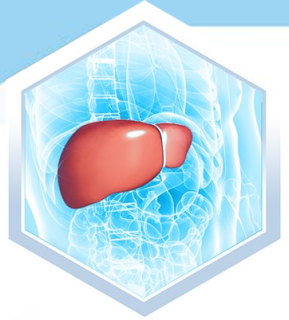
PROVEN GLOBAL CONTRACT RESEARCH EXPERTISE
FROM DISCOVERY THROUGH CLINICAL SUPPORT

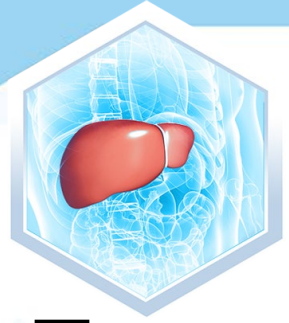
Understanding P-gp and BCRP Inhibition Assay Design and Outcomes

Andrea Wolff
XenoTech
Services Logistics Division Director

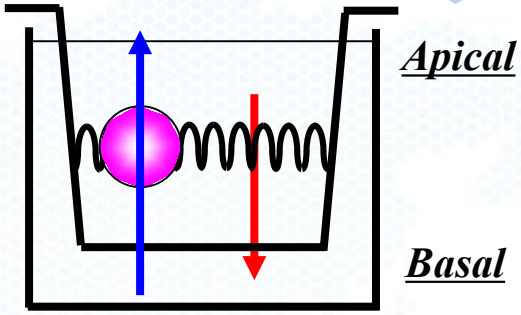


Presentation outline

- **P-gp and BCRP assays**
- **Test system qualification**
- **Test system comparison**
- **IC_{50} versus K_i**
- **Clinical relevance**

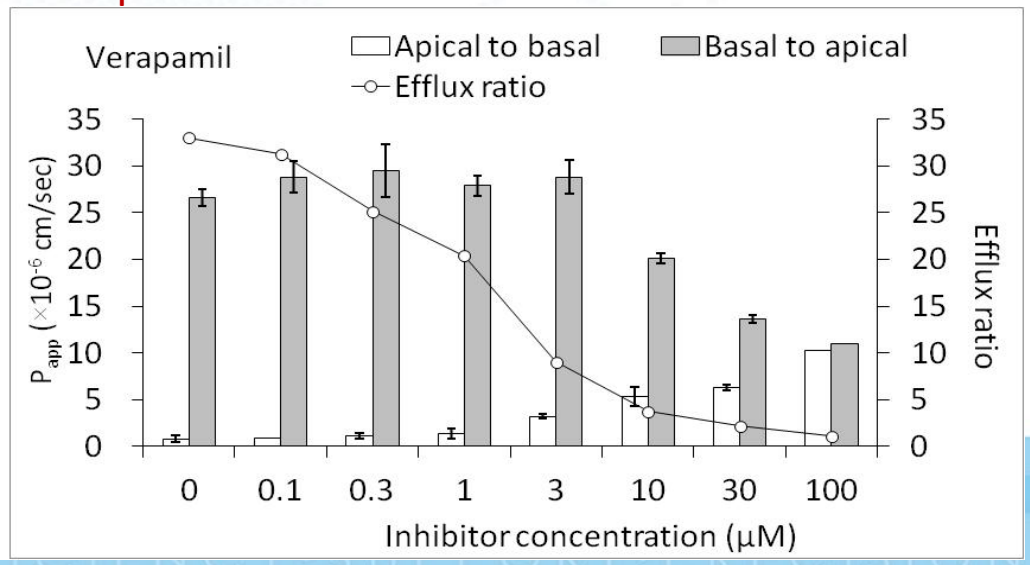


P-gp and BCRP Transwell assays



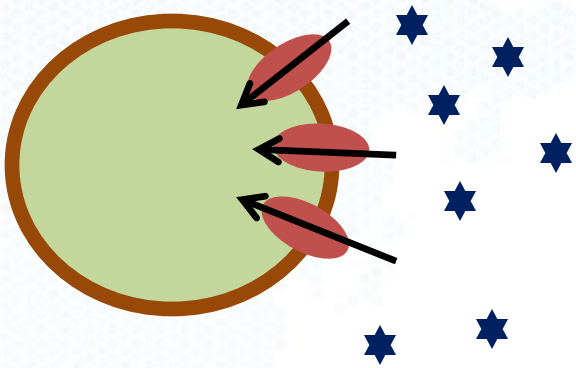
- **Test system:** Polarized cells grown on transwell plates
- P-gp and BCRP pump in basal to apical direction. Restrict permeation in apical to basal direction.
- **Substrate:** Measure bidirectional permeability of test article across cells
- **Inhibition:** Measure effect of test article on bidirectional permeability of probe substrate
- P-gp test systems: Caco-2 or MDCKII-P-gp
- BCRP test system: MDCKII-BCRP
- Efflux ratio = Papp B to A ÷ Papp A to B

