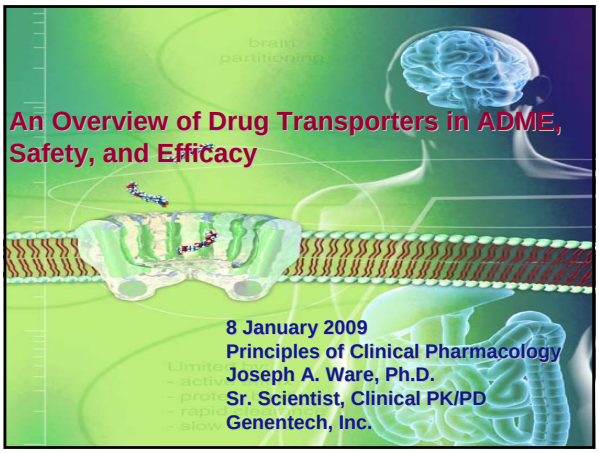
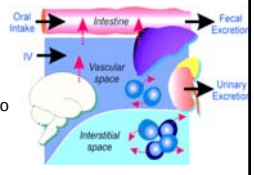


Presentation Outline

- 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

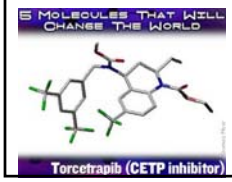
"To understand the transporter-mediated drug-drug interaction, we have to know the rate determining process of a substrate in the overall clearance."

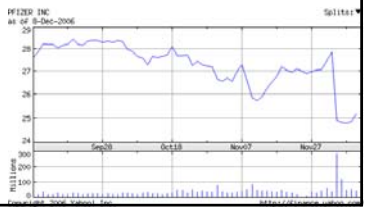
uptake, basolateral efflux, apical excretion, metabolism

Professor Sugiyama, Keynote address AAPS, November 2007

Cost of Drug Attrition (somewhat recent example)

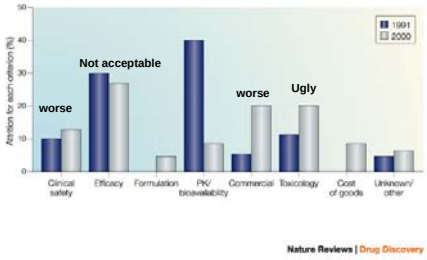
- Torcetrapib: Phase III-nearly 1-billion dollars spent on development.
- Safety
- Impact
  - Immediate
  - R&D





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

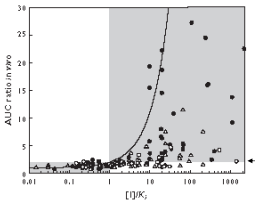
Speed/Quality impact
Discovery predictions
to early Clinical Development
Program

	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

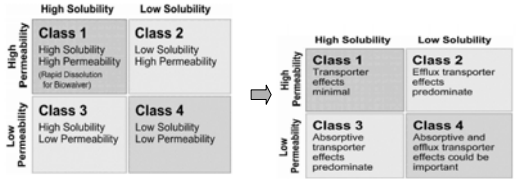
AUCi/AUC related to P-gp DDI

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?

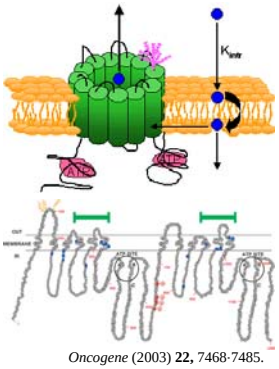
Permeability is an important determinant of In vitro-in vivo extrapolation for both Metabolism and Transport



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



- 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.

