Epacadostat

Transport and Metabolism of An Investigational IDO1 Inhibitor for Immuno-Oncology

Yan Zhang, Ph.D.

Meet the Experts: The Transporter Conference

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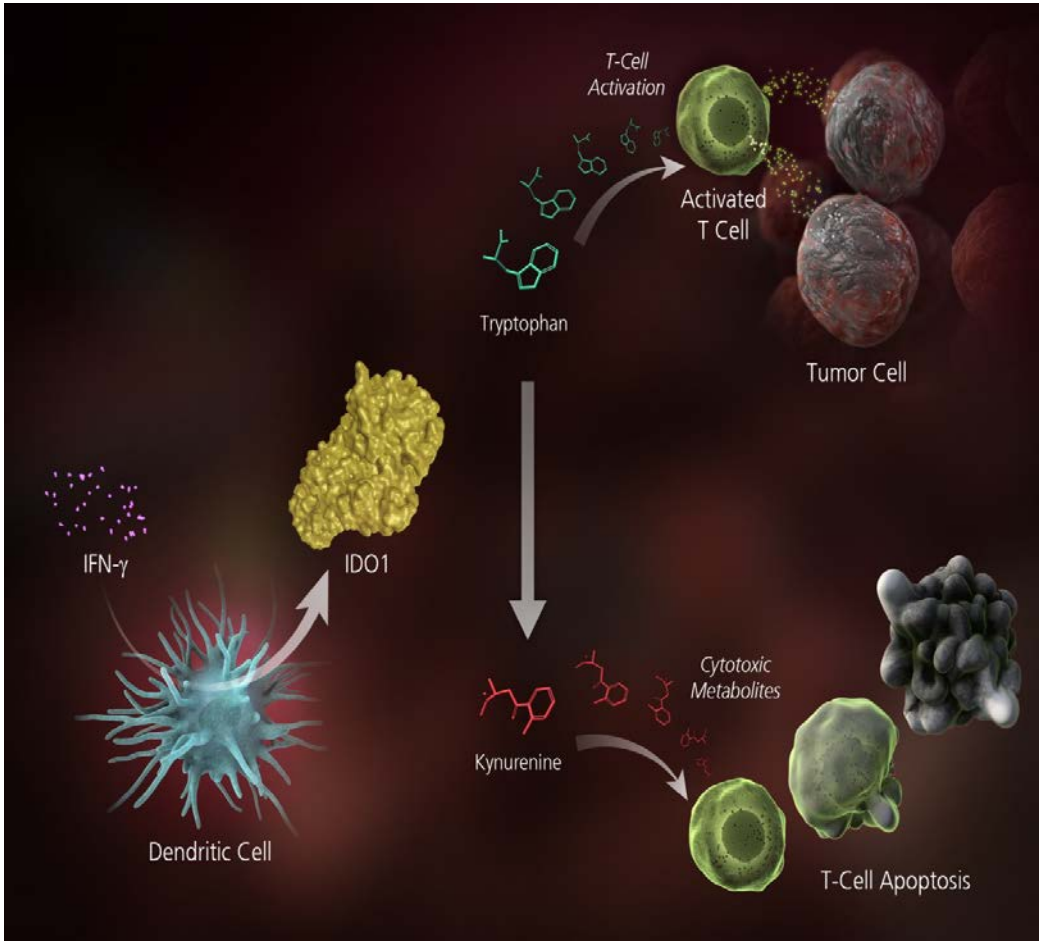
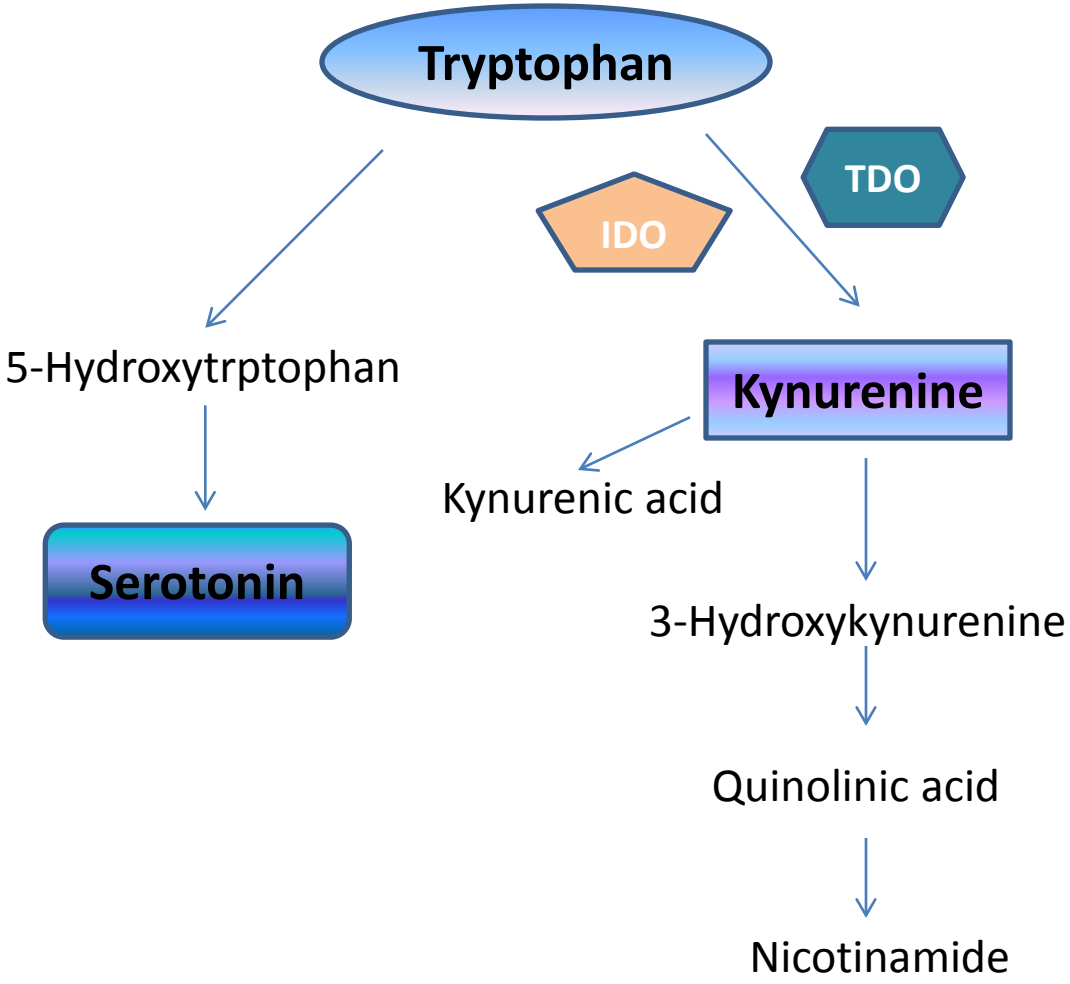
Indoleamine-2,3-Dioxygenase 1 (IDO1) Negatively Regulates the T Cell Responses



