Drug Transporters: Report from the International Transporter Consortium; Decisions, Impact and Future Directions

New England Drug Metabolism Discussion Group
March 3rd, 2010

Donald Tweedie
Director, Drug Metabolism
Drug Transporter White Paper


‘Membrane Transporters in Drug Development’

The International Transporter Consortium.

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The International Transporter Consortium considers this report as a work in progress, and is highly interested in obtaining feedback. Please send any comments, including areas that have not been included in this report but should be considered in the next version as well as controversial concepts, to the corresponding authors (highlighted by asterisk).
Outline

- International Transporter Consortium (ITC)
  - Genesis
  - Goals
  - White paper
    - How we got there
    - What it is
    - What it is not
    - Examples, MDR1, OATP, decisions tree(s)
  - Current Issues, Challenges, and Actions
  - Future Activities
- Conclusions
- Acknowledgements
PhRMA Pharmaceutical and Research Manufacturers of America

- Advocacy forum for the industry to influence the FDA (PhRMA – America)

- Drug Metabolism Technical Group (DMTG)
  - Subgroup responsible for DMPK issues
  - (MIST, DDI, pharmacogenomics, time-dependent inhibition)\(^1\)
  - Nov 2007, transporters identified as a key topic

- Academic group headed by Kathy Giacomini and Toshi Ishikawa were considering initiating a global committee to generate a white paper providing preferred approaches to conduct transporter studies


Grimm SW et al. (2009) The conduct of in vitro studies to address time-dependent inhibition of drug-metabolizing enzymes: a perspective of the pharmaceutical research and manufacturers of America. Drug Metabolism and Disposition 37(7):1355-1370
Key goals for the ITC

• Provide an update on current thinking on transporters.

• For in vitro studies, provide a focus on studies that can have a viable clinical interpretation (avoid raising red flags with in vitro studies that cannot be addressed in vivo in the clinic).

• Explore gaps and suggest ways forward.

• Provide a coordinated approach (Academia, Industry and Regulatory).

• Help to move the science forward.
  – Decision trees to assist drug development and regulatory
  – Consensus on current scientific status
Workshop
Bethesda North Marriott
October 2\textsuperscript{nd} and 3\textsuperscript{rd}, 2008

- Sponsored by FDA Critical Path
- Workshop organized by Drug Information Association (DIA)
- Co-sponsorship by AAPS, ISSX, PhRMA
- Provide a focus to initiate a White Paper for completion in 2009
## ITC Original Members

### Academia:
- Kim Brouwer, UNC
- Kathy Giacomini, UCSF
- Toshi Ishikawa, OSC, Tokyo
- Dietrich Keppler, Heidelberg
- Richard Kim, W. Ontario
- Peter Swann, Maryland

### Industry:
- Raymond Evers, Merck
- Volker Fischer, Abbott
- Kate Hillgren, Lilly
- Joe Polli, GSK
- Donald Tweedie, BI
- Joe Ware, Genentech

### Regulatory:
- Shiew Mei Huang, FDA
- Lei Zhang, FDA
## ITC author list

### Academia:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Les Benet</td>
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<td>Steve Wright</td>
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<td>Sook Wah Yee</td>
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### Industry:

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<td>FDA</td>
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Drug Transporters in Drug Development

The International Transporter Consortium, ITC

1. Basic Introduction and Summary of Transporters
   – Highlights what we know

2. Methods for Studying Transporters
   – Current solutions and future prospects

3. Drug Development Issues
   – Decision trees
Section 1

Transporters covered
- Efflux: P-gp, BCRP
- Renal: OAT/OCT
- Hepatic uptake: OATP

Other transporters not discussed in detail
- MRPs
- MATEs
- Considered less critical in the overall view
- But could be important for specific drugs?
## Tables of Substrates and Inhibitors

