# An Overview of Drug Transporters in ADME, Safety, and Efficacy

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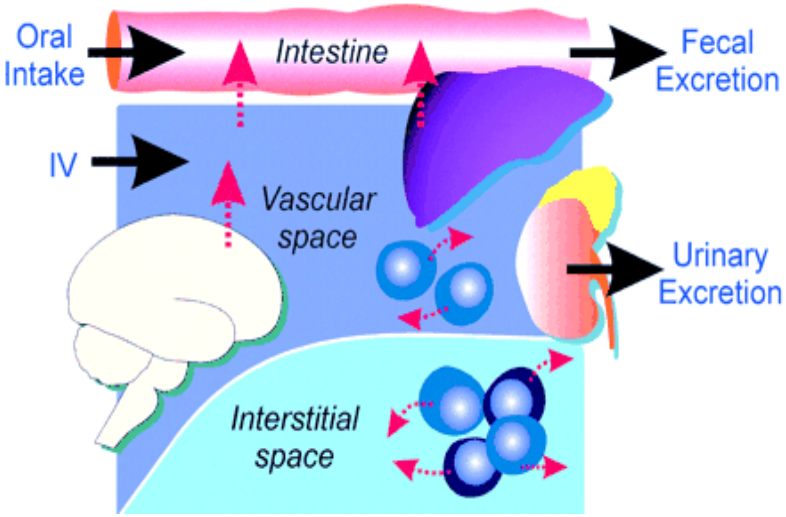
Principles of Clinical Pharmacology
Joseph A. Ware, Ph.D.
Sr. Scientist, Clinical PK/PD
Genentech, Inc.

# Presentation Outline

- Provide examples of when transport is the rate-limiting step in ADME
  - Absorption
  - Distribution
  - Metabolism and Transporter Interplay
  - Elimination (kidney and liver)
- Transporter biology investigations using preclinical models and GeMMs
- Variability in drug transport function
- Examples of when drug transport is a primary determinant of drug-induced toxicity.

# 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 pro
- 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 (Lilly)
- Xenoport.com 'transport by design'



# The rate determining process

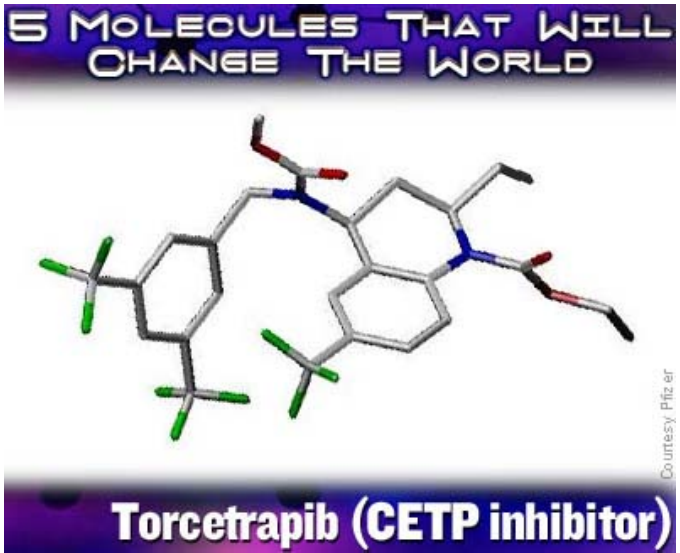
"To understand the transporter-mediated drug-drug interaction, we have to know the rate determining process of a substrate in the overall clearance."

uptake, basolateral efflux, apical excretion, metabolism

**Professor Sugiyama, Keynote address AAPS, November 2007**

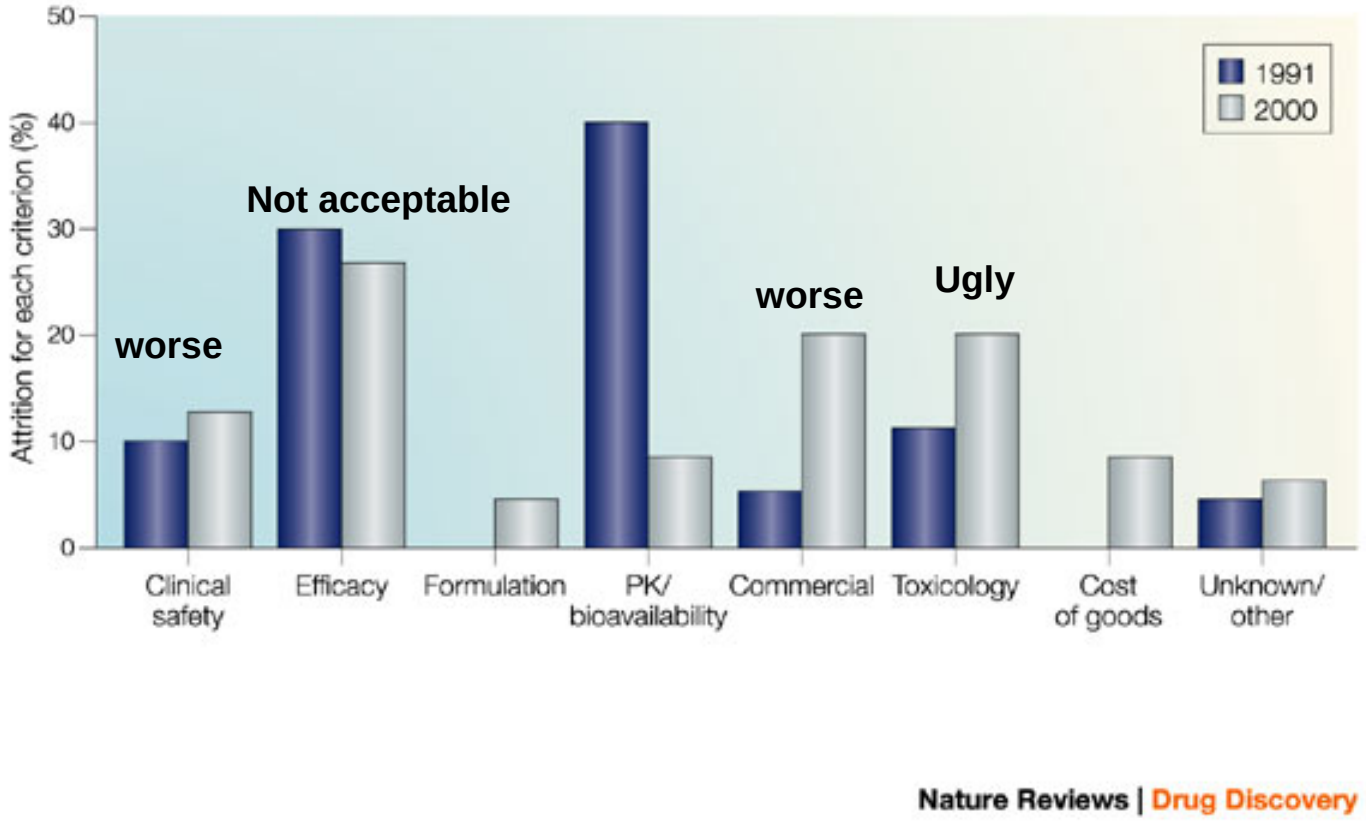
# Cost of Drug Attrition
# (somewhat recent example)

- Torcetrapib:  Phase III-nearly 1-billion dollars spent on development.
- Safety
- Impact
  - Immediate
  - R&D




# Reasons for Drug Attrition

(1991-2000)




*I. Kola and JB Landis, Nature Reviews Drug Discovery* **3**, 711 -716 (2004)

| | *CYPs* |
|---|---|
| *Importance in Drug Disposition* | *High* |
| *Substrate Specificity and Overlap* | *Very Good* |
| *Enzyme Kinetics: Specific In Vitro Probes* | *Very Good* |
| *Selective Clinical Probes* | *Good* |
| *Species Differences and Similarities* | *Good* |
| *Organ/Cellular Localization and regulation* | *Good* |
| *Relative Abundance* | *Very Good* |
| *Clearance and DDI Predictions* | *Very Good* |
| *Genetic Variability* | *Good* |
| *Functional Polymorphisms* | *Good* |

**Speed/Quality impact**

**Discovery predictions**

**to early Clinical Development**

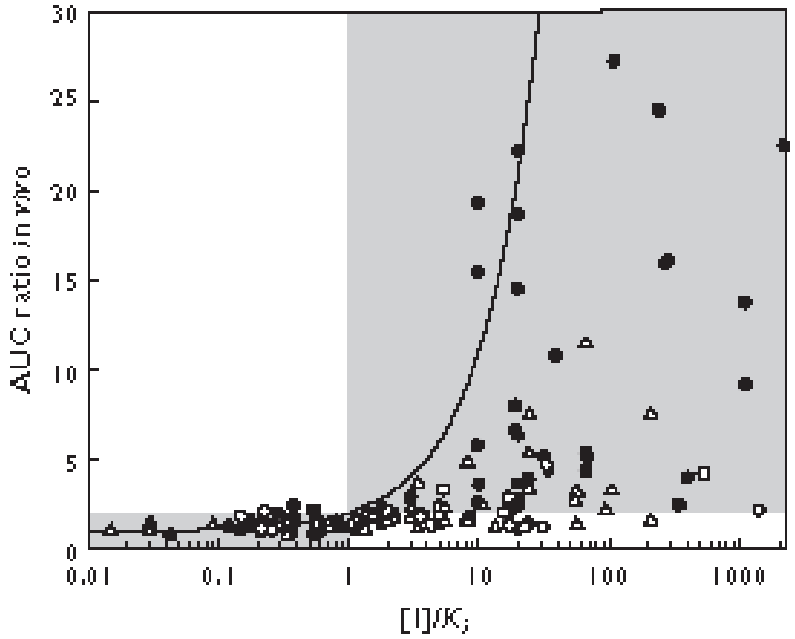
**Program**

| | *phase II enzymes* |
|---|---|
| *Importance in Drug Disposition* | *Moderate* |
| *Substrate Specificity and Overlap* | *Moderate-Good* |
| *Enzyme Kinetics: Specific In Vitro Probes* | *Good* |
| *Selective Clinical Probes* | *Moderate* |
| *Species Differences and Similarities* | *Poor* |
| *Organ/Cellular Localization and regulation* | *Moderate* |
| *Relative Abundance* | *Poor* |
| *Clearance and DDI Predictions* | *Poor* |
| *Genetic Variability* | *Moderate* |
| *Functional Polymorphisms* | *Moderate* |

| | *Transporters* |
|---|---|
| *Importance in Drug Disposition* | *Moderate?* |
| *Substrate Specificity and Overlap* | *Poor* |
| *Enzyme Kinetics: Specific In Vitro Probes* | *Moderate* |
| *Selective Clinical Probes* | *Poor-Moderate* |
| *Species Differences and Similarities* | *Poor* |
| *Organ/Cellular Localization and regulation* | *Moderate* |
| *Relative Abundance* | *Poor* |
| *Clearance and DDI Predictions* | *Poor* |
| *Genetic Variability* | *Poor* |
| *Functional Polymorphisms* | *Poor* |

# Drug Interactions: CYP Mediated

- Significant CYP mediated drug interactions based on AUC ratio




N= 115 Studies
CYP2C9, 2D6, 3A4

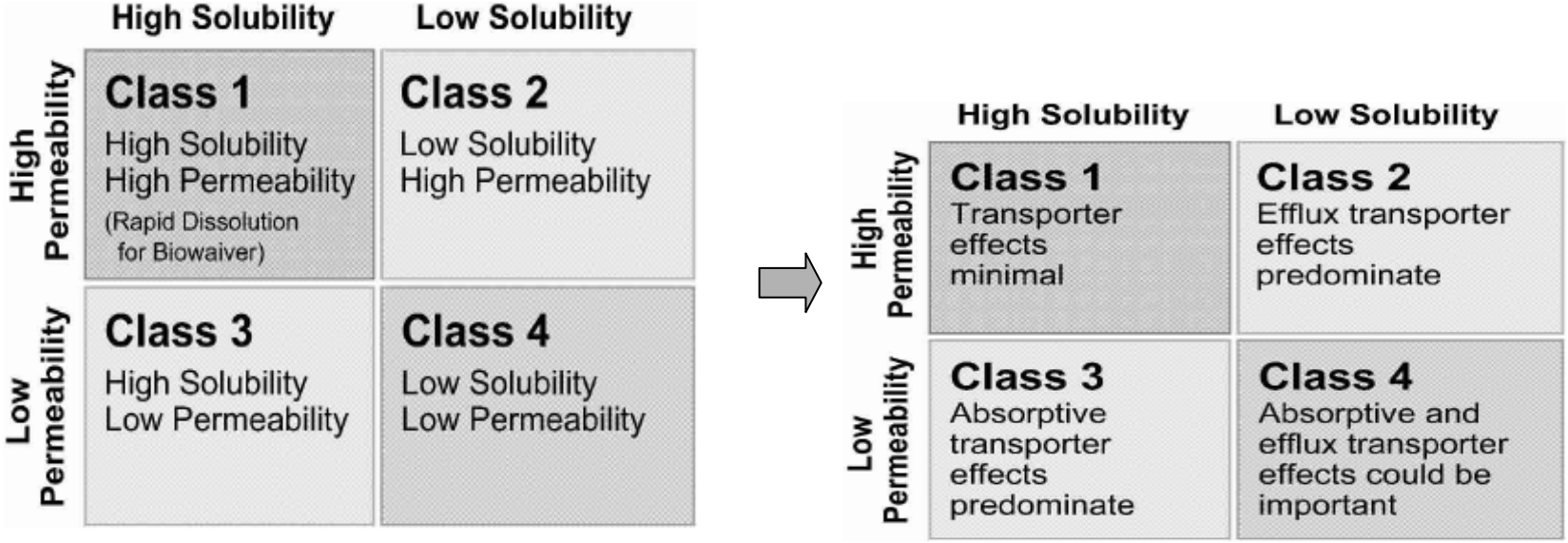
← AUCi/AUC related to P-gp DDI

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

# 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?

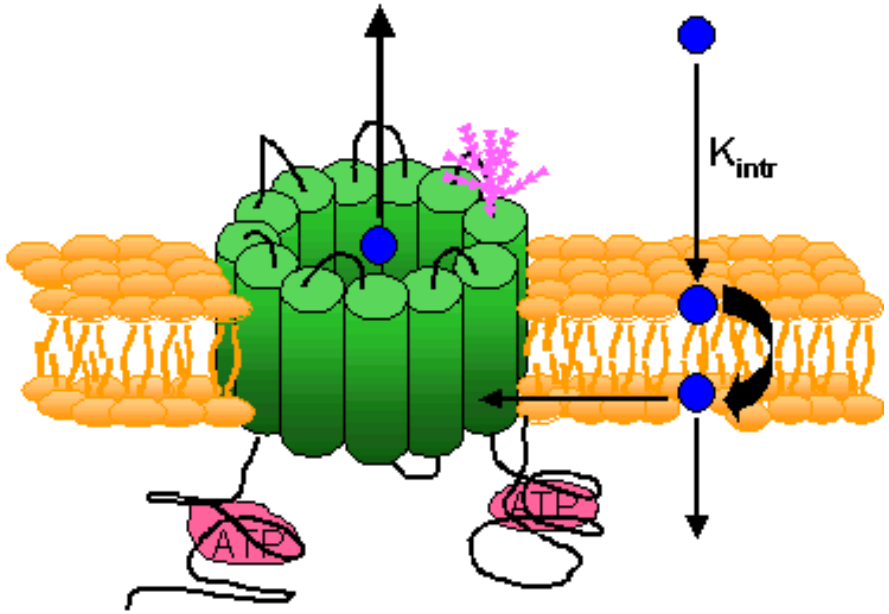
# *Permeability is an important determinant of In vitro-in vivo* extrapolation for both Metabolism and Transport

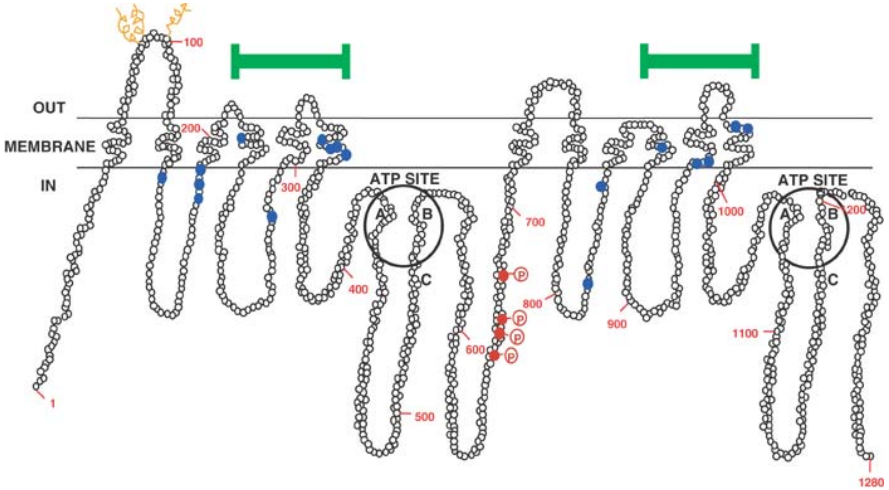



***Amidon et al., Pharm. Res. 12:413 (1995)***

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

# P-glycoprotein Structure & Function: ATP Binding and Hydrolysis are Coupled to Drug Transport




*Oncogene* (2003) **22,** 7468-7485.

- P-gp is distributed in the following organs: Intestine, kidney, liver, brain, adrenal gland, lymphocytes, and placenta
- Hypothetical MOA
  - "vacuum cleaner"
  - Membrane partitioning
- Walker A and Walker B binding motif
- Drug-stimulatable and inhibitable
- High basal activity present in P-gp ATPase assay.

# Role of mdr1a in the Blood-Brain Barrier and the Placenta

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

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

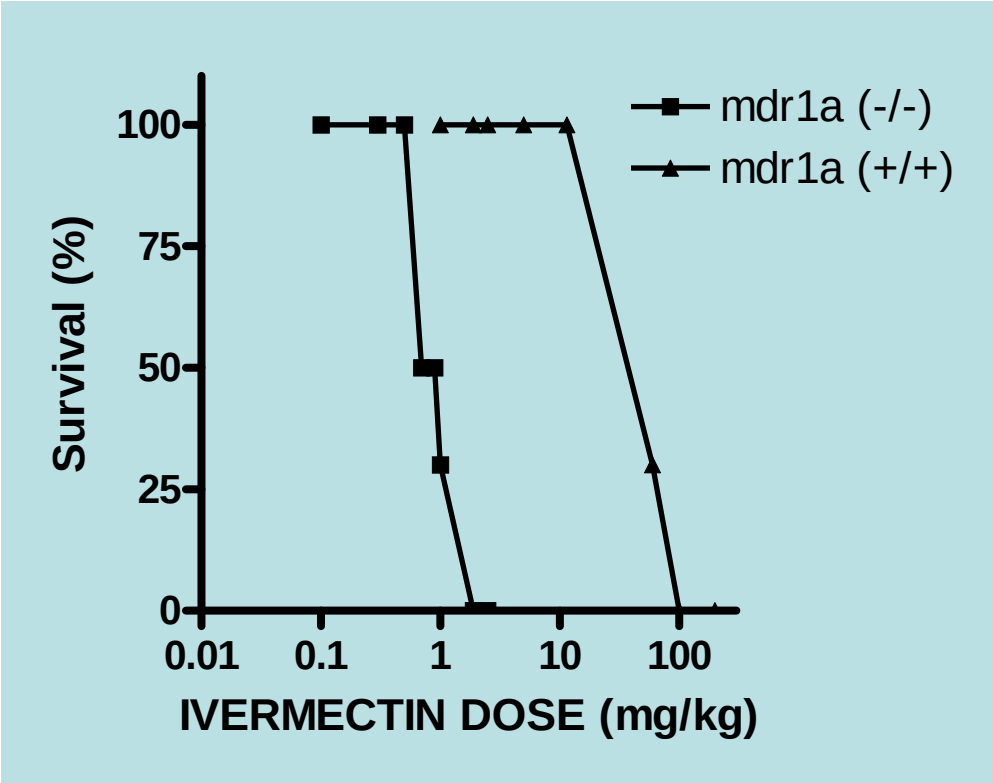



**Figure from A.H. Schinkel et al., Cell, Vol.77, 491-501, 1994**

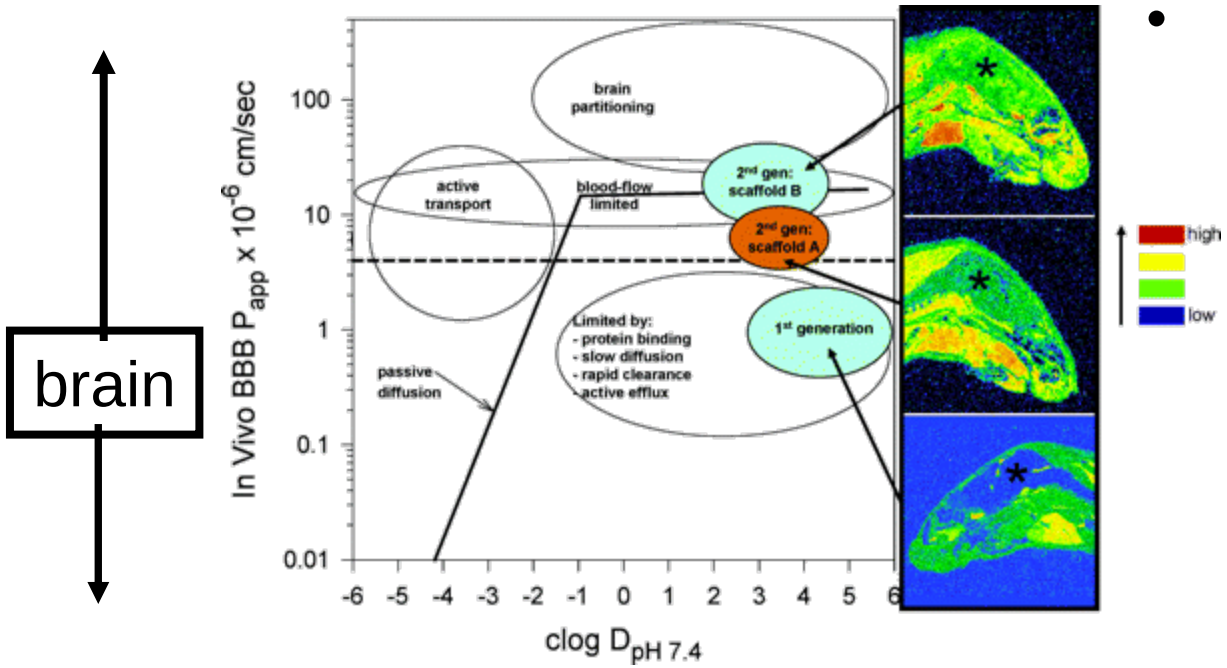
# Ivermectin Toxicity in the Collie

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

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

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

# P-gp at the Blood-Brain Barrier

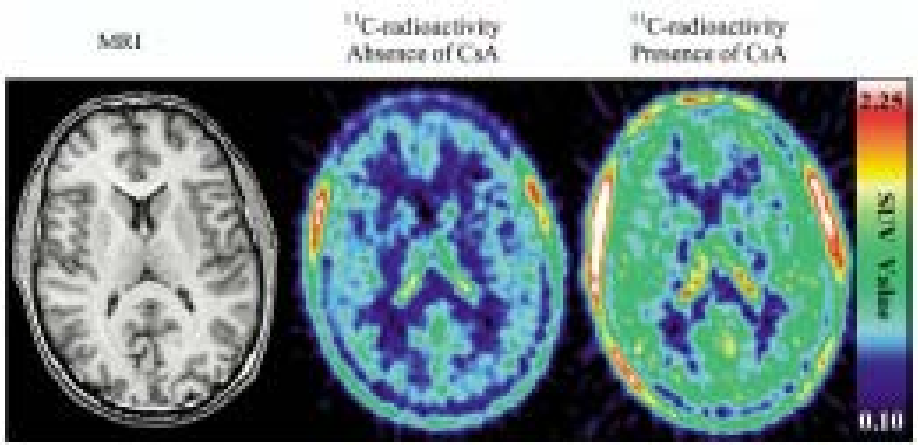


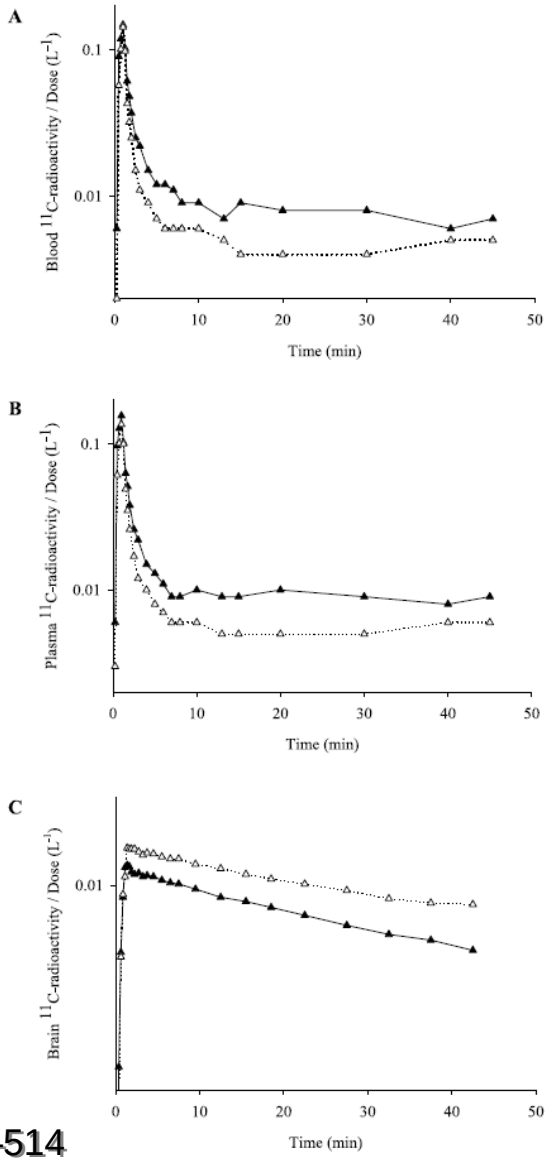

- Many Examples of Drugs whereby BBB Entry is Not Desirable
  - Ivermectin
  - Digoxin
  - Non-sedating antihistamines
    - Fexofenadine
    - Loratadine
    - Cetirizine

