The Application of hTERT-immortalized Primary Cells in Toxicological Assays

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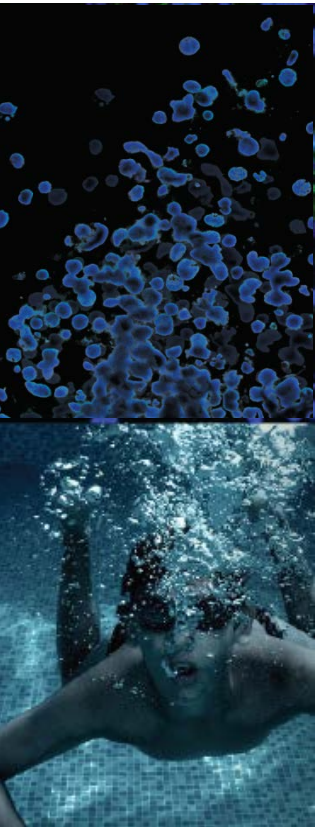
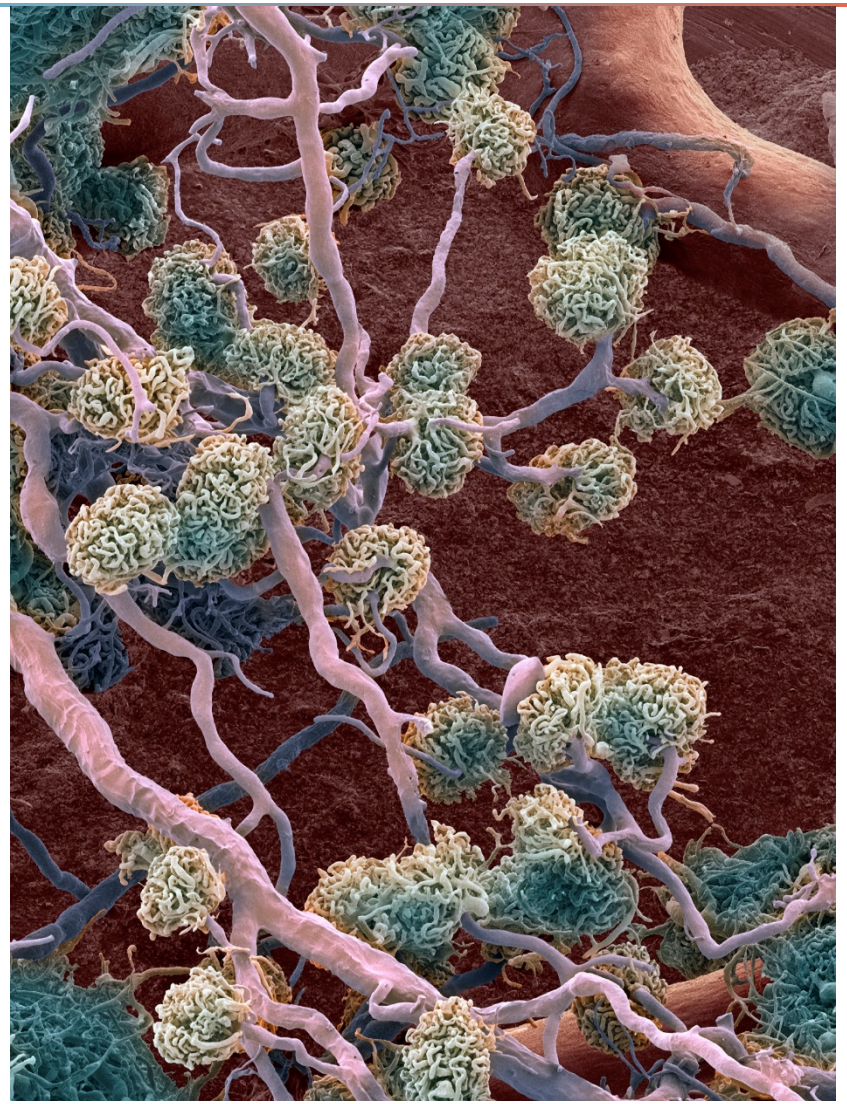


Credible Leads to Incredible™

Agenda

- ATCC mission and future direction
- ATCC toxicology portfolio
- hTERT primary cell portfolio
  - Kidney models
  - Skin models
  - Airway models
  - Angiogenesis system



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

Established partner to global researchers

The ATCC mission

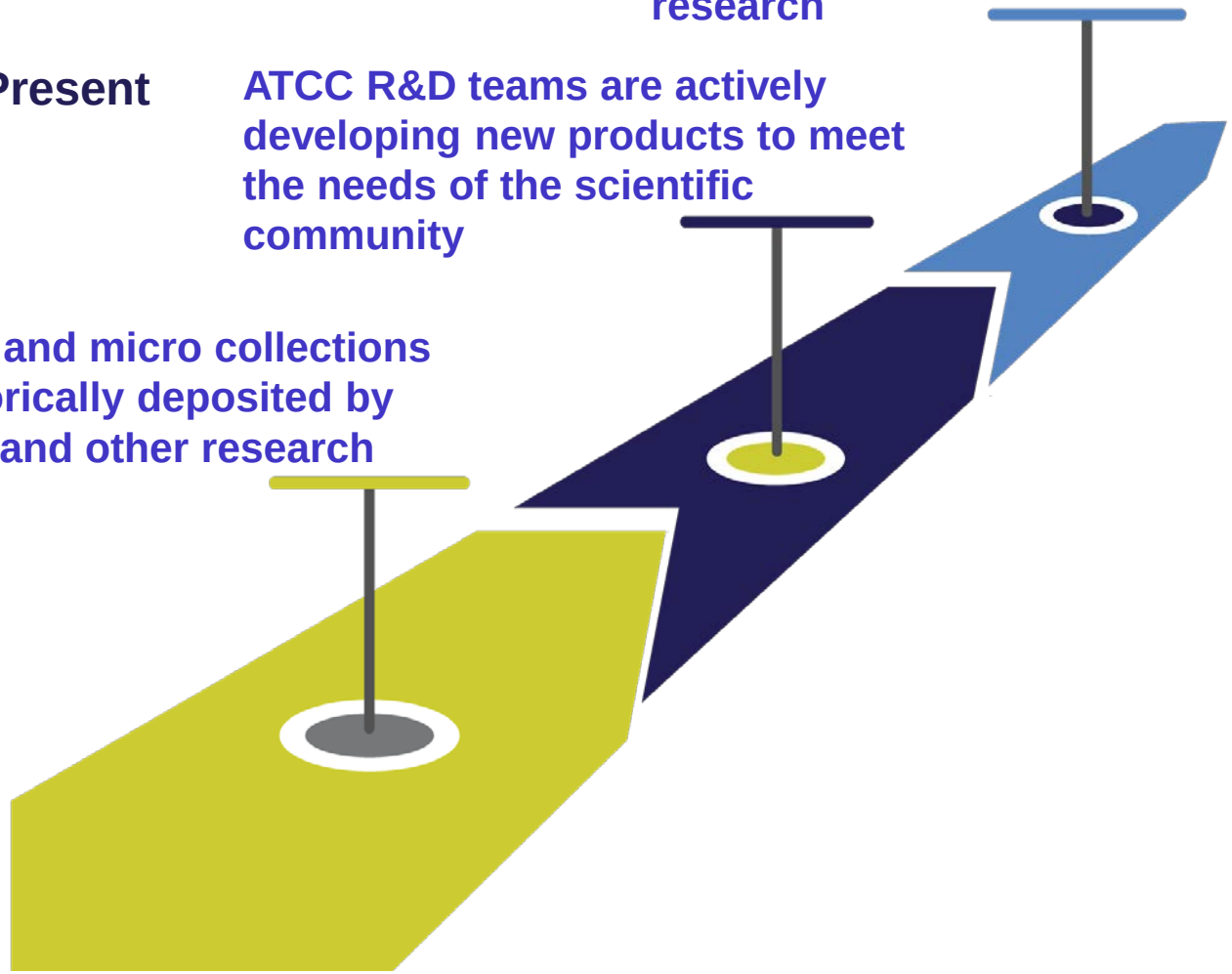
"Our mission focuses on the acquisition, authentication, production, preservation, development, and distribution of standard reference microorganisms, cell lines, and other materials."

Modernization of the ATCC portfolio

Ensuring the Future — ATCC is investing in key technologies to ensure its products and services remain the definitive standards in biological research

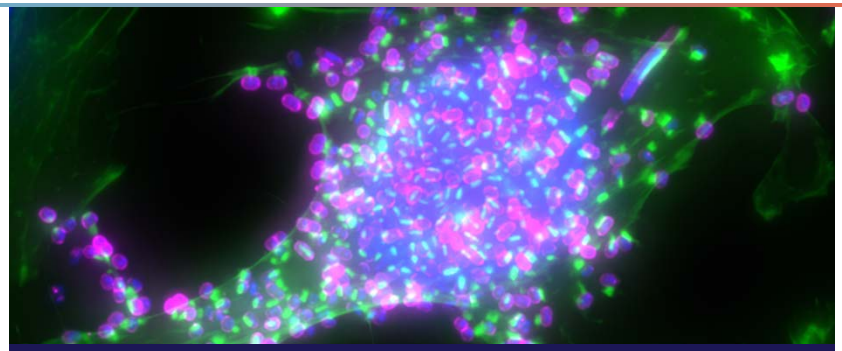
Present — ATCC R&D teams are actively developing new products to meet the needs of the scientific community

Past — ATCC cell and micro collections were historically deposited by academic and other research scientists

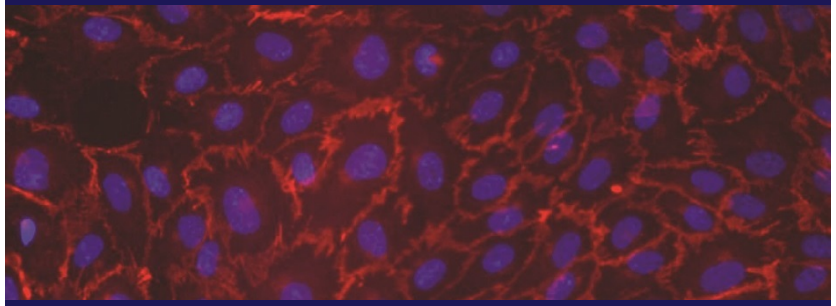


ATCC products for toxicology

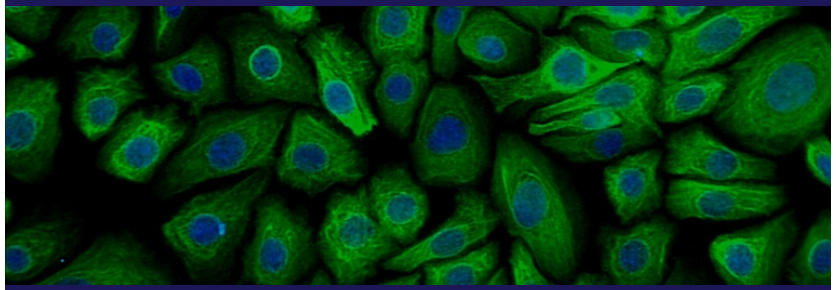
- ATCC is the complete solution supplier for toxicology
- From basic research through discovery and development to product testing
  - Continuous cell lines
  - Primary cells
  - hTERT-immortalized primary cells
  - upcyte® hepatocytes (ATCC® ACS-9000™)
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
  - Scalabilty into all aspects of the toxicology workflow
  - Biological relevancy



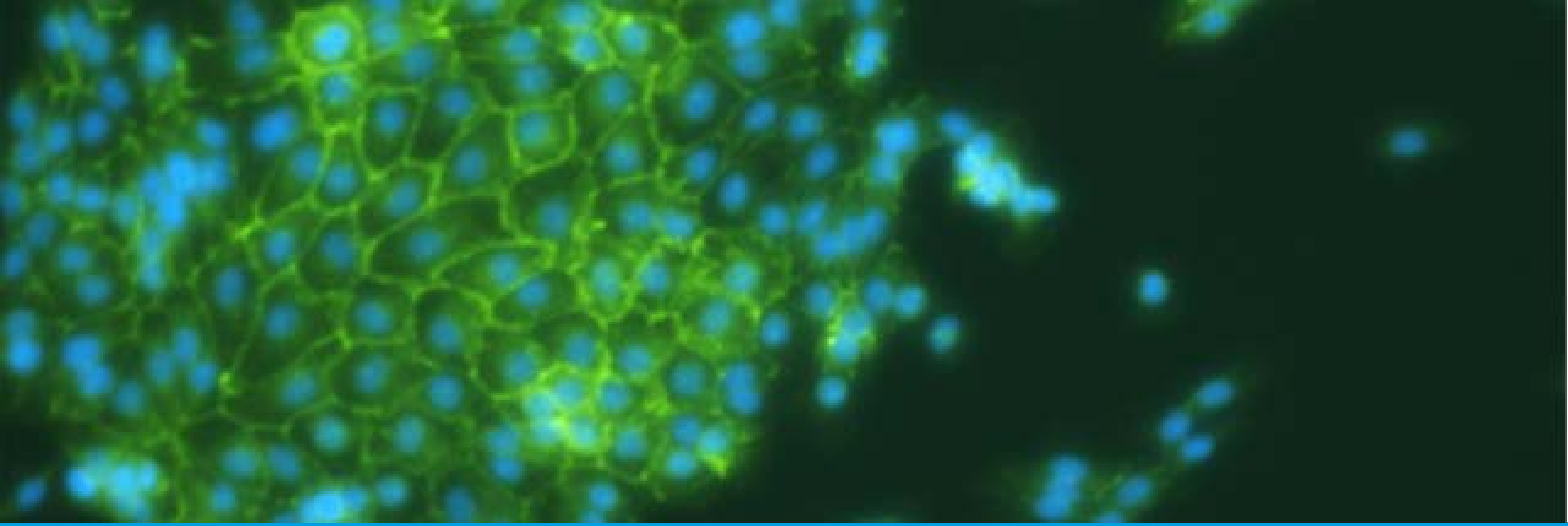
Continuous cell line: HeLa (ATCC® CCL-2™)



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



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

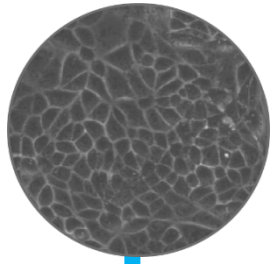


hTERT Immortalization Technology

hTERT Primary Cell Models

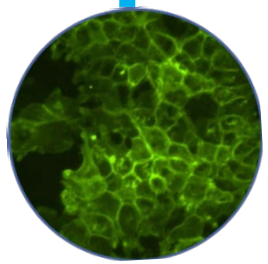
ATCC®

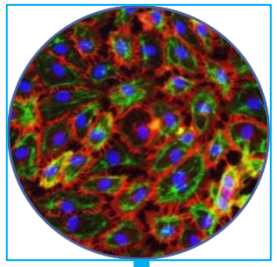
Evolution of *in vitro* cell models



First generation
Continuous cell lines

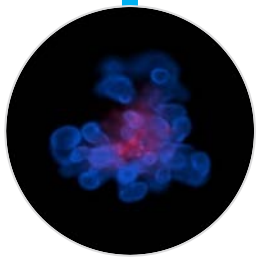
Second generation
Transfected cell lines
Reporter cell lines
Primary cells





Third generation
Immortalized primary cells
Gene edited cell lines

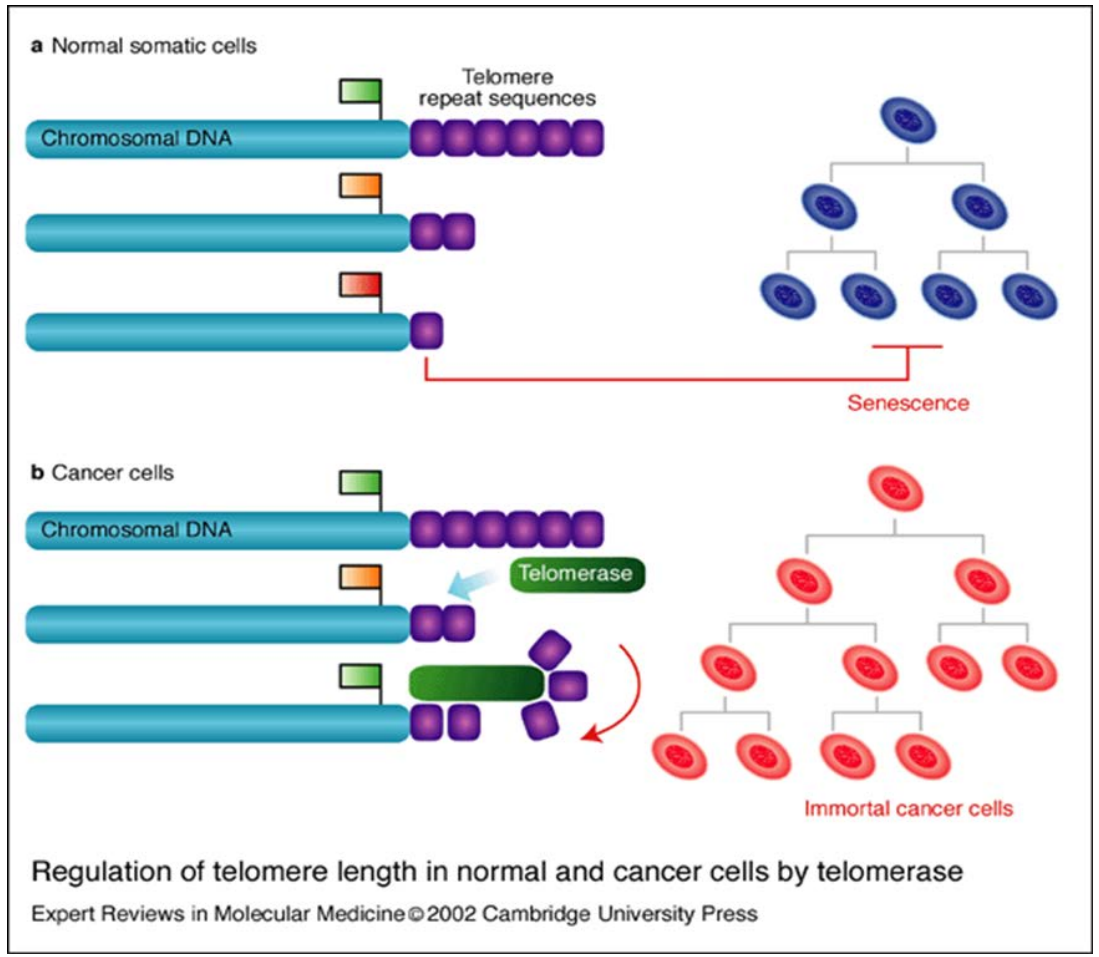
Fourth generation
3D models
Organoids



Characteristics of various cell models

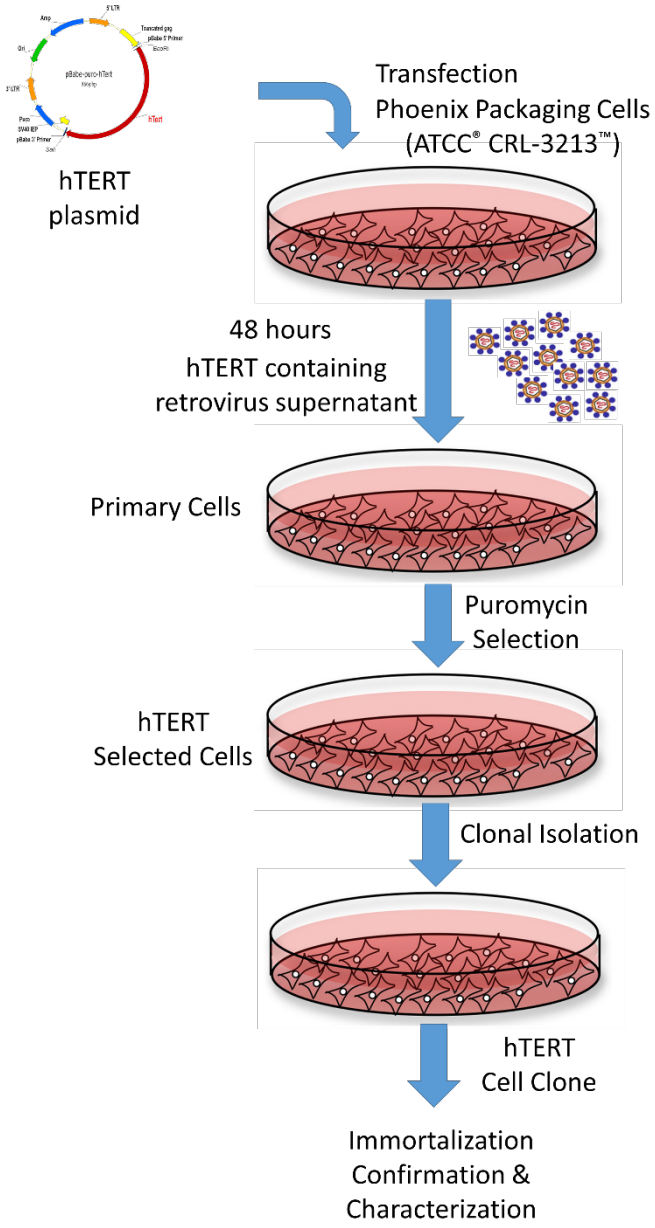
	Continuous (cancer) cell lines	Viral oncogene-immortalized cells	Primary cells	hTERT-immortalized cells
Mimic *in vivo* characteristics	+	++	++++	+++
Proliferative capacity	+++	+++	+	+++
Experimental reproducibility	+++	+++	+	+++
Predictability in toxicological studies	+	++	+++	+++
Genomic stability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

