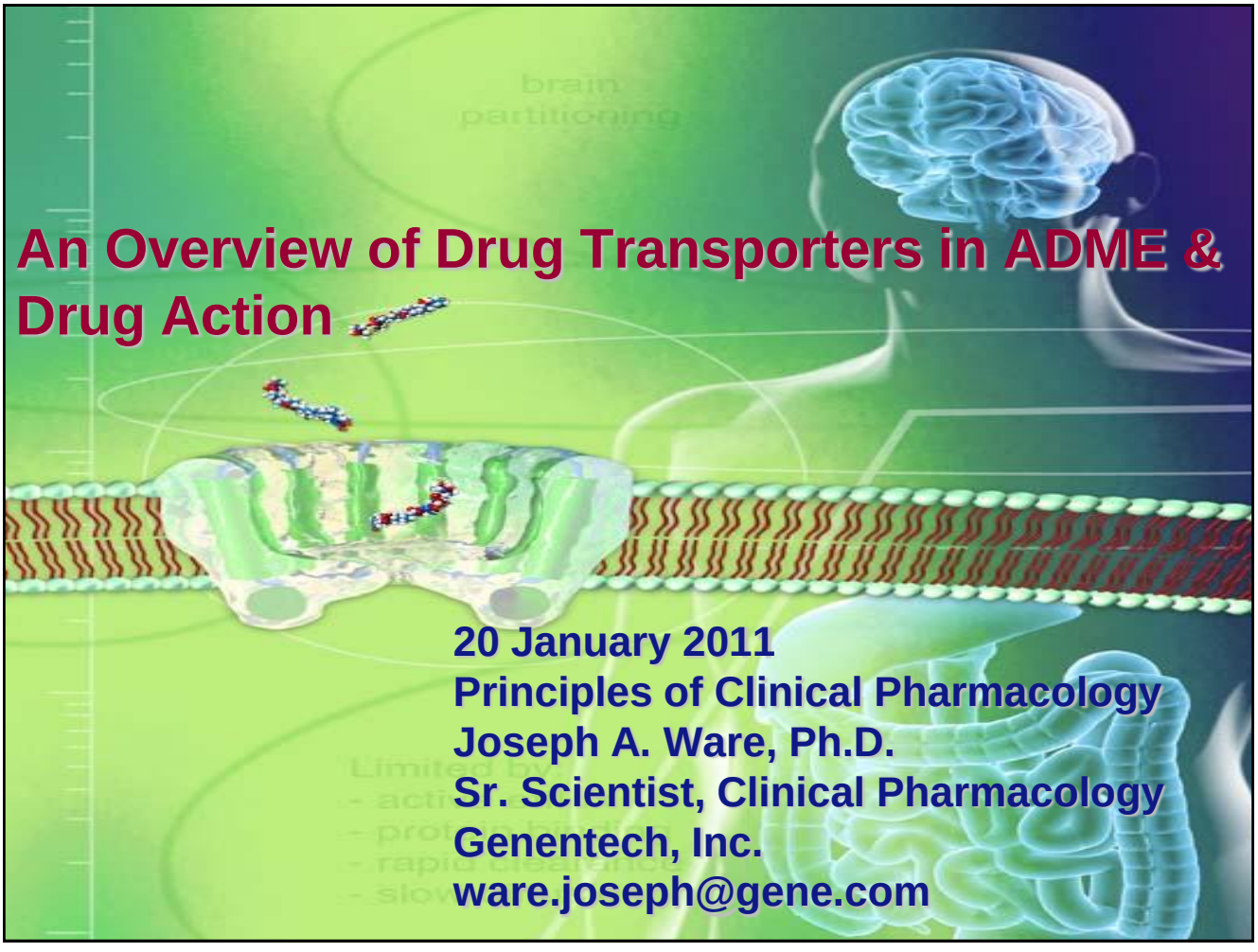

An Overview of Drug Transporters in ADME & Drug Action

20 January 2011
Principles of Clinical Pharmacology
Joseph A. Ware, Ph.D.
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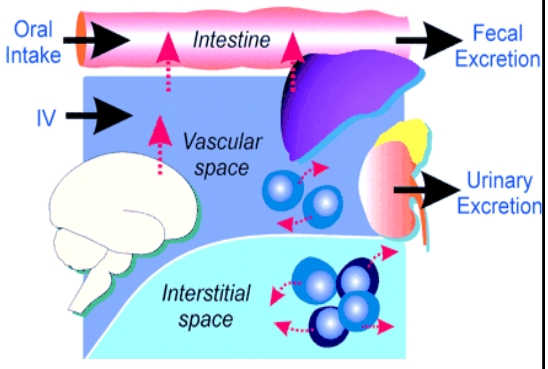
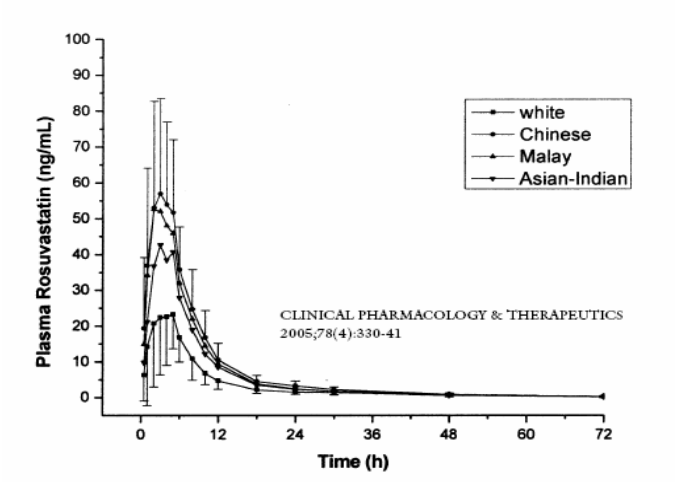
Implications of Drug Transport in Drug Discovery and Development

- 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).
- Transporters as Targets
  - Zosuquidar and Tariquidar
  - SGLT2 Na-Glucose contransporter



Rosuvastatin Calcium (Crestor) Pharmacokinetics and Prescribing Information



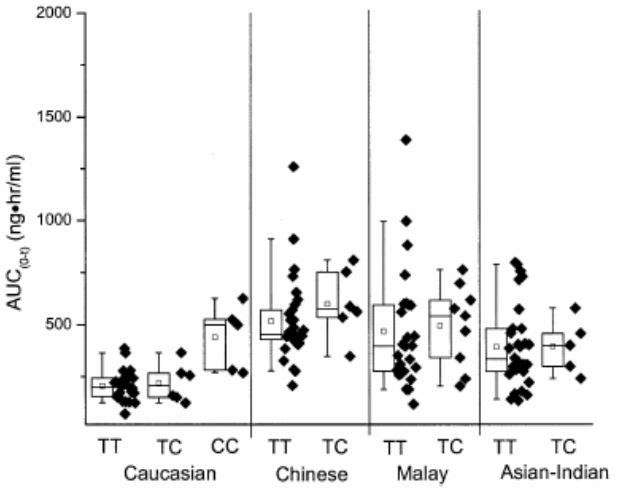
Rosuvastatin Calcium (marketed as Crestor) Information

FDA ALERT [03/2005]

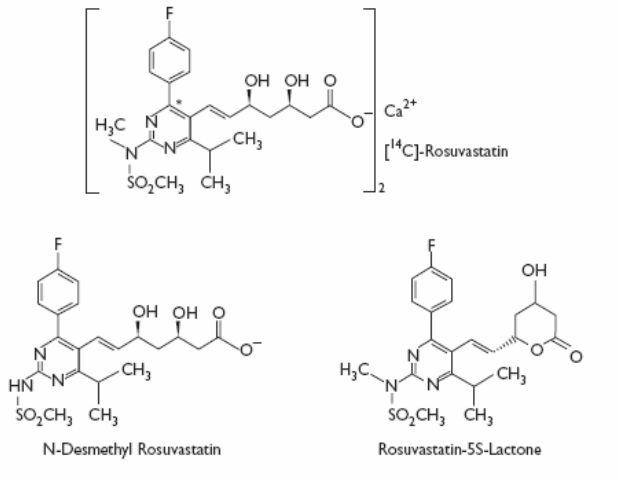
Rhabdomyolysis (serious muscle damage) has been reported in patients taking Crestor as well as other statin drugs. To date, it does not appear that the risk is greater with Crestor than with other marketed statins. However, the labeling for Crestor is being revised to highlight important information 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 *SLCO1B1* 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

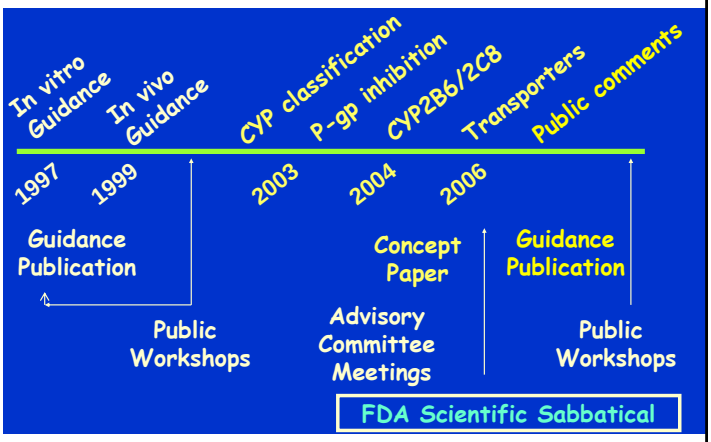
- Provide an Integrated approach to transporter biology
- Review when drug transport is the rate-limiting step of
  - **A**bsorption
  - **D**istribution
  - **M**etabolism and Transporter Interplay
  - **E**limination (kidney and liver)
- Examples of when drug transport is a primary determinant of drug action and drug-induced toxicity.
- Provide examples of drug-drug and drug-transporter interactions
- Functional consequences of genetic variations in transporter genes

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]/Ki > 0.1)
  - Inducer (40% control)

New Molecular Entity (NME)
International Transport Consortium (ITC)



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

Drug Transporter White Paper

Nature Reviews Drug Discovery 9, 215 - 236, (2010)

'Membrane Transporters in Drug Development'

The International Transporter Consortium.

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COMMENT

Transporters in drug development: advancing on the Critical Path

A new report from an international consortium provides comprehensive scientific recommendations for studies of transporter-related drug interactions in drug development.

In March 2004, the US FDA published a report[1] entitled "*Innovation or Stagnation: Challenges and Opportunities on the Critical Path to New Medical Products*". This paper focused on the concern that the timely translation of advances in biomedical research into more effective, affordable and safe innovative medical products was being impeded because drug development was becoming increasingly challenging, inefficient and costly. Recognizing that regulators have a key role to play in addressing this problem, the FDA launched the Critical Path Initiative to identify and prioritize the most pressing drug development issues and the greatest opportunities to address them, emphasizing the importance of collaboration among stakeholders.

In this issue, the International Transporter Consortium (ITC) — comprising experts in industry, academic groups and the FDA from the United States, Europe and Japan (see page 215) — presents a report[2] on membrane transporters in drug development that exemplifies the aims of the Critical Path Initiative. The report, which is based on discussions before, during and after a 2008 workshop supported by the FDA Critical Path Initiative and the Drug Information Association, has three goals. The first is to provide an overview of key transporters that are involved in drug absorption and disposition. The second is to provide examples of various technologies in studies of transporter-related drug–drug interactions, including computational methods that have been used to construct models for predicting such interactions. The third is to provide criteria for the design and conduct of clinical studies of transporter-related drug–drug interactions. These include decision trees to assist drug development scientists and regulatory personnel in determining when to conduct clinical studies to investigate transporter-related drug–drug interactions.

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doi:10.1038/nrd3124

Transporters and drug safety

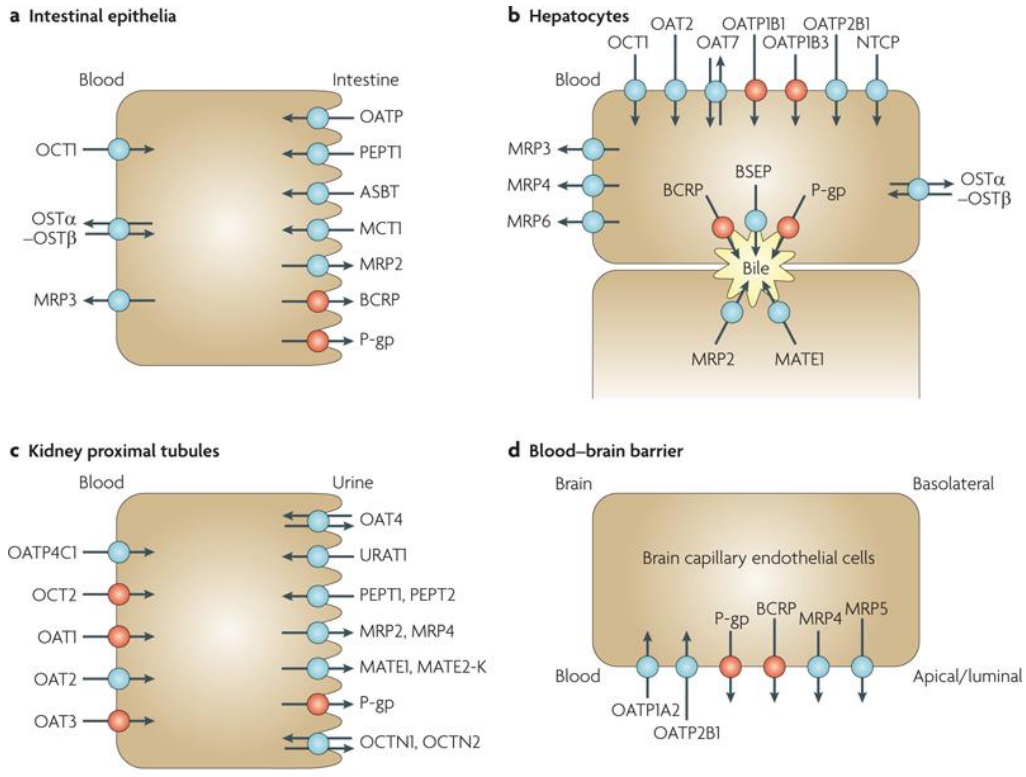
Drug–drug interactions are particularly important in the growing ageing populations in many countries, given the number of different drugs older people may be taking. For example, a recent survey indicated that more than 30% of the elderly population in the United States takes at least five prescription drugs at any given time[3]. Drug–drug interactions can result in reduced efficacy or increased toxicity. Indeed, several drugs that have been withdrawn from the US market for safety reasons — such as terfenadine, astemizole and cisapride — demonstrated major drug–drug interactions.

