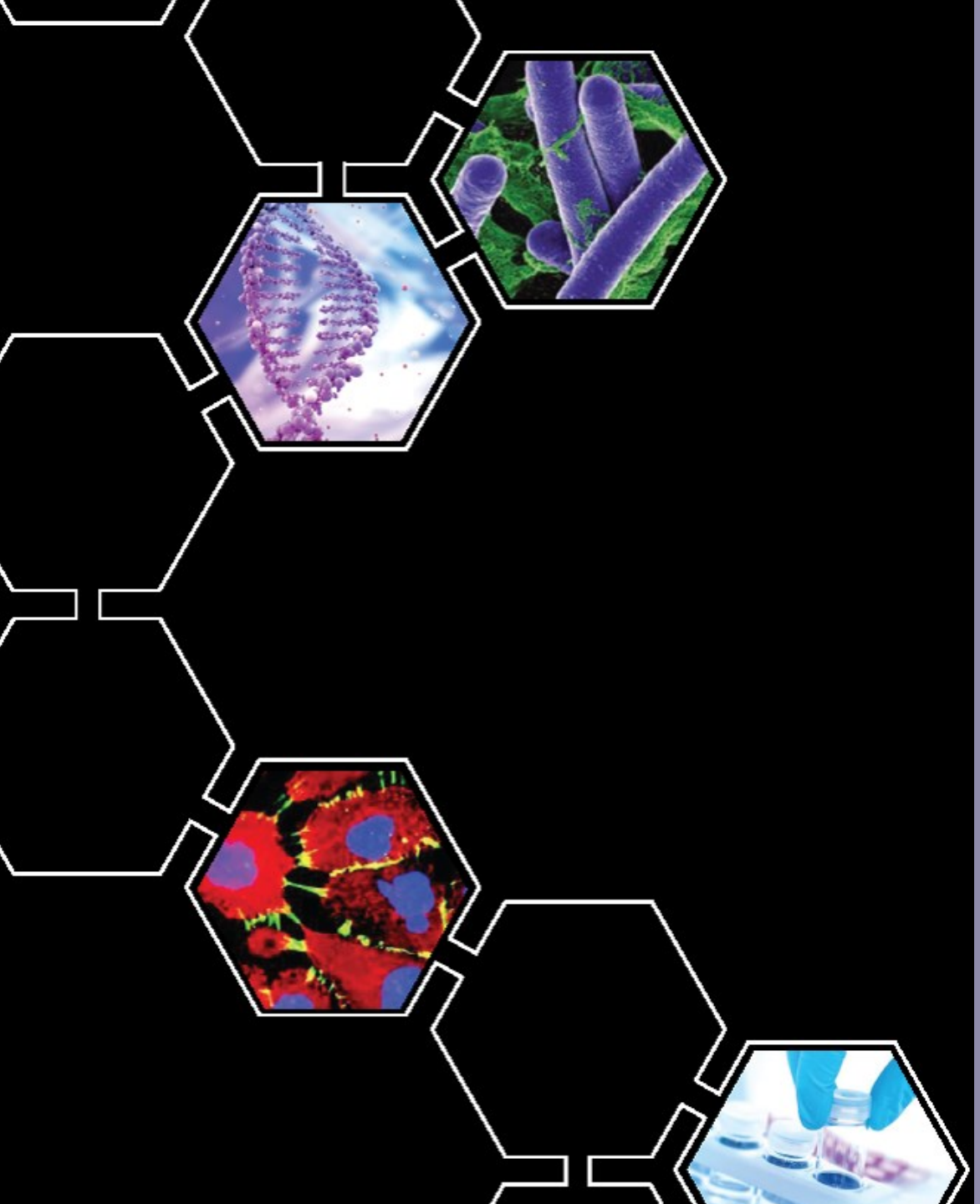


Best of Both Worlds – hTERT-immortalized Primary Cells

Kevin Grady, B.S., *Senior Product Line Business Manager*, ATCC

Chaozhong Zou, Ph.D., *Senior Scientist*, ATCC

ATCC Today

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA and an R&D & Services center in Gaithersburg, MD
- World wide brand name and quality recognition

- World's premiere biological materials resource and standards development organization
  - 4,000 cell lines
  - 70,000 microbes

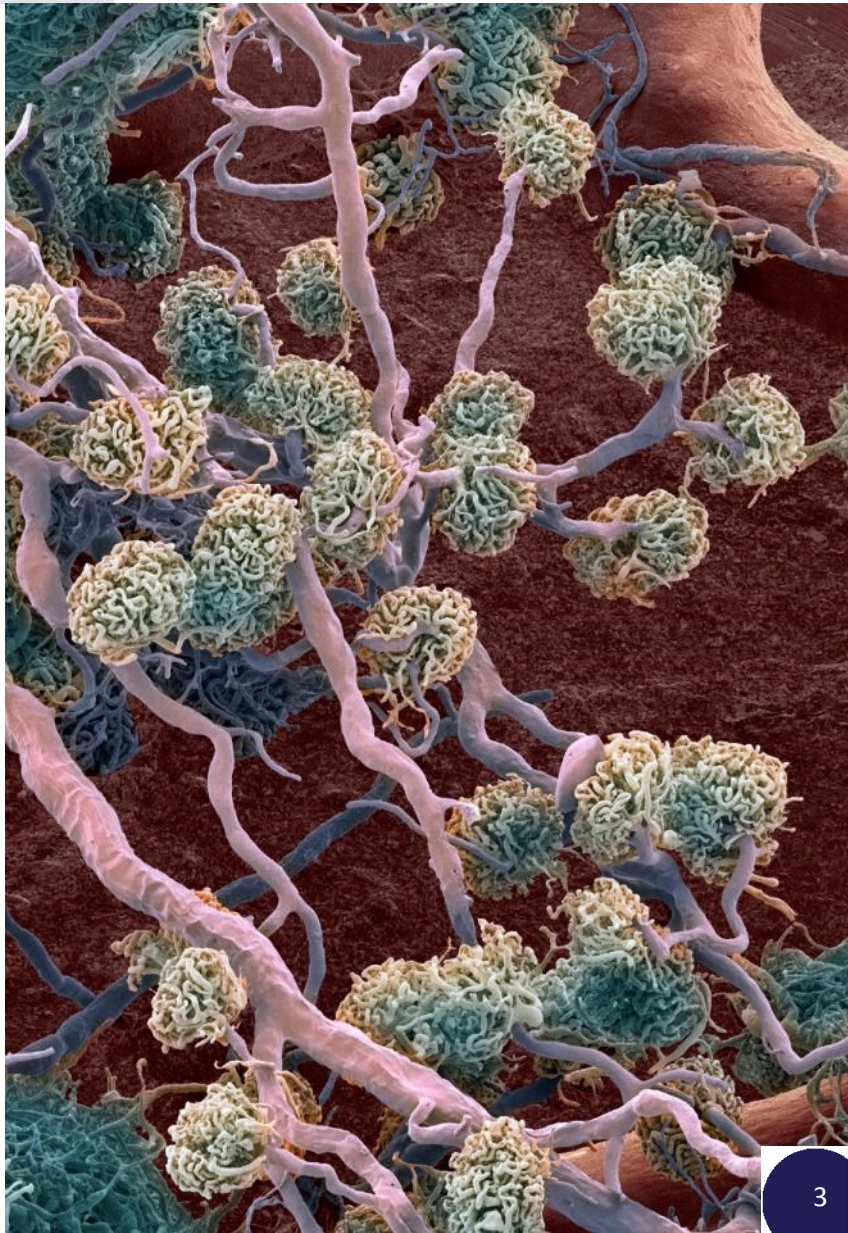
- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
  - Growing portfolio of products and services
  - Sales and distribution in 140 countries, 12 International distributors

- Talented team of 475+ employees; > one third with advanced degrees

- Multiple accreditations including ISO 9001 and ISO 13485

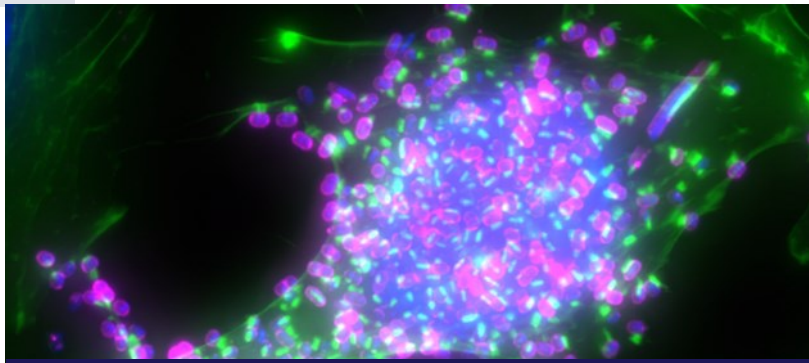
Agenda

- hTERT-immortalized Primary Cells Portfolio
- Kidney transporter models
  - Current *in vitro* transporter cell models
  - Generation of RPTEC renal transporter models
  - Application data

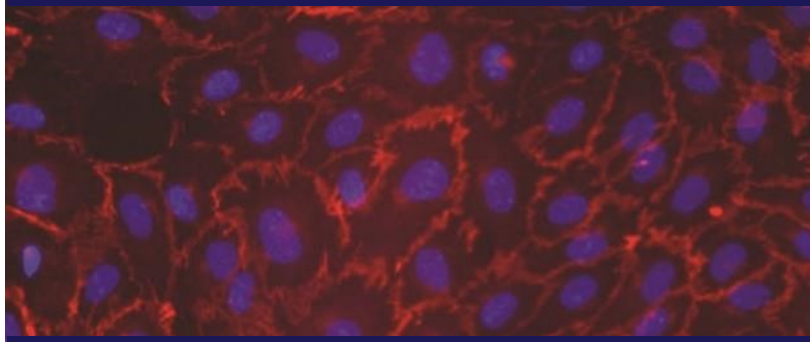


ATCC classic and advanced cell models

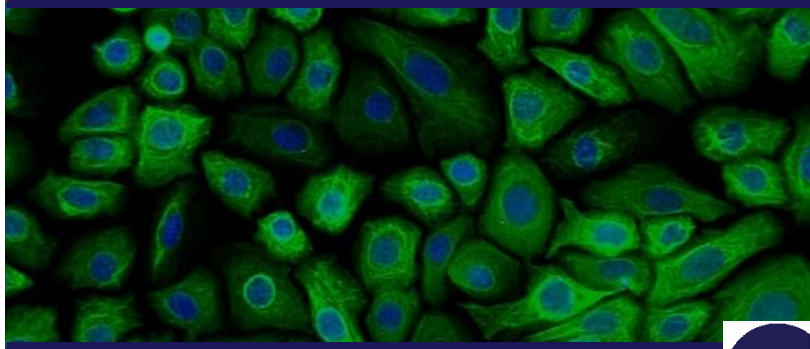
- ATCC is the complete solution supplier
- From basic research through discovery and development to product testing
  - Continuous cell lines
  - Primary cells
  - hTERT immortalized primary cells
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols



Continuous cell line: HeLa (ATCC® CCL-2™)



Primary: Umbilical Endothelial Cells (ATCC® PCS-100-010™)



Immortalized lung cells; NuLi-1 (ATCC® CRL-4011™)

Pros and cons of different cell models

	Primary cells	hTERT immortalized	Oncogene, virally immortalized	Cancer cell lines
Mimic *in vivo* tissue phenotype	++++	+++	++	+
Genotypic stability	Diploid	Diploid / Near diploid	Near diploid / Aneuploid	Aneuploid
Proliferative capacity	+	+++	+++	+++
Supply	+	+++	+++	+++
Inter-experimental reproducibility	+	+++	+++	+++
Cost	+++	++	+	+
Ease-of-use	+	++	++	+++
Predictability in toxicological studies	+++	+++	++	+

Pros and cons of different cell models

	Primary cells	hTERT immortalized	Oncogene, virally immortalized	Cancer cell lines
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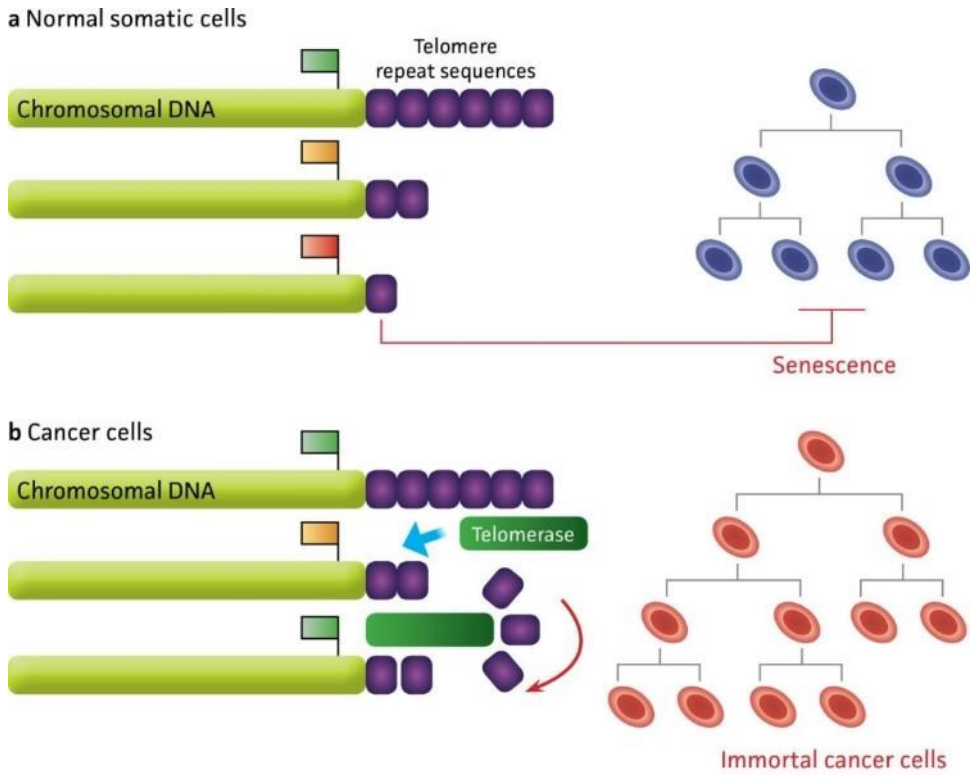
Inter-experimental reproducibility	+	+++	+++	+++
Cost	+++	++	+	+
Ease-of-use	+	++	++	+++
Predictability in toxicological studies	+++	+++	++	+

hTERT-immortalized cells are unique tools

hTERT-immortalized cells combine:

- The *in vivo* nature of primary cells
- The ability to be continuously cultured

hTERT-immortalized cells avoid the limitations of primary cells while still reaping their benefits



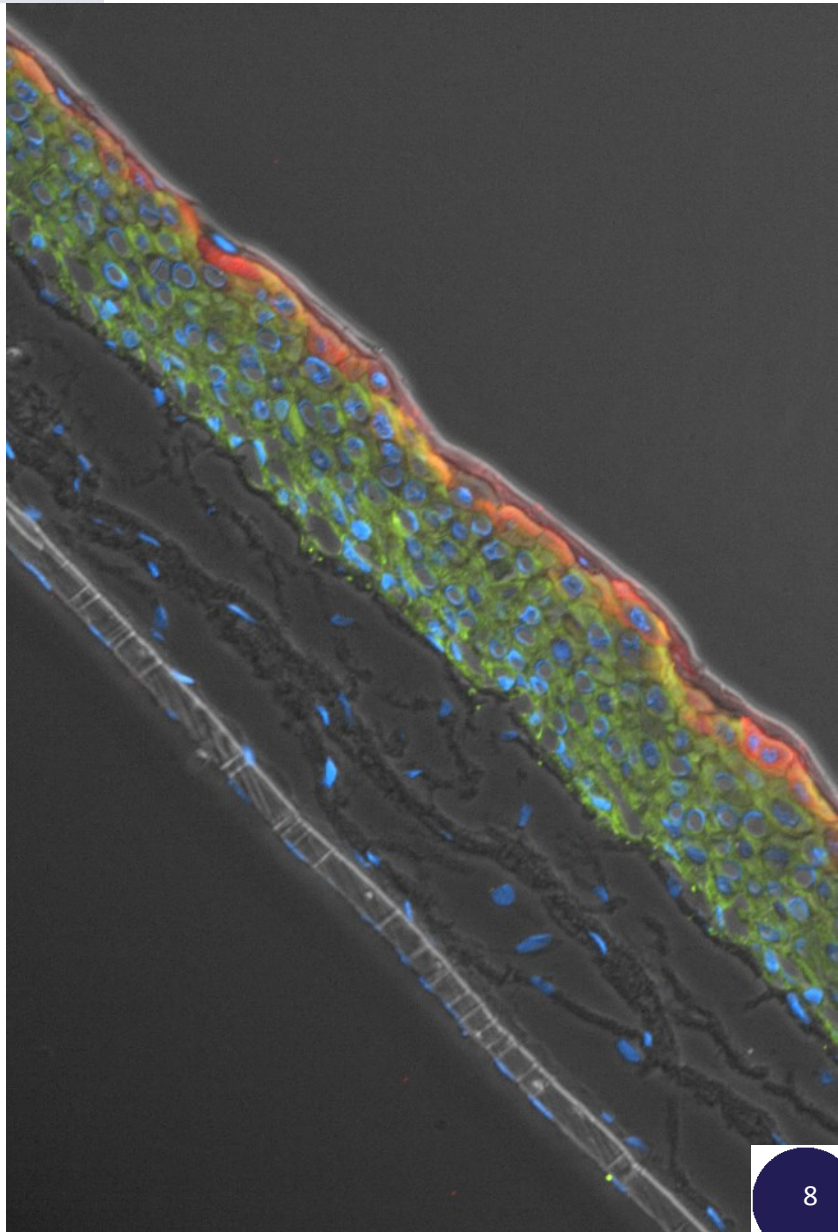
ATCC portfolio – skin models

Primary Cells

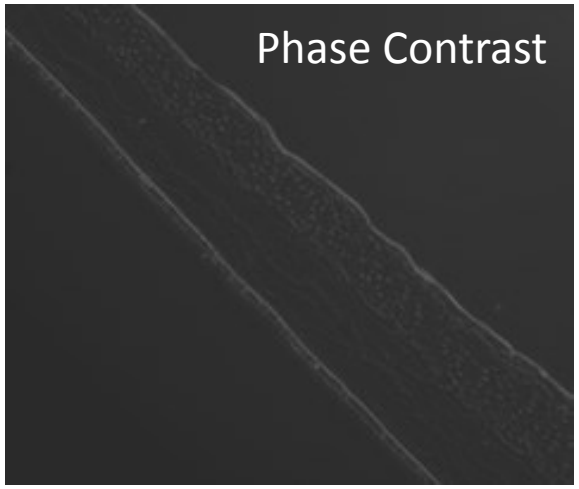
- Keratinocytes
- Dermal Fibroblasts
- Melanocytes
- Dermal Microvascular Endothelial