**Table 1 | Solute carrier transporters of emerging clinical importance in the disposition of drugs**

<table>
<thead>
<tr>
<th>Transporter/alias (Gene)</th>
<th>Selected substrates</th>
<th>Selected inhibitors</th>
<th>Organs/cells</th>
<th>Comments</th>
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</table>
| OATP1B1/OATP-C, OATP2, LST-1 (SLCO1B1) | Bromosulphophthalein, oestrone-3-sulphate, oestradiol-17β-glucuronide, statins*, repaglinide*, valsartan, olmesartan*, bilirubin glucuronide, bilirubin, bile acids | Saquinavir*, ritonavir*, lopinavir*, rifampicin*, cyclosporine* | Hepatocytes (sinusoidal) | • Has a role in disposition and excretion  
• Has clinically relevant polymorphisms  
• Has known clinical drug–drug interactions at the transporter level |
| OATP1B3/OATP-8 (SLCO1B3) | Bromosulphophthalein, cholecystokinin 8, statins*, digoxin, fexofenadine, telmisartan glucuronide, telmisartan*, valsartan, olmesartan, oestradiol-17β-glucuronide, bile acids | Rifampicin*, cyclosporine*, ritonavir, lopinavir* | Hepatocytes (sinusoidal) | • Has a role in disposition and excretion |
| OAT1 (SLC22A6) | Para-aminohippurate, adefovir, cidofovir, zidovudine*, lamivudine*, zalcitabine*, acyclovir*, tenofovir*, ciprofloxacin*, methotrexate* | Probencid*, novobiocin | Kidney proximal tubule, placenta | • Has a role in disposition and excretion  
• Has known clinical drug–drug interactions at the transporter level |
| OAT3 (SLC22A8) | Oestrone-3-sulphate, non-steroidal anti-inflammatory drugs, cefaclor, ceftizoxime, furosemide*, bumetanide* | Probencid*, novobiocin | Kidney proximal tubule, choroidplexus, blood–brain barrier | • Has a role in disposition and excretion  
• Has known clinical drug–drug interactions at the transporter level |
Section 2. Methods for Studying Transporters

A. Cell and Membrane Models

B. Intact Organ/In Vivo Models

C. Methods to Measure the Contribution of Transporters to Tissue Distribution and Excretion

D. Interplay of Efflux Transporters and Enzymes

E. Coordination of Influx and Efflux Transporters and Enzymes in the Clearance of Drugs

F. Computational Models
Section 3. Drug Development Issues

Box 2. Decision trees for P-gp or BCRP substrate interactions
Box 3. Decision trees for P-gp or BCRP inhibitor interactions
Box 4. Decision trees for OCT or OAT substrate interactions
Box 5. Decision trees for OCT or OAT inhibitor interactions
Box 6. Decision trees for OATP interactions
Box 7. OATP1B1 Decision Analysis: Case Studies

Summary and Conclusions
In bi-directional transporter assays, for example, in Caco-2 or P-gp-overexpressing polarized epithelial cell lines, is the net flux ratio of NME ≥ 2?

- **a** Net flux ratio ≥ 2
  - **b** Is efflux significantly inhibited by 1 or more P-gp inhibitors?
    - Yes: Probably P-gp substrate
    - No: Other efflux transporters are responsible for observed data
  - **c** Net flux ratio < 2
    - Poor or non-P-gp substrate

Complete an assessment of preclinical and clinical information to determine whether an in vivo DDI study is warranted.
Current issues?

Decision Trees

**Pros**
- evolution of concepts
- highlight discussion points
- offers flexibility

**Cons**
- rigid interpretation – prescriptive and overly cautious
- insufficient knowledge to populate the decision points
- lack of selective substrates and inhibitors

‘The evolution and appropriate application of these decision trees will require constant monitoring. How can this be achieved with an assured and encompassing measure of success?’
False Positives (unnecessary clinical studies)

Alert for $[I_1]/IC_{50} \geq 0.1$ or $[I_2]/IC_{50} \geq 10$,
- $[I_1]$ is steady-state total Cmax at the highest clinical dose
- $[I_2]$ is the GI concentration calculated as dose (mg)/250 mL

$[I_2]/IC_{50} > 10$ will be exceeded at a dose of ~12 mg for a drug with an inhibition potency of ~10 µM in vitro (MW ~ 500).

False Negatives (safety concerns)
White Paper - What it is

- A consensus view on the current thinking on drug transporters
  - What are the current realities

- The known knowns
  - What do we know about the relative importance of all transporters?
  - Where do you put your effort?

- The known unknowns
  - What facts are known to be untrue (dispelling myths)?
  - Where are our gaps in knowledge (so where should we focus short and long term to increase our knowledge)?

- A guideline (not a guidance) towards what we should focus on currently
  - What are we capable of addressing?
White Paper - What it is not

- A complete literature review.

- A prescriptive guidance on what to do and how to do it, with a clear description of what it will mean.

- A consensus document that everyone agrees to.

- A description of all of the exceptions.
  - Your experience is important to you and we would certainly appreciate you sharing that with the scientific community to educate us all.

