Immortalized Primary Cells: The Best of Both Worlds

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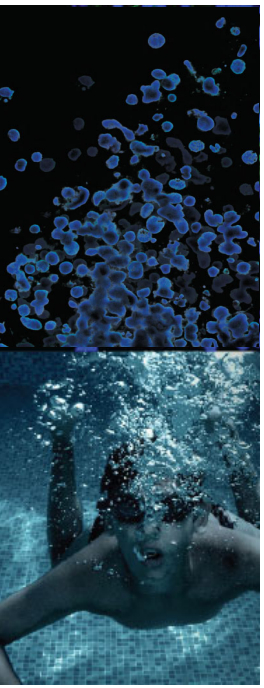
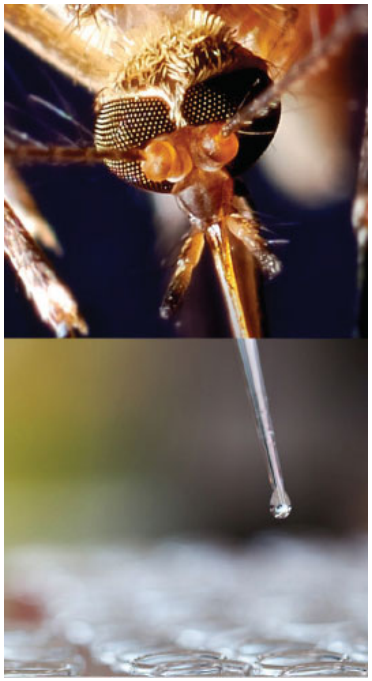
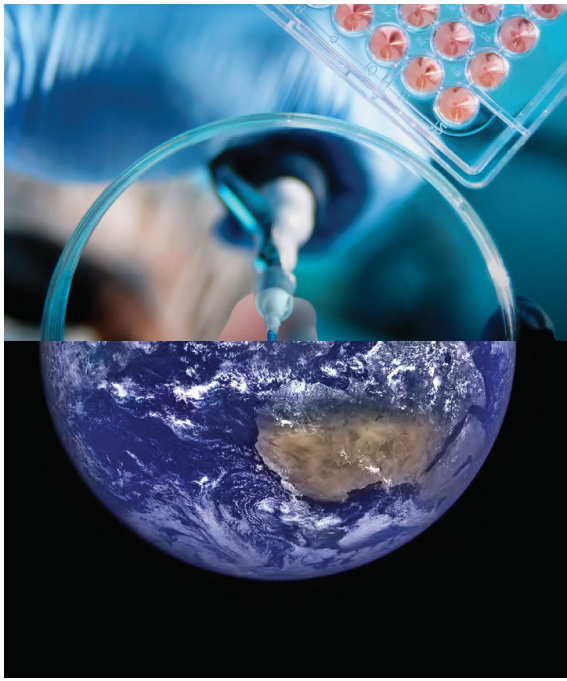


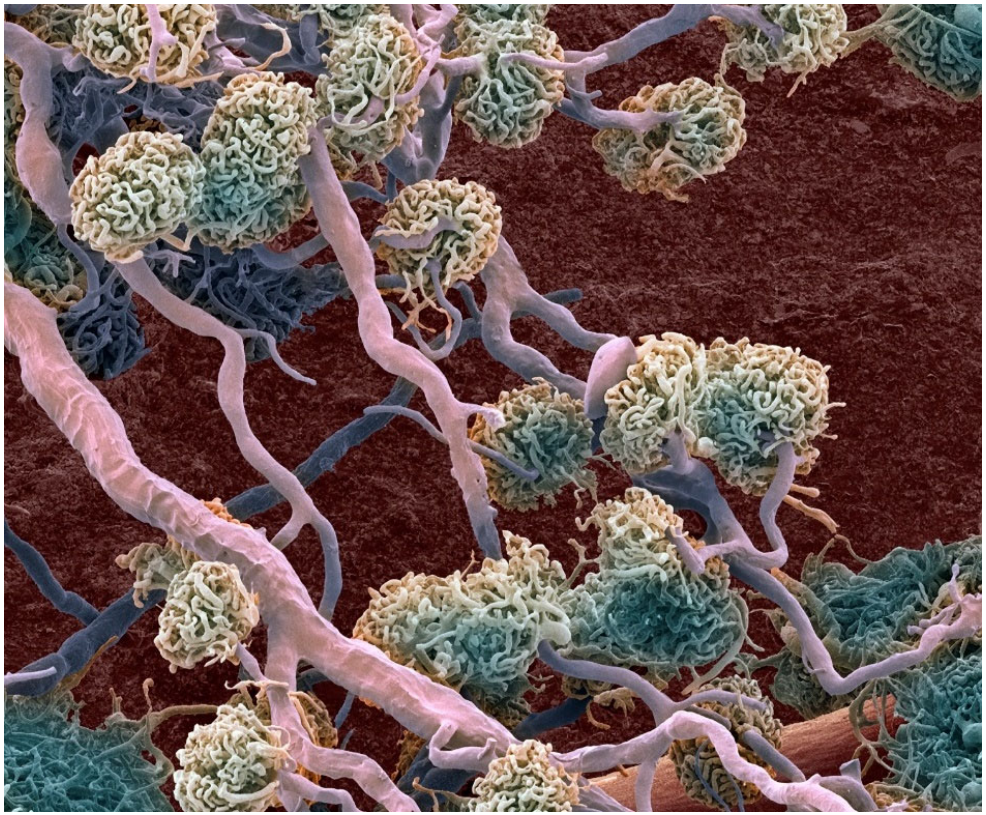




Credible Leads to Incredible™

Agenda

- ATCC® mission and future direction
- ATCC® immortalized primary cell portfolio
- Case studies
  - Kidney models
  - Cardiovascular models

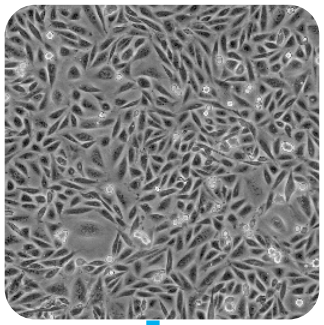


About ATCC®

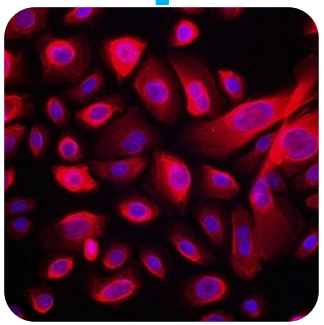
- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring gene editing, differentiated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

ATCC®

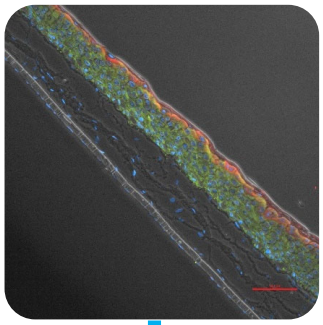
Evolution of in vitro cell models



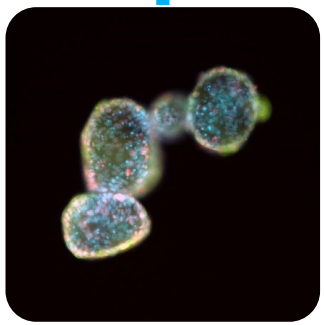
First generation
Continuous cell lines



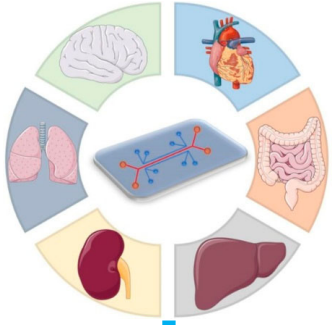
Second generation
Transfected cell lines
Reporter cell lines
Primary cells



Third generation
Immortalized primary cells
Gene-edited cell lines
iPSC derived cells



Fourth generation
3-D models
Organoids

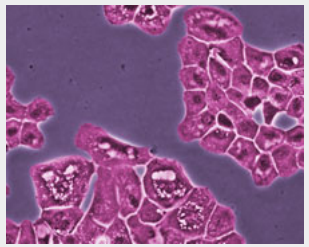
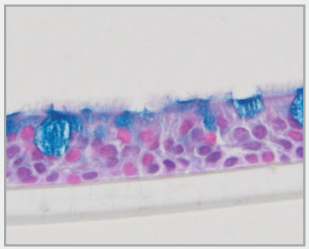
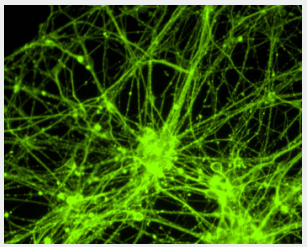
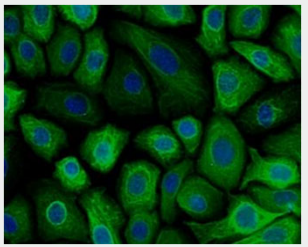


Fifth generation
Organ-on-a-chip

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8866728/

Sources of cell models

Cells are one of the most important resources for predicative in vitro models

Cell lines	Primary cells	iPS-derived cells	Immortalized primary cells
▪ Highly proliferative ▪ Easy to culture and transfect ▪ Differ genetically and phenotypically from their tissue origin ▪ Accepted for specific functions, i.e., Caco-2 is well accepted for drug transporters studies; however, it lacks physiological CYP activities	▪ Most accepted cell types; physiological relevance ▪ Maintain many of the important markers and functions ▪ Higher predictability ▪ Donor-donor variability ▪ Finite lifespan and limited expansion capacity ▪ Difficult to source or preserve some tissue types (alveolus, cardiomyocytes, neurons)	▪ Advantages of precision medicine ▪ It might maintain many of the important markers and functions ▪ Multiple cell types from the same donor ▪ Need experienced users to culture and maintain ▪ Cryopreservation is an issue ▪ Poor differentiation/ maturation	▪ Maintain physiological feature of primary cells ▪ Allows for extended cultivation ▪ Improved supply and reproducibility ▪ Amenable to genetic editing ▪ Need experienced users to generate and characterize
			
Chordoma Cell Line	Bronchial Epithelial Cells	Neural Progenitor Cells	hTERT Bronchial Epithelial Cells

