

***What's All the Flux About?***  
***An Industrial Perspective on the Drug  
Transporter Whitepaper and Recent  
Regulatory Guidances***

Joseph W. Polli, Ph.D.

GlaxoSmithKline, Inc  
Drug Metabolism and Pharmacokinetics  
Room: N1.407  
P.O. Box 13398  
Research Triangle Park, NC 27709  
[joseph.w.polli@gsk.com](mailto:joseph.w.polli@gsk.com)

1

## **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?

2

# 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

'Membrane Transporters in Drug Development', NRDD 9:215 - 236 (2010)

## 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Draft guidance published 22April2010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.

Applicant	BOEHRINGER INGELHEIM PHARMACEUTICALS INC
Product	PRADAXA (DABIGATRAN ETEXILATE MESYLATE)
NDA/BLA Number	22512
NDA/BLA Approval Date	10/19/2010
Annual Report Due Date <small>(must be submitted within 90 days of this date)</small>	10/18/2011
Annual Report Received	
Requirement/Commitment Number 1	
Required Under	FDAAA 505
Original Projected Completion Date	11/30/2011
Description	An in vitro study profiling of dabigatran as a substrate or inhibitor of a panel of drug Solute Carrier (SLC) transporters (OATPs, OATs, and OCTs) that are proposed as being relevant by the recently published ITC white paper (Giacomini R, Huang S-H, Tweedle G, et al. Membrane transporters in drug development. <i>Nature Review Drug Discovery</i> , 2010, 9: 215-236.)

Post Marketing Commitments



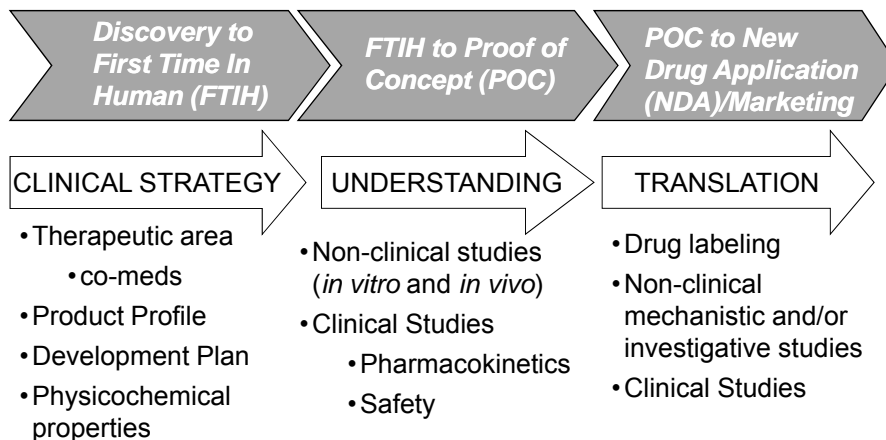
A very robust response from CROs to the ITC Whitepaper

## Drug Label Statistics

Approximate number of unique prescription drug labels which mention specific transporters		Drug (approval)	Pgp	BCRP	MRP	BSEP	OATP	OAT	OCT	MCT
		Topotecan	*	*	*					
		Ixabipelone	*	*	*					
		Ambrisentan	*	*			*			
		Eltrombopag	*	*			*			
Pgp	50	Lapatinib	*	*			*	#	#	
BCRP	7	Pazopanib	*	*			*			
MRP	4	Sitagliptin	*					*	*	
OATP	8	Saxagliptin	*		*				*	
OAT	1	Gabapentin							*	*
OCT	1	Pralatrexate	*					*		
MCT	1	Cabazitaxel	*	*	*					
BSEP	1	Fingolimod	*	*	*	*	*			
		Dabigatran	*				*	*	*	

pdr3d.com: ~36,000 labels (Jun2011; current in use and prior versions)

## Drug Transporter Assessment Strategy



**Central tenet is the clinical plan, which considers the therapeutic area, co-medicines and the patient population**

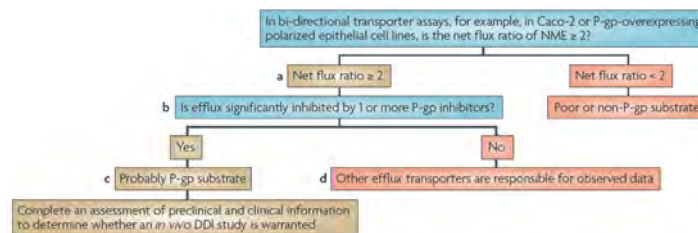
## Response to Regulatory Question

“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)

