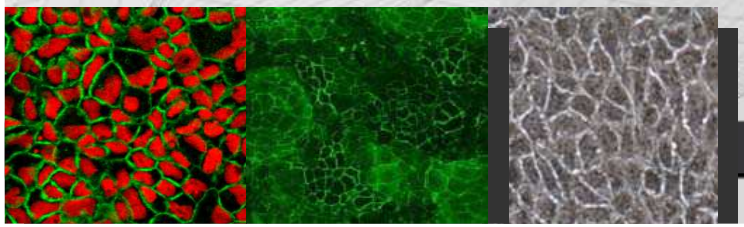
LEITAT Technological Center
managing your technologies
member of TECNIO

Modelos *in vitro* de barreras celulares utilizados para el desarrollo de nuevos fármacos.

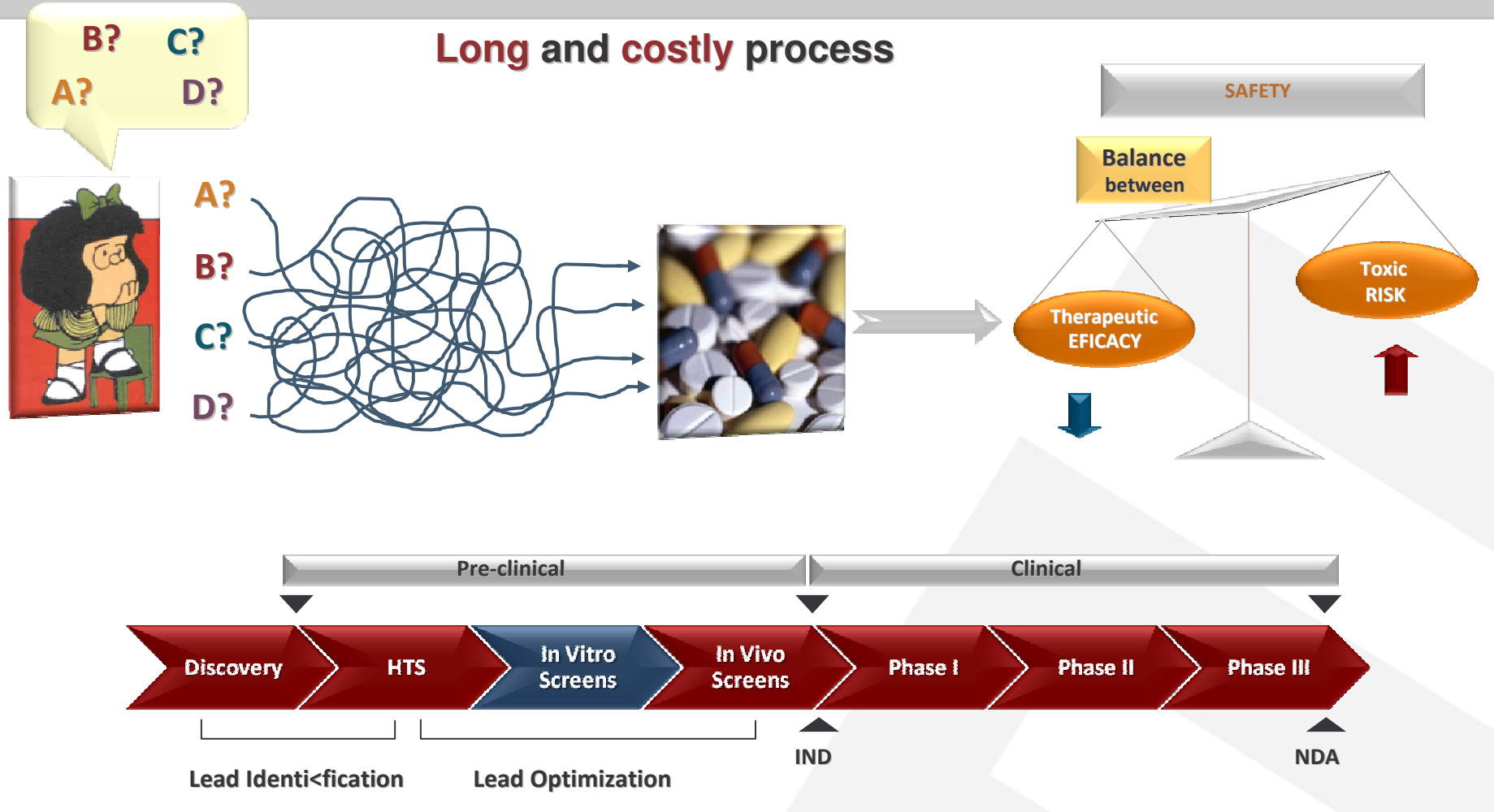
BioInVitro Research Area



Maya Vilà, PhD

5 Abril 2011

Development of new pharmaceutical compounds



Investigational New Drug (IND). New Drug Application (NDA)

