

# An Overview of Drug Transporters in ADME & Safety

14 January 2010

Principles of Clinical Pharmacology

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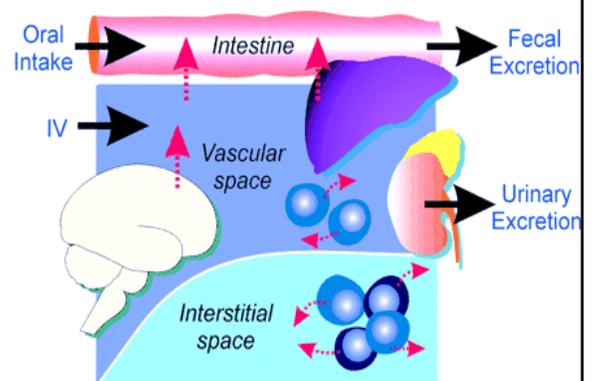
Sr. Scientist, Clinical Pharmacology

Genentech, Inc.

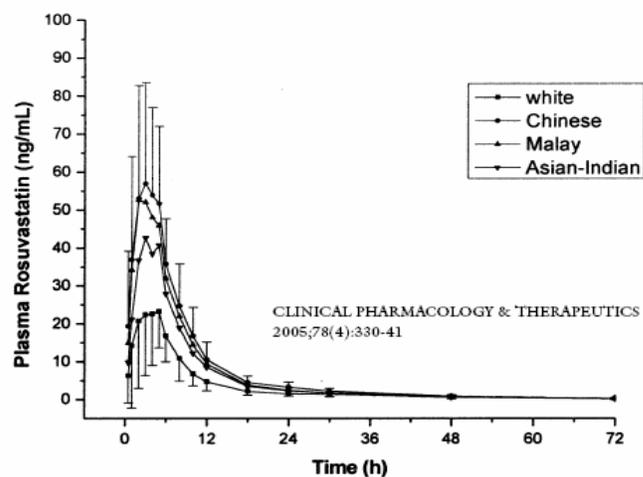
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# Implications of Drug Transport in Drug Discovery and Development

- 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).
- Drug Transporters as Targets
  - LY335979, Zosuquidar



## Rosuvastatin Calcium (Crestor) Pharmacokinetics and Prescribing Information



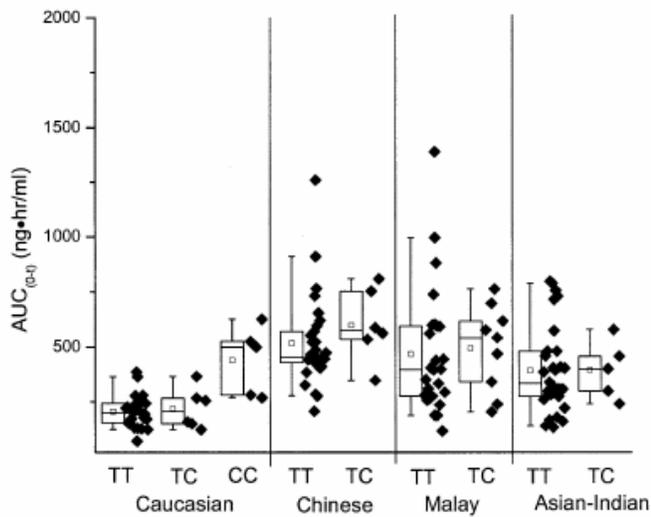
### Rosuvastatin Calcium (marketed as Crestor) Information

#### FDA ALERT [03/2005]

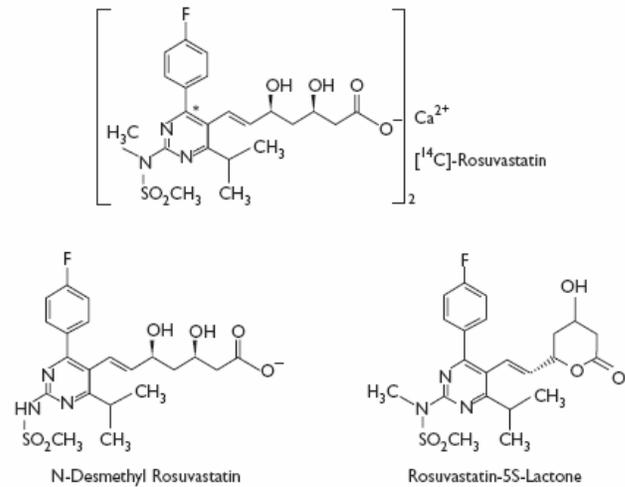
Rhabdomyolysis (serious muscle damage) has been reported in patients taking Crestor as well as other statin drugs. To date, it does not appear that the risk is greater with Crestor than with other marketed statins. However, the labeling for Crestor is being revised to highlight important information 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 *SLC01B1* T521>C Genotype on Rosuvastatin AUC



CLINICAL PHARMACOLOGY & THERAPEUTICS  
2005;78(4):330-41



PD Martin et al., *Clinical Therapeutics*, vol 25, No. 11, 2003

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 ADME
  - Absorption
  - Distribution
  - Metabolism and Transporter Interplay
  - Elimination (kidney and liver)
- Provide examples of drug-drug and drug-transporter interactions
- Inter-Individual variability as a determinant of drug transport
- Examples of when drug transport is a primary determinant of drug-induced toxicity.

# 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

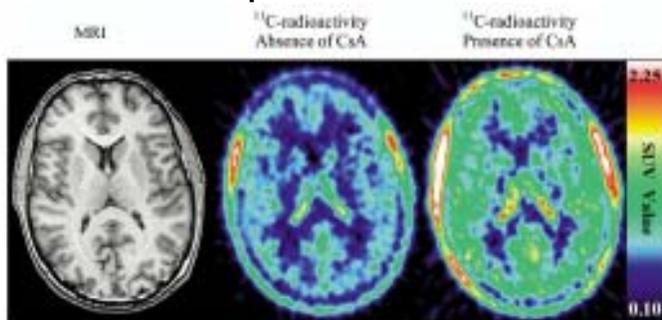
- Ivermectin
- Abamectin

## ⚡ Miscellaneous

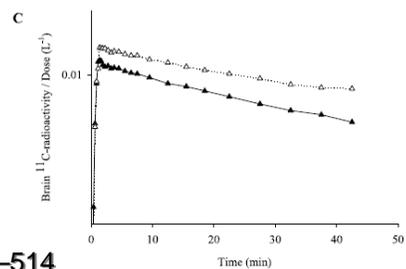
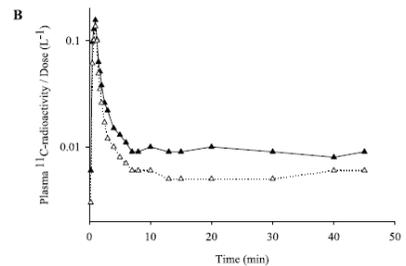
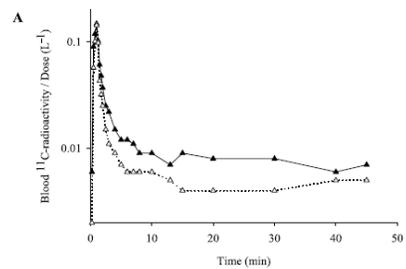
- Loperamide
- Colchicine
- Ondansetron
- Erythromycin

## Clinical Translation of P-gp Inhibition at the BBB

- N=12 subjects  
[<sup>11</sup>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<sub>50</sub> = 0.7 mg/kg
    - mdr1a +/+ LD<sub>50</sub> = 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%

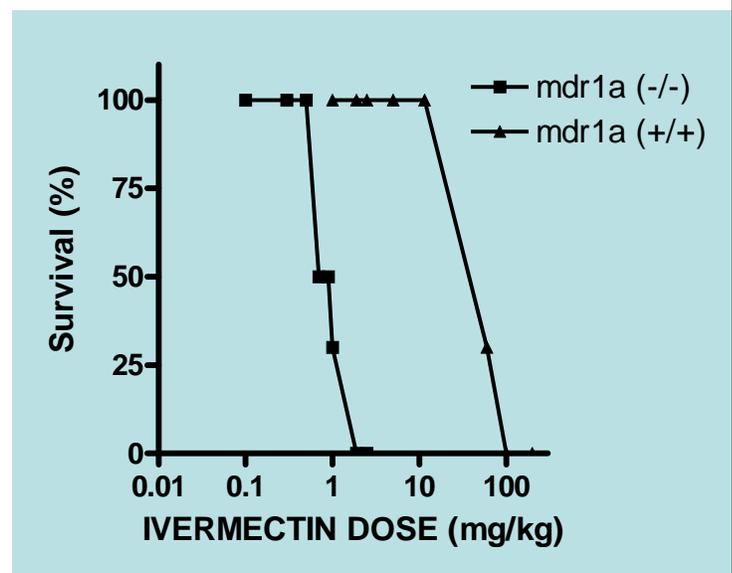
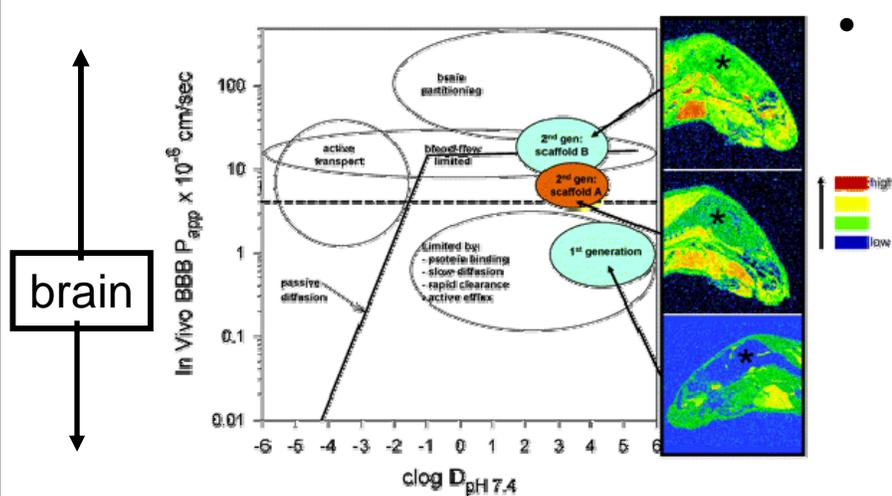


Figure from A.H. Schinkel et al., Cell, Vol.77, 491-501, 1994

## P-gp at the Blood-Brain Barrier



- Many Examples of Drugs whereby BBB Entry is Not Desirable

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

TJ Raub Mol. Pharmaceutics, 3 (1), 3 -25, 2006

## Ivermectin Toxicity in the Collie



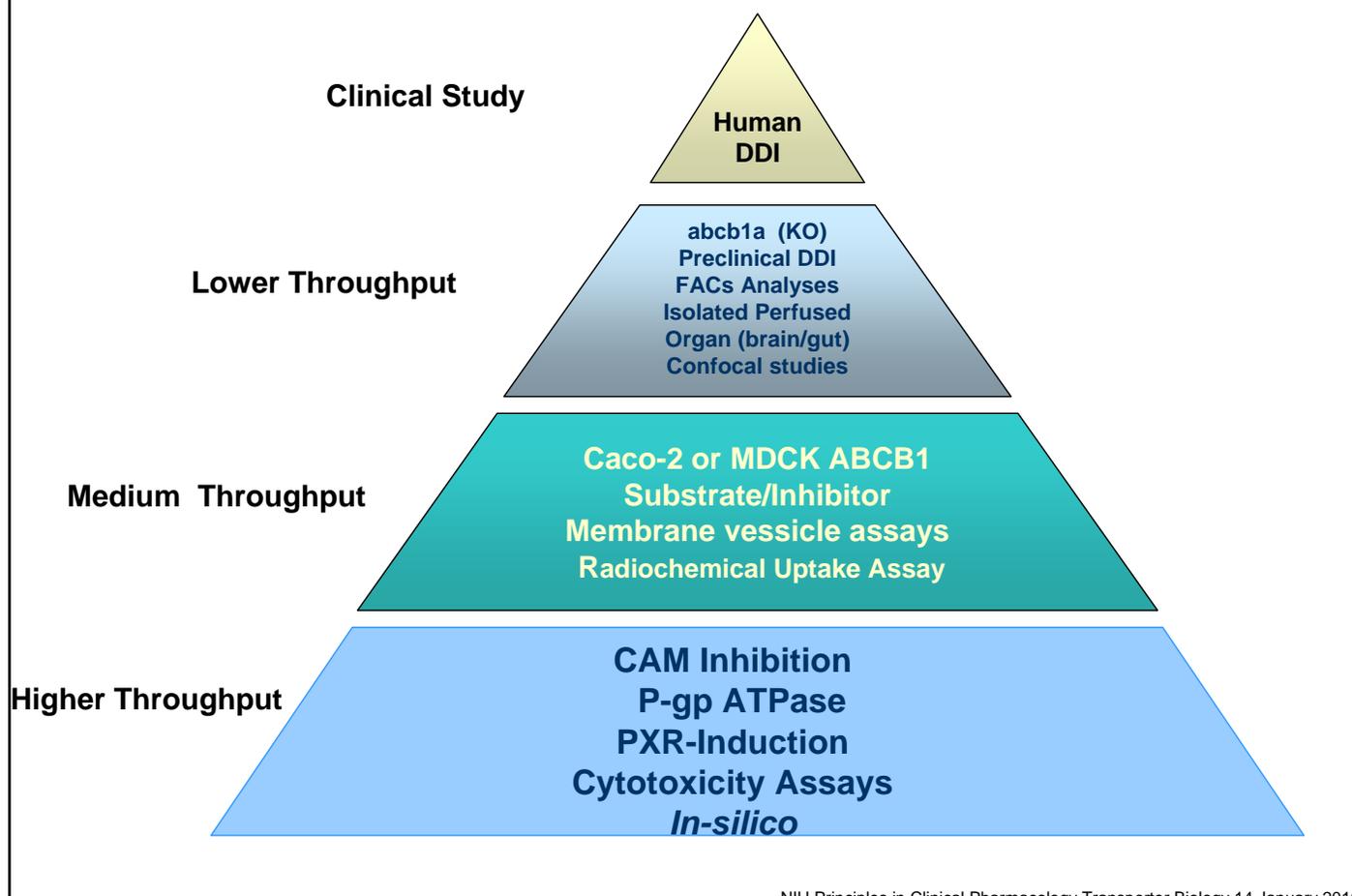
<http://www.awca.net/drug.htm>

- 50% of Collies display CNS toxicity when treated with normal doses of IVM (>60  $\mu\text{g}/\text{kg}$ ).
- 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

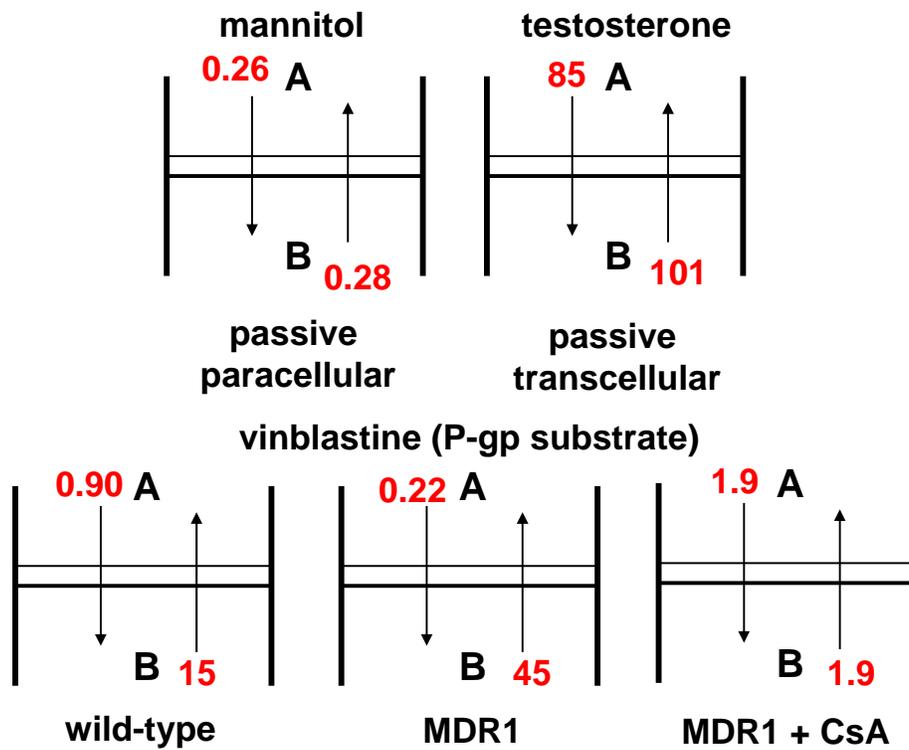
From Mealy et al. Pharmacogenetics. 2001 Nov;11(8):727-33.