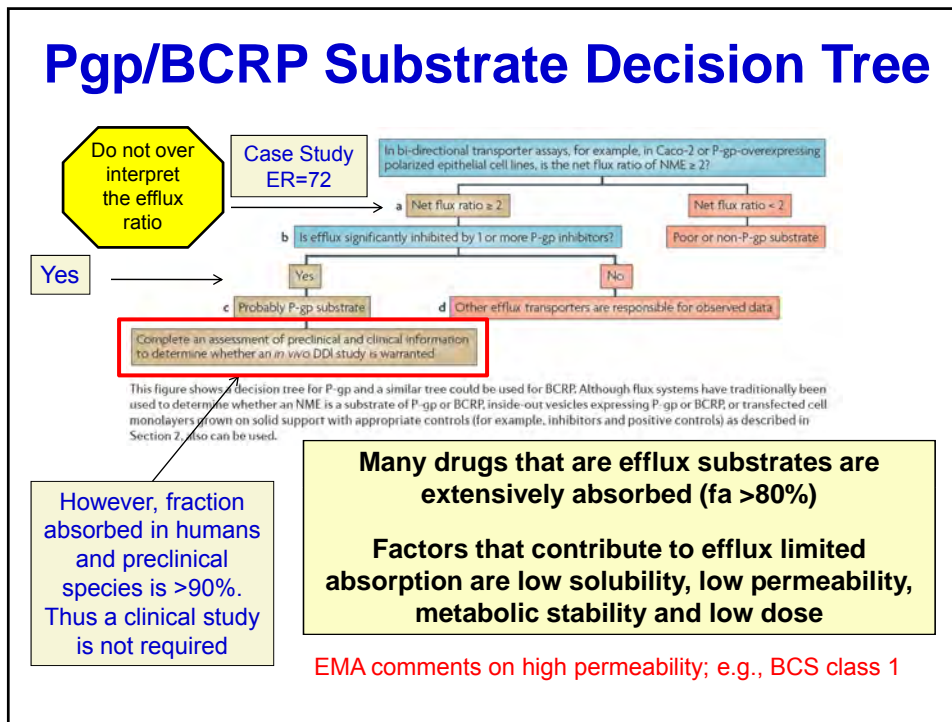
.....We are **aware of this article** as well as the discussions from the FDA Advisory Committee meeting held in March 2010. We **have investigated the potential interactions with Pgp and OATPs**, two of the most studied transporters with reported clinical drug interactions. There are **no apparent indications 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

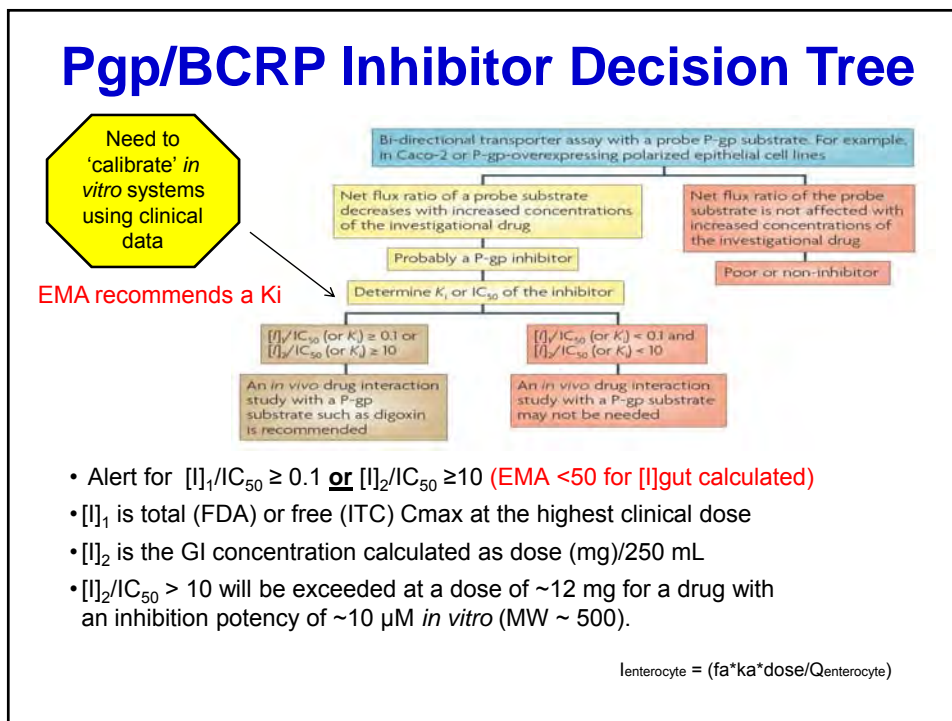


This figure shows a decision tree for P-gp and a similar tree could be used for BCRP. Although flux systems have traditionally been used to determine whether an NME is a substrate of P-gp or BCRP, inside-out vesicles expressing P-gp or BCRP, or transfected cell monolayers grown on solid support with appropriate controls (for example, inhibitors and positive controls) as described in Section 7, also can be used.

