Role of Drug Efflux Transporters in the Permeability of the HIV-1 Integrase Inhibitor, Raltegravir (RAL), across Blood-Tissue Barriers

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Building Better Therapeutics
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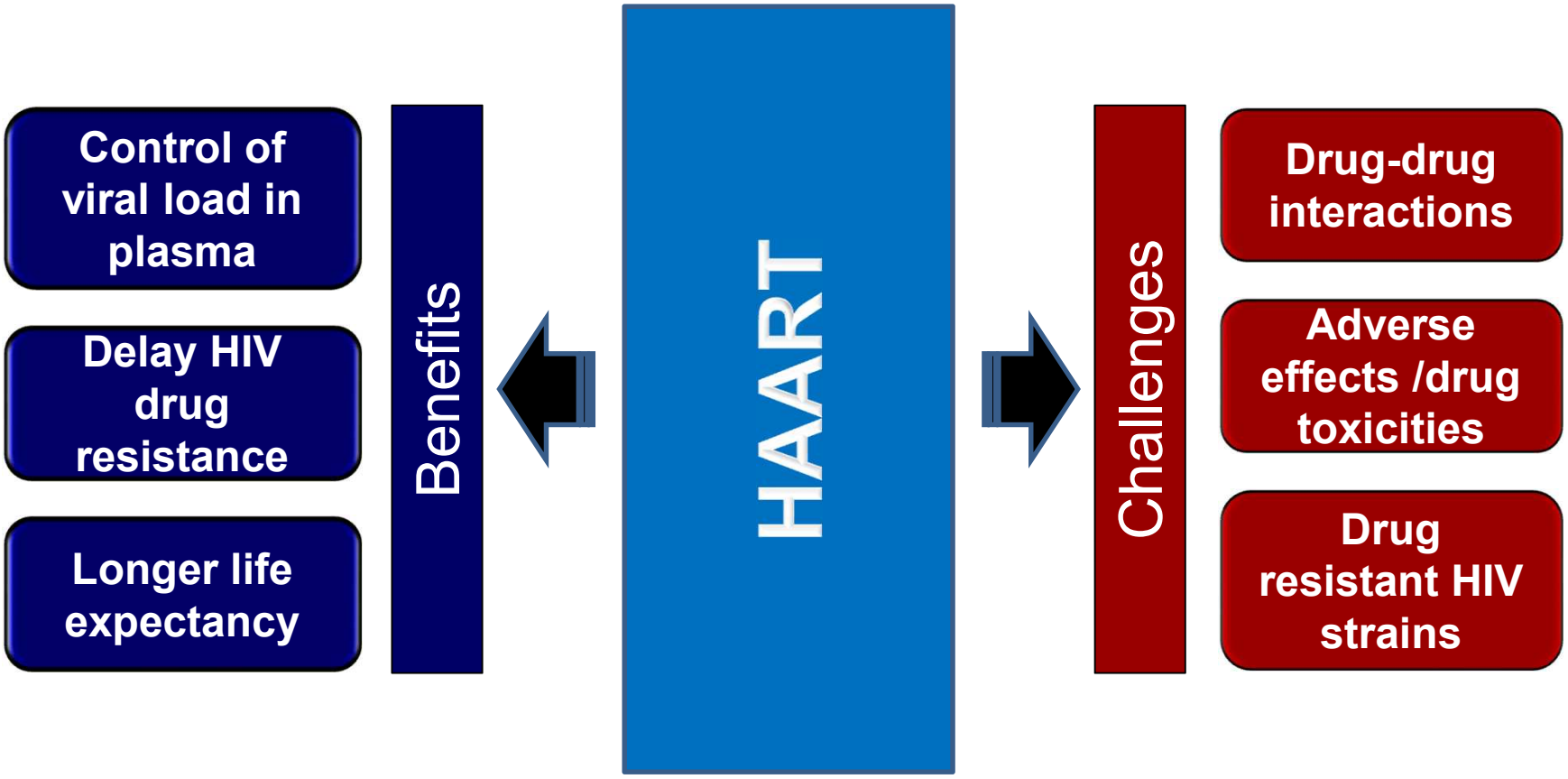
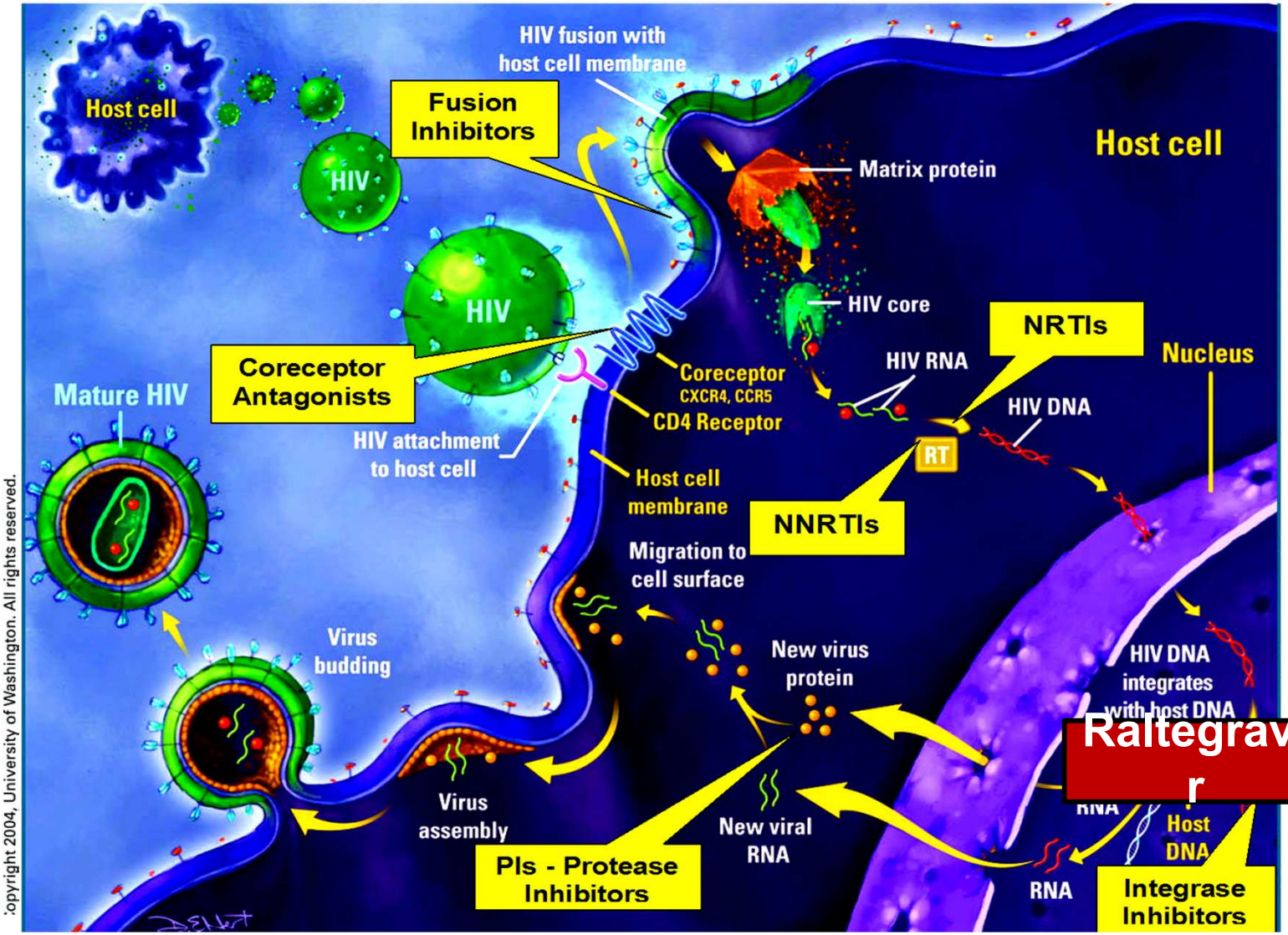
CHANGING THE COURSE OF THE HIV PREVENTION, ENGAGEMENT AND TREATMENT CASCADE

