Clarity on Transporters:
Navigating the 2012 FDA Draft Guidance
Through the Looking Glass

Preparation

Execution

Keys to Navigating Transporter Science
Clinical Significance of Drug Transporters

Why DDIs Occur

- Altered Pharmacokinetics
- Altered Pharmacodynamics

P-gp by 2006

The Jabberwocky...

Fearsome

Misunderstood

Can be Harmless
Why P-gp?

- Increased frequency in identification of substrates and inhibitors

- Clinical DDI mediated by P-gp
  - Digoxin and quinidine
  - Fexofenadine and ketoconazole (erythromycin)
  - Colchicine and clarithromycin

- Withdrawals/reapprovals

- Increased PMCs and PMRs

Adapted from Zhang L et al., Mol Pharmaceutics 2006; 3(1):62-69
Regulatory Response: 2006 Draft Guidance
Evolving Outlook: A New Dimension

In many cases, negative findings from early in vitro and early clinical studies can eliminate the need for later clinical investigations.

<table>
<thead>
<tr>
<th>Interacting Drug</th>
<th>PK</th>
<th>Fold Change and 90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP3A4/P-gp Inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconazole 100 mg, twice daily</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine 600 mg</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine 200 mg</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Ketoconazole 200 mg, twice daily</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Verapamil 240 mg, once daily</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
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<tr>
<td><strong>Antihypertensives</strong></td>
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<tr>
<td>Hydrochlorothiazide 25 mg, once daily</td>
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<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Ramipril 10 mg, once daily</td>
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</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Amlodipine 10 mg, once daily</td>
<td>Cmax</td>
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</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
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<tr>
<td>Valsartan 320 mg, once daily</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Furosemide 20 mg, once daily</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Irbesartan 300 mg, once daily</td>
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<tr>
<td></td>
<td>AUC</td>
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<tr>
<td>Atenolol 100 mg</td>
<td>Cmax</td>
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<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td><strong>Others</strong></td>
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<td></td>
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<tr>
<td>Metformin 1000 mg, once daily</td>
<td>Cmax</td>
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<td></td>
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<tr>
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<td></td>
<td>AUC</td>
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<td>Citrerdine 600 mg, once daily</td>
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<tr>
<td></td>
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<tr>
<td>Dofetilide 0.25 mg, once daily</td>
<td>Cmax</td>
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<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin 80 mg, once daily</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
<tr>
<td>Lovastatin 40 mg</td>
<td>Cmax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
</tr>
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</table>

P-gp: 2006-2012

Rising Prevalence of Testing

<table>
<thead>
<tr>
<th>Year</th>
<th>Count</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>2003-2006</td>
<td>22</td>
<td>25%</td>
</tr>
<tr>
<td>2007-2010</td>
<td>30</td>
<td>42%</td>
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</table>

P-gp Inhibition Assessment

Frequency of Tools

<table>
<thead>
<tr>
<th>Test System</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caco-2</td>
<td>57%</td>
</tr>
<tr>
<td>MDR1-MDCKII</td>
<td>21%</td>
</tr>
<tr>
<td>Other Cells</td>
<td>22%</td>
</tr>
</tbody>
</table>

In Vitro Cell-Based Test System

Agarwal S, et al, J Clin Pharm XX(X) 1-6
P-gp: 2006-2012

- Reliable Classification
- Potential for Clinical Exceptions

Agarwal S, et al, J Clin Pharm XX(X) 1-6
**In Vitro Choices**

- Inhibitor
- CsA
- Solubility
- Basolateral
- Apical
- Vesicles Test
- Systems
- Caco-2
- Knockdown
- MDR1-MDCK
- FTC
- Ko143
- Assay Format
- Non-specific Binding
- Vectoria
- Substrate
- Time Points
- Digoxin Uptake
- Compound Characteristics
- Cladribine
- Rosuvastatin
- E3S
- Safety Testing
- Safety Testing

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P-gp: 2006 - 2012

- Improving Translatability

- **In Vitro**
  - Improve IV/IVC
    - Maturation of Considerations
      - Evolution of Criteria
  
- **In Vivo**
  - Consistent Recommendations
    - Better Study Designs
      - Standardized Labeling
  
- **Label**
  - Actionable Steps
    - General to Specific Instructions

- **Prescriber/ Patient**
Ongoing Research: 2006 - 2012

- FDA Draft Guidance: P-gp
- EMA: P-gp, BCRP, OATP1B1, OATP1B3, OCT1, BSEP, OAT1, OAT3, and OCT2

- Formation of International Transporter Consortium (ITC)
- ITC White Paper: P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2
- FDA decision trees
Rising Prominence: 2006 - 2012
## Why Hepatic and Renal Transporters?

