In Vitro DDI Drug Transporter Studies: Efflux and Uptake Transporters

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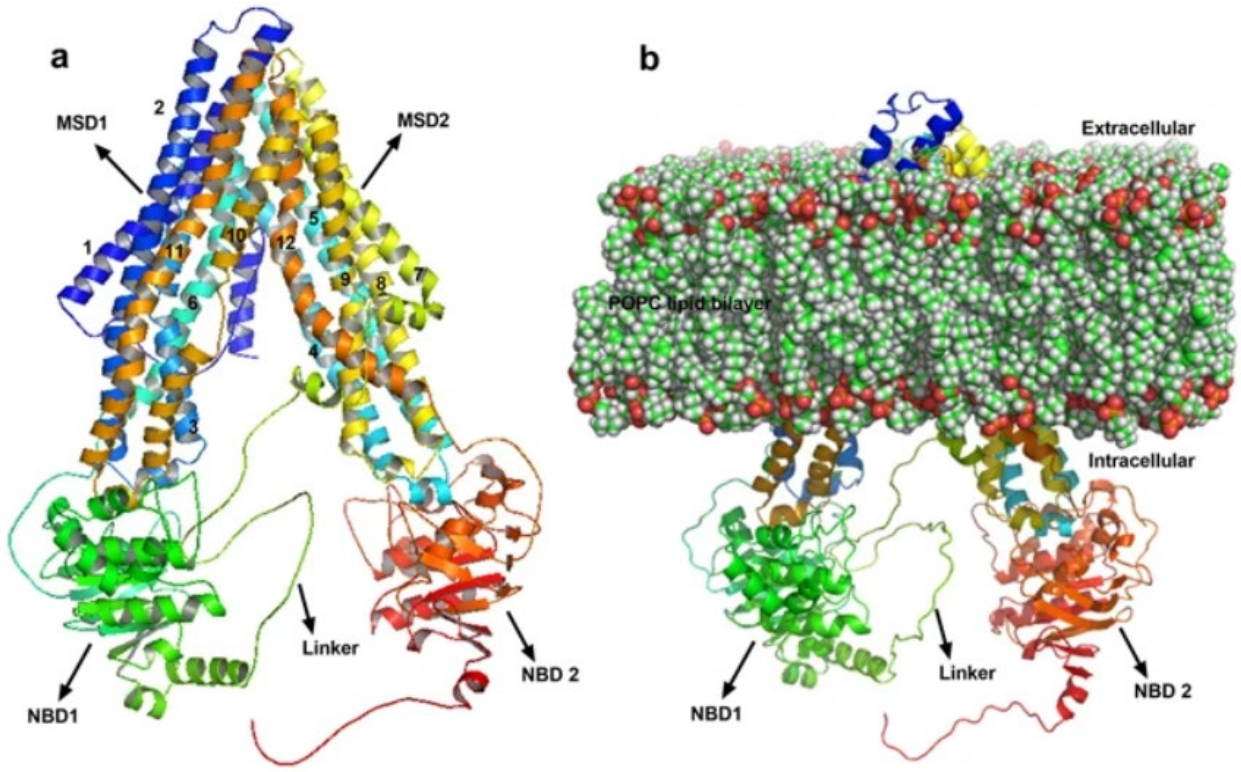
XenoTech

What are Drug Transporters?

- Proteins bound to membranes that prevent or assist in movement of drugs in and out of cells
- Located throughout the body
  - Intestine
  - Liver
  - Kidney
  - Blood-brain barrier
- Two superfamilies
  - ATP-binding cassette (ABC) Transporters (Efflux)
    - P-gp (MDR1), BCRP, BSEP, MRPs
  - Solute carrier (SLC) Transporters (Uptake)
    - OATP1B1/3, OCT1/2, OAT1/3, MATE1/2K