- IDO1 is an intracellular enzyme that plays an important role in the negative regulation of T-cell responses via localized metabolism of tryptophan
  - ✓ IDO1 depletes tryptophan levels and leads to decreased T-cell proliferation and activation
  - ✓ Tryptophan metabolites including kynurenine contribute to a local immunosuppressive environment

Spranger, Gajewski, *et al*. *Sci Trans Medicine* **2013**; Mbongue JC, *et al. Vaccines.* **2015**;3: 703-729

IDO and TDO:
Rate Limiting Enzymes in the Kynurenine Metabolic Pathway

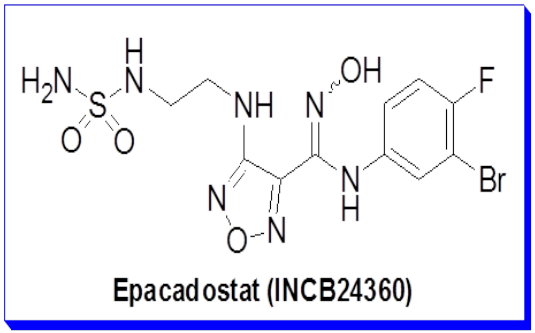


- **IDO1** metabolizes a variety of indoleamines, including tryptophan, 5-HT, melatonin and serotonin
  - IDO1 is expressed throughout the body and is overexpressed in multiple tumor types
- **IDO2** is similar with IDO1 by 42% at the amino acid level. However, its role in cancer is unclear
- **TDO** metabolizes only tryptophan and is predominantly expressed in the liver
  - TDO is responsible for the homeostasis of tryptophan levels in the body

Mbongue JC, *et al. Vaccines.* **2015**;3: 703-729; Brochez L, *et al. Eur J Cancer.* **2017**;76: 167-182

Epacadostat Preclinical *In Vitro* Profile

In Vitro	INCB24360
IDO1 IC_{50} (nM)	73
IDO2 IC_{50} (nM)	>10,000
TDO IC_{50} (nM)	>50,000
HeLa IC_{50} (nM)	7.4
WB IC_{50} (nM)	125
Fu (%)	3.1
Caco-2 Papp ($x10^{-6}$ cm/s)	4
Solubility @ pH 7.4 (μg/mL)	72



Epacadostat (INCB24360)

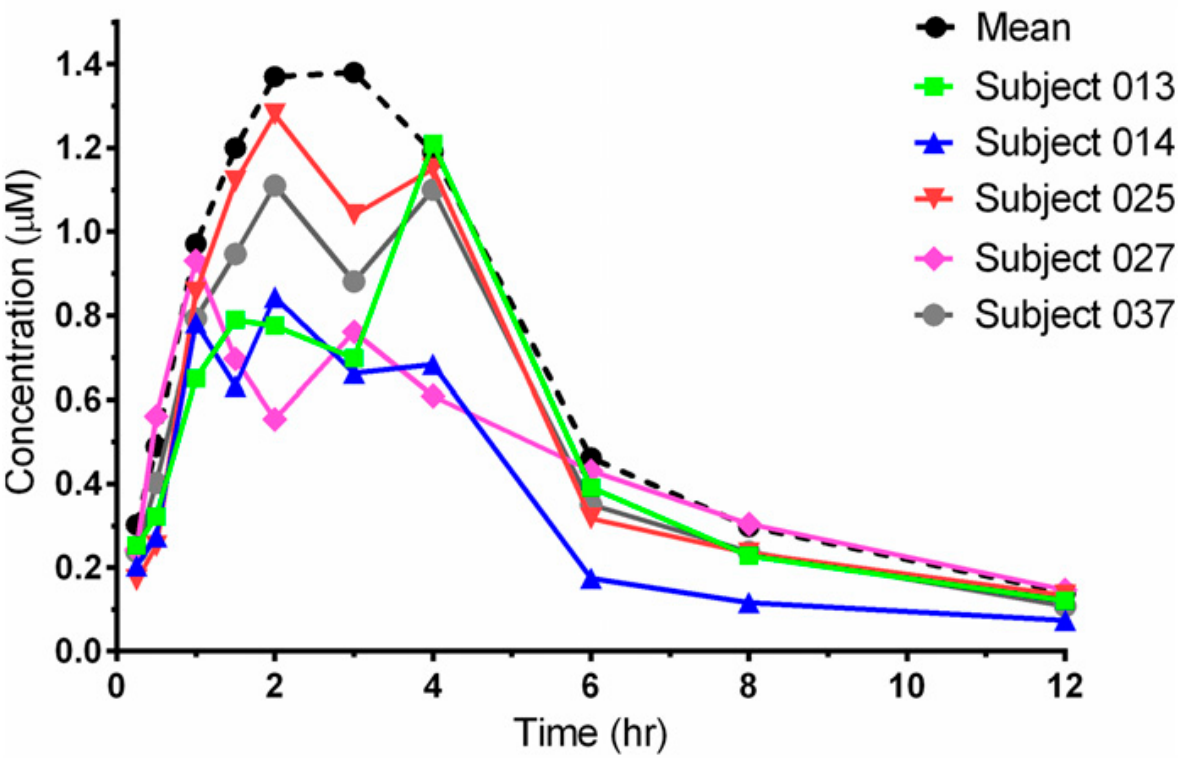
- Highly potent and selective IDO1 inhibitor
- No off-target activities
  - ✓ No hERG or PXR
  - ✓ No CYP inhibition
  - ✓ CEREP clean
  - ✓ Ames negative

Epacadostat Preclinical Pharmacokinetic Profile

	Rat	Dog	Cyno
Oral Dose (mg/kg)	**5**	**10**	**10**
AUC (μM*h)	**1.3**	**29**	**9.3**
$T_{1/2}$ (h)	**2.2**	**4.9**	**2.7**
I.V. Dose (mg)	**5**	**5**	**4**
Cl (L/h/kg)	**1.1**	**0.5**	**0.8**
Vss	**2.0**	**0.7**	**1.8**
%F	**11**	**59**	**33**

- Preclinical PK suggests that a clinical dose of ~50-100 mg BID would produce a steady-state trough value equal to the whole blood (WB) IC_{50}

