SSX India-2018
Oct 11th 2018

Navigating Transporter Sciences in Pharmacokinetics Characterization using Extended Clearance Classification System (ECCS)

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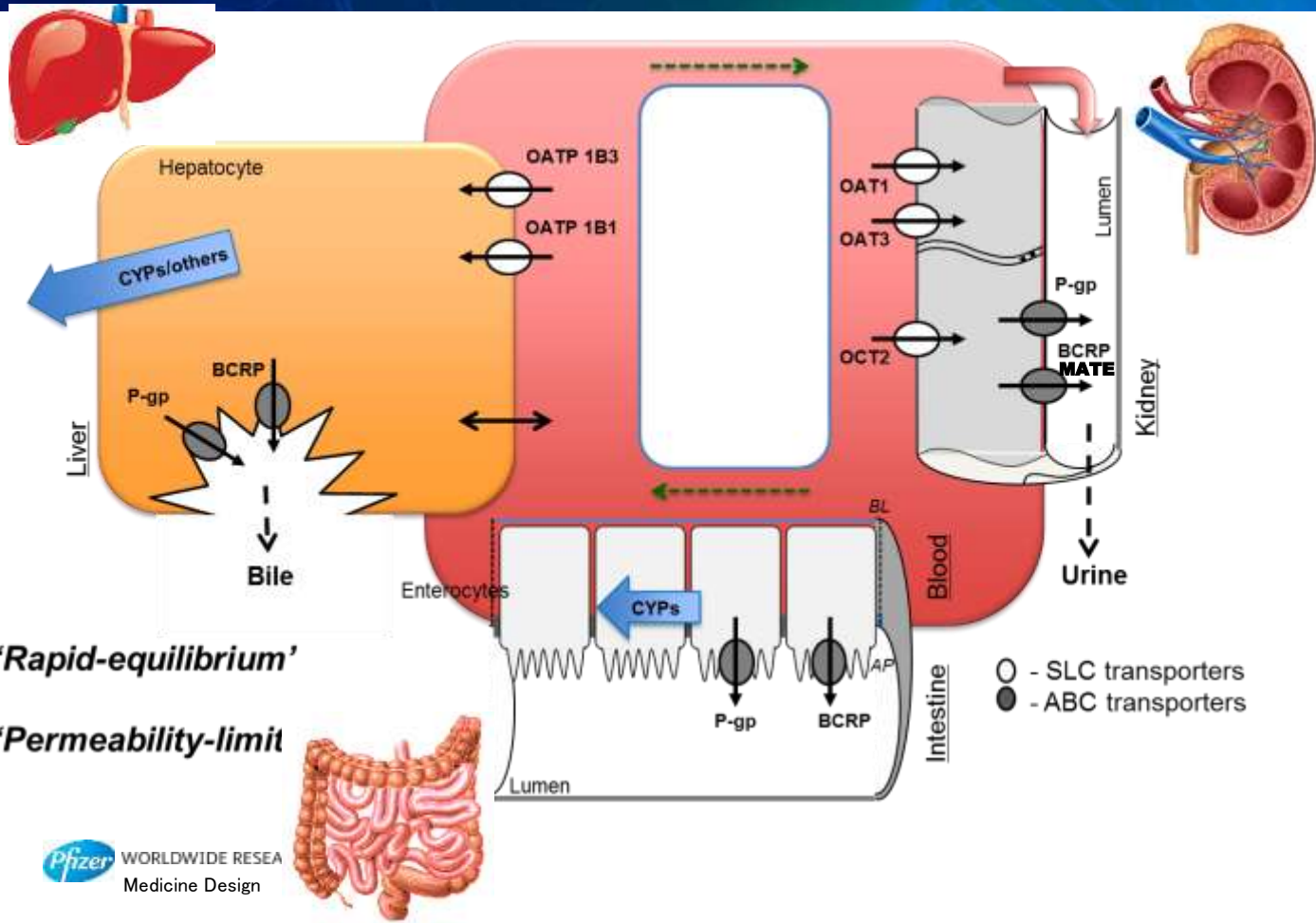
Med Design, Pfizer, Groton CT

Transporters and Enzymes – Drug Clearance

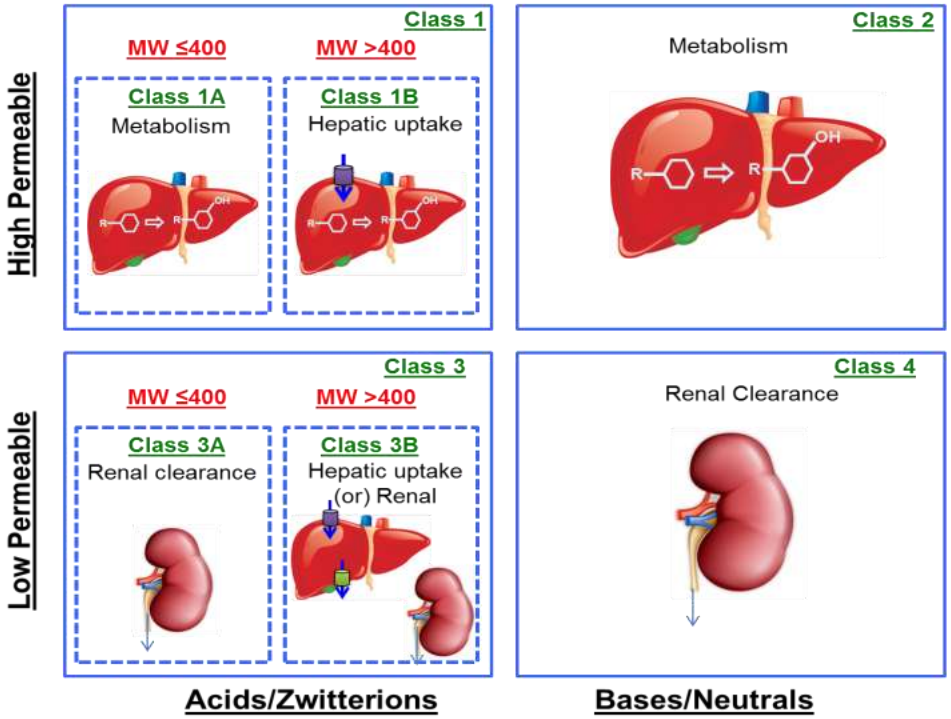


Major proteins

- Uptake transporters
- Efflux transporters
- CYPs
- UGTs
- Other enzymes

Extended Clearance Classification System [ECCS]

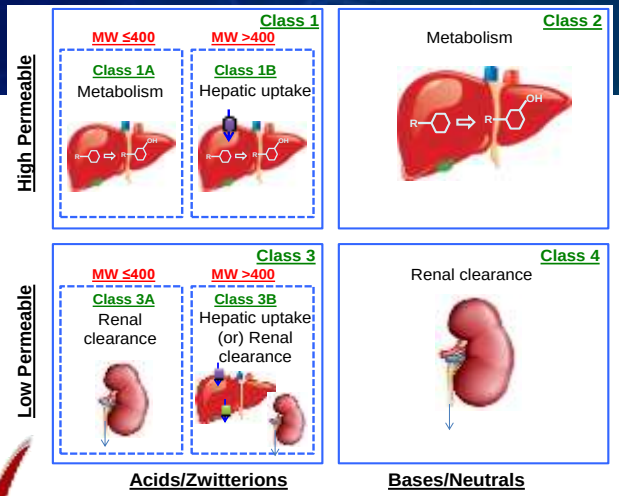
Clearance mechanism (rate-determining step)

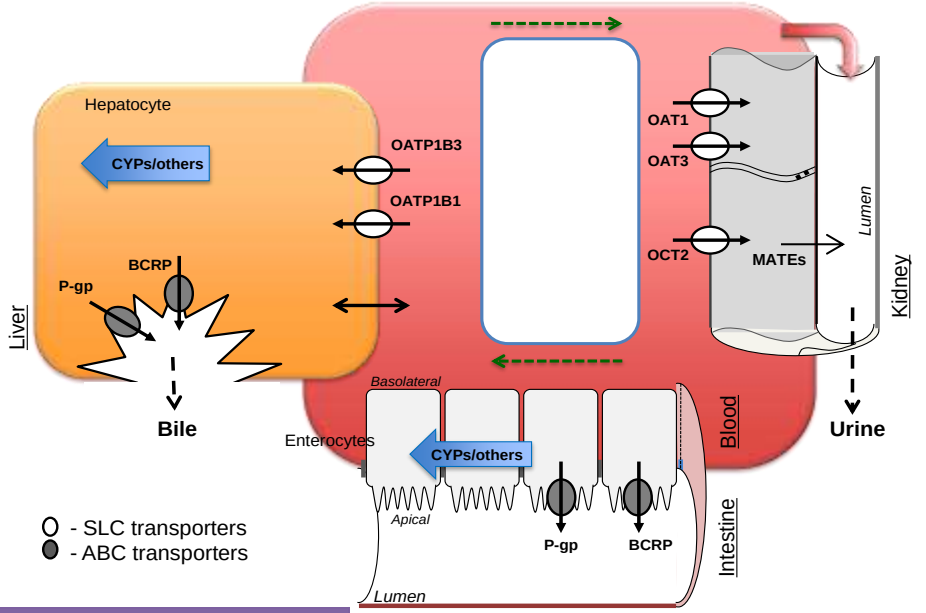


- Permeability cut off =
  - **5 X 10^{-6} cm/sec**
- Ionization:
  - **Acids/Zwits vs Bases/Neutrals**
- Molecular Weight cut off:
  - **400 [Class 1/3]**