Example inhibition data

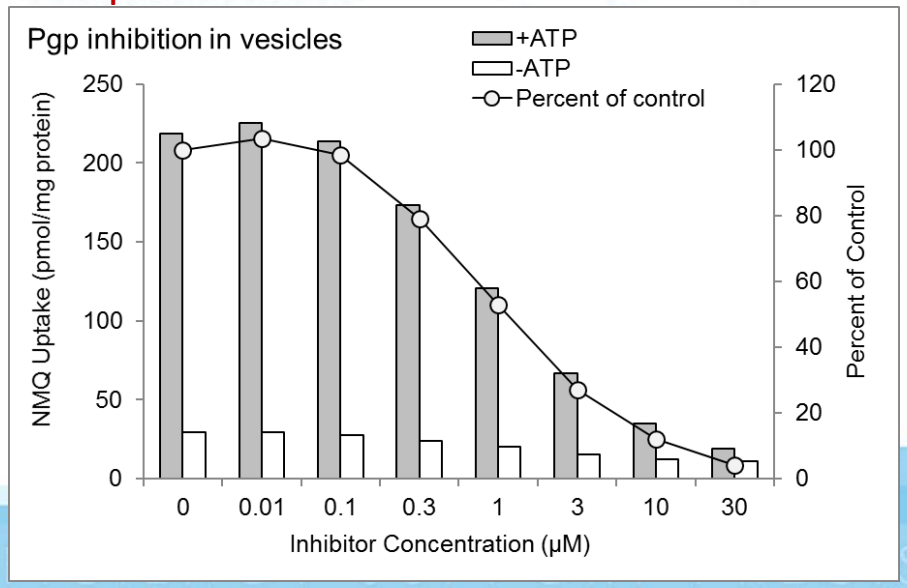


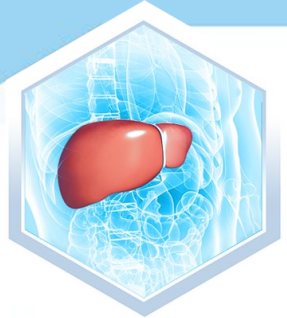
P-gp and BCRP Vesicle assays

- **Test system:** Inverted plasma membrane vesicles, from cells over expressing transporter
- **Substrate:** Measure accumulation of test article in transfected vesicles ± ATP
- **Inhibition:** Measure effect of test article on accumulation of probe substrate ± ATP
- Used for other efflux transporters (e.g., BSEP, MRPs)



Example inhibition data





Advantages/Disadvantages Transporters assays

Test system	Advantages	Disadvantages
Transwell	• Measure permeability and bidirectional transport • Stably transfected cells lines • Consistent transporter activity	• Endogenous transporter activity • Measure recovery • Non-specific binding • Cytotoxicity • Kinetics determinations are complicated • Long culture time (21 days for Caco-2) • Longer incubation times (up to 120 min)
Vesicles	• Not limited by cytotoxicity • Readily available • Short incubation times (<10 min) • Direct access to transporter binding site • Kinetics determinations are easy (similar to microsomes)	• Not useful for highly permeable compounds • Non-specific binding • Transporter activity can vary from batch to batch (kinetics determination with each batch)