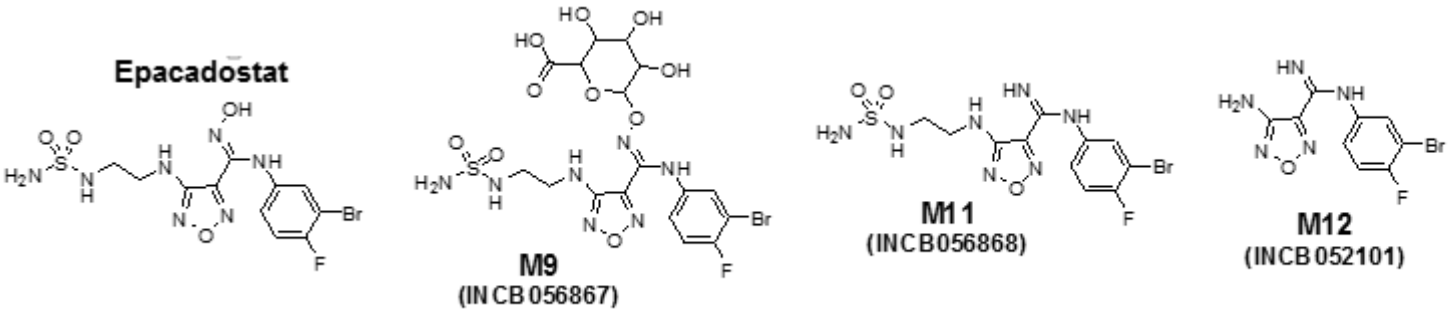
Oral PK Profiles of Epacadostat in Healthy Volunteers Suggests Enterohepatic Circulation



Plasma concentration-time profiles of EPAC in individual healthy subjects after 5 days of oral administration of 300 mg EPAC twice a day

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

Three Major Plasma Metabolites of Epacadostat Identified in Humans



Area Under the Curve (AUC_{0-12h}) Ratios of Major Plasma Metabolites Relative to Epacadostat in Humans

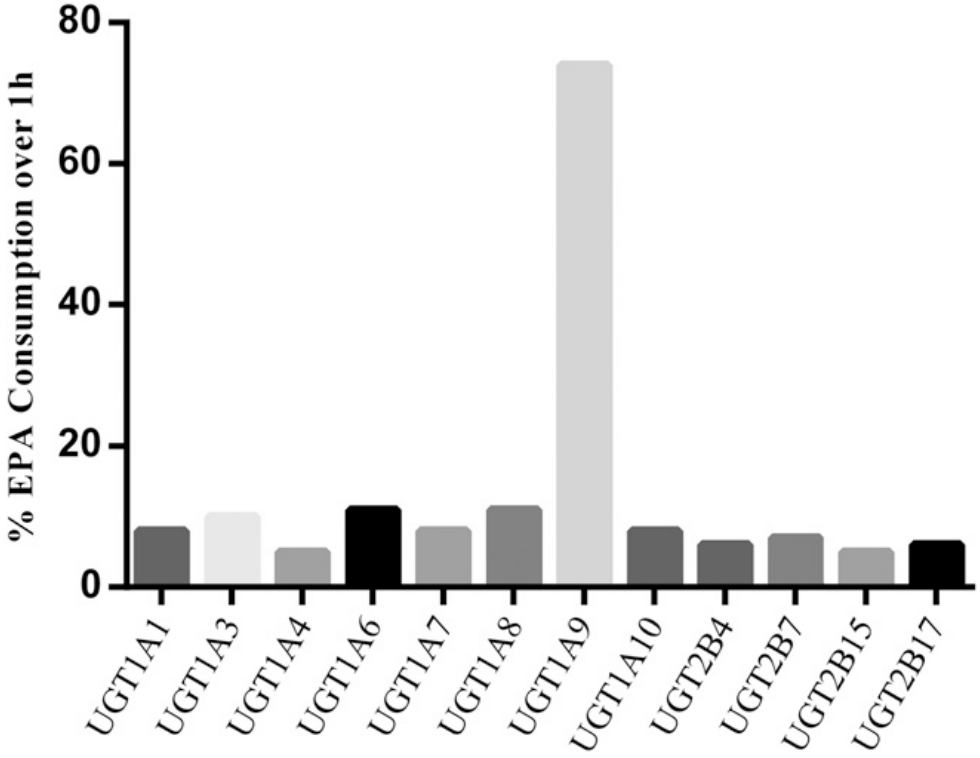
Dose (mg)	Day	EPAC	M9	M11	M12
50 BID	1	1	8.2	NC	NC
	10	1	8.1	0.3	0.8

NC, Not calculable because most plasma concentrations were BQL.

- M9: glucuronide metabolite
- M11: amidine metabolite
- M12: N-dealkylated metabolite of M11

Boer J., *et al*. *Drug Metabolism and Disposition* **2016** 44; 1668-1674

UGT1A9 Is Responsible for Glucuronidation of Epacadostat



In vitro consumption of Epacadostat (5 mM) by individual recombinant human UGTs (0.5 mg/ml) over 1 hour.

- EPA was extensively metabolized to form M9 by only UGT1A9
  - 74% of the initial concentration of EPA consumed over the 60-minute incubation
- The other recombinant isozyme preparations showed little to no EPA turnover
- M9 was deconjugated to EPCA by gut microbiota

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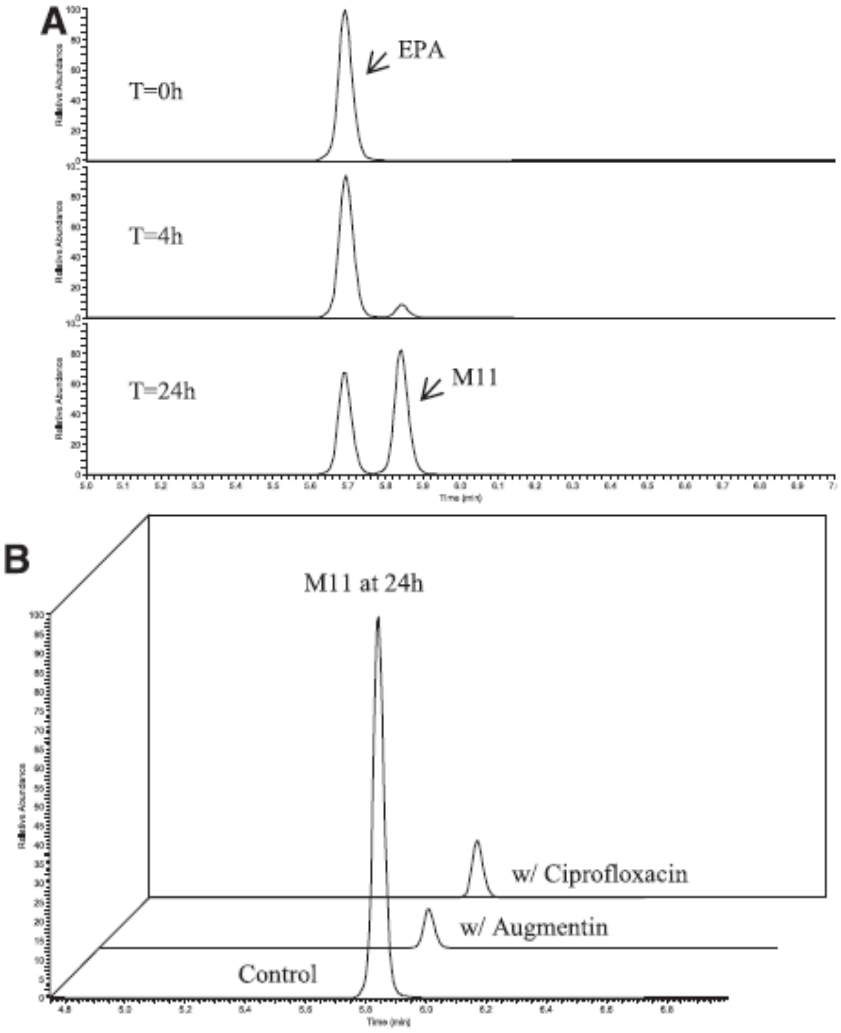
Boer J., *et al*. *Drug Metabolism and Disposition* **2016** 44; 1668-1674