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

# Clinical Translation of P-gp Inhibition at the BBB

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



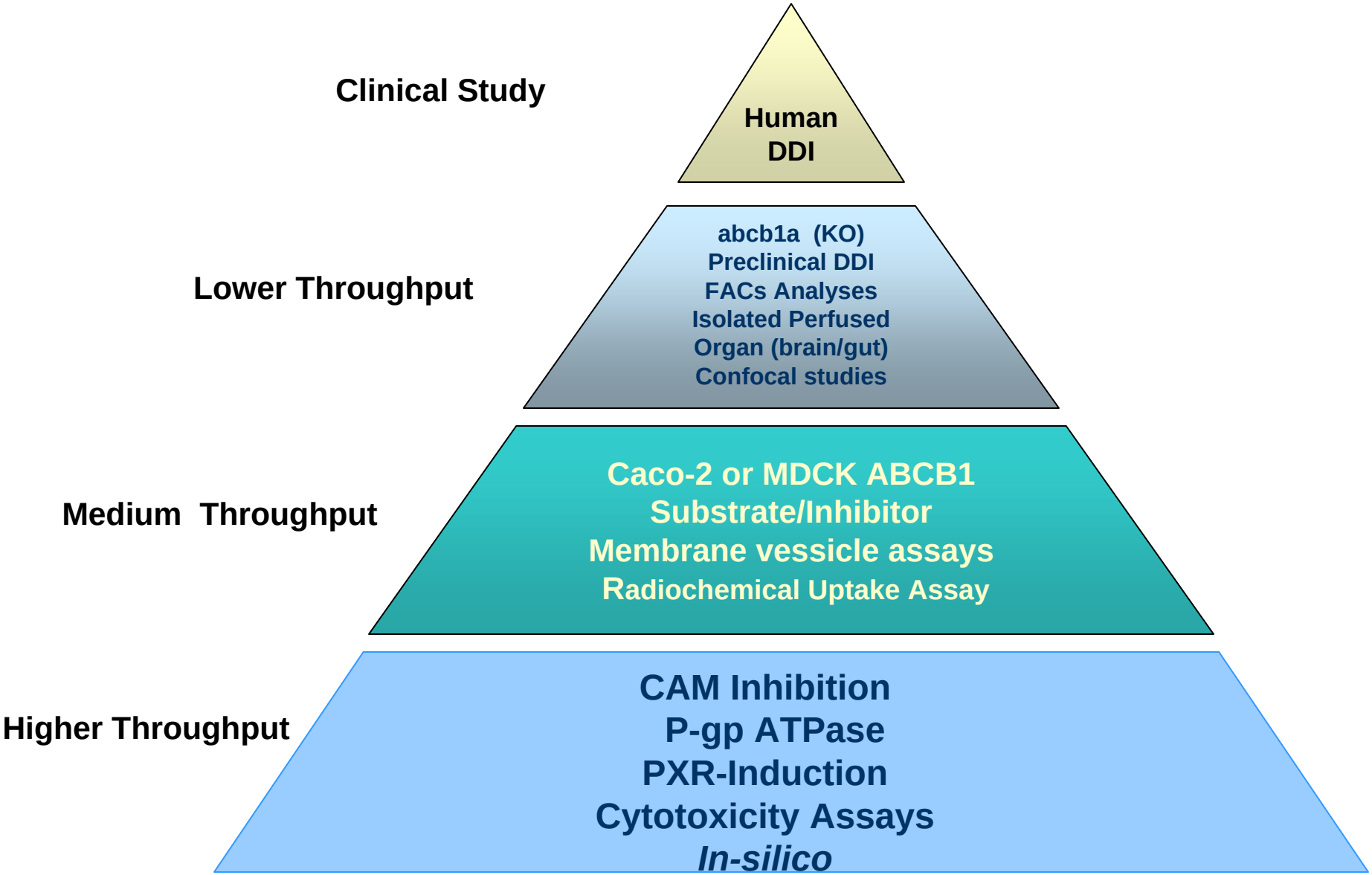




Clinical Pharmacology & Therapeutics (2005) 77, 503–514

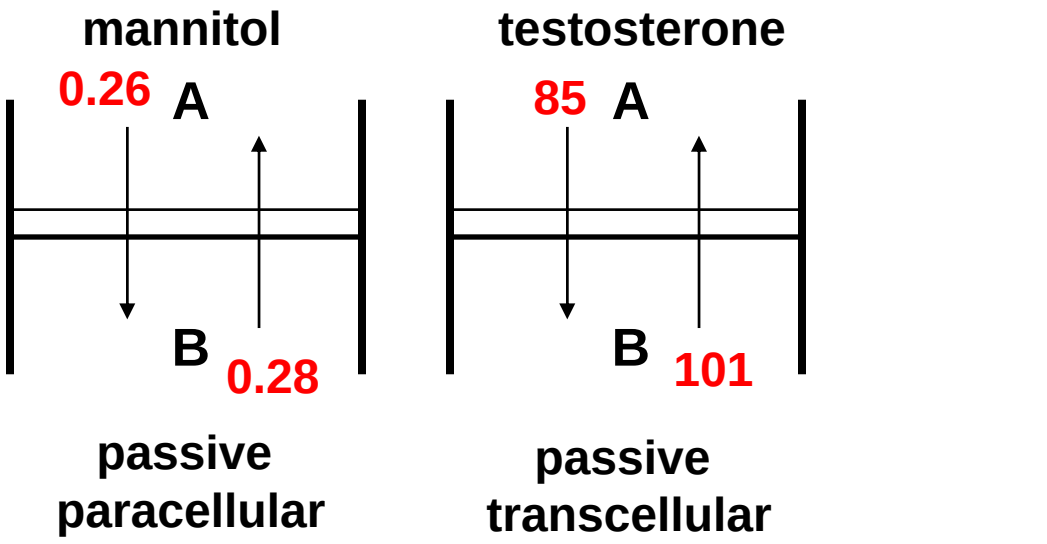
# P-glycoprotein Substrates

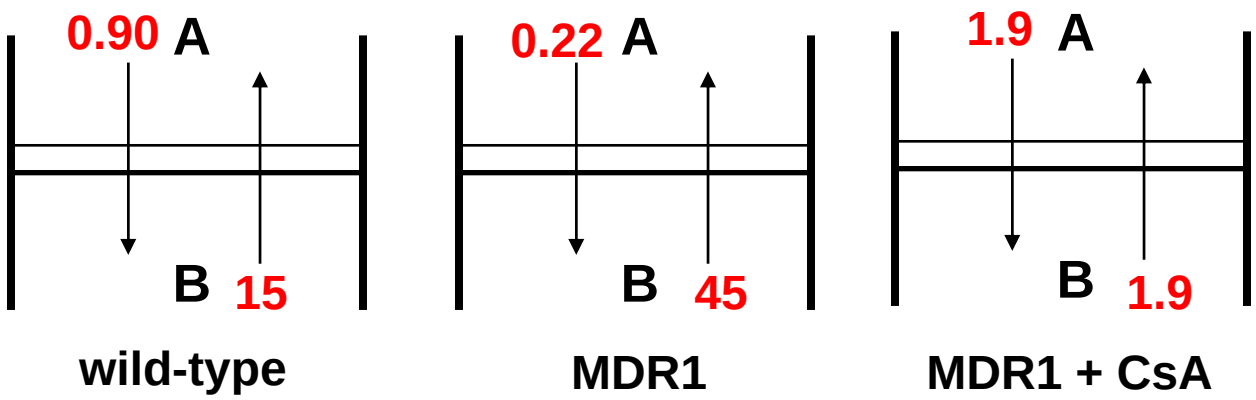
- Cancer Chemotherapy
  - Doxorubicin
  - Daunorubicin
  - Vinblastine
  - Vincristine
  - Paclitaxel
  - Teniposide
  - Etoposide
- Immunosuppressive Drugs
  - Cyclosporine A
  - FK506
- Antihistamine
  - Terfenadine
- Steroid-like
  - Aldosterone
  - Hydrocortisone et al.
- HIV Protease Inhibitors
  - Amprenavir
  - Indinavir
  - Ritonavir
  - Saquinavir
- Cardiac Drugs
  - Digoxin
  - Quinidine
  - Posicor
  - Most statins
- Anti-thelmintics
  - Ivermectin
  - Abamectin
- Miscellaneous
  - Loperamide
  - Colchicine
  - Ondansetron
  - Erythromycin

# P-glycoprotein (ABCB1) Cluster Evaluation



# In Vitro Permeabilities



# Caco-2 and MDCK cell comparison

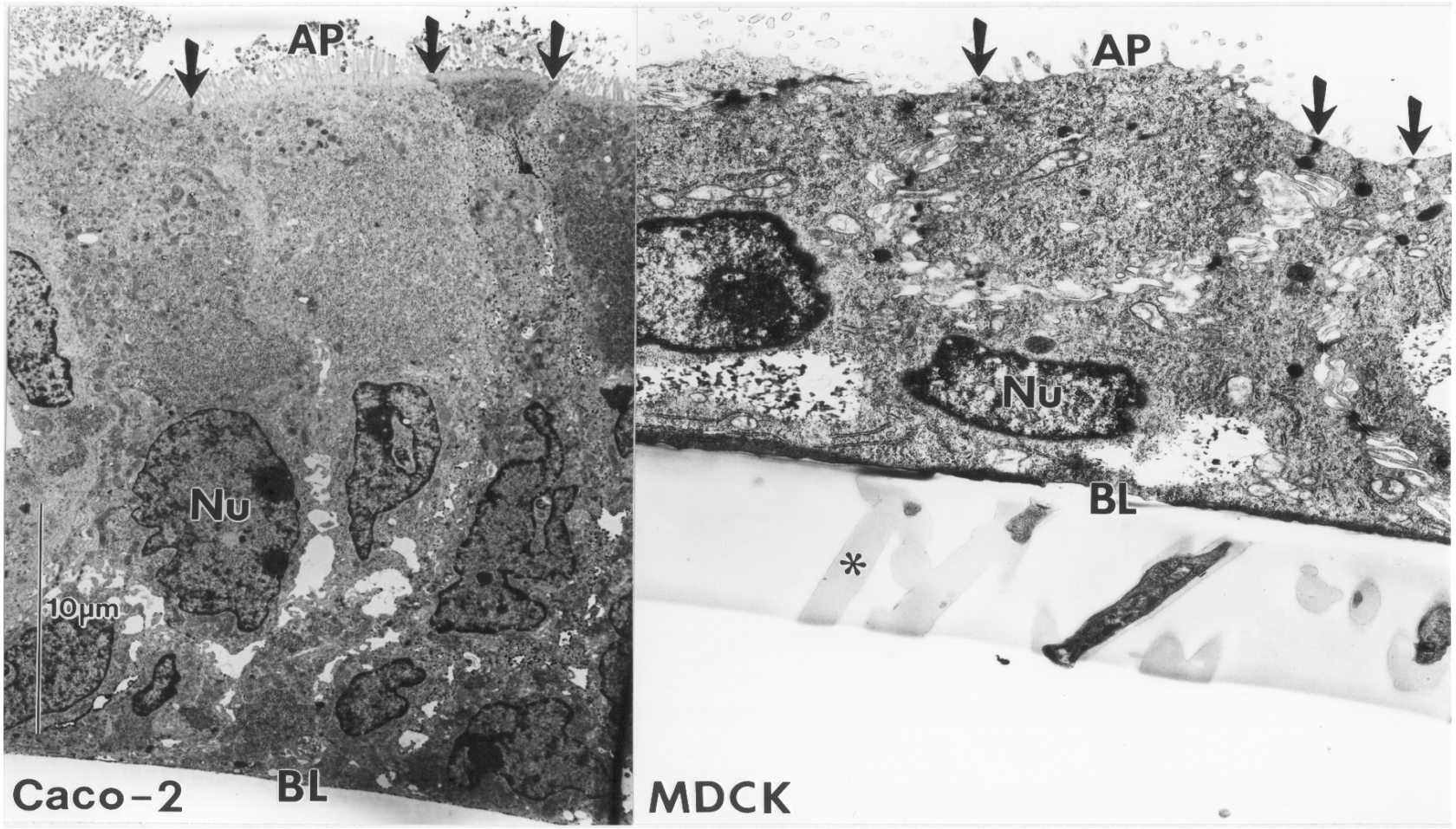
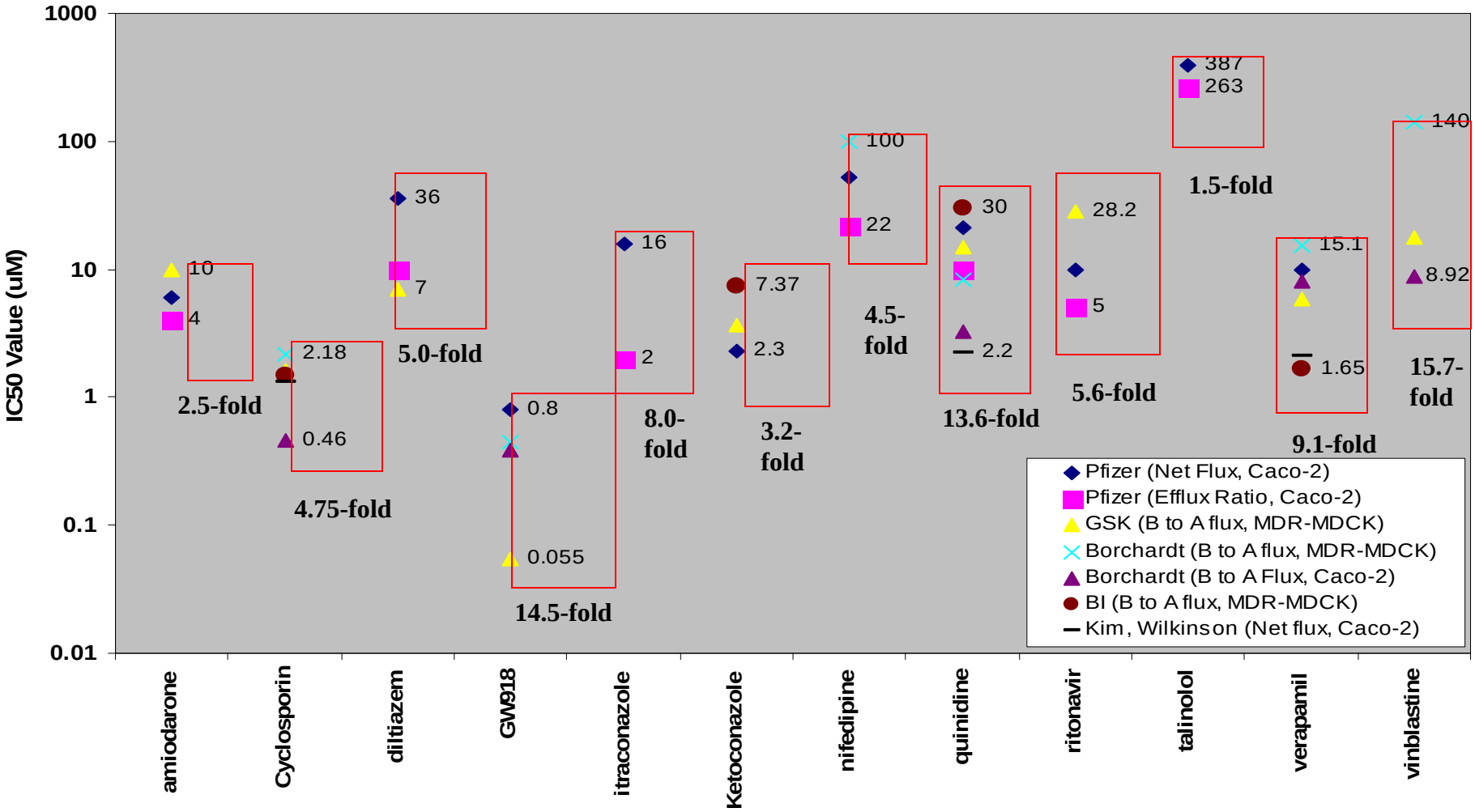



Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub

# In Vitro P-gp $IC_{50}$ for Inhibition of Digoxin Efflux Data from Multiple Labs / Techniques

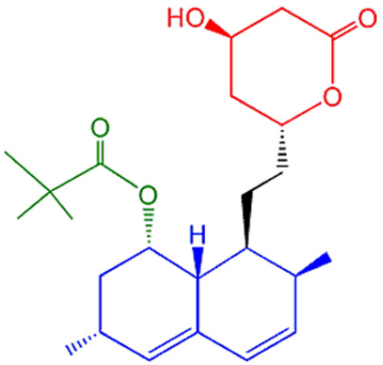



Slide courtesy of M. Troutman/C. Lee Pfizer

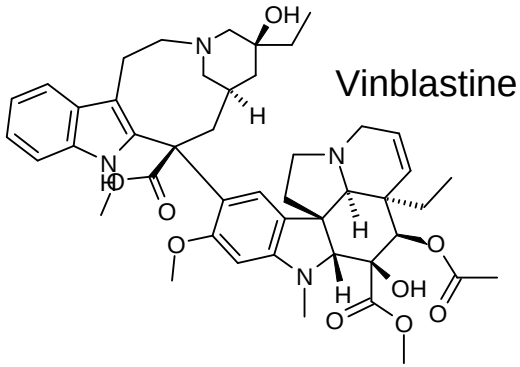
# Chemical Features of P-gp Substrates

## General Attributes:

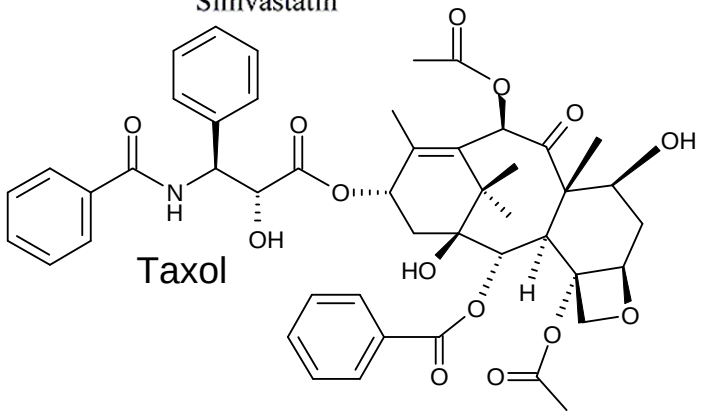
- lipophilic
- large MW (volume)
- amphiphilic
- cationic at pH 7.4
- cyclic
- electron donating groups
  - nitrogen, H-bonding



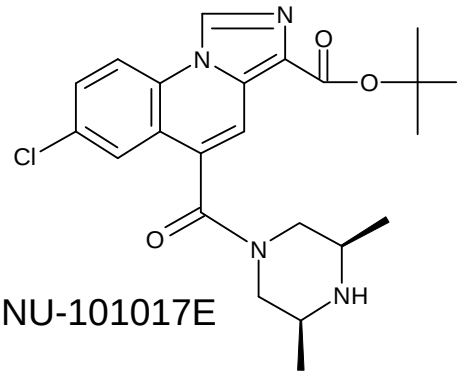
Simvastatin



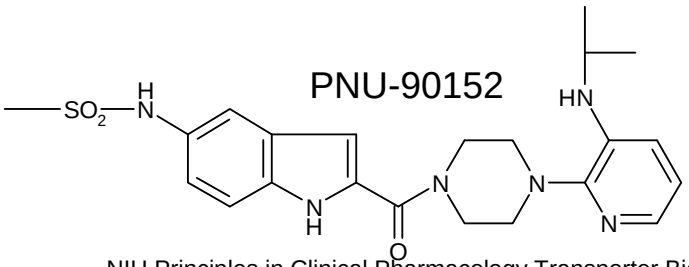
Vinblastine



Taxol

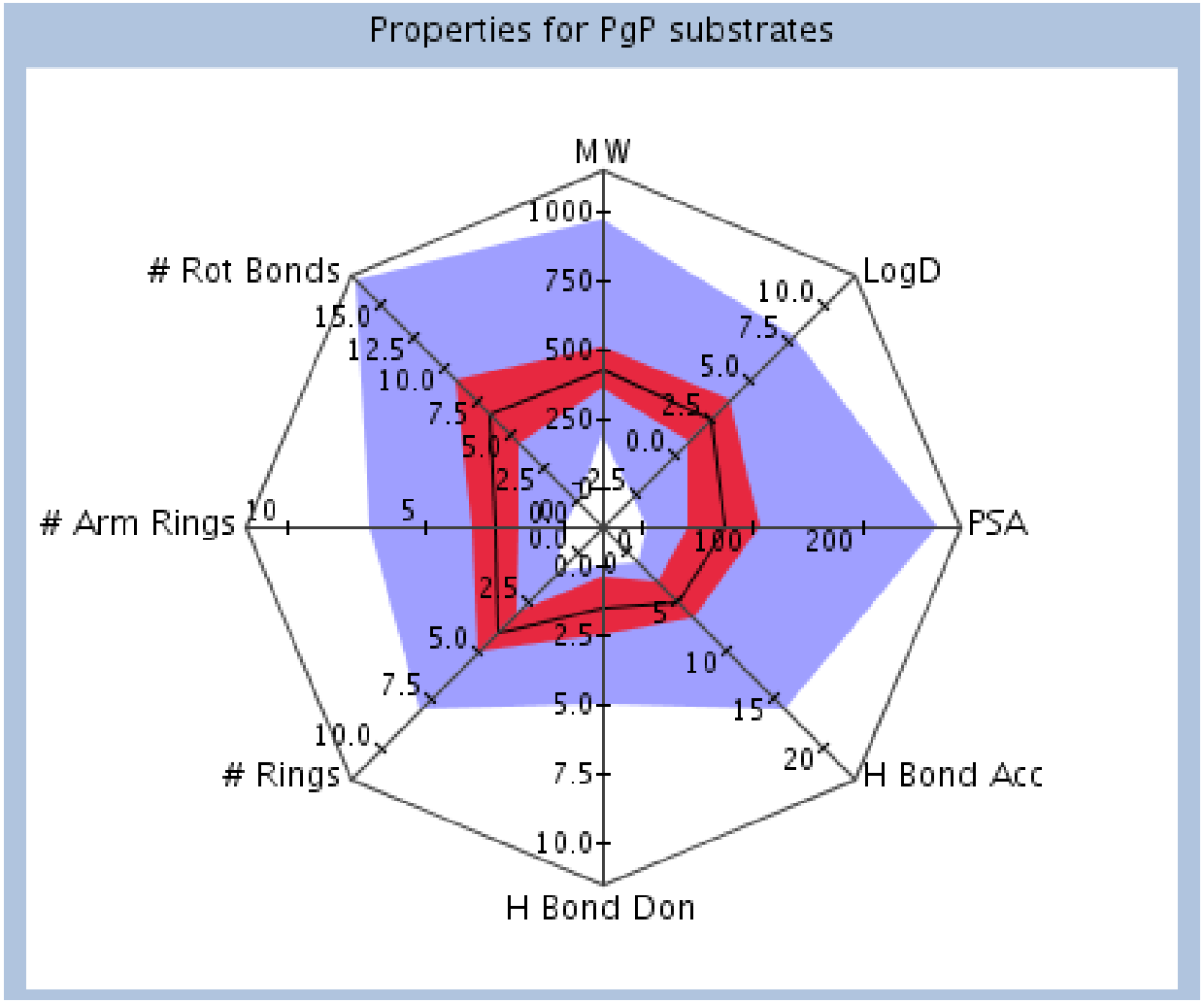


PNU-101017E



PNU-90152

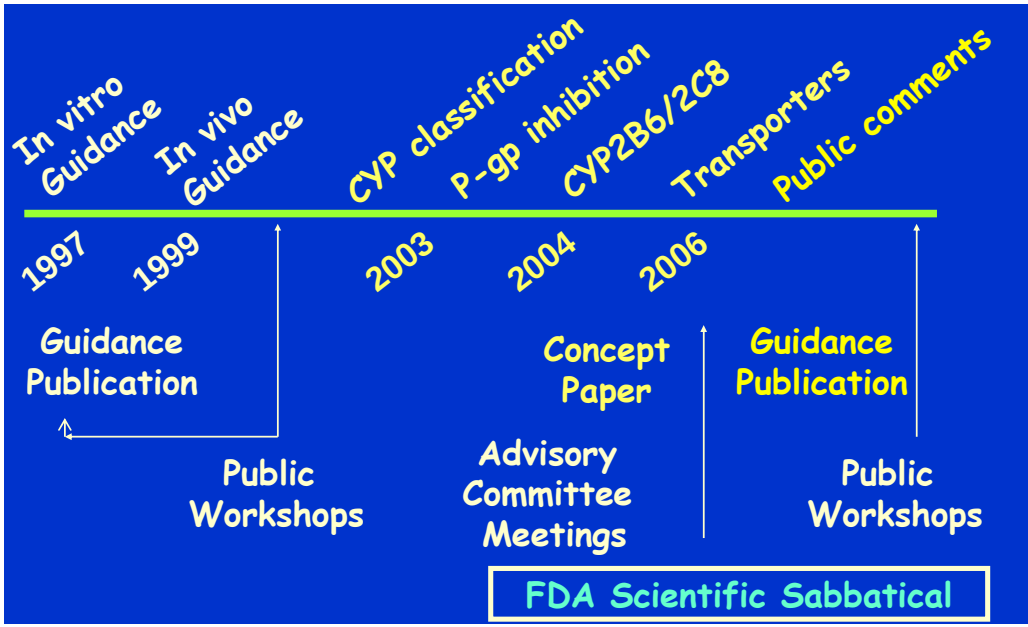
# Chemical Features of P-gp Substrates




N = 8463 (MDR1: $P_{app}$ BA/ $P_{app}$ AB >= 2.5)