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# P-glycoprotein (ABCB1) Cluster Evaluation



## In Vitro Permeabilities



## Caco-2 and MDCK cell comparison

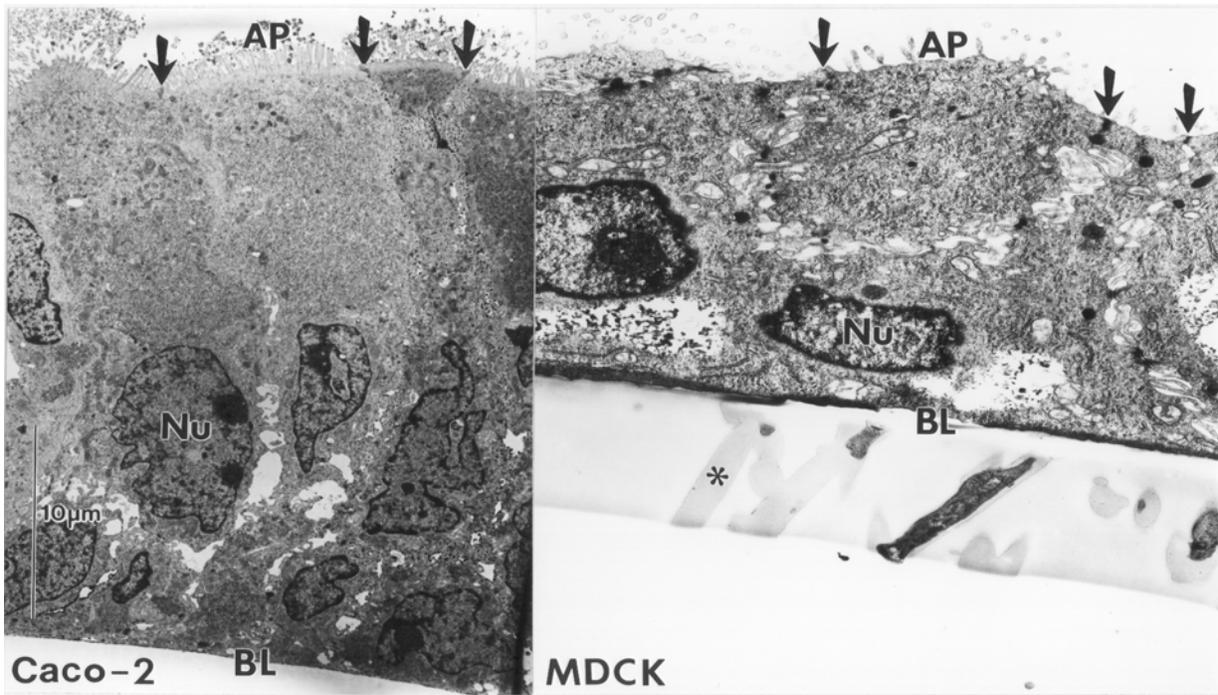
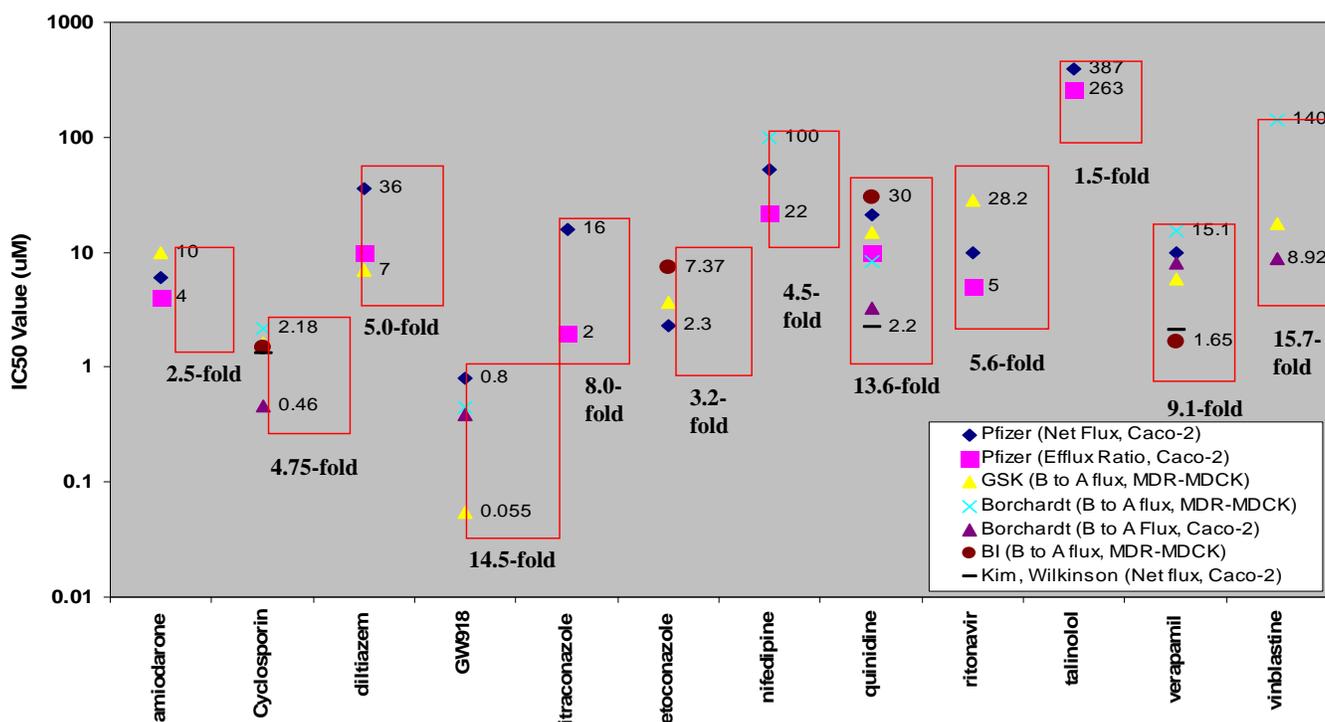


Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub

# In Vitro P-gp IC<sub>50</sub> for Inhibition of Digoxin Efflux Data from Multiple Labs / Techniques

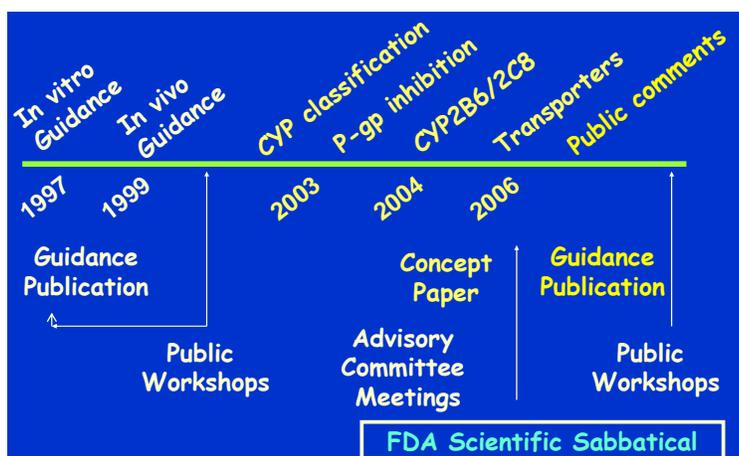


Slide courtesy of M. Troutman/C. Lee Pfizer

## 2006 FDA Draft Guidance, International Transport Consortium and FDA Critical Path Workshop

### 2006 FDA Draft Guidance

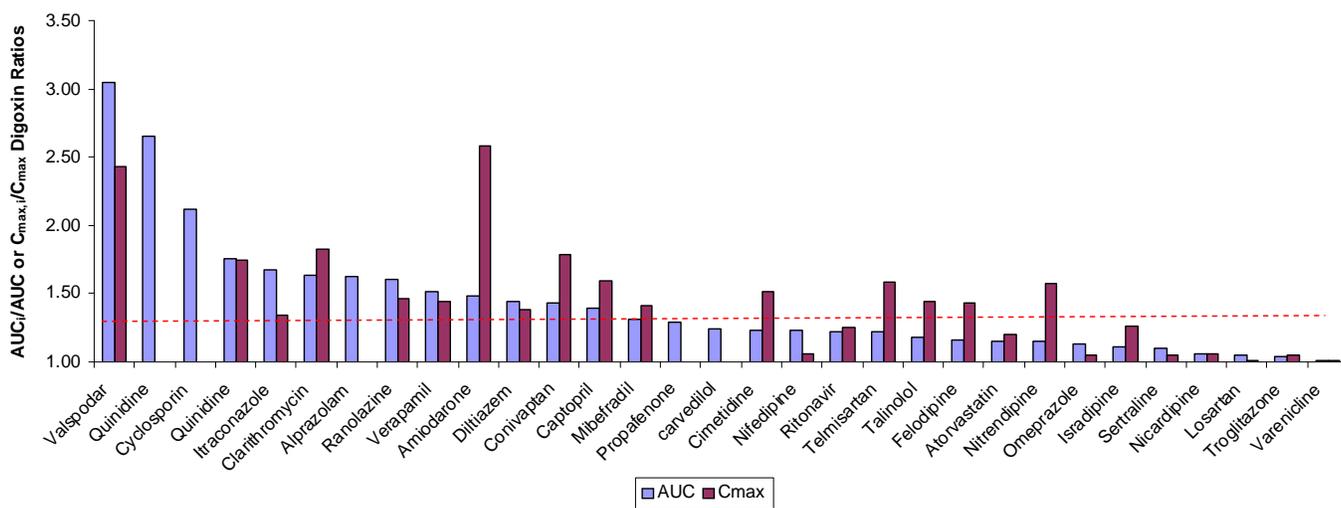
- Knowledge of NME metabolic pathways, interactions, and influence of active transport on drug disposition with respect to DDI potential is key to benefit/risk assessment.
- Integrated approach may reduce number of unnecessary studies and optimize clinical pharmacology studies.
- Classification of CYP inhibitors and substrates can aid in study design and labeling.
  - Substrate (25% metabolism)
  - Inhibitor ( $[I]/K_i > 0.1$ )
  - Inducer (40% control)



Slide adapted from Shiew-Mei Huang, Ph.D., FDA

### International Transport Consortium

## Digoxin: Safety Concerns



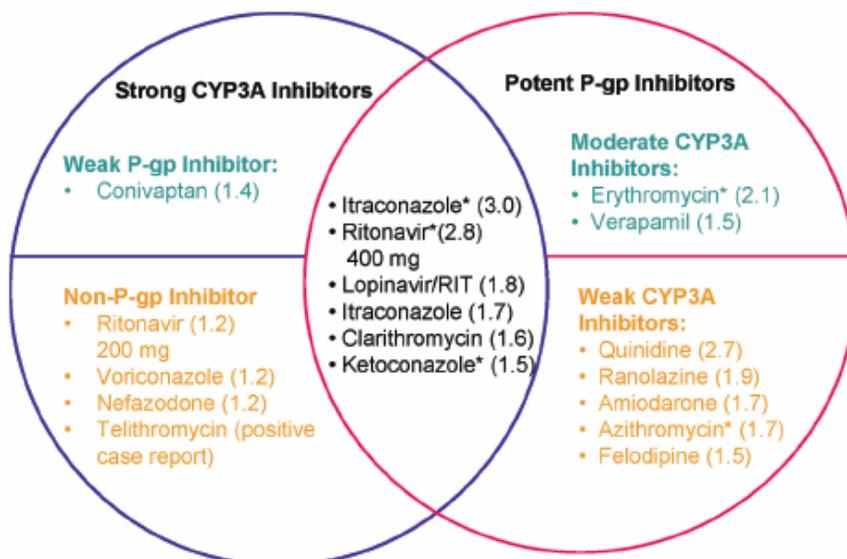
- Therapeutic conc ~ 1.5 ng/mL
- 33% change in Digoxin Exposure (C<sub>max</sub>) ~ 2.0 ng/mL → Safety concerns
- 25% change in exposure might be clinically relevant

Clinical Pharmacology & Therapeutics (2009); 85, 173–181

## P-gp Mediated Digoxin DDIs

- <2-fold change in digoxin C<sub>max</sub> or exposure were observed in the majority of published cases
  - I/IC<sub>50</sub> > 0.1 is predictive of positive clinical digoxin DDI related to P-gp
  - I<sub>2</sub>/IC<sub>50</sub> < 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/IC<sub>50</sub> or false (+)'s with I<sub>2</sub>/IC<sub>50</sub>

## Drug Metabolizing Enzyme - Drug Transporter Interplay



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 or itraconazole represents the worse case scenario for a Clinical DDI study

Mol. Pharmaceutics, 2009, 6 (6), pp 1766–1774

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## 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**

# ABC Substrate/Inhibitor Overlap

*Distinct but Overlapping Substrate Specificities*

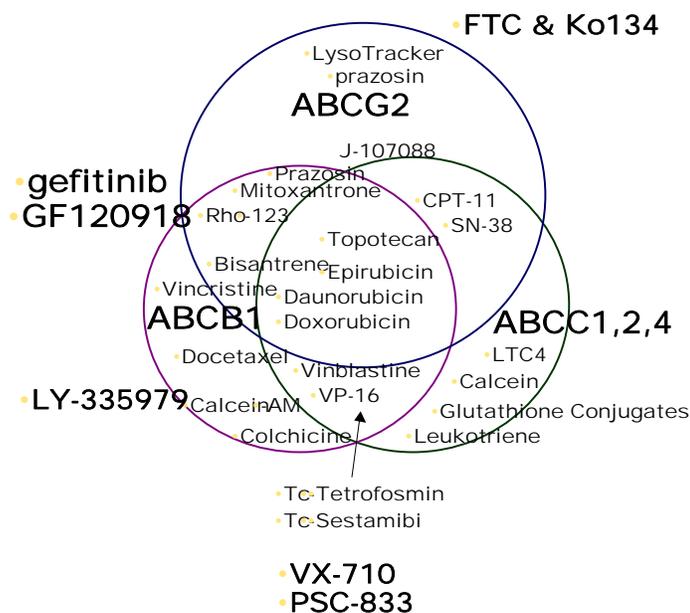


Figure adapted from Thomas Litman

## 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

### Drugs/NMEs

- Topotecan
- CPT-11/SN-38
- J-107088
- Mitoxantrone
- Flavoperidol
- Diflomotecan
- Methotrexate
- Sulfasalazine
- Prazosin
- Benzoylphenylurea
- Cimetidine
- Imatinib

### Xenobiotics Endobiotics

- PhIP
- Pheophorbide A
- Estrogen SO<sub>4</sub>
- lysotracker (green)
- H33342
- Rhodamine 123
- Bodipy-prazosin
- Riboflavin (vitamin B2)

### Inhibitors

- FTC
  - Ko134, 143
- Tryprostatin A
- GF120918
- Lapatinib
- Erlotinib
- Gefitinib
- CI-1033
- Novobiocin
- Imatinib
- Ritonavir

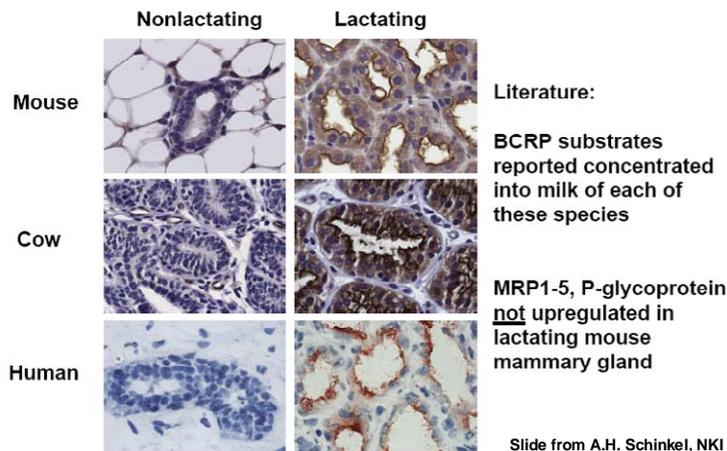
The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyrin.