- *Varma et al., Pharm Res. 2015, 3785-3802*
- El-Kattan and Varma. Drug Metab Dispos. 2018, 729-739.
- Varma et al., Adv Drug Deliv Rev. 2017 Jul 1;116:92-99.
- Varma et al., Clin Pharmacol Ther. 2017, 33-36
- El-Kattan et al., Pharm Res. 2016, 3021-3030.
- Varma and El-Kattan. J Clin Pharmacol. 2016, S99-S109.

ECCS Class and Drug Transporters





ECCS Class	Intestinal Transporters	Hepatic uptake transporters	Renal secretory transporters
IA	P-gp, BCRP	(OAT2)	
1B	P-gp, BCRP	OATP1B1, OATP1B3	
2	P-gp, BCRP		
3A	P-gp, BCRP (PEPT1)		OAT1, OAT3
3B	P-gp, BCRP (PEPT1, OATP2B1)	OATP1B1, OATP1B3	OAT1, OAT3
4	P-gp, BCRP (OATP2B1)		OAT1, OAT3, OCT2, MATEs

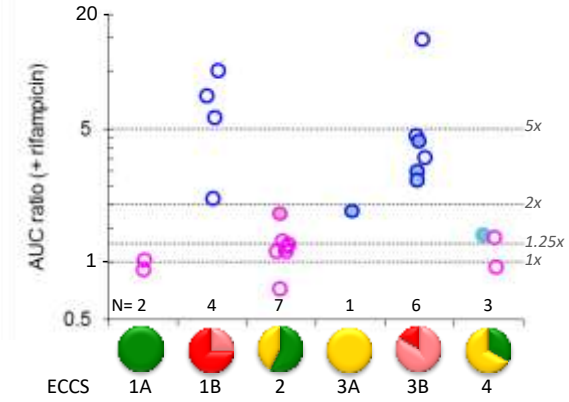
Varma et al. CPT 2017, 33-36.

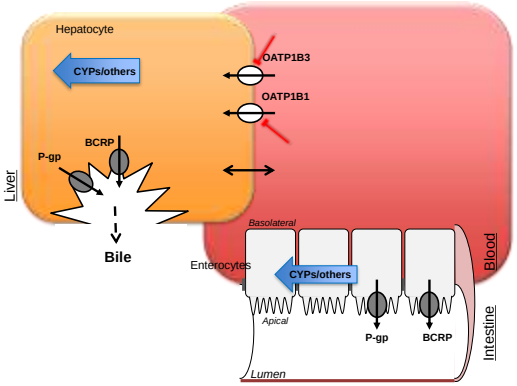
Transporter Victim DDIs with probe inhibitors

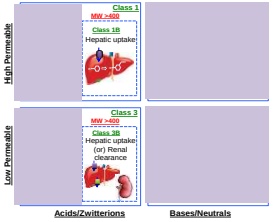
Clinical probe inhibitors

A) DDIs with rifampicin, an OATP1B1/1B3 probe inhibitor

Rifampicin
Inh- OATP1B1/1B3

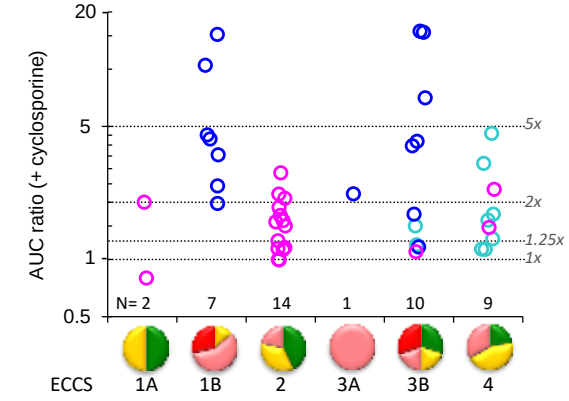


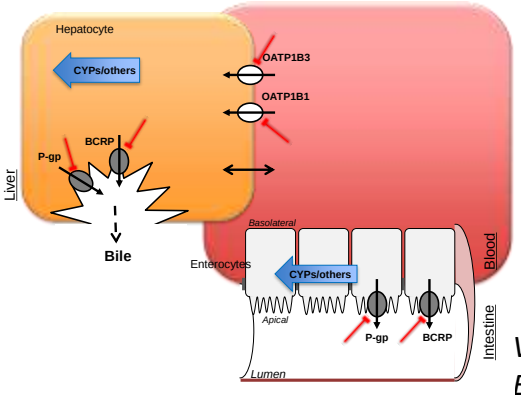




B) DDIs with cyclosporine, an OATP1B1/1B3 and P-gp/BCRP probe inhibitor

Cyclosporine
Inh- OATP1B1/1B3
- P-gp, BCRP
- intestinal CYP





Pfizer WORLDWI
Medicine [

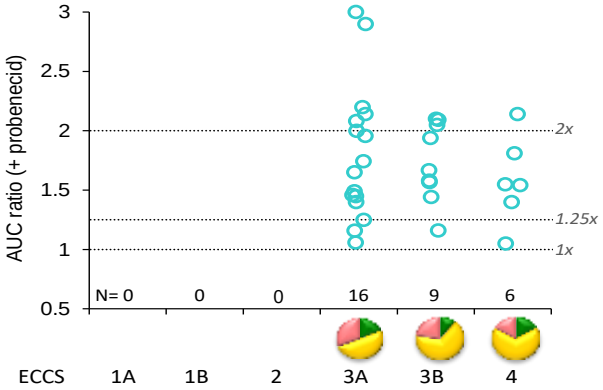
Varma et al. CPT 2017, 33.
El-Kattan & Varma, DMD, 2018, 729.

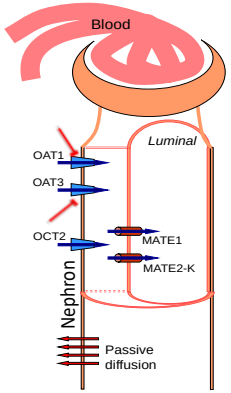
Transporter Victim DDIs with probe inhibitors

Clinical probe inhibitors

Probenecid
Inh- OAT1/3
- UGTs

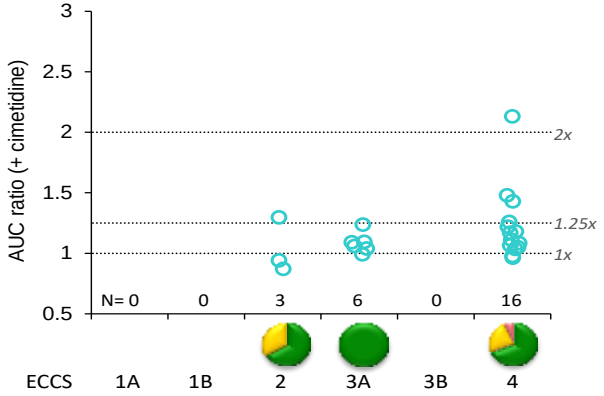
A) DDIs with porbenecid, an OAT1/3 probe inhibitor

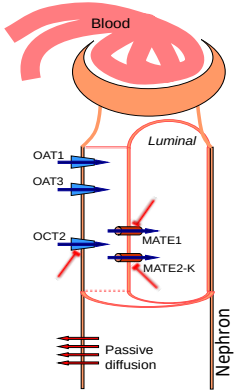




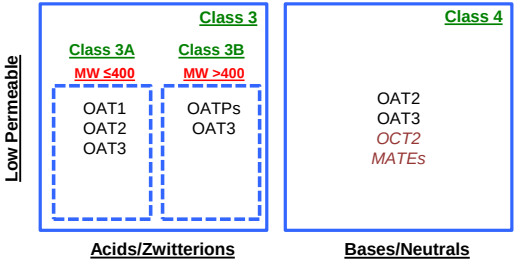
Cimetidine
Inh- OCT2/MATE
- CYP1A2/2C19/3A/2D6