M11 Is Formed by Gut Microbiota

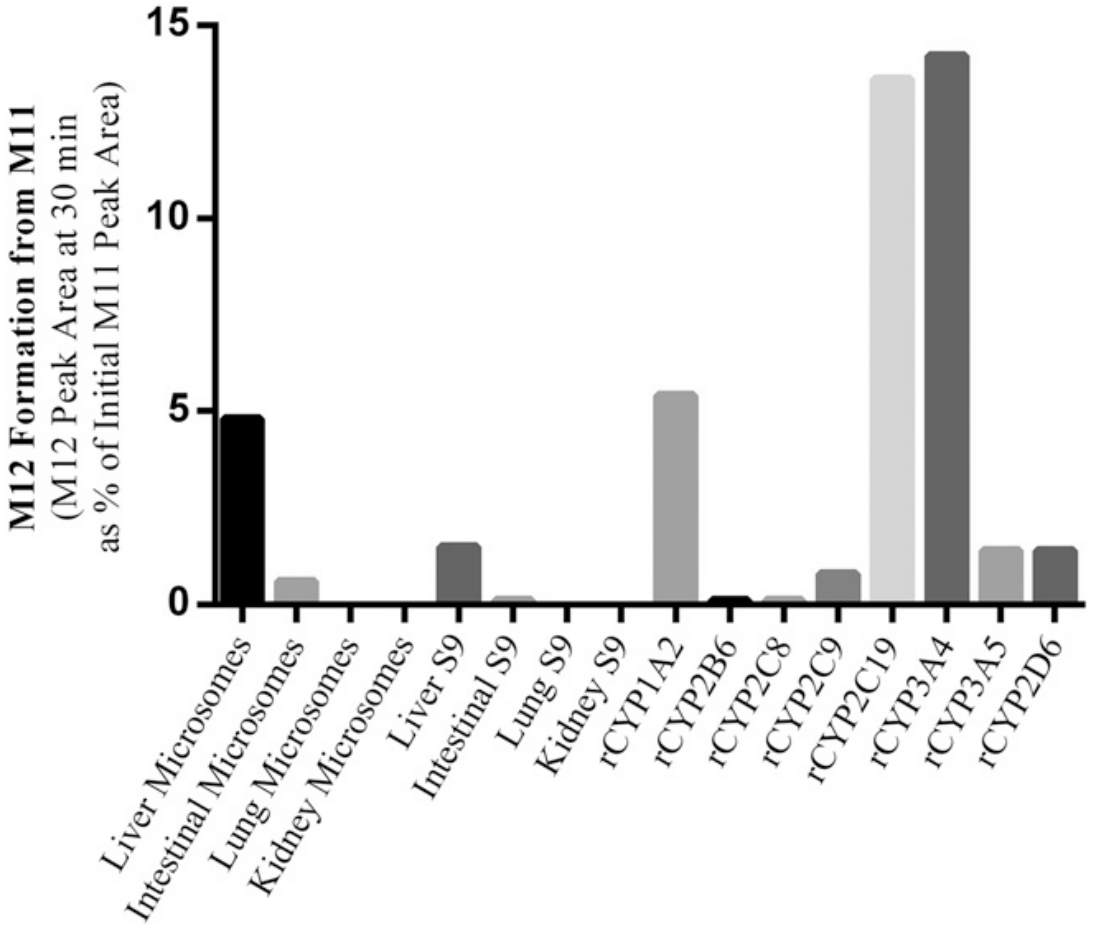


- Epacadostat was incubated with human feces homogenate under aerobic and anaerobic conditions
  - M11 was detected at level comparable to parent at the end of the 24h incubation
  - M11 was formed to similar extents under both aerobic and anaerobic conditions
  - M12 was detected in trace quantities
- Addition of antibiotics amoxicillin/clavulanate (Augmentin) and ciprofloxacin to the fecal incubations under aerobic conditions decreased M11 formation by 91% and 94%, respectively

Representative extracted ion chromatograms showing ex vivo formation of M11 from EPA in human feces homogenate (A) and decrease in M11 formation from EPA in human feces homogenate by commonly prescribed antibiotics (B).

Boer J., *et al*. *Drug Metabolism and Disposition* **2016** 44; 1668-1674

M12 Is a Secondary Metabolite of Epacadostat Formed from M11



Formation of M12 from M11 (10 uM) by human tissue microsomes (2 mg/ml protein) and S9 fractions (3 mg/ml protein) and recombinant cytochrome P450s (0.3 nmol/ml).

