**What’s All the Flux About?**

*An Industrial Perspective on the Drug Transporter Whitepaper and Recent Regulatory Guidances*

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**Outline**

- The Challenge and Strategy to Study Drug Transporters
- Case Studies and ITC Decision Trees
  - Rosuvastain Model
- Discussion- which transporter and when.
  - What do you think?
Challenge of Drug Transporters

Rapidly growing scientific area with many *in vitro*, preclinical and clinical publications

- More than 30 transporters ‘involved’ in ADME
- Few agreed clinical translation approaches
- Limited tools/reagents relative to CYP enzymes
- Measuring drug exposure in plasma may not reflect impact on a drug’s disposition (e.g., toxicity)
- Conflicting messages to prescribers, patients, and regulatory bodies

Drug Transporter White Paper


EMA Guideline on the Investigations of Drug Interactions-
Draft guidance published 22April2010

Acceptance and Application of ITC Whitepaper Came Quickly

“Provide the plan to evaluate the potential transporter-based drug interactions mediated by Pgp, OATP1B1, OATP1B3, BCRP, OAT1, OAT3 and OCT2--- See the reference “Giacomini et al. Nature Reviews Drug Discovery 9, 215-236, March 2010”.

--Comments from a regulatory agency on a Phase I briefing document in June 2010.

A very robust response from CROs to the ITC Whitepaper
Drug Label Statistics

<table>
<thead>
<tr>
<th>Drug (approval)</th>
<th>Pgp</th>
<th>BCRP</th>
<th>MRP</th>
<th>BSEP</th>
<th>OATP</th>
<th>OAT</th>
<th>OCT</th>
<th>MCT</th>
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</tbody>
</table>

Approximate number of unique prescription drug labels which mention specific transporters

Pdr3d.com: ~36,000 labels (Jun2011; current in use and prior versions)

Drug Transporter Assessment Strategy

**Discovery to First Time In Human (FTIH)**
- Therapeutic area
- co-meds
- Product Profile
- Development Plan
- Physicochemical properties

**FTIH to Proof of Concept (POC)**
- Non-clinical studies *(in vitro and in vivo)*
- Clinical Studies
- Pharmacokinetics
- Safety

**POC to New Drug Application (NDA)/Marketing**
- Drug labeling
- Non-clinical mechanistic and/or investigative studies
- Clinical Studies

CLINICAL STRATEGY

UNDERSTANDING

TRANSLATION

Central tenet is the clinical plan, which considers the therapeutic area, co-medicines and the patient population
Response to Regulatory Question

“Provide the plan to evaluate the potential transporter-based drug interactions mediated by Pgp, OATP1B1, OATP1B3, BCRP, OAT1, OAT3 and OCT2--- See the reference “Giacomini et al. Nature Reviews Drug Discovery 9, 215-236, March 2010”.

Response (summarized)

…..We are aware of this article as well as the discussions from the FDA Advisory Committee meeting held in March 2010. We have investigated the potential interactions with Pgp and OATPs, two of the most studied transporters with reported clinical drug interactions. There are no apparent indications for drug-interaction risk with other transporters at this time. We will continually evaluate potential interactions with other transporters during development. The evaluation of drug interactions with transporters and enzymes will be driven by the clinical plan, addition of co-meds and safety evaluation……..

Pgp/BCRP Substrate Decision Tree
Do not over interpret the efflux ratio

Case Study
ER=72

Yes

Net flux ratio = 2
Net flux ratio < 2

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

This figure shows a decision tree for P-gp and a similar tree could be used for BCRP. Although flux systems have traditionally been used to determine whether an NME is a substrate of P-gp or BCRP, in vivo systems (e.g., using human intestinal or rat intestine) may provide valid support for appropriate controls (e.g., inhibitors and positive controls) as described in Section 3.1.

Many drugs that are efflux substrates are extensively absorbed (fa > 80%).

Factors that contribute to efflux limited absorption are low solubility, low permeability, metabolic stability, and low dose.

