The Gut-Brain-Microbiome Axis:

Epacadostat Transport and Metabolism on Brain Serotonin Concentration

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Meet the Experts: The Transporter Conference

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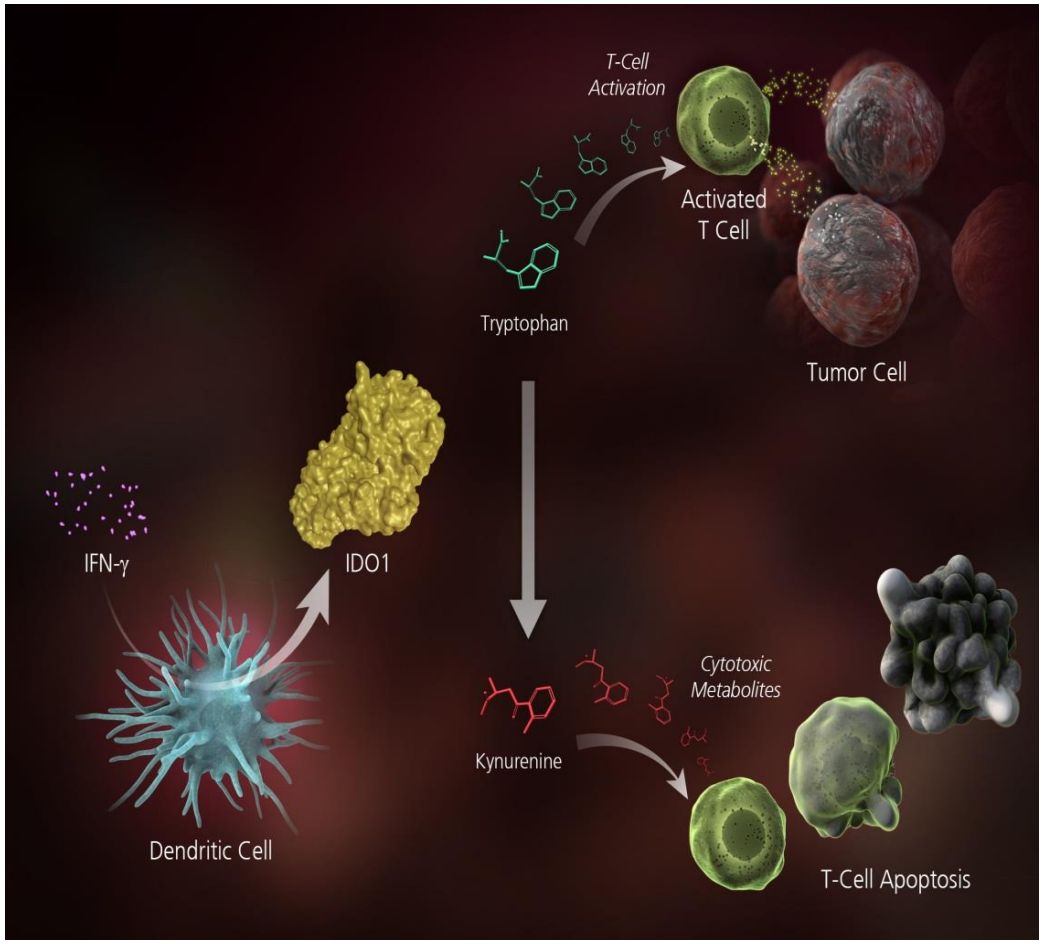
Indoleamine-2,3-Dioxygenase 1 (IDO1) Negatively Regulates the T Cell Responses

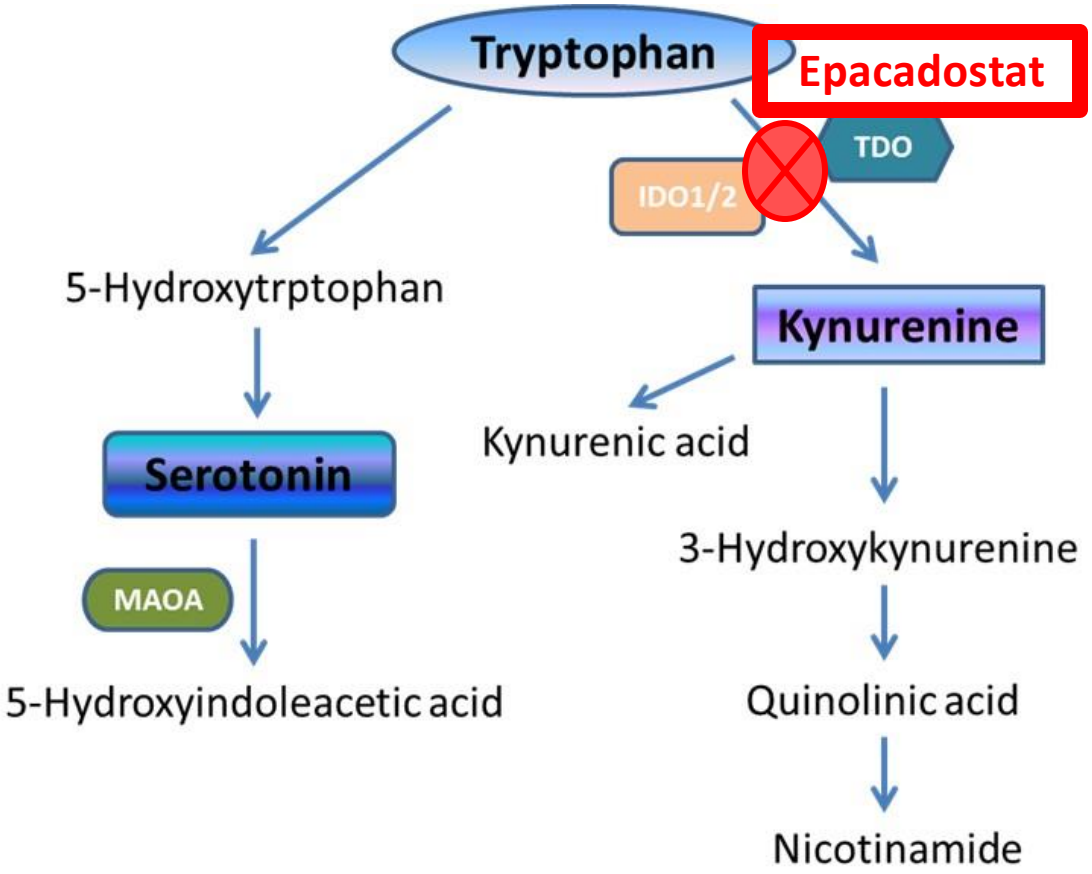


- IDO1 is an intracellular enzyme that plays an important role in the negative regulation of T-cell responses via localized metabolism of tryptophan
  - ✓ IDO1 depletes tryptophan levels and leads to decreased T-cell proliferation and activation
  - ✓ Tryptophan metabolites including kynurenine contribute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

Spranger., *et al*. *Sci Trans Medicine* **2013**; 5: 200ra116. doi:10.1126/scitranslmed.3006504;
Mbongue., *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

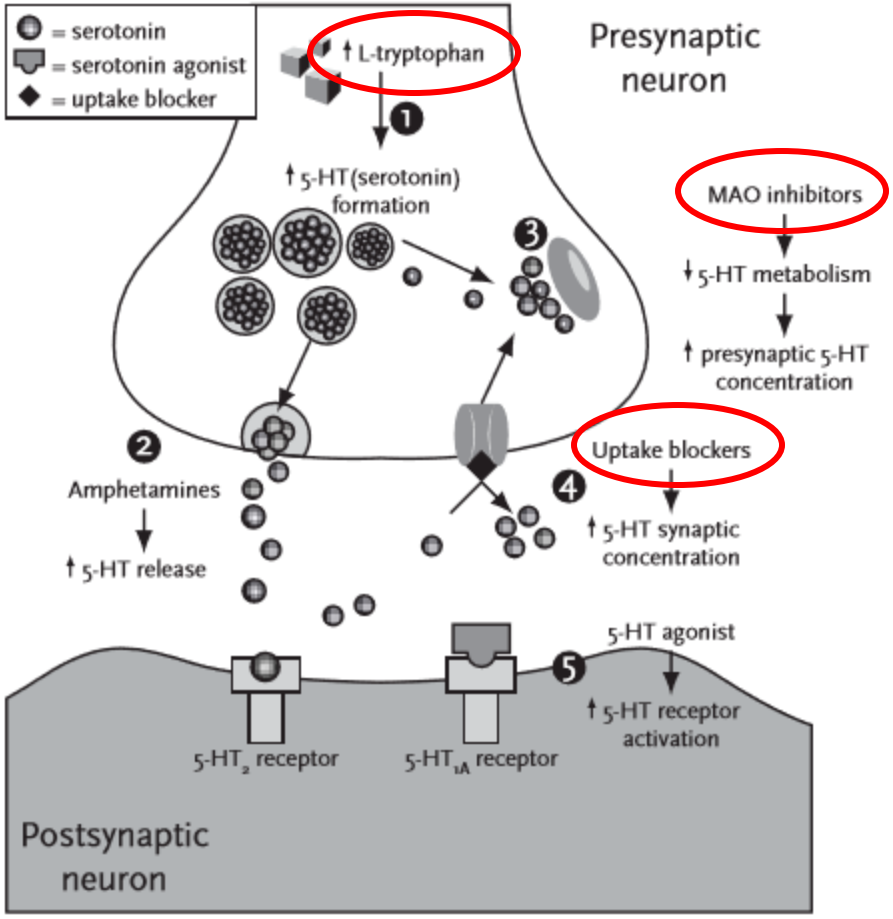
IDO2 is similar with IDO1 by 41% at the amino acid level. However, its role in cancer is unclear

