

Solvo Biotechnology Meet the Experts Transporter Conference
Cambridge, Boston
Sept 4th-5th, 2019

ADME Biomarkers: Vision and Status Report

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WORLDWIDE RESEARCH & DEVELOPMENT



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Thanks to numerous colleagues

- Andrew Rowland (Flinders)
- Michael Sorich (Flinders)
- Hiroyuki Kusuhsara (Tokyo U.)
- Yuichi Sugiyama (Riken Inst.)
- Daiki Mori (Tokyo U.)
- Yusuke Kondo (Tokyo U.)
- Kenichi Furihata (P1 Clinic)

Pfizer

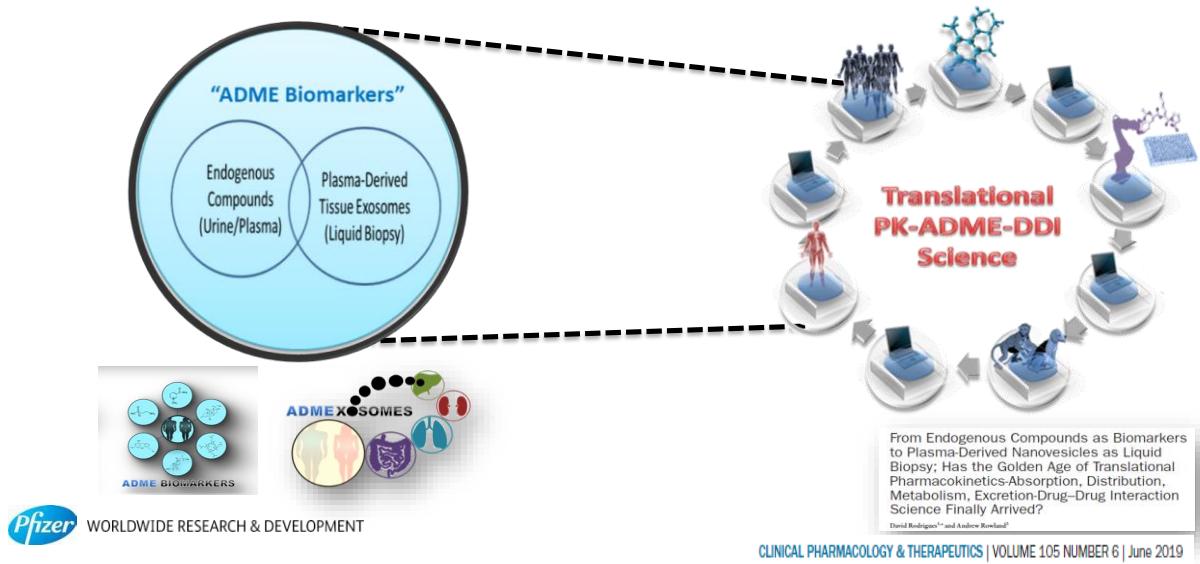
- Manoli Vourvahis
- Ragu Ramanathan
- Brian Rago
- Amanda King-Ahmad
- Lina Luo
- Emi Kimoto
- Chieko Muto
- Manthena Varma
- Sumathy Mathialagan
- Yi-An Bi
- Chester Costales
- Susan Mahoney
- Jack Cook
- Olga Kavetska
- Dennis Scott

- Sarah Lazzaro
- Christine Orozco
- Matt Cerny
- Mark West
- Adam Ogden
- Ted Johnson
- Martin Dowty
- Art Bergman
- Jillian Johnson
- Linda Wood
- James Gosset
- David Tess



WORLDWIDE RESEARCH & DEVELOPMENT

PK-ADME-DDI in the age of translation



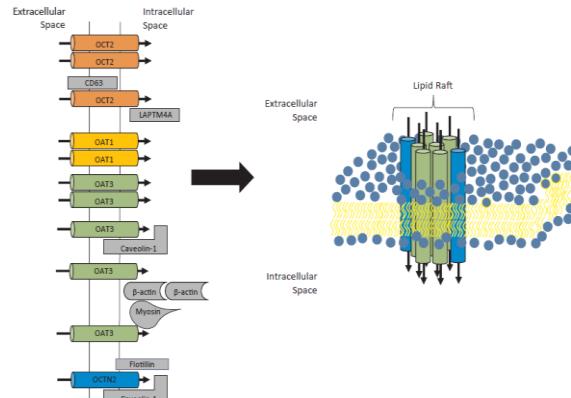
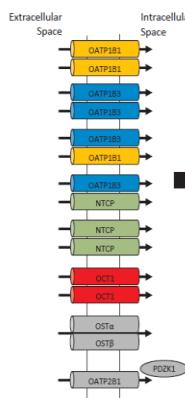
PK-ADME-DDI in the age of translation

Commentary

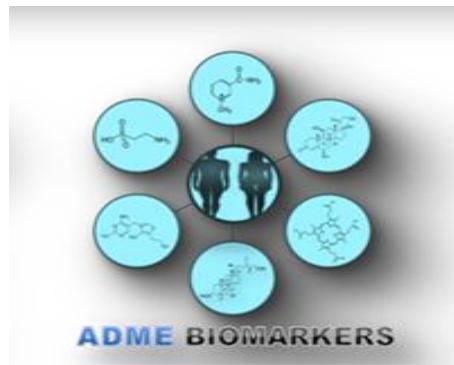
Protein-protein interactions of drug uptake transporters that are important for liver and kidney

Yuchen Zhang^a, Bruno Hagenbuch^{b,*}

Biochemical Pharmacology 168 (2019) 384–391



PK-ADME-DDI in the age of translation



WORLDWIDE RESEARCH & DEVELOPMENT

Introduction

- Acknowledged that DDI involving transporters are important (e.g., patient safety concerns or when there is impact on PD)
 - Especially statins involving inhibition of liver OATP1B1/3 (AUC ratio > 3)
 - But agencies also focused on non-OATP1B DDI (AUC ratio >1.25 to ≤ 3)
 - Metformin (renal OCT2 + MATEs, liver OCT1)
 - Digoxin (gut Pgp)
 - Rosuvastatin (gut BCRP)
 - Furosemide (kidney OAT1/3)



WORLDWIDE RESEARCH & DEVELOPMENT

[Clinical Aspects of Transporter-Mediated Drug-Drug Interactions](#). Gessner A, König J, Fromm MF. Clin Pharmacol Ther. 2019 Jan 16. doi: 10.1002/cpt.1360. [Epub ahead of print]

Introduction

- Transporter DDIs can be complicated by interplay with enzymes, genotype, metabolic disease (e.g., T2DM, NAFLD), organ impairment age, pregnancy, infection, cancer, etc
- Acknowledged that DDI IVIVEs are complex and current agency DDI risk cut offs are conservative (significant number of FP)
- Greater emphasis on development/validation of tools to facilitate clinical DDI assessment and de-risk DDI in Phase 1
 - E.g., Drug probe cocktails, micro-dosing, imaging, and biomarkers, etc



Introduction

- Endogenous compounds in plasma and urine present as transporter substrates that can serve as biomarkers. Therefore....
- Characterization of biomarkers ongoing**
 - In vivo, GWAS, transgenic animals, in vitro
- Development & publication of bioanalytical methods to support biomarker quantitation in biofluids**
- Push for tools to support biomarker M&S (PBPK)**
- Clinical validation of biomarkers is underway**
 - Versus drug probes (e.g., tool inhibitors such as CsA & RIF)
 - Leveraging of ongoing Phase 1 (SAD/MAD) studies and formal DDI studies (NCEs)



Introduction; examples of publications

Reviews/commentaries

- ❑ Chu et al., J Pharm Sci. 2017 Sep;106(9):2357-2367
 - ❑ Rodrigues et al., Clin Pharmacol Ther. 2018 Mar;103(3):434-448
 - ❑ Mariappan et al., Curr Drug Metab. 2017 Oct 16;18(8):757-768
 - ❑ Chu et al., Clin Pharmacol Ther. 2018 Nov;104(5):836-864
 - ❑ Muller et al., Pharmacol Rev. 2018 Apr;70(2):246-277

Animal studies (e.g., Cyno monkey)

- ❑ Shen et al., Drug Metab Dispos. 2018 Feb;46(2):178-188
 - ❑ Shen et al., Drug Metab Dispos. 2016 Feb;44(2):238-49
 - ❑ Shen et al., J Pharmacol Exp Ther. 2013 Mar;344(3):673-85.
 - ❑ Chu et al., Drug Metab Dispos. 2015 Jun;43(6):851-63
 - ❑ Thakare et al., Drug Metab Dispos. 2017 Jul;45(7):721-733

Clinical studies

- Yee SW, et al. Clin Transl Sci. 2019 Apr 13. doi: 10.1111/cts.12625. [Epub ahead of print]
 - Shen et al., Drug Metab Dispos. 2017 Aug;45(8):908-919
 - Barnett et al., J Pharmacol Exp Ther. 2019 Jan;368(1):125-135.
 - Takehara et al., Pharm Res. 2017 Aug;34(8):1601-1614
 - Ito et al., Clin Pharmacol Ther. 2012 Nov;92(5):635-41
 - Liu et al., J Clin Pharmacol. 2018 Nov;58(11):1427-1435
 - Mori et al., Drug Metab Pharmacokinet. 2019 Feb;34(1):78-86
 - Tsuruya et al., Drug Metab Dispos. 2016 Dec;44(12):1925-1933
 - Imamura et al., Drug Metab Dispos. 2014 Apr;42(4):685-94
 - Kato et al., Pharm Res. 2014 Jan;31(1):136-47
 - Shen et al., Drug Metab Dispos. 2018 Aug;46(8):1075-1082
 - Kunze et al., Clin Pharmacokinet. 2018 Dec;57(12):1559-1570

