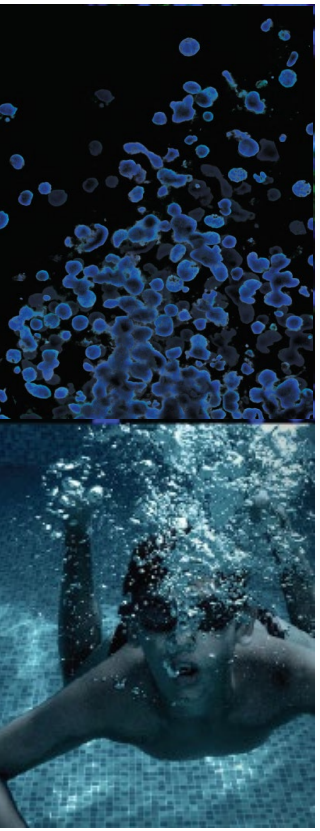
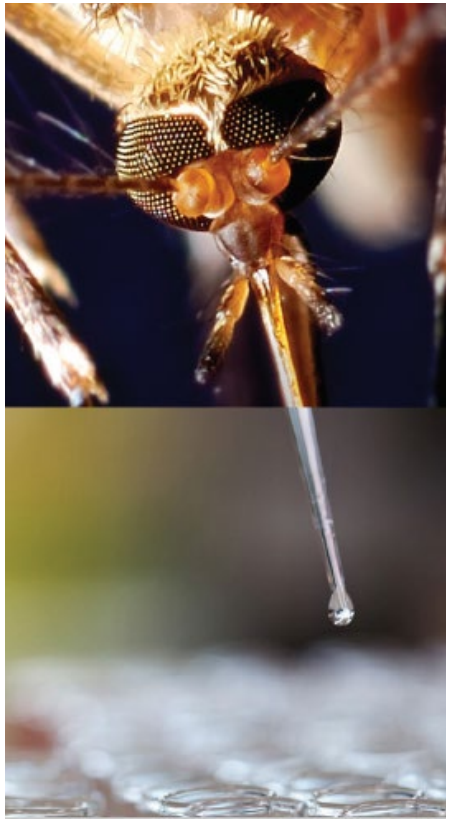
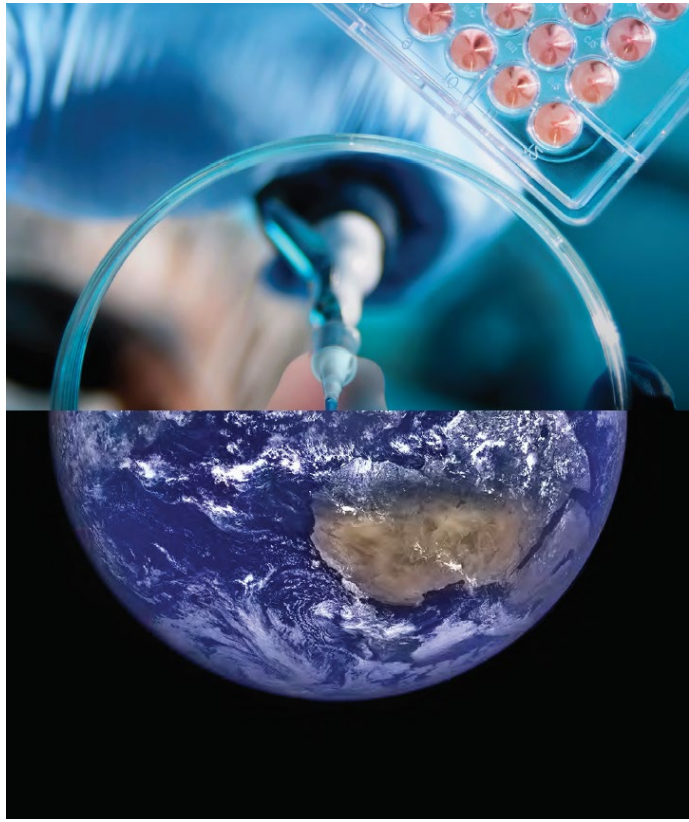
High-throughput, Predictive, and Reproducible Models for Hepatic, Cardiovascular, and Renal Toxicity Studies

Brian Shapiro, PhD
Marketing Segment Manager, ATCC

Sujoy Lahiri, PhD
Lead Scientist, R&D, ATCC







Credible Leads to Incredible™

ATCC®'s mission and future direction

Brian Shapiro, PhD
Market Segment Manager, Toxicology Segment, ATCC

Brian A Shapiro, PhD, works to drive revenue growth for ATCC's Cell Biology products through the development of relevant offerings, marketing strategies and execution of associated marketing plans. Previously, he worked at Virginia Commonwealth University, where he investigated the role of pre-mRNA splicing in the multi-drug resistance of lung cancer. Dr. Shapiro attended the Medical College of Georgia, where his research focused on adrenal physiology as well as diseases of the epidermis.

Agenda

1. ATCC’s mission and future direction
2. The ATCC immortalized primary cell portfolio
3. Case studies
   - Kidney models
   - Cardiovascular models
4. HepatoXcell™ – ATCC’s new offering of primary human hepatocytes

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, 20 international distributors
- Talented team of 600+ employees, over one-third with advanced degrees

bsi. ISO 9001 Quality Management

bsi. ISO 13485 Medical Devices Quality Management

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

ANAB ACCREDITED ISO 17034 REFERENCE MATERIAL PRODUCER

ATCC®

Modernization of the ATCC® portfolio

Past

The ATCC® cell and micro collections were historically deposited by academic and other research scientists

Present

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

Future

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

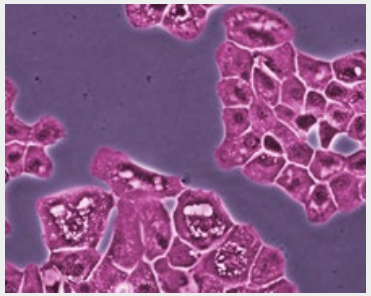
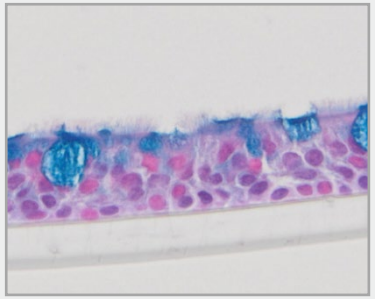
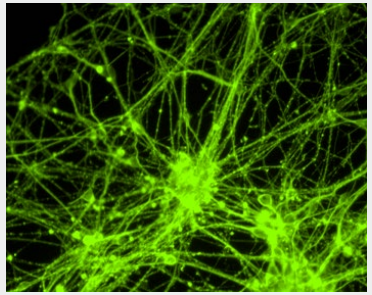
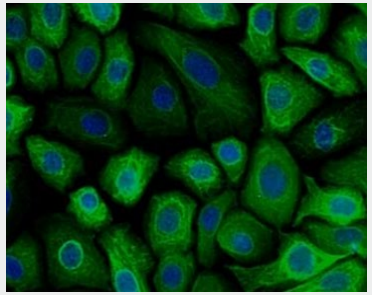
Cardiovascular, renal, and hepatic models

Sujoy Lahiri, PhD
Lead Scientist, ATCC

Sujoy Lahiri, PhD, is an R&D scientist in ATCC. He leads the primary cell division, working on advanced cellular models using primary cells as well as expansion of ATCC's immortalized primary cell portfolio. Dr. Lahiri has extensive knowledge in the field of toxicology and drug metabolism. Previously, Dr. Lahiri worked at National Institutes of Health, where his work focused on lipid biochemistry. Dr. Lahiri received his PhD from the Weizmann Institute of Science, where he studied sphingolipid biochemistry and metabolism.