Jonker et al., *Proc Natl Acad Sci U S A* 2002 Nov 26;99(24):15649-54

- **Bcrp -/- ADME Phenotype**

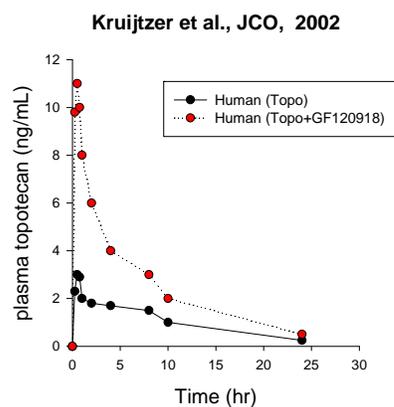
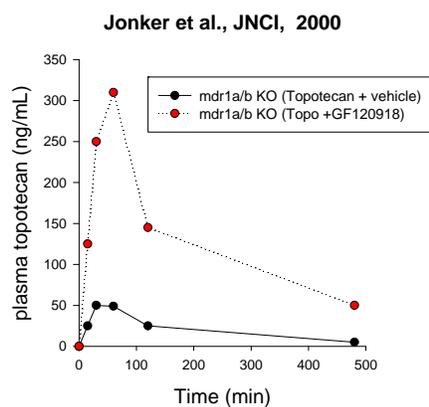
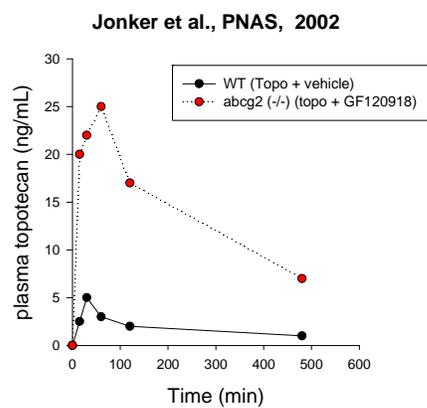
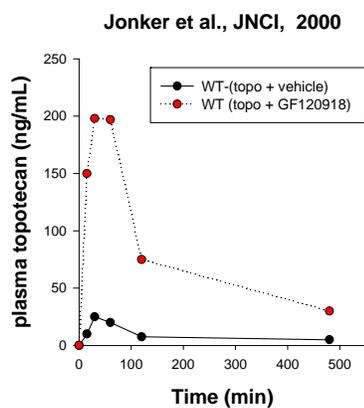
- Mice displayed diet-dependent phototoxicity
- Protoporphyrin
- Enhanced oral absorption of topotecan
- ABCG2 is expressed in bone marrow stem cells.
- Milk secretion of drugs and xenotoxins *Nat. Med.* 2005 Feb;11(2):127-9

**Expression BCRP in mammary gland across species**



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# Of mice and men: Topotecan:BCRP interaction



## Absorption, metabolism, and excretion of salicylazosulfapyridine in man

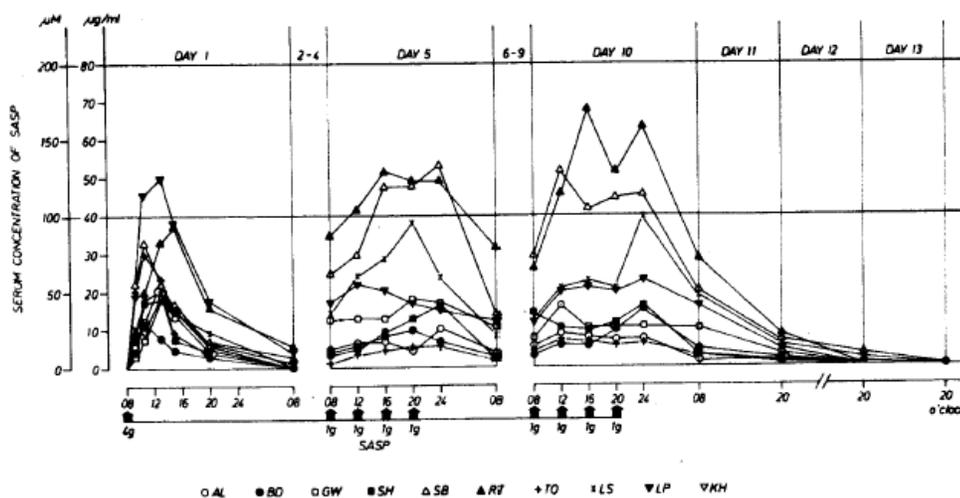
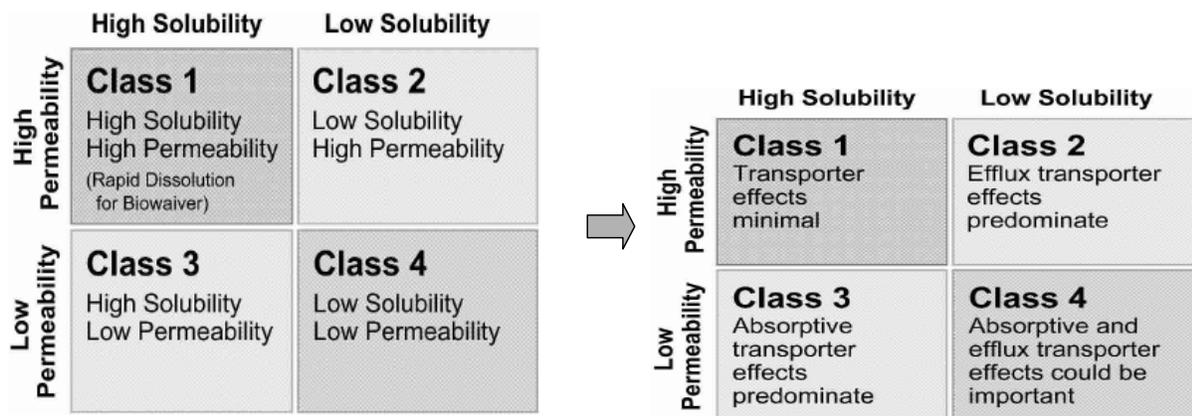


Fig. 2. Serum concentrations of SASP after ingestion of a single 4 Gm. dose of SASP on Day 1 (10 subjects) and 4 x 1 Gm. of SASP on Days 2 to 10 (9 subjects).

Hasse Schröder and Dag E. S. Campbell Uppsala, Sweden  
 Department of Zoophysiology, University of Uppsala, Pharmacia AB, Box 604, 751 25

*Permeability is an important determinant of In vitro-in vivo extrapolation for both Metabolism and Transport*



*Amidon et al., Pharm. Res. 12:413 (1995)*

*Wu and Benet, Pharm. Res. 22:11 (2005)*

## **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.*

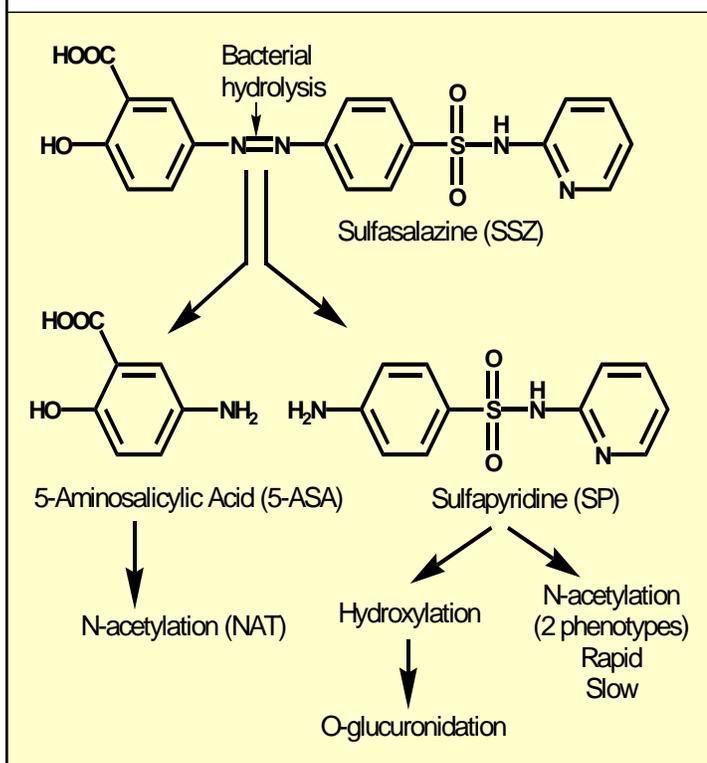
## ABCG2 Polymorphisms and Ethnic Distribution of SNPs.

- 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.

| Allelic variant | Caucasians | African-Americans | Asians | Hispanics | Africans | Middle Easterns |
|-----------------|------------|-------------------|--------|-----------|----------|-----------------|
| V12M            | 2          | 4                 | 20–45  | 40        |          | 5               |
| Q141K           | 11–14      | 2.3–5.0           | 15–35  | 10        | 1.0      | 13              |
| I206L           | 0          | 0                 | 0      | 10        |          | 0               |
| N590Y           | 1          |                   |        |           |          |                 |

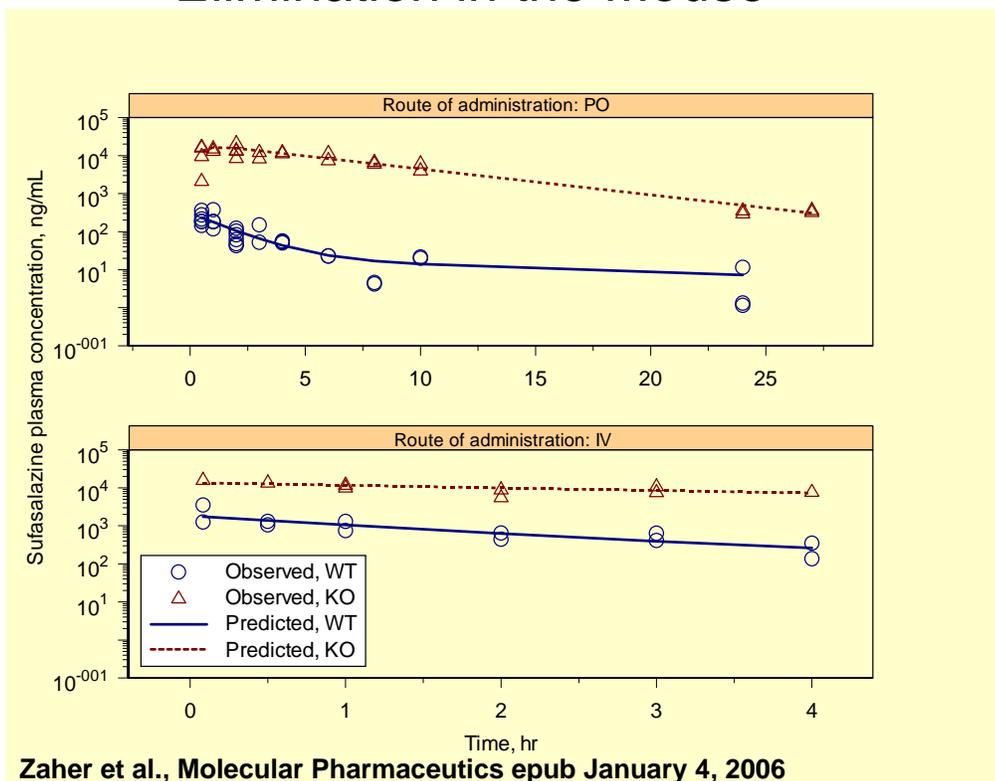
Figg et al., Anticancer Drugs. 2007

## Sulfasalazine (SASP) Disposition

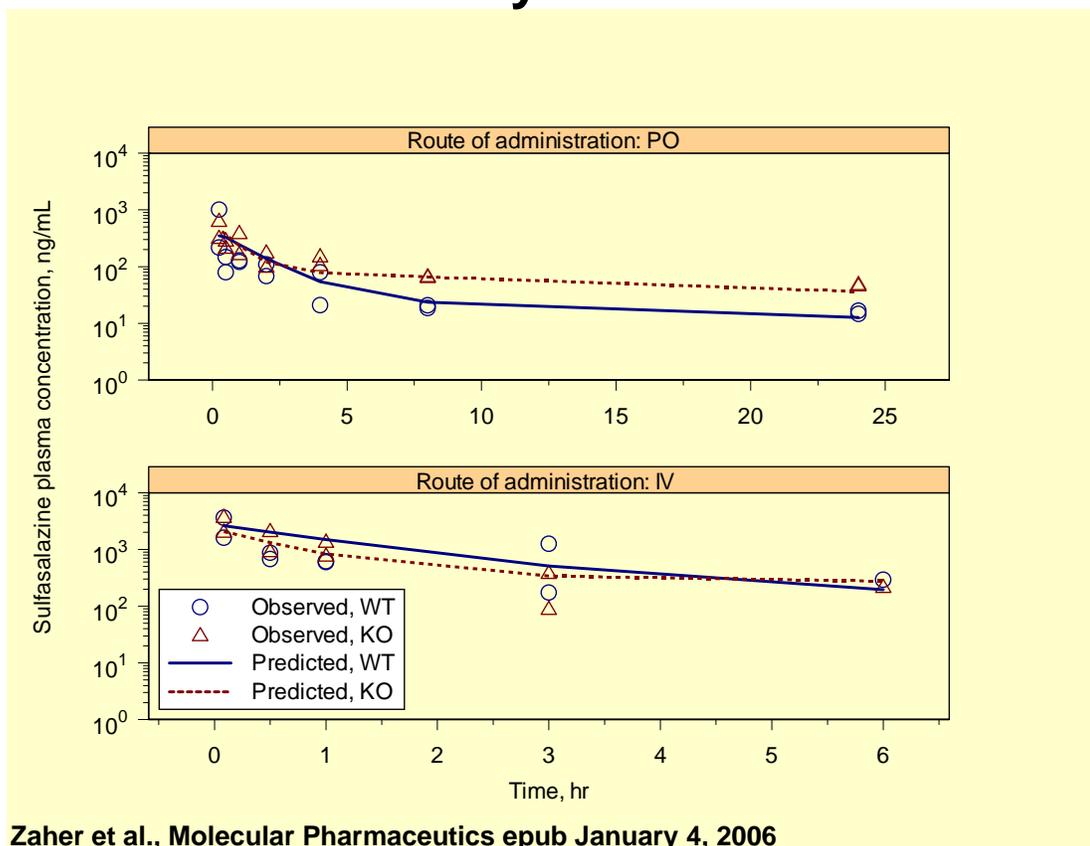


- **Indications: Rheumatoid arthritis (RA), Long term therapy of ulcerative colitis, and Crohn's disease**
- **Bioavailability (F) of SASP in humans is low (F < 15%) and highly variable**
- **Low %F primarily attributed to SASP's low permeability and poor solubility (thus, poor absorption)**
- **Azo-reduction 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



