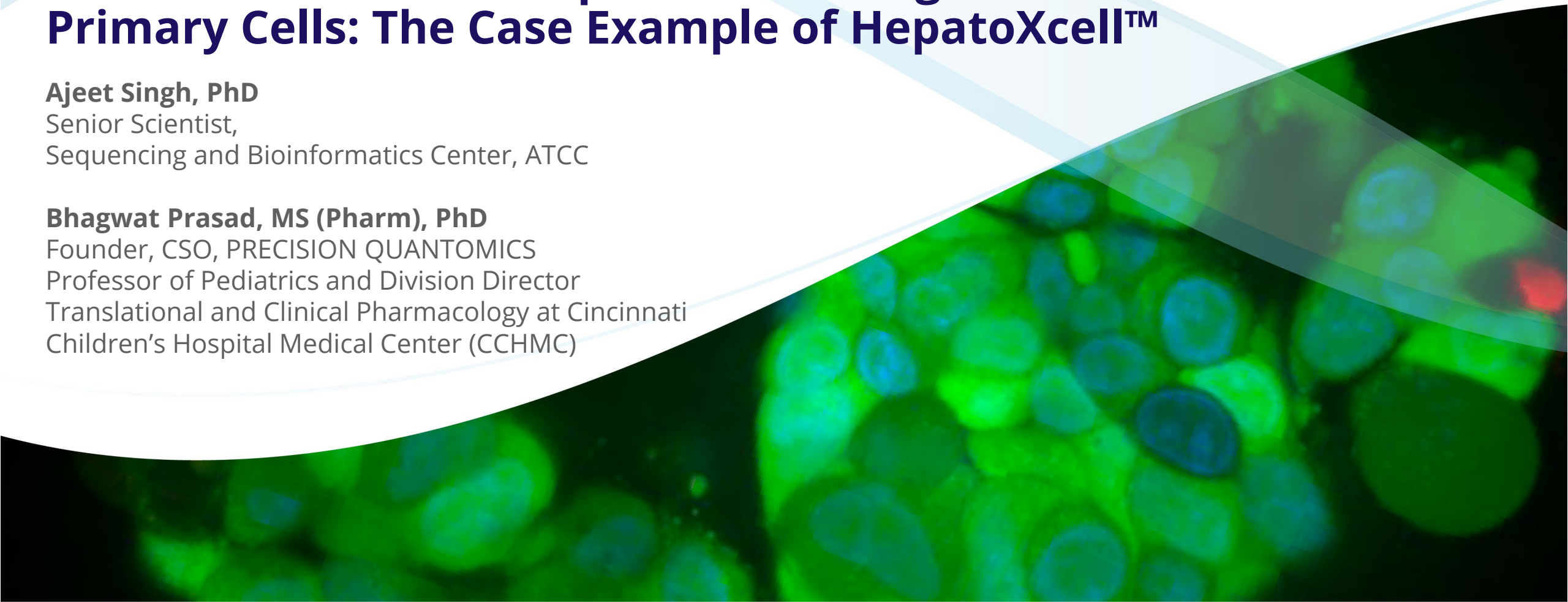
Authenticating Translational ADME Reagents for Gold-Standard Science Using Quantitative Proteomic and Transcriptomic Profiling of Primary Cells: The Case Example of HepatoXcell™

Ajeet Singh, PhD
Senior Scientist,
Sequencing and Bioinformatics Center, ATCC

Bhagwat Prasad, MS (Pharm), PhD
Founder, CSO, PRECISION QUANTOMICS
Professor of Pediatrics and Division Director
Translational and Clinical Pharmacology at Cincinnati
Children's Hospital Medical Center (CCHMC)



Agenda

1. ATCC and Precision Quantomics
2. Authentication of biological reagents through quantitative proteomics
3. HepatoXcell™ Primary Human Hepatocytes
4. Multiomics integration for improved predictive power of assay readouts
5. Conclusions

About Us

ATCC is a global leader in providing authenticated, high-quality biological resources and standards for industry, academia, and government.

- Founded in 1925, ATCC is a private, nonprofit, global biological resource center and standards organization that provides scientists with the biomaterials and resources they need to conduct critical life science research.
- World's trusted, premier biological materials resource and standards development organization:
  - 4,000+ cell lines
  - 80,000+ microorganisms
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents
  - Advanced cell models
  - Standards

Precision Quantomics, Inc.

Better reagents. Better models. Better predictions. Better therapies.

