An Overview of Drug Transporters in ADME & Drug Action

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Principles of Clinical Pharmacology
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Implications of Drug Transport in Drug Discovery and Development

- Impact of Drug Transport on ADME
  - Oral absorption of drug
  - Complex metabolism interaction(s)
  - Drug Distribution and elimination
  - Organ-selective delivery of drugs and prodrugs
- Impact of Drug Transport on Response and Toxicology
  - *Emerging Role in Toxicology*
  - Over expression of drug transporter may be a major factor in tumor, bacterial, and fungal multi-drug resistance (MDR).
- Transporters as Targets
  - Zosuquidar and Tariquidar
  - SGLT2 Na-Glucose contraporter
Rosuvastatin Calcium (Crestor) Pharmacokinetics and Prescribing Information

FDA ALERT [03/2003]
Rhabdomyolysis (serious muscle damage) has been reported in patients taking Crestor as well as other statin drugs. To date, it does not appear that the risk is greater with Crestor than with other marketed statins. However, the labeling for Crestor is being revised to highlight important information on the safe use of Crestor to reduce the risk for serious muscle toxicity (myopathy/rhabdomyolysis), especially at the highest approved dose of 40 mg. The labeling will also be revised to reflect the results of a large pharmacokinetic study involving a diverse population of Asian patients compared with a Caucasian control group that found drug levels to be elevated approximately 2-fold. Kidney failure of various types.

Impact: Start patients of Asian descent at lowest dose of Rosuvastatin (5 mg)
Influence of *SLCO1B1* T521>C Genotype on Rosuvastatin AUC

CYP2C9 responsible for formation of N-desmethyl rosuvastatin (10%)
Rosuvastatin also substrate for BCRP (ABCG2)
Presentation Objectives

• Provide an Integrated approach to transporter biology

• Review when drug transport is the rate-limiting step of
  – Absorption
  – Distribution
  – Metabolism and Transporter Interplay
  – Elimination (kidney and liver)

• Examples of when drug transport is a primary determinant of drug action and drug-induced toxicity.

• Provide examples of drug-drug and drug-transporter interactions

• Functional consequences of genetic variations in transporter genes
2006 FDA Draft Guidance, International Transport Consortium and FDA Critical Path Workshop

2006 FDA Draft Guidance

- Knowledge of NME metabolic pathways, interactions, and influence of active transport on drug disposition with respect to DDI potential is key to benefit/risk assessment.

- Integrated approach may reduce number of unnecessary studies and optimize clinical pharmacology studies.

- Classification of CYP inhibitors and substrates can aid in study design and labeling.
  - Substrate (25% metabolism)
  - Inhibitor ([I]/Ki > 0.1)
  - Inducer (40% control)

New Molecular Entity (NME)
International Transport Consortium (ITC)

Slide adapted from Shiew-Mei Huang, Ph.D., FDA
The ITC considers this report as a work in progress, and is highly interested in obtaining feedback, including areas that have not been included in this report but should be considered in the next version as well as controversial concepts. Please send any comments to the corresponding authors.
Transporters covered

- Efflux: P-gp, BCRP
- Renal: OAT/OCT
- Hepatic uptake: OATPs
P-glycoprotein Substrates

- **Cancer Chemotherapy**
  - Doxorubicin
  - Daunorubicin
  - Vinblastine
  - Vincristine
  - Paclitaxel
  - Teniposide
  - Etoposide

- **Immunosuppressive Drugs**
  - Cyclosporine A
  - FK506

- **Antihistamine**
  - Terfenadine

- **Steroid-like**
  - Aldosterone
  - Hydrocortisone et al.

- **HIV Protease Inhibitors**
  - Amprenavir
  - Indinavir
  - Ritonavir
  - Saquinavir

- **Cardiac Drugs**
  - Digoxin
  - Quinidine
  - Posicor
  - Most statins

- **Anti-thelmintics**
  - Ivermectin
  - Abamectin

- **Miscellaneous**
  - Loperamide
  - Colchicine
  - Ondansetron
  - Erythromycin
Clinical Translation of P-gp Inhibition at the BBB

- N=12 subjects
  $[^{11}\text{C}]$verapamil +/- CsA.
- Mean 88% increase in BBB exposure (range 62-148%).
- Clinical observation significantly less than mouse prediction.