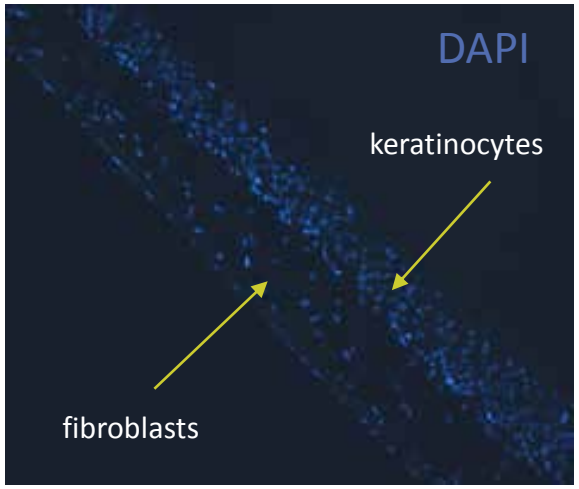
hTERT Immortalized Primary Cells

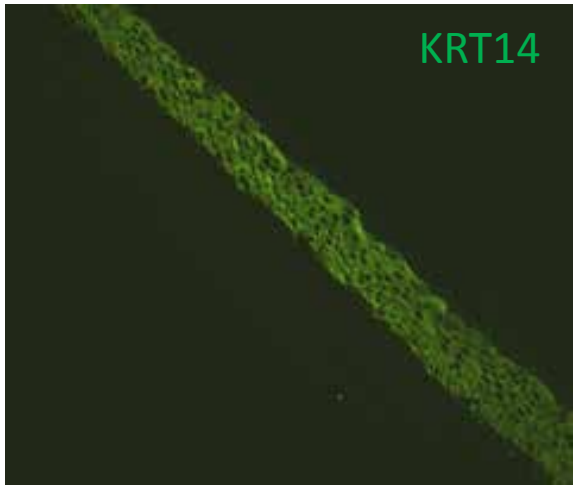
- Keratinocytes
- Dermal Fibroblasts
- Dermal Microvascular Endothelial
- Melanocytes

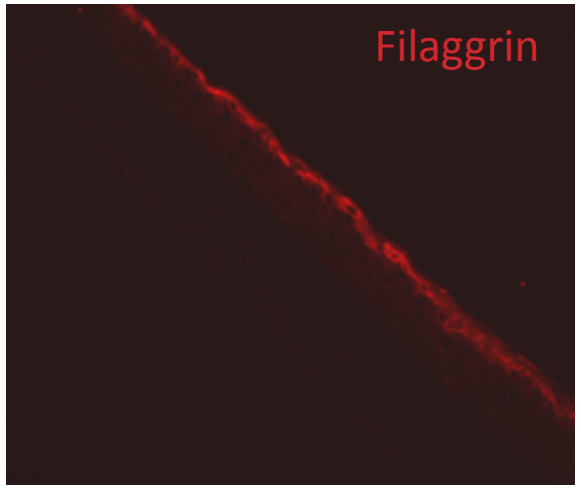


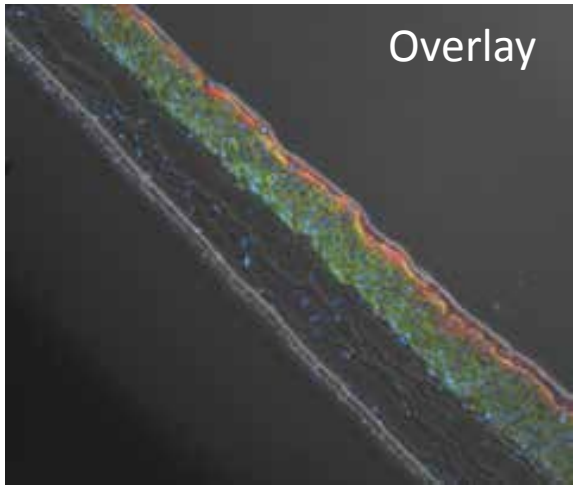
ATCC keratinocytes cultured at ALI display similar architecture to skin *in vivo*











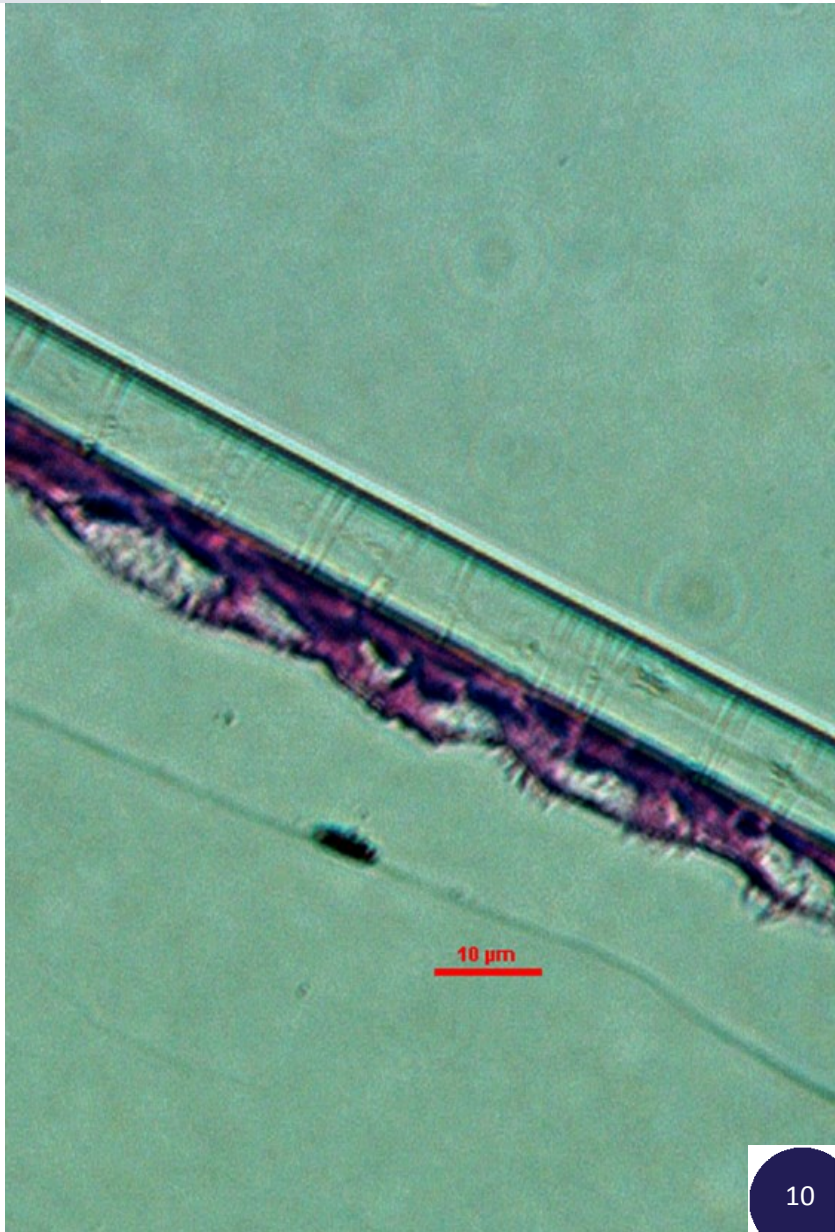
ATCC portfolio – airway models

Primary Cells

- Lung Fibroblasts
- Bronchial/Tracheal Epithelial Cells
- Small Airway Epithelial Cells
- Lung Smooth Muscle Cells
- Bronchial/Tracheal Smooth Muscle Cells

hTERT Immortalized Primary Cells

- Bronchial/Tracheal Epithelial Cells
- Small Airway Epithelial Cells
- Lung Fibroblasts



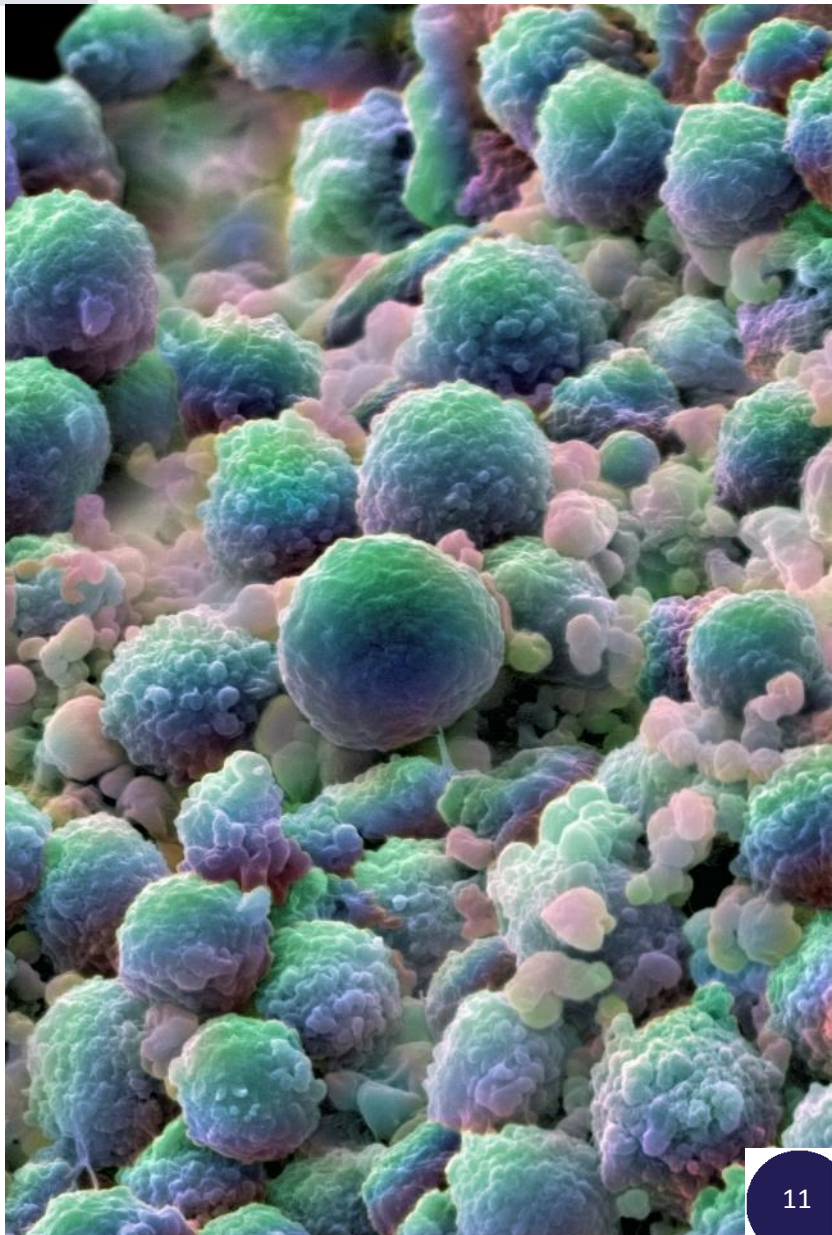
ATCC portfolio – urogenital models

Primary Cells

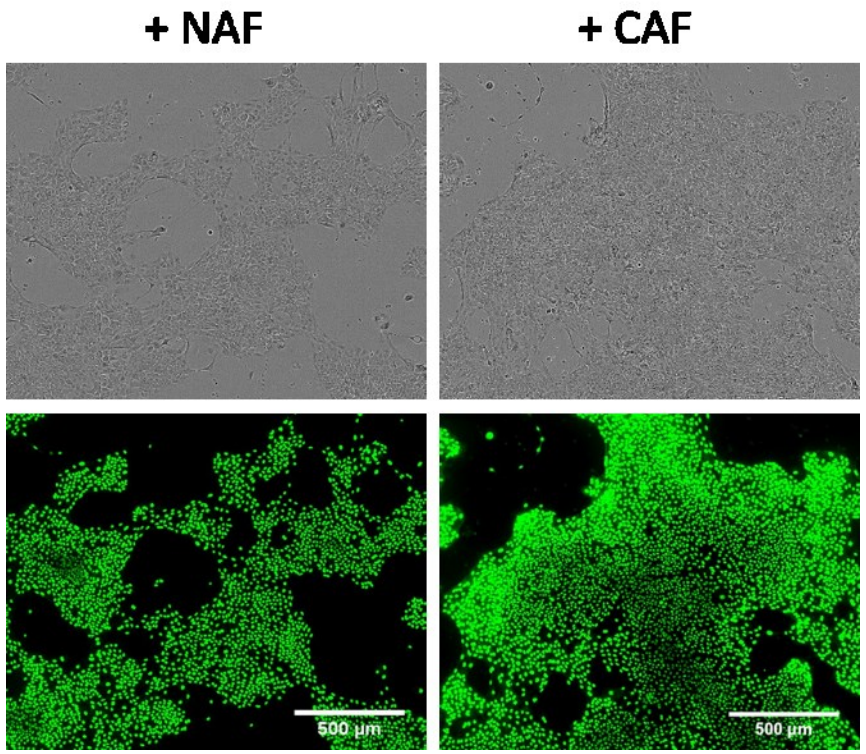
- Prostate Epithelial Cells
- Vaginal Epithelial Cells
- Uterine Fibroblast Cells
- Uterine Smooth Muscle Cells
- Bladder Epithelial (A/T/N) Cells
- Bladder Smooth Muscle Cells
- Bladder Fibroblast Cells

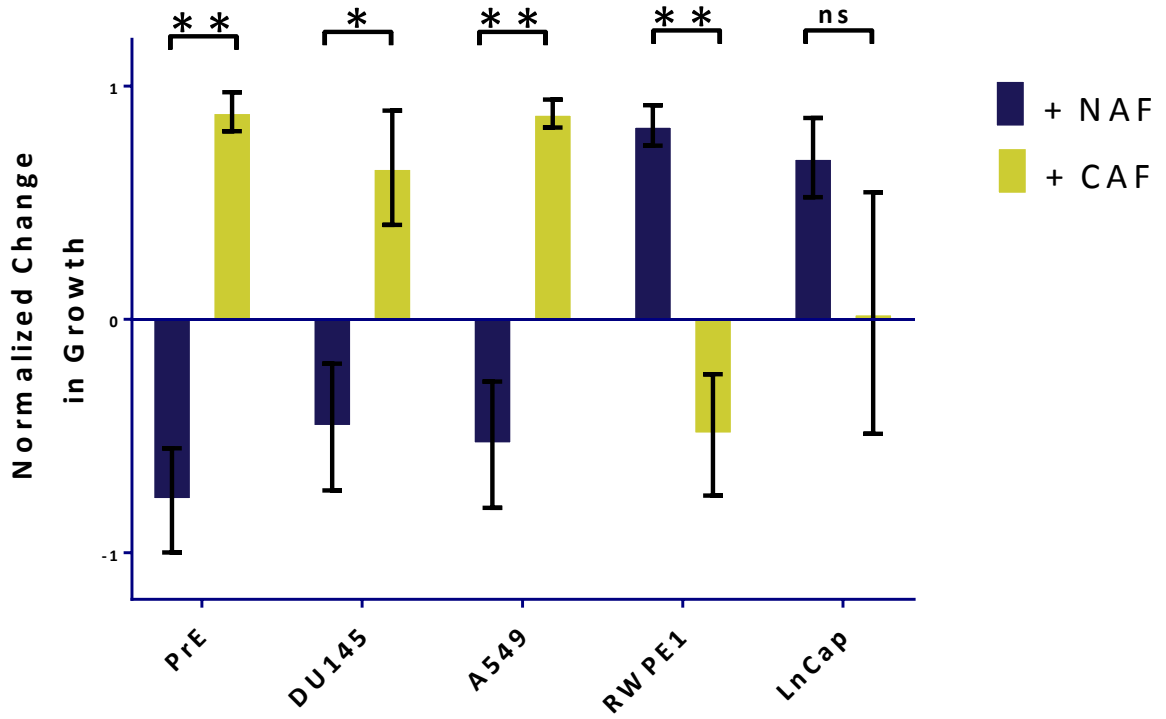
hTERT Immortalized Primary Cells

- Prostate Fibroblast Cells
- Prostate Cancer-associated Fibroblast Cells
- Prostate Epithelial Cells
- Endometrial Fibroblast Cells



hTERT-immortalized prostate cells differentially influence the tumor microenvironment





A representative image of growth and staining of normal prostate epithelial cells in the presence of prostate normal-associated fibroblast (NAF) and prostate cancer-associated fibroblast (CAF). Data were then analyzed for percent change of growth of the experimental cells based on cell densities between the cells in the presence and absence of fibroblast cells.