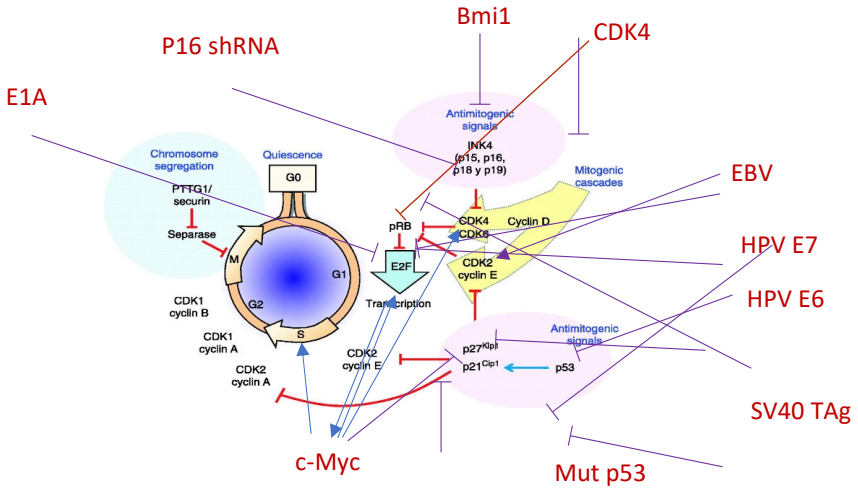
Immortalization - Cell cycle regulation

- Primary cells can only replicate a handful of times, or not at all. Cellular senescence, donor availability, and donor-to-donor variability limit the applications

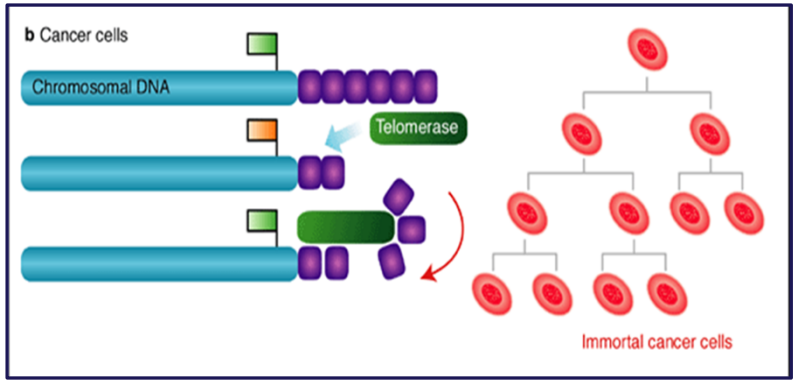
Immortalizing genes:

- **hTERT**
- Viral oncogenes: SV40T Antigen, EBV, HPV E6/E7, E1A
- Other cellular genes: CDK4, **Bmi1**, P16, c-Myc , mutant p53

Cell cycle regulation by immortalizing genes:

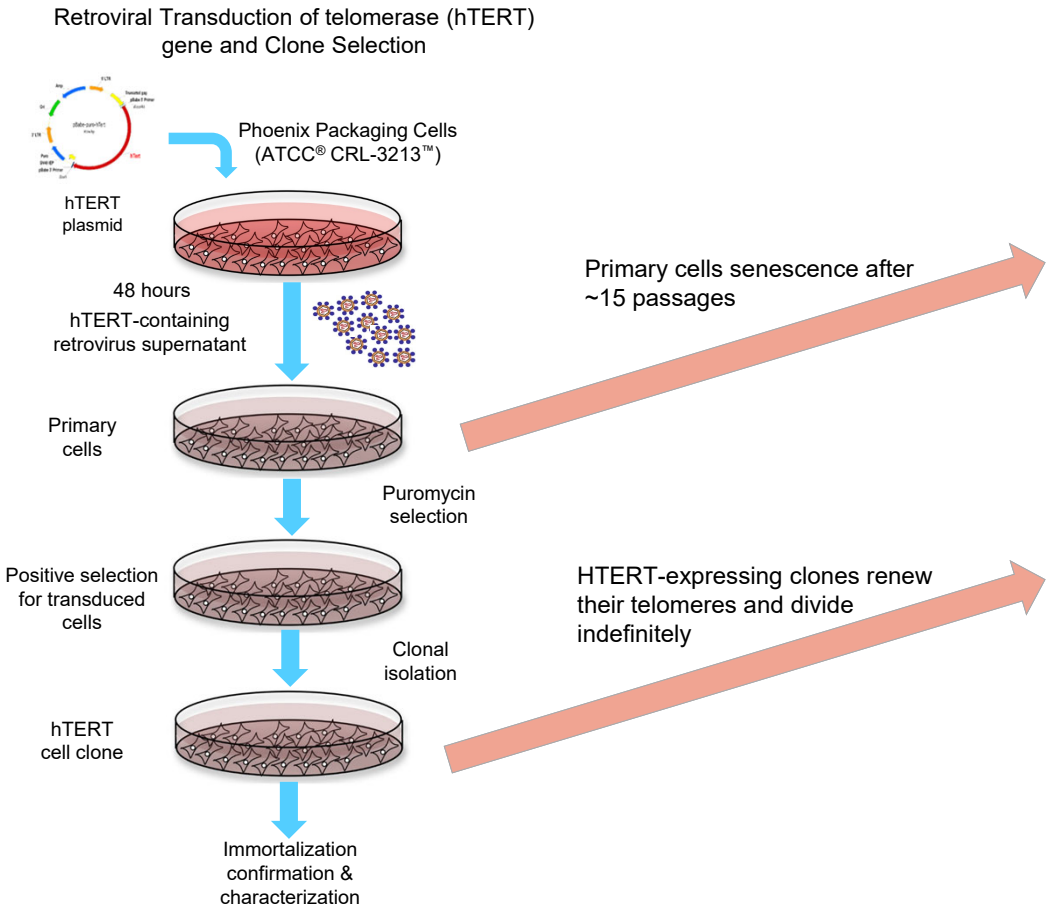


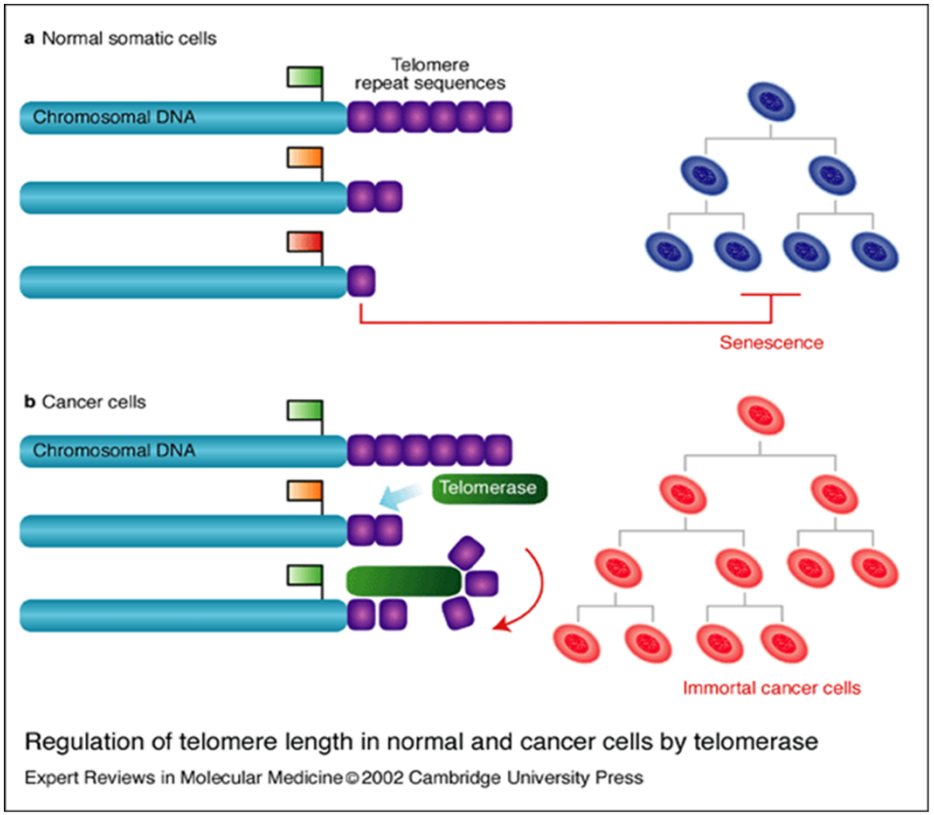
Modified from Victor Q, Malumbres M. J Mol Endocrinol 42(2):75-86, 2009.



Keith W, et al. Expert Rev Mol Med 22;4(10):1-25, 2002.

Cell immortalization process - hTERT alone



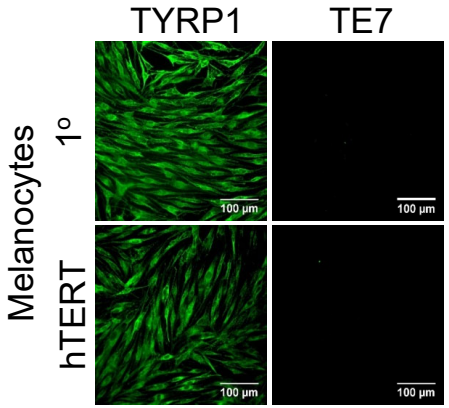


Keith W, et al. Expert Rev Mol Med 22;4(10):1-25, 2002.

