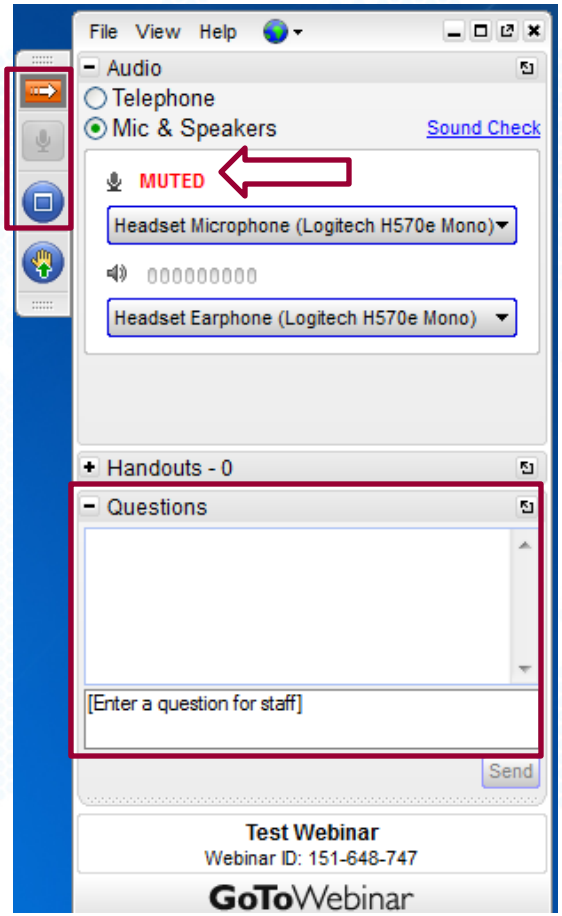
Evaluation of Ketoconazole and Its Alternative Clinical CYP3A4/5 Inhibitors as Inhibitors of Drug Transporters: The In Vitro Effects of Ketoconazole, Ritonavir, Clarithromycin and Itraconazole on 13 Clinically-Relevant Drug Transporters

Lydia Vermeer, Ph.D.
Senior Scientist, Drug Transport
lvermeer@xenotechllc.com

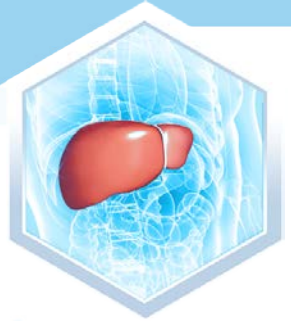
Before we begin…

- User console features
- Audience audio is muted
- Post questions to our staff anytime via “Questions” message pane
- Slides and recorded session available soon. Watch your e-mail for both.
- A short survey will appear at the conclusion of the webinar, we value your feedback!



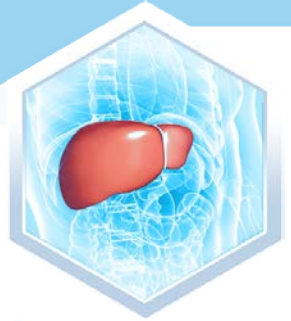
Evaluation of Ketoconazole and Its Alternative Clinical CYP3A4/5 Inhibitors as Inhibitors of Drug Transporters: The In Vitro Effects of Ketoconazole, Ritonavir, Clarithromycin and Itraconazole on 13 Clinically-Relevant Drug Transporters

Lydia Vermeer, Ph.D.
Senior Scientist, Drug Transport
lvermeer@xenotechllc.com



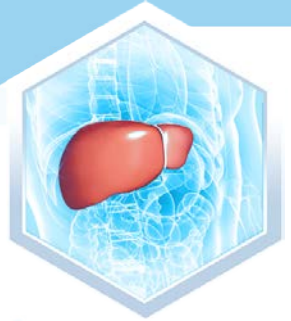
Drug Transport Technology at XenoTech

Transporter Category	Transporter	Experimental system	
SLC	OAT1, 3	HEK	
	OCT1, 2	HEK	
	OATP1B1, 1B3,	HEK	
ABC	MDR1 (human)	MDCK Caco-2 (BCRP also present)	Vesicle
	MRP2-4	Vesicle	
	BCRP (human)	MDCK	Vesicle
	BSEP	Vesicle	



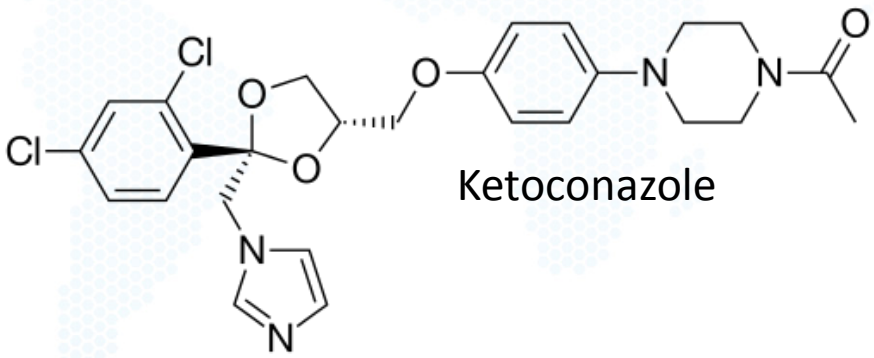
Drug Transport Technology at XenoTech

	FDA (2012)	EMA (2013)
Recommended transporters	P-gp, BCRP OATP1B1, OATP1B3, OAT1, OAT3, OCT2	P-gp, BCRP, **BSEP** OATP1B1, OATP1B3, OAT1, OAT3, OCT2
Transporters to consider	BSEP, MATE, MRP2 Others as necessary	OCT1, MATE1, MATE2 Others as necessary
Substrate evaluation	Yes (P-gp, BCRP) Others based on clearance/elimination routes	
Inhibitor evaluation	Yes, all of the recommended transporters (EMA and PDMA: in vitro data prior to Phase III trials)	



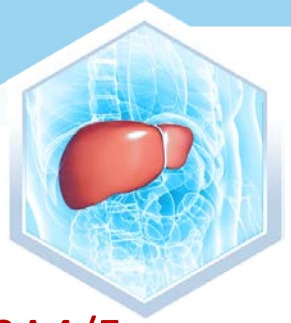
Ketoconazole

- Orally available
- Synthetic, broad spectrum, antifungal agent (imidazole)
- Approved in 1982 by FDA for use in fungal infections
- Known substrate and inhibitor of CYP3A4/5