No conflict of interest to declare

Highly Active Antiretroviral Therapy (HAART)

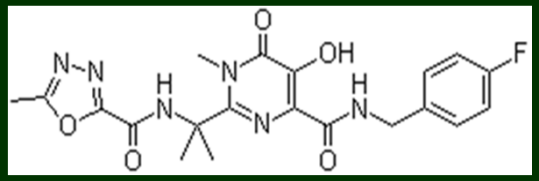


Anti-HIV Drugs: 6 mechanistic classes



Structural/clinical pharmacokinetic parameters of RAL

Chemical structure:



Molecular formula:
$C_{20}H_{21}FN_6O_5$

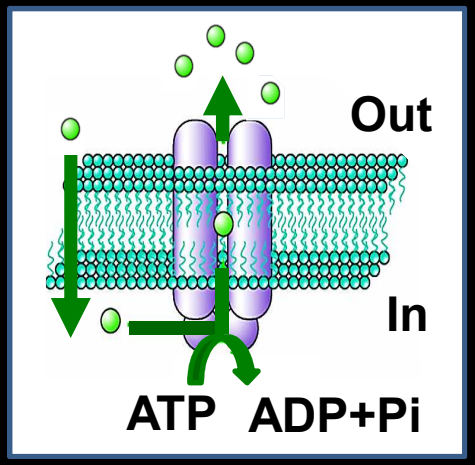
Molecular weight:
444.15 g/mole

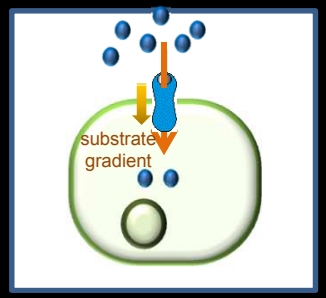
Dosing	400 mg bid
IC_{95}	16 ng/ml
Protein binding	83%
Metabolism	UGT1A1
Excretion	Feces (51%); Urine (32%)
C_{max}, C_{min} and AUC	C_{max}: 2 µg/ml, C_{min}: 67 ng/ml AUC: 7 µg/ml.h
Half-life ($T_{1/2}$)	~9 hours
CSF to plasma ratio	0.01 – 0.61
Semen to plasma ratio	0.52 – 6.66

Kassahun *et al, Drug Metab. Dis.* 35:1657-16663 (2007); Adams *et al., Curr Opin HIV AIDS* 7:390-400 (2012); Barau *et al.*, 2010; Yilmaz *et al*, 2009)
http://www.merck.com/product/usa/pi_circulars/i/isentress/isentress_pi.pdf.

