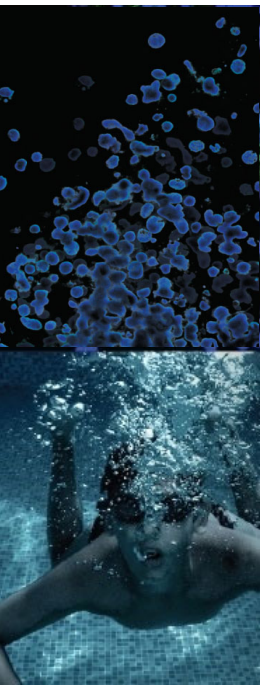
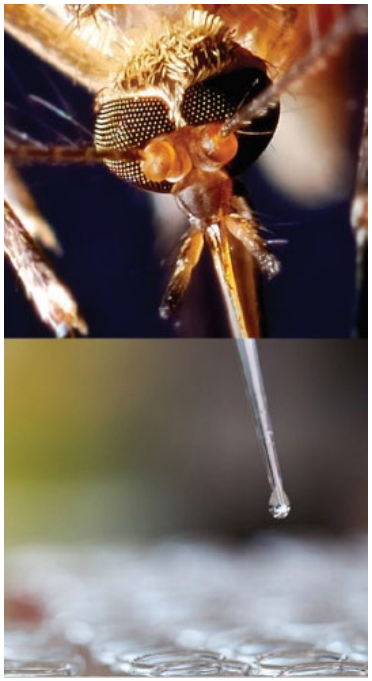
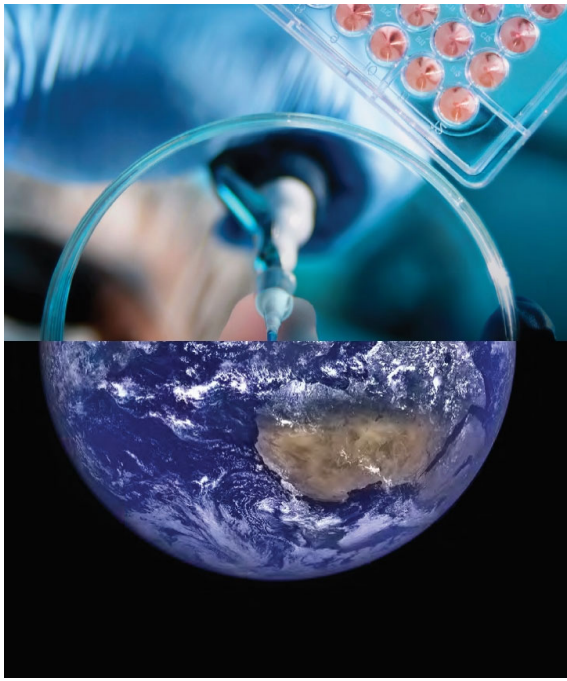
Kidney SLC Transporter Cells – Reliable Tools for Assessing Renal Solute Passage and Drug Toxicity

Kevin Grady, BS
Manager, Product Management, ATCC

Credible Leads to Incredible™







About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring gene editing, differentiated stem cells, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

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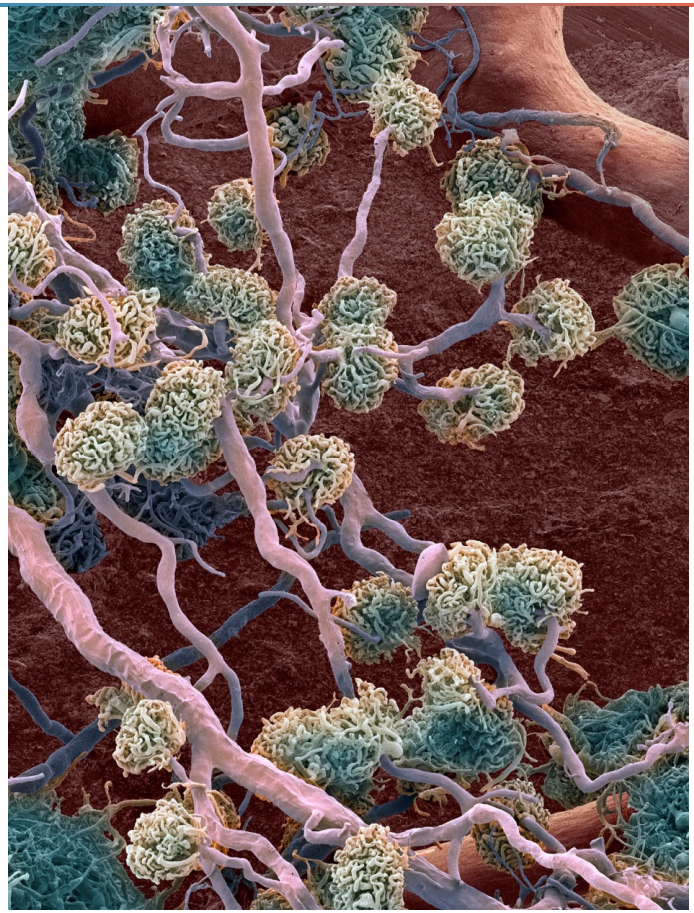
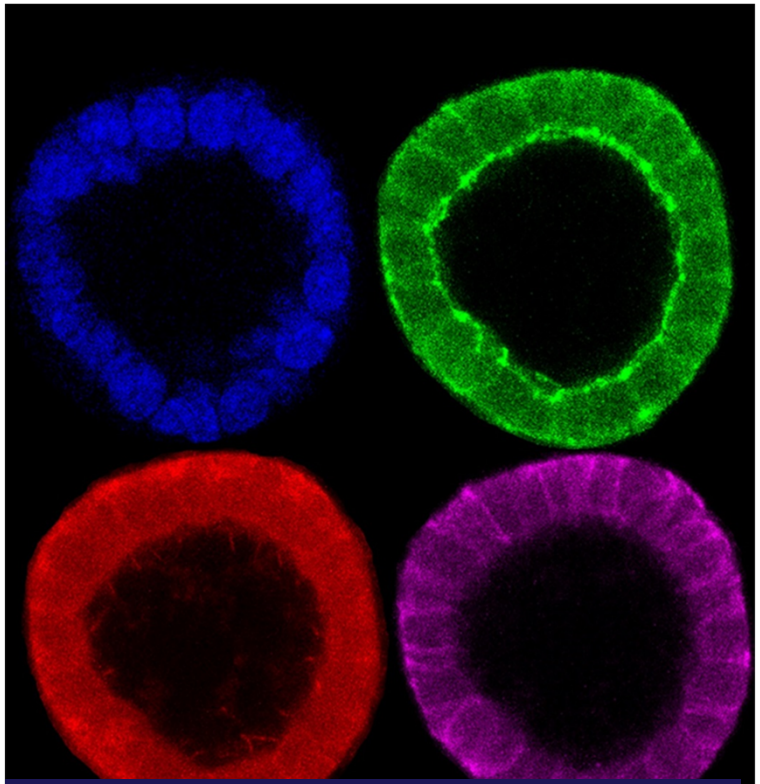
Agenda

- Renal transport
- Current renal models
- Generation of RPTEC renal uptake models
- Application data
- Summary



Renal transport proteins

- Play important roles in drug absorption, distribution, and elimination
- Can be divided into 2 classes:
  - The ATP-binding cassette (ABC) family, most are efflux transporters
  - The solute carrier (SLC) family, most are influx transporters, some are efflux and bidirectional
- Expression and activities are significant determinants for:
  - Drug disposition
  - Drug-drug interactions
  - Variability in drug response and toxicity
- Focus of today's study:
  - Organic anion transporter protein 1 (OAT1)
  - Organic cation transporter protein 2 (OCT2)
  - Organic anion transporter protein 3 (OAT3)