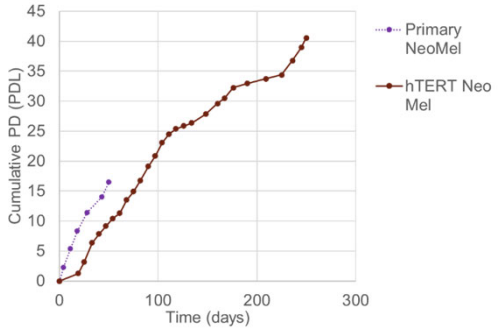
Immortalized primary cells – Key characteristics

ATCC® offers primary and immortalized cell solutions that are authenticated via rigorous QC and tested for common biomarker expression and cell performance

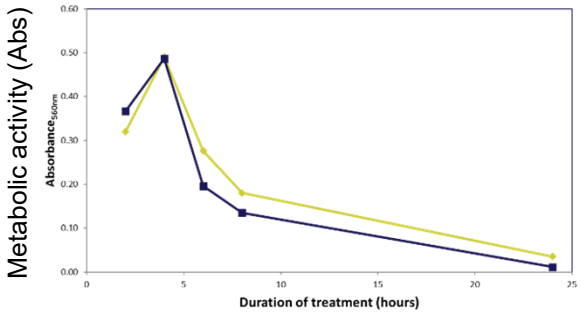
- **Growth**
  - Cells retain replicative capacity (“immortalized”)
  - Population doubling rate is comparable to primary cells
- **Characterization**
  - Morphology and marker expression similar to primary cells
  - Sterility testing
  - STR profile
- **Functional responses**
  - Within expected range
  - Analogous to primary cells

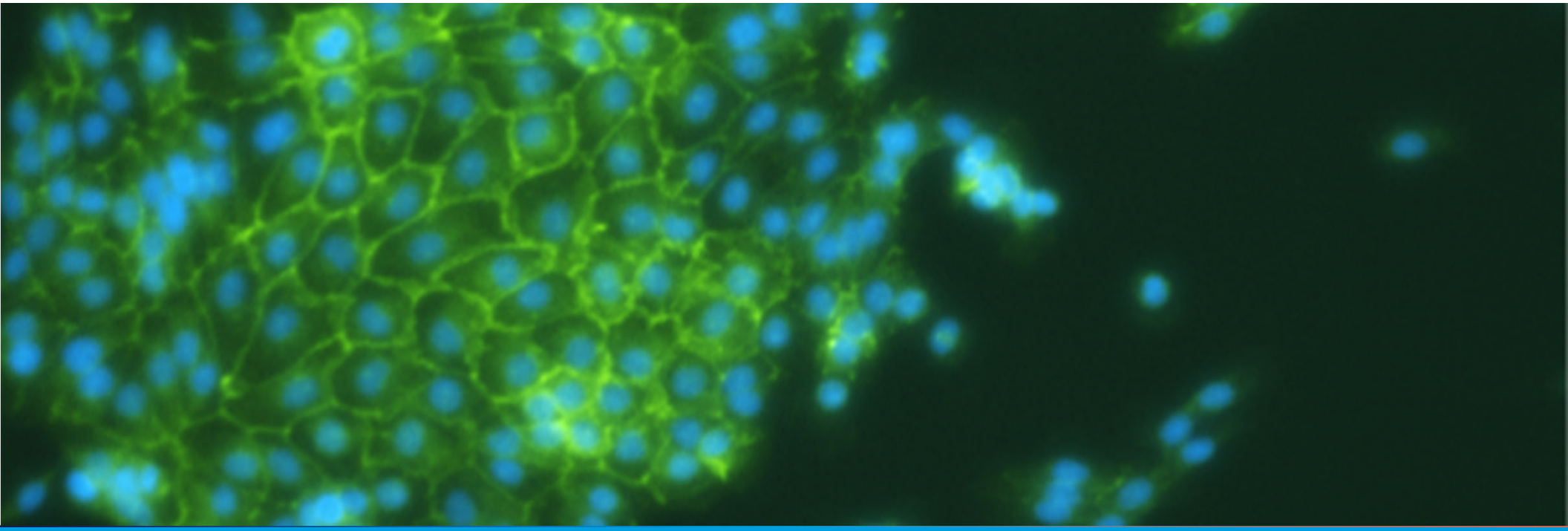


Growth of ATCC® CRL-4064™ Neonatal Dermal Melanocytes



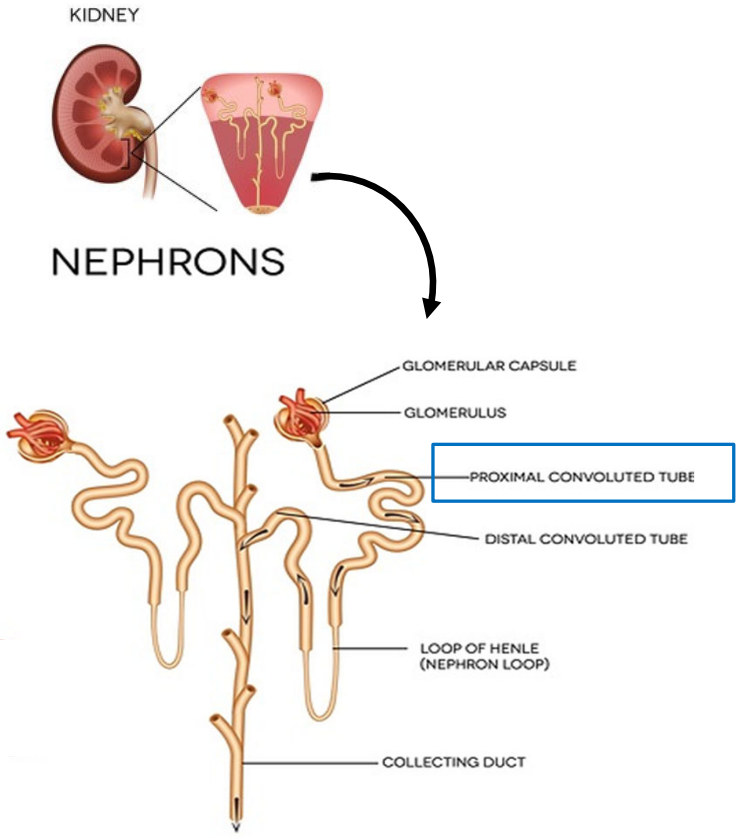
Metabolic reduction by 3-D organotypic skin culture in Triton-X





Kidney Models and Functionality

Kidney drug toxicity and transporters



Nephron, the functional unit of the kidney. Credit: Modified image by TefiM

- **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

ATCC

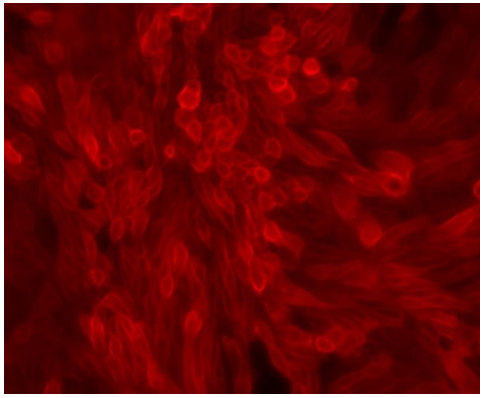
Kidney models

Renal proximal tubule epithelial cells

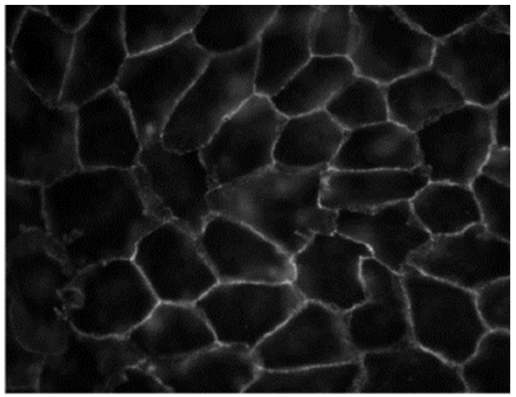
- hTERT-RPTEC–immortalized renal proximal tubule epithelial cells