Clinical Pharmacology & Therapeutics (2005) 77, 503–514
Role of Mdr1a in the Blood-Brain Barrier and the Placenta

- Mdr1a/b (-/-) were found to be:
  - Viable
  - Fertile
  - Without observable phenotype until pharmacological challenge with IVM.
    - mdr1a -/- LD$_{50}$ = 0.7 mg/kg
    - mdr1a +/- LD$_{50}$ = 60 mg/kg

- CF-1 mice were found to be spontaneously mutant in mdr1a by MSD Scientists. The degree of chemical exposure of fetuses within each litter was inversely related to expression of placental P-gp and cleft palate susceptibility
  - mdr1a -/- 100% cleft palate
  - mdr1a +/- 50% cleft palate
  - mdr1a +/- 0%

Figure from A.H. Schinkel et al., Cell, Vol.77, 491-501, 1994
Many Examples of Drugs whereby BBB Entry is Not Desirable

- Ivermectin
- Digoxin
- Non-sedating antihistamines
  - Fexofenadine
  - Loratadine
  - Cetirizine

Ivermectin Toxicity in the Collie

- 50% of Collies display CNS toxicity when treated with normal doses of IVM (>60 μg/kg).
- Ivm-sensitive Collies lack functional P-gp at the blood brain barrier.
- ABCB1 cDNA sequencing
  - Sensitive Collies (7/7)
    - 4-base pair deletion
    - homozygous
  - Non-sensitive Collies (6/6)
    - heterozygous (mutant/normal)
  - Other breeds (4/4)
    - normal/normal


http://www.awca.net/drug.htm
P-glycoprotein (ABCB1) Cluster Evaluation

**Clinical Study**
- Human DDI

**Lower Throughput**
- abcb1 (KO)
- Preclinical DDI
- FACs Analyses
- Isolated Perfused Organ (brain/gut)
- Confocal studies

**Medium Throughput**
- Caco-2 or MDCK ABCB1
- Substrate/Inhibitor
- Membrane vesicle assays
- Radiochemical Uptake Assay

**Higher Throughput**
- CAM Inhibition
- P-gp ATPase
- PXR-Induction
- Cytotoxicity Assays
  - *In-silico*
In Vitro Permeabilities

- Mannitol: Passive Transcellular 0.26, Passive Paracellular 0.28
- Testosterone: Passive Transcellular 85, Passive Paracellular 101
- Vinblastine (P-gp substrate): Wild-type 0.90, MDR1 0.22, MDR1 + CsA 1.9
Caco-2 and MDCK cell comparison

Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub
In Vitro P-gp IC$_{50}$ for Inhibition of Digoxin Efflux Data from Multiple Labs / Techniques

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pfizer (Net Flux, Caco-2)</th>
<th>Pfizer (Efflux Ratio, Caco-2)</th>
<th>GSK (B to A flux, MDR-MDCK)</th>
<th>Borchardt (B to A flux, MDR-MDCK)</th>
<th>Borchardt (B to A Flux, Caco-2)</th>
<th>BI (B to A flux, MDR-MDCK)</th>
<th>Kim, Wilkinson (Net flux, Caco-2)</th>
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<td>15.1</td>
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<td>Vinblastine</td>
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<td>8.92</td>
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</table>

Slide courtesy of M. Troutman/C. Lee Pfizer
Digoxin: Safety Concerns

- Therapeutic conc ~ 1.5 ng/mL
- 33% change in Digoxin Exposure ($C_{\text{max}}$) ~ 2.0 ng/mL \(\rightarrow\) Safety concerns
- 25% change in exposure might be clinically relevant

**Fenner et al., Clinical Pharmacology & Therapeutics (2009); 85, 173–181**
P-gp Mediated Digoxin DDIs

• <2-fold change in digoxin Cmax or exposure were observed in the majority of published cases
  – I/IC50 > 0.1 is predictive of positive clinical digoxin DDI related to P-gp
  – I2/IC50 < 10 is predictive of no clinical digoxin DDI
• For Digoxin or NMEs that have a narrow T.I. (similar to digoxin), P-gp may be an important determinant of PK and response.
• Additional work is needed to fully understand the mechanism of false (-)’s observed with I/IC50 or false (+)’s with I2/IC50
Substrate overlap with multiple CYPs and Drug Transporters complicates in vitro to in vivo predictions

However, if your drug is a substrate of CYP3A4 and P-gp, Ketoconazole oritraconazole represents the worse case scenario for a Clinical DDI study

Mol. Pharmaceutics, 2009, 6 (6), pp 1766–1774
P-gp Summary

• For some compounds, P-gp may hinder drug absorption, moderately change AUC/Cmax and be moderate to major determinant of CNS exposure.