ATP-binding cassette (ABC) membrane transporters

Efflux pump

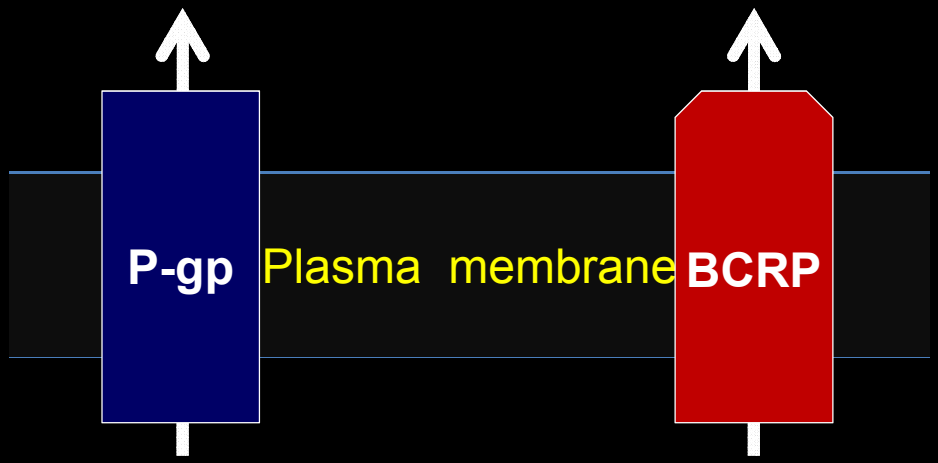




Uptake carrier

PIs, NRTIs
CCR5-antagonist

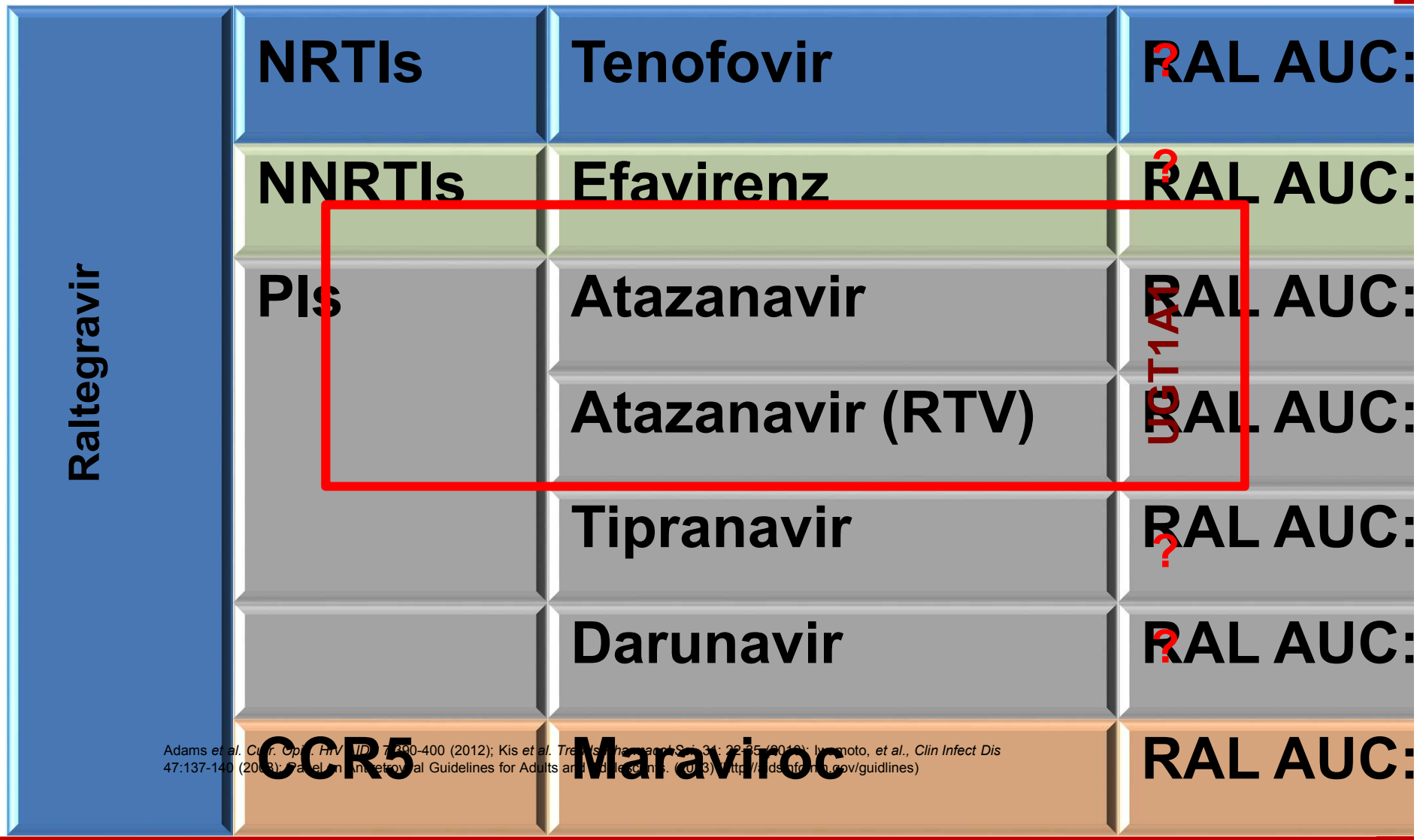
NRTIs



- Wide-range of substrate specificity including many xenobiotics and ARVs
- Expressed in different tissues such as small intestine, liver, kidney, brain, Testes, etc.

Ashraf T, Kis O, Banerjee N, Bendayan, R. *Landes Bioscience* (book chapter) (2012); Kis O, Robillard K, Chan GN, Bendayan R. *Trends Pharmacol.Sci.* 31: 22-35 (2010); Aller *et al., Science,* 323: 1718-1722 (2009); Ashraf T, Robillard K, Chan G, Bendayan R. *Curr Pharm Des*. (2013) [epub ahead of print]

Interactions between ARVs: RAL and NRTIs, NNRTIs, PIs, CCR5



Adams *et al. Curr. Opin. HIV AIDS* 7:390-400 (2012); Kis *et al. Treat Guidelines*. (2013) http://aidsinfo.nih.gov/guidlines)

Rationale

At present, limited *in vitro* data are available on the potential interaction between RAL and drug efflux/influx transporters. Recently, clinical studies demonstrated several RAL interactions with ARV drugs. Although some of these interactions are demonstrated to be regulated by UGT1A1, others remain unexplained, and could be mediated by drug transporters.

Hypothesis

RAL can act as an inhibitor, substrate and/or inducer of drug efflux and /or influx transporters, thus resulting in drug-drug interactions which could compromise anti-HIV pharmacotherapy in the clinic.

Objectives

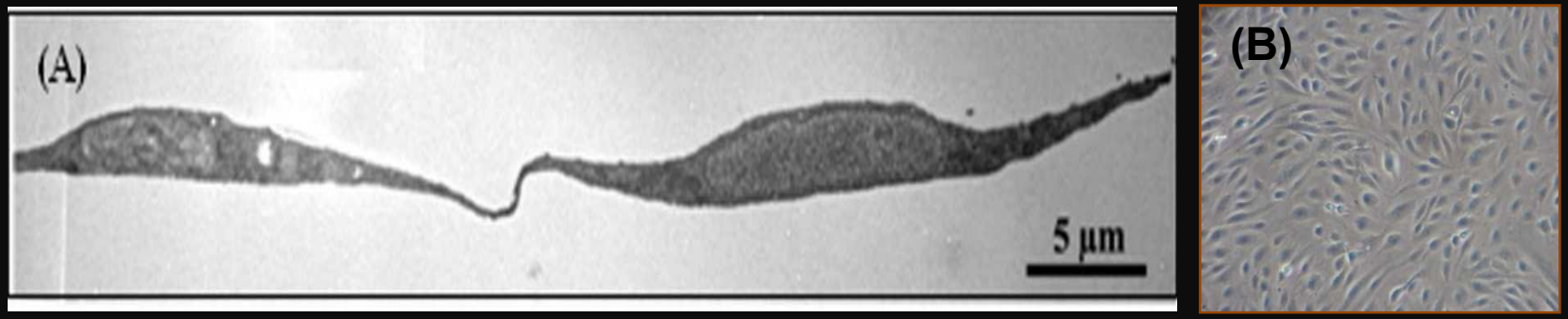
- To assess *in vitro*, potential interactions of RAL with drug efflux transporters in transporter over expressing cell culture systems.
- To investigate *in vitro and in situ*, the permeability of RAL, at blood-tissue barriers using cell cultures models well characterized in our laboratory for human blood-brain barrier (hCMEC/D3), mouse blood-testicular barrier (TM4), human blood-intestinal barrier (Caco2) and rat blood-intestinal barrier (*in situ* model).

Research Plan

P-gp	BCRP
P-gp overexpressing (MDA-MDR1)	BCRP overexpressing (HEK-ABCG2)
Raltegravir inhibitor properties	
Rhodamine-6G (R-6G)	[^{3}H]-Mitoxantrone
Raltegravir [^{3}H] substrate properties	
PSC833 Cyclosporine A (CSA)	Ko143 Fumitremorgin C (FTC)

Research plan, continued

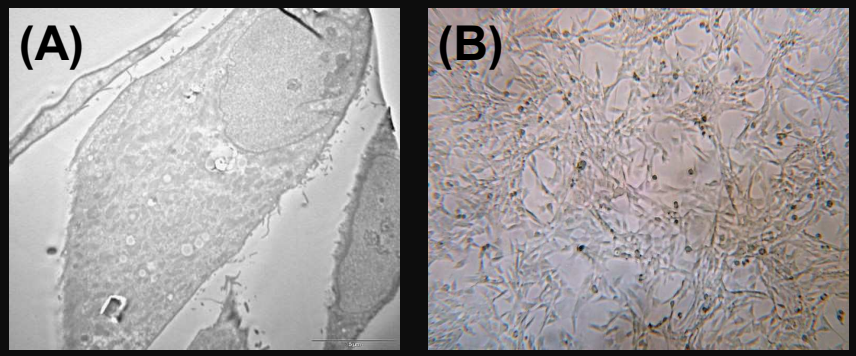
Human brain microvessel endothelial cells (hCMEC/D3), *an in vitro* cell culture model of BBB