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

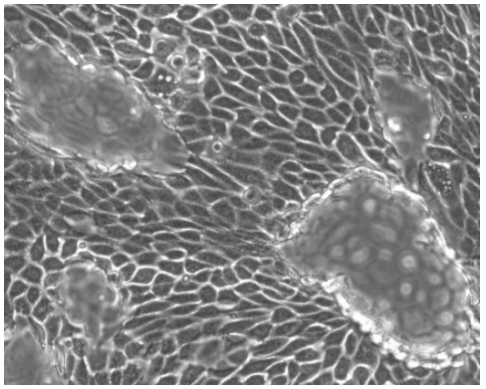
RPTEC/TERT1: CD13

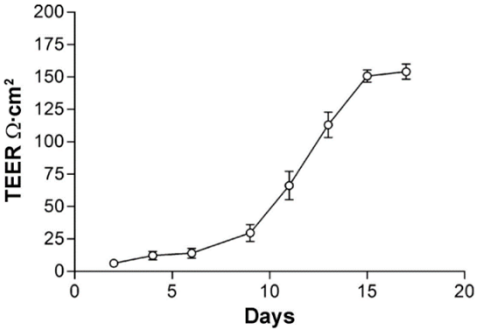


RPTEC/TERT1: E-cadherin

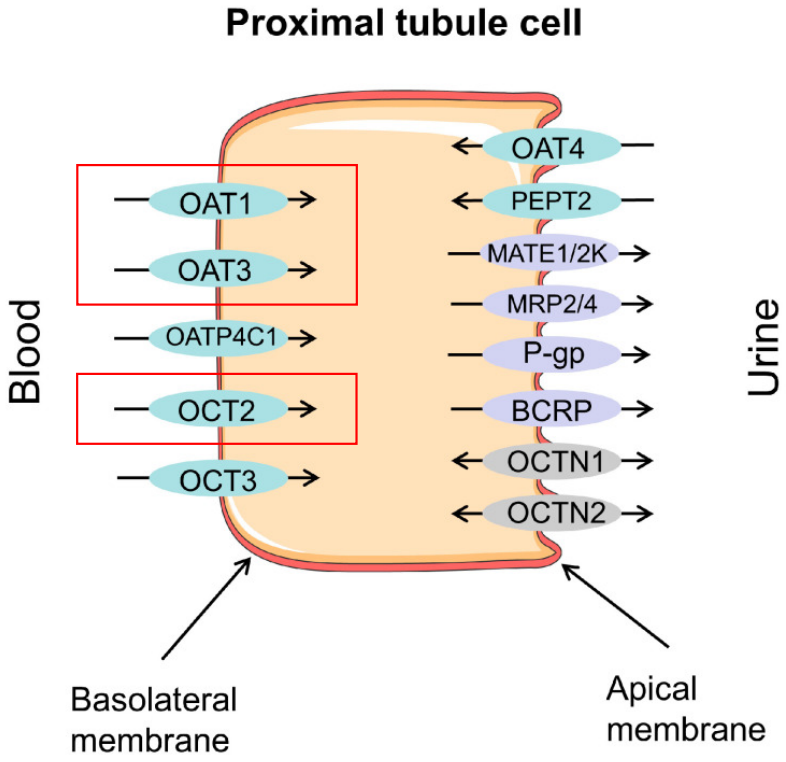


Dome formation

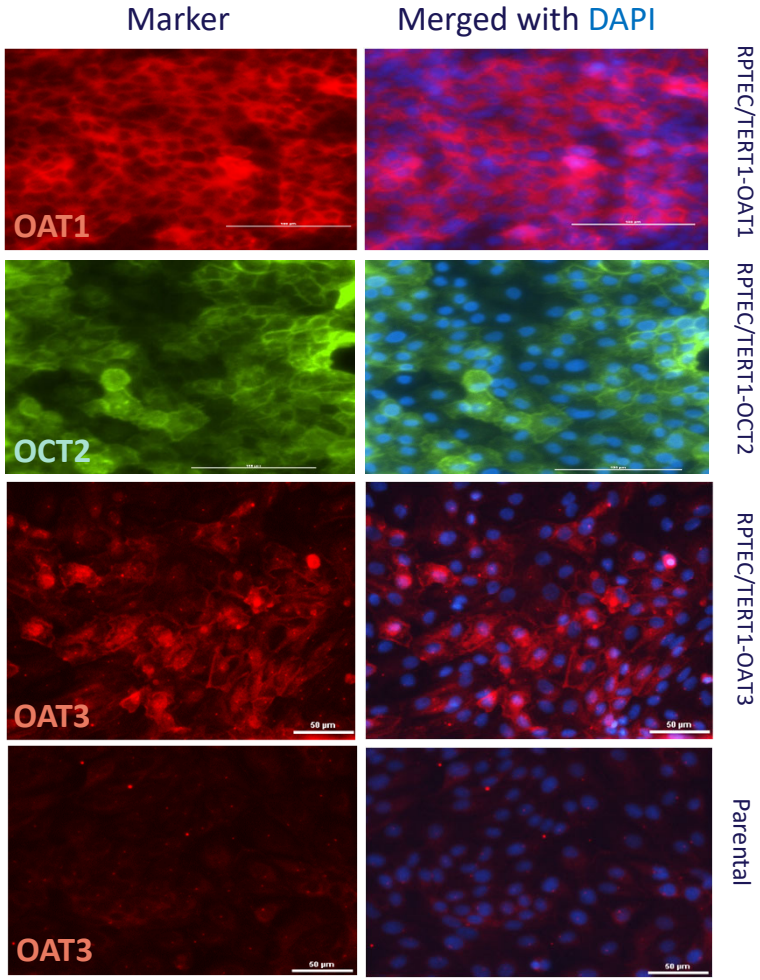




Kidney cells – Modeling solute transporters

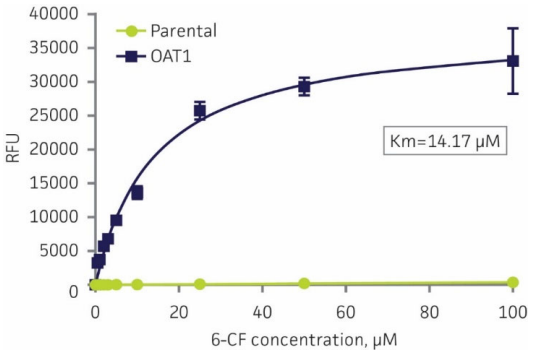


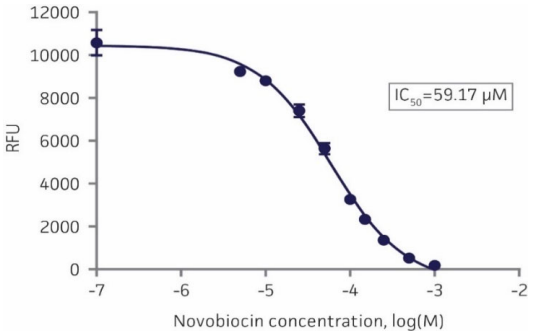
Lin K, et al. Molecules 28(13): 5252, 2023.



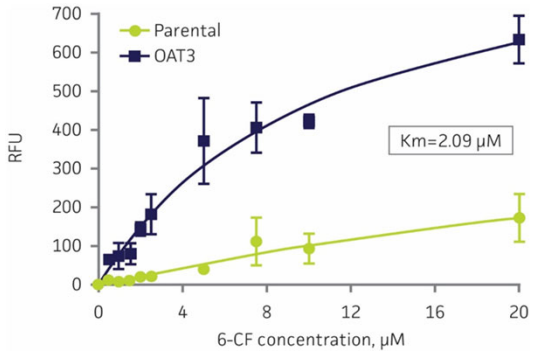
Functionality – Drug uptake & inhibition assay

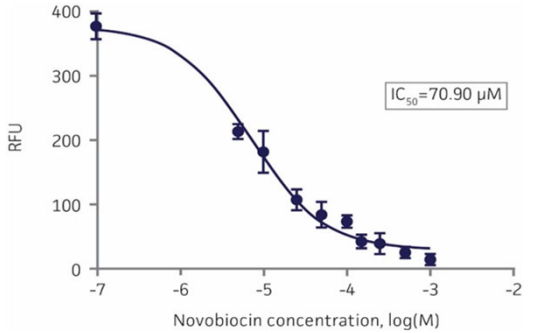
6-CF uptake inhibition in HTERT/RPTEC OAT-1



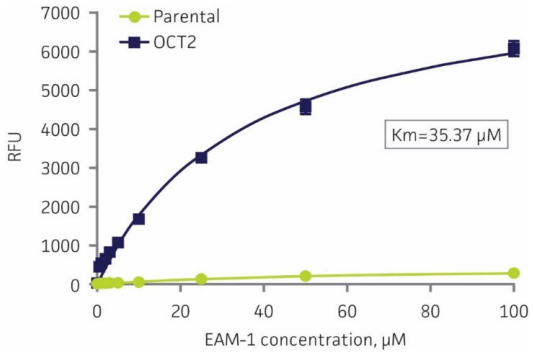


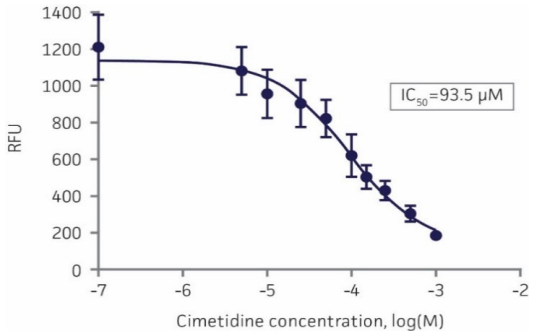
6-CF uptake inhibition in HTERT/RPTEC OAT-3





EAM-1 uptake inhibition in HTERT/RPTEC OCT-2





ASSAY PROTOCOL

- Equal numbers of both parental and transporter cells were seeded into 96-well plates in triplicate for 24 hours
- Increasing concentrations of 6-CF or EAM1 with or without inhibitors were added, and cells were incubated for 20 minutes at 37°C
- After washing with cold HBSS 4 times, cells were lysed and uptake intensity was measured

RPTEC-OCT2 – Drug-drug interactions (DDI) application

The problem

- A multitude of disease- and therapy-related factors drive the frequent development of renal disorders in cancer patients
- Many cancer patients have comorbidities such as urinary tract infections, tuberculosis, and diabetes
- Commonly prescribed medications such as levofloxacin (TEA) or metformin can interact with chemotherapeutics
- These common medications can block the renal uptake of the candidate via organic cation SLC transporters

The solution: Use SLC transporter cells to identify DDI

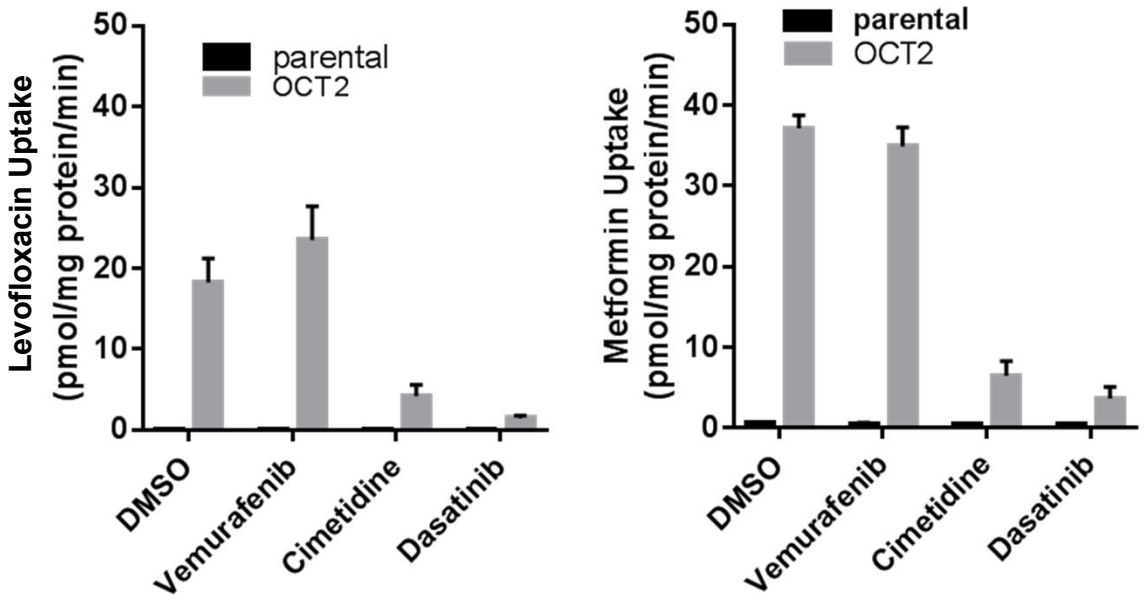
- Incubate candidate drugs with radiolabeled known SLC substrate drugs in RPTEC-OCT2 cultures then monitor uptake
- If uptake of radiolabeled compounds is inhibited, then DDI is indicated