• P-gp may be a target for Drug-Drug Interactions, optimal in-vitro to in-vivo or in-vivo to in-vitro strategy is needed. No Single in-vitro assay appears to be durable enough to perform within diverse chemical libraries and yield consistent ‘predictable’ in-vivo performance.
  – Multi-tiered Assay Cluster Approach used to define NCE/Drug-P-gp interaction.

• Use of mdr1a KO mouse appears to be the most sensitive method to define P-gp substrates, however, cross-species differences in P-gp remains a concern

• Overlap in CYP3A4 and P-gp inhibition may produce ‘worse case scenario’ for some drugs that are substrates for CYP3A4 and P-gp
Many drugs that are efflux substrates are extensively absorbed (fa >80%).

Factors that contribute to efflux limited absorption are high Km, Vmax, low solubility, low permeability, metabolic stability and low dose.
**Pgp/BCRP Inhibitor Decision Tree**

- **False Positives** (unnecessary clinical studies)
- Alert for $\frac{[I]}{IC_{50}} \geq 0.1$ or $\frac{[I]}{IC_{50}} \geq 10$,
  - $[I]$ is steady-state total Cmax at the highest clinical dose
  - $[I]$ is the GI concentration calculated as dose (mg)/250 mL
- $\frac{[I]}{IC_{50}} > 10$ 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 for NTI drugs like digoxin and topotecan)

*Slide courtesy from Joe Polli and ITC.*
ABCG2 (alias BCRP, MXR, ABCP, BMDP)

- Expressed endogenously in the intestine (small & large), liver, kidney, placenta, skeletal muscle, brain, and in hematopoietic stem cells
- In-vitro role in tumor drug resistance for Topo-1 and Topo-2 inhibitors (MXR, SN-38, Topotecan, J-107088)
- Emerging role in drug absorption of camptothecan analogues (Irinotecan and Topotecan).

- ABC subfamily 7 (G); member 2 (related to Drosophila White proteins)
- 655 amino acid protein
  - ABCP isolated from human placenta R482 WT (Allikmets, 1996)
  - BCRP breast cancer resistance protein R482 T (Doyle et al., 1998)
  - MXR: Mitoxantrone resistance protein R482G (Bates et al., 1999)
  - BMDP: Brain multidrug resistance protein (Eisenblatter et al., 2003)
### Substrates & Inhibitors of ABCG2

<table>
<thead>
<tr>
<th>Drugs/NMEs</th>
<th>Xenobiotics</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topotecan</td>
<td>PhIP</td>
<td>FTC</td>
</tr>
<tr>
<td>CPT-11/SN-38</td>
<td>Pheophorbide A</td>
<td>Ko134, 143</td>
</tr>
<tr>
<td>J-107088</td>
<td>Estrogen SO₄</td>
<td>Tryprostatin A</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>lysotracker (green)</td>
<td>GF120918</td>
</tr>
<tr>
<td>Flavoperidol</td>
<td>H33342</td>
<td>Lapatinib</td>
</tr>
<tr>
<td>Diflomotecan</td>
<td>Rhodamine 123</td>
<td>Erlotinib</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Bodipy-prazosin</td>
<td>Gefitinib</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>Riboflavin (vitamin B2)</td>
<td>CI-1033</td>
</tr>
<tr>
<td>Prazosin</td>
<td></td>
<td>Novobiocin</td>
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<tr>
<td>Benzoylphenylurea</td>
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<td>Imatinib</td>
</tr>
<tr>
<td>Cimetidine</td>
<td></td>
<td>Ritonavir</td>
</tr>
<tr>
<td>Imatinib</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endobiotics</td>
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</tr>
</tbody>
</table>

- **Bcrp**-/ADME Phenotype
  - Mice displayed diet-dependent phototoxicity
  - Protoporphyria
  - Enhanced oral absorption of topotecan
  - ABCG2 is expressed in bone marrow stem cells.