## Abcb1 (mdr1a) does not contribute to SASP Bioavailability or Clearance



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

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| Mice  | Route | Dose (mg/kg) | C <sub>max</sub> (ng/mL)* |       | AUC (ng.hr/mL) |      |        | Relative exposure, AUC <sub>KO</sub> /AUC <sub>WT</sub> |
|-------|-------|--------------|---------------------------|-------|----------------|------|--------|---|
|       |       |              | WT                        | KO    | Duration (hr)  | WT   | KO     |   |
| Bcrp1 | IV    | 5            | 1827                      | 13570 | 0-4            | 3015 | 40343  | <b>13</b>   |
|       | PO    | 20           | 233                       | 16176 | 0-24           | 1189 | 131822 | <b>111</b>  |
| Mdr1a | IV    | 5            | 2749                      | 2266  | 0-6            | 5131 | 3504   | <b>1</b>  |
|       | PO    | 20           | 349                       | 440   | 0-24           | 1098 | 1781   | <b>2</b>  |

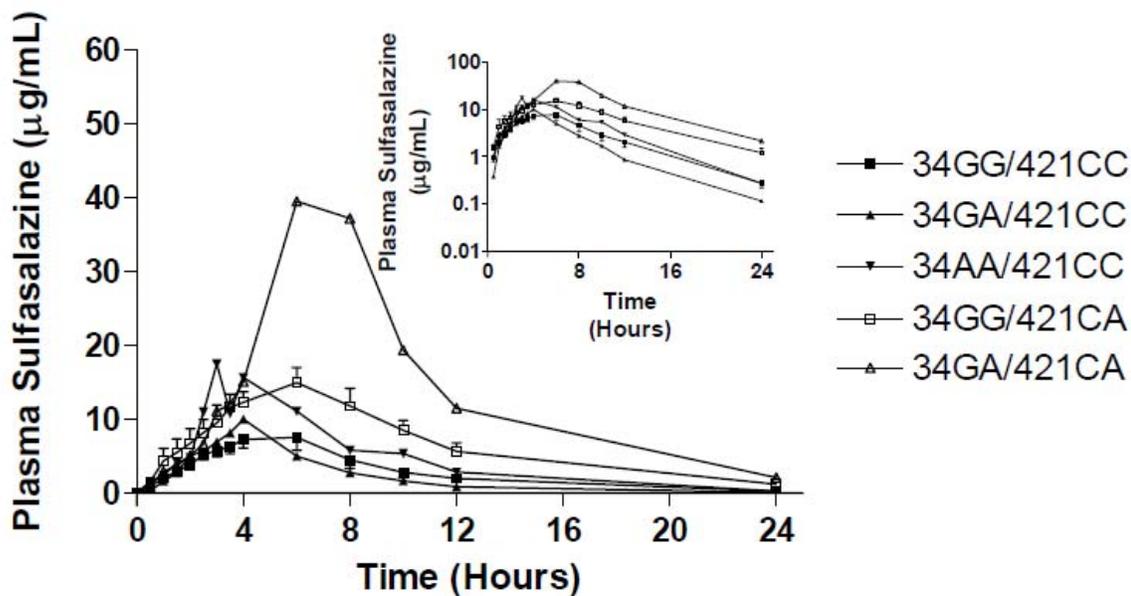
\* IV (intravenous) = C<sub>max</sub> at time zero was extrapolated from the model; PO (Oral) = visual C<sub>max</sub> from raw data

SASP 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

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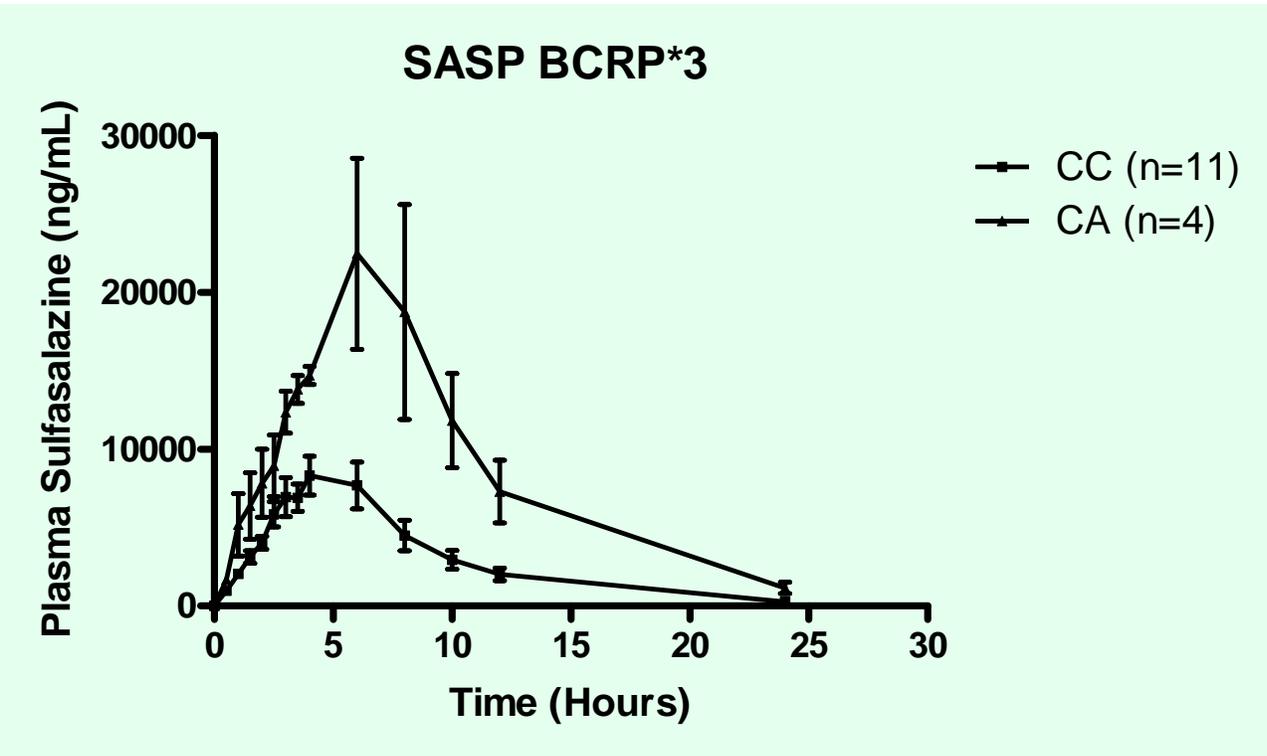
## SASP Disposition in North American Healthy Volunteers



Brad Urquhart et al., *Pharmacogenet Genomics*. 2008 May;18(5):439-48.

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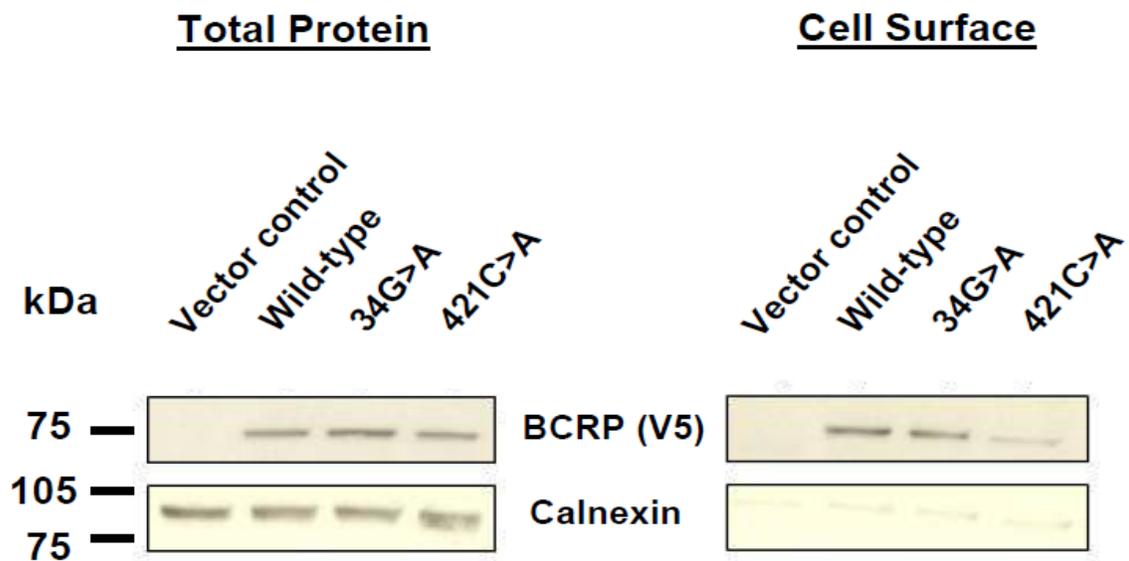
# Altered SASP Exposure in Q141K Subjects



Urquhart et al., Pharmacogenet Genomics. 2008 May;18(5):439-48.

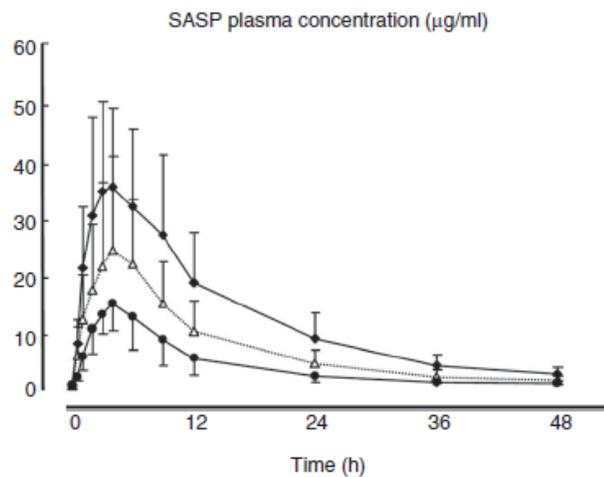
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## 421C>A SNP Changes Surface ABCG2 Expression



Pharmacogenet Genomics. 2008 May;18(5):439-48.

## SASP Disposition in Healthy Japanese Volunteers

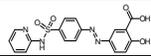
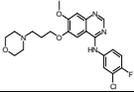
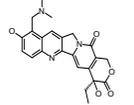
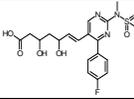
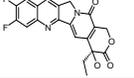
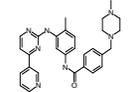
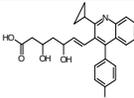


**Figure 2** Effect of *ABCG2* genotype on pharmacokinetics of sulfasalazine (SASP). Plasma concentration-time profiles of SASP after oral administration of a 2,000 mg conventional SASP tablet to 421C/C subjects (closed circles,  $n = 12$ ), 421C/A subjects (open triangles,  $n = 16$ ), and 421A/A subjects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

Yamasaki et al., CPT January 2, 2008

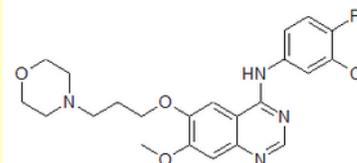
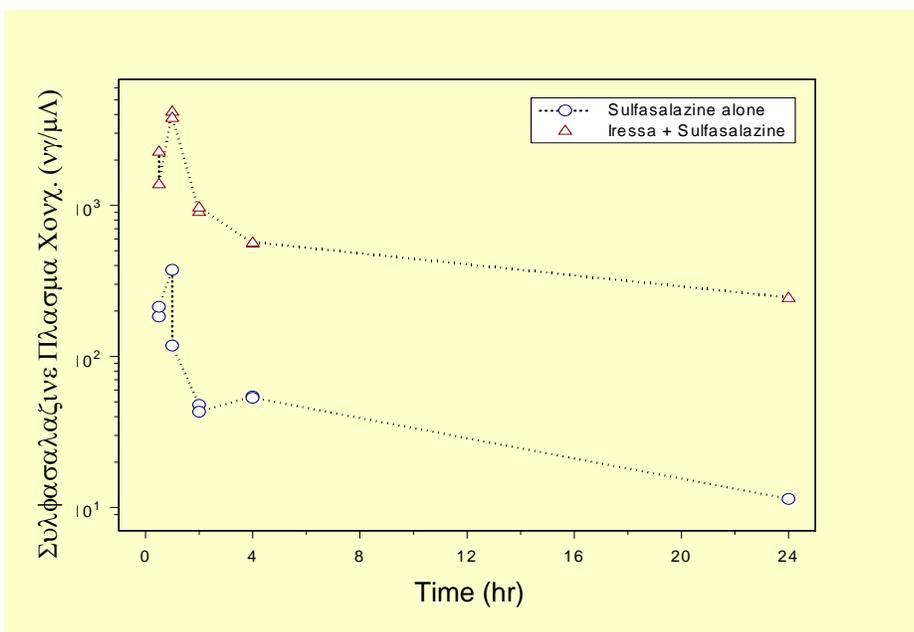
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# ABCG2 Pharmacogenomic Studies

| Formulation | Drug               | Structure   | Dose, Route    | # Patients | Ethnic Group, Gender | Result   | Reference  |
|-------------|--------------------|---|----------------|------------|----------------------|--|--|
| IR →        | Sulfasalazine      |    | 2000 mg po     | 37*        | Japanese Male        | 1.7-3.5X increase in AUC, Cmax                 | Yamasaki et al (2008) Clin Pharmacol Ther, ePub    |
| susp →      | Sulfasalazine      |   | 1000 mg po     | 17*        | Caucasian Both       | 1.7-2.4X increase in AUC, Cmax                 | Urquhart et al (2008) Pharmacogen & Genomics, ePub |
| SR →        | Sulfasalazine      |   | 500 mg po      | 36*        | Chinese Both         | No effect on AUC, Cmax                         | Adkison et al (2008) ASCPT mtg poster              |
|             | Gefitinib (IRESSA) |   | 250 mg po      | 124^       | Caucasian Both       | 44% with mutation had diarrhea vs. 12% with WT | Cusatis et al (2007) JNCI 98(23):1739              |
|             | Topotecan          |  | <2.5 mg po, iv | 18^        | Caucasian Both       | 1.35X increase in oral bioavailability         | Sparreboom et al (2005) Canc Biol Ther 4:650       |
|             | Rosuvastatin       |  | 20 mg po       | 14*        | Chinese Both         | 1.8X increase in AUC and Cmax                  | Zhang et al (2006) Clin Chim Acta 373:99           |
|             | Diflomotecan       |  | <0.5 mg po, iv | 22^        | Caucasian Both       | 3X increase in AUC and Cmax for iv only        | Sparreboom et al (2004) Clin Pharmacol Ther 76:38  |
|             | Imatinib (GLEEVEC) |  | 100-1000 mg po | 82^        | Caucasian Both       | No difference                                  | Gardner et al (2006) Clin Pharmacol Ther 80:192    |
|             | Pitavastatin       |  | 2 mg po        | 38*        | Japanese Male        | No difference                                  | Ieiri et al (2007) Clin Pharmacol Ther. 82:541     |