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

RPTEC-OCT2 – Drug-drug interactions (DDI) application

Drug-drug interactions



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

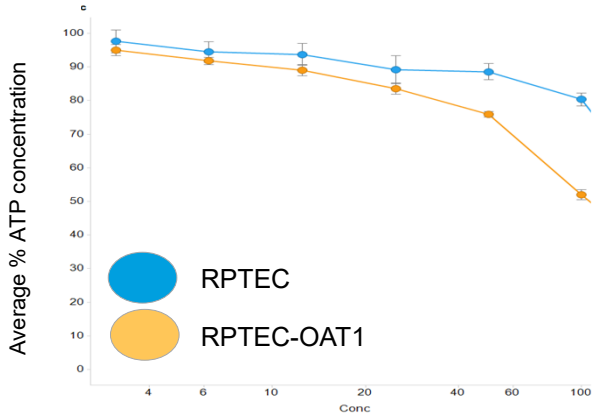
Nephron toxicity testing

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

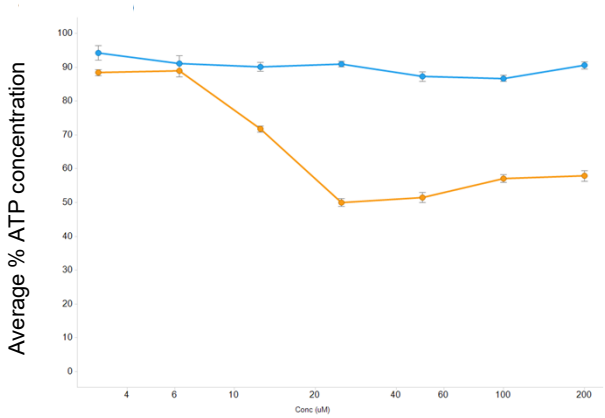
Use SLC transporter cells to identify nephron toxicity

- Targeted therapeutics can cause renal dysfunction through on- and off-target mechanisms
- Incubate candidate molecules with RPTEC-OAT1 or parental RPTEC cells, then monitor toxicity to identify mechanism of action

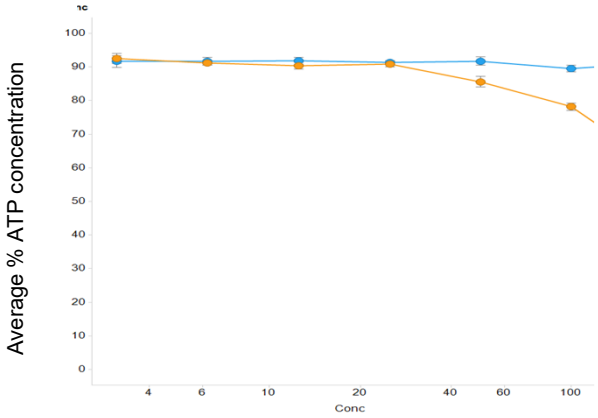
Tenofovir DF



Tenofovir



Cidofovir

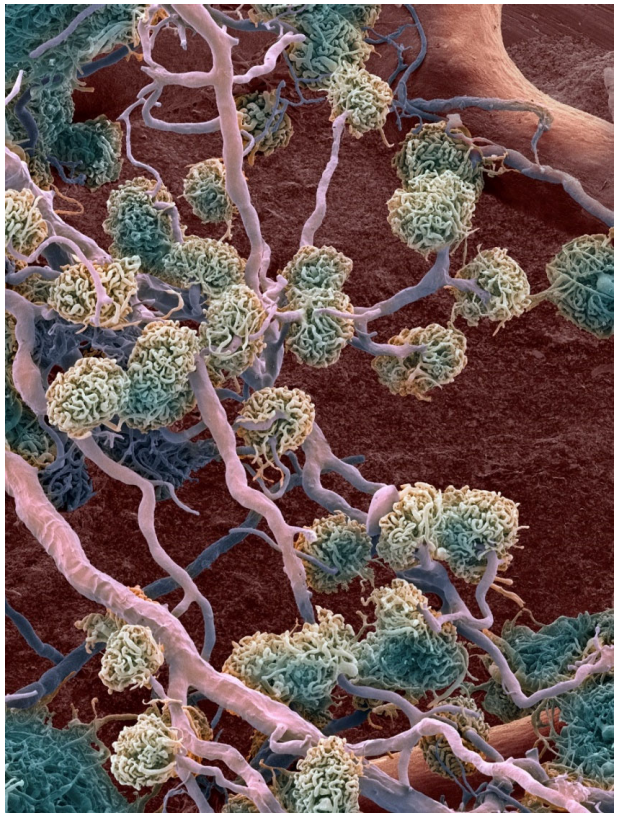


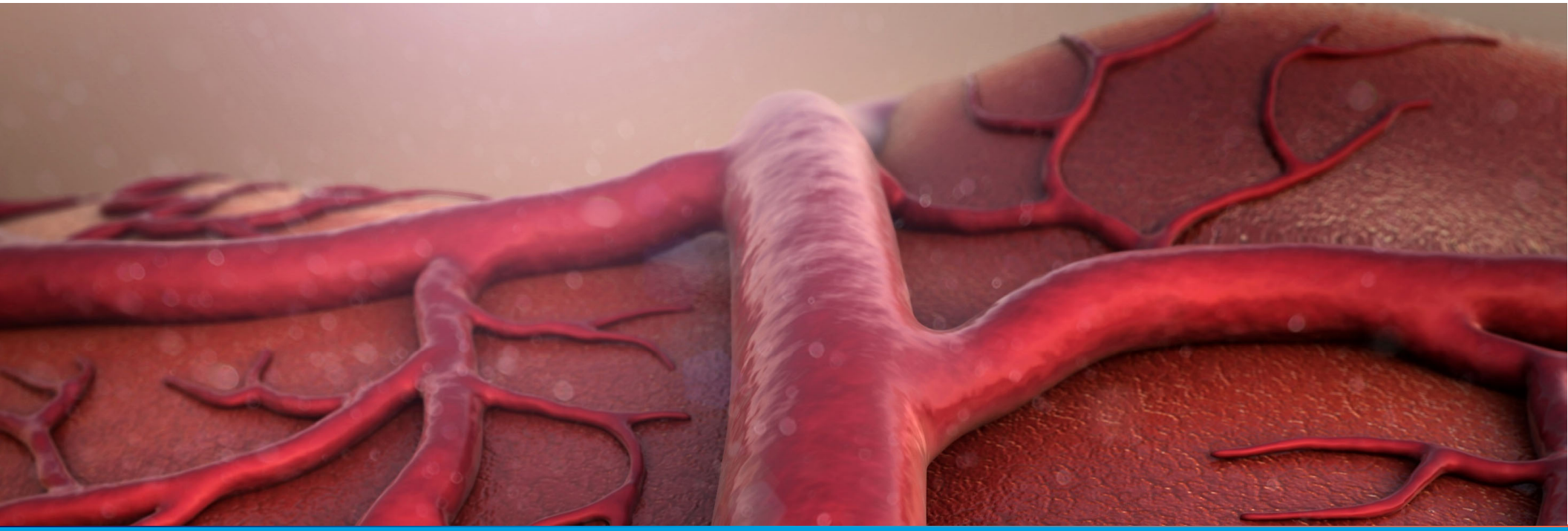
ASSAY PROTOCOL

- 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 the manufacturer's instructions

Summary of kidney models

- ATCC® primary and hTERT-immortalized RPTECs display many key in vivo characteristics
- We enhanced hTERT-immortalized RPTEC with organic anion/cation transporter proteins
- The hTERT-immortalized RPTEC models have been evaluated for
  - Uptake of specific fluorescent substrates
  - Selective substrate drug uptake effects by known transporter protein inhibitors
  - Drug-drug interactions (DDI) and nephron toxicity applications

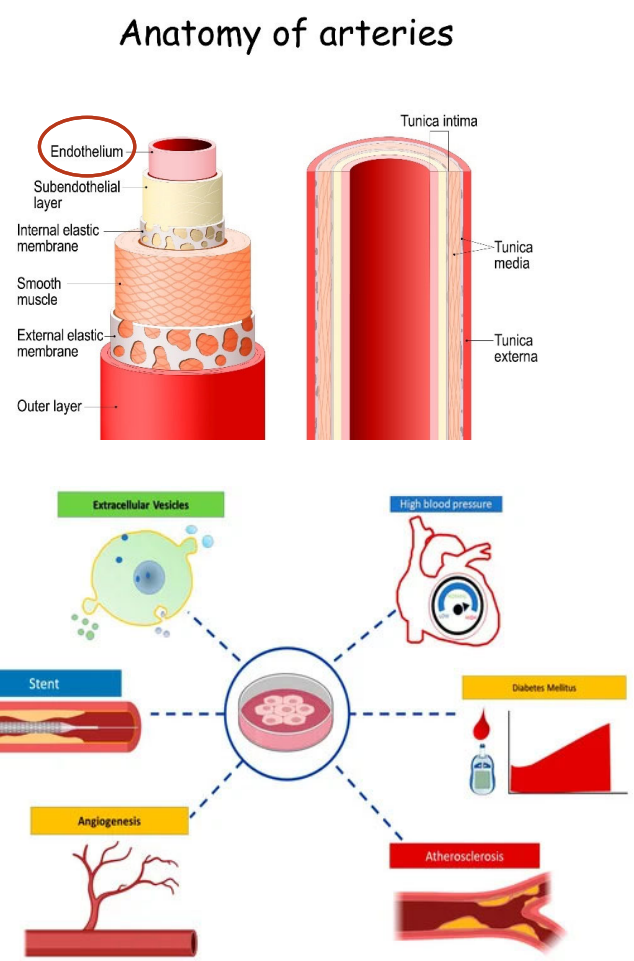




Cardiovascular Models and Functionality

Cardiovascular research

- Cardiovascular disease causes one-third of deaths worldwide and represents an urgent threat to global health
- Efforts to treat and cure cardiovascular disease depend upon advances in our understanding of the etiology and molecular mechanisms affecting the disease
- Areas of active research include:
  - Basic cardiovascular development and angiogenesis
  - Metabolism
  - Regenerative medicine, including tissue bioengineering
  - Cardiovascular and blood diseases
  - Cardiovascular effects of inflammation
  - Vascular and lymphatic disorders
  - Hypertension & atherosclerosis
- As a key cell in vascular tissue, endothelium cells are central to this research activity



Medina-Leyte DJ, et al. Appl Sci 10(3), 938, 2020.