# Pgp/BCRP Substrate Decision Tree



# Pgp/BCRP Inhibitor Decision Tree



# Pgp/BCRP Inhibitor Decision Tree

Transporter	IC <sub>50</sub> (μM)
Pgp	3.9

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<sub>i</sub> or IC<sub>50</sub> of the inhibitor
  - Criteria:  $([I]_1/IC_{50} \text{ (or } K_i) \geq 0.1 \text{ or } [I]_2/IC_{50} \text{ (or } K_i) \geq 10$
  - ITC Calculations:  $[I]_1/IC_{50} = 0.01$ ,  $[I]_2/IC_{50} = 2500$
  - 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 increased concentrations of the investigational drug → Poor or non-inhibitor

**Lapatinib (breast cancer)**  
 Dose=1250 mg daily dose  
 C<sub>max</sub> = 4.2 μM or 2432 ng/mL  
 PPB >99%

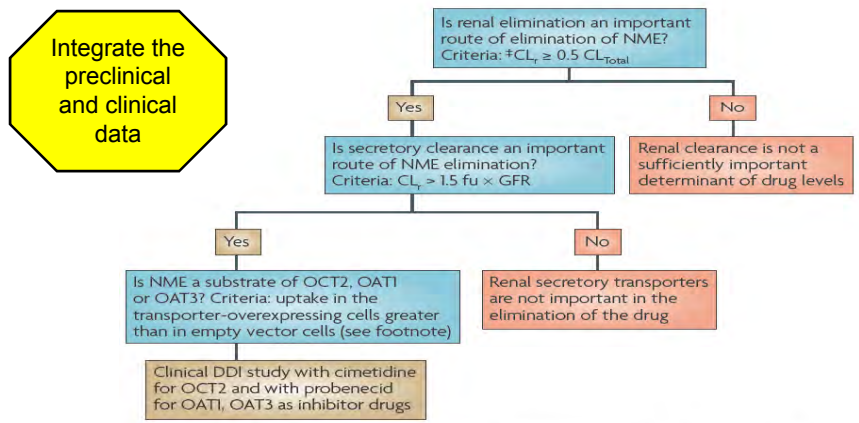
Digoxin PK Parameters (N=17)		
Parameter	Digoxin	Digoxin + Lapatinib
AUC <sub>0</sub>	15.9 (10.7, 23.6)	28.6 (21.7, 37.6)
CL <sub>r</sub>	82.7 (61.1, 112)	68.2 (52.1, 89.1)
C <sub>max</sub>	1.64 (1.40, 1.93)	3.50 (3.06, 4.01)
k <sub>a</sub>	4.78 (3.07, 7.45)	5.40 (4.41, 6.62)

Digoxin DDI (n=17)

Group	Dose
D1	Digoxin 0.5mg
D2-8	Lapatinib 1500 mg
D9	Lapatinib + digoxin

Approval letter (FDA Drugs)  
[http://www.accessdata.fda.gov/drugsatfda\\_docs/appletter/2007/022059s000ltr.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/appletter/2007/022059s000ltr.pdf)  
 GSK Clinical Trials  
<http://download.gsk-clinicalstudyregister.com/files/20694.pdf>

# OCT/OAT Substrate Decision Tree

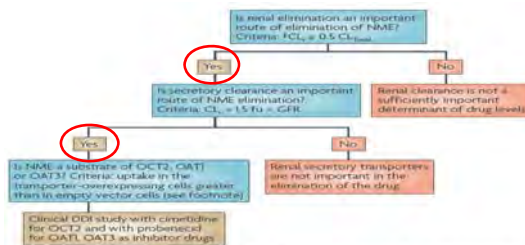


The ratio of NME uptake in the cells expressing the transporter versus the control (or empty vector) cells should be statistically greater than 1. No agreement was reached by the International Transporter Consortium regarding the magnitude of the ratio. However, it is important that uptake into the transfected cells be significantly greater than background in a control cell line and be inhibited by a known inhibitor of the transporter. A positive control should be included. <sup>#</sup>If the CL<sub>r</sub>/CL<sub>Total</sub> 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 Cl<sub>r</sub> (>GFR)