TDO metabolizes only tryptophan and is predominantly expressed in the liver

- TDO is responsible for the homeostasis of tryptophan levels in the body

Modified from Zhang., *et al. Drug Metabolism and Disposition.* **2019;** 47: 710-714

The Serotonin Syndrome

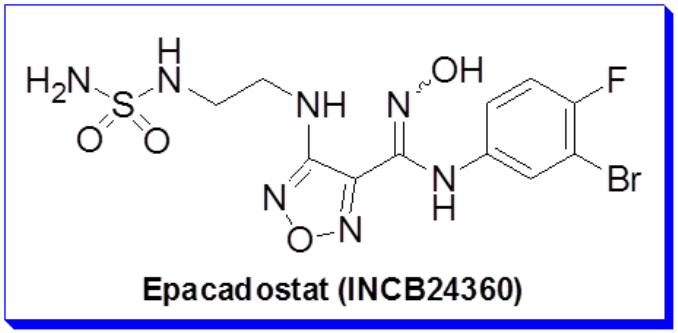


- Serotonin syndrome (SS):
  - An adverse, toxic drug reaction caused by excessive levels of circulating serotonin in the central nervous system (CNS)
  - Typically occurs following treatment with two or more drugs that enhance serotonin levels, but it can also be induced by an overdose of single drug use
- Post marketing report of SS when MAOIs was given with serotonergic drugs or soon after the discontinuation of serotonergic drugs
- Use of MAOIs including linezolid are prohibited with EPAC.

Bijl. *The Netherlands Journal of Medicine*. **2004**;62:309-313

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
%F	11	59	33
I.V. Dose (mg/kg)	5	5	4
Cl (L/h/kg)	1.1	0.5	0.8
Vss	2.0	0.7	1.8



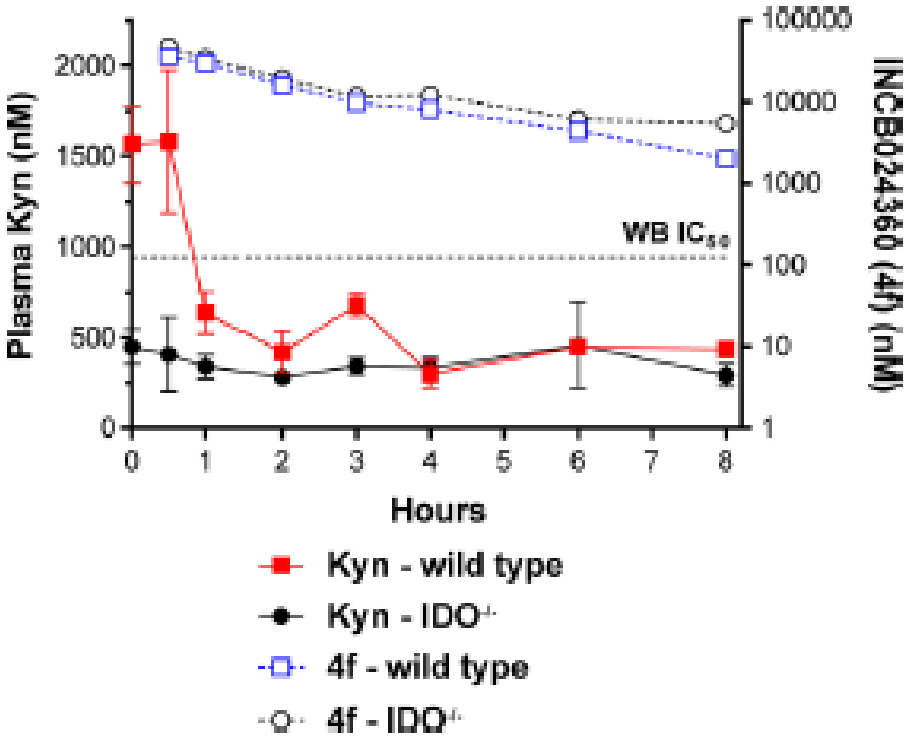
Epacadostat (INCB24360)

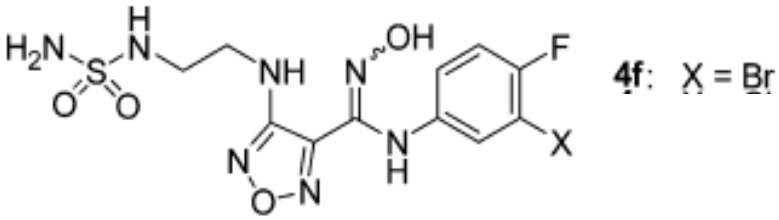
- Preclinical PK suggested that a clinical dose of ~50-100 mg BID would produce a steady-state trough value equal to the whole blood (WB) IC_{50}

Epacadostat is an investigational compound and its safety and efficacy have not been established. The preclinical data presented are not intended to imply clinical benefit.

Modified from Yue., *et al. ACS Med. Chem. Lett.* **2017**; 8: 486-491

Coverage of the WB IC_{50} at the Trough Correlated to the Reduction of Kynurenine Plasma Concentrations

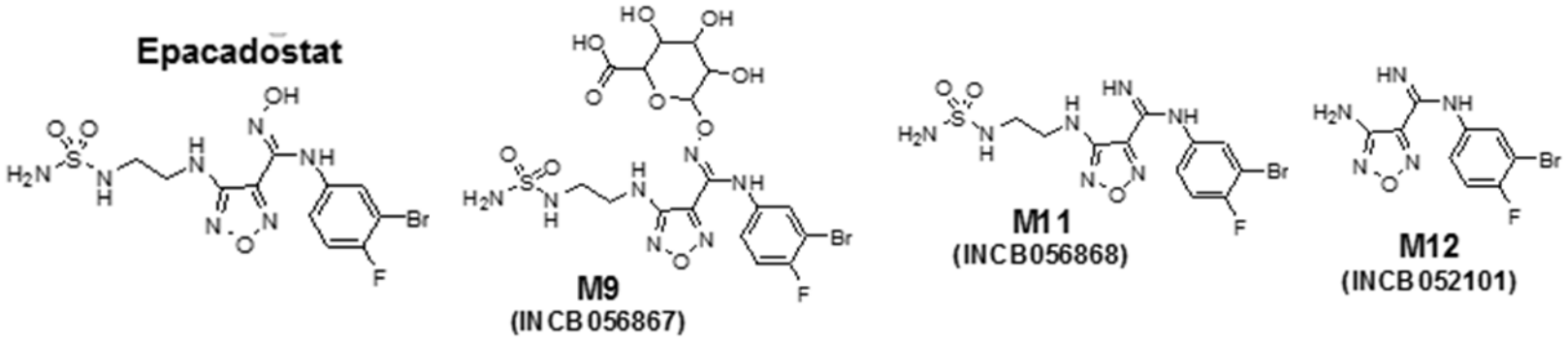




- Once daily oral dosing of EPAC at 50 mg/kg reduced kynurenine levels in wild-type mice to basal levels present in IDO null mice
- TDO metabolism is believed to be responsible for the observed baseline levels of kynurenine in these studies.
- Similar PK/PD correlations were observed in dogs and cynomologous monkeys.