hTERT immortalization technology



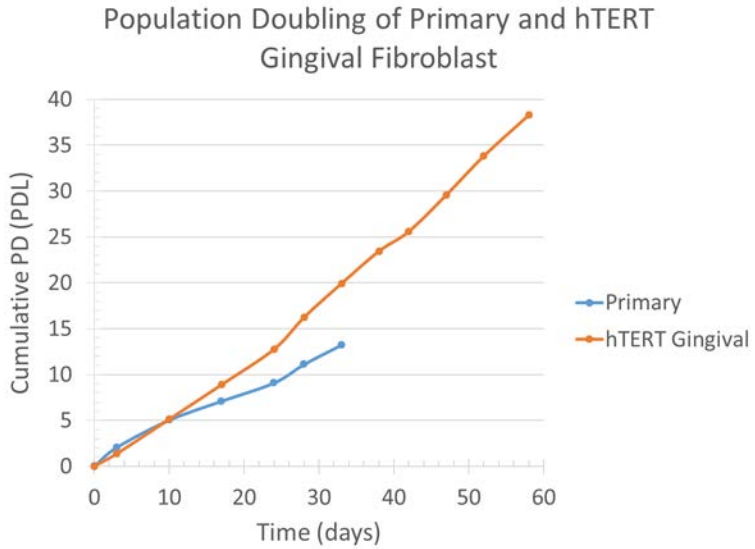
Regulation of telomere length in normal and cancer cells by telomerase

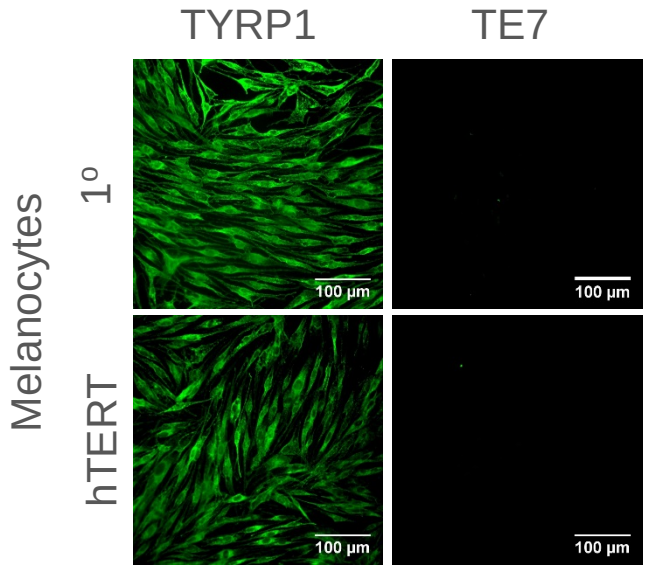
Expert Reviews in Molecular Medicine ©2002 Cambridge University Press



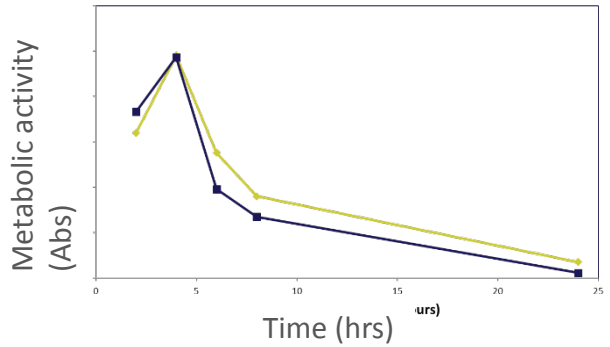
hTERT immortalized cells – Key characteristics

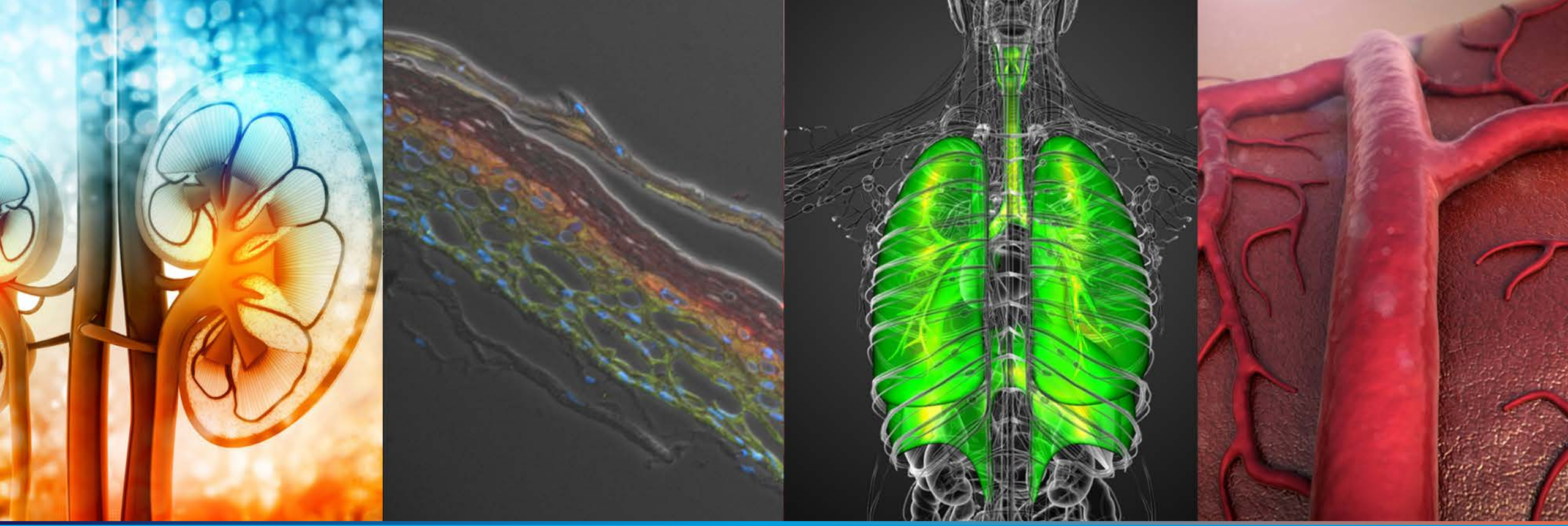
- Growth
  - Cells retain replicative capacity (“immortalized”)
  - Population doubling rate is comparable to primary cells
- Morphology and marker expression
  - Similar to primary cells
- Toxicology responses
  - Analogous to primary cells





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





hTERT Cell Models and Functionality

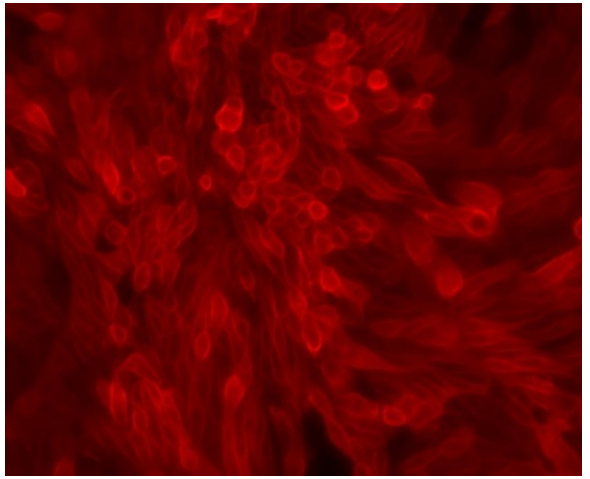
Kidney models

Renal proximal tubule epithelial cells

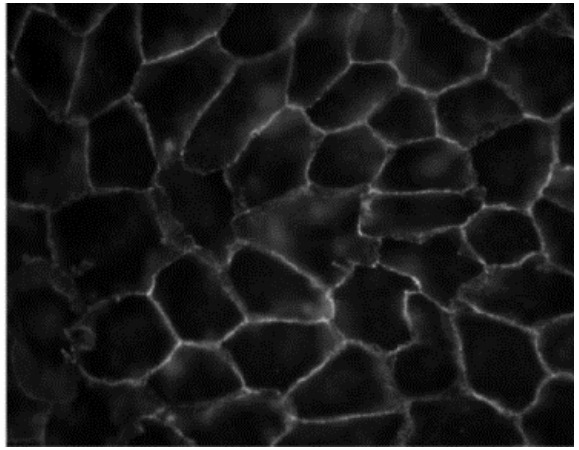
- hTERT-RPTEC – immortalized renal proximal tubule epithelial cells (ATCC® CRL-4031™)

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

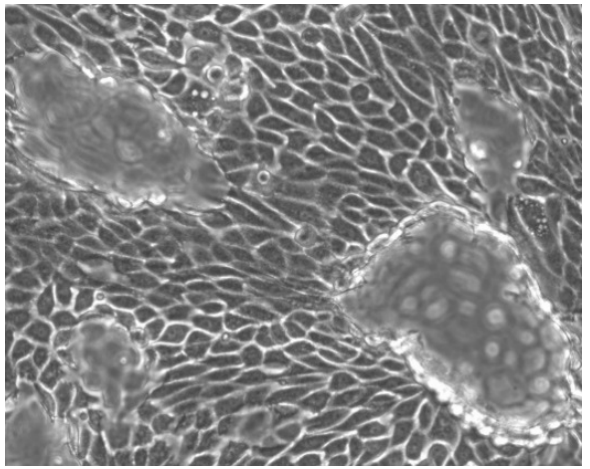
RPTEC/TERT1: CD13

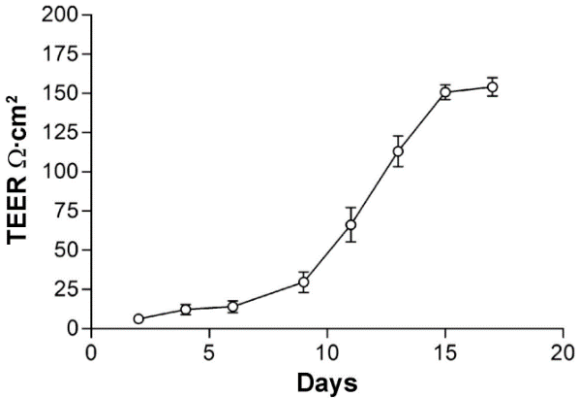


RPTEC/TERT1: E-cadherin



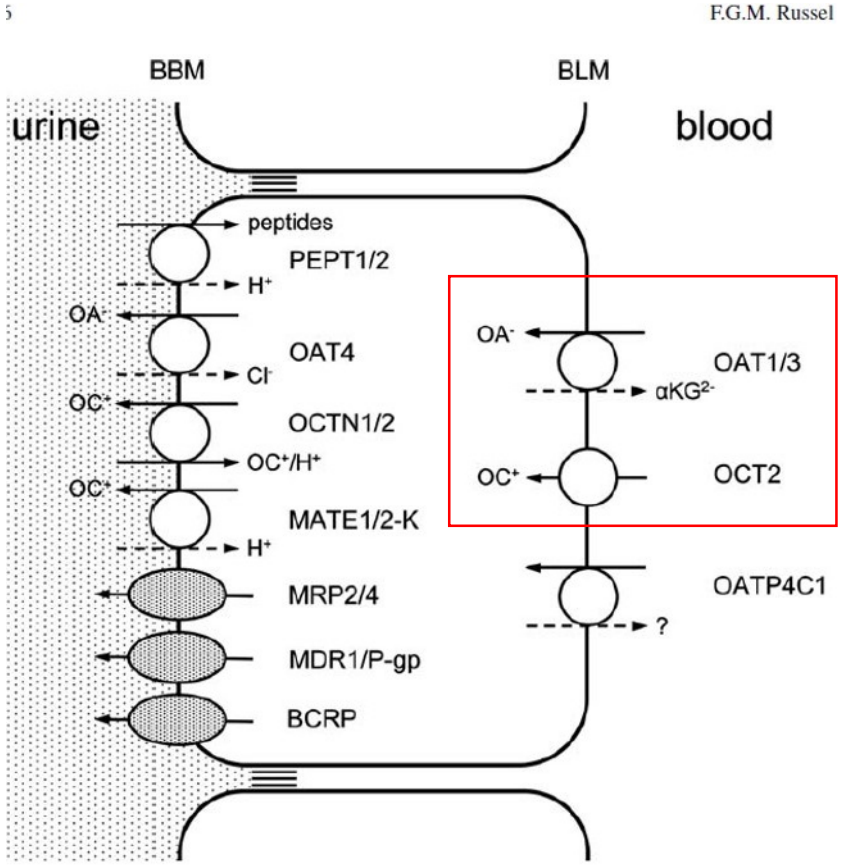
Dome formation





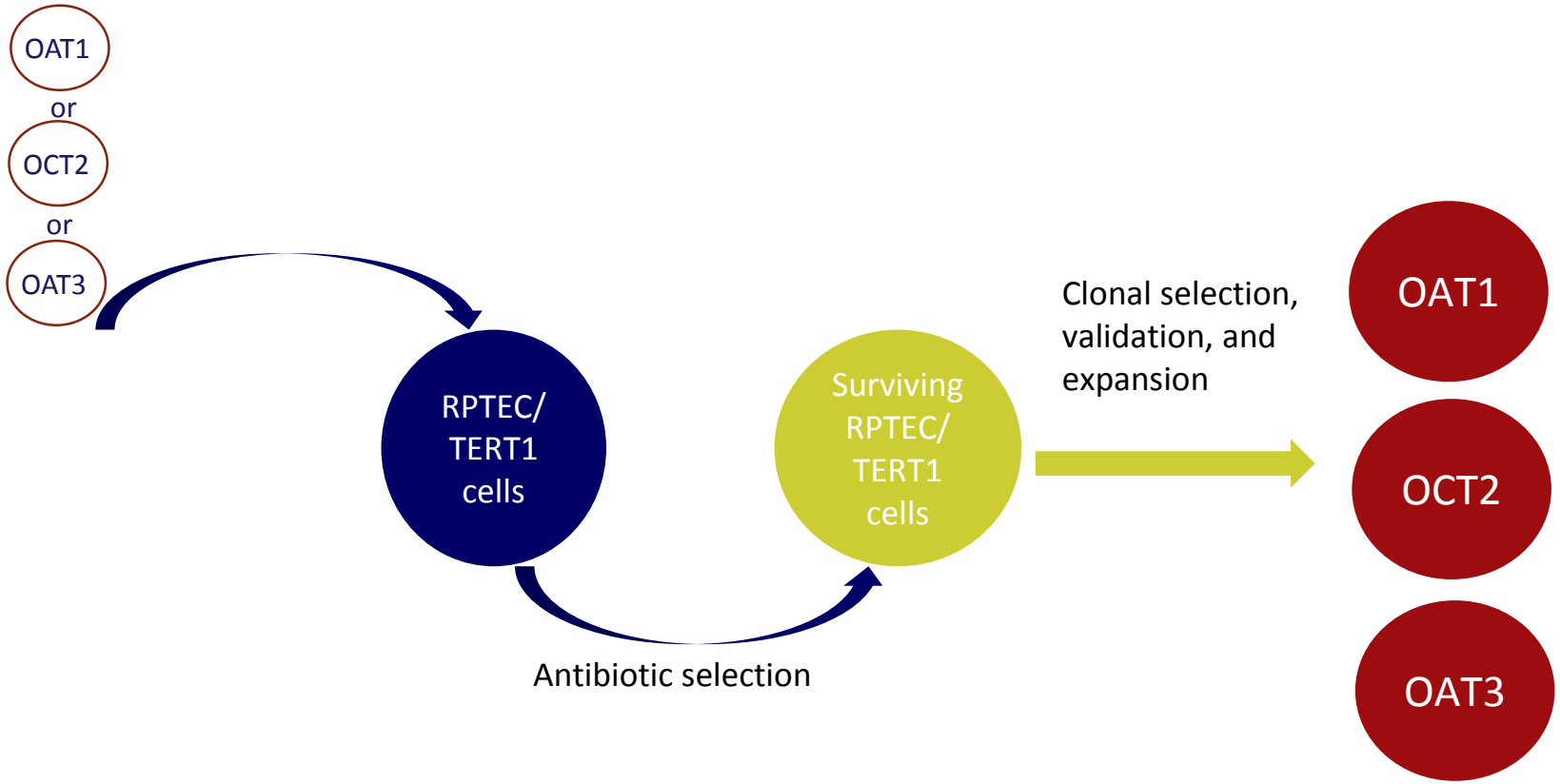
Kidney cells – Role of OAT1/OCT2/OAT3

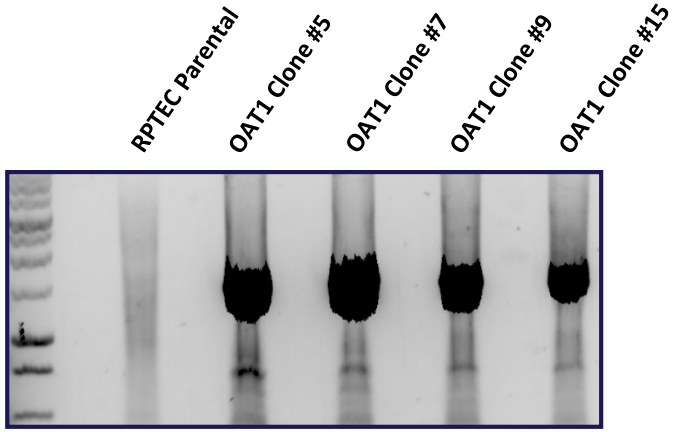
- Challenge: Expression of organic solute carrier transporters is lost in primary kidney cells
- Organic anion and cation transporters are vital in kidney metabolism
  - OAT1
  - OCT2
  - OAT3
- FDA guidance recommend evaluation of Oat/Oct transporter interactions:
  - In Vitro Metabolism-and Transporter- 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)