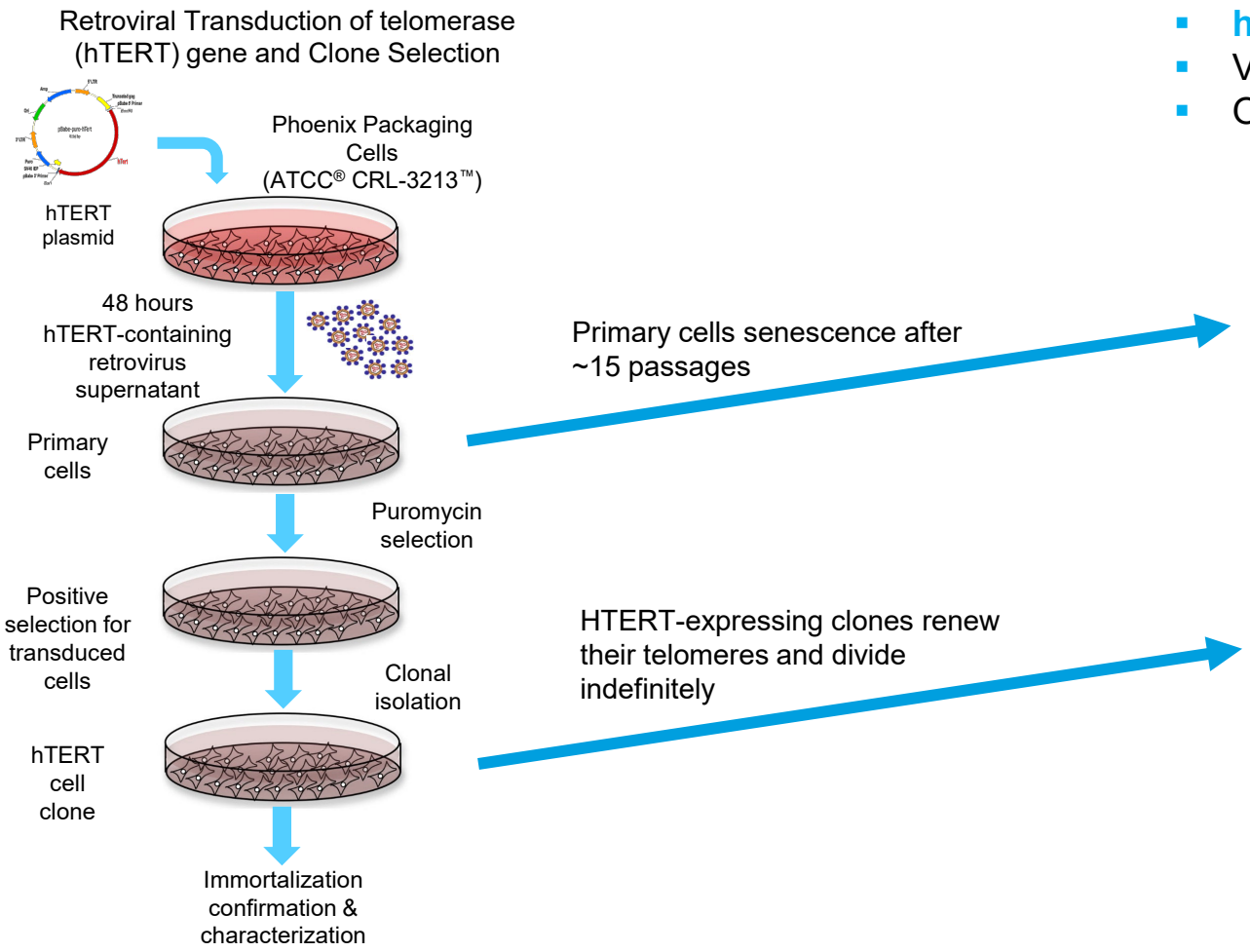
Sources of cell models

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

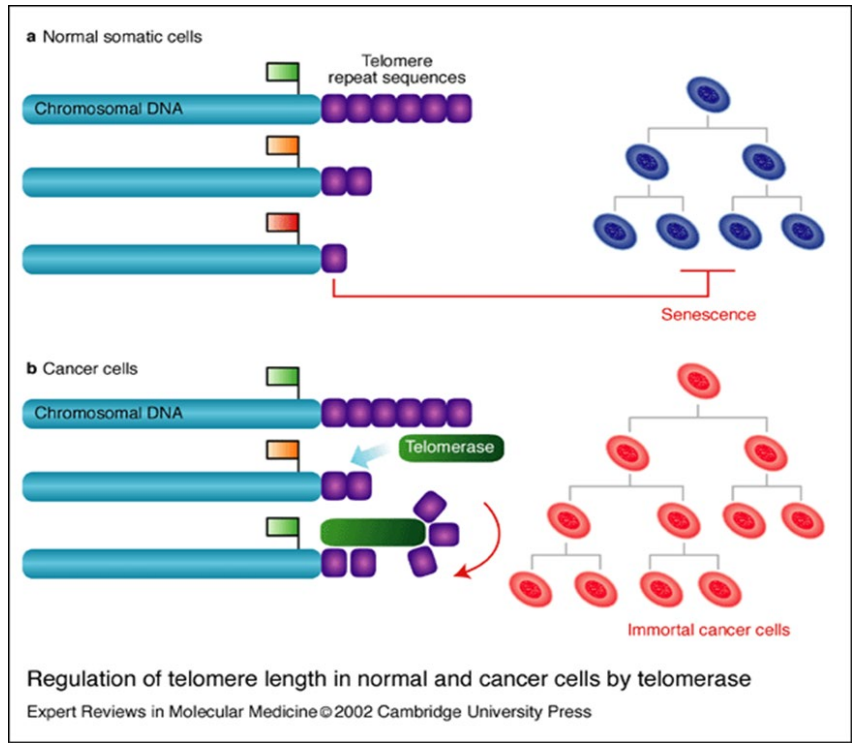
Immortalization – Cell cycle regulation

- 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



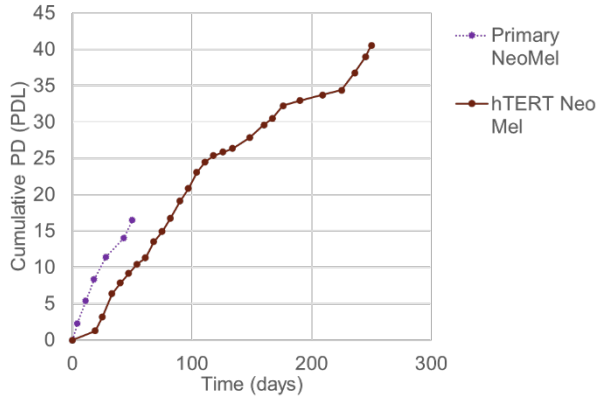
Keith W, et al. Expert Rev Mol Med 22;4(10):1-25, 2002.

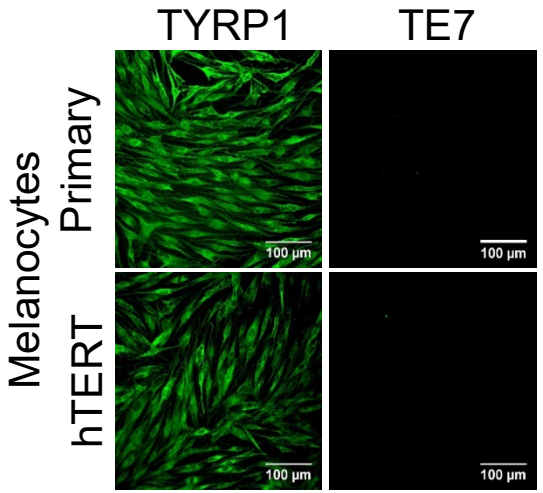
Immortalized primary cells – Key characteristics

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

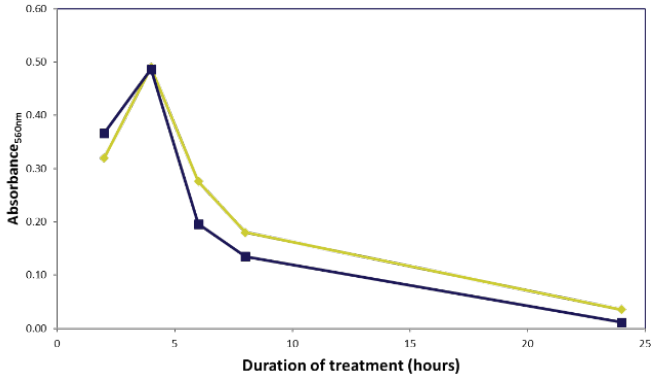
- **Growth**
  - Cells retain replicative capacity (“immortalized”)
  - Population doubling rate is comparable to primary cells
- **Characterization**
  - Morphology and marker expression similar to primary cells
  - Sterility testing
  - STR profile
- **Functional responses**
  - Within expected range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

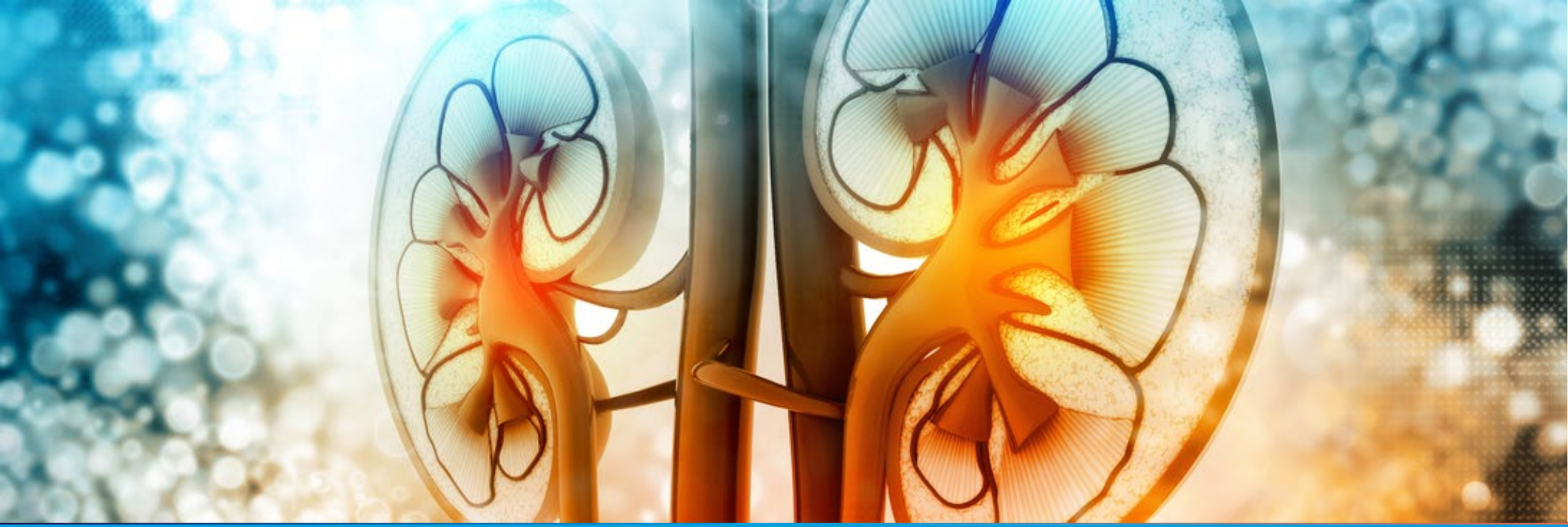
Growth of ATCC® CRL-4064™ Neonatal Dermal Melanocytes