Many of these drugs are metabolized by cytochrome P450 3A4 (CYP3A4), which has been estimated to be involved in the metabolism of ~50% of prescription drugs and is therefore a common cause of drug–drug interactions. Recent data suggest that transporters may also contribute to drug safety issues. For example, another withdrawn drug, mibefradil, which in combination with simvastatin caused several cases of rhabdomyolysis[4], is an inhibitor of transporters such as P-glycoprotein, as well as of CYP3A4. It is possible that transporter-mediated drug–drug interactions may have played a part in this serious adverse drug reaction.

Further evidence of the important role of transporters in drug safety was provided by a genome-wide association study showing that particular polymorphisms in the liver transporter protein organic anion transporting polypeptide 1B1 (OATP1B1) increase the risk for statin-induced myopathy[5]. Given this, the FDA has also recently revised the drug label for atorvastatin to include information that atorvastatin and its metabolites are substrates of OATP1B1. The label also states that the daily dose should not exceed 10 mg when given with cyclosporine, which is a nonspecific inhibitor of transporters including P-glycoprotein and OATP1B1.

In addition, recent new drug applications (NDAs) have included information on OATP1B1, which have been incorporated in the drug labels of approved new molecular entities (NMEs). For example, eltrombopag, a thrombopoietin receptor agonist, was recently approved for the treatment of thrombocytopaenia in patients with chronic immune (idiopathic) thrombocytopaenic purpura who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy. The label for eltrombopag, which is an inhibitor of OATP1B1, notes the importance of monitoring patients for potential overexposure to other drugs that are substrates of OATP1B1. It is also important to highlight when a certain drug interaction is not present or expected.

The ITC considers this report as a work in progress, and is highly interested in obtaining feedback, including areas that have not been included in this report but should be considered in the next version as well as controversial concepts. **Please send any comments to the corresponding authors**.



Transporters covered

- Efflux: P-gp, BCRP
- Renal: OAT/OCT
- Hepatic uptake: OATPs

Nature Reviews | Drug Discovery

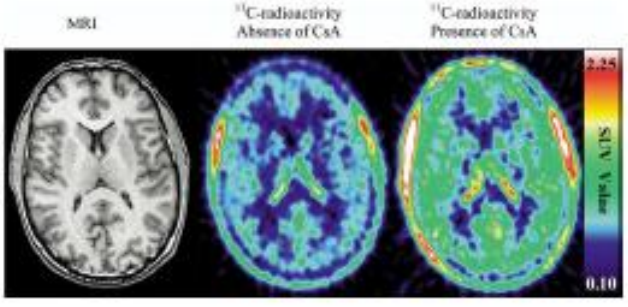
NIH Principles in Clinical Pharmacology Transporter Biology 20 January 2011

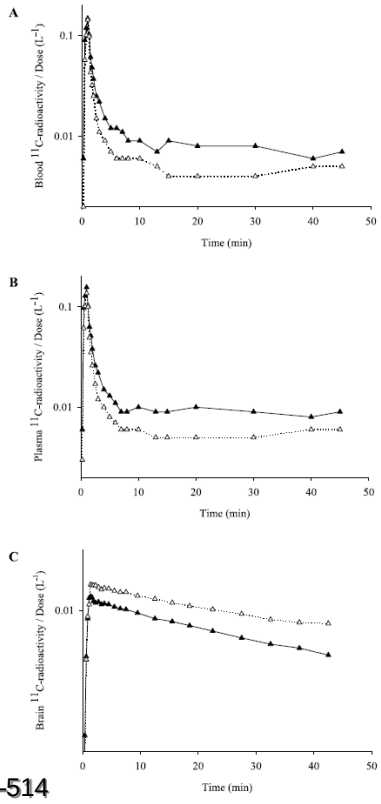
P-glycoprotein Substrates

- 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 [^{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

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%

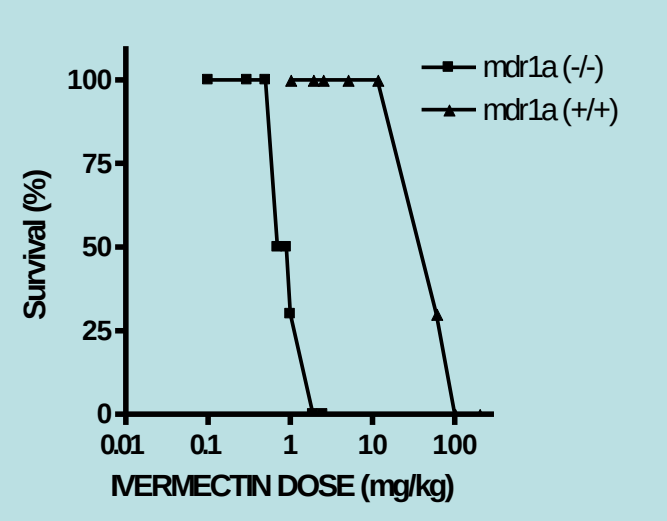
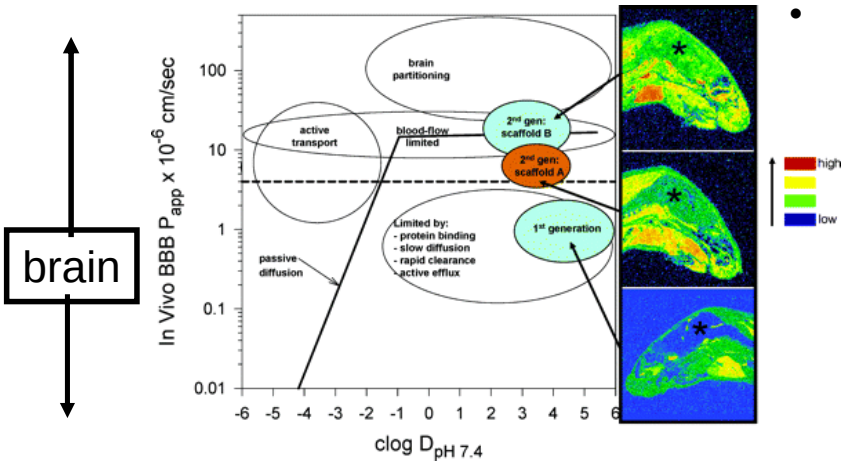


Figure from A.H. Schinkel et al., Cell, Vol.77, 491-501, 1994

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

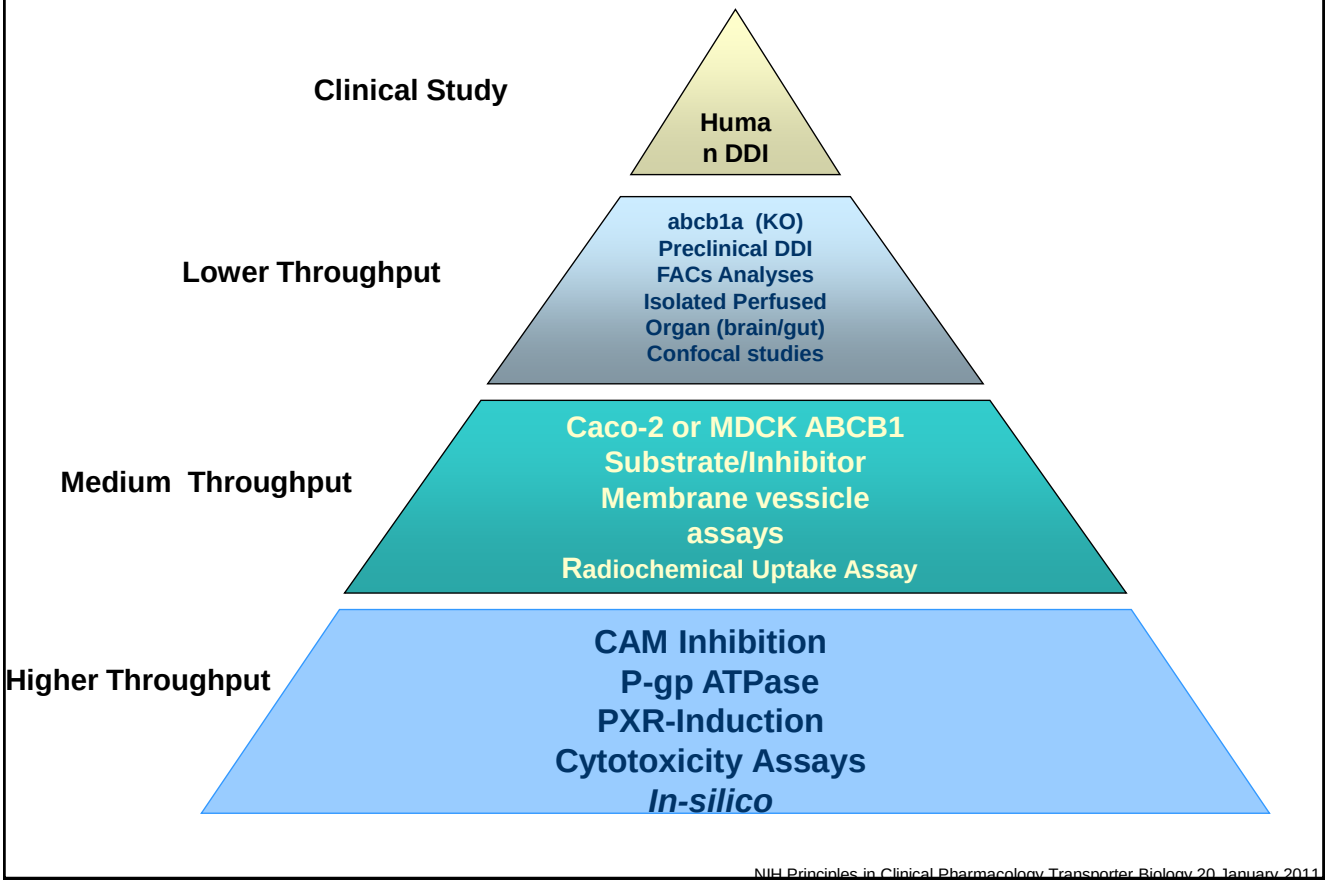
Ivermectin Toxicity in the Collie

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

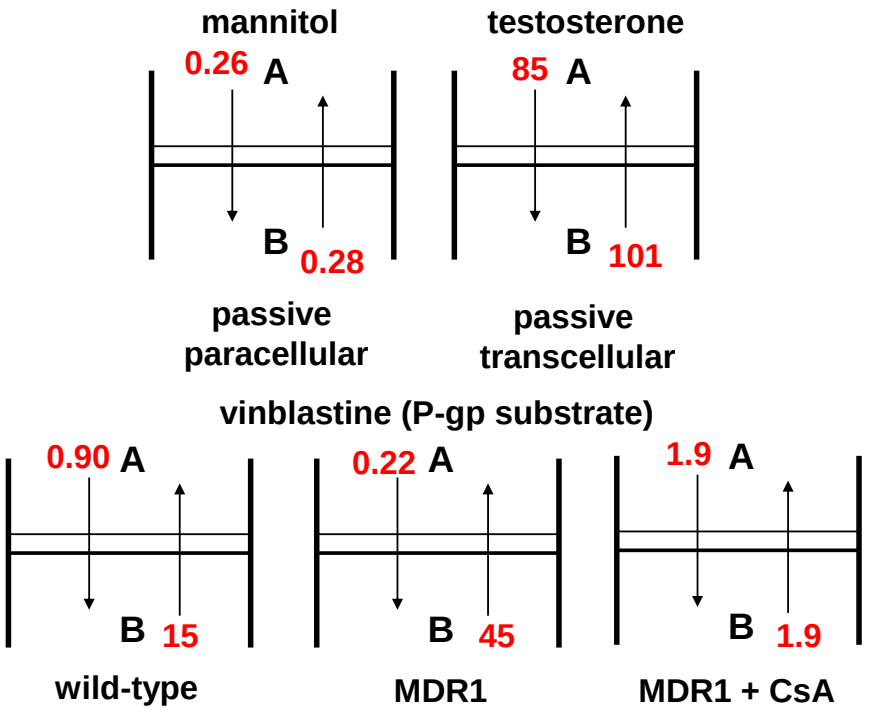
- 50% of Collies display CNS toxicity when treated with normal doses of IVM (>60 μg/kg).
- Ivm-sensitive Collies lack functional P-gp at the blood brain barrier.
- ABCB1 cDNA sequencing
  - Sensitive Collies (7/7)
    - 4-base pair deletion
    - homozygous
  - Non-sensitive Collies (6/6)
    - heterozygous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