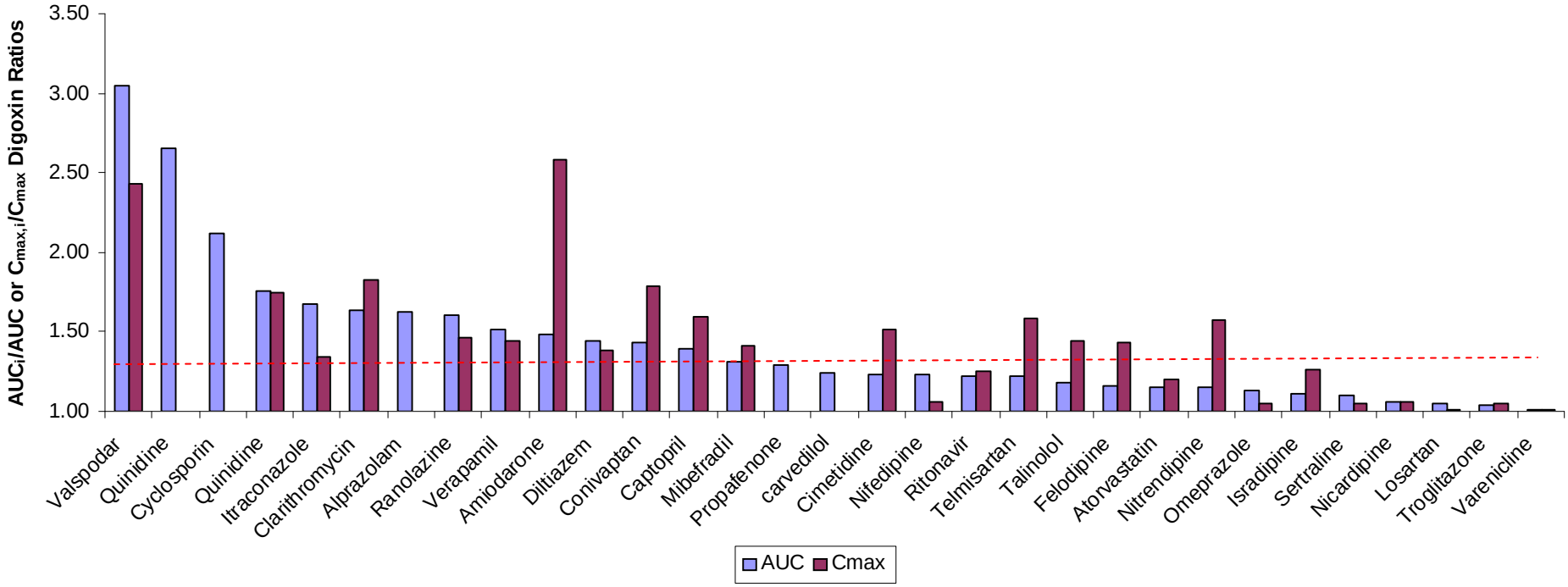
# Evolution of 2006 Draft Guidance

- 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 (in vitro and in vivo) may reduce number of unnecessary studies and optimize clinical pharmacology studies.
- Classification of CYP inhibitors and substrates can aid in study design and labeling.
  - Substrate (25% metabolism)
  - Inhibitor ([I]/Ki > 0.1)
  - Inducer (40% control)




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

# Digoxin: Safety Concerns




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

# P-gp Mediated Digoxin DDIs

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

# 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.

- 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 an area of debate (JPharmacol Toxicol Methods. 2006 Mar 15 and Feng et al., DMD 2008)

- 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 in a case by case basis.

# ABC Substrate/Inhibitor Overlap

*Distinct but Overlapping Substrate Specificities*

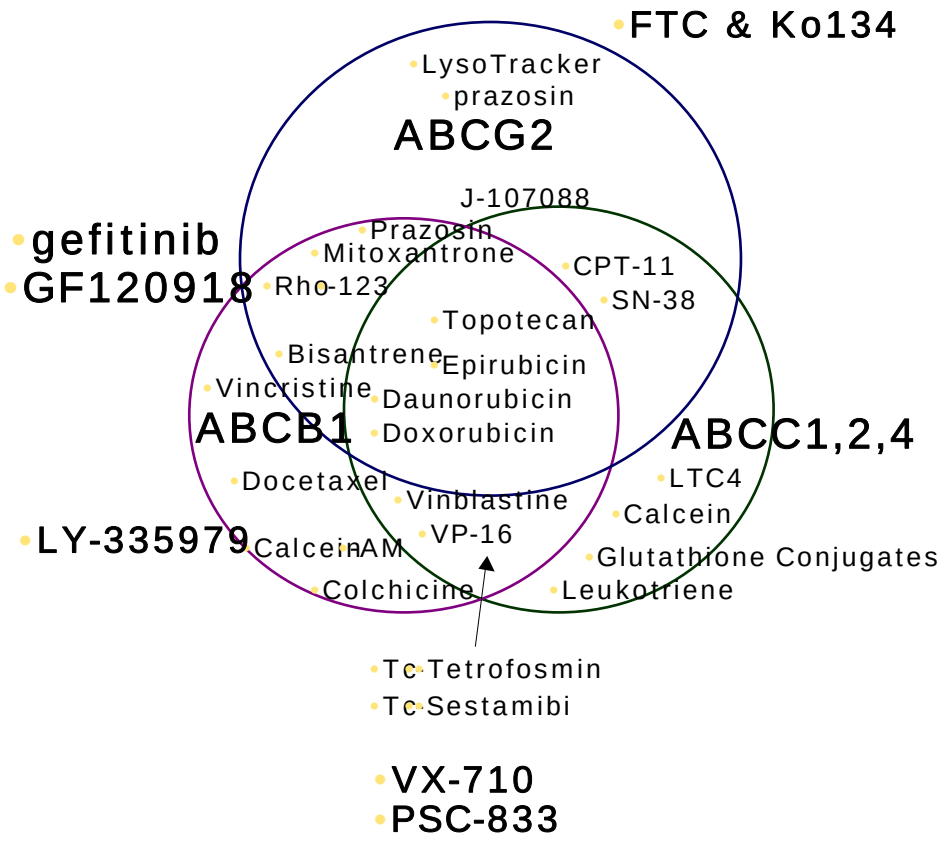
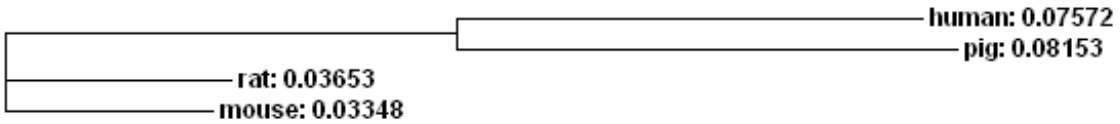



**Figure adapted from Thomas Litman**

# 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)

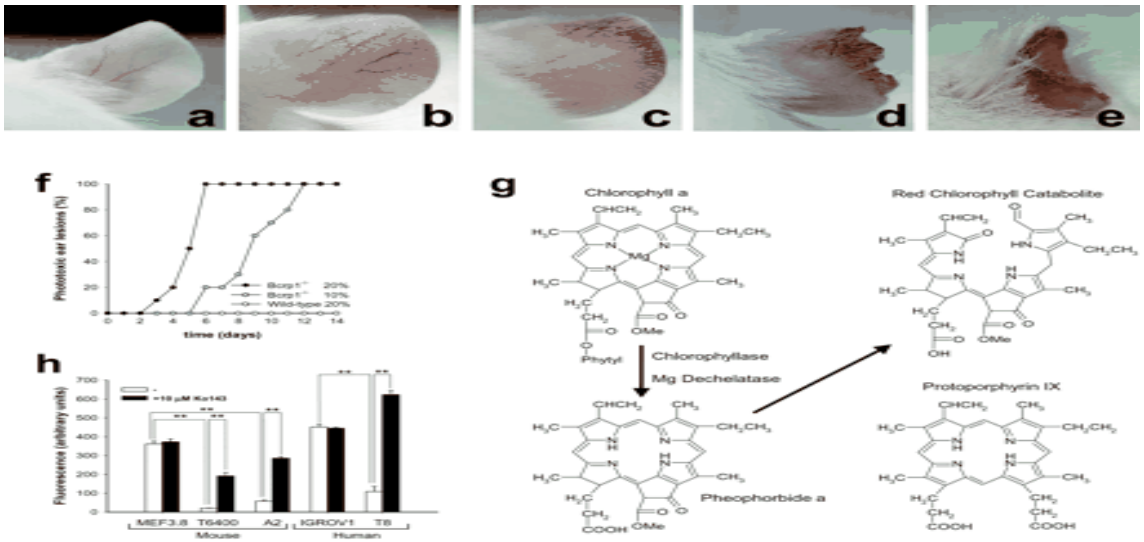
Phylogram with distances



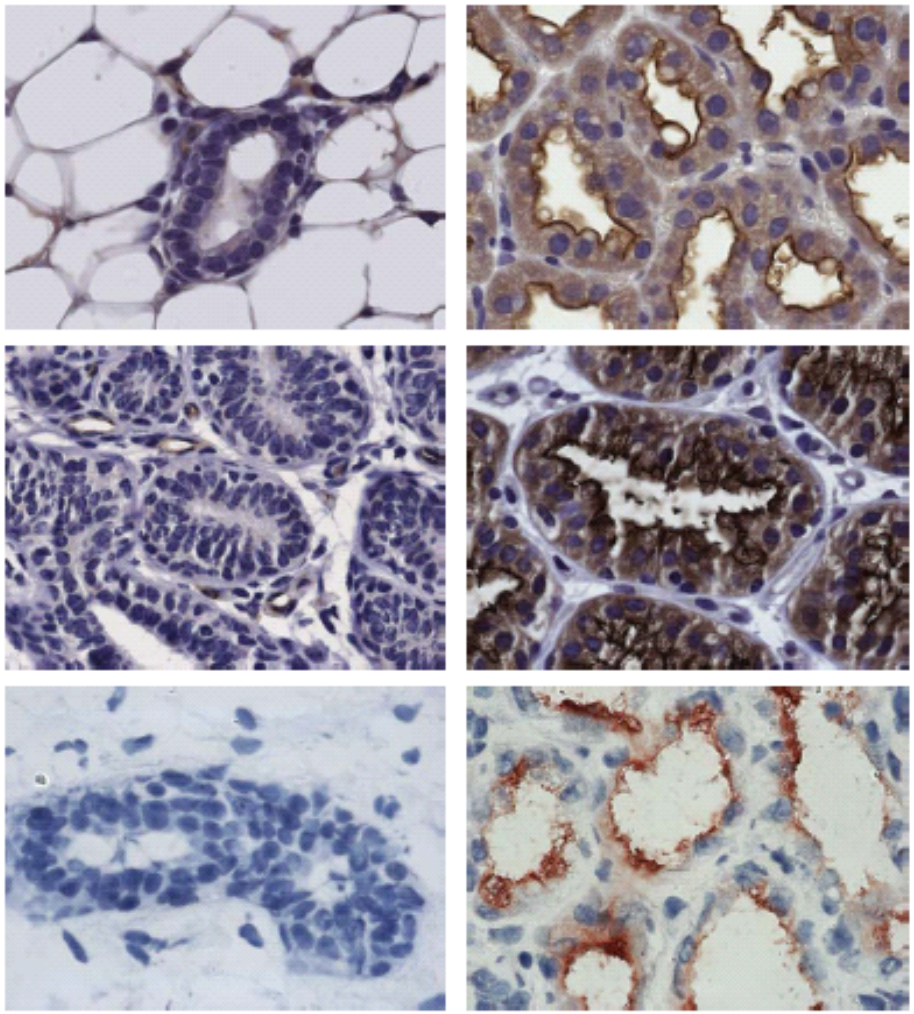
The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria.

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

- Bcrp -/- ADME Phenotype
  - Diet-dependent phototoxicity
  - Protoporphyria
  - Enhanced oral absorption of topotecan
  - Milk secretion of drugs and xenotoxins Nat. Med. 2005 Feb;11(2):127-9
  - ABCG2 is expressed in bone marrow stem cells.



# Expression BCRP in mammary gland across species




Literature:

BCRP substrates reported concentrated into milk of each of these species

MRP1-5, P-glycoprotein not upregulated in lactating mouse mammary gland

Slide from A.H. Schinkel, NKI

# Substrates & Inhibitors of ABCG2

**Drugs/NMEs**

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

**Xenobiotics**
**Endobiotics**

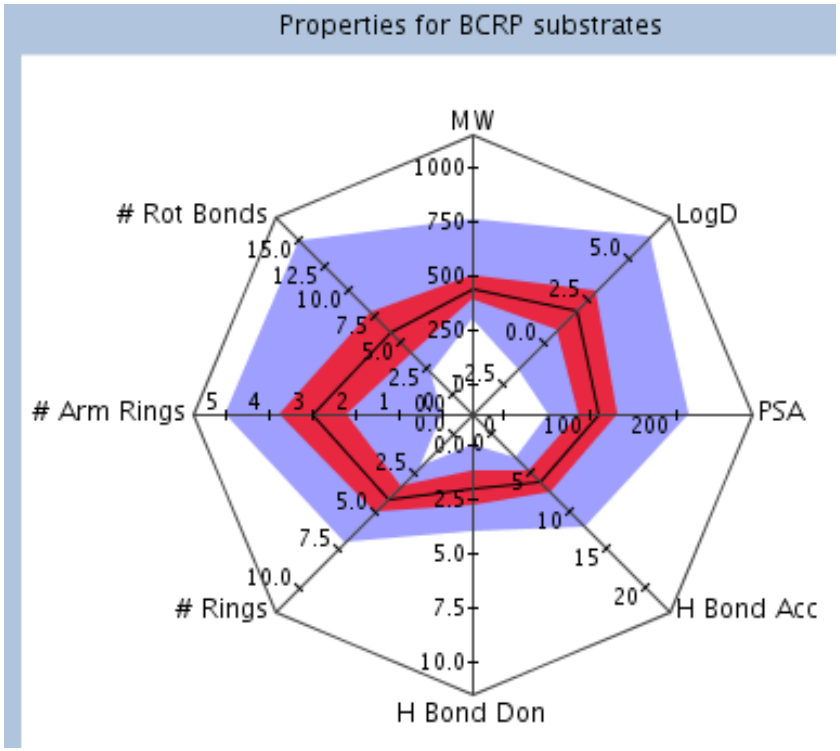
- PhIP
- Pheophorbide A
- Estrogen $SO_4$
- lysotracker (green)
- H33342
- Rhodamine 123
- Bodipy-prazosin
- Riboflavin (vitamin B2)

**Inhibitors**

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

# Physicochemical properties of BCRP substrates

**N = 609 (BCRP: $P_{app}$ BA/ $P_{app}$ AB > 2.0)**



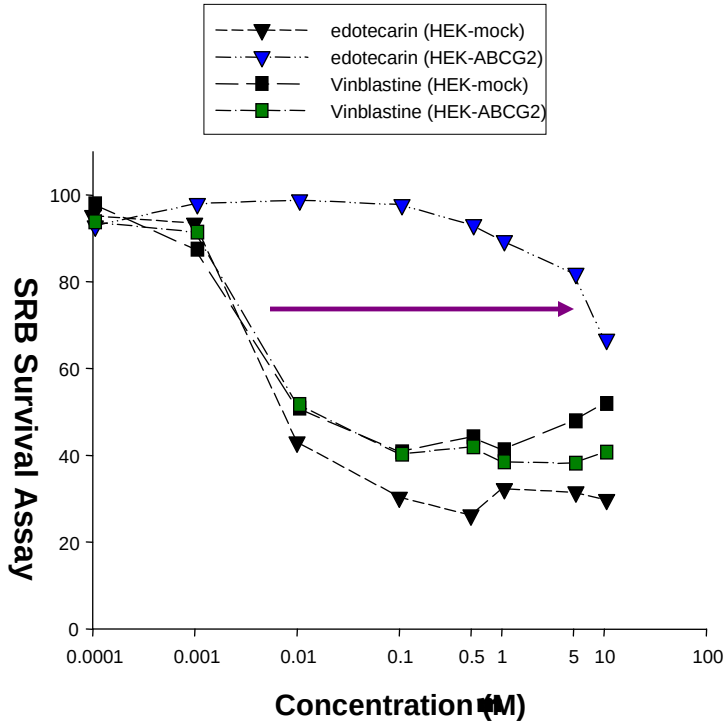

Pil Lee and Eric Reyner, SMI 2007

- Pipeline Pilot program 5.1.0.100

- Molecular Weight (MW)
- logD
- Polar surface area (PSA)
- # hydrogen bond acceptor (H Bond Acc)
- # hydrogen donor (H Bond Don)
- # Rings
- # Arm Rings
- # Rot Bonds

- Blue region: the range of each property
- Black line inside the red region is the average value for each property
- Red region: the standard deviation from the average value.

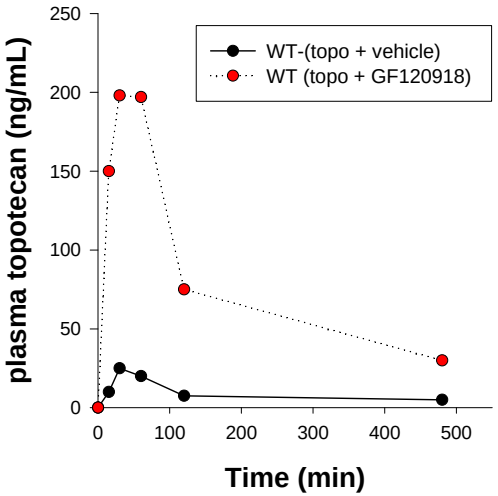
# Influence of BCRP (ABCG2) Expression on Cytotoxicity



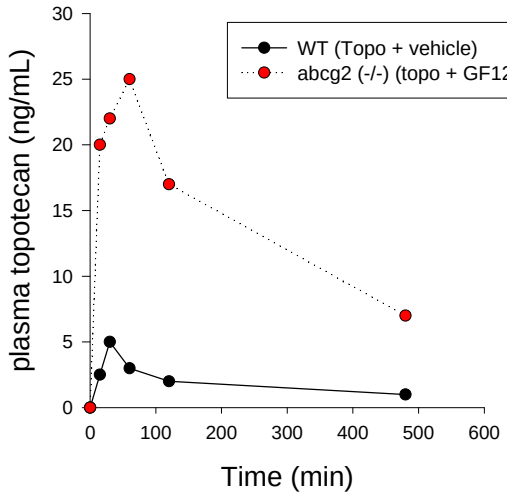

- Edotecarin (J-107088) is an excellent substrate of ABCG2 (Kotani et al., Cancer Res. 2001)
- In vitro combination studies of gefitinib suggest complete reversal of J-107088 in drug resistance.
- How may ABCG2 alter ADME and PD in vivo?
- *Project terminated before impact of transport biology fully characterized.*

# Of mice and men: Topotecan:BCRP interaction

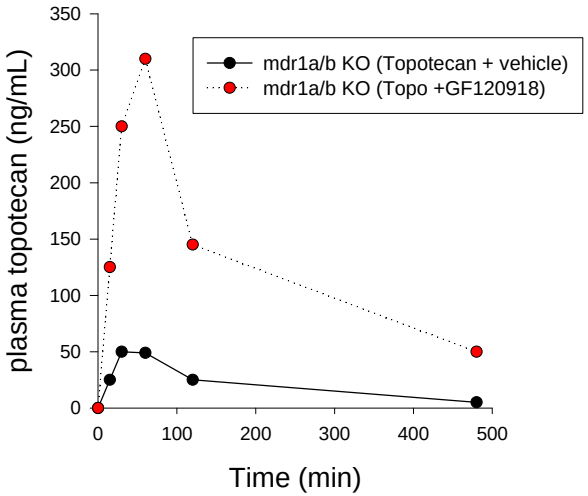
**Jonker et al., JNCI, 2000**



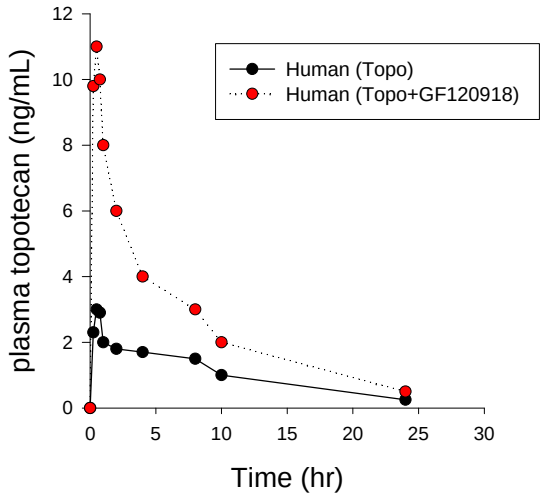

**Jonker et al., PNAS, 2002**




**Jonker et al., JNCI, 2000**




**Kruijtzer et al., JCO, 2002**



# Oral Topotecan

**A Phase I Study Of Oral Topotecan And Lapatinib In Subjects With Advanced Solid Tumors**

**This study is not yet open for participant recruitment.**
Verified by GlaxoSmithKline, May 2008

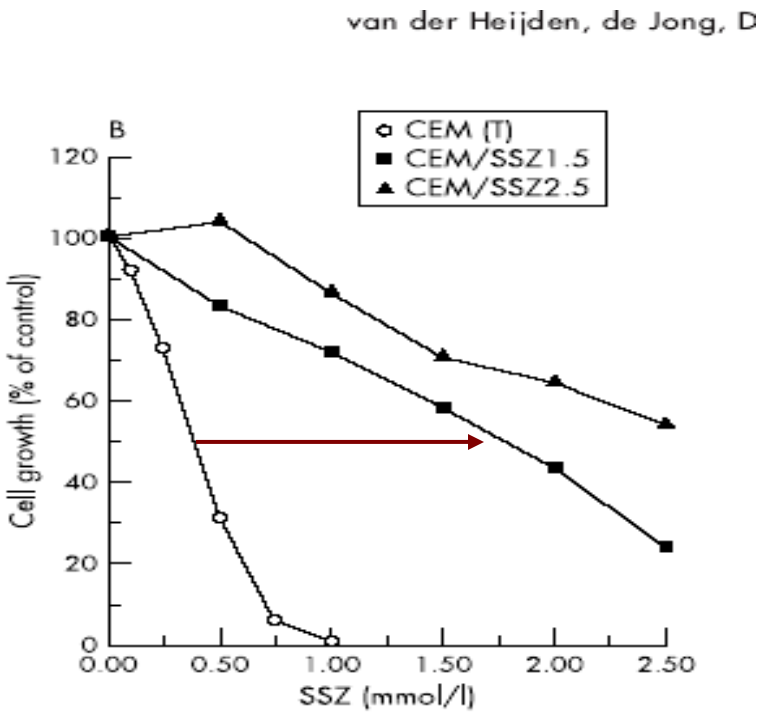
**Sponsored by: GlaxoSmithKline**
**Information provided by:**GlaxoSmithKline**ClinicalTrials.gov Identifier:**NCT00682279