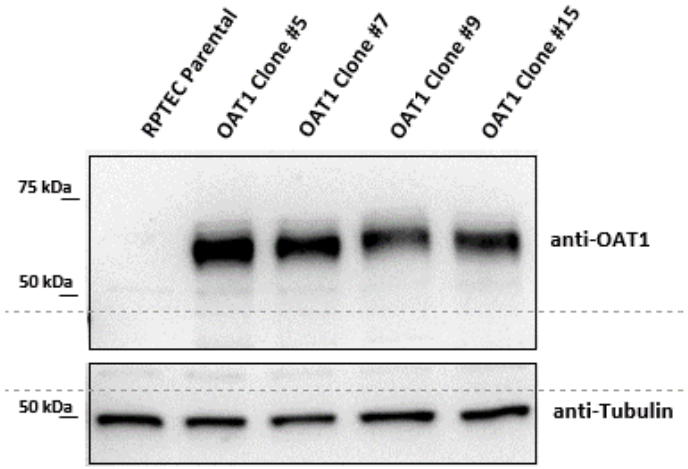


Pang K, et al. Enzyme- and Transporter-Based Drug–Drug Interactions. DOI 10.1007/978-1-4419-0840-7_2,C Am Assoc Pharmaceut Sci 2010

Enhanced kidney cellular models





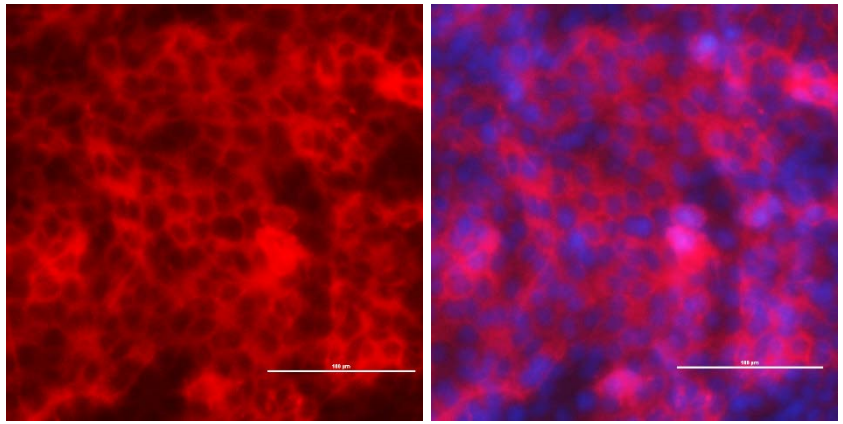


- RPTEC/TERT1 (ATCC® CRL-4031™)
- RPTEC/TERT1 OAT1 (ATCC® CRL-4031-OAT1™)
- RPTEC/TERT1 OCT2 (ATCC® CRL-4031-OCT2™)
- RPTEC/TERT1 OAT3 (ATCC® CRL-4031-OAT3™)

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

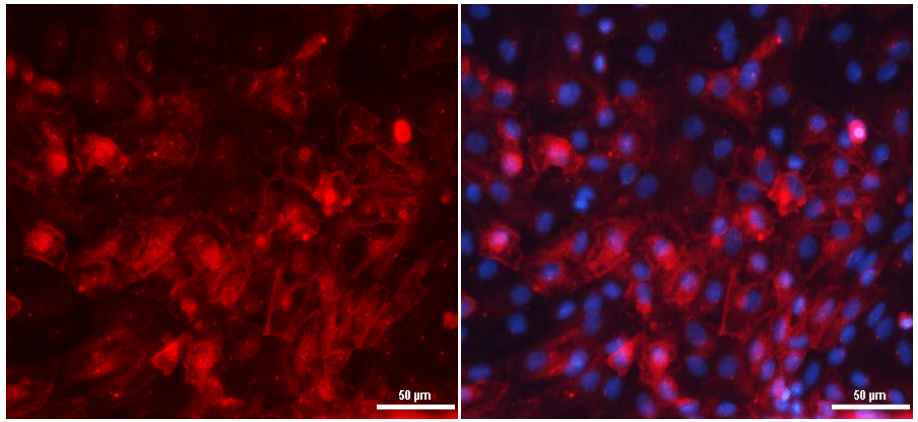
Expression and localization of OAT1/OCT2/OAT3

OAT1 Merged with DAPI



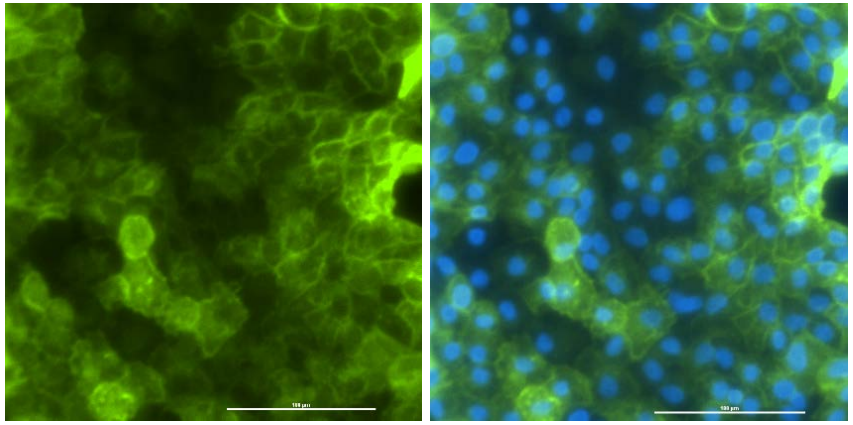
RPTEC/TERT1-OAT1

OAT3 Merged with DAPI



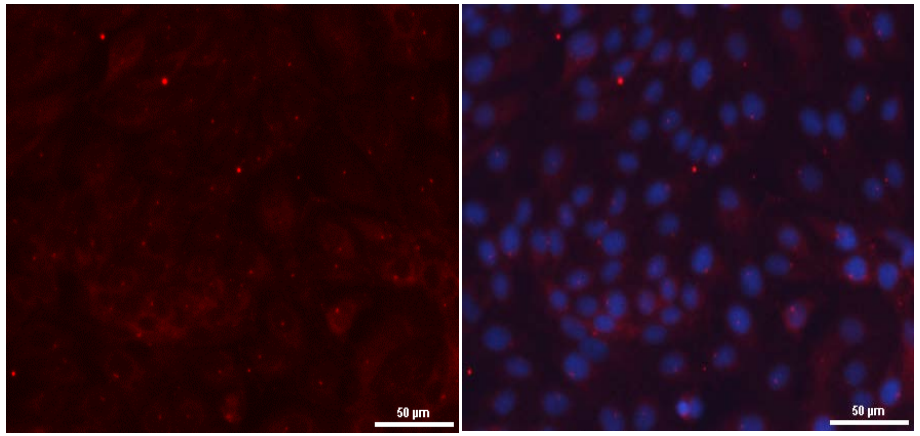
RPTEC/TERT1-OAT3

OCT2 Merged with DAPI



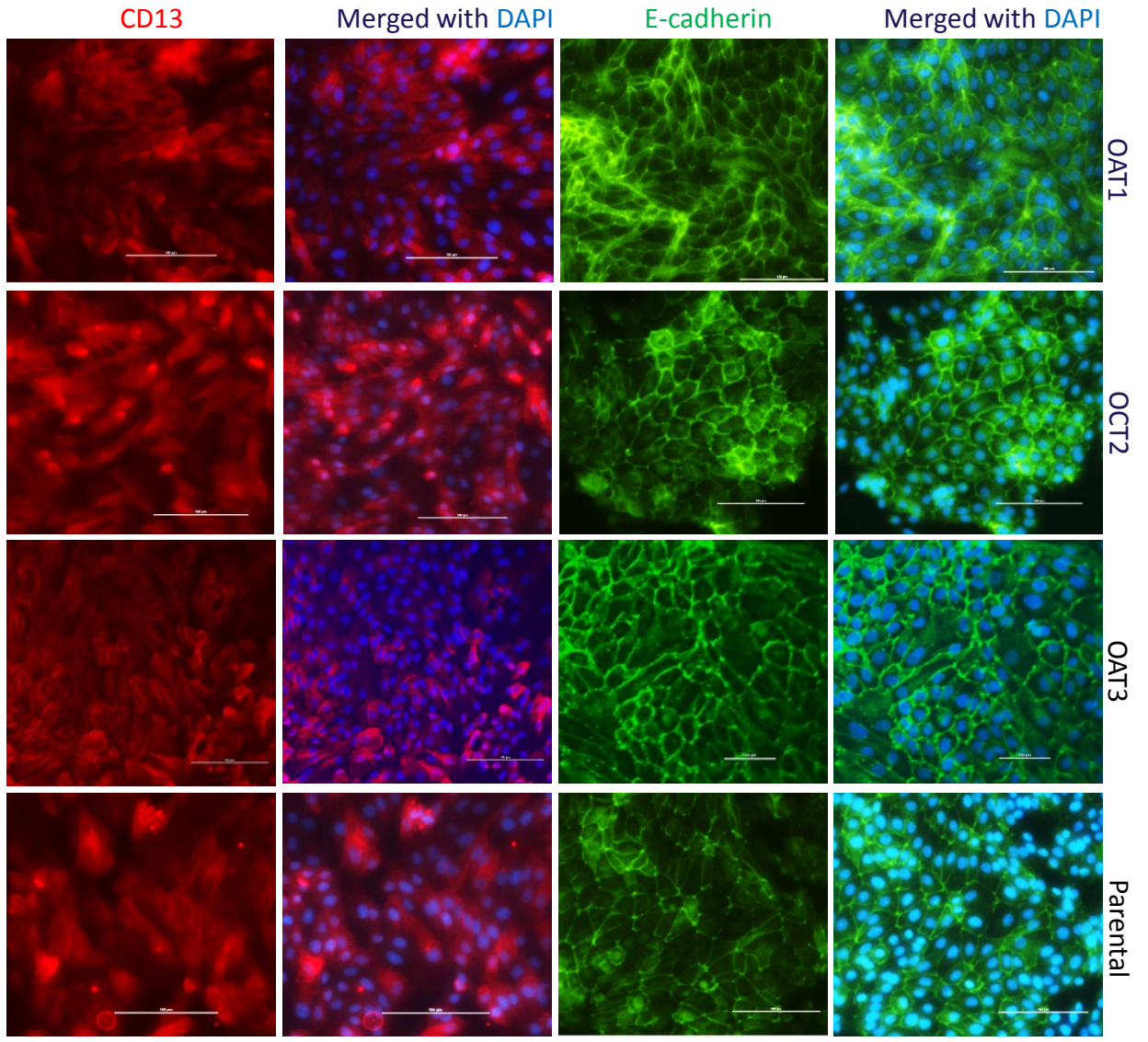
RPTEC/TERT1-OCT2

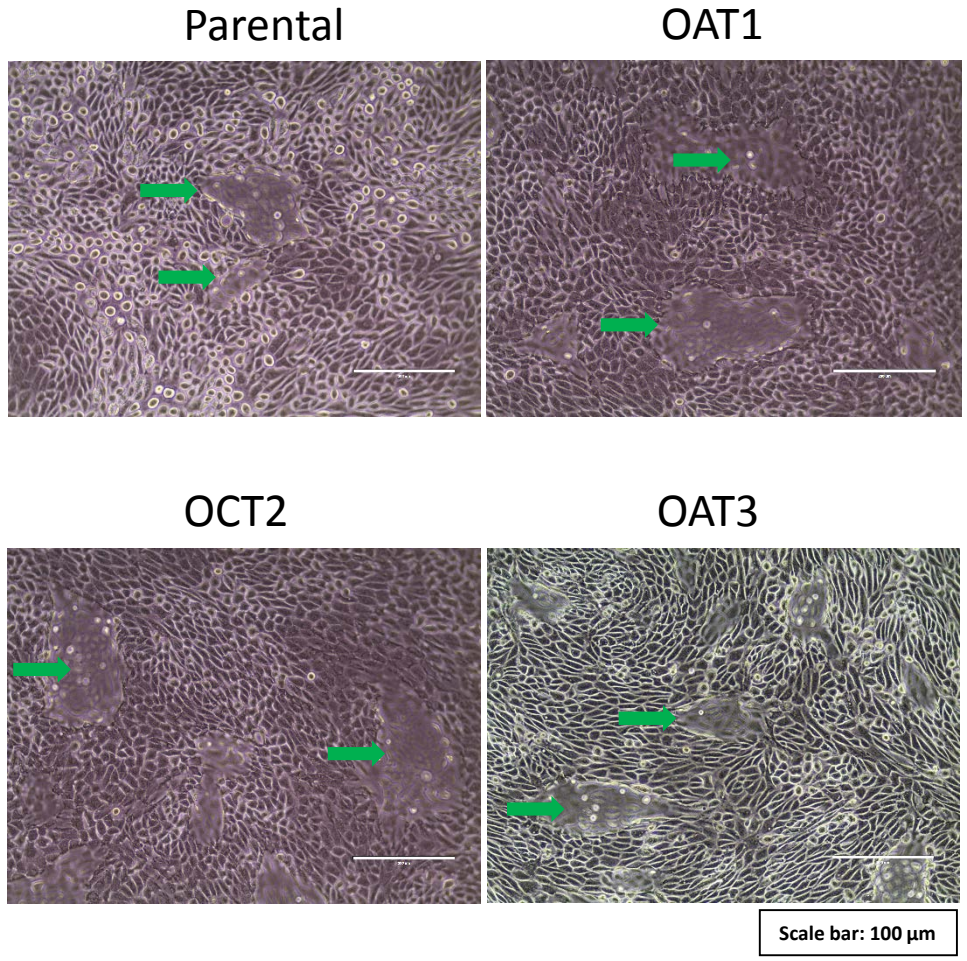
OAT3 Merged with DAPI



Parental

Endogenous marker expression and dome formation

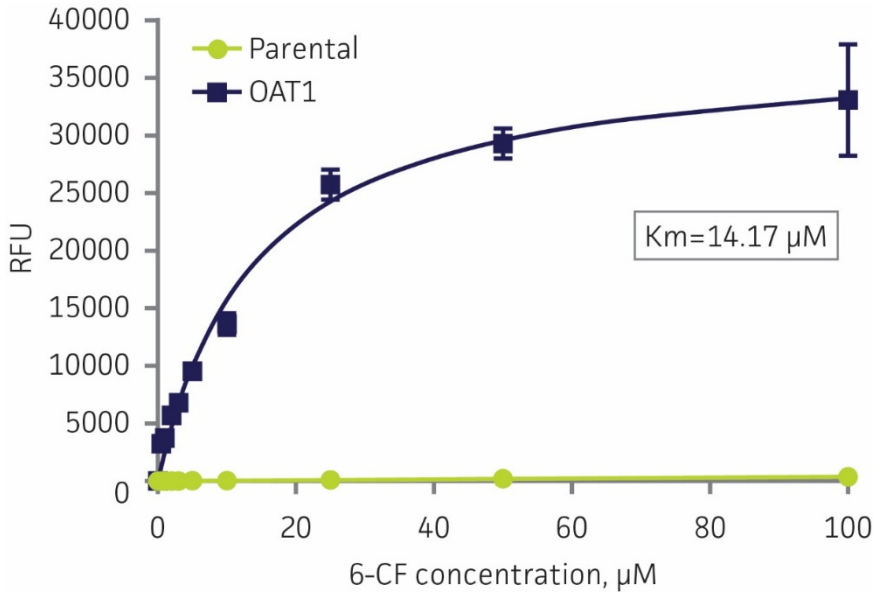


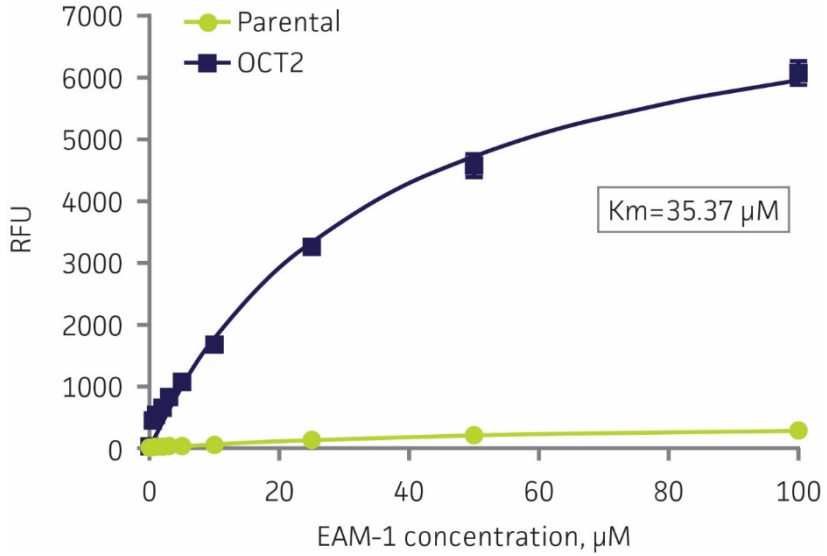


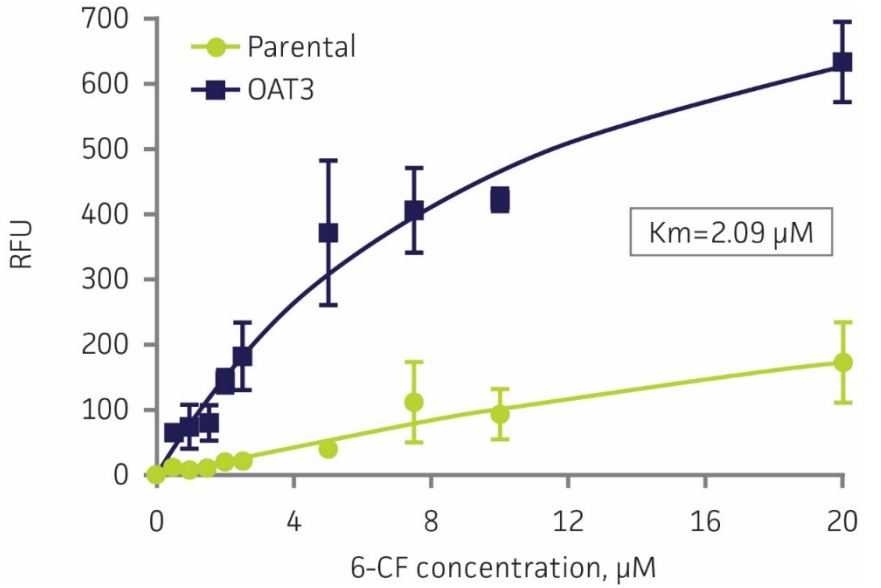
Functionality – Drug uptake assay

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





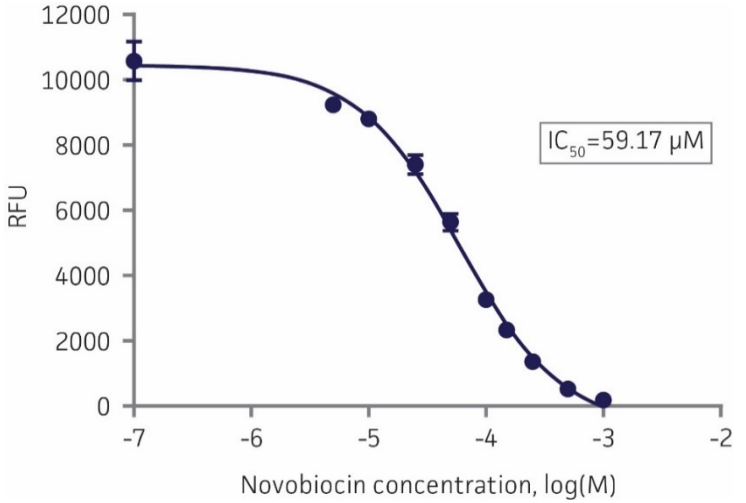


Functionality – Drug uptake inhibition assay

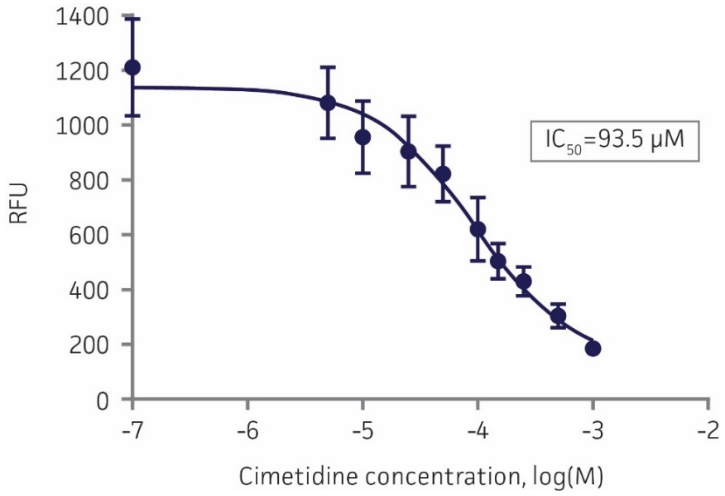
UPTAKE INHIBITION ASSAY PROTOCOL

- 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

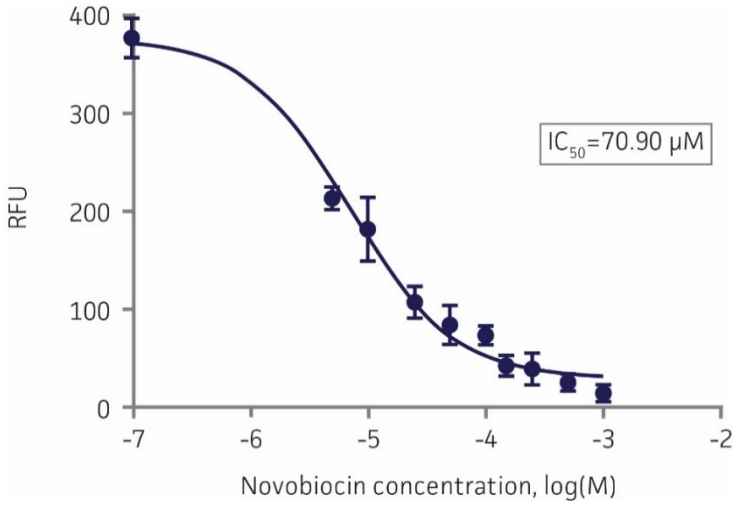
6-CF uptake inhibition in OAT-1 expressing RPTEC