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

P-glycoprotein (ABCB1) Cluster Evaluation



In Vitro Permeabilities



Caco-2 and MDCK cell comparison

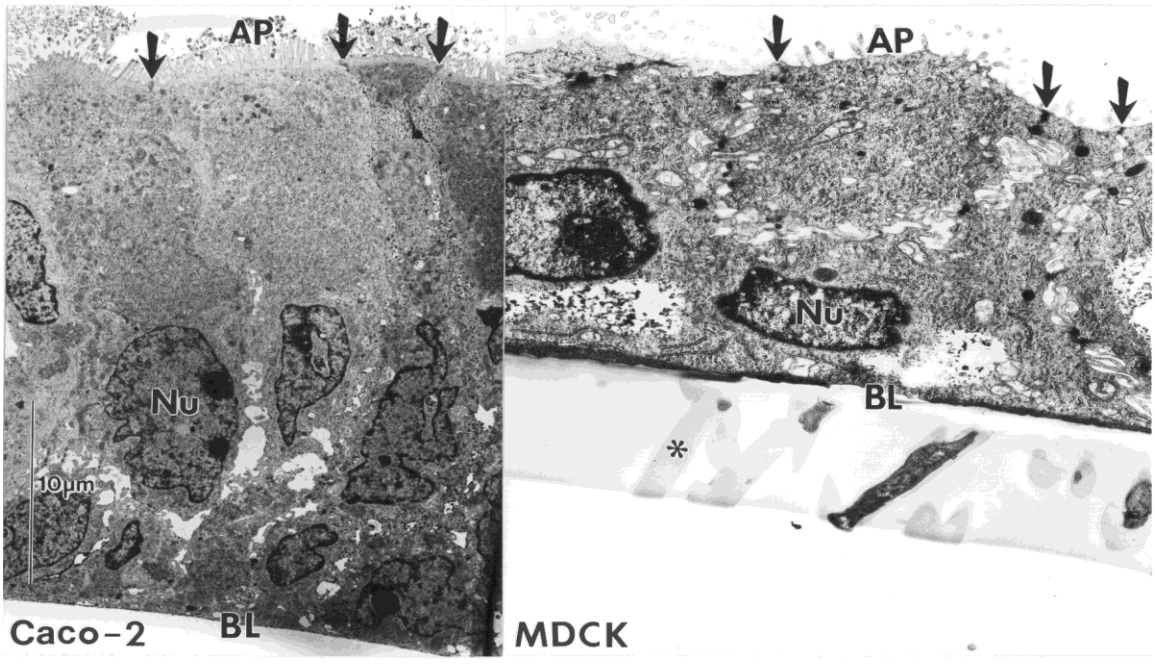
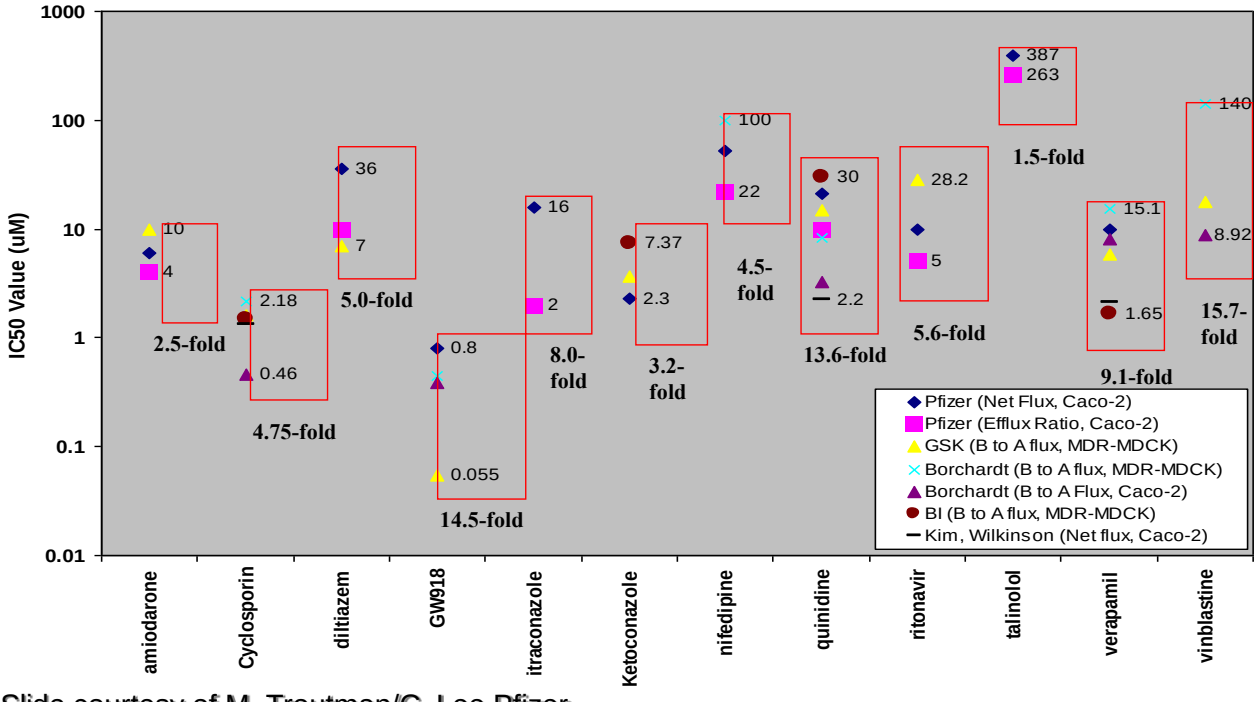


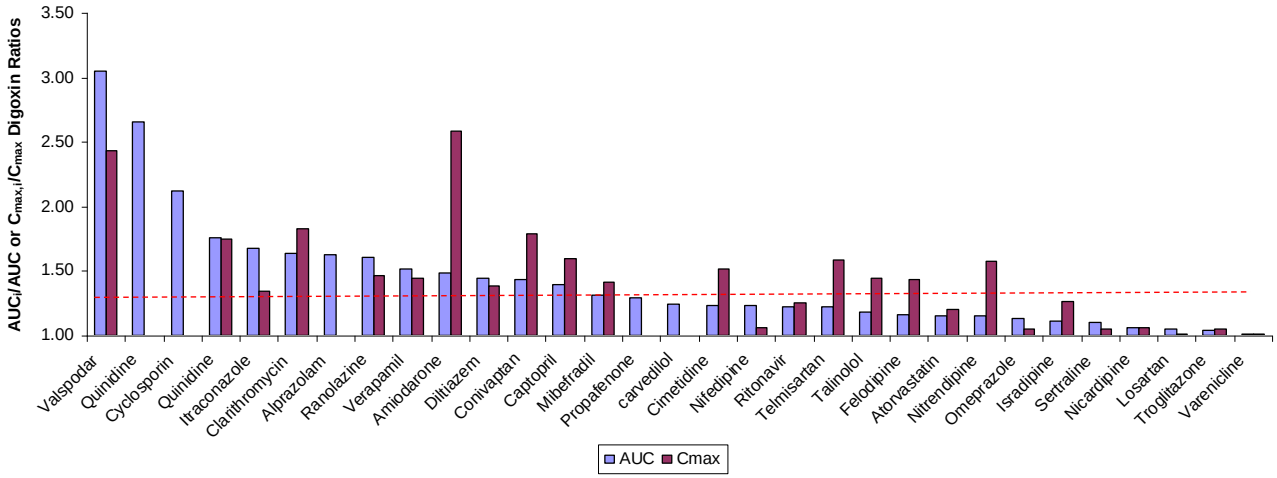
Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub

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



Slide courtesy of M. Troutman/C. Lee Pfizer

Digoxin: Safety Concerns



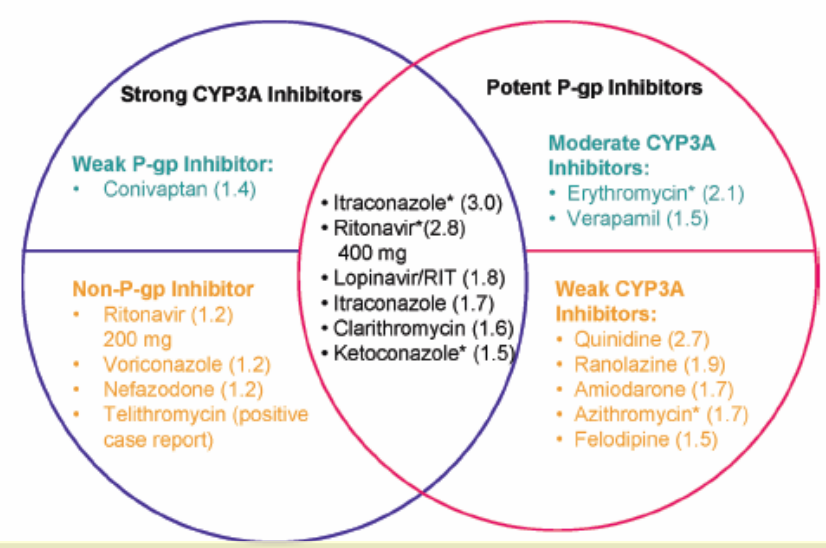
- **Therapeutic conc ~ 1.5 ng/mL**
- **33% change in Digoxin Exposure (C_{max}) ~ 2.0 ng/mL → Safety concerns**
- **25% change in exposure might be clinically relevant**

Fenner et al., Clinical Pharmacology & Therapeutics (2009); 85, 173–181

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

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 itraconaz represents the worse case scenario for a Clinical DDI study

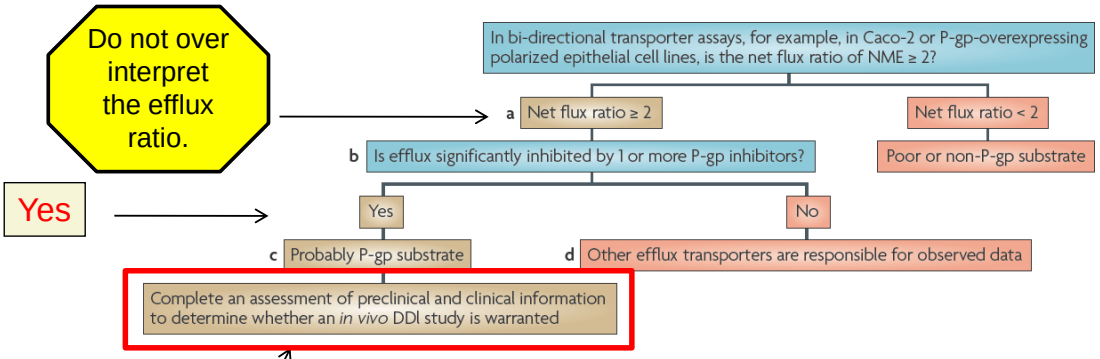
Mol. Pharmaceutics, 2009, 6 (6), pp 1766–1774

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**

Pgp/BCRP Substrate Decision Tree

Do not over interpret the efflux ratio.



This figure shows a decision tree for P-gp and a similar tree could be used for BCRP. Although flux systems have traditionally been used to determine whether an NME is a substrate of P-gp or BCRP, inside-out vesicles expressing P-gp or BCRP, or transfected cell monolayers grown on solid support with appropriate controls (for example, inhibitors and positive controls) as described in Section 2, also can be used.

Yes

However, fraction absorbed in humans and preclinical species is >90%. Thus a clinical study not required.