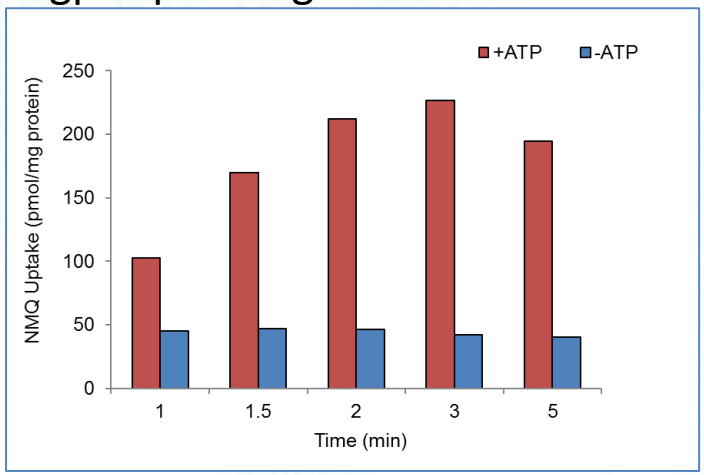
Brower, et al. Clin Pharmacol Ther 2013

Vesicle assay qualification

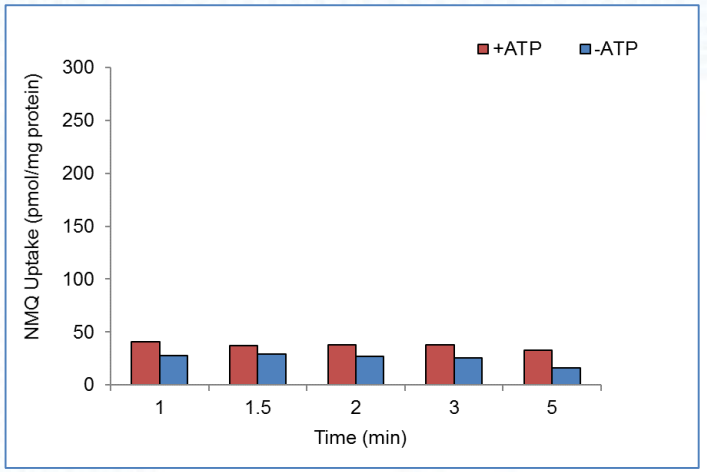
Step 1: Time course determination

N-methyl quinidine

P-gp expressing vesicles

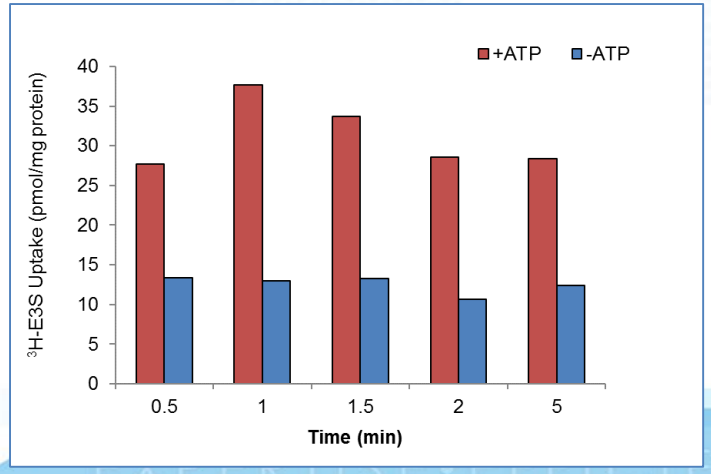


Control vesicles

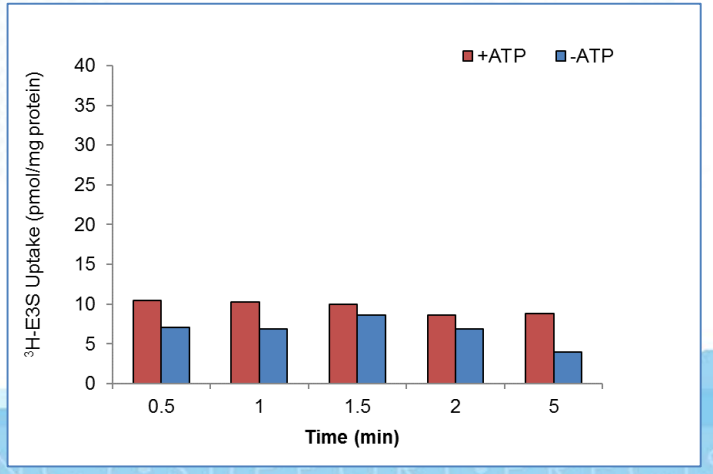


Estrone-3-sulfate

BCRP expressing vesicles

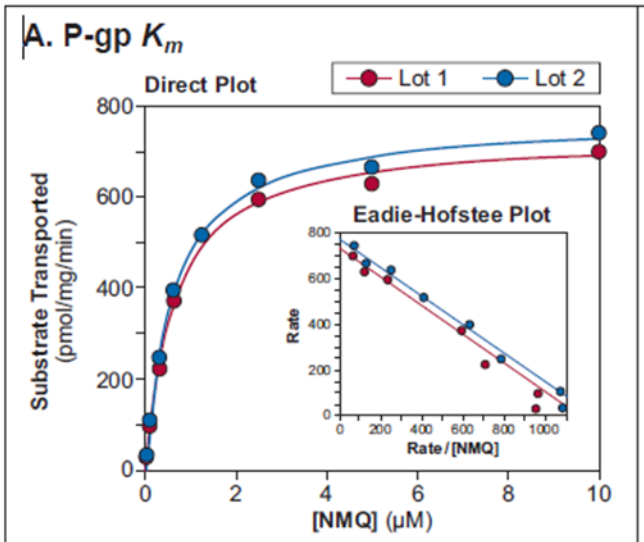


Control vesicles



Vesicle assay qualification