Ketoconazole

https://www.nlm.nih.gov/



Statement of the problem

High dose ketoconazole was previously the gold-standard strong CYP3A4/5 inhibitor used in clinical DDI studies

- Ketoconazole was essentially banned by FDA from clinical use in 2013
  - Typically asymptomatic, reversible liver function test abnormalities
  - 1984 estimate by Van Tyle: DILI in 0.1 to 1.0% of patients. No association with dosage, but increase with duration (e.g., months)
  - Later estimates: 1) 134.1 per 100,000 person-months; 2) 4.9 cases per 10,000 patients; 3) 3.6 to 4.2% (Reviewed by Greenblatt and Greenblatt, 2014)
- The FDA specifically recommended clarithromycin or itraconazole as alternatives for DDI studies, but noted other drugs may be used
- Ritonavir has been suggested as an alternative CYP3A4/5 inhibitor by some (Greenblatt et al., 2014 and 2015)
  - Excluded by Ke et al., due to non-specific CYP inhibition and induction

Ke et al. (2014) CPT **95**:473; Greenblatt and Greenblatt (2014) JCP **54**:1321; Greenblatt and Harmatz (2015) BJCP **80**: 342

Statement of the problem (continued)

- 19 strong CYP3A inhibitors systematically evaluated by Ke and colleagues (2014). Only itraconazole and clarithromycin were deemed acceptable. Others excluded because:

  1) Drug not approved in U.S.

  2) Known to be a non-specific CYP inhibitor

  3) Significant safety issues

  4) Used exclusively in combination with ritonavir

  5) Are only moderate CYP3A4/5 inhibitors

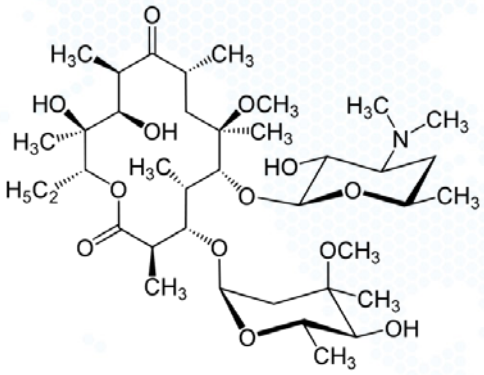
- Some transporter data, but no previous comprehensive investigation of these alternative CYP3A4/5 inhibitors as transporter inhibitors
  - Ketoconazole IC_{50} values vary 37-fold for P-gp inhibition, 6.4-fold for OATP1B1 inhibition
  - Ritonavir: 85-fold variation for P-gp, 117-fold for OATP1B1 inhibition

Ke et al. (2014) CPT **95**:473; University of Washington DIDB (2015)

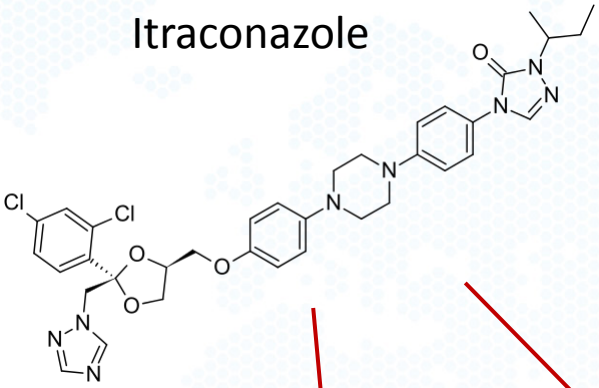
Goals of the study

Define the inhibition profile of the following compounds:

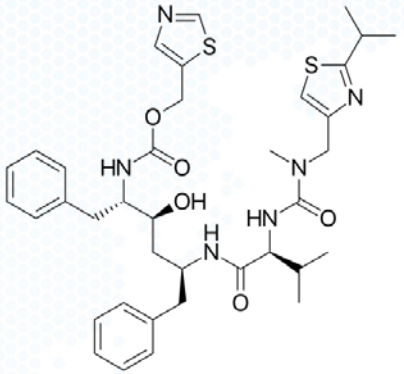
Clarithromycin

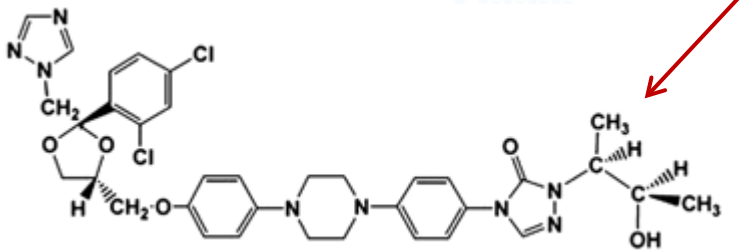


Itraconazole

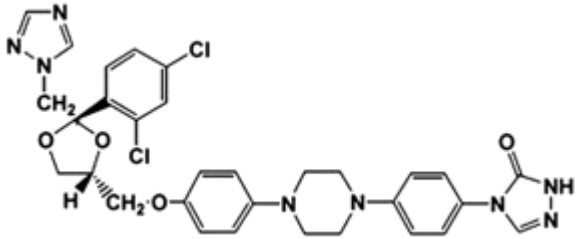


Ritonavir

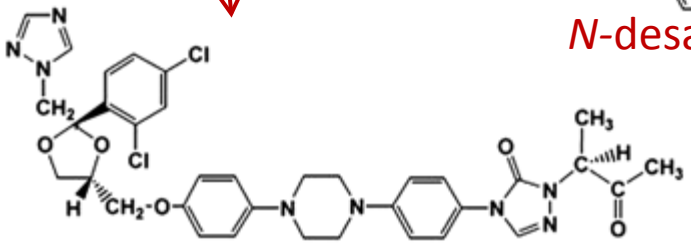




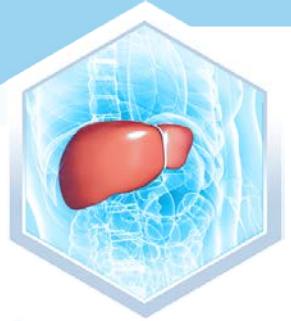
Hydroxyitraconazole



N-desalkylitraconazole



Ketoitraconazole



Goals of this study

To define the inhibition profile towards the following transporters:

OATP1B1	MATE2-K
OATP1B3	P-gp
OAT1	BCRP
OAT3	MRP2
OCT1	MRP3
OCT2	BSEP
MATE1	

To allow a more informed choice of a strong clinical CYP3A4/5 inhibitor for clinical DDI studies with a drug candidate known to be a substrate of one or more of these transporters: reduce confounding DDI results