- The decision trees are clearly not definitive.
  - Included to help move the science forward by acting as templates for discussion
  - P-gp most mature but not perfect
The issues presented by transporters are significantly more complex than for DMEs

- Involved in absorption, distribution and excretion, so multiple processes of concern

- Broad tissue distribution; different effects at different sites, e.g. P-gp at intestine and BBB

- Redundancy; different transporters (P-gp and BCRP) and different subfamilies (OATP1B1 and 1B3)

- Uptake and efflux transporters (need to consider both to assess the overall effect)

- Applicability of kinetic parameters and their interpretation
Transporter Interaction Redundancy:

- Drugs that are shown to interact with one transporter typically interact with multiple transporters.

- Thus, multiple pathways for clearance are possible for transporter substrates.

Ieiri et al. (2009) Expert Opinion in Drug Metabolism and Toxicology, 5: 703-729.
Lack of selective inhibitors of drug transporters

- **LY 335979 (zosuquidar)** is a potent inhibitor/modulator of P-gp, but does not inhibit MRP1 or MRP2.
  - Selectivity over inhibition of CYP3A4 is ~60-fold.  
  [Reference 1]

- **Discovery, cloning, and publication of OATP superfamily of uptake transporters**  
  [References 2-5]

- **OATP1B1-mediated uptake of anticancer drugs gimatecan and BNP1350** were inhibited by zosuquidar.
  - The effect of modulators on the plasma pharmacokinetics of OATP1B1 substrate drugs may not be solely ascribed to inhibition of P-gp  
  [Reference 6]


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Slide courtesy of Dr. Mitchell Taub
## P-gp at the Blood-Brain Barrier: Species Differences

<table>
<thead>
<tr>
<th>P-gp inhibitor</th>
<th>P-gp inhibitor dosage</th>
<th>Drug (P-gp substrate)</th>
<th>Clinical usage of the drug</th>
<th>CNS exposure index</th>
<th>Plasma AUC and C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>CNS effect</th>
<th>Brain inhibition</th>
<th>P-gp inhibition</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Quinidine</td>
<td>600 mg</td>
<td>Fentanyl</td>
<td>Synthetic opioid</td>
<td>Pupil diameter</td>
<td>Oral AUC ↑171%, C&lt;sub&gt;max&lt;/sub&gt; ↑162%</td>
<td>Quinidine had no major influence on fentanyl pharmacodynamics in humans.</td>
<td>No</td>
<td>1</td>
<td></td>
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<tr>
<td>Quinidine</td>
<td>600 mg</td>
<td>Loperamide</td>
<td>A peripherally acting opioid receptor agonist for treatment of chronic diarrhea</td>
<td>Respiratory response to CO2 rebreathing</td>
<td>AUC ↑148%</td>
<td>Respiratory depression occurred when loperamide was given with quinidine.</td>
<td>Yes?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>800 mg</td>
<td>Loperamide</td>
<td>A peripherally acting opioid receptor agonist for treatment of chronic diarrhea</td>
<td>Pupil size</td>
<td>AUC ↑80%</td>
<td>Pupil size decreased with co-administration of quinidine.</td>
<td>Yes?</td>
<td>3</td>
<td></td>
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<tr>
<td>Quinidine</td>
<td>600 mg</td>
<td>Methadone</td>
<td>Opioid</td>
<td>Pupil diameter</td>
<td>i.v. AUC and C&lt;sub&gt;max&lt;/sub&gt; no changes</td>
<td>No effect on methadone miosis after i.v. administration</td>
<td>No</td>
<td>4</td>
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<tr>
<td>Quinidine</td>
<td>600 mg</td>
<td>Morphine</td>
<td>Opioid</td>
<td>Pupil diameter</td>
<td>Oral AUC ↑60%, C&lt;sub&gt;max&lt;/sub&gt; ↑88%</td>
<td>No effect on i.v. morphine miosis, Difference in oral morphine miosis were commensurated with changes in plasma morphine concentration.</td>
<td>No</td>
<td>5</td>
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</tr>
<tr>
<td>Quinidine</td>
<td>800 mg</td>
<td>Morphine</td>
<td>Opioid</td>
<td>Pupil diameter and respiratory response to CO2 rebreathing</td>
<td>Plasma concentration, Not result in an enhancement of central nervous opioid effects.</td>
<td>No</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>800 mg</td>
<td>Morphine 6-glucuronide</td>
<td>An active metabolite of morphine</td>
<td>Pupil size</td>
<td>No effect on the pharmacokinetics of morphine 6-glucuronide</td>
<td>No effect.</td>
<td>No</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Can digoxin be used as a clinical P-gp probe substrate?