### Hepatic

<table>
<thead>
<tr>
<th>Compound</th>
<th>Clinical Consequence</th>
<th>Transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lopinavir/Ritonavir</td>
<td>5-48 fold increase in Bosentan Exposure</td>
<td>OATP1B1</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>7.1 fold increase in Rosuvastatin Exposure</td>
<td>OATP1B3</td>
</tr>
</tbody>
</table>

### Renal

<table>
<thead>
<tr>
<th>Compound</th>
<th>Clinical Consequence</th>
<th>Transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimetidine</td>
<td>1.5 fold increase in Pindolol Exposure</td>
<td>OCT2</td>
</tr>
<tr>
<td>Probenecid</td>
<td>3.6 fold increase in Cephradine Exposure</td>
<td>OAT1</td>
</tr>
<tr>
<td>Probenecid</td>
<td>2.9 fold increase in Furosemide Exposure</td>
<td>OAT3</td>
</tr>
</tbody>
</table>

Enzyme-Transporter Interplay

Adapted from Gut 2003; 52: 1788-95
“In vitro data indicate that repaglinide metabolism may be inhibited by antifungal agents like ketoconazole and miconazole, and antibacterial agents like erythromycin (cytochrome P-450 enzyme system 3A4 inhibitors). **Drugs that induce the cytochrome P450 enzyme system 3A4 may increase repaglinide metabolism;** such drugs include rifampin, barbiturates, and carbamezepine.”

Niemi al, Diabetologia. 2003 Mar;46(3): M et 347-51
Complex DDI: CYPs and Transporters

**Repaglinide**
- Metabolized by: CYP2C8, CYP3A4
- Substrate of: OATP1B1

**Gemfibrozil**
- MODERATE inhibitor of: CYP2C8
- Glucuronide
- Irreversible inhibitor of: CYP2C8

**Itraconazole**
- Inhibitor of: CYP3A4
- Inhibitors of: OATP1B1

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Repaglinide: What We Now Know

Comparative PK with Various Genotypes

- Concurrent inhibition of enzyme and transporter
- Increased inhibition of drug elimination by use of inhibitors of more than one enzyme that metabolizes the drug.
- Metabolite-mediated DDI

Drug-Drug Interactions (June 2009)

Repaglinide appears to be a substrate for active hepatic uptake transporter (organic anion transporting protein OATP1B1). Drugs that inhibit OATP1B1 (e.g. cyclosporine) may likewise have the potential to increase plasma concentrations of repaglinide.
Parallel Drug Transporter Work: 2006 - 2012

Emerging 7 Transporters

- P-gp
- BCRP
- OATP1B1
- OATP1B3
- OAT1
- OAT3
- OCT2

Complex DDI:

- Concurrent inhibition and induction
- Parallel inhibition of transporter and metabolism pathways
- Increased inhibition with co-dosed inhibitors
- Metabolite-mediated DDI
- Inhibition of non-polymorphic enzymes in poor metabolizers
- Inhibition in subjects with organ impairment
Drug Transporters: circa 2012

“It seems very pretty, but is hard to understand.”

-Alice, Through the Looking Glass, Lewis Carroll
Clarity on Transporters

Substrate Assessment

Inhibition Assessment

Induction Potential
Evaluation of Substrate Potential

All NMEs

- Determine whether NME is a P-gp and/or BCRP substrate *in vitro*
- Hepatic or biliary secretion major? e.g., ≥25% of total clearance *or unknown*?
- Renal active secretion major? e.g., ≥25% of total clearance *or unknown*?
  - Determine whether NME is an OATP1B1 and/or OATP1B3 substrate *in vitro*
  - Determine whether NME is an OAT1, OAT3 and/or OCT2 substrate *in vitro*

Other transporters (e.g. MRP2) may need to be studied based on knowledge of other drugs in the same therapeutic class.
P-gp and BCRP Substrate

In bi-directional transporter assays (e.g., in Caco-2 or MDR1-overexpressing polarized epithelial cell lines) is the net flux ratio of an investigational drug ≥ 2?