EAM-1 uptake inhibition in OCT-2 expressing RPTEC



6-CF uptake inhibition in OAT-3 expressing RPTEC

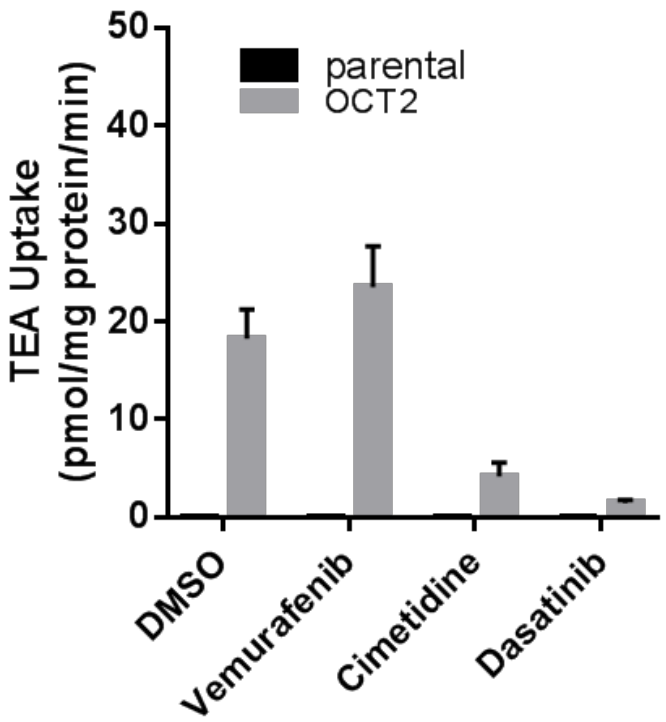


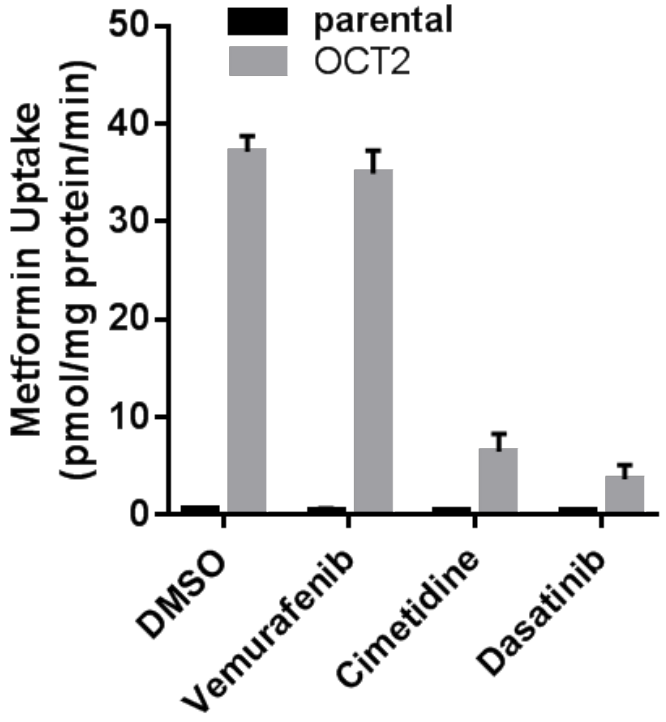
RPTEC-OCT2 – Drug-drug Interactions (DDI)

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



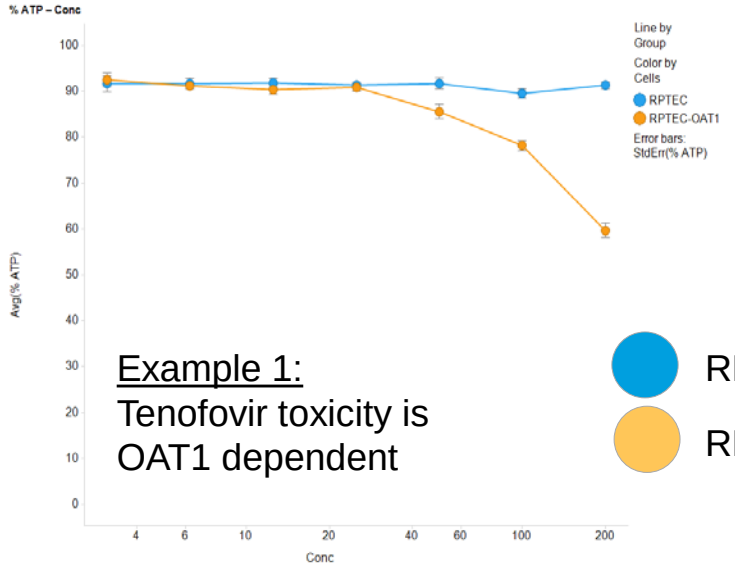


Data kindly provided by:
Kevin Huang, *Graduate Research Associate,* Ohio State University, College of Pharmacy
Alice Gibson, Ph.D., *Senior Research Specialist,* Ohio State University, College of Pharmacy

Application for nephron toxicity studies

CELL VIABILITY ASSAY PROTOCOL

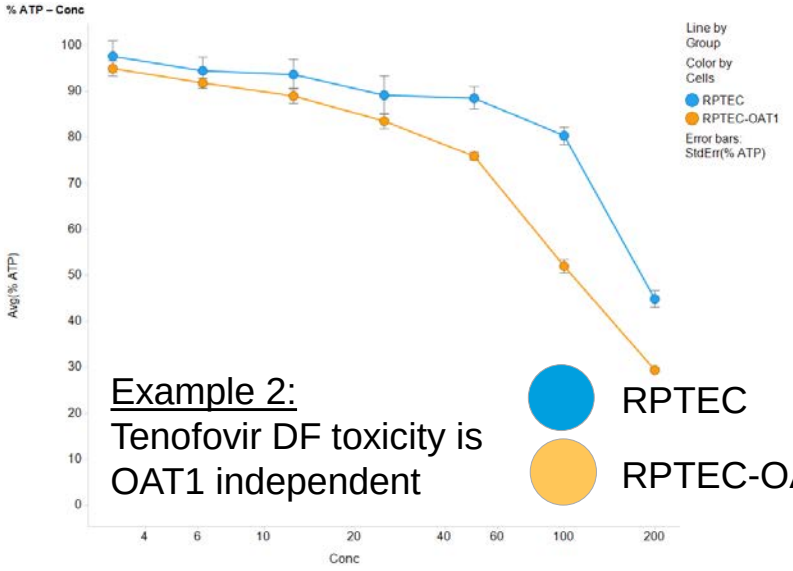
- About 35000 cells were seeded per well in triplicate in a 96-well plate and incubated overnight
- Cells were incubated with a series of compounds at various concentrations for 3 days
- Cell viability was determined using a cell viability assay per manufacturer's instructions



Example 1:
Tenofovir toxicity is OAT1 dependent

RPTEC

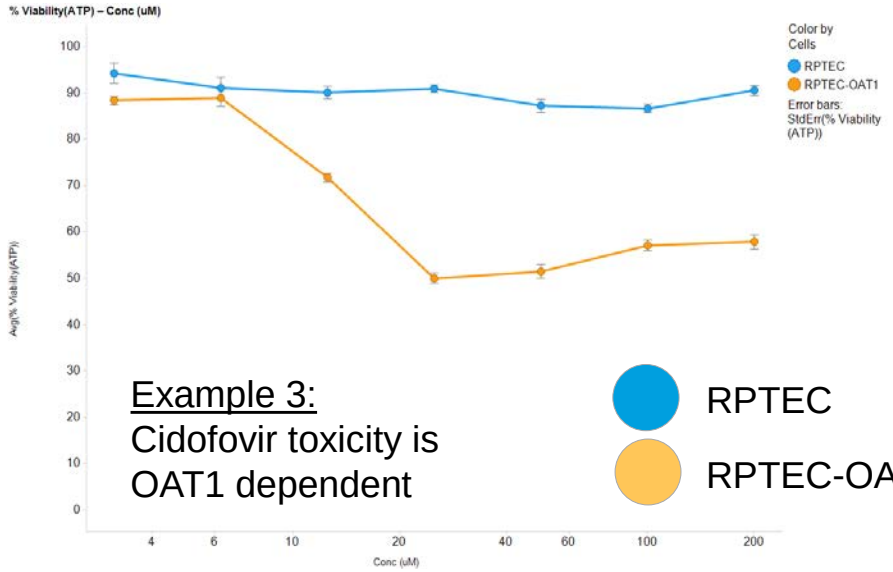
RPTEC-OAT1



Example 2:
Tenofovir DF toxicity is OAT1 independent

RPTEC

RPTEC-OAT1



Example 3:
Cidofovir toxicity is OAT1 dependent

RPTEC

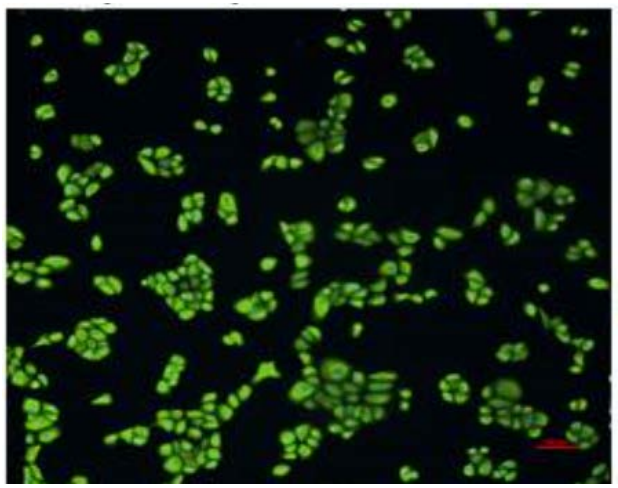
RPTEC-OAT1

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

Skin models

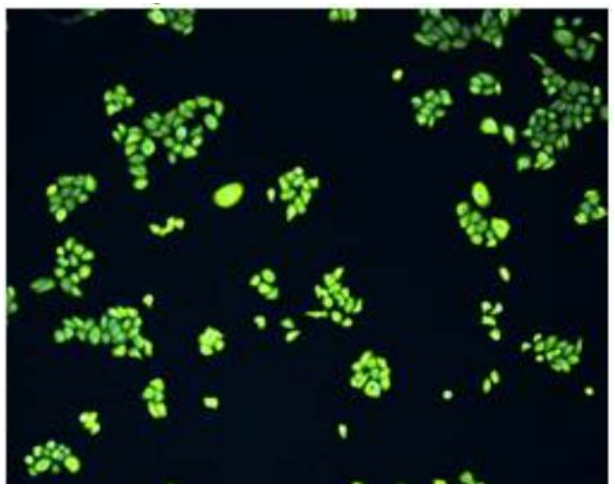
- BJ-5ta (Skin fibroblasts; ATCC® CRL-4001™)
- Ker-CT (Epidermal keratinocytes; ATCC® CRL-4048™)
- hTERT-immortalized Dermal Melanocyte (ATCC® CRL-4059™)
- Complementary primary cells:
  - Primary epidermal keratinocytes (ATCC® PCS-200-010™)

Ker-CT



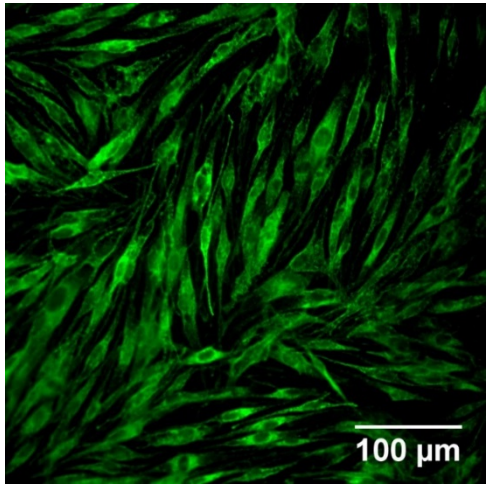
KRT5(FITC) + DAPI

Primary Epidermal Keratinocytes



KRT5(FITC) + DAPI

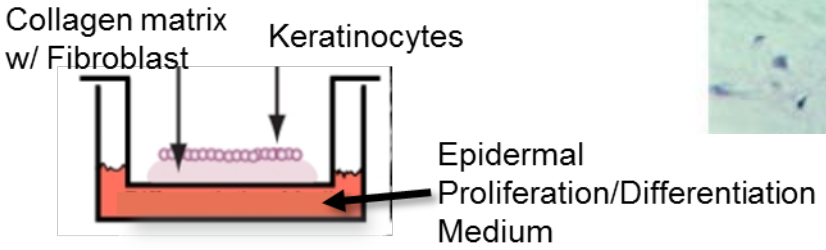
hTERT Melanocytes

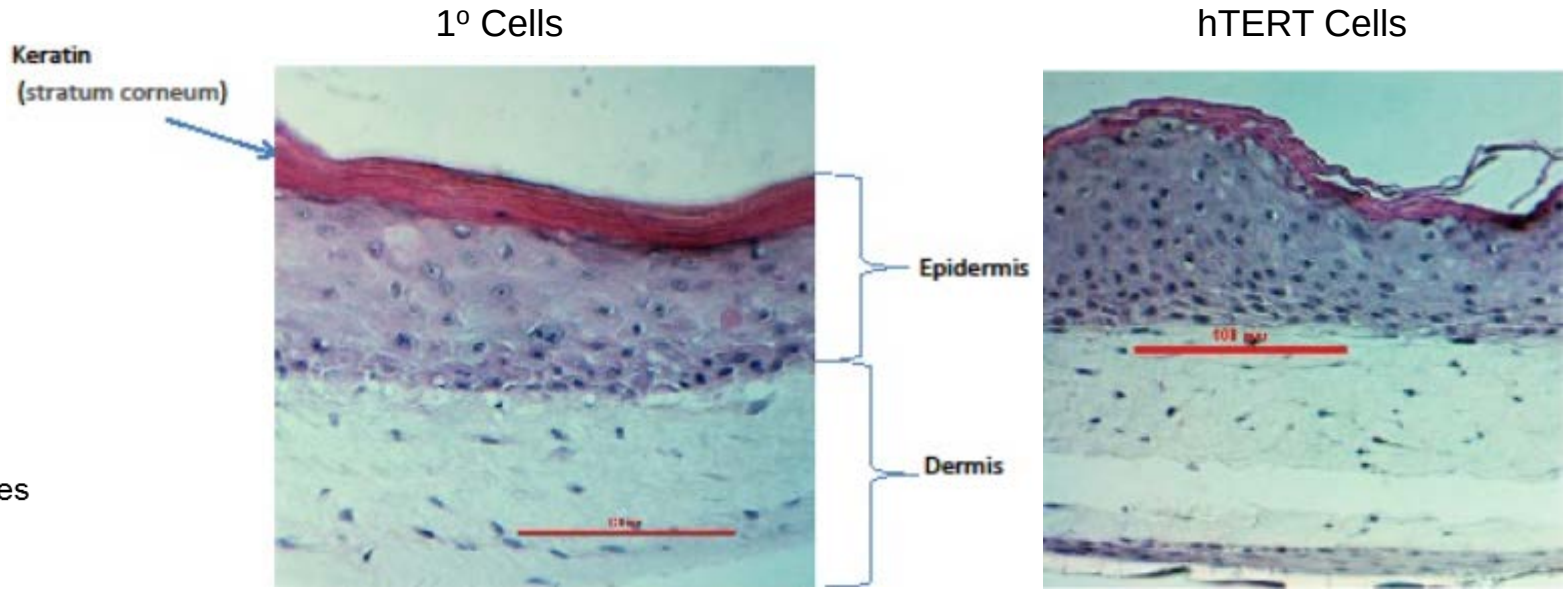


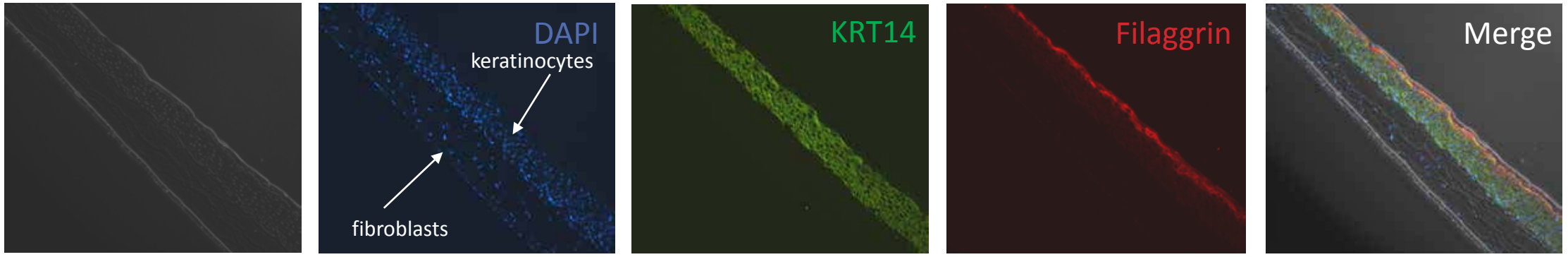
hTERT keratinocytes and hTERT fibroblasts