B) DDIs with cimetidine, an OCT2/MATEs probe inhibitor



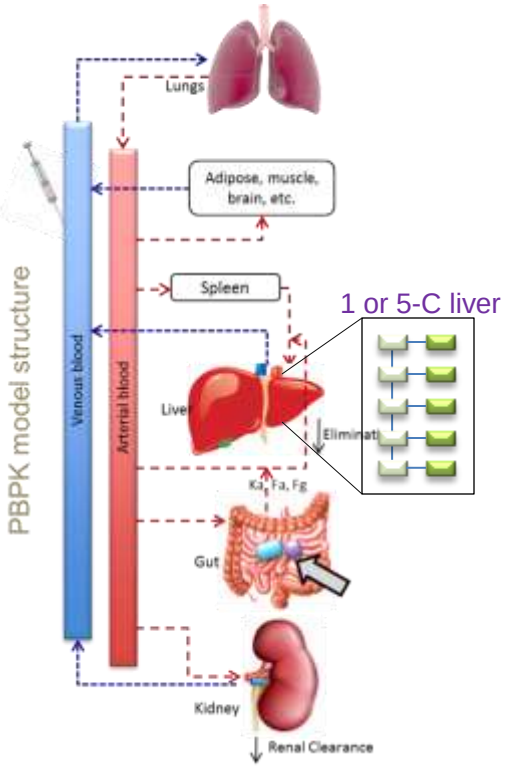


ECCS and renal transporters



Varma et al. CPT 2017, 33.
El-Kattan & Varma, DMD, 2018, 729.

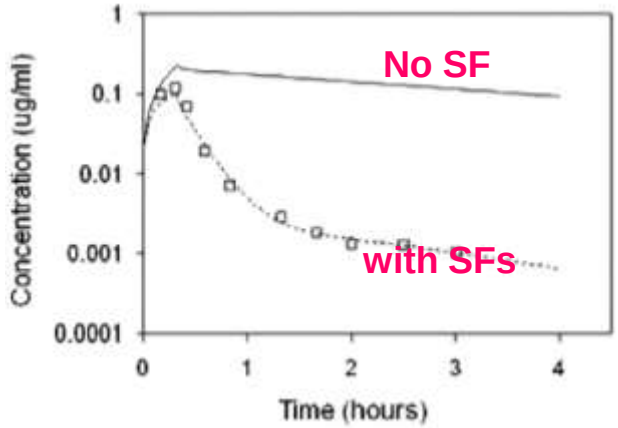
In vitro – in vivo translation (PBPK)



"Middle-out" PBPK reports

Compound	Uptake ESF	Source
valsartan	5	Poirier A, et al, (2009) *JPKPD* 36
fexofenadine	10	Poirier A, et al, (2009) *Mol Pharm* 6
pravastatin	3.7	Watanabe T, et al, (2009) *JPET* 328
pravastatin	31	Varma MV, et al, (2012) *Pharm Res* 29
repaglinide	12	Gertz M, et al, (2013) *Pharm Res* 30
repaglinide	17	Varma MV, et al, (2013) *Pharm Res* 30
glyburide	2	Varma MV, et al, (2014) *AAPS J* 16
rosuvastatin	-	Jamei M, et al. (2014) Clin Pk 73
cerivastatin	30	Varma MV, et al, (2015) *DMD* 1108
Montelukast	22	Varma MV, et al, (2016) *CPT*

PK profile prediction



- SF are estimated by fitting clinical data
- Can we use such ESF to predict PK of other compounds?

Jones HM et al. DMD, 2012, 1007.

In vitro – in vivo translation (intrinsic hepatic CL)

SCHH

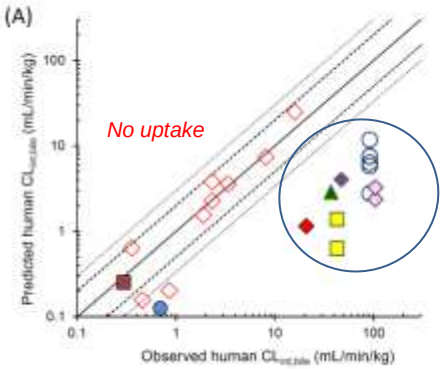
CL_{uptake}, CL_{pssive}, CL_{bile}

HLM/Heps

CL_{met}

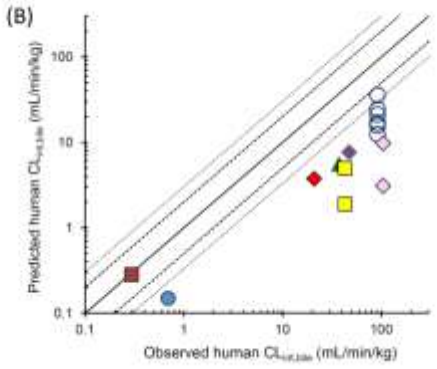
'no consideration to uptake'

Direct scaling



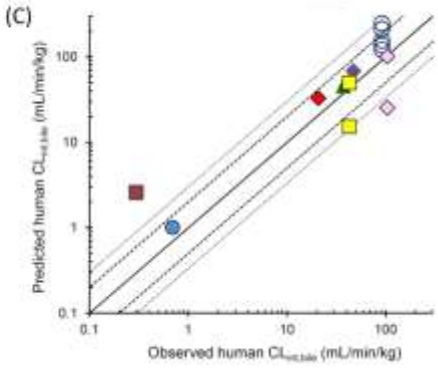
'Bottom-up'

Extended CL



'Middle-out'

Extended CL with SF_{active}



Empirical SF of 10.6

ECCS 1B	ECCS 3B
Atorvastatin	Pravastatin
Bosantan	Rosuvastatin
Cerivastatin	Valsartan
Fluvastatin	
Pitavastatin	
Repaglinide	
Glyburide	

$$CL_{int,h} = (SF_{active} \cdot CL_{active} + CL_{passive}) \cdot \frac{(\sum CL_{int,CYP} + CL_{int,bile})}{(CL_{passive} + \sum CL_{int,CYP} + CL_{int,bile})}$$

Varma et al. JPET, 2014, 214.
Kimoto et al. JPS, 2017

In vitro – In vivo Extrapolation for Metabolic CL compds

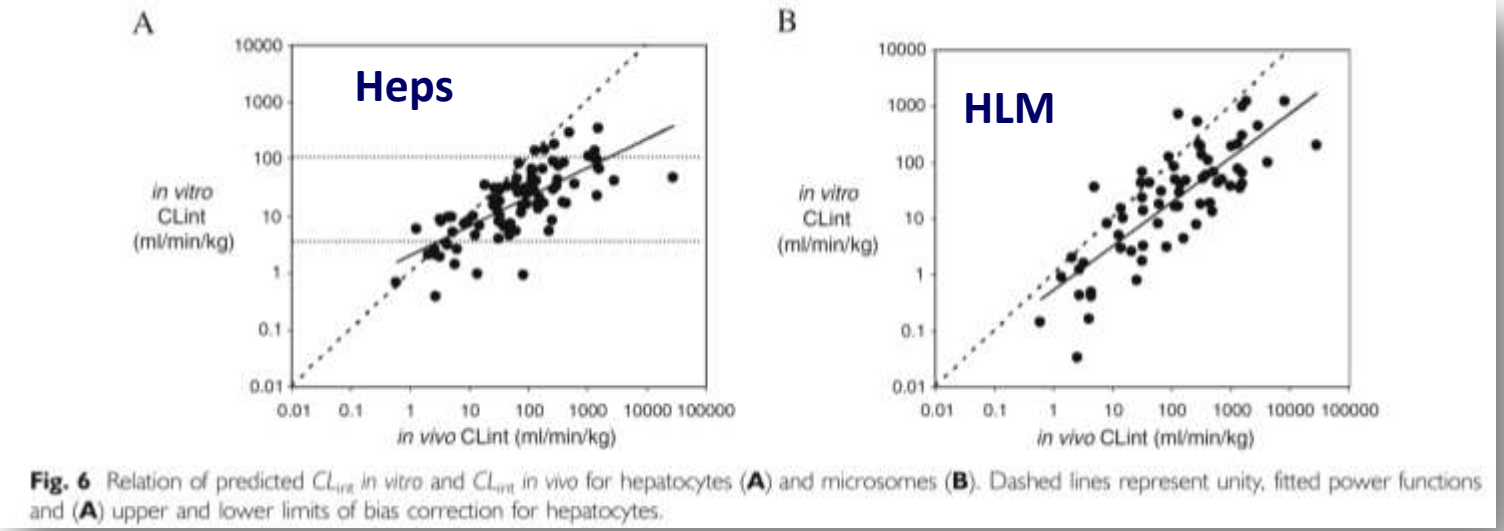
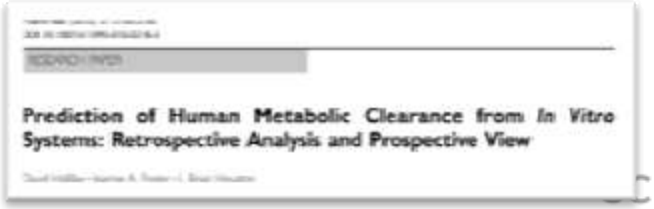


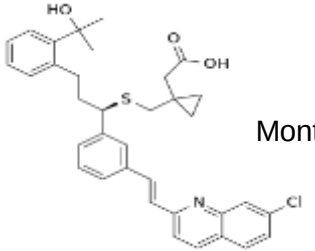
Fig. 6 Relation of predicted CL_{int} *in vitro* and CL_{int} *in vivo* for hepatocytes (**A**) and microsomes (**B**). Dashed lines represent unity, fitted power functions and (**A**) upper and lower limits of bias correction for hepatocytes.