**Expression BCRP in mammary gland across species**

<table>
<thead>
<tr>
<th></th>
<th>Nonlactating</th>
<th>Lactating</th>
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<tbody>
<tr>
<td>Mouse</td>
<td><img src="image" alt="Mouse Nonlactating" /></td>
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<tr>
<td>Cow</td>
<td><img src="image" alt="Cow Nonlactating" /></td>
<td><img src="image" alt="Cow Lactating" /></td>
</tr>
<tr>
<td>Human</td>
<td><img src="image" alt="Human Nonlactating" /></td>
<td><img src="image" alt="Human Lactating" /></td>
</tr>
</tbody>
</table>

**Literature:**
- BCRP substrates reported concentrated into milk of each of these species
- MRP1-5, P-glycoprotein not upregulated in lactating mouse mammary gland

*Slide from A.H. Schinkel, NKI*
Of mice and men: Topotecan:BCRP interaction

Jonker et al., JNCI, 2000

Jonker et al., PNAS, 2002

Jonker et al., JNCI, 2000

Kruijter et al., JCO, 2002
Absorption, metabolism, and excretion of salicylazosulfapyridine in man

Fig. 2. Serum concentrations of SASP after ingestion of a single 4 Gm. dose of SASP on Day 1 (10 subjects) and 4 × 1 Gm. of SASP on Days 2 to 10 (9 subjects).

Hasse Schröder and Dag E. S. Campbell  Uppsala, Sweden
Department of Zoophysiology, University of Uppsala, Pharmacia AB, Box 904, 751 25
Permeability is an important determinant of *in vitro-in vivo* extrapolation for both Metabolism and Transport


Sulfasalazine (SASP) Hypothesis

Inter-individual differences in intestinal expression and function of ABCG2 (BCRP) contribute to variability in drug bioavailability, exposure and pharmacological response to SASP.
Sulfasalazine (SASP) Disposition

- Indications: Rheumatoid arthritis (RA), Long term therapy of ulcerative colitis, and Crohn’s disease
- Bioavailability (F) of SASP in humans is low (F< 15%) and highly variable
- Low %F primarily attributed to SASP’s low permeability and poor solubility (thus, poor absorption)
- Azo-reduction is the primary route of metabolic clearance
- Metabolism occurs in distal small intestine and large intestine via bacterial flora
- Studies in T-cells (CEM) demonstrate SASP is an ABCG2 (BCRP) substrate
Abcg2 is Major Determinant of SASP Absorption and Elimination in the Mouse

Zaher et al., Molecular Pharmaceutics epub January 4, 2006
Abcb1 (mdr1a) does not contribute to SASP Bioavailability or Clearance

Zaher et al., Molecular Pharmaceutics epub January 4, 2006
**SASP C<sub>max</sub> and exposure (AUC) in Bcrp1 (abcg2) and mdr1a (WT and KO) mice following intravenous (IV) and oral (PO) administration.**

Zaher et al., Molecular Pharmaceutics epub January 4, 2006

<table>
<thead>
<tr>
<th>Mice</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)*</th>
<th>AUC (ng.hr/mL)</th>
<th>Relative exposure, AUC&lt;sub&gt;KO&lt;/sub&gt;/AUC&lt;sub&gt;WT&lt;/sub&gt;</th>
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<td></td>
<td></td>
<td>WT</td>
<td>KO</td>
<td>Duration (hr)</td>
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<td>IV</td>
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<td>1827</td>
<td>13570</td>
<td>0-4</td>
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<tr>
<td></td>
<td>PO</td>
<td>20</td>
<td>233</td>
<td>16176</td>
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<td>Mdr1a</td>
<td>IV</td>
<td>5</td>
<td>2749</td>
<td>2266</td>
<td>0-6</td>
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<tr>
<td></td>
<td>PO</td>
<td>20</td>
<td>349</td>
<td>440</td>
<td>0-24</td>
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</table>

* IV (intravenous) = C<sub>max</sub> at time zero was extrapolated from the model; PO (Oral) = visual C<sub>max</sub> from raw data
SASP Disposition in North American Healthy Volunteers

Altered SASP Exposure in Q141K Subjects

421C>A SNP Changes Surface ABCG2 Expression

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<th>kDa</th>
<th>Total Protein</th>
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<td>Vector control</td>
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<tr>
<td>105</td>
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<tr>
<td>75</td>
<td>BCRP (V5)</td>
<td>Calnexin</td>
<td>421C&gt;A</td>
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</table>

SASP Disposition in Healthy Japanese Volunteers

Figure 2  Effect of ABCG2 genotype on pharmacokinetics of sulfasalazine (SASP). Plasma concentration-time profiles of SASP after oral administration of a 2,000 mg conventional SASP tablet to 421C/C subjects (closed circles, n = 12), 421C/A subjects (open triangles, n = 16), and 421A/A subjects (closed diamonds, n = 9).