- M12 was not detected in vitro when EPA was incubated with human microsomes, S9 fractions, or recombinant P450s.
- M12 was formed when M11 was incubated in human liver microsomes and S9 fraction.
- CYPs 3A4, 2C19, and 1A2 catalyze the N-dealkylation of M11 to form M12.
  - CYP3A4 is primarily responsible for the metabolism of M11

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Boer J., *et al*. *Drug Metabolism and Disposition* **2016** 44; 1668-1674

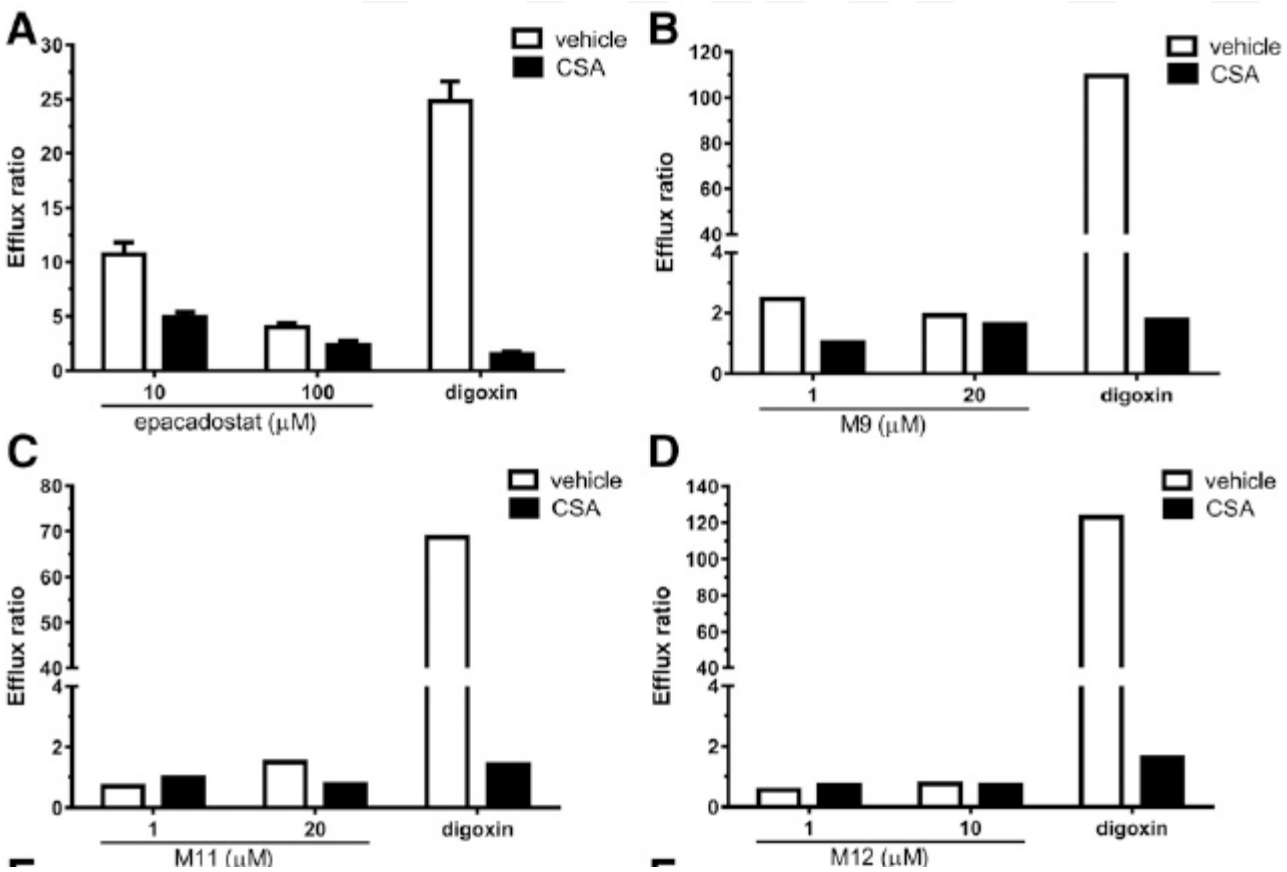
Physicochemical Properties and Permeability of Epacadostat and Its Major Metabolites

Compound	Mol. Wt.	Log P[a]	PSA[a]	Caco-2 P_{app}
	g/mol			*10^{-6} cm/s*
EPAC	438.2	2.17	168	4.0
M9	614.4	−2.1	264	<0.1
M11	422.2	0.61	159	3.2
M12	300.1	1.9	101	9.3

PSA, polar surface area.
[a]Calculated by ADMET Predictor software (Simulations Plus, Lancaster, CA).

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

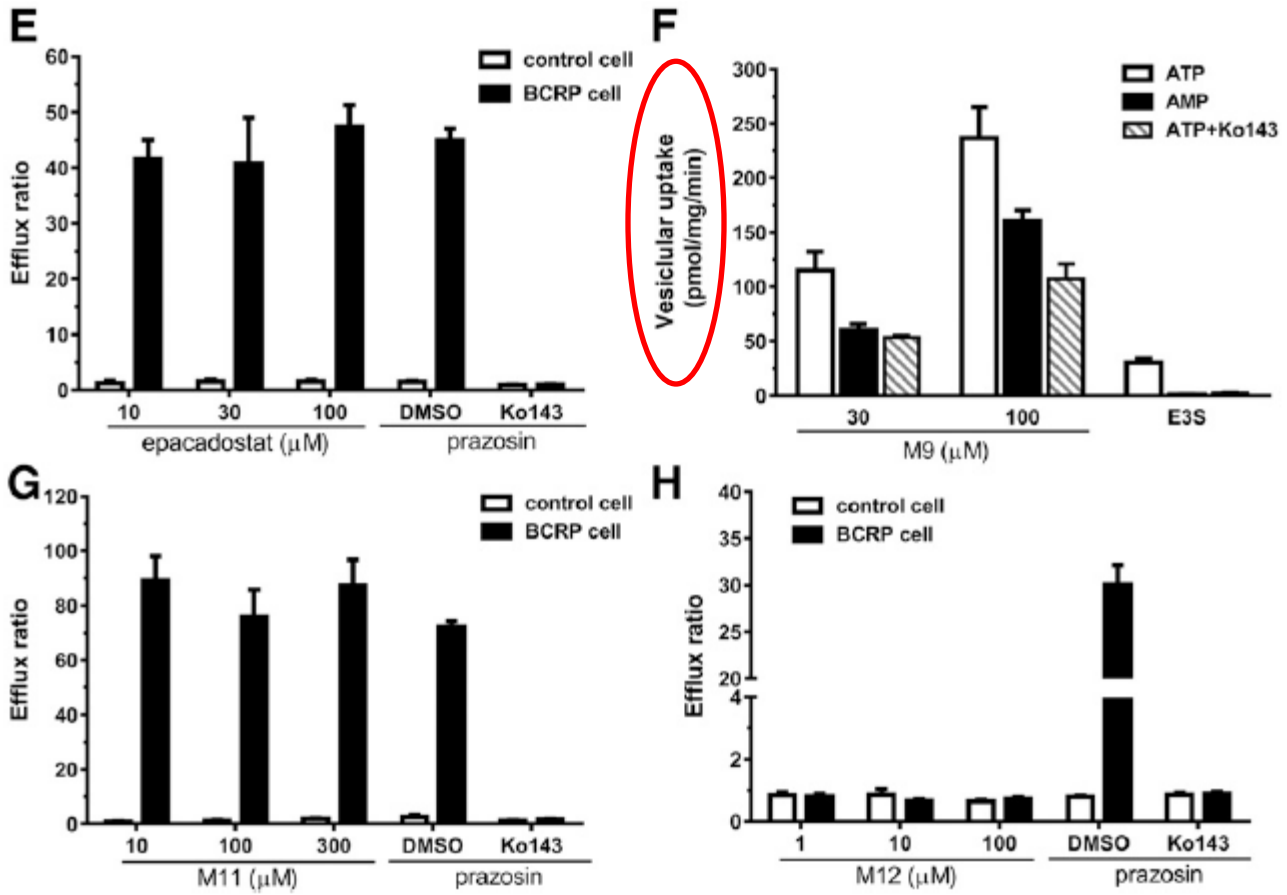
Transport of Epacadostat in Caco-2 Cell Lines