ADME/T Properties

A bsorption

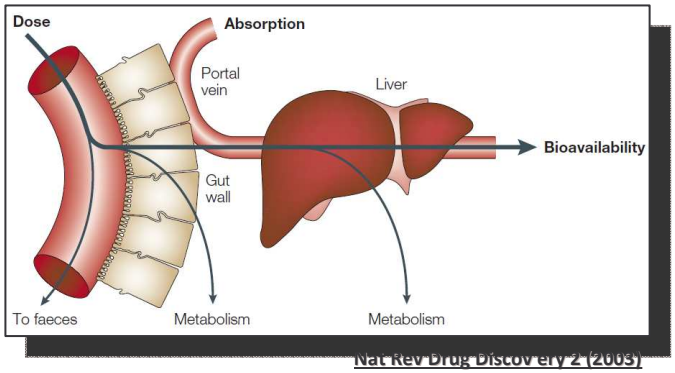
D istribution

M etabolism

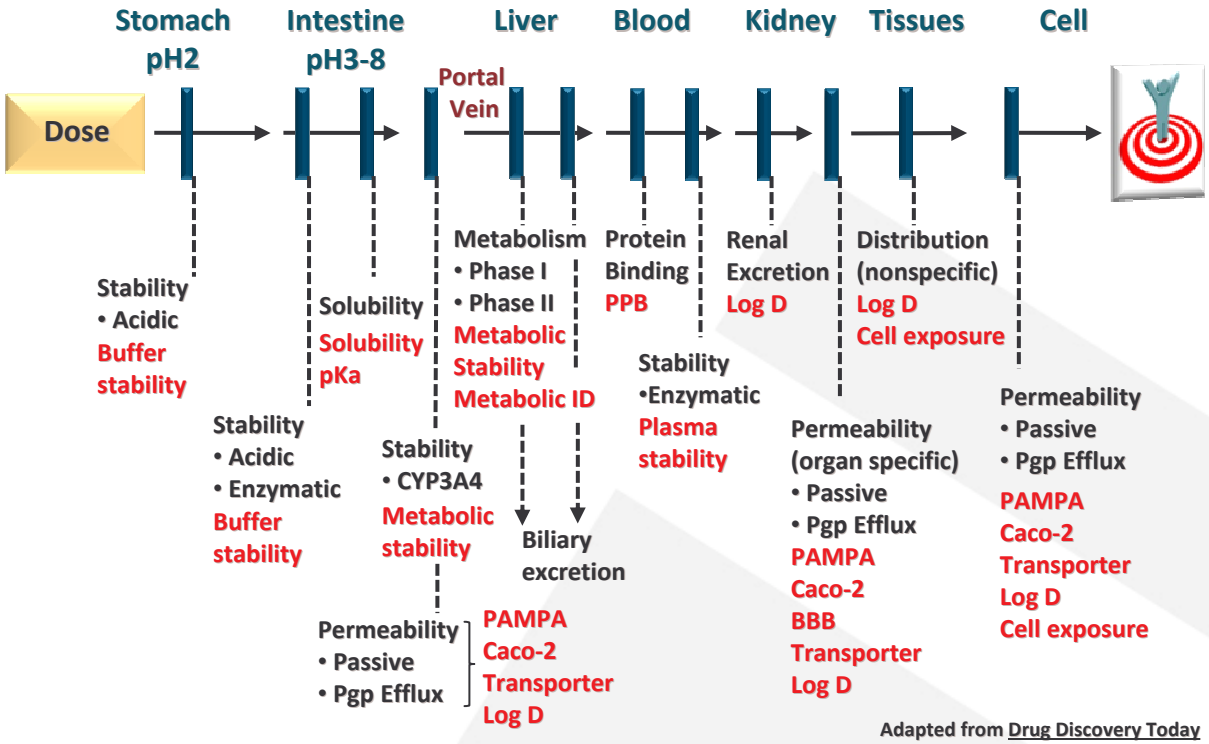
E xcretion

T oxicity

The barriers to target



Nat Rev Drug Discov ery 2 (2003)



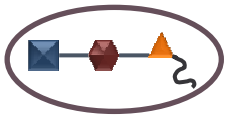
Adapted from Drug Discovery Today

The knowledge of the drug behavior in front of these barriers can help us to know which are the properties we have to improve in the selected candidates

→ ***IN VITRO*** **MODELS**

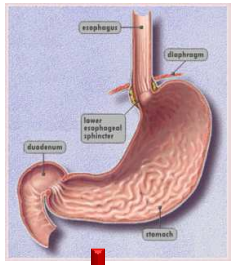
ADME/T Properties

Solubility (A)



Formulated Pill

Solubility (A)
Acidic Stability (A)



Mucosa permeability (A, D)
Efflux transporters (A, D)
Intestinal Metabolism (M)

Intestine

Feces

Bile

Gallbladder

Excretion Inhibition (E)

Portal Vein

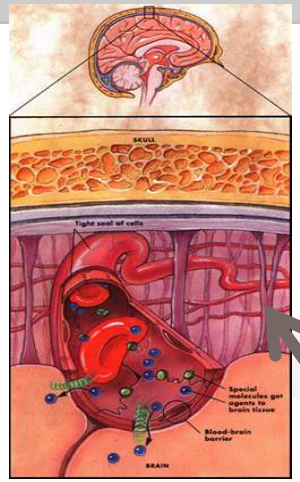
Intestinal wall

Biliary clearance (E)

Phase I and II metabolism (M)

CYP Inhibition (M)
CYP Induction (M)
CYP Phenotyping (M)
Reactive Metabolite (M)

Brain Tissue



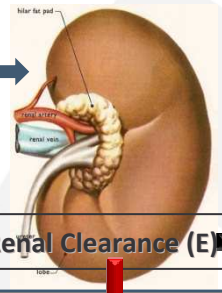
Target neuron cell

BBB

Tissue protein binding(D)
Cell permeability (D)
Efflux Transporters (D)
Tissue metabolisms (M)

Toxic Metabolite (M)

Systemic Circulation



Renal Clearance (E)

Artery

Vein

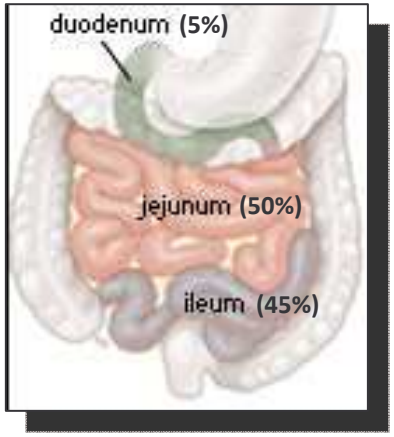
Urine

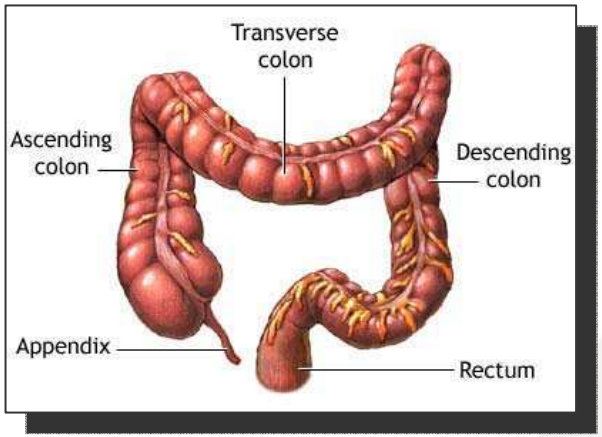
Plasma protein binding (D)
Plasma Stability (M)

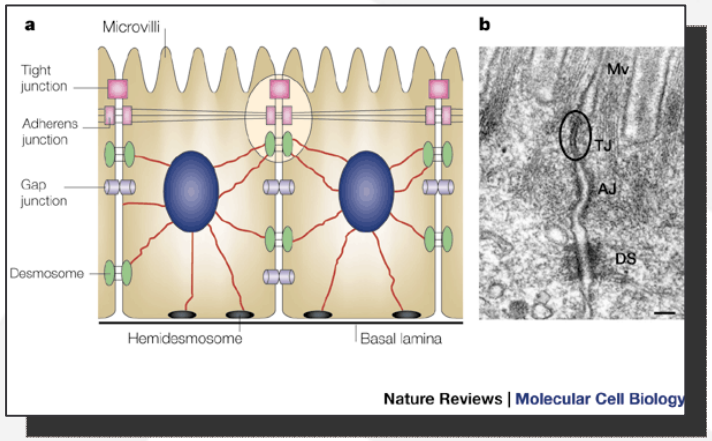
The Gastrointestinal Barrier

Oral absorption

- **Dissolution**
  - **Solubility**
  - **Ionization (pKa)**
  - **Lipophilicity (log D, Log P)**
- **Permeation in the GI tract**







Small Intestine (abpsortive)

Large Intestine (mucosecretor)

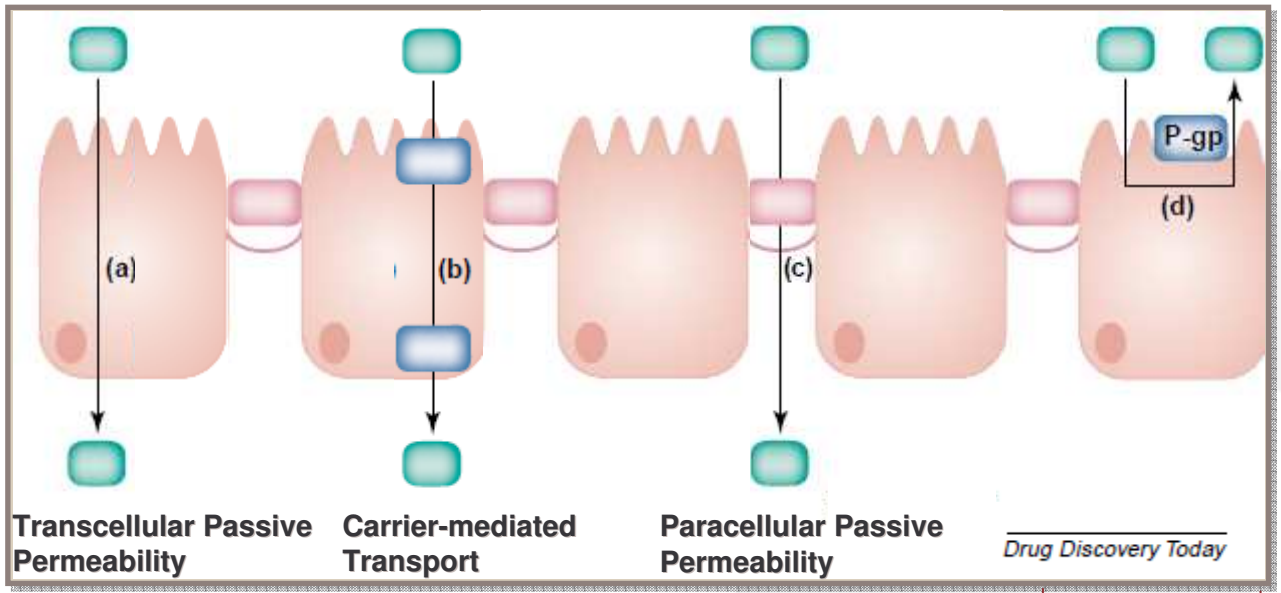
Pathways for intestinal absorption of a compound