Biomarker PK modeling (e.g., CP-I)

- ❑ Yoshikado et al., CPT Pharmacometrics Syst Pharmacol. 2018 Nov;7(11):739-747
 - ❑ Yoshida et al., CPT Pharmacometrics Syst Pharmacol. 2018 Aug;7(8):517-524
 - ❑ Barnett et al., Clin Pharmacol Ther. 2018 Sep;104(3):564-574



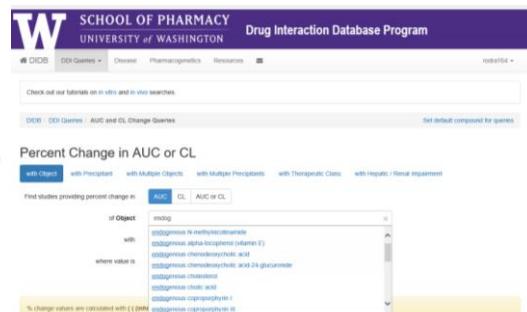
Introduction; examples of publications

Bioanalytical methods (e.g., CP-I, NMN, bile acids)

- Luo et al., *Bioanalysis*. 2018 May 1;10(9):673-689 (NMN)
 - Rago et al., *Bioanalysis*. 2018 May 1;10(9):645-657 (bile acids)
 - King-Ahmad et al., *Bioanalysis*. 2018 May 1;10(9):691-701
 - Ramanthan et al., *Bioanalysis*. 2017 Nov;9(22):1787-1806
 - Kandousi et al., *Bioanalysis*. 2018 May 1;10(9):633-644
 - Niumbe et al., *J Chromatogr B Analyt Technol Biomed Life Sci*. 2018 Jan 15;1073:80-89

Biomarker information appearing in Wash U DDI db !

<https://didb.druginteractioninfo.org/>



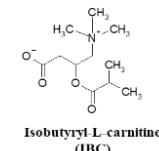
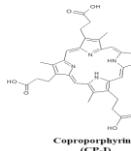
Introduction; examples of biomarkers

ADME Target	Biomarker(s)	Drug Probe(s)
OATP1B, MRP2	Coproporphyrin-I (CP-I), Coproporphyrin-III (CP-III)	Various Statins
OATP1B	Glycochenodeoxycholic Acid 3-O-Sulfate (GCDCA-S) Glycochenodeoxycholic Acid 3-O-Glucuronide (GCDCA-G)	
OCT1	Isobutyryl-L-Carnitine (IBC)?	Sumatriptan? (Metformin?)
OCT2/MATE1/MATE2K	N ¹ -Methylnicotinamide (NMN), Creatinine	Metformin
OAT3	6β-Hydroxycortisol (6βHC) Glycochenodeoxycholic Acid 3-O-Sulfate (GCDCA-S)	Benzylpenicillin (Rosuvastatin)
OAT1	Taurine	Adefovir
OAT1 + OAT3	Pyridoxic Acid (PDA)	Furosemide
BCRP (ABCG2 PgX subjects)	??	Sulfasalazine (Rosuvastatin)
Pgp	??	Digoxin, DABE
CYP3A	6βHC/Cortisol Ratio 4β-Hydroxycholesterol	Midazolam (IV/PO)



Introduction; examples of biomarkers

ADME Target	Biomarker(s)	Drug Probe(s)
OATP1B, MRP2	Coproporphyrin-I (CP-I), Coproporphyrin-III (CP-III)	Various Statins
OATP1B	Glycochenodeoxycholic Acid 3-O-Sulfate (GCDCA-S) Glycochenodeoxycholic Acid 3-O-Glucuronide (GCDCA-G)	
OCT1	Isobutyryl-L-Carnitine (IBC)?	Sumatriptan? (Metformin?)
OCT2/MATE1/MATE2K	N ¹ -Methylnicotinamide (NMN), Creatinine	Metformin



Plasma AUCR or ΔPlasma Conc

Plasma AUCR + ΔCL_{renal}

- Pfizer has developed methods to support bioanalysis
- Select portfolio compounds, that trigger agency DDI risk cut off, prioritized for clinical biomarker assessment (SAD/MAD)
- Biomarkers incorporated into select number of formal DDI studies (e.g., OATP-statin; OCT2/MATE-metformin)

Balanced view of biomarkers

Pros (utility)

- Rapid assessment of DDI in SAD/MAD studies
- Can prioritize formal DDI studies
- DDI assessment at rising doses of perpetrator (e.g., SAD)
- Generation of in vivo K_i
- Avoid/minimize probe drug dosing
- Study full DDI time course (onset, washout)
- Can multiplex BA across biomarkers
- DDI assessment in non-NHVs

Cons (considerations)

- Cannot study effect of victim-perpetrator dose interval on DDI
- Diurnal variation
- SLC/ABC PgX impact
- Inter-subject variability
- Dynamic range to differentiate none, weak, moderate, strong DDI
- Largely reflect liver/kidney
- Impact of disease, pregnancy
- ABC/SLC profile vs probe drug
- Knowledge of PK-ADME profile
- BA assay; availability of stds, sample handling, etc
- Substrate-dependent inhibition
- Food effect



WORLDWIDE RESEARCH & DEVELOPMENT

Agency Transporter DDI Risk Cutoffs

Transporter(s)	Parameter	Cutoff	
		FDA	EMA
Intestinal Efflux Transporters (Pgp/BCRP)	$I_2/IC_{50}(K_i)$	≥ 10	> 10
System Efflux Transporters (Pgp/BCRP)	$C_{max,u}/K_i$	n/a	> 0.02
Hepatic OATP1B1 + OATP1B3	<i>R</i> -Value	≥ 1.1	> 1.04
Hepatic OCT1		n/a	
Renal Basolateral SLCs (OAT1/OAT3/OCT2)	$C_{max,u}/IC_{50}(K_i)$	≥ 0.1	> 0.02
Renal Apical SLCs (MATE1/MATE2K)		≥ 0.02	

$$I_{in,max} = I_{max} + \frac{F_a * F_g * k_a * Dose}{Q_h} / R_B$$

$$R\text{-value} = 1 + \frac{f_{u,p} * I_{in,max}}{IC_{50}}$$

$$I_2 = \frac{\text{Molar Dose}}{250 \text{ mL}}$$

$$IC_{50}/2 = K_i \text{ when } [S] = K_m$$

$$IC_{50} \sim K_i \text{ when } [S] \ll K_m$$

% Inhibition

$$\text{Fractional inhibition} = 1 - \frac{CL_{int'}}{CL_{int}} = \frac{[I]}{[I] + K_i}$$

$CL_{int'}$ = intrinsic active uptake CL in presence of inhibitor



$$\% \text{ Inhibition} = \frac{[I]}{[I] + IC_{50}} * 100$$

$[I]$ = Free C_{max} (renal SLCs)
= Free $I_{max, inlet}$ (liver SLCs)

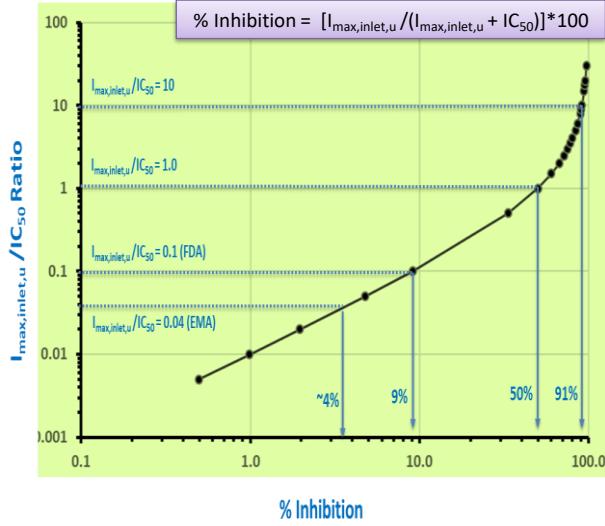
When $[S]/K_m$ ratio < 0.1

- ◻ $IC_{50} \sim K_i$
- ◻ Applies to competitive, non-competitive and mixed inhibition