- EPAC is a substrate of P-gp in Caco-2 cells

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

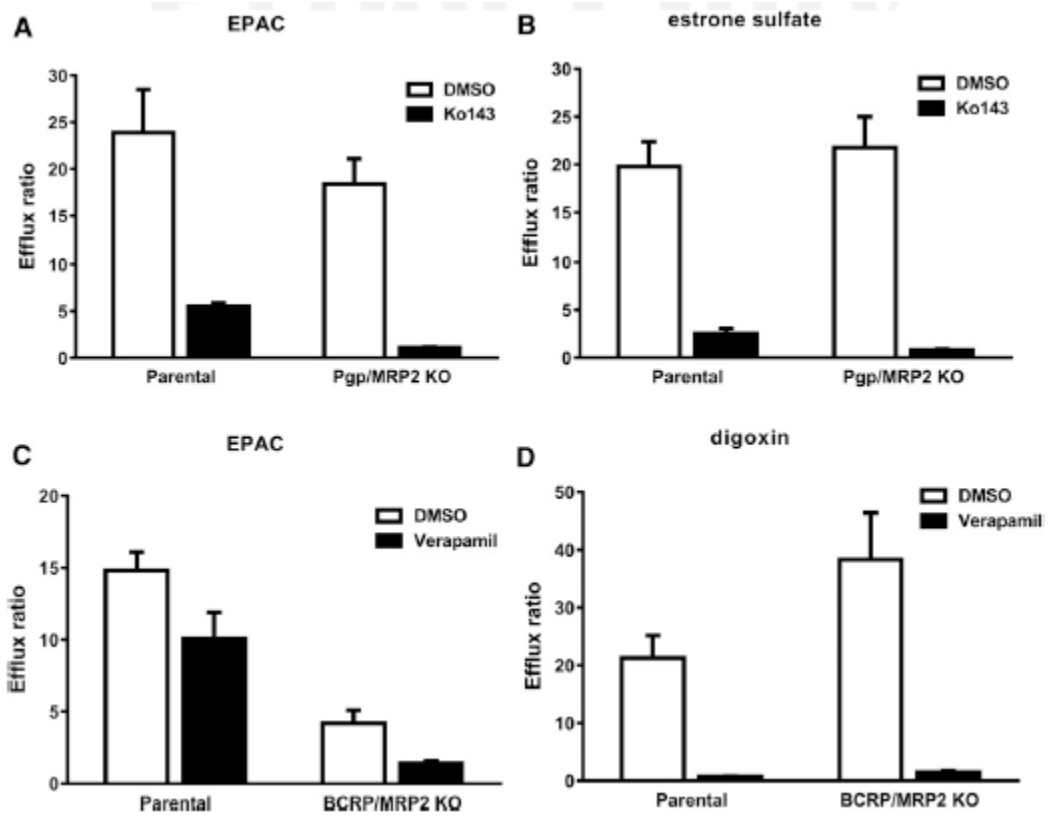
Transport of Epacadostat in BCRP-MDCKII Cell Lines and BCRP-Expressin Membrane Vesicles



- EPAC and M11 are substrates of BCRP in BCRP-MDCKII cells
- M9 is a BCRP substrate based on the MV study

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

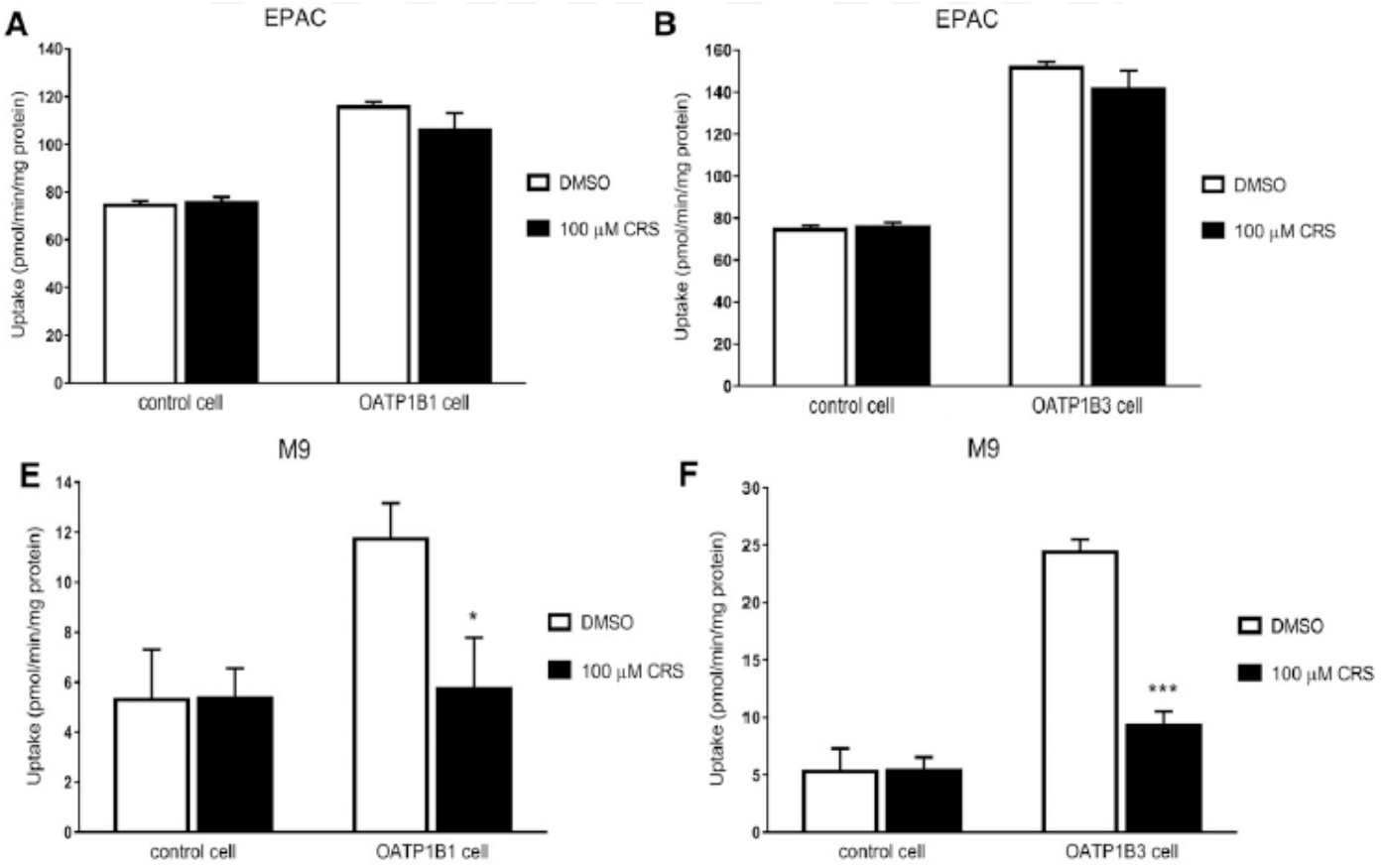
Transport of Epacadostat in P-gp / MRP2 and BCRP / MRP2 double-KO Caco-2 cell lines