Publication details / acknowledgements

1521-009X/44/3/1–7$25.00
DRUG METABOLISM AND DISPOSITION

http://dx.doi.org/10.1124/dmd.115.067744
Drug Metab Dispos 44:1–7, March 2016

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 [S]

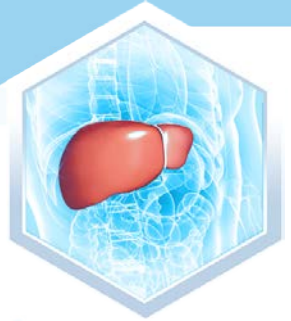
Lydia M. M. Vermeer,[1] Caleb D. Isringhausen,[1] Brian W. Ogilvie, and David B. Buckley

XenoTech, Kansas City, Kansas

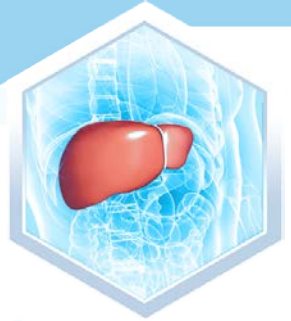
Received October 8, 2015; accepted December 11, 2015

Drug Metab Dispos; **Published ahead of print December 14, 2015**;
doi:10.1124/dmd.115.067744

Methods – test systems and substrates



- HEK-293 assays:
  - Transfected (immortalized cell line with stable transfection) and control (wild type) cells were utilized (OATP1B1, OATP1B3, OAT1, OAT3, OCT1, and OCT2)
  - Transient transfection for MATE1 and MATE2-K
  - Seeded and cultured for 24 hours and supplemented with 2 mM sodium butyrate (OATP1B1, OAT1B3, MATE1, and MATE2-K) or fed with fresh supplemented DMEM (OAT1, OAT3, OCT1, and OCT2)
- Vesicle assays:
  - Membrane vesicles expressing P-gp, BCRP, MRP2, MRP3, and BSEP
  - ATP-dependent test system



Methods – test systems and substrates

HEK-293 assays

Transporter	Substrate
OATP1B1	[^{3}H]-Estradiol-17β-glucuronide (50 nM)
OATP1B3	
OAT1	[^{3}H]-*p*-Aminohippurate (1 µM)
OAT3	[^{3}H]-Estrone 3-sulfate (50 nM)
OCT1	[^{3}H]-Tetraethyl ammonium bromide (5 µM)
OCT2	[^{14}C]-Metformin (10 µM)
MATE1	
MATE2-K	

Membrane vesicle assays

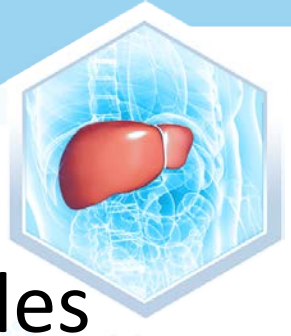
Transporter	Substrate
P-gp	*N*-Methylquinidine (0.5 µM)
BCRP	[^{3}H]-Estrone-3-sulfate (1 µM)
MRP2	[^{3}H]-Estradiol -17β-glucuronide (50 nM)
MRP3	
BSEP	[^{3}H]-Taurocholic acid (0.4 µM)

- Inhibitors were pre-incubated with HEK-293 cells or vesicles for 15 min prior to addition of substrates to minimize effects of time-dependency and non-specific binding
- [Substrate] = ~1-10% of K_m for each assay (such that $IC_{50} \approx K_i$)

Methods – inhibitors in HEK-293 cells

CYP3A4 inhibitor	OATP1B1	OATP1B3	OAT1	OAT3	OCT1	OCT2	MATE1	MATE2-K
Ketoconazole	0.1 - 20 µM	0.1 - 20 µM	0.1 - 20 µM	0.1 - 20 µM	0.1 - 20 µM	0.1 - 20 µM	0.01 -2 µM	0.01 -2 µM
Itraconazole	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM
Hydroxy-itraconazole	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.001 - 0.3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM
Keto-itraconazole	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM
N-deskalkyl itraconazole	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM
Clarithromycin	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM
Ritonavir	0.03 - 10 µM	0.3 - 30 µM	0.3 - 30 µM	0.3 - 30 µM	0.3 - 30 µM	0.3 - 30 µM	0.1 - 20 µM	0.1 - 20 µM

- Concentrations based on clinical PK data for each inhibitor in commonly used dosing regimens in clinical CYP3A4/5 DDI studies, up to 10-fold higher than the average $C_{max,ss}$
- Where concentrations are lower, an initial range-finding IC_{50} experiment was performed, and concentrations adjusted lower, as needed (values in red)



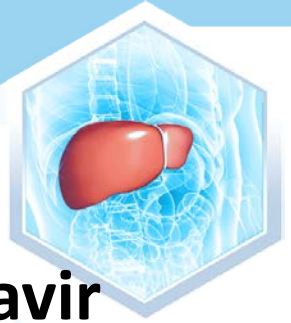
Methods – inhibitors in membrane vesicles

CYP3A4 inhibitor	P-gp	BCRP	MRP2	MRP3	BSEP
Ketoconazole	0.1 - 20 µM	0.1 - 20 µM	0.1 - 20 µM	0.1 - 20 µM	0.1 - 20 µM
Itraconazole	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM	0.03 - 10 µM
Hydroxyitraconazole	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM
Ketoitraconazole	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM	0.01 - 3 µM
N-deskalkyl itraconazole	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM	0.001 - 0.2 µM
Clarithromycin	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM	0.3 - 50 µM
Ritonavir	0.01 - 3 µM	0.3 - 30 µM	0.3 - 30 µM	0.3 - 30 µM	0.3 - 30 µM