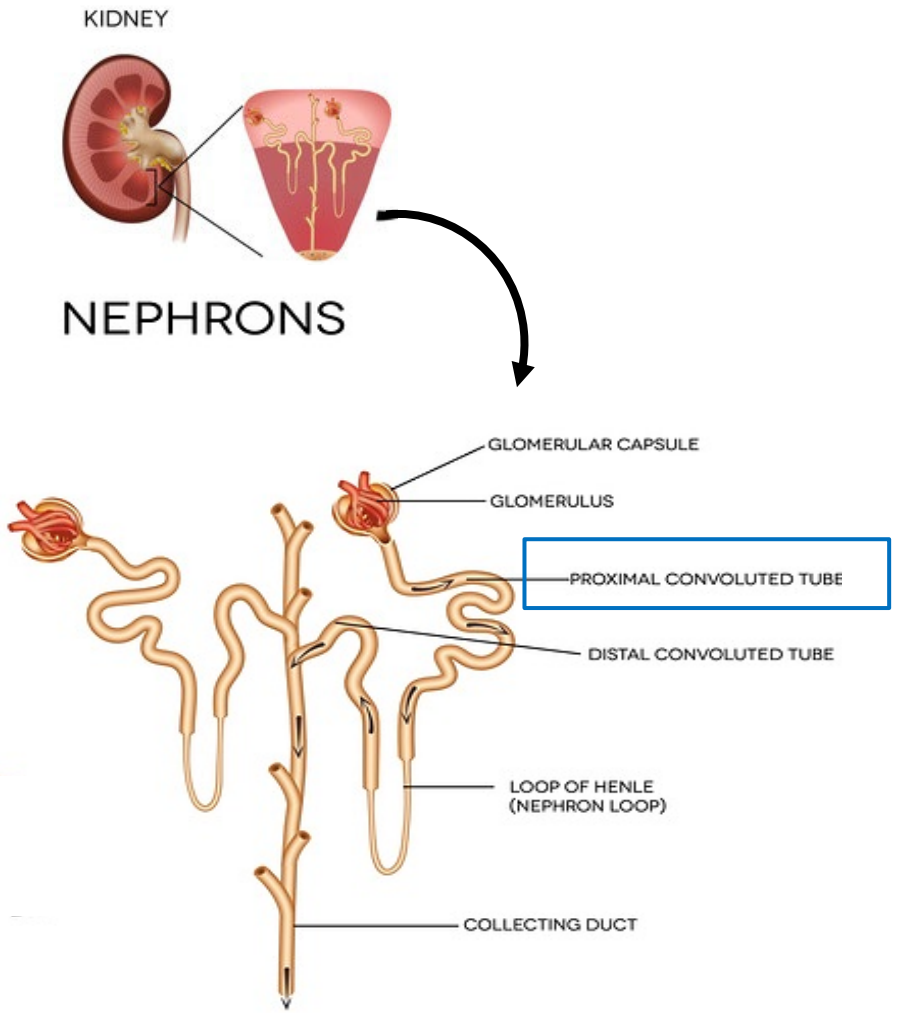
Metabolic reduction by 3-D organotypic skin culture in Triton-X





Kidney models and functionality

Kidney drug toxicity and transporters



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

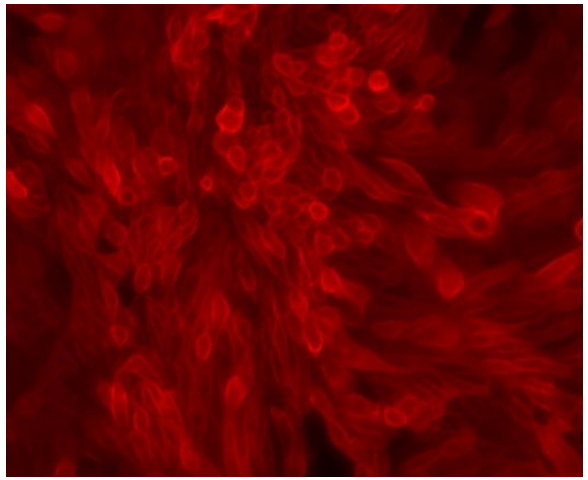
- **The kidney is one of the major target organs for drug-induced toxicity**
  - Large functional reserve of the kidney
  - Nephrotoxic effects become obvious only after regulatory approval
- **Nephrotoxic potential**
  - Often underestimated when new drugs are available
  - Leads to clinical complications 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

Kidney models

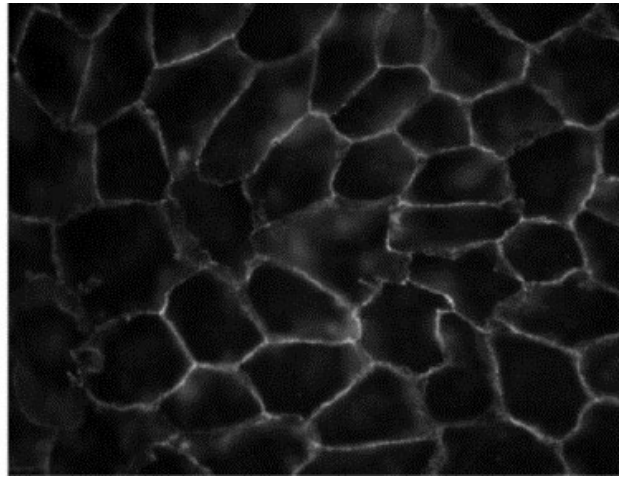
Renal proximal tubule epithelial cells (RPTEC)

- 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)