EMA comments on high permeability; e.g., BCS class 1

Pgp/BCRP Inhibitor Decision Tree

Need to ‘calibrate’ in vitro systems using clinical data

EMA recommends a Ki

- Alert for $[I_1]/IC_{50} \geq 0.1$ or $[I_2]/IC_{50} \geq 10$ (EMA <50 for [I]gut calculated)
- $[I_1]$ is total (FDA) or free (ITC) 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).

$$I_{enterocyte} = (fa'ka'dose/Q_{enterocyte})$$
Pgp/BCRP Inhibitor Decision Tree

<table>
<thead>
<tr>
<th>Transporter</th>
<th>IC50 (μM)</th>
</tr>
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<tbody>
<tr>
<td>Pgp</td>
<td>3.9</td>
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</tbody>
</table>

Lapatinib (breast cancer)
Dose=1250 mg daily dose
Cmax = 4.2 μM or 2432 ng/mL
PPB >99%

Digoxin PK Parameters (N=17)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Digoxin</th>
<th>Digoxin + Lapatinib</th>
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</thead>
<tbody>
<tr>
<td>AUC0</td>
<td>15.9 (10.7, 23.6)</td>
<td>28.6 (21.7, 37.6)</td>
</tr>
<tr>
<td>Clr</td>
<td>68.2 (52.1, 89.1)</td>
<td>68.2 (52.1, 89.1)</td>
</tr>
<tr>
<td>Cmax</td>
<td>3.50 (3.06, 4.01)</td>
<td>3.50 (3.06, 4.01)</td>
</tr>
<tr>
<td>ka</td>
<td>4.78 (3.07, 7.45)</td>
<td>4.78 (3.07, 7.45)</td>
</tr>
</tbody>
</table>

ITC Calculations:
\[ \frac{[I]}{IC50} = 0.01 \]
\[ \frac{[I]}{IC50} = 2500 \]

Approved letter (FDA Drugs)
http://www.accessdata.fda.gov/drugsatfda_docs/appletter/2007/022059s000ltr.pdf
GSK Clinical Trial

OCT/OAT Substrate Decision Tree

Integrate the preclinical and clinical data

Is renal elimination an important route of elimination of NME?
Criteria: \[ \frac{CL_r}{CL_{tot}} = 0.5 \]

Yes

Is secretory clearance an important route of NME elimination?
Criteria: \[ Cl_r = 1.5 \times GFR \]

Yes

Renal secretion transporters are not important in the elimination of the drug

No

No

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 gemcitabine for OCT2 and with probenecid for OAT1, OAT3 as inhibitor drugs

The ratio of NME uptake in the cells expressing the transporter versus the control (or empty vector) cells should be statistically greater than 1. No agreement was reached by the International Transporter Consortium regarding the magnitude of the ratio. However, it is important that the uptake into the transfected cells be significantly greater than background in a control cell line and be inhibited by a known inhibitor of the transporters. A positive control should be included. If the \( \frac{CL_r}{CL_{tot}} \) is unknown, go to "Yes".
**OCT/OAT Substrate Decision Tree**

Integrate Preclinical and Clinical data
- Drug class typically undergoes renal secretion
- Physico-chemical properties
  - Low mw (<400)
  - Hydrophilic
  - Moderate Papp
- ADME
  - Well absorbed
  - Eliminated in urine as parent (>70%)
  - High Clr (>GFR)

### Perpetrator Drug

<table>
<thead>
<tr>
<th>Perpetrator Drug</th>
<th>Dose (mg)</th>
<th>Cmax (uM)</th>
<th>Unbound Cmax (uM)</th>
<th>IC50 (uM) OAT1, OAT3</th>
<th>Unbound Cmax/IC50</th>
<th>DDI Risk?</th>
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<tbody>
<tr>
<td>Probenecid</td>
<td>1000</td>
<td>246</td>
<td>22.1</td>
<td>7.5, 7.8</td>
<td>2.9, 2.8</td>
<td>high</td>
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<tr>
<td>Ibuprofen</td>
<td>800</td>
<td>297</td>
<td>2.97</td>
<td>2.0, 7.5</td>
<td>1.5, 0.4</td>
<td>high</td>
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<tr>
<td>Ketoprofen</td>
<td>50</td>
<td>7.9-15.4</td>
<td>0.08-0.015</td>
<td>1.4, &gt;100</td>
<td>&lt;0.1</td>
<td>low</td>
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<tr>
<td>Simvastatin</td>
<td>40</td>
<td>0.11</td>
<td>0.007</td>
<td>6.0, &gt;100</td>
<td>&lt;0.1</td>
<td>low</td>
</tr>
</tbody>
</table>

**Recommendations:**
- Exclude probenecid, ibuprofen, and other high-dose NSAIDs.
- Conduct probenecid DDI study after Phase IIA
- No risk with statins

**OCT/OAT Inhibitor Decision Tree**

Driven by clinical plan and co-meds

Is the NME an inhibitor of OCT2, OAT1 or OAT3? Criteria: determine the IC50 of NME against MPP, for OCT2, PAR for OAT1 or OS for OAT3 or other model substrates.