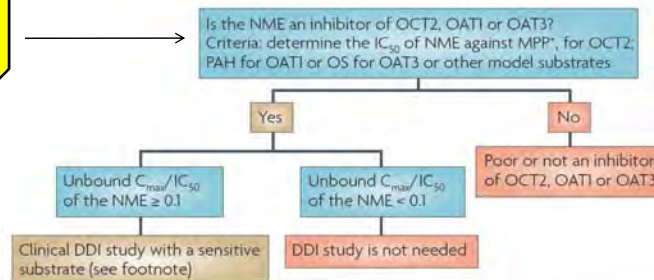
Perpetrator Drug	Dose (mg)	C <sub>max</sub> (uM)	Unbound C <sub>max</sub> (uM)	IC <sub>50</sub> (uM) OAT1, OAT3	Unbound C <sub>max</sub> /IC <sub>50</sub>	DDI Risk?
Probenecid	1000	246	22.1	7.5, 7.8	2.9, 2.8	high
Ibuprofen	800	297	2.97	2.0, 7.5	1.5, 0.4	high
Ketoprofen	50	7.9-15.4	0.08-0.015	1.4, >100	<0.1	low
Simvastatin	40	0.11	0.007	6.0, >100	<0.1	low

### 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



For NMEs that are OAT inhibitors, multiple candidate probe substrates could be used in clinical DDI studies, including zidovudine, lamivudine, zalcitabine, acyclovir, ciprofloxacin, tenofovir 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 OATs<sup>10,18</sup>, MPP<sup>+</sup>, 1-methyl-4-phenylpyridinium; OS, oestrone-3-sulphate; PAH, para-aminhippuric acid.

# OCT/OAT Inhibitor Decision Tree

## Background

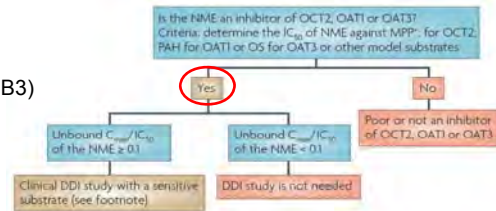
- ~30% of dose excreted in the urine
- High permeability, Pgp substrate
- Not an inhibitor of Pgp, OATPs (1B1,1B3)
- Plasma protein binding: >99%

## Observation:

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

## DDI Hypothesis:

Inhibition of OCT2 or MATE1/2.

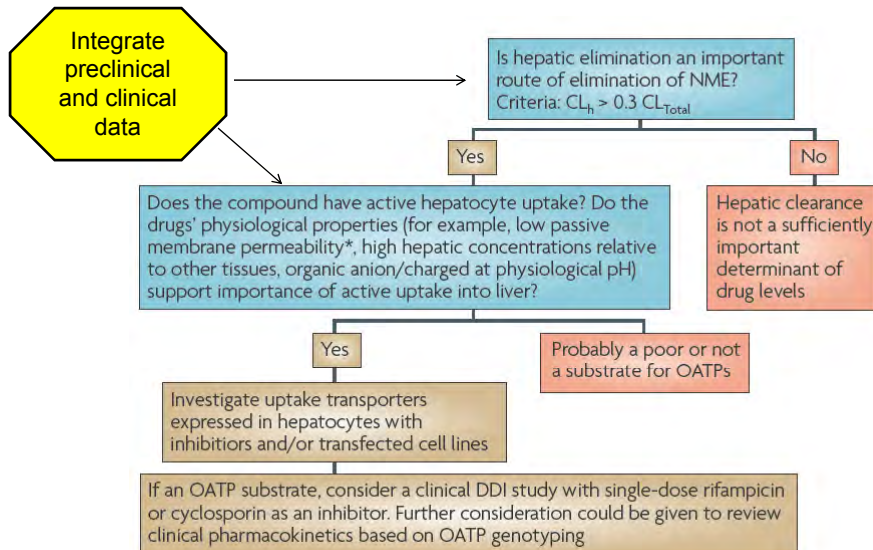


	IC <sub>50</sub> (μM)	C <sub>max</sub> (μM)	Free C <sub>max</sub> (μM)	[I]/IC <sub>50</sub>	
				C <sub>max</sub>	Free C <sub>max</sub>
Cmp A	1.9	11	0.11	5.8	0.06
Cimetidine	73-120	12	9.72	0.16	0.13

## 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.

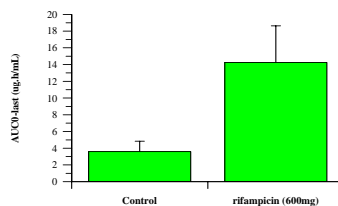
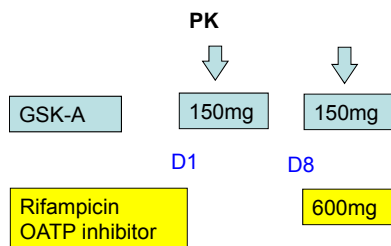
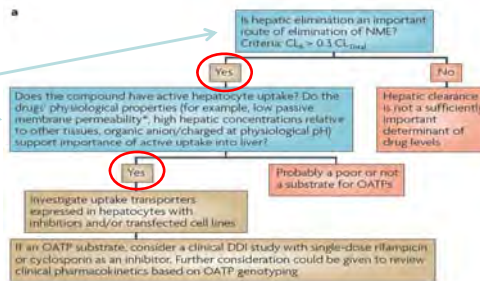
# OATP Substrate Decision Tree