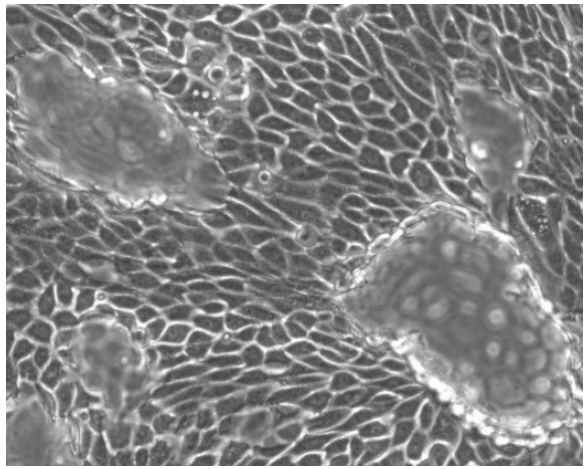
RPTEC/TERT1: CD13

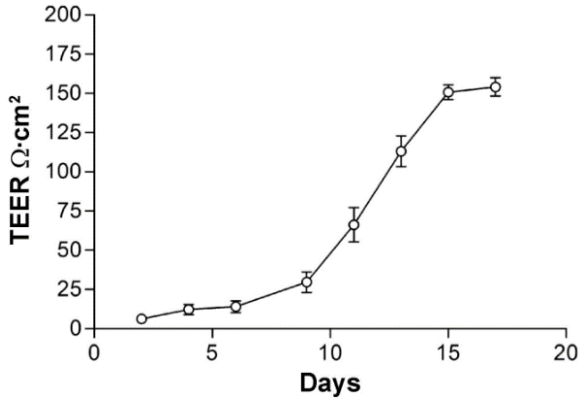


RPTEC/TERT1: E-cadherin

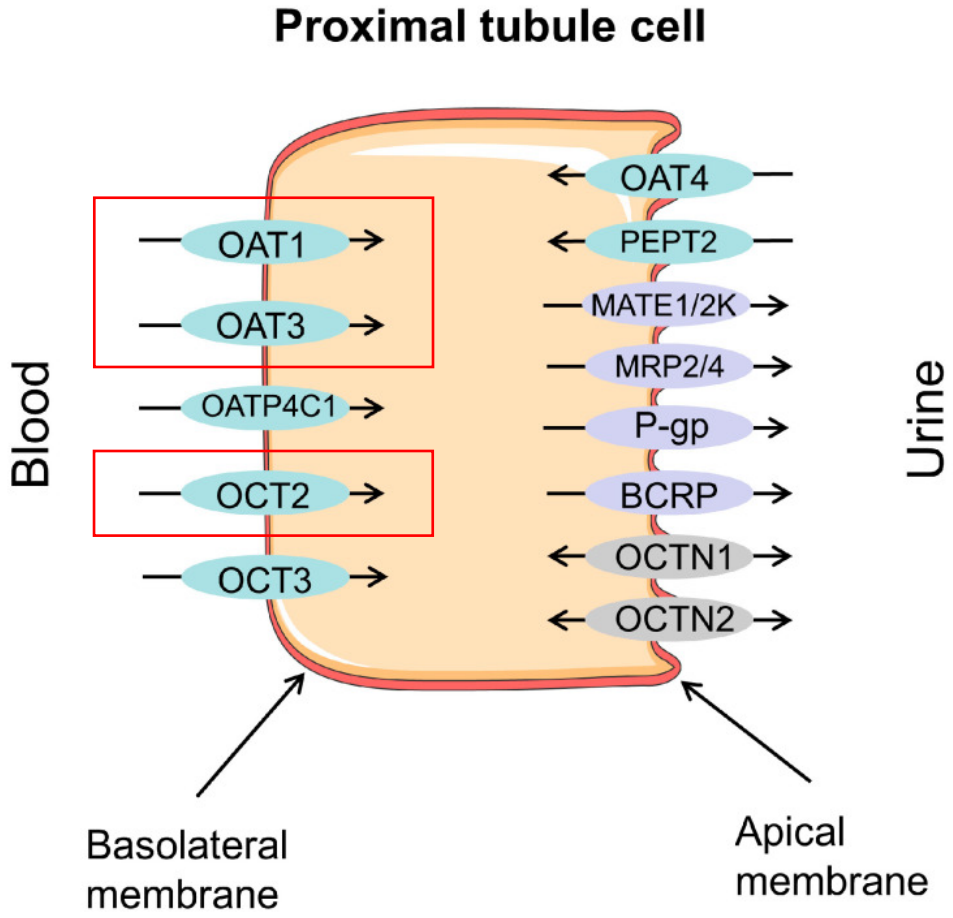


Dome formation

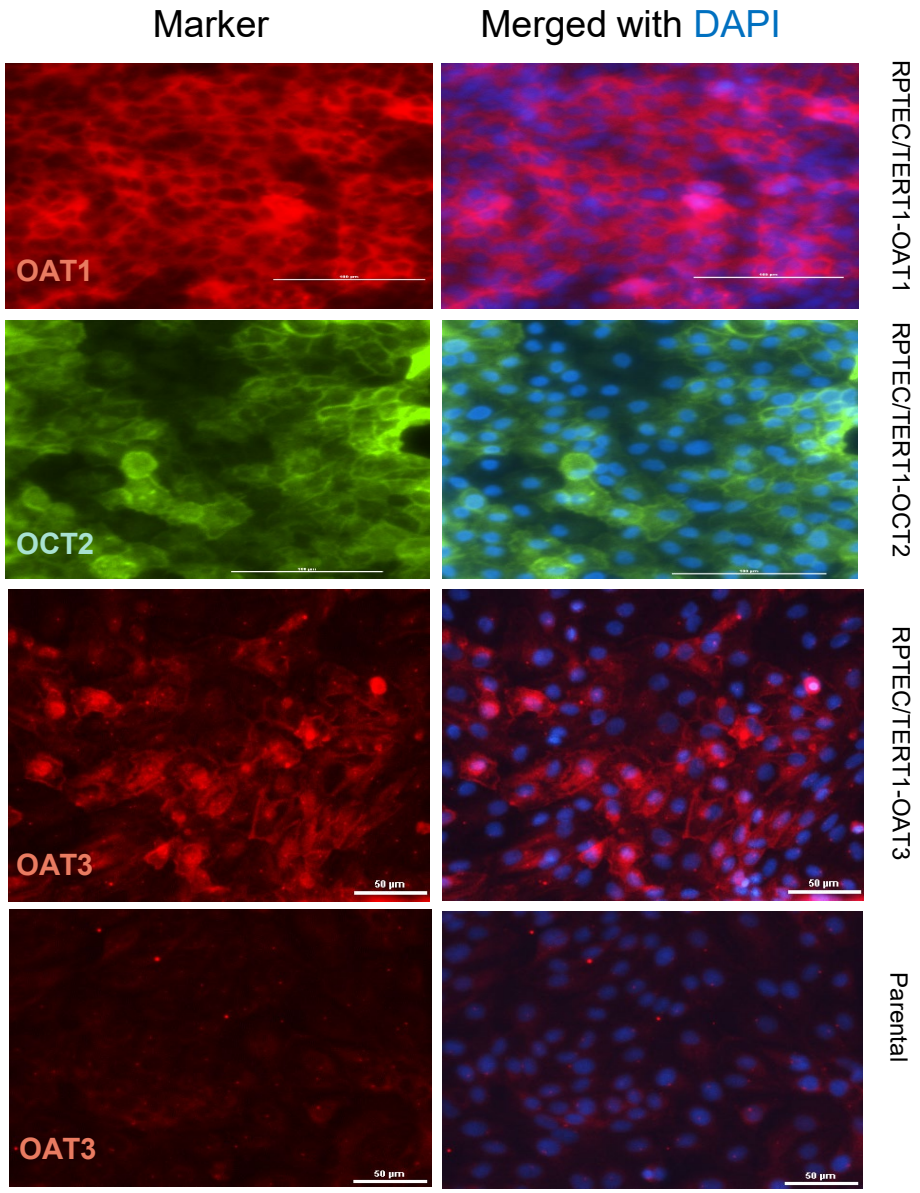




Kidney cells – Modeling solute transporters

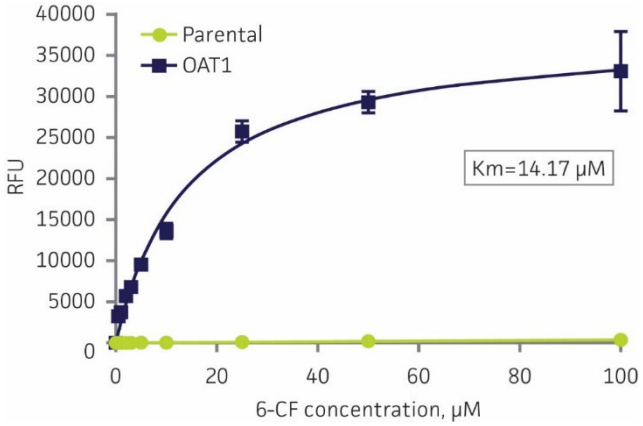


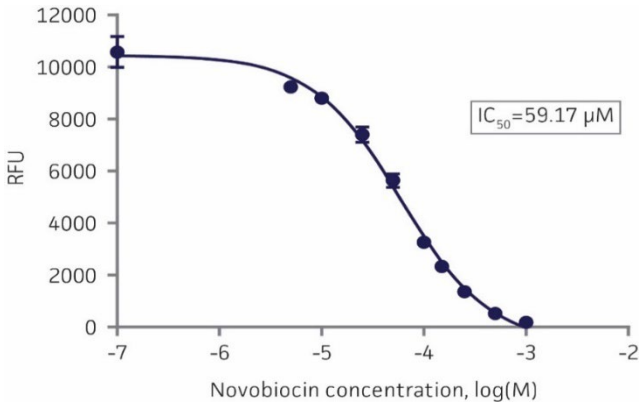
Lin K, et al. Molecules 28(13): 5252, 2023.



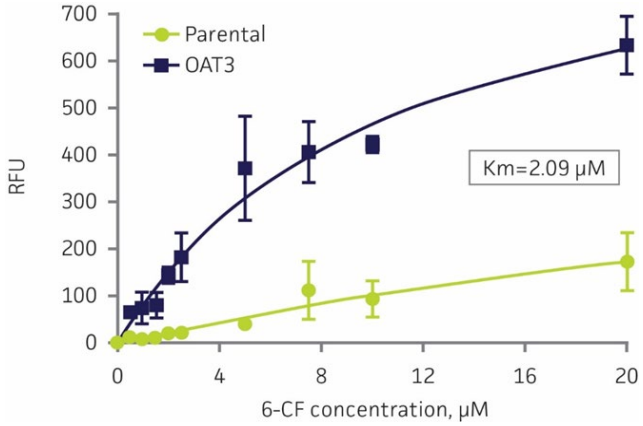
Functionality – Drug uptake & inhibition assay

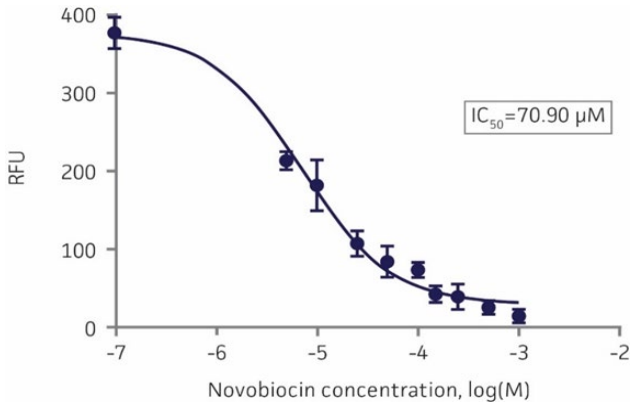
6-CF uptake inhibition in HTERT/RPTEC OAT-1



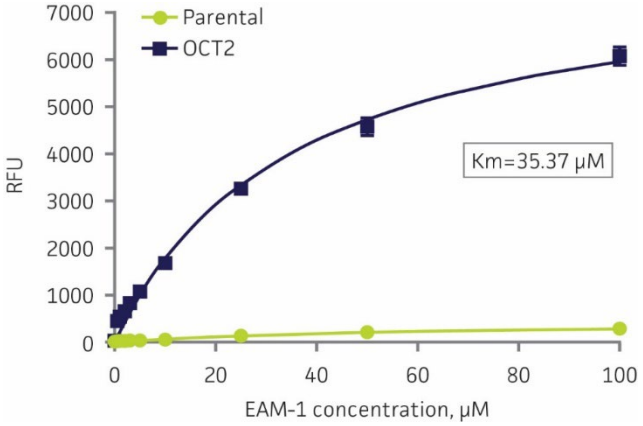


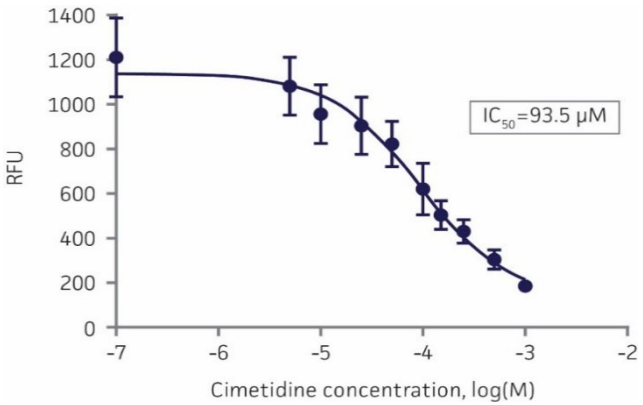
6-CF uptake inhibition in HTERT/RPTEC OAT-3





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



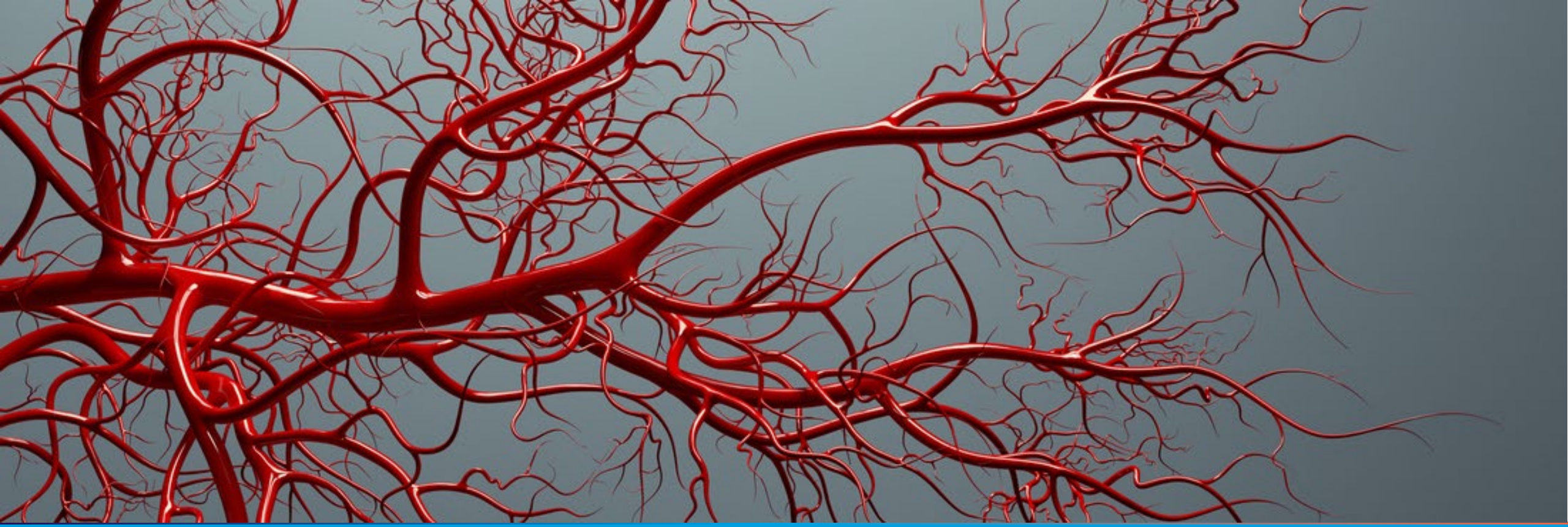


ASSAY PROTOCOL

- 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

Summary of kidney models

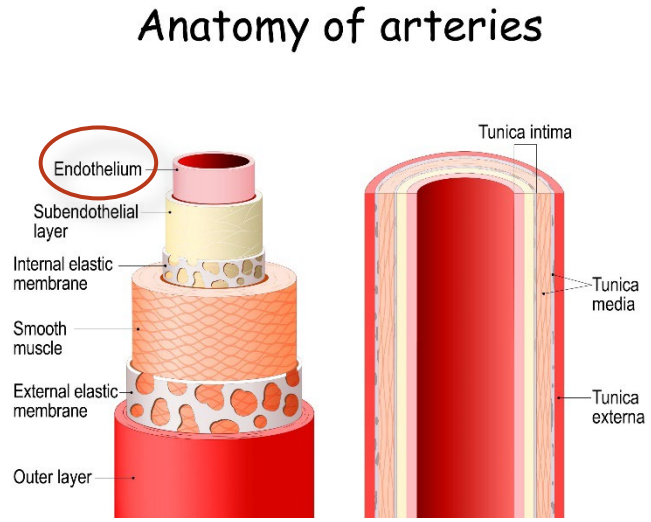
- ATCC® primary and hTERT-immortalized RPTECs display many key in vivo characteristics
- We enhanced hTERT-immortalized RPTEC with organic anion/cation transporter proteins
- The hTERT-immortalized RPTEC models have been evaluated for:
  - Uptake of specific fluorescent substrates
  - Selective substrate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