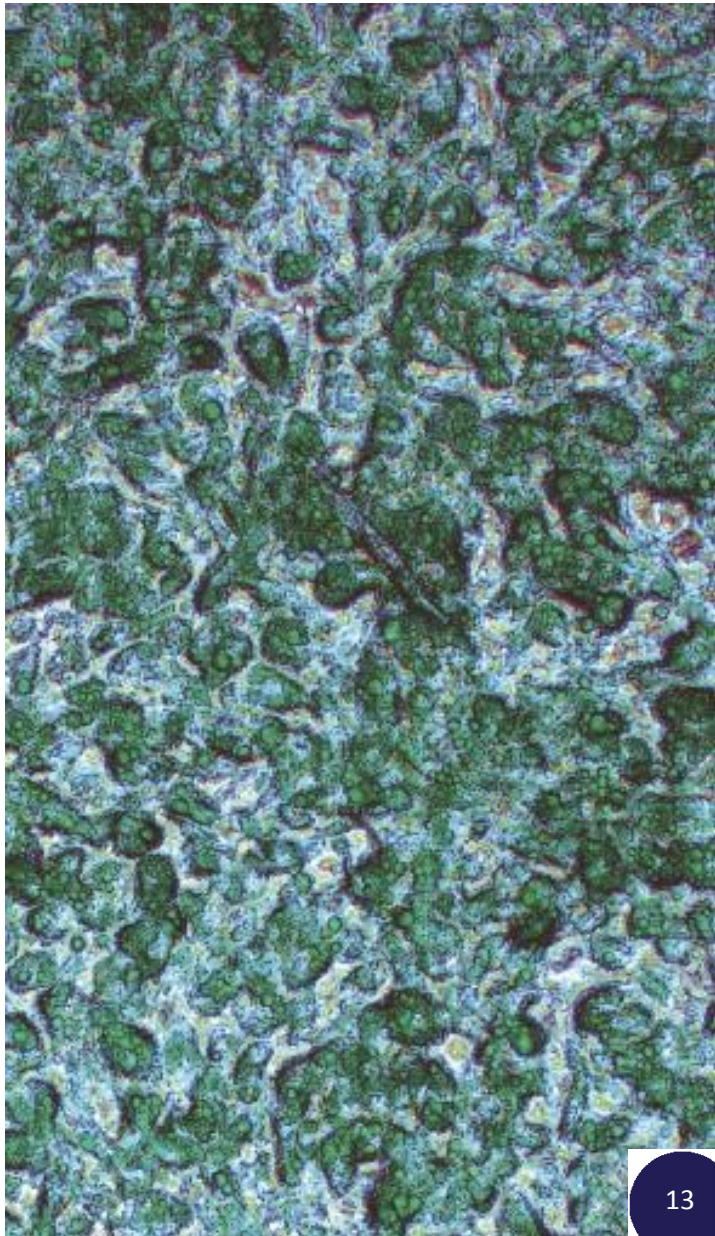
ATCC portfolio – mesenchymal stem cells

Primary Stem Cells

- Adipose-derived Mesenchymal Stem Cells
- Subcutaneous Pre-adipocytes
- Bone Marrow-derived Mesenchymal Stem Cells
- Umbilical Cord-derived Mesenchymal Stem Cells

hTERT Immortalized Primary Cells

- Adipose-derived Mesenchymal Stem Cells



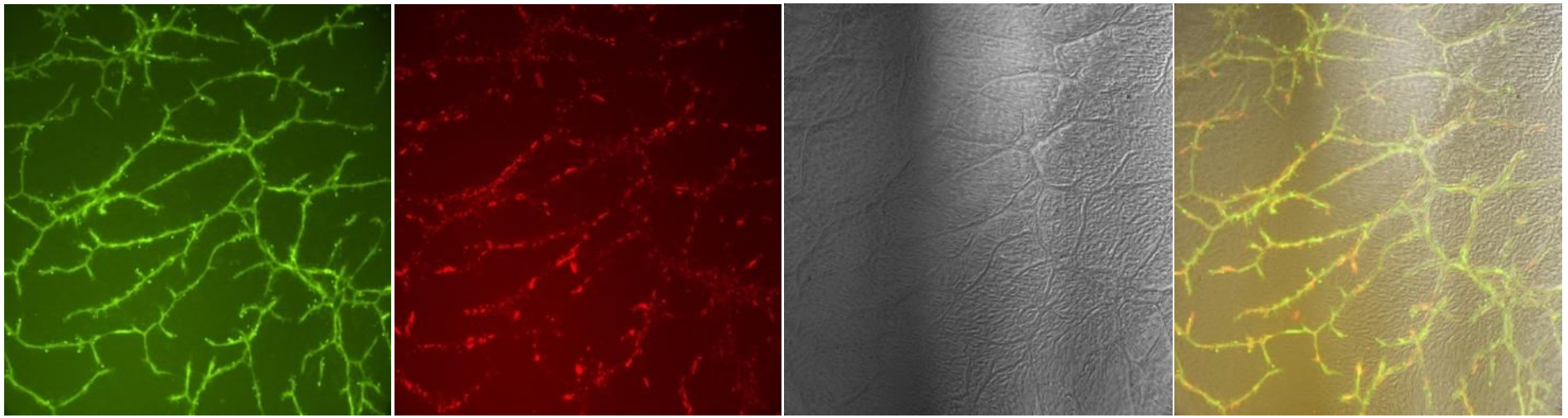
hTERT-immortalized and primary mesenchymal stem cells express equivalent markers

Flow cytometric analysis of surface marker expression determined ATCC hTERT-mesenchymal stem cells (MSCs) were equivalent to primary MSCs:

- Bone marrow (BM), adipose tissue (AT), umbilical cord (UC), and hTERT-derived MSCs were tested
- **These data meet International Society for Cellular Therapy (ISCT) guidelines**

CD Marker	CD14 (%)	CD19 (%)	CD34 (%)	CD45 (%)	CD29 (%)	CD44 (%)	CD73 (%)	CD90 (%)	CD105 (%)	CD166 (%)
BM-MSC	0.26	0.07	2.91	0.15	100	99	100	100	100	94
AT-MSC	0.55	0.23	2.86	0.29	100	100	100	99	100	90
UC-MSC	0.52	0.79	1.50	0.47	100	90	95	96	94	95
hTERT-MSC	0.25	0.23	0.99	0.55	100	100	100	100	99	96

What is the Angio-*Ready*™ System?

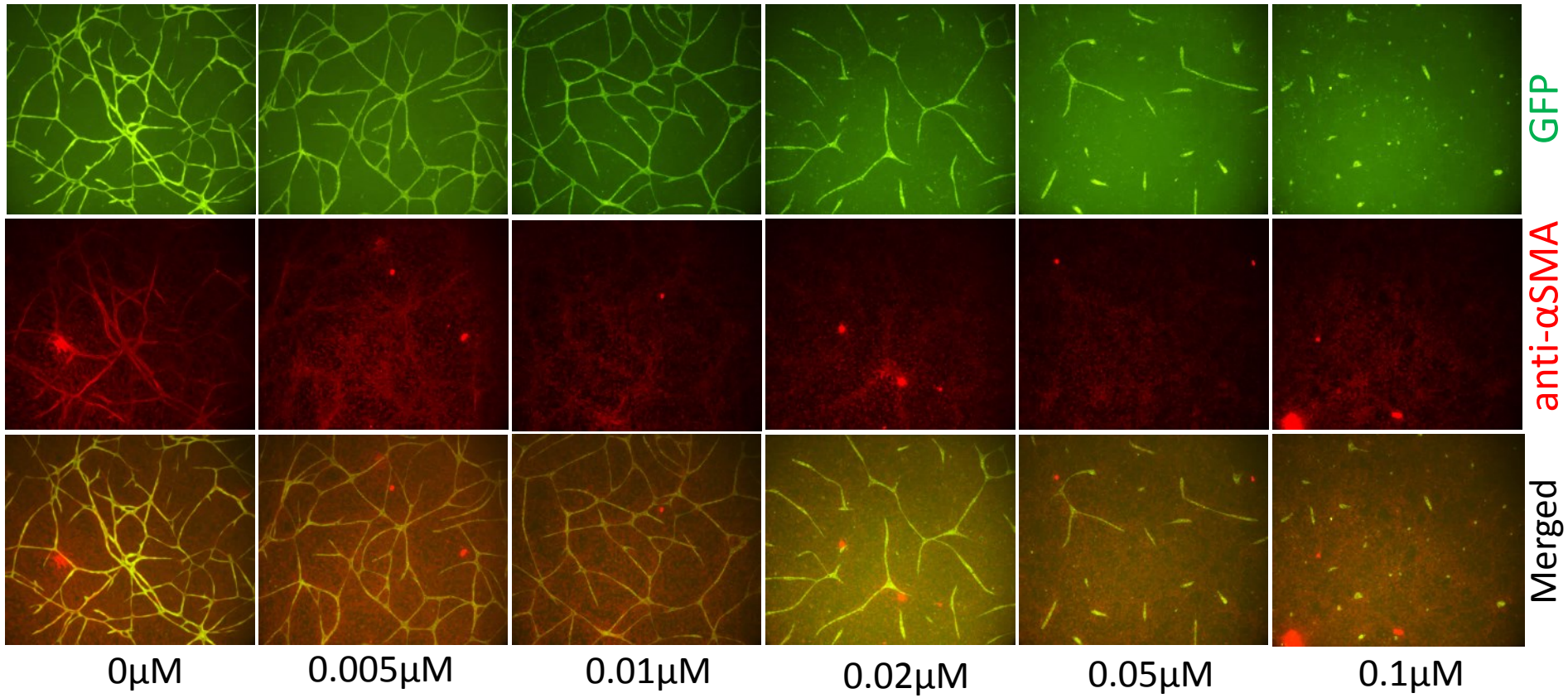


GFP | anti-αSMA | Phase contrast | Merged

A co-culture system consisting of telomerase-immortalized endothelial cells and mesenchymal stem cells, providing an *in vitro* angiogenesis system that is more close to the *in vivo* situation

- A mix of TeloHAEC-GFP (ATCC® CRL-4054™) and ASC52telo (ATCC® SCRC-4000™) provided with an optimized medium formulation

Sunitinib blocks tubular structure growth in dose- dependent manner in the Angio-*Ready*™ System



TeloHAEC-GFP and hTERT-MSC cells premixed, thawed and seeded immediately into wells of a 96-well plate, and treated with different doses of sunitinib; fixed and stained with anti-αSMA antibody at day 8

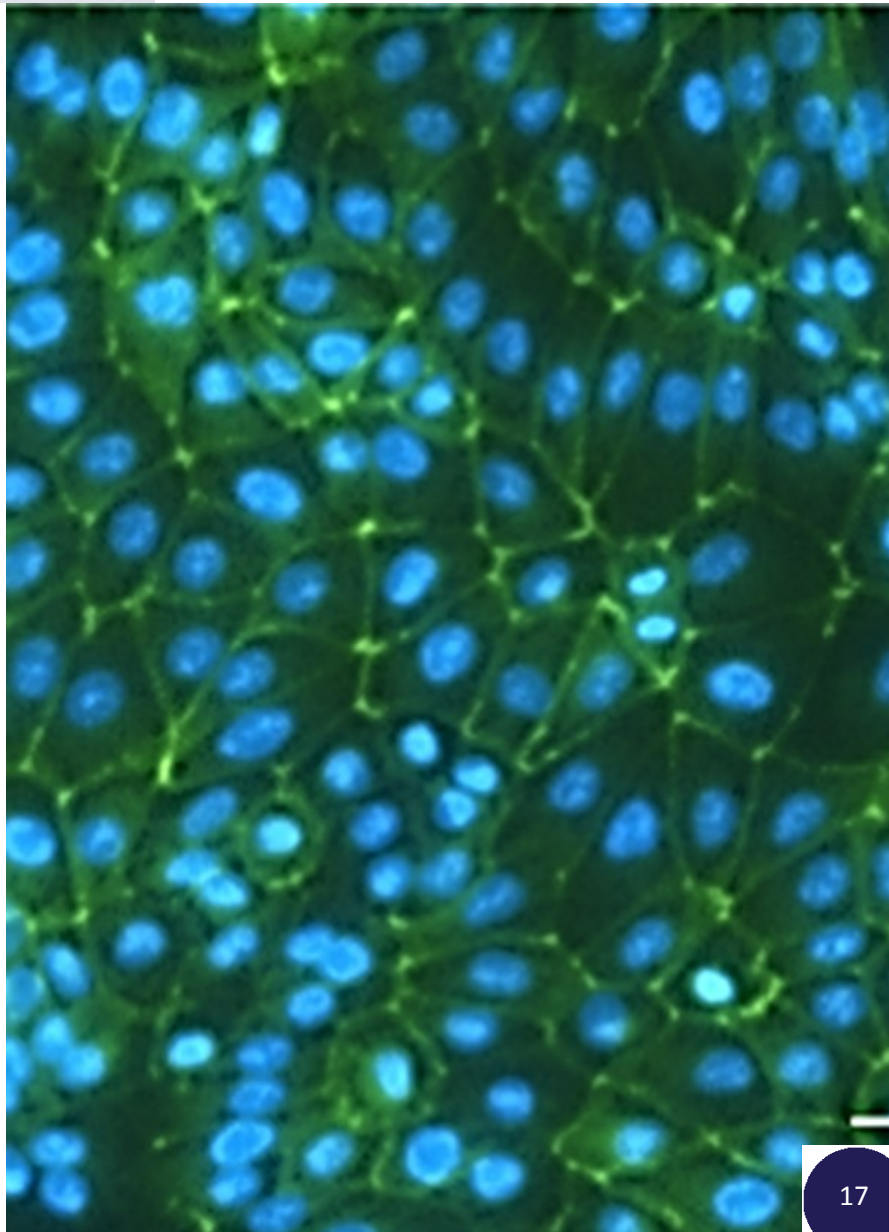
ATCC portfolio – kidney models

Primary Cells

- Renal Proximal Tubule Epithelial Cells
- Renal Cortical Epithelial Cells
- Renal Mixed Epithelial Cells

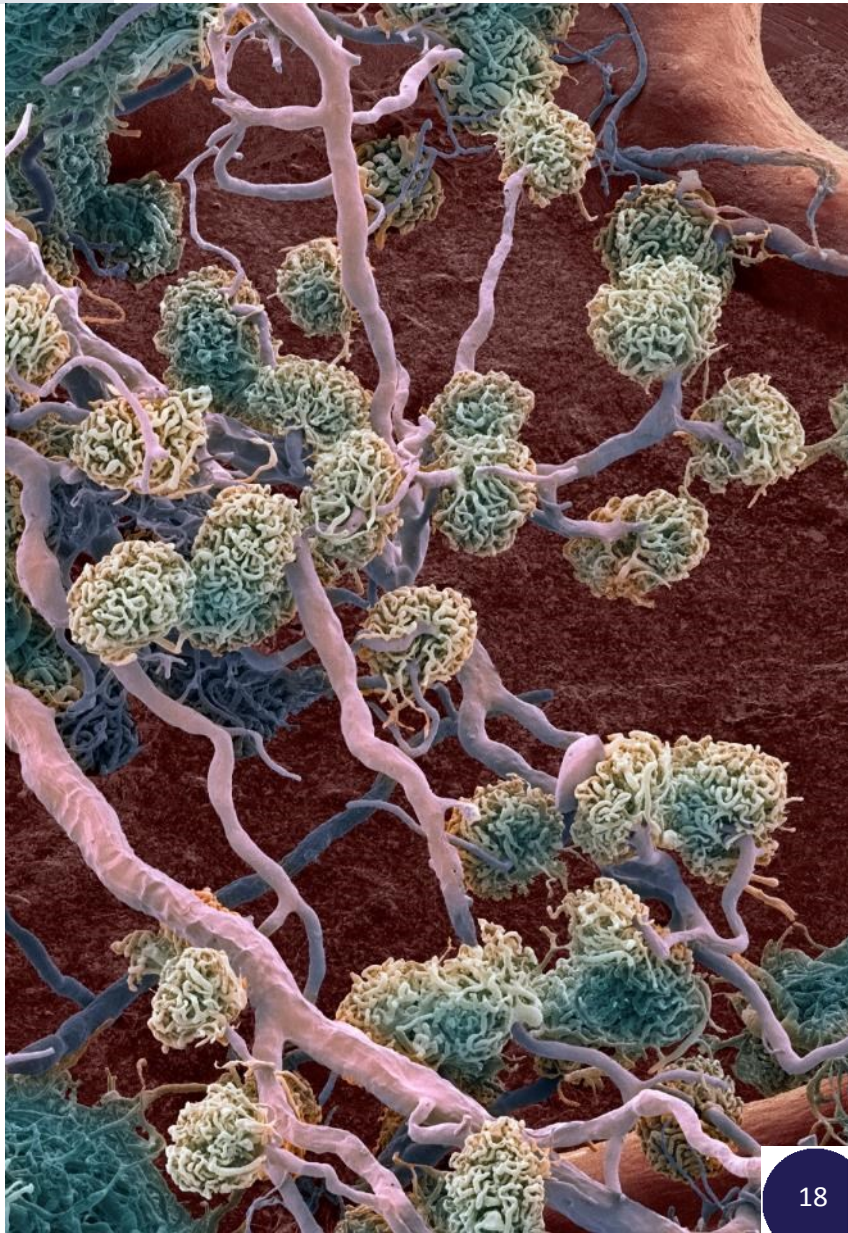
hTERT Immortalized Primary Cells

- Renal Proximal Tubule Epithelial Cells
- Renal Proximal Tubule Epithelial Cells-OAT1
- Renal Proximal Tubule Epithelial Cells-OCT2
- Renal Proximal Tubule Epithelial Cells-OAT3 (coming soon)

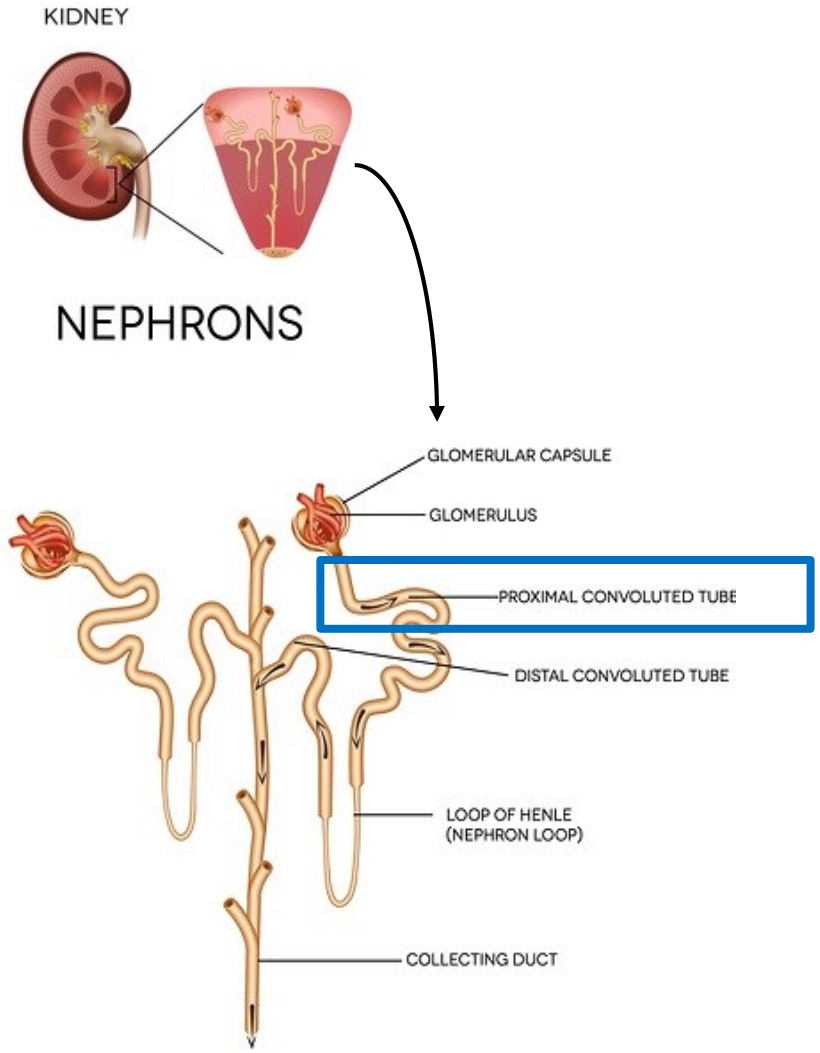


Agenda

- hTERT-immortalized Primary Cells Portfolio
- Kidney transporter models
  - Current *in vitro* transporter cell models
  - Generation of RPTEC renal transporter models
  - Application data



Kidney drug toxicity and transporters



https://ghr.nlm.nih.gov/condition/renal-hypouricemia

The kidney is one of the major target organs for drug-induced toxicity

- Large functional reserve of the kidney
- Nephrotoxic effects become obvious only after regulatory approval