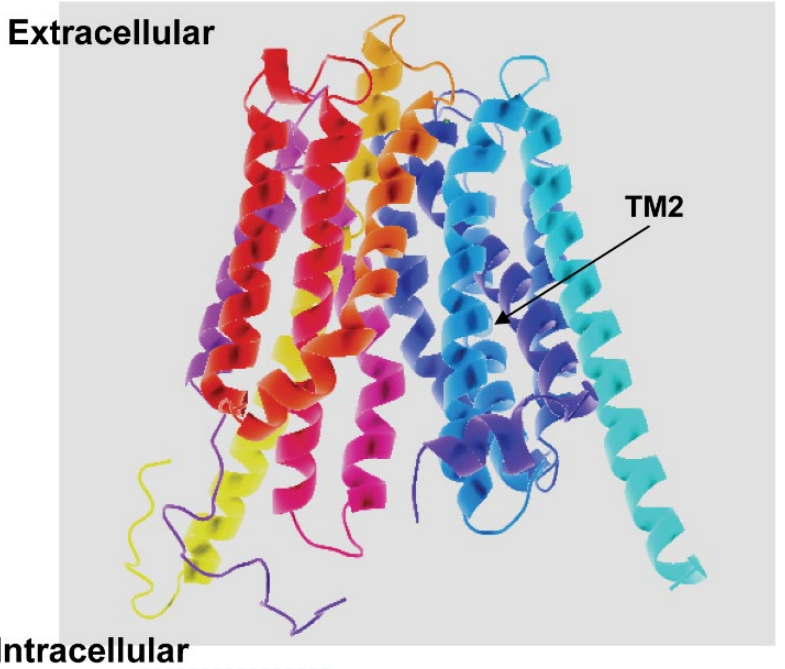


Syed, S.B., Arya, H., Fu, I. *et al.* Targeting P-glycoprotein: Investigation of piperine analogs for overcoming drug resistance in cancer. *Sci Rep* **7,** 7972 (2017). https://doi.org/10.1038/s41598-017-08062-2

Why are Transporters Important?

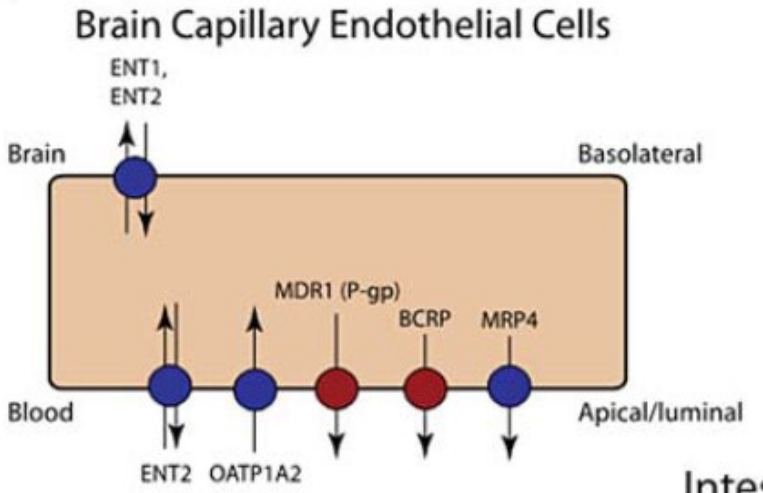
The AD&E in ADME

- Drug absorption, distribution, tissue-specific drug targeting, and elimination
- Drug-drug interactions
  - Clearance of transporter substrates (Victims) can be impacted by transporter inhibitors or inducers (Perpetrators)
  - Toxicity or loss of efficacy
- Real world example - Statins
  - Hepatic uptake transporter (OATPs) substrates: taken up in the liver, reduce cholesterol
  - Cyclosporine inhibits OATPs: up to 10-fold increase in statin exposure
  - Toxic side effect: rhabdomyolysis (skeletal muscles break down, cells released into bloodstream, can lead to kidney failure and possibly death)

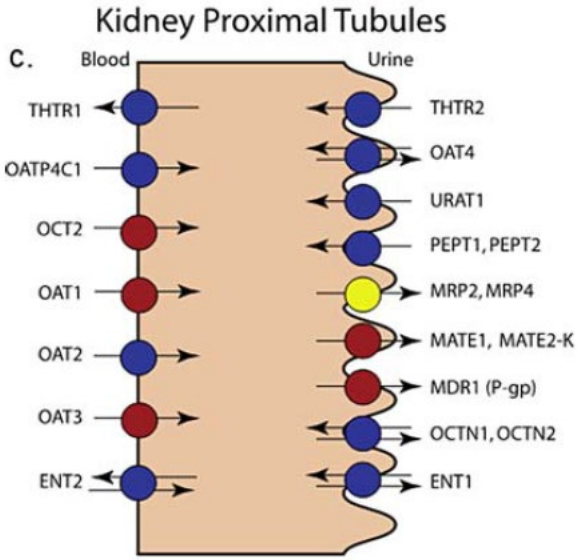


Li N, Hong W, Huang H, Lu H, Lin G, et al. (2013) Correction: Identification of Amino Acids Essential for Estrone-3-Sulfate Transport within Transmembrane Domain 2 of Organic Anion Transporting Polypeptide 1B1. PLOS ONE 8(5): 10.1371/annotation/35943d4c-3455-4818-976a-be73a2e07fff.