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

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%

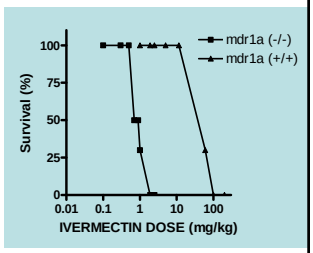


Figure from A.H. Schinkel et al., Cell, Vol.77, 491-501, 1994

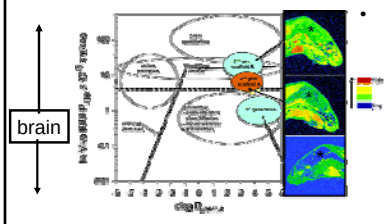
Ivermectin Toxicity in the Collie

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

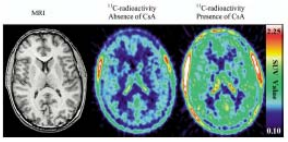


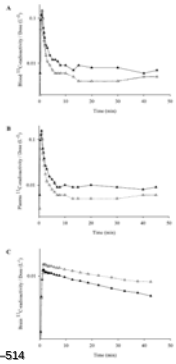
- Many Examples of Drugs whereby BBB Entry is Not Desirable
  - Ivermectin
  - Digoxin
  - Non-sedating antihistamines
    - Fexofenadine
    - Loratadine
    - Cetirizine

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.



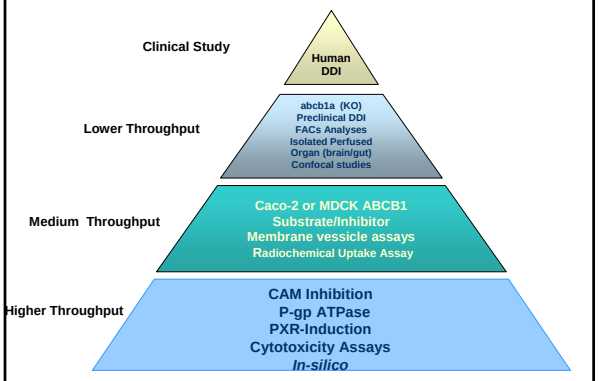


Clinical Pharmacology & Therapeutics (2005) 77, 503–514

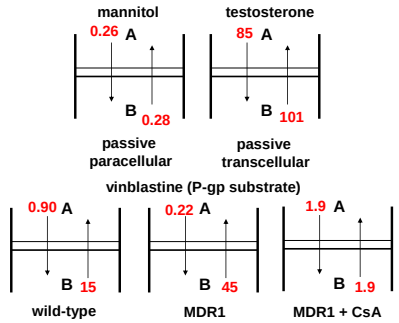
P-glycoprotein Substrates

- **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

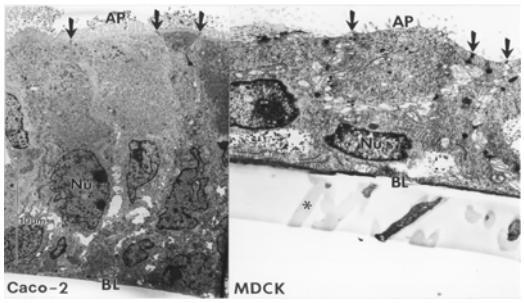
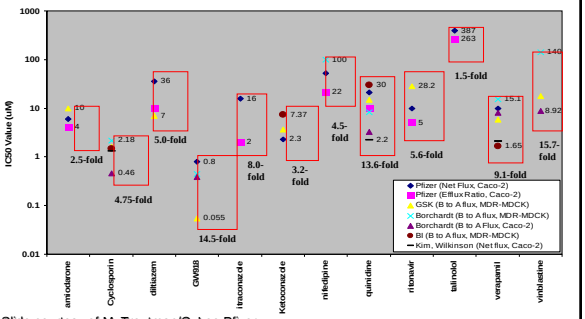


Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub

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

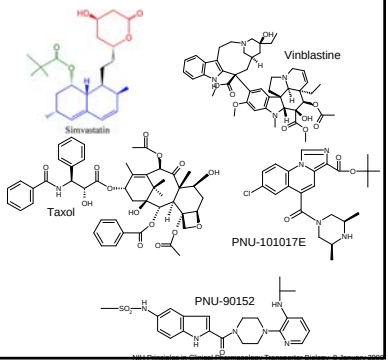


Slide courtesy of M. Troutman/C. Lee Pfizer

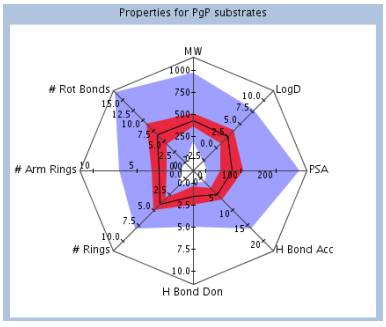
Chemical Features of P-gp Substrates

General Attributes:

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



Chemical Features of P-gp Substrates

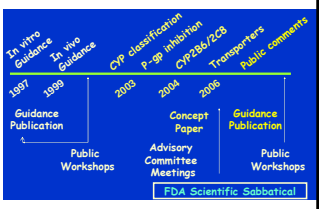


N = 8463 (MDR1: P_{app} BA/ P_{app} AB >= 2.5)

Pil Lee and Ralph Davidson, PFE Groton

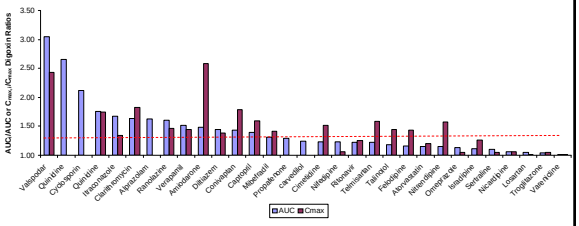
Evolution of 2006 Draft Guidance

- 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

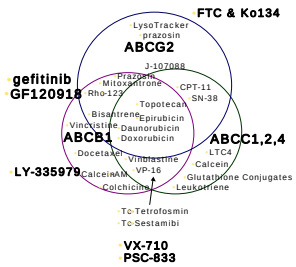


Figure adapted from Thomas Litman

ABCG2 (*alias* BCRP, MXR, ABCP,BMDP)

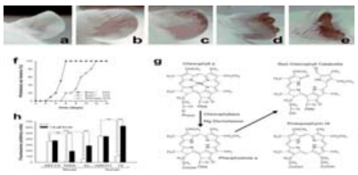
- Expressed endogenously in the intestine (small & large), liver, kidney, placenta, skeletal muscle, brain, and in hematopoietic stem cells
- In-vitro role in tumor drug resistance for Topo-1 and Topo-2 inhibitors (MXR, SN-38, Topotecan, J-107088)
- Emerging role in drug absorption of camptothecan analogues (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.



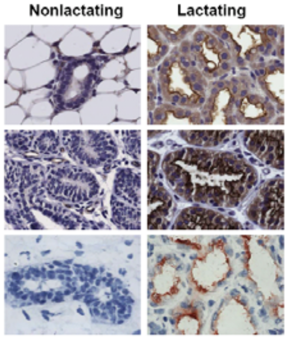
Expression BCRP in mammary gland across species

Nonlactating | Lactating

Mouse

Cow

Human



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

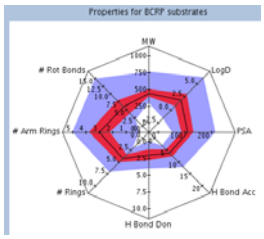
- PhIP
- Pheophorbide A
- Estrogen SO_4
- lysotracker (green)
- H33342
- Rhodamine 123
- Bodipy-prazosin
- Riboflavin (vitamin B2)

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)



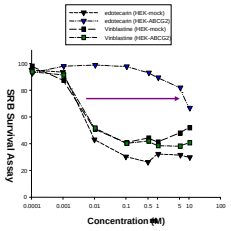
Pil Lee and Eric Reyner, SMI 2007

- 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

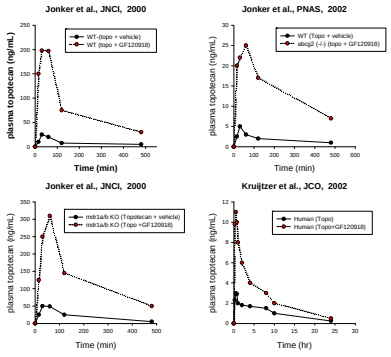
- Blue region: the range of each property
- Black line inside the red region is the average value for each property
- Red region: the standard deviation from the average value.