Yue., *et al. ACS Med. Chem. Lett.* **2017**; 8: 486-491

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

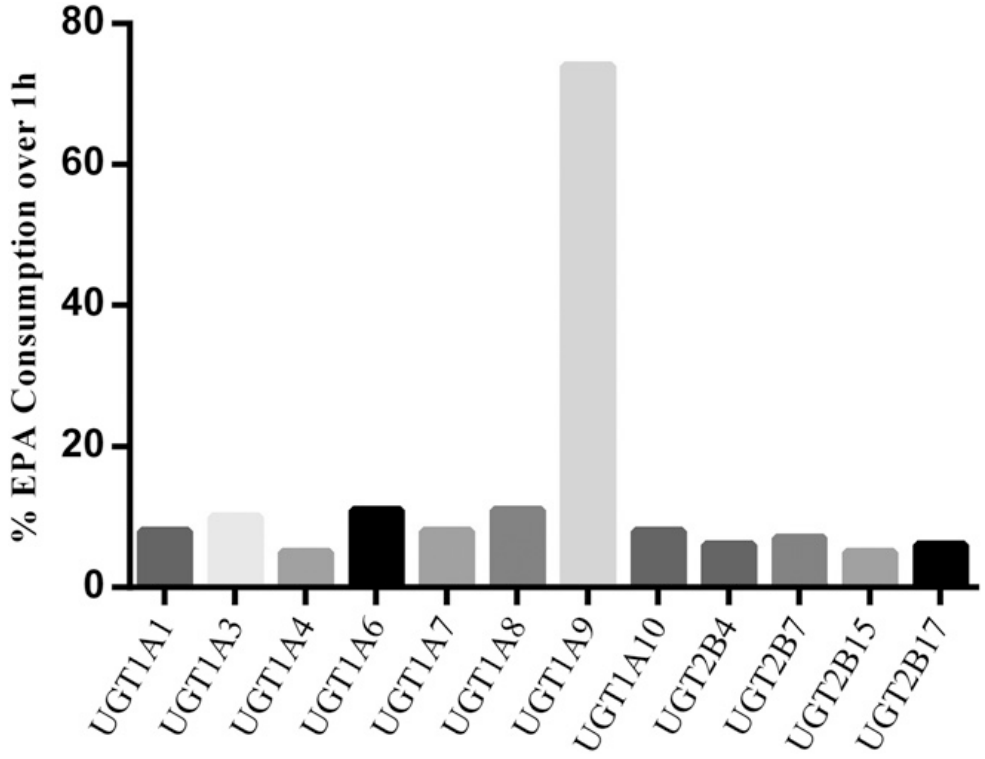
Dose (mg)	Day	EPAC	M9	M11	M12
50 BID	1	1	8.2	NC	NC
	10	1	8.1	0.3	0.8

NC, Not calculable because most plasma concentrations were BQL.

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

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

UGT1A9 Was Shown To Be Responsible for Glucuronidation of Epacadostat (M9)

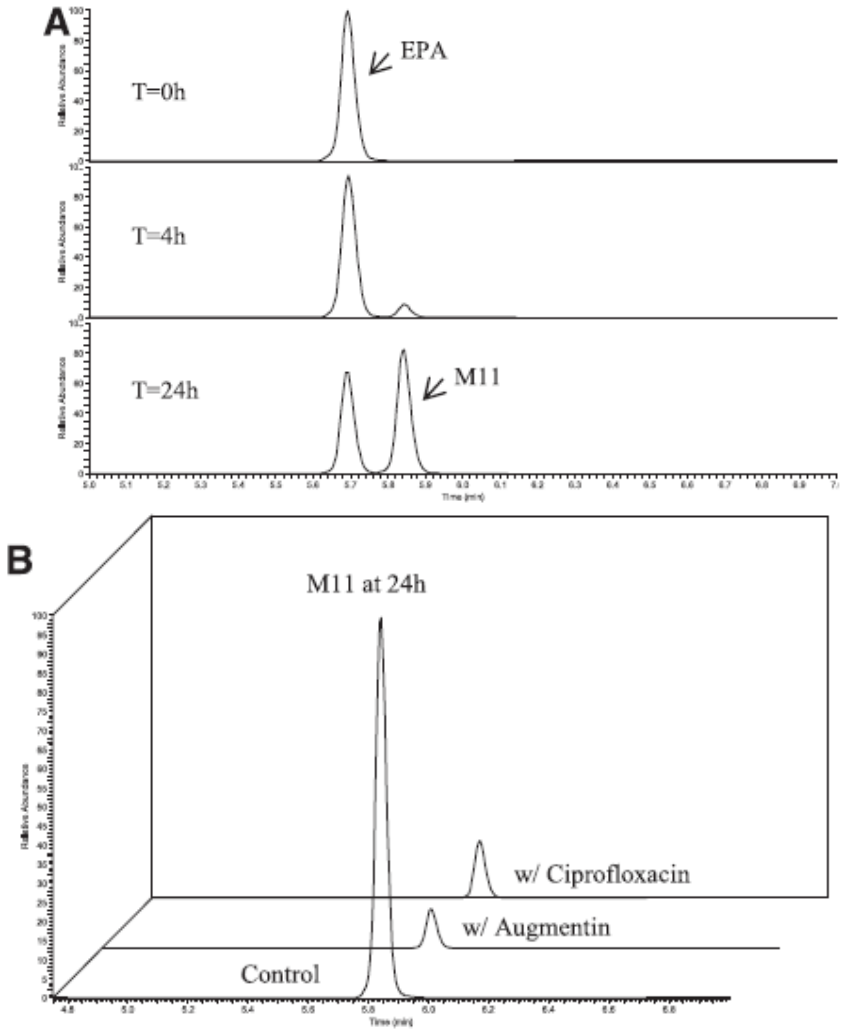


In vitro consumption of Epacadostat (5 uM) by individual recombinant human UGTs (0.5 mg/ml) over 1 hour.

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

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

M11 Was Shown To Be Formed by Gut Microbiota



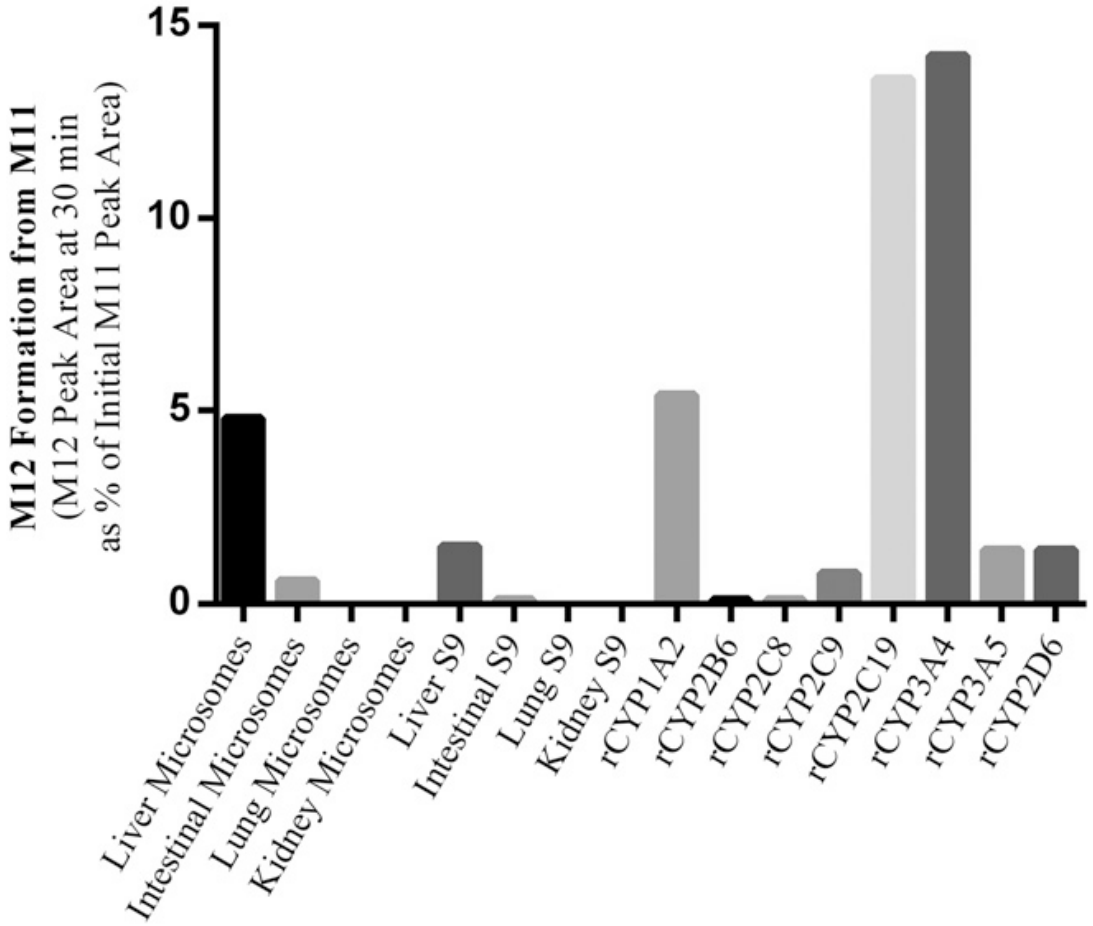
- Epacadostat was incubated with human feces homogenate under aerobic and anaerobic conditions
  - M11 was detected at level comparable to parent at the end of the 24h incubation
  - M11 was formed to similar extents under both aerobic and anaerobic conditions
  - M12 was detected in trace quantities
- 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 EPAC in human feces homogenate (A) and decrease in M11 formation from EPA in human feces homogenate by commonly prescribed antibiotics (B).