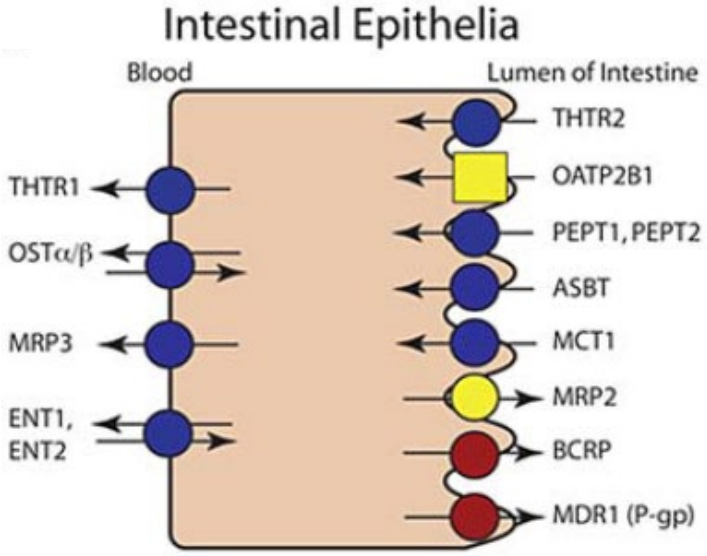
Transporter Expression and Function

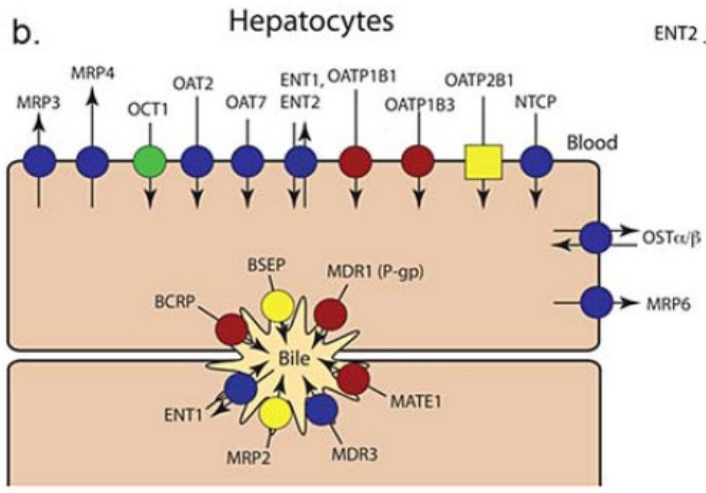


Liver (hepatocytes) and **kidney** (proximal tubules): **uptake** transporters (SLC) remove compounds from the blood and **efflux** (ABC) or SLC transporters pump them into the bile or urine



Intestine and **BBB: efflux** (ABC) transporters predominate (particularly **P-gp** & **BCRP)** regulating absorption or entry into the brain





Figures apted from Zamek-Gliszczynski et al., Clin Pharm Ther 2018

Regulatory Guidance on Transporters

	FDA (2020)	EMA (2013)	PMDA (2014)
Recommended transporters	P-gp, BCRP OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K	P-gp, BCRP, BSEP OATP1B1, OATP1B3, OAT1, OAT3, OCT2	P-gp, BCRP OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE-2K
Transporters to consider	BSEP, MRP2, OCT1 Others as necessary	OCT1, MATE1, MATE-2K Others as necessary	Others as necessary
Substrate evaluation	Yes (P-gp, BCRP) Others based on clearance/elimination routes and knowledge of drugs in same therapeutic class EMA: Two systems for P-gp, 4 conc (100-fold range)		
Inhibitor evaluation	Yes, all of the recommended transporters (EMA and PMDA: in vitro data prior to Phase III trials) EMA: Two inhibitors as positive controls		
Induction	FDA and EMA: In vivo studies Yes, for CYP3A4, PXR, CAR inducers (P-gp)		
Metabolites	≥ 25% of parent drug AUC		

General Transporter Study Design: Inhibition

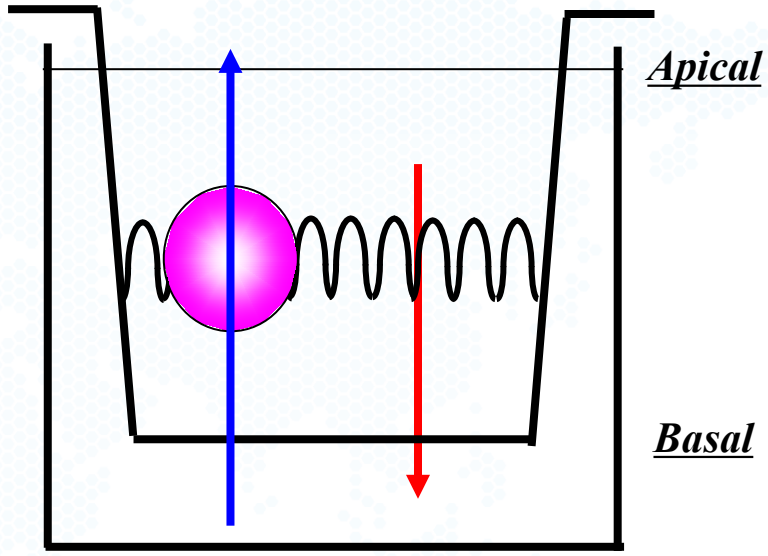
- Solubility: Typically DMSO, 0.1% v/v (avoid 1% or more)
- Preliminary experiments
  - Cytotoxicity, stability in media, non-specific binding to plates
- Inhibition
  - Probe substrate concentration << Km (IC_{50} ≈ Ki)
  - Short incubation time (in linear range with acceptable fold uptake)
  - Highest [TA] based on EMA/FDA guidance
  - Minimal design: 2 [TA] + no solvent and solvent control
  - Definitive design: 7 [TA] + no solvent and solvent control to determine IC_{50}