Kidney epithelial cells recapitulate *in vivo* tubule formation, image courtesy of Moe Mahjoub

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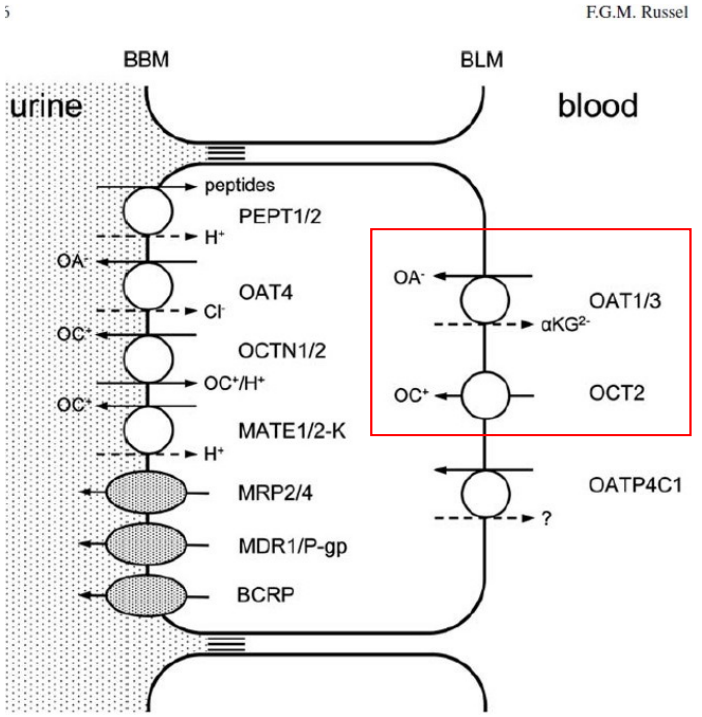
Kidney cells – Role of OAT1/OCT2/OAT3

- Challenge: Expression of organic solute carrier transporters is lost in primary kidney cells
- Organic anion and cation transporters are vital in kidney metabolism
  - OAT1
  - OCT2
  - OAT3
- FDA guidance recommend evaluation of Oat/Oct transporter interactions:
  - In Vitro Metabolism-and Transporter- Mediated Drug-Drug Interaction Studies - (*draft)* Guidance for Industry (2017)
  - Clinical Drug Interaction Studies: Study Design, Data Analysis, and Clinical Implications – (*draft*) Guidance for Industry (2017)



Pang K, et al. Enzyme- and Transporter-Based Drug–Drug Interactions. DOI 10.1007/978-1-4419-0840-7_2,C Am Assoc Pharmaceut Sci 2010

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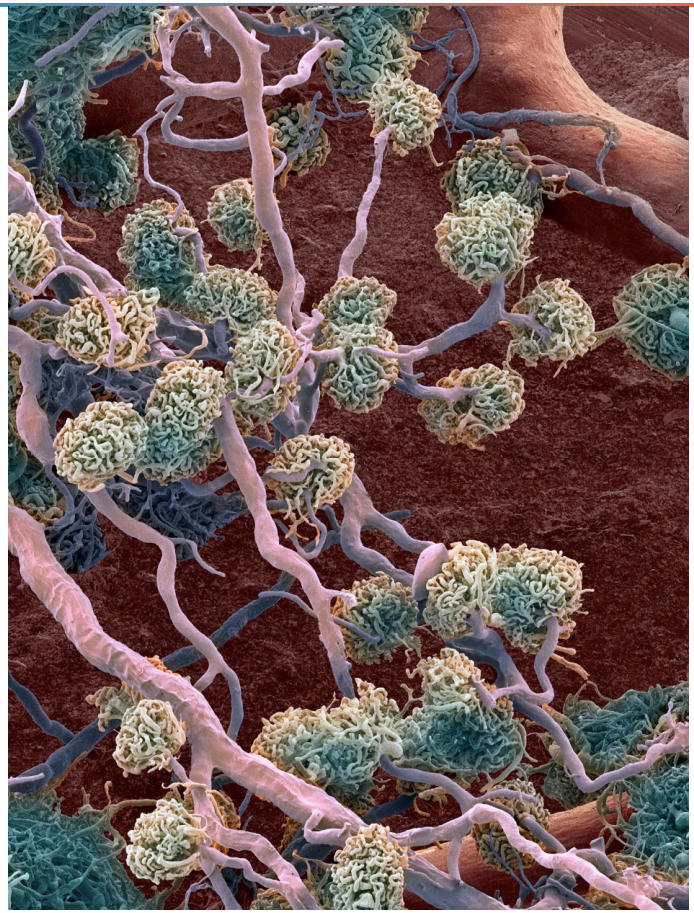
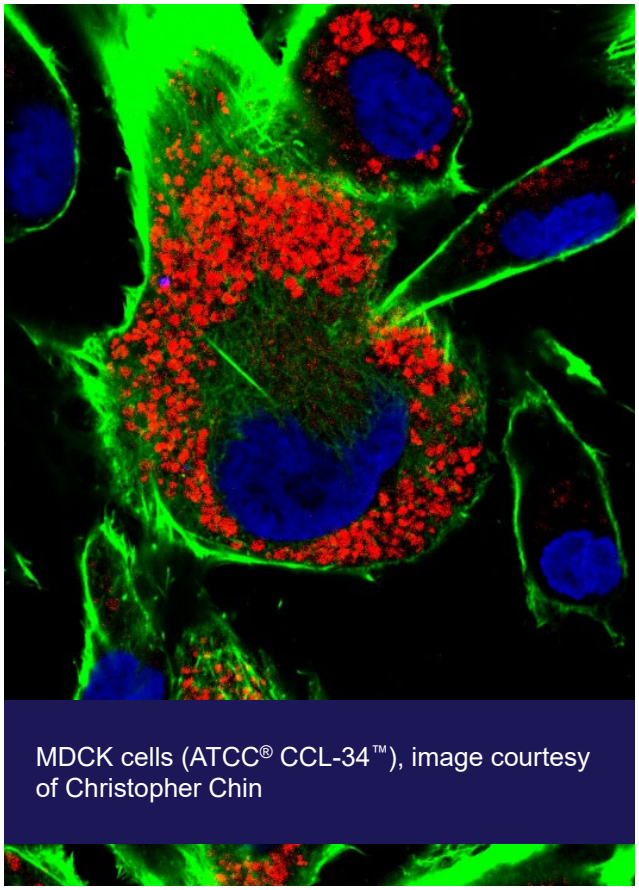


Cell lines and primary cell-derived models

Problems with primary kidney cell models:



MDCK cells (ATCC® CCL-34™), image courtesy of Christopher Chin

Cell Lines:

- Problems with these lines:
  - Do not have the human kidney tissue origination
  - The cell line itself is a cancer line
- Therefore, the clinical predictability is greatly compromised

Primary Cells:

- Obtaining primary cultures is difficult
  - Homogeneous cultures retaining physiological functions are hard to obtain
- Primary RPTEC lose OAT1, OCT2, and OAT3 expression in culture
- Transiently expressing transporters in primary RPTEC show large variations between production lots
  - Makes the data hard to interpret