Efflux Transporters

Lumen

Intestinal barrier

Blood

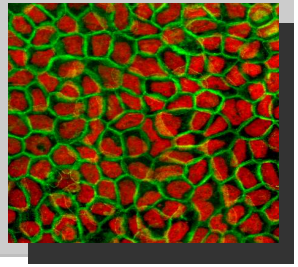


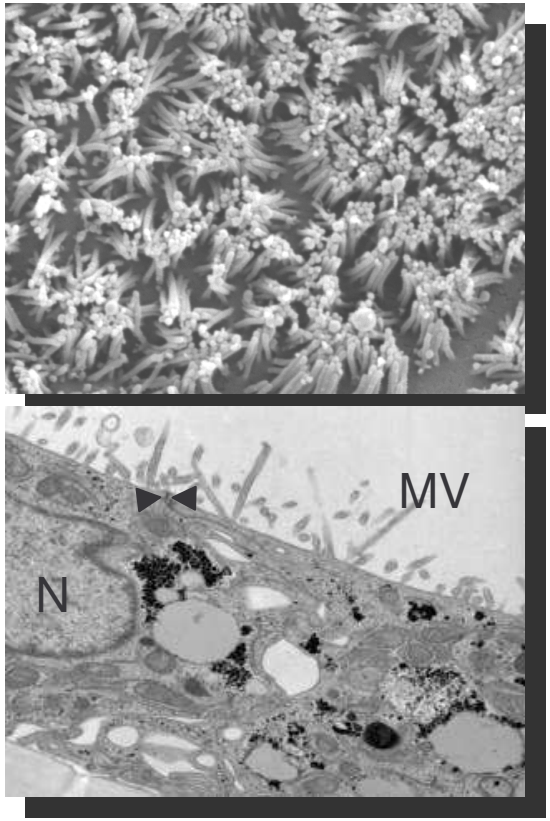
- Lipophilic compounds
- Certain Nutrients
- Vitamins
- Hydrophilic compounds
- Peptides

Limit intestinal absorption

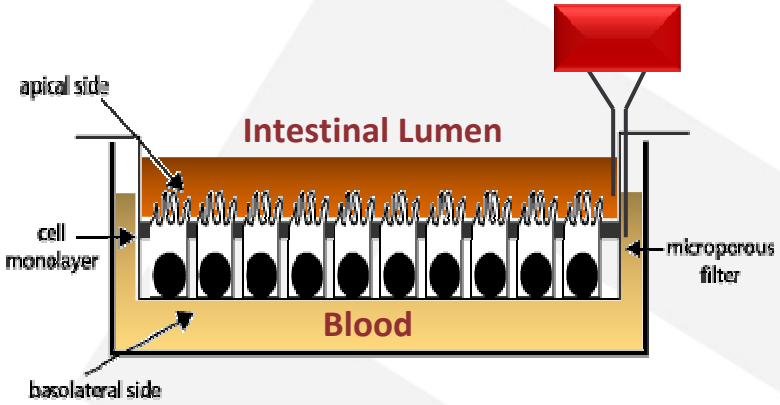
- Substrates of ABC Transporters

Caco-2 Characteristics

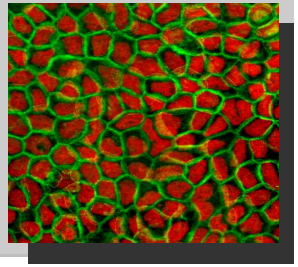




- Grown on polycarbonate filters during 21 days
- Spontaneous differentiation
- Polarized intestinal barrier (absorptive phenotype)
- Barrier integrity parameters
  - TEER
  - LY



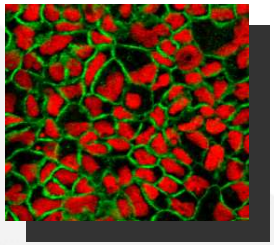
Caco-2 Applications



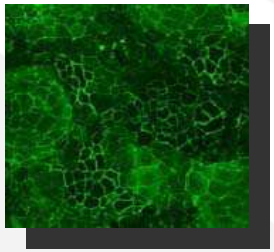
- Evaluation of
  - Oral Absorption efficiency
  - Oral bioavailability
  - Oral toxicity
- Study of mechanisms involved in oral and intestinal absorption
- Study the effects of transporters on permeability
- Evaluation of substrates and inhibitors of Pgp
- Accepted by EMEA and FDA as a predictive model of human *in vivo* intestinal permeability