- Keratinocytes and fibroblast can form 3D organotypic skin cultures *in vitro*

- hTERT 3D organotypic skin cultures are positive for dermal skin markers





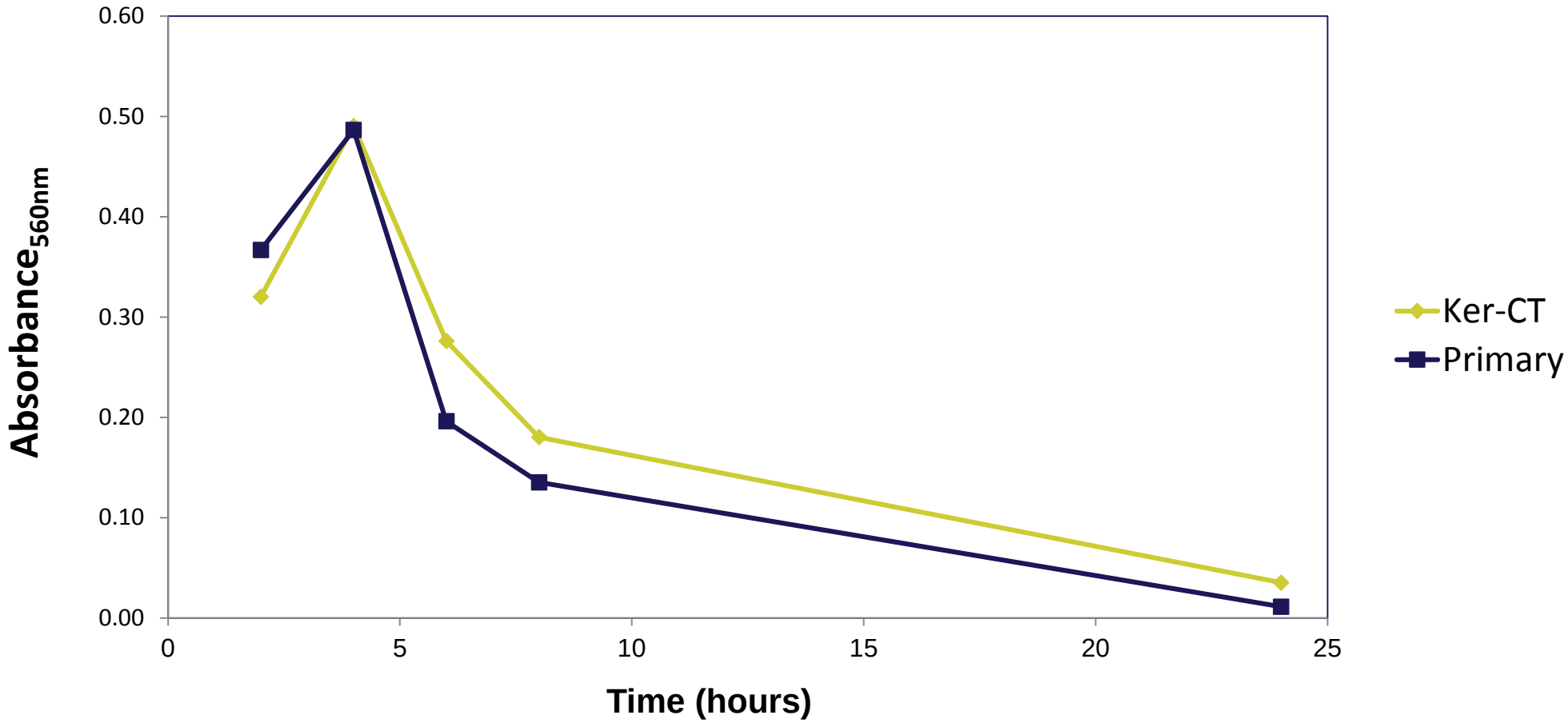


Phase contrast

Stratum granulosum/corneum

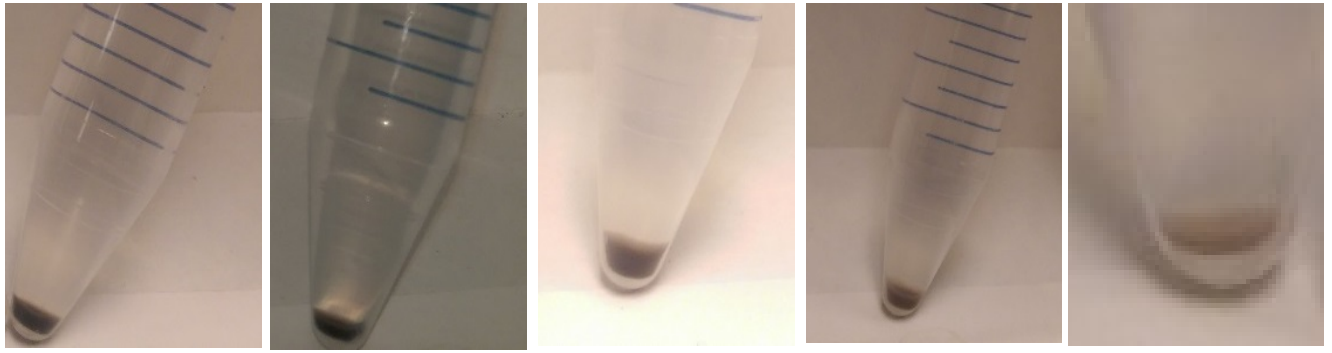
Keratinocyte 3D skin model of toxicity

3D organotypic skin culture in presence of Triton X-100. Viability monitored via MTT Assay (ATCC® 30-1010K™)



hTERT adult melanocyte characterization

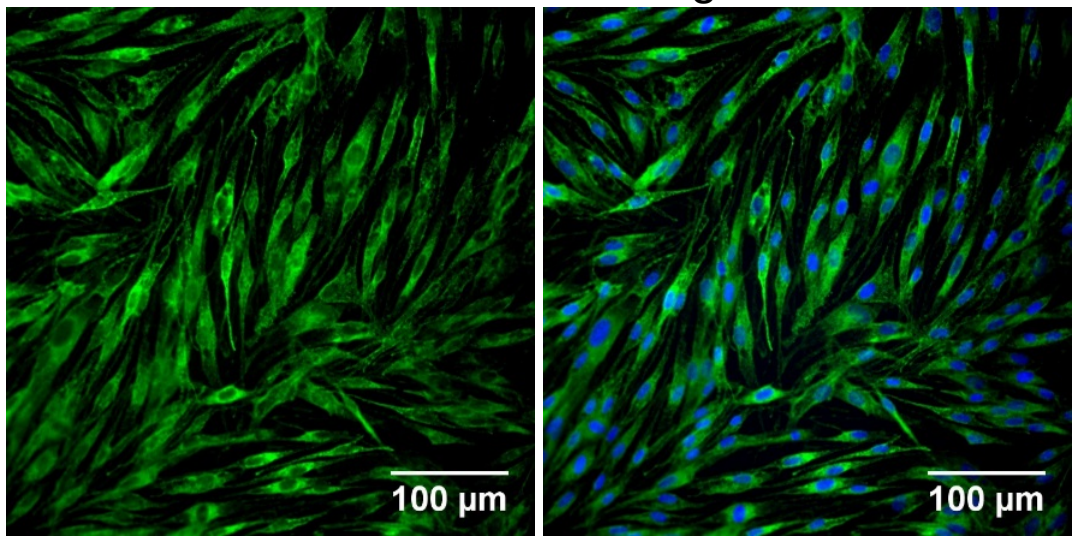
hTERT melanocytes maintain melanin production



PDL	6	15	24	40	53

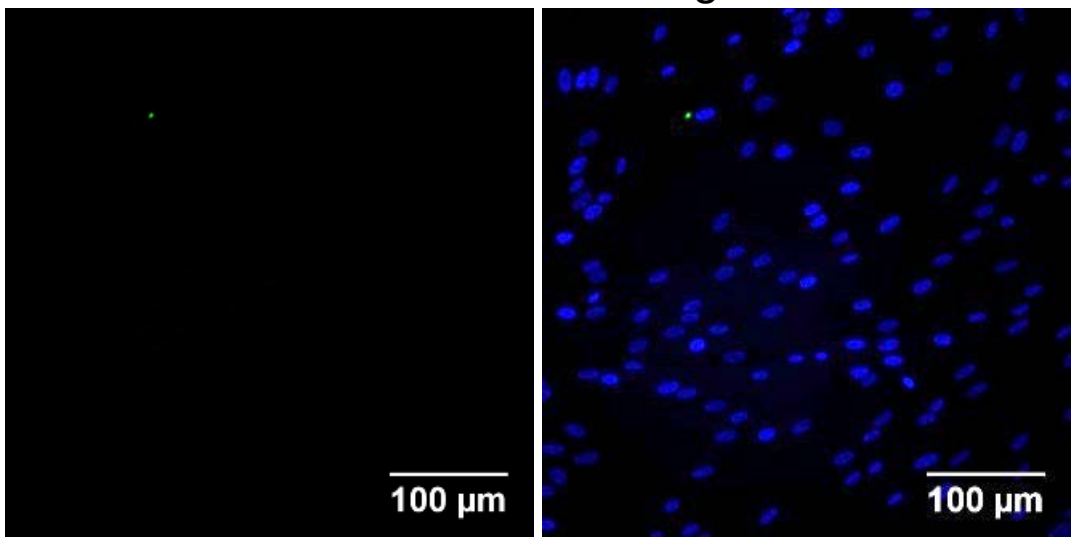
hTERT melanocytes retain positive melanin marker

TYRP1 | Merged with DAPI



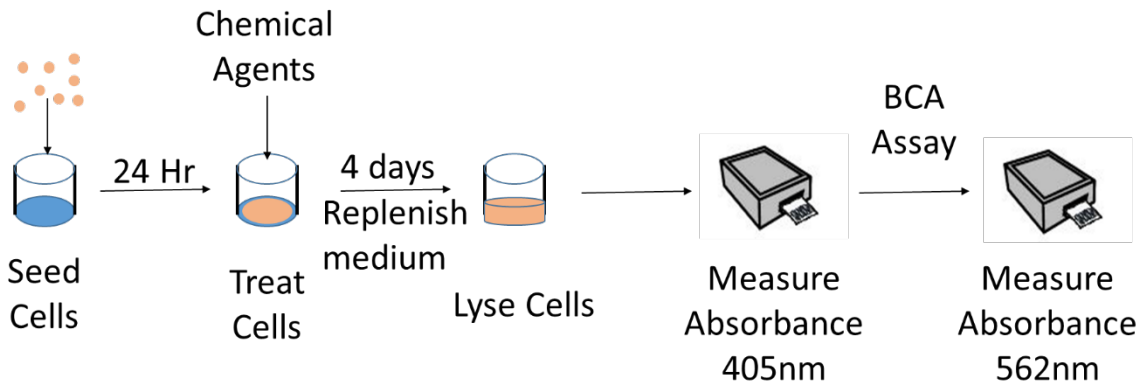
hTERT melanocytes lack fibroblast cell marker

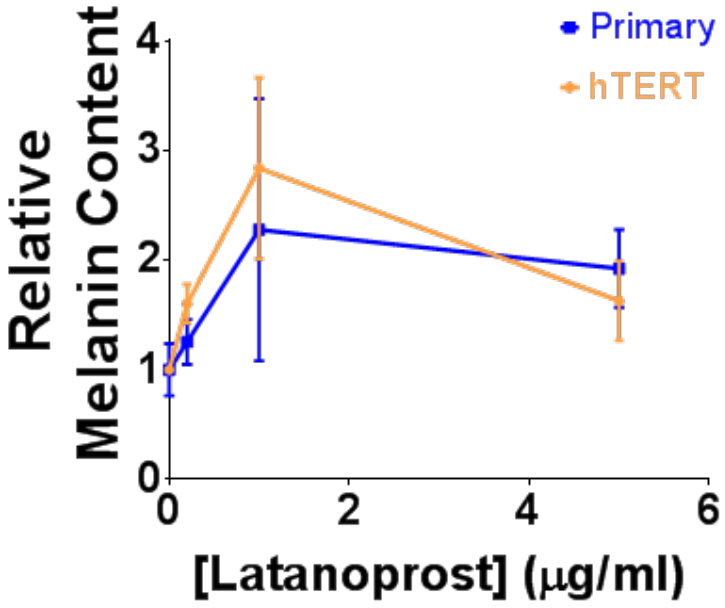
TE7 | Merged with DAPI

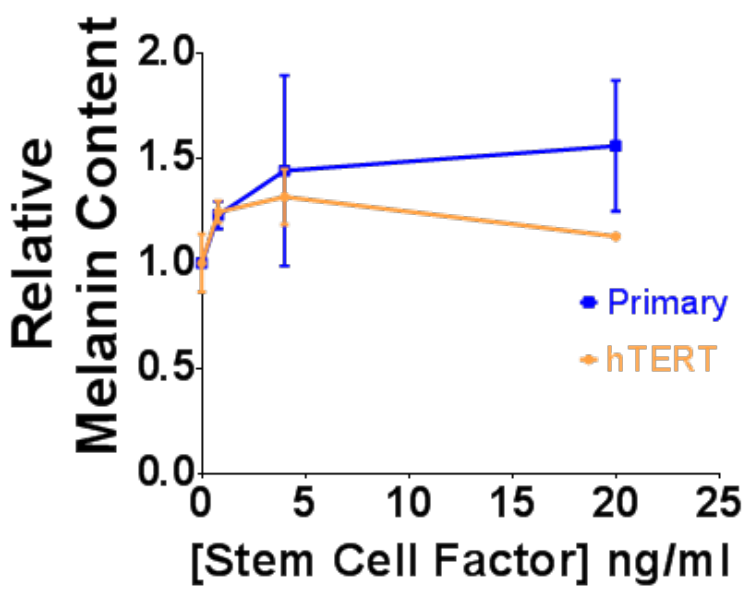


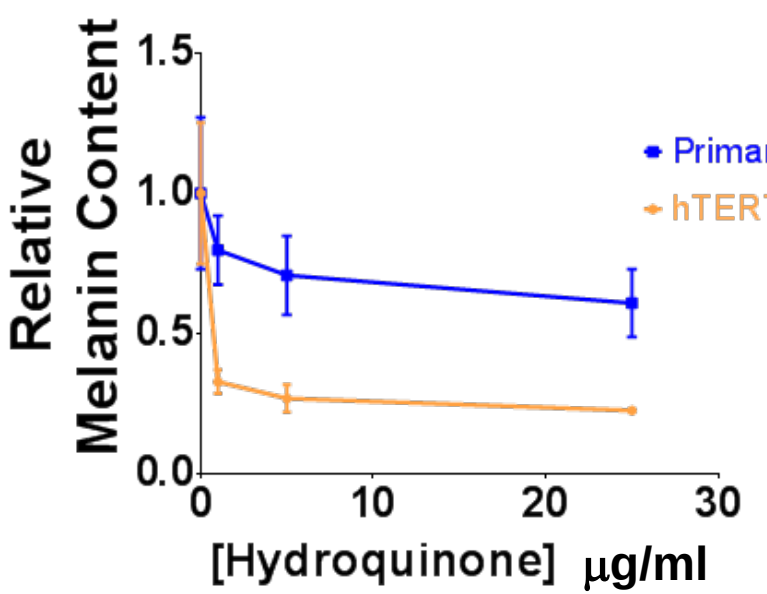
hTERT adult melanocytes sustain functionality

Stimulation and inhibition of melanin









3D skin model – Combination of cell types

hTERT melanocytes pigment a 3D organotypic skin model

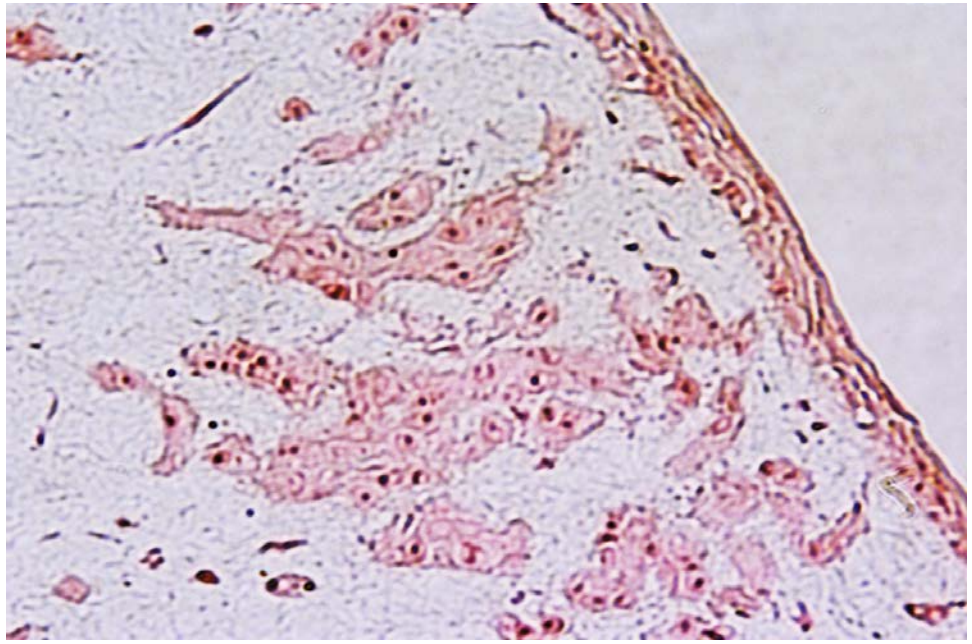
hTERT BJ-5ta/KerCT

Epidermis

Dermis

+ hTERT Melanocytes

hTERT BJ-5ta/KerCT

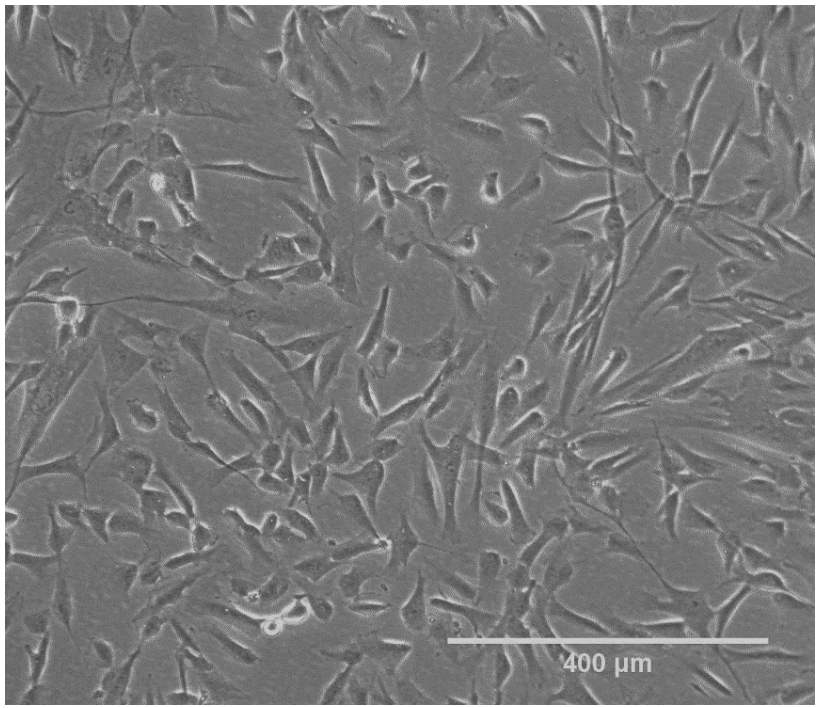


- hTERT Melanocytes

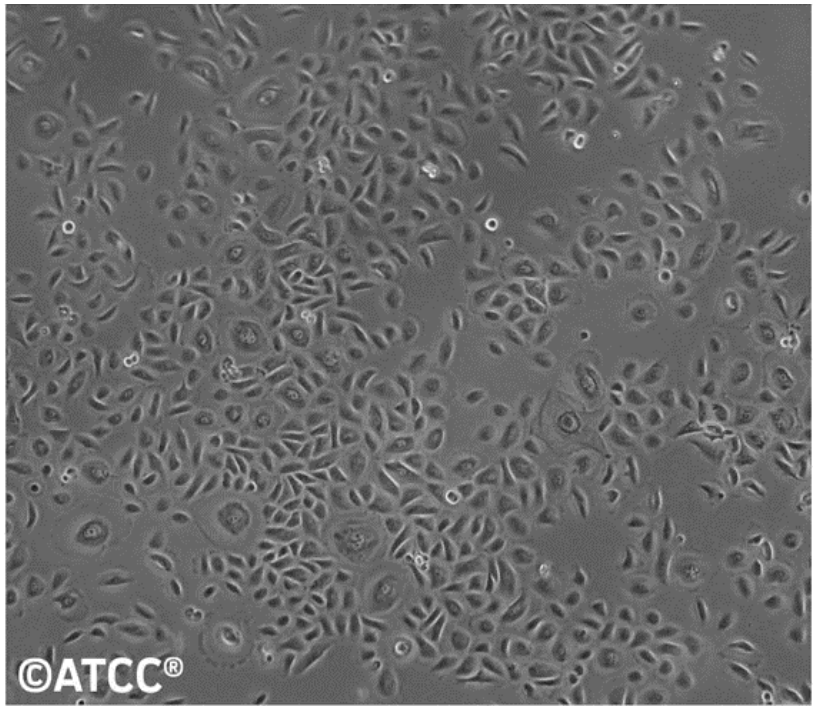
Gingival model

- hTERT Gingival Fibroblast (ATCC® CRL-4061™, *coming soon!*)
- Complementary primary cells
  - Primary Gingival Keratinocytes (ATCC® PCS-200-014™)

hTERT Gingival Fibroblast



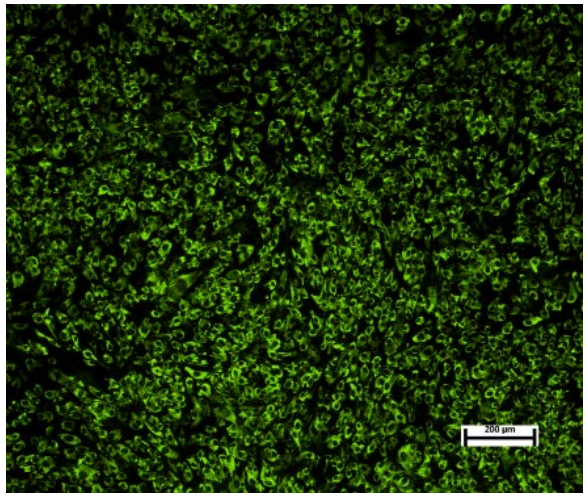
Primary Gingival Keratinocytes



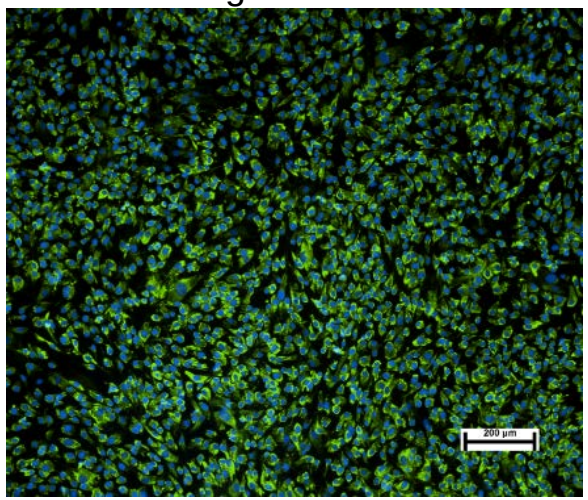
hTERT gingival fibroblasts characterization