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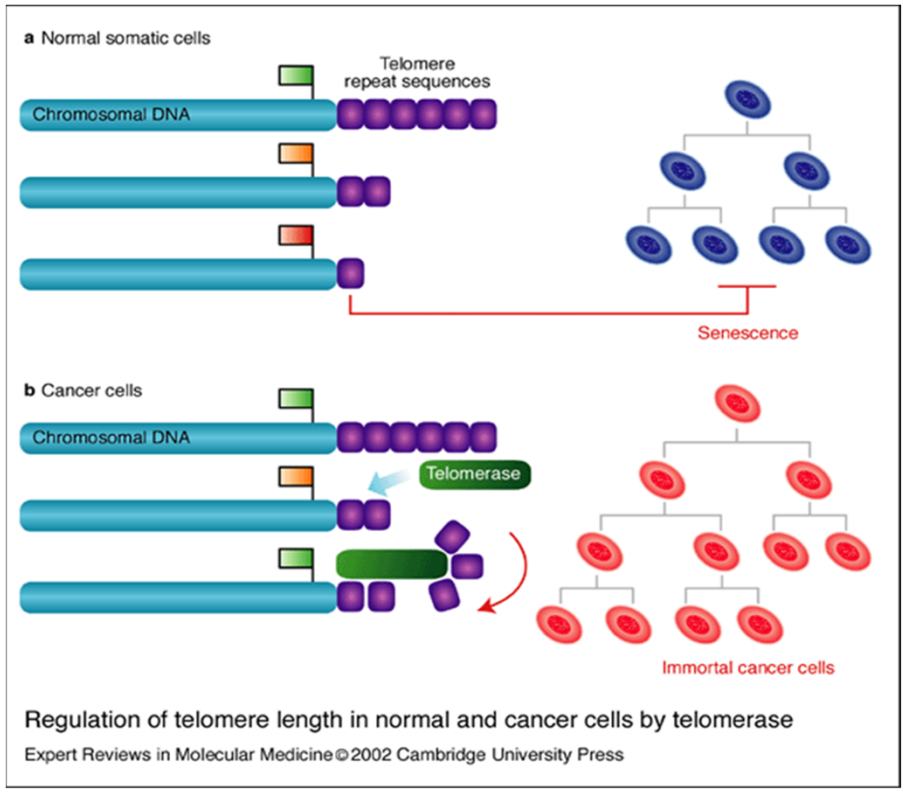
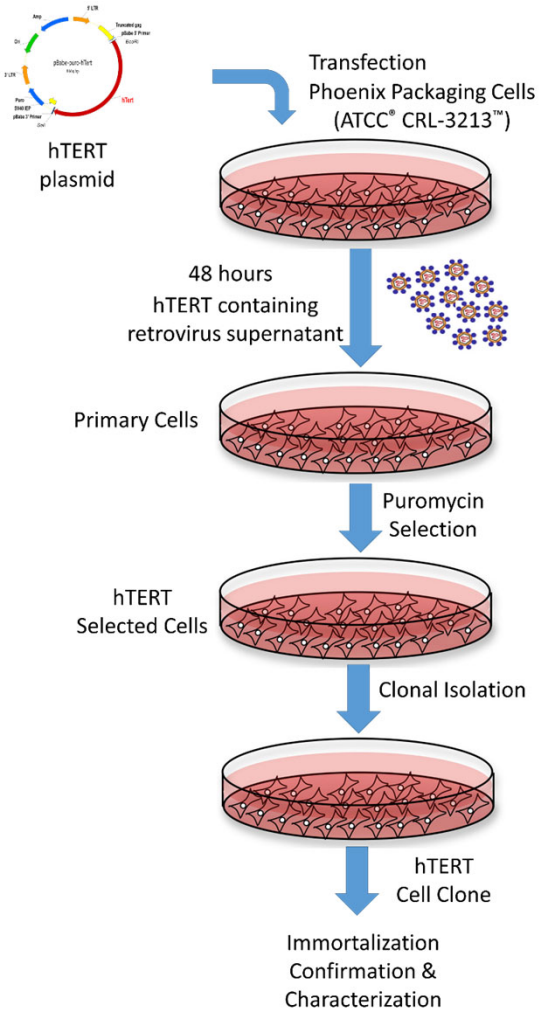
hTERT immortalization technology



Regulation of telomere length in normal and cancer cells by telomerase

Expert Reviews in Molecular Medicine ©2002 Cambridge University Press



Characteristics of various cell models

	Continuous (cancer) cell lines	Primary cells	hTERT-immortalized primary cells
Mimic *in vivo* characteristics	+	++++	+++
Proliferative capacity	+++	+	+++
Experimental reproducibility	+++	+	+++
Predictability in toxicological studies	+	+++	+++
Genomic stability	Aneuploid	Diploid	Diploid/near diploid
Supply	+++	+	+++
Cost	+++	+	++
Ease of use	+++	+	++

hTERT-immortalized primary cells avoid the limitations of primary cells and continuous cell lines while reaping the benefits of both!

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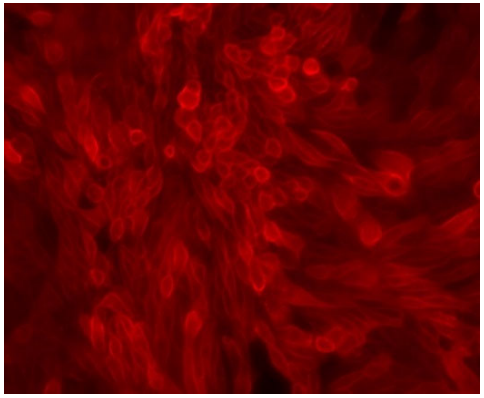
Kidney models

Renal proximal tubule epithelial cells

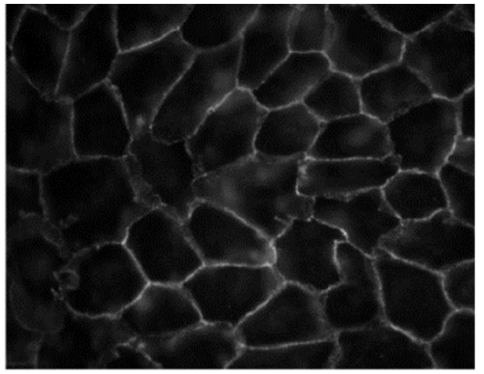
- Primary renal proximal tubule epithelial cells
- hTERT-RPTEC – immortalized renal proximal tubule epithelial cells

- Key characteristics:
  - Uniform expression of E-cadherin and CD13 (aminopeptidase N)
  - Formation of dome-like structures
  - Stabilized transepithelial electrical resistance (TEER)