In Vitro Models of GI Barriers

CACOREADY™ — Differentiated Caco-2 cell barrier



CACOGOBLET — Differentiated co-culture Caco-2 and human goblet cells



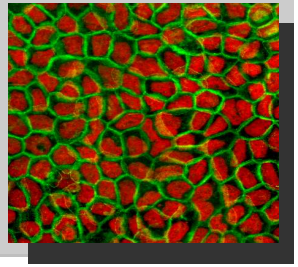
READY-TO-USE

- **Solid** Shipping Medium
- Packaging system

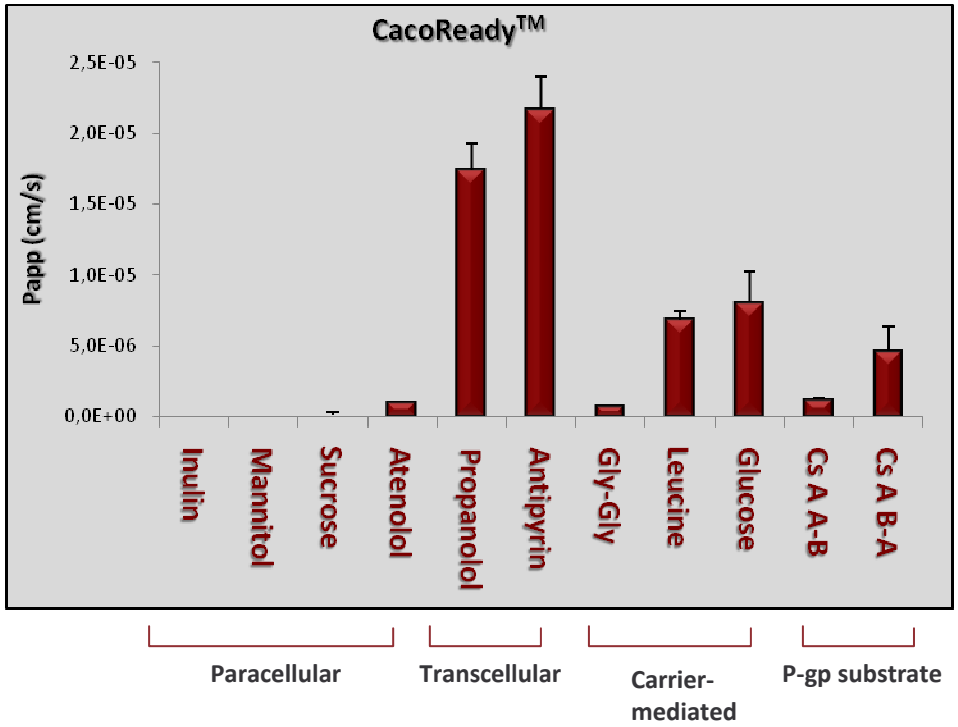
→ Patented ADVANCELL's Technology

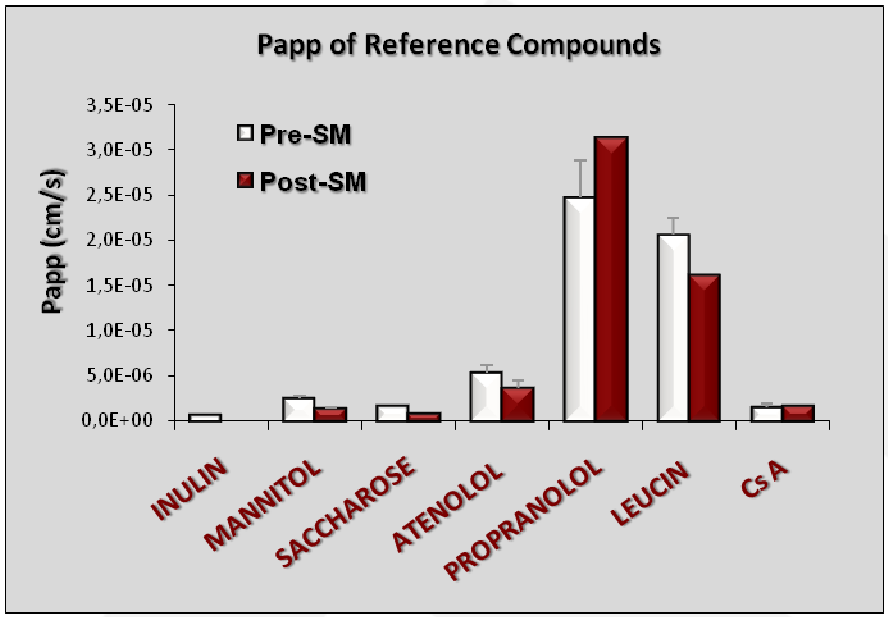
- Storage and Transportation at RT
- Cell functional properties maintained
- Easy-to-use and handle
- Avoiding in house cell culture
- Standard internal quality controls
- Available on demand

BENEFITS



- **Validated system under quality standards**
- **Full cell functionality after transportation**

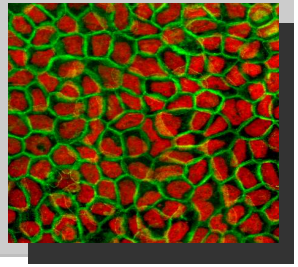




$$P_{app} = \frac{dQ}{Dt \cdot A \cdot C_0}$$

CACO READY™

BENEFITS



MON	TUES	WED	THUR	FRID			MON	TUES	WED	THUR	FRID
D14	D15	D16	D17	D18			D21	D22	D23	D24	D25

TEER/LY control

SM Application

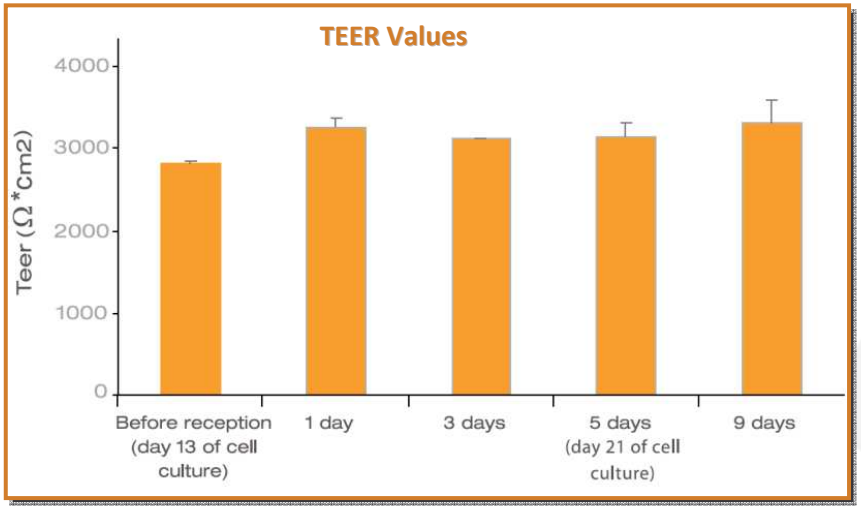
Shipment → Removal of SM

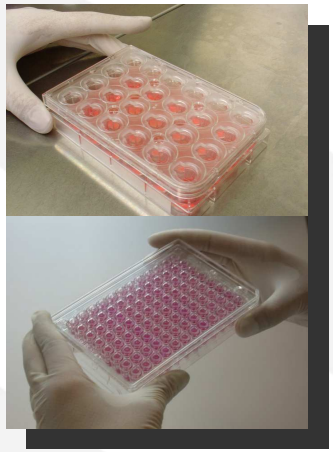
TEER control

Medium change

Permeability Assay

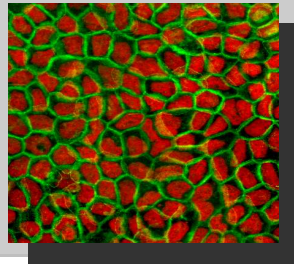
Flexibility: up to 5 days to perform the experiment





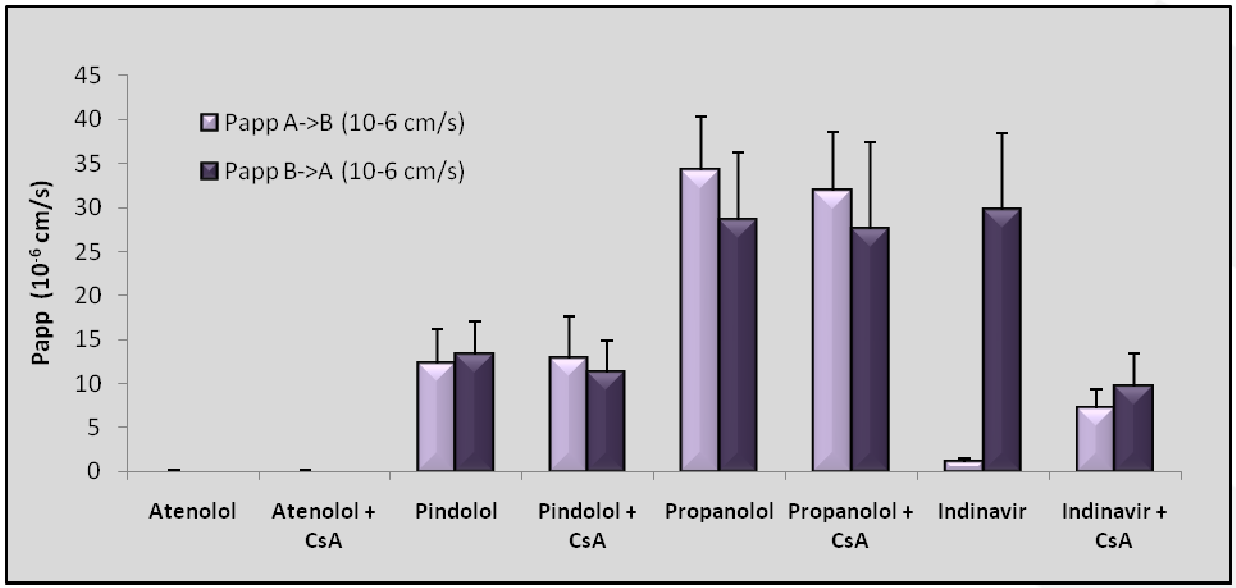
CACO READY™

BENEFITS



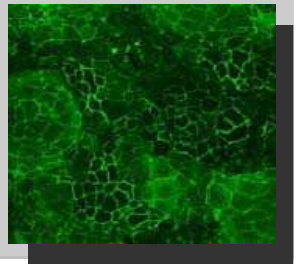
High reproducibility among different batches