SF ~4-5 needed to recover CLint.

Skew in IVIVE

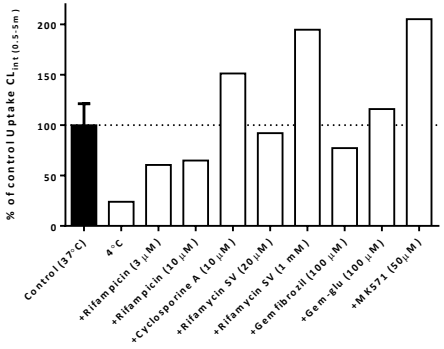

Prediction of Human Metabolic Clearance from *In Vitro* Systems: Retrospective Analysis and Prospective View

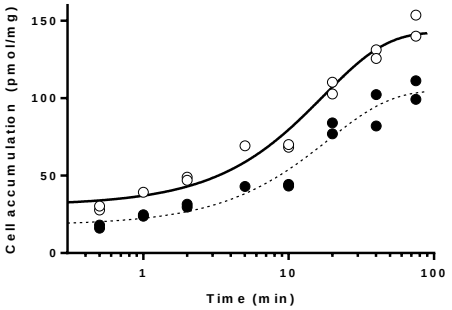
Challenging Class 1B compds: Montelukast case



Montelukast

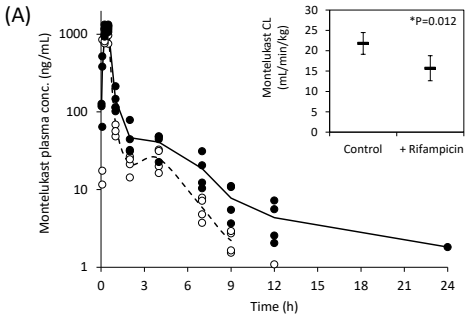
ECCS 1B



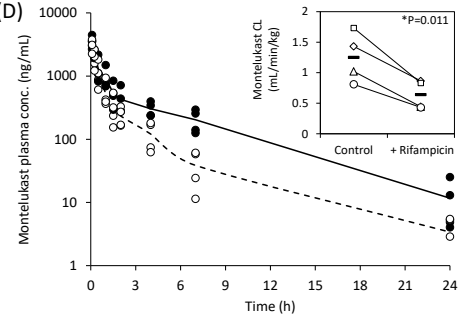


Weak in vitro activity

Interaction with rifampicin in rats



Interaction with rifampicin in Cynomolgus monkeys

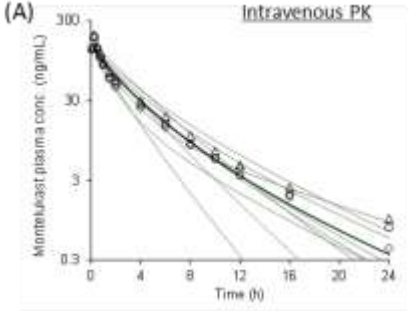


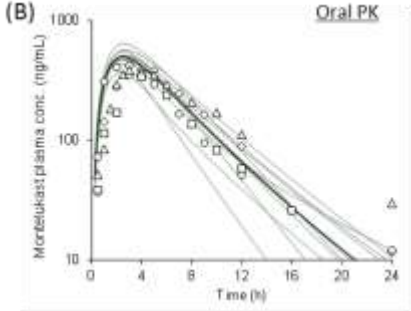
CL reduced with Rifampicin in rat and monkey

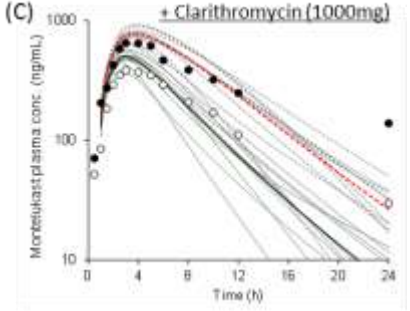
Varma et al., CPT 2017, 406-415.

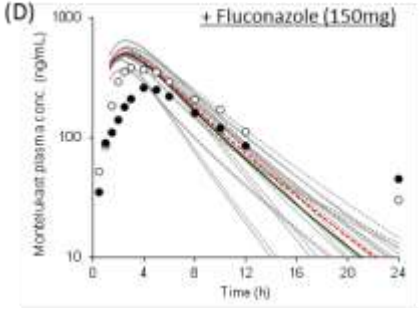
Montelukast clinical DDIs - PBPK modeling

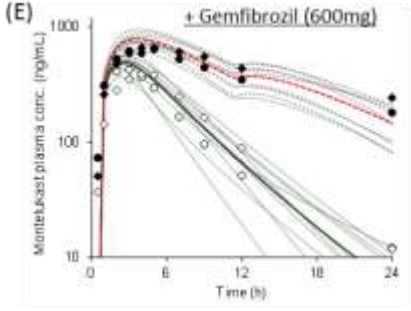
Case example

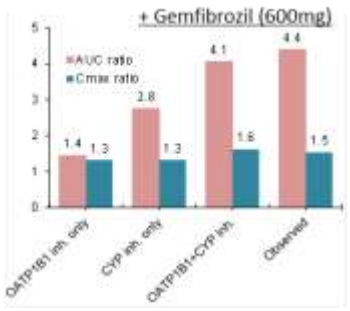


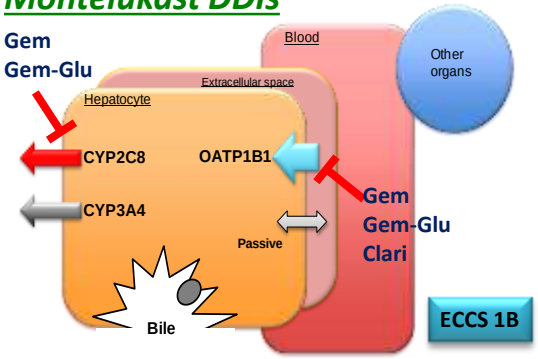












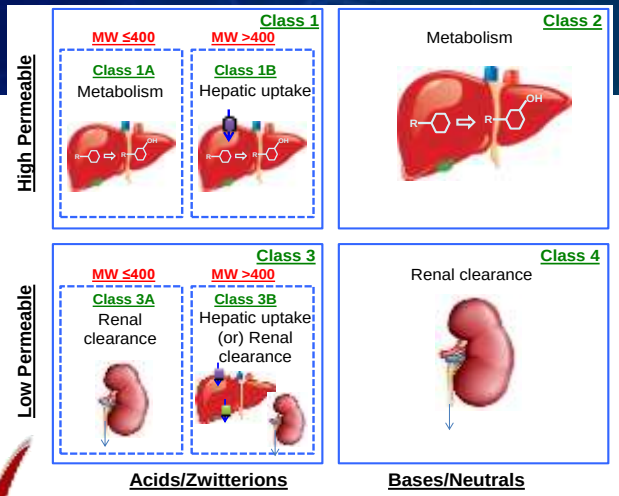
Control simulated mean
Control simulated individual trial
+Inhibitor simulated mean
+Inhibitor simulated individual trial