A, Electron micrograph; ***B,*** Light microscopic image

Mouse sertoli cells (TM4), an *in vitro* cell culture model of BTB

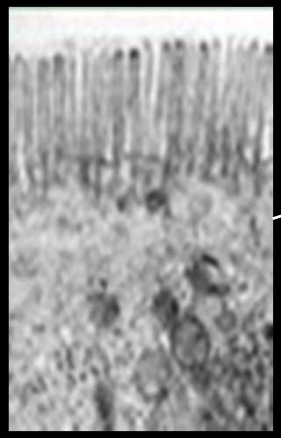
A, Electron micrograph;
B, Light microscopic image

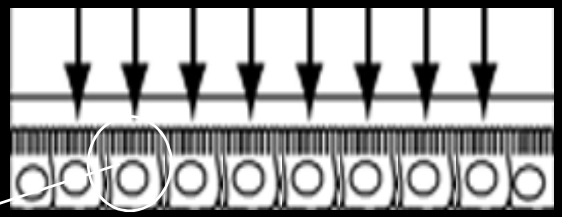


Zastre *et al*., *J. Neurosci. Res.,* 2009; Weksler *et al., FASEB J.,* 2005; Robillard *et a*l., *J. Pharm. Exp. Ther*. 340:96–108, 2012

Research plan, continued

Human intestinal Caco2 cells, an *in vitro* cell culture model of blood-intestinal barrier



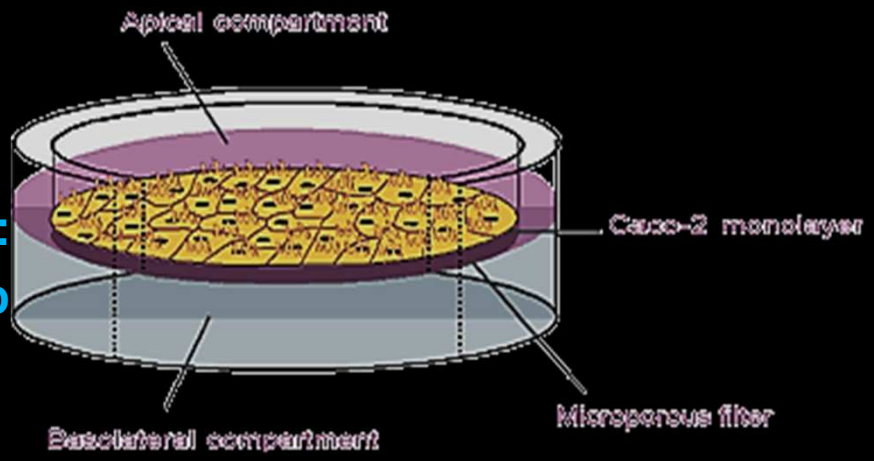


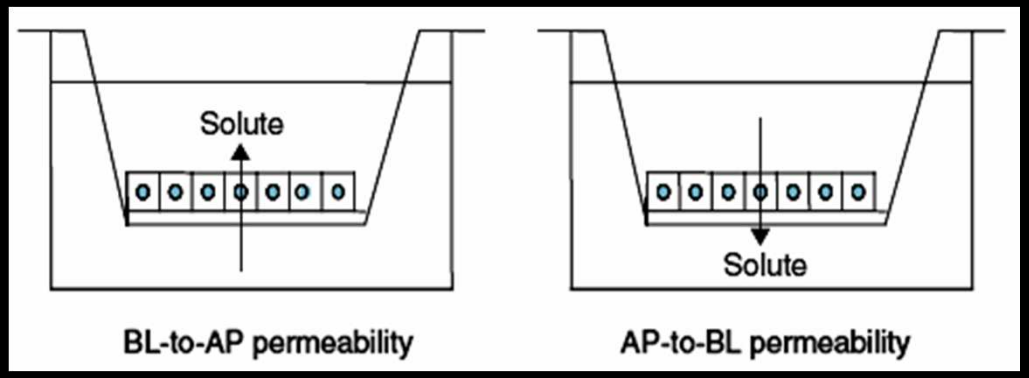
Measure drug accumulation:

- cells grown on solid supp
- apical drug transport

Measure drug permeability

Transwell membrane inserts





$$P_{app} = \frac{\Delta Q}{\Delta t} \frac{1}{AC_0}$$

Efflux Ratio:

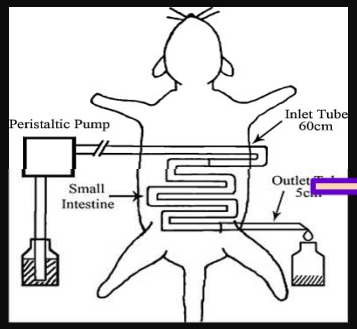
$ER = P_{app\ (B\text{-}A)} / P_{app\ (A\text{-}B)}$

Hilgendorf et al (2007) *DMD* 35

Research plan, continued

In situ intestinal perfusion in rat

$$P_{eff} = \frac{Q(1 - C_{out}/C_{in})}{2\pi RL}$$



- Each animal used as its own control
- Paired data analysis of steady-state P_{eff} values

Pump

C_{in} (mol/mL)

Glass Cannula

Flow rate Q (mL/min)

Glass Cannula

R (cm)

L (cm)