Yamasaki et al., CPT January 2, 2008
### ABCG2 Pharmacogenomic Studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Dose, Route</th>
<th># Patients</th>
<th>Ethnic Group, Gender</th>
<th>Result</th>
<th>Reference</th>
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<td>1000 mg po</td>
<td>17</td>
<td>Caucasian Both</td>
<td>1.7-2.4X increase in AUC, Cmax</td>
<td>Urquhart et al (2008) Pharmacogen &amp; Genomics, ePub</td>
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<tr>
<td>Sulfasalazine</td>
<td><img src="image3" alt="Structure" /></td>
<td>500 mg po</td>
<td>36</td>
<td>Chinese Both</td>
<td>No effect on AUC, Cmax</td>
<td>Adkinson et al (2008) ASCPT mtg poster</td>
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<td>Gefitinib (IRESSA)</td>
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<td>250 mg po</td>
<td>124</td>
<td>Caucasian Both</td>
<td>44% with mutation had diarrhea vs. 12% with WT</td>
<td>Cusatis et al (2007) JNCI 98(23):1739</td>
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<td>Topotecan</td>
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<td>≤2.5 mg po, iv</td>
<td>18</td>
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<td>1.35X increase in oral bioavailability</td>
<td>Sparreboom et al (2005) Canc Biol Ther 4:650</td>
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<td>Diflomotecan</td>
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<td>≤0.5 mg po, iv</td>
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<td>Caucasian Both</td>
<td>3X increase in AUC and Cmax for iv only</td>
<td>Sparreboom et al (2004) Clin Pharmacol Ther 76:38</td>
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<td>Imatinib (GLEEVEC)</td>
<td><img src="image8" alt="Structure" /></td>
<td>100-1000 mg po</td>
<td>82</td>
<td>Caucasian Both</td>
<td>No difference</td>
<td>Gardner et al (2006) Clin Pharmacol Ther 80:192</td>
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</table>
ABCG2 Polymorphisms and Ethnic Distribution of SNPs.

- The ABCG2 Q141K genotype significantly affected the pharmacokinetics of diflomotecan (Clin Pharmacol Ther. 2004).
- Gefitinib-induced diarrhea correlates with Q141K (J Natl Cancer Inst. 2006).
- ABCG2 expression correlates with flavopiridol-induced myelotoxicity.

<table>
<thead>
<tr>
<th>Allelic variant</th>
<th>Caucasians</th>
<th>African-Americans</th>
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<th>Hispanics</th>
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<td>V12M</td>
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Figg et al., Anticancer Drugs. 2007
Plasma concentrations versus time curve after oral administration of SASP (20 mg/kg) alone or combined with gefitinib (50 mg/kg) gavage 2 hrs prior to SASP administration in wt-type mice.
Curcumin increases SASP Bioavailability

ABCG2 Summary

- ABCG2 (BCRP/ABCP) has a role in the absorption and the elimination of a growing list of drugs, endobiotics, and xenobiotics.
- Additional probe substrates and inhibitors are needed to investigate cross-species to human comparisons and to improve *in-vitro* to *in-vivo* predictions.
  - SASP dose and formulation are important determinants of ABCG2’s influence on F.
- ABCG2-transfected LLC-PK1 or MDCK cells may be useful to evaluate the interaction of this transporter with NCEs or Drugs, however, many BCRP (ABCG2) substrates require a basolateral uptake transporter.
- The abcg2 KO mouse in combination with ABCG2 (BCRP) assay cluster may be best way to define ABCG2 substrates and inhibitors.
The SLC Superfamily

- Solute Carrier (SLC) superfamily contains
  - 43 families
  - 298 genes
- HUGO database (see http://www.gene.ucl.ac.uk/nomenclature/)
  - SLC root symbol
  - Followed by numeral (family)
  - Followed by letter
  - Followed by numeral (ie SLC22A1)
  - Further elaborated in the SLC21/SLCO

<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>OAT1 (SLC22A1)</td>
<td>Free amino acids, amines, nucleosides, organic anions, aromatic acids</td>
<td>Prasugrel, ursodeoxycholic acid</td>
<td>Kidney proximal tubule, colon</td>
<td>Has role in disposition and excretion</td>
</tr>
<tr>
<td>OAT2 (SLC22A2)</td>
<td>Ostrone-3-sulfate, norepinephrine, serotonin, dopamine, histamine, catecholamines, salicylates</td>
<td>Prasugrel, ursodeoxycholic acid</td>
<td>Kidney proximal tubule, colon</td>
<td>Has role in disposition and excretion</td>
</tr>
<tr>
<td>OCT1 (SLC22A1)</td>
<td>Ostrone-3-sulfate, norepinephrine, serotonin, dopamine, histamine, catecholamines, salicylates</td>
<td>Cinacalcet, glibenclamide, verapamil</td>
<td>Kidney proximal tubule, colon</td>
<td>Has role in disposition and excretion</td>
</tr>
<tr>
<td>OCT2 (SLC22A2)</td>
<td>Ostrone-3-sulfate, norepinephrine, serotonin, dopamine, histamine, catecholamines, salicylates</td>
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</tr>
</tbody>
</table>

*Courses not tested for in vivo clinical studies.