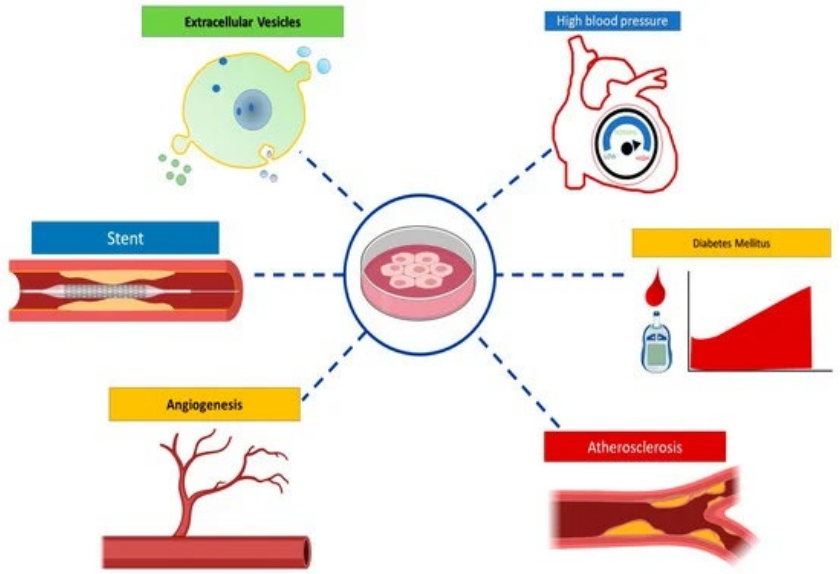


Cardiovascular models and functionality

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





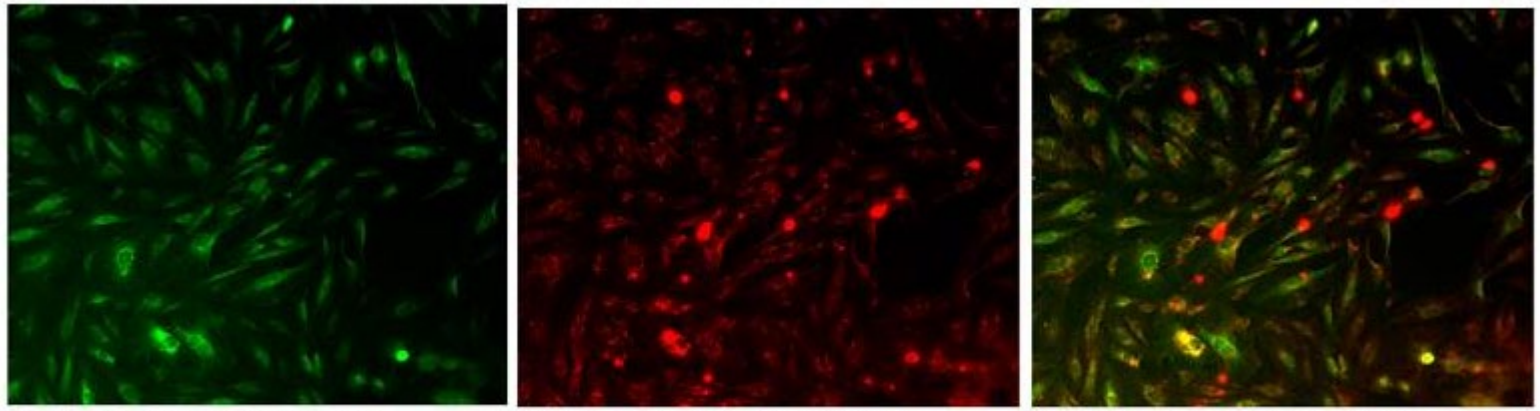
Medina-Leyte DJ, et al. Appl Sci 10(3), 938, 2020.

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)



GFP (ATCC® CRL-4045™) | Ac-LDL (ATCC® CRL-4045™) | Merge of GFP and Ac-LDL (ATCC® CRL-4045™)

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

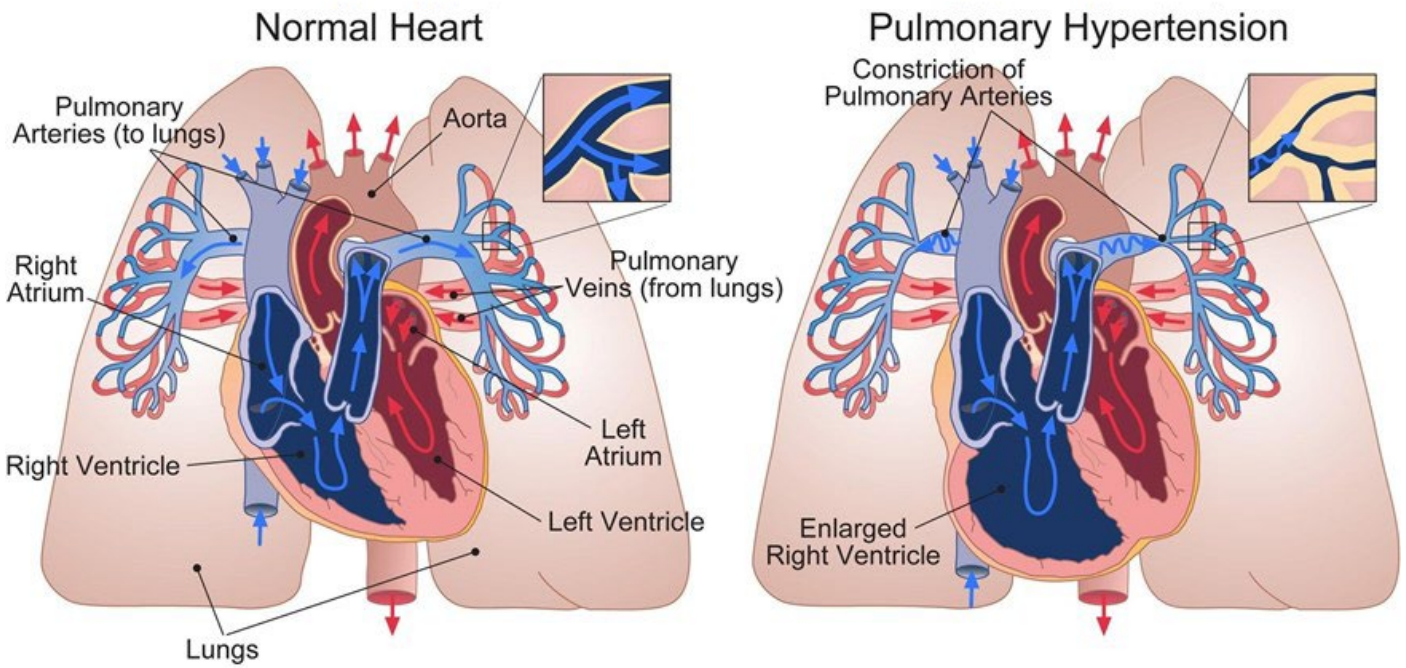


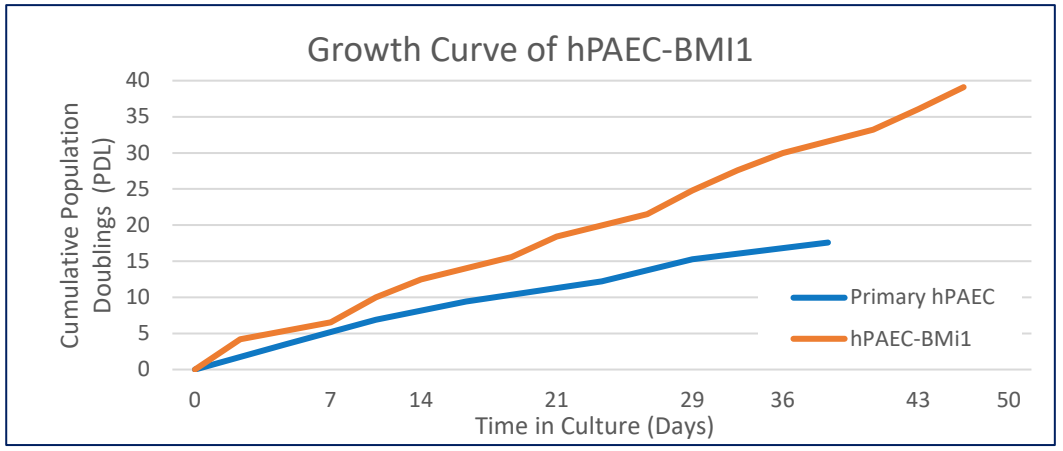
Image courtesy of the Columbus Ohio Adult Congenital Heart Disease Program at Nationwide Children's Hospital Heart Center, Columbus, Ohio

HPAEC-BMI1– Key characteristics

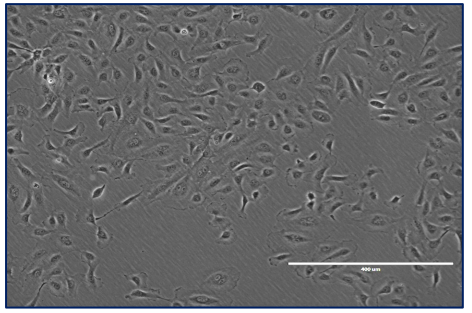
ATCC® offers primary and immortalized cell solutions that are authenticated with our rigorous QC and tested for common biomarker expression and cell performance