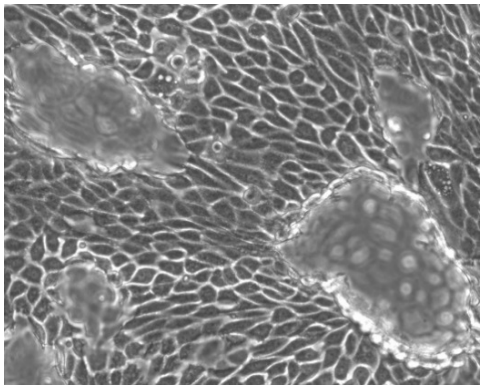
RPTEC/TERT1: CD13

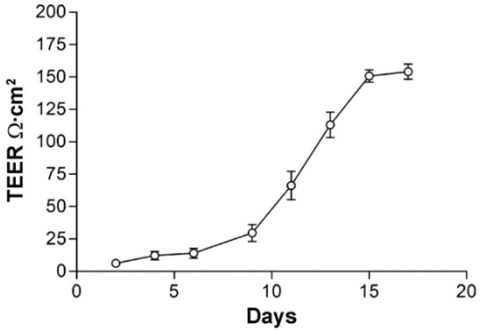


RPTEC/TERT1: E-cadherin



Dome formation





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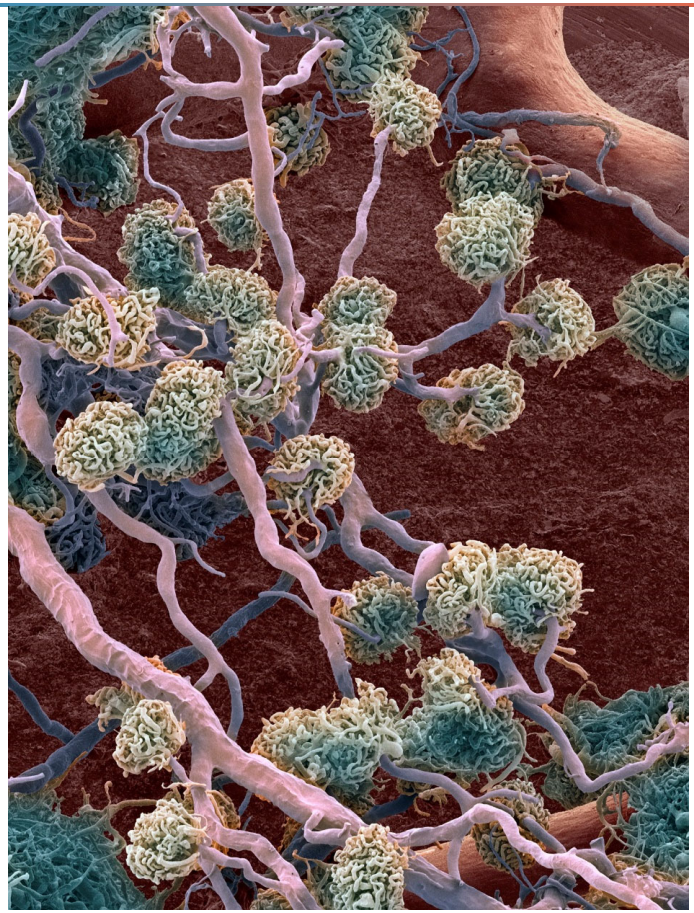
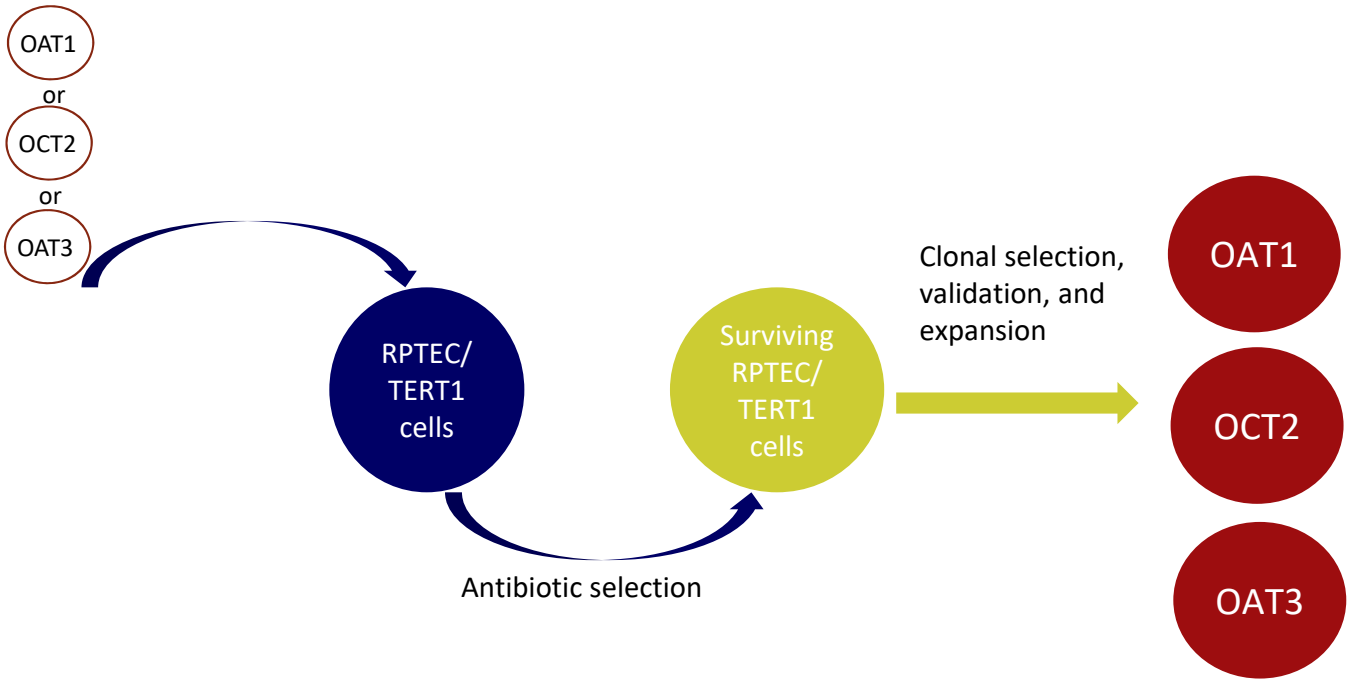
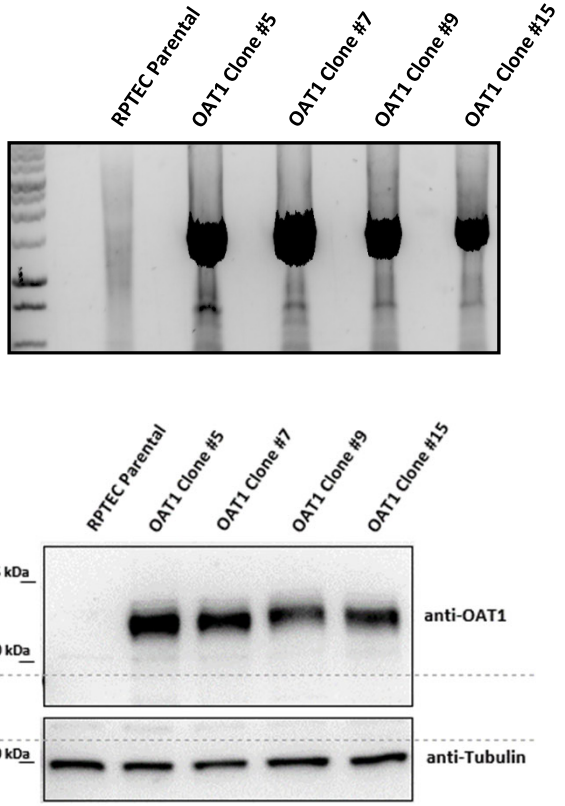


Enhanced kidney cellular models





- RPTEC/TERT1
- RPTEC/TERT1 OAT1
- RPTEC/TERT1 OCT2
- RPTEC/TERT1 OAT3

Characterized by
RT-PCR, WB, sequencing
(copy number verified)