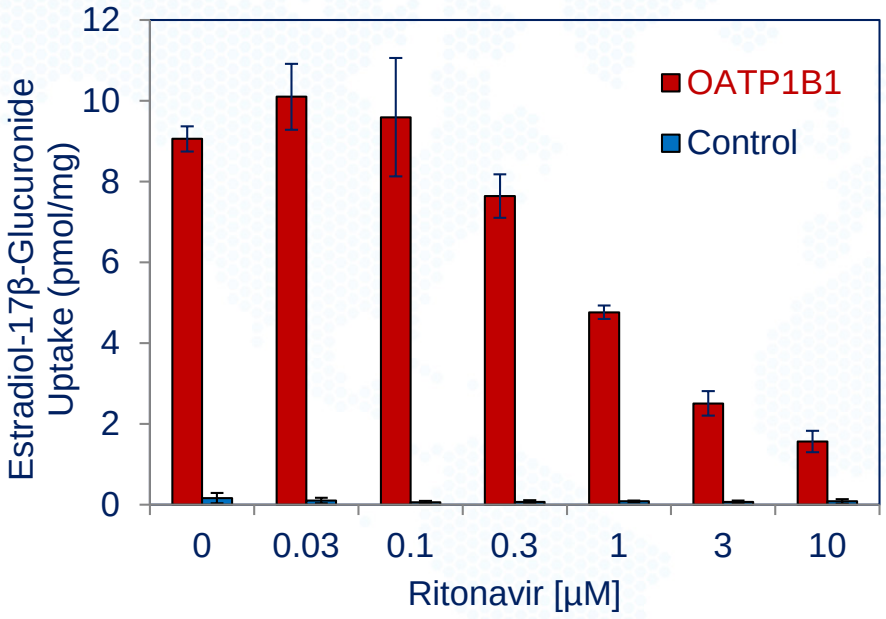
- Prototypical inhibitors used as positive controls for all assays

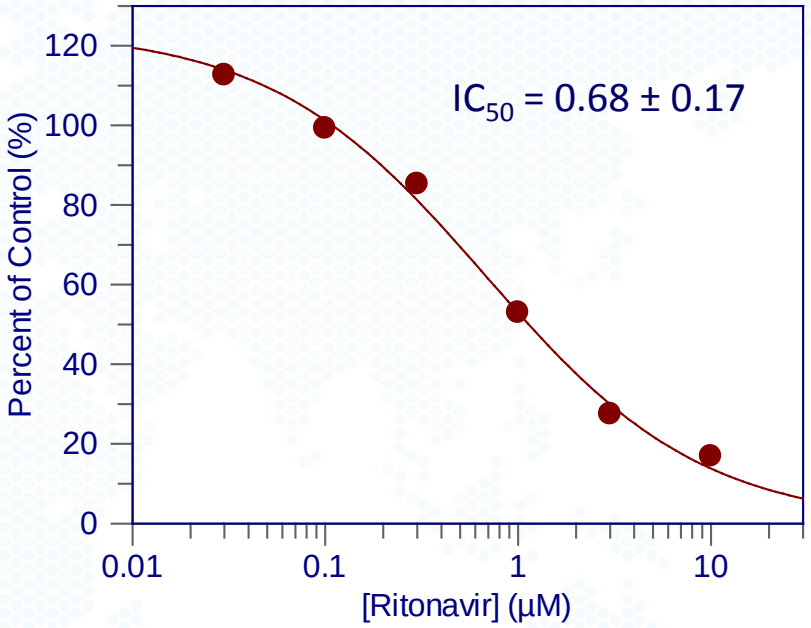
Methods – prototypical inhibitors

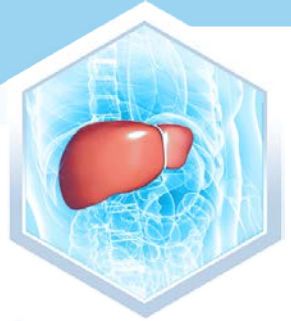
Transporter	Inhibitor
OATP1B1	Rifampin (10 µM)
OATP1B3	
OAT1	Probenecid (100 µM)
OAT3	
OCT1	Quinidine (300 µM)
OCT2	
MATE1	Cimetidine (1000 µM)
MATE2-K	
P-gp	Verapamil (60 µM)
BCRP	Ko143 (1 µM)
MRP2	Benzbromarone (100 µM)
MRP3	
BSEP	Cyclosporine (20 µM)



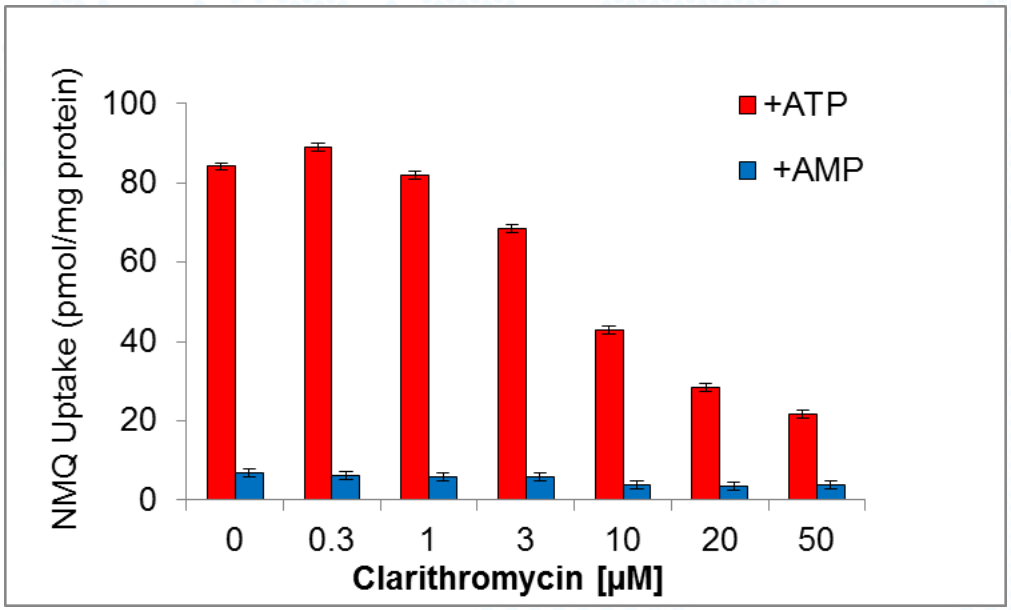
Results – Example: Inhibition of OATP1B1 by ritonavir