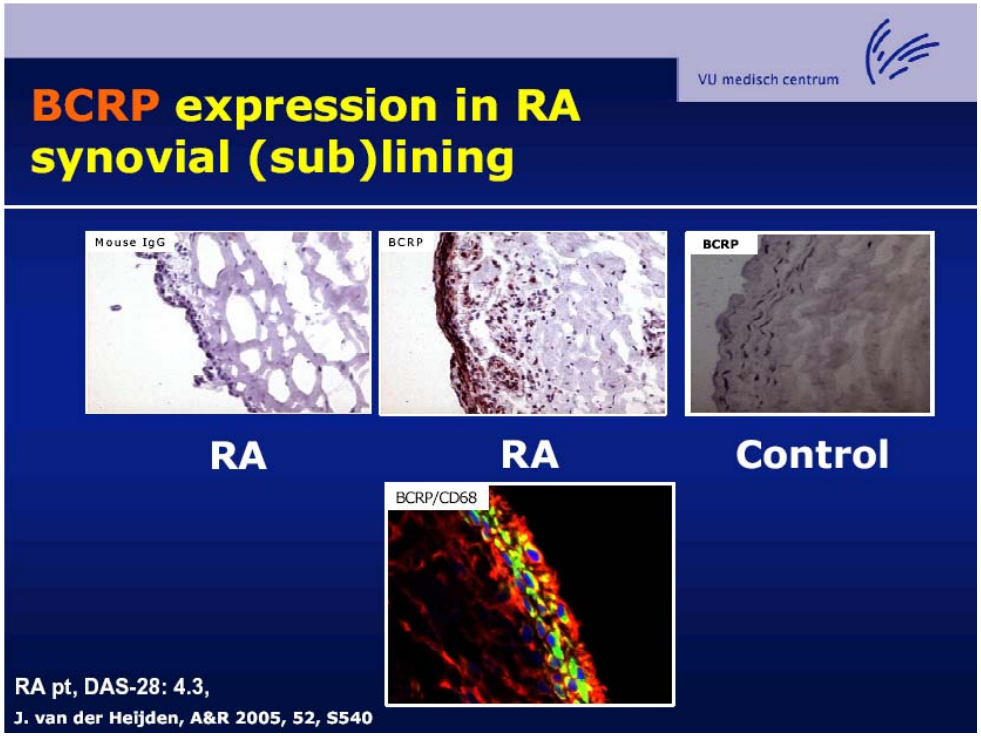
**Purpose**

This is an open-label, Phase I study of oral **topotecan** administered in combination with lapatinib in subjects with advanced solid tumors. This Phase I study will evaluate the safety, tolerability, and pharmacokinetics of oral **topotecan** administered in combination with lapatinib. This study will be conducted in two parts. Part 1 of the study will investigate the impact of lapatinib on the bioavailability of oral **topotecan** (bioavailability phase) and Part 2 of the study will consist of dose finding to determine the maximum-tolerated dose (MTD) regimen of the combination (dose escalation phase). In Part 2 of the study, the dose of oral **topotecan** will be escalated while lapatinib will be given initially as fixed doses. The primary objective of the study is to determine the MTD regimen of oral **topotecan** administered for five-consecutive days every 21 days in combination with daily lapatinib in subjects with advanced solid tumors.

**Source: clinicaltrials.gov**

# BCRP (ABCG2) Modulates Sulfasalazine (SASP) Resistance in-vitro




*van der Heijden et al., Ann Rheum Dis. 2004*

# Absorption, metabolism, and excretion of salicylazosulfapyridine in man

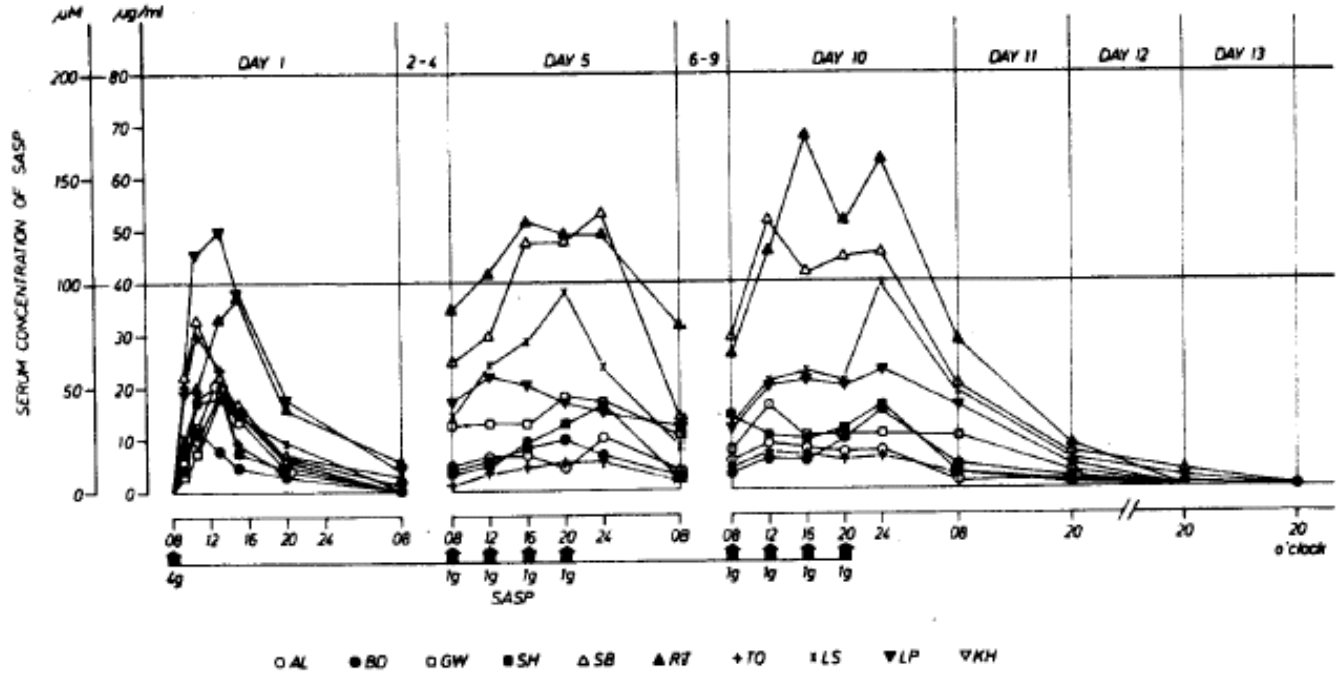



Fig. 2. Serum concentrations of SASP after ingestion of a single 4 Gm. dose of SASP on Day 1 (10 subjects) and 4 × 1 Gm. of SASP on Days 2 to 10 (9 subjects).

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

## 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.*

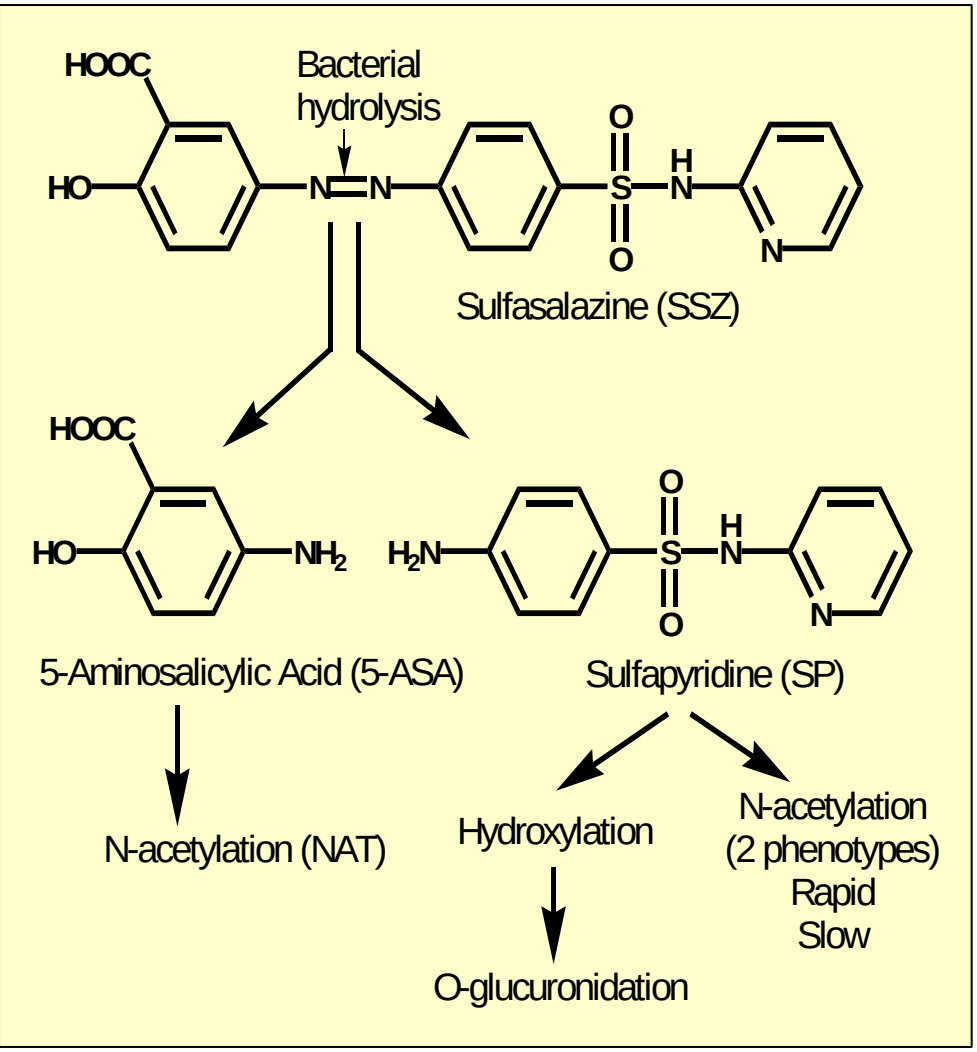
## ABCG2 Polymorphisms and Ethnic Distribution of SNPs.

- 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



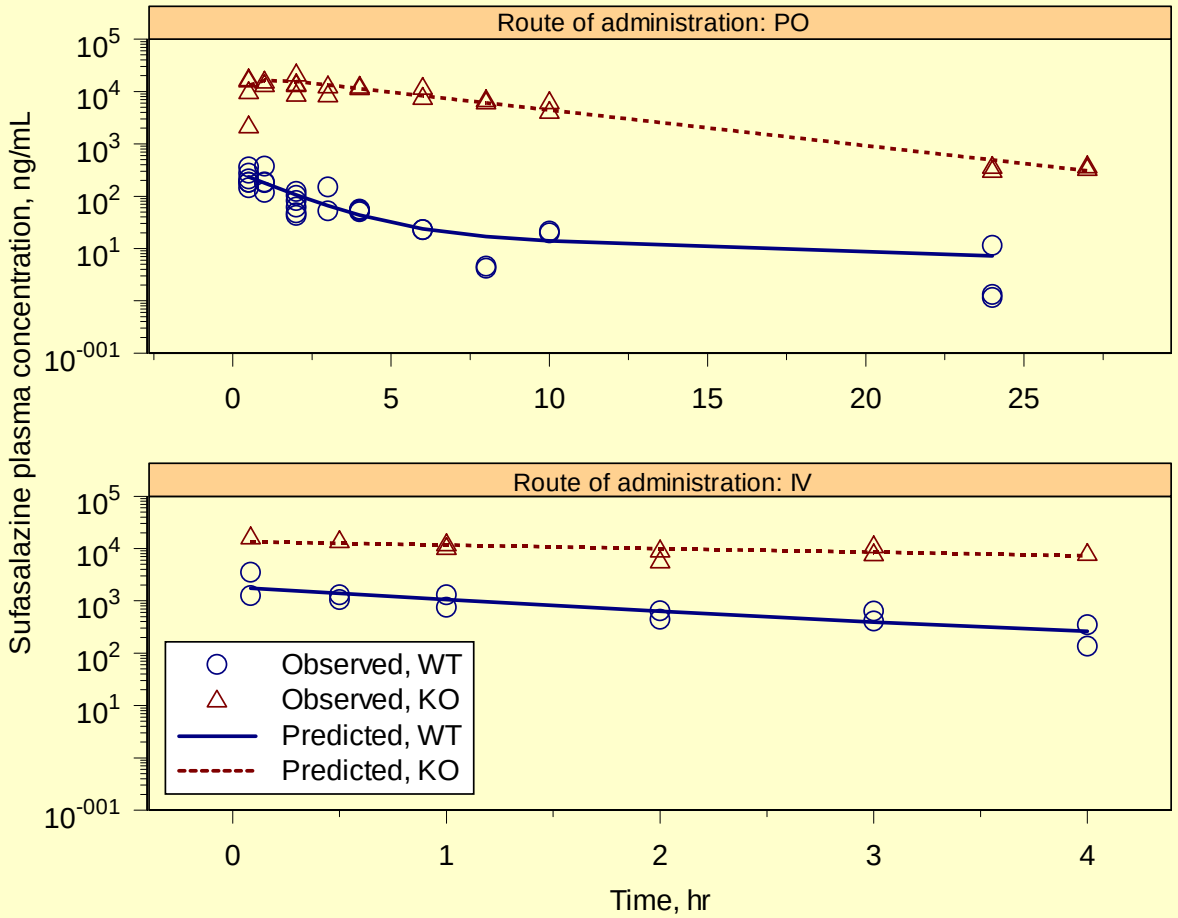

- **Indications: Rheumatoid arthritis (RA), Long term therapy of ulcerative colitis, and Crohn's disease**
- **Bioavailability (F) of SASP in humans is low (F< 15%) and highly variable**
- **Low %F primarily attributed to SASP's low permeability and poor solubility (thus, poor absorption)**
- **Azo-reduction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

## In vitro Permeability of SASP with ABCG2 ( BCRP)

| | |
|---|---|
| MDCK-ABCG2 B>A/MDCK B>A | 2.1 |
| MDCK-MDR1 B>A/MDCK B>A | 2.3 |
| Caco-2 B>A | 160 |

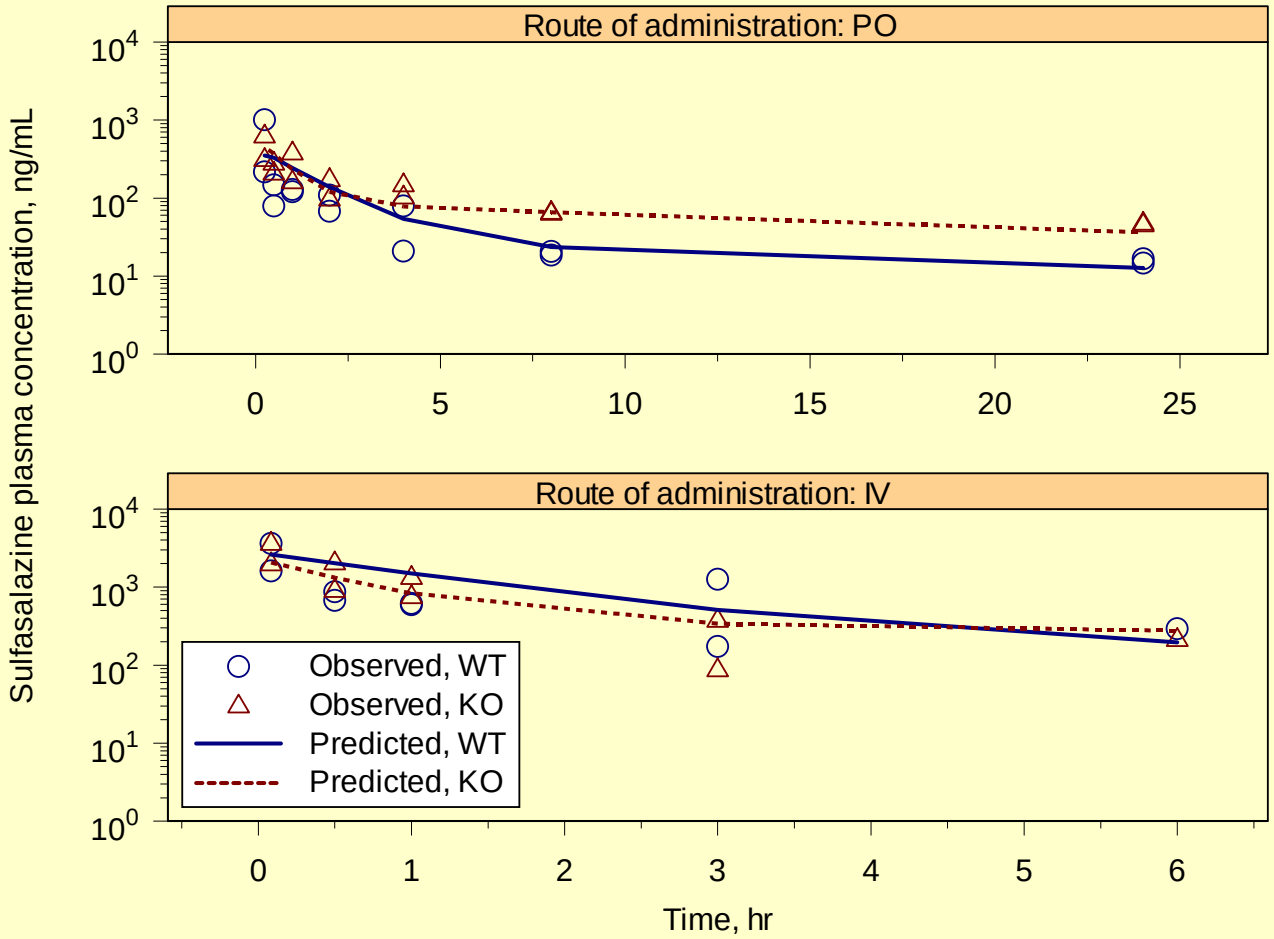
**Why the discordance in assays?**

# Abcg2 is Major Determinant of SASP Absorption and Elimination in the Mouse




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

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




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

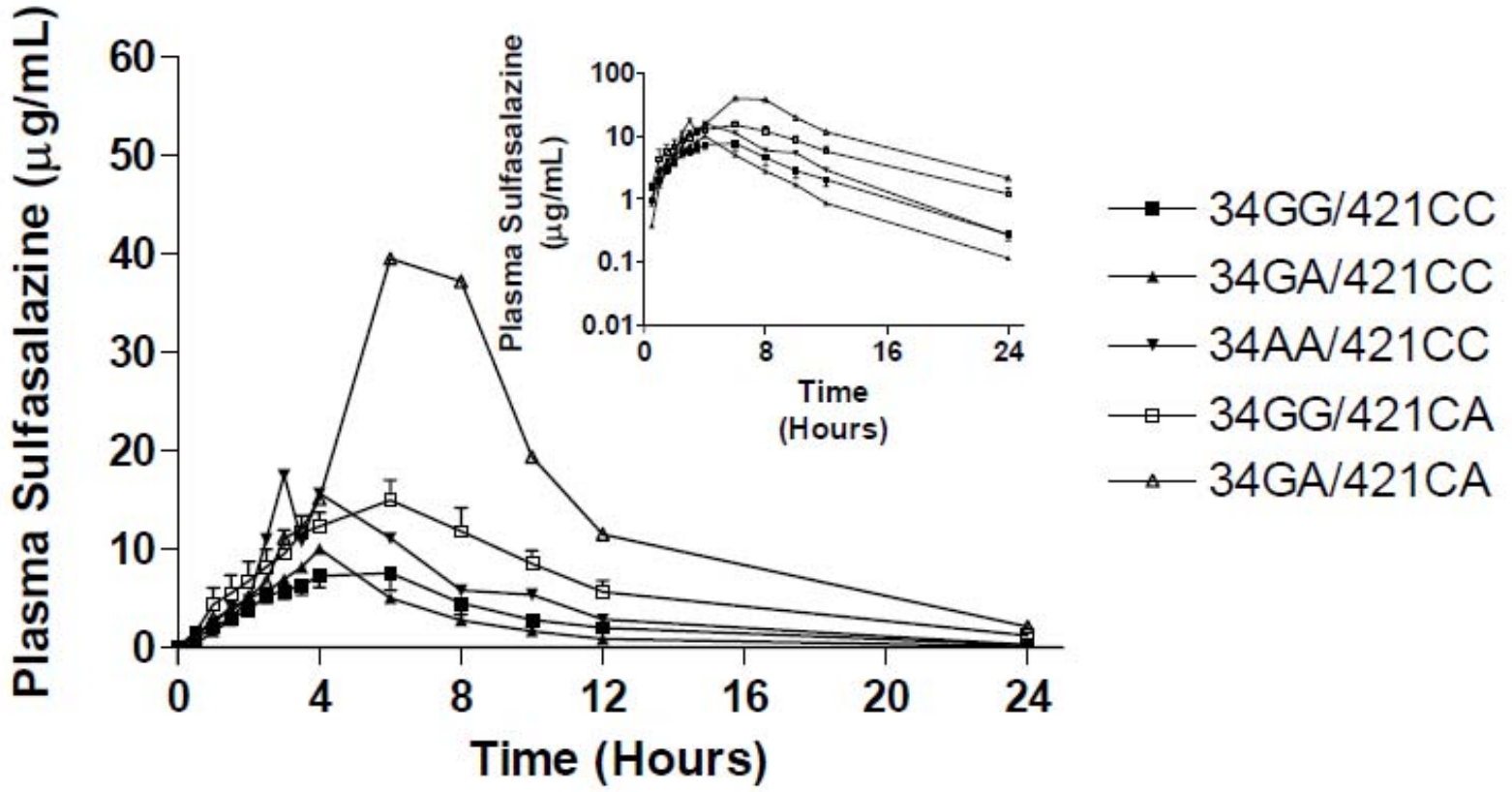
| Mice | Route | Dose (mg/kg) | $C_{max}$ (ng/mL)* | | AUC (ng.hr/mL) | | | Relative exposure, $AUC_{KO}/AUC_{WT}$ |
|---|---|---|---|---|---|---|---|---|
| | | | WT | KO | Duration (hr) | WT | KO | |
| Bcrp1 | IV | 5 | 1827 | 13570 | 0-4 | 3015 | 40343 | **13** |
| | PO | 20 | 233 | 16176 | 0-24 | 1189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

* IV (intravenous) = $C_{max}$ at time zero was extrapolated from the model; PO (Oral) = visual $C_{max}$ from raw data

SASP $C_{max}$ and exposure (AUC) in Bcrp1 (abcg2) and mdr1a (WT and KO) mice following intravenous (IV) and oral (PO) administration.