- Narrow therapeutic window of digoxin requires close monitoring
- Abundant digoxin clinical DDI study data – especially for relatively new drugs
- Digoxin currently viewed as the “gold standard” probe for studying clinical P-gp–related DDIs
  - recent data may indicate that digoxin interacts with other transporters; OATPs

Fenner et al. (2009) *CPT* 85, 173-181

- 123 Clinical Digoxin DDIs
- 30 Digoxin trials $\text{AUC}_i/\text{AUC} \geq 1.25$
- 75% of the Digoxin DDIs showed no change

- 123 Clinical Digoxin DDIs
- 30 Digoxin trials $\text{AUC}_i/\text{AUC} \geq 1.25$
- 75% of the Digoxin DDIs showed no change
• $^{11}$C-verapamil and CsA dosed IV
• $\text{AUC}_{\text{brain}}/\text{AUC}_{\text{blood}}$ of $^{11}$C-radioactivity $\uparrow 88\%$ in the presence of CsA
• $\uparrow 770\%$ in similar study in mouse

## P-gp at the Blood-Brain Barrier: Mouse KO


<table>
<thead>
<tr>
<th>Drug</th>
<th>Brain level ratio mdr1a (−/−):mdr1a(+/+)</th>
<th>Therapeutic category</th>
</tr>
</thead>
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<tr>
<td>Amprenavir</td>
<td>27</td>
<td>HIV protease inhibitor</td>
</tr>
<tr>
<td>Asimadoline*</td>
<td>11</td>
<td>analgesic</td>
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<td>Azasetron</td>
<td>7</td>
<td>anti-emetic</td>
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<tr>
<td>Carebastin</td>
<td>8</td>
<td>antihistamine</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>17</td>
<td>immune suppressant</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>3</td>
<td>glucocorticoid</td>
</tr>
<tr>
<td>Digoxin</td>
<td>35</td>
<td>cardiotonic</td>
</tr>
<tr>
<td>Doxorubicin*</td>
<td>3</td>
<td>antineoplastic</td>
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<tr>
<td>Ebastine</td>
<td>7</td>
<td>antihistamine</td>
</tr>
<tr>
<td>Grepafloxacin</td>
<td>3</td>
<td>antibacterial</td>
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<tr>
<td>Indinavir</td>
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<td>HIV protease inhibitor</td>
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<td>Ivermectin</td>
<td>87</td>
<td>anthelmintic</td>
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<td>Loperamide</td>
<td>14</td>
<td>antidiarrheal</td>
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<td>Morphine</td>
<td>2</td>
<td>analgesic</td>
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<td>Nelfinavir</td>
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<td>Ondansetron</td>
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<td>immunosuppressant</td>
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<td>antihypertensive</td>
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<tr>
<td>Vinblastine*</td>
<td>22</td>
<td>antineoplastic</td>
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Potential OATP1B1 probe substrates in vivo

- **Atorvastatin** (Km 12.4 μM, Kameyama et al 2005)
  - OATP2B1, P-glycoprotein and BCRP substrate
  - Metabolized (CYP3A4, CYP2C8)

- **Pitavastatin** (Km 3.0-6.7 μM, Hirano et al 2004, Deng et al 2008)
  - OATP1B3, P-glycoprotein and BCRP substrate
  - Metabolized to a minor extent only (CYP2C9)

  - OATP2B1, P-glycoprotein, BCRP and MRP2 substrate
  - Metabolized to a minor extent only (non-CYP mediated)

- **Repaglinide** (Km ?)
  - Metabolized (CYP2C8, CYP3A4)

  - OATP1A2, OATP1B3, OATP2B1, NTCP and BCRP substrate
  - Metabolized to a minor extent only (CYP2C9)

- **Simvastatin (acid)** (Km ?)
  - P-glycoprotein substrate
  - Metabolized (CYP3A4, CYP2C8)
Transporters are a very dynamic field – the white paper is intended to be a snapshot

White paper will need to be updated (timeline?)

White paper provides framework for FDA to add to current guidance(s) – DDI

Emphasizes the need for flexibility
• which provides some realistic challenges for regulatory agencies

Has identified areas of highest immediate need
• decision trees for other transporters
• relevance of unbound drug concentrations

Never intended to be a panacea

Focus group for collating new data
Moving Forward

Committee of FDA and Pharma

- Lei Zhang (leik.zhang@fda.hhs.gov) and Donald Tweedie (donald.tweedie@boehringer-ingelheim.com)

Main committee with sub-committees for specific topics

- Identify experts for different transporters
- Identify experts for selected topics (P-gp and digoxin, kinetics)

Outcome

- Provide feedback on discussions, action items
- Make recommendations
  - change current practices
  - monitor specific practices
- Publish mini-white papers
Acknowledgements