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

- Net flux ratio < 2
  - Poor or non-P-gp substrate

Complete an assessment of nonclinical and clinical information to determine whether an in vivo DDI study is warranted

Considerations: Test Compound Characteristics

**Non-Specific Binding**

- Compound A
- Before: 5
- After: 35
- Average Minoxidil: 10

- 1% BSA
- Pre-incubation

**Intrinsic Permeability**

- Compound B
- MDR1-MDCK: Favorable drug-specific property
- Caco-2: Potentially enables biowaiver
Considerations: Test System Choices

Relevant Organ Phenotype

Fexofenadine
- Multiplicity of transporters
- Clinical P-gp probe substrate

Cladribine
- Clinically relevant interaction with BCRP
Considerations: Design Elements

Reagents vs. Knockdown
Rosuvastatin

![Bar chart showing the comparison of reagents vs. knockdown for Rosuvastatin in different conditions.](chart.png)
Clinical Waiver of Transporter Studies

FDA Perspective

“For drugs that are highly permeable and highly soluble, the intestinal absorption is not a rate-limiting step, and, therefore it may be appropriate to exempt such drugs from the in vivo evaluation with a P-gp or BCRP inhibitor.”

\[ J_e = J_c + J_m \]

\[ \text{when } J_m \gg J_c \]

\[ J_e = J_m \]

\[ J_e : \text{effective flux} \]
\[ J_c : \text{carrier-mediated flux} \]
\[ J_m : \text{passive flux} \]
Exceptions: Consider the Rate-Limiting Step

- Minoxidil: $P_{app} \approx 4$
- Pindolol: $P_{app} \approx 17$
- Metoprolol: $P_{app} \approx 28$
- Antipyrine: $P_{app} \approx 55$

**Graph Details:**
- Quadrant 1
- Quadrant 2
- Quadrant 3
- Quadrant 4
- Human $F_{abs} (%)$
- Caco-2 $P_{app} (x10^4 \text{ cm/s})$
Best Practices

Considerations
1. Stage: Discovery or Development?
2. Properties: Non-specific binding?
3. Intrinsic Permeability
4. Organ Phenotype
5. Specificity
6. Exclusions

Actions
1. Test System Selection: Reagents vs. Knockdown
2. Recovery
3. Calculations
4. Appropriate High Permeability Standard
OATP1B1 and OATP1B3 Substrate

Does the compound have active hepatocyte uptake, do the drug’s 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

Is uptake in OATP1B1- or OATP1B3-transfected cells greater than 2-fold of empty vector transfected cells and inhibitable by known inhibitor?

No

Likely a poor or not a substrate for OATPs

Yes

Consider an in vivo drug interaction study with single dose rifampin or cyclosporin as perpetrator. Comparative PK study in subjects with various genotypes of OATP1B1 can help identify the importance of this pathway

OAT1, OAT3, and OCT2 substrate

Is uptake of the investigational drug in the OCT2-, OAT1- or OAT3-overexpressing cells vs. empty vector cells greater than 2 and inhibitable by known inhibitor?

- Yes
  - Likely a substrate. *In vivo* DDI study with cimetidine for OCT2 and with probenecid for OAT1, OAT3 as perpetrators

- No
  - Poor or not a substrate of OCT2, OAT1, or OAT3
Considerations: Pre-Defined Conditions
Considerations: False Negatives

- Non-specific binding
- Transporter properties
- Robustness of transfection
Considerations: Matrix Evaluation

![Bar chart showing influx rate ratio of Pindolol (OCT2-HEK293 vs HEK293) at different incubation times (1 uM, 2 min incubation, 1 uM, 5 min incubation, 1 uM, 20 min incubation).]
'I see you're admiring my little box.' the Knight said in a friendly tone. 'It's my own invention -- to keep clothes and sandwiches in. You see I carry it upside-down, so that the rain can't get in.'

'But the things can get out,' Alice gently remarked. 'Do you know the lid's open?'