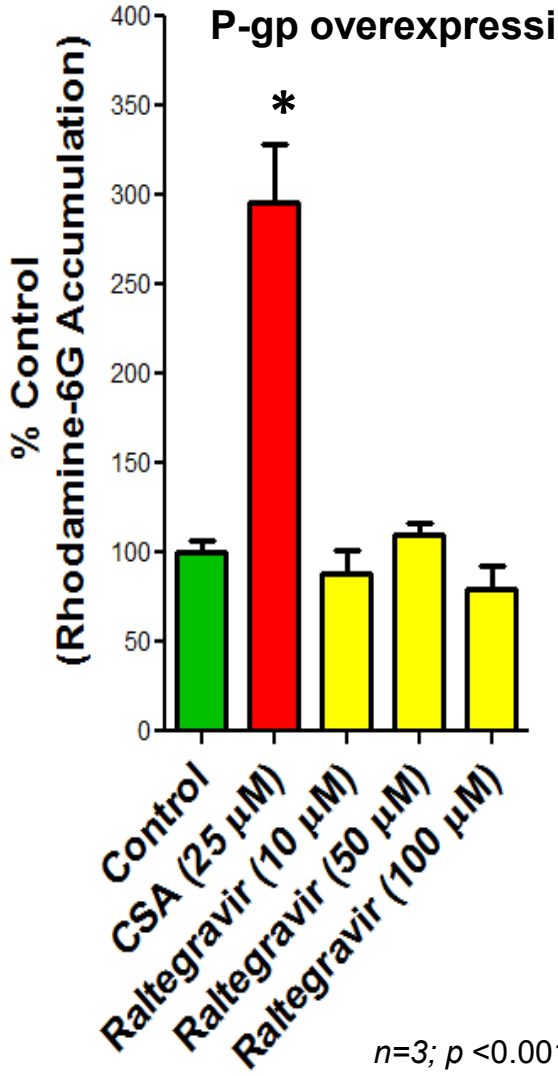
Intestinal segment

Outlet Tygon tubing

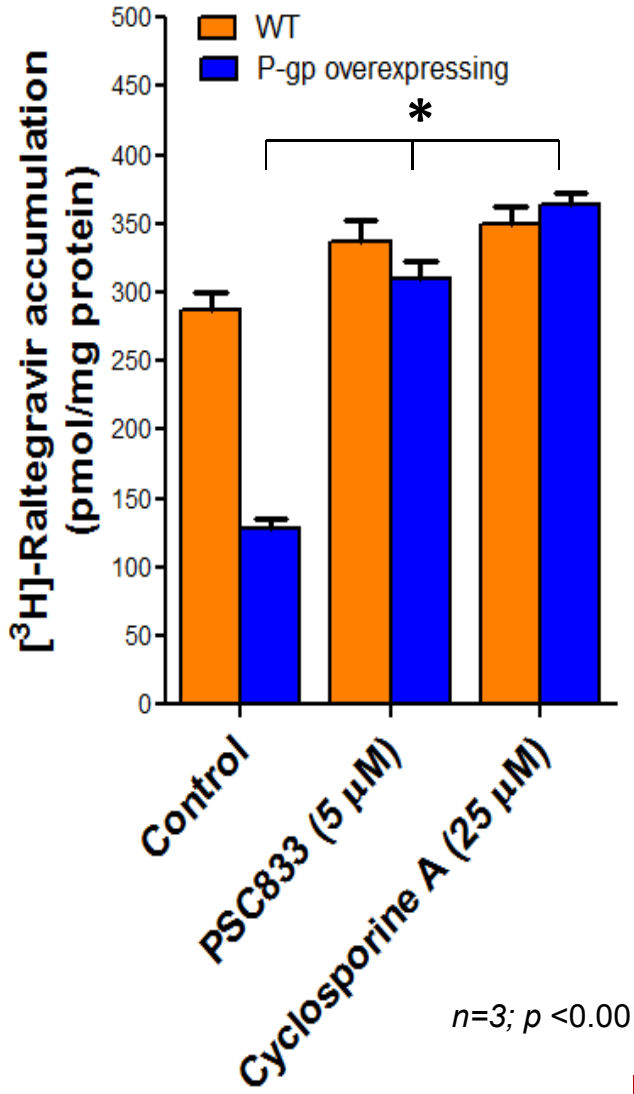
C_{out} (mol/mL)