Step 2: Kinetics determination



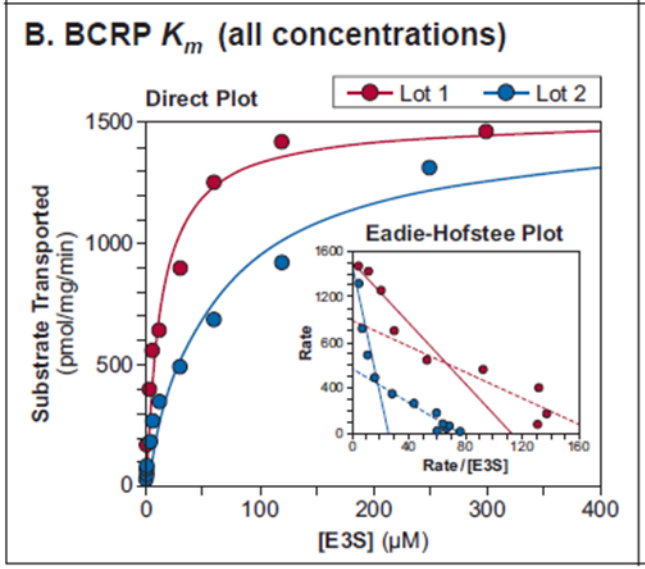
Tabular Summary of Values

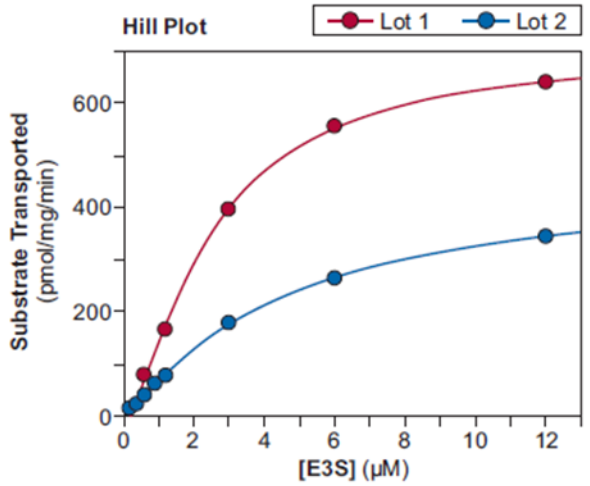
P-pg:

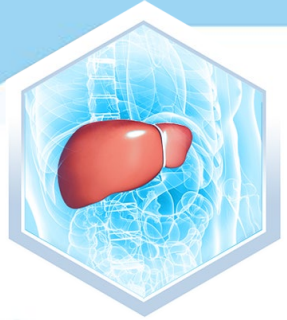
Lot	K_m (μM)	V_{max} (pmol/mg/min)
1	0.628 ± 0.051	735 ± 16
2	0.627 ± 0.038	774 ± 12

BCRP:

Low Affinity		
Lot	K_m (μM)	V_{max} (pmol/mg/min)
1	13.4 ± 2.5	1510 ± 70
2	58.4 ± 14.2	1500 ± 140
High Affinity		
Lot	K_m (μM)	V_{max} (pmol/mg/min)
1	2.53 ± 0.11	702 ± 15
2	4.39 ± 0.28	451 ± 14