Cardiovascular research - Angiogenesis

- Angiogenesis, or blood vessel formation, is the sprouting of new capillaries from pre-existing vessels
- Plays a central role in numerous human diseases, including cancer and age-related macular degeneration
- Numerous anti-angiogenesis drugs have been developed, and several have received FDA approval

In vitro angiogenesis models can be used for

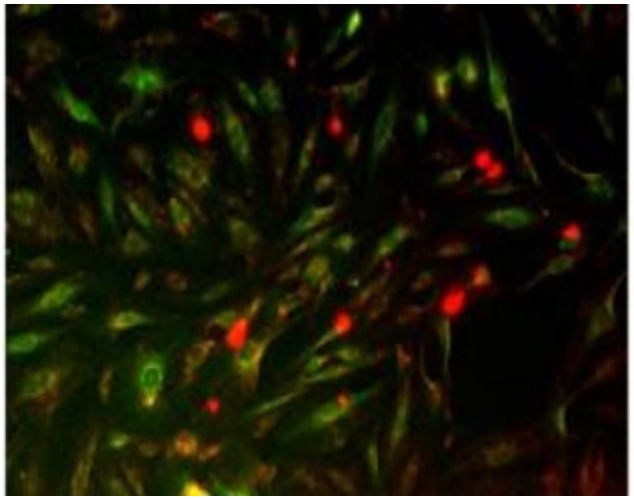
- Screening of activation and inhibition compounds
- Vessel formation in wound healing study and regenerative compound screening
- Angiogenesis under hypoxia situation study and compounds screening

ATCC®

Immortalized primary endothelial cells

Broad and growing collection of cell models

- HUVEC/TERT2 (ATCC® CRL-4053™)
- TIME (Microvascular endothelial cells; ATCC® CRL-4025™)
- TIME-GFP (GFP-expressing microvascular endothelial cells; ATCC® CRL-4045™)
- NFκB-TIME (ATCC® CRL-4049™)
- TeloHAEC (ATCC® CRL-4052™)
- TeloHAEC-GFP (Aortic endothelial cells; ATCC® CRL-4054™)
- HPAEC-BMI1 (ATCC® CRL-4065™) ***New!***



TIME-GFP stained for Ac-LDL (red) and CD31

Pulmonary hypertension and hPAEC

- Pulmonary arterial hypertension (PAH) is a medical condition with a 1% global incidence rate
  - 100,000 patients in US/year
- High blood pressure in the lungs occurs due to narrowed and stiffened pulmonary arteries.
- Human Pulmonary Artery Endothelial Cells (hPAEC) play a key role in the etiology of the disease

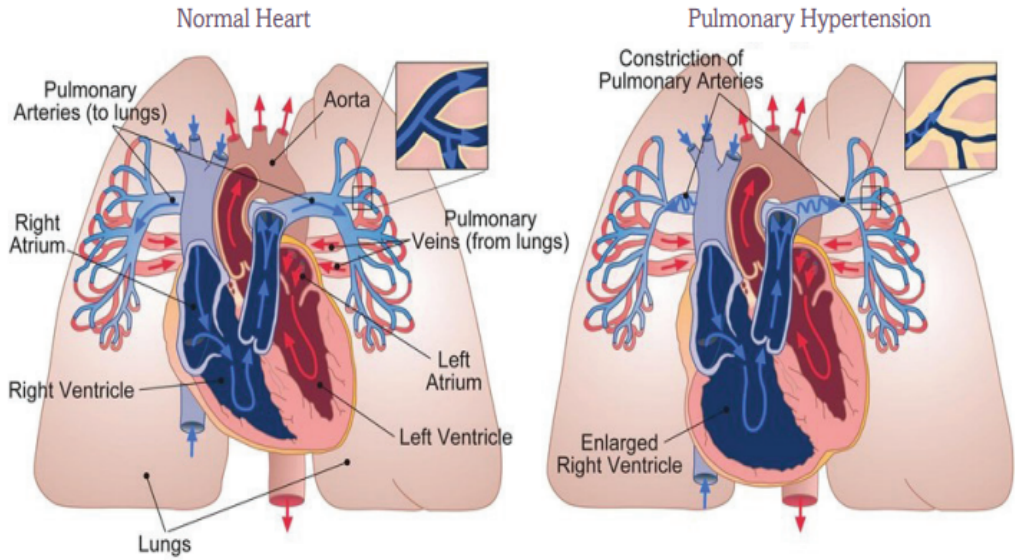


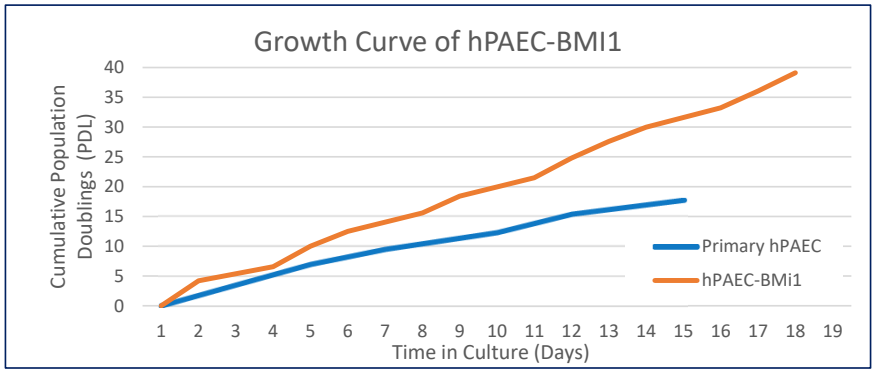
Image courtesy of the Columbus Ohio Adult Congenital Heart Disease Program at Nationwide Children's Hospital Heart Center, Columbus, Ohio

HPAEC-BMI1– Key characteristics

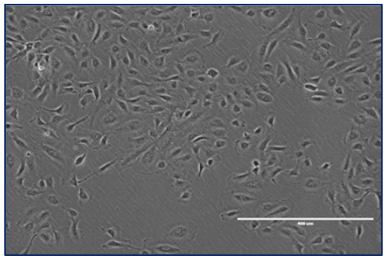
ATCC® offers primary and immortalized cell solutions that are authenticated with our rigorous QC and tested for common biomarker expression and cell performance

Growth & Morphology

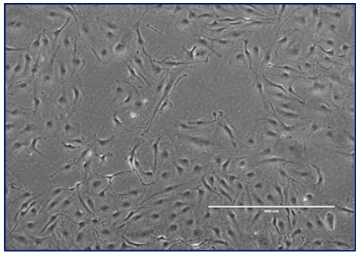
- Cells retain replicative capacity (“immortalized”)
  - Morphology similar to primary cells
- Key biological functions similar to primary cells
  - Formation of capillary-like tubes
  - 82% of cell uptake acLDL



Primary hPAEC

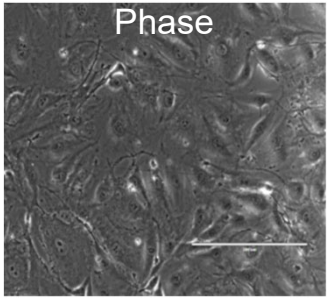


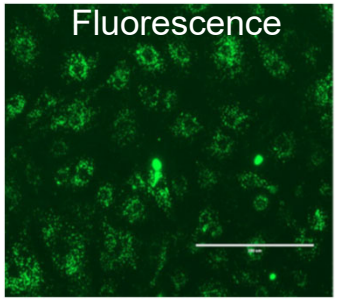
hPAEC BMI1

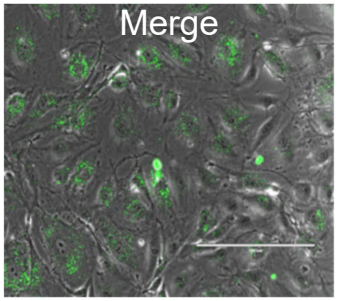


10x magnification

acLDL Uptake







20x magnification

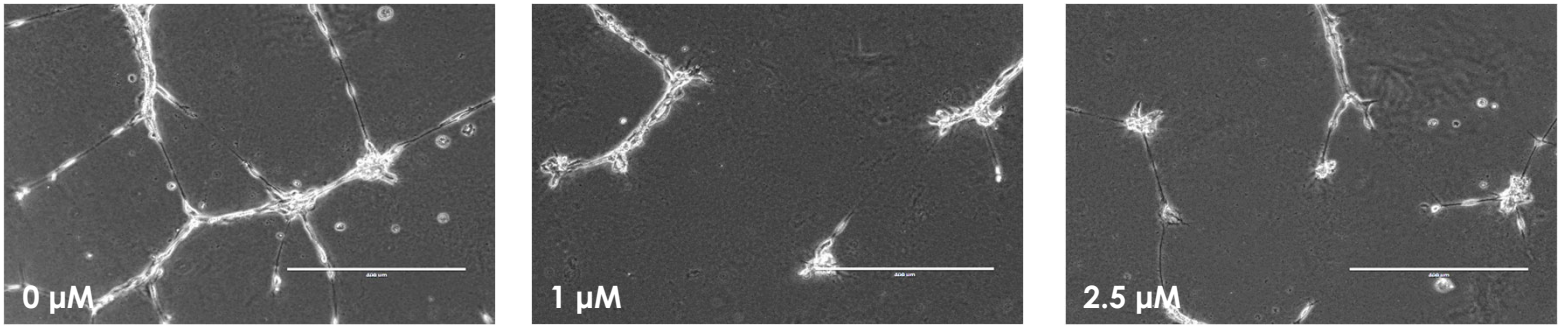
HPAEC-BMI1– Key characteristics

ATCC® offers primary and immortalized cell solutions that are authenticated with our rigorous QC and tested for common biomarker expression and cell performance

Application Data

- Capillary-like tube formation assay is responsive to well know stimulatory and inhibitory agents