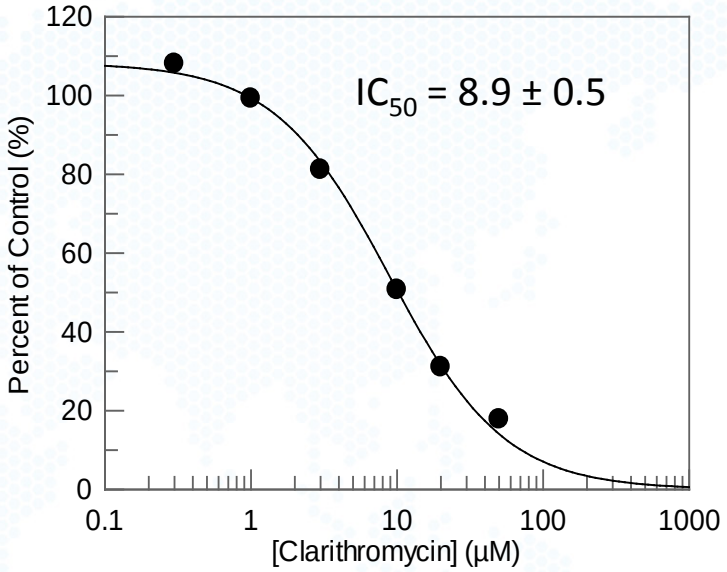






Results – Example: Inhibition of P-gp by Clarithromycin



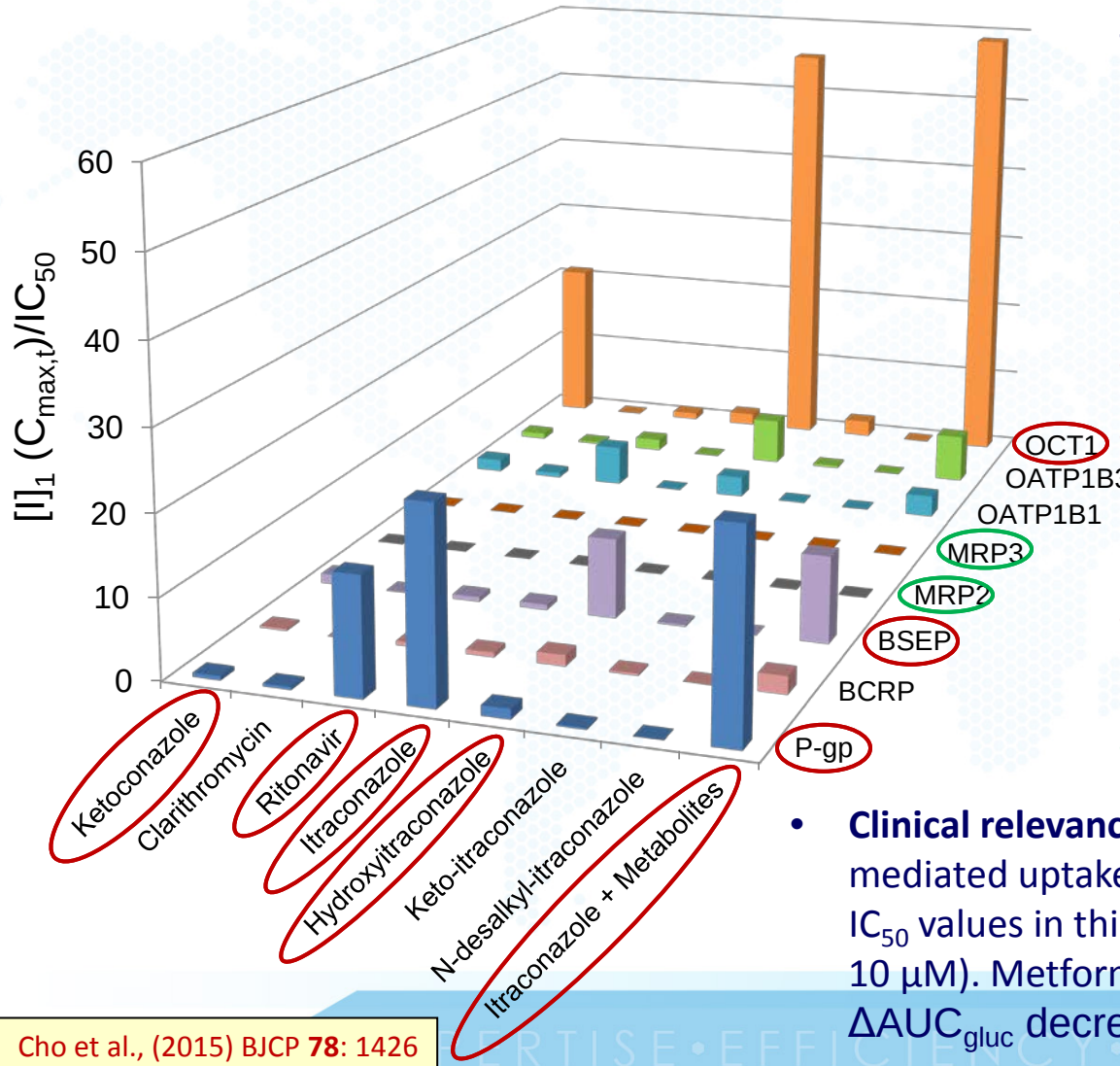


Results – IC50 values (µM)

Transporter	Ketoconazole	Itraconazole	Hydroxy-itraconazole	Keto-itraconazole	N-Desalkyl-itraconazole	Clarithromycin	Ritonavir
OATP1B1	1.8 ± 0.2	>10	0.23 ± 0.03	0.29 ± 0.04	>0.2	5.3 ± 1.3	0.68 ± 0.17
OATP1B3	3.9 ± 0.6	>10	0.10 ± 0.01	0.088 ± 0.035	>0.2	14 ± 2	2.3 ± 0.4
OAT1	5.7 ± 0.5	>10	>3	>3	>0.2	>50	17 ± 3
OAT3	0.86 ± 0.68	>10	2.0 ± 0.3	>3	>0.2	>50	>30
OCT1	0.13 ± 0.03	0.74 ± 0.24	0.01 ± 0.00	0.04 ± 0.01	>0.2	>50	4.1 ± 0.6
OCT2	0.89 ± 0.35	>10	>3	>3	>0.2	>50	>30
MATE1	0.37 ± 0.03	>10	0.84 ± 0.21	1.1 ± 0.2	>0.2	>50	1.2 ± 0.2
MATE2-K	>2	>10	>3	>3	>0.2	>50	15 ± 2
P-gp	5.6 ± 0.4	0.048 ± 0.04	0.49 ± 0.14	0.12 ± 0.12	0.26 ± 0.05	8.9 ± 0.5	0.24 ± 0.02
BCRP	12 ± 9	1.9 ± 0.3	0.44 ± 0.03	0.10 ± 0.01	>0.2	>50	6.6 ± 0.5
MRP2	>20	>10	>3	>3	>0.2	>50	>30
MRP3	>20	>10	>3	>3	>0.2	>50	>30
BSEP	2.4 ± 0.6	1.8 ± 0.0	1.2 ± 0.8	0.11 ± 0.02	>0.2	59 ± 8	6.1 ± 0.9