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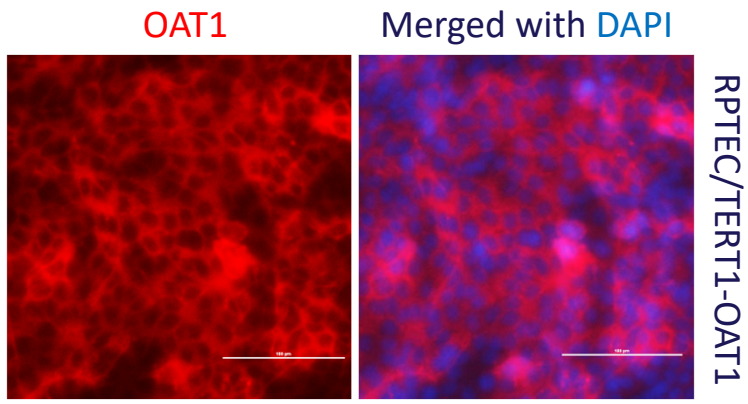
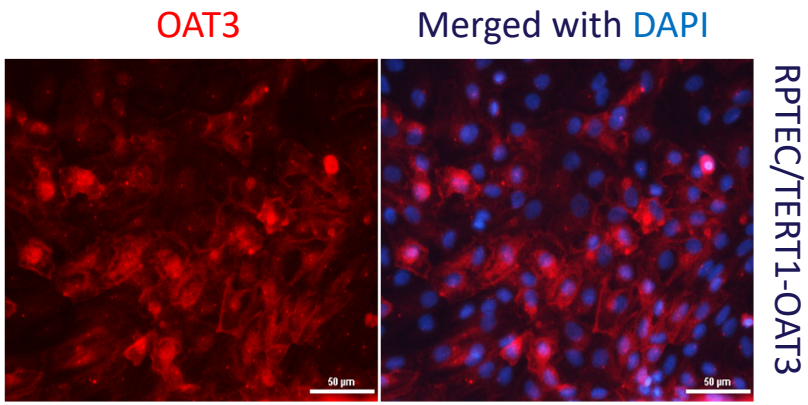
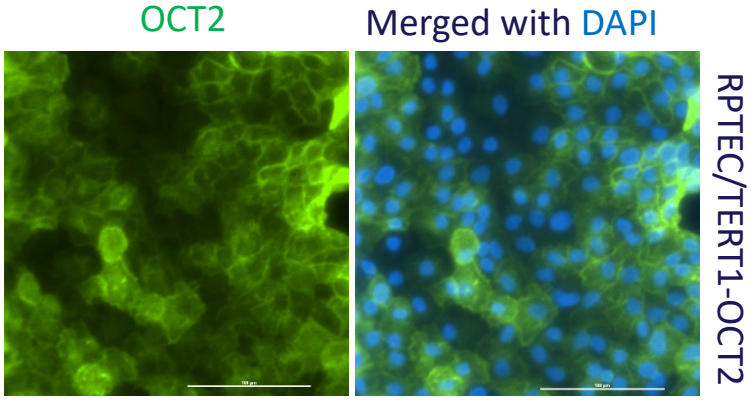
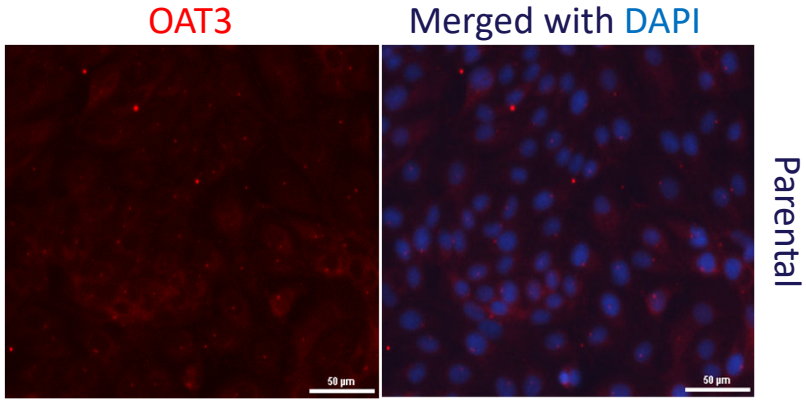
Expression and localization of OAT1/OCT2/OAT3