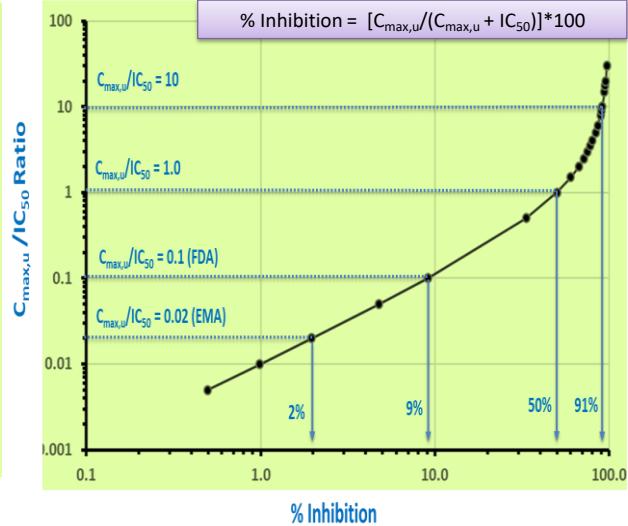


% Inhibition

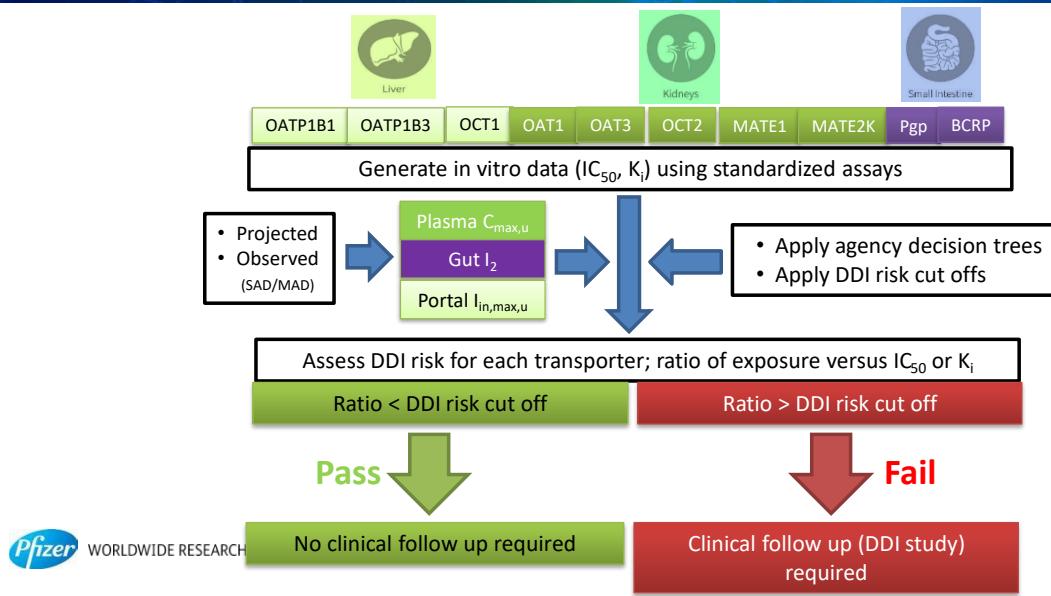
Liver SLCs



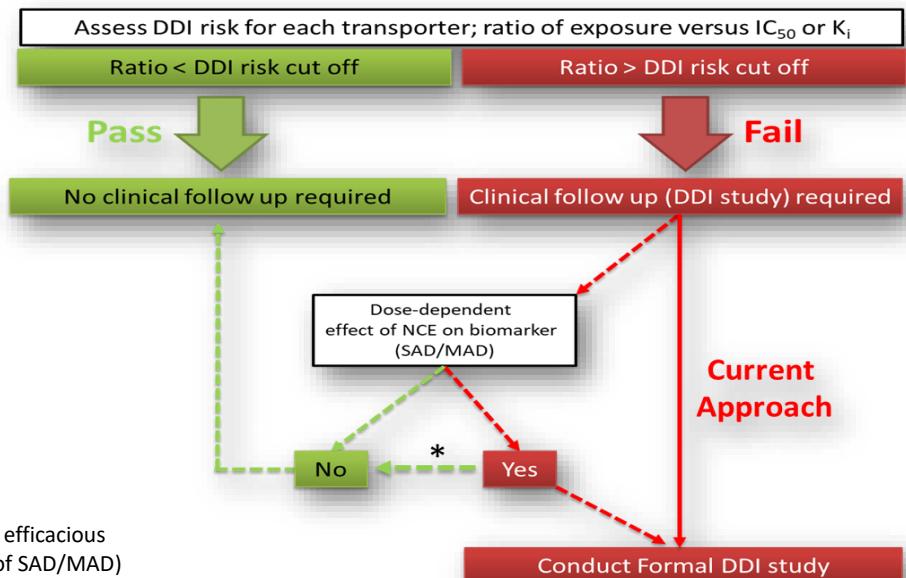
Renal SLCs



Pfizer portfolio transporter DDI risk assessment

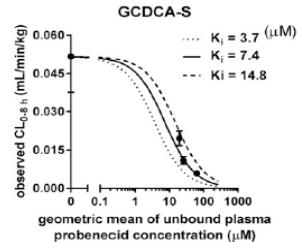
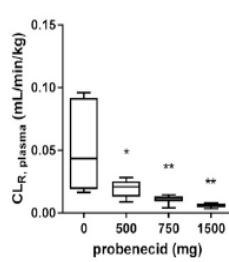
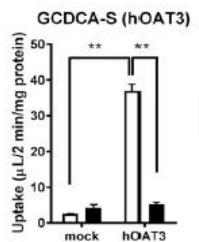
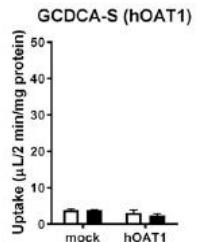
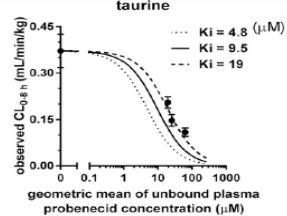
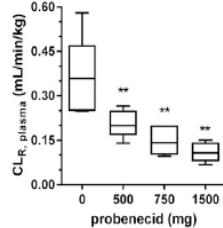
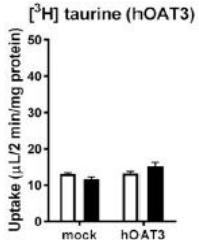
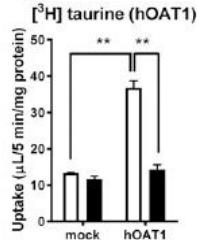


Concept of biomarker integration with agency decision trees



Examples of OAT1 & OAT3 Biomarkers

Drug Metab Dispos 44:1925–1933, December 2016

A

CP-I as OATP1B biomarker

CP-I vs statins at all doses, single + cocktail (literature)

- N = 4 statins
- N = 9 perpetrators

- Antimicrob Agents Chemother. 2014;58(8):4555-64
- Clin Transl Sci. 2019 Apr 13. doi: 10.1111/cts.12625
- Biopharm Drug Dispos. 2016;37(8):479-490

- J Clin Pharmacol. 2018;58(11):1427-1435.
- Clin Pharmacokinet. 2018 Apr 16. [Epub ahead of print]
- CPT (2012) 91: 1053

- Bioanalysis. 2018 May 1;10(9):691-701
- JPET. 2016 Sep;358(3):397-404
- DMD 2018 Aug;46(8):1075-1082.
- Clin Pharmacol Ther. 2019 Aug 2. doi: 10.1002/cpt.1599



WORLDWIDE RESEARCH & DEVELOPMENT

CP-I as OATP1B biomarker

CP-I vs single statin at therapeutic dose only (literature)

- N = 4 statins
- N = 9 perpetrators

- Antimicrob Agents Chemother. 2014;58(8):4555-64
- Clin Transl Sci. 2019 Apr 13. doi: 10.1111/cts.12625
- Biopharm Drug Dispos. 2016;37(8):479-490
- J Clin Pharmacol. 2018;58(11):1427-1435.
- Clin Pharmacokinet. 2018 Apr 16. [Epub ahead of print]
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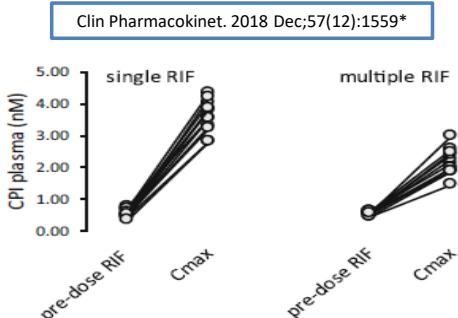


WORLDWIDE RESEARCH & DEVELOPMENT

CP-I as OATP1B biomarker

CP-I to assess OATP induction?

- Evidence that multi-dose RIF administration impacts OATP substrate AUC
 - One report describing decreased CP-I plasma levels with multi-dose RIF*
 - But what of induction of P450s, ABC transporters ?
- Also RIF is a known auto-inducer (decreased OATP inhibition vs induction?)



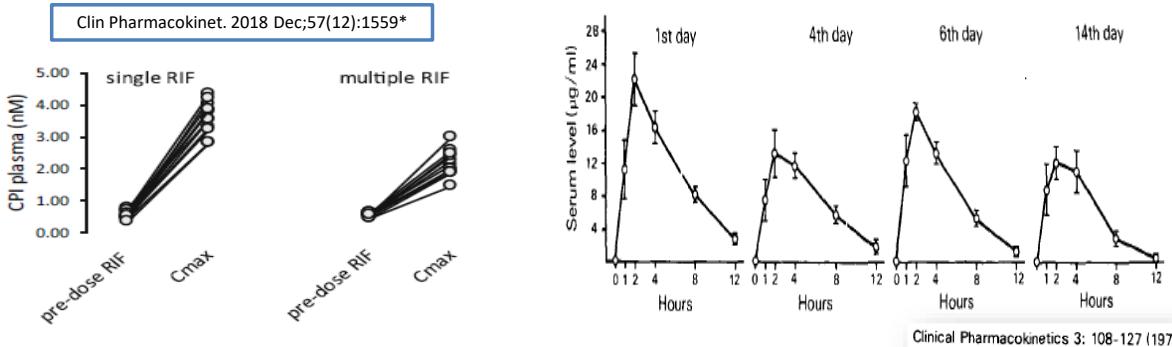
Precipitant	Object	% Change AUC	Precipitant Dose	Object Dose
rifampin	asunaprevir	-21	600 mg (7 days)	600 mg (14 days)
rifampin	atorvastatin	-80.3	600 mg (5 days)	40 mg
rifampin	pravastatin	-14.9	2 mg (17 days)	20 mg
rifampin	pravastatin	-19.6	10 mg (17 days)	20 mg
rifampin	pravastatin	-30.9	600 mg (5 days)	40 mg
rifampin	pravastatin	-50.5	600 mg (17 days)	20 mg
rifampin	pravastatin	-55	75 mg (17 days)	20 mg
rifampin	rosuvastatin	-4.2	450 mg (6 days)	20 mg
rifampin	rosuvastatin	-8.8	2 mg (17 days)	10 mg
rifampin	rosuvastatin	-21.6	10 mg (17 days)	10 mg
rifampin	rosuvastatin	-53.5	75 mg (17 days)	10 mg
rifampin	rosuvastatin	-56.2	600 mg (17 days)	10 mg
rifampin	simvastatin	-86.1	600 mg (5 days)	40 mg
rifampin	simvastatin	-91	600 mg (9 days)	40 mg