Compounds in yellow inhibited the fewest transporters
Transporters in green were inhibited by the fewest compounds

DDI predictions for hepatic uptake and efflux transport based on the FDA's basic model



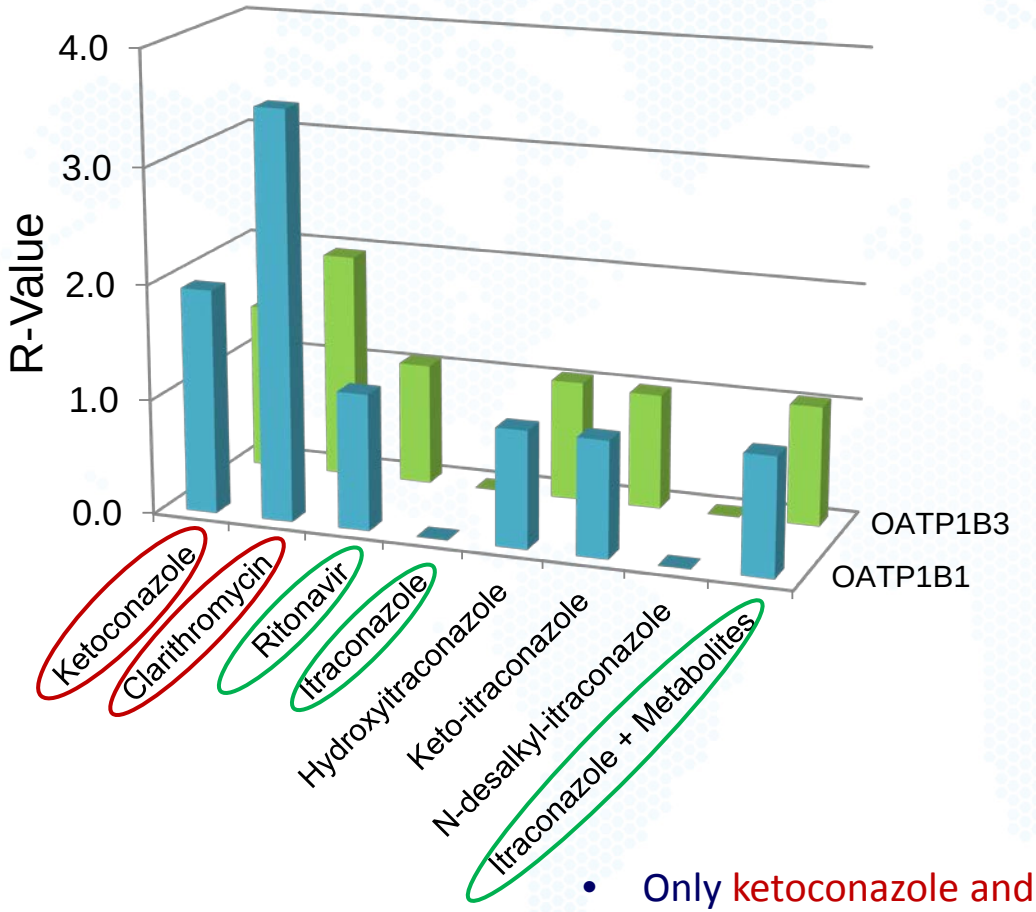
Some very high $[I]_1/IC_{50}$ values (>100-fold the 0.1 cut-off):

- Ketoconazole for OCT1 (~200x)
- Ritonavir for P-gp (~150x)
- Itraconazole for P-gp (~240x)
- OH-Itraconazole for OCT1 (550x)
- Sum of itraconazole + metabolites >250-fold cut-off for P-gp, BSEP and OCT1
- No inhibitors with all values <0.1
- MRPs not affected by any

- **Clinical relevance**? Verapamil inhibits OCT1-mediated uptake of metformin (IC_{50}=13 µM; Most IC_{50} values in this study much less than 10 µM). Metformin plasma PK not altered, but ΔAUC_{gluc} decreased 240%.

Cho et al., (2015) BJCP **78**: 1426

DDI predictions for OATP-mediated hepatic uptake: R-values

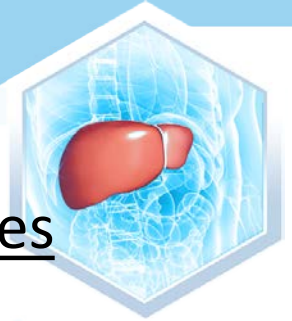


$$\boldsymbol{R\ value} = 1 + (f_u * \frac{I_{in,max}}{IC_{50}})$$

Where:

- f_u = fraction unbound of the inhibitor
- $I_{in,max}$ = estimated maximum inhibitor concentration at the inlet to the liver
- $I_{in,max} = C_{max} + (k_a * Dose * \frac{F_a F_g}{Qh})$
- k_a = absorption rate constant of the inhibitor (assumed 0.1)
- $F_a F_g$ = fraction of the dose of inhibitor absorbed (assumed 1)
- C_{max} = maximum systemic plasma concentration of inhibitor

- Only ketoconazole and clarithromycin predicted to affect OATP1B1 and 1B3 using the R-value method with cut-off ≥1.25.
- Neither ritonavir nor itraconazole had values >1.25