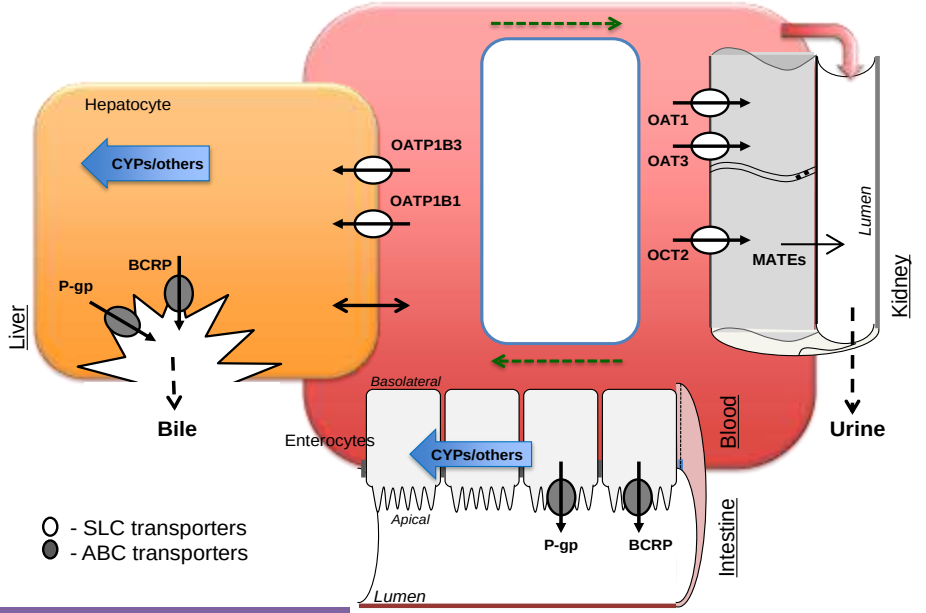
Open points → Control observed mean

Closed points → +Inhibitor observed mean

Varma et al., CPT 2017, 406-415.

ECCS Class and Drug Transporters





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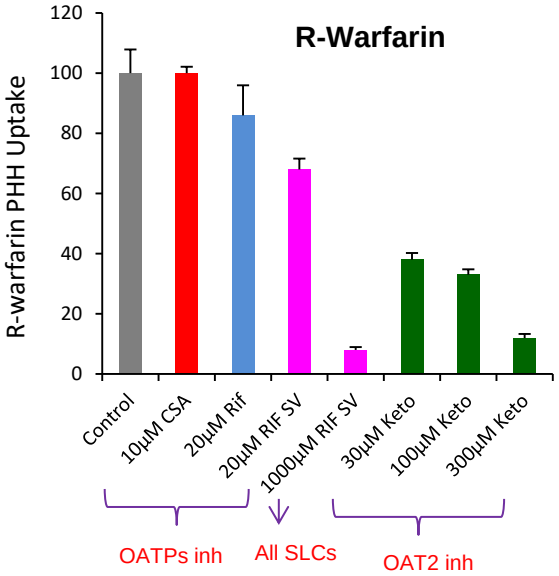
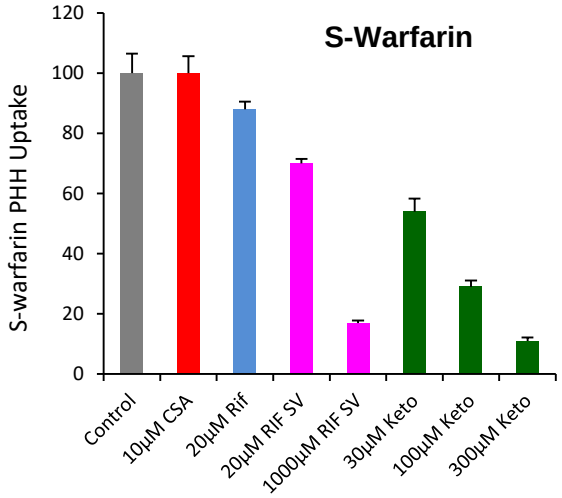
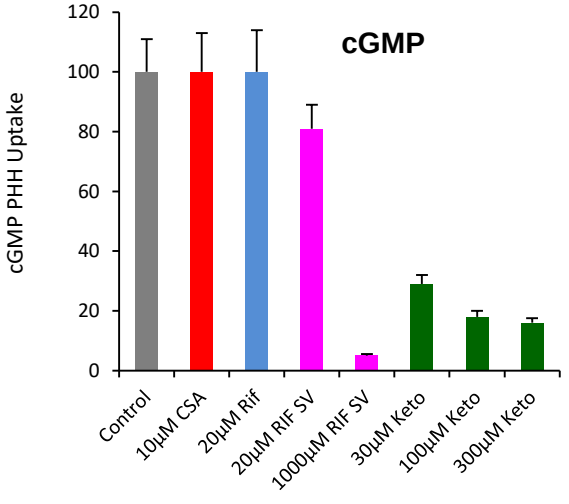
Varma et al. CPT 2017, 33-36.

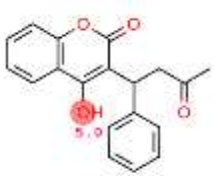
Warfarin uptake by Human Hepatocytes



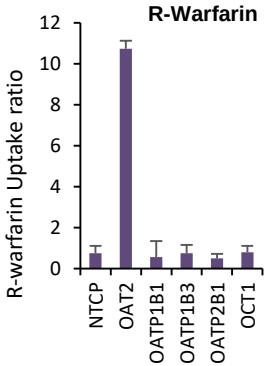


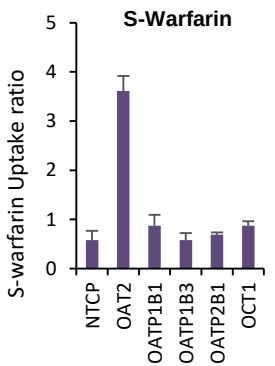


- **Both R/S-warfarin behave like cGMP – Known to be selective for OAT2**

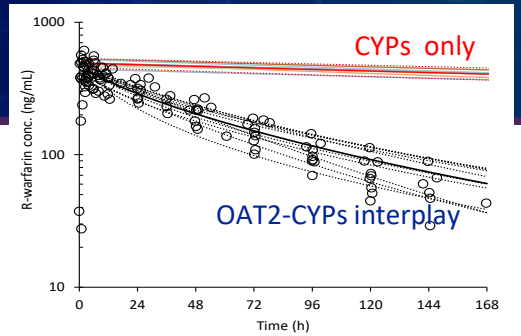


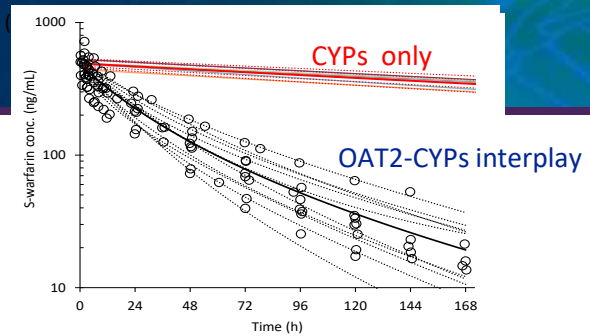
OAT2 selectivity was confirmed using singly transfected HEK293 cells

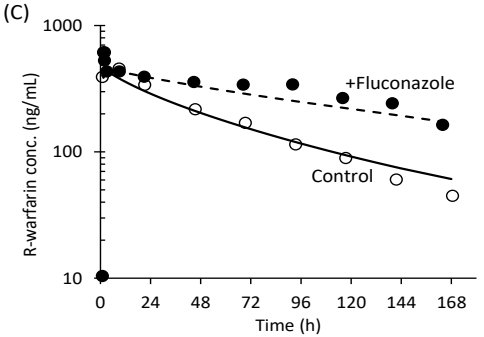


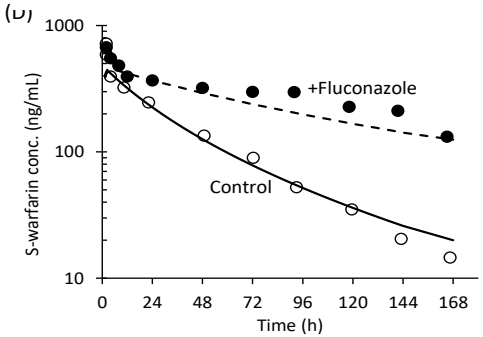


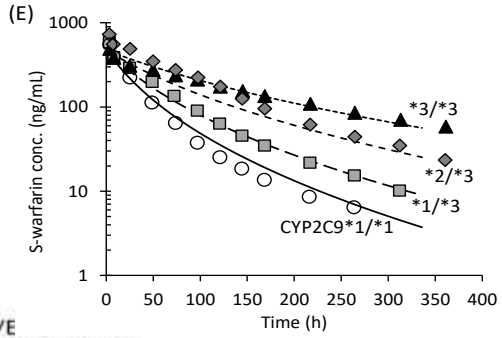
R/S-warfarin PK analysis



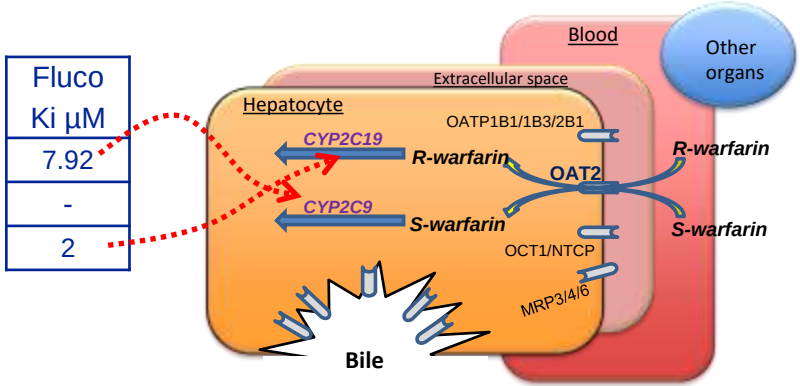






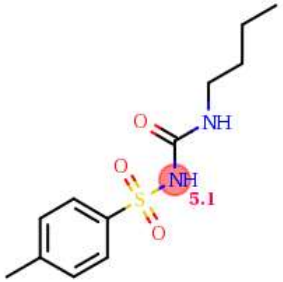


Fluco Ki μM
7.92
-
2



Bi et al. Mol Pharma 2018, 1284-1295.