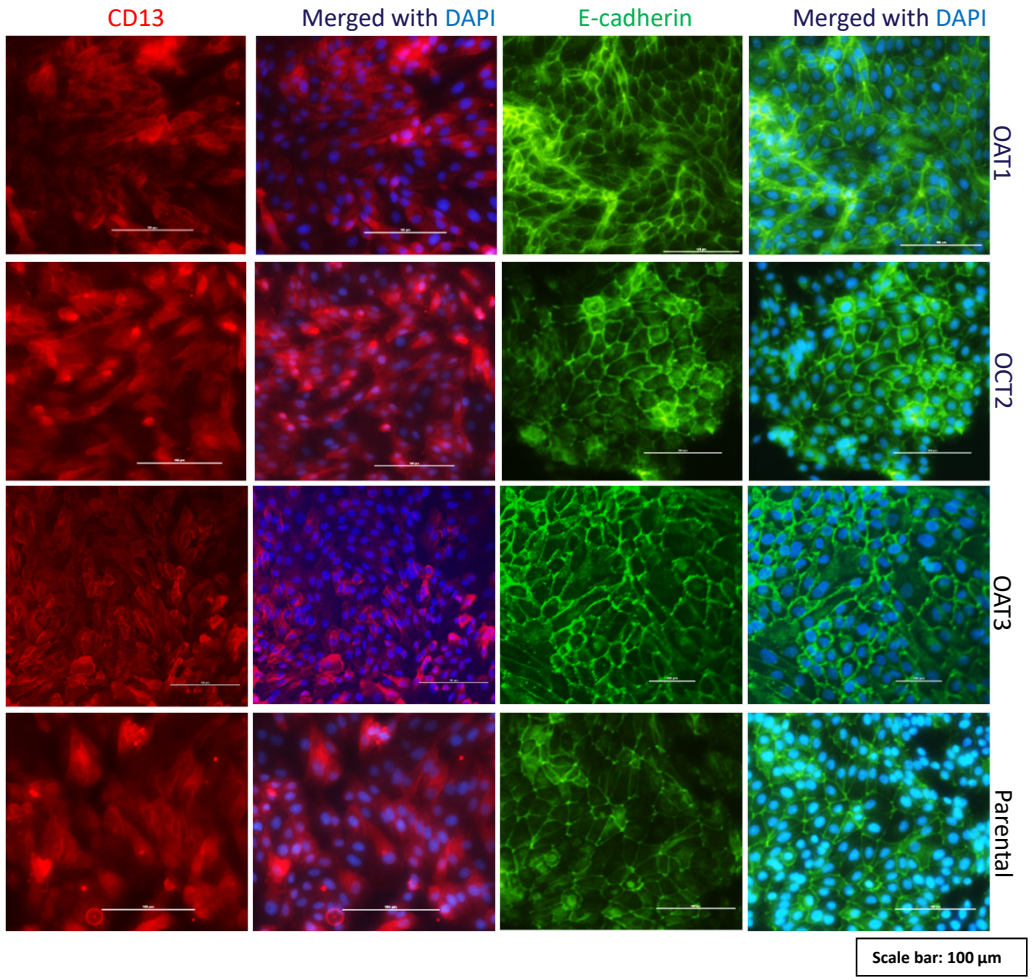


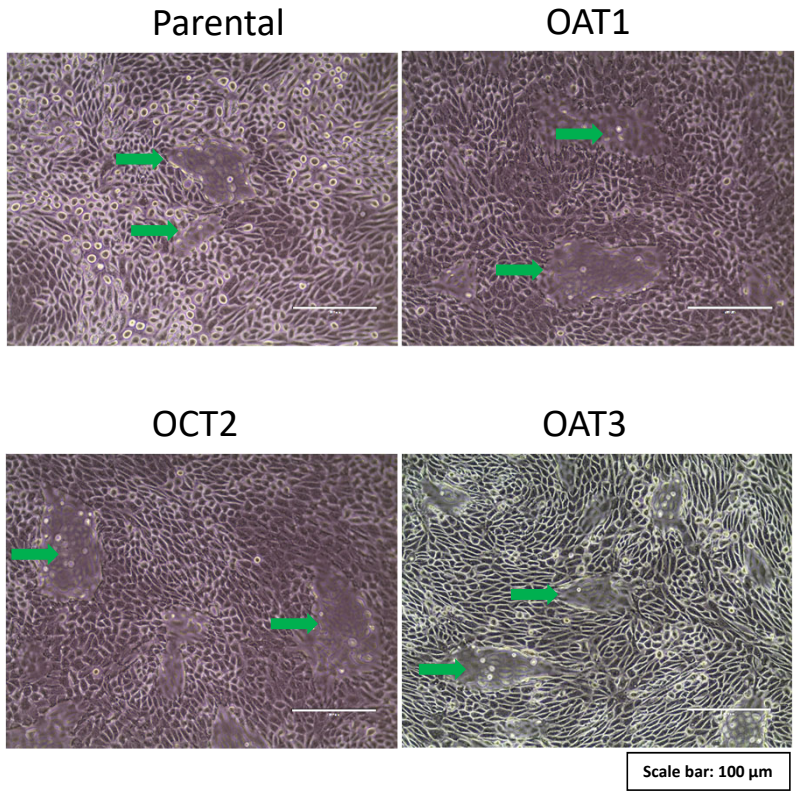






Endogenous marker expression and dome formation





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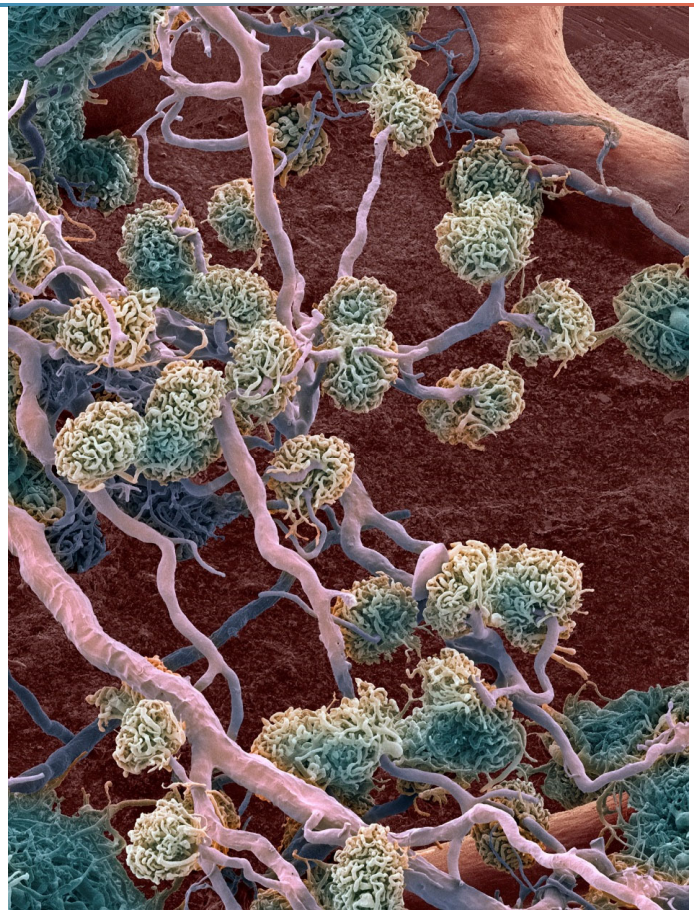
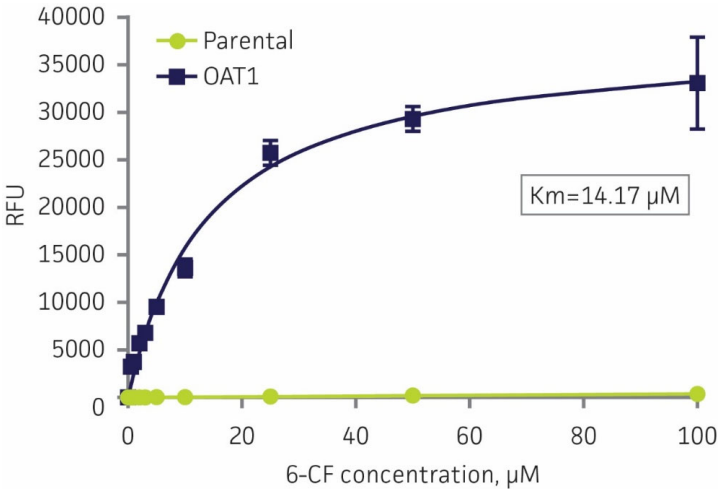
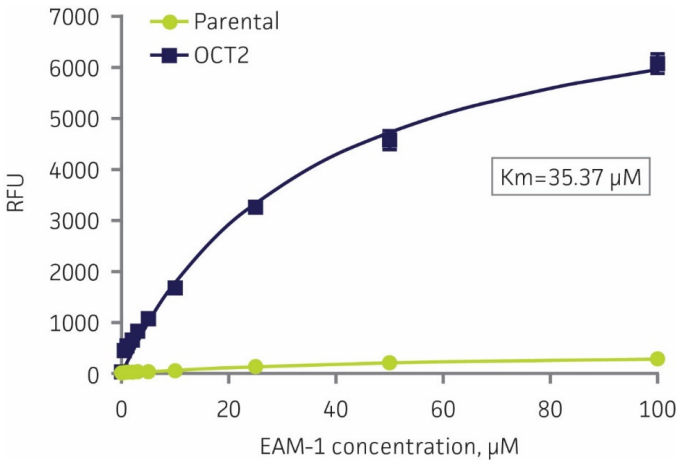


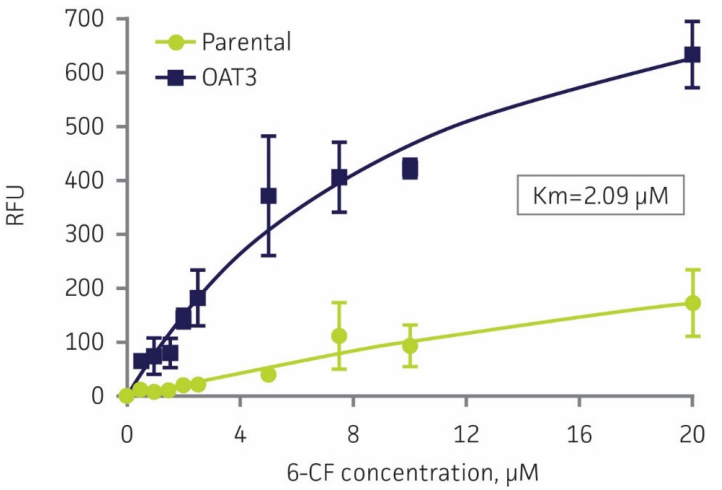
Functionality – Drug uptake assay

UPTAKE ASSAY PROTOCOL

- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate for 24 hours
- Increasing concentration of 6-CF or EAM1 were added and incubated for 20 minutes at 37°C
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured





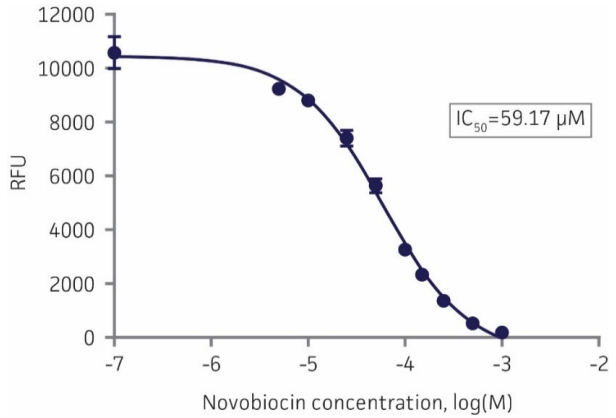


Functionality – Drug uptake inhibition assay

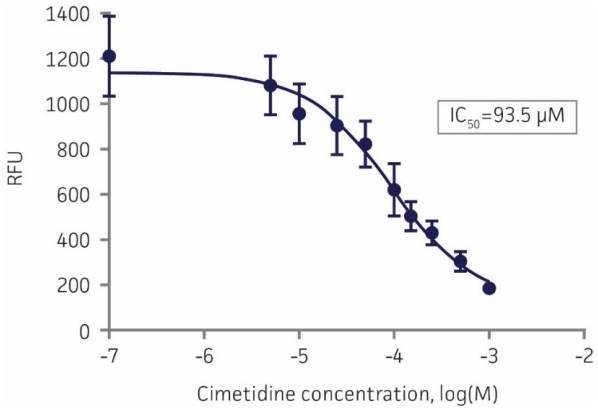
UPTAKE INHIBITION ASSAY PROTOCOL

- Equal numbers of both parental and transporter cells were seeded into 96-well plate in triplicate for 24 hours
- Increasing concentration of inhibitors were added together with constant concentrations of the uptake substrate and incubated for 20 mins at 37°C
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured

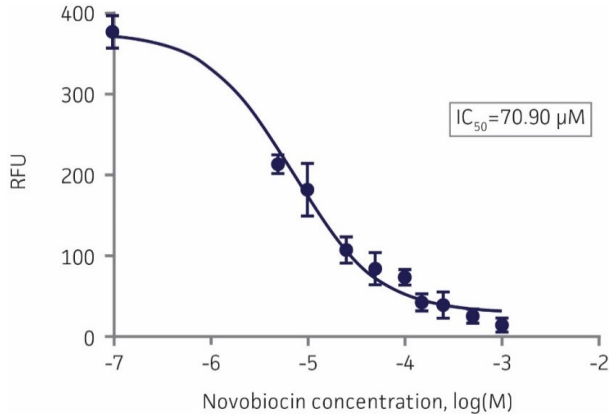
6-CF uptake inhibition in OAT-1 expressing RPTEC



EAM-1 uptake inhibition in OCT-2 expressing RPTEC



6-CF uptake inhibition in OAT-3 expressing RPTEC

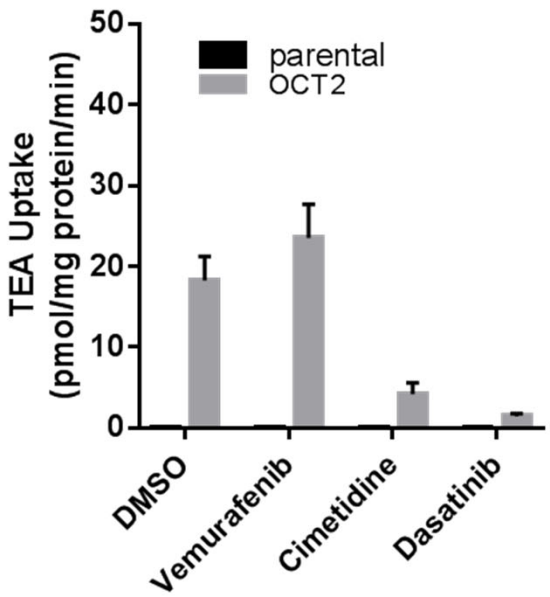


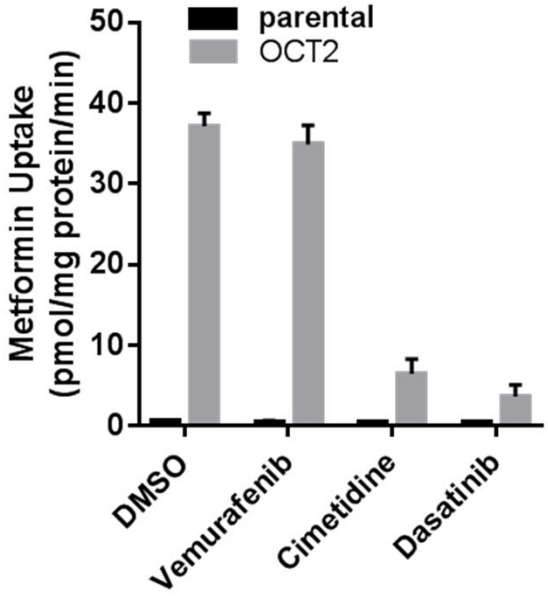
RPTEC-OCT2 – Drug-drug Interactions (DDI)

Drug-Drug Interactions

UPTAKE INHIBITION ASSAY PROTOCOL

- Aspirate growth media and wash once with warm 1X PBS; remove PBS and add 250 µL of cold inhibitors (prepared serum free DMEM, 0.5 µM) and incubate for 15 minutes
- Remove inhibitors and add 250 µL of radio-labeled TEA or metformin (prepared serum free DMEM, 4.5 µM) and incubate for 15 minutes
- Remove drug and wash 3 times with cold PBS; lyse the cells and count





Data kindly provided by:
Kevin Huang, *Graduate Research Associate,* Ohio State University, College of Pharmacy
Alice Gibson, Ph.D., *Senior Research Specialist,* Ohio State University, College of Pharmacy