Wash U DDI db

CP-I as OATP1B biomarker

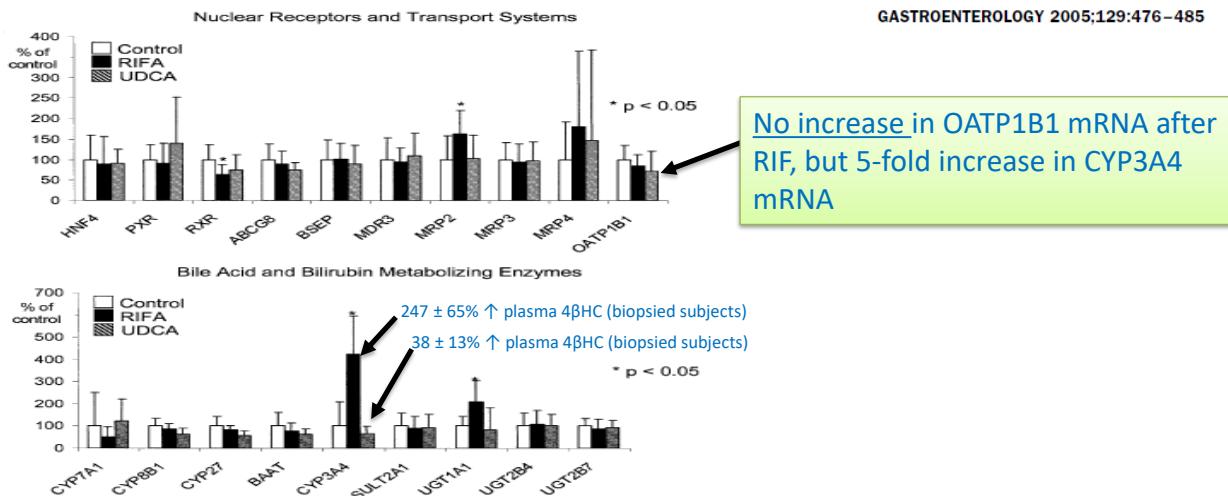
CP-I to assess OATP induction?

- ❑ Evidence that multi-dose RIF administration impacts OATP substrate AUC
 - ❑ One report describing decreased CP-I plasma levels with multi-dose RIF*
 - ❑ But what of induction of P450s, ABC transporters ?
- ❑ Also RIF is a known auto-inducer (decreased OATP inhibition vs induction?)



CP-I as OATP1B biomarker

Human liver biopsy pre/post rifampicin (600 mg x 7 days) or ursodeoxycholic acid (1 g/day x 3 weeks); mRNA data



CP-I as OATP1B biomarker

Weak induction of gut OATPs!! (gut biopsy data)



Cite This: Mol Pharmacol XXXX, XXX, XXX-XXX
pubs.acs.org/molecularpharmacology

Transcriptional and Post-Transcriptional Regulation of Duodenal P-Glycoprotein and MRP2 in Healthy Human Subjects after Chronic Treatment with Rifampin and Carbamazepine

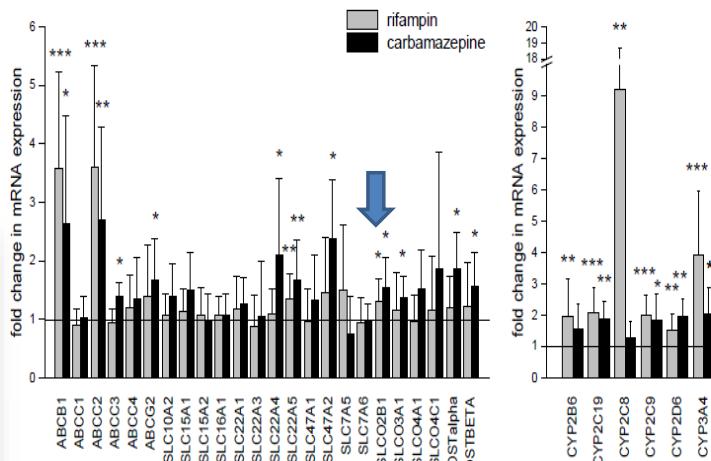
Susanne Brueck,¹ Henrike Bruckmuller,² Danilo Wegner,¹ Diana Busch,¹ Paul Martin,² Stefan Oswald,¹ Ingolf Cascorbi,¹ and Werner Siegmund^{1,2}

None of the organic anion transporting polypeptides (OATPs) or organic cation transporting polypeptides were affected in their expression to a significant extent by rifampicin. OATP2, which is regulated by PXR in mouse

Effects of rifampicin on global gene expression in human small intestine

Mikael Oscarson^a, Oliver Burk^{b,d}, Stefan Winter^c, Matthias Schwab^{b,d}, Renzo Wolbold^{b,d}, Juergen Dippon^c, Michel Eichelbaum^{b,d} and Urs A. Meyer^b

Pharmacogenetics and Genomics 2007, Vol 17 No 11



CP-I as OATP1B biomarker

What??

Citation: CPT Pharmacometrics Syst. Pharmacol. (2018) 7, 647-659; doi:10.1002/cpt.12343

PBPK Models for CYP3A4 and P-gp DDI Prediction: A Modeling Network of Rifampicin, Itraconazole, Clarithromycin, Midazolam, Alfentanil, and Digoxin

Induction EC ₅₀	μmol/L	0.34
E _{max} OATP1B1		0.38
E _{max} AADAC		0.99
E _{max} P-gp		2.5
E _{max} CYP3A4		9.0

Articles

Open Access

Expanded PBPK Model of Rifampicin for Predicting Interactions with Drugs and an Endogenous Biomarker via Complex Mechanisms including OATP1B Induction

Ryuta Asaumi, Karsten Menzel, Wooin Lee, Ken-ichi Nunoya, Haruo Imawaka, Kusuhara Hiroyuki, Yuichi Sugiyama

First Published: 16 August 2019

Abstract | PDF

CPT: Pharmacometrics & Systems Pharmacology

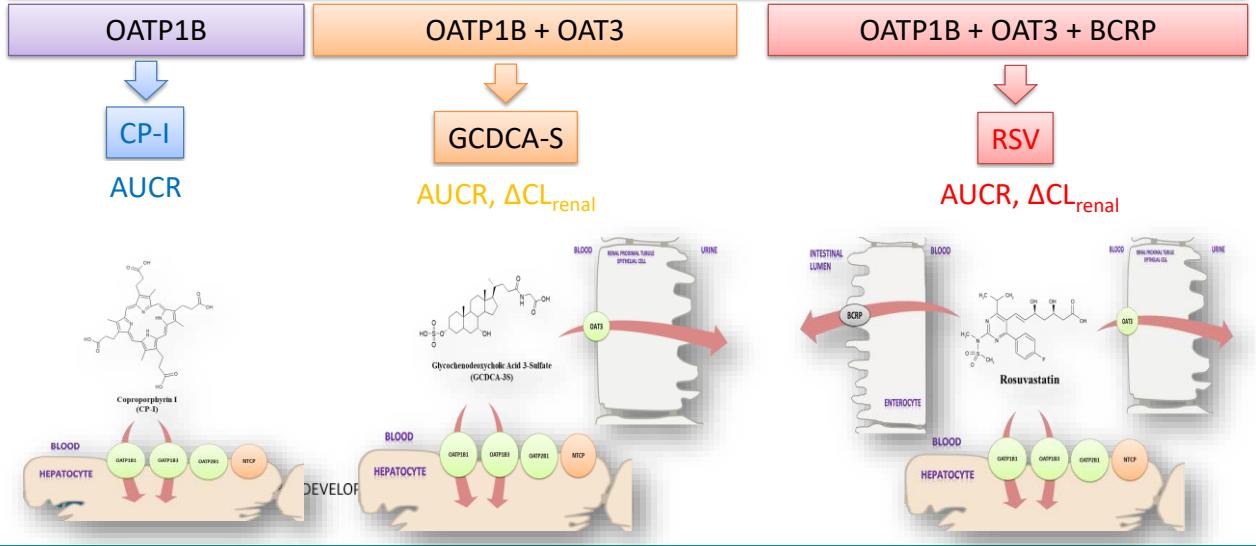
Parameter	Rifampicin
E _{max} for OATP1B	2.32 ± 0.18 / 2.26 ± 0.18 / 2.23 ± 0.19