-Through the Looking Glass, Lewis Carroll
Evaluation of Inhibition Potential

Current Perspective:
Inhibition Potential of all 7 transporters should be assessed as part of Safety test

- P-gp and BCRP inhibitor evaluation
- OATP1B1 and OATP1B3 inhibitor evaluation
- Likely to be co-administered with known anionic drugs that are substrates for OAT1/3 (methotrexate, tenofovir, acyclovir, etc.)
- Likely to be co-administered with cationic drugs that are OCT2 substrates (e.g. metformin)?

OAT1 and OAT3 inhibition evaluation
OCT2 inhibition evaluation

• FDA Clinical Pharmacology Advisory Committee Meeting, March 17, 2010
• The International Transporter Consortium, Nature Reviews, March 2010
• FDA Comment on the Report from the ITC, Lei Zhang, AAPS Webinar, July 29, 2010
P-gp and BCRP Inhibition

Bidirectional transport assay with a probe P-gp substrate (e.g. in Caco-2 or MDR1-overexpressing polarized epithelial cell lines)

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

Probably a P-gp inhibitor

Determine Ki or IC$_{50}$ of the inhibitor

$\frac{[I]}{IC_{50}}$ (or Ki) ≥ 0.1 or $\frac{[I]}{IC_{50}}$ (or Ki) ≥ 10

An in vivo drug interaction study with a P-gp substrate such as digoxin is recommended

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

Poor or non-inhibitor

$\frac{[I]}{IC_{50}}$ (or Ki) < 0.1 and $\frac{[I]}{IC_{50}}$ (or Ki) < 10

An in vivo drug interaction study with a P-gp substrate such as digoxin is not needed.

$[I]_1$ is total C$_{max}$ (different from ITC whitepaper)

$[I]_2$ (gut concentration) is new (not in 2006 Guidance)
[I]dentity Crisis

- [I₁] Total plasma Cmax
- [I₂] Dose in 250 mL
- [I₂] Highest clinical dose in 250 mL
- [I₁] Unbound plasma Cmax
- [I₂] Highest clinical dose in 250 mL
- [I₁] Total plasma Cmax
- [I₂] Dose of inhibitor in 250 mL

References:
- FDA Guidance for the Industry 2006
- A regulatory viewpoint on transporter based drug interactions, Xenobiotica 2008
- Predicting drug-drug interactions: An FDA Perspective, AAPS Journal 2009
- EMA Guideline on the Investigation of Drug Interactions, 2010
- Membrane Transporters in Drug Development, Nature Review 2010
- Membrane Transporters in Drug Development: Comment on the Report from the ITC; Lei Zhang, AAPS Webinar July 2010

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[I] Matter: *In Vitro* and *In Vivo* Digoxin Data

<table>
<thead>
<tr>
<th>Drug</th>
<th>$[I_1]/IC_{50}$ (unbound)</th>
<th>$[I_1]/IC_{50}$ (total)</th>
<th>$[I_2]/IC_{50}$</th>
<th>Digoxin $C_{\text{max}}$ (% change)</th>
<th>Digoxin AUC (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lapatinib</td>
<td>&lt; 0.1</td>
<td>1</td>
<td>1355</td>
<td>N/A</td>
<td>180</td>
</tr>
<tr>
<td>Dronedarone</td>
<td>&lt; 0.1</td>
<td>0.09</td>
<td>1349</td>
<td>N/A</td>
<td>150</td>
</tr>
<tr>
<td>Ranolazine</td>
<td>&lt;0.1</td>
<td>0.04</td>
<td>2987</td>
<td>46</td>
<td>60</td>
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<tr>
<td>Darunavir</td>
<td>&lt;0.1</td>
<td>0.33</td>
<td>146</td>
<td>15</td>
<td>58</td>
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<tr>
<td>Tolvaptan</td>
<td>&lt;0.1</td>
<td>0.23</td>
<td>109</td>
<td>30</td>
<td>30</td>
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<tr>
<td>Etravirine</td>
<td>&lt;0.1</td>
<td>0.04</td>
<td>76</td>
<td>19</td>
<td>18</td>
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<tr>
<td>Tetrabenzone</td>
<td>&lt;0.1</td>
<td>0.01</td>
<td>6</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Maraviroc</td>
<td>&lt;0.1</td>
<td>0.01</td>
<td>13</td>
<td>4</td>
<td>0.5</td>
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<td>Deferasirix</td>
<td>&lt;0.1</td>
<td>0.003</td>
<td>4.3</td>
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<td>-8</td>
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<td>Lacosamide</td>
<td>&lt;0.1</td>
<td>0.01</td>
<td>1</td>
<td>4.8</td>
<td>2.4</td>
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<tr>
<td>Sitagliptin</td>
<td>&lt;0.1</td>
<td>0.02</td>
<td>2</td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>