Maintain positive fibroblast marker

TE7

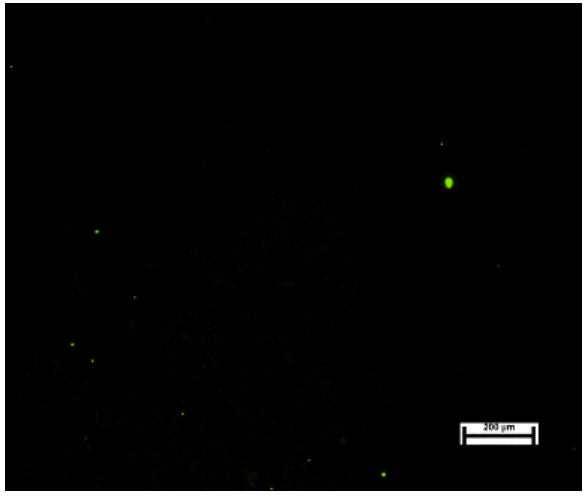


Merged w/ DAPI

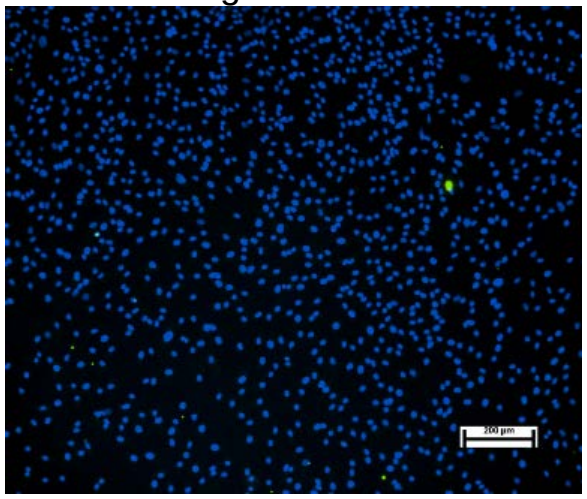


Lack epithelial cell markers

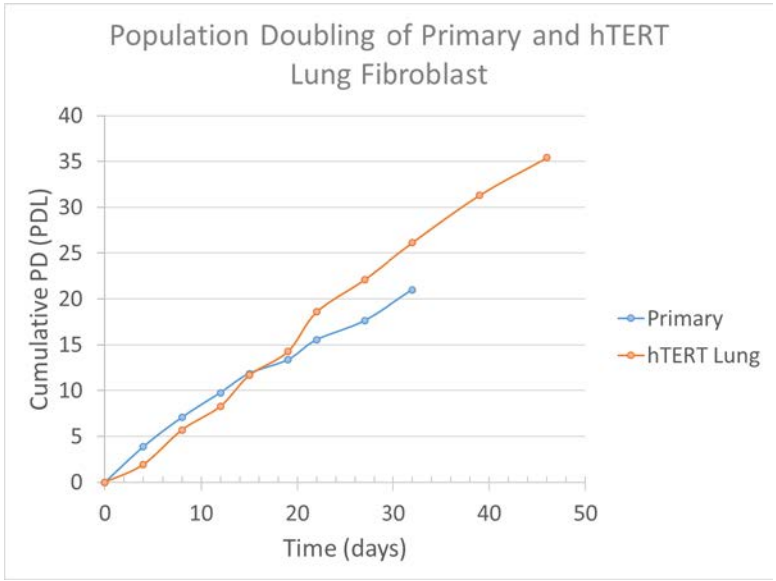
Pan-CK

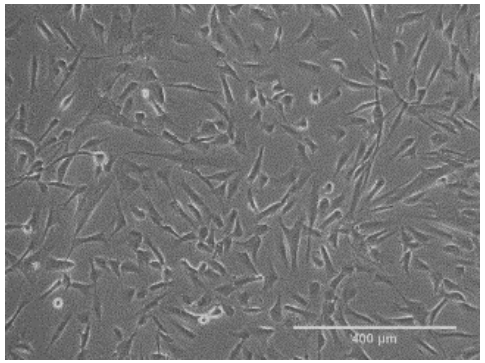


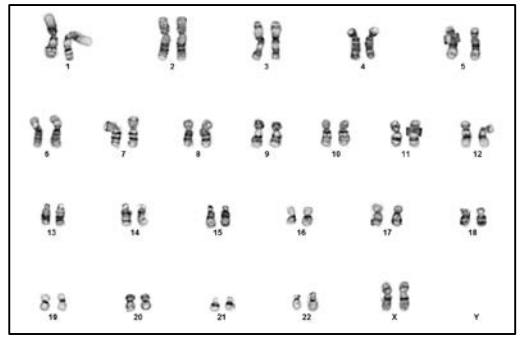
Merged w/ DAPI



Gingival fibroblasts growth, morphology, karyotype compares to primary.

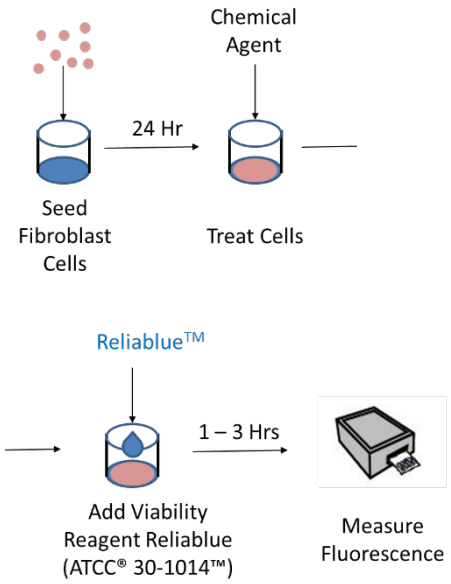


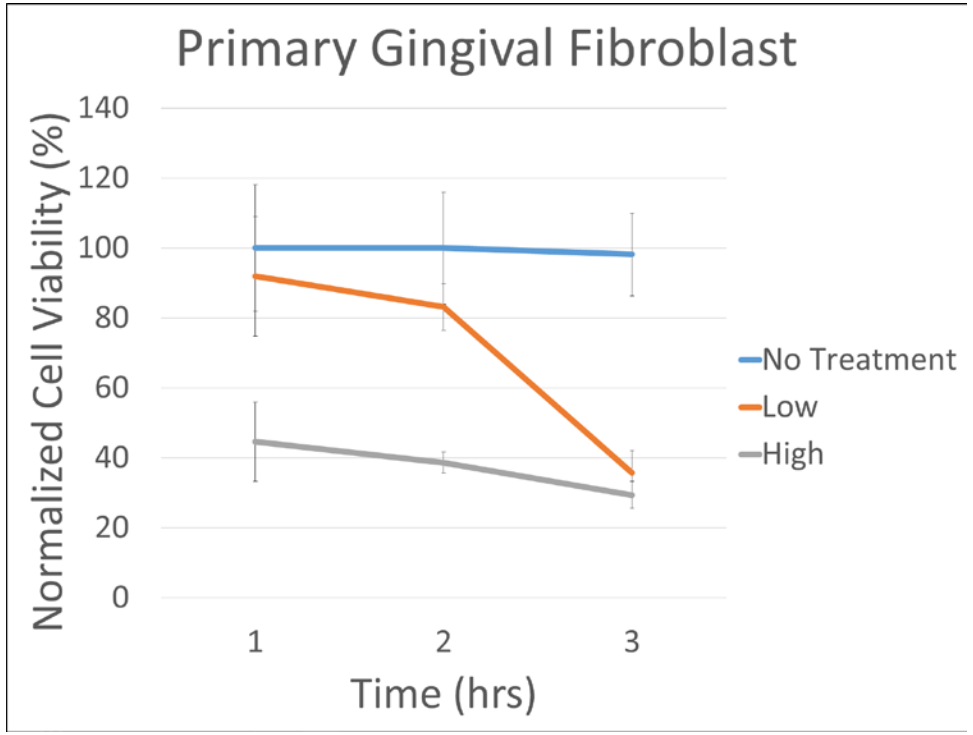


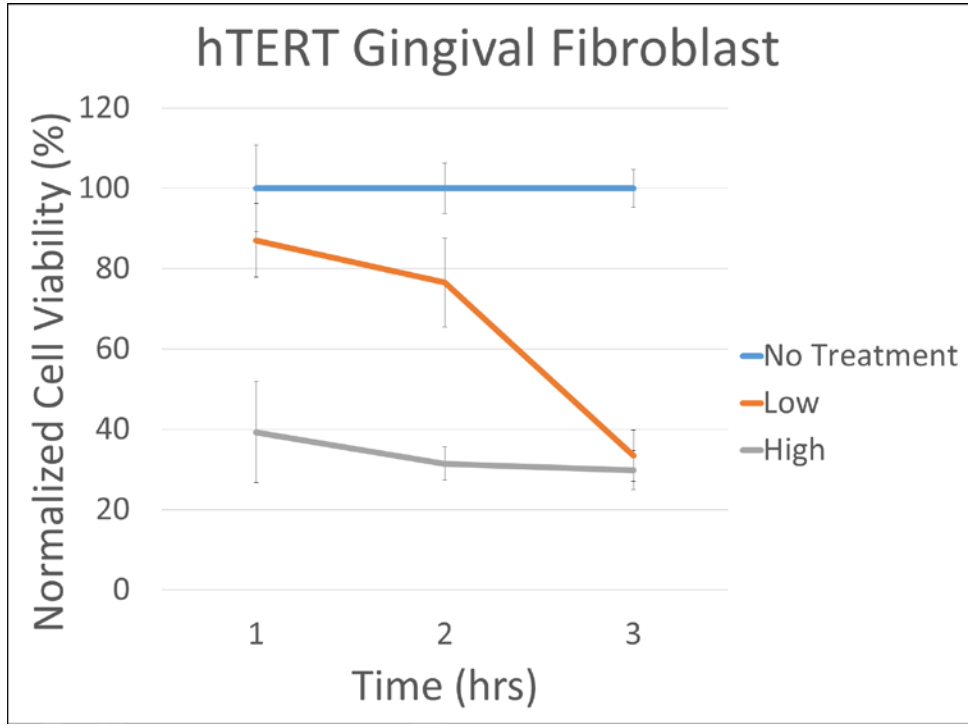


hTERT gingival fibroblasts respond to chlorhexidine

Cellular cytotoxicity of gingival fibroblast by chlorhexidine is dose-dependent





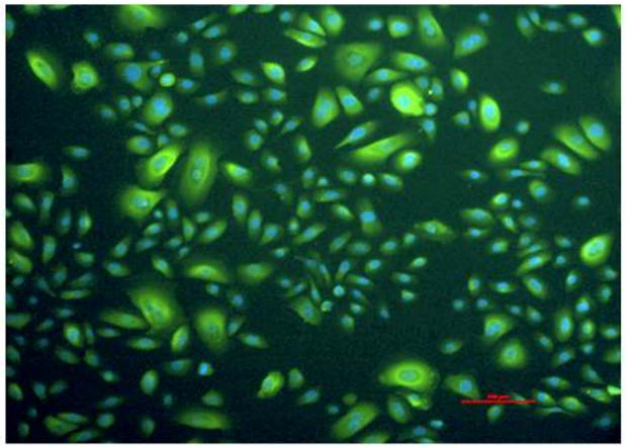


Airway models

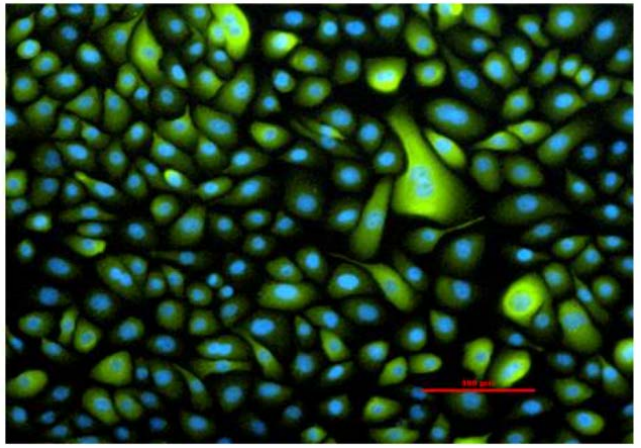
- HBEC-3KT (Bronchial epithelial cells; ATCC® CRL-4051™)
- hTERT Lung Fibroblast (ATCC® CRL-4058™)
- HSAEC1-KT (Small airway epithelial cells; ATCC® CRL-4050™)
- NuLi-1 (Bronchial epithelial cells; ATCC® CRL-4011™)
- Complementary primary cells
  - Primary bronchial/tracheal epithelial cells
  - Lung smooth muscle cells
  - Bronchial tracheal smooth muscle cells
  - Disease airway cells
    - Asthma, COPD, Cystic Fibrosis, Fibrosis

CCSP + DAPI

Primary Bronchial/Tracheal Epithelial Cells

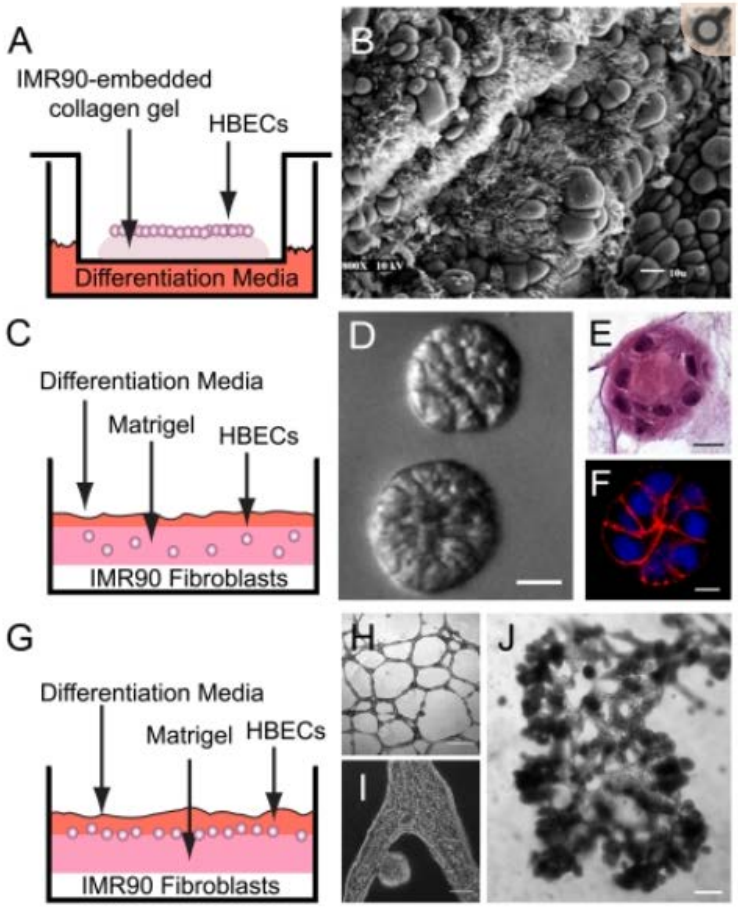


HSAEC1-KT



Bronchial epithelial cells - Differentiate

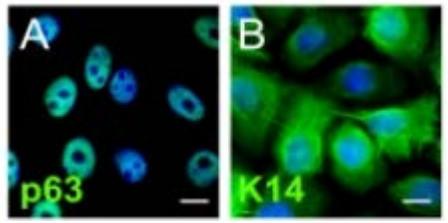
Bronchial epithelial cells are multipotent in multiple 3D systems.



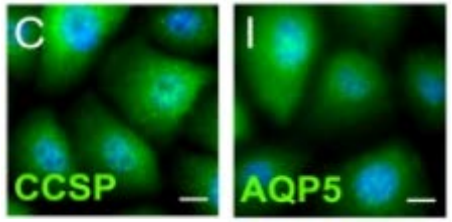
HBEC-3KT can be studied in a variety of 3D culture models

Immortalized HBECs express markers of multiple cell types of the lung.