- Unbound Cmax/KIC of the NME > 0.1
  - Clinical DDI study with a sensitive substrate (see footnote)
- Unbound Cmax/KIC of the NME ≤ 0.1
  - Poor or not an inhibitor of OCT2, OAT1 or OAT3
  - DDI study is not needed

For NMEs that are OAT inhibitors, multiple candidate probe substrates could be used in clinical DDI studies, including zidovudine, lamivudine, zidovudine, acyclovir, ciprofloxacin, terephosphate and methotrexate. Some of these substrates may be transported by multiple transporters. For example, some data suggest that methotrexate renal elimination may be affected by co-administration of non-steroidal anti-inflammatory drugs; however, the mechanisms may include other transporters in addition to OAT1, OAT2, MRP2, 1-methyl-4-phenylpyridinium (OS), oestrone-3-sulfate, PAM, para-aminohippuric acid.
**OCT/OAT Inhibitor Decision Tree**

**Background**
- ~30% of dose excreted in the urine
- High permeability, Pgp substrate
- Not an inhibitor of Pgp, OATPs (1B1,1B3)
- Plasma protein binding: >99%

**Observation:**
Serum creatinine elevated (~10-15%) in clinical studies.
- Toxicity or a DDI?

**DDI Hypothesis:**
Inhibition of OCT2 or MATE1/2.

<table>
<thead>
<tr>
<th></th>
<th>IC₅₀ (µM)</th>
<th>Cmax (µM)</th>
<th>Free Cmax (µM)</th>
<th>Cmax</th>
<th>Free Cmax</th>
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<tbody>
<tr>
<td>Cmp A</td>
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<td>11</td>
<td>0.11</td>
<td>5.8</td>
<td>0.06</td>
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<td>Cimetidine</td>
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<td>12</td>
<td>9.72</td>
<td>0.16</td>
<td>0.13</td>
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</table>

**Conclusions**
- In vitro data support the hypothesis of inhibition of creatinine active secretion, reducing creatinine clearance and raising serum creatinine concentrations
- Dofetilide, an OCT2 substrate and renally cleared drug with narrow therapeutic index is contraindicated.

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**OATP Substrate Decision Tree**

Integrate preclinical and clinical data

Is hepatic elimination an important route of elimination of NME?
- Criteria: CLᵢ > 0.3 CL_total

Does the compound have active hepatocyte uptake? Do the drug’s 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
- No

Hepatic clearance is not a sufficiently important determinant of drug levels

- Yes
- Not a substrate for OATPs

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.
OATP Substrate Decision Tree

Background
- Organic anion
- Low permeability
- Low Vd (< 1 L/kg)
- Liver:blood ratio ~20:1
- Metabolism minor
- Supra-proportional PK

![Decision Tree Diagram]

OATP Inhibition Decision Tree

\[ R = 1 + \left( \frac{fu \cdot lin_{\text{max}}}{IC_{50}} \right) \]
- \( fu \) – unbound fraction
- \( lin_{\text{max}} \) - estimated maximum concentration at the inlet of the liver
  - \( lin_{\text{max}} = Imax + (fa \cdot ka \cdot dose/Qh) \)
  - \( Imax \) is maximum total plasma concentration
  - \( fa \) = fraction of drug absorbed; often \( fa \) is assumed to be 1.0
  - \( Ka \) = absorption rate constant, often assumed to be 0.03/min
  - \( Qh \) = hepatic blood flow, 1500 mL/min

These estimations assume that the fraction of the substrate transported by OATP1B1 is 100%- likely not true.

Validation of Decision Tree
- 13 drugs with clinical data
- Two approaches
  - R value
  - ft (fraction transported)

Special thanks to Drs. Sugiyama, Polli and Evers
Rosuvastatin Static Model

\[
\frac{AUC\ (\text{inhibited})}{AUC\ (\text{control})} = \frac{ft1 + ft3}{1 + \frac{ft1}{K_i}} + \frac{ft2 + ft3}{1 + \frac{ft2}{K_i}} + 1 - \frac{ft1 + ft2}{K_i}
\]

- Fraction transported (ft) values for rosuvastatin were determined by Kitamura et al. DMD 2008 using known OATP1B1/3 probes.
  - Relative activity factors calculated (ratio of uptake clearance in hepatocytes vs. expressed cell lines) and applied to rosuvastatin.
- The sum of ft's gives ~0.7 as a total fraction uptake to systemic clearance, remainder (~0.3) is mediated by a renal component (Fs calculated from clinical IV data).

What inhibitor concentration should be used (e.g., systemic or portal vein Cmax, free (unbound) or total)?