**False Negatives**  **False Positives**

### [I] Matter: *In Vitro* and *In Vivo* Digoxin Data

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$[I_1]/IC_{50}$ (unbound)</td>
<td>6/11 (55%)</td>
<td>5 False Negatives</td>
<td></td>
</tr>
<tr>
<td>$[I_1]/IC_{50}$ (total)</td>
<td>9/11 (82%)</td>
<td>2 False Negatives</td>
<td></td>
</tr>
<tr>
<td>$[I_2]/IC_{50}$</td>
<td>9/11 (82%)</td>
<td>2 False Positives</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>✦ Solubility</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>✦ <em>In vitro/in vivo</em> probe substrate differences</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>✦ Possible P-gp induction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>✦ Weak <em>in vitro</em> inhibitor</td>
<td></td>
</tr>
</tbody>
</table>
IC₅₀ Working Group: 2008-Present

**Objective:** To standardize IC₅₀ evaluation

**Considerations:**
- Choice of test system
- Calculation techniques
- Intra- and inter-lab variability
- Calibration to enhance predictability

**Findings:**
- No “best” test system/equation
- Caco-2 and vesicles produce lower IC₅₀ values than over-expressed systems.
- Use of AUC’ plots, accuracy plots, and ROC analysis enhances accuracy.

IC\textsubscript{50} Working Group: ROC Analysis

<table>
<thead>
<tr>
<th></th>
<th>[I\textsubscript{1}]/IC\textsubscript{50}</th>
<th>[I\textsubscript{2}]/IC\textsubscript{50}</th>
<th>Accuracy</th>
<th>FN</th>
<th>FP</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Company</td>
<td>0.03</td>
<td>45</td>
<td>9/10</td>
<td>0/4</td>
<td>1/6 (17%)</td>
</tr>
<tr>
<td>FDA Guidance</td>
<td>0.1</td>
<td>10</td>
<td>8/10</td>
<td>0/4</td>
<td>2/6 33%</td>
</tr>
</tbody>
</table>

Application of “refined” \textit{in vitro} cut-off:

- [I\textsubscript{1}]/IC\textsubscript{50} = 0.03
- [I\textsubscript{2}]/IC\textsubscript{50} = 45

Considerations: Solubility

**Challenge**
- Low solubility

**Recommended Excipients**
- PEG400
- HP-B-CD
- Tween 80
- NMP
- DMSO

**Suitability Criteria**
- Integrity (lucifer yellow)
- Rank order permeability
- P-gp function
- BCRP function

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PS80</th>
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<tbody>
<tr>
<td>Integrity</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Digoxin Efflux</td>
<td>16</td>
<td>11.4</td>
</tr>
<tr>
<td>E3S Efflux</td>
<td>33.6</td>
<td>16.7</td>
</tr>
</tbody>
</table>
Considerations: Impact vs. Sensitivity

### Comparison of HBSS to Polysorbate 80

<table>
<thead>
<tr>
<th>Solute</th>
<th>Buffer</th>
<th>$P_{A-B ; \text{transco}}$</th>
<th>$J_{\text{max}}/K_M$ (10^{-5} \text{ cm/sec})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loperamide</td>
<td>HBSS</td>
<td>35.5</td>
<td>313</td>
</tr>
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<td>0.05% Polysorbate 80</td>
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<td>Quinidine</td>
<td>HBSS</td>
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<td>Talinolol</td>
<td>HBSS</td>
<td>43.7</td>
<td>6.8</td>
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<td>0.05% Polysorbate 80</td>
<td>3.6</td>
<td>0.8</td>
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Adapted from Burton, P.S. et al Strategy for Characterizing P-Glycoprotein Substrate and Inhibitor Potential for Sparingly Soluble Drugs, Poster, AAPS, 2008
OATP1B1 and OATP1B3 Inhibition

Is total $C_{\text{max}}/IC_{50}$ of the investigational drug $\geq 0.1$ for OATP1B1 or OATP1B3?

Yes

Is the AUC of statin (e.g., rosvastatin, pravastatin, pitavastatin) predicted to increase $\geq 1.25$-fold in the presence of the investigational drug using extrapolation?