Nephrotoxic potential

- Often underestimated when new drugs are available
- Leads to clinical complications such as COX2 inhibitors

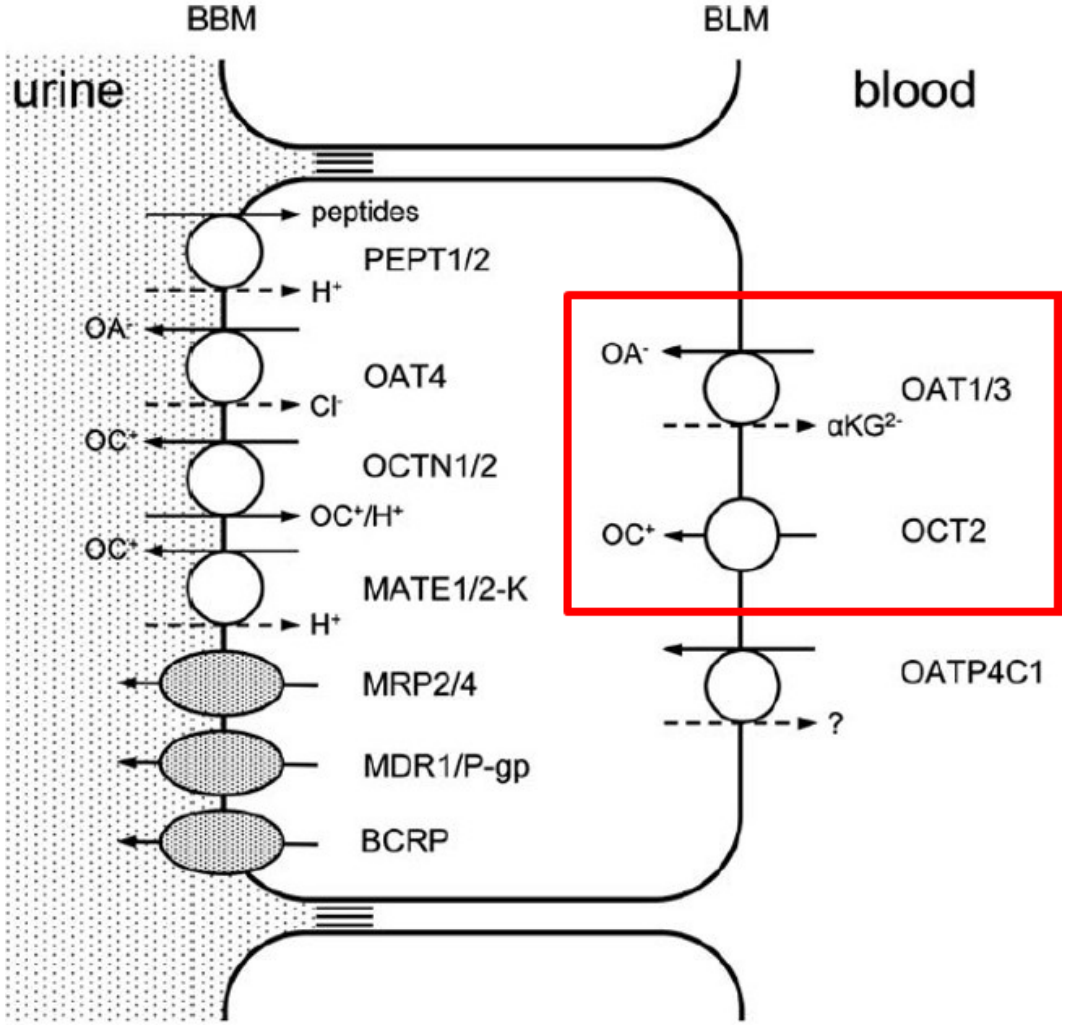
Renal proximal tubule (PT, blue box) is a major target for drug-induced toxicity due to its role in:

- Glomerular filtrate concentration
- Transport of drugs and organic compounds

Kidney drug toxicity and transporters

- 2 major transporters (ATP-binding Cassette transporters and **Solute Carrier transporters**)
- My talk today will only focus on SLC transporters
- New (draft) regulatory documents published by FDA, EMA, and ITC (2010 & 2012):

"evaluate OAT1, OCT2, and OAT3 as major SLC targets for drug interaction testing expressed in the kidney"



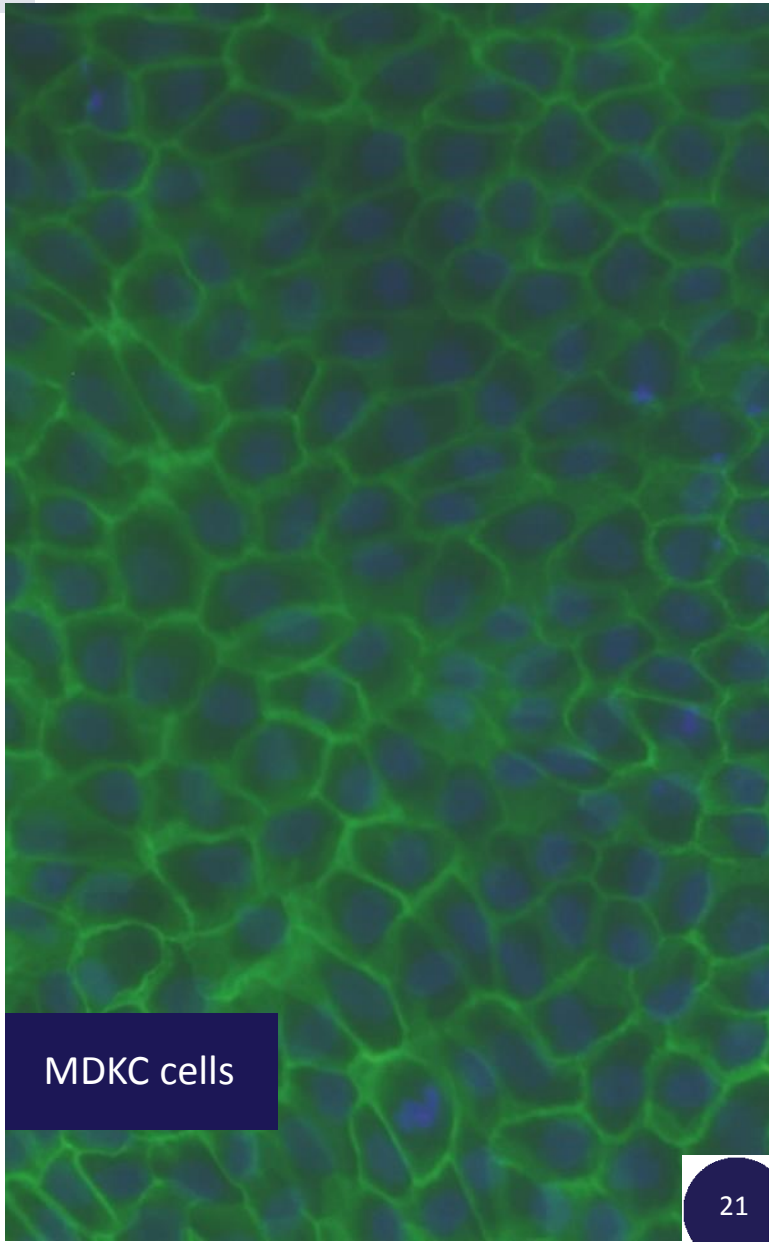
Pang K, et al. Enzyme- and Transporter-Based Drug–Drug Interactions. DOI 10.1007/978-1-4419-0840-7_2,C Am Assoc Pharmaceut Sci 2010

Searching for the best *in vitro* models

To evaluate the drug transporter interactions, we need a good in vitro model with:

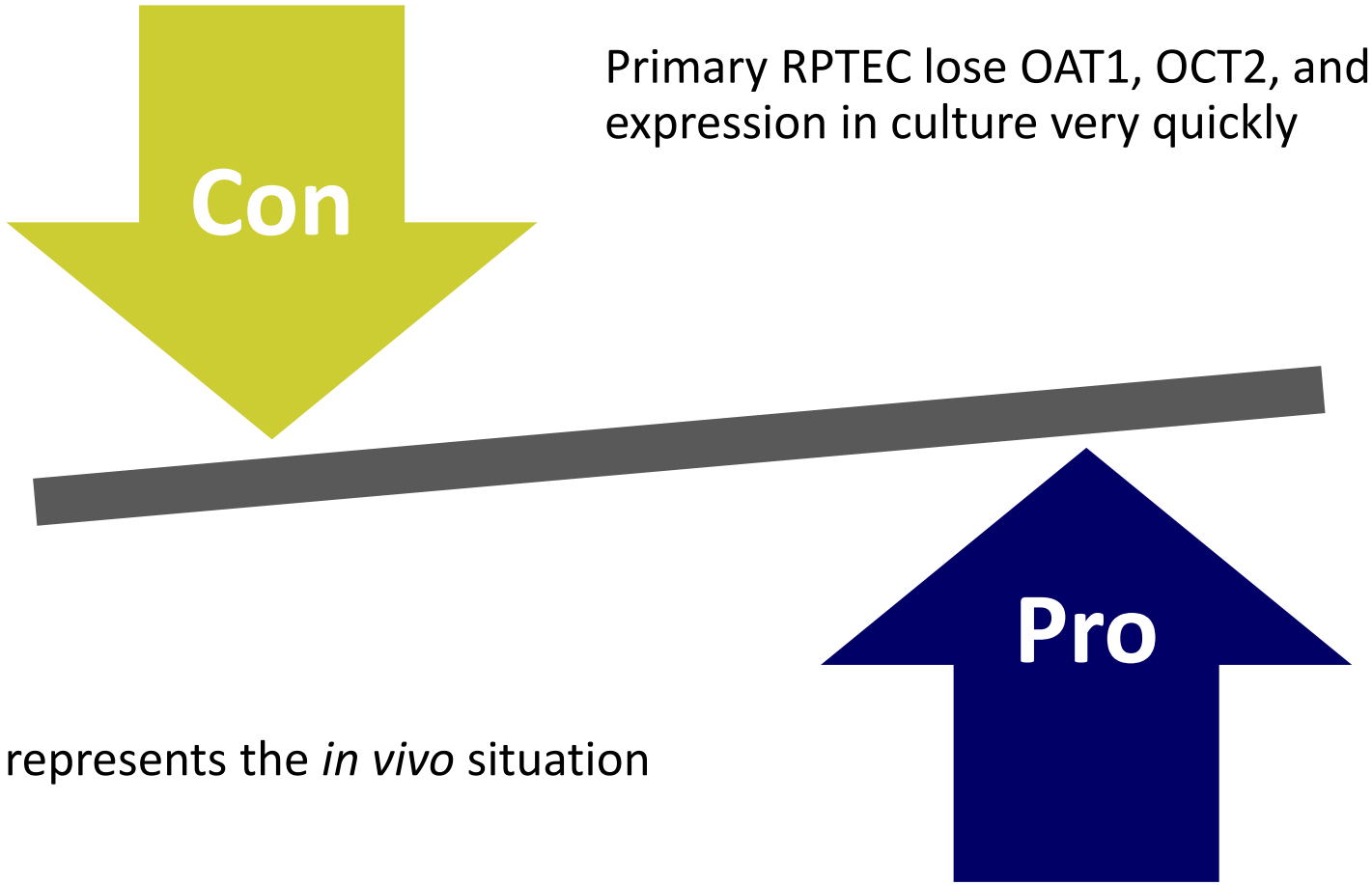
- High predictability
- Mature kidney PT origin
- Best mimics PT micro-environment

The central problem is the lack of good pre-clinical cell-based models



MDKC cells

Searching for the best models: Primary cells



Primary RPTEC lose OAT1, OCT2, and OAT3 expression in culture very quickly

Best represents the *in vivo* situation

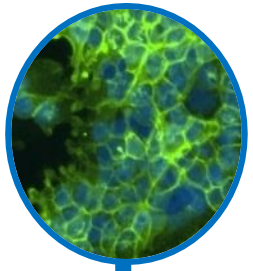
Searching for the best models: Renal cell lines

Cell Line	ATCC® No.	Species of origin	Nephron segment of origin
LLC-PK1	CL-101™	Yorkshire Pig	Proximal nephron
OK	CRL-1840™	North American Opossum	Proximal nephron
JTC-12	N/A	Monkey	Proximal nephron
MDCK	CCL-34™	Dog	Collecting duct
A6	CCL-102™	*Xenopus*	Distal tubule
HK-2	CRL-2190™	Human	HPV16-transformed, Proximal/Distal
Caki-1	HTB-46™	Human	Kidney carcinoma
HEK293	CRL-11268™	Human	Embryonic, SV40T

None of the continuous renal epithelial cell lines have been fully characterized for recapitulating the functions of the PT cells *in vivo*

Searching for the best models: gene modification

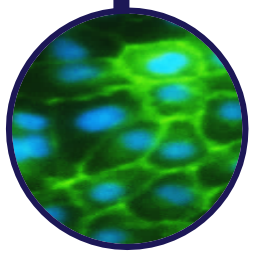
Non-kidney or embryonic kidney transformed models (U2OS, CHO, etc…)



First-generation models

HEK293T17 overexpressing OAT1 (ATCC® CRL-11268-OAT1™)

Next-generation models



ATCC hTERT-immortalized RPTEC, stably transfected

Immortalized human adult proximal tubular cells: RPTEC/TERT1

RPTEC/TERT1 (ATCC® CRL-4031™)

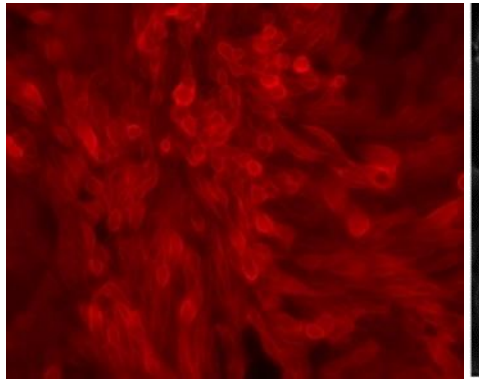
- Derived from normal renal PT epithelium
- Immortalized using only hTERT

RPTEC/TERT1 exhibit:

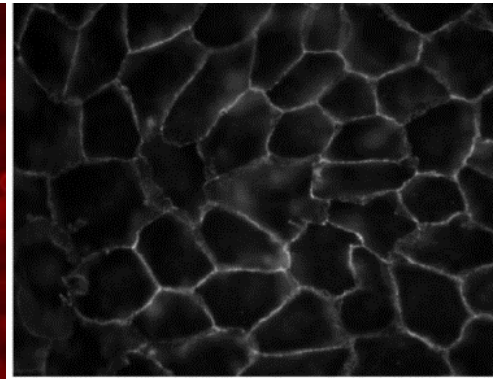
- Uniform expression of E-cadherin and CD13 (aminopeptidase N)
- Dome*-like structures
- Stabilized TEER (Trans-Epithelial Electrical Resistance)

*Domes are multicellular, cystic structures, which have been described previously in epithelia cultured from various tissues that have a known transport or secretory function *in vivo* and *in vitro*