Rosuvastatin Prediction Tool

Using estimated total systemic Cmax in the prediction tool, we predict all clinical DDI (using >2fold AUC change) but over predict one low clinical DDI.
GSK Rosuvastatin DDI prediction
Since an IC50 could not be calculated, the boundaries for the steep slope in the IC50 curve between 0.3 and 1 µM were used to estimate the potential interaction using a dose of 300 mg

**Summary statistics for simulation**

<table>
<thead>
<tr>
<th></th>
<th>OATP1B1 ft</th>
<th>AUC/AUC</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>95% CI Upper</th>
<th>95% CI Lower</th>
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<tbody>
<tr>
<td>Mean</td>
<td>0.558</td>
<td>2.031</td>
<td>0.106</td>
<td>0.438</td>
<td>0.147</td>
<td>3.582</td>
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<td>SD</td>
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</tbody>
</table>

Prediction supports potential for DDI (>1.5 to <2-fold) for GSK123456

**Clinical Results - Rosuvastatin**

- Cmax and AUC both increase ~ 40% with no change in t_{1/2}
- Drug appears to impact mainly bioavailability of RSV
- Many "OATP" interactions with RSV demonstrate a marked difference in effect on Cmax and AUC

Patients with the BCRP 421C>A variant allele exhibit a 1.8- and 1.9-fold change in AUC and Cmax, respectively, with no change in t_{1/2}

<table>
<thead>
<tr>
<th>Co-med</th>
<th>Cmax fold change</th>
<th>AUC fold change</th>
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<tr>
<td>Cyclosporine A</td>
<td>11</td>
<td>7</td>
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<tr>
<td>Lopinavir/ritonavir</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Atazanavir/ritonavir</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Rifampin</td>
<td>7.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>2.2</td>
<td>1.9</td>
</tr>
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</table>

These drugs inhibit OATP - note bias to Cmax>>AUC.
**Summary Statin DDIs**

Clinical data

- In vitro experiments and the results from statin clinical DDI studies indicate that BCRP is important in interactions of GSK123456 with statins.
- Atorvastatin is a less sensitive substrate for BCRP than both rosuvastatin and simvastatin.
  - Significant change in atorvastatin exposure or efficacy not anticipated.

Clinical study confirmed the lack of interaction.

**How Does This Approach Compare To The White Paper?**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Rosuvastatin: Parameter</th>
<th>Clinical (Fold AUC Change)</th>
<th>RBV Static Total Cmax</th>
<th>[I1]IC50 Total 2</th>
<th>[I1]IC50 Free 2</th>
<th>R value Total Cmax</th>
<th>R-value Portal Free 1</th>
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<tr>
<td>GSK1</td>
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<td>1.5</td>
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<td>0.02</td>
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<td>Ketoconazole</td>
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<td>Itraconazole*</td>
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<td>Cyclosporine</td>
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<td>2.8</td>
<td>11</td>
<td>0.8</td>
<td>12</td>
<td>5.8</td>
</tr>
</tbody>
</table>

1. Using a prediction threshold of ≥ 2 to trigger a clinical DDI study (AUC Change >1.5-fold) Correct (<1.5 or >2.0), borderline (1.5 and <2.0), Incorrect
2. Using a prediction threshold of ≥ 6.1 to trigger a clinical DDI study (AUC Change >1.5-fold) Correct (<0.07 or >0.3), borderline (0.07 and <0.3), Incorrect

- Rosuvastatin as substrate
  - Not just OATP substrate
- The static model predicts less false negatives than the white paper R value but more false positives.
- Large doses, with high Cmax (>20 uM) seemed to be ‘missed by R value’.
- ‘noise’ around fu and IC50 values.

All approaches have their limitations but both are good for guidance.
The Future: Transporters and Toxicity

ABCC2, ABCC3, and ABCB1, but not CYP3A, Protect against Trabectedin-Mediated Hepatotoxicity

Robert A.B. van Waterschoot,1 Rharidy M. Emray,1 Ellis Wagenaar,2 Cornello M.M. van der Kuijlen,1 Hilde Reesing,1 Jos H. Beijnen,2 and Alfred H. Schinkel1

Abstract

Purpose: Trabectedin (Yondelis, ET-743) is a novel anticancer drug with potent activity against various tumors. However, dose-limiting hepatotoxicity was observed during clinical trials. Because recent reports have suggested that cytochrome P450 3A (CYP3A), as well as the drug transporters ABCB1, ABCC2, and ABCC3 might protect against trabectedin-mediated hepatotoxicity, we investigated the individual and combined roles of these detoxifying systems.