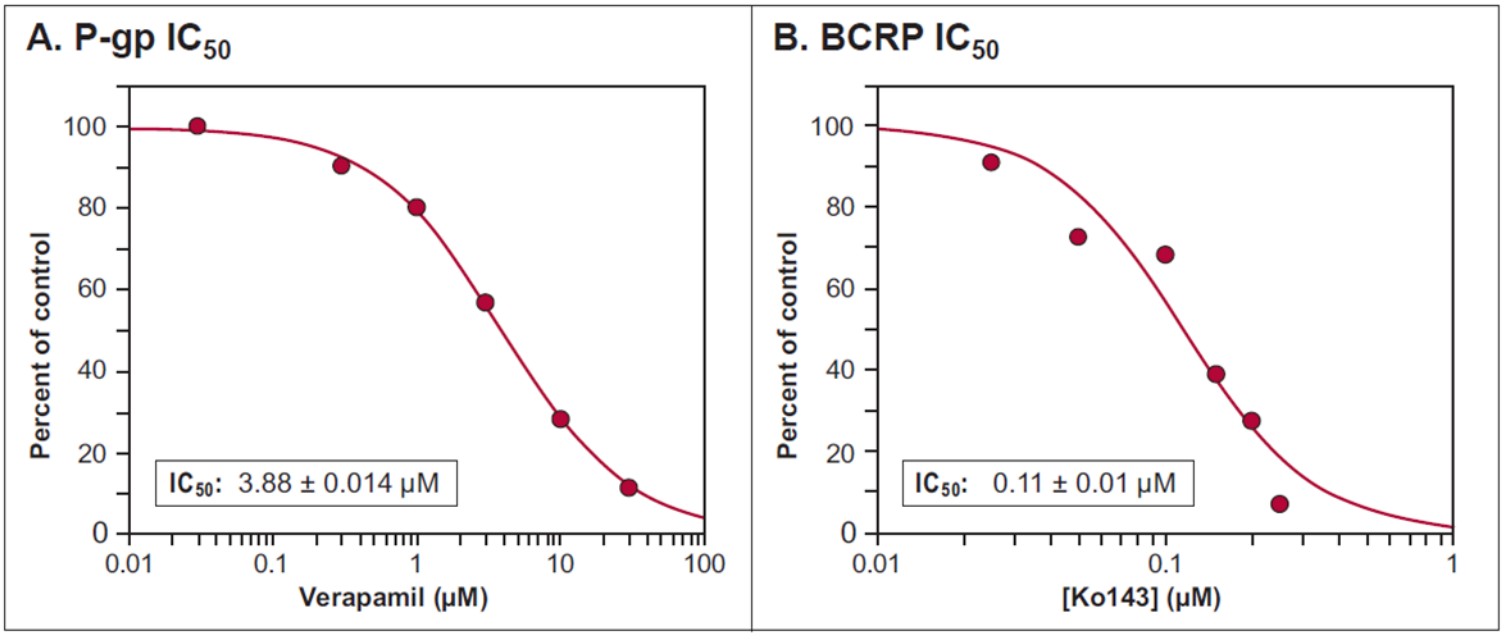






Vesicle assay qualification

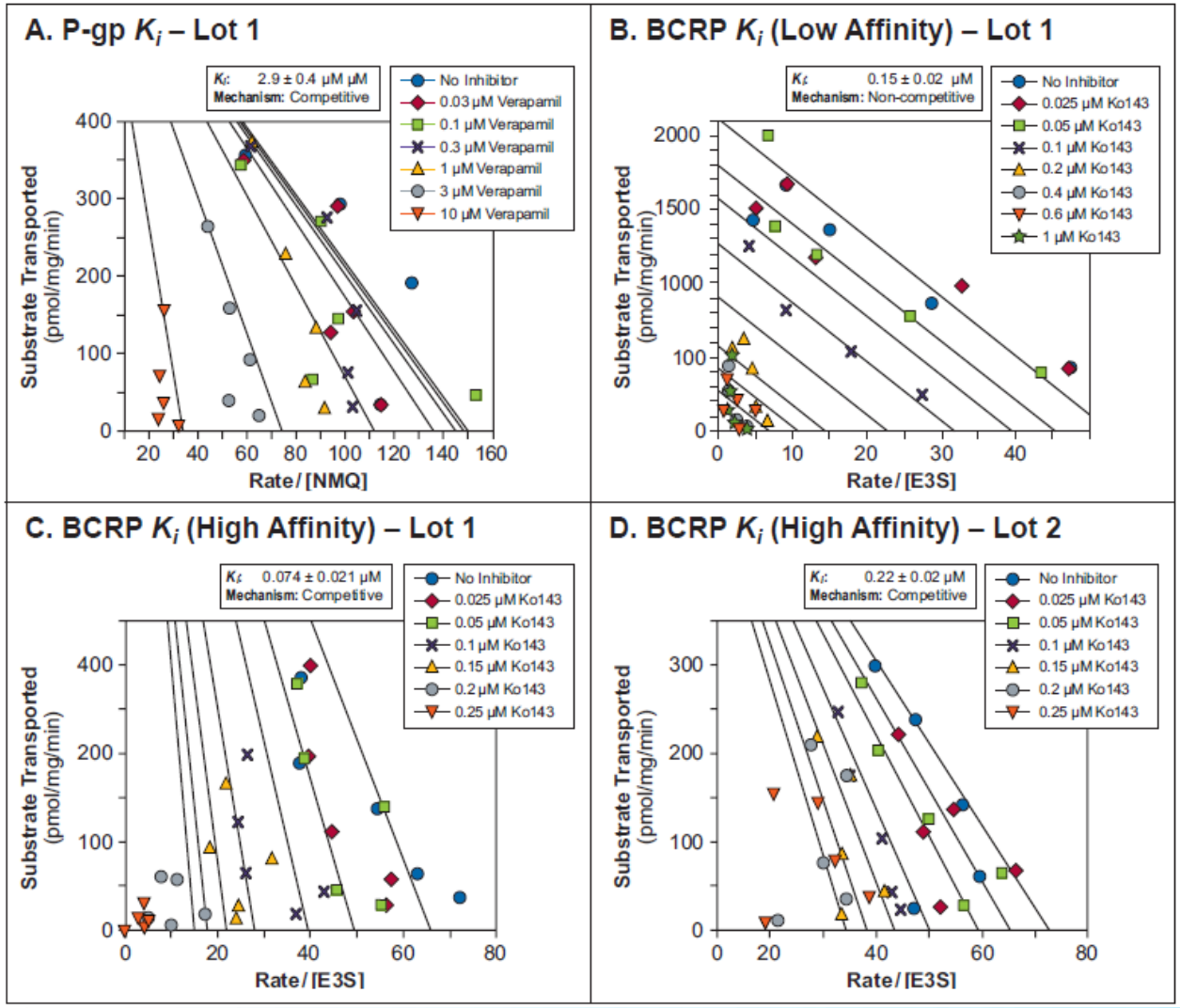
Step 3: IC_{50} determination (positive control inhibitor)