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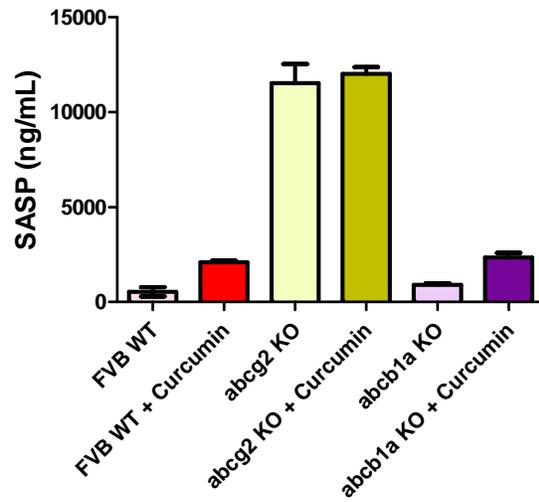
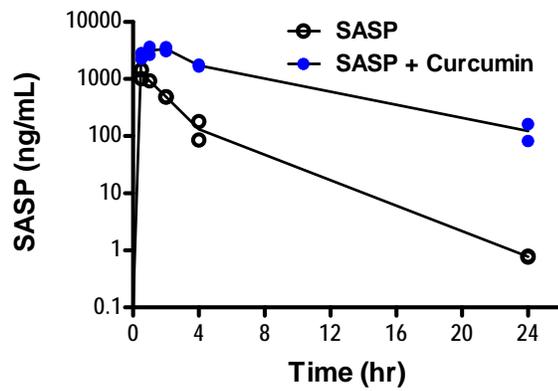
### Gefitinib (Iressa)-enhanced SASP Bioavailability



Gefitinib (Iressa)

**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

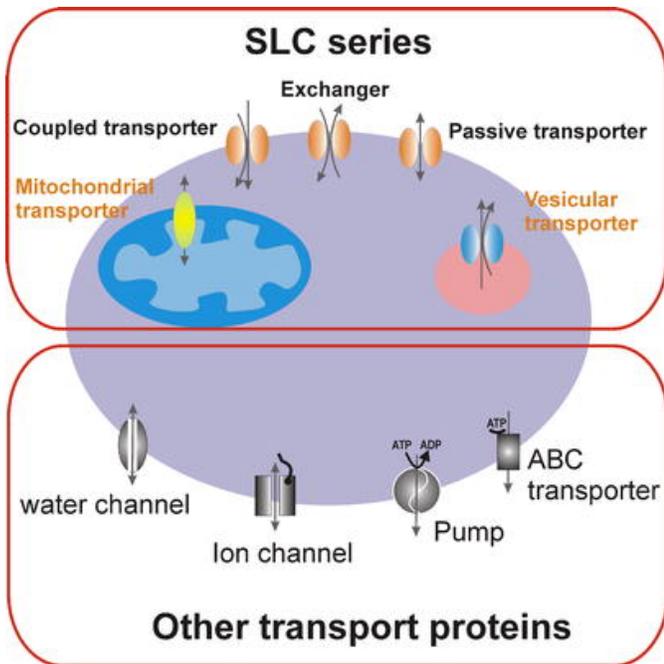


Suneet Shukla et al. Pharm Res. 2008 Oct 9.

## 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

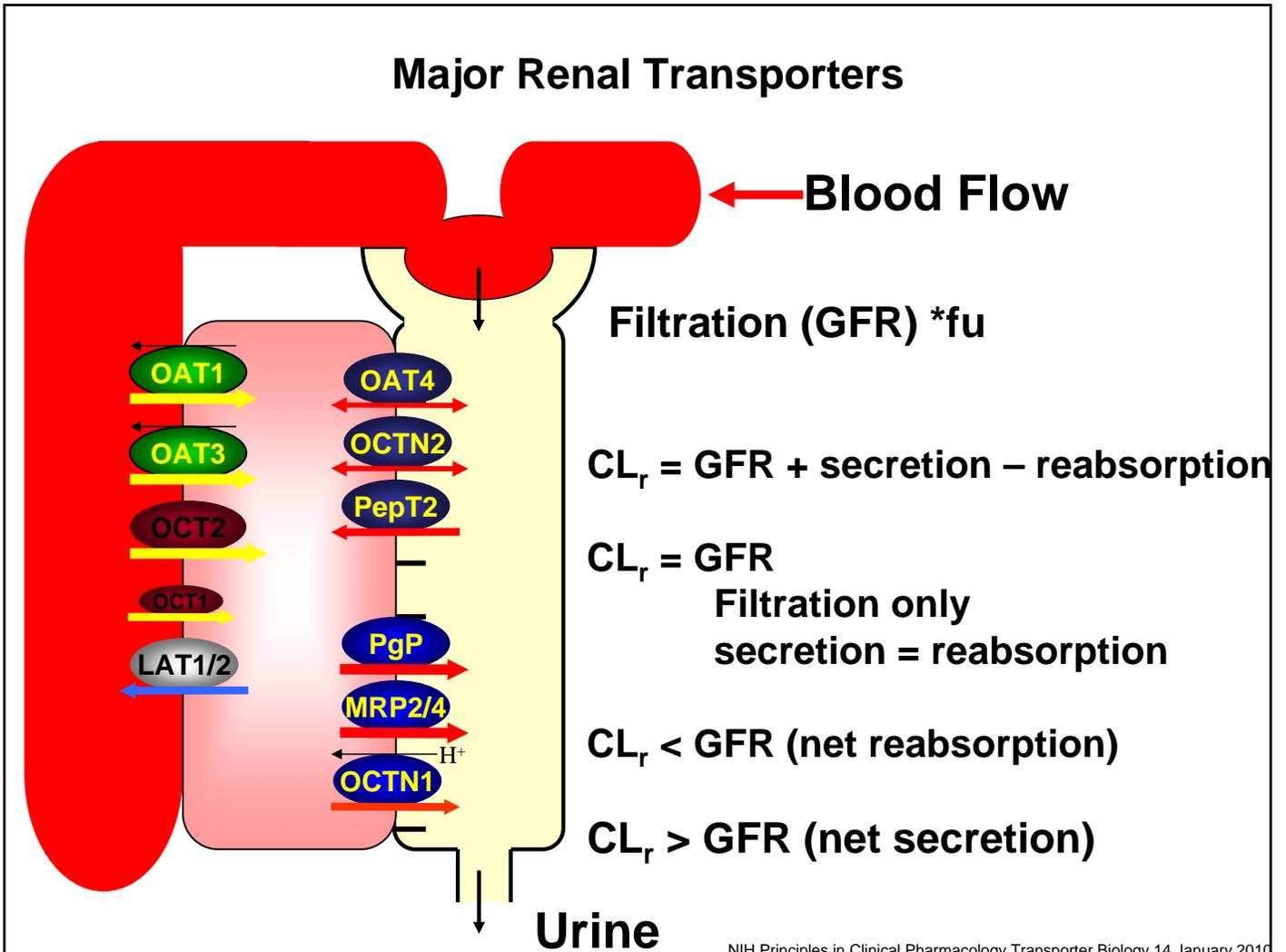


- Solute Carrier (SLC) superfamily contains
  - 43 families
  - 298 genes
- HUGO database (see <http://www.gene.ucl.ac.uk/nomenclature/>)
  - SLC root symbol
  - Followed by numeral (family)
  - Followed by letter
  - Followed by numeral (ie SLC22A1)
  - Further elaborated in the SLC21/SLCO

References: Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA. Introduction. *Pflügers Arch.* 2004 Feb;447(5):465-8.

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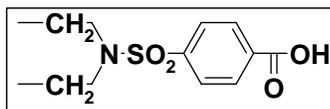
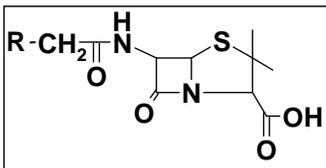
## Major Renal Transporters



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## 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

## 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
- What is the optimal in vitro and in vivo strategy that will bridge preclinical to Clinical Development Plan?
- 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?

## Package Inserts: Clinical Studies and DDI Potential

| <b>Drug (CL<sub>R</sub>)</b>                         | <b>Results (Bedside)</b>   |
|--|--|
| Mirapex (400 mL/min)<br>+ cimetidine<br>+ probenecid | N=12 subjects/treatment arm.<br>50% ↑ in AUC; 40% ↑ in T 1/2<br>No effect on PK                    |
| Tikosyn (420 mL/min)<br>+ cimetidine<br>+ probenecid | Narrow TDI<br>40% ↑ in AUC; CLR ↓ 33%; QTc ↑17-19 ms<br>No effect                                  |
| Oseltamivir<br>+cimetidine<br>+probenecid            | N=12-18/treatment (see Hill et al.)<br>No change on PK<br>2.5-fold AUC of Ro64-0802 (active metab) |
| Axid (500 mL/min)                                    | Not currently defined, however TDI very high   |

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## Transporter Nomenclature

### SLC Family

- **Basolateral**
  - OCT2 = SLC22A2
  - OAT1 = SLC22A6
  - OAT3 = SLC22A8
  - System L = SCL7A5/8
- **Apical**
  - PepT2 = SLC15A2
  - OCTTN1 = SLC22A4
  - OCTN2 = SLC22A5
  - OAT4 = SLC22A11

### ABC Family

- **Apical**
  - MDR1 = ABCB1
  - MRP2 = ABCC2
  - MRP4 = ABCC4
  - BCRP = ABCG2

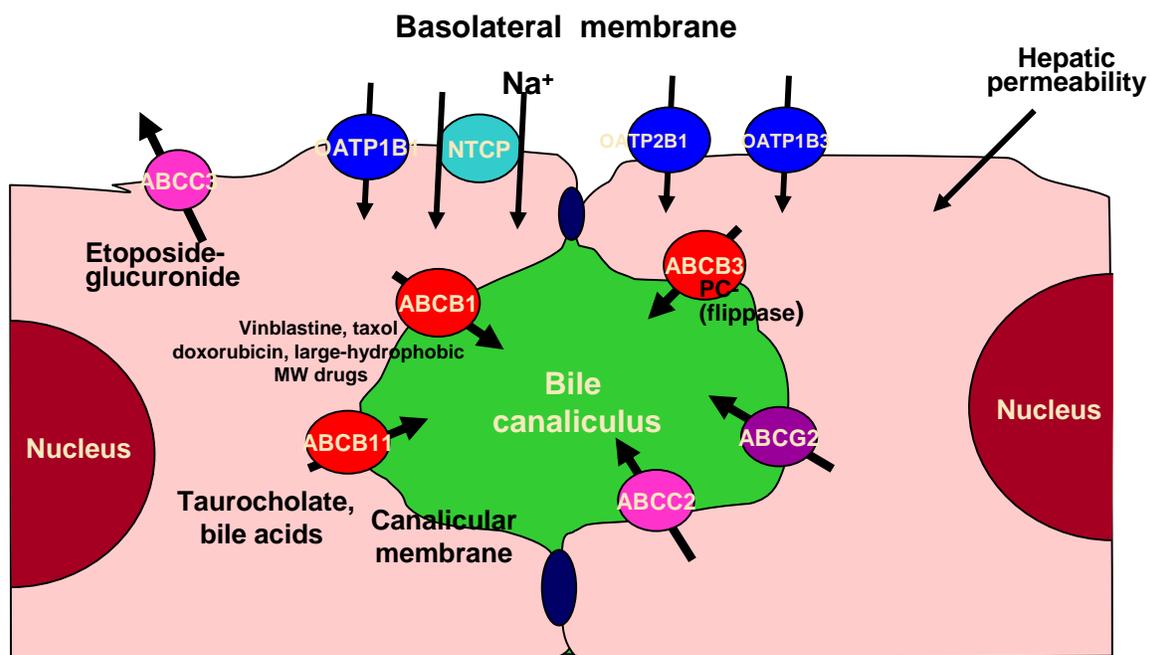
## Hepatic Transporters

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?

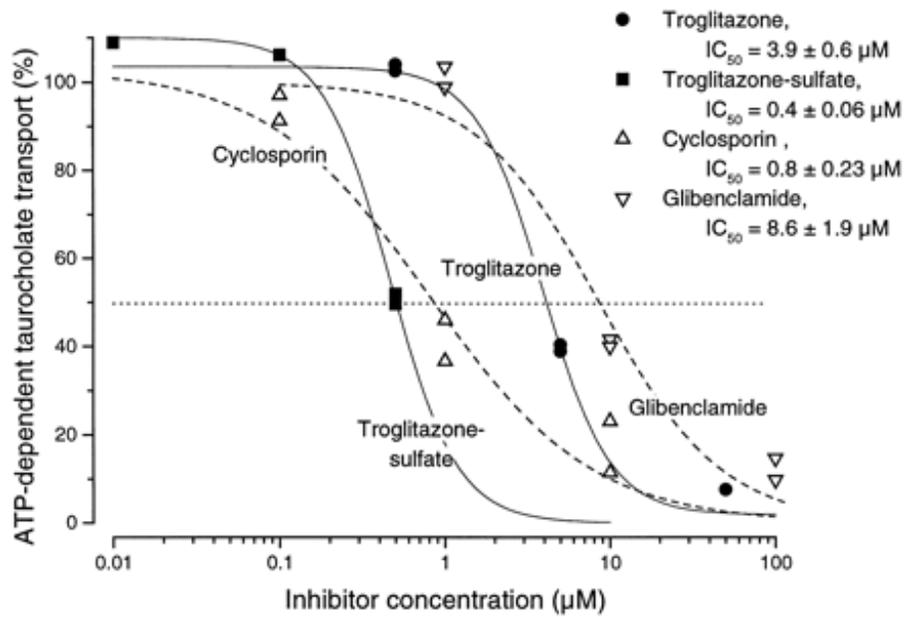
Question 3. Toxicological significance of bile acid uptake, synthesis, or efflux inhibition

# Hepatic Uptake/Efflux Transporters



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## Hepatic Transport and Liver Injury



Funk et al., Mol. Pharm. Vol. 59, Issue 3, 627-635, March 2001

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## OATP Substrates

### OATP1B1 (OATP-C, LST-1, OATP2)

**Endogenous Substrates:**

Estrone Sulfate, PGE<sub>2</sub>, Bilirubin, thyroid hormone (T<sub>3</sub>, T<sub>4</sub>) Bilirubin-glucuronides Estradiol 17β-d-glucuronide, bile acids

**Drug Substrates:**

Atorvastatin, Cerivastatin, Pravastatin  
Rosuvastatin, Pitavastatin, Caspofungin,  
Troglitazone-sulfate, Rifampin, Arsenic,  
Atrasentan, Valsartan, Olmesartan, Enalapril,  
MTX, Temocaprilat, SN-38

**Toxins:**

Phalloidin, Microcystin-LR

### OATP1B3 (OATP8, LST-2)

**Endogenous Substrates:**

CCK-8, PGE<sub>2</sub> Thyroid hormone (T<sub>3</sub>, T<sub>4</sub>) Estradiol  
17 β -d-glucuronide, Bile acids, Deltophin, DPDPE,

**Drug Substrates:**

Pravastatin, Pitavastatin, Rosuvastatin,,  
Fexofenadine, BQ-123, Oubain,, Digoxin,  
Doxotaxel, Paclitaxel,, Rifampin, MTX, Bilirubin,  
Repaglinide, Telmisartan, Valsartan,  
Olmesartan, Enalapril, Temocaprilat, SN-38

**Toxins:**

Phalloidin, Microcystin-LR

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# The NEW ENGLAND JOURNAL of MEDICINE

## SLCO1B1 Variants and Statin-Induced Myopathy — A Genomewide Study

The SEARCH Collaborative Group\*

### ABSTRACT

#### BACKGROUND

Lowering low-density lipoprotein cholesterol with statin therapy results in substantial reductions in cardiovascular events, and larger reductions in cholesterol may produce larger benefits. In rare cases, myopathy occurs in association with statin therapy, especially when the statins are administered at higher doses and with certain other medications.