WORLDWIDE RESEARCH & DEVELOPMENT

CP-I as OATP1B biomarker

Rosuvastatin + biomarkers to cover OATP1B + BCRP + OAT3



CP-I as OATP1B biomarker

Rosuvastatin + CPI to cover OATP1B + BCRP

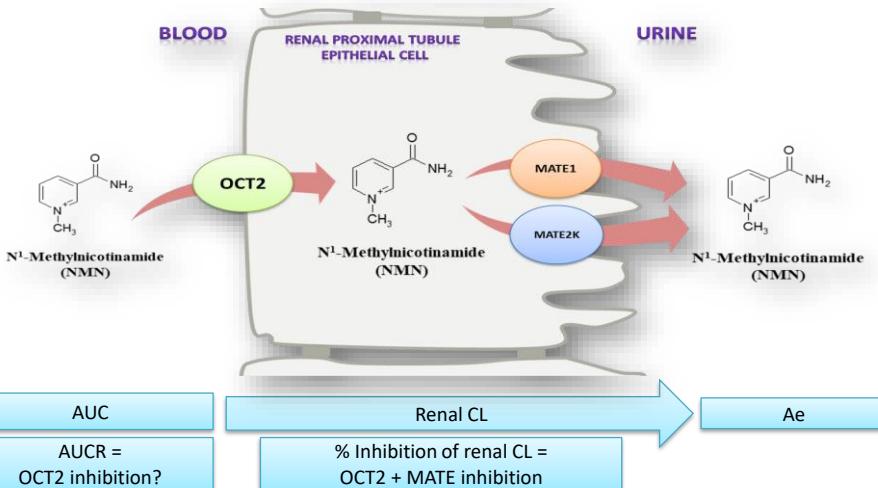
- ❑ Fenebrutinib is an OATP1B and BCRP inhibitor in vitro and triggers agency DDI cutoffs
 - ❑ Minimal inhibition of OAT3 at conc. tested in vitro
- ❑ Clinical follow up with fenebrutinib (200mg BID X 6 days)
 - ❑ No impact on plasma CPI & CPIII
 - ❑ Rosuvastatin AUCR = 2.66; C_{max} ratio = 4.99

Table S1: In Vitro to In Vivo Extrapolation of Fenebrutinib Interaction Potency

	Inhibition potency	Criteria ¹	Predicted numbers
BCRP	9.4	$I_2/IC_{50} \geq 10$	I_2/IC_{50}
OATP1B1	$IC_{50} (\mu M)$	$I_{unbound, inlet, max}/IC_{50}$	0.2
OATP1B3	7.15	≥ 0.1	0.5

Complex drug-drug interaction (DDI) by fenebrutinib and the use of transporter endogenous biomarker to elucidate the mechanism of DDI. Jones N, Yoshida K, Salphati L, Kenny JR, Durk M, Chinn L. Clin Pharmacol Ther. 2019 Aug 2. doi: 10.1002/cpt.1599. [Epub ahead of print].

NMN as SLC biomarker



WORLDWIDE RESEARCH & DEVELOPMENT

NMN as SLC biomarker

Comparison of Metformin, Creatinine and NMN as Renal SLC Substrates
(Single Transfect HEK Cell Panel; [S] << K_m)

Substrate	OAT1	OAT2	OAT3	OCT2	MATE1	MATE2K
Creatinine	0.6 ± 0.1	7.4 ± 0.9	0.8 ± 0.1	28 ± 4.5	21 ± 0.9	7.7 ± 0.2
Metformin	0.8 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	39 ± 0.7	22 ± 0.2	7.8 ± 0.6
NMN	1.4 ± 0.3	0.7 ± 0.1	1.1 ± 0.2	72 ± 4.3	8 ± 0.4	9.0 ± 0.6

Sumathy Mathialagan, Pfizer, Unpublished

Only creatinine presents as OAT2 substrate

Considerable uncertainty regarding the role of OAT2 vs OCT2 in creatinine renal CL_{sec} **

NMN as viable biomarker for metformin (vs creatinine?)

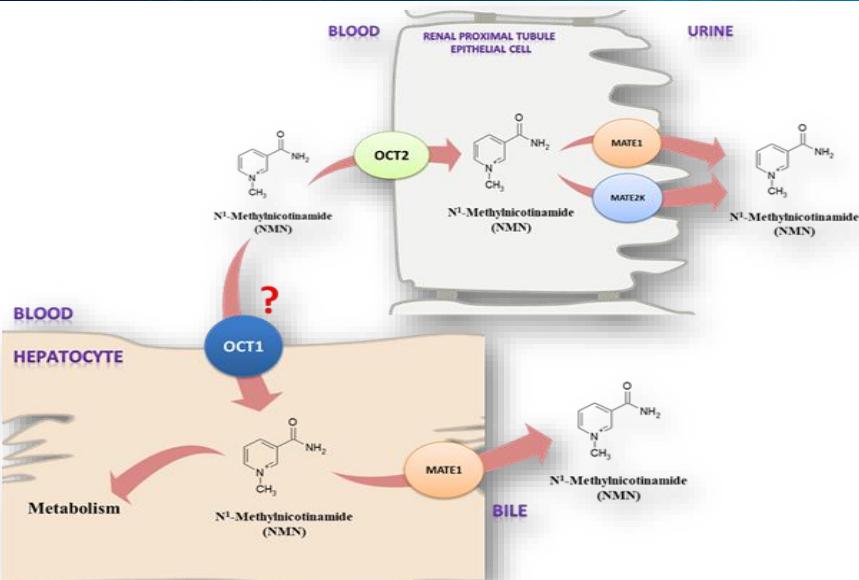
- SLC phenotype
- Reported renal CL_{sec} vs total CL_{renal} (> creatinine)

$$CL_{renal} = \frac{A_{e,0-t(urine)}}{AUC_{o-t}(plasma)} = (1 - FR) * (f_u * GFR + CL_{sec})$$

↓ Fraction Reabsorbed
↑ Tubular secretion

**Clin Pharmacol Ther. 2016 Nov;100(5):437-440
Drug Metab Dispos. 2016 Sep;44(9):1498-509
Drug Metab Dispos. 2017 Feb;45(2):228-236
Drug Metab Dispos. 2015 Jul;43(7):984-93.

NMN as SLC biomarker



Older publication has described
NMN as hOCT1 substrate

DNA AND CELL BIOLOGY
Volume 16, Number 7, 1997
Mary Ann Liebert, Inc.
Pp. 871-881

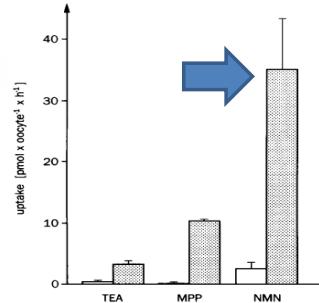
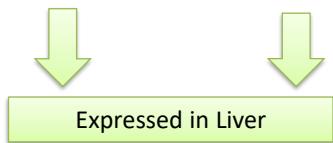


FIG. 7. Expression of cyanine inhibitable cation uptake by hOCT1. Oocytes of *Xenopus laevis* were injected with water (open bars) or with cRNA of hOCT1 (dotted bars) and incubated for 2 days. Uptake of 100 μ M [³H]-labeled TEA, 14 μ M [³H]-labeled MPP, and 300 μ M [³H]-labeled NMN was tested in the absence or presence of 36 μ M cyanine863, and the cyanine-inhibited fraction was calculated. Medians \pm SEM values of 10 parallel measurements with and without cyanine863 are presented.

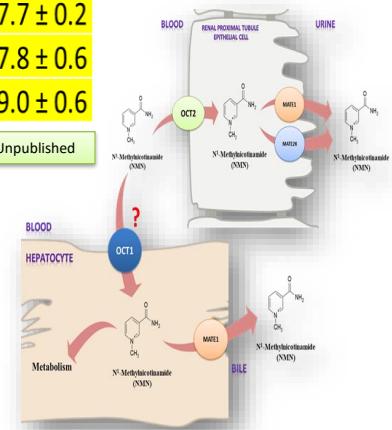
NMN as SLC biomarker

Comparison of Metformin, Creatinine and NMN as Renal SLC Substrates (Single Transfect HEK Cell Panel; [S] << K_m)

Substrate	OAT1	OAT2	OAT3	OCT1	OCT2	MATE1	MATE2K
Creatinine	0.6 \pm 0.1	7.4 \pm 0.9	0.8 \pm 0.1	1.7 \pm 0.1	28 \pm 4.5	21 \pm 0.9	7.7 \pm 0.2
Metformin	0.8 \pm 0.1	1.6 \pm 0.1	1.1 \pm 0.1	19 \pm 1.4	39 \pm 0.7	22 \pm 0.2	7.8 \pm 0.6
NMN	1.4 \pm 0.3	0.7 \pm 0.1	1.1 \pm 0.2	11 \pm 0.4	72 \pm 4.3	8 \pm 0.4	9.0 \pm 0.6



Sumathy Mathialagan, Pfizer, Unpublished



- NMN profile similar to metformin
(implies hepatic OCT1 role also?)