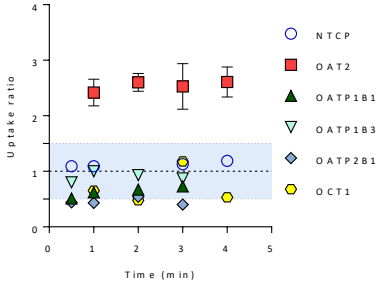
Tolbutamide



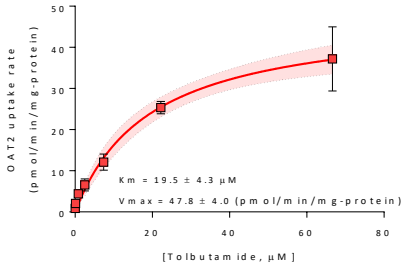
MW – 270
pKa – 5.2
RRCK – 31

- Low CLmet
- IVIVE disconnect with CLmet
- CYP2C9 clinical probe

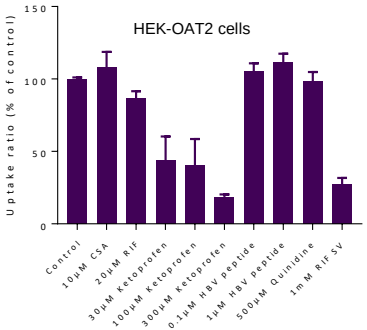
HEKs



Time-course

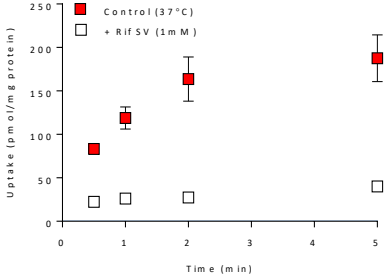


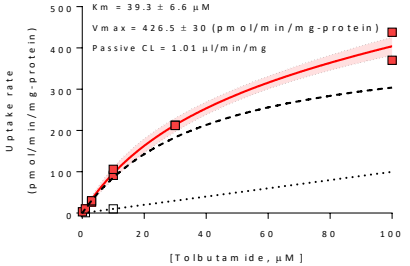
Transport kinetics

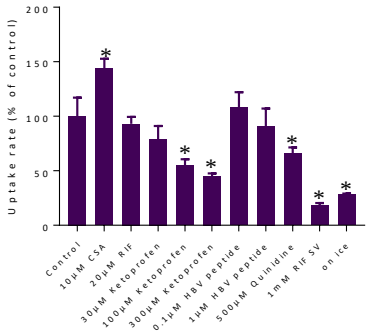


Phenotyping

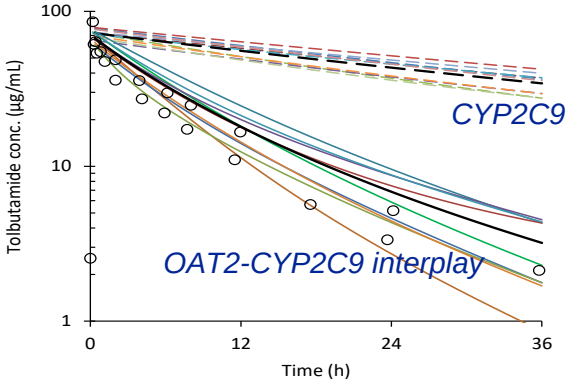
PHH

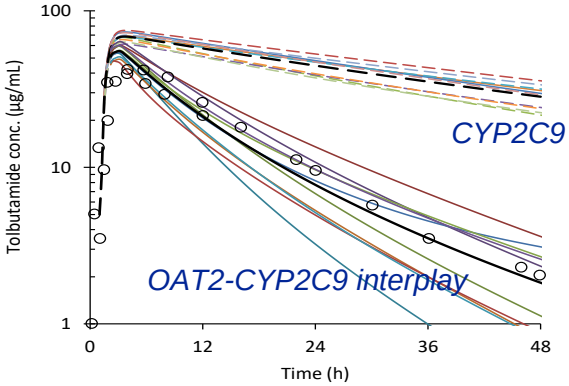


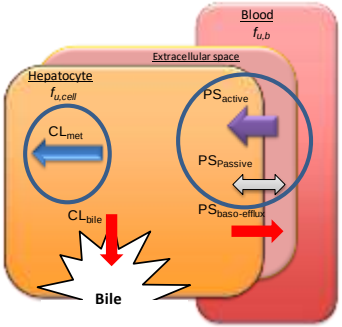




Tolbutamide: OAT2-CYP2C9 interplay

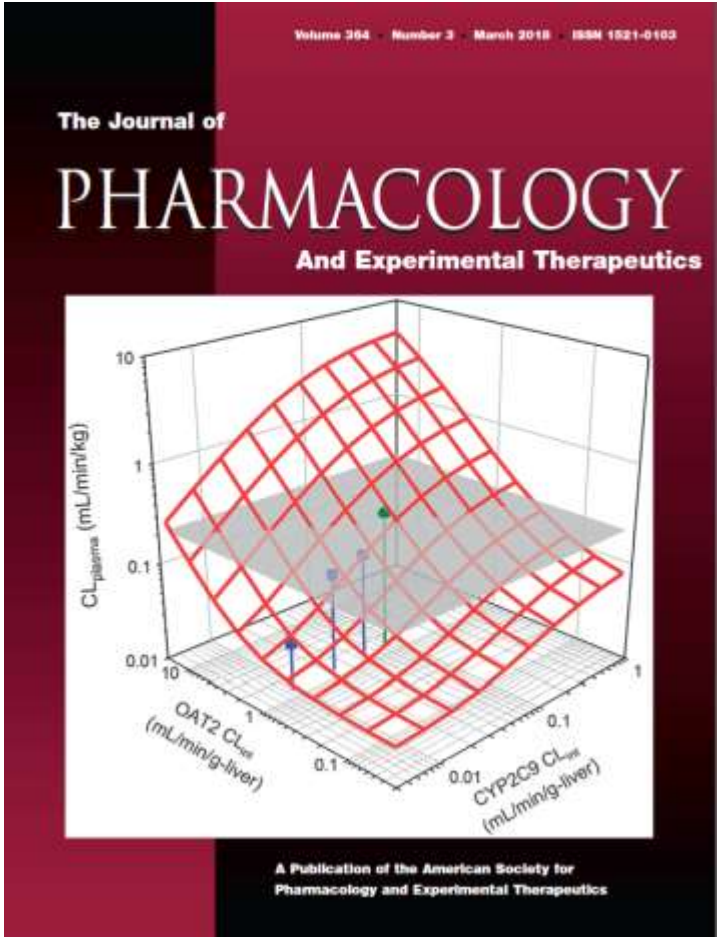


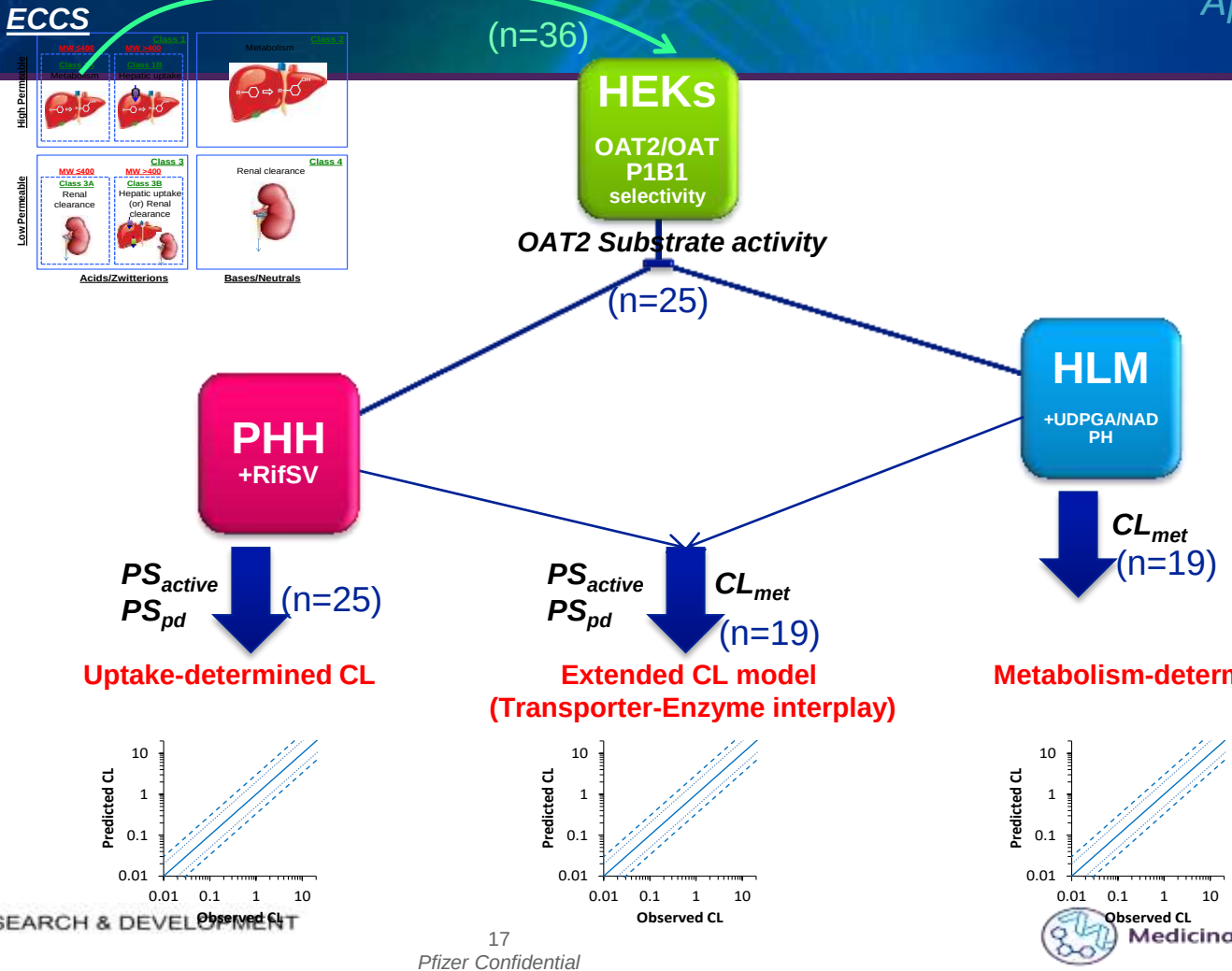




Sensitivity analysis
Green point – WT
Blue points - *1/*3, *2/*3, *3/*3 variants

Bi et al. J Pharmacol Exp Ther. 2018, 390-398.

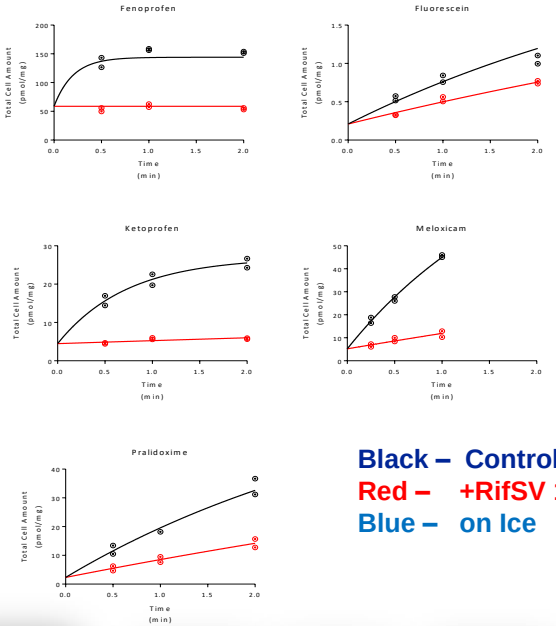




CL prediction for low MW, high permeability A/Z (ECCS 1A)

ECCS 1A drugs - List of drugs

Drug	Permeability (10^{-6} cm/s)[a]	Molecular weight	Acidic pKa	Ionization	Uptake Transporters	Main Enzyme[c]	Plasma free fraction ($f_{u,p}$)[d]
Bromfenac	17.8	334	4.1	A	OATP1B1, OAT2	CYP2C9/UGT	0.002
Entacapone	6.14	305	4	A	OATP1B1, OAT2	UGT	0.02
Fluorescein	5.82	332	4.5	A	OATP1B1, OAT2	-	0.11
Nateglinide	4.24	317	3.8	A	OATP1B1	CYP2C9	0.011
Diclofenac	18.5	296	4.4	A	OAT2	CYP2C9, UGT2B7	0.004