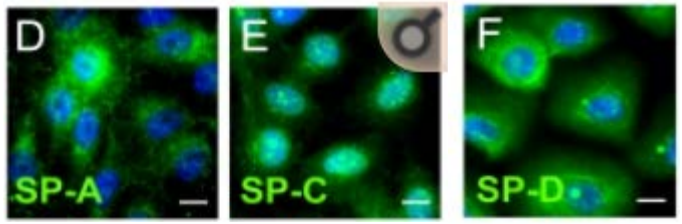
Epithelial cell markers



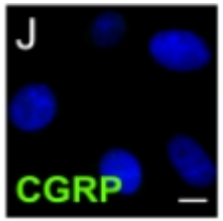
Bronchial cell markers



Secreting cell markers



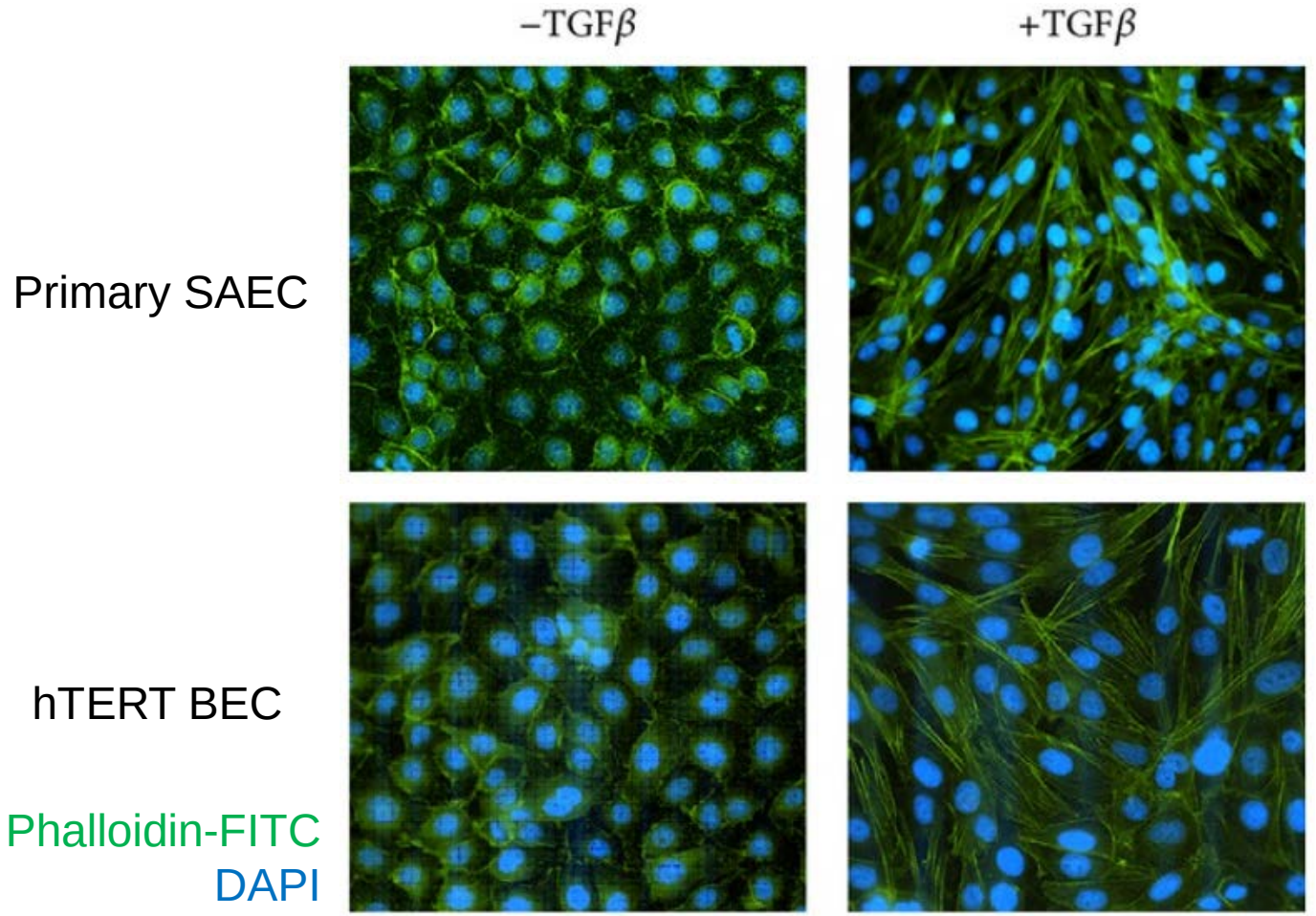
Negative cell marker



Delgado et al. Multipotent capacity of immortalized human bronchial epithelial cells. PLoS One 6(7):e22023, 2011.

Bronchial epithelial cells – Respond to TGFβ

- Respiratory mucosa coordinates the inflammatory response in chronic airway diseases, including asthma and COPD
- Signals produced by the chronic inflammatory process induce epithelial mesenchymal transition (EMT)

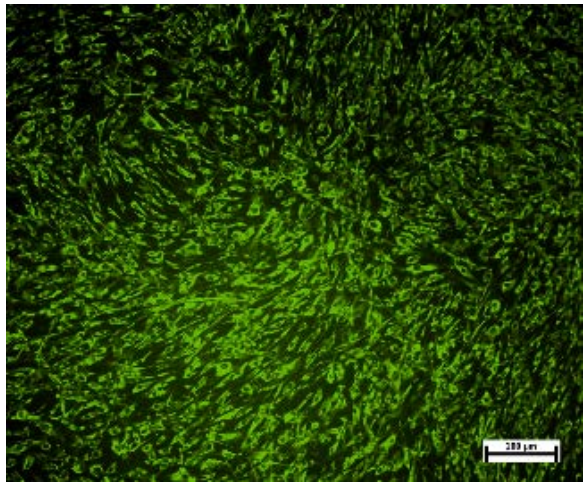


Kalita M, et al. Systems Approaches to Modeling Chronic Mucosal Inflammation. Biomed Res Int doi: 10.1155/2013/505864, 2013.

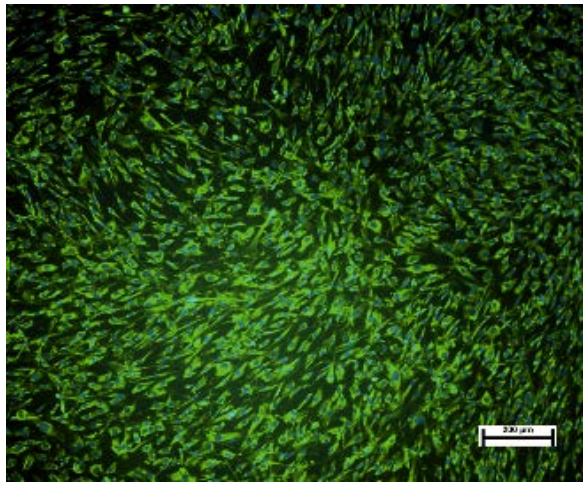
hTERT lung fibroblast characteristics and functionality

Maintain a positive fibroblast marker

TE7



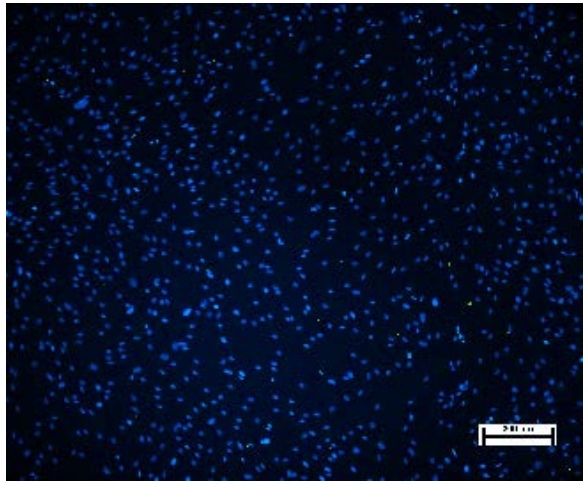
Merged w/ DAPI



Lack epithelial cell markers

Pan-CK

Merged w/ DAPI

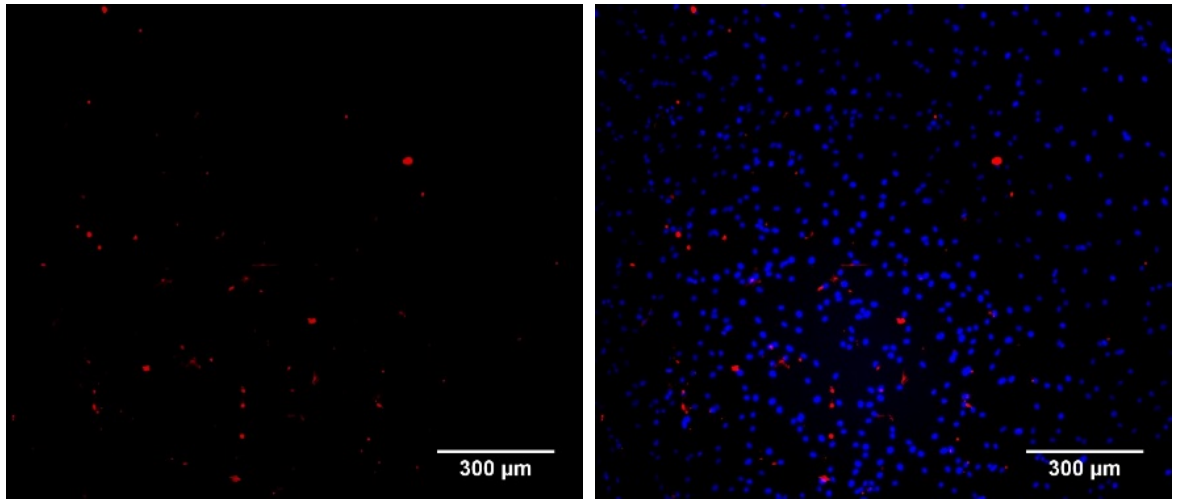


Respond to TGF-β1

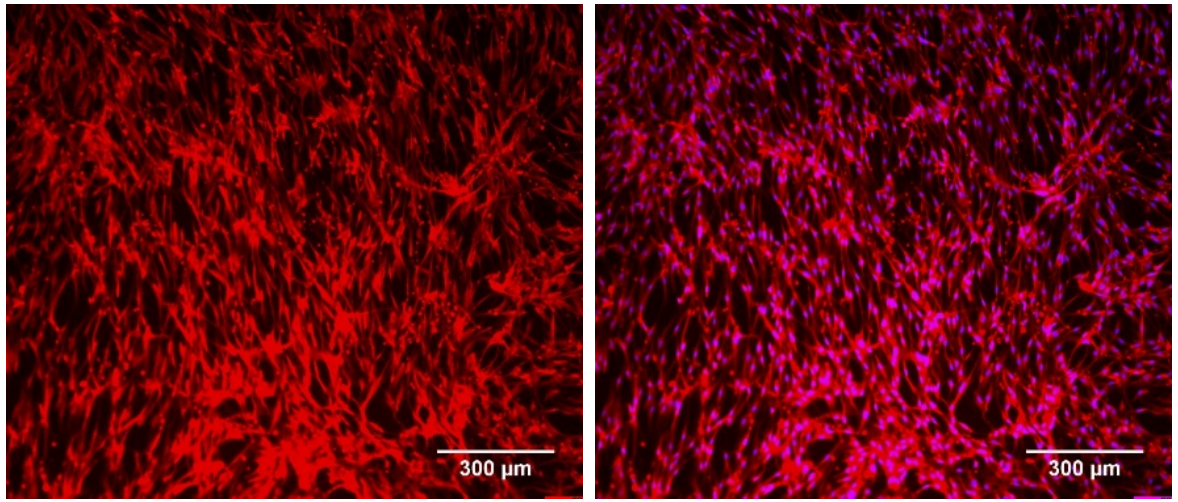
α-SMA

Merged w/ DAPI

Untreated



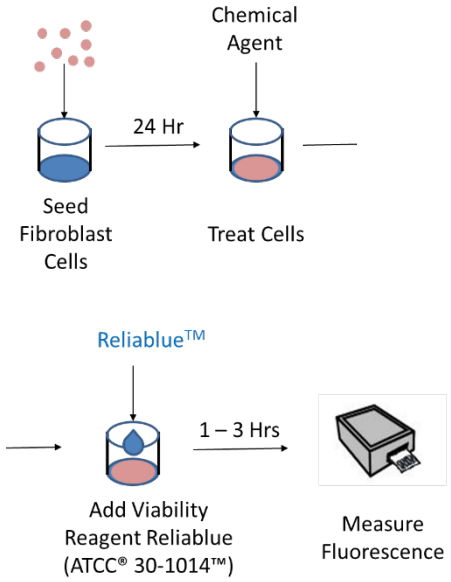
TGF-β1

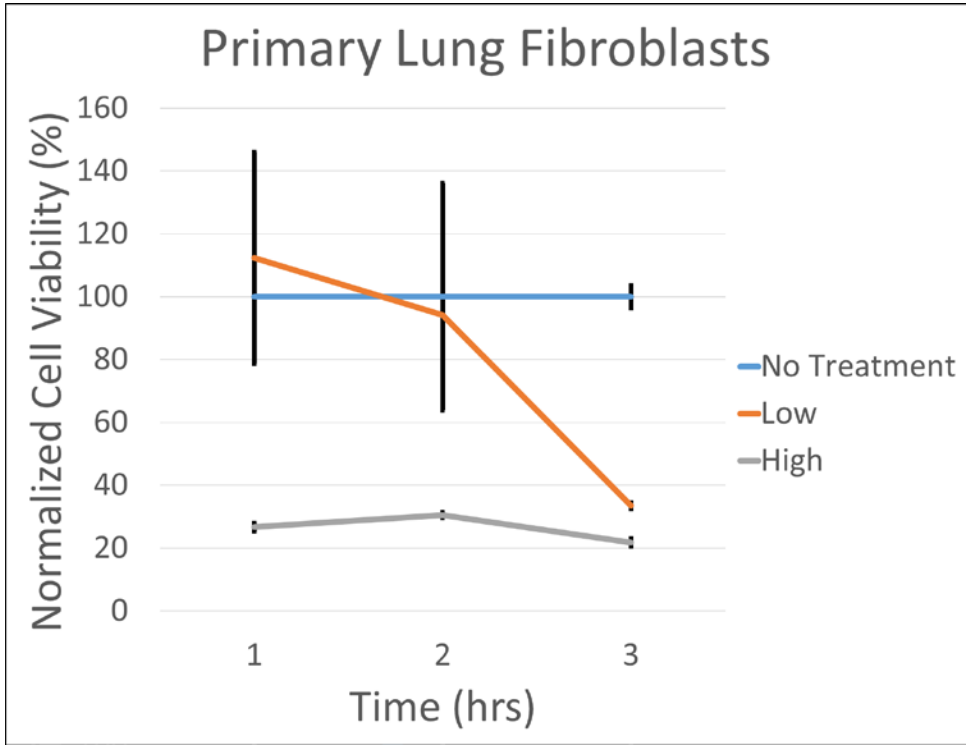


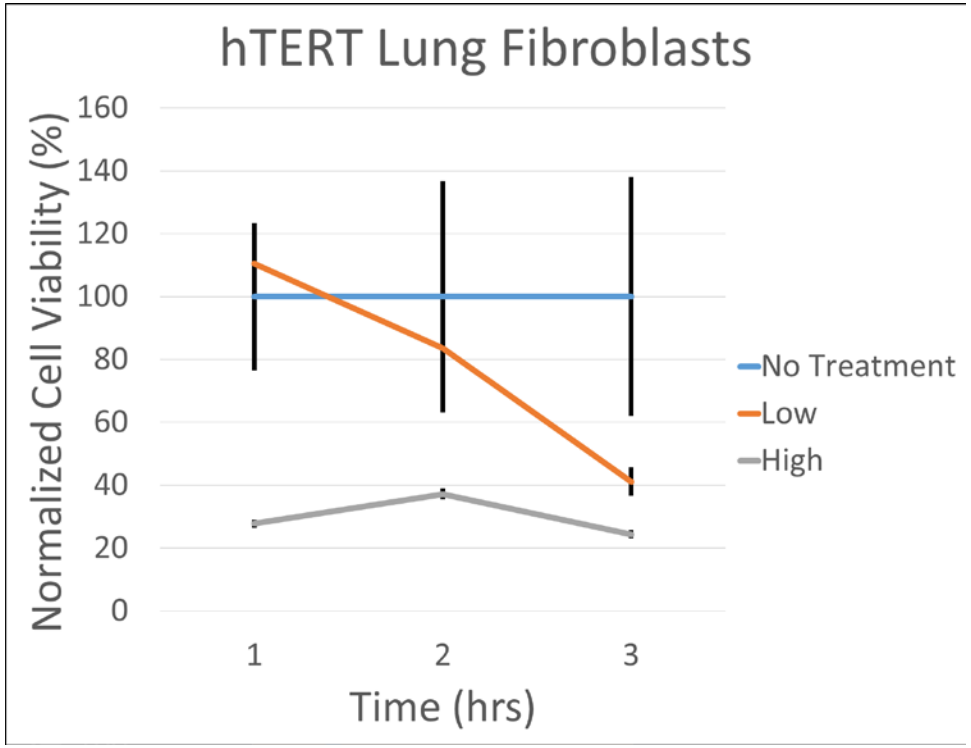
Lung Fibroblast untreated or treated with 1ng/mL TGF-β1 for one hour.

hTERT lung fibroblasts respond to chlorhexidine

Cellular cytotoxicity of lung fibroblasts by chlorhexidine is dose-dependent





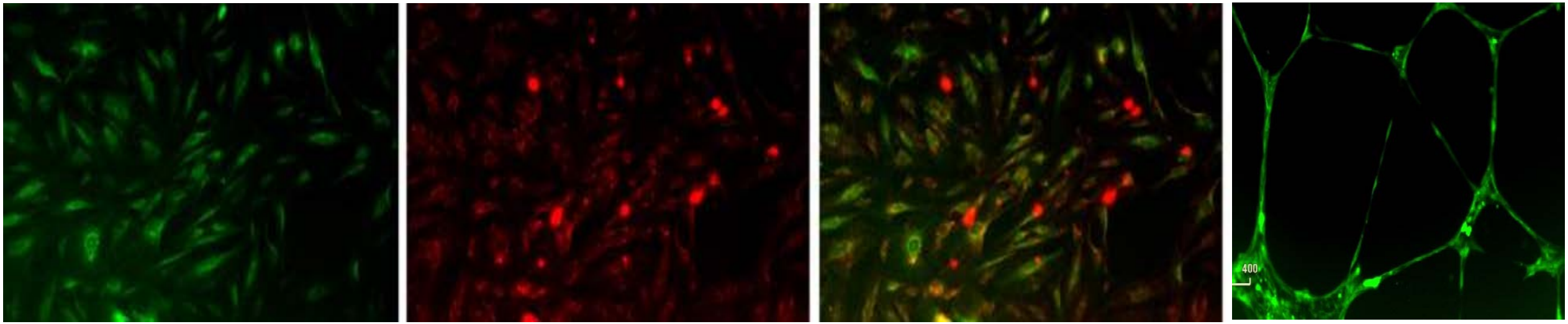


Angiogenesis – Endothelial cells

- TIME (Microvascular endothelial cells; ATCC® CRL-4025™)
- TIME-GFP (GFP-expressing microvascular endothelial cells; ATCC® CRL-4045™)
- HUVEC/TERT2 (ATCC® CRL-4053™)
- TeloHAEC-GFP (Aortic endothelial cells; ATCC® CRL-4054™)

TIME-GFP

HUVEC/TERT2



GFP | AC-LDL | Merge of GFP and Ac-LDL | GFP

Angio-*Ready*™ – An advanced angiogenesis system

- Angio-***Ready***™ Angiogenesis Assay System (ATCC® ACS-2001-2™/ ACS-2001-10™)
  - Two pre-mixed, assay-ready immortalized cell lines
  - Physiologically relevant microenvironment:
    - Mesenchymal stem cells to provide stroma
    - GFP-labeled aortic endothelial cells
  - Responsive to positive and negative angiogenic stimuli
  - Compatible with HTP screening in 96, 384, and 1536 well plates