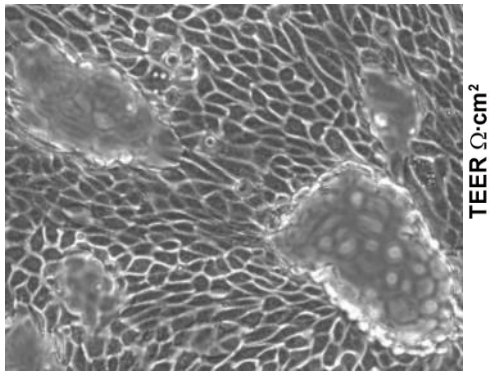
RPTEC/TERT1: CD13

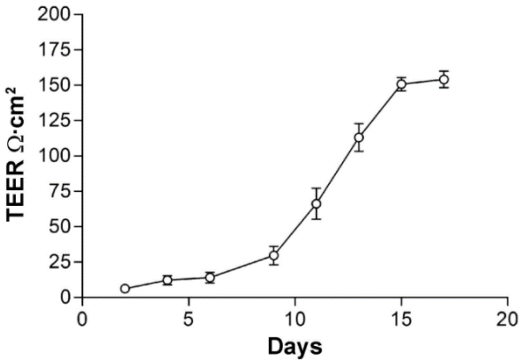


RPTEC/TERT1: E-cadherin



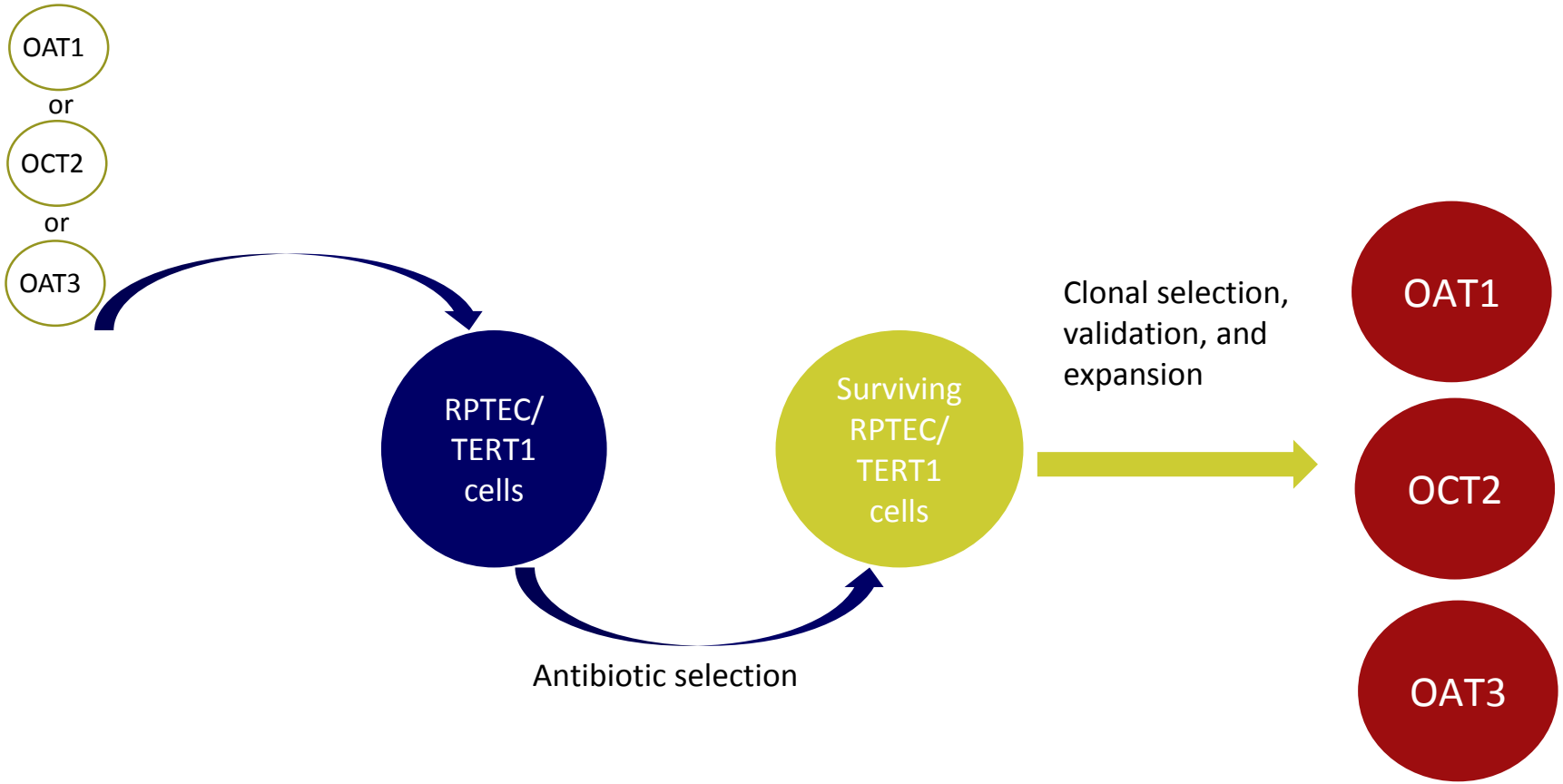
Dome formation





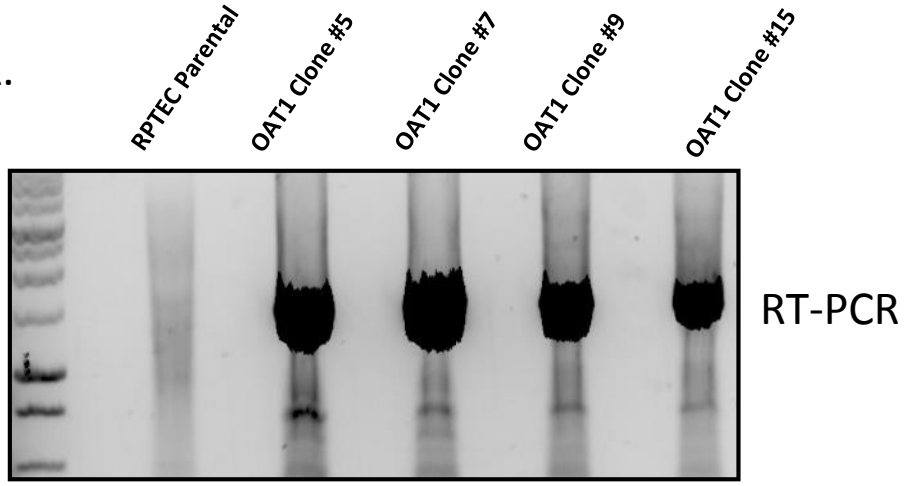
Stable cell line generation

RPTEC/TERT1 cells, like its primary counterparts, lost OAT1, OCT2, and OAT3 expression in culture



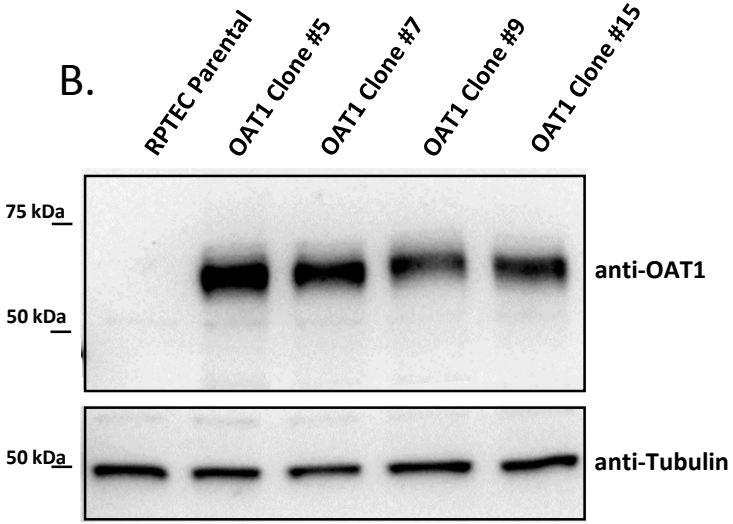
Characterization of RPTEC/TERT1-OAT1

A.



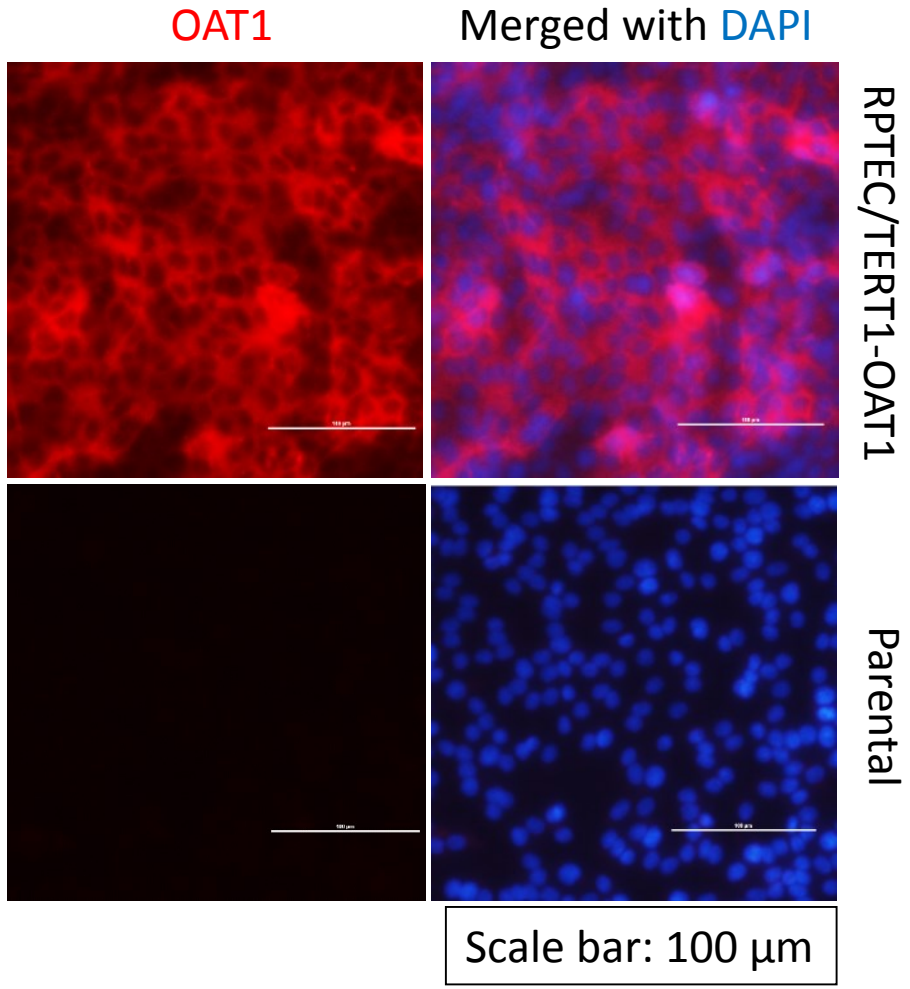
RT-PCR

B.



Western Blot

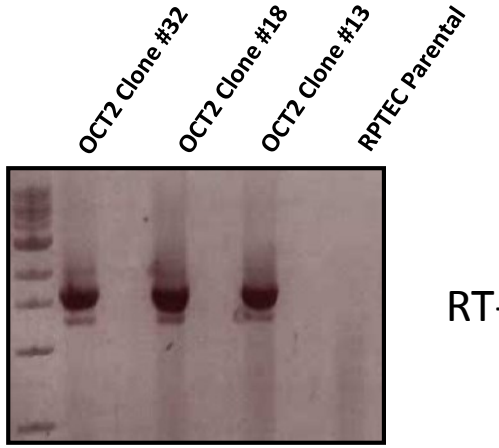
C.



Sequencing shows no mutation and 7 copies of OAT1 (vs 2 in the parental line)

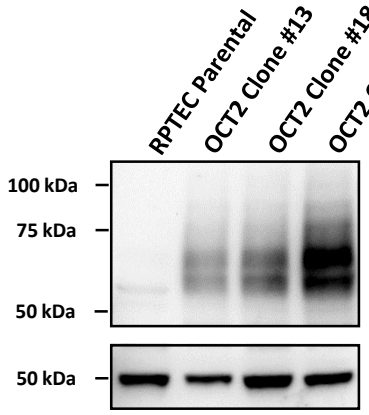
Characterization of RPTEC/TERT1-OCT2

A.



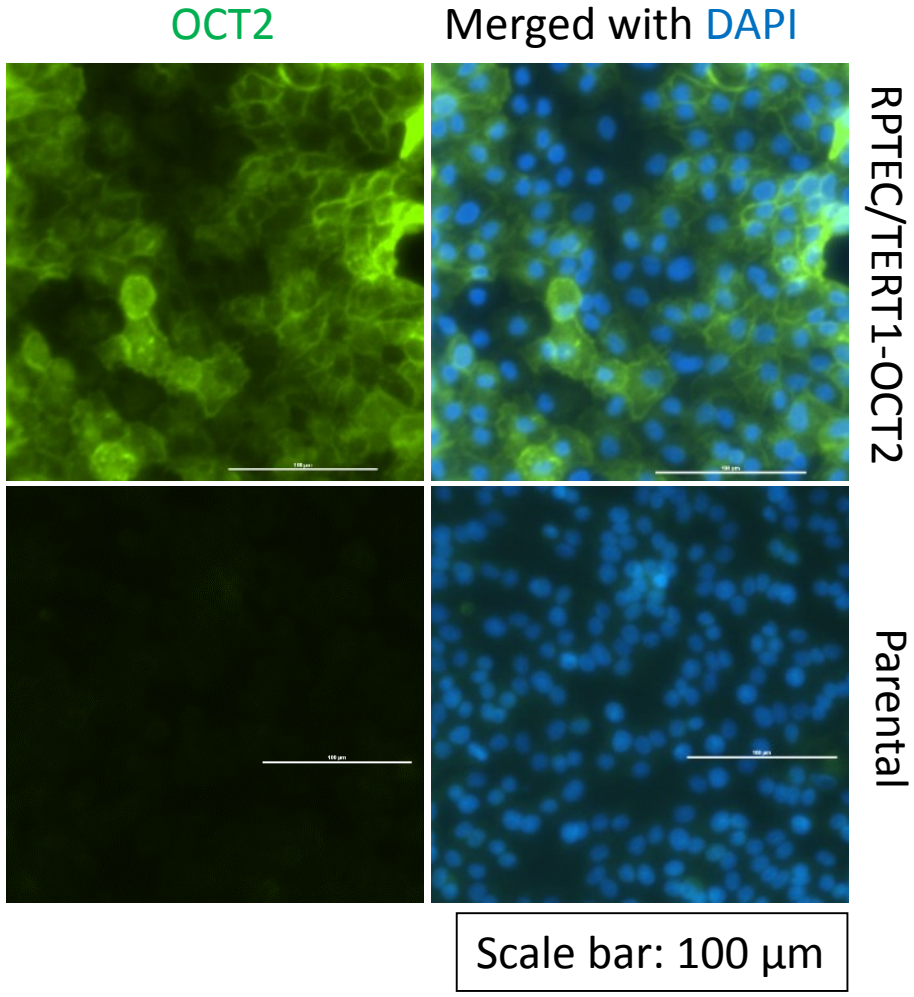
RT-PCR

B.



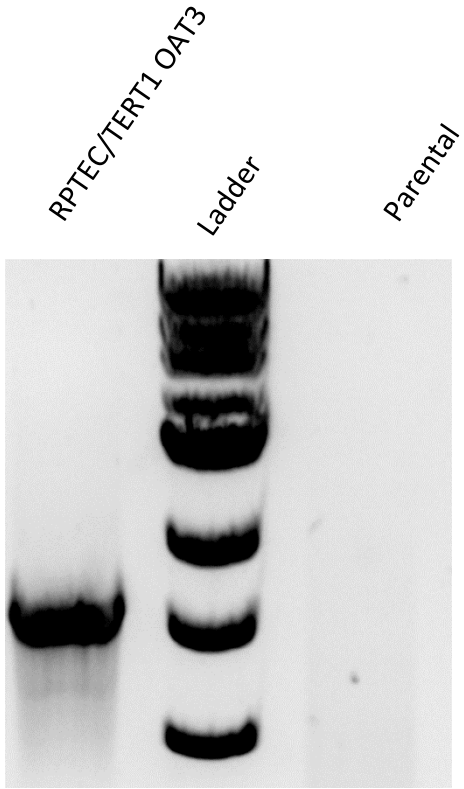
Western Blot

C.

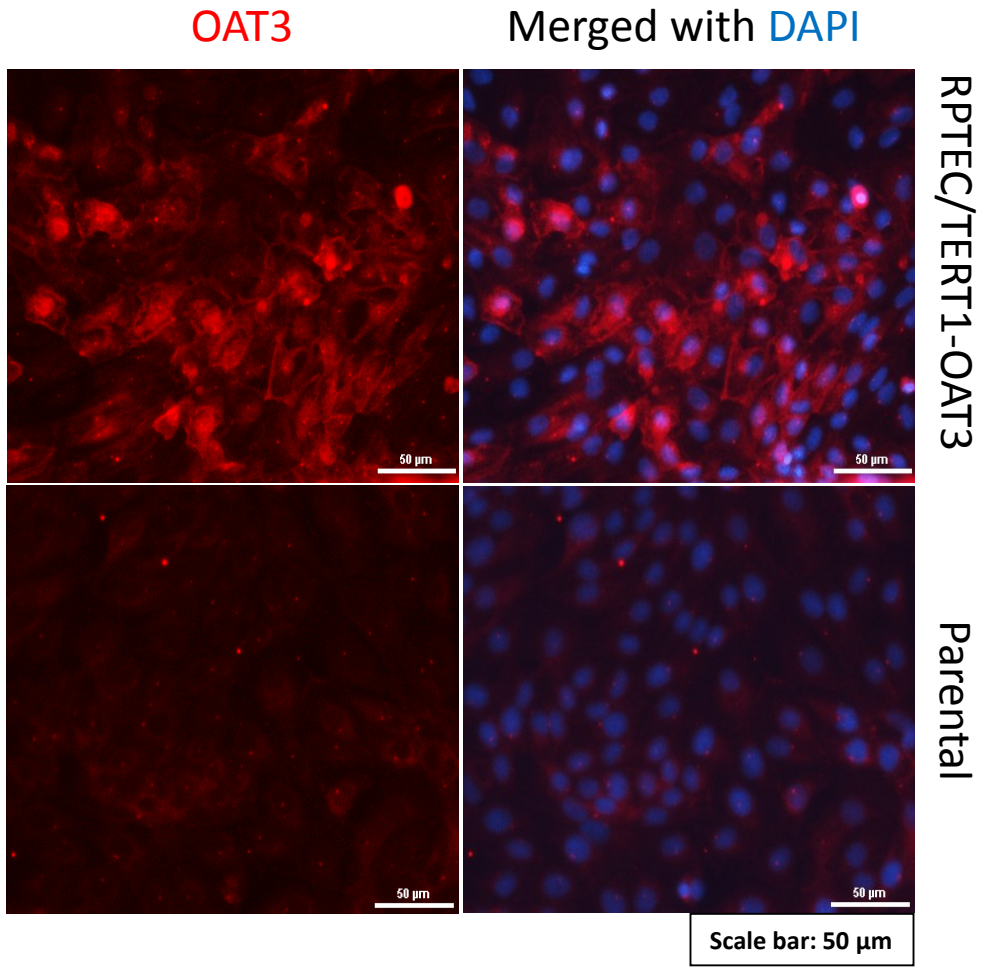


Sequencing shows no mutation and 5 copies of OCT2 (vs 2 in the parental line)