$$K_i = \frac{IC_{50}}{1 + \left(\frac{[S]}{K_m}\right)}$$

If [Substrate] << K_m, then $K_i = IC_{50}$

K_i determinations



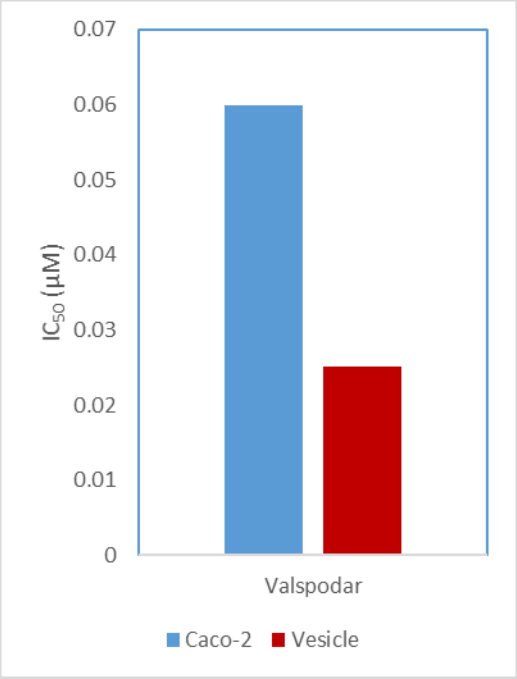
P-gp:
Estimated K_i = 2.6 µM
Determined K_i = 2.9 µM
(competitive fit)

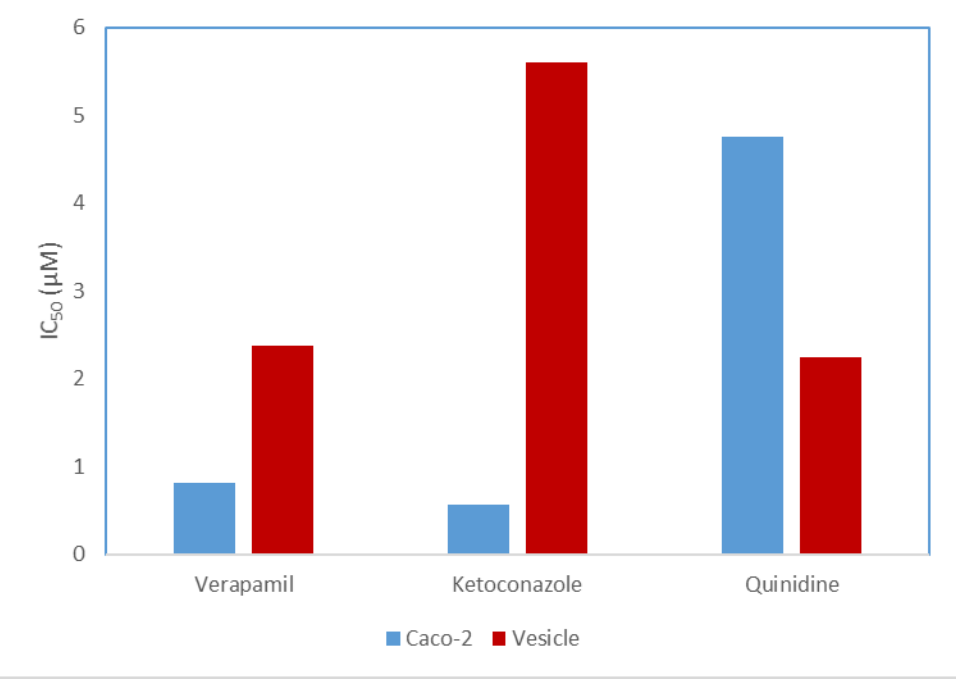
BCRP:
All concentrations
Estimated K_i = 0.10 µM
High affinity
Estimated K_i = 0.079 µM

Low affinity (lot 1)
Determined K_i = 0.15 µM
High affinity (lot 1)
Determined K_i = 0.079 µM
High affinity (lot 2)
Determined K_i = 0.22 µM