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



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

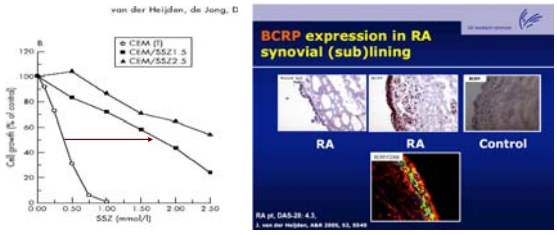
Sponsored by: GlaxoSmithKline
Information provided by: GlaxoSmithKline**ClinicalTrials.gov Identifier:**NCT00682279

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

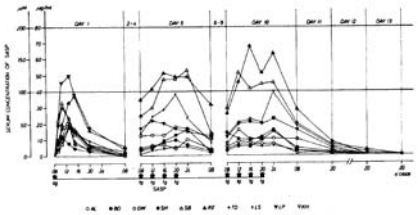


Fig. 2. Serum concentrations of SASP after ingestion of a single 4 Gm. dose of SASP on Day 1 (10 subjects) and 4 × 1 Gm. of SASP on Days 2 to 10 (9 subjects).

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.

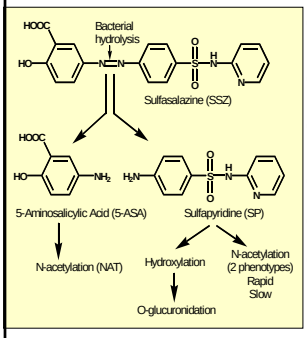
ABCG2 Polymorphisms and Ethnic Distribution of SNPs.

- 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



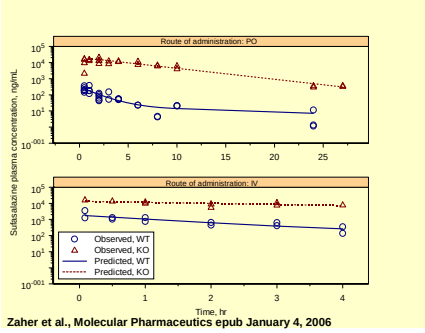
- **Indications: Rheumatoid arthritis (RA), Long term therapy of ulcerative colitis, and Crohn's disease**
- **Bioavailability (F) of SASP in humans is low (F< 15%) and highly variable**
- **Low %F primarily attributed to SASP's low permeability and poor solubility (thus, poor absorption)**
- **Azo-reduction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

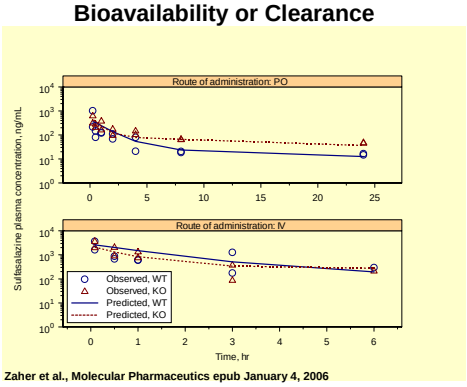
Why the discordance in assays?

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

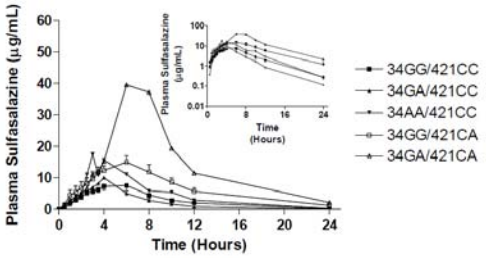
Mice	Route	Dose (mg/kg)	C_{max} (ng/mL)*		AUC (ng.hr/mL)			Relative exposure, AUC_{KO}/AUC_{WT}
			WT	KO	Duration (hr)	WT	KO	
Bcrp1	IV	5	1827	13570	0-4	3015	40343	**13**
	PO	20	233	16176	0-24	1189	131822	**111**
Mdr1a	IV	5	2749	2266	0-6	5131	3504	**1**
	PO	20	349	440	0-24	1098	1781	**2**

* 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.