Preliminary characterization of RPTEC/TERT1-OAT3



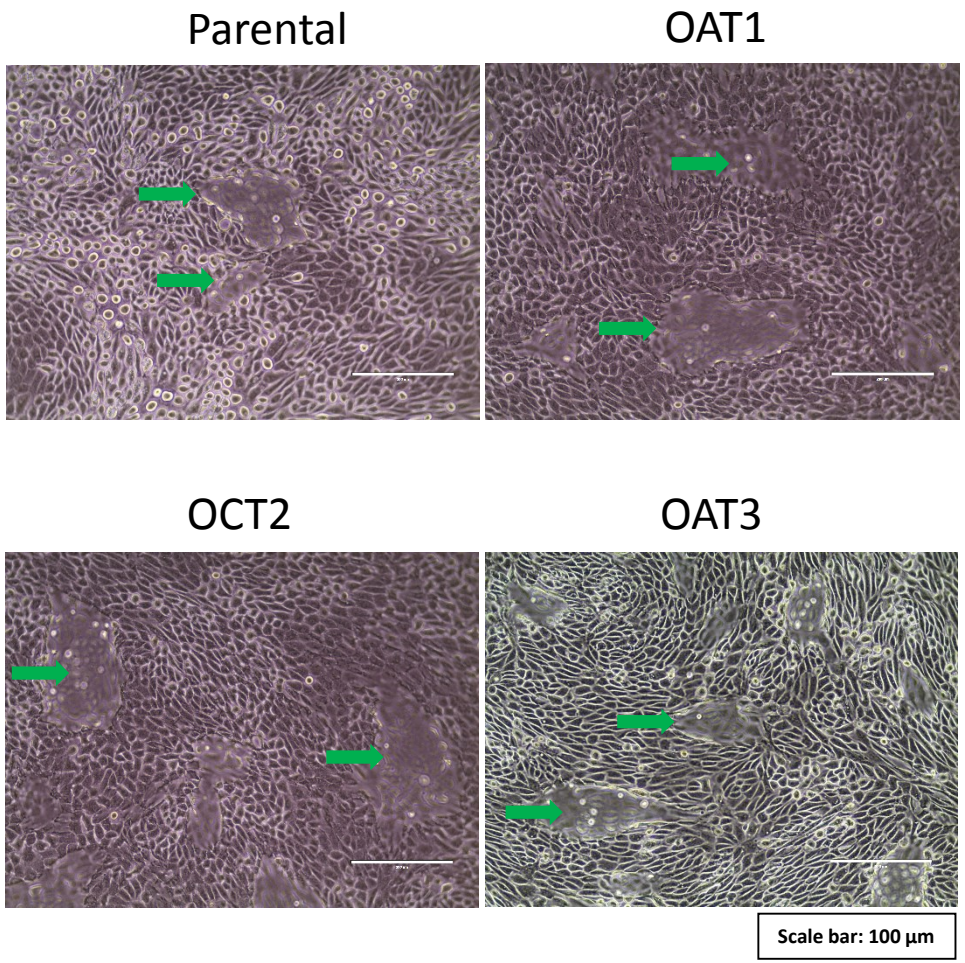
RT-PCR



5 copies of OAT3 vs 2 parental

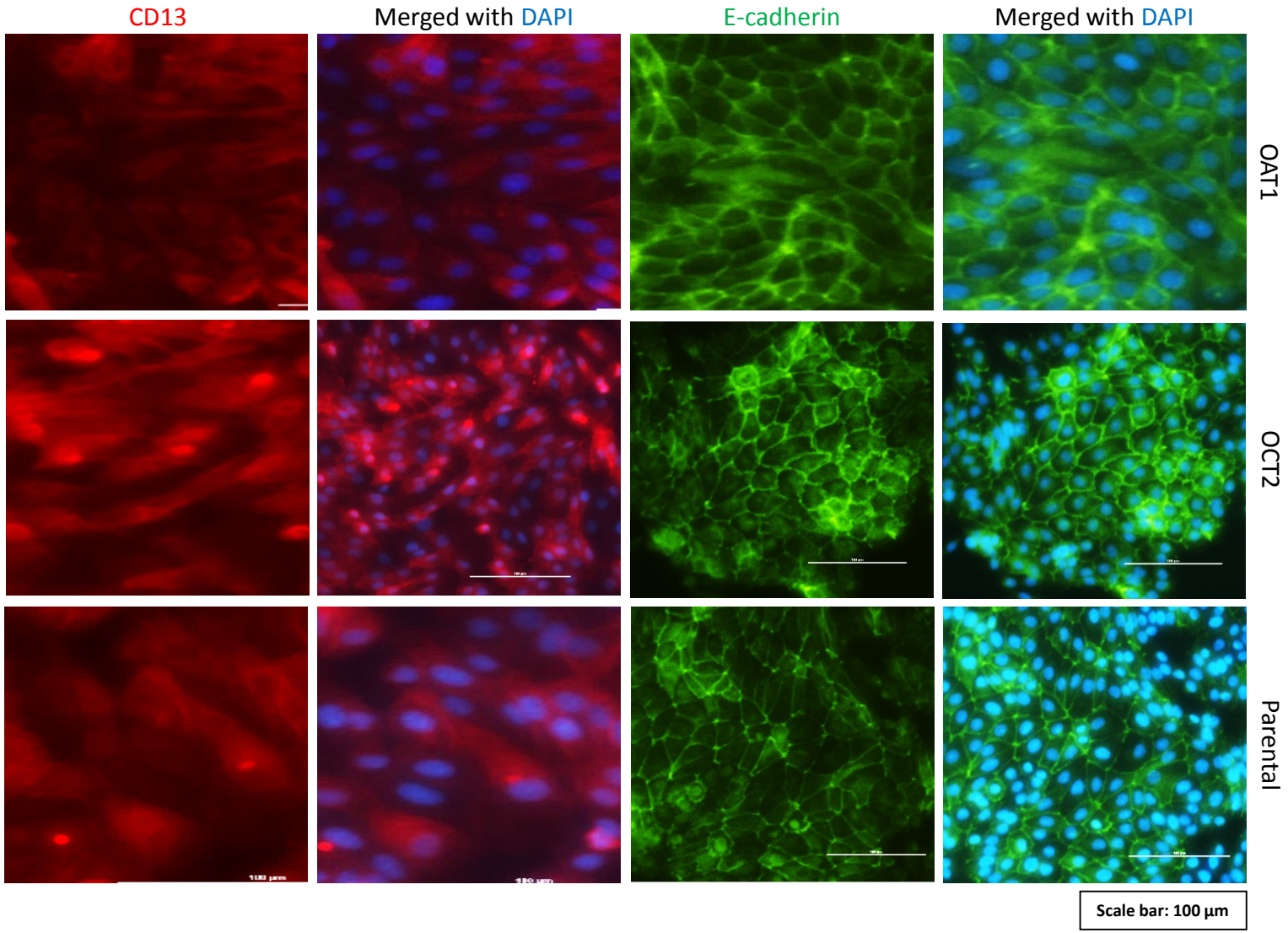
Dome formation – cells maintain original function

Domes are shown by the green arrows

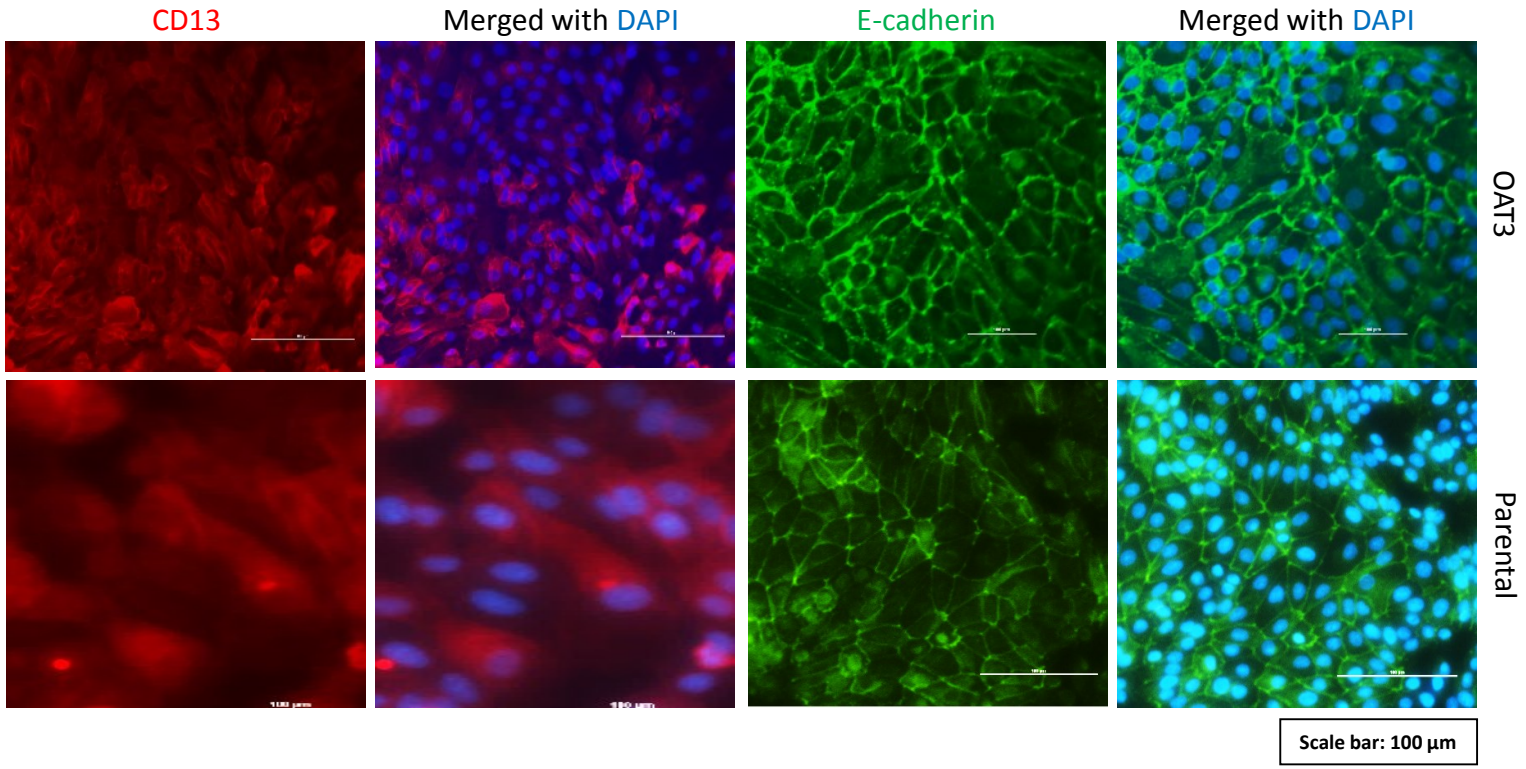


*Domes are multicellular, cystic structures, which have been described previously in epithelia cultured from various tissues that have a known transport or secretory function *in vivo* and *in vitro*

Key renal epithelial marker staining

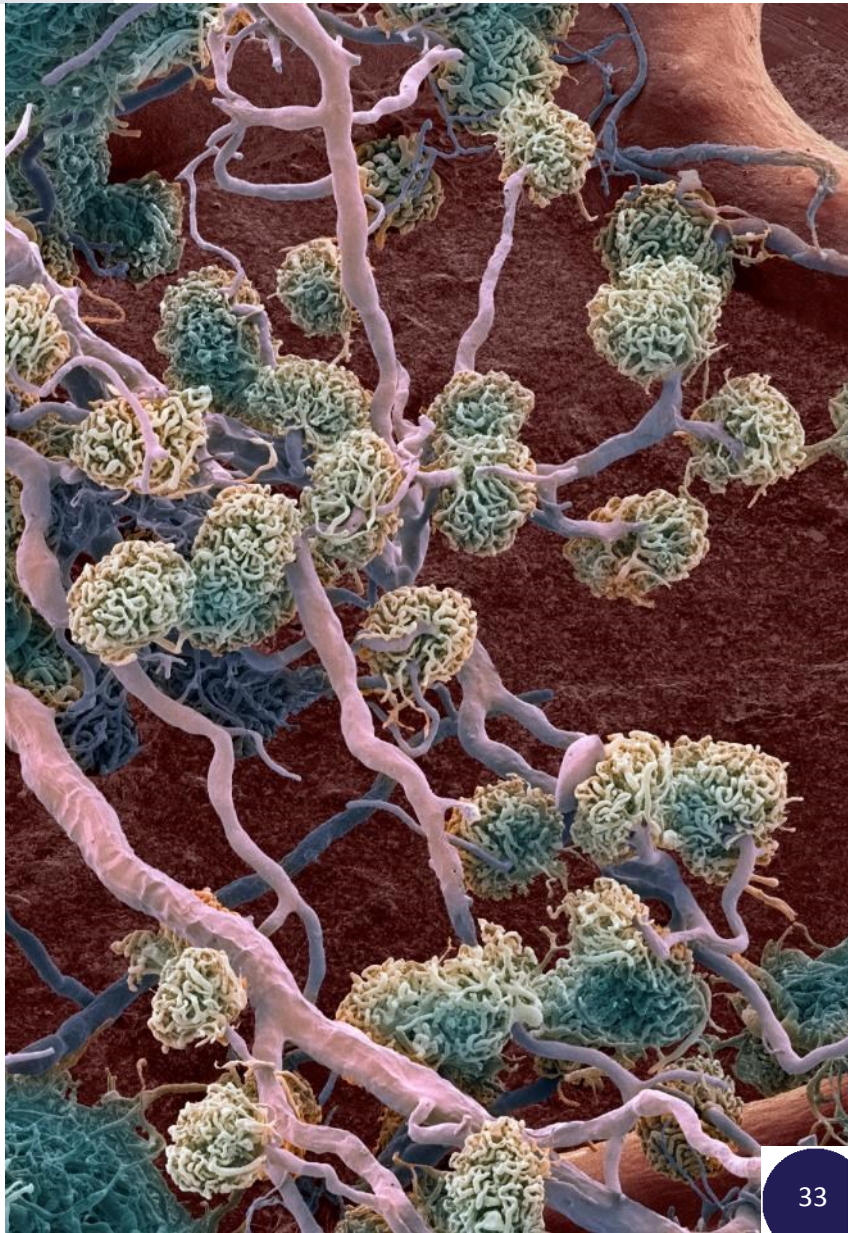


Key renal epithelial marker staining



Agenda

- ATCC toxicology tools
- Kidney transporter models
  - Current *in vitro* transporter cell models
  - Generation of RPTEC renal transporter models
  - Application data



Experimental design – brief assay protocol

Uptake assay:

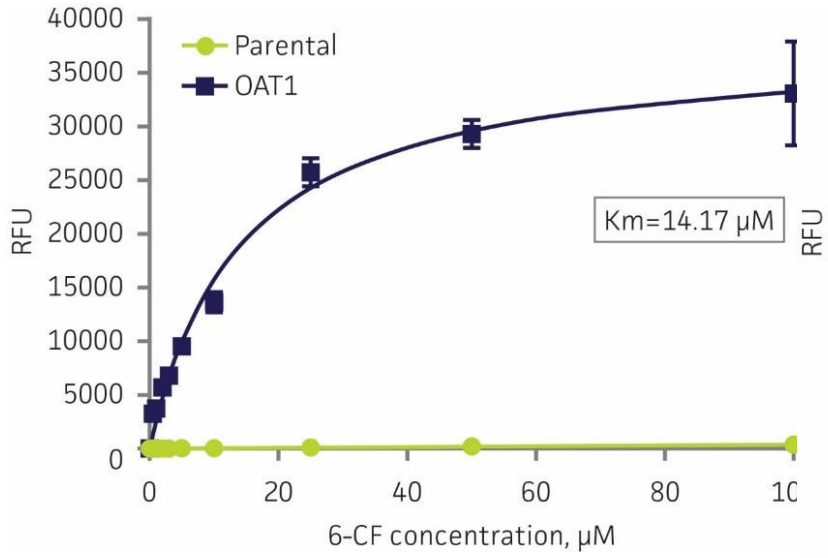
- **Equal numbers of both parental and transporter cells** were seeded into 96-well plate in triplicate and placed in an incubator at 37°C, 5.0% CO_2
- After 24 hours assays were performed
- **Increasing concentration of 6-CF** or **EAM1** were added and incubated for 20 minutes at 37°C
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured

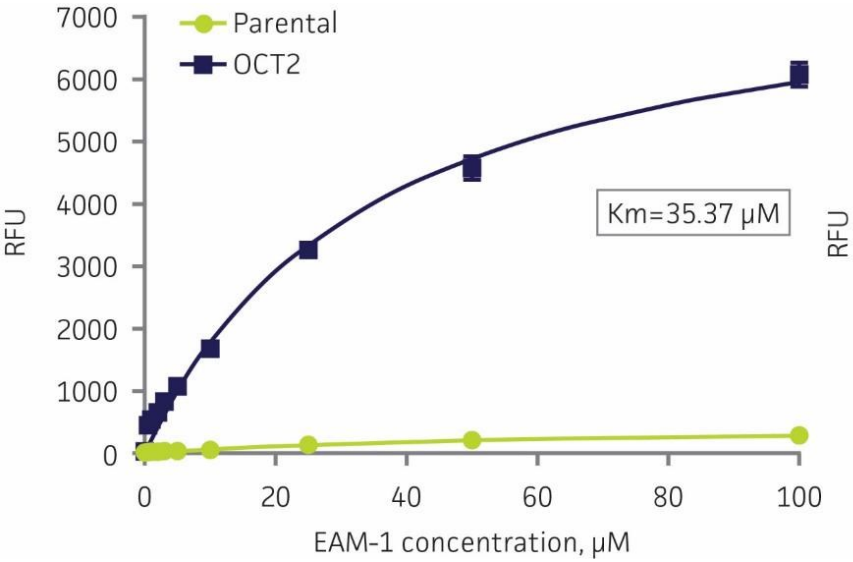
Inhibition assay:

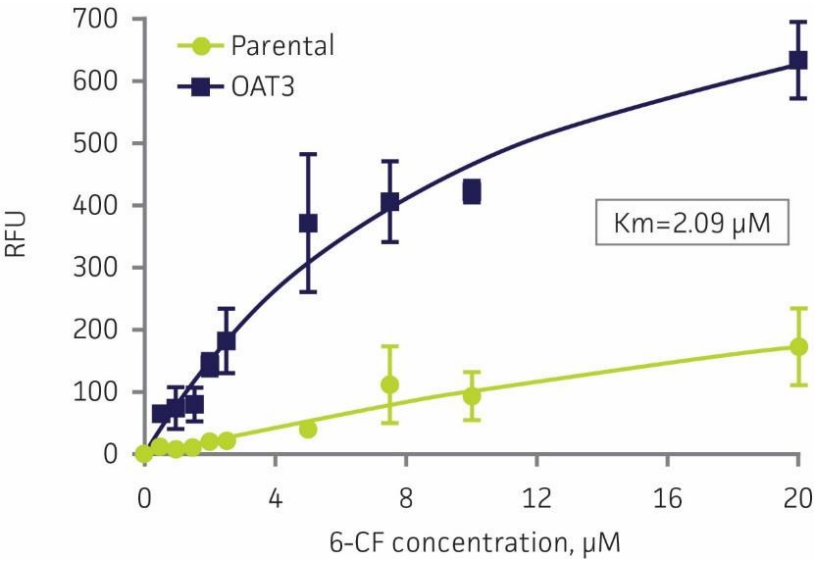
- **Equal numbers of both parental and transporter cells** were seeded into 96-well plate in triplicate and placed in an incubator at 37°C, 5.0% CO_2
- After 24 hours, assays were performed
- **Increasing concentration of inhibitors** were added together with **constant concentrations of the uptake substrate** and incubated for 20 mins at 37°C
- After wash with cold HBSS 4 times, cells were lysed and uptake intensity were measured

These protocols apply to the following series of experiments

OAT1, OCT2 and OAT3 displays high uptake sensitivity







6-CF concentration	0 μM	0.5 μM	1 μM	2 μM	3 μM	5 μM	10 μM	25 μM	50 μM	100 μM
Uptake ratio	1.16	169.67	226.94	239.13	260.74	274.13	287.70	198.19	146.07	84.16

OAT1 6-CF

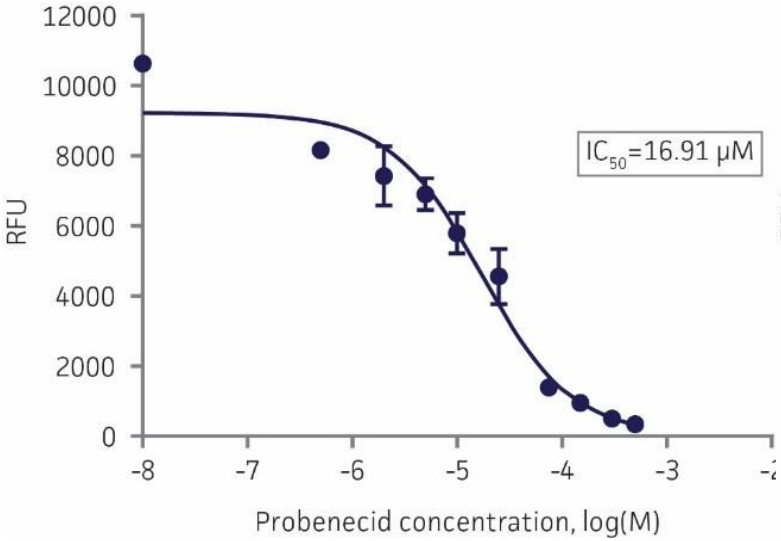
EAM-1 concentration	0 μM	0.5 μM	1 μM	2 μM	3 μM	5 μM	10 μM	25 μM	50 μM	100 μM
Uptake ratio	2.27	21.56	23.01	22.65	22.73	28.13	29.92	23.72	21.58	21.22