General Transporter Study Design: Substrate

- Substrate
  - [TA] pharmacologically relevant (too low, then BLQ; too high, then saturate)
  - Transwell
    - Minimal design: 1 [TA] , 3 times, ± inhibitor
    - Definitive design: 4 [TA] , 3 times, ± inhibitor and control cells at 1 [TA]
  - Uptake
    - Minimal design: 1 [TA] , 2 times, ± inhibitor, transporter and control cells
    - Definitive design: 4 [TA] , 3 times, transporter and control cells, ± inhibitor at 1 [TA]

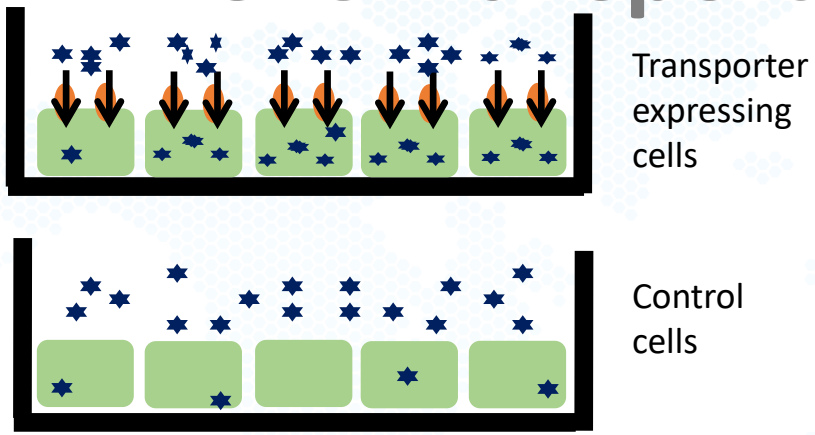
Efflux Transporter: Transwell Assays

- **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 and MDCKII-MDR1
- BCRP test system: MDCKII-BCRP



Transporter	Probe substrate	Positive control inhibitors
P-gp	Digoxin	Valspodar, Verapamil
BCRP	Prazosin	Ko143, Lopinavir

SLC Transporter Uptake Assays

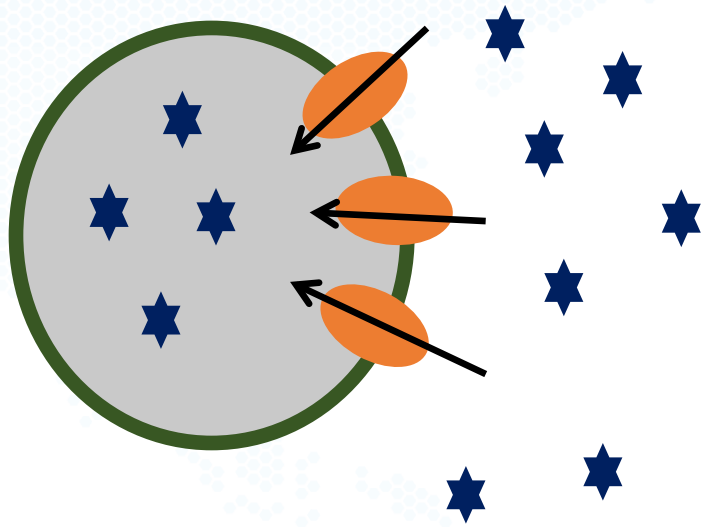


Transporter	Probe substrate	Positive control inhibitors
OATP1B1/ OATP1B3	^{3}H-Estradiol-17β-glucuronide	Rifampin, Cyclosporine
OAT1	^{3}H-p-Aminohippurate	Probenecid, Novobiocin
OAT3	^{3}H-Estrone-3-sulfate	Probenecid, Ibuprofen
OCT2	^{14}C-Metformin	Quinidine, Cimetidine
OCT1	^{14}C-Tetraethylammonium bromide	Quinidine, Verapamil
MATE1/2K	^{14}C-Metformin	Cimetidine, Pyrimethamine

- **Test system:** transfected HEK293 cells grown in cell culture plates
- **Substrate:** measure the accumulation of the test compound in transfected and control cells
- **Inhibition:** measure the effect of the test article on the accumulation of a probe substrate