#### METHODS

We carried out a genomewide association study using approximately 300,000 markers (and additional fine-mapping) in 85 subjects with definite or incipient myopathy and 90 controls, all of whom were taking 80 mg of simvastatin daily as part of a trial involving 12,000 participants. Replication was tested in a trial of 40 mg of simvastatin daily involving 20,000 participants.

#### RESULTS

The genomewide scan yielded a single strong association of myopathy with the rs4363657 single-nucleotide polymorphism (SNP) located within *SLCO1B1* on chromosome 12 ( $P = 4 \times 10^{-9}$ ). *SLCO1B1* encodes the organic anion–transporting polypeptide OATP1B1, which has been shown to regulate the hepatic uptake of statins. The noncoding rs4363657 SNP was in nearly complete linkage disequilibrium with the nonsynonymous rs4149056 SNP ( $r^2 = 0.97$ ), which has been linked to statin metabolism. The prevalence of the rs4149056 C allele in the population was 15%. The odds ratio for myopathy was 4.5 (95% confidence interval [CI], 2.6 to 7.7) per copy of the C allele, and 16.9 (95% CI, 4.7 to 61.1) in CC as compared with TT homozygotes. More than 60% of these myopathy cases could be attributed to the C variant. The association of rs4149056 with myopathy was replicated in the trial of 40 mg of simvastatin daily, which also showed an association between rs4149056 and the cholesterol-lowering effects of simvastatin. No SNPs in any other region were clearly associated with myopathy.

#### CONCLUSIONS

We have identified common variants in *SLCO1B1* that are strongly associated with an increased risk of statin-induced myopathy. Genotyping these variants may help to achieve the benefits of statin therapy more safely and effectively. (Current Controlled Trials number, ISRCTN74348595.)

Address reprint requests to the SEARCH Collaborative Group at the Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Richard Doll Bldg., Old Road Campus, Roosevelt Dr., Oxford OX3 7LF, United Kingdom, or at [search@ctu.ox.ac.uk](mailto:search@ctu.ox.ac.uk).

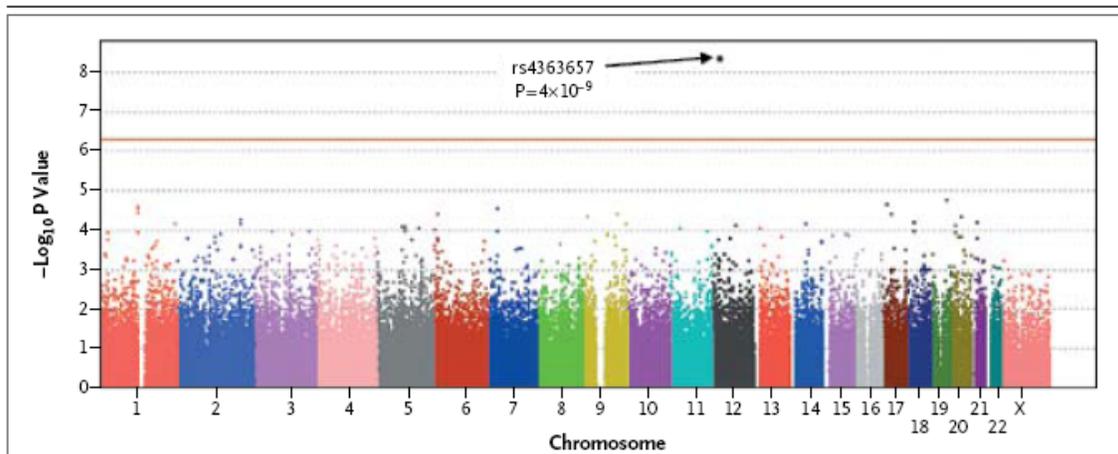
\*The investigators and institutions participating in the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) are listed in the Appendix and in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org).

This article (10.1056/NEJMoa0801936) was published at [www.nejm.org](http://www.nejm.org) on July 23, 2008.

N Engl J Med 2008;359.

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SLCO1B1 VARIANTS AND STATIN-INDUCED MYOPATHY



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

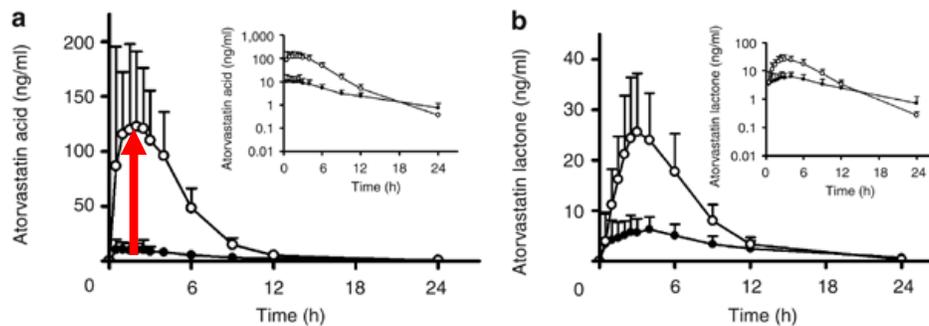
P values are shown for each SNP measured among 85 participants with myopathy and 90 matched controls who were taking 80 mg of simvastatin daily. Analyses are based on 316,184 of the 318,237 SNPs (99.4%) on the Sentrix HumanHap300-Duo BeadChip (Illumina). A result above the horizontal red line indicates strong evidence of an association ( $P < 5 \times 10^{-7}$ ).

**N Engl J Med. 2008 Aug 21;359(8):789-99**

## 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

## Rifampicin Inhibits Atorvastatin through OATP

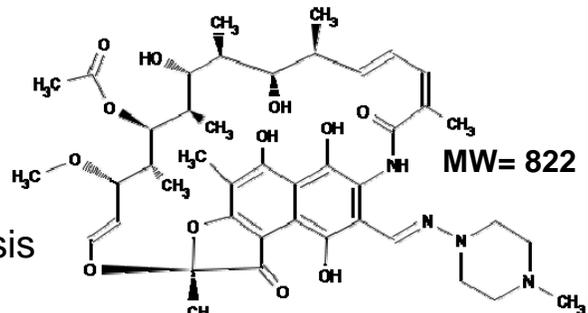


- 600 mg rifampicin IV increases atorvastatin acid AUC 7-fold.
- Acutely, single dose rifampicin may inhibit OATP1B3, CYP3A4, and CYP2C8.

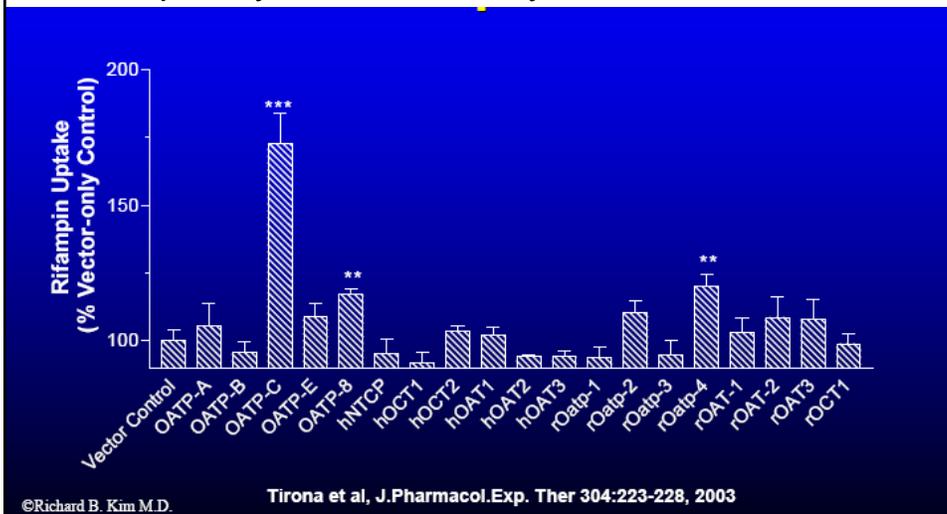
(Lau YY et al., Clin Pharmacol Ther, 81, 194-204 (2007), slide courtesy of Dr. L.Z. Benet)

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# 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)
- Recently identified as an inhibitor of OATPs and entry into human hepatocytes mediated by OATP1B1

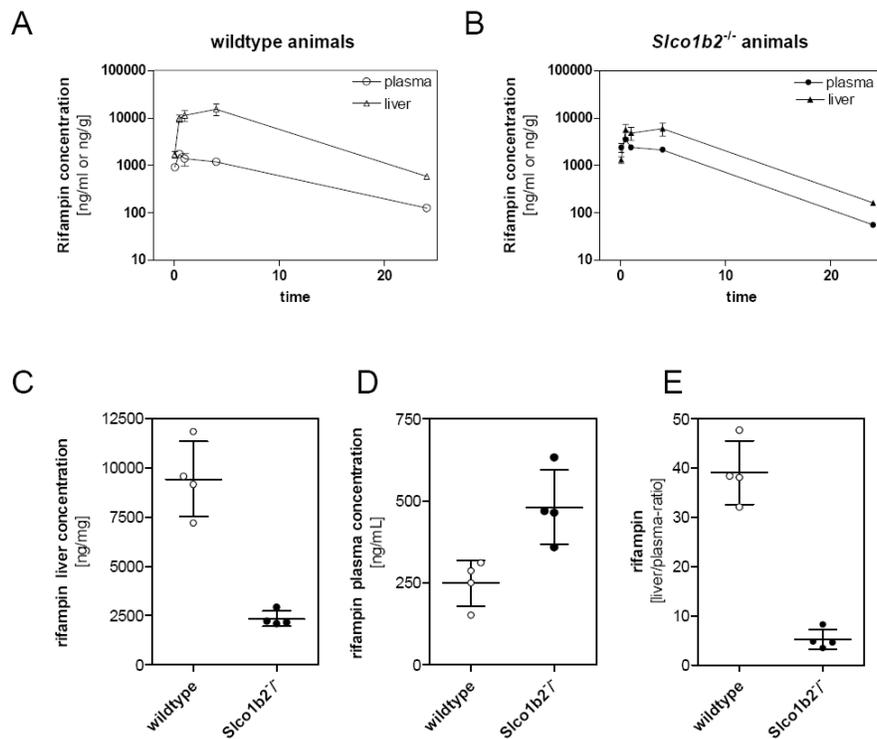


©Richard B. Kim M.D.

Tirona et al, J.Pharmacol.Exp. Ther 304:223-228, 2003

Clinical Pharmacology Transporter Biology 14 January 2010

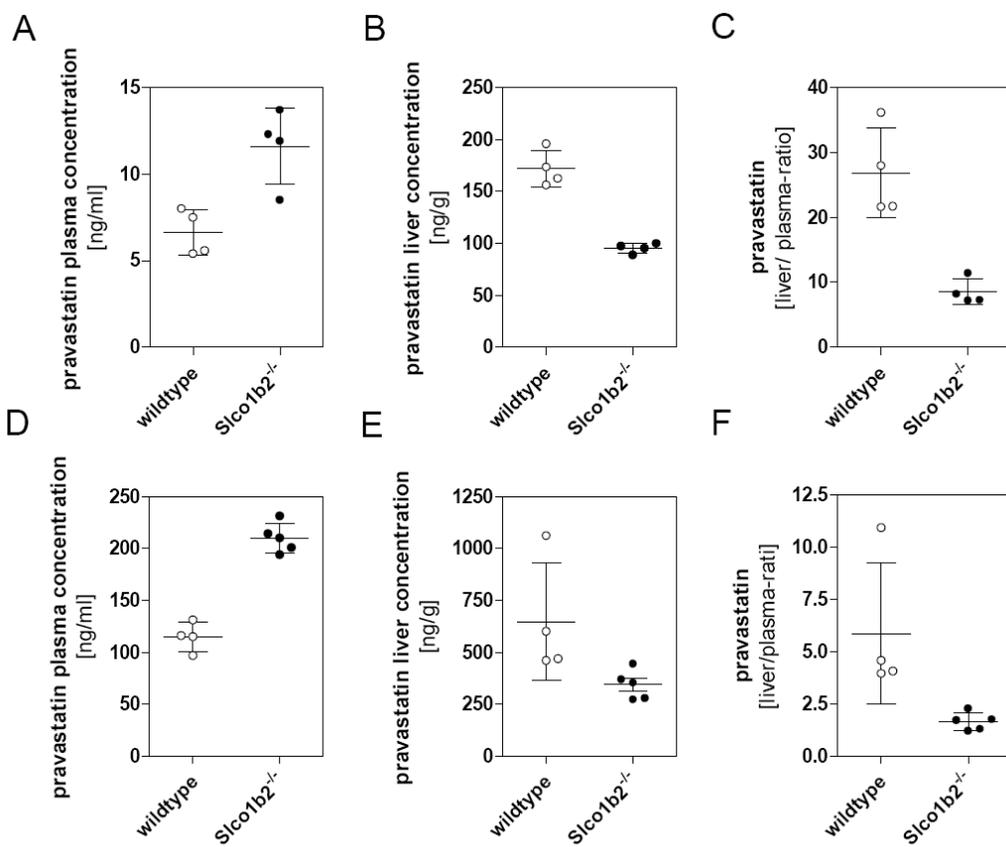
# Rifampacin Disposition in WT vs *Slco1b2*<sup>-/-</sup> KO Mice



Zaher et al., Mol Pharmacol 74: 320-329, 2008

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## Pravastatin Css Dispositon in WT vs Slco1b2<sup>-/-</sup> Mice



Zaher et al., *Mol Pharmacol* 74: 320-329, 2008

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## Ongoing work with *Oatp1b2* KO

- Understand the physiologic role of *Oatp1b2*
- Further characterize translatability of murine *Oatp*'s to human ADME and disease

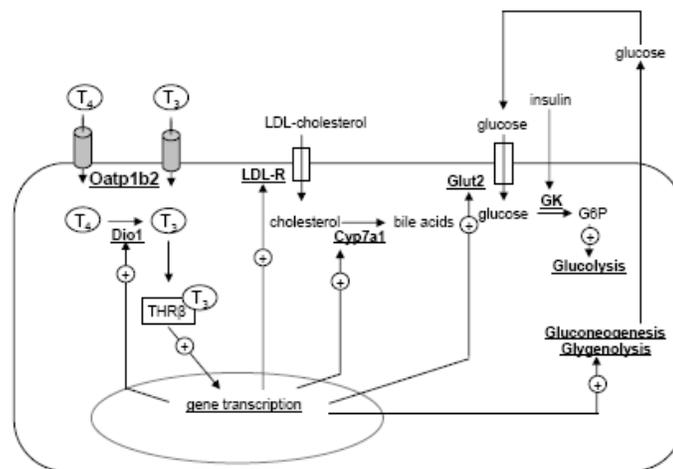


Figure from Henriette E. Meyer zu Schwabedissen

## Future Direction of Drug Transport in Preclinical Development and Clinical Pharmacology

- DDIs mediated through drug transporter(s) have received increased attention, however, at present one can define the likelihood of a DDI for well characterized transporters only qualitatively (Likely, Possible, and Not Likely).
- Significant 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 regulatory awareness. Therefore, the accuracy of the predicted DDI is dependent on the **Quality** of the *in-vitro* assay.
- Greater emphasis on Clinical Translation with respect to PK/PD of select transport probes is needed.
- Preclinical and clinical differences in transporter expression may be a determinant of drug-induced toxicity and a developing area of research for drug-induced diseases.
  - Additional KO and Tg mice to investigate the *in-vivo* contribution of drug transporters are needed.