Nature Reviews Drug Discovery 9, 215-236 (March 2010)

NIH Principles in Clinical Pharmacology: Transporter Biology 20 January 2011
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.

Major Renal Transporters

Blood Flow

Filtration (GFR) *fu

\[ \text{CL}_f = \text{GFR} + \text{secretion} - \text{reabsorption} \]

\[ \text{CL}_f = \text{GFR} \]

Filtration only

secretion = reabsorption

\[ \text{CL}_f < \text{GFR} \] (net reabsorption)

\[ \text{CL}_f > \text{GFR} \] (net secretion)

*fu
When is it Important to Study Renal Transporters?

• Does scientific evidence suggest that it is necessary to investigate renal transport DDI potential for NMEs?
  – Toxicologic significance
  – Primary determinant of systemic CL
  – NME inhibits the $CL_R$ of compound with narrow TDI

• Is there a need to perform both probenecid and cimetidine studies in healthy volunteers if in vitro and preclinical data support that compound is a prototypical transport substrate?
Renally-Mediated DDIs

Penicillin/Probenecid one of the earliest examples of ATS (Active Tubular Secretion) inhibition.

Drugs that have labeling precautions relating to renally-mediated drug transport:

Dofetilide (Tikosyn™)
> Concomitant administration OCT inhibitors increase potential for cardiac toxicity

Cidofovir (Vistide™)
> Concomitant administration of OAT inhibitors decrease potential for nephrotoxicity
Package Inserts: Clinical Studies and DDI Potential

<table>
<thead>
<tr>
<th>Drug (CL&lt;sub&gt;R&lt;/sub&gt;)</th>
<th>Results (Bedside)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirapex (400 mL/min) + cimetidine + probenecid</td>
<td>N=12 subjects/treatment arm. 50% ↑ in AUC; 40% ↑ in T 1/2. No effect on PK</td>
</tr>
<tr>
<td>Tikosyn (420 mL/min) + cimetidine + probenecid</td>
<td>Narrow TI 40% ↑ in AUC; CLR ↓ 33%; QTc ↑ 17-19 ms. No effect</td>
</tr>
<tr>
<td>Metformin (600 mL/min) + cimetidine + probenecid</td>
<td>Narrow TI 40% ↑ in AUC and 60% ↑ in Cmax. No effect</td>
</tr>
<tr>
<td>Oseltamivir + cimetidine + probenecid</td>
<td>N=12-18/treatment (see Hill et al.). No change on PK 2.5-fold AUC of Ro64-0802 (active metab)</td>
</tr>
</tbody>
</table>
Evaluation of OCT or OAT inhibitors requires determination of an IC50 in an *in vitro* study

Nature Reviews Drug Discovery 9, 215-236 (March 2010)
**Hepatic Uptake/Efflux Transporters**

- **Hepatic permeability**
- **Basolateral membrane**
- **Hepatic uptake**
- **Efflux transporters**

- **ABCB1**
- **ABCB3**
- **ABCC2**
- **ABCC3**
- **Bile canaliculus**
- **ABCG2**
- **NTCP**
- **OATP1B1**
- **OATP2B1**
- **OATP1B3**

- **Etoposide-glucuronide**
- **Vinblastine, taxol, doxorubicin, large-hydrophobic MW drugs**
- **Taurocholate, bile acids**

- **PC (flippase)**

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*NIH Principles in Clinical Pharmacology Transporter Biology 20 January 2011*
Hepatic Transporters

Question 1. Is uptake transport the rate-Limiting Step of total clearance (assume low/no metabolism).

Question 2. Is it possible to predict the DDI potential mediated through hepatic uptake or efflux or are we only able to define potential mechanisms of a PK observation?

Question 3. Toxicological significance of bile acid uptake, synthesis, or efflux inhibition
Hepatic Transport and Liver Injury

SLCO1B1 Variants and Statin-Induced Myopathy — A Genomewide Study

The SEARCH Collaborative Group

ABSTRACT

Leveraging how drug transporter substrates with limited energy metabolism interplay with transporters in comorbid or chronic diseases, and larger variations in environment may produce large results, in rare cases, particularly recent in association with drug delivery, reports. When the variance may be less detectable and within certain environments.