Fenoprofen	34.2	242	4.4	A	OAT2	UGT2B7	0.005
Gliclazide	16.7	323	6	A	OAT2	CYP2C9	0.05
Ibuprofen	29.3	206	4.4	A	OAT2	CYP2C9	0.012
Indomethacin	22.12	356	4.4	A	OAT2	CYP2C9	0.012
Isoxicam	26.9	335	3.9	A	OAT2	-	0.035
Ketoprofen	18	254	4.1	A	OAT2	CYP2C9, UGT2B7	0.021
Meloxicam	23.9	351	3.8	A	OAT2	CYP2C9/3A4	0.008
Pioglitazone	23.3	356	6.1	A	OAT2	CYP2C8	0.012
Piroxicam	32.9	331	2.2	A	OAT2	CYP2C9, UGT2B7	0.024
Pralidoxime	31.9	137	7.9	A	OAT2	-	0.515
Rosiglitazone	27.3	357	6.3	Z	OAT2	CYP2C8	0.003
R-Warfarin	24.2	308	5	A	OAT2	CYP2C9	0.013
Sulfamethoxazole	8.38	253	5.6	A	OAT2	CYP2C9	0.325
S-Warfarin	24.2	308	5	A	OAT2	CYP2C9	0.013
Tolbutamide	31.3	270	5.1	A	OAT2	CYP2C9	0.027
Tolcapone	20.9	273	5	A	OAT2	UGT	0.002
Clinafloxacin	6.84	366	5.9	Z	-	-	0.96
Naproxen	22.1	230	4.3	A	OAT2	UGT2B7	0.026
Thalidomide	11.1	258	7.8	A	OAT2	-	0.4
Tiagabine	19.9	376	3.3	Z	OAT2	CYP3A4	0.16



Black – Control
Red – +RifSV 1mM
Blue – on Ice

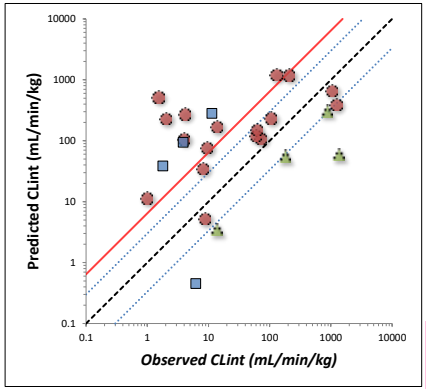


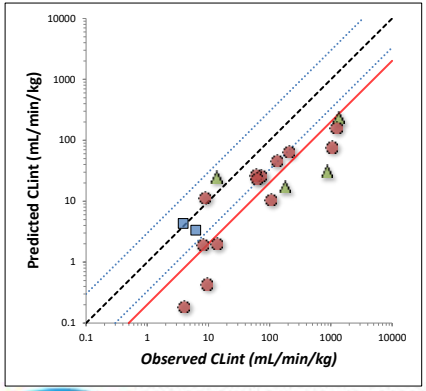


Emi Kimoto *Sumathy & Laurie* **Mark Niosi & Jian Lin**

Kimoto et al. JPET, 2018, inpress

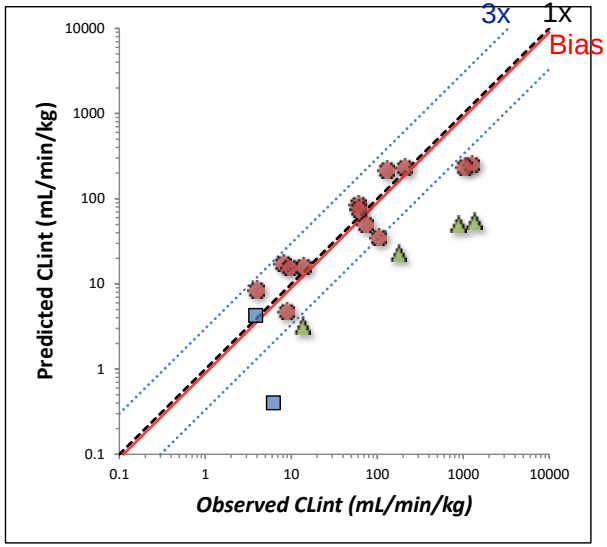
OAT2-mediated uptake → IVIVE to Hepatic CL_{int}





$$CL_{h,int} = \frac{CL_{uptake} \cdot CL_{met}}{CL_{passive} + CL_{met}}$$

Extended CL (Uptake + metabolism)