Substance	Papp A->B (10^{-6} cm/s)	STD (AB)	Papp B->A (10^{-6} cm/s)	STD (BA)	Efflux Ratio	Permeability	Efflux	Recovery A->B	Recovery B->A
Atenolol	0,1	0,04	0,2	0,04	1,4	LOW	NO	105	100
Atenolol + CsA	0,1	0,1	0,1	0	0,8	LOW	NO	100	99
Pindolol	12,3	3,9	13,3	3,7	1,1	HIGH	NO	103	103
Pindolol + CsA	12,8	4,9	11,3	3,6	0,9	HIGH	NO	104	102
Propanolol	34,3	6	28,6	7,7	0,8	HIGH	NO	96	96
Propanolol + CsA	31,9	6,7	27,6	9,9	0,9	HIGH	NO	94	96
Indinavir	1	0,5	29,7	8,7	28,7	LOW	YES	105	91
Indinavir + CsA	7,1	2,2	9,7	3,7	1,4	Medium	NO	101	98



Data obtained from n=13 independent experiments

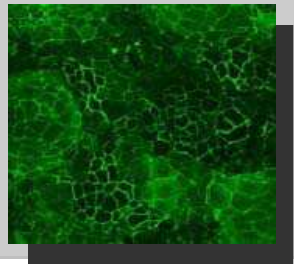
CACOGOBLET



- *In vivo vs. In vitro* absorption of compounds that cross the barrier in a paracellular passive diffusion way: 20-80% lower in Caco-2
- Colonic epithelia is characterized by the presence of mucus

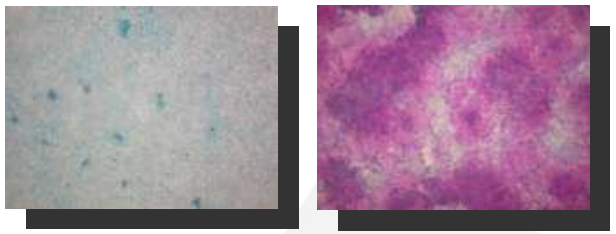
- ADVANCELL has developed a more physiological barrier that mimics the intestinal epithelium based on the co-culture of Caco-2 and Goblet cells

CACOGOBLET



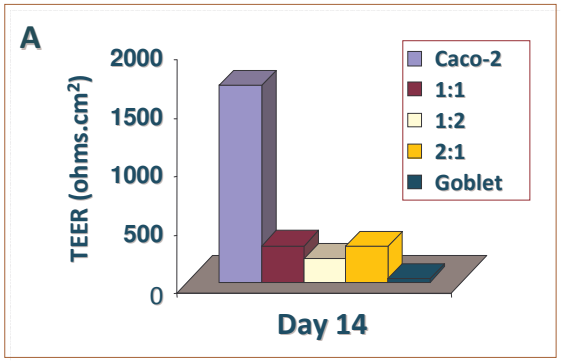
	Human intestine	CacoReady™	CacoGoblet
Composition	absorptive (80%), mucus-secreting (10-30%)	absorptive (100%)	absorptive (50%), mucus-secreting (50%)
Presence of mucus	YES	NO	YES
Paracellular permeability	More permissive epithelium	Very tight epithelium	More permissive epithelium
TEER (ohm.cm²)	20-110	1000-2000	50-120

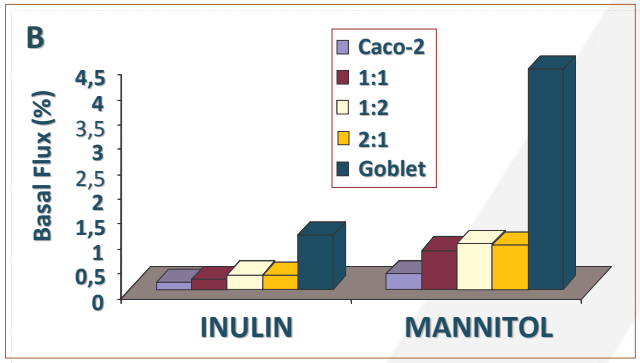
MUCUS SECRETION



Alcian Blue staining CacoReady™ 21 days

Alcian Blue staining CacoGoblet 21 days





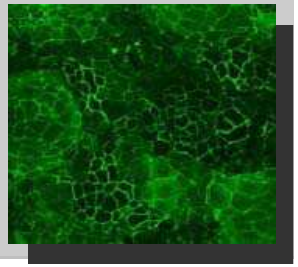
TEER (Variability Study)

Mean	SD	CV (%)
59.19	0.87	1.47

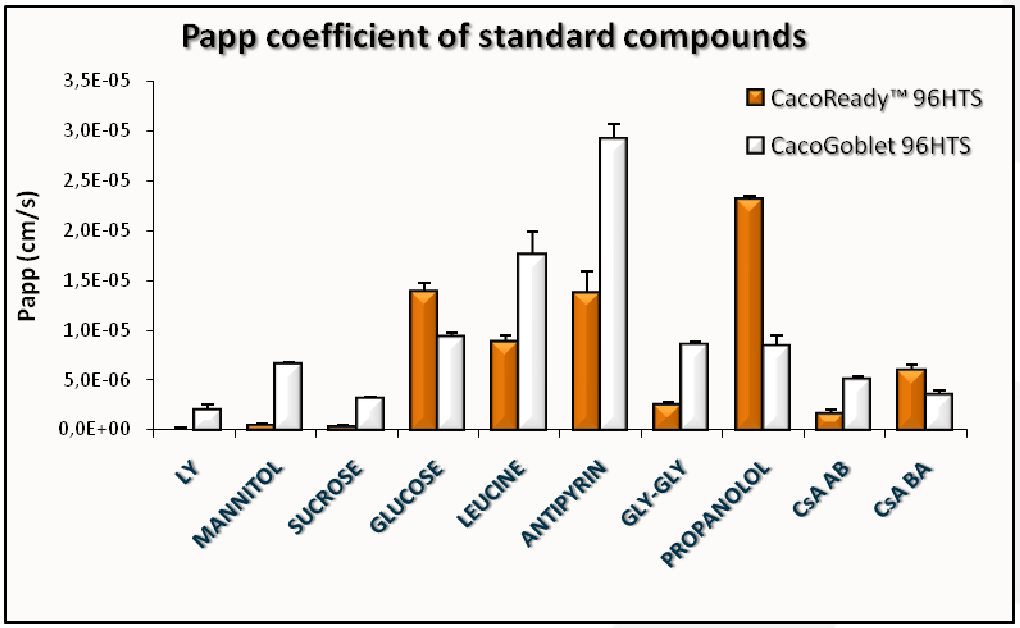
LY (Variability Study)