Growth & morphology

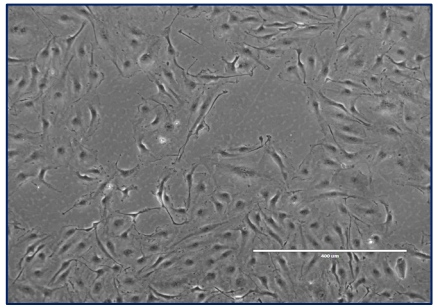
- Cells retain replicative capacity (“immortalized”)
  - Morphology similar to primary cells
- Key biological functions similar to primary cells
  - Formation of capillary-like tubes
  - 82% of cell uptake acLDL



Primary hPAEC

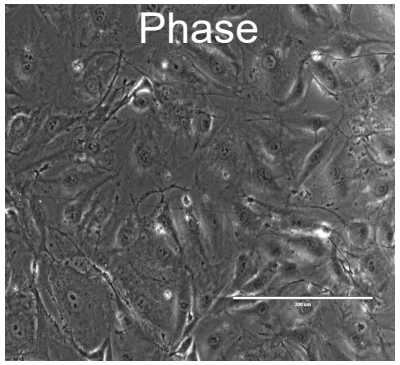


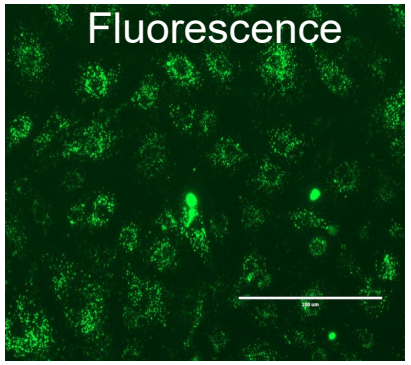
hPAEC BMI1

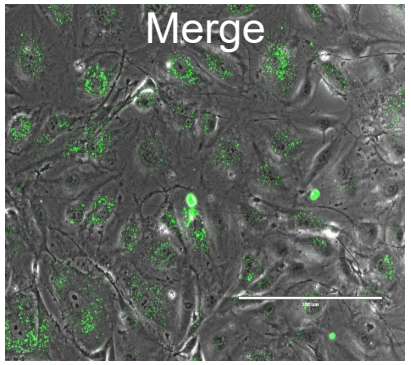


10x magnification

acLDL Uptake



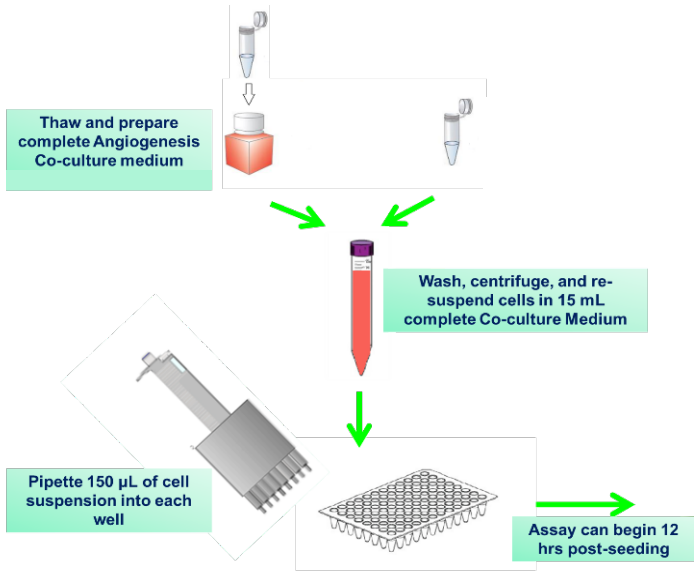




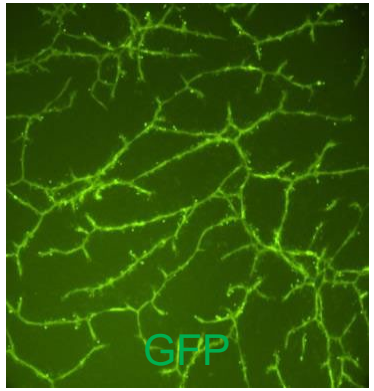
20x magnification

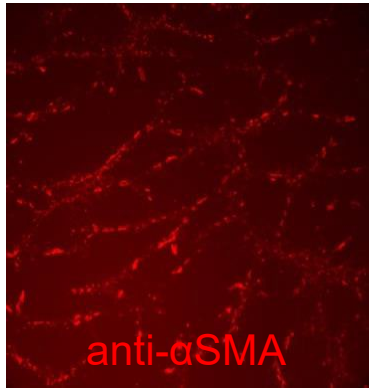
An advanced co-culture system

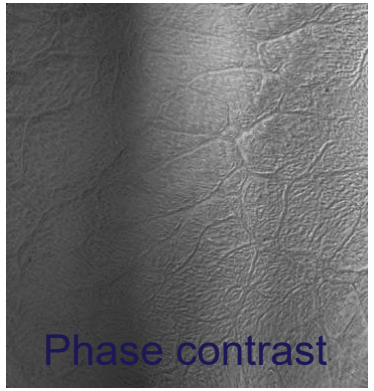
- Co-culture of endothelial cells with fibroblasts allows for:
  - Formation of more heterogeneous tubules
  - More defined culture conditions, no need for ECM coating



- 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

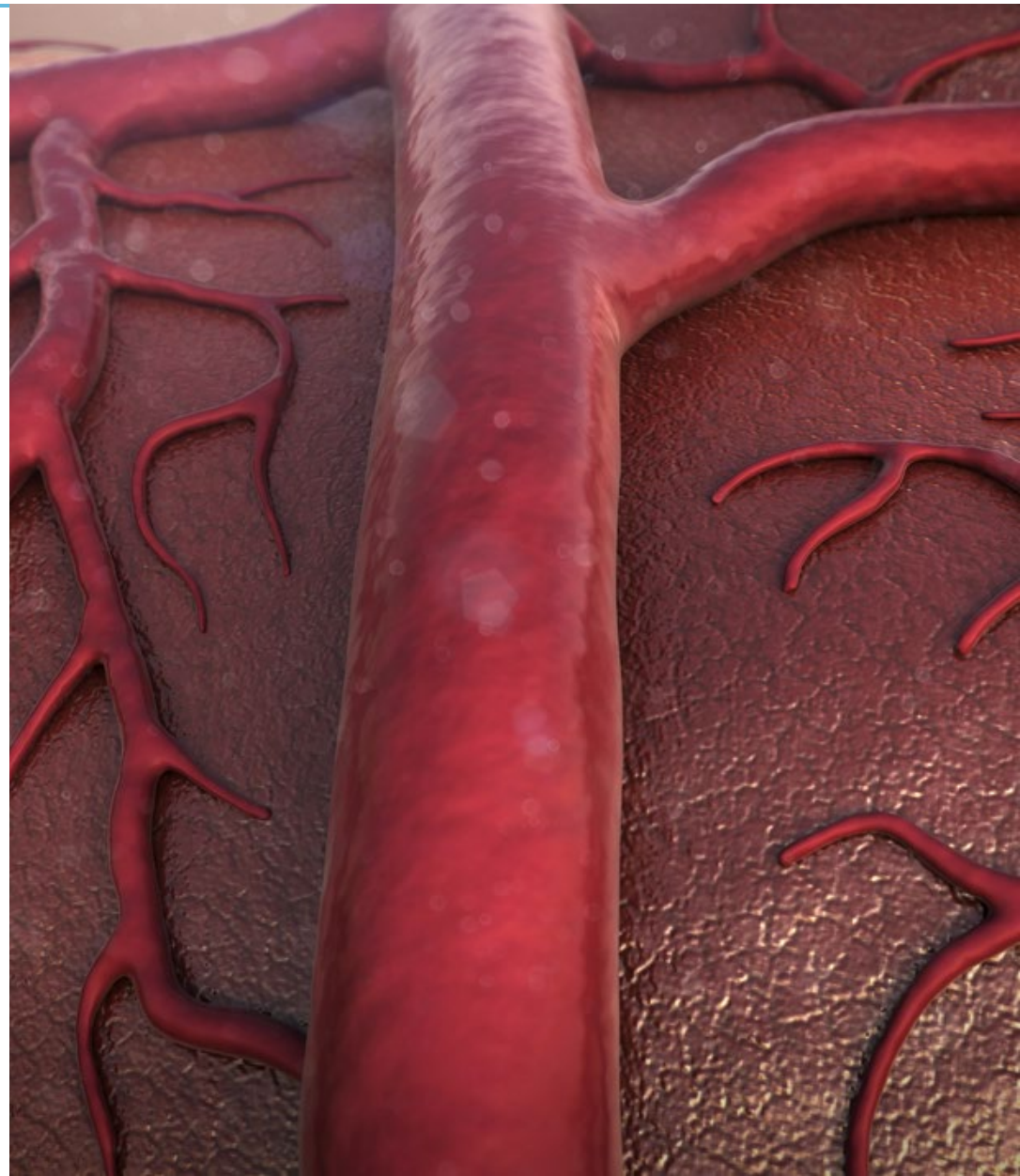






Summary of cardiovascular models

- ATCC® immortalized endothelial cells display in 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
  - Endothelial Cell Tubule Formation Assay
  - Screening of activation and inhibition compounds
  - Vessel formation in wound healing study and regenerative compound screening