# OATP Substrate Decision Tree

## Background

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



Average 3.9-fold increase in AUC

# OATP Inhibition Decision Tree

$$R = 1 + (f_u \cdot \text{lin}_{\text{max}} / IC_{50})$$

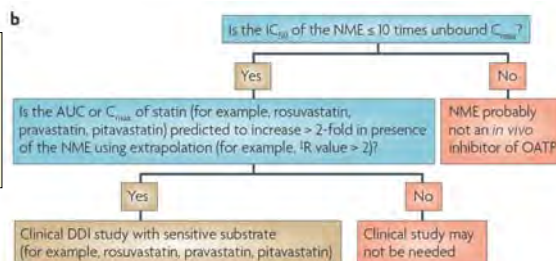
- $f_u$  – unbound fraction
- $\text{lin}_{\text{max}}$  - estimated maximum concentration at the inlet of the liver
  - $\text{lin}_{\text{max}} = I_{\text{max}} + (f_a \cdot k_a \cdot \text{dose} / Q_h)$
  - $I_{\text{max}}$  is maximum total plasma concentration
  - $f_a$  = fraction of drug absorbed; often  $f_a$  is assumed to be 1.0
  - $k_a$  = absorption rate constant, often assumed to be 0.03/min
  - $Q_h$  = hepatic blood flow, 1500 mL/min

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

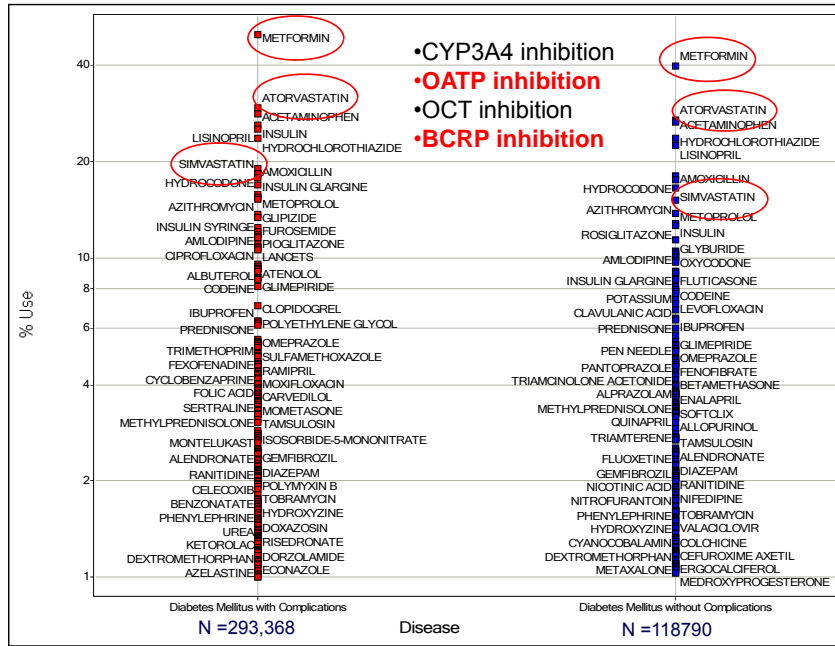
## Validation of Decision Tree

- 13 drugs with clinical data
- Two approaches
  - R value
  - $f_t$  (fraction transported)