Mean	SD	CV (%)
2.09E-6	2.62E-7	12.54

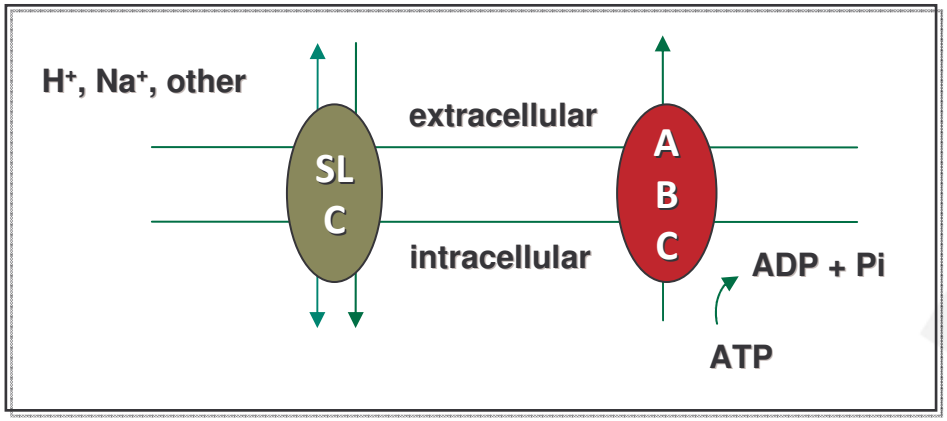
CacoGoblet system leads to a more permissive epithelium which is more similar to physiological conditions



Comparison of std compounds



The Role of Transporters in ADME/T



Intestinal absorption

Distribution

- CNS (BBB, blood – CSF barrier)
- Fetus (B-placenta barrier in syncytiotrophoblast)
- Testis (Btestis barrier)

Modulation of metabolism

Drug-drug interaction

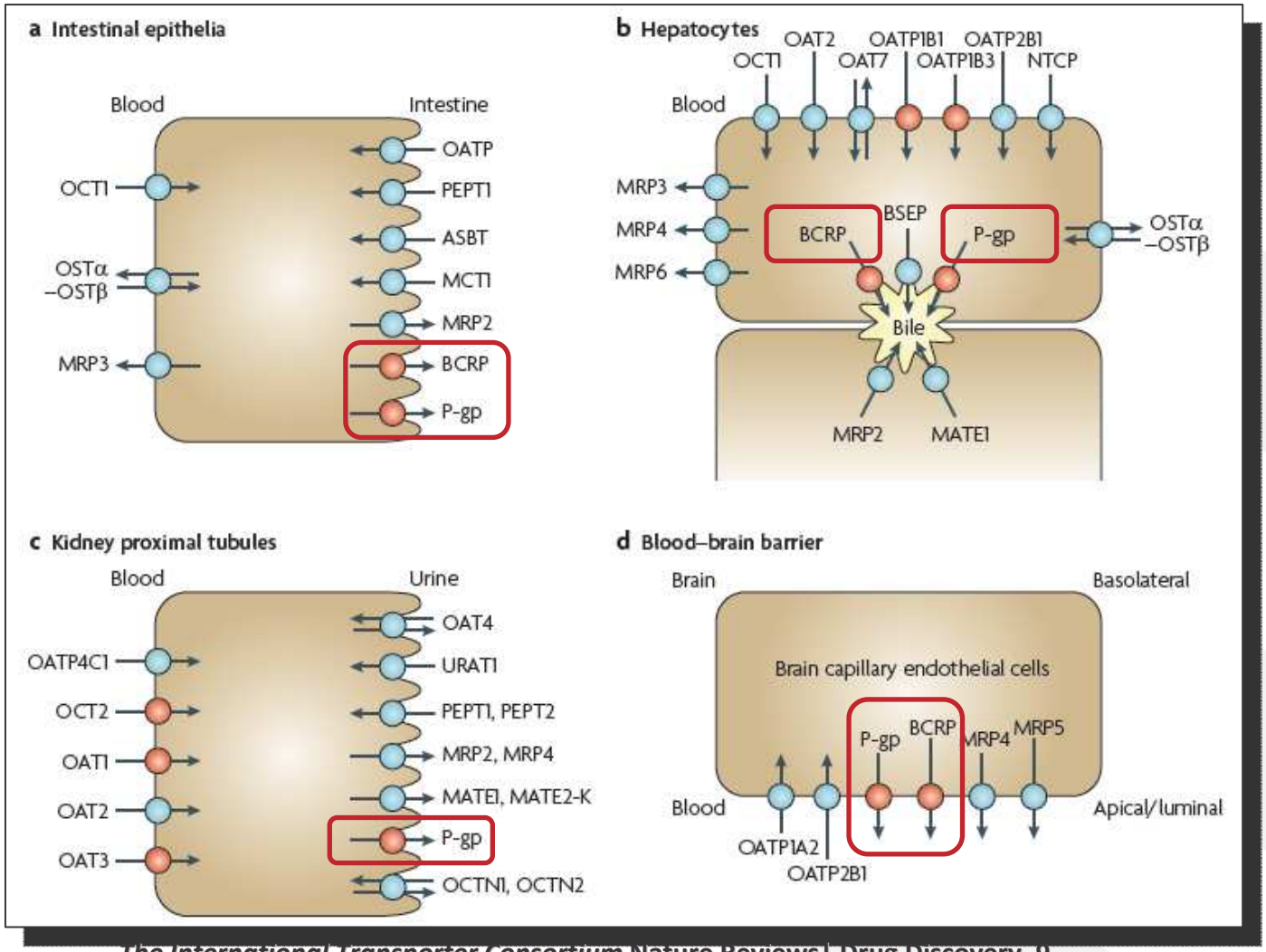
Excretion

- Intestinal
- Liver
- Kidney

Toxicity

Interference with transport and metabolism of endogenous substrates

Role of Transporters in physiological barriers



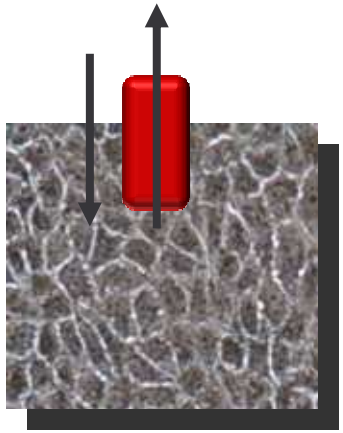
The International Transporter Consortium Nature Reviews| Drug Discovery, 9 (2010)