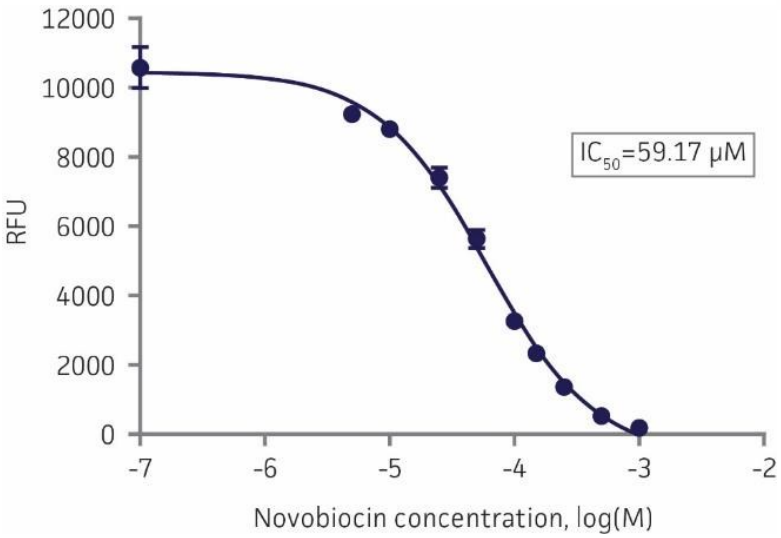
OCT2 EAM-1

6-CF concentration	0μM	0.5μM	1.0μM	1.5μM	2.0μM	2.5μM	5.0μM	7.5μM	10μM	20μM
Uptake ratio	1.00	5.47	9.05	7.50	7.46	8.58	9.12	3.60	4.51	3.66

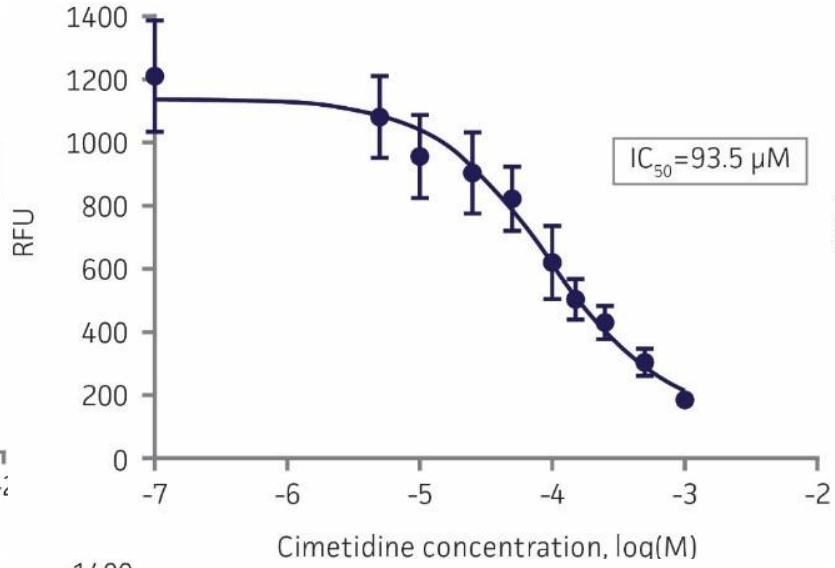
OAT3 6-CF

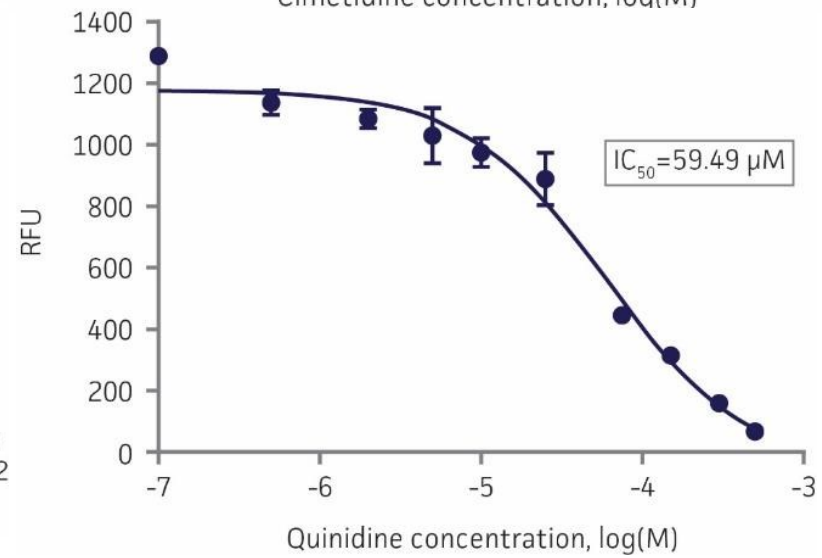
OAT1, OCT2 and OAT3 show accurate uptake specificity



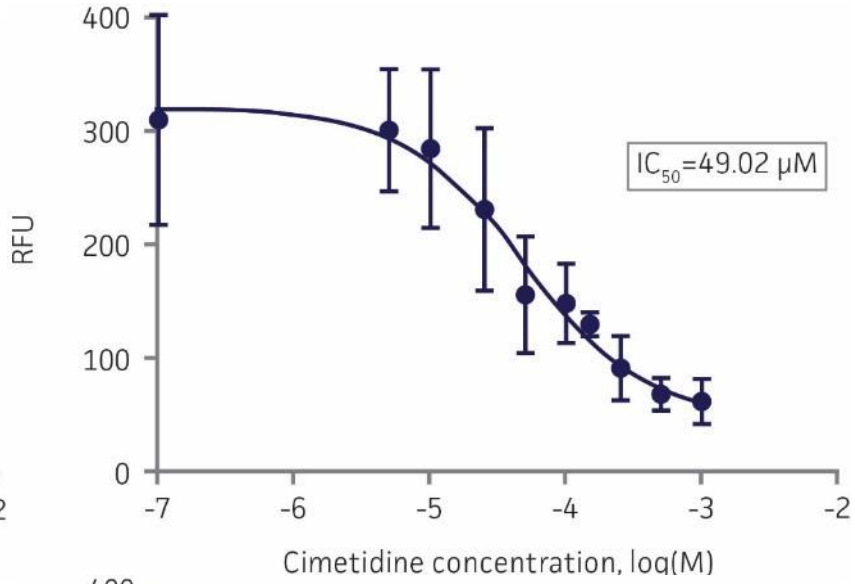


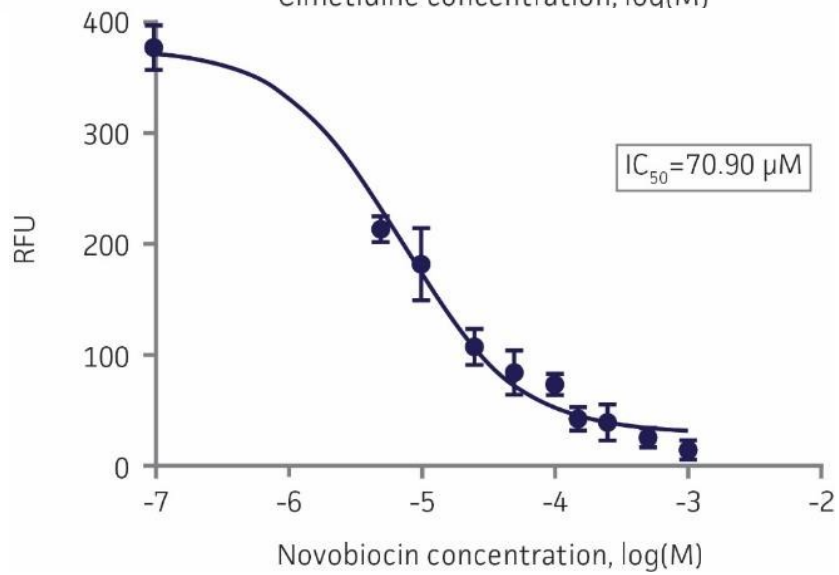
OAT1





OCT2

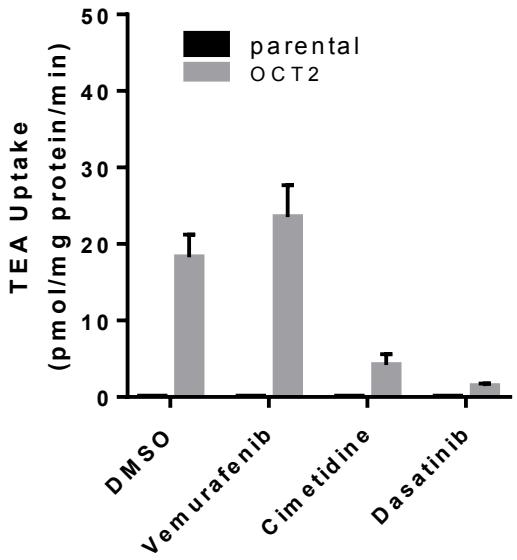


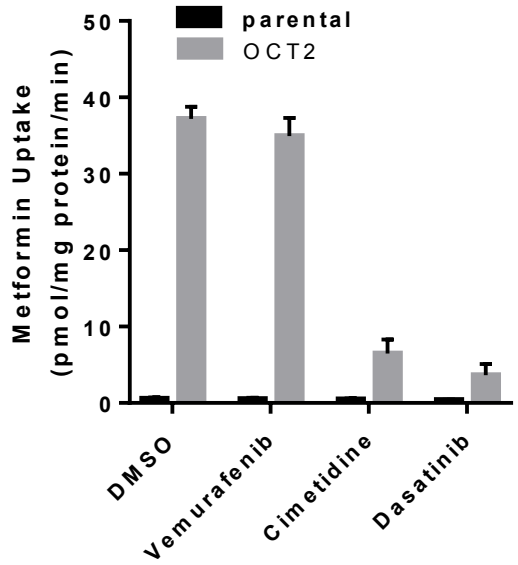


OAT3

Independent confirmation of the solute transport ability of RPTEC/TERT1-OCT2

Brief 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; after 15 minutes, remove drug and wash 3 times with cold PBS; lyse the cells and count.





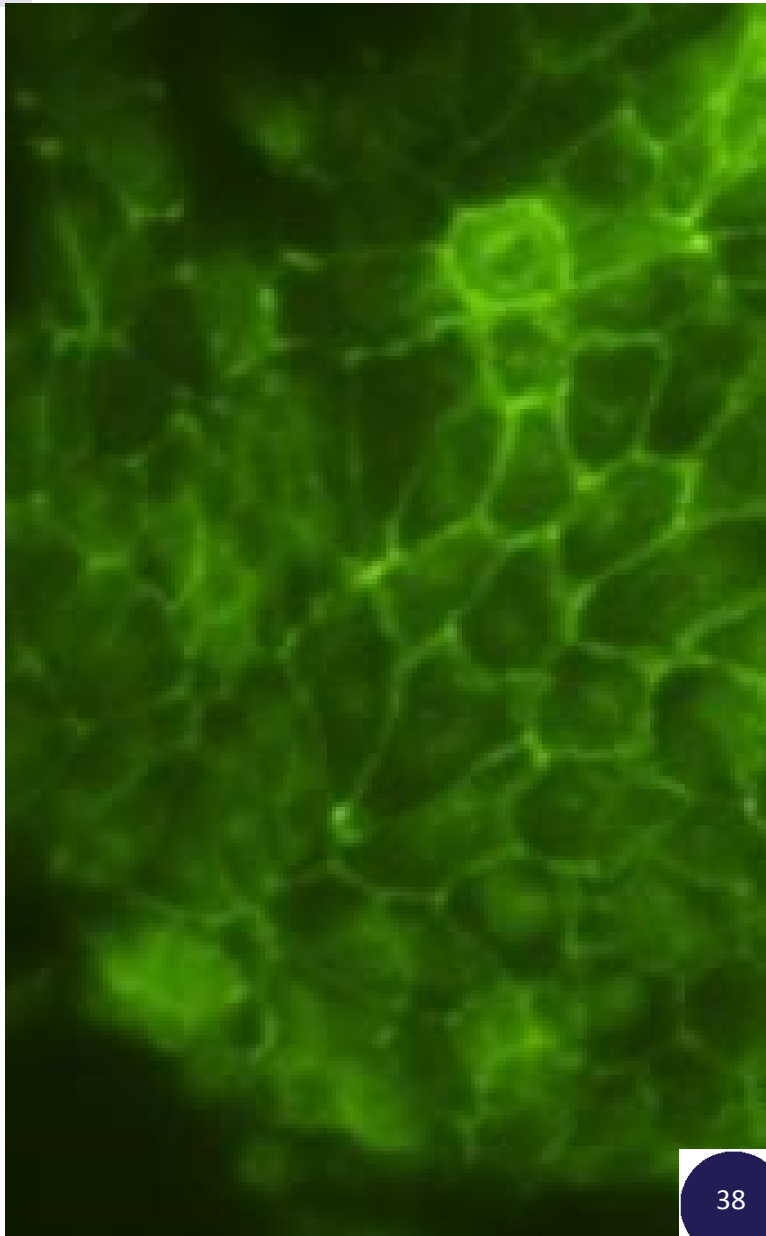
Data kindly provided by:
Kevin Huang, *Graduate Research Associate,* Ohio State University, College of Pharmacy
Alice Gibson, Ph.D., *Senior Research Specialist,* Ohio State University, College of Pharmacy

Applications for nephron toxicity studies

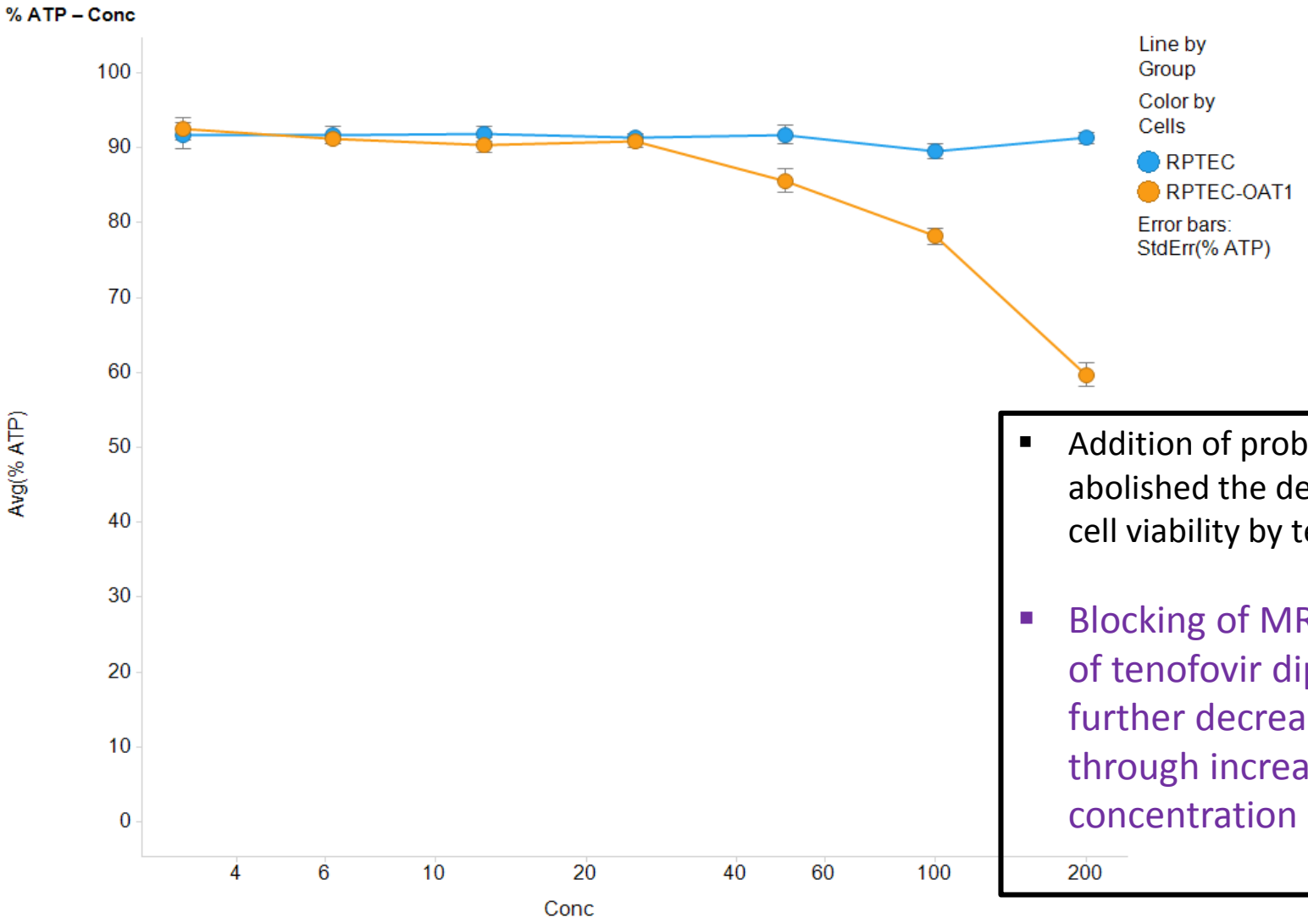
The following data was kindly provided by Merck & Co., Inc.