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

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

Slide courtesy from Joe Polli and ITC

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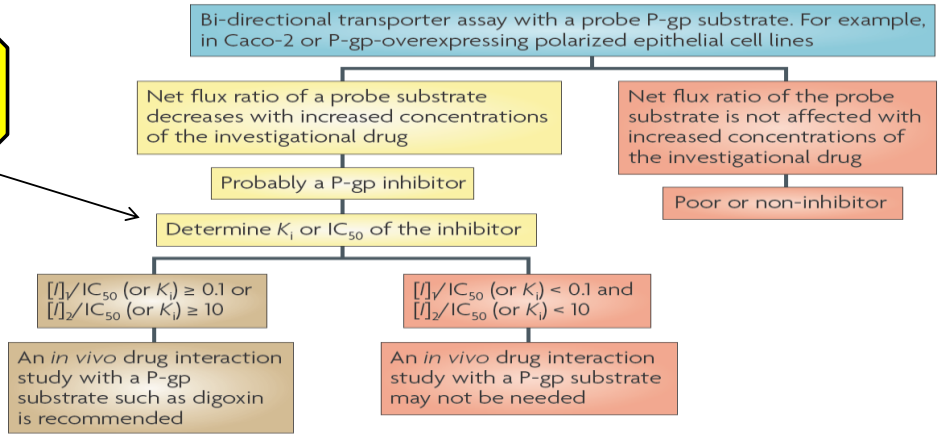
Pgp/BCRP Inhibitor Decision Tree

Need to 'calibrate' in vitro systems using clinical data



- False Positives (unnecessary clinical studies)
- Alert for $[I]_1/IC_{50} \geq 0.1$ **or** $[I]_2/IC_{50} \geq 10$,
  - $[I]_1$ is steady-state total Cmax at the highest clinical dose
  - $[I]_2$ is the GI concentration calculated as dose (mg)/250 mL
- $[I]_2/IC_{50} > 10$ will be exceeded at a dose of ~12 mg for a drug with an inhibition potency of ~10 µM *in vitro* (MW ~ 500).
- False Negatives (safety concerns for NTI drugs like digoxin and topotecan)

Special Cases

Slide courtesy from Joe Polli and ITC

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_4
- lysotracker (green)
- H33342
- Rhodamine 123
- Bodipy-prazosin
- Riboflavin (vitamin B2)

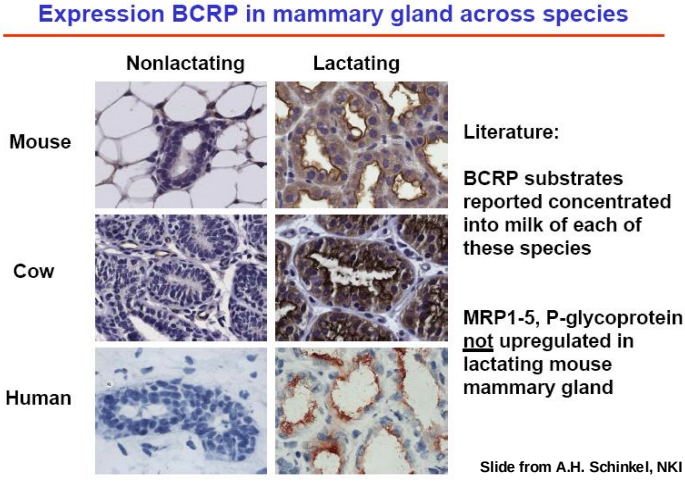
Inhibitors

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

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

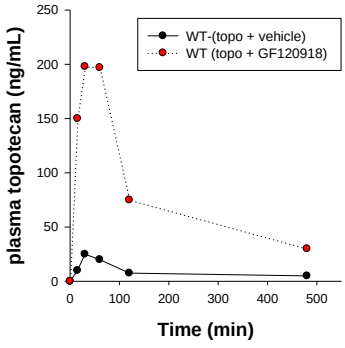
Jonker et al., *Proc Natl Acad Sci* U S A 2002 Nov 26;99(24):15649-54

- Bcrp -/- ADME Phenotype
  - Mice displayed diet-dependent phototoxicity
  - Protoporphyria
  - 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

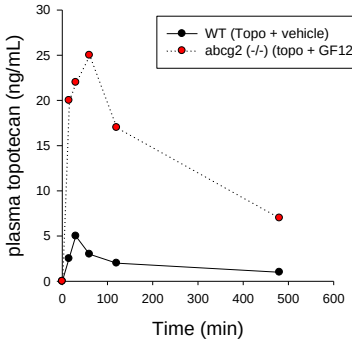


Of mice and men: Topotecan:BCRP interaction

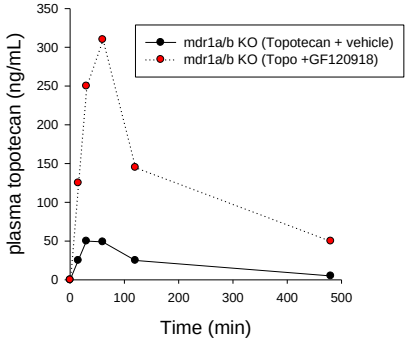
Jonker et al., JNCI, 2000



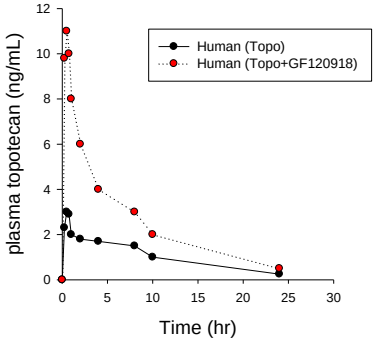
Jonker et al., PNAS, 2002



Jonker et al., JNCI, 2000



Kruijtzer et al., JCO, 2002



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Absorption, metabolism, and excretion of salicylazosulfapyridine in man

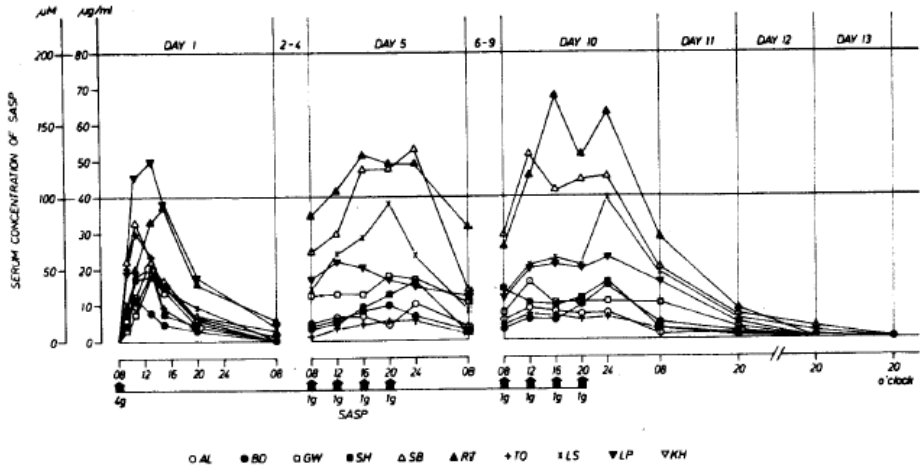
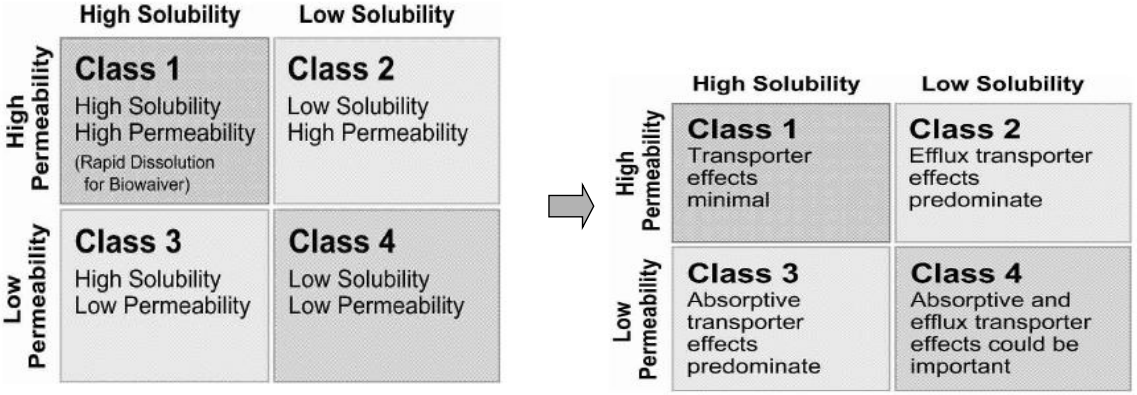


Fig. 2. Serum concentrations of SASP after ingestion of a single 4 Gm. dose of SASP on Day 1 (10 subjects) and 4 × 1 Gm. of SASP on Days 2 to 10 (9 subjects).

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

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



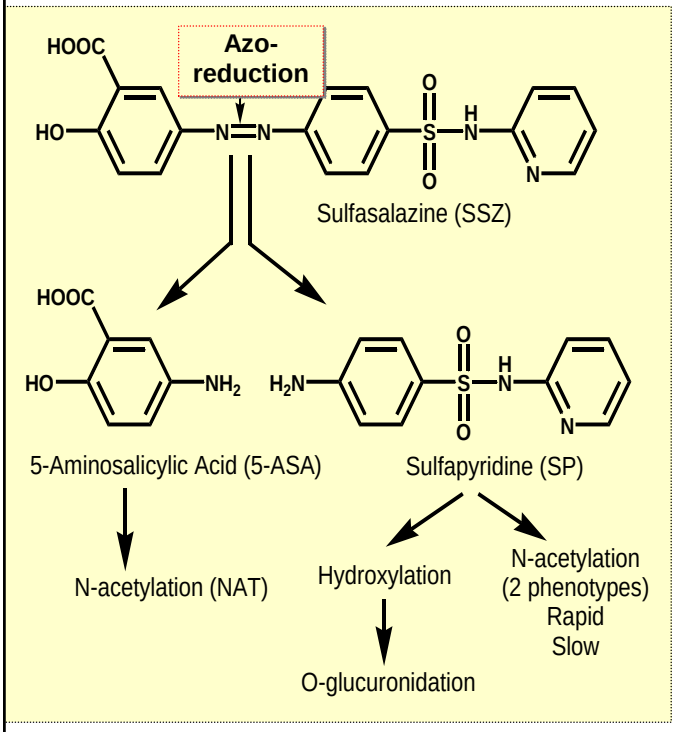
Amidon et al., Pharm. Res. 12:413 (1995)

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

Sulfasalazine (SASP) Hypothesis

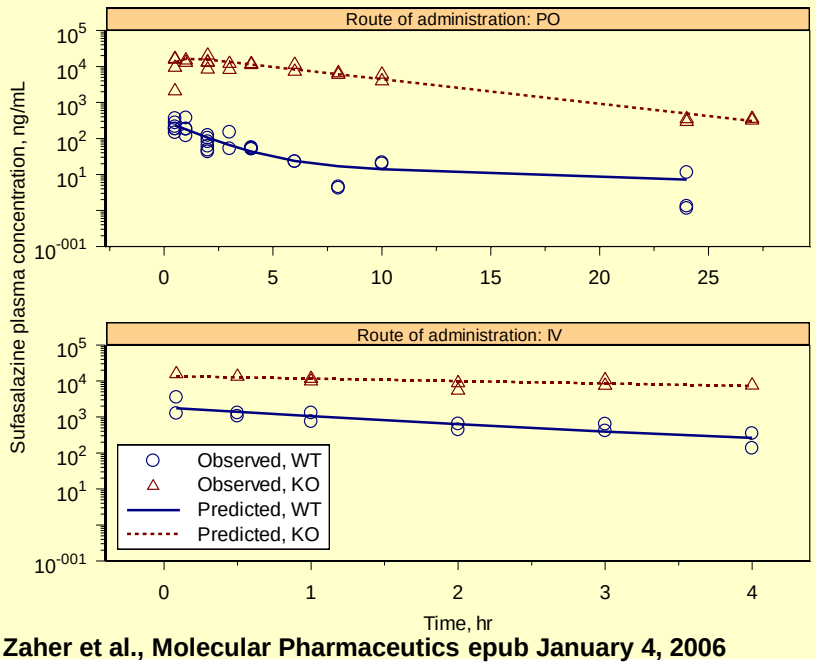
Inter-individual differences in intestinal expression and function of ABCG2 (BCRP) contribute to variability in drug bioavailability, exposure and pharmacological response to SASP.