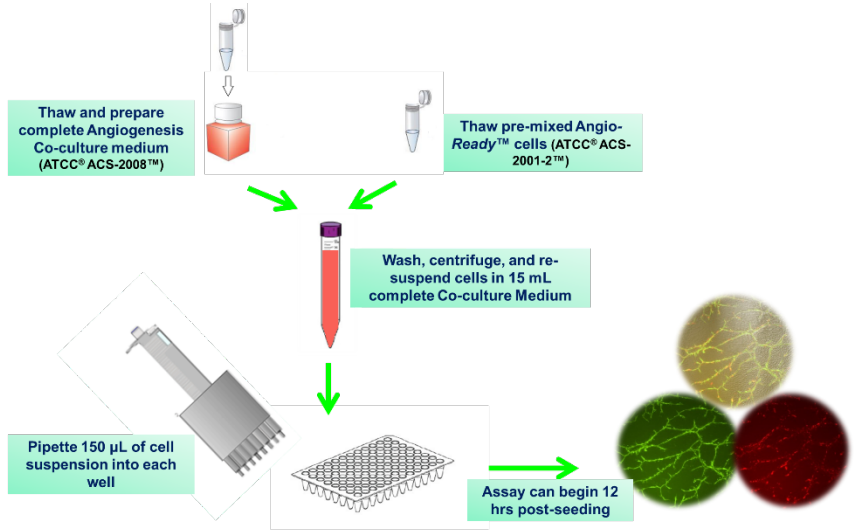
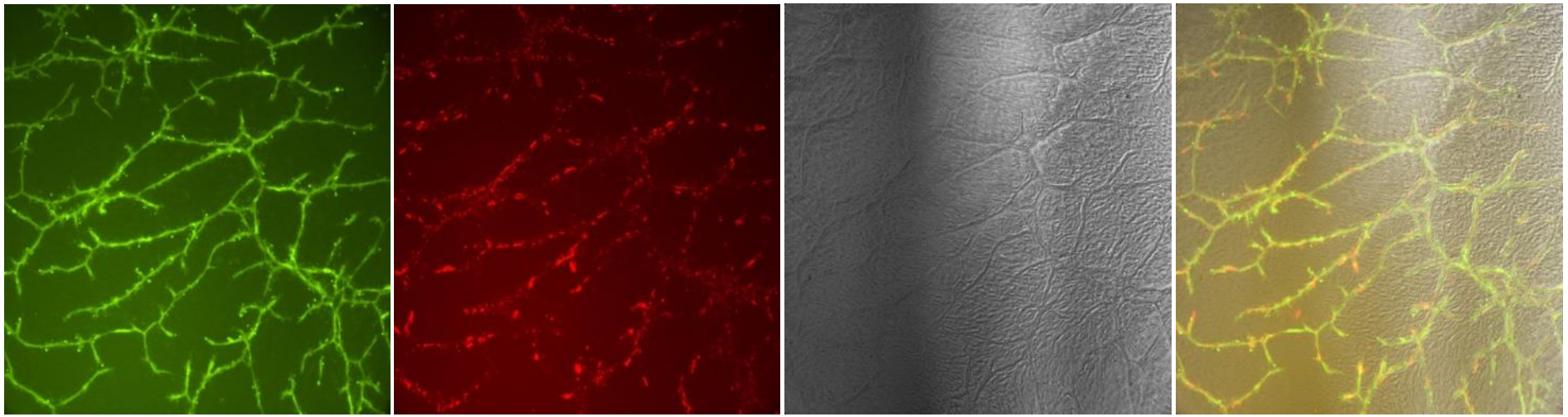


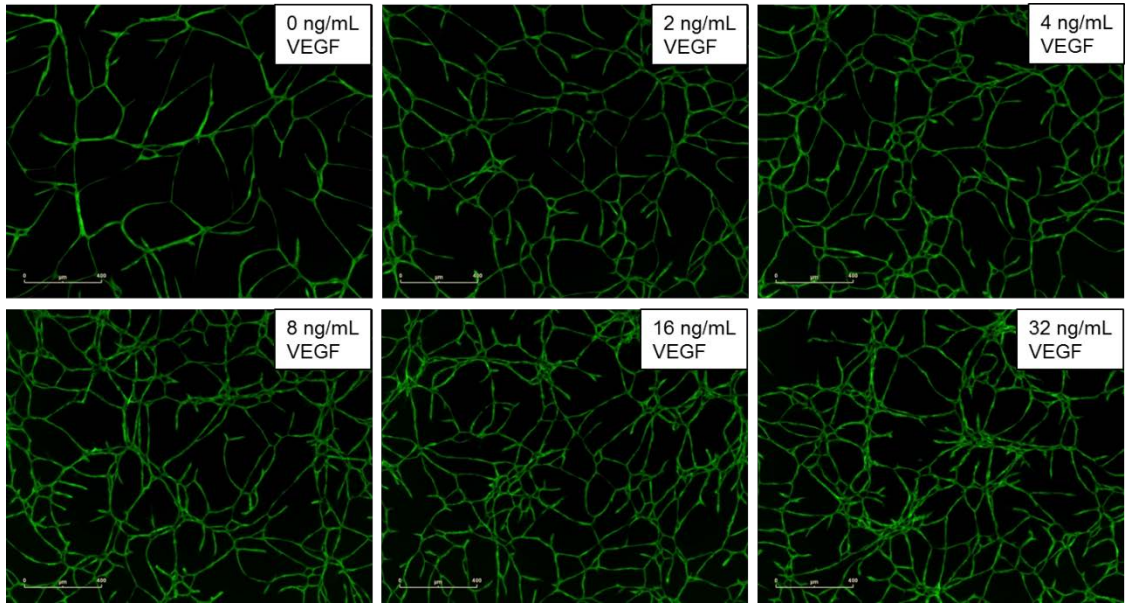
Figure 1. Angio-*Ready*™(ATCC® ACS-2001-2™) Assay overview: "thaw, seed, and assay".

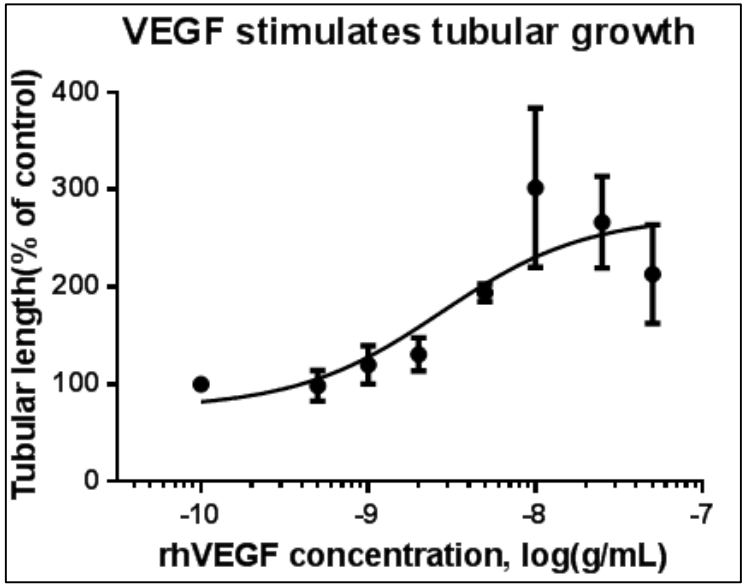


GFP | anti-αSMA | Phase contrast | Merged

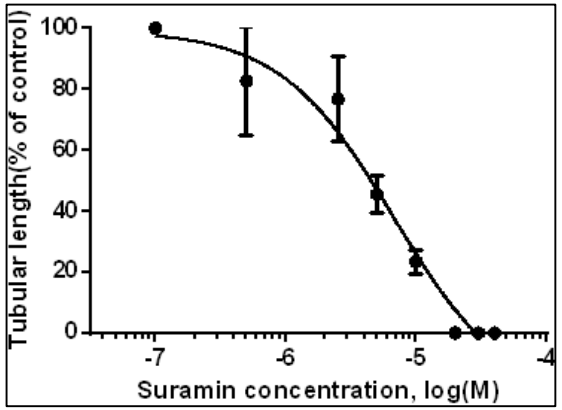
Angio-*Ready*™ – Response to stimuli

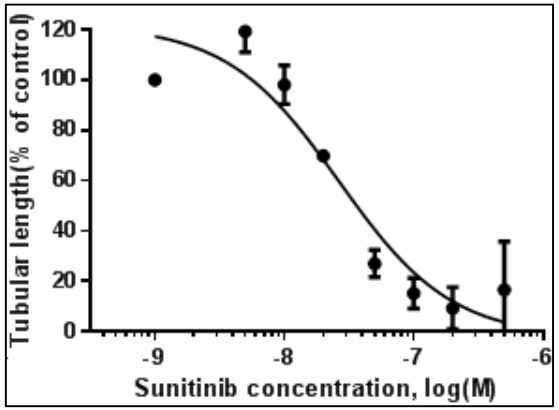
Positive response to VEGF stimulation

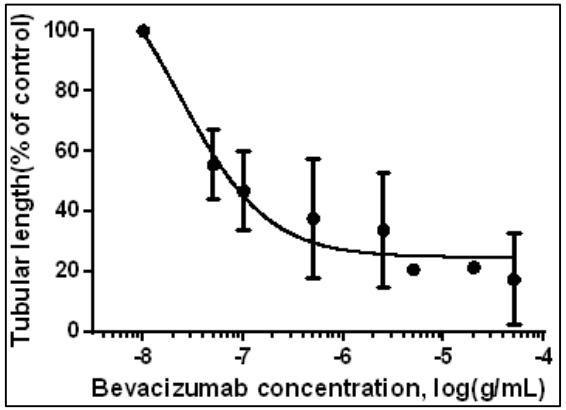




Response to angiogenesis inhibitors

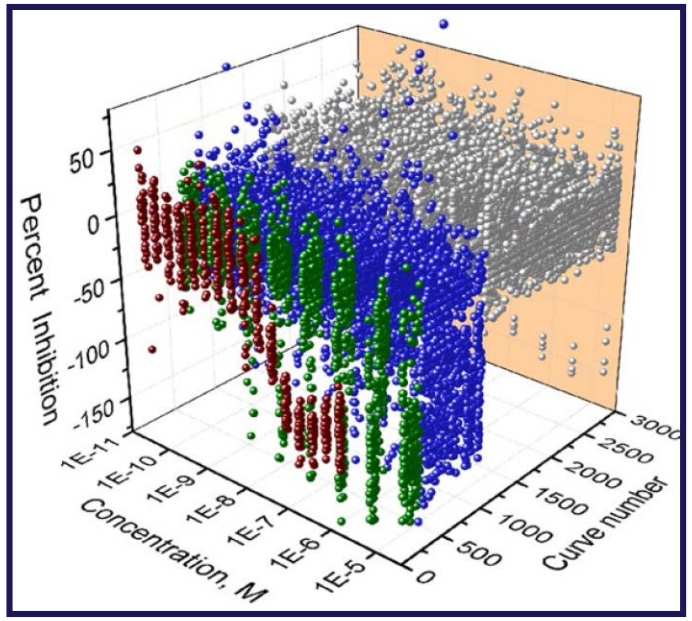


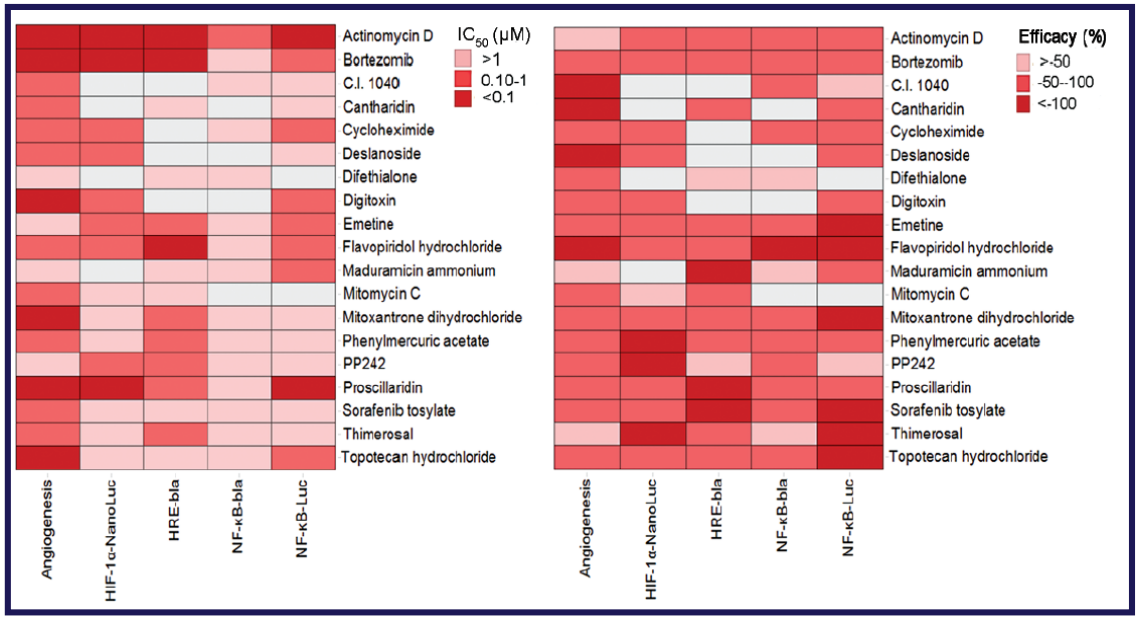




Angio-*Ready*™ – High-throughput screening

- Li et al. Identification of Angiogenesis Inhibitors Using a Co-culture Cell Model in a High-Content and High-Throughput Screening Platform. *SLAS Technology* 23(3): 217-225, 2018.
  - The 2019 SLAS Technology Ten
- Screening of 2,816 drugs on 1,536-well format





Data kindly supplied by:
Menghang Xia, Ph.D., National Institutes of Health, National Center for Advancing Translational Sciences, Bethesda, MD

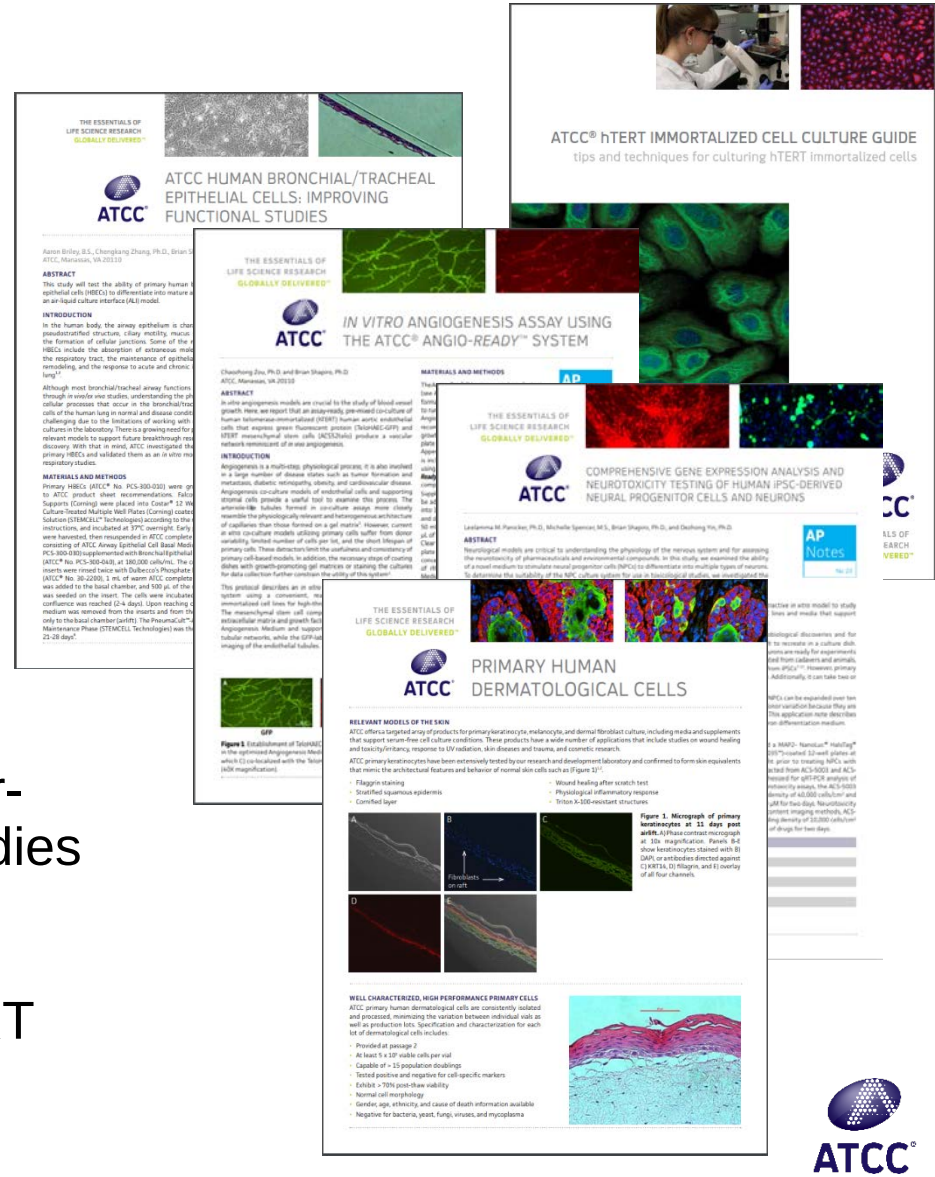
Summary and resources

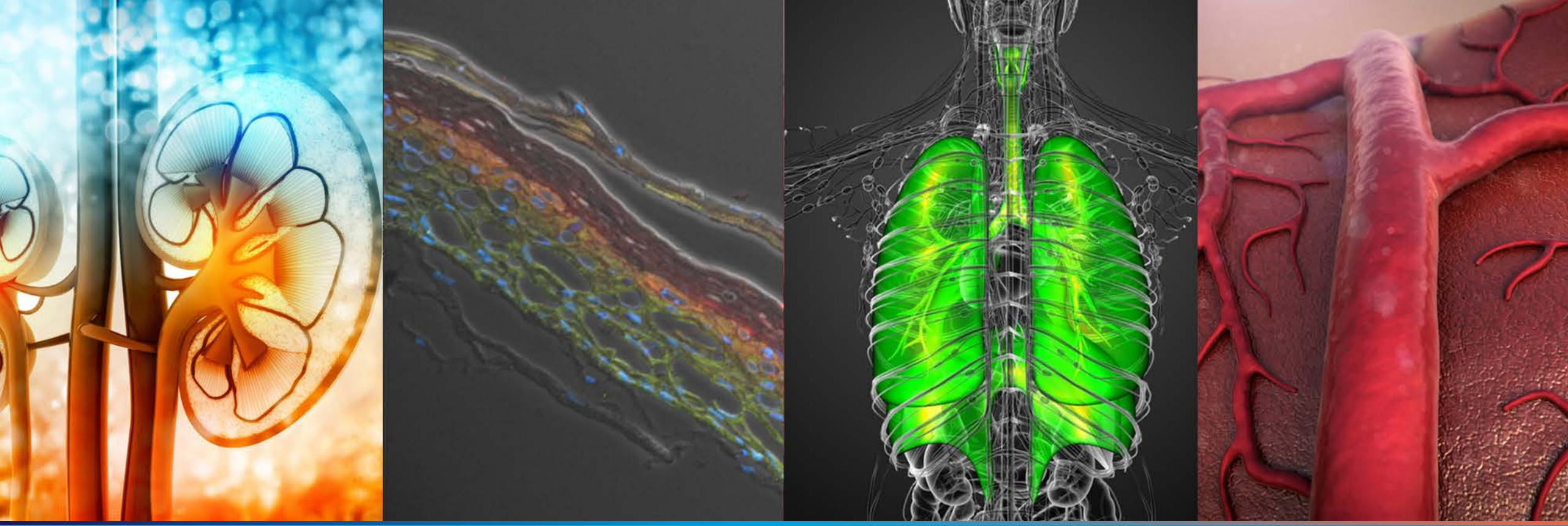
- ATCC offers a variety of cell models for toxicology research:
  - Continuous cell lines
  - Human primary cells
  - hTERT-immortalized primary cells

- hTERT immortalized cells offer:
  - Primary cell functionality
  - Continuous cell line longevity

- hTERT cells alone or in combination with other cells are a user-friendly solution for building reliable cell models for toxicity studies

- Multiple hTERT resources are available at www.atcc.org/hTERT





Thank you and questions?

Upcoming webinar

CRISPR/Cas9-engineered 3D Tissue Culture Models of Drug-resistant Melanoma

Elizabeth Gillies, Ph.D.

May 30 | 12:00 PM ET