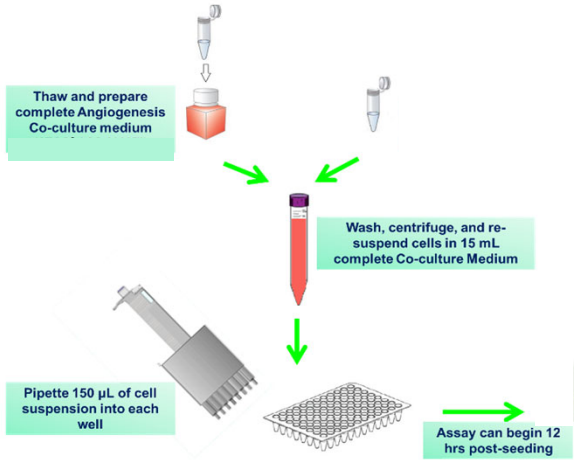
Sunitinib Inhibition

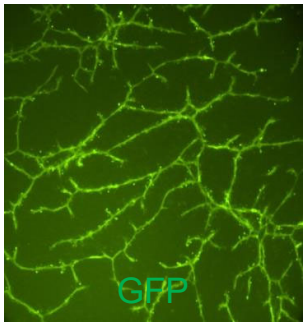


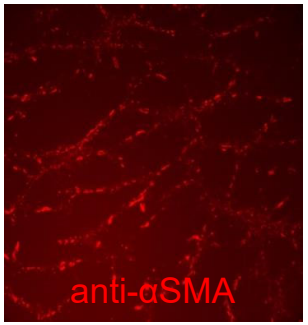
10x magnification

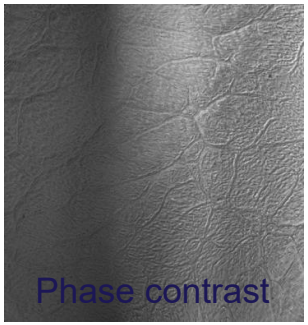
An advanced co-culture system

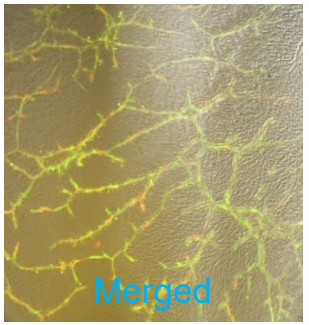
- Co-culture of endothelial cells with fibroblasts allows for
  - Formation of more heterogeneous tubules
  - More defined culture conditions, no need for ECM coating
- Scientists at ATCC® optimized a co-culture system using:
  - hTERT-immortalized mesenchymal stem cells ASC52telo
  - GFP-labeled TeloHAEC, an immortalized aortic endothelial cell
- Can form tubular structures in < 7 days instead of 14 days compared with standard co-culture methods
- Immunofluorescence shows MSCs surrounding the microvascular structures differentiate into smooth muscle





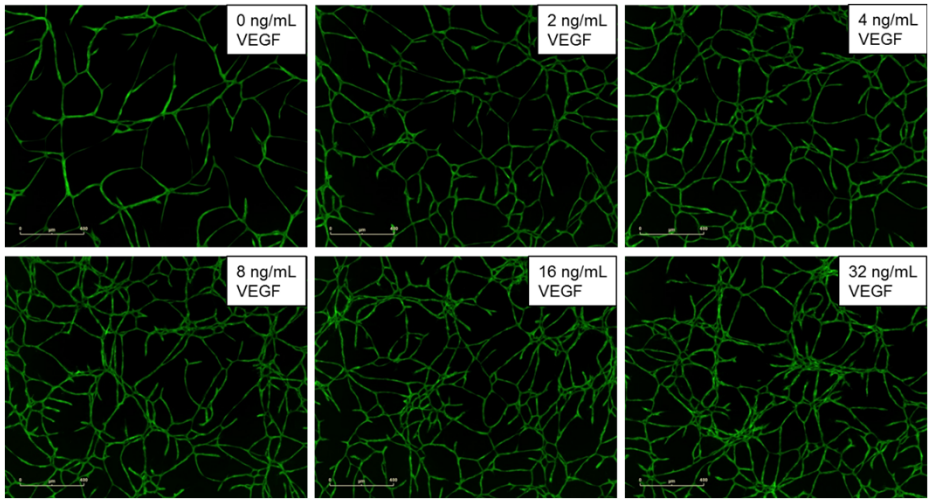


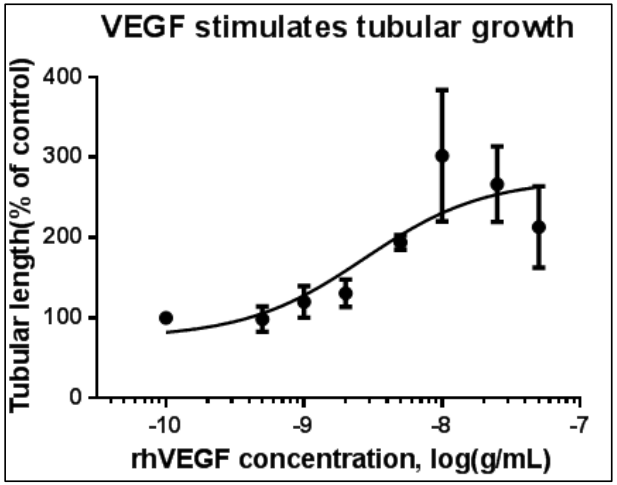




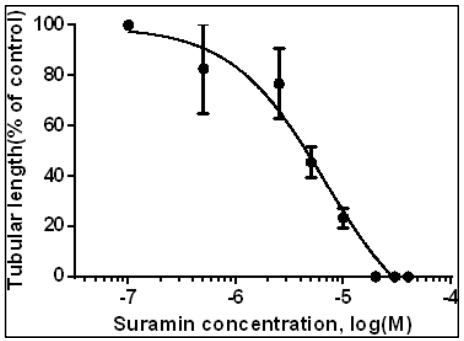
An advanced co-culture system – Response to stimuli