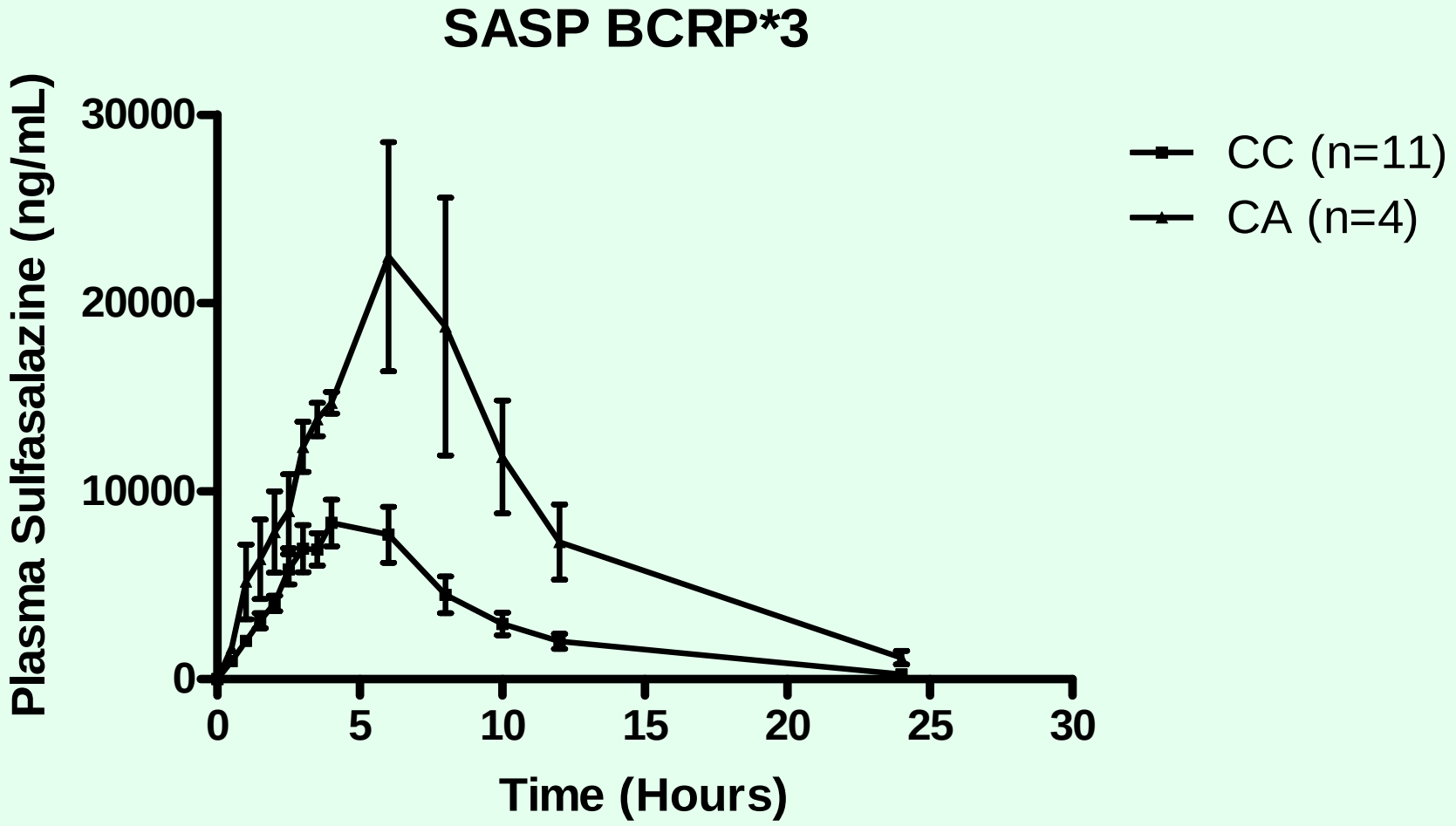
**Zaher et al., Molecular Pharmaceutics epub January 4, 2006**

# SASP Disposition in North American Healthy Volunteers



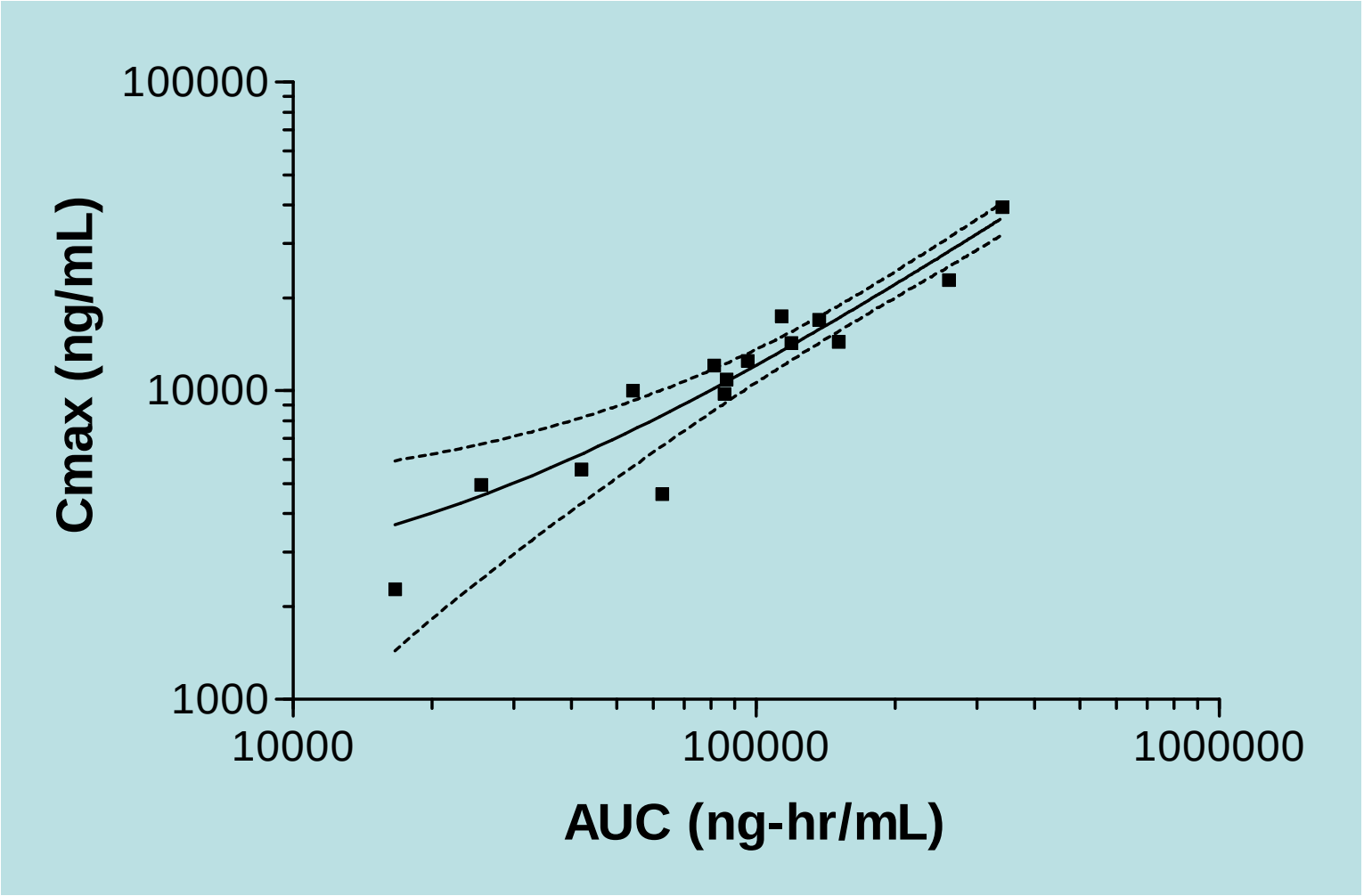

*Brad Urquhart et al., Pharmacogenet Genomics. 2008 May;18(5):439-48.*

# Altered SASP Exposure in Q141K Subjects



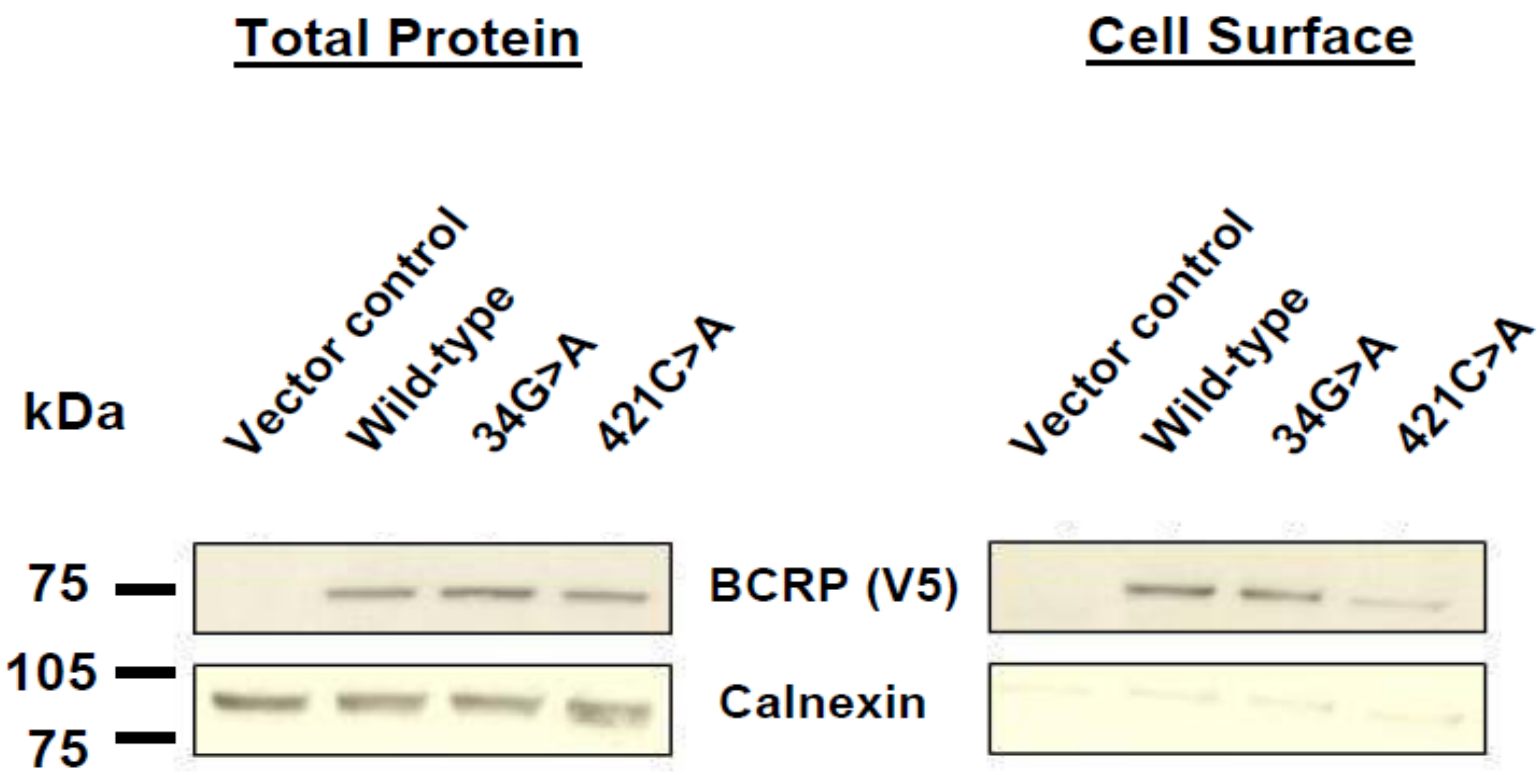

Urquhart et al., Pharmacogenet Genomics. 2008 May;18(5):439-48.

# Correlation between SASP Cmax and AUC for Healthy Subjects




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

# 421C>A SNP Changes Surface ABCG2 Expression




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

# SASP Disposition in Healthy Japanese Volunteers

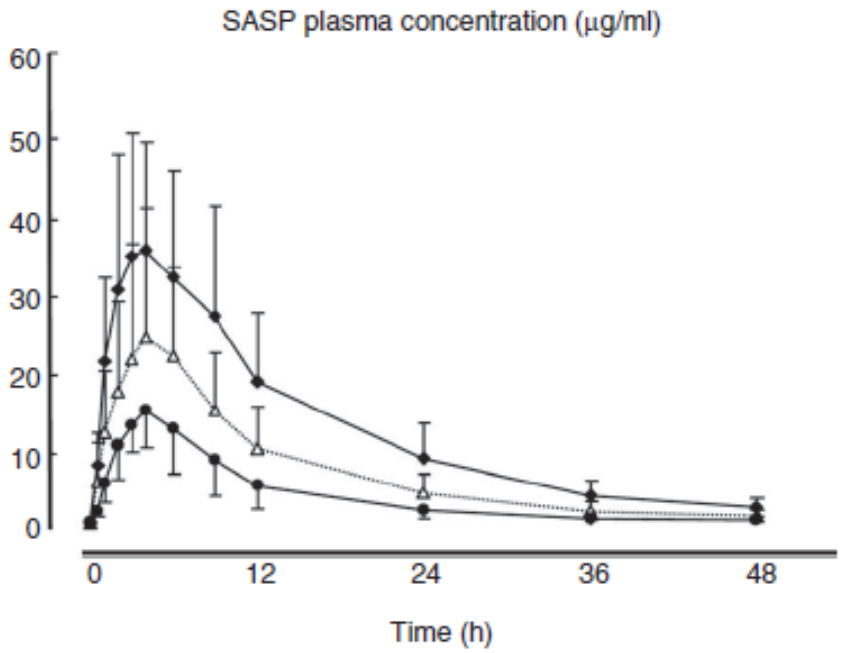
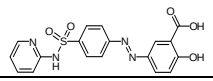
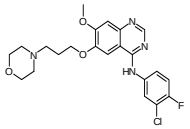
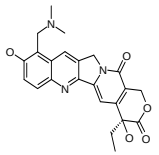
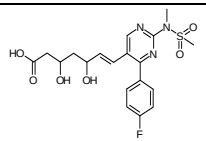
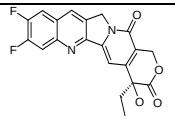
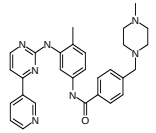
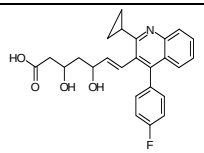



**Figure 2** Effect of *ABCG2* genotype on pharmacokinetics of sulfasalazine (SASP). Plasma concentration-time profiles of SASP after oral administration of a 2,000 mg conventional SASP tablet to 421C/C subjects (closed circles, $n = 12$), 421C/A subjects (open triangles, $n = 16$), and 421A/A subjects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

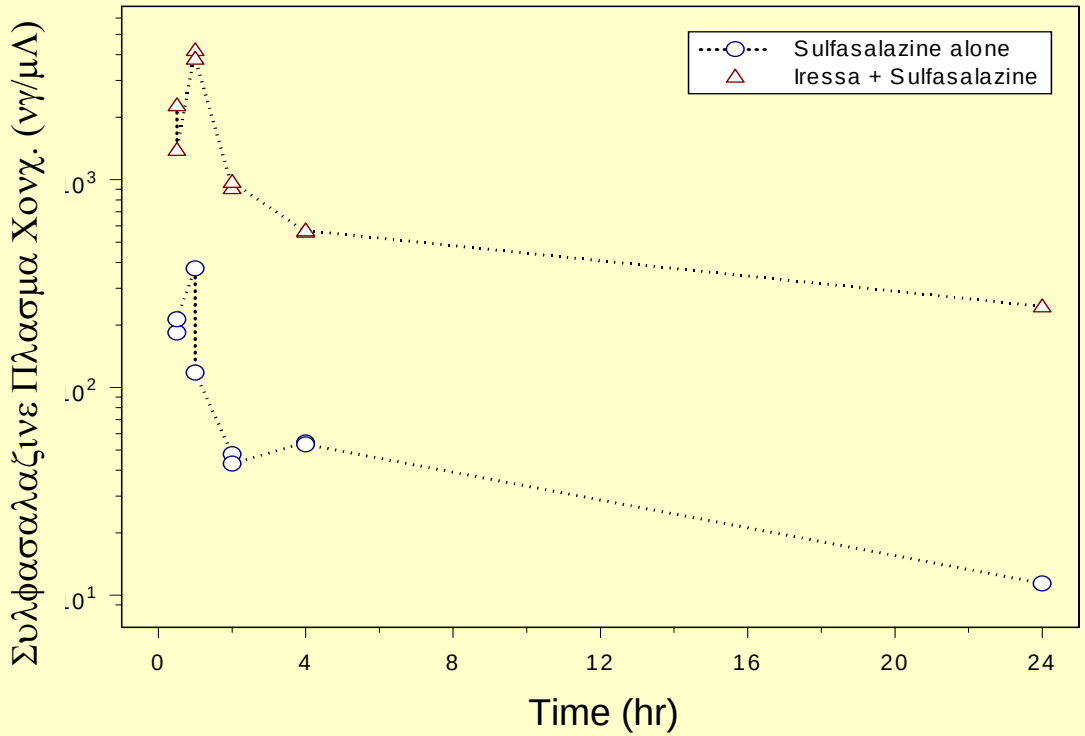
**Yamasaki et al., CPT January 2, 2008**

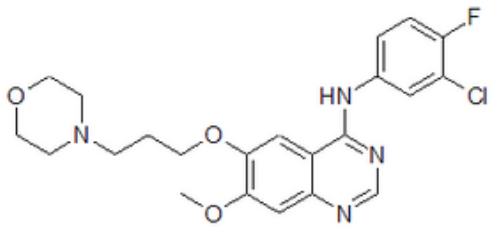
# ABCG2 Pharmacogenomic Studies

Formulation
IR →
susp →
SR →

| Drug | Structure | Dose, Route | # Patients | Ethnic Group, Gender | Result | Reference |
|---|---|---|---|---|---|---|
| Sulfasalazine |  | 2000 mg po | 37* | Japanese Male | 1.7-3.5X increase in AUC, Cmax | Yamasaki et al (2008) Clin Pharmacol Ther, ePub |
| Sulfasalazine | | 1000 mg po | 17* | Caucasian Both | 1.7-2.4X increase in AUC, Cmax | Urquhart et al (2008) Pharmacogen & Genomics, ePub |
| Sulfasalazine | | 500 mg po | 36* | Chinese Both | No effect on AUC, Cmax | Adkison et al (2008) ASCPT mtg poster |
| Gefitinib<br><br>(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<br><br>(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 |

# Gefitinib (Iressa)-enhanced SASP Bioavailability

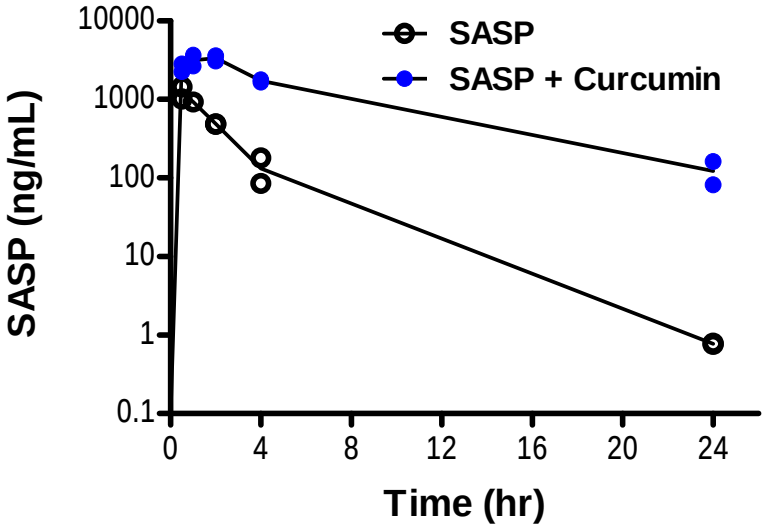


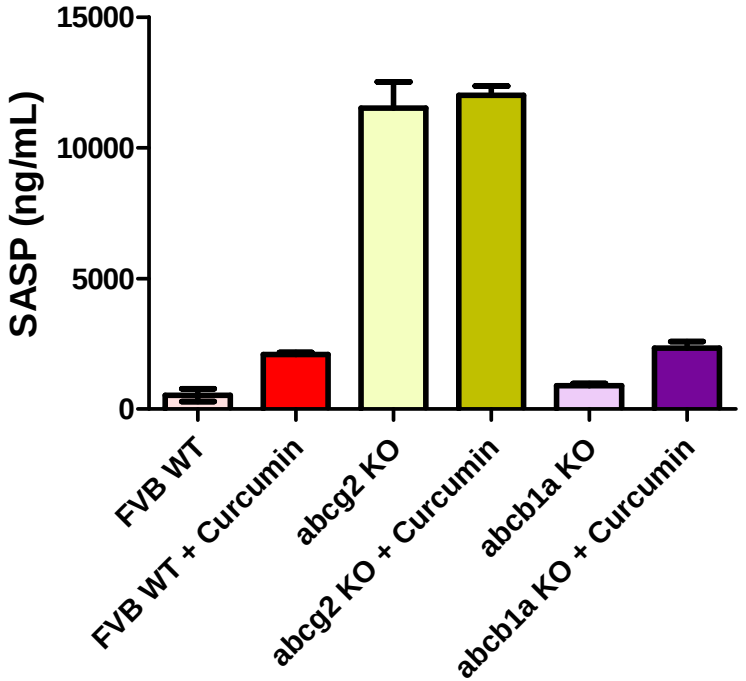




Gefitinib (Iressa)

**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



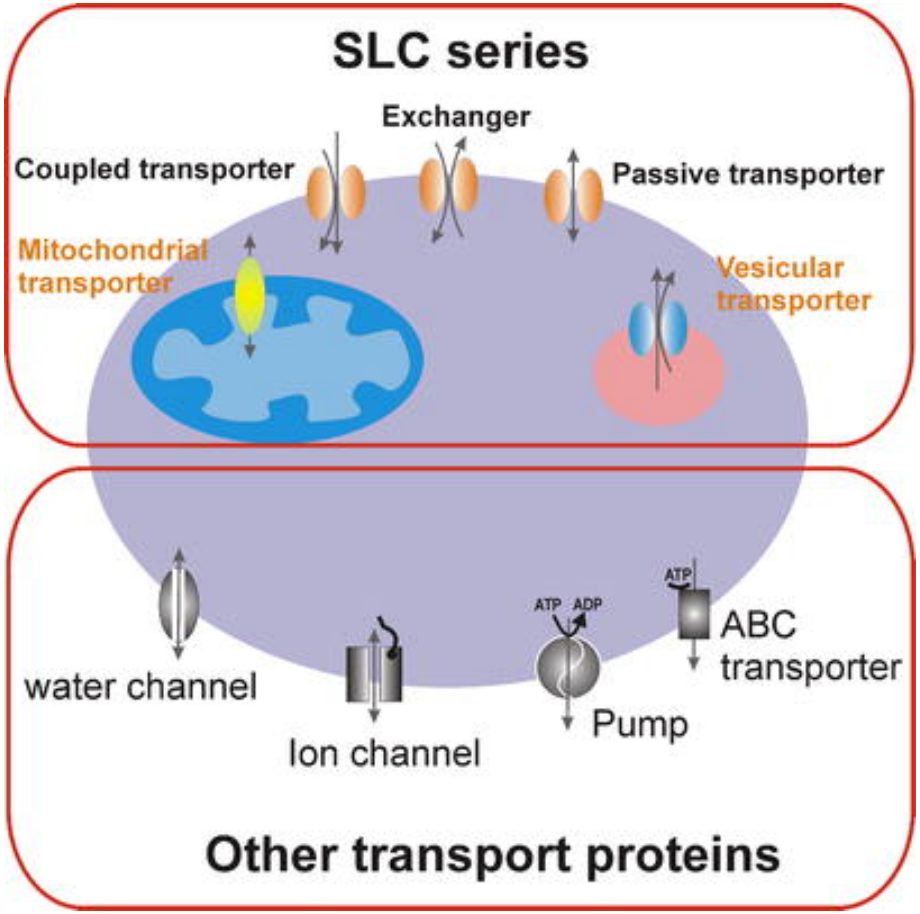




***Suneet Shukla et al. Pharm Res. 2008 Oct 9.***

# 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

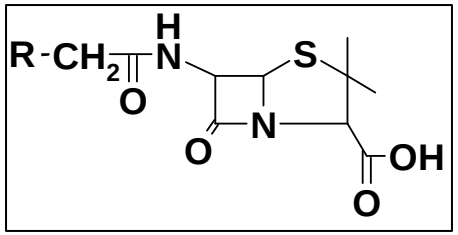


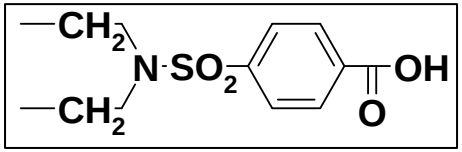

- Solute Carrier (SLC) superfamily contains
  - 43 families
  - 298 genes
- HUGO database (see http://www.gene.ucl.ac.uk/nomenclature/)
  - SLC root symbol
  - Followed by numeral (family)
  - Followed by letter
  - Followed by numeral (ie SLC22A1)
  - Further elaborated in the SLC21/SLCO

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

# 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 preclinicial data support that compound is a prototypical transport substrate?