PREADYPORT™

In collaboration with SOLVO Biotechnology

PREADYPORT™-MDR1

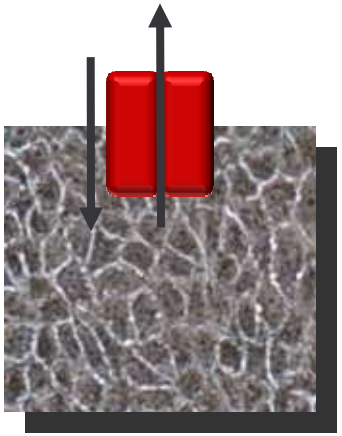
MDR1



Intestine
Liver
BBB
Kidney

PREADYPORT™-BCRP

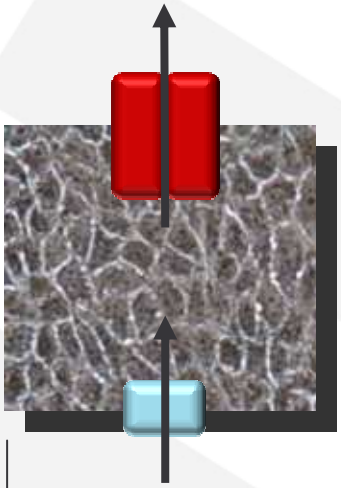
BCRP



Intestine
Liver
BBB
Testis

can be used for regulatory submission studies

BCRP/OATP2B1



Liver
Placenta

suitable for studying low permeability substrates

SOLVO BIOTECHNOLOGY

PREADYPORT™

The kit can be used up to 7 days after reception

D1			D4	D5	D6	D7	D8			D11	D12	D13	D14	D15
FRID			MON	TUES	WED	THUR	FRID			MON	TUES	WED	THUR	FRID

Seeding

TEER int control

SM Application

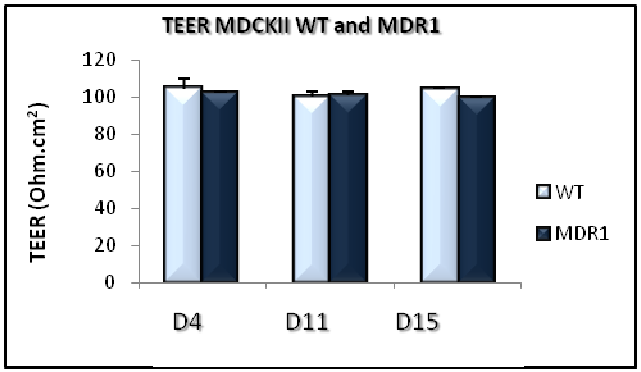
Shipment → Removal of SM

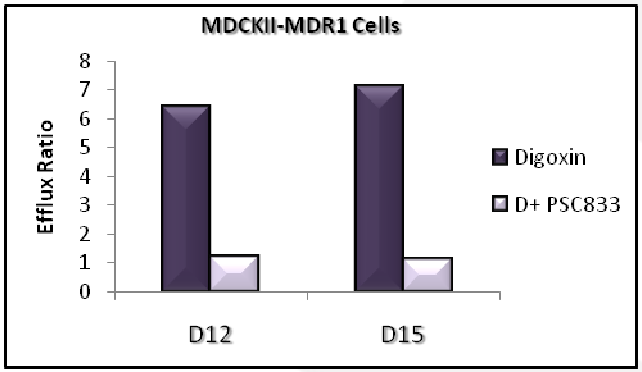
TEER control

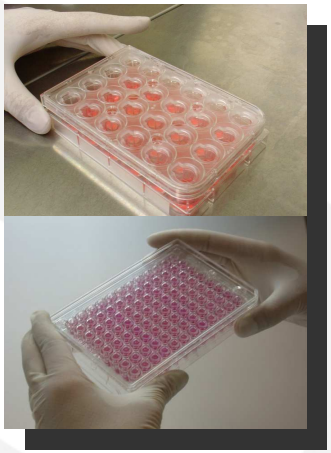
Medium change

Transport Assay

Flexibility: up to 4 days for transport measurements



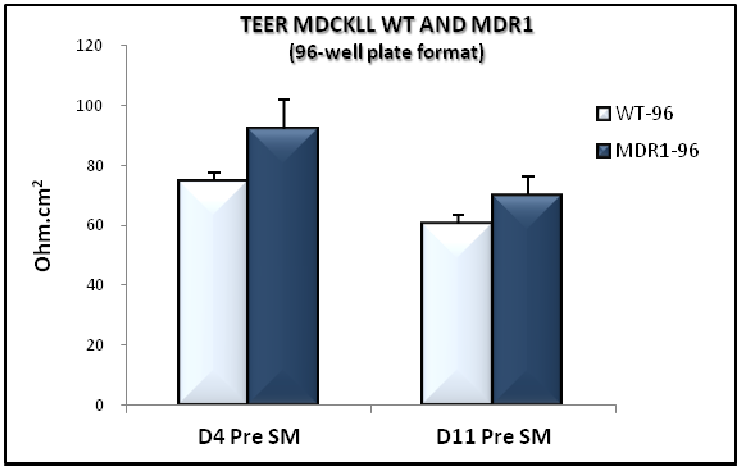




