

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

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**Principles of Clinical Pharmacology**

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## 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

Graphic illustration of drug transport in drug discovery and development.

## Impact of Drug Transport on ADME

- Oral absorption of drug

- Complex metabolism interaction(s)

- Drug Distribution and elimination

- Organ-selective delivery of drugs and prodrugs

## Impact of Drug Transport on Response and Toxicology

- Emerging Role in Toxicology*

- Over expression of drug transporter may be a major factor in tumor, bacterial, and fungal multi-drug resistance (MDR).

## Drug Transporters as Targets

- LY335979, Zosuquidar (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

Chemical structure of Torcetrapib (CETP inhibitor)

Chart for Pfizer Inc. drug development expenditures as of 8-Dec-2006

## Reasons for Drug Attrition (1991-2000)

Bar chart showing drug attrition due to poor clinical safety, efficacy, formulation, PK/bioavailability, commercial value, toxicology, cost of goods, unknown/other reasons.

From Nature Reviews/Drug Discovery

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

Chart illustrating the significance of CYP drug metabolizing enzymes regarding drug disposition, genetic variability, and prediction of in vivo clearance and drug-drug interactions.

Chart illustrating the significance of phase II enzymes regarding drug disposition, genetic variability, and prediction of in vivo clearance and drug-drug interactions.



Chart illustrating the significance of transporters regarding drug disposition, genetic variability, and prediction of in vivo clearance and drug-drug interactions.

## Drug Interactions: CYP Mediated

Significant CYP mediated drug interactions based on AUC ratio

Chart showing AUC ratio in vivo for CYP2C9, 2D6 and 3A4 substrates

**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*

Chart showing permeability and solubility of Class 1, 2, 3. and 4 drugs

*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**

Graphic illustration

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

P-gp is distributed in the following organs: Intestine, kidney, liver, brain, adrenal gland, lymphocytes, and placenta

Hypothetical MOA

“vacuum cleaner”

Membrane partitioning

Walker A and Walker B binding motif

Drug-stimulatable and inhibitable

High basal activity present in P-gp ATPase assay.

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

Chart showing Ivermectin dose (mg/kg) and % survival of exposed mice.

Mdr1a/b (-/-) were found to be:

Viable

Fertile

Without observable phenotype until pharmacological challenge with IVM.

mdr1a -/- LD<sub>50</sub>= 0.7 mg/kg

mdr1a +/- LD<sub>50</sub>= 60 mg/kg

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

mdr1a -/- 100% cleft palate

mdr1a +/- 50% cleft palate

mdr1a +/- 0%

## Ivermectin Toxicity in the Collie

Photo of a group of five collies with the following web address beneath it:  
<http://www.awca.net/drug.htm>.

50% of Collies display CNS toxicity when treated with normal doses of IVM (>60 microgram/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

Graphic illustration of in vivo BBB  $P_{app} \times 10^{-6}$  cm/sec by  $\text{clog } D_{\text{pH } 7.4}$

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 [ $^{11}\text{C}$ ]verapamil +/- CsA.

Mean 88% increase in BBB exposure (range 62-148%).

Clinical observation significantly less than mouse prediction.

### MRI images

Three charts showing [ $^{11}\text{C}$ ]verapamil levels in blood, plasma and brain before and after cyclosporine A.

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**

Graphic illustration of a pyramid showing from top

(narrow part) – Clinical Study of drug-drug interactions in humans.

Followed by Lower,

Medium and

(wide part of pyramid at bottom) Higher Throughput pre-clinical screening assays.

## In Vitro Permeabilities

Graphic illustration for mannitol, (passive paracellular), testosterone, (passive transcellular), and vinblastine (P-gp substrate).