Experimental Design: Madin-Darby canine kidney cells expressing ABCC2 and ABCC3 were used to study in vitro trabectedin transport. We investigated the hepatotoxicity of trabectedin, and the plasma and liver levels of this drug and its metabolites in mice deficient for CYP3A, Abcb1a/b, Abcc2, and/or Abcc3 after i.v. trabectedin administration.

Results: Trabectedin was transported by ABCC2 but only modestly by ABCC3. Contrary to our expectations, absence of CYP3A resulted in only a marginal increase in hepatotoxicity. Some hepatotoxicity was observed in Abcc2−/− and Abcc3−/− mice, but very little in Abcb1a/b−/− and Abcc3−/− mice. Strikingly, severe hepatotoxicity was found in Abcb1a/b−/− and Abcc2−/−Abcc3−/− mice. However, hepatotoxicity was dramatically decreased in Cyp3a−/−Abcb1a/b−/−Abcc2−/− compared with Abcb1a/b−/−Abcc2−/− mice. This suggests that the formation of CYP3A-specific metabolites in an important prerequisite for trabectedin-mediated hepatotoxicity. Further studies revealed that there is increased accumulation of metabolites of trabectedin, but not of trabectedin itself, in the livers of mice that lack Abcc2 but are CYP3A proficient.

Conclusions: Our data show that ABCB1, ABCC2, and ABCC3 have a profound and partially redundant function in protection from trabectedin-mediated hepatotoxicity, presumably by clearing the liver from hepatotoxic trabectedin metabolites that are primarily formed by CYP3A. J Clin Cancer Res 2009;15(24):7816–23

Transports more likely to alter toxicity profile rather than PK profile, and potentially involves multiple transporters.

LC/MS Imaging Provides Spatial Distribution Information

Optical Image of Region Analyzed
Discussion

• Fact or Myth
• Which Transporter and when?
• Is transporter information helpful in labelling?

Facts or Myths

• I must analyze all 7 transporters described in the ITC Whitepaper for my drug. 
  Myth

• Pgp is generally not very important for the intestinal absorption of a substrate. Therefore, one should rarely do a clinical study.
  Fact

• There are no agreed timings for transporter studies. Timing of these studies should be driven by the regulatory authorities.
  Both (Fact-Myth)
**Which Transporter and When?***

### CLINICAL STRATEGY
- Pgp (ABCB1)
  - Neurology (PK/PD)
  - Digoxin early co-med?
- OATP1B1 (SLC01B1)
  - Statin—clinical enrollment commercial view
- Other transporters?

### UNDERSTANDING
- Safety and Enrollment
  - BCRP (ABCG2)
  - Topotecan (oral)
- OCT 1 and 2 (SLC22)
  - Metformin (efficacy?)
- OAT
  - Sitagliptin, methotrexate

### TRANSLATION
- Drug labeling
- Mechanistic and/or investigative studies
- Other transporters (MATEs, MRPs, etc)

There are no agreed timings for transporter studies. Timing of these studies should be driven by the clinical plan, with the objective of characterizing key transporters prior to start of Phase III.

* One example of prioritizing transporters

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**Lapatinib (Tykerb®): Labeling and Drug Transporters (2010)**

**Drug Metabolizing Enzymes and Drug Transport Systems (7.1 Drug Interactions)**
Lapatinib inhibits human P-glycoprotein. If TYKERB is administered with drugs that are substrates of Pgp, increased concentrations of the substrate drug are likely.....

**Drugs that Inhibit Drug Transport Systems (7.3 Drug Interactions):** Lapatinib is a substrate of the efflux transporter P-glycoprotein. If TYKERB is administered with drugs that inhibit Pgp, increased concentrations of lapatinib are likely, .......

**Distribution (12.3 Clinical Pharmacology):** In vitro studies indicate that lapatinib is a substrate for the transporters breast cancer resistance protein (BCRP, ABCG2) and P-glycoprotein (Pgp, ABCB1). Lapatinib has also been shown in vitro to inhibit these efflux transporters, as well as the hepatic uptake transporter OATP1B1, at clinically relevant concentrations.

Value of drug transporter information in drug labels?
Requires further education of prescribers, patients and payers

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* One example of prioritizing transporters
Conclusions

• Clinical plan and patient population drive DDI strategy, which should drive the work on drug transporters.
• In vitro and clinical data, in combination with extrapolation and modeling, are powerful tools for understanding DDIs and toxicity.
• Statin DDIs predictions evolving, and include a number of transport and metabolic pathways.
  – Review the relationship between Cmax and AUC

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Discussion - What’s on your mind?

- Fact or Myth
- Which Transporter and when?
- Is transporter information helpful in labelling?