Augmentin is the registered trademark of GlaxoSmithKline.

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

M12 Was Shown To Be a Secondary Metabolite of Epacadostat Formed from M11

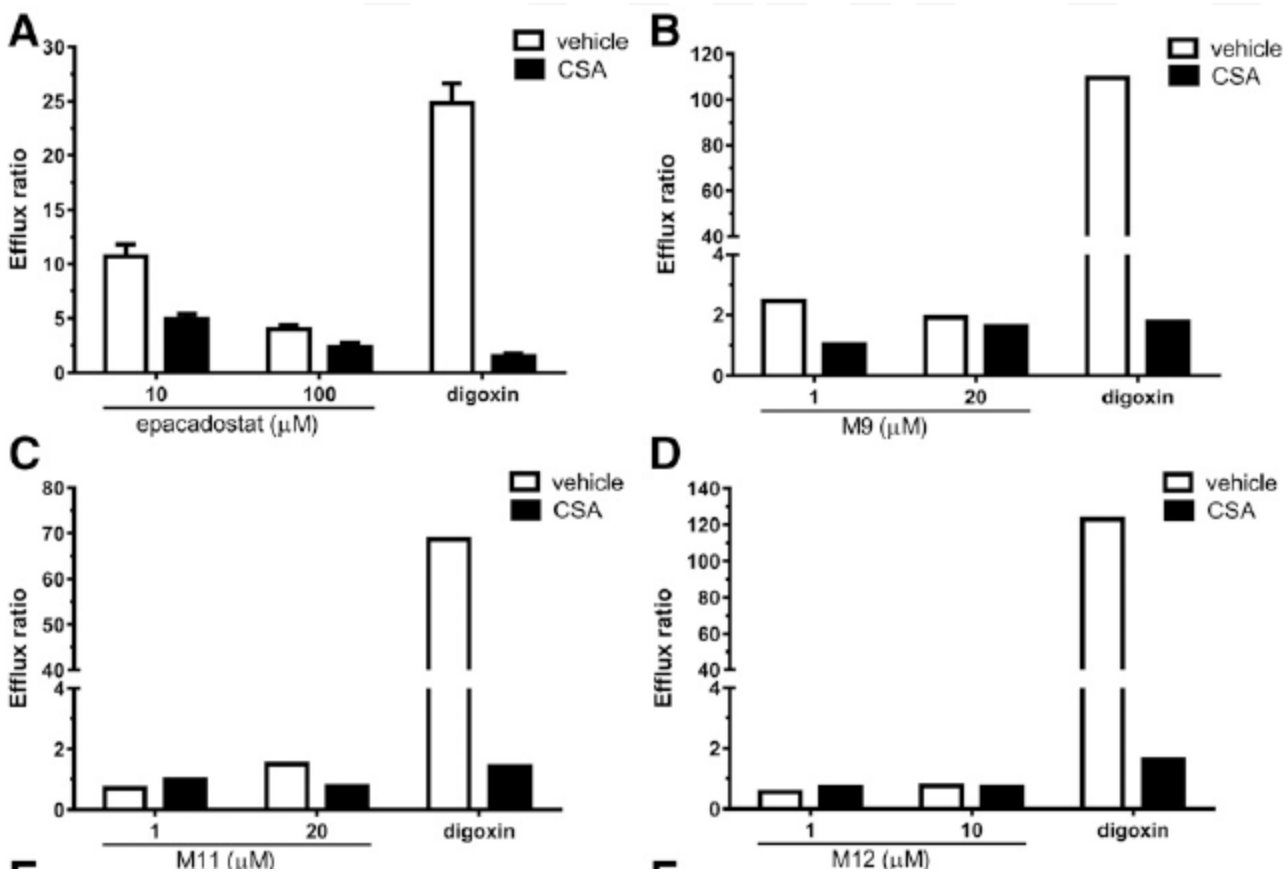


Formation of M12 from M11 (10 uM) by human tissue microsomes (2 mg/ml protein) and S9 fractions (3 mg/ml protein) and recombinant cytochrome P450s (0.3 nmol/ml).

- M12 was not detected *in vitro* when EPAC 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 catalyzed the N-dealkylation of M11 to form M12.
  - CYP3A4 was shown to be primarily responsible for the metabolism of M11

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

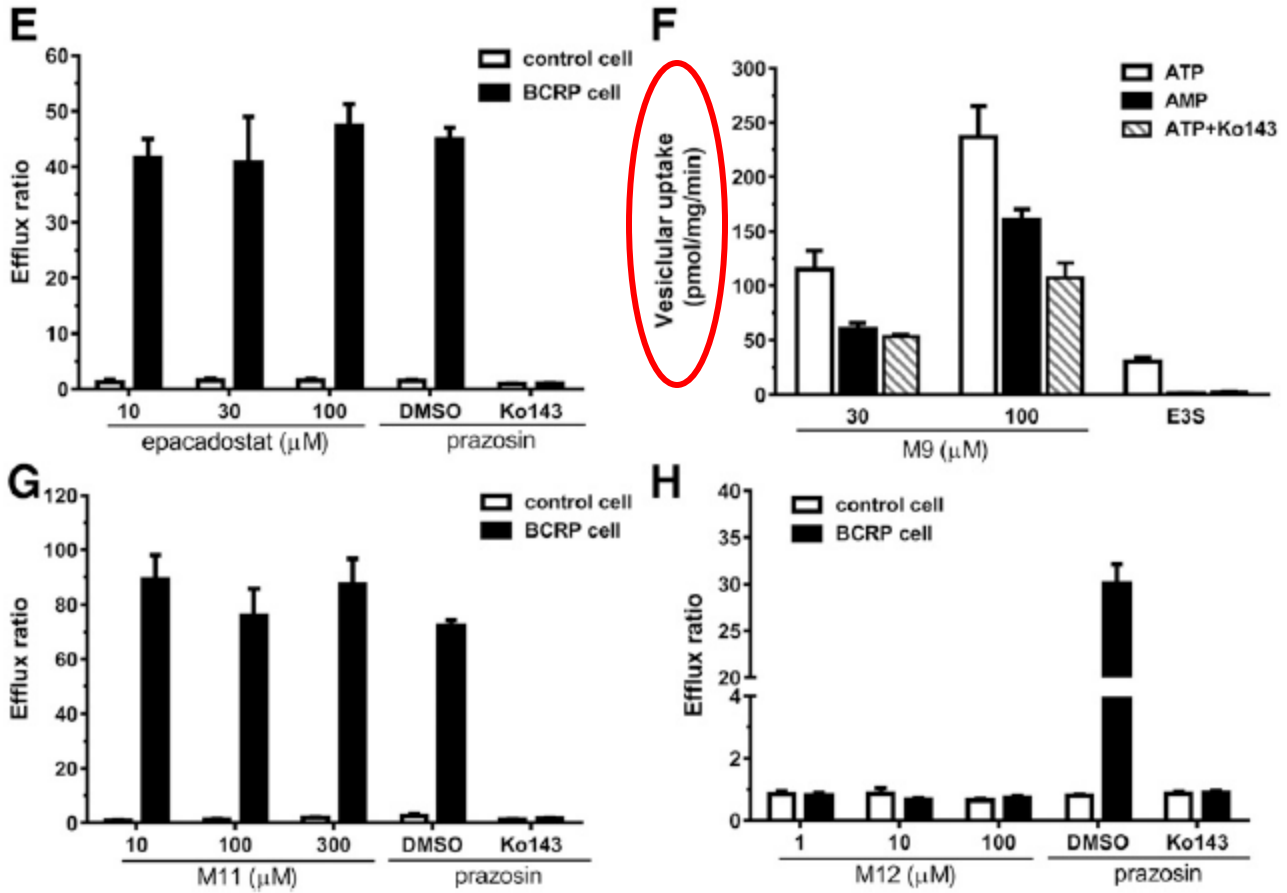
Transport of Epacadostat and Its Metabolites in Caco-2 Cell Lines