# Package Inserts: Clinical Studies and DDI Potential

| **Drug ($CL_R$)** | **Results (Bedside)** |
|---|---|
| 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 |

# Transporter Nomenclature

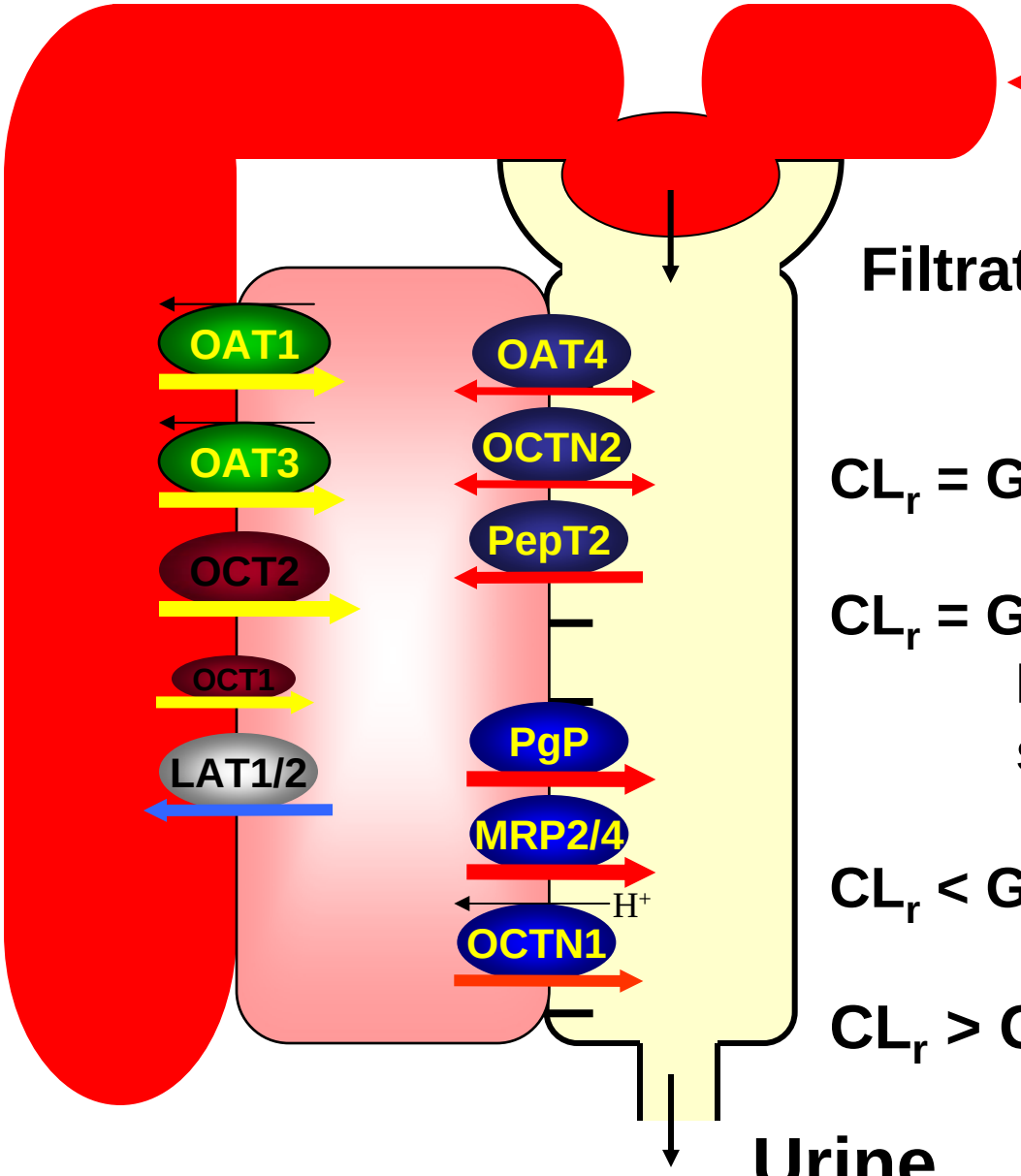
## SLC Family

- **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**

# Major Renal Transporters




Blood Flow

Filtration (GFR) *fu

$CL_r$ = GFR + secretion – reabsorption

$CL_r$ = GFR

Filtration only

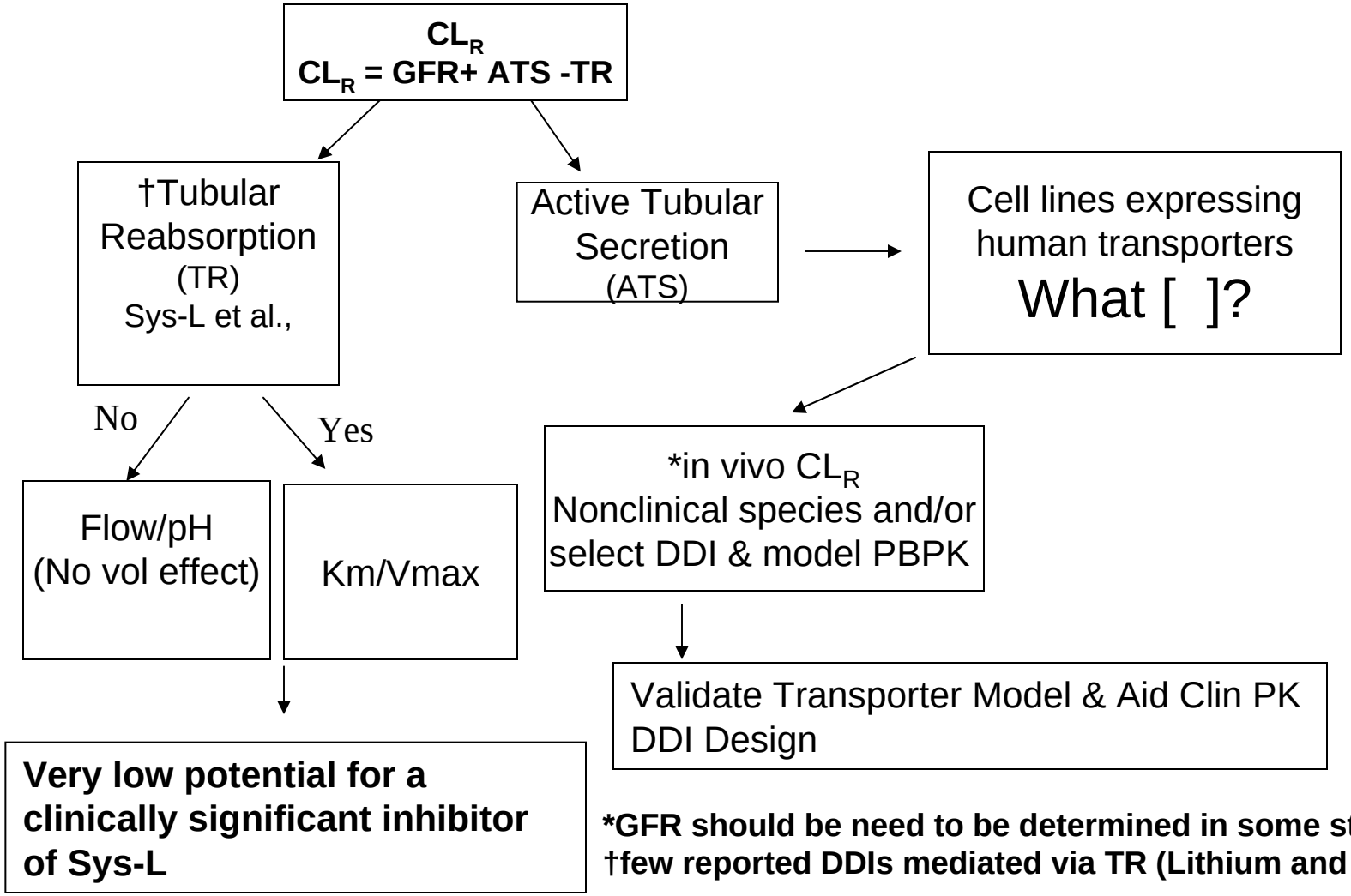
secretion = reabsorption

$CL_r$ < GFR (net reabsorption)

$CL_r$ > GFR (net secretion)

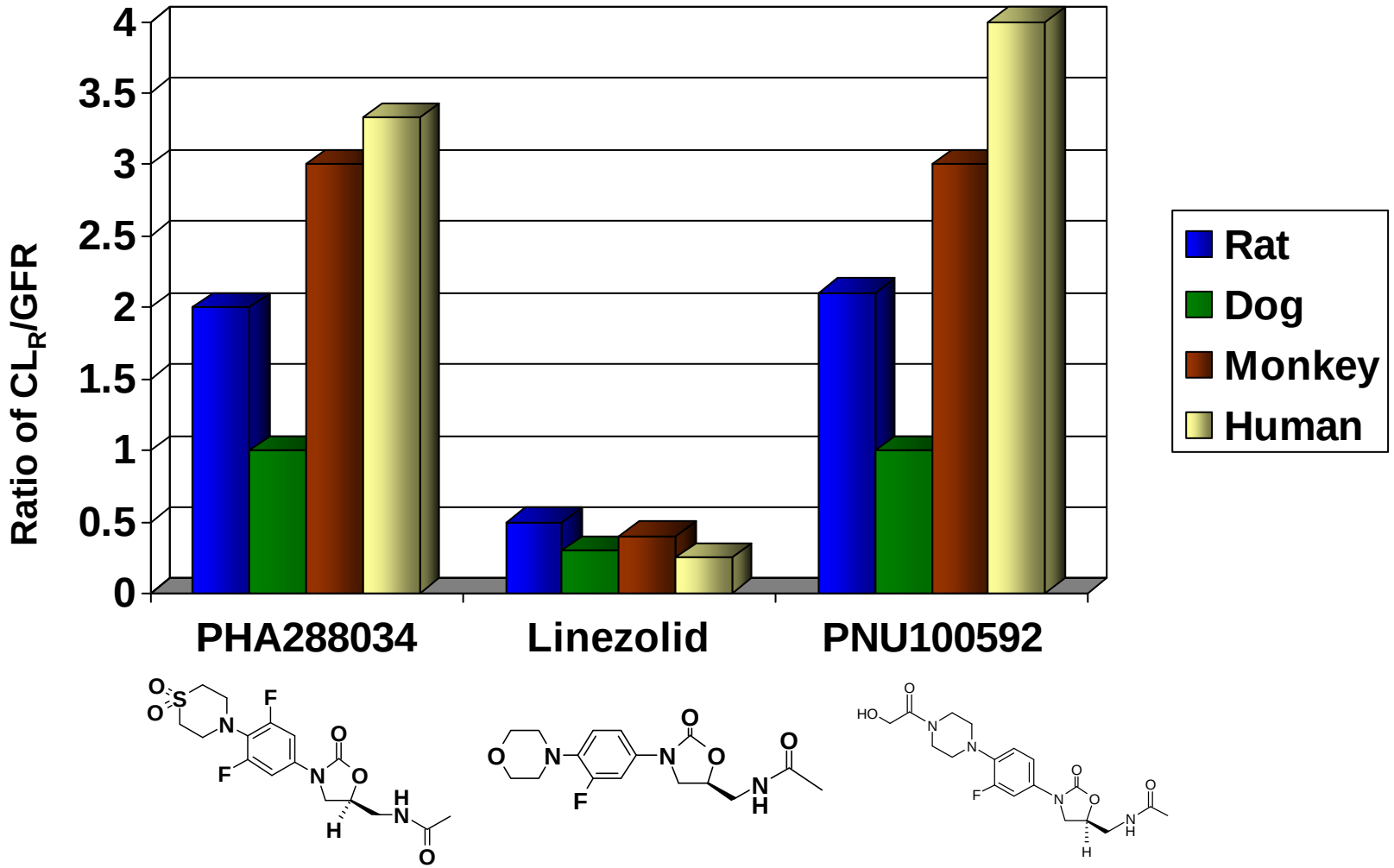
Urine

# Renally-Mediated DDIs

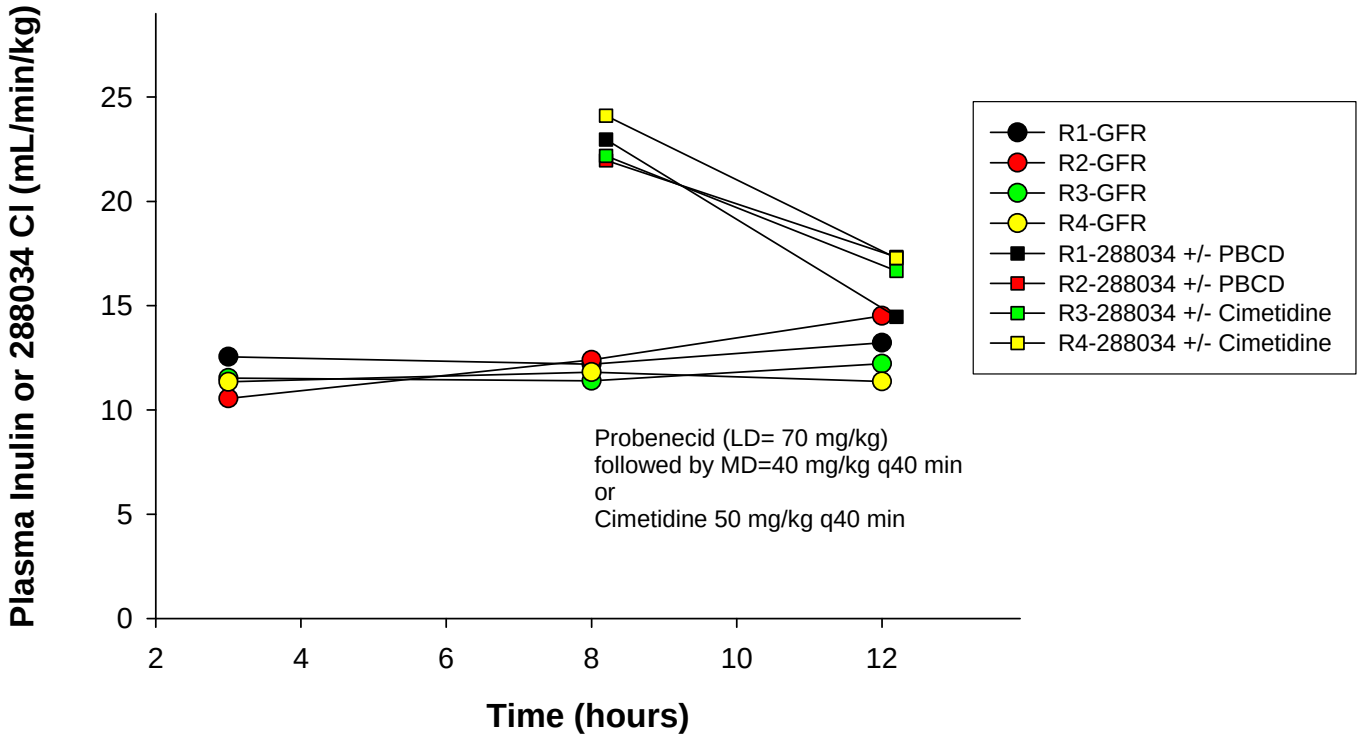



***GFR should be need to be determined in some studies**
**†few reported DDIs mediated via TR (Lithium and amiloride)**

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



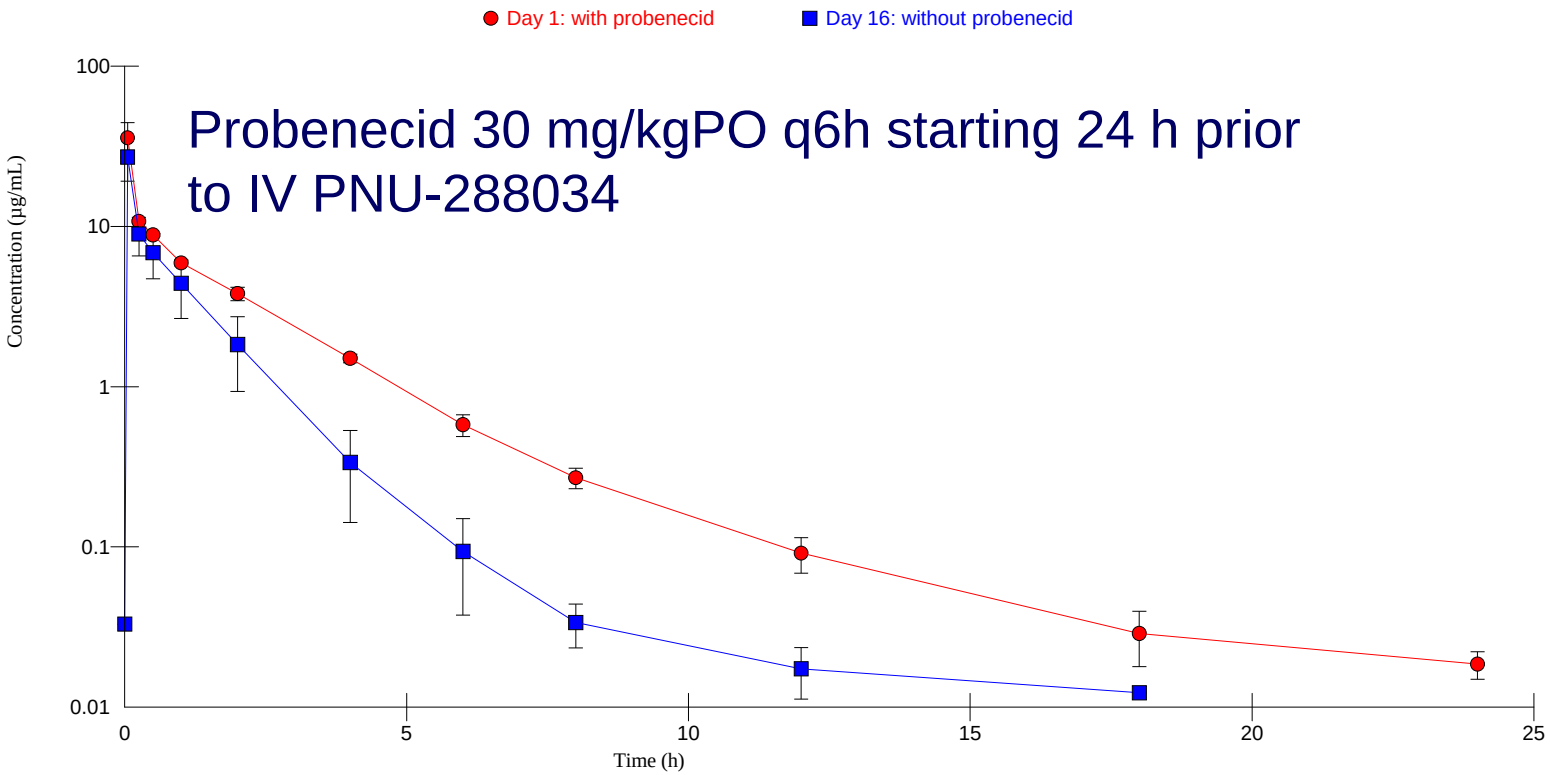
# Inhibition of PHA-288034 Clearance via Probenecid or Cimetidine in the Rat




| | | 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 | |

009

# Monkey PHA288034 Probenecid Interaction Study



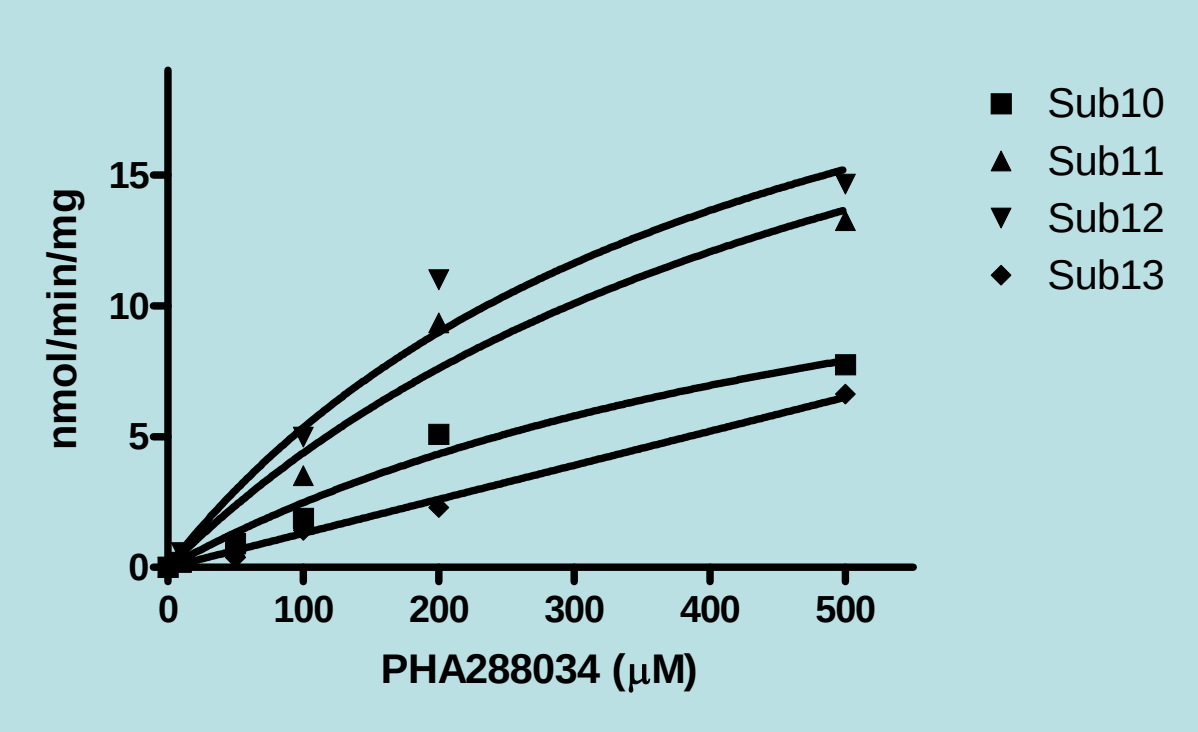

Study by WJ Adams et al.