P-gp test system comparison

Internal data

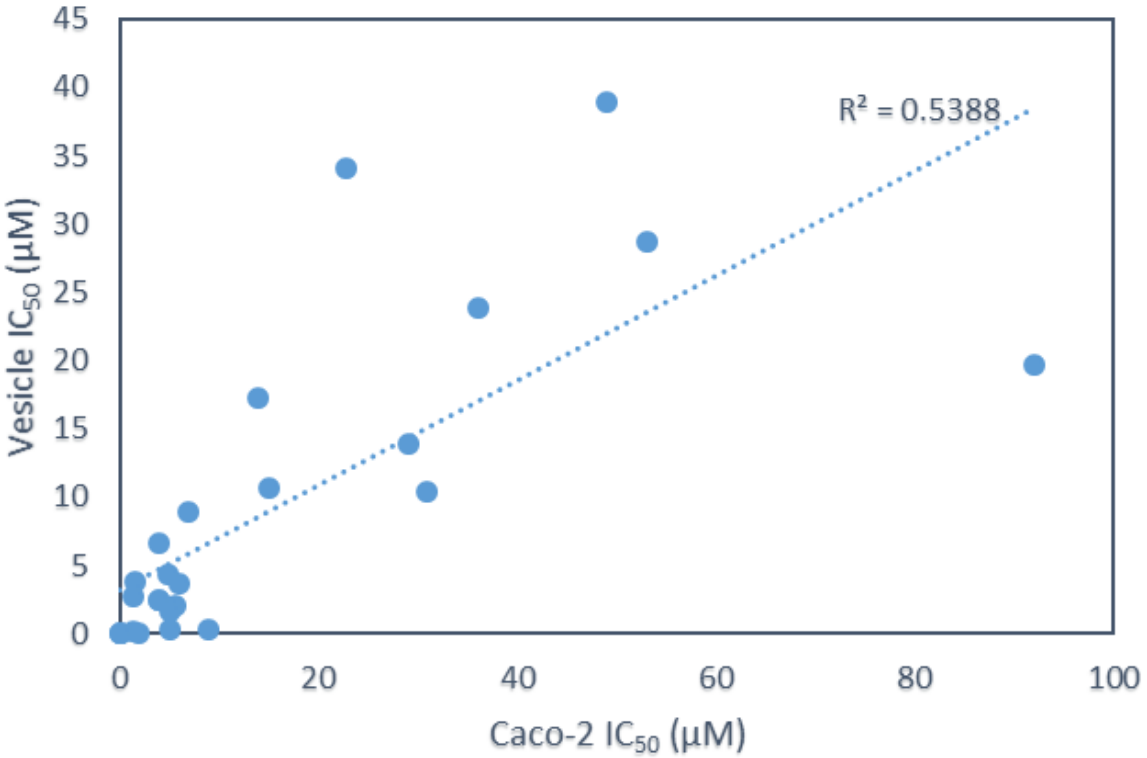




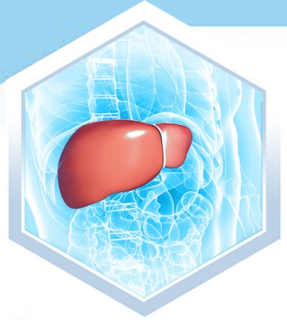
Inhibitor	Caco-2 IC_{50} (µM)	Vesicle IC_{50} (µM)	Fold difference
Valspodar	0.0599	0.0252	0.4
Verapamil	0.814	2.37	2.9
Ketoconazole	0.562	5.60	10
Quinidine	4.75	2.24	0.5

P-gp test system comparison

External data (UW Drug Interaction Database)

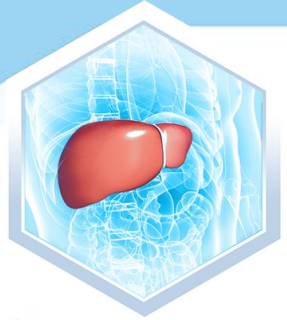


- 26 inhibitors
- Same probe substrates (digoxin and N-methyl quinidine)
- Fold-difference: 0.2 – 42
- 23 of 26 had a fold-difference < 10



Regulatory agencies: Transporter inhibition – Cutoffs for clinical DDIs

Transporter expression	EMA criteria	Relevant concentration	FDA criteria	Relevant concentration
P-gp, BCRP intestinal	K_i < 0.1×dose/250 mL	0.1 × dose/250 mL	I_{gut} /IC_{50} is ≥ 10	0.1 × dose/250 mL
P-gp, BCRP systemic	K_i ≤ 50 × unbound C_{max}	50 × unbound C_{max}	Not applicable	
OATP1B1, OATP1B3 (hepatic uptake)	50 × unbound C_{max} for iv drugs or K_i ≤ 25 × $[I]_{u,\ in,\ max}$	50 × unbound C_{max} of iv drugs or 25 × $[I]_{u,\ in,\ max}$	R ≥ 1.1 (equivalent to 10 × $f_{u,p}$ x $I_{in,max}$)	10 × $f_{u,p}$ x $I_{in,max}$
OAT1, OAT3, OCT2 (renal uptake)	K_i ≤ 50 × unbound C_{max}	50 × unbound C_{max}	$I_{max,u}$/IC_{50} is ≥ 0.1 (equivalent to IC_{50} ≤ 10 × unbound C_{max})	10 × unbound C_{max}
MATE1, MATE2-K	K_i ≤ 50 × unbound C_{max}	50 × unbound C_{max}	$I_{max,u}$/IC_{50} is ≥ 0.02 (equivalent to IC_{50} ≤ 50 × unbound C_{max})	50 × unbound C_{max}