- Quantitative Proteomics Assay Kits
- Quantitative Proteomics Services
- Translational Proteomics Atlas™
- Scientific Interpretation and Translational Support

TRANSLATIONAL PROTEOMICS ATLAS™

Authentication of Biological Reagents through Quantitative Proteomics

Bhagwat Prasad, PhD

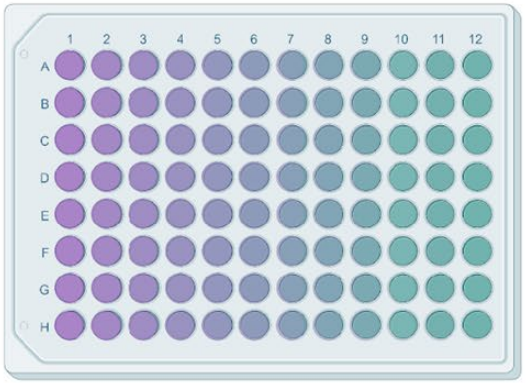
bhagwat.prasad@cchmc.org

bhagwat.prasad@precisionquantomics.com

Conflict of Interest Disclosure

Bhagwat Prasad is cofounder and CSO of Precision Quantomics, Inc.

Lost in Translation



Reference population

$CL_{int} \alpha [E]$

[E] = protein abundance

Specific populations

The Core Problem in Drug Development

The industry challenge

- In vitro models often **lack biological authenticity**
- In silico models often **scale uncertainty instead of truth**

Reproducibility crisis driven by:

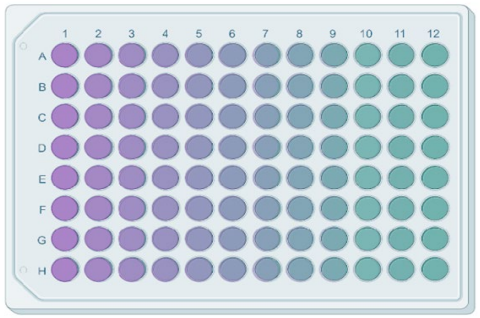
- Poor model characterization
- Hidden biological drift
- Unverified assumptions

If the biology is wrong, the model will fail.

Authentication of Biological Reagents
Reliable Reagents, Reproducible Science.

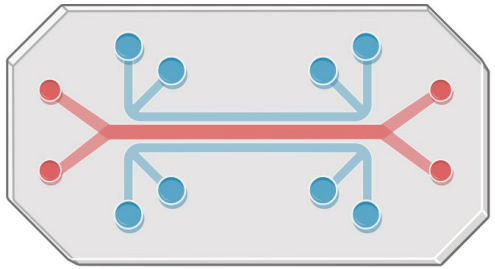
Gold standard science through authentic reagents

- Strengthens data reliability
- Reduces scientific variability and unintended bias
- Prevents costly setbacks