Sulfasalazine (SASP) Disposition



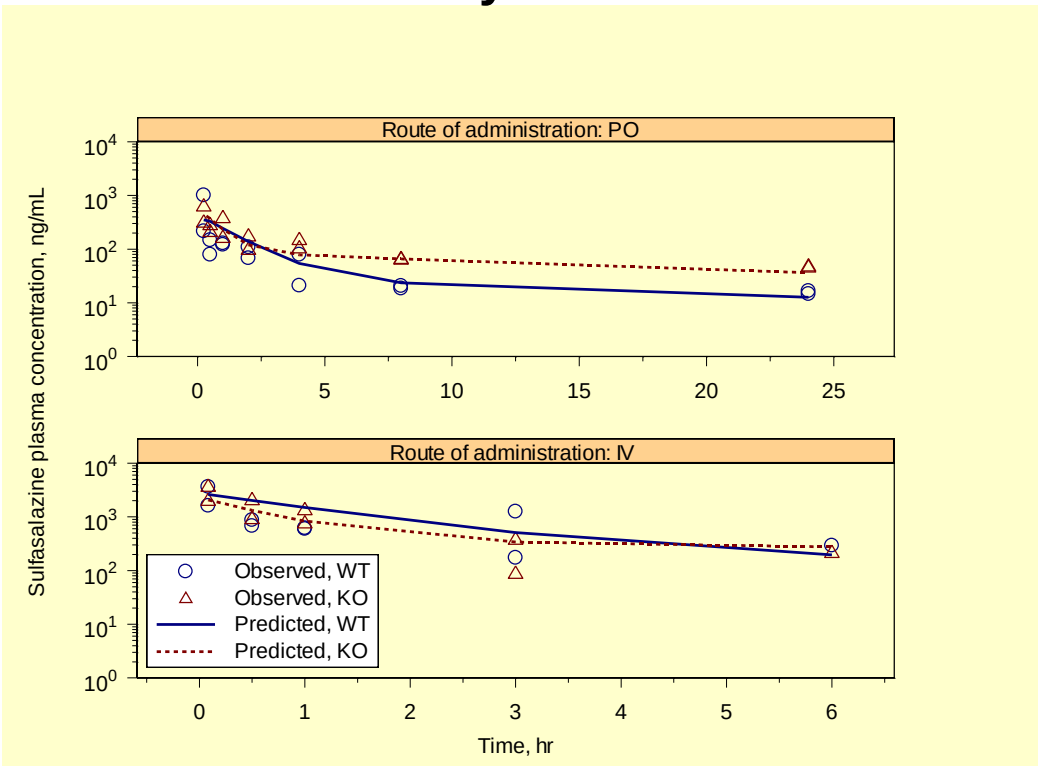
- **Indications: Rheumatoid arthritis (RA), Long term therapy of ulcerative colitis, and Crohn's disease**
- **Bioavailability (F) of SASP in humans is low (F< 15%) and highly variable**
- **Low %F primarily attributed to SASP's low permeability and poor solubility (thus, poor absorption)**
- **Azo-reduction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



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

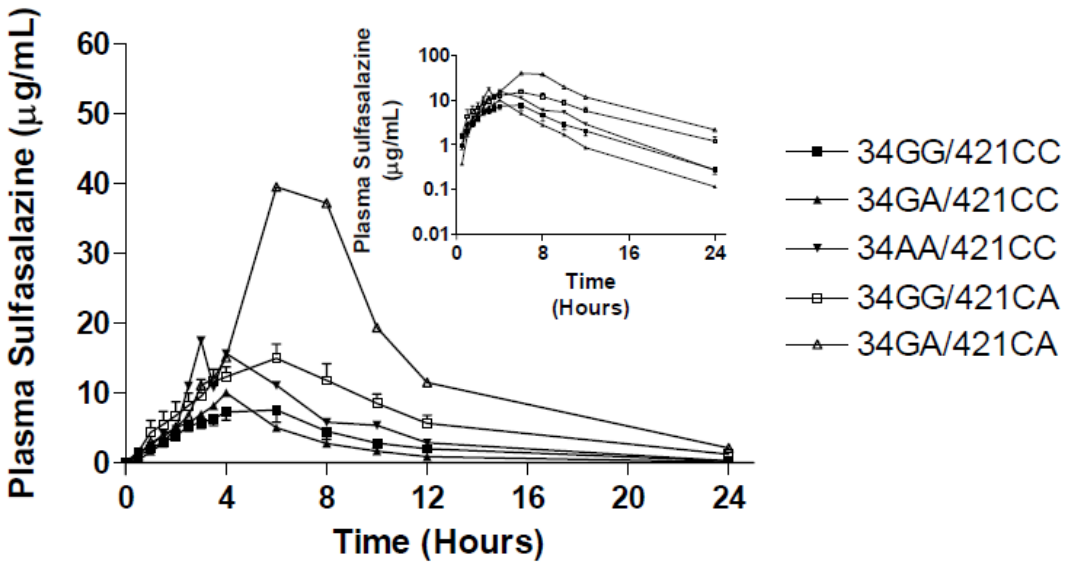
Mice	Route	Dose (mg/kg)	C_{max} (ng/mL)*		AUC (ng.hr/mL)			Relative exposure, AUC_{KO}/AUC_{WT}
			WT	KO	Duration (hr)	WT	KO	
Bcrp1	IV	5	1827	13570	0-4	3015	40343	**13**
	PO	20	233	16176	0-24	1189	131822	**111**
Mdr1a	IV	5	2749	2266	0-6	5131	3504	**1**
	PO	20	349	440	0-24	1098	1781	**2**

* 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.

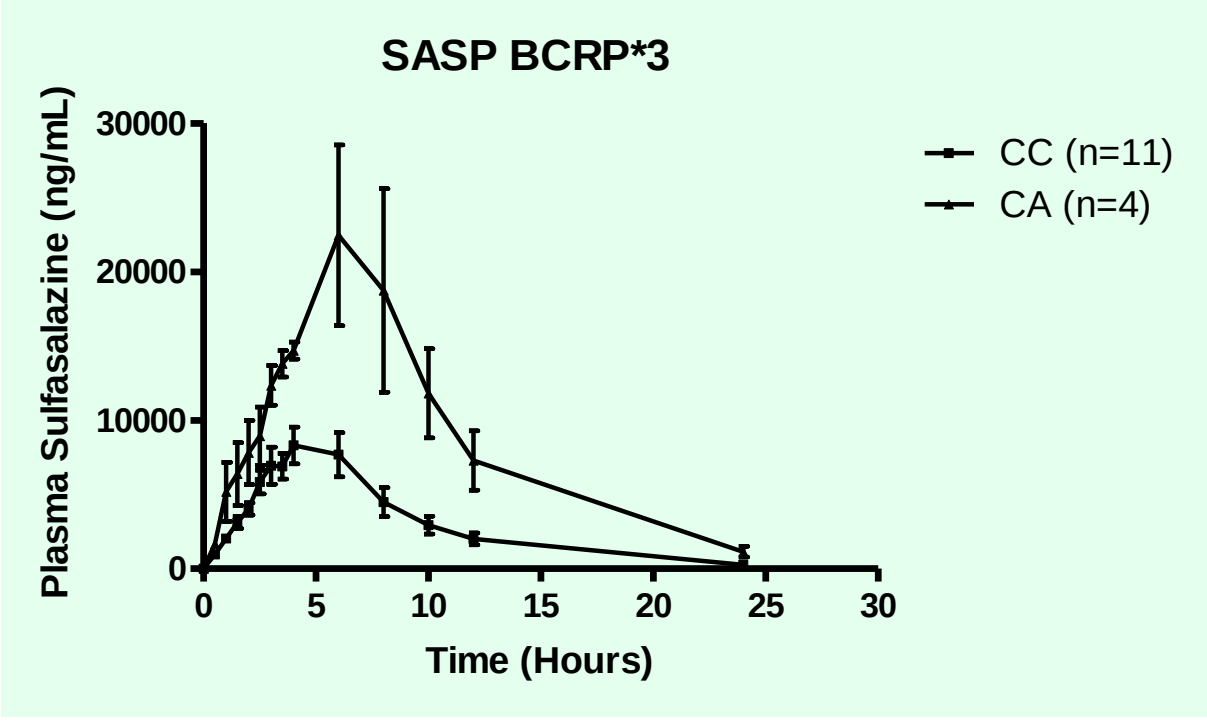
Zaher et al., Molecular Pharmaceutics epub January 4, 2006

SASP Disposition in North American Healthy Volunteers



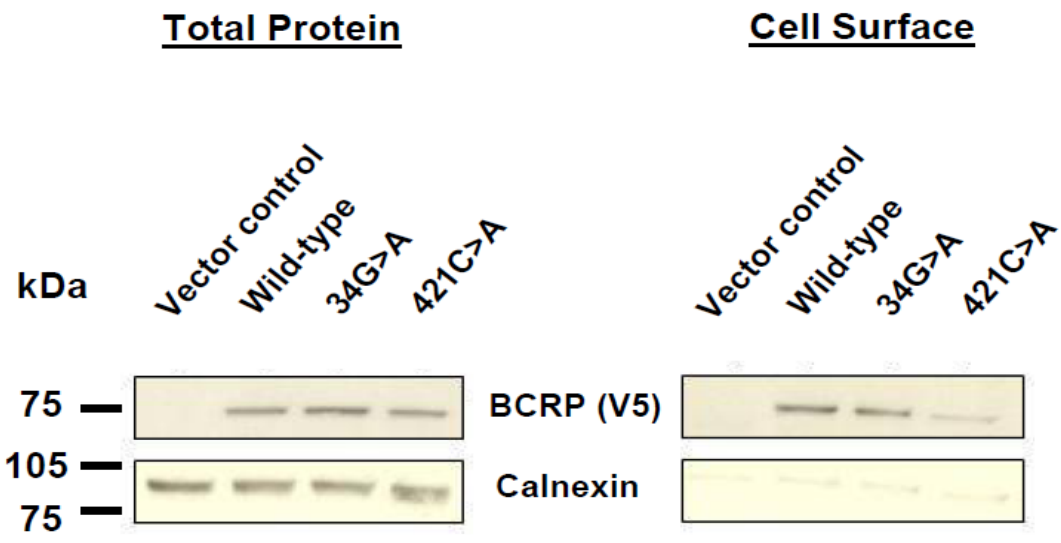
Brad Urquhart et al., Pharmacogenet Genomics. 2008 May;18(5):439-48.

Altered SASP Exposure in Q141K Subjects



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

421C>A SNP Changes Surface ABCG2 Expression



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

SASP Disposition in Healthy Japanese Volunteers

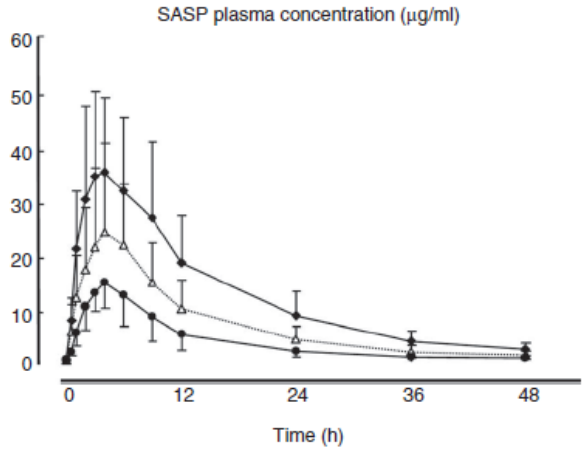
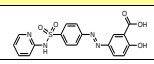
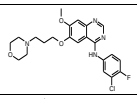
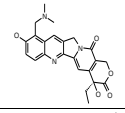
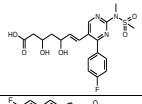
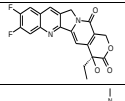
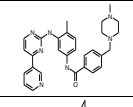
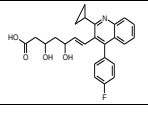


Figure 2 Effect of *ABCG2* genotype on pharmacokinetics of sulfasalazine (SASP). Plasma concentration-time profiles of SASP after oral administration of a 2,000 mg conventional SASP tablet to 421C/C subjects (closed circles, $n = 12$), 421C/A subjects (open triangles, $n = 16$), and 421A/A subjects (closed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

Yamasaki et al., CPT January 2, 2008

ABCG2 Pharmacogenomic Studies

Formulation	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

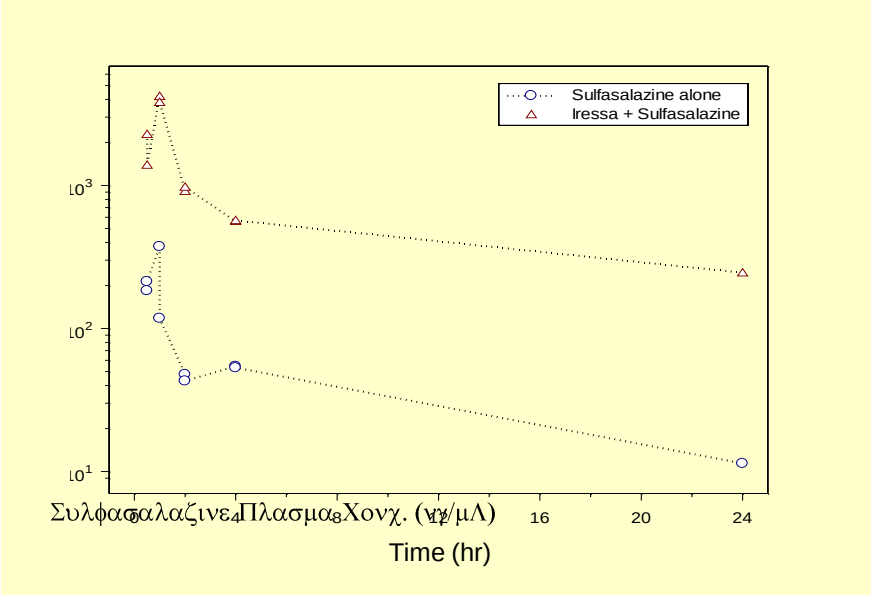
ABCG2 Polymorphisms and Ethnic Distribution of SNPs.

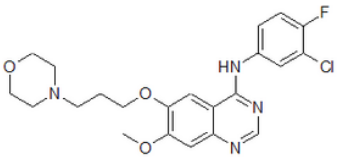
- The ABCG2 Q141K genotype significantly affected the pharmacokinetics of diflomotecan (Clin Pharmacol Ther. 2004)
- Gefitinib-induced diarrhea correlates with Q141K (J Natl Cancer Inst. 2006).
- ABCG2 expression correlates with flavopiridol-induced myelotoxicity.