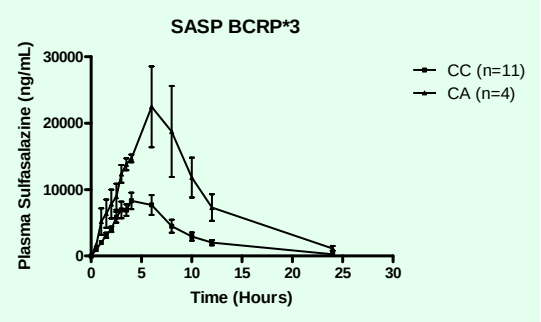
Zaher et al., Molecular Pharmaceutics epub January 4, 2006

SASP Disposition in North American Healthy Volunteers



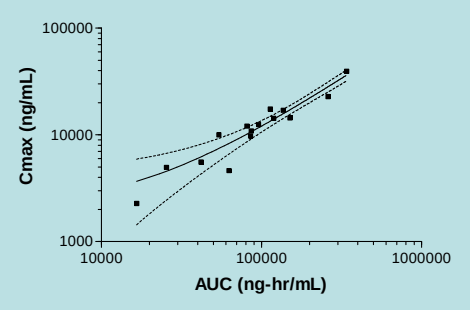
Brad Urquhart et al., Pharmacogenet Genomics. 2008 May;18(5):439-48.

Altered SASP Exposure in Q141K Subjects



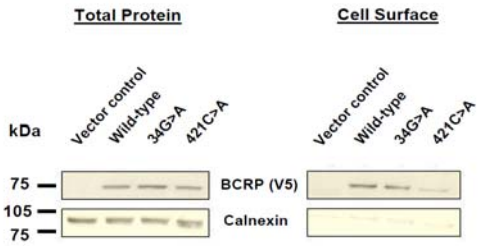
Urquhart et al., Pharmacogenet Genomics. 2008 May;18(5):439-48.

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

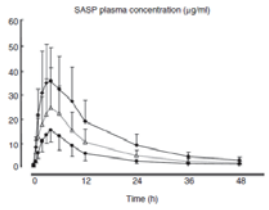


Figure 2 Effect of *ABCG2* genotype on pharmacokinetics of sulfasalazine (SASP). Plasma concentration-time profiles of SASP after oral administration of a 2,000 mg conventional SASP tablet to 421C/C subjects (closed circles, $n = 12$), 421C/A subjects (open triangles, $n = 16$), and 421A/A subjects (closed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

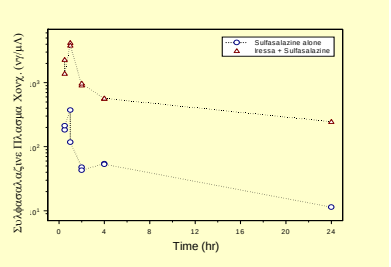
Yamasaki et al., CPT January 2, 2008

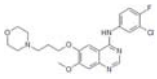
ABCG2 Pharmacogenomic Studies

Formulation
IR →
susp →
SR →

Drug	Structure	Dose, Route	# Patients	Ethnic Group, Gender	Result	Reference
Sulfasalazine		2000 mg po	37ª	Japanese Male	1.7-3.5X increase in AUC, Cmax	Yamasaki et al (2008) Clin Pharmacol Ther, ePub
Sulfasalazine		1000 mg po	17ª	Caucasian Both	1.7-2.4X increase in AUC, Cmax	Urquhart et al (2008) Pharmacogen & Genomics, ePub
Sulfasalazine		500 mg po	36ª	Chinese Both	No effect on AUC, Cmax	Adkison et al (2008) ASCPT mtg poster
Gefitinib (IRESSA)		250 mg po	124ª	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	18ª	Caucasian Both	1.35X increase in oral bioavailability	Sparreboom et al (2005) Canc Biol Ther 4:650
Rosuvastatin		20 mg po	14ª	Chinese Both	1.8X increase in AUC and Cmax	Zhang et al (2006) Clin Chim Acta 373:99
Diflomotecan		<0.5 mg po, iv	22ª	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	82ª	Caucasian Both	No difference	Gardner et al (2006) Clin Pharmacol Ther 80:192
Pitavastatin		2 mg po	38ª	Japanese Male	No difference	Ieiri et al (2007) Clin Pharmacol Ther. 82:541

Gefitinib (Iressa)-enhanced SASP Bioavailability

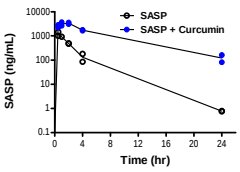


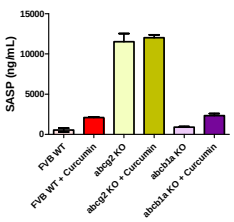


Gefitinib (Iressa)

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



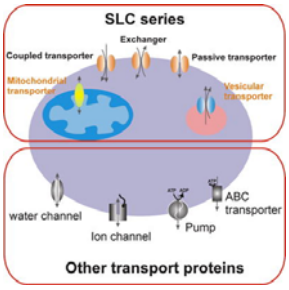


Suneet Shukla et al. Pharm Res. 2008 Oct 9.