Caco-2 and MDCK cell comparison  
In Vitro P-gp IC<sub>50</sub> for Inhibition of Digoxin Efflux Data from Multiple  
Labs / Techniques

Electron microscopy

Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub

In Vitro P-gp IC50 for Inhibition of Digoxin Efflux Data from Multiple Labs /  
Techniques

Graphic illustration

IC50Value (uM)

Amiodarone  
Cyclosporin  
Diltiazem  
GW918  
Itraconazole  
Ketoconazole  
Nifedipine  
Quinidine  
Ritonavir  
Talinolol  
Verapamil  
Vinblastine

## 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 structures of Simvastatin, Vinblastine, Taxol, and PNU-101017E

## Chemical Features of P-gp Substrates

Properties for Pgp substrates

Graphic illustration

N = 8463 (MDR1: Papp BA/ Papp AB  $\geq 2.5$ )

**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 ( $[I]/K_i > 0.1$ )

- Inducer (40% control)

Graphic illustration

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

## Digoxin: Safety Concerns

Bar chart showing  $AUC_i/AUC$  or  $C_{MAX,J}$  Digoxin Ratios over

Valspodar  
Quinidine  
Cyclosporin  
Quinidine  
Itraconazole  
Clarithromycin  
Alprazolam  
Ranolazine  
Verapamil  
Amiodarone  
Diltiazem  
Conivaptan  
Captopril  
Mibefradil  
Propafenone  
Carvedilol  
Cimetidine  
Nifedipine  
Ritonavir  
Telmisartan  
Talinolol  
Felodipine  
Atorvastatin  
Nitrendipine  
Omeprazole  
Isradipine  
Sertraline  
Nicardipine  
Losartan  
Troglitazone  
Varenicline

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 C<sub>max</sub> or exposure were observed in the majority of published cases

I/IC<sub>50</sub> > 0.1 is predictive of positive clinical digoxin DDI related to P-gp

I<sub>2</sub>/IC<sub>50</sub> < 10 is predictive of no clinical digoxin DDI

For Digoxin or NMEs that have a narrow T.I. (similar to digoxin), P-gp may be an important determinant of PK and response.

Additional work is needed to fully understand the mechanism of false (-)'s observed with I/IC<sub>50</sub> or false (+)'s with I<sub>2</sub>/IC<sub>50</sub>

## 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*

Graphic illustration

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.

Electron microscopy

Charts

## Expression BCRP in mammary gland across species

Electronmicroscopy showing nonlactating and lactating mouse, cow, and human tissue.

Literature:

BCRP substrates reported concentrated into milk of each of these species

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

Slides 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<sub>4</sub>  
lysotracker (green)  
H33342  
Rhodamine 123  
Bodipy-prazosin  
Riboflavin (vitamin B<sub>2</sub>)

### Inhibitors

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

## Physicochemical properties of BCRP substrates

Graphic illustration depicting properties for BCRP substrates

**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.**

Pil Lee and Eric Reyner, SMI 2007

## Influence of BCRP (ABCG2) Expression on Cytotoxicity

Chart indicating SRB Survival Assay over concentration

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

Four separate line charts indicating the following:

Plasma topotecan (ng/mL) over time (Jonker et al, JNCI, 2000)

Plasma topotecan (ng/mL) over time (min) (Jonker et al., PNAS, 2002)

Plasma topotecan (ng/mL) over time (min) (Jonker et al., JNCI, 2000)

Plasma topotecan (ng/mL) over time (hr) in humans (Kruijtzer et al., JCO, 2002)

# Oral Topotecan

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

**This study is not yet open for participant recruitment.**

Verified by GlaxoSmithKline, May 2008

**Sponsored by: GlaxoSmithKline**

**Information provided by:** [GlaxoSmithKlineClinicalTrials.gov](http://GlaxoSmithKlineClinicalTrials.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](http://clinicaltrials.gov)

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

Graph of cell growth (% of control) over SSz (mmol/l)

BCRP expression in rheumatoid arthritis (RA) synovial (sub)lining

Electron microscopy of RA, RA and control.

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

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

Chart

Serum concentrations of SASP after ingestion of a single 4Gm. Dose of SASP on Day 11 (10 subjects) and 4 x 1 Cm. 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.

