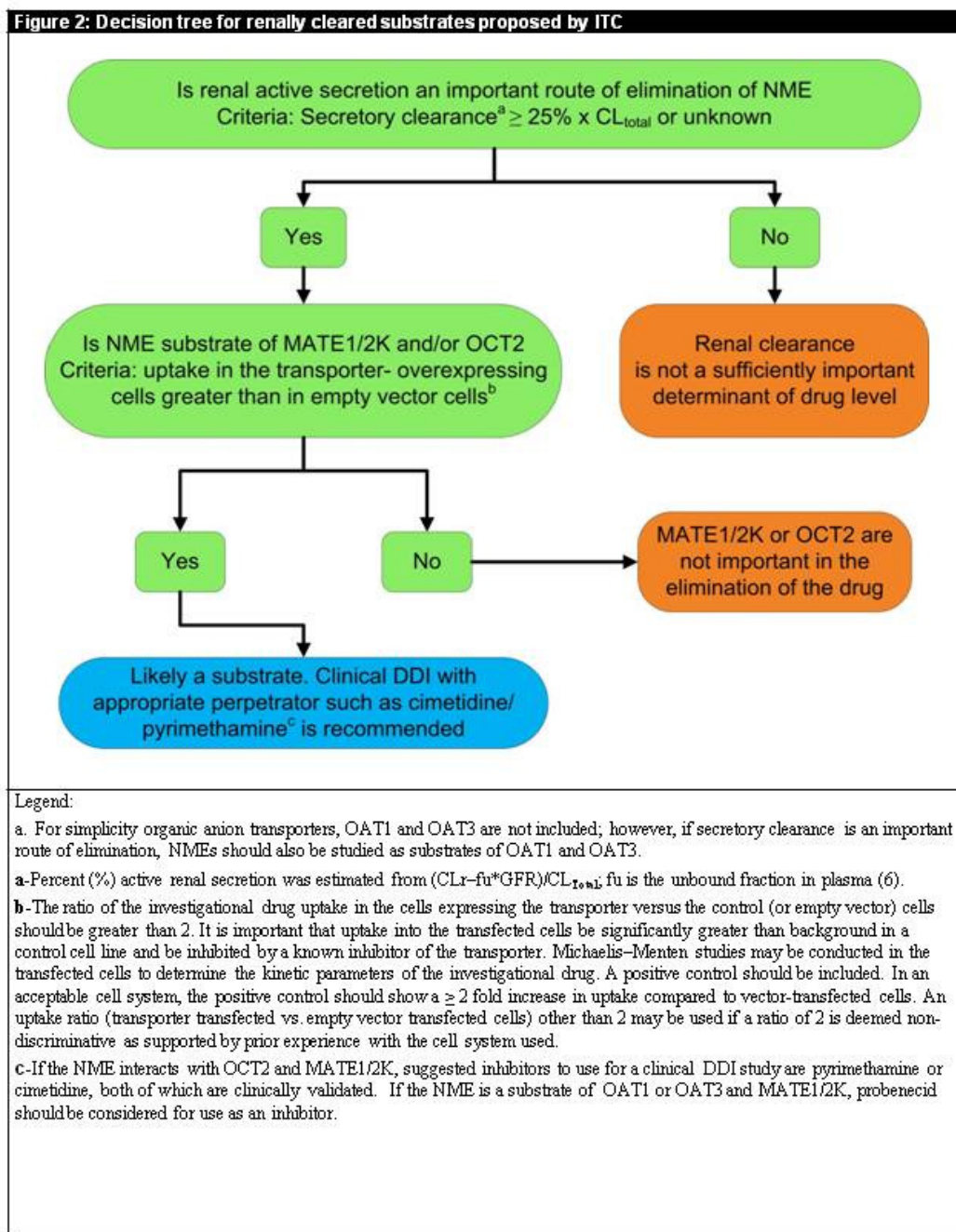


Figure 3