NMN as SLC biomarker

Inhibition of uptake in the presence of plated human hepatocytes

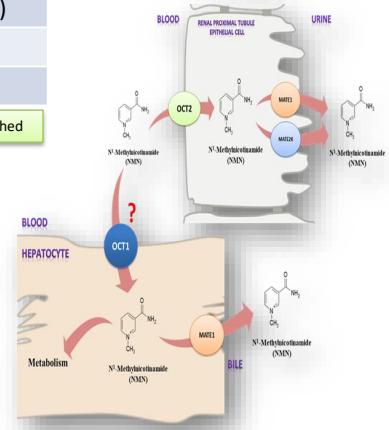
Substrate	All SLCs	OAT2	OCT1	OCT1 + OAT2	Other
	RIFsv (1 mM)	Ketoprofen (0.3 mM)	Quinidine (0.3 mM)	KETO + QND (0.3 + 0.3 mM)	RIFsv - (KETO+QND)
Creatinine	84%	36%	19%	48%	36%
NMN	80%	<5%	73%*	76%	4%

*Similar to metformin

J Pharmacol Exp Ther. 2019 Jul;370(1):72-83

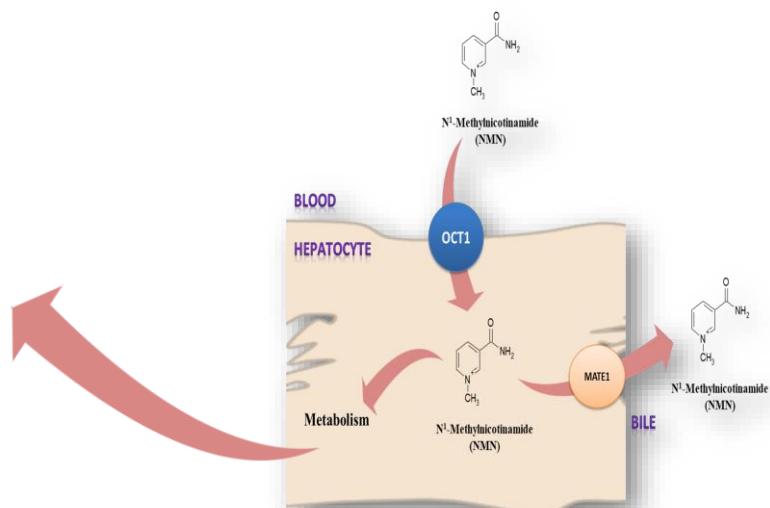
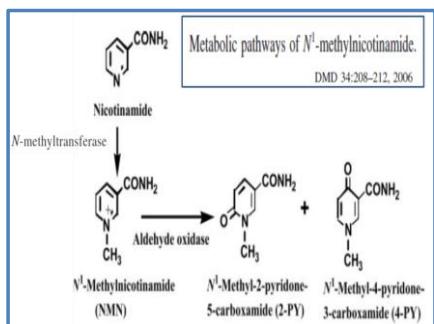
Sumathy Mathialagan, Pfizer, Unpublished

- Evidence for NMN active uptake (80%) with plated human primary hepatocytes (PHH)
- OCT1 plays a major role in NMN uptake in PHH (73%)
- PHH inhibitor profile similar to metformin but different from creatinine
- But what is contribution of hepatic CL to overall NMN CL (vs renal CL)?



NMN as SLC biomarker

For NMN need to consider inhibition of NMT and/or AO?

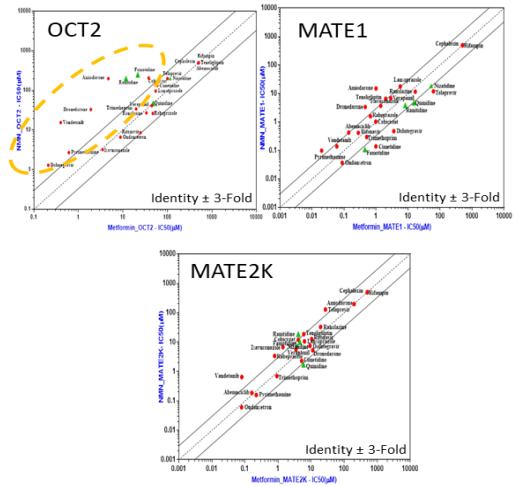


WORLDWIDE RESEARCH & DEVELOPMENT

NMN as SLC biomarker

Substrate-dependent inhibition observed for some compounds (NMN vs MET), especially for OCT2

- ❑ Pfizer has determined in vitro IC₅₀s for NMN vs metformin for tool inhibitors ([S] << K_m)
- ❑ Wide range of IC₅₀s <1 to >100 μM
- ❑ Consistent with the literature, OCT2 presents substrate-dependent effect with some inhibitors (> 3-fold difference)
 - ❑ NMN IC₅₀ right shift (vs metformin)
- ❑ To date, DDI risk assessment for Pfizer compounds based on metformin IC₅₀s
 - ❑ Beginning to use NMN as second OCT2 and MATE substrate with portfolio compounds



Sumathy Mathialagan, Pfizer, Unpublished

NMN as SLC biomarker

- ❑ NMN data available for two tool compounds that present as more potent MATE inhibitors (vs OCT2)
- ❑ Only one reference (Trimethoprim) describing NMN & MET in same study³ !!!!
- ❑ Metformin (MET) data for Pyrimethamine more variable!

Precipitant	% Inhibition Metformin % Inhibition NMN		
	OCT2	MATE1	MATE2K
Pyrimethamine (PYR)	32 10	94 75	85 65
Trimethoprim (TRIM)	16 10	88 93	96 85

Precipitant	NMN		Metformin	
	Plasma AUC % Increase	CL _{renal} % Decrease	Plasma AUC % Increase	CL _{renal} % Decrease
PYR 50 mg SD ^{1,2}	< 1	70 ¹	35 ² , 170 ²	35 ² , 70 ²
TRIM 200 mg BID X 4 days ³	< 1	20	30	26

¹CPT (2012) 92:635 ; CPT (2011) 89: 837; ²UWash DDI db.

³Eur J Clin Pharmacol (2015) 71: 85

$$\% \text{ Inhibition} = [C_{\max,u}/(C_{\max,u} + IC_{50})]*100$$

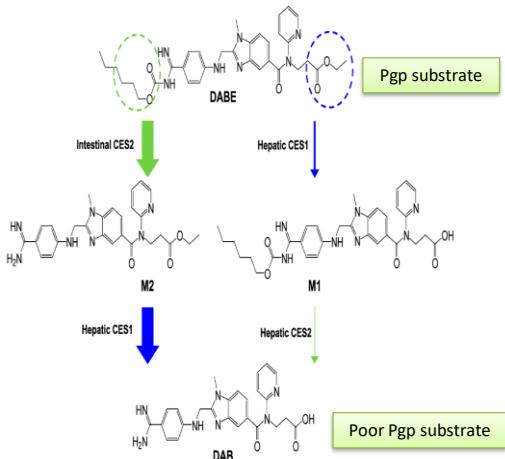


WORLDWIDE RESEARCH & DEVELOPMENT

NMN as SLC biomarker

Dabigatran etexilate (DABE) + NMN for Pgp and MATEs

Drug Metab Dispos. 2014 Feb;42(2):201-6

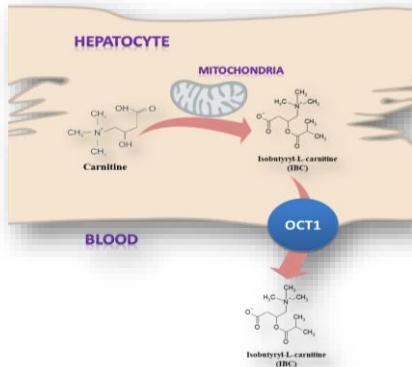


- ❑ DABE (not dabigatran) presents as a Pgp substrate
- ❑ Typically, Pgp DDI reported as a dabigatran AUCR
- ❑ Majority of a DABE dose (~85%) is recovered in urine as unchanged dabigatran
- ❑ renal active secretion ~25% of total clearance
- ❑ Dabigatran identified as MATE1 + MATE2K substrate
- ❑ Should inhibition of renal MATEs be considered when evaluation Pgp inhibition with DABE?
- ❑ Consider running NMN as renal MATE biomarker to check for MATE inhibition?

Shen H, Yao M, Sinz M, Marathe P, Rodrigues AD, Zhu M. **Renal Excretion of Dabigatran: The Potential Role of Multidrug and Toxin Extrusion (MATE) Proteins**. Mol Pharm. 2019 Jul 23. doi: 10.1021/acs.molpharmaceut.9b00472. [Epub ahead of print]

IBC as OCT1 biomarker

- ❑ Plasma IBC conc associated with *SLC22A1* LOF allele (GWAS)
 - ❑ ~50% decrease in plasma IBC ($p = 3 \times 10^{-18}$)
- ❑ Proposed that OCT1 mediates efflux of acylcarnitine (AC) species out of liver

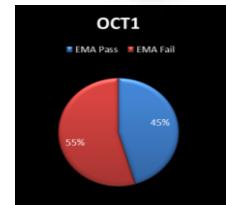
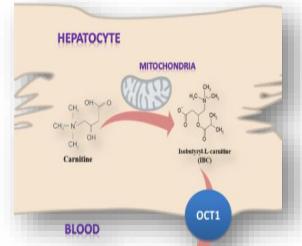


[Emerging Clinical Importance of Hepatic Organic Cation Transporter 1 \(OCT1\) in Drug Pharmacokinetics, Dynamics, Pharmacogenetic Variability, and Drug Interactions](#). Zamek-Gliszczynski et al. Clin Pharmacol Ther. 2018 May;103(5):758-760.