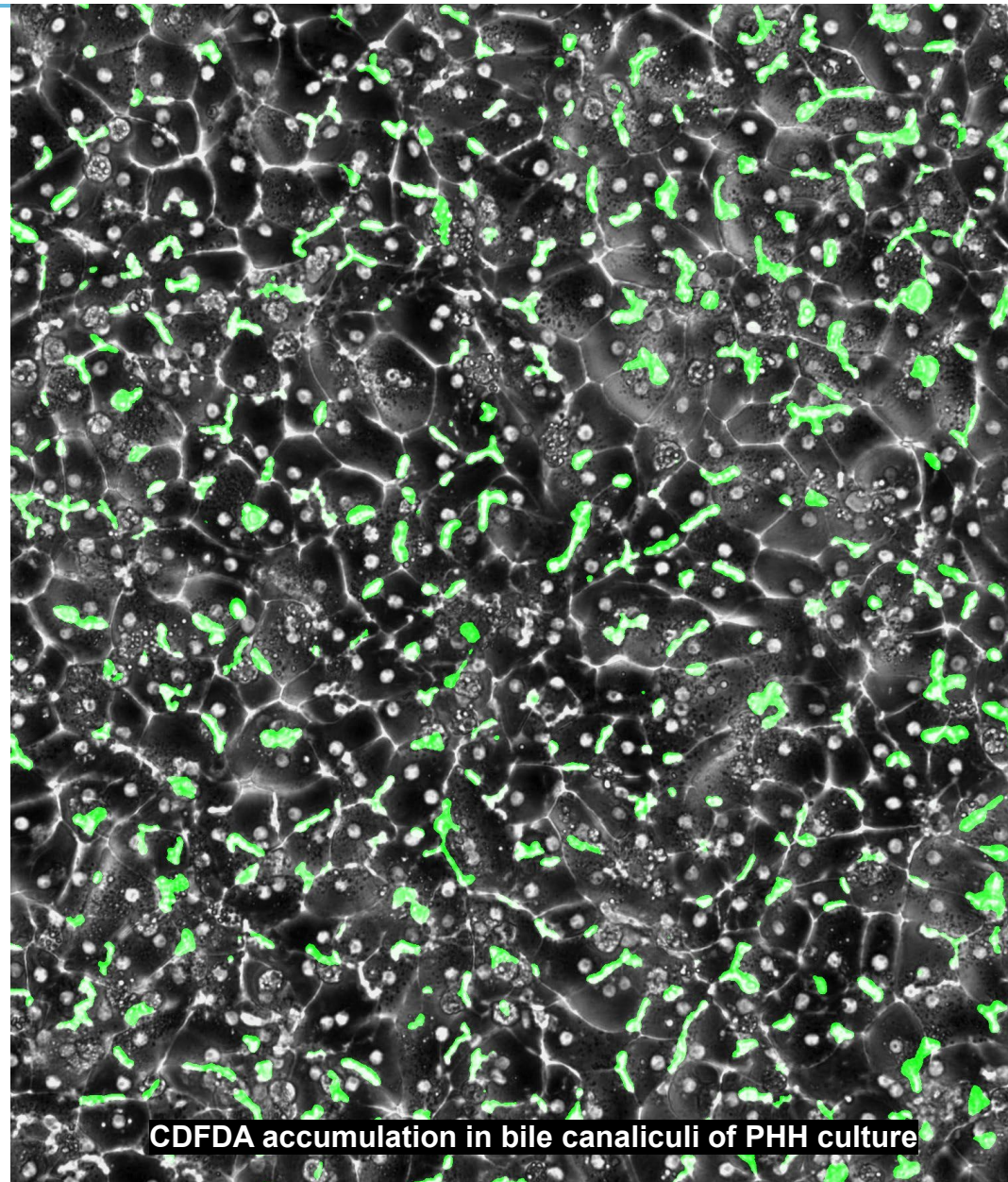
HepatoXcell™: Primary human hepatocytes from ATCC

HepatoXcell by ATCC

ATCC

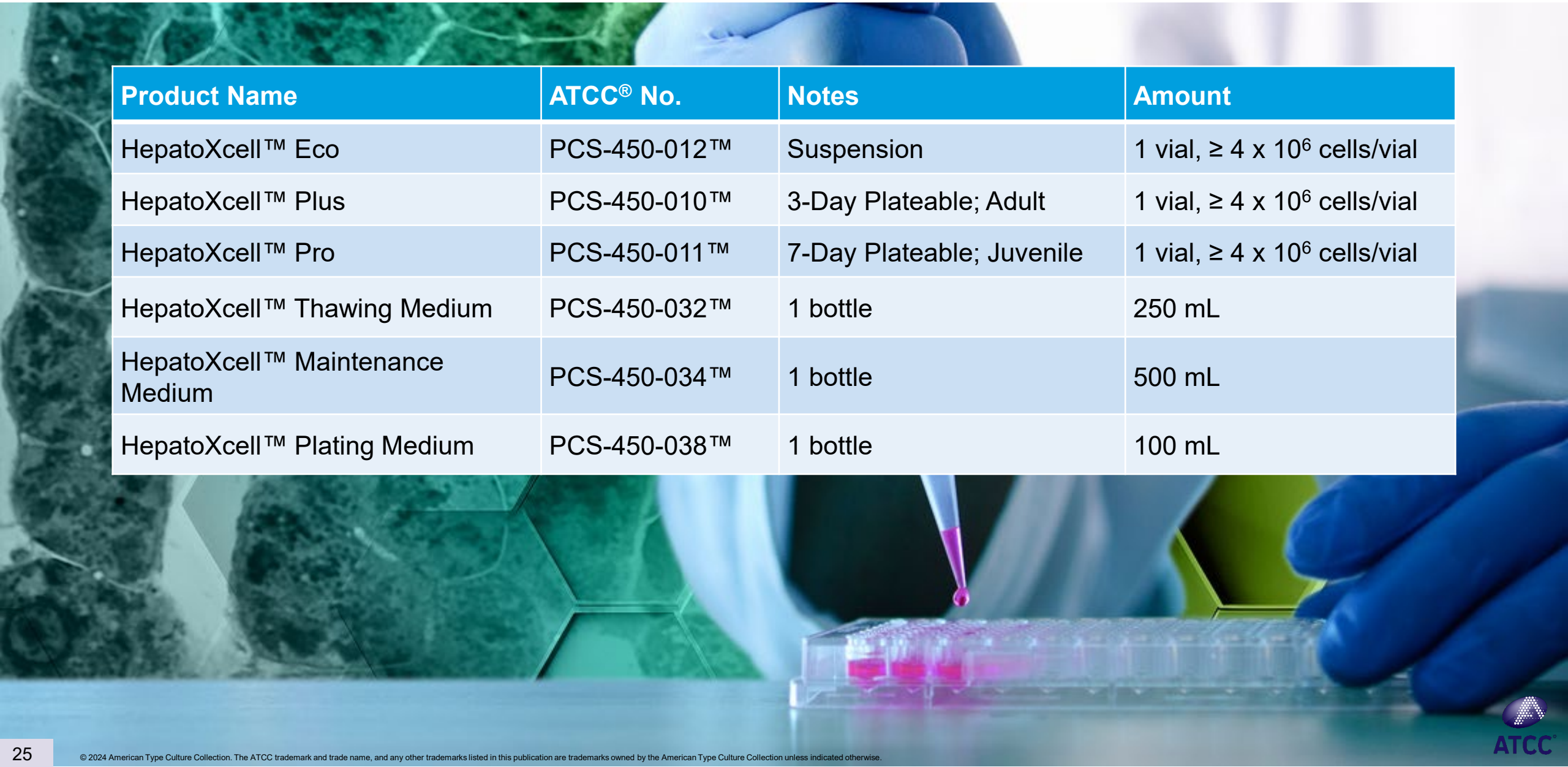
Challenges in ADME-Tox testing

- Limited models for in vitro ADME-Tox testing
  - Over dependence on primary human hepatocytes (PHH)
  - Limited success with immortalized hepatic cell lines or iPSC-derived hepatocytes
- Industry shortage of primary human hepatocytes for toxicological testing
  - Higher number of successful liver transplants
  - Difficult to acquire healthy liver tissue
- Choosing and ordering the right lot can be difficult
  - Access to donor information
  - Number of available vials per lot
- Characterization information of PHH offerings can be lacking
  - Lots not prequalified
  - Genomic data isn’t available
- Prohibitive Cost



CDFDA accumulation in bile canaliculi of PHH culture

HepatoXcell™ hepatocytes and media



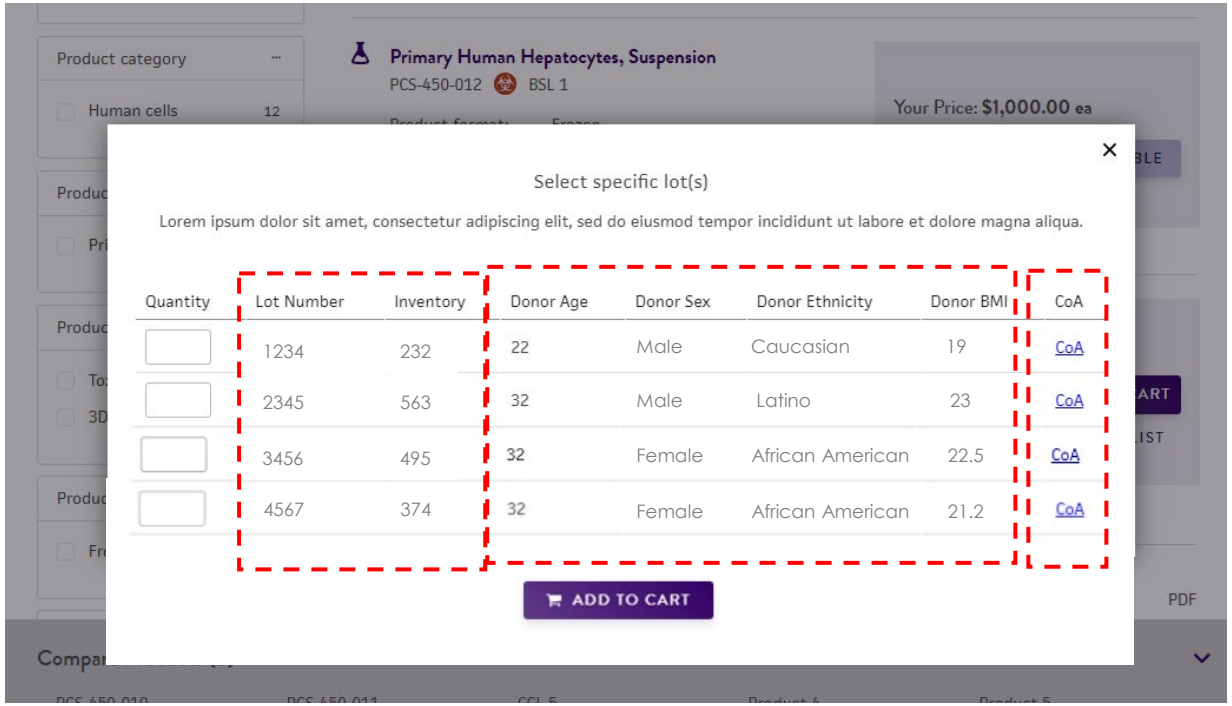
Product Name	ATCC® No.	Notes	Amount
HepatoXcell™ Eco	PCS-450-012™	Suspension	1 vial, ≥ 4 x 10^6 cells/vial
HepatoXcell™ Plus	PCS-450-010™	3-Day Plateable; Adult	1 vial, ≥ 4 x 10^6 cells/vial
HepatoXcell™ Pro	PCS-450-011™	7-Day Plateable; Juvenile	1 vial, ≥ 4 x 10^6 cells/vial
HepatoXcell™ Thawing Medium	PCS-450-032™	1 bottle	250 mL
HepatoXcell™ Maintenance Medium	PCS-450-034™	1 bottle	500 mL
HepatoXcell™ Plating Medium	PCS-450-038™	1 bottle	100 mL