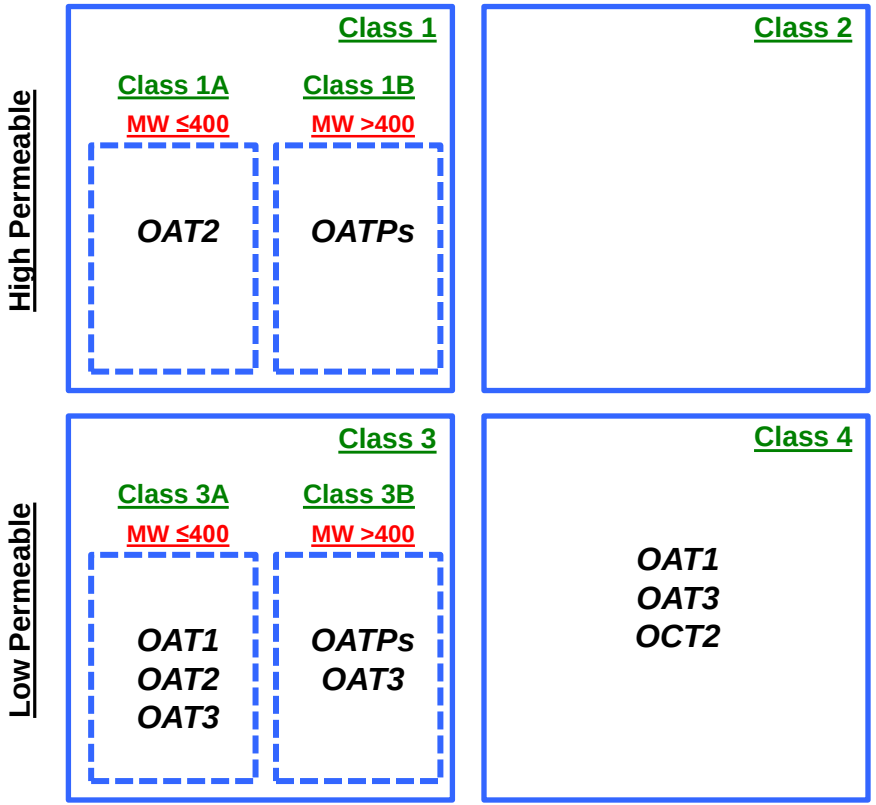


Stats †	n	13	$CI_{90\%}$
$DI_{90\%}$ (±fold)		3.91	[2.6 - 6.9]
Bias ‡ (fold)		0.9	[0.6 - 1.4]

Circles - OAT2 substrates with Hep uptake in PHH
Triangles - OAT2/OATP1B1 substrates with Hep uptake in PHH
Square - no Hep uptake in PHH

Kimoto et al. JPET, 2018, inpress

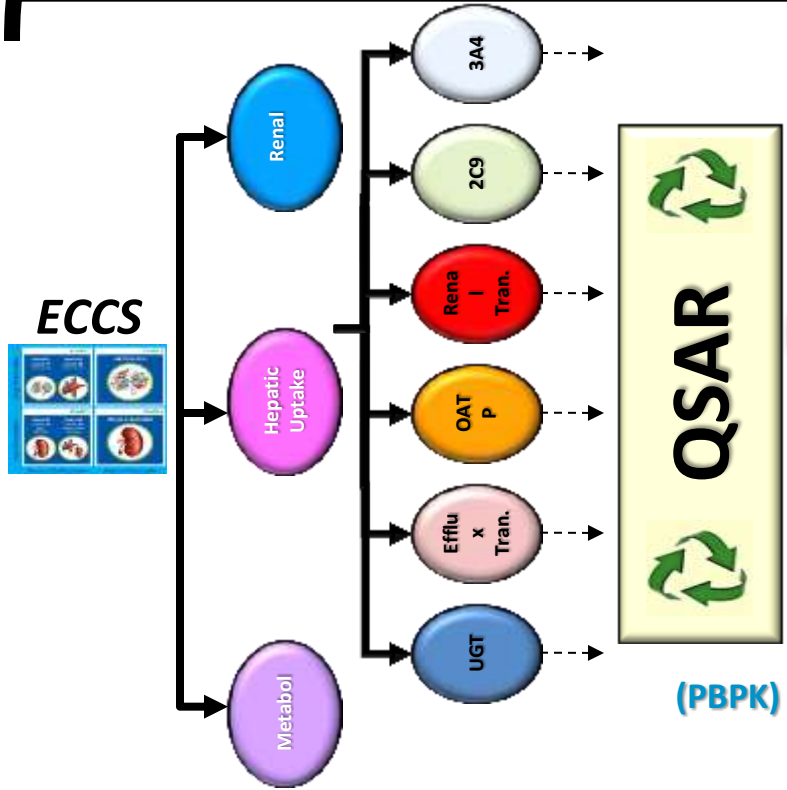
Transporter phenotype per class

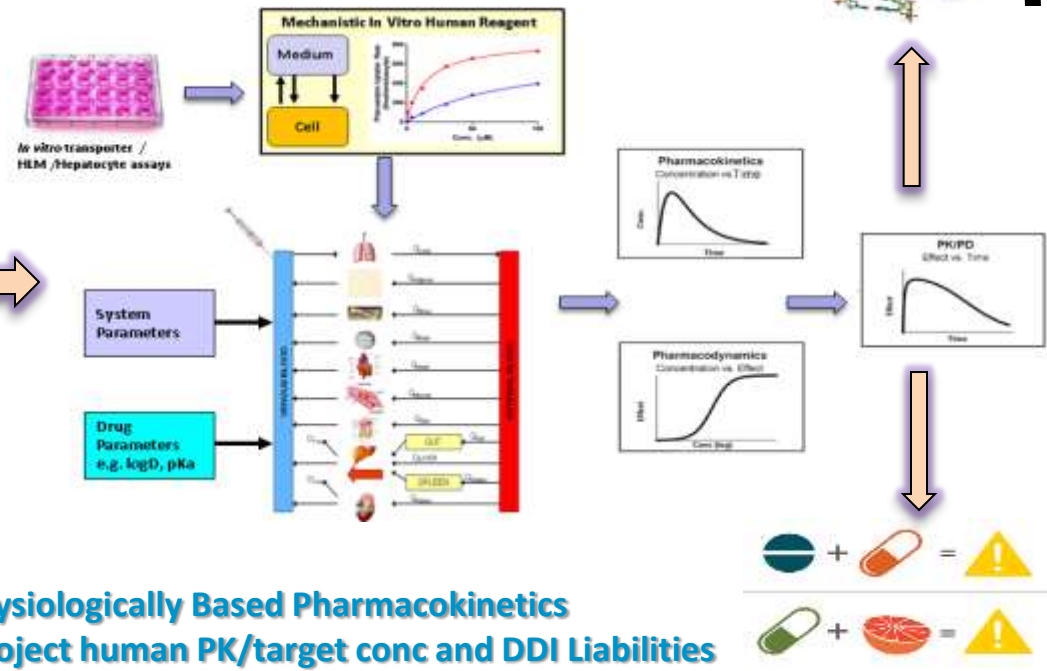


Invigoration of Human Based In Vitro Reagents as Driver for Accurate Human Clearance and DDI Prediction

Lead Identification and Optimization

Lead Development





Physiologically Based Pharmacokinetics (PBPK) to project human PK/target conc and DDI Liabilities

Varma, Lai and El-Kattan. Adv Drug Deliv Rev 2017, 92-99.

Acknowledgments

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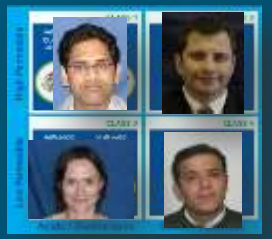
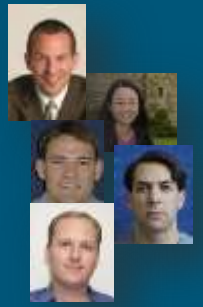


Project leads & ADME CoE



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