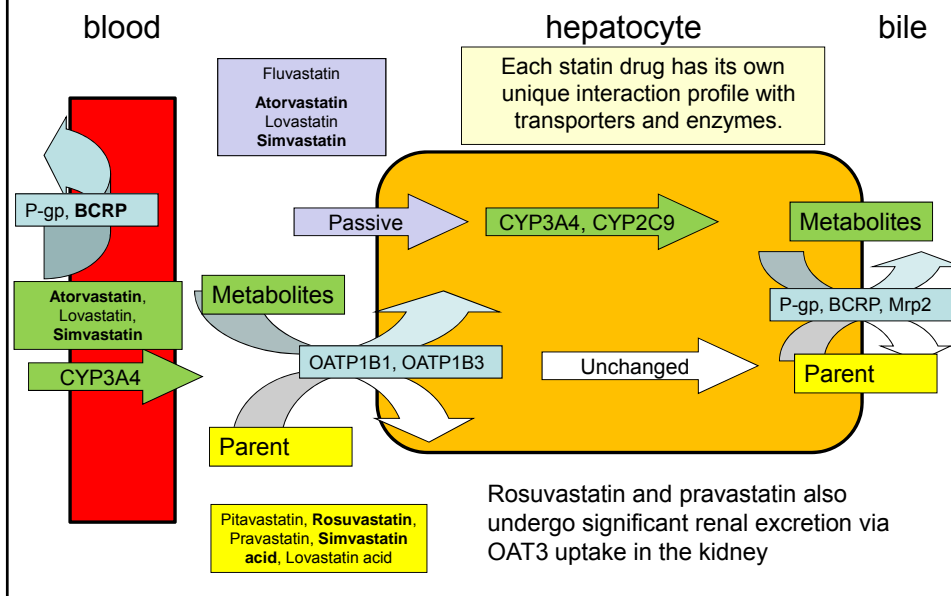
Special thanks to Drs. Sugiyama, Polli and Evers



### US prescriptions- Diabetes Mellitus



## Statin Metabolism and Disposition



# Rosuvastatin Static Model

Equation adapted from Ito *et al*, DMD 2005

$$\frac{AUC (inhibited)}{AUC (control)} = \frac{ft1}{1 + \frac{[Inhibitor]}{K_{i1}}} + \frac{ft2}{1 + \frac{[Inhibitor]}{K_{i2}}} + 1 - (ft1 + ft2)$$

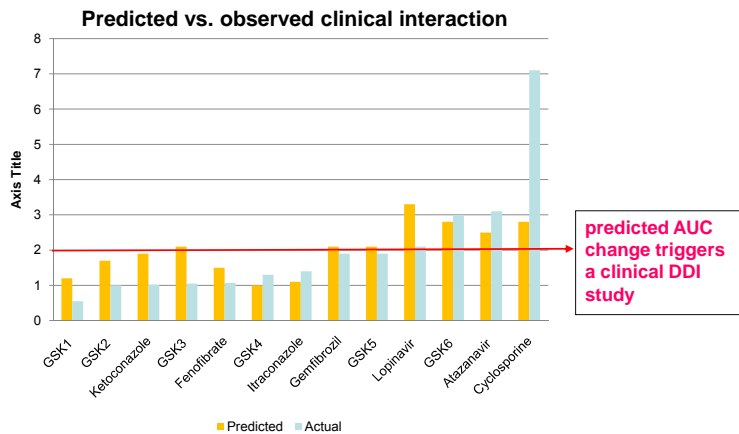
Fraction Transported OATP1B1 (0.520)      Fraction Transported OATP1B3 (0.191)

Inhibitor concentration.       $K_i = IC_{50}$  because of low concentration of probe substrate used in assays.

- 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 fts gives ~0.7 as a total fraction uptake to systemic clearance, remainder (~0.3) is mediated by a renal component ( $F_e$  calculated from clinical IV data).

**What inhibitor concentration should be used (e.g., systemic or portal vein  $C_{max}$ , free (unbound) or total)?**

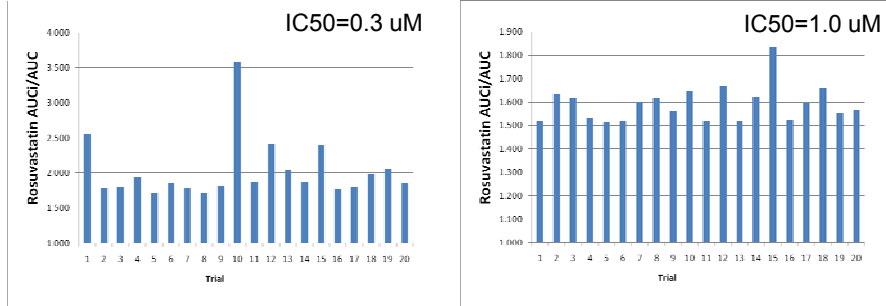
# Rosuvastatin Prediction Tool



Using estimated **total systemic  $C_{max}$**  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  $\mu\text{M}$  were used to estimate the potential interaction using a dose of 300 mg

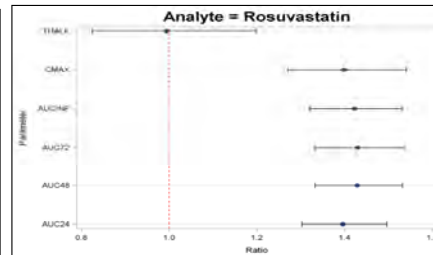
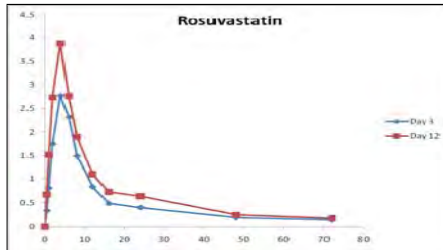