[Fine Mapping and Functional Analysis Reveal a Role of SLC22A1 in Acylcarnitine Transport](#). Kim HI, et al. Am J Hum Genet. 2017 Oct 5;101(4):489-502

IBC as OCT1 biomarker

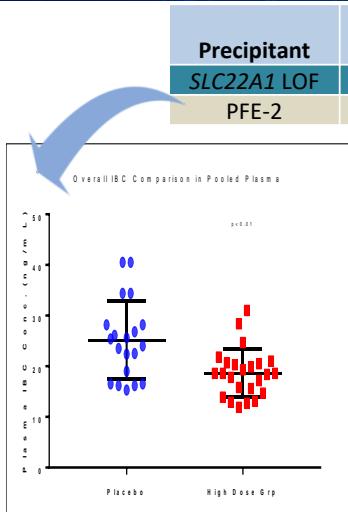
- ❑ Plasma IBC conc associated with *SLC22A1* LOF allele (GWAS)
 - ❑ ~50% decrease in plasma IBC ($p = 3 \times 10^{-18}$)
- ❑ Proposed that OCT1 mediates efflux of acylcarnitine (AC) species out of liver
- ❑ AC species present as substrates of human & mouse OCT1
 - ❑ Transfected protein (vs mock)
 - ❑ Primary hepatocytes (murine) after addition of carnitine
- ❑ Preliminary Pfizer in vitro data with human hepatocytes consistent with IBC as OCT1 substrate
- ❑ Pfizer has developed BA method for plasma IBC to support selected SAD/MAD studies and deployment in certain DDI studies
 - ❑ Significant % of portfolio compounds trigger EMA OCT1



[Emerging Clinical Importance of Hepatic Organic Cation Transporter 1 \(OCT1\) in Drug Pharmacokinetics, Dynamics, Pharmacogenetic Variability, and Drug Interactions](#). Zamek-Gliszczynski et al. Clin Pharmacol Ther. 2018 May;103(5):758-760.

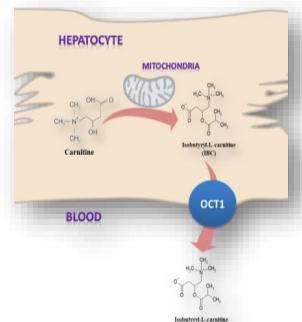
[Fine Mapping and Functional Analysis Reveal a Role of SLC22A1 in Acylcarnitine Transport](#). Kim HI, et al. Am J Hum Genet. 2017 Oct 5;101(4):489-502

IBC as OCT1 biomarker



PFE-2 decreases plasma IBC levels 30% ($p < 0.01$)

- ❑ Directionally correct (vs impact of *SLC22A1* PgX)
- ❑ Magnitude consistent with high (~90%) inhibition ($R\text{-value} = 15.7$)
- ❑ Subjects in study were NOT *SLC22A1* genotyped



*Same approach as for OATP1B

**Clin Pharmacol Ther. 2016 Jun;99(6):633-41; Am J Hum Genet. 2017 Oct 5;101(4):489-502

***Statistically significant ($p < 0.01$)

[Simultaneous Measurements of N1-Methylnicotinamide, Creatinine, Isobutyryl-L-carnitine by Liquid Chromatograph- High Resolution Mass Spectrometry for Assessing the Activities of Multiple Cationic Transporters in a First-in-Human Clinical Trial](#) Lina Luo, et al; 2018 North American ISSX Meeting Poster

IBC as OCT1 biomarker

Precipitant	Metformin OCT1 IC ₅₀ (μM)	R-Value*	Sumatriptan# (AUC % ↑)	OCT Biomarker (% ↓ Plasma IBC)
SLC22A1 LOF	N/A	-	115**	~50**
PFE-2	0.38	15.7	-	30***
Telcagepant	3.6	2.4	23	-
Propranolol	3.2	1.2	2.5	-
Clarithromycin	500	1.0	7.0	-
Naproxen	500	1.0	0.4 - 12.8	-
Flunarizine	227	1.0	<1.0	-
Atorvastatin	103	1.0	0.9 - 3.1	-



Sumatriptan as possible OCT1 selective drug probe? CL_{renal} ≤20%
Clin Pharmacokinet. 1994 Nov;27(5):337-44; J Pharm Sci. 1993 Jan;82(1):73-6

*Same approach as for OATP1B

**Clin Pharmacol Ther. 2016 Jun;99(6):633-41; Am J Hum Genet. 2017 Oct 5;101(4):489-502

***Statistically significant (p < 0.01); Lina Luo, Pfizer, ISSX NA 2018 mtg, poster

#UWash DDI db

PK-ADME-DDI in the age of translation



WORLDWIDE RESEARCH & DEVELOPMENT

Plasma-derived tissue exosomes as liquid biopsy

Various extracellular vesicles (EV) constitute the “secretome”

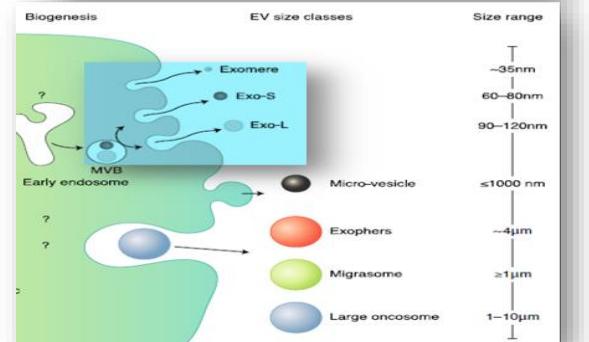
- Present in most human biofluids (blood, saliva, urine, feces)

EV carry cargo and support intercellular communication

- “Remote sensing” between cells, organs, people, species (e.g., microbiome)



NATURE CELL BIOLOGY | VOL 20 | MARCH 2018 | 225–232



[From Endogenous Compounds as Biomarkers to Plasma-Derived Nanovesicles as Liquid Biopsy: Has the Golden Age of Translational PK-ADME-DDI Science Finally Arrived?](#) Rodrigues D, Rowland A. Clin Pharmacol Ther. 2018 Dec 16. doi: 10.1002/cpt.1328. [Epub ahead of print] Review.

[Plasma extracellular nanovesicle \(exosome\)-derived biomarkers for drug metabolism pathways: a novel approach to characterize variability in drug exposure.](#) Rowland A, Ruanglertboon W, et al., Br J Clin Pharmacol. 2019 Jan;85(1):216-226

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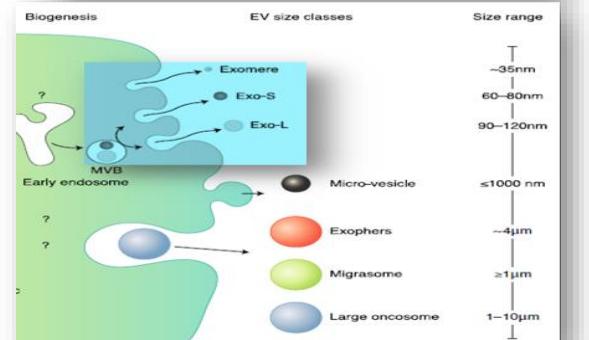
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[Mol Pharmacol.](#) 2011 May;79(5):795-805. **Remote communication through solute carriers and ATP binding cassette drug transporter pathways: an update on the remote sensing and signaling hypothesis.** Wu W, Dnyanmote AV, Nigam SK

[The drug transporter OAT3 \(SLC22A8\) and endogenous metabolite communication via the gut-liver-kidney axis.](#) Bush KT, Wu W, Lun C, Nigam SK. J Biol Chem. 2017 Sep 22;292(38):15789-15803

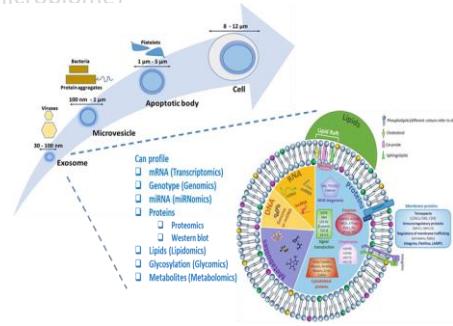
NATURE CELL BIOLOGY | VOL 20 | MARCH 2018 | 225–232



WORLDWIDE RESEARCH & DEVELOPMENT

Plasma-derived tissue exosomes as liquid biopsy

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 - Present in most human biofluids (blood, saliva, urine, feces)
- EV carry cargo and support intercellular communication
 - “Remote sensing” between cells, organs, people, species (e.g., microbiome)
- Differentiated by size, mechanism of formation
 - Nano- vs micro-vesicles, MVB-mediated exocytosis vs budding
 - Protein marker signatures (exosomal TSG101, CD9, CD63, Alix)
- EV cargo can be subjected to analysis
 - microRNA (miR), mRNA, DNA, protein, lipids, activity

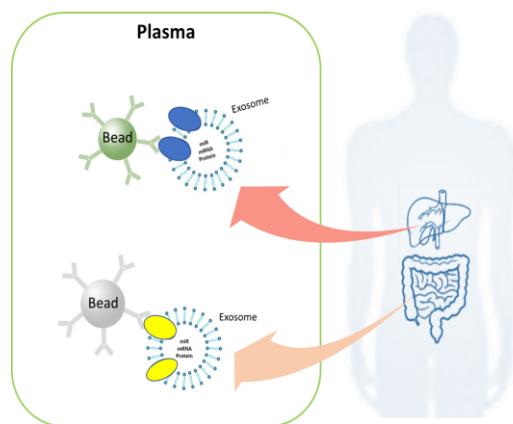


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Plasma-derived tissue exosomes as liquid biopsy