**Figg et al., Anticancer Drugs. 2007**

## **Sulfasalazine (SASP) Disposition**

Chemical structure of SASP and metabolites (5-ASA and sulfapyridine).

**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

Charts showing comparison between WT and KO mice.

Route of administration: PO over time, hr

Route of administration: IV over time, hr

Sufasalazine plasma concentration, ng/mL

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

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

Two charts showing Sulfasalazine plasma concentration, ng/mL, comparing the route of administration, PO, with the route of administration, IV, over time in WT and KO mice..

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

Chart showing that exposure to SASP is in Bcrp1 KO mice.

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

Chart showing Plasma Sulfasalazine ( $\mu\text{g}/\text{mL}$ ) over time (Hours) in subjects with variant genotypes.

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

## Altered SASP Exposure in Q141K Subjects

SASP BCRP\*3

Chart

Plasma Sulfasalazine (ng/mL) over time (hours).

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



## Correlation between SASP Cmax and AUC for Healthy Subjects

Chart showing Cmax (ng/mL) over AUC (ng-hr/mL)

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

## 421C>A SNP Changes Surface ABCG2 Expression

Chart comparing total protein with Cell surface

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

## SASP Disposition in Healthy Japanese Volunteers

Chart showing SASP plasma concentration ( $\mu\text{g/ml}$ ) over time.

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

DRUG	REFERENCE
Sulfasalazine	Yamasaki et al (2008) Clin Pharmacol Ther, ePub
Sulfasalazine	Urquhart et al (2008) Pharmacogen & Genomics, ePub
Sulfasalazine	Adkison et al (2008) ASCPT mtg poster
Gefitinib (IRESSA)	Cusatis et al (2007) JNCI 98(23):1739
Topotecan	Sparreboom et al (2005) Canc Biol Ther 4:650
Rosuvastatin	Zhang et al (2006) Clin Chim Acta 373:99
Diflomotecan	Sparreboom et al (2004) Clin Pharmacol Ther 76:38
Imatinib (GLEEVEC)	Gardner et al (2006) Clin Pharmacol Ther 80:192
Pitavastatin	Ieiri et al (2007) Clin Pharmacol Ther. 82:541

## Gefitinib (Iressa)-enhanced SASP Bioavailability

Chart

Chemical structure of 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

One chart showing SASP (ng/mL) over time.

Another chart (a bar chart) showing SASP (ng/mL) over

FYB WT

FVB WT = Curcumin

abcg2 KO

abcg2 KO + Curcumin

abcb1aKO

abcb1a KO + Curcumin

*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**

SLC root symbol

Followed by numeral (family)

Followed by letter

Followed by numeral (ie SLC22A1)

Further elaborated in the SLC21/SLCO

Graphic illustration

**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.

Chemical structure

Drugs that have labeling precautions relating to renally-mediated drug transport:

Dofetilide (Tikosyn™)

- > Concomitant administration OCT inhibitors *increase* potential for cardiac toxicity

Cidofovir (Vistide™)

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

## When is it Important to Study Renal Transporters?

Does scientific evidence suggest that it is necessary to investigate renal transport DDI potential for NMEs?

- Toxicologic significance

- Primary determinant of systemic CL

- NME inhibits the  $CL_R$  of compound with narrow TDI

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

Is there a need to perform both probenecid and cimetidine studies in healthy volunteers if in vitro and preclinical data support that compound is a prototypical transport substrate?

## Package Inserts: Clinical Studies and DDI Potential

Chart showing drugs ( $CL_R$ ) with Results (Bedside) for Mirapex, Tikosyn, Oseltamivir and Axid and their interaction with cimetidine and probenecid.

# Transporter Nomenclature

## SLC Family

### Basolateral

OCT2 = SLC22A2

OAT1 = SLC22A6

OAT3 = SLC22A8

System L = SCL7A5/8

### Apical

PepT2 = SLC15A2

OCTN1 = SLC22A4

OCTN2 = SLC22A5

OAT4 = SLC22A11

## ABC Family

### Apical

MDR1 = ABCB1

MRP2 = ABCC2

MRP4 = ABCC4

BCRP = ABCG2

## Major Renal Transporters

Graphic illustration of a nephron unit.