METHODS

We carried out a genomewide association study using approximately 200 million markers in a total of 50,000 patients with different chronic diseases and 45,000 controls. All-cause deaths were censored at 94 mg of formulation daily as part of a trial involving 32,000 participants. In total, 437 was noted in a trial of 437 mg of formulation daily involving 32,000 participants.

RESULTS

The genomewide scan yielded a single strong association of sensitivity with the n444G/C single nucleotide polymorphism (SNP) located within the SLCO1B1 gene in an odds ratio (OR) of 2.43 (95% CI 1.29–4.50). The finding of the n444G/C SNP correlated with the prevalence of the n444G/C SNP was 8.5% in the population. The OR for 10 mg of formulation daily was 1.29 (95% CI 1.13–1.48) per copy of the C allele, and 1.69 (95% CI 1.47–1.93) for 10 mg of formulation daily, which was also an association between the n444G/C SNP and the drug-related change in the results. For SLCO1B1, no severe regions were strongly associated with an increased risk of liver injury.

CONCLUSIONS

We have identified common variants in SLCO1B1 that are strongly associated with an increased risk of liver injury. Identifying these variants may help to achieve the data of liver injury, bone injury, and disability. (Citation Controlled Trials register, NCT00747249595)
**SLC01B1 Variants and Statin-Induced Myopathy**

![Graph](image)

**Figure 1.** Results of Tests for a Trend in the Association between Myopathy and Each SNP Measured in the Genome-wide Association Study.

P values are shown for each SNP measured among 85 participants with myopathy and 90 matched controls who were taking 80 mg of simvastatin daily. Analyses are based on 316,184 of the 318,237 SNPs (99.4%) on the Sentrix HumanHap300-Duo BeadChip (Illumina). A result above the horizontal red line indicates strong evidence of an association (P<5x10^{-9}).

Rifampicin

- Antibiotic used in treatment of tuberculosis
- Known for its ability to induce drug metabolizing enzymes and transporters through activation of pregnane X receptor (PXR)
- Identified as an inhibitor of OATPs and entry into human hepatocytes mediated by OATP1B1

MW = 822
Rifampicin Inhibits Atorvastatin through OATP

- 600 mg rifampicin IV increases atorvastatin acid AUC 7-fold.
- Acutely, single dose rifampicin may inhibit OATP1B3, CYP3A4, and CYP2C8.

(Lau YY et al., Clin Pharmacol Ther, 81, 194-204 (2007), slide courtesy of Dr. L.Z. Benet)
Rifampacain Disposition in WT vs Slco1b2⁻/⁻ KO Mice

In multivariate analyses, the rifampin AUC0-24 was significantly affected by rifampin dosage (in mg/kg), SLCO1B1 c.463C>A polymorphism, and presence of tuberculosis by the region of enrollment.

Hepatic Uptake Substrate Decision Tree

Is hepatic elimination an important route of elimination of NME?
Criteria: $CL_{ir} > 0.3 CL_{total}$

Yes

Does the compound have active hepatocyte uptake, do the drugs physiological properties (e.g. 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 inhibitions and/or transfected cell lines.

If an OATP substrate, consider a clinical DDI study with single dose rifampicin or cyclosporin as perpetrator. Further consideration could be given to review clinical PK based on OATP genotyping.

Likely a poor or not a substrate for OATPs

No

Likely a poor or not a substrate for OATPs

* Low permeability needs to be defined by each lab based on standards, such as atenolol (BCS reference drug). A general guide would be $10^{-6}$ cm/sec (10 nm/sec) or lower is ‘low’ permeability.

Nature Reviews Drug Discovery 9, 215-236 (March 2010)
OATP Inhibitor Decision Tree

Is the IC_{50} of the NME ≤ 10 times unbound Cmax?

Yes

Is the AUC or Cmax of statin (e.g. rosvastatin, pravastatin, pitavastatin) predicted to increase > 2 fold in presence of the NME using extrapolation (e.g. R-value > 2)?

Yes

Clinical DDI study with sensitive substrate (e.g. rosvastatin, pravastatin, pitavastatin)

No

Clinical study may not be needed

No

NME likely not to be an in vivo inhibitor of OATP.