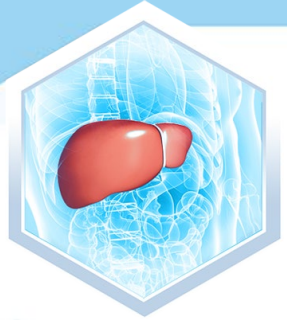


Clinical relevance

Compound	IC_{50} (µM) in P-gp vesicles	Dose	C_{max} (µM)	Fraction unbound	$[I]_2/IC_{50}$ (≥10)	Unbound C_{max}/IC_{50} (≥0.02)
Ketoconazole	5.6	400 mg qd 4 days	2.82	0.032	541	0.016
Clarithromycin	8.9	500 mg bid 7 days	3.12	0.028	301	0.10
Ritonavir	0.24	100 mg bid 15 days	3.50	0.02	2332	0.29
Itraconazole	0.048	100 mg bid 4 days	4.34	0.036	11860	0.87

- Recommended CYP3A4/5 inhibitors for DDI studies (FDA and EMA)
- Also inhibit P-gp, as well as other transporters

Vermeer LMM, Isringhausen CD, Ogilvie BW, Buckley DB (2015) Evaluation of ketoconazole and its alternative clinical CYP3A4/5 inhibitors as inhibitors of drug transporters: The in vitro effects of ketoconazole, ritonavir, clarithromycin, and itraconazole on 13 clinically-relevant drug transporters. DMD: 44 (3) 453-459.



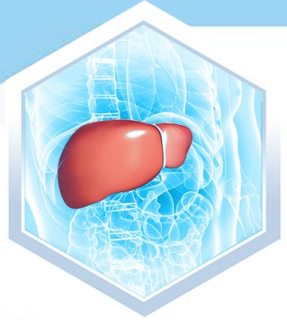
Clinical relevance
Example: Ketoconazole

Drug	IC_{50} (µM) in P-gp vesicles	Dose	C_{max} (µM)	Fraction unbound	$[I]_2/IC_{50}$ (≥10)	Unbound C_{max}/IC_{50} (≥0.02)
Ketoconazole	5.6	400	2.82	0.032	**541**	0.016

In vivo inhibition (P-gp and CYP3A4):

- 26 compounds listed in UW Drug Interaction Database with inhibition > 20%
- 1 compound listed with no inhibition (lenvatinib)

Co-administered drug	Dose	Increase in AUC (%)	Ref.
Alisporivir	600 mg	687	*Barve, et al. Clin Pharmacol Drug Dev 2015*
Fexofenadine	120 mg	174	*FDA NDA 1996*
Apixaban	10 mg	98.8	*Frost et al. Br J Clin Pharmacol 2015*
Naloxegol	25 mg	1141	*FDA NDA 2014*
Venetoclax	50 mg	540	*FDA NDA 2016*
Voclosporin	0.4 mg/kg	1713	*Ling et al. Br J Clin Pharmacol 2014*



Conclusions

- Qualified assays are utilized
- Some variability between vesicle lots, recommend K_m determinations
- K_i can be predicted from IC_{50}
- Results between Caco-2 and vesicles can be similar, but there is notable variability
- In vitro data are useful for predicting interactions

www.xenotech.com

Featuring publications, posters, webinars and other useful resources.

Webinar Topic Request Form:

www.xenotech.com/scientific-resources/upcoming-webinars

XenoTech's Services & Products:

In Vitro ADMET PK & DDI
- Drug Transport
- Drug Metabolism
- Enzyme Inhibition & Induction
- Protein Binding
- Metabolite Identification
- ADME Screening
- Toxicology

In Vivo ADME/PK & Distribution
- QWBA
- Microautoradiography
- Excretion / Mass Balance
- Tissue Distribution
- Blood / Plasma & Lymphatic Partition Rate

Bioanalytical Pharmacology
- In Vitro Ligand Binding & Radioreceptor Assays
- Immunoassays

Chemical Synthesis
- Radiolabeled Synthesis
- Metabolite Synthesis
- Peptide Synthesis

Consulting...

Cellular Products
- Hepatocytes (Cryo/Fresh, Genotyped...)
- Non-Parenchymal Cells (Kupffer Cells)

Subcellular Fractions
- Liver Microsomes
- S9 Fractions
- Cytosol
- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
- Extrahepatic Fractions

Custom Products
- Various Species, Tissues & Preparations

Research Biobank
- Normal & Diseased Tissue Samples

Recombinant Enzymes

Substrates & Metabolites

Metabolite Production Kits

JCRB Cell Lines...