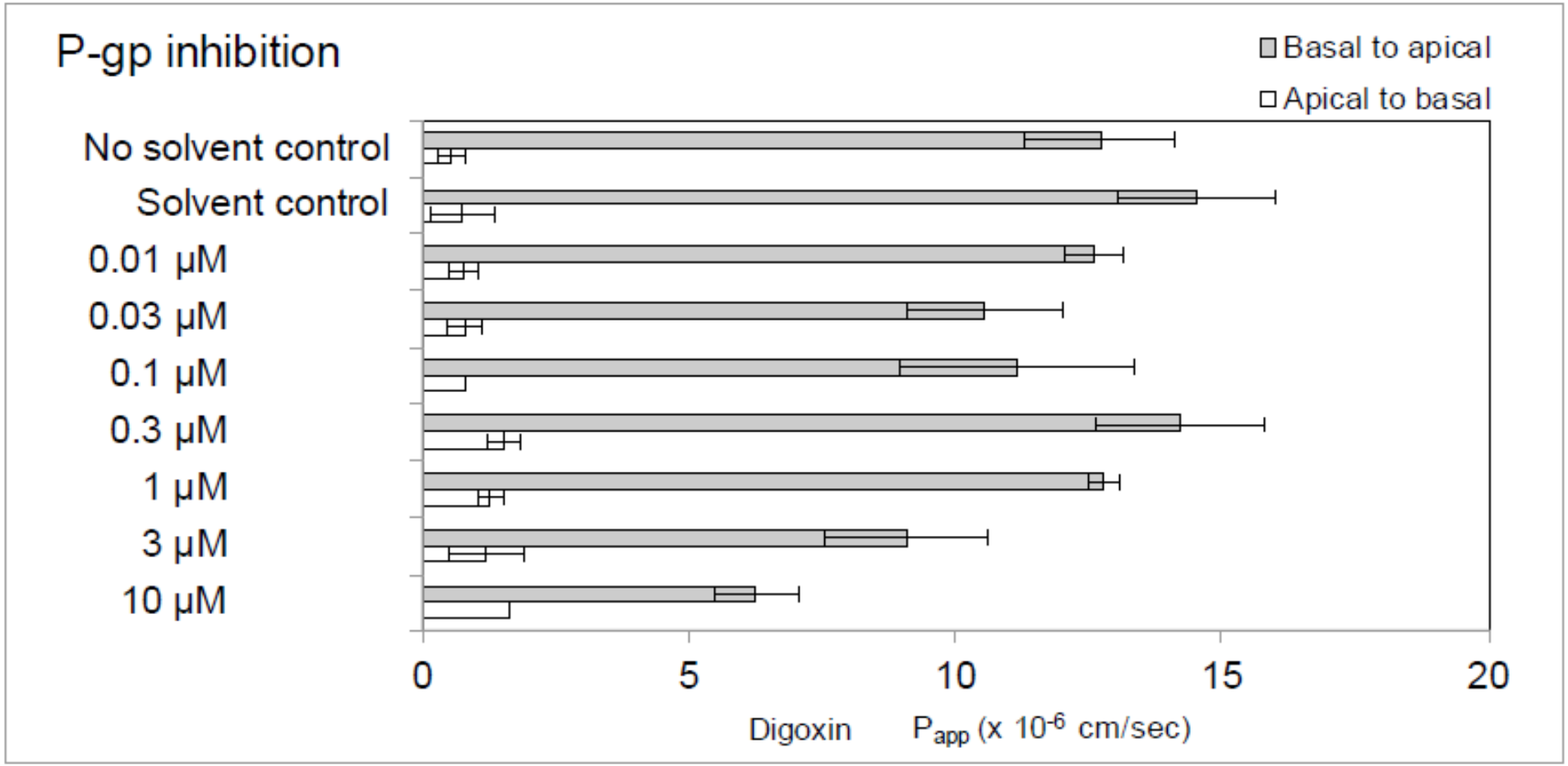
BSEP and MRP2 (Vesicle assays)

- **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., P-gp, BCRP, MRP1, MRP3, MRP4)