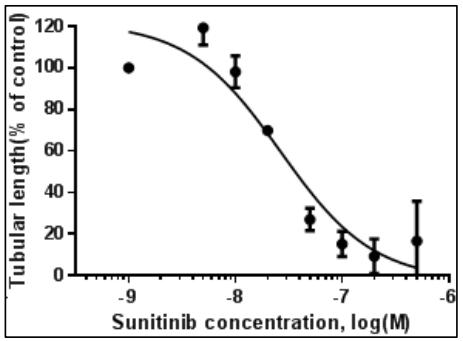
Positive response to VEGF stimulation

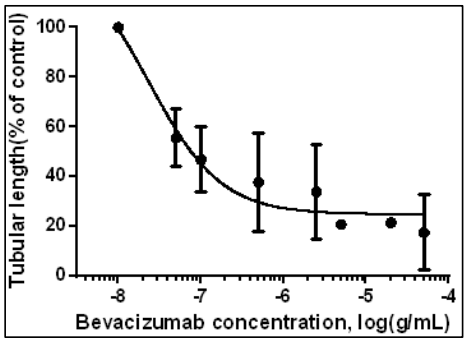




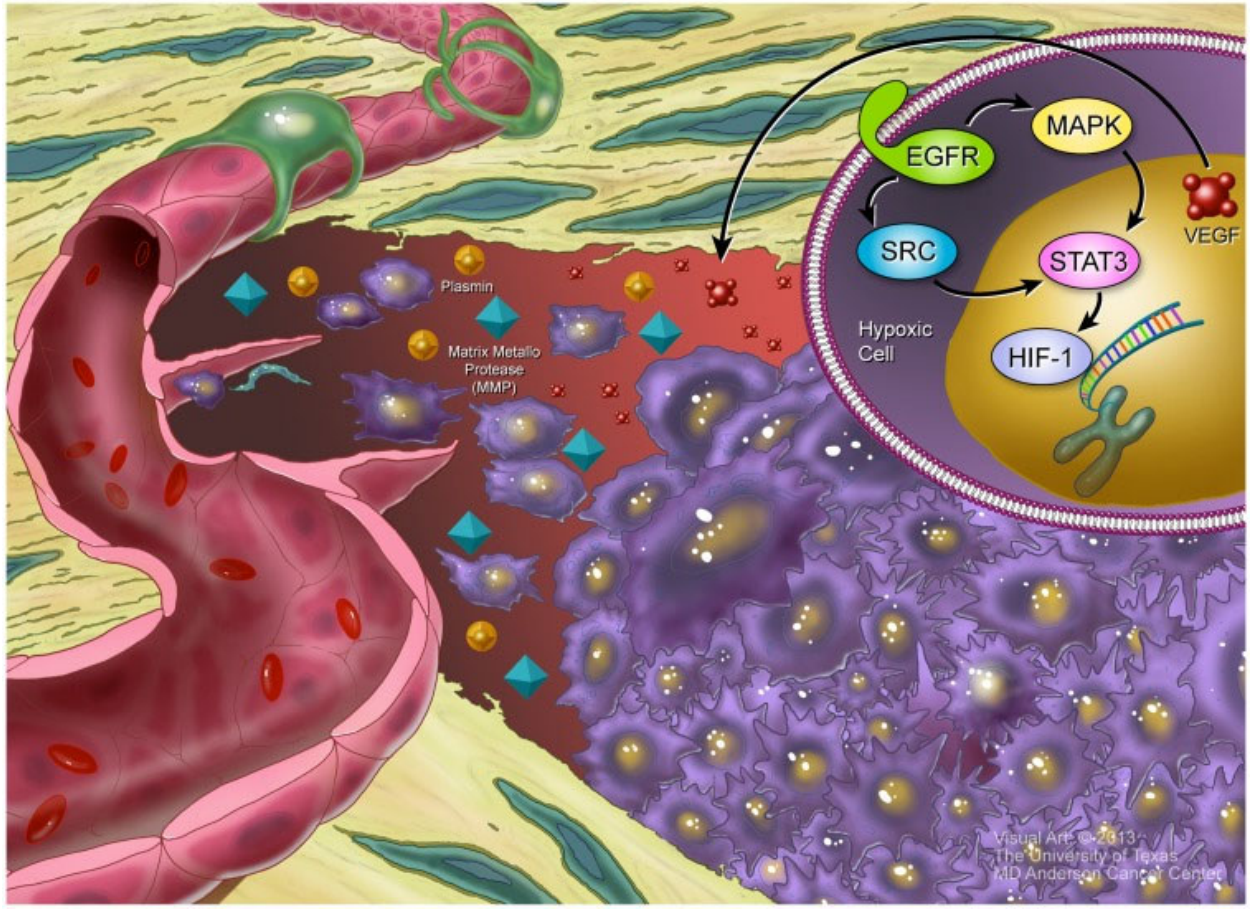
Response to angiogenesis inhibitors







Screening tumor angiogenesis inhibitors



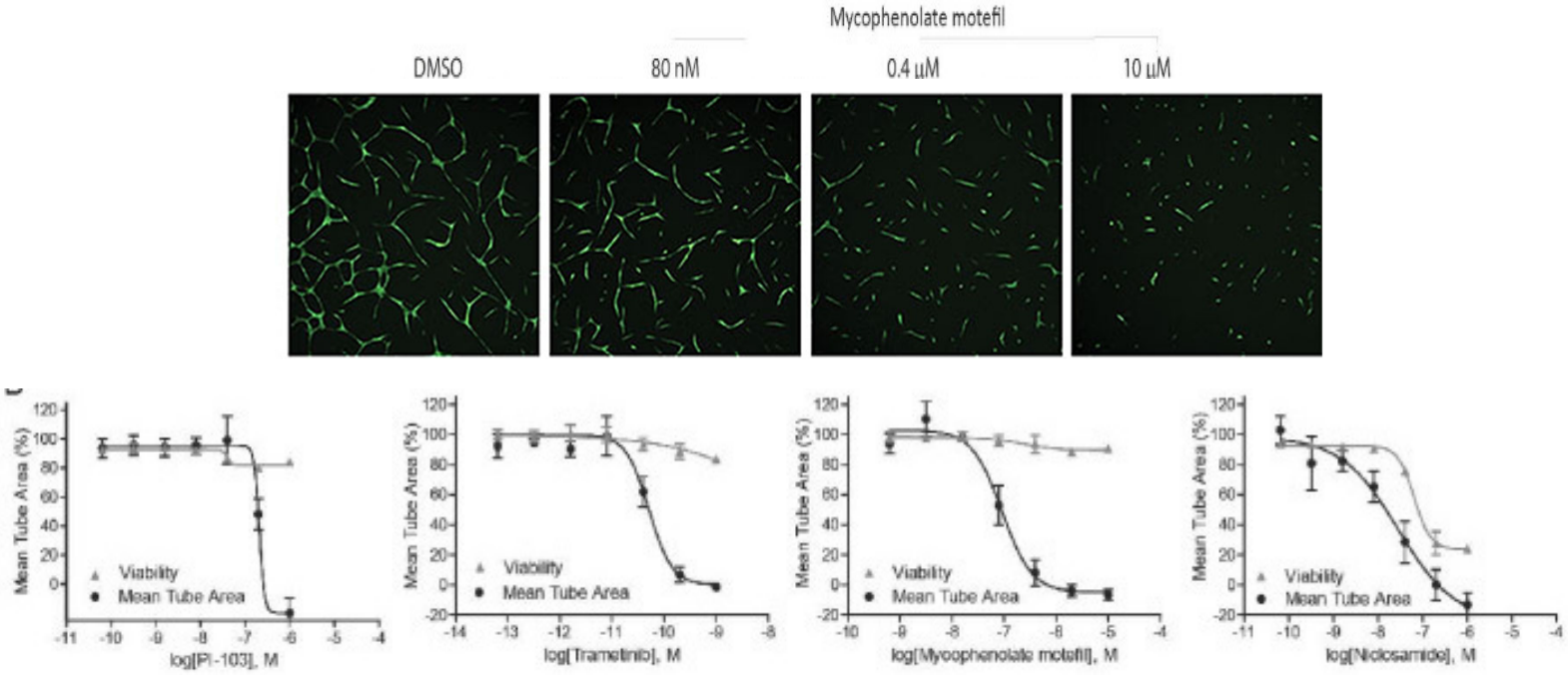
Zhu L, et al. Front Oncol 3: 230, 2013.

Modelling Hypoxia and tumor angiogenesis

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 7

Identification of approved and investigational drugs that inhibit hypoxia-inducible factor-1 signaling

Chia-Wen Hsu, Ruili Huang, Thai Khuc, David Shou, Joshua Bullock, Suzanne Grooby, Sue Griffin, Chaozhong Zou, Annette Little, Holly Astley, Menghang Xia



Advanced co-culture system– High-throughput screening

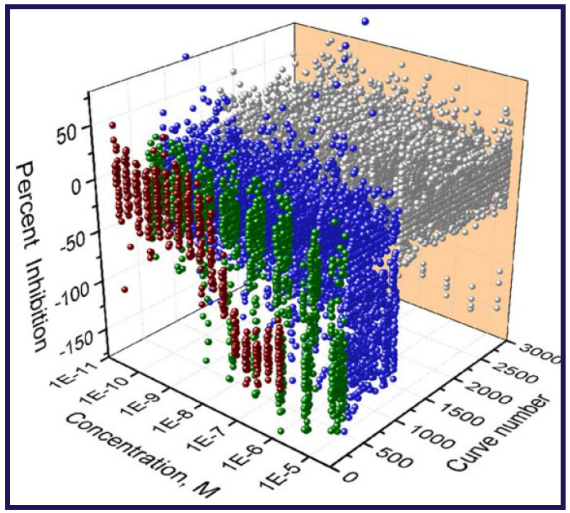
Identification of Angiogenesis Inhibitors Using a Co-culture Cell Model in a High-Content and High-Throughput Screening Platform

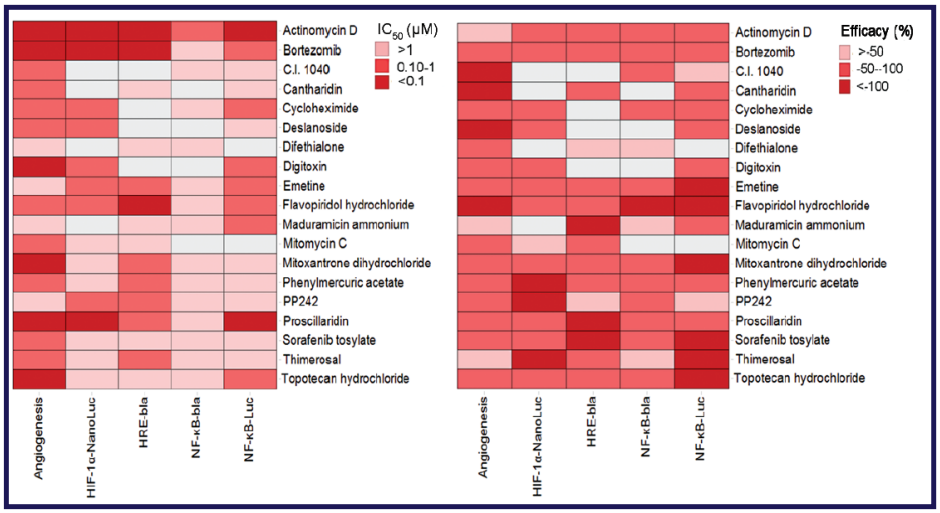
Shuaizhang Li , Chia-Wen Hsu , Srilatha Sakamuru, Chaozhong Zou, Ruili Huang, and Menghang Xia

SLAS Technology
2018, Vol. 23(3) 217–225

DOI: 10.1177/2472630317729792
journals.sagepub.com/home/jla
SAGE

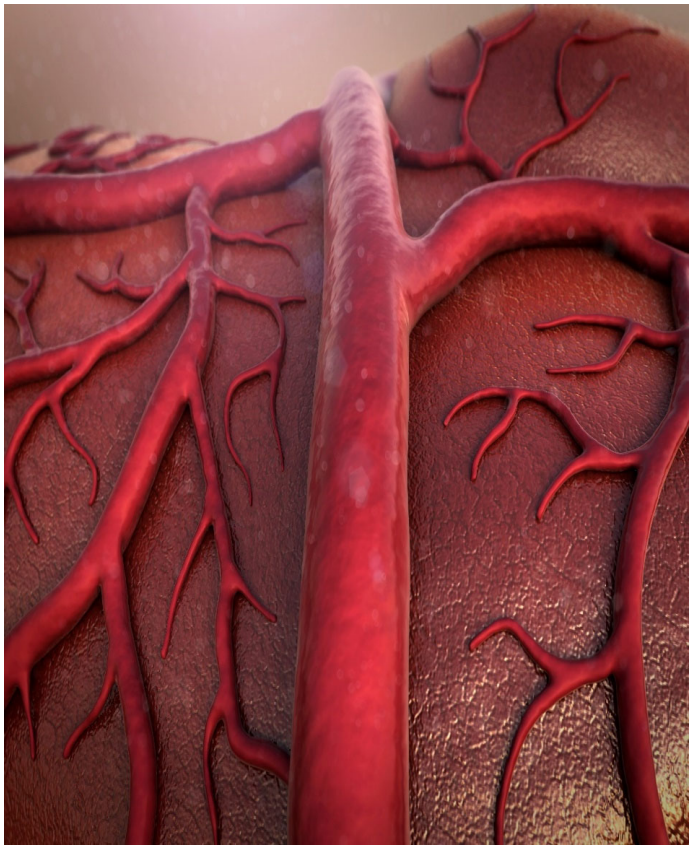
Screening of 2,816 drugs on 1,536-well format using ATCC's angiogenesis co-culture model.





Summary of cardiovascular models

- ATCC® immortalized endothelial cells display vivo characteristics of primary cells
- Immortalized primary cell alone or in combination with other cells are a user-friendly solution for building reliable and predictive cell models
- In vitro co-culture models provide physiologically relevant tool to examine angiogenesis
  - The tubules formed in co-culture assays more closely resemble capillaries than those formed on a gel matrix
  - Screening of activation and inhibition compounds
  - Vessel formation in wound healing study and regenerative compound screening
  - Angiogenesis under hypoxia situation study and compounds screening



ATCC®

Summary and resources

- ATCC® offers a variety of immortalized cell models
  - Cell lines are authenticated for immortalization and karyotype stability
  - Including several difficult to immortalize cell types
  - *Time and costs are saved in trying to immortalize these difficult cell types on one's own*
- Immortalized primary cells offer:
  - Primary cell functionality
  - Continuous cell line longevity
  - Improved supply and reproducibility for a standardized model
  - A suitable cell model for stable genetic modifications
  - *Immortalization eliminates donor variability*
- Immortalized primary cells alone or in combination with other cells are a user-friendly solution for nephrotoxicity or cardiovascular studies
- Multiple resources are available at www.atcc.org/hTERT