# Model Systems to Study Renal Transport

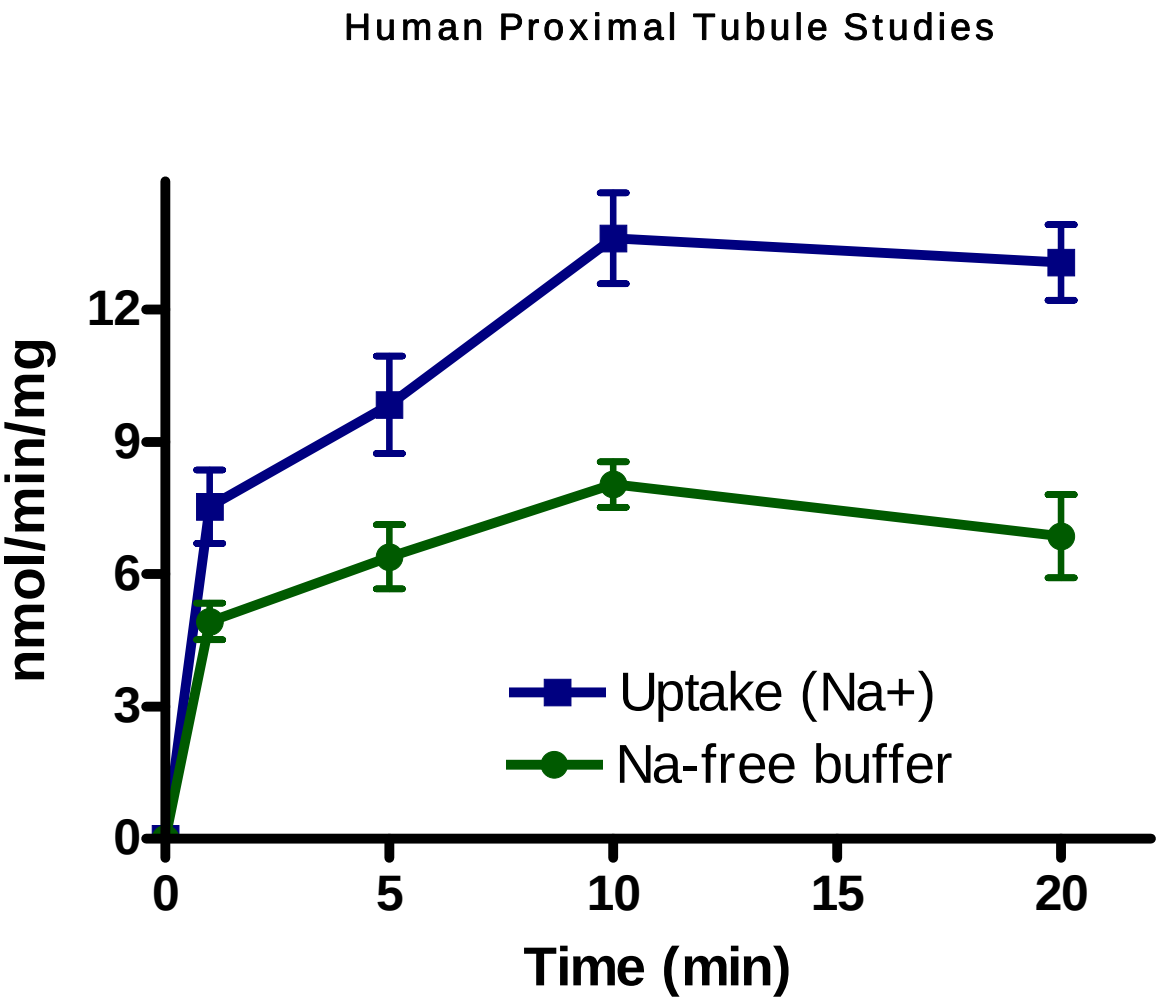
- Isolated Perfused kidney
- Kidney Slices
- **Isolated Renal Tubules (PCTs)**
- Isolated BBMVs
- **Individual Transporter Clones**
  - **Transient**
  - **Stable**
- GeMMs

# In Vitro Uptake Models

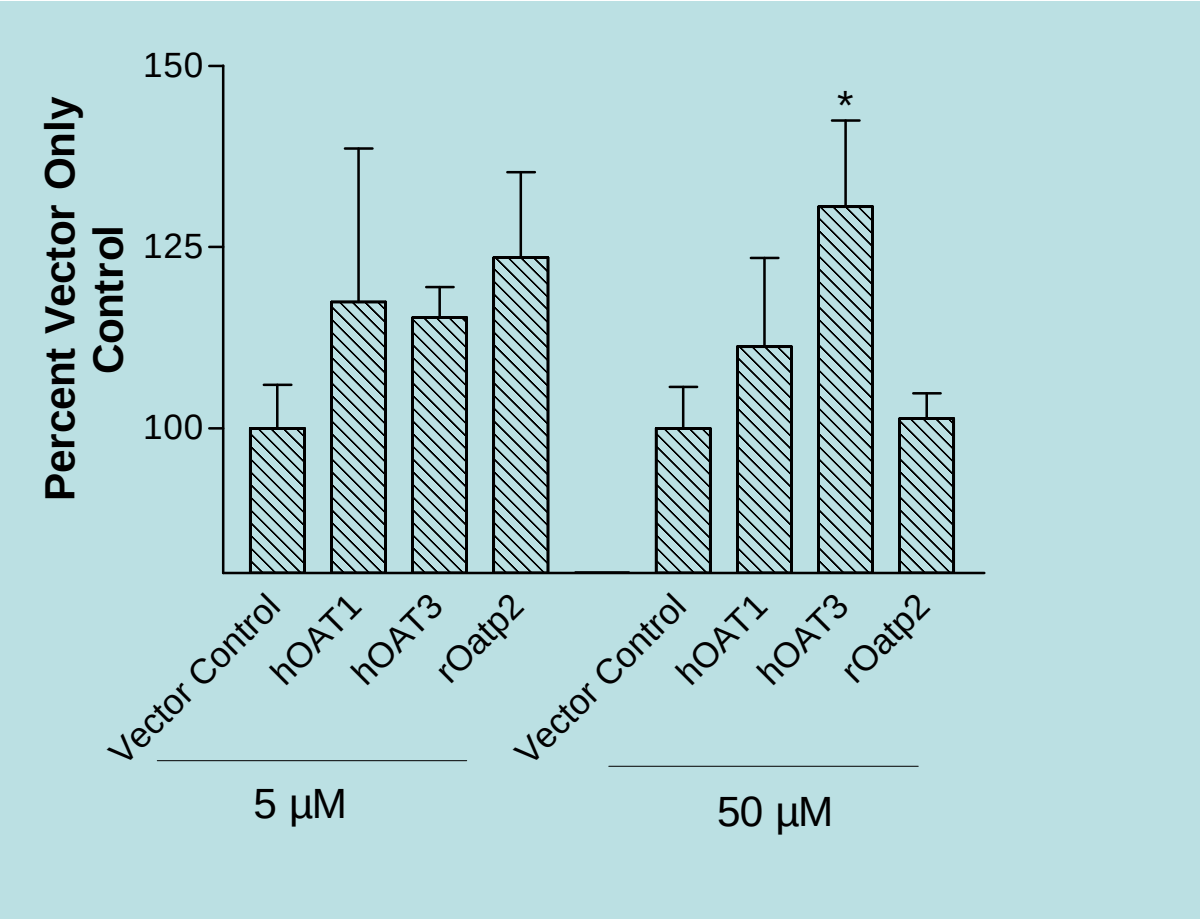
- Transport of PHA-288034 in human proximal tubules.
  - Drug uptake in cell suspension of hPTs.
  - Determine kinetics, substrate specificity, energy & ion dependence
  - Preliminary study suggested no metabolism in hPTs



# $Na^+$-dependent Uptake of PHA288034

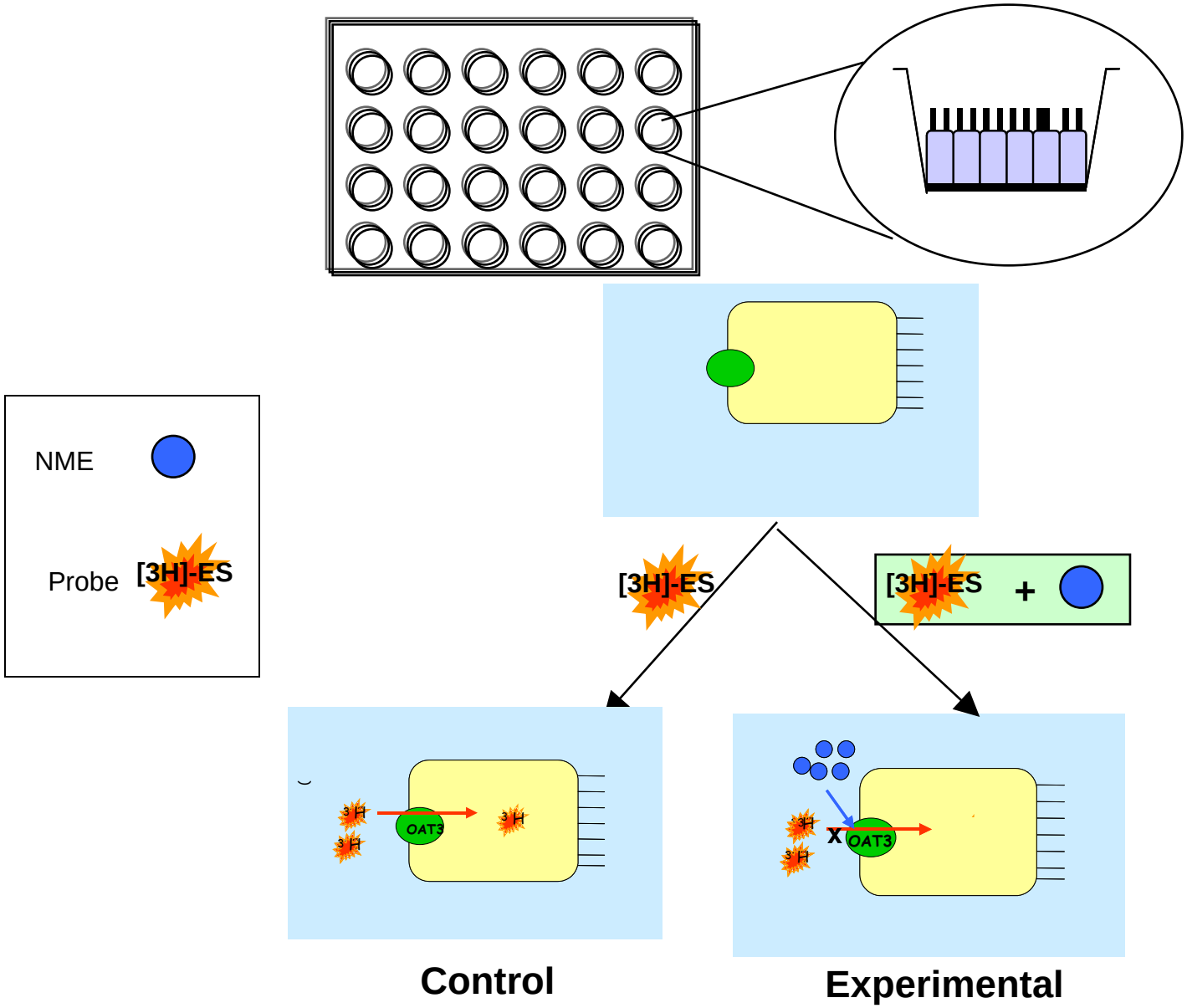


# PHA-288034 Uptake in HeLa cells Transfected with Transporter cDNAs



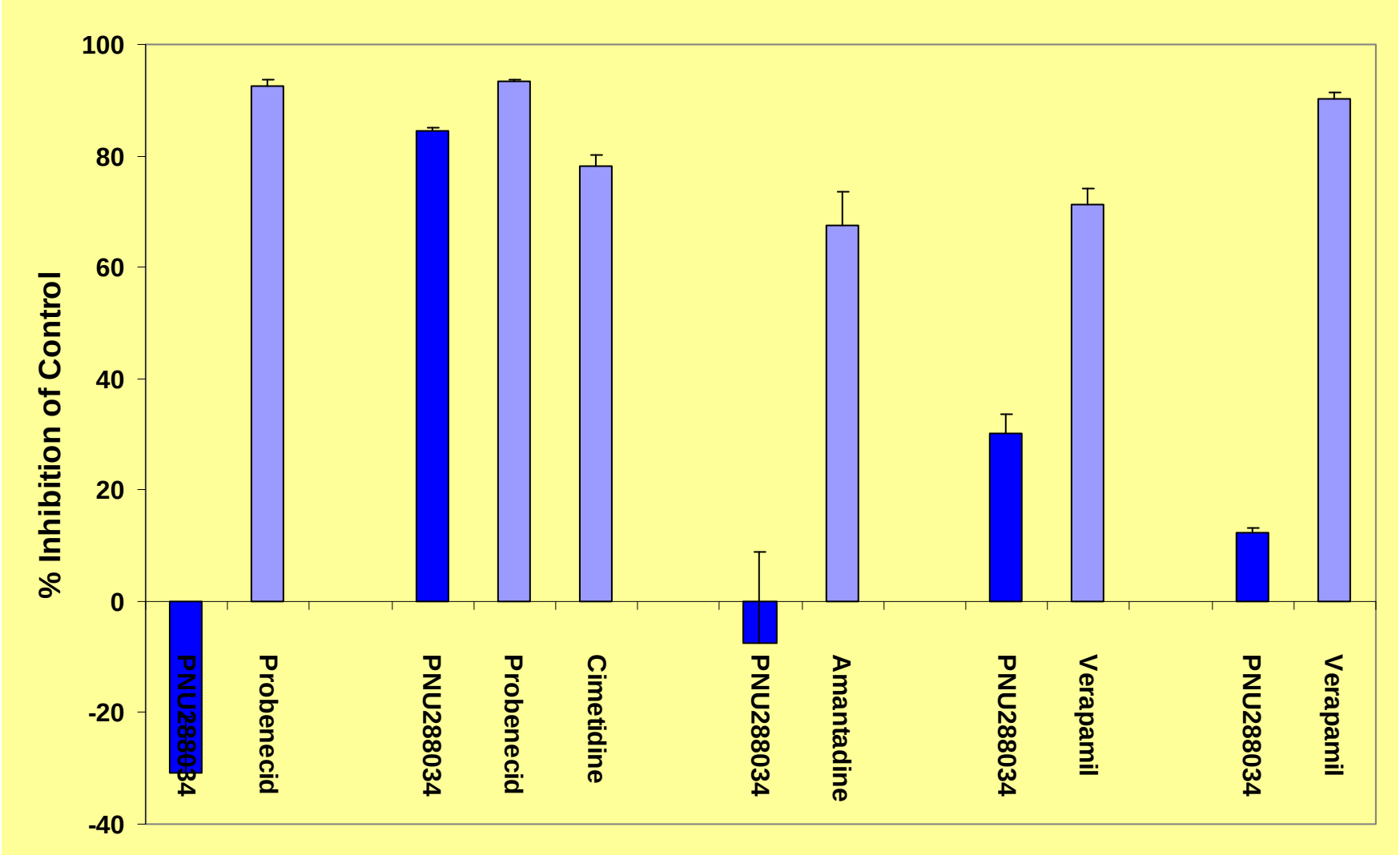

Richard Kim and Brenda Leake

# Experimental Protocol: Interaction Assay in Stable Transfectants



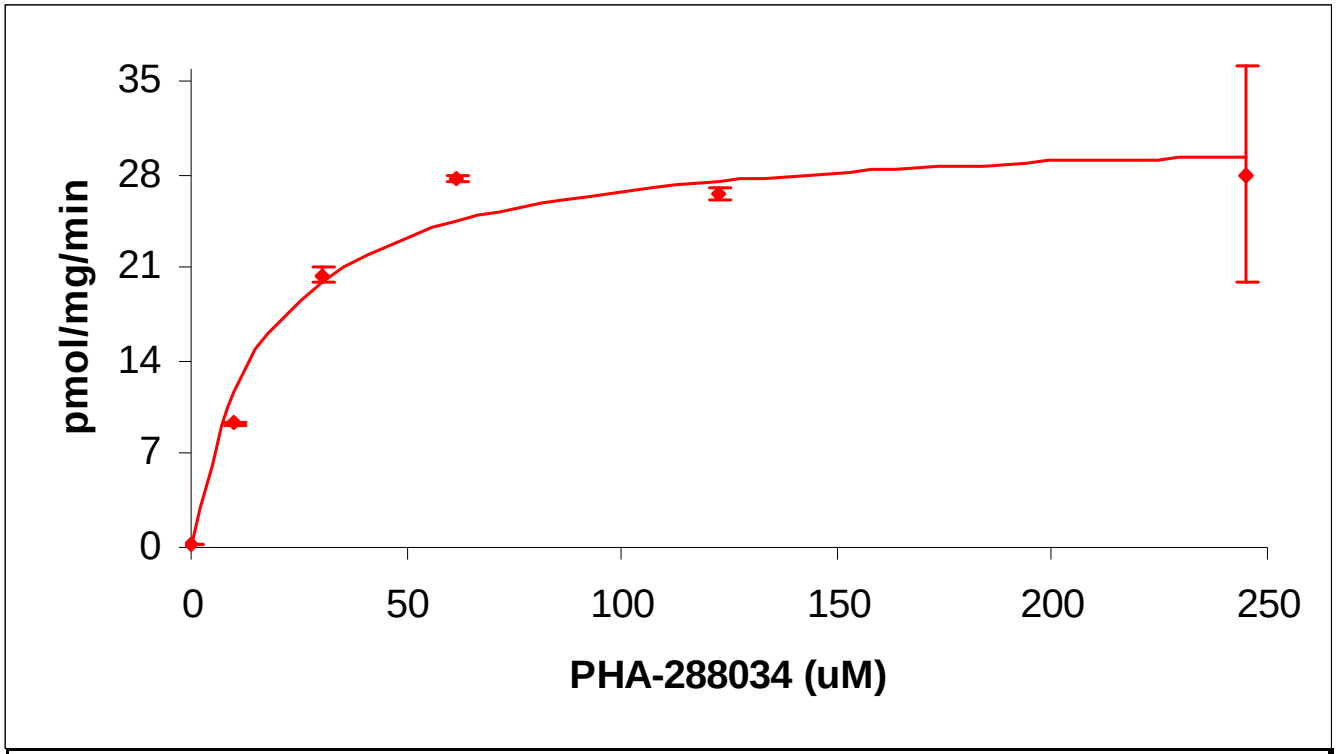

**Result/calculations = Inhibition of [3H]-ES uptake (% of control) in presence of NME**

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




*HEK cell lines created by Prof. KM Giacomini*

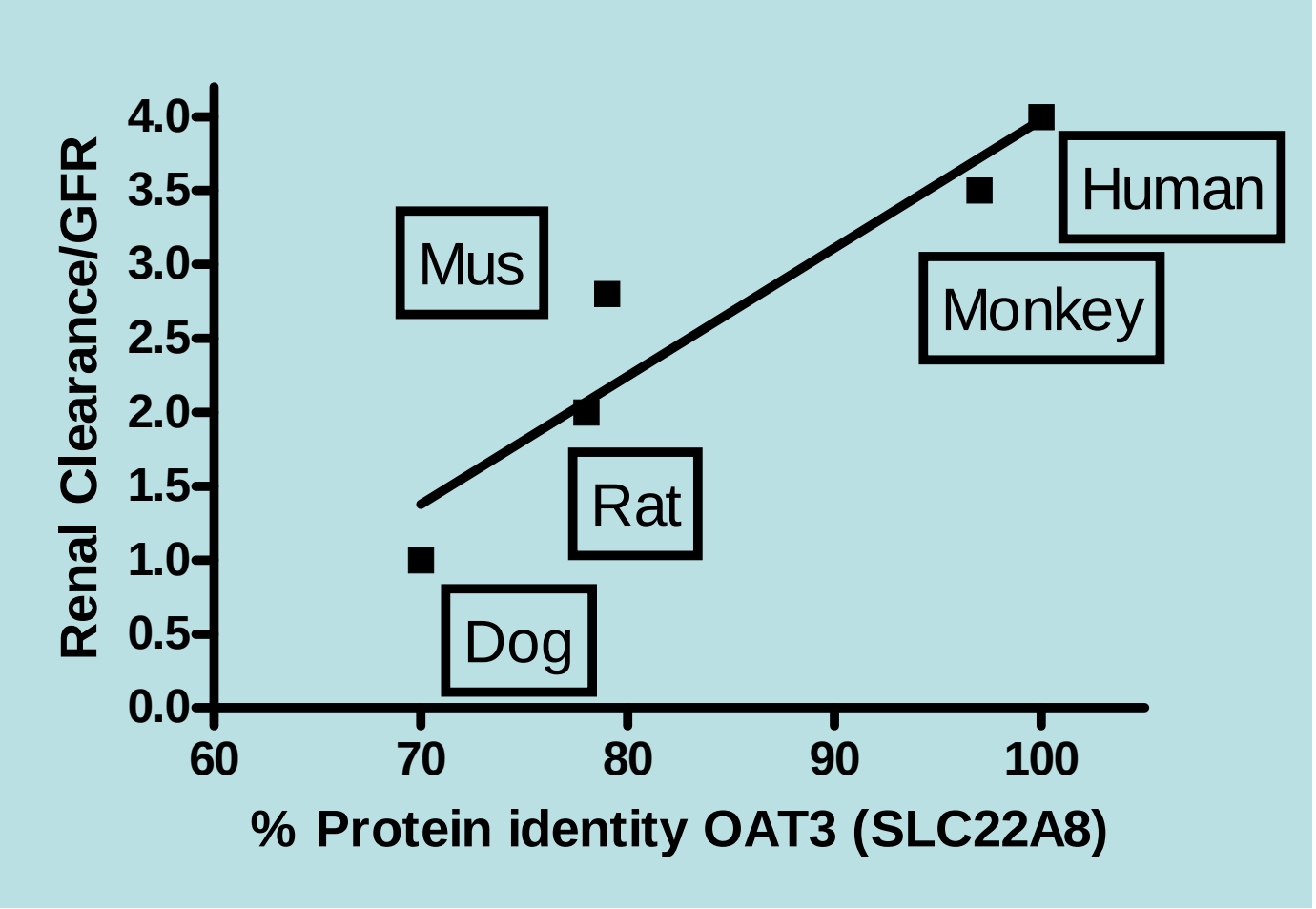
# PHA-288034 uptake in hOAT3 cells




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 $CL_R$



# Summary of PHA288034 Studies

Multi-tier approach appears to 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.

# For MW >400

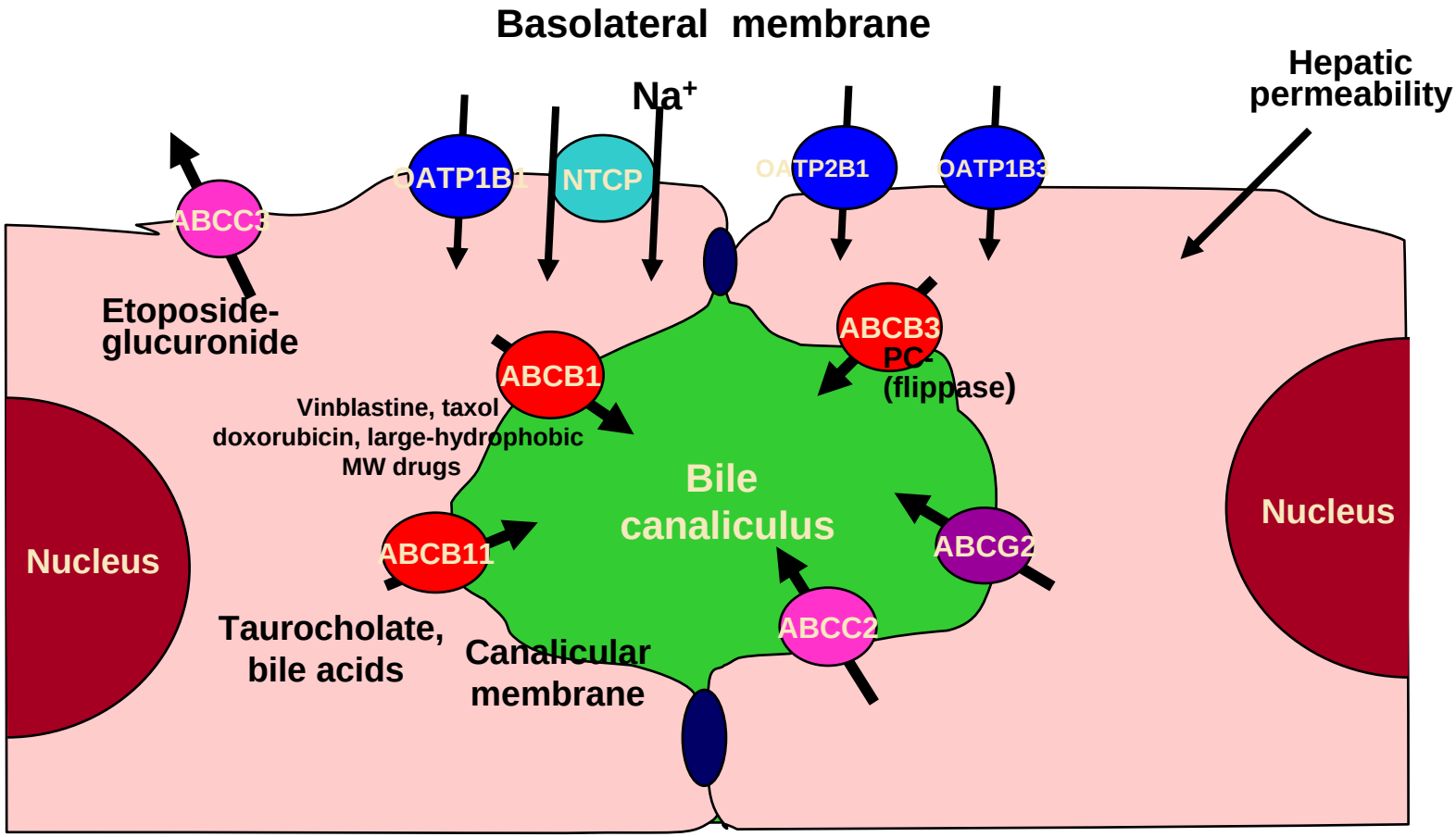
# Hepatic Transporters

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

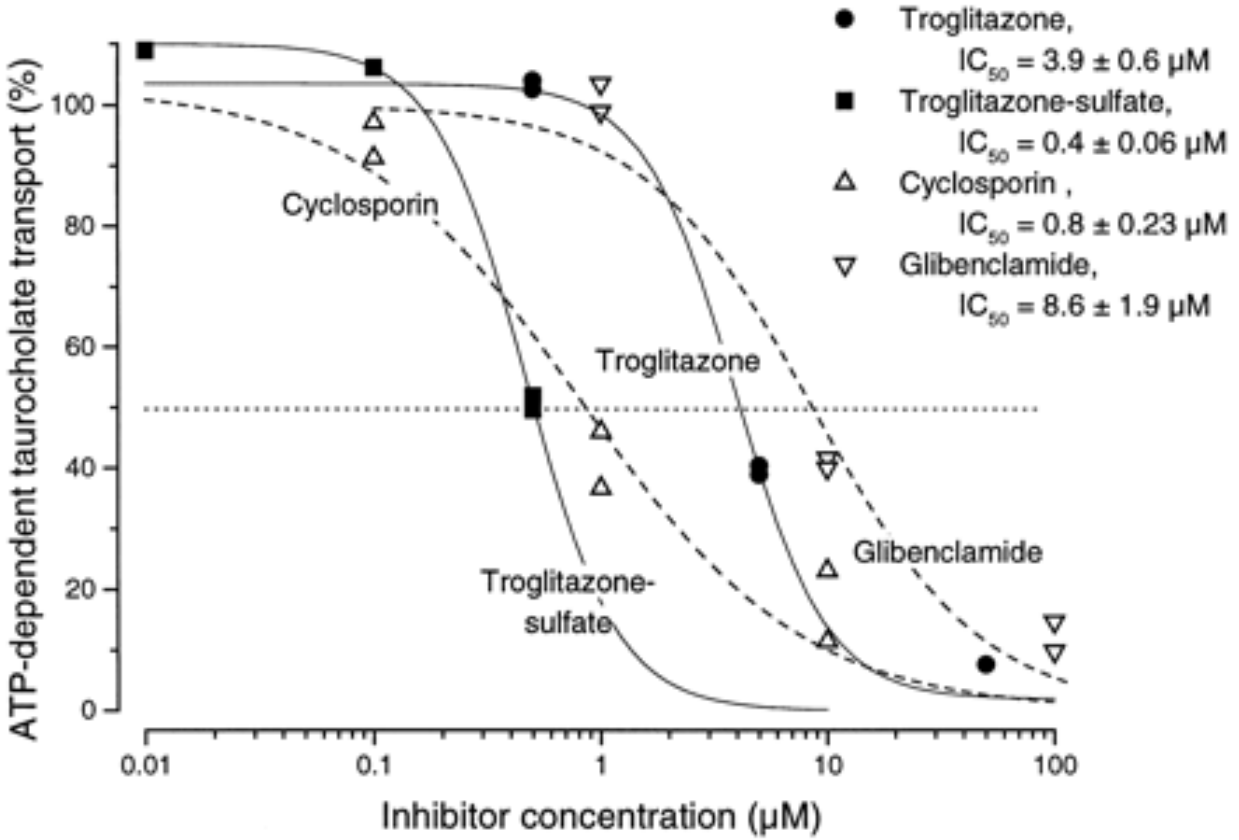
Question 2. Is it possible to predict the DDI potential mediated through hepatic uptake or efflux or are we only able to define potential mechanisms of a PK observation?