- EPAC was shown to be a substrate of P-gp in Caco-2 cells

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

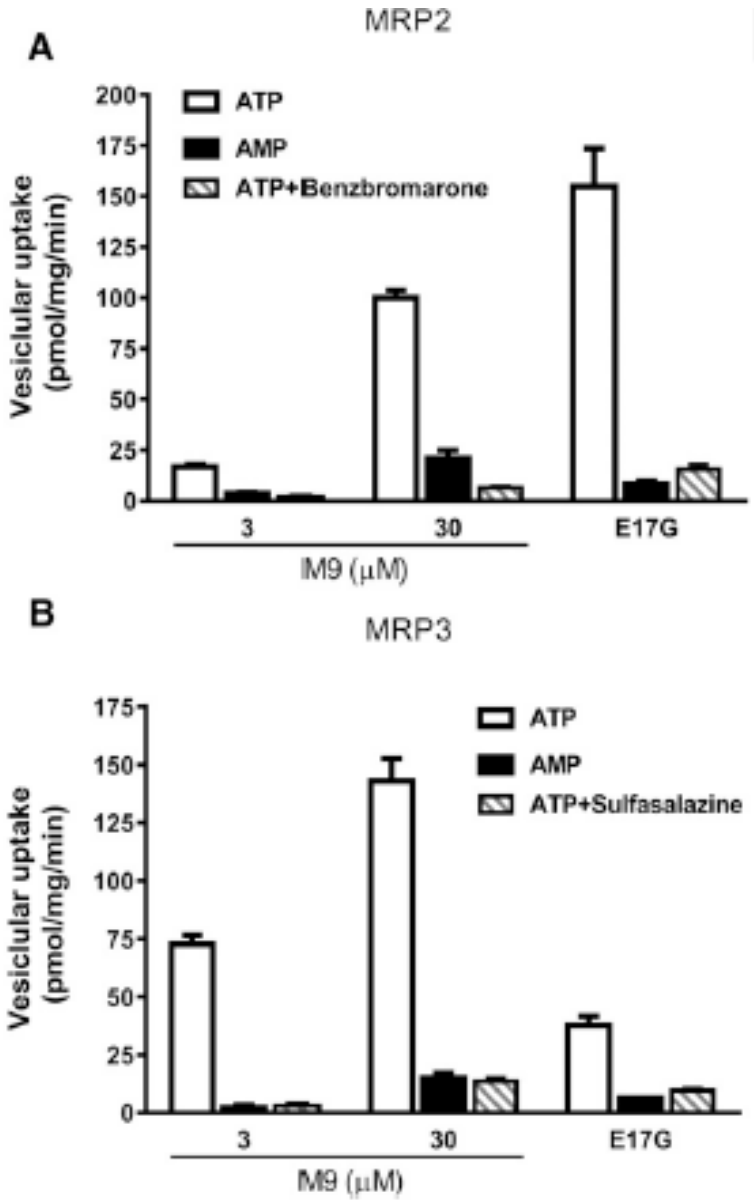
Transport of Epacadostat and Its Metabolites in BCRP-MDCKII Cell Lines and BCRP-Expressing Membrane Vesicles



- EPAC and M11 were shown to be substrates of BCRP in BCRP-MDCKII cells
- M9 was shown to be a BCRP substrate based on the MV study

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

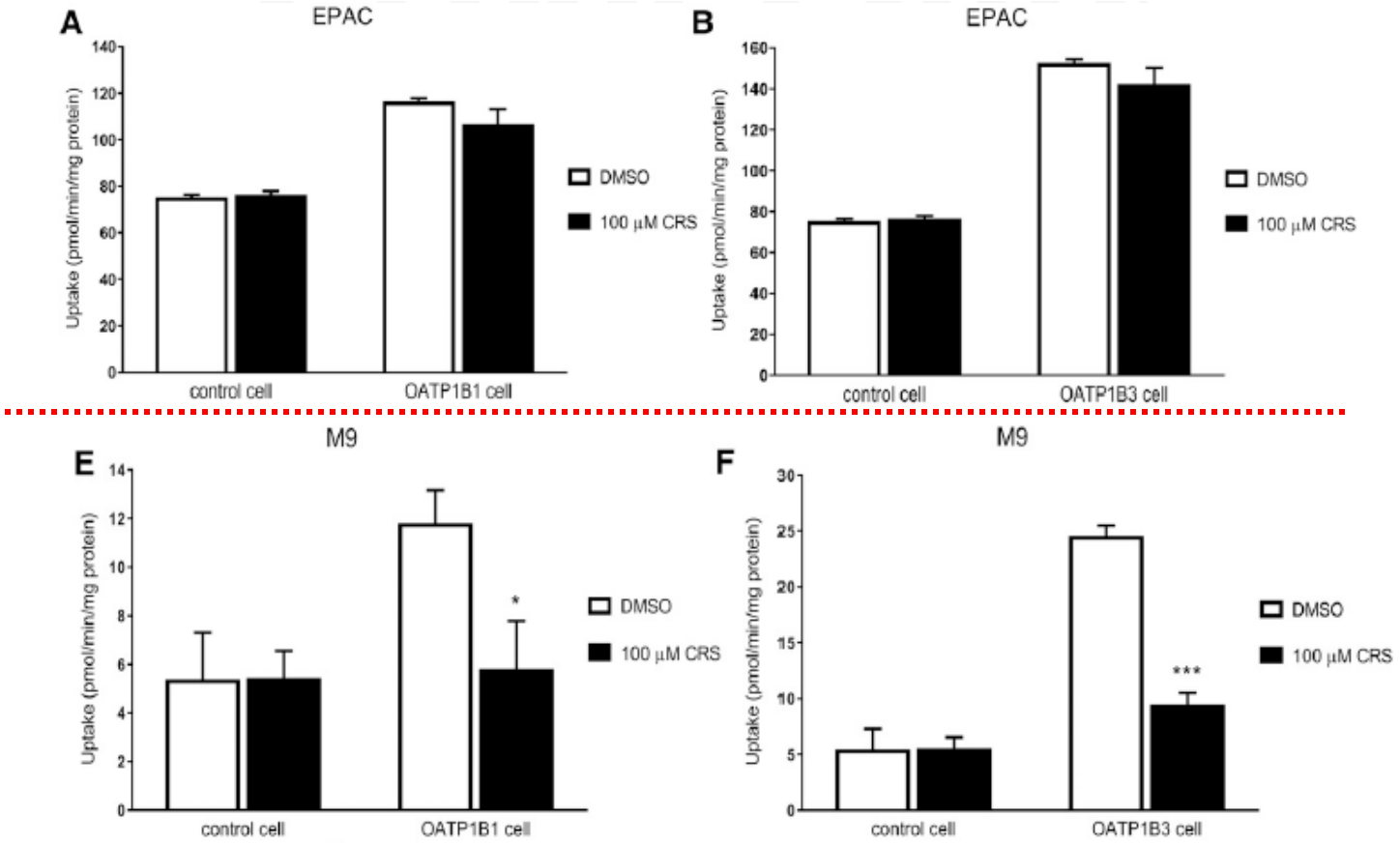
Transport of M9 by Human MRP2 and MRP3 in Membrane Vesicles Containing Human MRP2 or MRP3