- ITC members
  - Shiew Mei Huang, FDA
  - Kathy Giacomini, UCSF
- DMTG, PhRMA
  - Volker Fischer
- DIA (Drug Information Association)
- Mitch Taub, Boehringer Ingelheim
- Yongmei Li, Boehringer Ingelheim
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<td>Bromosulphophthalein, oestraone-3-sulphate, oestradiol-17β-glucuronide, statins*, repaglinide*, valsartan, olmesartan*, bilirubin glucuronidase, bilirubin, bile acids</td>
<td>Sequevin, ritonavir*, lopinavir*, rifampicin*, cyclosporine*</td>
<td>Hepatocytes (sinusoidal)</td>
<td>Has a role in disposition and excretion, Has clinically relevant polymorphisms, Has a role in clinical drug—drug interactions</td>
</tr>
<tr>
<td>OATP1B1/OATP-8 (SLCO1B3)</td>
<td>Bromosulphophthalein, cholecyctokinin β, statins*, digoxin, furosemide, telmisartan glucuronide, telmisartan*, valsartan, olmesartan, oestradiol-17β-glucuronide, bile acids</td>
<td>Rifampicin*, cyclosporine*, ritonavir, lopinavir*</td>
<td>Hepatocytes (sinusoidal)</td>
<td>Has a role in disposition and excretion</td>
</tr>
<tr>
<td>OAT1 (SLC22A6)</td>
<td>Pare-aminosalicyclic acid, adeclovir, cidofovir, zidovudine*, lamivudine*, zalcitabine*, acyclovir*, tenofovir*, ciprofloxacin*, methotrexate*</td>
<td>Probenicid*, novobiocin</td>
<td>Kidney proximal tubule, plasma</td>
<td>Has a role in disposition and excretion, Has a role in clinical drug—drug interactions</td>
</tr>
<tr>
<td>OAT3 (SLC22A8)</td>
<td>Oestrone-3-sulphate, non-steroidal anti-inflammatory drugs, cefadrox, ciprofloxacin, furosemide*, bumetanide*</td>
<td>Probenicid*, novobiocin</td>
<td>Kidney proximal tubule, choroid plexus, blood-brain barrier</td>
<td>Has a role in disposition and excretion, Has a role in clinical drug—drug interactions</td>
</tr>
<tr>
<td>OCT2 (SLC22A8)</td>
<td>N-Methylpyridinum, taetraethylammonium, mercurin*, pilocarpil, procarbazine, amitadine, amiloride, oxaliplatin, vanadate*</td>
<td>Cimetidine*, pilocarpin, cetirizine*, testosteron, quinidine</td>
<td>Kidney proximal tubule, neurons</td>
<td>Has a role in disposition and excretion, Has clinically relevant genetic polymorphisms, Has a role in clinical drug—drug interactions</td>
</tr>
<tr>
<td>OATP1A2/OATP-A (SLCO1A2)</td>
<td>Oestrone-3-sulphate, dehydroepiandrosterone sulphate, furosemide*, bile salts, methotrexate, bromosulphophthalein, oestrone, digoxin, levofloxacine, statins*</td>
<td>Naringin, ritonavir, lopinavir, saquinavir, rifampicin*</td>
<td>Brain capillaries endothelia, chelatorigocytes, distal nephron</td>
<td>Has a role in disposition and excretion</td>
</tr>
<tr>
<td>OATP2B1/OATP-B (SLCO1B1)</td>
<td>Oestrone-3-sulphate, bromosulphophthalein, teurnichlole, *statins, furosemide, glyburide, teurnichlole</td>
<td>Rifampicin, cyclosporine*</td>
<td>Hepatocytes (sinusoidal), endothelia</td>
<td>Has a role in disposition and excretion, Has a role in clinical drug—drug interactions</td>
</tr>
<tr>
<td>OCT1 (SLC22A1)</td>
<td>Tetraethylammonium, N-methylpyridinum, mercurin*, oxalate</td>
<td>Quinine, quinidine, disopyramide</td>
<td>Hepatocytes (sinusoidal), intestinal enterocytes</td>
<td>Has a role in disposition and excretion, Has clinically relevant genetic polymorphisms, Has a role in clinical drug—drug interactions</td>
</tr>
<tr>
<td>PEP1F1 (SLC15A1)</td>
<td>Glycylproline</td>
<td>Intestinal enterocytes, kidney proximal tubule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEP2F2 (SLC15A2)</td>
<td>Glycylproline</td>
<td>Kidney proximal tubule, choroid plexus, lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MATE1 (SLC47A1)</td>
<td>Mercurin, N-methylpyridinum, tetraethylammonium</td>
<td>Quinidine, cimetidine, procarbazine</td>
<td>Kidney proximal tubule, liver (canicular membrane), skeletal muscle</td>
<td>Has a role in disposition and excretion, Has a role in clinical drug—drug interactions</td>
</tr>
<tr>
<td>MATE2 (SLC47A2)</td>
<td>Mercurin, N-methylpyridinum, tetraethylammonium</td>
<td>Cimetidine, quinidine, procarbazine</td>
<td>Kidney proximal tubule</td>
<td>Has a role in disposition and excretion</td>
</tr>
</tbody>
</table>