Brief Protocol: Cell Viability Assay

- About 35000 cells were seeded per well in triplicate in a 96 well plate
  - Incubated overnight
  - Then incubated with a series of compounds at various concentrations as indicated for 3 days
- Cell viability was subsequently determined using CellTiter-Glo® Luminescent Cell Viability (ATP) Assay per Promega's instructions



Tenofovir decreases cell viability in RPTEC/TERT1-OAT1 but not RPTEC/TERT1 cells

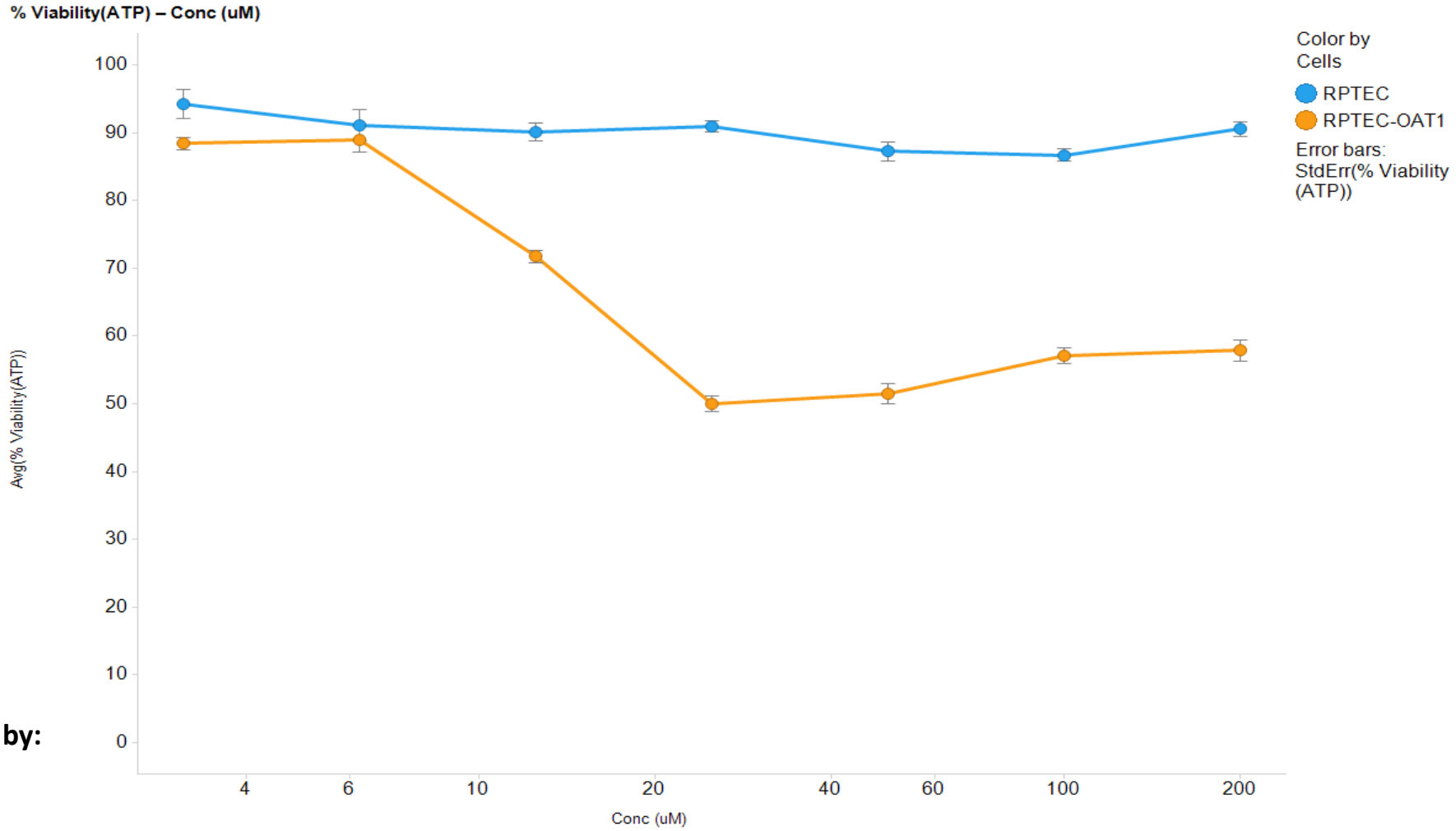


- Addition of probenecid, an inhibitor of OAT1, abolished the decrease in RPTEC/TERT1-OAT1 cell viability by tenofovir
- Blocking of MRP4, the efflux transporter of tenofovir diphosphate, by ceefourin 2, further decreases cell viability presumably through increase of tenofovir diphosphate concentration in the cell.

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

Cidofovir treatment decreases cell viability of RPTEC/TERT1-OAT1 but not RPTEC/TERT1 cells

Cidofovir is an OAT1 substrate

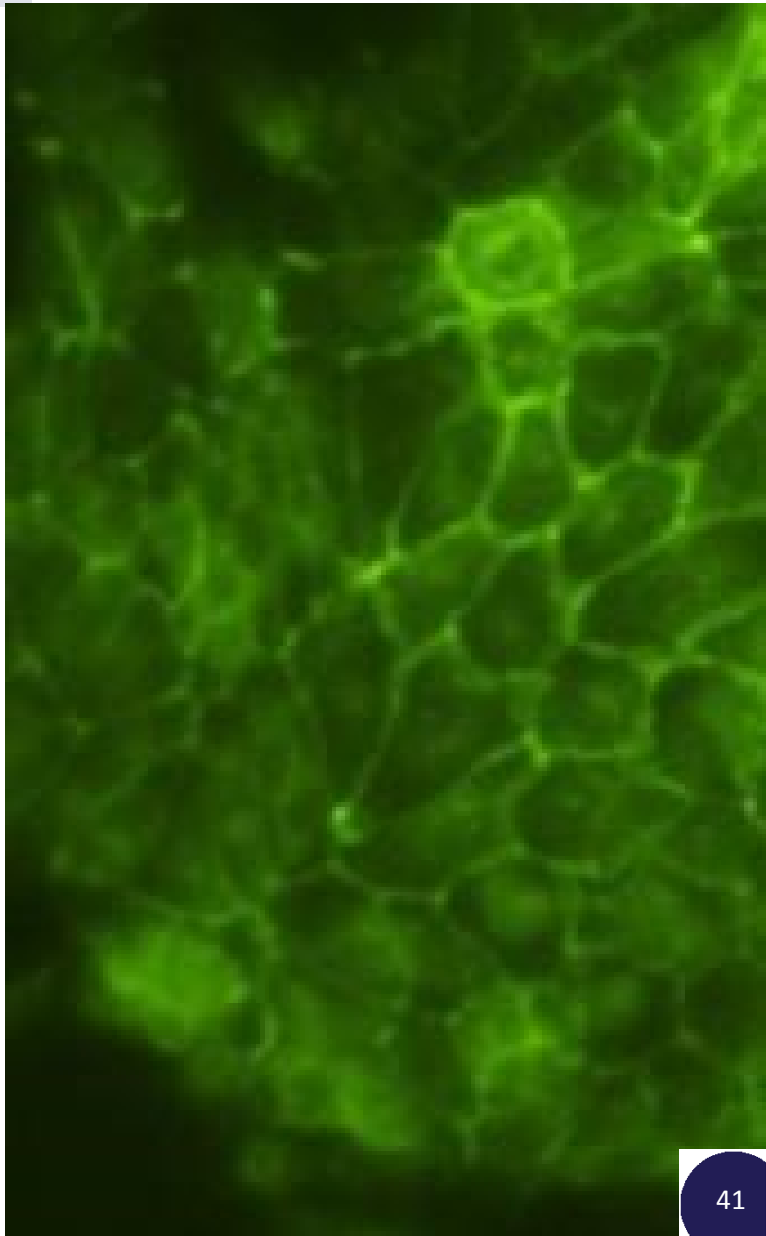


Data kindly provided by:
Merck & Co., Inc.

Conclusions – Merck study

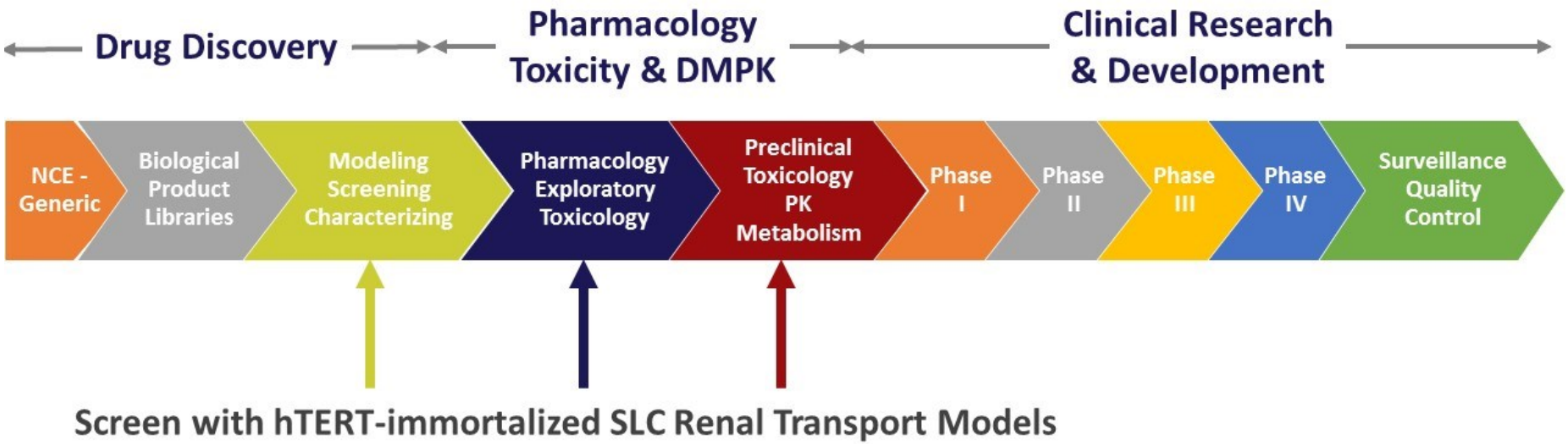
Modulation of cell viability with various test articles further indicates active transporter uptake

- RPTEC/TERT1-OAT1 cells but not the parent RPTEC/TERT1 cells demonstrate decreased cell viability following treatment of tenofovir, a substrate of OAT1 and 3.
- Blocking of MRP4, the efflux transporter of tenofovir diphosphate, by ceefourin 2, further decreases cell viability presumably through increase of tenofovir diphosphate concentration in the cell.
- Addition of probenecid, an inhibitor of OAT1, abolished the decrease in RPTEC/TERT1-OAT1 cell viability by tenofovir
- Tenofovir, and cidofovir dose response curves show
  - All 3 compounds are toxic to RPTEC/TERT1-OAT1 cells
  - Tenofovir and cidofovir are not toxic to RPTEC/TERT1, since these compounds cannot enter the cells.



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

Utility of ATCC next-gen models in drug development



Candidate Compound
+ RPTEC/TERT1-OAT1
+ RPTEC/TERT1-OCT2
+ RPTEC/TERT1-OAT3

Uptake or inhibition assay screening

If **no change in transport**:
Further testing to determine transport mechanism

If **positive or negative change in transport**:
Transport mechanism has been identified

Summary

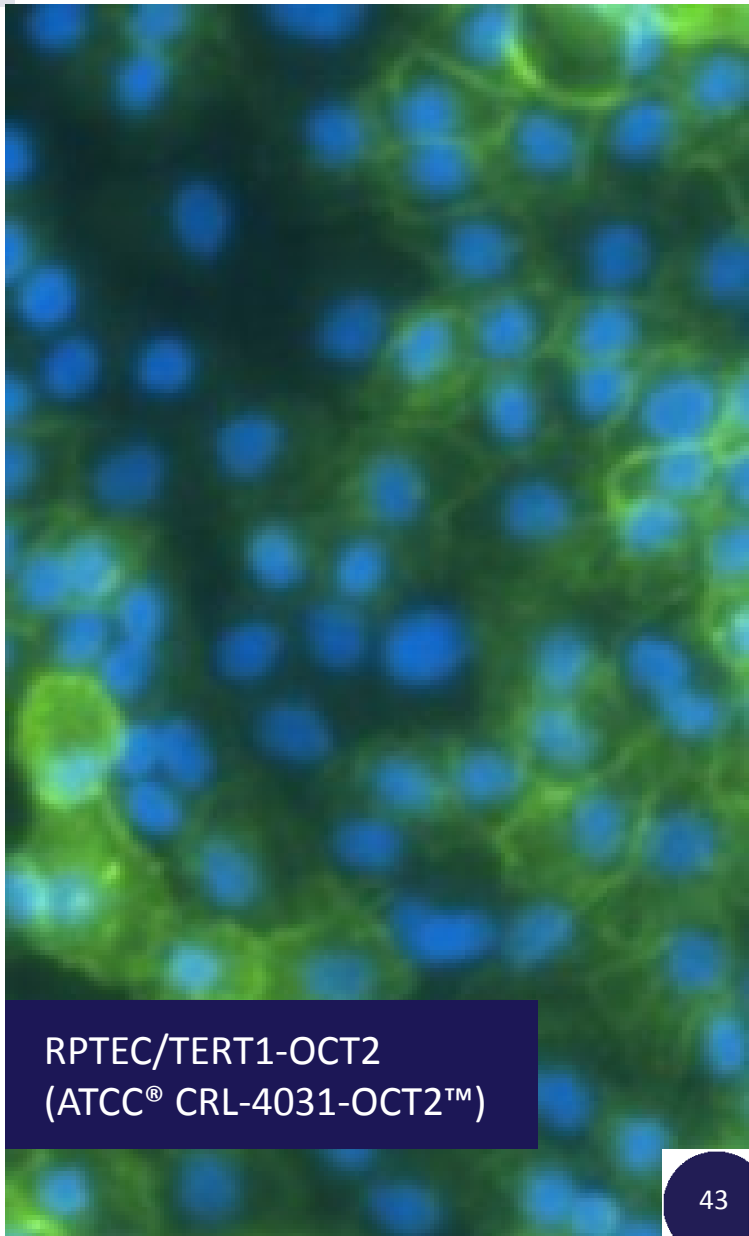
ATCC RPTEC/TERT1 renal uptake cell models stably overexpress OAT1, OCT2, and OAT3

- Expression has been confirmed by:
  - PCR
  - Western blot
  - Immunocytochemistry
  - Copy number-verified

The clonal stable cells retain important characteristics of *in vivo* adult renal cells

The performance of these stable cells are well characterized by:

- 6-CF and EAM-1 uptake assays
- Inhibition assays
- Confirmed by TEA, Metformin and PAH uptake assays
- Tested against the well know renal toxic drugs



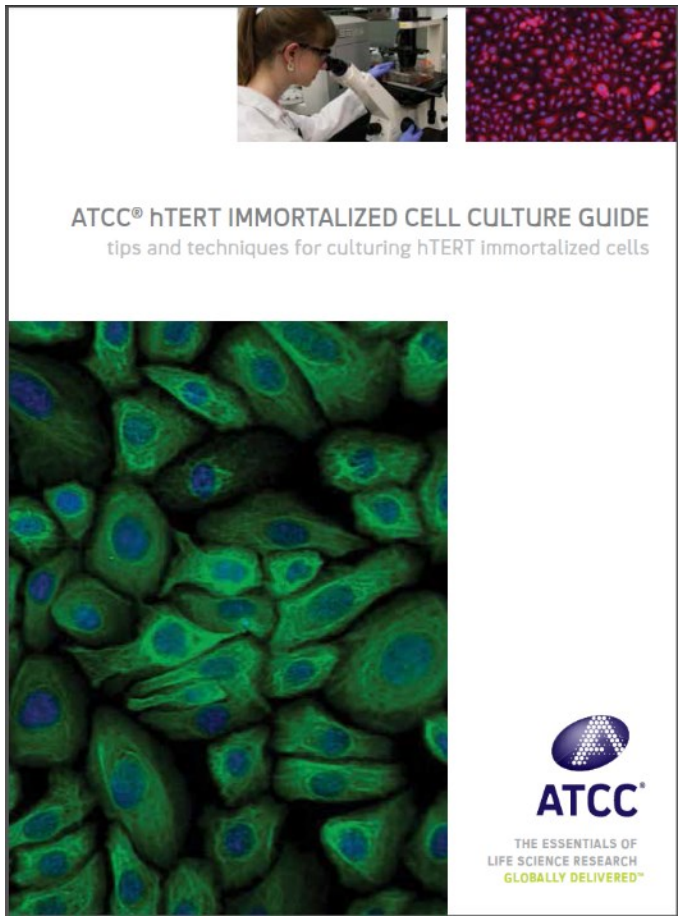
RPTEC/TERT1-OCT2
(ATCC® CRL-4031-OCT2™)

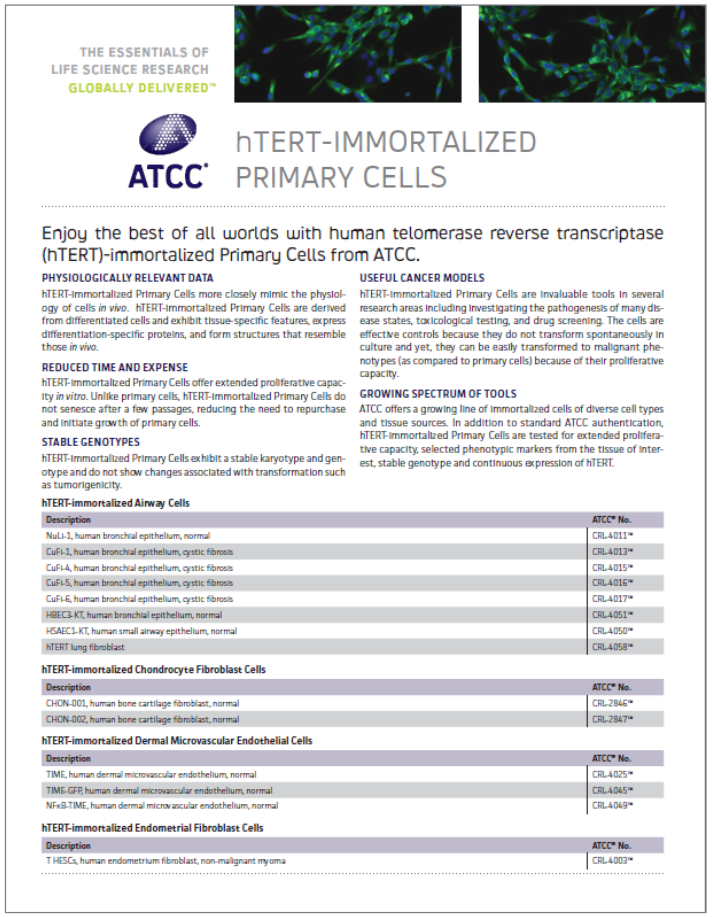
Resources

We have other hTERT cells – for a complete list download either:

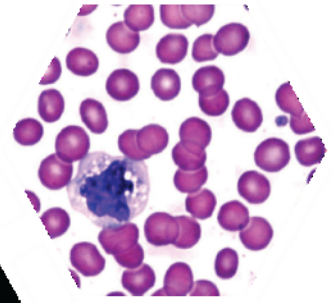
- **hTERT-immortalized Cell Culture Guide**
- **hTERT-immortalized Cells Brochure**

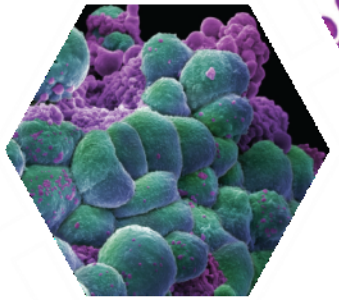
For more information visit www.atcc.org/hTERT





Thank you





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