SOLVO BIOTECHNOLOGY

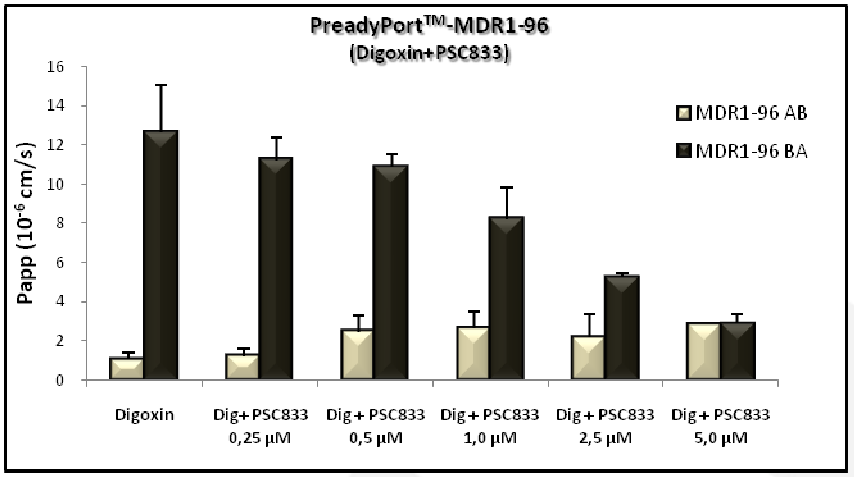
PreadyPort™-MDR1

TEER values

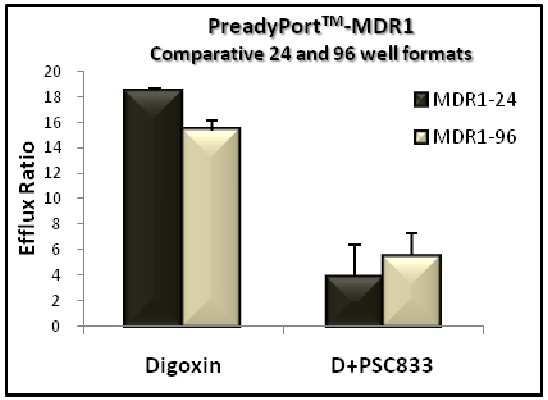


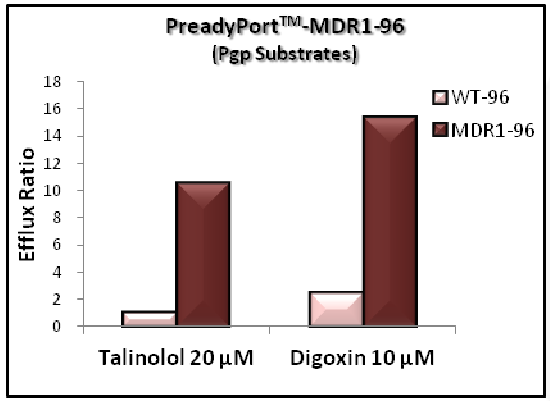
n=3 independent experiments

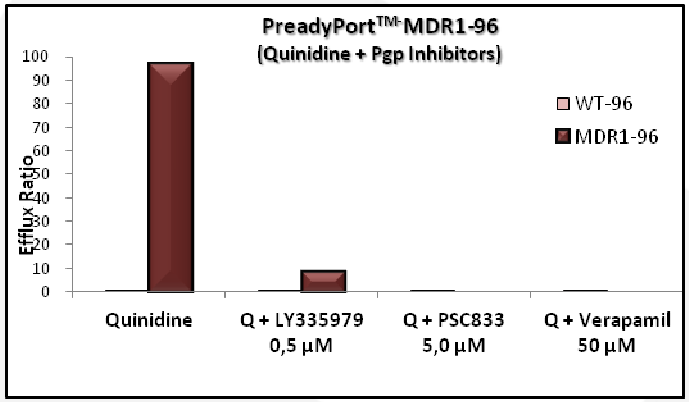
Inhibition Curve



Efflux ratio values for known P-gp/MDR1 Substrates and Inhibitors

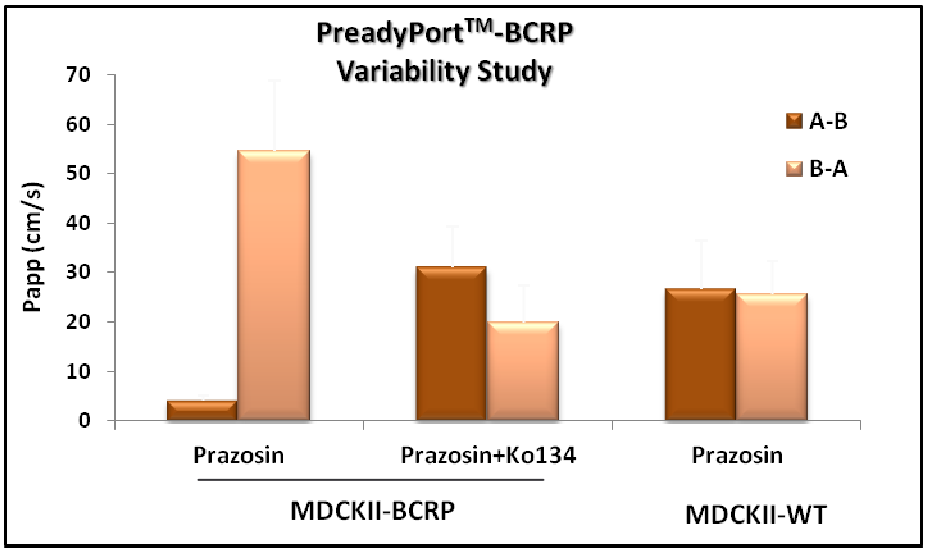


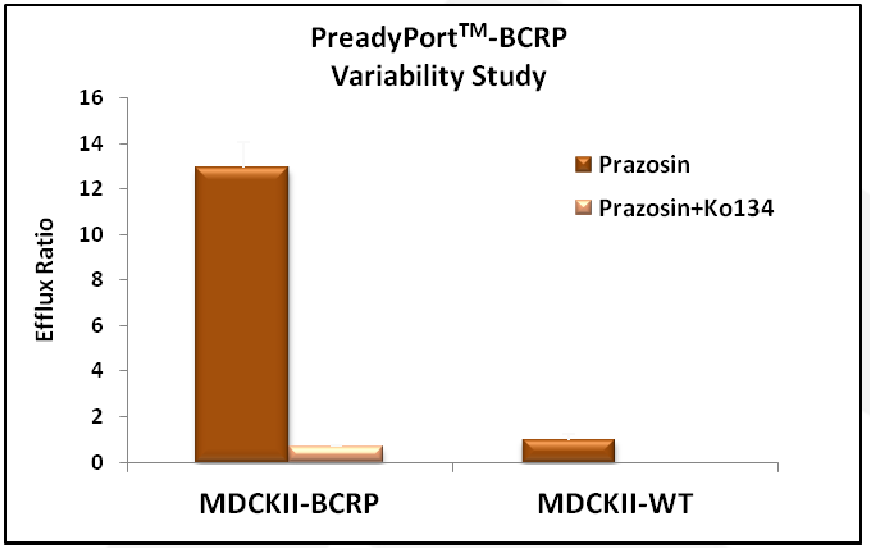




n=3 independent experiments

PreadyPort™-BCRP

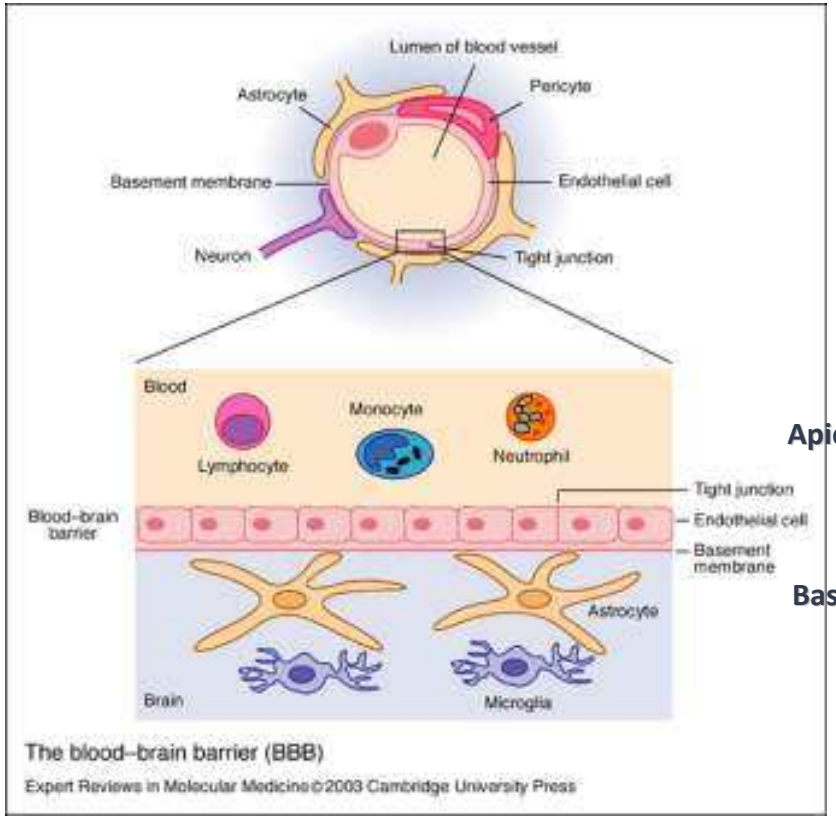




Blood Brain Barrier (BBB)

BBB *in vitro* model

Coculture of endothelial cells with astrocytes (12 days)



The blood–brain barrier (BBB)

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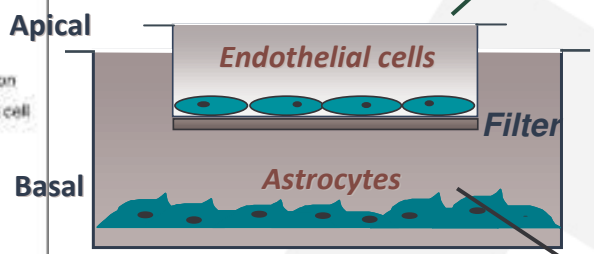

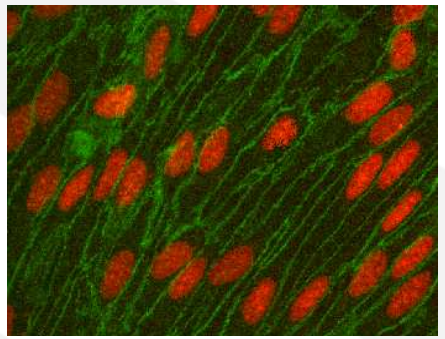

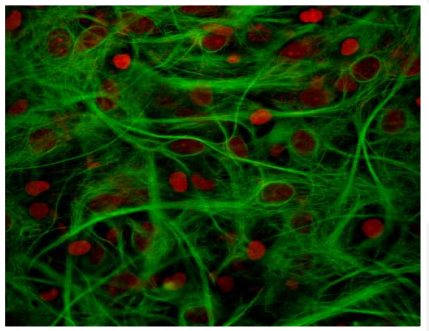

Thanks for your attention!