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

Gefitinib (Iressa)-enhanced SASP Bioavailability

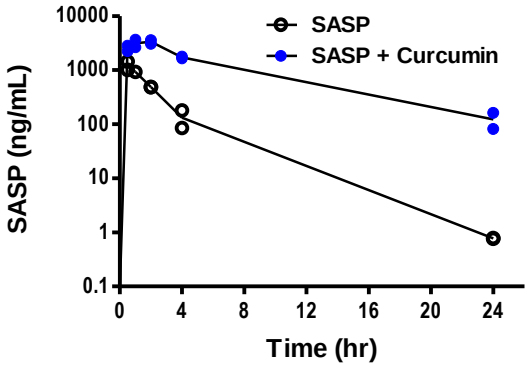


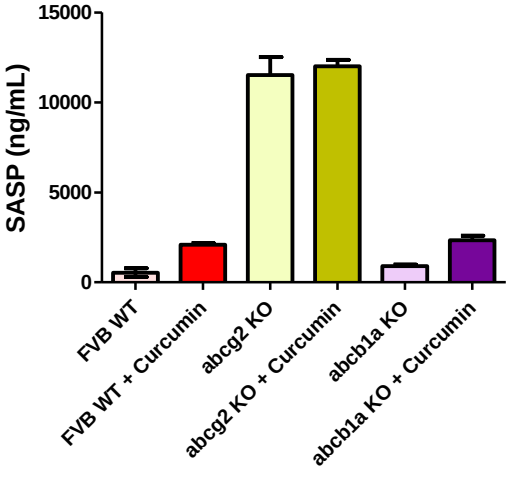


Gefitinib (Iressa)

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



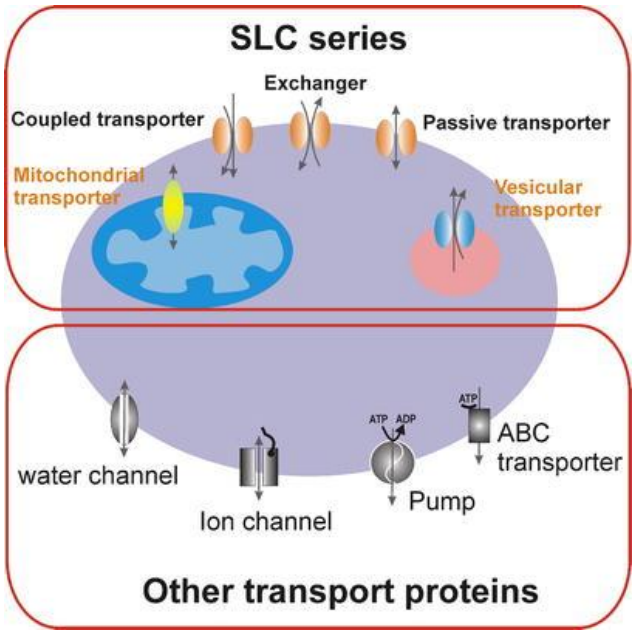


Suneet Shukla et al. Pharm Res. 2008 Oct 9.

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.

Transporter/alias (Gene)	Selected substrates	Selected inhibitors	Organs/cells	Comments
OATP1B1/OATP-C, OATP2, LST-1 (*SLCO1B1*)	Bromosulphophthalein, oestrone-3-sulphate, oestradiol-17β-glucuronide, statins*, repaglinide*, valsartan, olmesartan*, bilirubin glucuronide, bilirubin, bile acids	Saquinavir, ritonavir*, lopinavir*, rifampicin*, cyclosporine*	Hepatocytes (sinusoidal)	• Has a role in disposition and excretion • Has clinically relevant polymorphisms • Has a role in clinical drug–drug interactions
OATP1B3/OATP-8 (*SLCO1B3*)	Bromosulphophthalein, cholecystokinin 8, statins*, digoxin, fexofenadine, telmisartan glucuronide, telmisartan*, valsartan, olmesartan, oestradiol-17-β-glucuronide, bile acids	Rifampicin*, cyclosporine*, ritonavir, lopinavir*	Hepatocytes (sinusoidal)	• Has a role in disposition and excretion
OAT1 (*SLC22A6*)	Para-aminohippurate, adefovir, cidofovir, zidovudine*, lamivudine*, zalcitabine*, acyclovir*, tenofovir*, ciprofloxacin*, methotrexate*	Probenecid*, novobiocin	Kidney proximal tubule, placenta	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
OAT3 (*SLC22A8*)	Oestrone-3-sulphate, non-steroidal anti-inflammatory drugs, cefaclor, ceftizoxime, furosemide*, bumetanide*	Probenecid*, novobiocin	Kidney proximal tubule, choroid plexus, blood–brain barrier	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
OCT2 (*SLC22A2*)	*N*-Methylpyridinium, tetraethylammonium, metformin*, pindolol, procainamide, ranitidine amantadine, amiloride, oxaliplatin, varenicline*	Cimetidine*, pilsicainide, cetirizine*, testosterone, quinidine	Kidney proximal tubule, neurons	• Has a role in disposition and excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions
OATP1A2/OATP-A (*SLCO1A2*)	Oestrone-3-sulphate, dehydroepiandrosterone sulphate, fexofenadine*, bile salts, methotrexate, bromosulphophthalein, ouabain, digoxin, levofloxacin, statins*	Naringin, ritonavir, lopinavir, saquinavir, rifampicin*	Brain capillaries endothelia, cholangiocytes, distal nephron	• Has role in disposition and excretion
OATP2B1/OATP-B (*SLCO2B1*)	Oestrone-3-sulphate, bromosulphophthalein, taurocholate, *statins, fexofenadine, glyburide, taurocholate	Rifampicin, cyclosporine*	Hepatocytes (sinusoidal), endothelia	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
OCT1 (*SLC22A1*)	Tetraethylammonium, *N*-methylpyridinium, metformin*, oxaliplatin	Quinine, quinidine, disopyramide	Hepatocytes (sinusoidal), intestinal enterocytes	• Has a role in disposition and excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions
PEPT1 (*SLC15A1*)	Glycylsarcosine, cephalexin, cefadroxil, bestatin, valacyclovir, enalapril, aminolevulinic acid, captopril, dipeptides, tripeptides	Glycyl-proline	Intestinal enterocytes, kidney proximal tubule	• Has a role in absorption, disposition and excretion • Has a role in clinical drug–drug interactions
PEPT2 (*SLC15A2*)	Glycylsarcosine, cephalexin, cefadroxil, bestatin, valacyclovir, enalapril, aminolevulinic acid, captopril, dipeptides, tripeptides	Zofenopril, fosinopril	Kidney proximal tubule, choroid plexus, lung	• Has a role in excretion
MATE1 (*SLC47A1*)	Metformin, *N*-methylpyridinium, tetraethylammonium	Quinidine, cimetidine, procainamide	Kidney proximal tubule, liver (canalicular membrane), skeletal muscle	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
MATE2-K (*SLC47A2*)	Metformin, *N*-methylpyridinium, tetraethylammonium	Cimetidine, quinidine, pramipexole	Kidney proximal tubule	• Has a role in disposition and excretion

*Can potentially be used for *in vivo* (clinical) studies.

Nature Reviews Drug Discovery 9, 215-236 (March 2010)

Table 1. Substrates (clinically relevant drugs) [11,13,20,24,32].

OATP1B1	OATP1B3	BCRP	MRP2
Anticancer drugs	***Anticancer drugs***	***Anticancer drugs***	***Anticancer drugs***
Methotrexate	Docetaxel	Daunorubicin	Cisplatin
SN-38	Methotrexate	Doxorubicin	Daunorubicin
Antibiotics	Paclitaxel	Epirubicin	Doxorubicin
Benzylpenicillin	***Antibiotics***	Etoposide	Etoposide
Rifampicin	Rifampicin	Gefitinib	Imatinib
Antihypertensive drugs	***Antihypertensive drugs***	Imatinib	Irinotecan
Bosentan	Bosentan	Irinotecan	Methotrexate
Olmesartan	Olmesartan	Methotrexate	SN-38
Valsartan	Telmisartan	Mitoxantrone	Topotecan
Statins	Valsartan	SN-38	Vincristine
Pitavastatin	***Antiallergic drugs***	Topotecan	Vinblastine
Pravastatin	Fexofenadine	***Antibiotics***	***Antibiotics***
Rosuvastatin	***Cardioactive drugs***	Ciprofloxacin	Ampicillin
Others	Digoxin	Ofloxacin	***Statins***
Bilirubin	***Statins***	Norfloxacin	Pravastatin
Leukotriene C4	Pitavastatin	***Antiviral drugs***	***Glucuronide (-G) conjugates***
Leukotriene E4	***Others***	Zidovudine	Bilirubin-G
Prostaglandin E2	Bilirubin	Lamivudine	Estradiol-17-β-D-glucuronide
T3 (triiodothyronine)	Leukotriene C4	***Flavonoids***	SN-38-G
T4 (thyroxine)	T3 (triiodothyronine)	Genestein	Acetaminophen-G
Thromboxane B2	T4 (thyroxine)	Quercetin	Diclofenac-G
Conjugates	***Conjugates***	***Statins***	Indomethacin-G
Estrone-3-sulfate	Estrone-3-sulfate	Pravastatin	
Estradiol-17-β-D-glucuronide	Estradiol-17-β-D-glucuronide	Rosuvastatin	
Troglitazone sulfate		***Others***	
		Prazosin	
		Nitrofurantoin	
		Cimetidine	
		Conjugates	
		Estrone-3-sulfate	
		Dehydroepiandrosterone sulfate	
		Estradiol-17-β-D-glucuronide	
		dinitrophenyl-S-glutathione	

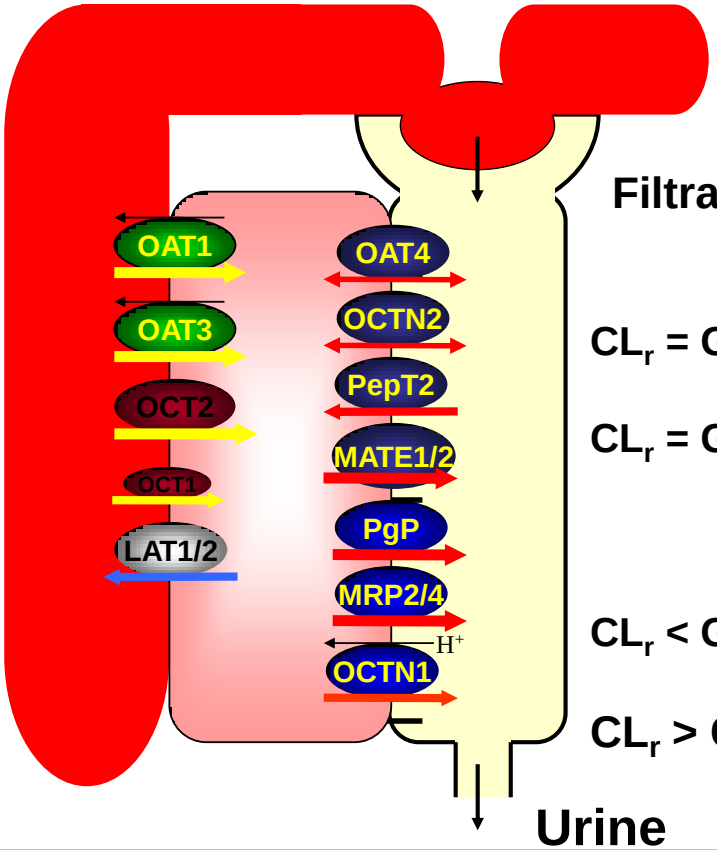
BCRP: Breast cancer resistance protein; MRP2: Multi-drug resistance-associated protein 2; OATP: Organic anion transporting polypeptide.

Transporter Interaction Redundancy:

- Drugs that are shown to interact with one transporter typically interact with multiple transporters.
- Thus, multiple pathways for clearance are possible for transporter substrates.

Ieiri et al. (2009) Expert Opinion in Drug Metabolism and Toxicology, 5: 703-729.

Major Renal Transporters



Filtration (GFR) *fu

CL_r = GFR + secretion – reabsorption

CL_r = GFR
Filtration only
secretion = reabsorption

CL_r < GFR (net reabsorption)

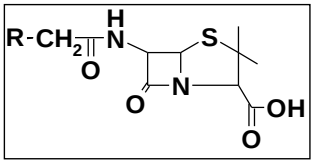
CL_r > GFR (net secretion)

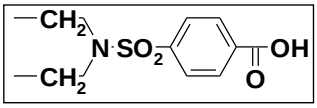
When is it Important to Study Renal Transporters?

- 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
- 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?