*Can potentially be used for in vivo (clinical) studies.
<table>
<thead>
<tr>
<th>Transporter/alias (Gene)</th>
<th>Selected substrates</th>
<th>Selected inhibitors</th>
<th>Organs/cells</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1/P-gp, ABCB1 (ABCB1)</td>
<td>Digoxin*, loperamide*, berberine, irinotecan, doxorubicin, vinblastine, paclitaxel, fexofenadine</td>
<td>Cyclosporine*, quinidine*, tantricular, verapamil</td>
<td>Intestinal enterocytes, kidney proximal tubule, hepatocytes (canalicular), brain endothelie</td>
<td>• Has a role in absorption, disposition and excretion • Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>BCRP/MXR (ABCG2)</td>
<td>Mitoxantrone, methotrexate, topotecan, imatinib, irinotecan, statins*, sulfae conjugates, porphyrins</td>
<td>Oestrone, 17β-oestradiol, fumitremorgin C</td>
<td>Intestinal enterocytes, hepatocytes (canalicular), kidney proximal tubule, brain endothelie, placenta, stem cells, mammary glands (lactating)</td>
<td>• Has a role in absorption, disposition and excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>BSEP/SPGP, cBAT, ABCB11 (ABCB11)</td>
<td>Taurocholic acid, pravastatin, bile acids</td>
<td>Cyclosporin A, rifampicin, glibenclamide</td>
<td>Hepatocytes (canalicular)</td>
<td>• Has a role in excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>MRP2/ABCC2, cMOAT (ABCC2)</td>
<td>Glutathione and glucuronide conjugates, methotrexate, etoposide, mitoxantrone, valsartan, olmesartan, glucuronidated SN-38</td>
<td>Cyclosporine, delavirdine, efavirenz, emtricitabine</td>
<td>Hepatocytes (canalicular), kidney (proximal tubule, luminal), enterocytes (luminal)</td>
<td>• Has a role in absorption, disposition and excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>MRP3/ABCC3 (ABCC3)</td>
<td>Oestradiol-17β-glucuronide, methotrexate, fexofenadine, glucuronate conjugates</td>
<td>Delavirdine, efavirenz, emtricitabine</td>
<td>Hepatocytes (sinusoidal), intestinal enterocytes (basolateral)</td>
<td>• Has a role in disposition</td>
</tr>
<tr>
<td>MRP4/ABCC4 (ABCC4)</td>
<td>Adefovir, tenofovir, cyclic AMP, dehydroepiandrosterone sulphate, methotrexate, topotecan, furosemide, cyclic GMP, bile acids plus glutathione</td>
<td>Celecoxib, diclofenac</td>
<td>Kidney proximal tubule (luminal), choroid plexus, hepatocytes (sinusoidal), platelets</td>
<td>• Has a role in disposition and excretion</td>
</tr>
<tr>
<td>MDR3/ABCB4 (ABCB4)</td>
<td>Phosphatidylcholine, paclitaxel, digoxin, vinblastine</td>
<td>Verapamil</td>
<td>Hepatocytes (canalicular)</td>
<td>• Has a role in disposition • Has a role in clinical drug–drug interactions</td>
</tr>
</tbody>
</table>