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

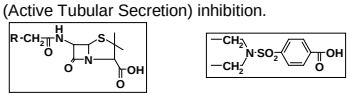


- Solute Carrier (SLC) superfamily contains
  - 43 families
  - 298 genes
- HUGO database (see http://www.gene.ucl.ac.uk/nomenclature/)
  - SLC root symbol
  - Followed by numeral (family)
  - Followed by letter
  - Followed by numeral (ie SLC22A1)
  - Further elaborated in the SLC21/SLCO

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) + 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 TDI 40% ↑ in AUC; CLR ↓ 33%; QTc ↑17-19 ms 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)
Axid (500 mL/min)	Not currently defined, however TDI very high

Transporter Nomenclature

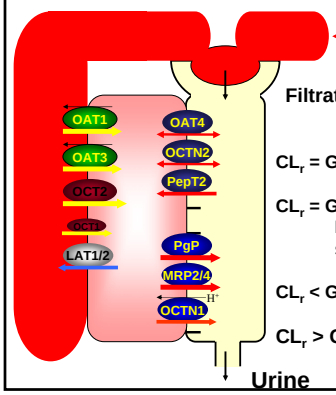
SLC Family

- **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

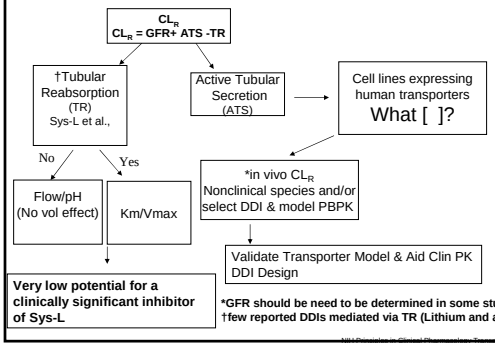
CL_r = GFR
Filtration only
secretion = reabsorption

CL_r < GFR (net reabsorption)

CL_r > GFR (net secretion)

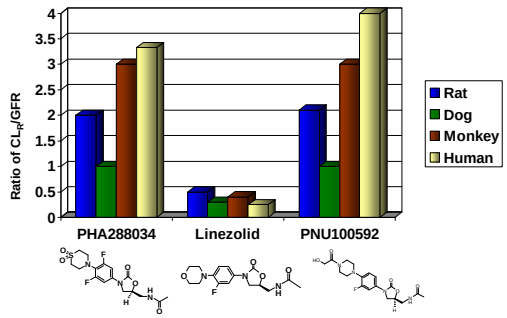
Urine

Renally-Mediated DDIs

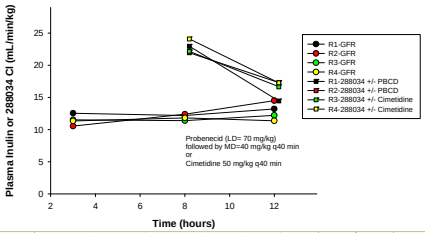


*GFR should be need to be determined in some studies
†few reported DDIs mediated via TR (Lithium and amiloride)

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

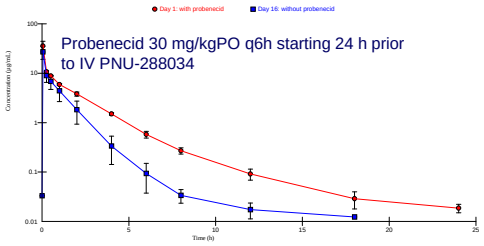


Inhibition of PHA-288034 Clearance via Probenecid or Cimetidine in the Rat



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

Monkey PHA288034 Probenecid Interaction Study



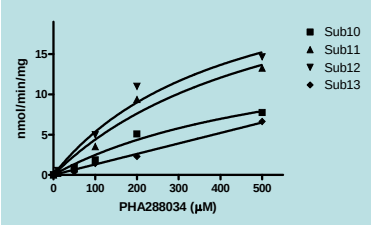
Study by WJ Adams et al.