- M9 was shown to be a substrate for MRP2 and MRP3

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

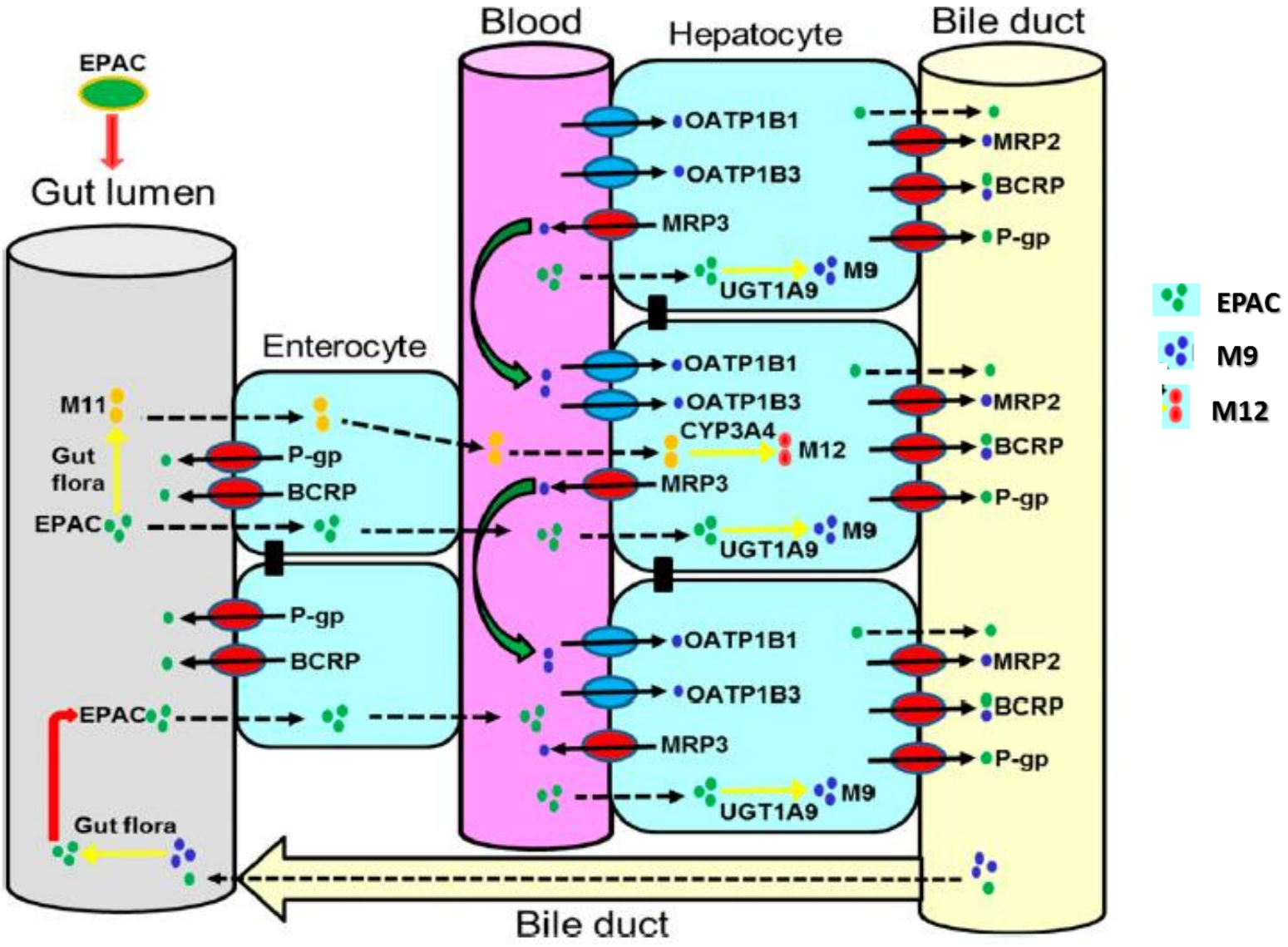
Uptake of Epacadostat and M9 into Human OATP1B1- and OATP1B3-Transfected CHO cells



- EPAC was not a substrate of OATP1B1 or OATP1B3
- M9 was shown to be a substrate of both OATP1B1 and OATP1B3

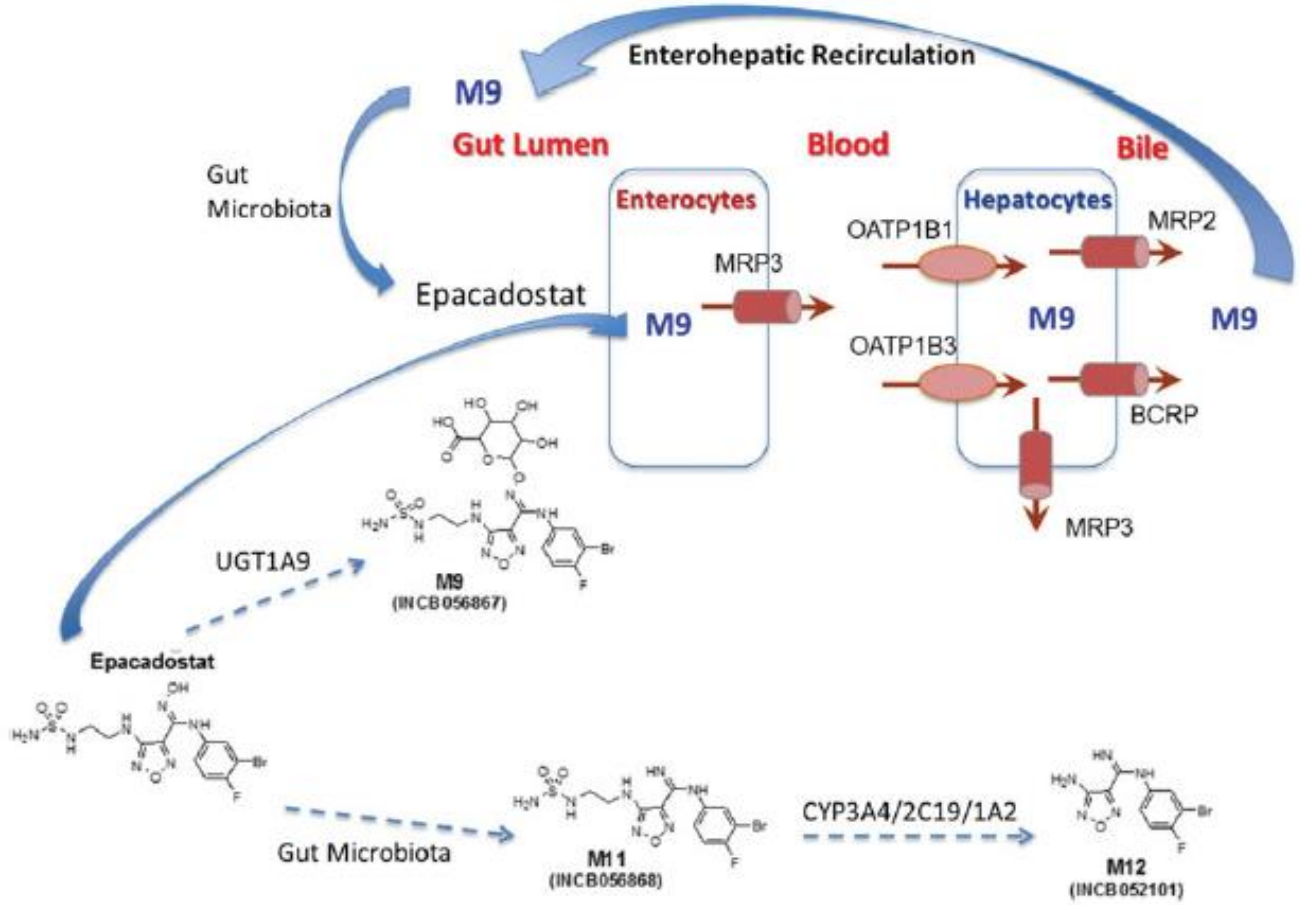
Zhang., *et al*. *Drug Metabolism and Disposition* **2017** 45; 1-12

Transporter-Mediated Disposition of Epacadostat and Its Metabolites in Humans



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

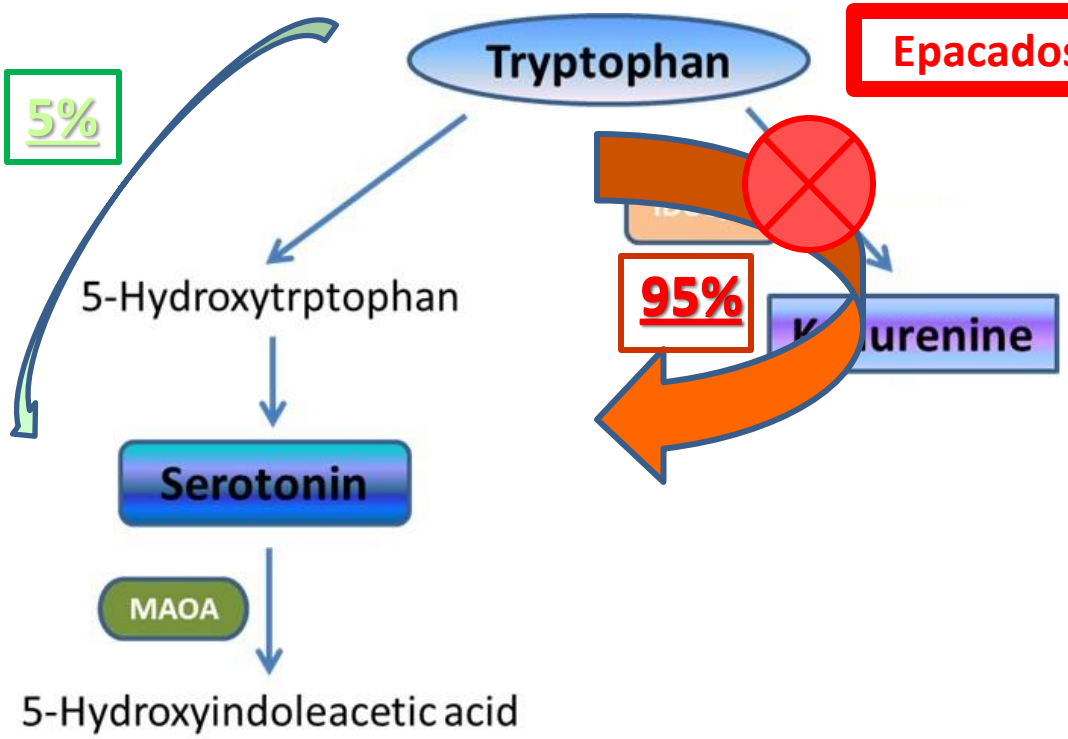
Epacadostat Metabolism and Transporter Mediated Enterohepatic Recirculation of M9