Li et al, 2010, *International Journal of Pharmaceutics*

Raltegravir is not an inhibitor but a substrate of P-gp

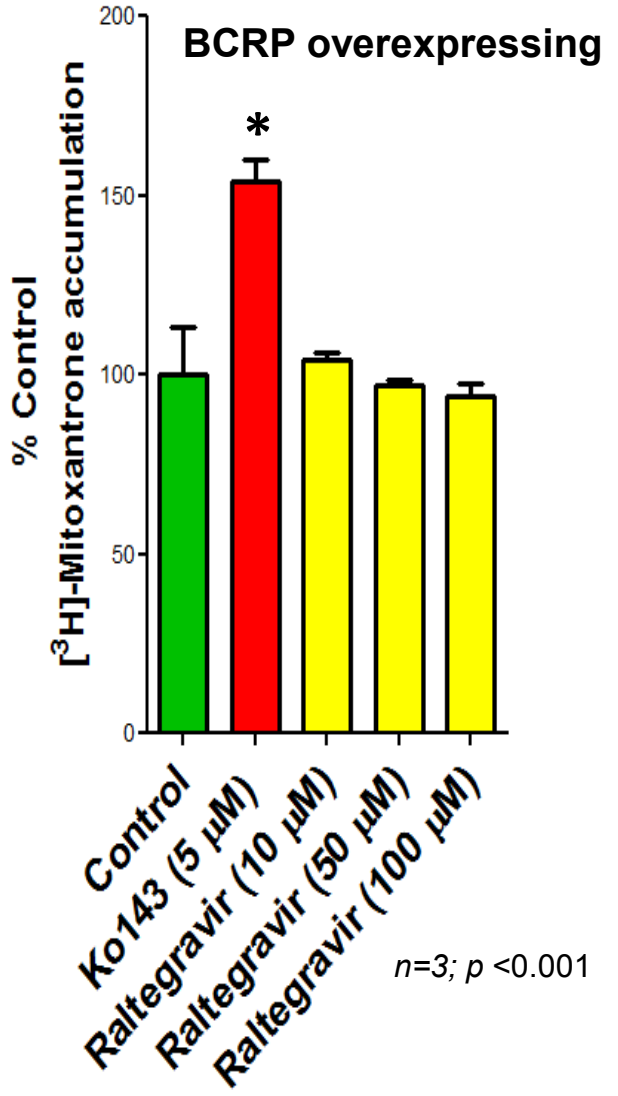


n=3; p <0.001

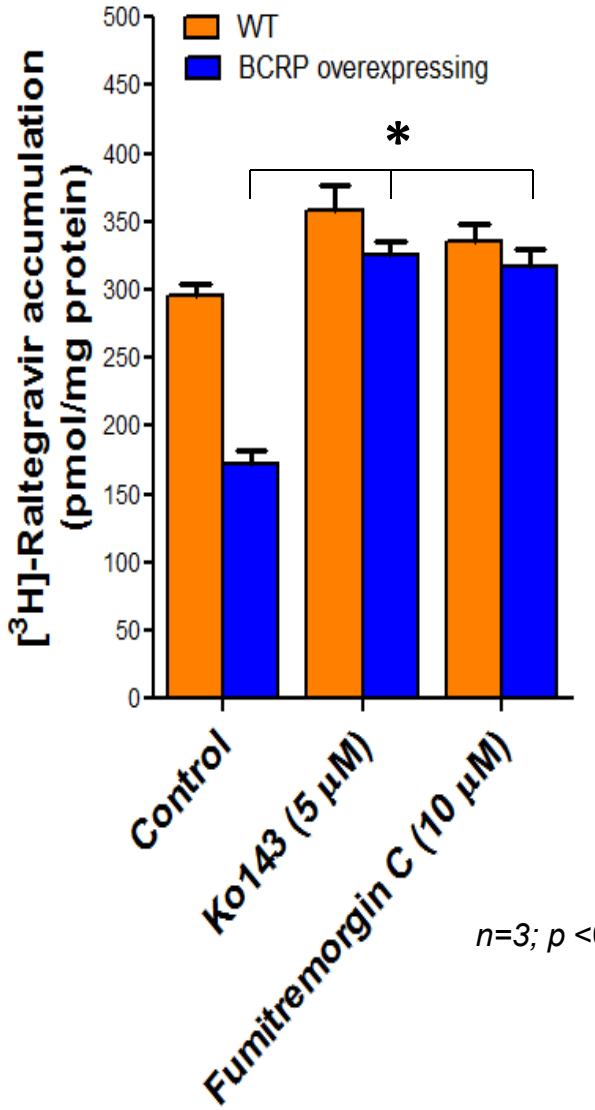


n=3; p <0.001

Raltegravir is not an inhibitor but a substrate of BCRP

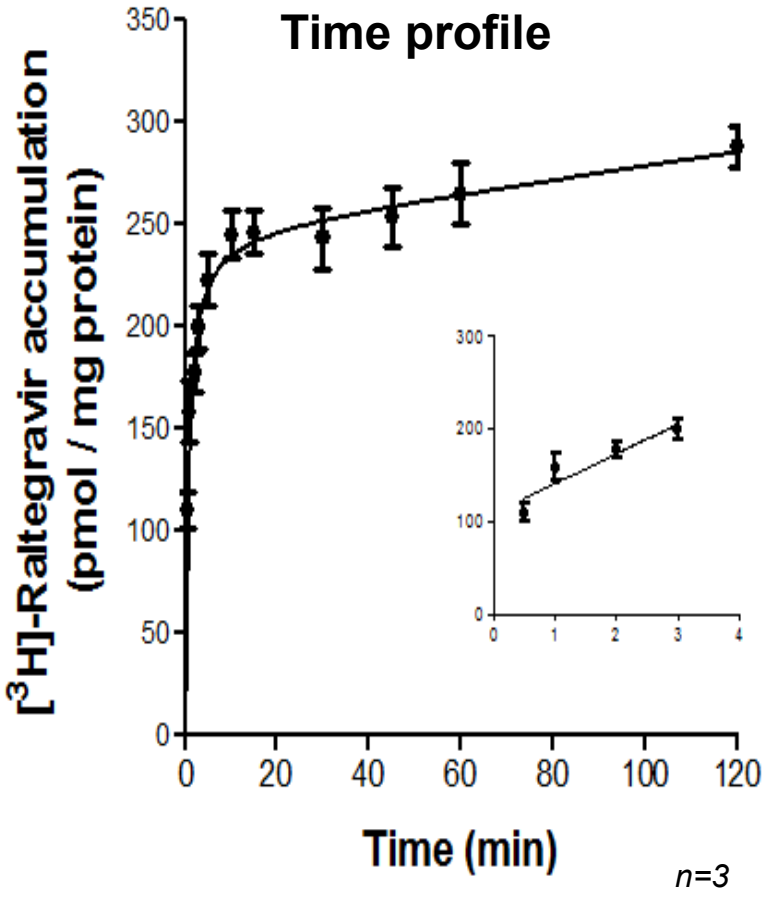


n=3; p <0.001

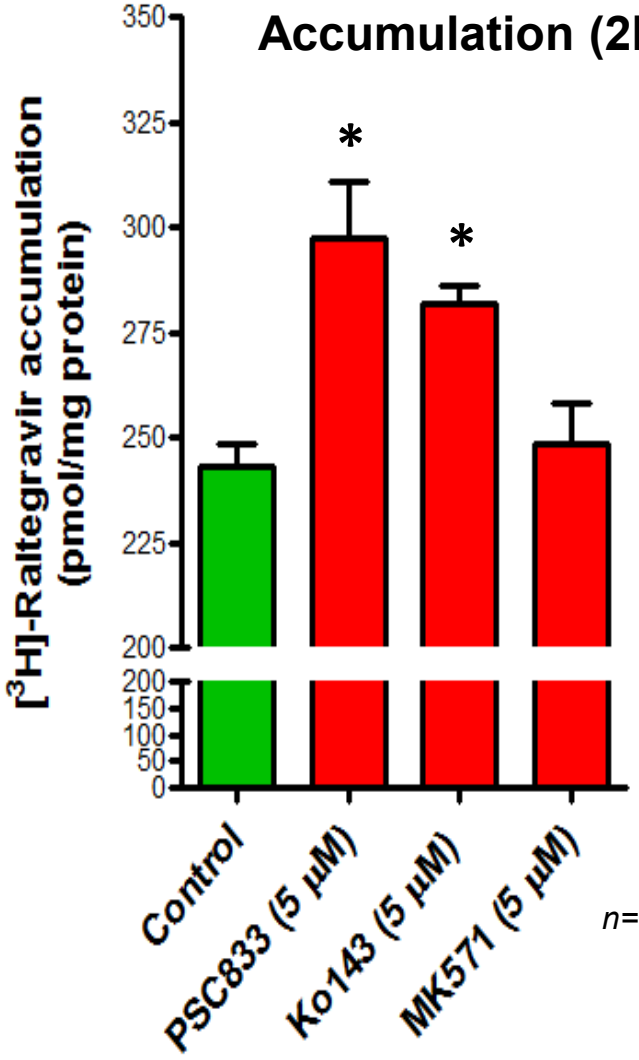


n=3; p <0.001

Raltegravir uptake and accumulation by hCMEC/D3, an *in vitro* cell culture model of human BBB