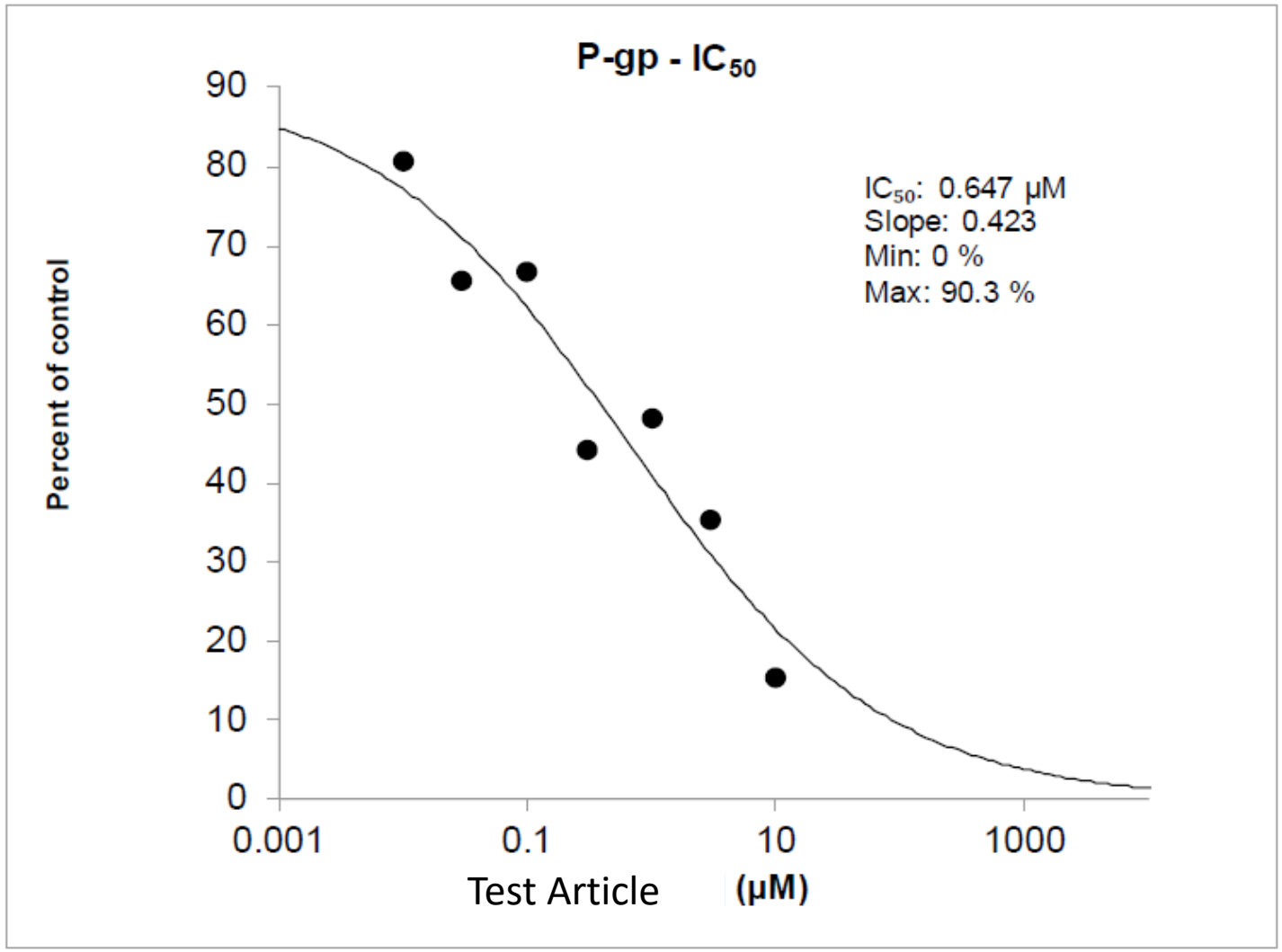


Transporter	Probe substrate	Positive control inhibitors
BSEP	^{3}H-Taurocholate	Cyclosporine, Glyburide
MRP2	^{3}H-Estradiol-17β-glucuronide	Benzbromarone