Yes

In vivo DDI study with a sensitive substrate (e.g., rosvastatin, pravastatin, pitavastatin)

No

In vivo study is not needed

No

In vivo study may not be needed

R-value of 1.25 instead of 2 (different from ITC whitepaper)

Total $C_{\text{max}}$ different from ITC whitepaper
# R-value Cutoff

## ITC Whitepaper

\( [I_1]_{\text{unbound}}/IC_{50} \geq 0.1 \)

<table>
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<tr>
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<th>FN</th>
<th>TP</th>
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<tbody>
<tr>
<td>FN</td>
<td>5 (21%)</td>
<td>19</td>
</tr>
<tr>
<td>TN</td>
<td>2</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Using total \( C_{\text{max}} \) reduces false negatives

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<th>FN</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN</td>
<td>5 (21%)</td>
<td>13</td>
</tr>
<tr>
<td>TN</td>
<td>2</td>
<td>0 (0%)</td>
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</tbody>
</table>

11 FN, 0 FP
61% accuracy

## FDA Guidance

\( [I_1]_{\text{total}}/IC_{50} \geq 0.1 \)

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<tbody>
<tr>
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<tr>
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<td>2 (50%)</td>
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<tr>
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<td>19</td>
</tr>
<tr>
<td>TN</td>
<td>0</td>
<td>2 (100%)</td>
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</table>

5 FN, 2 FP
75% accuracy

\( R > 2 \)

\( R > 1.25 \)

Including \( R \) introduces additional false negatives

Using total \( C_{\max} \) reduces false negatives
OAT1, OAT3, and OCT2 inhibition

Is the investigational drug an inhibitor of OCT2, OAT1, or OAT3?

**Criteria:** Uptake of model substrates (e.g., MPP+, for OCT2; PAH for OAT1, or ES for OAT3) decreases with increased concentrations of the investigational drug

- **Yes**
  - Determine the IC$_{50}$
  - **Unbound** $C_{\text{max}}$/IC$_{50}$ of the investigational drug ≥ 0.1
    - *In vivo* DDI study with a sensitive substrate
  - **Unbound** $C_{\text{max}}$/IC$_{50}$ of the investigational drug < 0.1
    - *In vivo* DDI study is not needed

- **No**
  - Poor or not an inhibitor of OCT2, OAT1, or OAT3
Considerations: Linearity and IC$_{50}$
Induction

“Transporters can be induced by mechanisms similar to those for CYP enzymes…they share common nuclear factors…”

“…lack of a validated in vitro systems to study transporter induction, the definitive determination of induction potential…on transporters in based on in vivo induction studies…”

Improving Translatability: 2012 and Beyond

**In Vitro**
- Improve IVIVC
  - R-value
  - PKPD
  - Fraction Transported
  - ROC (binary classification)
  - Test System Calibration
  - Tools for Complex DDI

**In Vivo**
- Consistent Recommendations
  - Broad to Specific
  - Extrapolate to unstudied drugs
  - Actionable labeling

**Label**
- Actionable Steps
  - Specificity in Dose Adjustments and Nuances
  - PMC/PMR?
  - Stakeholder Education
Closing Thoughts

“…one can’t believe impossible things.”
-Alice, Through the Looking Glass, Lewis Carroll

“Why, sometimes I’ve believed as many as six impossible things before breakfast.”
-The White Queen, Through the Looking Glass, Lewis Carroll
Therapeutic Proteins

Therapeutic Proteins (TP)

Cytokine or cytokine modulator (that has known effects on CYPs or transporters)

- In vitro study: TP → Drug: effect of CYPs or transporters
- Label may indicate the potential for interaction with CYP or transporter pathways with a particular focus on its effect on narrow therapeutic range drugs (e.g. warfarin)

TPs intended to be used in combination with Drug

- In vitro study: TP → Drug: effect of CYPs or transporters (cocktail or individual studies)
- In vivo interaction studies, e.g. crossover study, population PK, or parallel study
- TP → Drug
- Drug → TP
- Label describes study result and any important clinical actions

Cases where studies can be considered important because of known mechanism or general concern other than its possible effect on CYPs or Transporters

- Drug → TP
- In vitro or in vivo interaction study
- Population PK as initial assessment; may follow up with formal study