Summary statistics for simulation			
OATP1B1 ft		AUCi/AUC	
Mean	0.558	Mean	2.031
SD	0.106	SD	0.438
OATP1B3 ft		Min	1.715
Mean	0.147	Max	3.582
SD	0.055	95% CI	
		Upper	2.223
		Lower	1.839

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

Summary statistics for simulation			
OATP1B1 ft		AUCi/AUC	
Mean	0.518	Mean	1.592
SD	0.071	SD	0.078
OATP1B3 ft		Min	1.513
Mean	0.164	Max	1.836
SD	0.025	95% CI	
		Upper	1.626
		Lower	1.558

## Clinical Results- Rosuvastatin



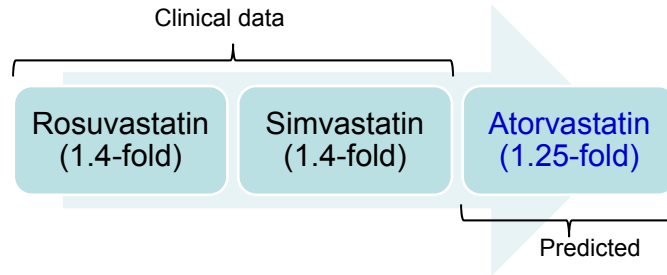
- Cmax and AUC both increase ~ 40% with no change in  $t_{1/2}$ 
  - Drug appears to impact mainly bioavailability of RSV
  - Many "OATP" interactions with RSV demonstrate a marked difference in effect on Cmax and AUC

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  $t_{1/2}$

Co-med	Cmax fold change	AUC fold change
Cyclosporine A	11	7
Lopinavir/ritonavir	5	2
Atazanavir/ritonavir	7	3
Rifampin	7.3	2.8
Gemfibrozil	2.2	1.9

These drugs inhibit OATP- note bias to Cmax>>AUC

## Summary Statin DDIs



- 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?

Inhibitor	Rosuvastatin: Parameter					
	Clinical (Fold AUC Change)	RSV Static Total Cmax 1	[1]/IC50 Total 2	[1]/IC50 Free 2	R value Total Cmax 1	R-value Portal free 1
GSK1	0.6	1.2	0.4	0.02	1.2	1.1
Ketoconazole	1.0	1.9	4.3	0.04	1.9	1.0
GSK2	1.1	1.7	2.2	0.2	3.2	1.7
GSK3	1.1	2.1	6.1	0.2	2.1	1.2
Fenofibrate	1.1	1.5	1.2	0.01	2.2	1.0
GSK4	1.3	1.0	<0.01	<0.01	1.0	1.0
Itraconazole*	1.4	1.1	0.3	<0.01	1.3	1.0
GSK5	1.6	2.1	115	<0.01	2.1	1.0
Gemfibrozil*	1.9	2.1	4.8	0.14	5.8	1.2
Lopinavir	2.1	3.3	200	2.0	201	4.3
GSK6	3.0	2.8	25	2.8	13	1.3
Atazanavir	3.1	2.5	14	2.0	44	7.0
Cyclosporine	7.1	2.8	11	0.8	12	5.8

1. Using a prediction threshold of  $\geq 2$  to trigger a clinical DDI study (AUC Change  $>1.5$ -fold) **Correct** ( $<1.5$  or  $>2.0$ ), **border line**  $>1.5$  and  $<2.0$ ), **Incorrect**
2. Using a prediction threshold of  $\geq 0.1$  to trigger a clinical DDI study (AUC Change  $>1.5$ -fold) **Correct** ( $<0.07$  or  $>0.3$ ), **border line**  $>0.07$  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,<sup>1</sup> Rhandy M. Eman,<sup>1</sup> Els Wagenaar,<sup>1</sup> Cornelia M.M. van der Kruijssen,<sup>1</sup> Hilde Rosing,<sup>2</sup> Jos H. Beijnen,<sup>2</sup> and Alfred H. Schinkel<sup>1</sup>