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

# Hepatic Uptake/Efflux Transporters



# Hepatic Transport and Liver Injury




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

# OATP Substrates

| OATP1B1 (OATP-C, LST-1, OATP2) | OATP1B3 (OATP8, LST-2) |
|---|---|
| **Endogenous Substrates:**<br>Estrone Sulfate, $PGE_2$, Bilirubin, thyroid hormone ($T_3$, $T_4$) Bilirubin-glucuronides Estradiol 17β-d-glucuronide, bile acids | **Endogenous Substrates:**<br>CCK-8, $PGE_2$ Thyroid hormone ($T_3$, $T_4$) Estradiol 17 β -d-glucuronide, Bile acids, Deltophin, DPDPE, |
| **Drug Substrates:**<br>Atorvastatin, Cerivastatin, Pravastatin Rosuvastatin, Pitavastatin, Caspofungin, Troglitazone-sulfate, Rifampin, Arsenic, Atrasentan, Valsartan, Olmesartan, Enalapril, MTX, Temocaprilat, SN-38 | **Drug Substrates:**<br>Pravastatin, Pitavastatin, Rosuvastatin,, Fexofenadine, BQ-123, Oubain,, Digoxin, Doxotaxel, Paclitaxel,, Rifampin, MTX, Bilirubin, Repaglinide, Telmisartan, Valsartan, Olmesartan, Enalapril, Temocaprilat, SN-38 |
| **Toxins:**<br>Phalloidin, Microcystin-LR | **Toxins:**<br>Phalloidin, Microcystin-LR |

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 ($P=4\times10^{-9}$). *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@ctsu.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.

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

N Engl J Med 2008;359.

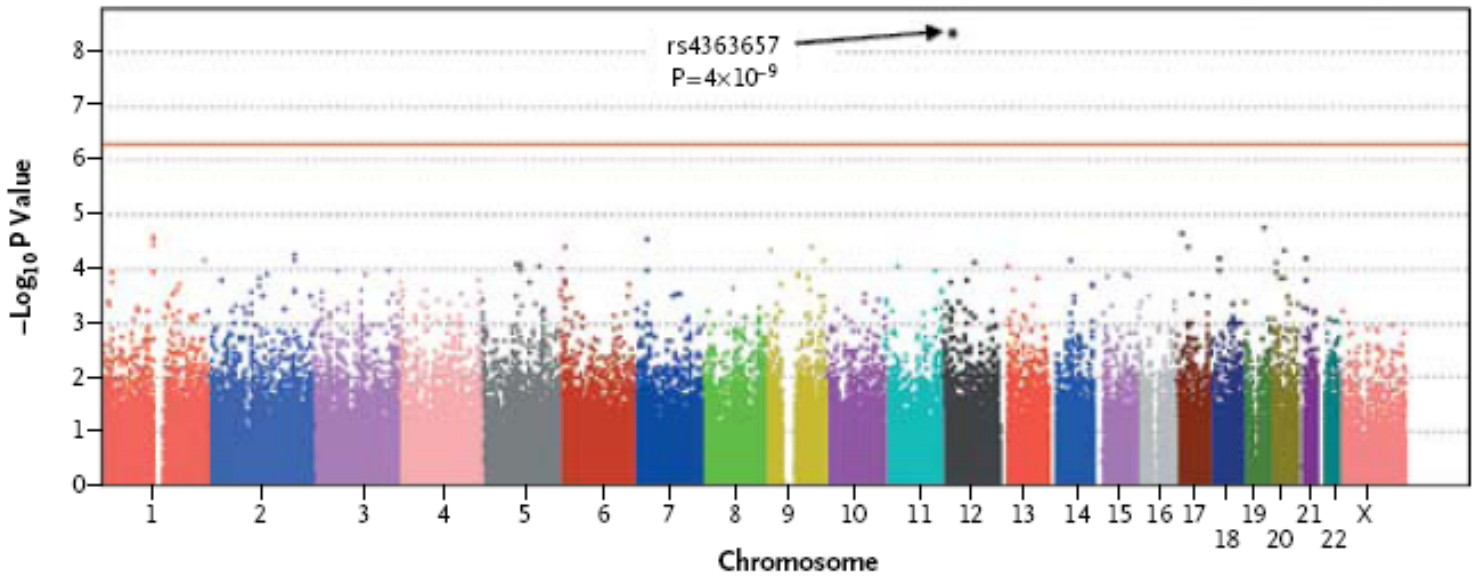



**Figure 1. Results of Tests for a Trend in the Association between Myopathy and Each SNP Measured in the Genome-wide Association Study.**

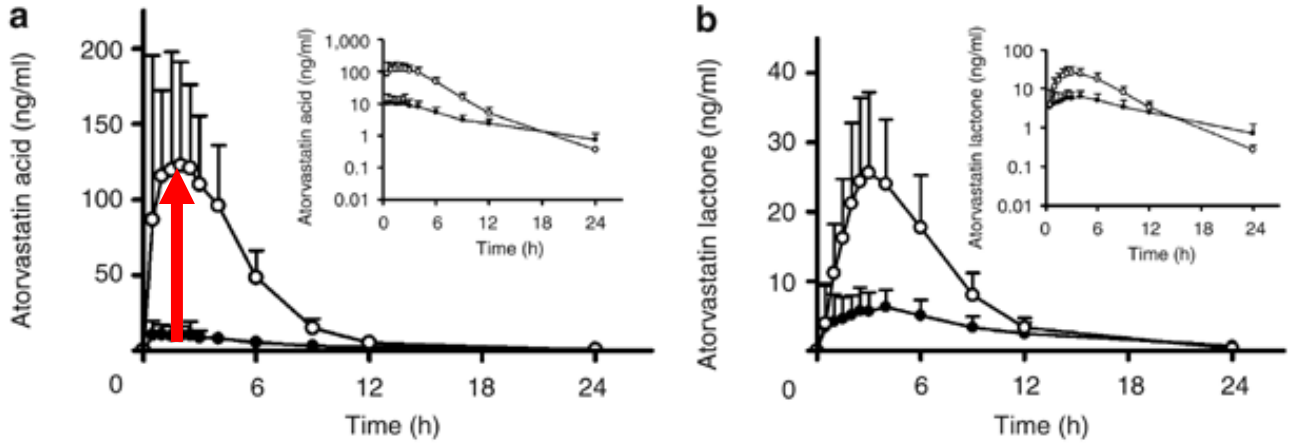
P values are shown for each SNP measured among 85 participants with myopathy and 90 matched controls who were taking 80 mg of simvastatin daily. Analyses are based on 316,184 of the 318,237 SNPs (99.4%) on the Sentrix HumanHap300-Duo BeadChip (Illumina). A result above the horizontal red line indicates strong evidence of an association ($P<5\times10^{-7}$).

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

# Hepatic Drug-Drug and Drug Transporter Interaction Potential

- Is NME 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 NME a substrate of uptake and efflux transporters
  - Multiplicity (ABCB1, ABCC2, and ABCG2)
- Uptake/efflux synergy

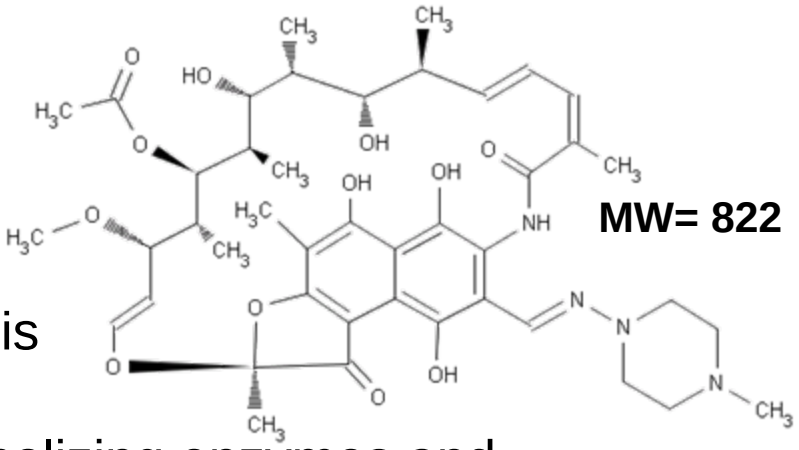
# Rifampicin Inhibits Atorvastatin through OATP



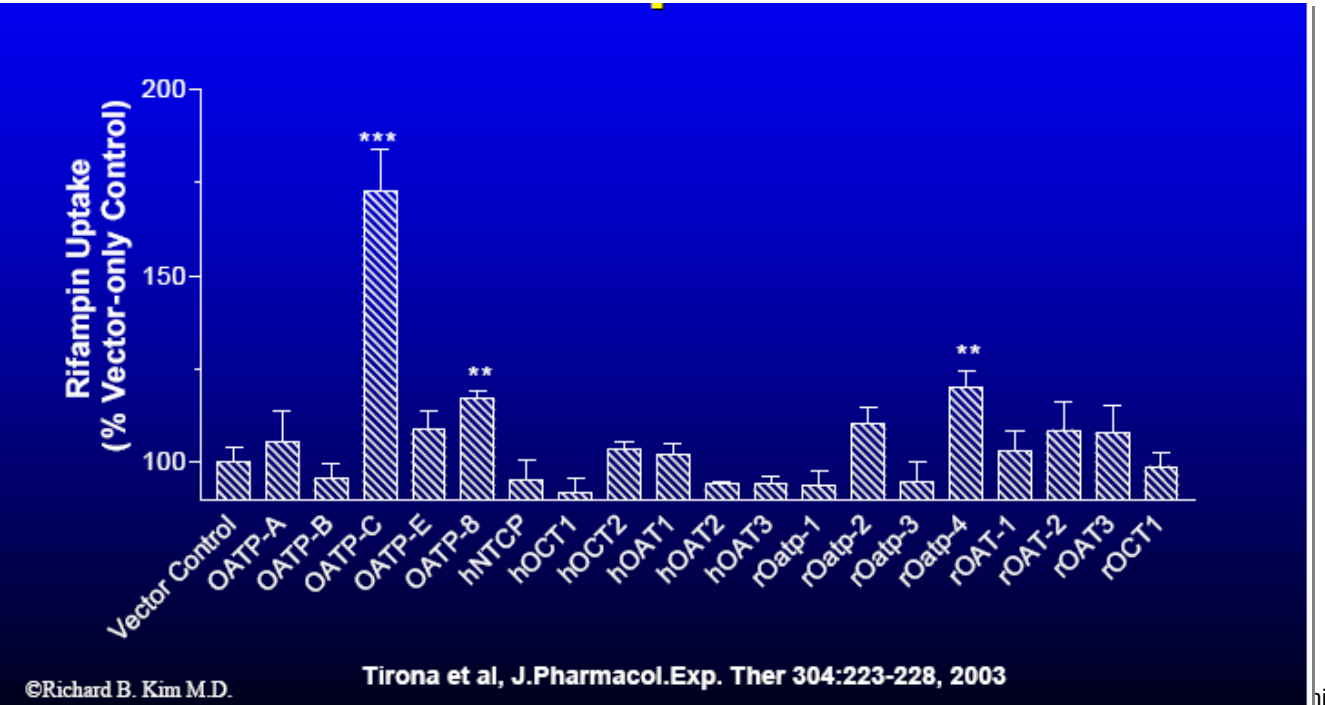

- 600 mg rifampacin IV increases atorvastatin acid AUC 7-fold.
- Acutely, single dose rifampacin may inhibit OATP1B3, CYP3A4, and CYP2C8.

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

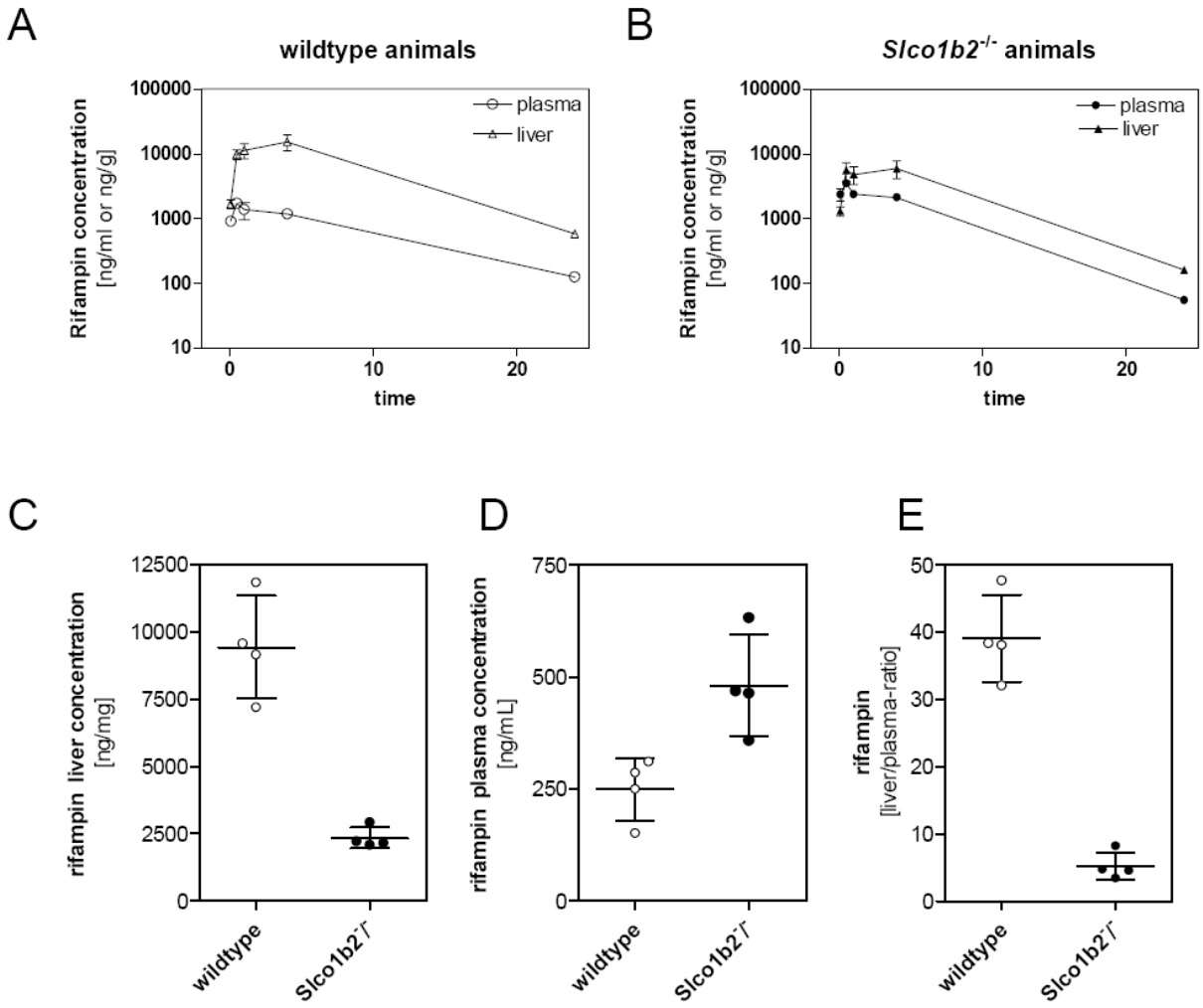
# Rifampicin




- 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

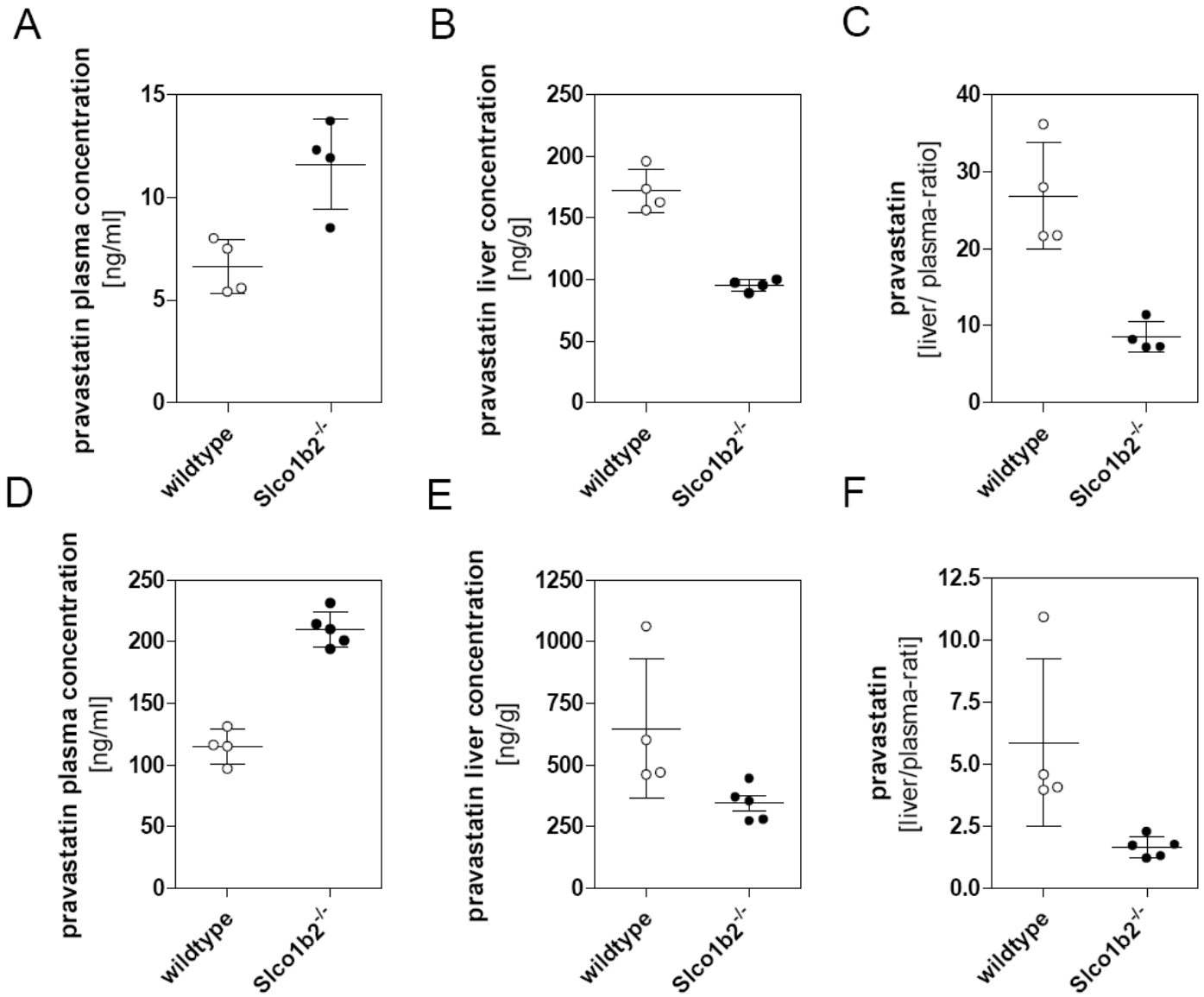


# Rifampacin Disposition in WT vs *Slco1b2*$^{-/-}$ KO Mice




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

# Pravastatin Css Dispositon in WT vs Slco1b2$^{-/-}$ Mice




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

# Ongoing work with *Oatp1b2* KO

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

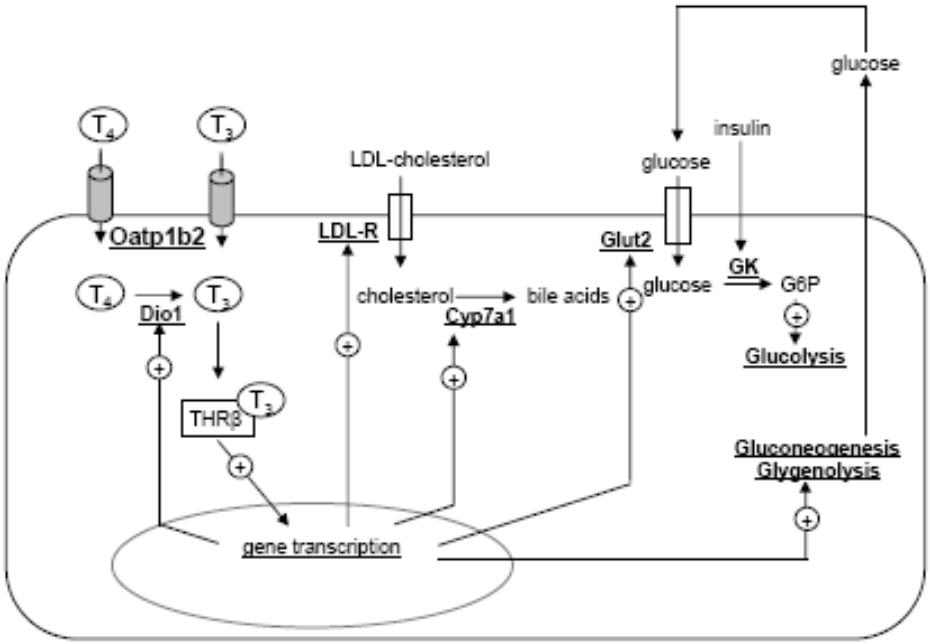



**Figure from Henriette E. Meyer zu Schwabedissen**

# 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.