Transporter Results Example



Transporter Results Example



Transporters Available

Description of construct	Parental Cell line	Gene	Substrates	Kinetic parameters	Kinetic parameters for inhibitors
MDR1	LLC-PK1	ABCB1	1 µM Digoxin	Km: >100 µM	IC50 Verapamil :1.6 µM
					IC50 Cyclosporin A: 0.94uM
BCRP	LLC-PK1	ABCG2	10 nM Prazosin	KM: 19.6 µM	IC50 Ko143: 0.0344 µM
	Vesicle		1 µM Estrone sulfate	KM: 6.77 µM (14.2)	IC50 Ko143: 0.0186 µM (0.026)
BSEP	Vesicle	ABCB11	2 µM Taurocholic acid	KM: 19.1 µM	IC50 Cyclosporine A: 6.68 µM
MRP2	Vesicle	ABCC2	0.1 µM Leukotriene C_4	KM: 5.79 µM (0.24-5.9)	IC50 BSP: 10.0 µM
					IC50 Benzbromarone: 11.1 µM (7.3-8.3)
OATP1B1	HEK	SLCO1B1	50 nM Estradiol glucuronide	KM: 3.94 µM (3.7-8.3)	IC50 Rifampicin: 0.243 µM(0.94)
					IC50 Cyclosporin A: 0.215 µM
OATP1B3	HEK	SLCO1B3	50 nM Estradiol glucuronide	KM: 26.5 µM (5-25)	IC50 Rifampicin: 0.388µM (1.5)
					IC50 Cyclosporin A: 0.386 µM
			20 nM CCK-8	KM: 18.6 µM	IC50 Rifampicin: 1.06 µM
					IC50 Cyclosporin A: 0.612 µM
OATP2B1	HEK	SLCO2B1	50 nM Estrone sulfate	KM: 14.5 µM (7-21)	IC50 Rifampicin: 20.6µM (90)
					IC50 Cyclosporin A: 1.05 µM
OAT1	HEK	SLC22A6	1000 nM p-Aminohippurate	KM: 11.9 µM (4-22)	IC50 Probenecid: 4.70 µM (6.3-12.5)
					IC50 Indomethacin: 1.95 µM
	S_2		1000 nM p-Aminohippurate	KM: 24.6 µM (4-22)	IC50 Probenecid: 11.7 µM (6.3-12.5)
OAT2	S_2	SLC22A7	50 nM PGF2α	KM: 0.137 µM (0.4-0.8)	IC50 Mefenamic acid: 11.7 µM (21.7)
OAT3	HEK	SLC22A8	50 nM Estrone sulfate	KM: 22.8 µM (2-7)	IC50 Probenecid: 2.70 µM (4-30)
					IC50 Cimetidine: 264 µM
	S_2		50 nM Estrone sulfate	KM: 1.59 µM (2-7)	IC50 Probenecid: 5.33 µM (4-30)
OAT4	HEK	SLC22A11	50 nM Estrone sulfate	KM: 15.8 µM (1-10)	IC50 BSP: 0.377 µM (<5)
	S_2		50 nM Estrone sulfate	KM: 7.42 µM (1-10)	IC50 Probenecid: 81.8 µM (25.4)
OAT7	HEK	SLC22A9	50 nM Estrone sulfate	KM: 12.9 µM (8.7-40)	IC50 BSP: 0.121 µM
OCT1	HEK	SLC22A1	5 µM Tetraethylammonium bromide	KM: 576 µM (160-1600)	IC50 Quinidine: 4.57 µM (5.4-23.4)
					IC50 Verapamil: 0.272 µM
			10 µM Metformin	KM: 899 µM	IC50 Quinidine: 13.8 µM
	S_2		5 µM Tetraethylammonium bromide	KM: 453 µM (160-1600)	IC50 Quinidine: 17.9 µM (5.4-23.4)
OCT2	HEK	SLC22A2	10 µM Metformin	KM: 3600 µM (990)	IC50 Quinidine: 88.6 µM (1 mM 5-20%)
			5 µM Tetraethylammonium bromide	KM: 723 µM	IC50 Quinidine: 93.7 µM
					IC50 Verapamil : 192 µM
	S_2		10 µM Metformin	KM: 12500 µM (990)	IC50 Quinidine: 104 µM
			5 µM Tetraethylammonium bromide	KM: 35.1 µM	IC50 Verapamil : 298 µM
OCT3	HEK	SLC22A3	5 µM Tetraethylammonium bromide	KM: 536 µM (+)	IC50 Verapamil: 6.21 µM (24)
					IC50 Quinidine : 12.7 µM
	S_2		5 µM Histamine	KM: 261 µM(180-420)	IC50 Verapamil: 11.6 µM (24)
OCTN1	S_2	SLC22A4	5 µM Tetraethylammonium bromide	KM: 315 µM (199-1280)	IC50 Verapamil: 12.3 µM (8.4)
OCTN2	S_2	SLC22A5	30 nM Carnitine	KM: 4.73 µM (2-66)	IC50 Verapamil: 5.19 µM (500 µM 2.4%)
NTCP1	HEK	SLC10A1	50 nM Taurocholate	KM: 10.2 µM (6.2-10)	IC50 Cyclosporin A: 1.20 µM (1)
NTCP2 (ASBT)	HEK	SLC10A2	200 nM Taurocholate	KM: 15.8 µM (9.4-20)	IC50 Chenodeoxycholic acid: 3.48 µM (Ki 3.3)
NPT1	HEK	SLC17A1	50 nM Estradiol glucuronide	KM: 6.68 µM	IC50 Indomethacin: 2.17 µM
					IC50 Probenecid : 69.1 µM
PEPT1	HEK	SLC15A1	100 nM Glycylsarcosine	KM: 556 µM (290-3130)	IC50 Cephalexin: 1460 µM (5200-13700)
PEPT2	HEK	SLC15A2	100 nM Glycylsarcosine	KM: 59.4 µM (74)	IC50 Cephalexin: 124 µM (10mM 0%)
URAT1	HEK	SLC22A12	50 µM Uric acid	KM: 1050 µM (371)	IC50 Benzbromarone: 0.0334 µM (0.3)
					IC50 Probenecid : 80.0 µM
MATE1	HEK	SLC47A1	10 µM Metformin	KM: 73.4 µM (220)	IC50 Cimetidine: 1.32 µM (<20)
			5 µM Tetraethylammonium bromide	KM: 29.9 µM	IC50 Cimetidine : 1.10 µM
					IC50 Quinidine : 1.40 µM
MATE2-K	HEK (Transient)	SLC47A2	10 µM Metformin	KM: 252 µM	IC50 Cimetidine: 4.95 µM
			5 µM Tetraethylammonium bromide	KM: 109 µM	IC50 Cimetidine : 36.6 µM
					IC50 Quinidine : 16.3 µM
rUrat1	HEK	Slc22a12	Uric acid	KM: 677 µM	IC50 Benzbromarone : 1.88 µM
OSTα/β	HEK	SLC51	Taurocholic acid	KM: 7960 µM	IC50 Indomethacin : 262 µM