- EPAC is a substrate of both P-gp and BCRP is further confirmed in the transporter double KO Caco-2 cell lines
- BCRP may have more pronounced effect on the efflux of EPAC in Caco-2 cells

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

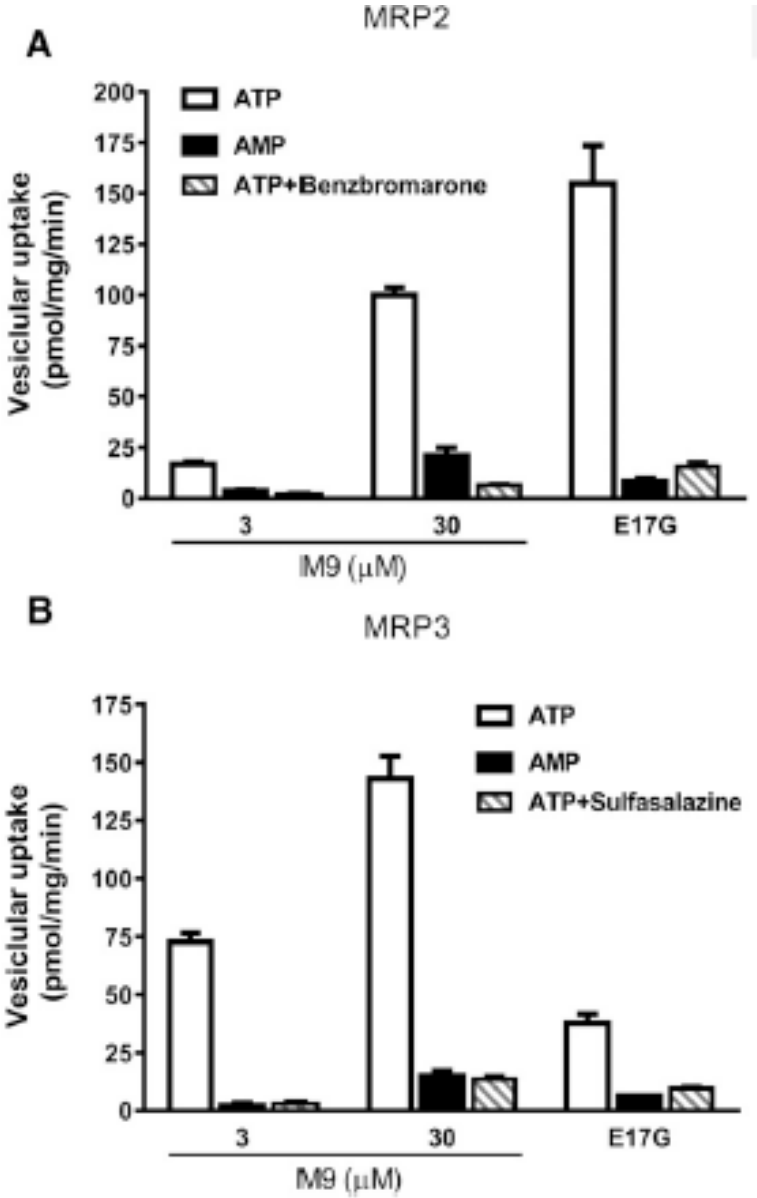
Uptake of Epacadostat and M9 into Human OATP1B1- and OATP1B3-Transfected CHO cells



- Epacadostat is not a substrate of OATP1B1 or OATP1B3
- M9 is a substrate of both OATP1B1 and OATP1B3

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

Transport of M9 by Human MRP2 and MRP3 in Membrane Vesicles Containing Human MRP2 or MRP3



- M9 is a substrate for MRP2 and MRP3

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

In Vitro Evaluation of Epacadostat and Its Metabolites as a Potential Perpetrator for P-gp- or BCRP-Mediated DDIs

Compound	I_1 (μM)	I_2 (μM)	P-gp			BCRP		
			IC_{50}	I_1/IC_{50}	I_2/IC_{50}	IC_{50}	I_1/IC_{50}	I_2/IC_{50}
			μM			μM		
EPAC	1.57	2740	>500	<0.004	<5.5	N/I	N/A	N/A
M9	10.2	Unknown	N/I	N/A	N/A	N/I	N/A	N/A
M11	2.10	Unknown	N/I	N/A	N/A	>300	<0.007	N/A
M12	2.10	Unknown	N/I	N/A	N/A	32	0.066	N/A

I_1, Mean steady-state total C_{max} after 300-mg twice daily dose; I_2, 300-mg (0.685 mmol) dose divided by 250 ml; N/A, not applicable; N/I, no inhibition was observed over the range of the concentrations tested.

- The potential of both EPAC and its metabolites to cause clinical DDIs via inhibition of P-gp or BCRP is low

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

In Vitro Evaluation of Epacadostat and Its Metabolites as a Potential Perpetrator for OATP1B1- or OATP1B3-Mediated DDIs

Compound	Total C_{max}	OATP1B1			OATP1B3		
		IC_{50}	C_{max}/IC_{50}	*R* Value	IC_{50}	C_{max}/IC_{50}	*R* Value
	μM	*μM*			*μM*		
EPAC	2.5	59	0.042	1.008	51	0.049	1.009
M9	10.2	262	0.039	1.007[a]	27	0.38	1.072[a]
M11	2.1	68	0.031	1.004[a]	19	0.11	1.014[a]
M12	2.1	49	0.043	1.002[a]	228	0.009	1.000[a]

[a] Values were calculated by using the maximum total plasma concentrations in the clinical studies instead of using Iin,max owing to a lack of certain parameters for the estimation of the Iin,max value.