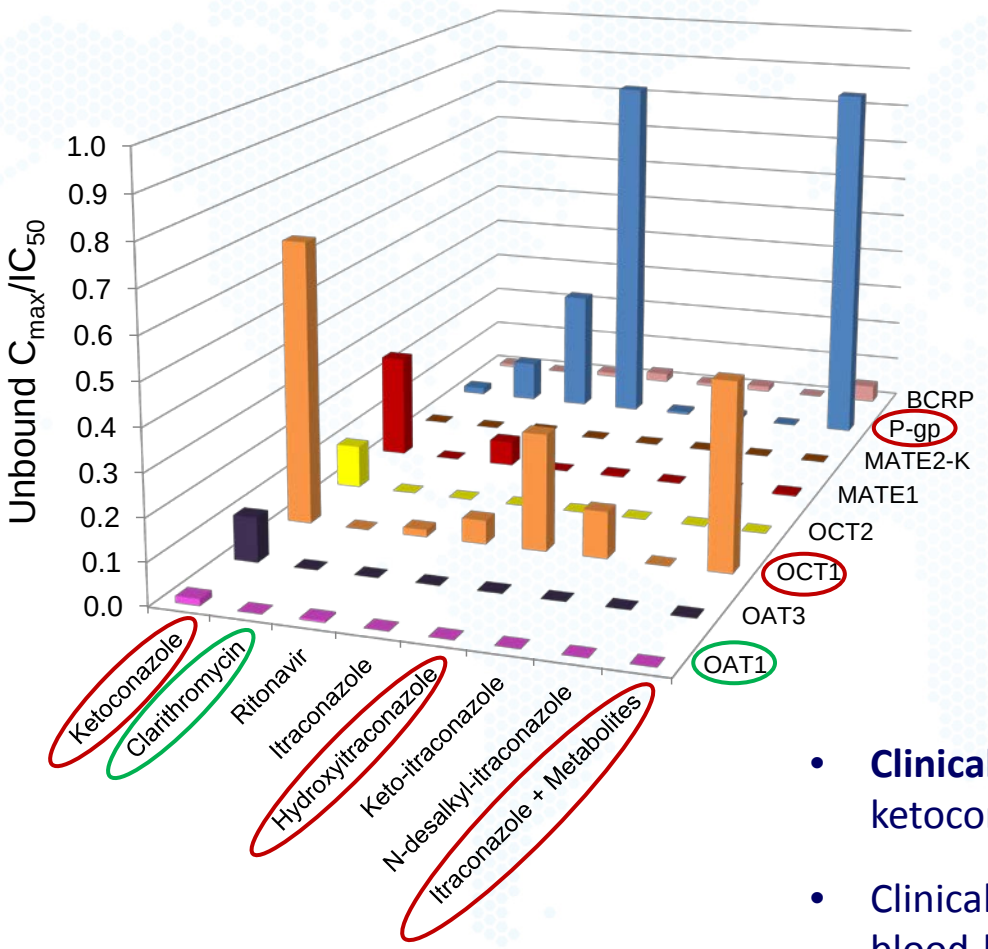
DDI predictions for OATP-mediated hepatic uptake: R-values

- Clinical relevance: Ketoconazole ↑ bosentan AUC by 122%
  - Clarithromycin and:
    - Bosentan (273% ↑ AUC)
    - Glyburide (33% ↑ AUC)
    - Pravastatin (111% ↑ AUC)

- OATP inhibition explains only part of these interactions, with CYP3A4 inhibition also playing a significant role

University of Washington DIDB (2015)

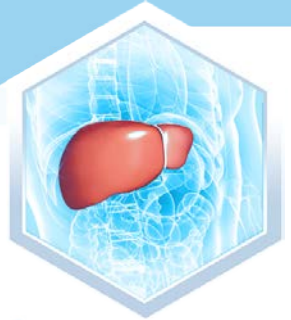
DDI predictions for renal or BBB transporters based on the FDA's basic model



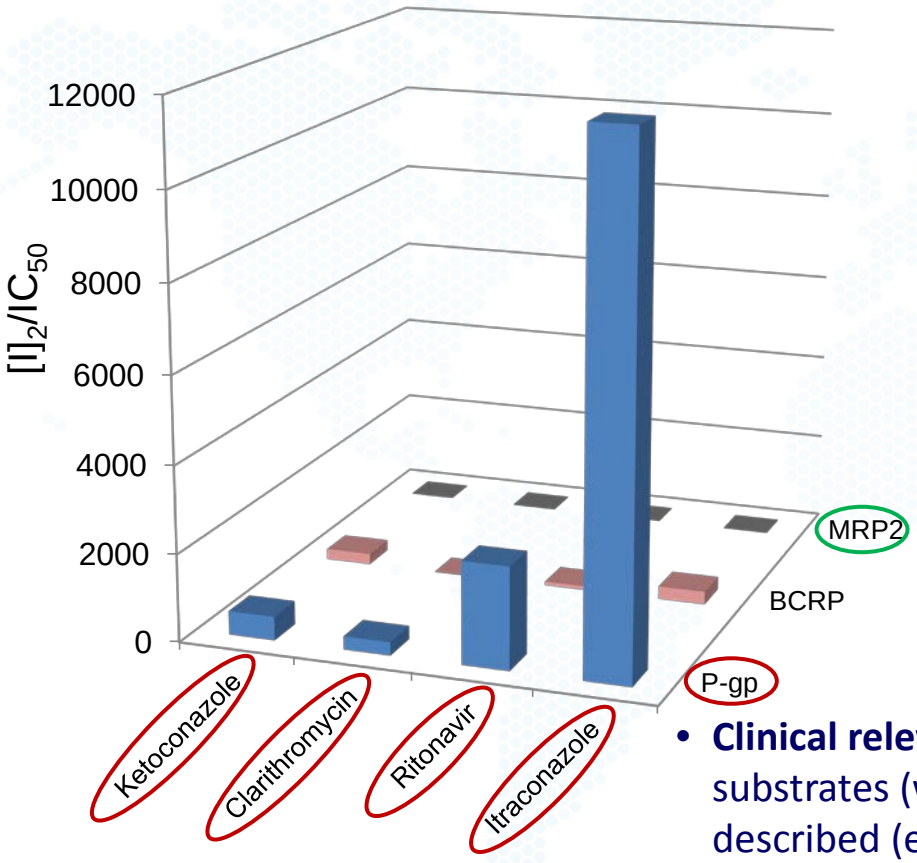
- With the unbound C_{max}/IC_{50} value cut-off >0.1 all transporters except OAT1 were predicted to be affected.
- All values were <10-fold higher than the cut-off
- P-gp and OCT1 were most affected
- Ketoconazole and OH-itraconazole had the greatest impacts
- Clarithromycin did not have any values >0.1

- **Clinical relevance**? Only MATE1 inhibition by ketoconazole mentioned as possibly relevant
- Clinically relevant inhibition of drug efflux at the blood-brain-barrier is unlikely to occur

Kalvass JC et al., (2013) CPT **94**: 80; University of Washington DIDB (2015); Matsson, EM, et al, (2013) Mol Pharm **10,** 11;



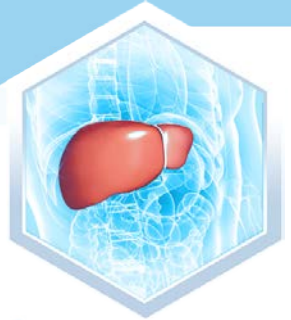
DDI predictions for intestinal efflux transporters based on the FDA's basic model



