Transporters have become increasingly important in drug development due to the major role they play in absorption, distribution and excretion of endogenous and exogenous compounds, inasmuch as transporter-mediated drug-drug interactions (DDI) are associated with potential toxicological and pharmacological consequences. As evidenced in numerous publications, the effects of transporters on the pharmacokinetics of several drugs, and associated DDI, have been reported. Consequently, recent guidance documents released by the US FDA, European Medicines Agency (EMA) and Pharmaceuticals & Medical Devices Agency (PMDA) of Japan emphasize the importance of evaluating the potential of new drug candidates for transporter-mediated DDI with a determination of victim (substrate) and perpetrator (inhibition) potential.

Sekisui XenoTech performs in vitro transporter studies to determine if compounds are substrates or inhibitors of clinically-relevant transporters with validated, and industry accepted, test systems. In addition to a dedicated drug transport team, Sekisui XenoTech employs an experienced analytical department with capabilities to conduct studies with labeled or unlabeled drug candidates, a Quality Assurance department to oversee GLP studies and a knowledge management group to facilitate and expedite production of high quality data reports.
P-gp (MDR1/ABCB1), BCRP (ABCG2), MRP2 (ABCC2) and BSEP (sPgp/ABCB11) are members of the ATP-binding cassette superfamily of transporters and are expressed on the apical membrane of cells in a number of tissues. P-gp and BCRP are expressed in the luminal membrane of enterocytes, endothelial cells in the brain, brush border membrane of renal proximal tubules and canalicular membrane of hepatocytes where they limit the intestinal absorption, blood-brain barrier penetration and facilitate excretion into the bile and urine. MRP2 and BSEP are mainly expressed in the canalicular membrane of hepatocytes where they facilitate excretion into the bile.

To determine if a compound is a substrate of P-gp or BCRP, the bidirectional permeability of the compound across MDCKII-MDR1 or MDCKII-BCRP and MDCKII control cells is measured. Prazosin efflux across BCRP-expressing MDCKII cells is linear over time and saturable. The prototypical P-gp substrate, digoxin, is not saturatable in Caco-2 or MDCKII cells (Caco-2 data shown below). BSEP and MRP2 substrate experiments are evaluated in membrane vesicles by measuring the effect of the test compound on the accumulation of a probe substrate. IC50 curves for inhibition of digoxin P-gp inhibition is evaluated by measuring the bidirectional permeability of digoxin across Caco-2 cells in the presence of the test compound. BCRP inhibition is evaluated by measuring the bidirectional permeability of prazosin across MDCKII-BCRP cells in the presence of the test compound. BSEP and MRP2 inhibition experiments are evaluated in membrane vesicles by measuring the effect of the test compound on the accumulation of a probe substrate. IC50 curves for inhibition of digoxin bidirectional permeability in Caco-2 cells and prazosin in MDCKII cells and Taurocholate in BSEP vesicles in the presence of the test compound are shown below.

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Cell Line</th>
<th>Substrate</th>
<th>Incubation Time (min)</th>
<th>Inhibitor</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp</td>
<td>Caco-2</td>
<td>10 µM Digoxin</td>
<td>120</td>
<td>Verapamil / Valspodar</td>
<td>0.81 ± 0.33 / 0.06 ± 0.02</td>
</tr>
<tr>
<td>P-gp</td>
<td>MDCKII-MDR1</td>
<td>10 µM Digoxin</td>
<td>120</td>
<td>Verapamil / Valspodar</td>
<td>21.7 ± 18.6 / 2.3</td>
</tr>
<tr>
<td>BCRP</td>
<td>MDCKII-BCRP</td>
<td>1 µM Prazosin</td>
<td>120</td>
<td>Ko143 / Lopinavir</td>
<td>0.012 ± 0.003 / 4.6</td>
</tr>
<tr>
<td>BSEP</td>
<td>Vesicles</td>
<td>0.4 µM Taurocholate</td>
<td>5</td>
<td>Cyclosporin A</td>
<td>0.4 ± 0.03</td>
</tr>
</tbody>
</table>

*Error bars represent means ± SD from 2-3 independent experiments*
OATP1B1 (OATP-C/OATP2/SLCO1B1), OATP1B3 (OATP-8/SLCO1B3) and OCT1 (SLC22A8) are expressed on the sinusoidal membrane of hepatocytes and facilitate the uptake of endogenous and xenobiotic compounds into hepatocytes for further metabolism or excretion into the bile. OAT1 (SLC22A6), OAT3 (SLC22A8) and OCT2 (SLC22A2) are expressed on the basolateral membrane of renal proximal tubules and facilitate the uptake of compounds into the proximal tubule for further excretion into the urine.

To determine if a compound is a substrate of an uptake transporter, the accumulation of the compound in cells over-expressing a single uptake transporter is measured. Inhibition of uptake transporters is evaluated by measuring the accumulation of a probe substrate in the cells in the presence of the test compound. Examples of substrate accumulation and concentration-dependent inhibition are presented below.