- Intestine:
  - UGT1A9
  - MRP3
- Liver and bile:
  - UGT1A9
  - OATP1B1 and OATP1B3
  - BCRP and MRP2
- Intestine:
  - Gut microflora

Zhang. *Current Protocols in Pharmacology,* **2019** 82, e46. doi: 10.1002/ cpph.46

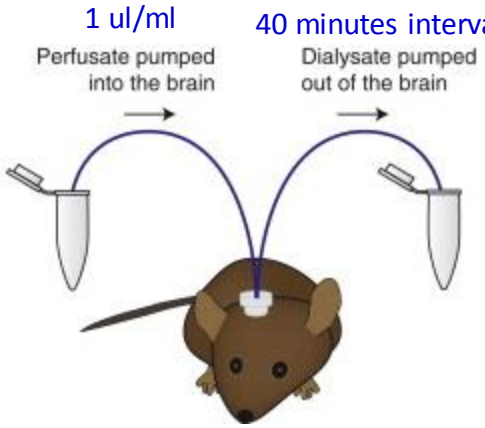
Epacadostat Transport and Metabolism on Brain Serotonin Concentration



- Effect of oral EPAC on tryptophan concentrations:
  - Peripheral (systemic)
    - Plasma concentrations
  - Local (CNS)
    - Local concentration of tryptophan?
    - BBB penetration of EPAC?

Modified from Zhang., et al *Drug Metabolism and Disposition*, **2019** 47; 710-714

Intracerebral Microdialysis Study Design



Sprague-Dawley Rats (male, 10 wks)

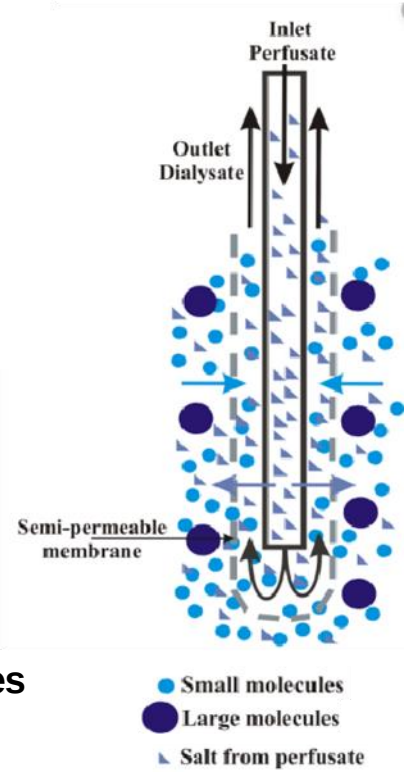
6 hr Post-dose Plasma samples

Pre-dose Dialysate Samples

Time 0 hr Dosing

Sample Interval 40 minutes

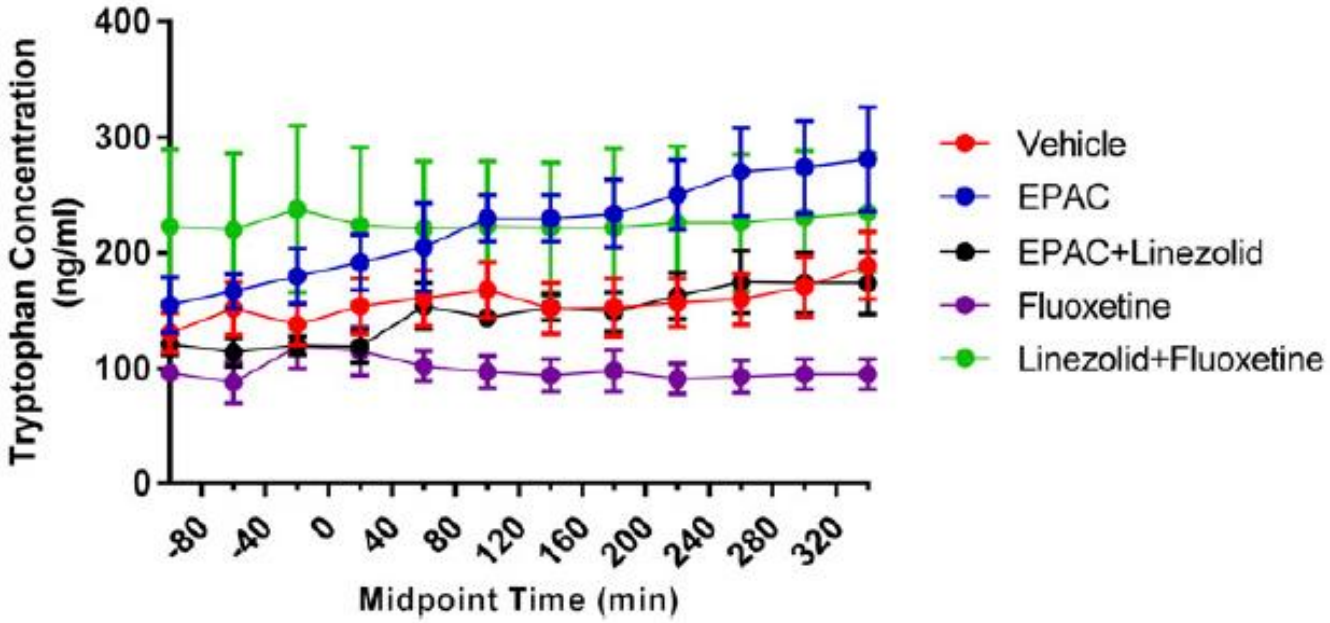
6 hr Post-dose Dialysate Samples



- Vehicle group
- EPAC, 25 mg/kg, oral
- EPAC, 25 mg/kg, oral + Linezolide, 100 mg/kg, oral
- Fluoxetine, 10 mg/kg ip
- Fluoxetine, 10 mg/kg ip + Linezolide, 100 mg/kg, oral

Modified from Zhang., et al *Drug Metabolism and Disposition,* **2019** 47; 710-714

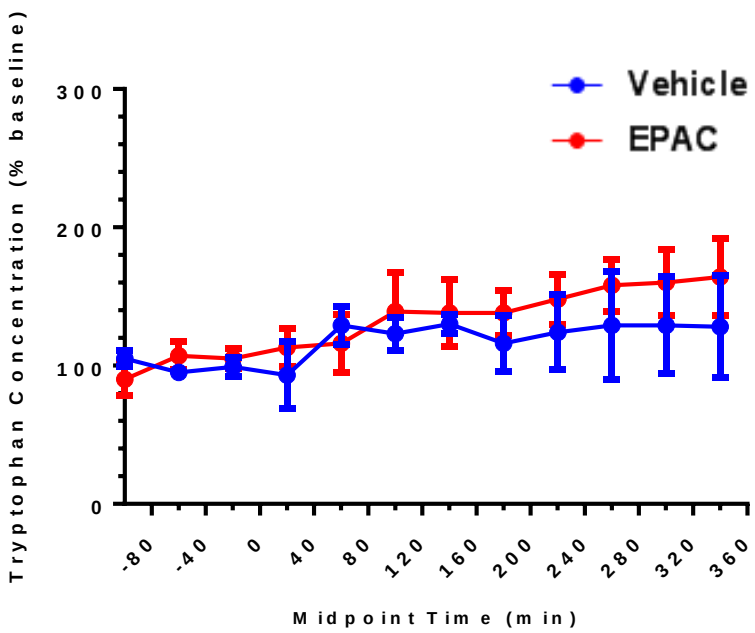
Tryptophan Concentrations in Rat Brain Extracellular Fluid following Treatment with Various Test Compounds



- Fluoxetine, combination of fluoxetine plus linezolid, combination of EPAC plus linezolid did not have significant effect on the brain ECF concentrations of tryptophan in rats.
- Following administration of EPAC alone, there was a trend towards an increase in tryptophan concentrations in brain ECF, however, the changes were well within the normal range for tryptophan across studies.