- With the $[I]_2/IC_{50}$ value cut-off >10, MRP2 was not predicted to be affected by any inhibitors.
- For P-gp, $[I]_2/IC_{50}$ values were:
  ~50-, 30-, 230- and 1000-fold the cut-off for ketoconazole, clarithromycin, ritonavir and itraconazole, respectively
- For BCRP $[I]_2/IC_{50}$ values were:
  25-, 8- and 30-fold the cut-off for ketoconazole, ritonavir and itraconazole, respectively
- Clarithromycin was not predicted to inhibit intestinal BCRP

- **Clinical relevance**? Interactions between all inhibitors and P-gp substrates (with minimal CYP3A4 contribution) are well described (e.g., fexofenadine, quinidine, dabigatran, digoxin - up to 2.5 fold AUC increase).
- BCRP mentioned in DDI between rosuvastatin and ritonavir

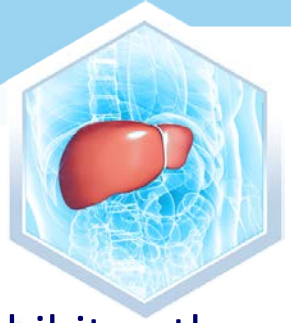
University of Washington DIDB (2015)



Potential limitations of the study

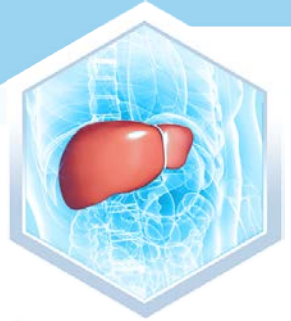
- The potential for non-specific binding of the inhibitors in the test systems was not directly measured
- If measured, should the free inhibitor concentration be evaluated with or without test system present and should pre-incubations be employed?
- Because of these questions, a consistent design was used throughout with a pre-incubation with inhibitor
- These studies were designed to use similar methodologies reported in recent publications (Brouwer et al., 2013; Izumi et al., 2013; Izumi et al., 2015; Shitara et al., 2013; Taub et al., 2011; Zamek-Gliszczynski et al., 2013)
- Designed to *minimize* effects of time-dependency and non-specific binding

Brouwer et al. (2013) CPT **94**: 95; Izumi et al. (2013) DMD **41**: 1859; Izumi et al. (2015) DMD **43**: 235; Shitara et al. (2013) JPS **102**: 3427; Taub et al. (2011) DMD **39**: 2093; Zamek-Gliszczynski et al. (2013) CPT **94**: 67.



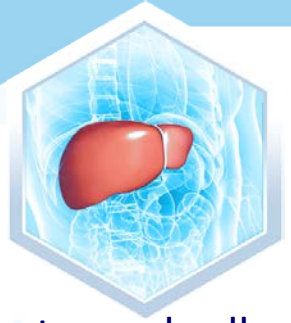
Summary – Part 1

- Of the alternatives to ketoconazole as a strong clinical CYP3A4/5 inhibitor, the following conclusions can be made:
  - MRP2 and MRP3 were not significantly inhibited by any at clinically relevant concentrations
  - MATE2-K was only inhibited by ritonavir
  - Itraconazole, *N*-desalkylitraconazole and clarithromycin inhibited the fewest transporters
- Based on the FDA's basic hepatic model $[I]_1/IC_{50}$:
  - OCT1 was the most potently inhibited transporter, with values >250-fold the cut-off of 0.1 for itraconazole + metabolites
  - P-gp was most potently inhibited by ritonavir
  - No inhibitors had $[I]_1/IC_{50}$ <0.1 for all transporters, except MRPs



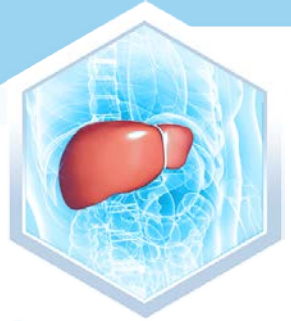
Summary – Part 2

- Based on the FDA's R-value hepatic model for OATP1B1 and 1B3:
  - Ritonavir and itraconazole have values <1.25
  - Clarithromycin most potently inhibits OATP1B1 (R value = 3.5)
- Based on the FDA's basic renal and BBB model (unbound C_{max}/IC_{50}):
  - OAT1 is predicted to be unaffected by all inhibitors
  - None of the unbound C_{max}/IC_{50} values were >10-fold the cut-off
  - Hydroxy-itraconazole had the greatest impacts
  - Clarithromycin did not have any unbound C_{max}/IC_{50} values >0.1
- Based on the FDA's basic intestinal model ($[I]_2/IC_{50}$):
  - MRP2 is predicted to be unaffected by all inhibitors
  - P-gp and BCRP were predicted to be significantly affected by the inhibitors with the exception of clarithromycin for BCRP



Conclusions

- None of the alternatives to ketoconazole provided a clean inhibition profile towards all 13 drug transporters evaluated
- The alternatives to ketoconazole each have unique transporter inhibition profiles
- MRP2 and MRP3 were not inhibited by any alternative inhibitors
- Ritonavir and itraconazole may be the best alternatives for CYP3A4/5 substrates that are transported by OATP1B1 and 1B3
- CYP3A4/5 substrates that are transported by OAT1 may not be affected by any of these alternative inhibitors
- Clarithromycin may be the best choice for substrates of renal transporters
- For substrates of intestinal P-gp or BCRP, clarithromycin may be the best choice (although P-gp is still predicted to be affected)
- The best choice for a strong clinical CYP3A4/5 inhibitor depends on the unique transporter substrate profile of the drug candidate



Acknowledgments

- Andrea Wolff
- XenoTech's Analytical Services group

Upcoming Show

AAPS ITC Drug Transporter Workshop

April 18-20, 2016 – Baltimore, MD

Booth # 4

Dr. Lydia Vermeer & Lindsey Harston

Senior Scientist, Drug Transport

lvermeer@xenotechllc.com

Technical Account Manager

lharston@xenotechllc.com

AAPS Drug Transporter Workshop Poster Presentation

Poster # M1032

Dr. Lydia Vermeer

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

Tuesday, April 19th

5:15 – 6:30pm

Drug Transporter Services at XenoTech

- Over 50 transporters assays available (Tokai & Kansas site)
- Variety of models available
  - Cell lines
  - Vesicles
  - Transfected cells
  - Oocytes
  - Hepatocytes
  - Animal models
- GLP & non-GLP study options


Questions