### OATP1B1 (HEK-293)

![Graph](image1)

**Time Course Evaluation**

**Kₘ/Vₐ₅ₐ**

**IC₅₀**

*Error bars represent means ± SD from 2-3 independent experiments*

### OAT1 (HEK-293)

![Graph](image2)

**Time Course Evaluation**

**Kₘ/Vₐ₅ₐ**

**IC₅₀**

### Transporter | Cell Line | Substrate | Kₘ (µM) | Incubation Time (min) | Inhibitor | IC₅₀ (µM)
---|---|---|---|---|---|---
OATP1B1 | HEK-293 | 50 nM Estradiol-17βglucuronide | 6.0 ± 1.8 | 2 | Rifampin / Cyclosporin A | 0.80 ± 0.13 / 0.04 ± 0.002
OATP1B3 | HEK-293 | 50 nM Estradiol-17βglucuronide | 13.2 ± 2.5 | 2 | Rifampin / Cyclosporin A | 0.19 ± 0.09 / 0.09 ± 0.002
OAT1 | HEK-293 | 1 µM p-Aminohippuric acid | 42.9 ± 8.9 | 1 | Probeneid / Novobiocin | 8.7 ± 3.2 / 8.2
OAT3 | HEK-293 | 50 nM Estrone-3-Sulfate | 24.4 ± 11.9 | 2 | Probeneid / Ibuprofen | 2.8 ± 1.1 / 4.5
OCT1 | HEK-293 | 5 µM Tetraethylammonium | 0.5 ± 1.8 | 15 | Quinidine / Verapamil | 15 ± 3 / 0.5
OCT2 | HEK-293 | 10 µM Metformin | 1840 ± 640 | 2 | Quinidine / Cimetidine | 25 ± 1 / 280
Selection of test article concentrations for *in vitro* transporter inhibition assays

The 2012 FDA Guidance for Industry (Drug Interaction Studies- Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations) and the 2013 EMA Guideline on the Investigation of Drug Interactions describe criteria for investigating possible DDIs based on *in vitro* inhibition data of compounds. The criteria relate pharmacokinetic data and the IC$_{50}$ or $K_i$ of the compound. Based on those criteria, the maximum concentration of the compound that needs to be tested in an *in vitro* inhibition experiment can be inferred. The highest concentration that can reasonably be tested in each experiment should also be considered as it may be limited by *in vitro* solubility or cell toxicity or estimated maximum *in vivo* solubility at gastrointestinal or physiological pH. The criteria outlined below are described in the EMA and FDA guidance documents.

### Selection of test article concentrations for *in vitro* transporter inhibition assays

<table>
<thead>
<tr>
<th>Transporter expression</th>
<th>EMA criteria</th>
<th>Relevant concentration</th>
<th>FDA criteria</th>
<th>Relevant concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp, BCRP intestinal</td>
<td>$K_i &lt; 0.1x\ dose/250 \text{ mL}$</td>
<td>0.1x dose/250 mL</td>
<td>$[I]<em>2/IC</em>{50}$ is &gt; 10</td>
<td>0.1x dose/250 mL</td>
</tr>
<tr>
<td>P-gp, BCRP systemic</td>
<td>$K_i \leq 50x\ unbound \ C_{\text{max}}$</td>
<td>50x unbound $C_{\text{max}}$</td>
<td>$[I]<em>1\ total/IC</em>{50}$ is $\geq 0.1$ (equivalent to $IC_{50} \leq 10x\ total\ C_{\text{max}}$)</td>
<td>10x total $C_{\text{max}}$</td>
</tr>
<tr>
<td>OATP1B1 and OATP1B3 (hepatic uptake)</td>
<td>$K_i \leq 25x\ unbound portal vein concentration or 50x the unbound $C_{\text{max}}$ for iv drugs</td>
<td>25x unbound portal vein concentration or 50x unbound $C_{\text{max}}$ of iv drugs</td>
<td>$[I]<em>1\ total/IC</em>{50}$ is $\geq 0.1$ (equivalent to $IC_{50} \leq 10x\ total\ C_{\text{max}}$)</td>
<td>10x total $C_{\text{max}}$</td>
</tr>
<tr>
<td>OAT1, OAT3 and OCT2 (renal uptake)</td>
<td>$K_i \leq 50x\ unbound \ C_{\text{max}}$</td>
<td>50x unbound $C_{\text{max}}$</td>
<td>$[I]<em>1\ unbound/IC</em>{50}$ is $\geq 0.1$ (equivalent to $IC_{50} \leq 10x\ unbound\ C_{\text{max}}$)</td>
<td>10x unbound $C_{\text{max}}$</td>
</tr>
</tbody>
</table>

For reference only:

- $[I]_1$ Concentration equal to the unbound or the total $C_{\text{max}}$
- $[I]_2$ Concentration equal to the maximum dose in 250 mL, apparent intestinal concentration

Contact us to learn more about our Drug Transporter offerings at [www.xenotech.com](http://www.xenotech.com) or by calling [913.438.7450](tel:913.438.7450)