- Although the ratios of total C_{max}/IC_{50} of M9 and M11 for OATP1B3 are greater than 0.1, the resulting R values are less than the cutoff value of 1.25 by FDA

Incyte

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

In Vitro Evaluation of Epacadostat and Its Metabolites as a Potential Perpetrator for OAT1-, OAT3-, or OCT2-Mediated DDIs

Compound	Free C_{max}	OAT1		OAT3		OCT2	
		IC_{50}	C_{max}/IC_{50}	IC_5	C_{max}/IC_{50}	IC_{50}	C_{max}/IC_{50}
	μM	μM		μM		μM	
EPAC	0.049	>300	<0.0002	21	0.002	203	0.0002
M9	1.95	N/I	N/A	>300	<0.007	N/I	N/A
M11	0.263	N/I	N/A	14	0.019	23	0.011
M12	0.080	8.6	0.009	21	0.004	21	0.004

N/A, Not applicable; N/I, No inhibition was observed over the range of the concentrations tested.

- The potential of EPAC and its metabolites to cause clinical DDIs via inhibition of OAT1, OAT3, or OCT2 is low.

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

Epacadostat and Its Metabolites as Perpetrators for Various Drug Transporters

Transporter	Epacadostat IC_{50} (μM)	M9 IC_{50} (μM)	M11 IC_{50} (μM)	M12 IC_{50} (μM)
Pgp	> 500	N/I	N/I	N/I
BCRP	N/I	N/I	> 300	32
OATP1B1	59	262	68	49
OATP1B3	51	27	19	228
OAT1	> 300	N/I	N/I	8.6
OAT3	21	> 300	14	21
OCT2	203	N/I	23	21

N/I, no inhibition was observed over the range of the concentrations tested

- The potential of EPAC and its metabolites to cause clinical DDIs via inhibition of all seven transporters is low

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

Epacadostat and Its Metabolites as Victims for Various Drug Transporters

Transporter	Epacadostat	M9	M11	M12
Pgp	Yes	No	No	No
BCRP	Yes	Yes [a]	Yes	No
OATP1B1	No	Yes	No	No
OATP1B3	No	Yes	No	No
MRP2	ND	Yes [a]	ND	ND
MRP3	ND	Yes [a]	ND	ND

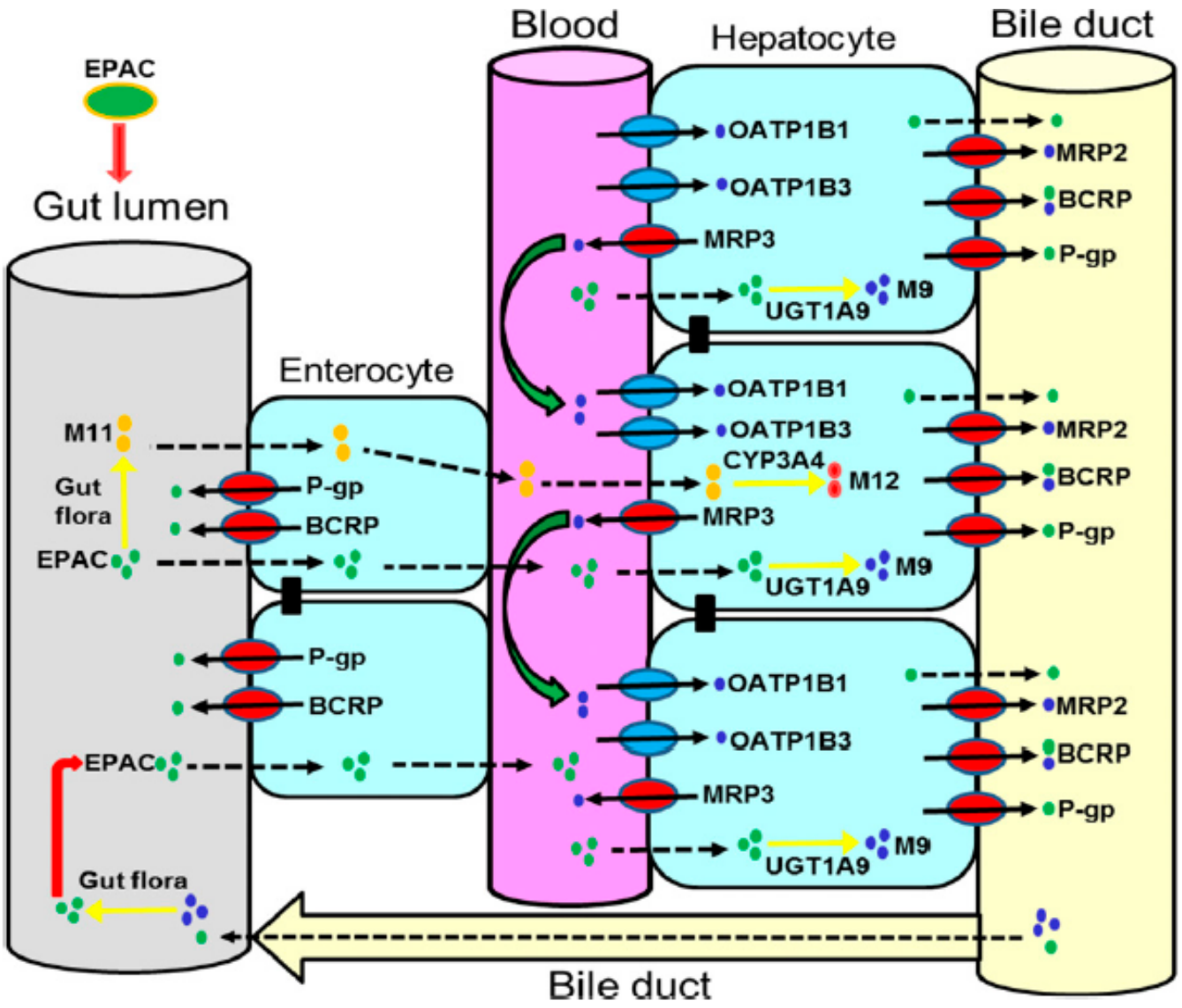
[a], Membrane vesicle data (BioReliance);

ND = Not determined;

Substrate studies were not conducted for OAT1, OAT3, and OCT2.

Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

Transporter-Mediated Disposition of Epacadostat and Its Metabolites in Humans



Zhang Q., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

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