Model Systems to Study Renal Transport

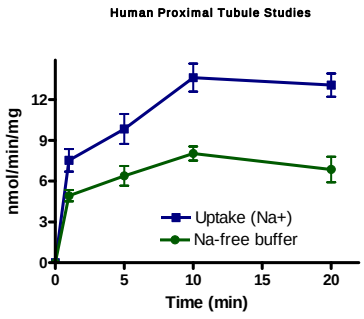
- Isolated Perfused kidney
- Kidney Slices
- **Isolated Renal Tubules (PCTs)**
- Isolated BBMVs
- **Individual Transporter Clones**
  - **Transient**
  - **Stable**
- GeMMs

In Vitro Uptake Models

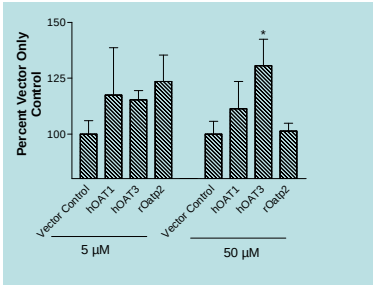
- Transport of PHA-288034 in human proximal tubules.
  - Drug uptake in cell suspension of hPTs.
  - Determine kinetics, substrate specificity, energy & ion dependence
  - Preliminary study suggested no metabolism in hPTs



Na⁺-dependent Uptake of PHA288034

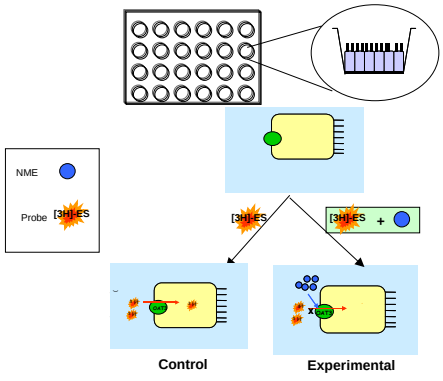


PHA-288034 Uptake in HeLa cells Transfected with Transporter cDNAs



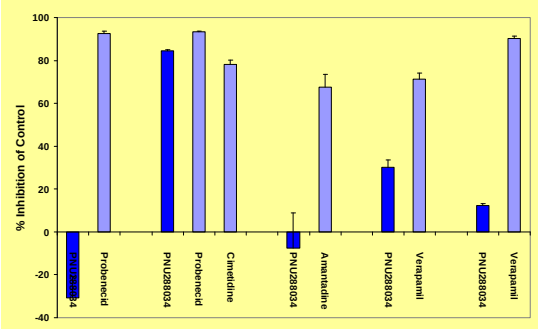
Richard Kim and Brenda Leake

Experimental Protocol: Interaction Assay in Stable Transfectants



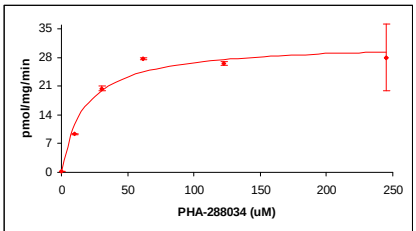
Result/calculations = Inhibition of [3H]-ES uptake (% of control) in presence of NME