Concept of immunocapture of tissue-derived exosomes in plasma



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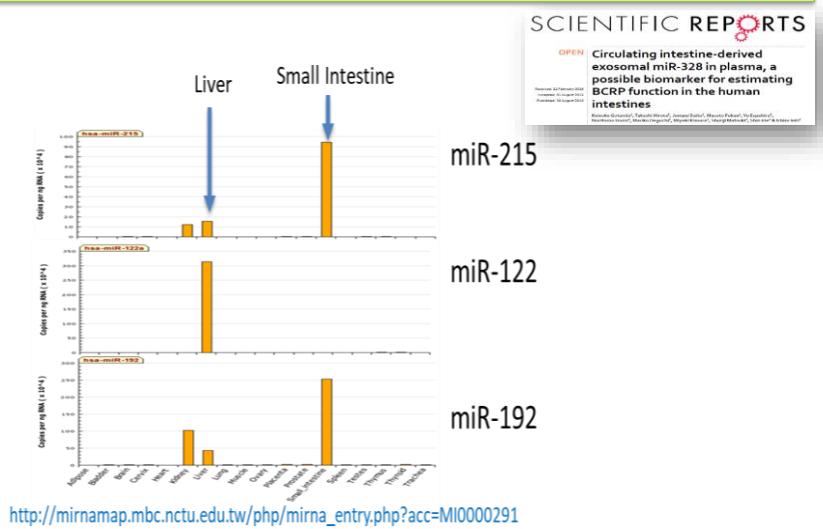
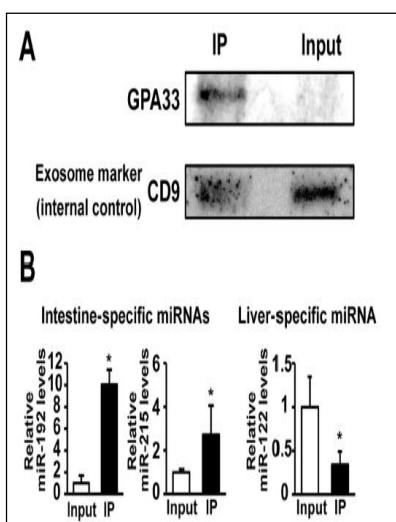
Plasma-derived tissue exosomes as liquid biopsy

Fascinating Captain....I see exosomes!!!



Plasma-derived tissue exosomes as liquid biopsy

Immunocapture of gut-derived exosomes from human plasma



Plasma-derived tissue exosomes as liquid biopsy

Immunocapture of gut-derived exosomes from human plasma

Correlation of sulfasalazine plasma AUC with miR-328 levels in plasma-derived gut (GPA33 IP) exosomes

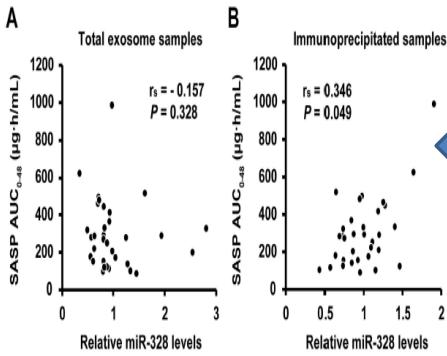


Figure 4. Relationship between miR-328 levels in total exosomes or intestine-derived exosomes in plasma and SASP AUC₀₋₄₈. MiR-328 levels were normalized with the most stable reference genes selected by geNorm for all samples. Significance was determined by Spearman's correlation test.

SCIENTIFIC REPORTS

OPEN Circulating intestine-derived exosomal miR-328 in plasma, a possible biomarker for estimating BCRP function in the human intestines

Konoko Gotoh¹, Takashi Horio², Junpei Kuroki³, Masao Fukao⁴, Yu-Equation⁵, Hidemitsu Horio⁶, Norio Miyazaki⁷, Ryosuke Matsuda⁸, Naoya Nakamura⁹

- miR-328 modulates expression of BCRP (ABCG2)
- Sulfasalazine (SASP) is intestinal BCRP probe
- Anticipated a correlation between SASP plasma AUC vs miR-328 levels

miR-328 ↑ ABCG2 ↓ BCRP ↓ SASP AUC↑

Plasma-derived tissue exosomes as liquid biopsy

Immunocapture of gut-derived exosomes from human plasma

Correlation of sulfasalazine plasma AUC with miR-328 levels in plasma-derived gut (GPA33 IP) exosomes

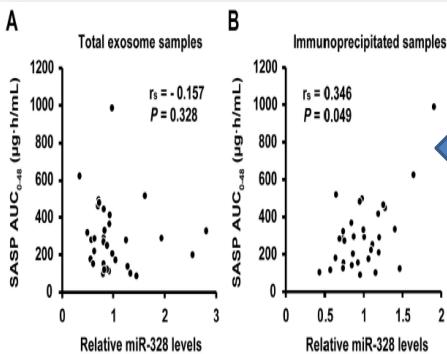


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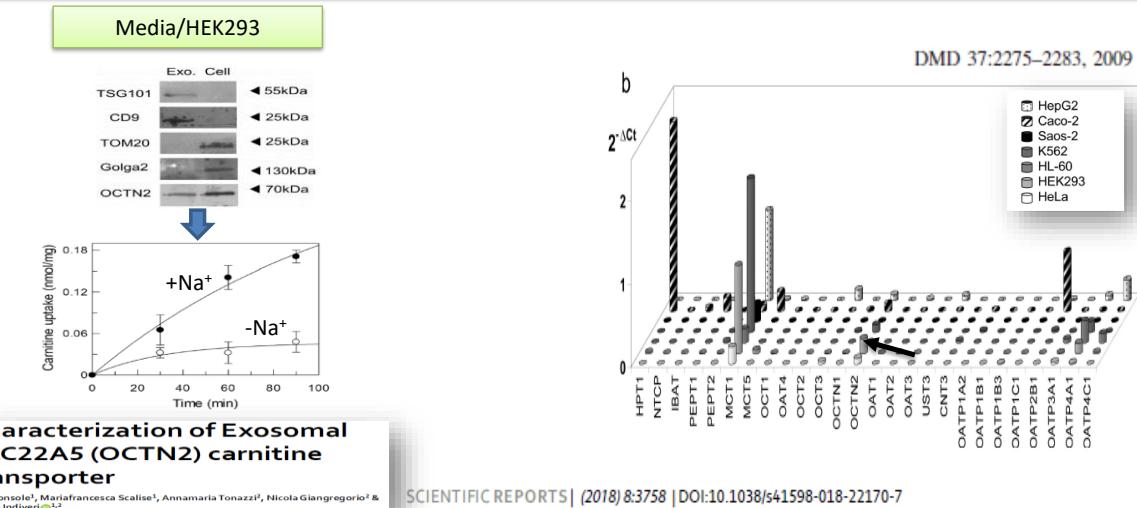
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- Sulfasalazine (SASP) is intestinal BCRP probe
- Anticipated a correlation between SASP plasma AUC vs miR-328 levels
- ABCG2 genotype not considered?
- Liver BCRP not considered?
- Liver miR-328 expression?
- BCRP protein expression not determined

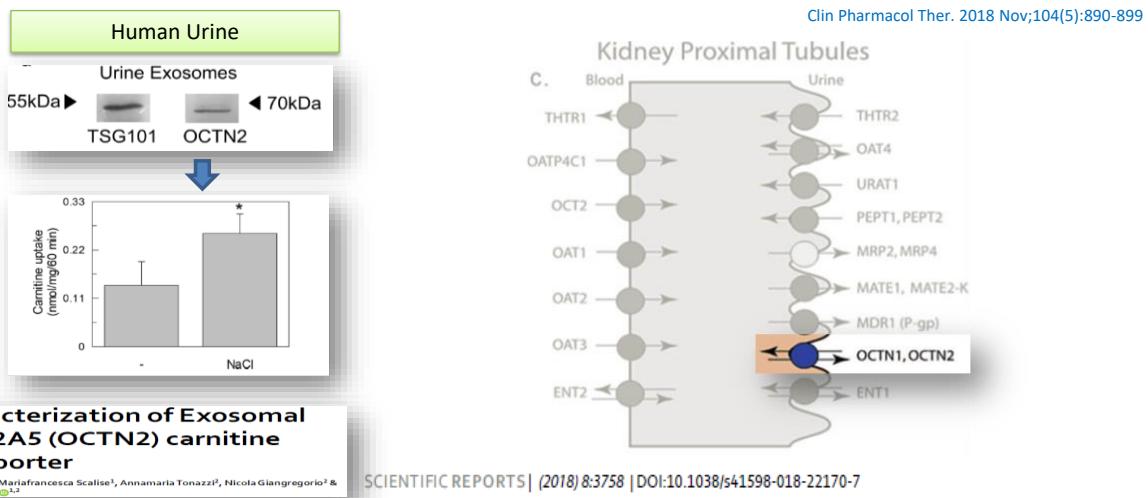
Urine/media-derived tissue exosomes!!

Isolation of exosomes from HEK293 cells and reconstituted in proteoliposomes to measure Na⁺-dependent OCTN2-mediated carnitine uptake



Urine/media-derived tissue exosomes!!

Isolation of exosomes from human urine and reconstituted in proteoliposomes to measure Na⁺-dependent OCTN2-mediated carnitine uptake



Thanks for your
attention !



WORLDWIDE RESEARCH & DEVELOPMENT