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

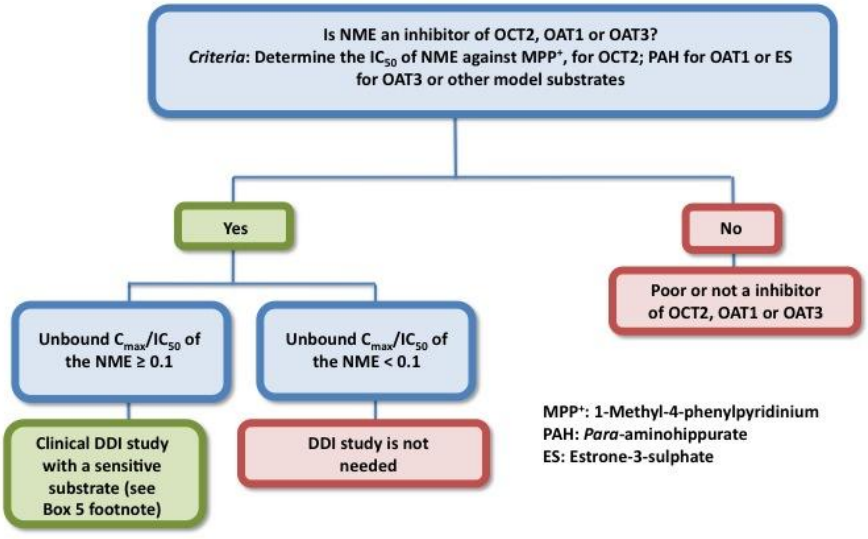
  Cidofovir (Vistide™)

  > Concomitant administration of OAT inhibitors *decrease* potential for nephrotoxicity

Package Inserts: Clinical Studies and DDI Potential

Drug (CL_R)	**Results (Bedside)**
Mirapex (400 mL/min) + cimetidine + probenecid	N=12 subjects/treatment arm. 50% ↑ in AUC; 40% ↑ in T 1/2 No effect on PK
Tikosyn (420 mL/min) + cimetidine + probenecid	Narrow TI 40% ↑ in AUC; CLR ↓ 33%; QTc ↑17-19 ms No effect
Metformin (600 mL/min) + cimetidine + probenecid	Narrow TI 40% ↑ in AUC and 60% ↑ in Cmax No effect
Oseltamivir +cimetidine +probenecid	N=12-18/treatment (see Hill et al.) No change on PK 2.5-fold AUC of Ro64-0802 (active metab)

Evaluation of OCT or OAT inhibitors requires determination of an IC50 in an *in vitro* study



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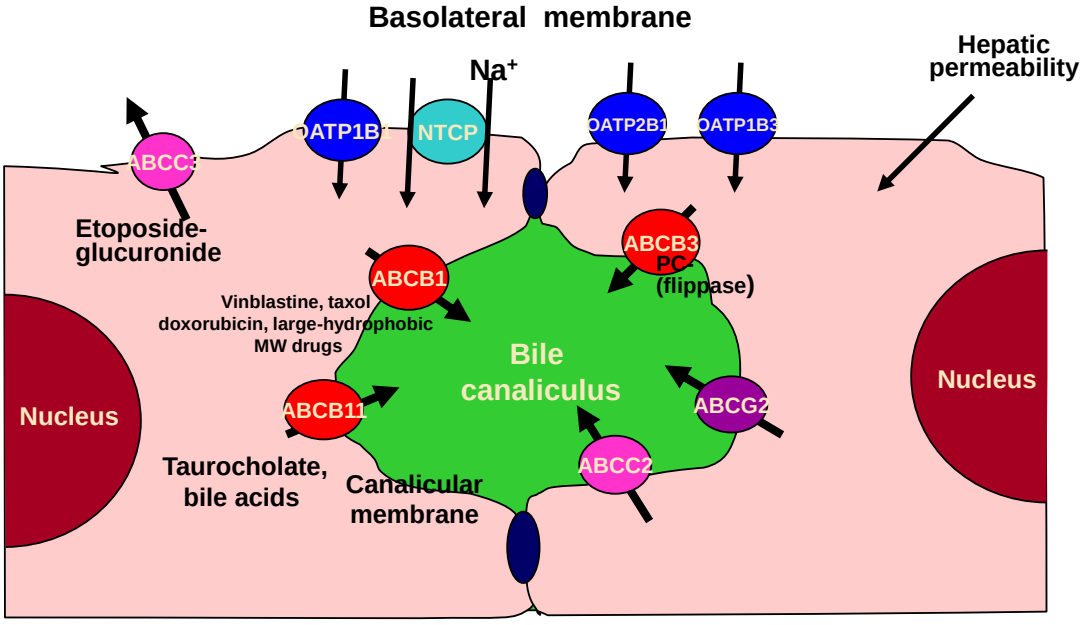
Hepatic Uptake/Efflux Transporters



NIH Principles in Clinical Pharmacology Transporter Biology 20 January 2011

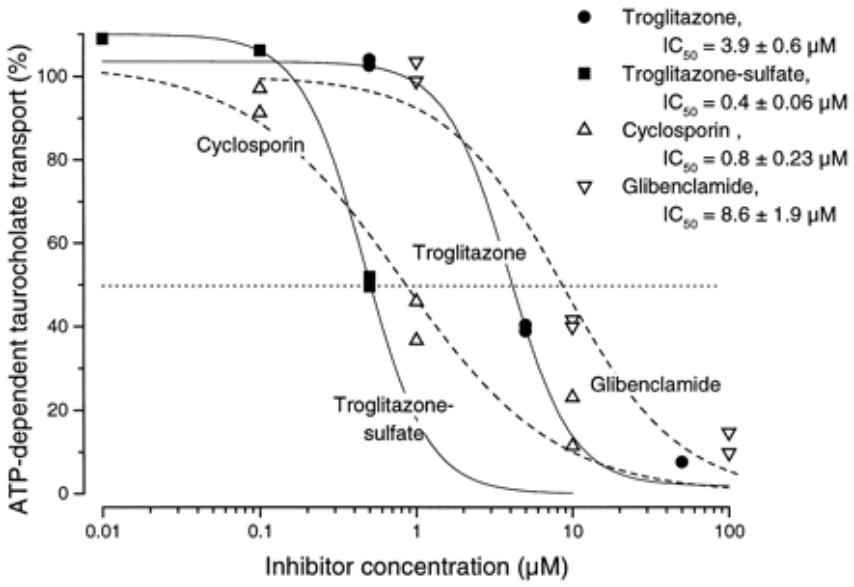
Hepatic Transporters

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 Transport and Liver Injury



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

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.)

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*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.

SLCO1B1 VARIANTS AND STATIN-INDUCED MYOPATHY

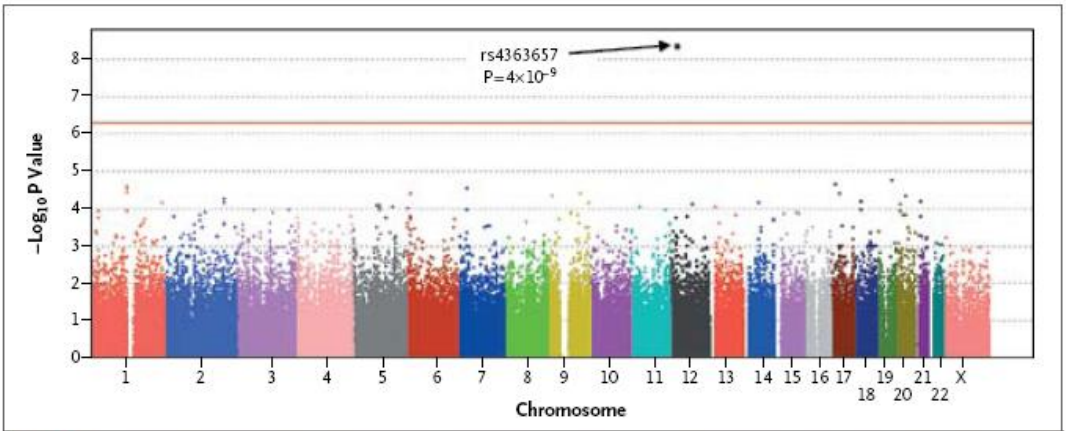
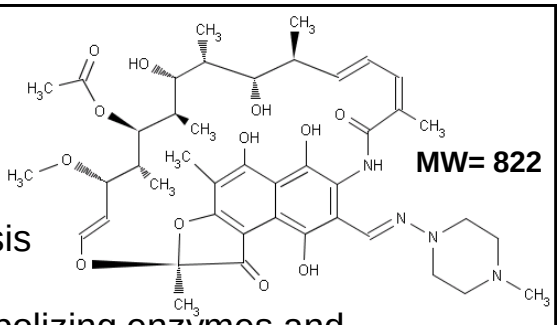


Figure 1. Results of Tests for a Trend in the Association between Myopathy and Each SNP Measured in the Genome-wide Association Study.

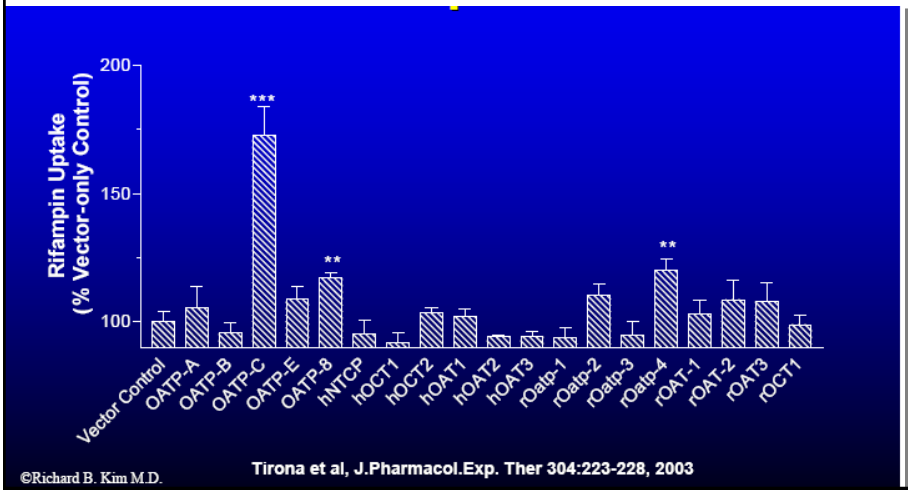
P values are shown for each SNP measured among 85 participants with myopathy and 90 matched controls who were taking 80 mg of simvastatin daily. Analyses are based on 316,184 of the 318,237 SNPs (99.4%) on the Sentrix HumanHap300-Duo BeadChip (Illumina). A result above the horizontal red line indicates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

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

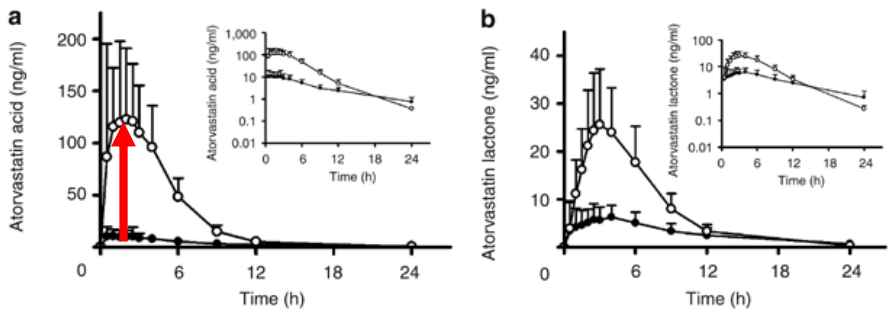
Rifampicin



- Antibiotic used in treatment of tuberculosis
- Known for its ability to induce drug metabolizing enzymes and transporters through activation of pregnane X receptor (PXR)
- Identified as an inhibitor of OATPs and entry into human hepatocytes mediated by OATP1B1



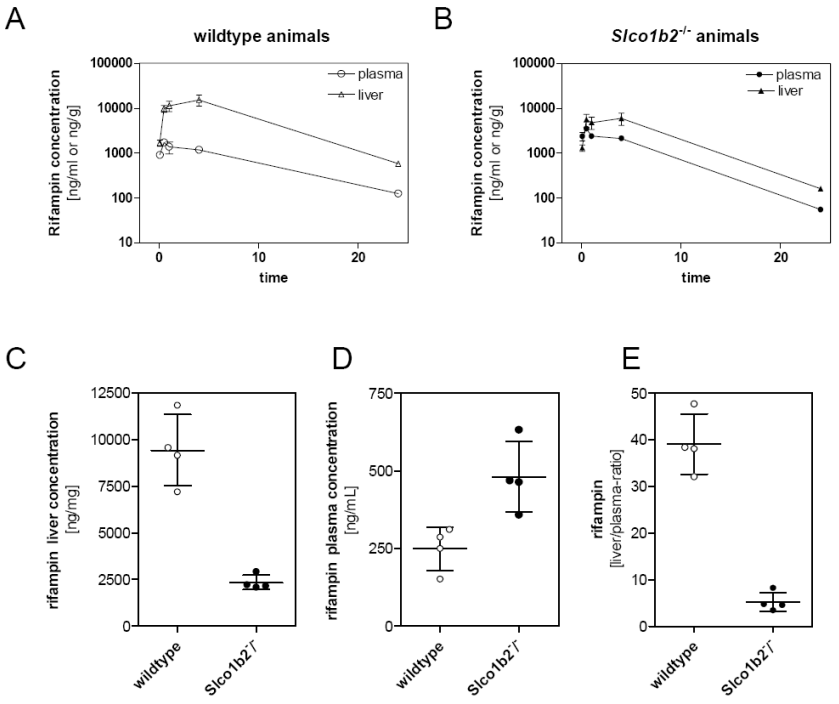
Rifampicin Inhibits Atorvastatin through OATP