Blood flow

Filtration (GFR) \*fu

**CLr = GFR + secretion – reabsorption**

**CLr = GFR**

**Filtration only**  
**secretion = reabsorption**

**CLr < GFR (net reabsorption)**

**CLr > GFR (net secretion)**

Urine

## Renally-Mediated DDIs

Flow chart illustrating renal tubular secretion and reabsorption and the potential for drug-drug interactions.

## **Interspecies Comparison of Oxazolidinone CLR/GFR in Rat, Dog, Monkey & Humans**

Bar chart showing ratio of  $CL_R/GFR$  for PHA288034, Linezolid, and PNU100592.

Chemical structures of PHA288034, Linezolid, and PNU100592.

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

Chart showing plasma inulin or 288034 CL (mL/min/kg) over time(hours)



## Monkey PHA288034 Probenecid Interaction Study

Chart of PNU-288034 with and without probenecid

Probenecid 30 mg/kgPO q6h starting 24 h prior  
to IV PNU-288034

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

Chart showing nmol/min/mg over PHA288034 ( $\mu\text{M}$ ).

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

### **Human Proximal Tubule Studies**

Chart showing uptake in nmol/min/mg over time (min) with and without sodium in the buffer.

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

Chart showing Percent of Vector Only Control for 5  $\mu$ M and 50 $\mu$ M PHA-288034

Richard Kim and Brenda Leake

Experimental Protocol: Interaction Assay in Stable Transfectants

Chart comparing controls with experimental.

**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.

Bar graph indicating percent inhibition by probenecid, cimetidine, amantadine, and verapamil.

*HEK cell lines created by Prof. KM Giacomini*

## PHA-288034 uptake in hOAT3 cells

Graphic illustration of Michaelis-Menten Kinetics



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

Chart showing renal clearance/GFR over % protein identity OAT3 (slc22a8) in dog, rat, monkey, and human.

## Summary of PHA288034 Studies

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

### **Active Tubular Secretion**

PHA-288034 appears to be a substrate and an inhibitor of hOAT3 (SLC22A8).

PHA-288034 does not appear to be a substrate for hOAT1, OCT2, OCTN1, or OCTN2.

Additional work is needed to fully appreciate OAT3 cross-species differences.

Cimetidine inhibits OAT3-mediated transport as well as OCT-2 mediated transport.

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

Graphic illustration of hepatic cell transporters at the basolateral and canalicular membrane.

## Hepatic Transport and Liver Injury

Chart showing ATP-dependent taurocholate transport (%) over inhibitor concentration ( $\mu\text{M}$ ) for troglitazone, troglitazone-sulfate, cyclosporine, and glibenclamide.

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

## OATP Substrates

Chart showing substrates for OATP1B1 (OATP-C, LST-1, OATP2) and OATP183 (OATP8, LST-2)

An abstract from The New England Journal of Medicine entitled  
SLC01B1 Variants and Statin-Induced Myopathy – A genomewide Study.



## SLCO1B1 Variants and Statin-Induced Myopathy

Chart - Figure 1. Results of tests for a trend in the association between myopathy and each SNP measured in the Genome-wide Association Study.

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

Two charts showing atorvastatin acid and lactone concentrations versus time.

600 mg rifampicin IV increases atorvastatin acid AUC 7-fold.

Acutely, single dose rifampicin may inhibit OATP1B3, CYP3A4, and CYP2C8.

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

## **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

Bar graph of rifampicin uptake.

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

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

Four charts illustrating plasma and liver concentrations

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

## Pravastatin C<sub>ss</sub> Dispositon in WT vs Slco1b2<sup>-/-</sup> Mice

6 charts showing plasma and liver concentrations.

*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

Graphic illustration

**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.