HepatoXcell™

ATCC's solution for your predictive drug development and toxicity testing

- HepatoXcell™ primary human hepatocytes come with ATCC®'s quality assurance
- Lot selection tool provides easy access to donor and lot information
- Lots are prequalified as per plate-ability or suspension viability
- CoA includes viability, metabolism, induction, and uptake data
- **Access to transcriptome and whole-exome data for individual lots**
- Competitive pricing

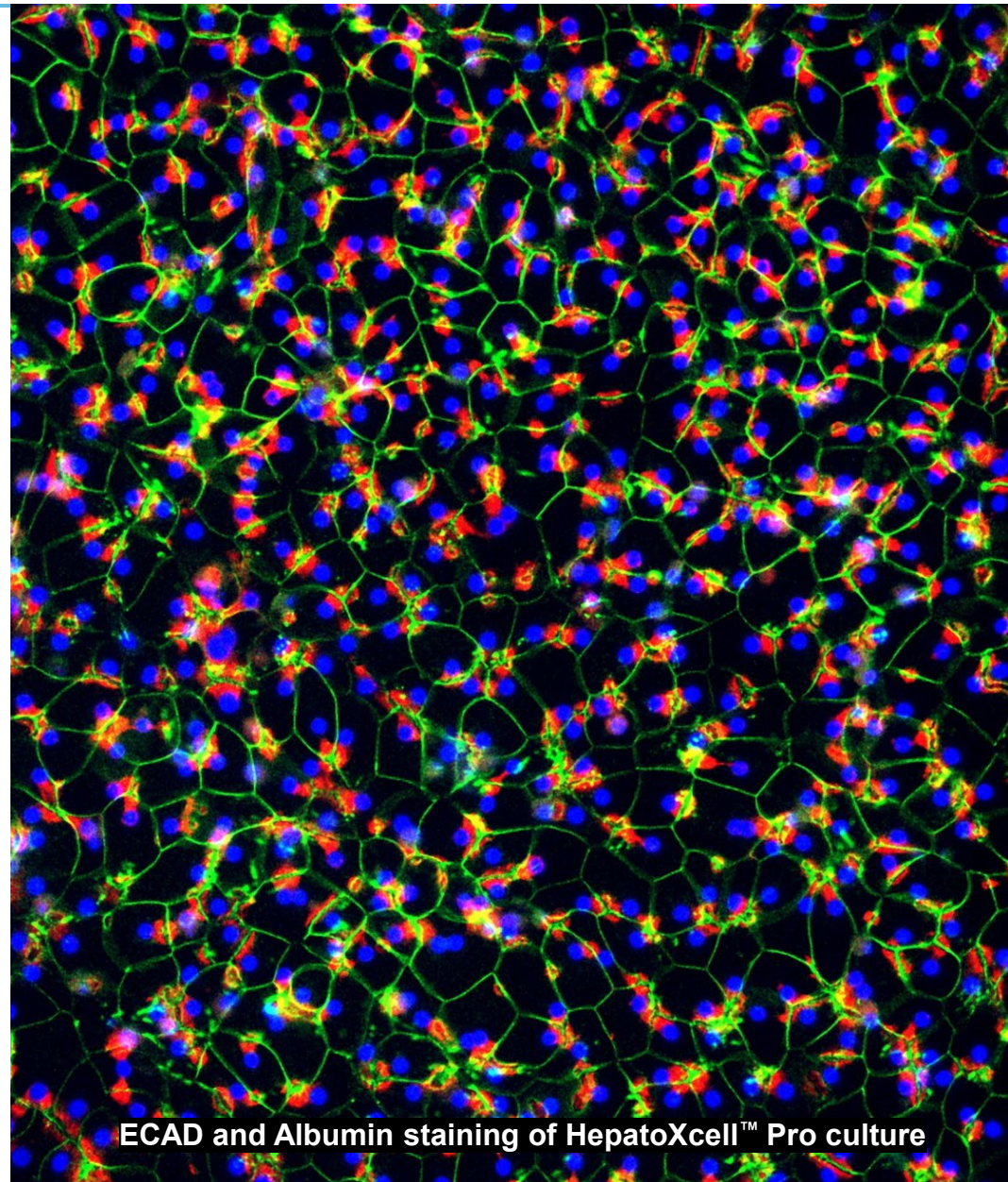
Lot Selection Web Tool



Select a specific lot to add to your shopping cart

Why choose our primary human hepatocytes?

- **High viability and functionality:** Our hepatocytes exhibit excellent viability and retain key liver functions, making them ideal for drug metabolism, toxicity studies, and liver disease research.
- **Comprehensive characterization:** Each batch undergoes rigorous testing to ensure consistency and reliability, including assessments of enzyme activity, protein expression, and metabolic function.
- **Genetic diversity:** These cells can be sourced from multiple donors, reflecting the genetic variability found in the human population. This diversity allows for more comprehensive studies on how different genetic backgrounds can influence liver function and drug response.


ECAD and Albumin staining of HepatoXcell™ Pro culture

ATCC®'s premium hepatocyte offering

Hepatocyte Premium Offering	**HepatoXcell™ Pro**: 7-day plateable hepatocytes
Application	Toxicology testing, ADME, drug development, disease research, advanced cellular modeling, co-culture, microphysiological system
Assays	Metabolism, hepatotoxicity, TEER, induction of CYP mRNA, transporter efflux, transporter uptake, metabolite formation, compound stability, inhibition, gene expression, clearance assay

ATCC®'s hepatocyte offering

Hepatocyte Plateable Offering	**HepatoXcell™ Plus**: 3-day plateable hepatocytes
Application	Toxicology testing, ADME, drug development
Assays	Metabolism, hepatotoxicity, transporter uptake, clearance assay

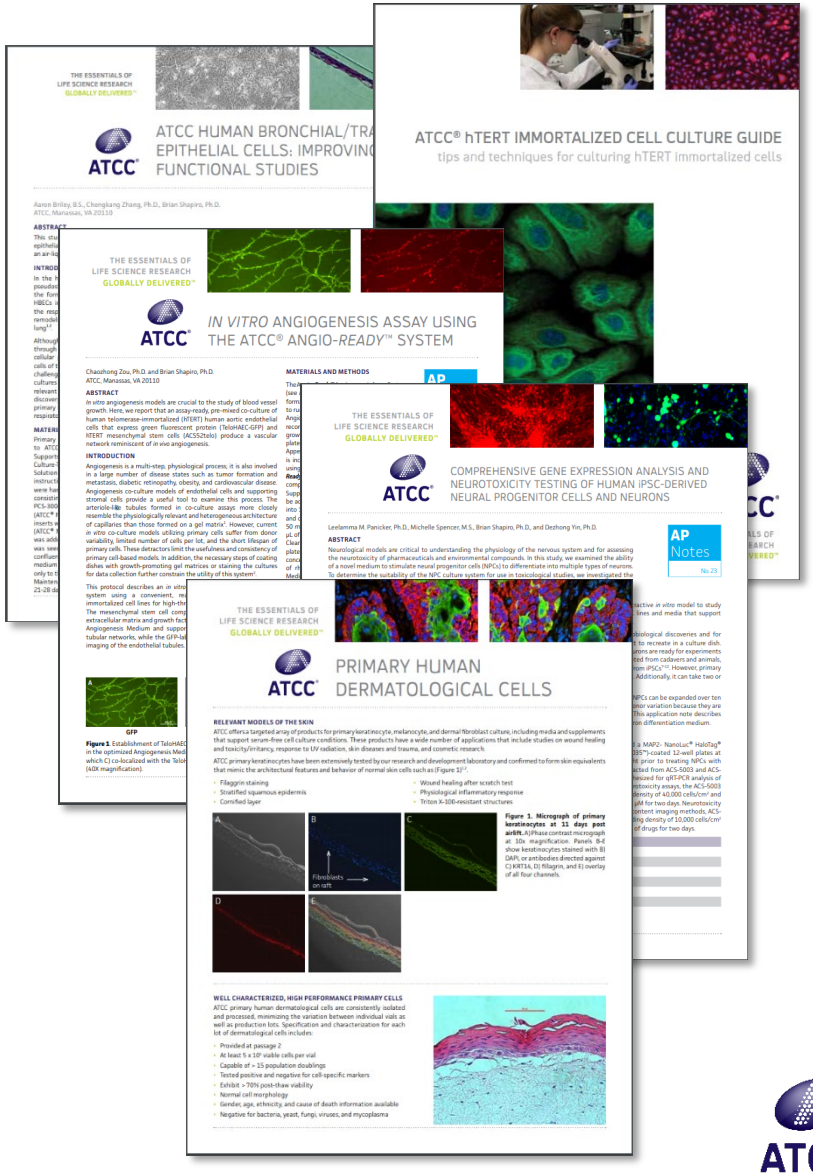
Suspension Hepatocyte Offering	**HepatoXcell™ Eco**: Suspension hepatocytes
Application	ADME, Drug development
Assays	Metabolism, hepatotoxicity, transporter uptake, clearance assay

ATCC®'s upcoming hepatic offerings

- Pooled primary human hepatocytes
- Spheroid/3-D qualified hepatocytes appropriate for MPS
- Non-parenchymal cells (NPC)
- Subcellular fractions – S9 microsomes and cytosol
- MPS application data using multiple platforms

Summary and resources

- ATCC® offers a variety of immortalized cell models
  - Cell lines are authenticated for immortalization and karyotype stability
  - Our portfolio includes several difficult-to-immortalize cell types
  - *Save time and money*
- Multiple resources are available at www.atcc.org/hTERT
- ATCC's newly launched HepatoXcell™ portfolio provides high-quality primary human hepatic products for ADME-Tox and advanced cellular modelling.
- With our upcoming offerings, ATCC will provide an end-to-end solution for hepatic workflow.



Questions