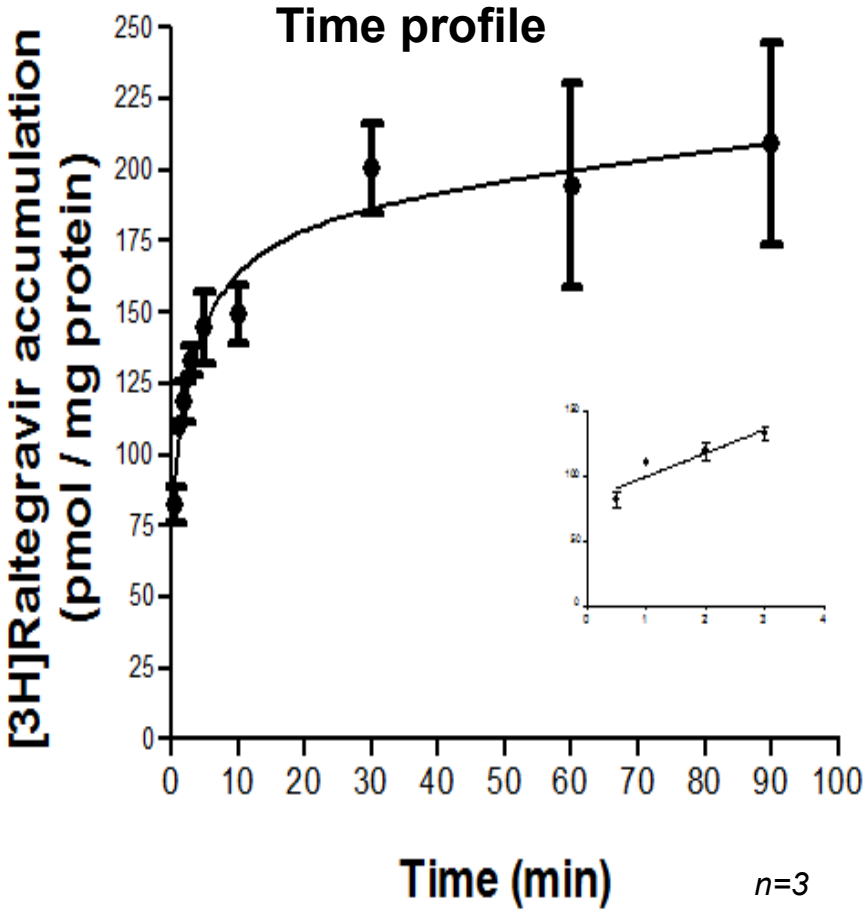


n=3

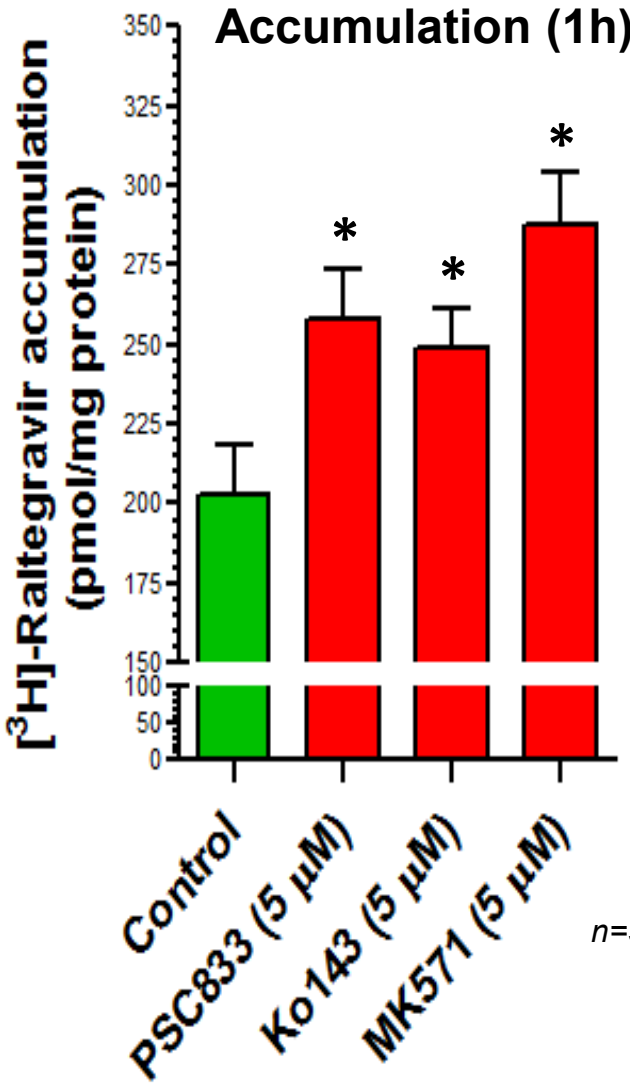


n=3; p <0.05

Raltegravir uptake and accumulation by TM4, an *in vitro* cell culture model of mouse BTB