## **Acknowledgment(s) and Contributors**

- /// Genentech Development Sciences Clinical Pharmacology, ED-PK/PD, SA, and DMPK**
- /// Collaborators: Richard Kim, Yuichi Sugiyama, Tim Tracy, Thomas Litman & Suresh Ambudkar and Hani Zaher**
- /// Timothy Brayman, Bob Conradi, Alla Karnovsky, Anis Khan, Joe Palandra, James P. Sams, and Kathy Sampson. Groton: Scott Campbell, Scott Obach, and Neil Duncan. La Jolla: Caroline Lee, Bill Smith and Eric Reyner. Cambridge: Muhammid Hashim.**
- /// PHA Legacy Collaborators: AZO: Tom Raub, Phil Burton, Larry Schaaf, Mark Grillo, Wade Adams, Jeff Stevens, Jim Bourdage, John Easter, Brad Maxwell, and Greg Winterrowd. Nerviano Medical Sciences (Congregazione dei Figli dell'Immacolata Concezione, CFIC): Pietro Grossi, Mario Monshouwer, Marina Ciomei, Erminia Fontana, Chris James, and Cinzia Pellizzoni**
- /// Legacy Pfizer PDMLT: Suri Surendran, Steven Michael, Simon Ball, Terry Smolarek, Madhu Cherukury, Phil Worboys, Cathy Knupp, Rob**

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THANK YOU !!



# Renally-Mediated DDIs

$$CL_R = GFR + ATS - TR$$

↑Tubular Reabsorption (TR)  
Sys-L et al.,

Active Tubular Secretion (ATS)

Cell lines expressing human transporters  
**What [ ]?**

No / Yes

Flow/pH  
(No vol effect)

Km/Vmax

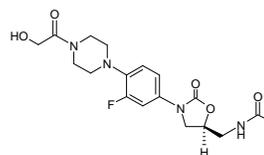
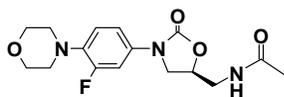
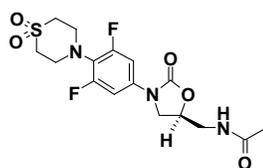
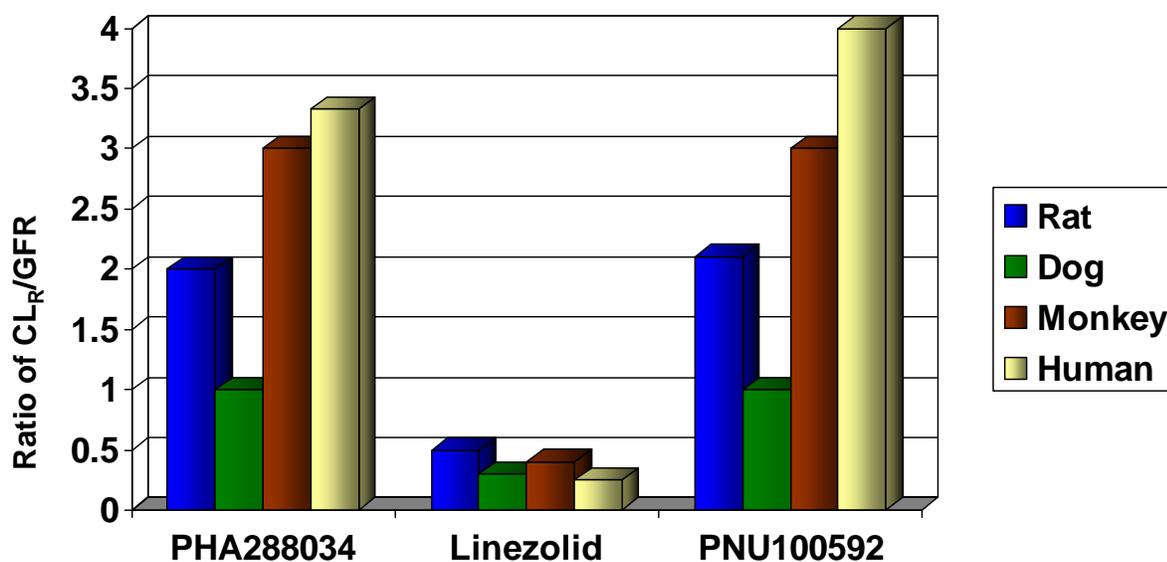
\*in vivo  $CL_R$   
Nonclinical species and/or  
select DDI & model PBPK

Validate Transporter Model & Aid Clin PK  
DDI Design

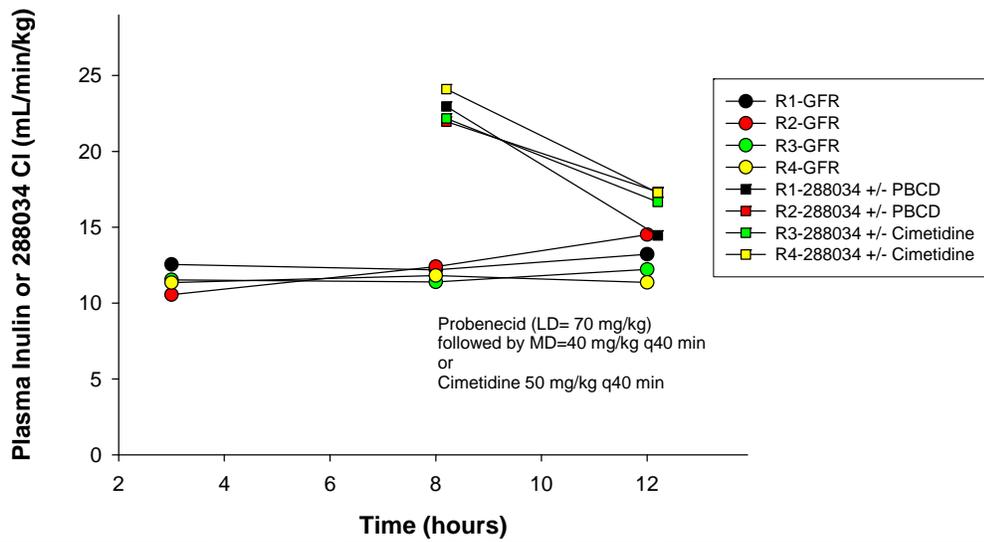
**Very low potential for a clinically significant inhibitor of Sys-L**

\*GFR should be determined in some studies  
†few reported DDIs mediated via TR (Lithium and amiloride)

## Interspecies Comparison of Oxazolidinone $CL_R$ /GFR in Rat, Dog, Monkey & Humans

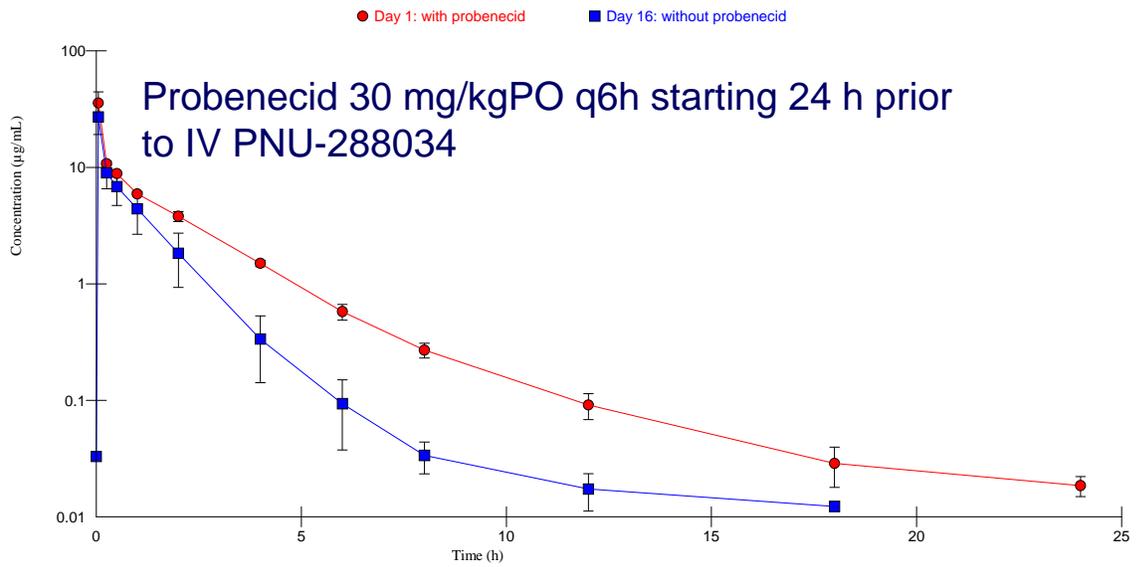


## Inhibition of PHA-288034 Clearance via Probenecid or Cimetidine in the Rat



| PLASMA 288034 or Inulin Clearance +/- PBCD or Cimetidine |            |                         |                 |       |       |  |           |           |
|--|------------|-------------------------|-----------------|-------|-------|--|-----------|-----------|
|  | 288034 CLr | 288034 + PBCD CLr       | GFR-1 (control) | GFR-2 | GFR-3 |  | CLR/GFR-2 | CLR/GFR-3 |
| Rat1   | 22.96      | 14.46                   | 12.55           | 12.19 | 13.22 |  | 1.88      | 1.09      |
| Rat 2  | 21.96      | 17.33                   | 10.55           | 12.40 | 14.51 |  | 1.77      | 1.19      |
|  | 288034 CLr | 288034 + Cimetidine CLr | GFR-1           | GFR-2 |       |  | CLR/GFR-2 | CLR/GFR-3 |
| Rat 3  | 22.18      | 16.67                   | 11.53           | 11.40 | 12.22 |  | 1.95      | 1.36      |
| Rat 4  | 24.11      | 17.28                   | 11.35           | 11.81 | 11.36 |  | 2.04      | 1.52      |

### Monkey PHA288034 Probenecid Interaction Study



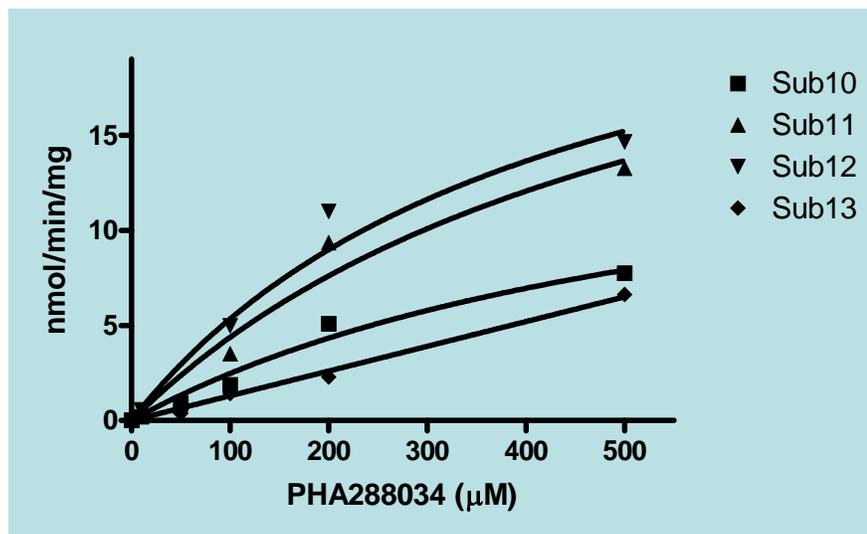
Study by WJ Adams et al.