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



HEK cell lines created by Prof. KM Giacomini

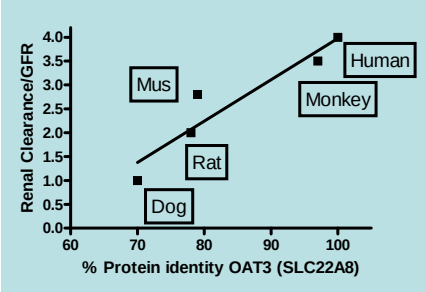
PHA-288034 uptake in hOAT3 cells



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

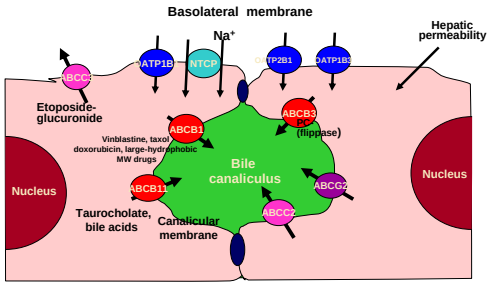
Hepatic Transporters

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

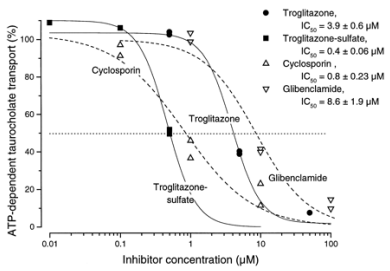
Question 2. Is it possible to predict the DDI potential mediated through hepatic uptake or efflux or are we only able to define potential mechanisms of a PK observation?

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


The NEW ENGLAND JOURNAL of MEDICINE

SLCO1B1 Variants and Statin-Induced Myopathy — A Genomewide Study

The SEARCH Collaborative Group*

ABSTRACT

SLCO1B1 VARIANTS AND STATIN-INDUCED MYOPATHY

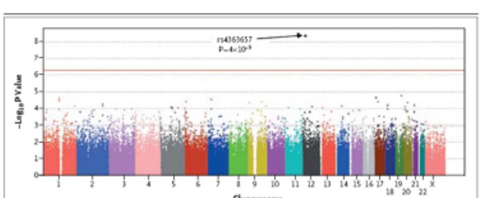


Figure 1. **Results of Tests for a Trend in the Association between Myopathy and Each SNP Measured in the Genome-wide Association Study.**

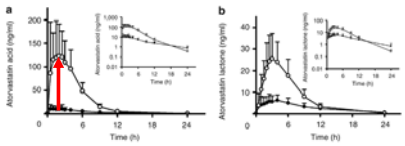
P values are shown for each SNP measured among 85 participants with myopathy and 90 matched controls who were taking 80 mg of simvastatin daily. Analyses are based on 316,184 of the 318,237 SNPs (99.4%) on the Sentrix HumanHap300-Duo BeadChip (Illumina). A result above the horizontal red line indicates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

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

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

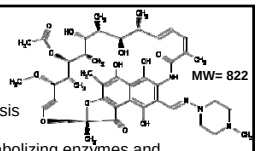
Rifampicin Inhibits Atorvastatin through OATP



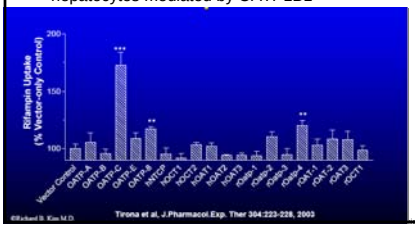
- 600 mg rifampacin IV increases atorvastatin acid AUC 7-fold.
- Acutely, single dose rifampacin may inhibit OATP1B3, CYP3A4, and CYP2C8.

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

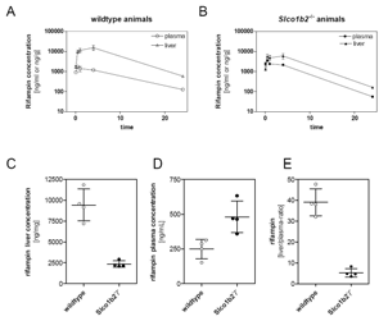
Rifampicin



- 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

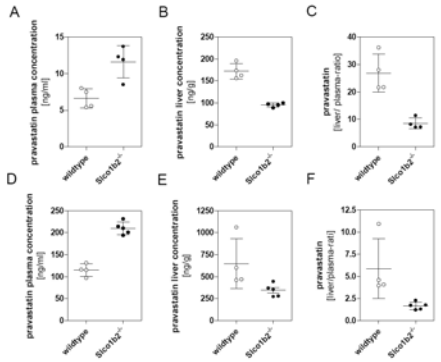


Rifampacin Disposition in WT vs *Slco1b2*$^{-/-}$ KO Mice



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

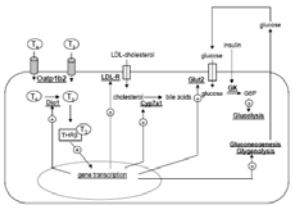


Figure from Henriette E. Meyer zu Schwabedissen

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.