P-gp (MDR1)	LLC-PK1, MDCKII, Caco-2	✓				
MRP1		✓				
MRP2		✓	OATP1B1			
MRP3		✓				
MRP4		✓				
BCRP	LLC-PK1, MDCKII, Caco-2	✓				
BSEP		✓				
OATP1A2				✓		
OATP1B1	HEK293		MRP2	✓	✓	
OATP1B3	HEK293			✓	✓	
OATP2B1	HEK293			✓		
OAT1	HEK293, S_2			✓		
OAT2	S_2					
OAT3	HEK293, S_2			✓		
OAT4	HEK293, S_2			✓		
OAT7	HEK293					
OCT1	HEK293, S_2			✓		
OCT2	HEK293, S_2			✓		
OCT3	HEK293, S_2					
OCTN1	S_2					
OCTN2	S_2					
OST α/β	HEK293					
PEPT1	HEK293					
PEPT2	HEK293					
URAT1	HEK293					
NTCP1	HEK293			✓	✓	
NTCP2 (ASBT)	HEK293					
MATE1	HEK293					
MATE2-K	HEK293 (TEA & Metformin)					
NPT1	HEK293					
ENT1	MDCKII					
ENT2	MDCKII					
ENT4	MDCKII					
CNT1	MDCKII					
CNT2	MDCKII					
CNT3	MDCKII					
GLUT1				✓		
GLUT2				✓		
Mouse Mdr1a	LLC-PK1					KO animal
Mouse Mdr1b	LLC-PK1					KO animal
Rat Mdr1a	LLC-PK1					KO animal
Rat Mdr1b	LLC-PK1					
Rat Urat1	HEK293			✓		
Rat Oatp4/Oatp1b2				✓		
Dog Mdr1	LLC-PK1					

XT Products (Transporters)

Primary Human Hepatocytes

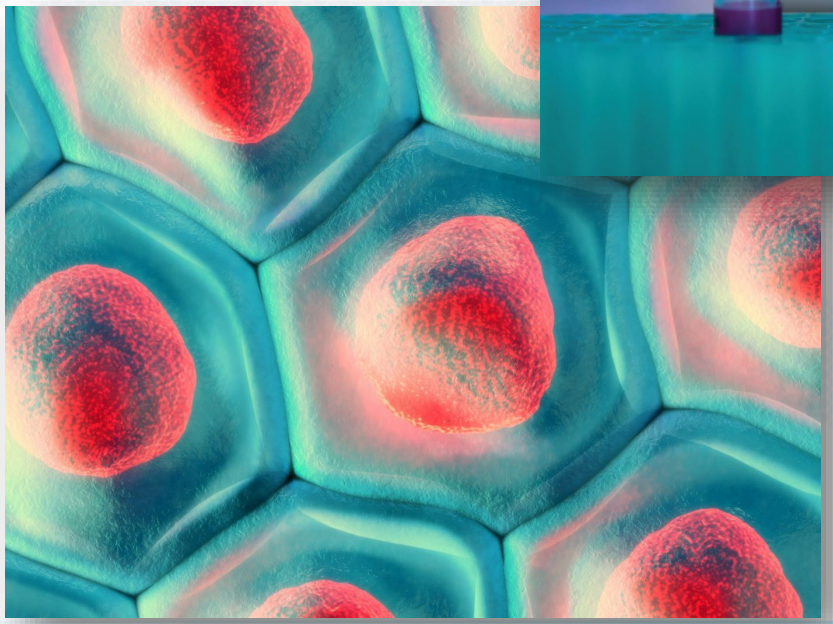
- Suspension (uptake)
  - Some lots with uptake characterization
    - Individual donor and Pooled CryostaX
- Plated
  - CryostaX (plated uptake)
    - No individual lots are characterized, but no reason not to believe that they do not maintain uptake activity.
    - No Efflux characterized products

Primary Animal Hepatocytes

- No lot characterization, but have R&D data to show that our cell preparation protocols maintain uptake activity (rat and mouse)
  - Suspension
  - Plated

Support Reagents

- PHH
  - OptiTHAW
  - OptiPLATE
  - OptiCULTURE
  - OptiINCUBATE



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