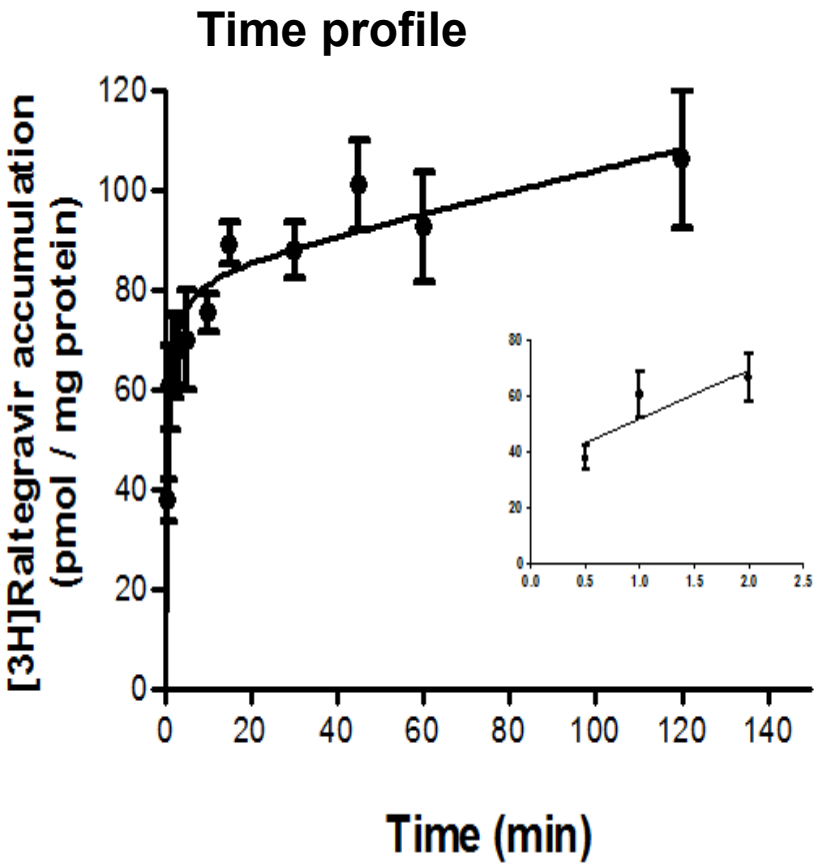


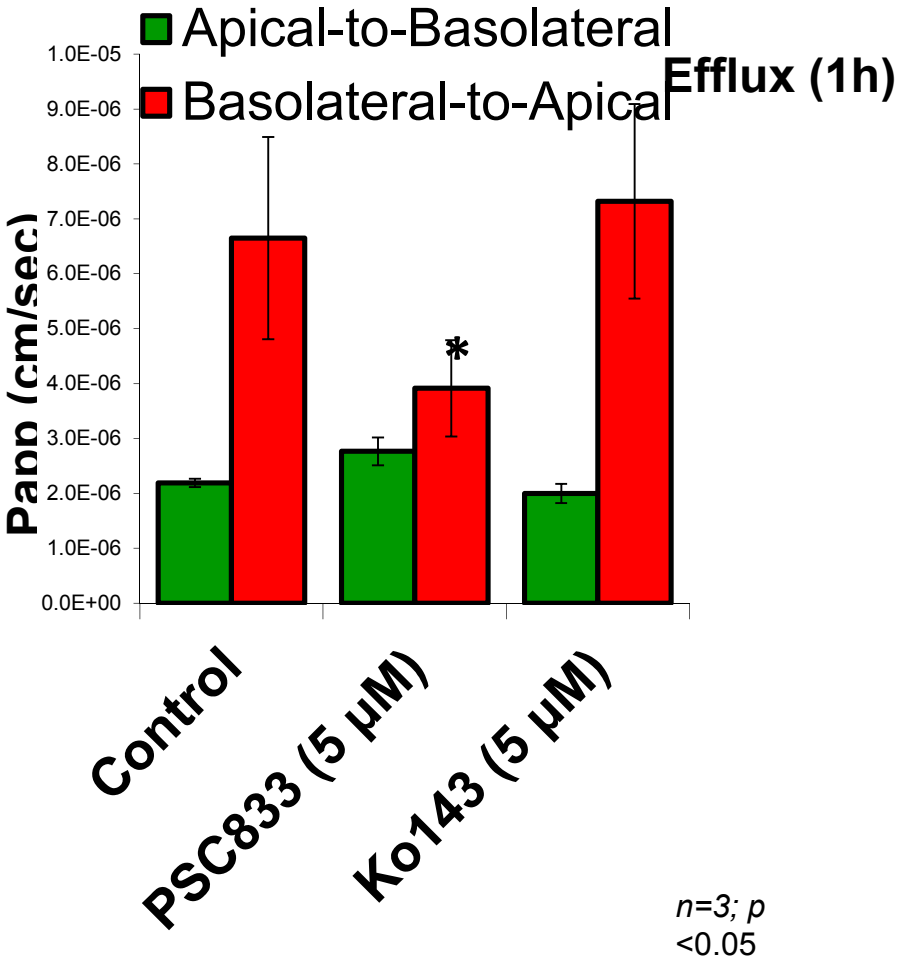
n=3



n=3; p <0.05

Raltegravir permeability across Caco2, an *in vitro* cell culture model of human intestinal barrier

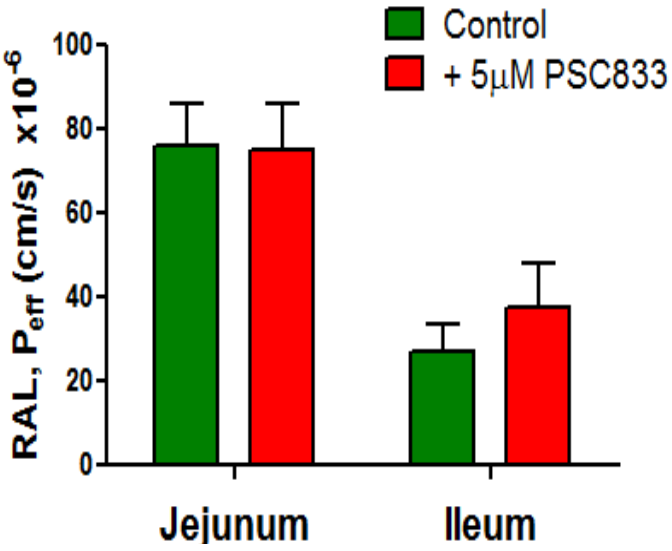


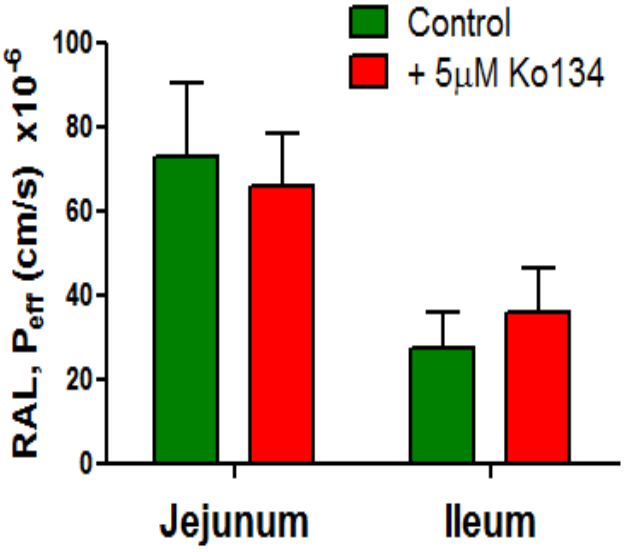


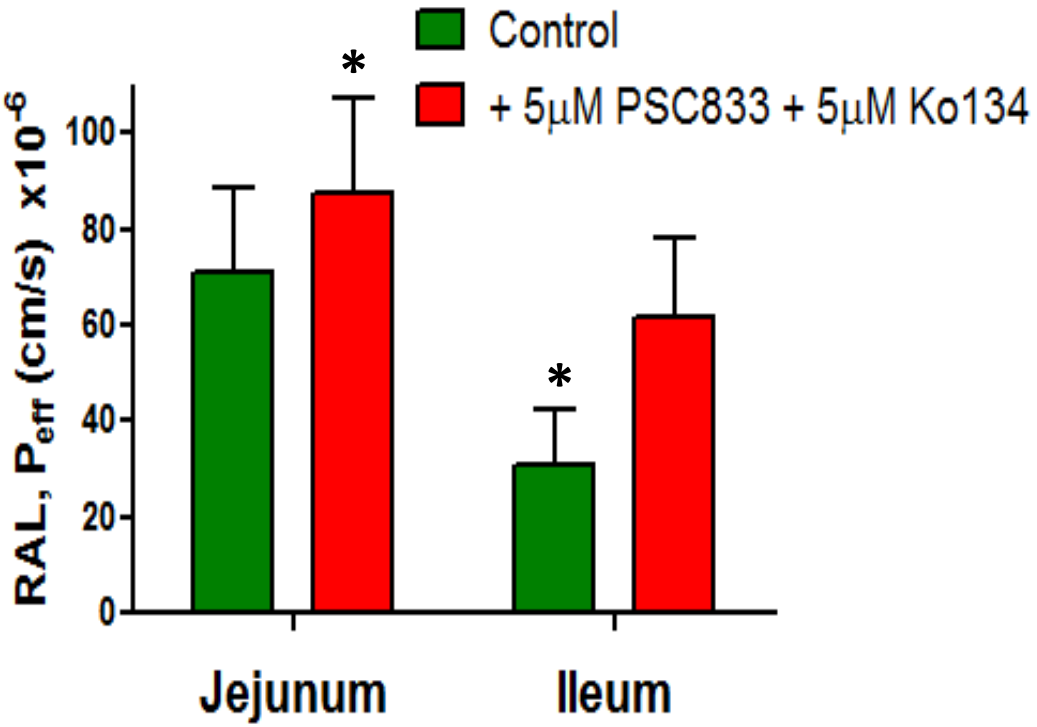
n=3; p <0.05

Efflux Ratio	**3.05**	**1.43**	**3.74**

P-gp and BCRP inhibition significantly increased RAL effective permeability (Peff) in rat







n=4-5 animals; p <0.05

Summary

- RAL does not inhibit P-gp and BCRP mediated transport, however RAL can serve as a substrate of both P-gp and BCRP drug efflux transporters.
- RAL accumulation is significantly enhanced in presence of P-gp or BCRP inhibitor in hCMEC/D3 and TM4 cells, while such an effect is only observed in presence of P-gp inhibitor in Caco2 cells.
- Concurrent administration of P-gp and Bcrp inhibitors, significantly inhibited RAL effective permeability in rat jejunum and ileum *in situ*.
- These results suggest that both P-gp and BCRP could play a role in RAL permeability at blood tissue barriers and penetration into target organs of HIV infection.

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Questions