R-value = 1 + (fu * I_{in,max}/IC_{50}), where, I_{in,max} is the estimated maximum inhibitor concentration at the inlet to the liver and is equal to: I_{in,max} = (F_{l}/Dose * k_{a} * Q_{l}). I_{in,max} is the maximum systemic plasma concentration of inhibitor; F_{l} is the fraction of the dose of inhibitor, Dose, which is absorbed; k_{a} is the absorption rate constant of the inhibitor and Q_{l} is the hepatic blood flow (e.g., 1500 mL/min)

Nature Reviews Drug Discovery 9, 215-236 (March 2010)
Future Direction of Drug Transport in Preclinical Development and Clinical Pharmacology

- Drug-Drug Interactions mediated through drug transporter(s) have received increased attention and are recognized as important contributors of ADME.
- Significant substrate overlap exists between drug metabolizing enzymes and drug transporters.
- Evaluation of *in-vitro* screens to predict *in-vivo* drug-drug interactions is an area of increased awareness during drug development. Therefore, the accuracy of the predicted DDI is dependent on the **Quality** of the *in-vitro* assay and our ability to translate the interaction into the Clinic.
  - **Clinical Translation** with respect to physiologic PK of transport probe substrates and inhibitors is needed.
- Preclinical and clinical differences in transporter expression remain important determinants of drug-induced toxicity and an important consideration in drug development.
  - Additional KO and Tg models to investigate the *in-vivo* contribution of drug transporters are needed.
## Acknowledgment(s) and Contributors

- **Genentech Research and Early Development, Development Sciences, Clinical Pharmacology, ED-PK/PD, SA, and DMPK**

- **ITC Collaborators**

<table>
<thead>
<tr>
<th>Academia:</th>
<th>Industry:</th>
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<tbody>
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<td>Shiew Mei Huang</td>
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<td>Lei Zhang</td>
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THANK YOU !!
Transporter Nomenclature

**SLC Family**

- **Basolateral**
  - OCT2 = SLC22A2
  - OAT1 = SLC22A6
  - OAT3 = SLC22A8
  - System L = SCL7A5/8

- **Apical**
  - PepT2 = SLC15A2
  - OCTTN1 = SLC22A4
  - OCTN2 = SLC22A5
  - OAT4 = SLC22A11
  - hMATE1 = SLC47A1
  - hMATE2 = SLC47A2

**ABC Family**

- **Apical**
  - MDR1 = ABCB1
  - MRP2 = ABCC2
  - MRP4 = ABCC4
  - BCRP = ABCG2
Hepatic Drug-Drug and Drug Transporter Interaction Potential

- Is Drug eliminated unchanged in the bile and is a substrate of uptake transporter or transporters?
  - Permeability
  - Multiplicity
  - Affinity and Capacity
    - Relative abundance of OATP1B1, OATP1B3, OAT2B1, NTCP
    - Selective vs pan-inhibitors (ie CsA)
- Is Drug a substrate of uptake and efflux transporters
  - Multiplicity (ABCB1, ABCC2, and ABCG2)
- Uptake/efflux synergy
Drug Interactions: CYP Mediated

- Significant CYP mediated drug interactions based on AUC ratio

N= 115 Studies
CYP2C9, 2D6, 3A4

CYP Summary

• CYP interactions were complex when first recognized
• Largest CYP-mediated DDIs
  – Increase AUC 20X, $C_{\text{max}}$ 12X
• Mechanism of CYP inhibition
  – Competitive or non-competitive
  – Potent inhibitors in sub-nanomolar range
• Many CYP liabilities are thought to be ‘screened’ out at an early stage of preclinical development, however, what liabilities are we selecting for?
The rate determining process

“To understand the transporter-mediated drug-drug interaction, we have to know the rate determining process of a substrate in the overall clearance.”

uptake, basolateral efflux, apical excretion, metabolism

Professor Sugiyama, Keynote address AAPS, November 2007
ABC Substrate/Inhibitor Overlap

Distinct but Overlapping Substrate Specificities

- FTC & Ko134
- LysoTracker
- Prazosin
- prazosin
- ABCG2
- J-107088
- gefitinib
- GF120918
- Mitoxantrone
- Rho-123
- Topotecan
- SN-38
- ABCB1
- CPT-11
- Bicalutamide
- Daunorubicin
- Doxorubicin
- Docetaxel
- LDH
- Vinblastine
- VP-16
- Calcein
- AM
- Colchicine
- Tc-Tetrofosmin
- Tc-Sestamibi
- VX-710
- PSC-833

Figure adapted from Thomas Litman
Pravastatin Css Disposition in WT vs Slco1b2\(^{-/-}\) Mice