ABC, ATP-binding cassette. *Can potentially be used for in vivo (clinical) studies.
<table>
<thead>
<tr>
<th>Implicated transporter*</th>
<th>Interacting drug</th>
<th>Affected drug</th>
<th>Clinical pharmacokinetic impact on affected drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic anion transporting polypeptides</td>
<td>Cyclosporine</td>
<td>Pravastatin</td>
<td>AUC ↑800% and C&lt;sub&gt;max&lt;/sub&gt; ↑678%&lt;sup&gt;102,204&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine</td>
<td>Rosuvastatin</td>
<td>AUC ↑610%&lt;sup&gt;205&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine</td>
<td>Pitavastatin</td>
<td>AUC ↑360% and C&lt;sub&gt;max&lt;/sub&gt; ↑560%&lt;sup&gt;206&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rifampicin (single dose)</td>
<td>Glyburide</td>
<td>AUC ↑125%&lt;sup&gt;207&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Rifampicin (single dose)</td>
<td>Bosentan</td>
<td>C&lt;sub&gt;trough&lt;/sub&gt; ↑500%&lt;sup&gt;208&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Lopinavir/ritonavir</td>
<td>Bosentan</td>
<td>Day 4: C&lt;sub&gt;trough&lt;/sub&gt; ↑4,700%&lt;sup&gt;208&lt;/sup&gt;; day 10: C&lt;sub&gt;trough&lt;/sub&gt; ↑400%&lt;sup&gt;208&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Lopinavir/ritonavir</td>
<td>Rosuvastatin</td>
<td>AUC ↑107% and C&lt;sub&gt;max&lt;/sub&gt; ↑365%&lt;sup&gt;209&lt;/sup&gt;</td>
</tr>
<tr>
<td>Organic anion transporters</td>
<td>Probenecid</td>
<td>Ciclofivir</td>
<td>CL&lt;sub&gt;r&lt;/sub&gt; ↓32%&lt;sup&gt;216,211&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Probenecid</td>
<td>Furosemide</td>
<td>CL&lt;sub&gt;r&lt;/sub&gt; ↓66%&lt;sup&gt;210&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Probenecid</td>
<td>Acyclovir</td>
<td>CL&lt;sub&gt;r&lt;/sub&gt; ↓32% and AUC ↑40%&lt;sup&gt;210,212&lt;/sup&gt;</td>
</tr>
<tr>
<td>Organic cation transporters</td>
<td>Cimetidine</td>
<td>Metformin</td>
<td>AUC ↑50% and CL&lt;sub&gt;r&lt;/sub&gt; ↓27%&lt;sup&gt;213,214&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cimetidine</td>
<td>Pindolol</td>
<td>CL&lt;sub&gt;r&lt;/sub&gt; ↓34%&lt;sup&gt;215&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cimetidine</td>
<td>Varenicline</td>
<td>AUC ↑25%&lt;sup&gt;216&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cimetidine</td>
<td>Pilsicainide</td>
<td>AUC ↑33%, CL&lt;sub&gt;r&lt;/sub&gt; ↓28%&lt;sup&gt;217&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cetirizine</td>
<td>Pilsicainide</td>
<td>CL&lt;sub&gt;r&lt;/sub&gt; ↓41%&lt;sup&gt;218&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cimetidine</td>
<td>Dofetilide</td>
<td>CL&lt;sub&gt;r&lt;/sub&gt; ↓33%&lt;sup&gt;219&lt;/sup&gt;</td>
</tr>
<tr>
<td>P-glycoprotein</td>
<td>Quinidine</td>
<td>Digoxin</td>
<td>CL&lt;sub&gt;r&lt;/sub&gt; ↓34–48%&lt;sup&gt;220,221&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ritonavir</td>
<td>Digoxin</td>
<td>AUC ↑86%&lt;sup&gt;222&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Dronedarone</td>
<td>Digoxin</td>
<td>AUC ↑157% and C&lt;sub&gt;max&lt;/sub&gt; ↑75%&lt;sup&gt;223&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ranolazine</td>
<td>Digoxin</td>
<td>AUC ↑60% and C&lt;sub&gt;max&lt;/sub&gt; ↑46%&lt;sup&gt;214&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breast cancer resistance protein</td>
<td>GF120918</td>
<td>Topotecan</td>
<td>AUC ↑143%&lt;sup&gt;225&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Implicated transporter refers to the likely transporter; however, because the studies are carried out in vivo it is not possible to assign specific transporters to the drug–drug interaction. Percent change refers to the difference between the area under the curve (AUC), or C<sub>max</sub>, in the presence and the absence of the inhibitor (interacting drug) normalized to the AUC in the absence of the inhibitor. For clearance values (CL<sub>r</sub>), the values are normalized for the absence of the inhibitor. C<sub>trough</sub> is the minimum drug concentration observed after administration of a dose of the drug and the concentration prior to the administration of a subsequent dose.
In bi-directional transporter assays, for example, in Caco-2 or P-gp-overexpressing polarized epithelial cell lines, is the net flux ratio of NME ≥ 2?

a. Net flux ratio ≥ 2
   b. Is efflux significantly inhibited by 1 or more P-gp inhibitors?
      - Yes: Probably P-gp substrate
      - No: Other efflux transporters are responsible for observed data

   c. Probably P-gp substrate
   d. Net flux ratio < 2
      - Poor or non-P-gp substrate