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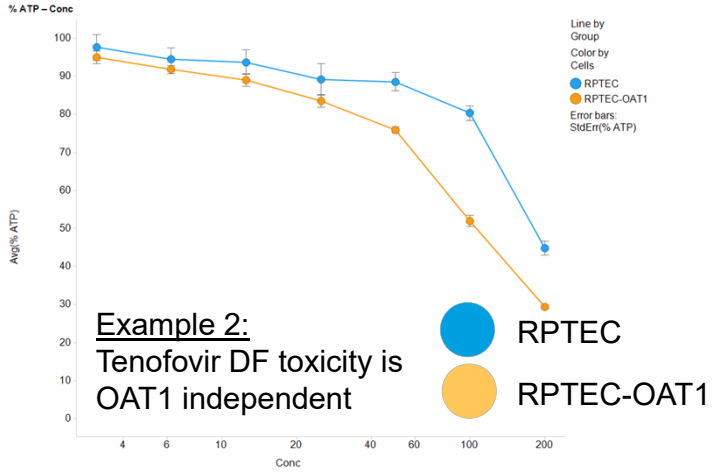
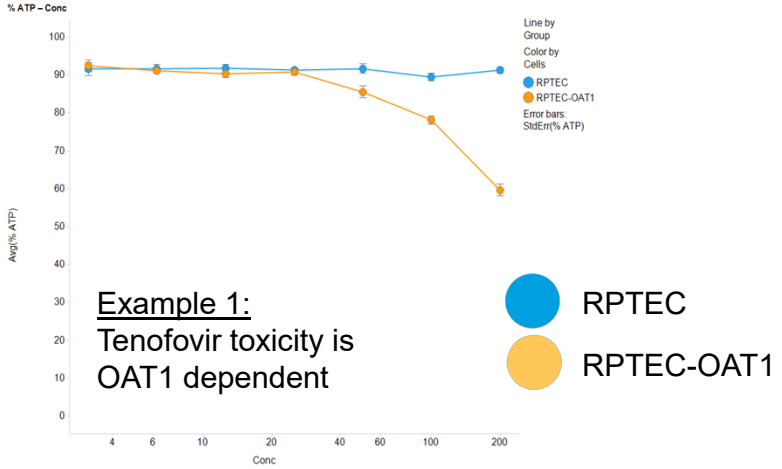
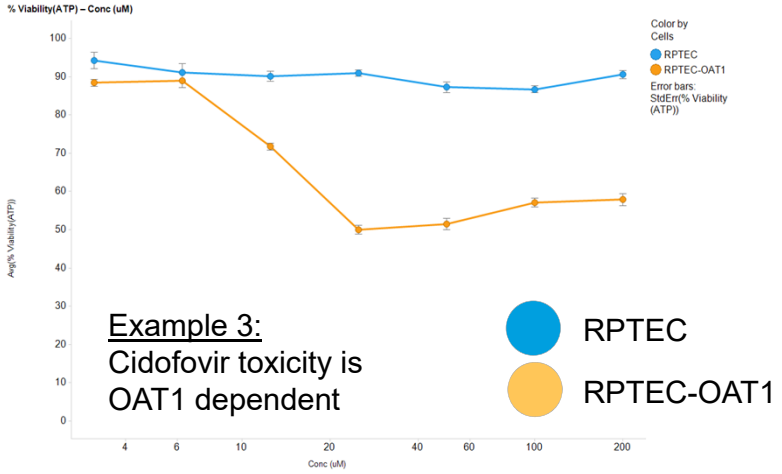
Application for nephron toxicity studies

CELL VIABILITY ASSAY PROTOCOL

- About 35000 cells were seeded per well in triplicate in a 96-well plate and incubated overnight
- Cells were incubated with a series of compounds at various concentrations for 3 days
- Cell viability was determined using a cell viability assay per manufacturer's instructions







Data kindly provided by: Merck & Co., Inc.

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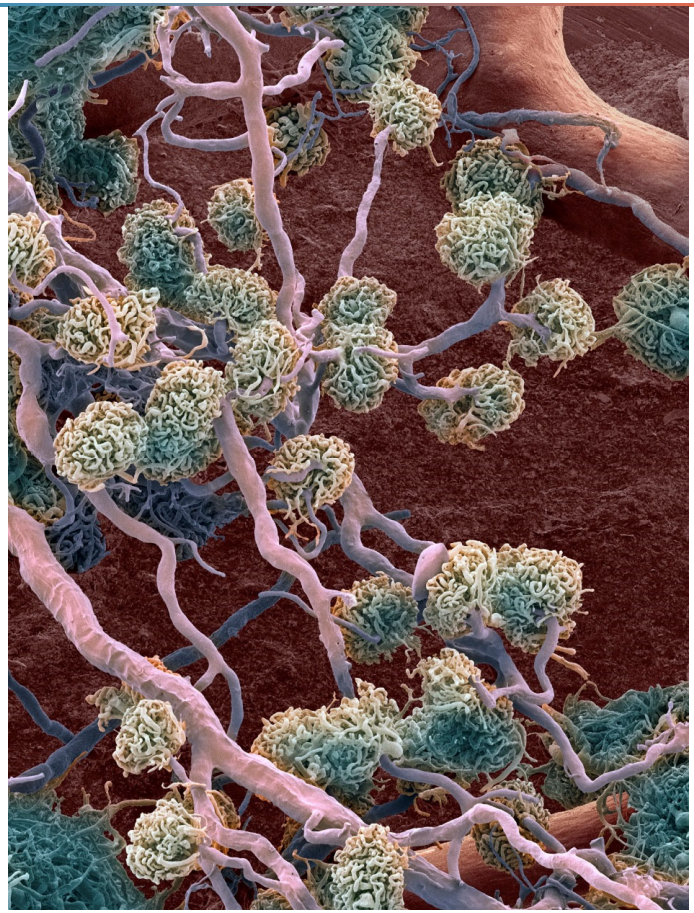
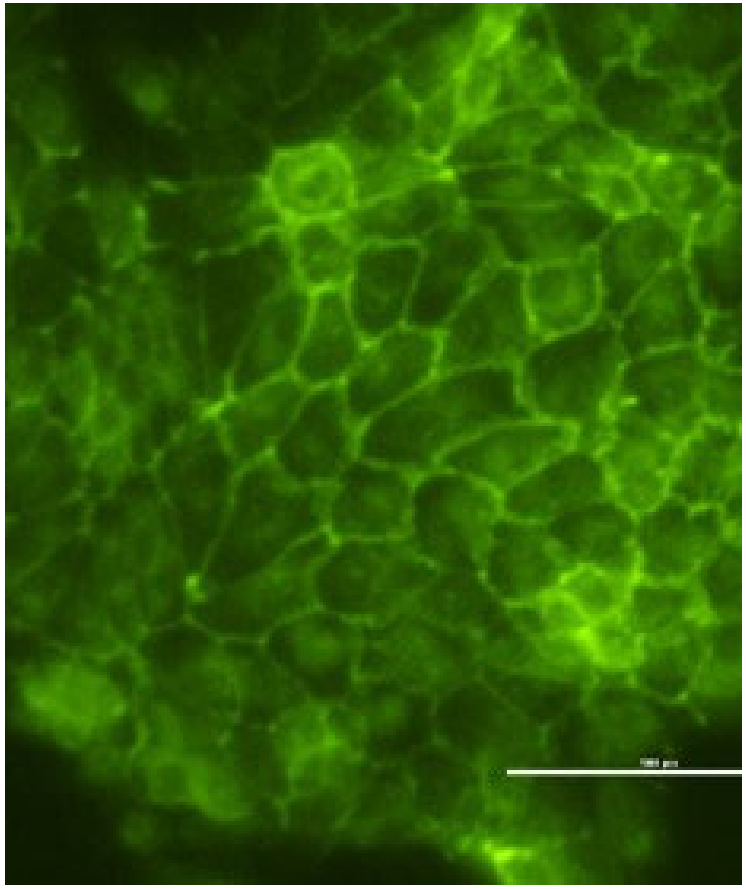


Summary

- hTERT immortalized primary cells provide primary cell functionality with continuous cell line longevity
- We generated the clonal RPTEC/TERT1 renal uptake cell models by stably overexpressing OAT1, OCT2, and OAT3
- Expression was confirmed via genomic and expression data
- Clonal stable cells retain the original characteristics of RPTEC/TERT1The performance of those stable cells was well-characterized
- Data from two outside collaborators indicating that the hTERT-immortalized primary RPTEC that express SLC transporters have multiple applications for toxicity testing.
- For more information visit

www.atcc.org/tox



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Thank you and questions?

Coming soon!
Modeling Toxicity with Neural Progenitor Cell-derived Neurospheres
Presented by Brian Shapiro
February 24, 12:00 PM EST

Reproducibility and Physiological Relevance: The ATCC Toxicology Portfolio Webinar
Presented by Kevin Grady and Kevin Tyo
March 3, 12:00 PM EST

Society of Toxicology 61st Annual Meeting and ToxExpo
San Diego Convention Center, San Diego, California
March 28-30, Booth #1810

www.atcc.org/TOX