**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.

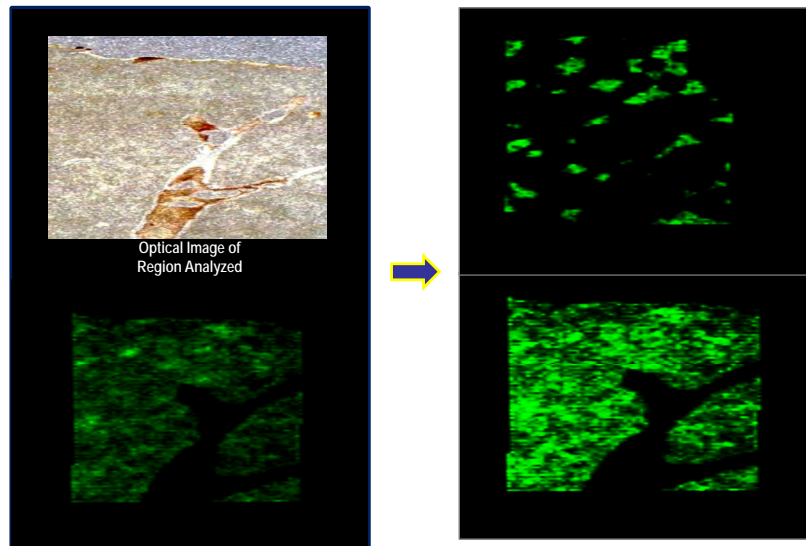
**Experimental Design:** Madin-Darby canine kidney cells expressing ABCC2 and ABCC3 were used to study *in vitro* trabectedin transport. We investigated the hepatotoxicity of trabectedin, and the plasma and liver levels of this drug and its metabolites in mice deficient for CYP3A, Abcb1a/1b, Abcc2, and/or Abcc3 after i.v. trabectedin administration.

**Results:** Trabectedin was transported by ABCC2 but only modestly by ABCC3. Contrary to our expectation, absence of CYP3A resulted in only a marginal increase in hepatotoxicity. Some hepatotoxicity was observed in Abcc2<sup>-/-</sup> mice, but very little in Abcb1a/1b<sup>-/-</sup> and Abcc3<sup>-/-</sup> mice. Strikingly, severe hepatotoxicity was found in Abcb1a/1b/Abcc2<sup>-/-</sup> and Abcc2/Abcc3<sup>-/-</sup> mice. However, hepatotoxicity was drastically decreased in Cyp3a/Abcb1a/1b/Abcc2<sup>-/-</sup> compared with Abcb1a/1b/Abcc2<sup>-/-</sup> mice. This suggests that the formation of CYP3A-specific metabolites is 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. (Clin Cancer Res 2009;15(24):7616–23)

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

## LC/MS Imaging Provides Spatial Distribution Information



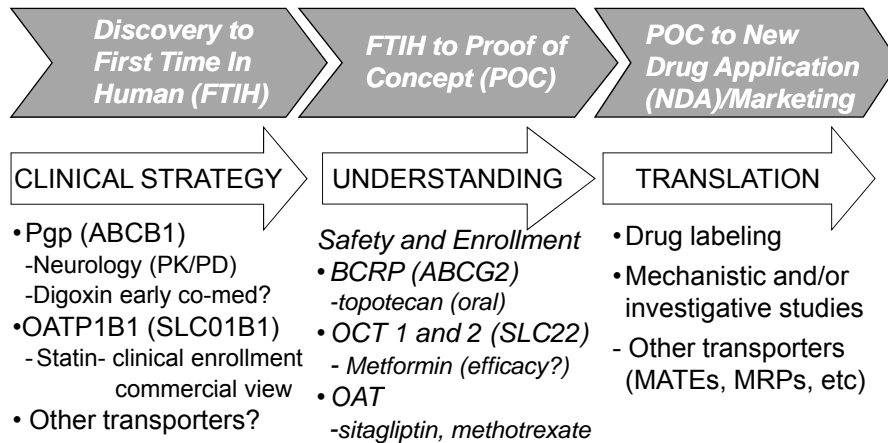
## Discussion

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

## Facts or Myths

- I [redacted] **Myth** [redacted] ibed in the MOC whitepaper for my drug.
- Pgp is generally not very important for the in [redacted] **Fact** [redacted] Therefore, one should rarely do a clinical study.
- There are no agreed timings for transporter s [redacted] **Both (Fact-Myth)** [redacted] be driven by the regulatory authorities.

## Which Transporter and When?\*



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

## 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

## 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 C<sub>max</sub> and AUC

## Acknowledgements

- Andy Ayrton
- Sandra Baldwin
- Jackie Bloomer
- Catherine Cartwright
- Liangfu Chen
- James Clarke
- Harma Ellens
- Grant Generaux
- Mike Hobbs
- Kelly Harmon
- Joan Humphreys
- Marta Johnson
- William Johnson
- John Keogh
- Kathryn Kenworthy
- Chris MacLauchlin
- Gail Nolan
- Ed Sternberg
- Mindy Reese
- Lindsey Webster

Special thanks to the ITC members.

## Discussion- What's on your mind?

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