## **Model Systems to Study Renal Transport**

- Isolated Perfused kidney
- Kidney Slices
- **Isolated Renal Tubules (PCTs)**
- Isolated BBMVs
- **Individual Transporter Clones**
  - **Transient**
  - **Stable**
- GeMMs

## In Vitro Uptake Models

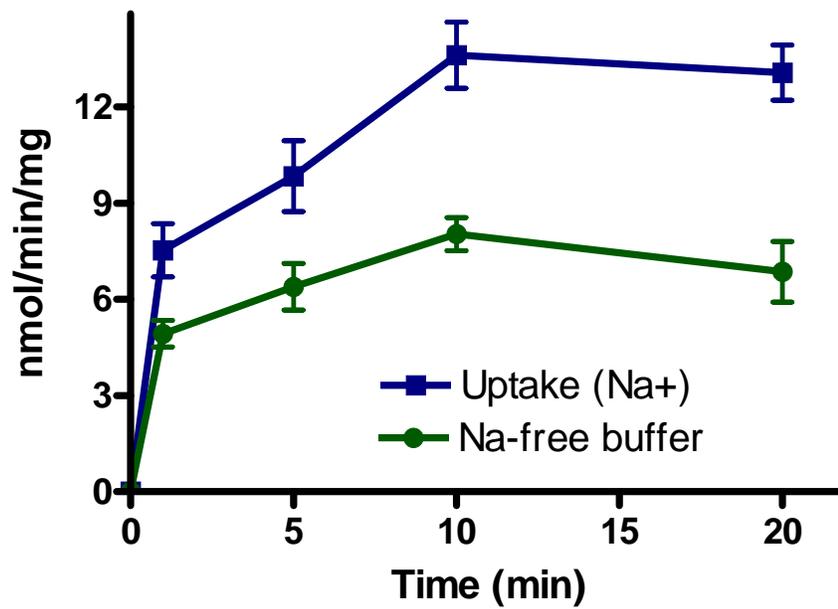
- Transport of PHA-288034 in human proximal tubules.
  - Drug uptake in cell suspension of hPTs.
  - Determine kinetics, substrate specificity, energy & ion dependence
  - Preliminary study suggested no metabolism in hPTs



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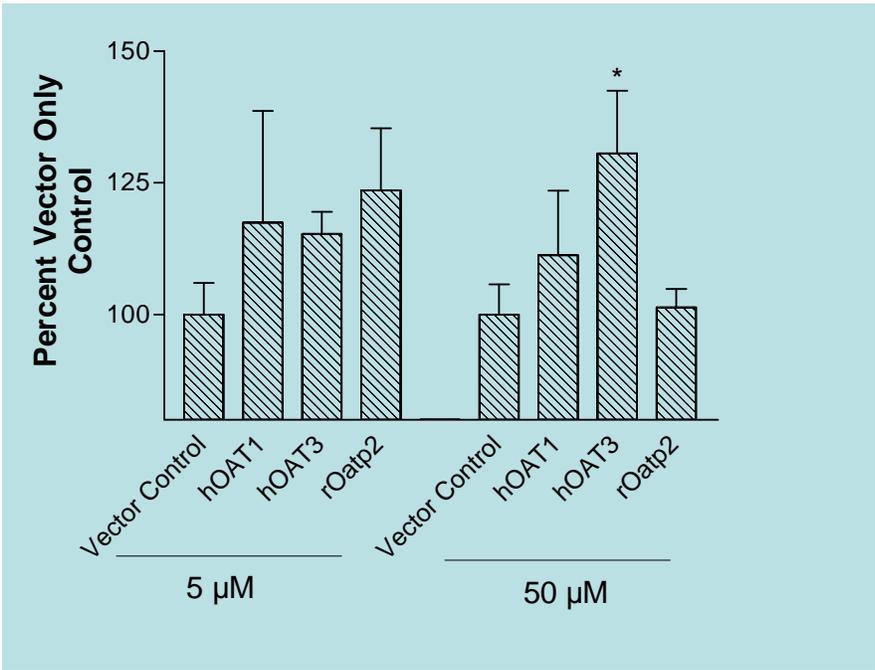
# Na<sup>+</sup>-dependent Uptake of PHA288034

Human Proximal Tubule Studies



Biology 14, January 2010

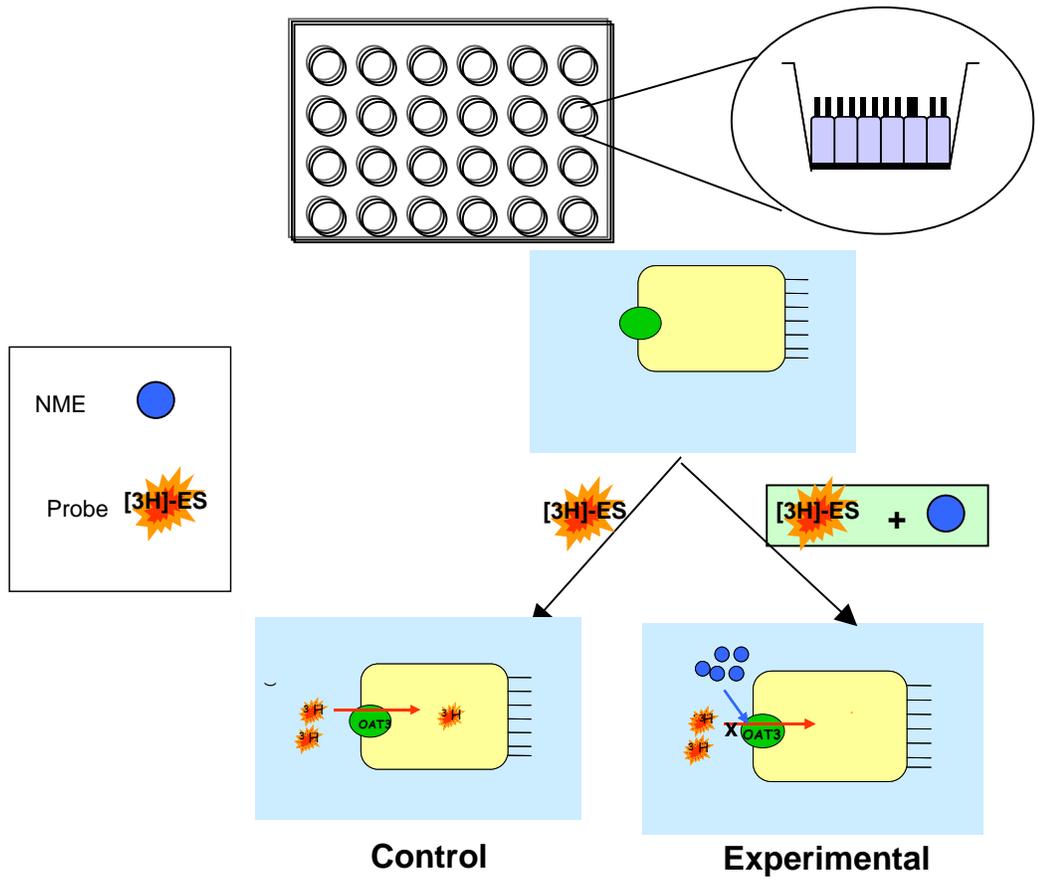
### PHA-288034 Uptake in HeLa cells Transfected with Transporter cDNAs



Richard Kim and Brenda Leake

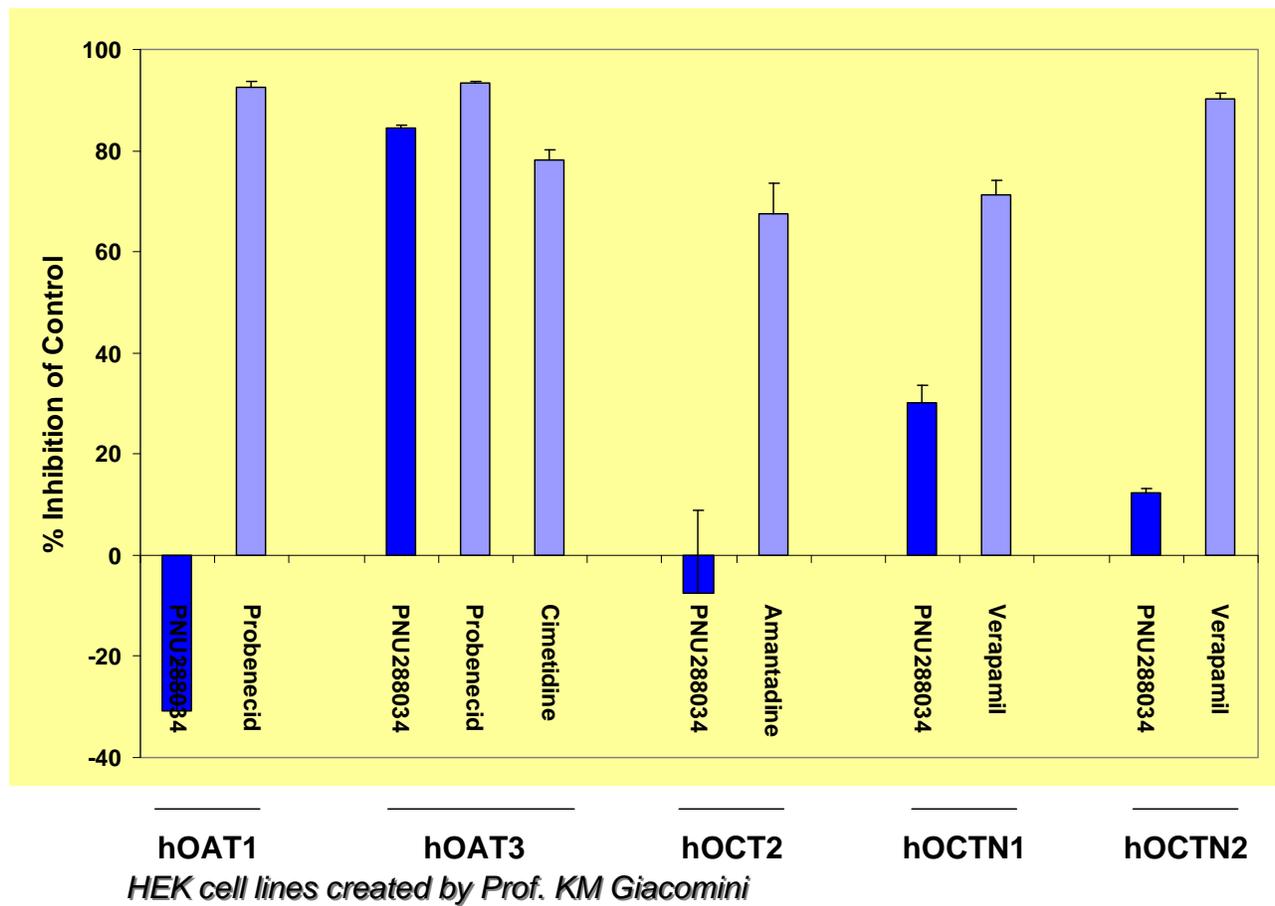
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# Experimental Protocol: Interaction Assay in Stable Transfectants



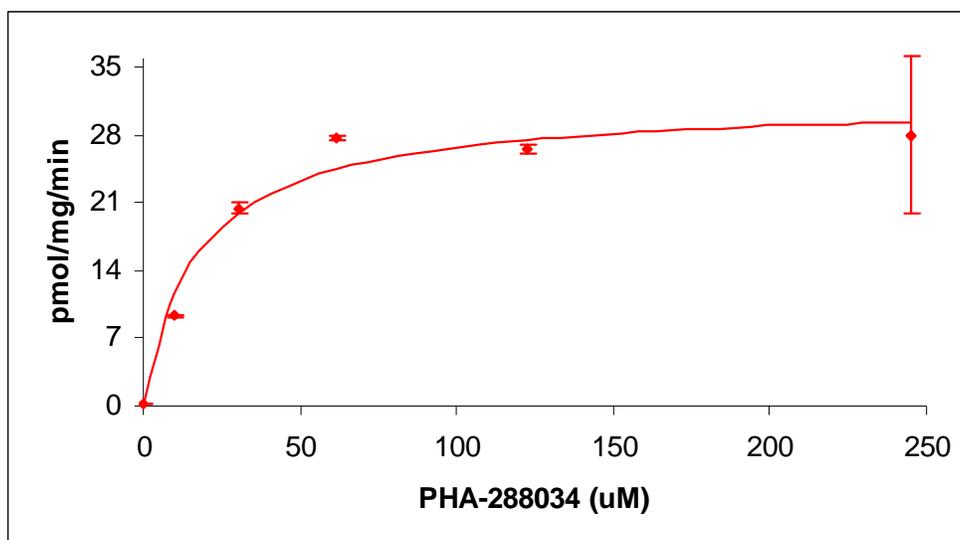
Result/calculations = Inhibition of [3H]-ES uptake (% of control) in presence of NME

PHA-288034 Interaction with hOAT1-HEK, hOAT3-HEK, hOCT2-HEK, hOCTN1-HEK and hOCTN2-HEK Cells.



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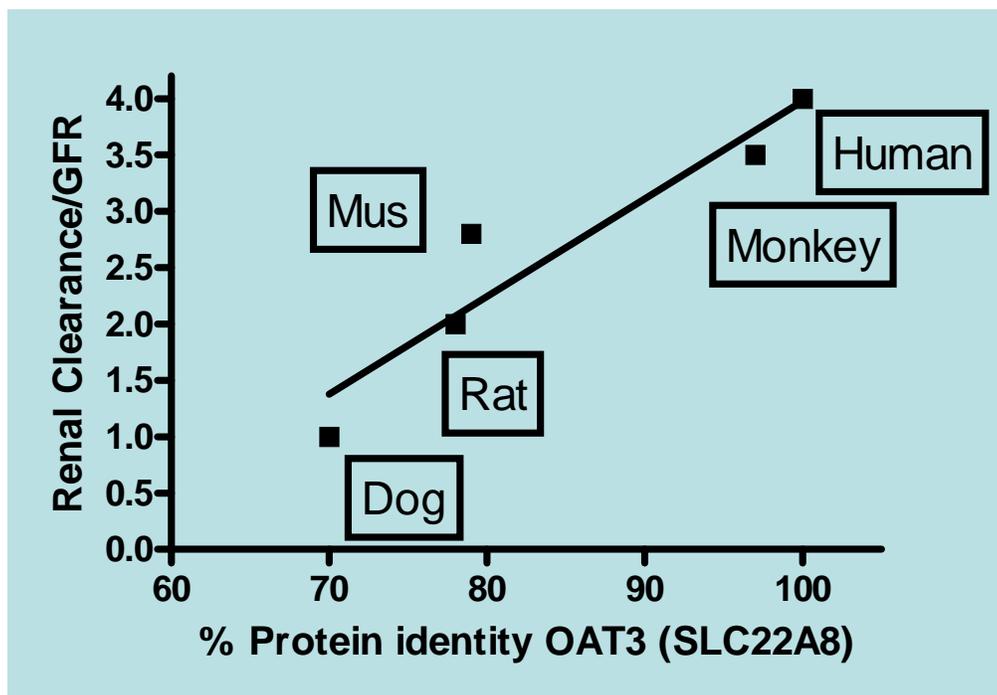
## PHA-288034 uptake in hOAT3 cells



Michaelis-Menten Model fitted to individual responses using OLS

|                    | Estimate | Standard Error | 95% Confidence Interval |
|--------------------|----------|----------------|-------------------------|
| Km (uM)            | 18       | 6.9            | 7 42                    |
| Vmax (pmol/mg/min) | 31.5     | 2.90           | 25.1 38.0               |

## Cross-species Homology of OAT3 (SLC22A8) vs PHA288034 CL<sub>R</sub>



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## Summary of PHA288034 Studies

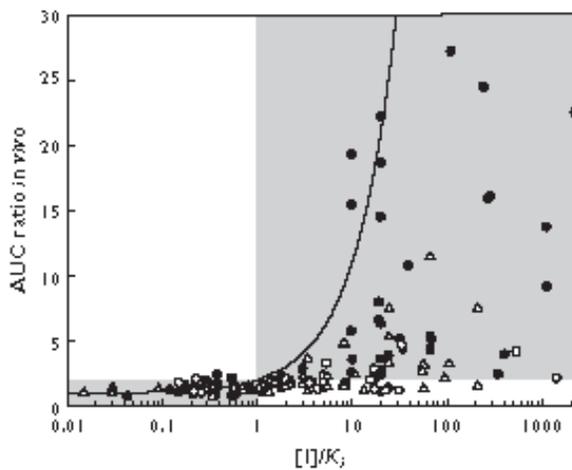
Multi-tier approach appears to be best way to identify substrates/inhibitors of uptake/efflux drug transporters.

### **Active Tubular Secretion**

- PHA-288034 appears to be a substrate and an inhibitor of hOAT3 (SLC22A8).
- PHA-288034 does not appear to be a substrate for hOAT1, OCT2, OCTN1, or OCTN2.
- Additional work is needed to fully appreciate OAT3 cross-species differences.
- Cimetidine inhibits OAT3-mediated transport as well as OCT-2 mediated transport.

## Drug Interactions: CYP Mediated

- Significant CYP mediated drug interactions based on AUC ratio



N= 115 Studies  
CYP2C9, 2D6, 3A4

← AUC<sub>i</sub>/AUC related to P-gp DDI

Brown et al., Br J Clin Pharmacol 60:508 (2005)

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## CYP Summary

- CYP interactions were complex when first recognized
- Largest CYP-mediated DDIs
  - Increase AUC 20X,  $C_{\max}$  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**