Complete an assessment of preclinical and clinical information to determine whether an in vivo DDI study is warranted.
Bi-directional transporter assay with a probe P-gp substrate. For example, in Caco-2 or P-gp-overexpressing polarized epithelial cell lines

Net flux ratio of a probe substrate decreases with increased concentrations of the investigational drug

- Probably a P-gp inhibitor
  - Determine $K_i$ or $IC_{50}$ of the inhibitor
    - $[\eta]/IC_{50}$ (or $K_i$) \(\leq 0.1\) or $[\eta]/IC_{50}$ (or $K_i$) \(\geq 10\)
      - An *in vivo* drug interaction study with a P-gp substrate such as digoxin is recommended
    - $[\eta]/IC_{50}$ (or $K_i$) \(< 0.1\) and $[\eta]/IC_{50}$ (or $K_i$) \(< 10\)
      - An *in vivo* drug interaction study with a P-gp substrate may not be needed

Net flux ratio of the probe substrate is not affected with increased concentrations of the investigational drug

- Poor or non-inhibitor
Is renal elimination an important route of elimination of NME? Criteria: \( \frac{\text{CL}_r}{\text{CL}_{\text{Total}}} \geq 0.5 \)

- Yes
  - Is secretory clearance an important route of NME elimination? Criteria: \( \text{CL}_r > 1.5 \text{fu} \times \text{GFR} \)
    - Yes
      - Is NME a substrate of OCT2, OAT1 or OAT3? Criteria: uptake in the transporter-overexpressing cells greater than in empty vector cells (see footnote)
      - Clinical DDI study with cimetidine for OCT2 and with probenecid for OAT1, OAT3 as inhibitor drugs
    - No
      - Renal secretory transporters are not important in the elimination of the drug
  - No
    - Renal clearance is not a sufficiently important determinant of drug levels
Is the NME an inhibitor of OCT2, OAT1 or OAT3?
Criteria: determine the IC\textsubscript{50} of NME against MPP+, for OCT2; PAH for OAT1 or OS for OAT3 or other model substrates

**Yes**

- Unbound $C_{\text{max}}/IC_{\text{50}}$
  - of the NME $\geq 0.1$
  - Clinical DDI study with a sensitive substrate (see footnote)

**No**

- Poor or not an inhibitor of OCT2, OAT1 or OAT3
  - Unbound $C_{\text{max}}/IC_{\text{50}}$
    - of the NME $< 0.1$
    - DDI study is not needed
a

Is hepatic elimination an important route of elimination of NME? Criteria: $CL_h > 0.3 \cdot CL_{Total}$

- Yes
  - Does the compound have active hepatocyte uptake? Do the drugs’ physiological properties (for example, low passive membrane permeability, high hepatic concentrations relative to other tissues, organic anion/charged at physiological pH) support importance of active uptake into liver?
    - Yes
      - Investigate uptake transporters expressed in hepatocytes with inhibitors and/or transfected cell lines
      - If an OATP substrate, consider a clinical DDI study with single-dose rifampicin or cyclosporin as an inhibitor. Further consideration could be given to review clinical pharmacokinetics based on OATP genotyping
    - No
      - Hepatic clearance is not a sufficiently important determinant of drug levels

- No

b

Is the $IC_{50}$ of the NME $\leq 10$ times unbound $C_{max}$?

- Yes
  - Is the AUC or $C_{max}$ of statin (for example, rosuvastatin, pravastatin, pitavastatin) predicted to increase $> 2$-fold in presence of the NME using extrapolation (for example, $R$ value $> 2$)?
    - Yes
      - Clinical DDI study with sensitive substrate (for example, rosuvastatin, pravastatin, pitavastatin)
    - No
      - Clinical study may not be needed
  - NME probably not an in vivo inhibitor of OATP

- No
  - Probably a poor or not a substrate for OATPs
Intestinal transporters

Intestinal Epithelia

Blood

OCT1

OSTα

OSTβ

MRP3

Intestine

OATP

PEPT1

ASBT

MCT1

MRP2

BCRP

MDR1
Kidney Proximal Tubules

Blood

OATP4C1

OCT2

OAT1

OAT2

OAT3

Urine

OAT4

URAT1

PEPT1, PEPT2

MRP2, MRP4

MATE1, MATE2

MDR1

OCTN1, OCTN2
Brain transporters

Blood Brain Barrier

Brain capillary endothelial cells

Blood  OATP1A2  OATP2B1  MDR1  BCRP  MRP4  MRP5  Apical/luminal