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

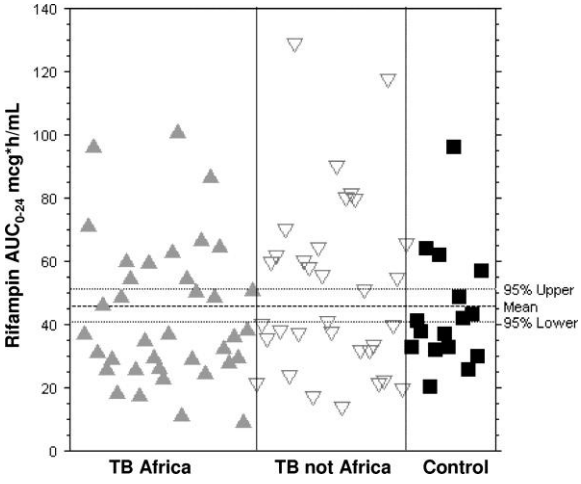
(Lau YY et al., Clin Pharmacol Ther, 81, 194-204 (2007), slide courtesy of Dr. L.Z. Benet)

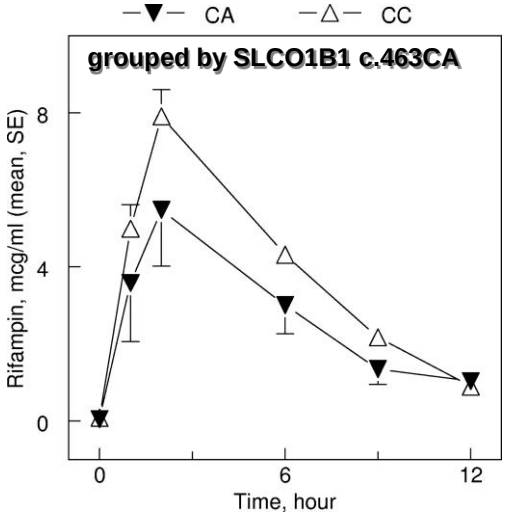
Rifampacin Disposition in WT vs *Slco1b2*$^{-/-}$ KO Mice



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

Rifampacin PKPD, Disease and PGx

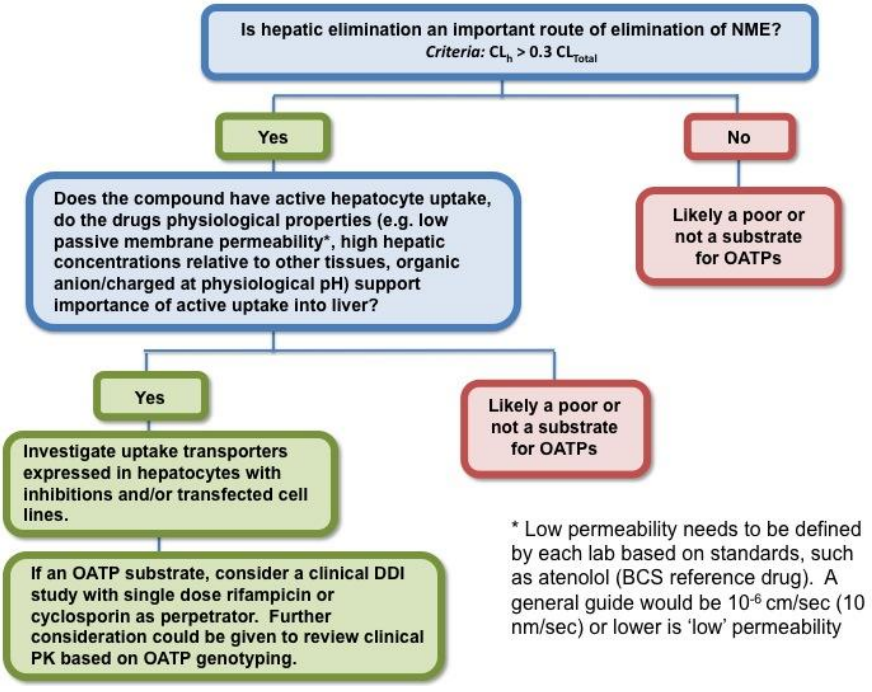




In multivariate analyses, the rifampin AUC0-24 was significantly affected by rifampin dosage (in mg/kg), SLCO1B1 c.463C>A polymorphism, and presence of tuberculosis by the region of enrollment

Weiner, M. et al. 2010. Antimicrob. Agents Chemother. 54(10):4192-4200

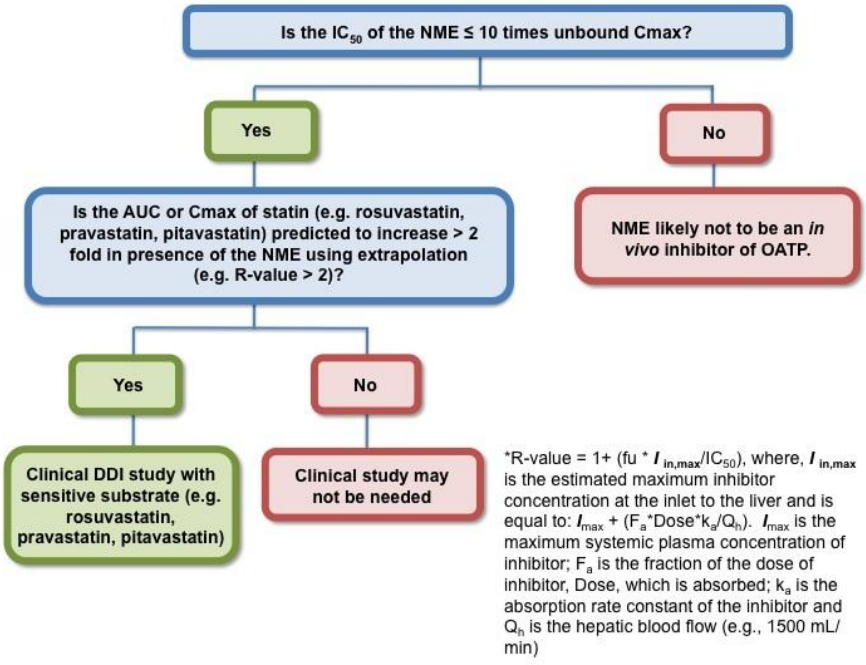
Hepatic Uptake Substrate Decision Tree



* Low permeability needs to be defined by each lab based on standards, such as atenolol (BCS reference drug). A general guide would be 10^{-6} cm/sec (10 nm/sec) or lower is 'low' permeability

Nature Reviews Drug Discovery 9, 215-236 (March 2010)

OATP Inhibitor Decision Tree



Nature Reviews Drug Discovery 9, 215-236 (March 2010)

Future Direction of Drug Transport in Preclinical Development and Clinical Pharmacology

- Drug-Drug Interactions mediated through drug transporter(s) have received increased attention and are recognized as important contributors of ADME
- Significant substrate 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 awareness during drug development. Therefore, the accuracy of the predicted DDI is dependent on the ***Quality*** of the *in-vitro* assay and our ability to translate the interaction into the Clinic
  - **Clinical Translation** with respect to physiologic PK of transport probe substrates and inhibitors is needed.
- Preclinical and clinical differences in transporter expression remain important determinants of drug-induced toxicity and an important consideration in drug development.
  - Additional KO and Tg models to investigate the *in-vivo* contribution of drug transporters are needed.

Acknowledgment(s) and Contributors

- **Genentech Research and Early Development, Development Sciences, Clinical Pharmacology, ED-PK/PD, SA, and DMPK**
- **ITC Collaborators**

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THANK YOU !!

Transporter Nomenclature

SLC Family

- **Basolateral**
  - **OCT2 = SLC22A2**
  - **OAT1 = SLC22A6**
  - **OAT3 = SLC22A8**
  - **System L = SCL7A5/8**
- **Apical**
  - **PepT2 = SLC15A2**
  - **OCTTN1 = SLC22A4**
  - **OCTN2 = SLC22A5**
  - **OAT4 = SLC22A11**
  - **hMATE1 =SLC47A1**
  - **hMATE2=SLC47A2**

ABC Family

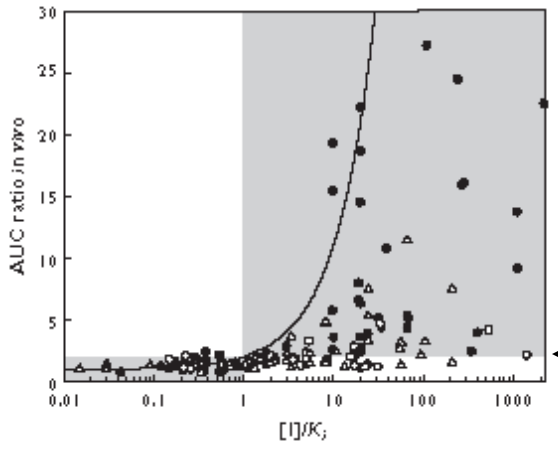
- **Apical**
  - **MDR1 = ABCB1**
  - **MRP2 = ABCC2**
  - **MRP4 = ABCC4**
  - **BCRP = ABCG2**

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

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?

The rate determining process

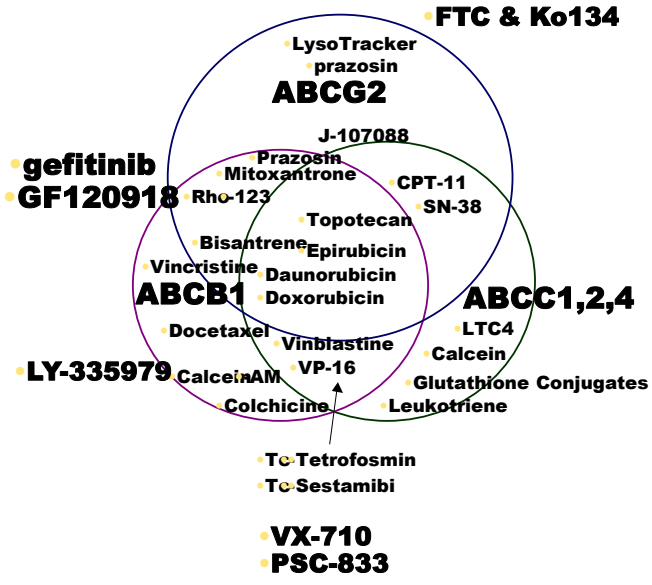
"To understand the transporter-mediated drug-drug interaction, we have to know the rate determining process of a substrate in the overall clearance."

uptake, basolateral efflux, apical excretion, metabolism

Professor Sugiyama, Keynote address AAPS, November 2007

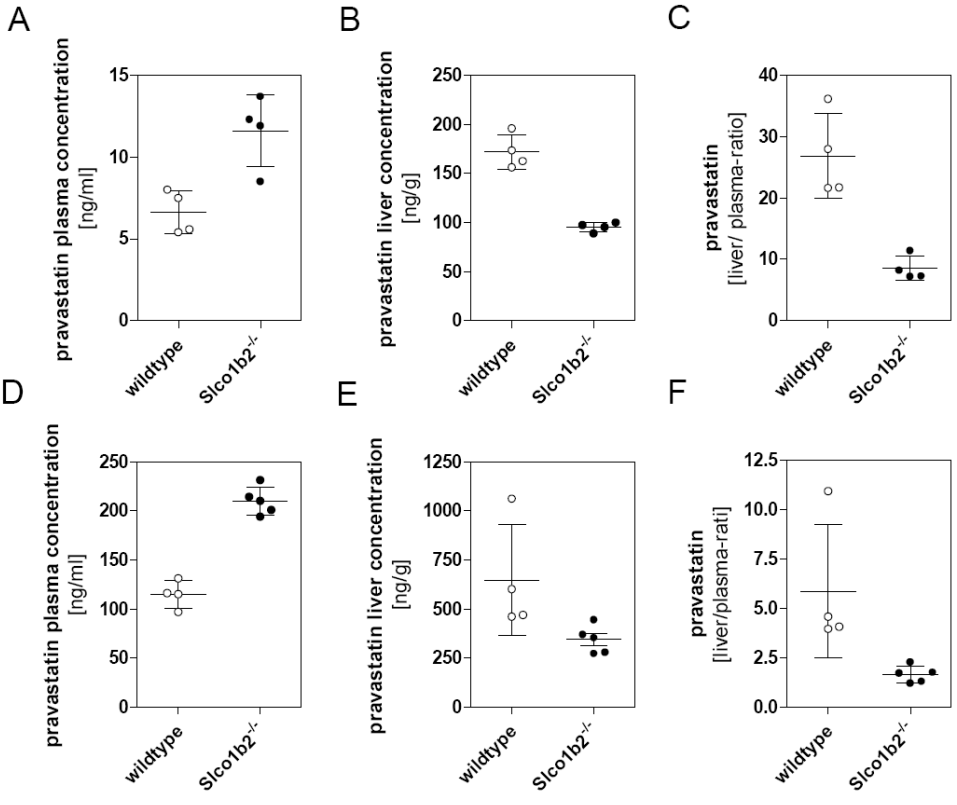
ABC Substrate/Inhibitor Overlap

Distinct but Overlapping Substrate Specificities



- Figure adapted from Thomas Litman

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



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