Zhang., et al *Drug Metabolism and Disposition*, **2019** 47; 710-714

Tryptophan Concentrations in Rat Brain Extracellular Fluid and Plasma

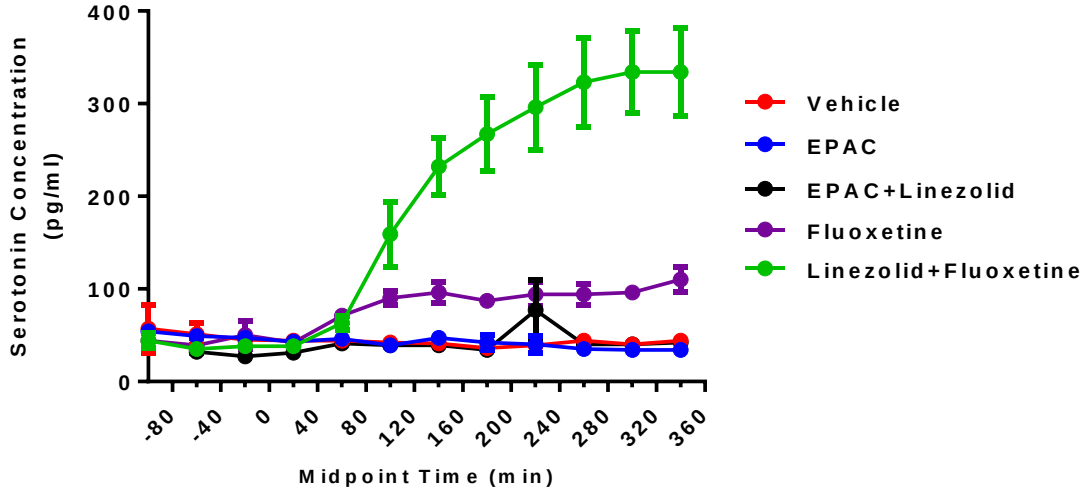


	Plasma Concentration (µM)	
Time	**1h**	**6h**
Vehicle	**121 ± 23**	**157 ± 30**
EPAC	**128 ± 36**	**220 ± 170**

- EPAC did not increase tryptophan concentrations in rat brain ECF
- Minimal impact of EPAC treatment on systemic concentration of tryptophan
- Serotonin concentration was BQL in plasma

Modified from Zhang., et al *Drug Metabolism and Disposition,* **2019** 47; 710-714

Serotonin Concentrations in Rat Brain Extracellular Fluid following Treatment with Various Test Compounds



- Moderate increase (~ 2-fold) in ECF serotonin concentrations following the treatment with fluoxetine
- Co-administration of linezolid with fluoxetine resulted in a pronounced (9-fold) elevation of serotonin ECF concentrations in rats

Zhang., et al *Drug Metabolism and Disposition,* **2019** 47; 710-714

Brain, CSF and Plasma Concentrations of EPAC in Rats following IV Infusion for 4 Hours

Plasma Concentration at 4h (nM)	CSF Concentration at 4h (nM)	Brain Concentration at 4h (nM)	Brain/Plasma (%)
851 ± 180	**BQL**	**127 ± 39**	**15 ± 6**

BQL, below quantifiable limitation of 4 nM
N = 4.

- The poor brain uptake for EPAC suggests that the potential for IDO1 inhibitory activity in the brain following EPAC oral dosing is limited

Modified from Zhang., et al *Drug Metabolism and Disposition*, **2019** 47; 710-714

Epacadostat Clinical Study Results (N = 2490)

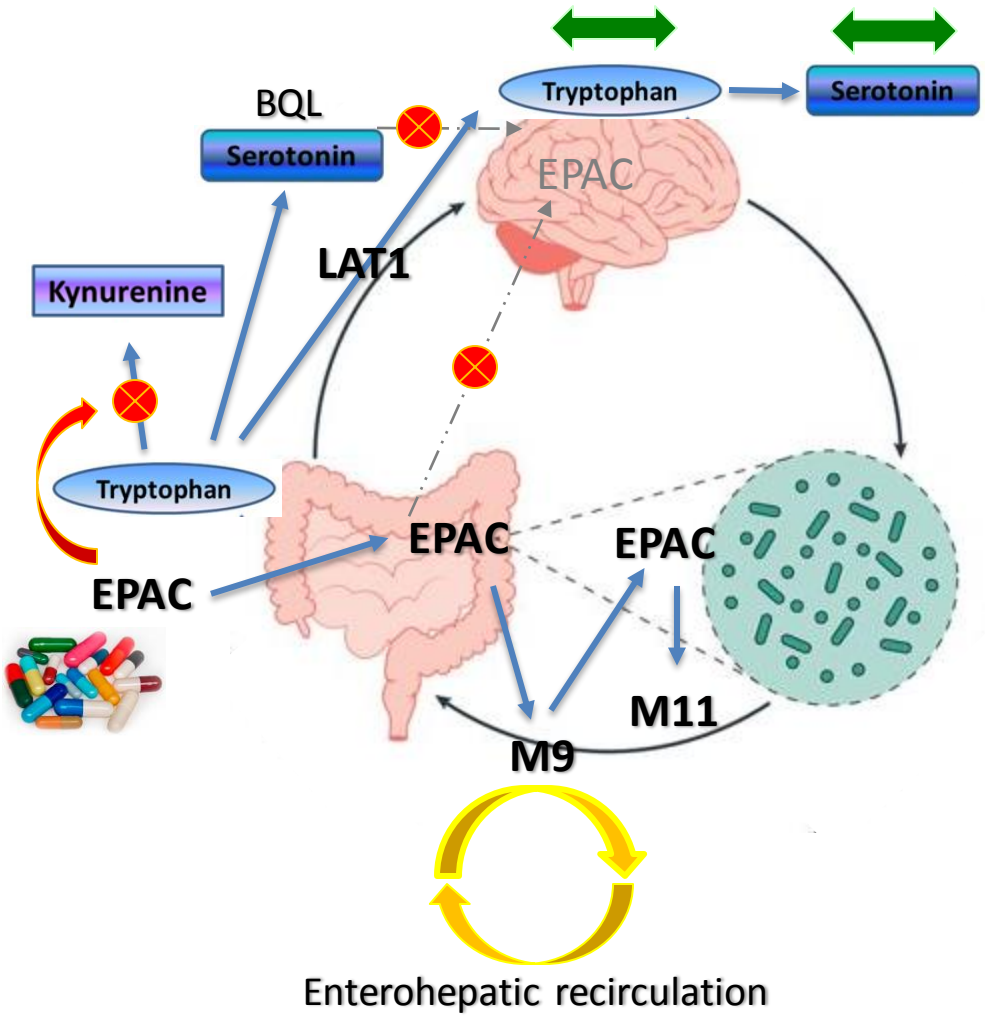
Clinical Trial Types	# Studies	Trial Name	Dose (mg)	Con Med	Outcome
Monotherapy	6	NA			
Combination Therapy	22	ECHO202 (Pembrolizumab)	100 BID	Escitalopram	The full constellation of SS was not observed nor could it be ruled out. The SSRI was discontinued. Restarted EPAC at the same dose level without further incidents
		ECHO202 (Durvalumab)	75 BID	Alprazolam	The full constellation of SS was not observed nor could it be ruled out. The events resolved and the retreatment started with EPAC of 50 mg BID
		ECHO306 (Pembrolizumab)	100 BID	Granisetron	The recovery was confirmed as the symptoms resolved after treatment with cyproheptadine. The subject stopped EPAC but continued on pembrolizumab.
		ONC-DPX-Survivac-06	300 BID	DPX-Survivac Vaccine cyclophosphamide alprazolam ondansetron	Lactated Ringers, lorazepam, and cyproheptadine were given to the subject and the symptoms were reduced within 30 minutes. The subject resumed treatment with no new symptoms reported.

- Four SS-like episodes occurred across multiple clinical studies. These episodes were confounded by other medical conditions, mild in severity and all events recovered after supportive care interventions took place.
- Three of the Four subjects were able to resume treatment with EPAC.
- None of the 4 reports was clinically substantiated to represent a true case of SS.

On April 6, 2018, Incyte Corporation announced that a review of the ECHO-301/KEYNOTE-252 study results determined that the study did not meet the primary endpoint of improving progression-free survival in the overall population compared to pembrolizumab monotherapy. Based on these results, the study was stopped.

Zhang., et al *Drug Metabolism and Disposition*, **2019** 47; 710-714

Summary



- In preclinical observations, serotonin syndrome was unlikely following oral treatment with either EPAC alone or with combination of MAOIs such as linezolid in rats.
- No true cases of serotonin syndrome were demonstrated clinically.
- Based on the preclinical and clinical observations, EPAC does not appear to cause serotonin syndrome.
- The exclusion of MAOIs from clinical studies with EPAC has been lifted.

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* former employees of Incyte