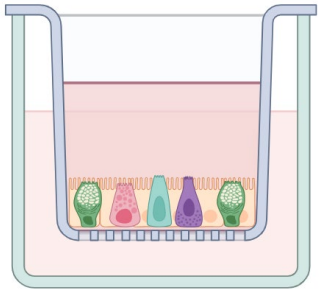


In vitro models

Organoids

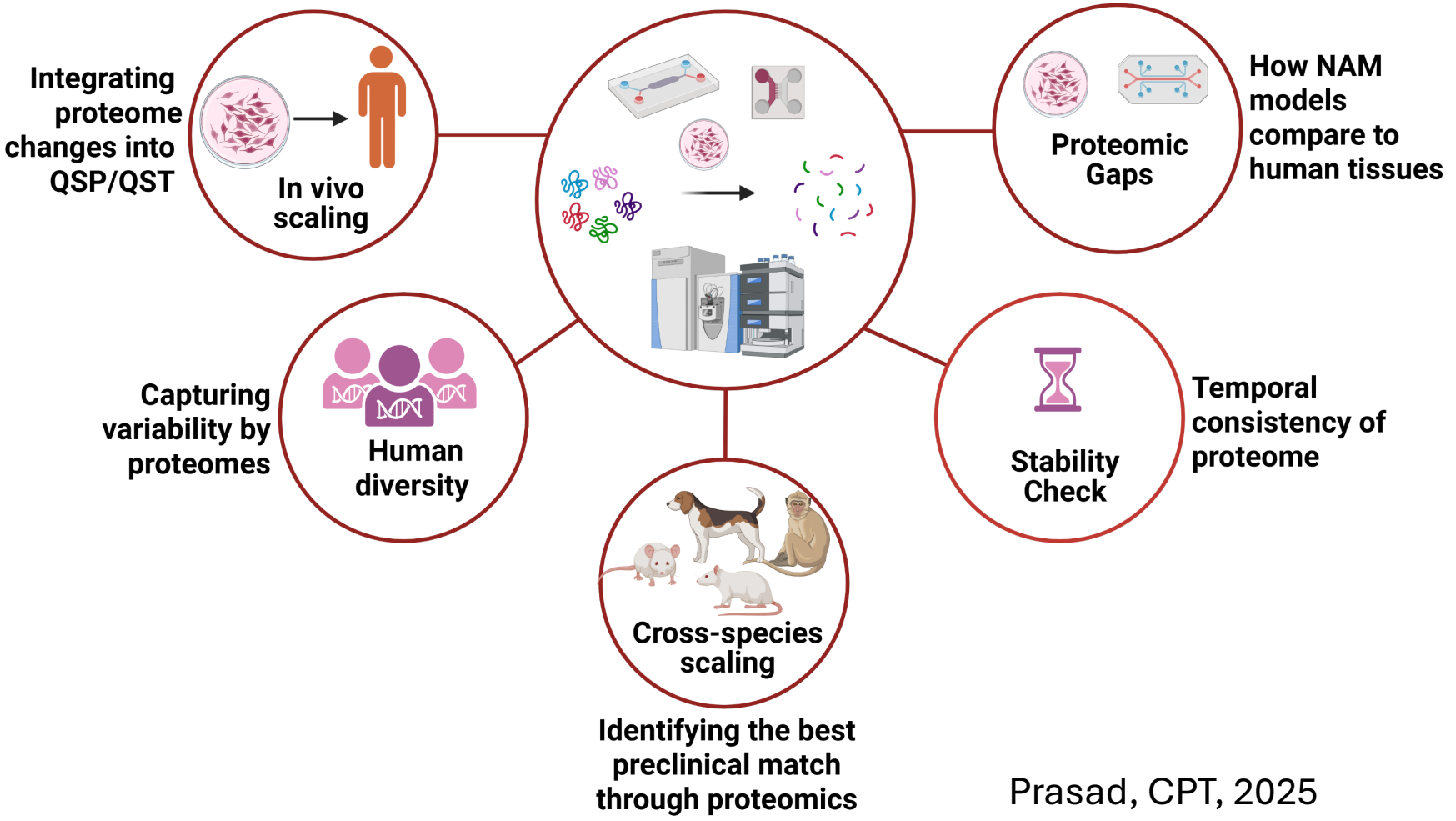


Microphysiological Systems (MPS)



3D Cultures

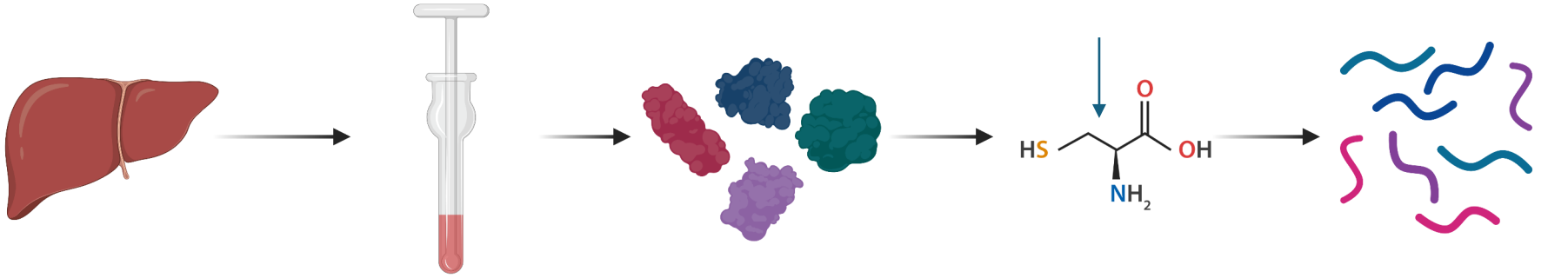
Promise of Quantitative Proteomics in Characterizing In Vitro Models



Prasad, CPT, 2025

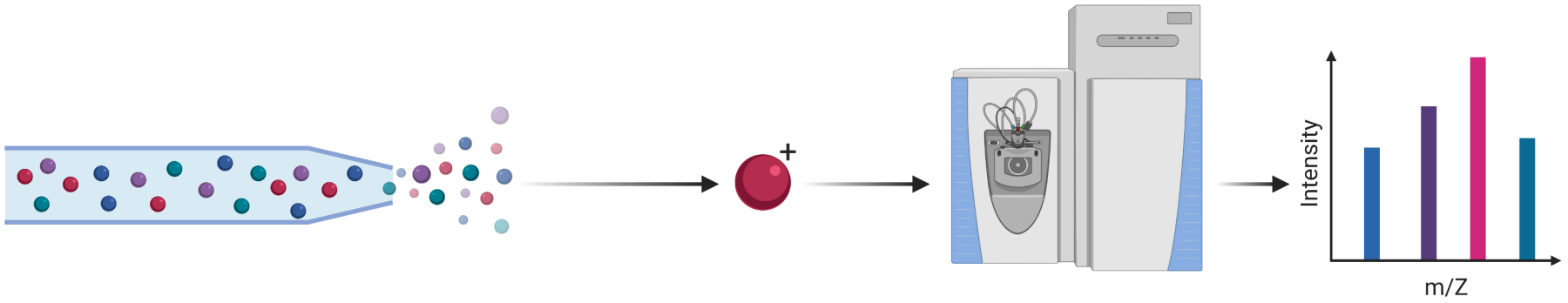
Quantitative Proteomics

Peptides are used as surrogates to quantify enzyme digested proteins



① Tissue ② Homogenization ③ Protein mixture ④ Denaturation/ alkylation ⑤ Digested peptides

1. Targeted
2. Global/untargeted

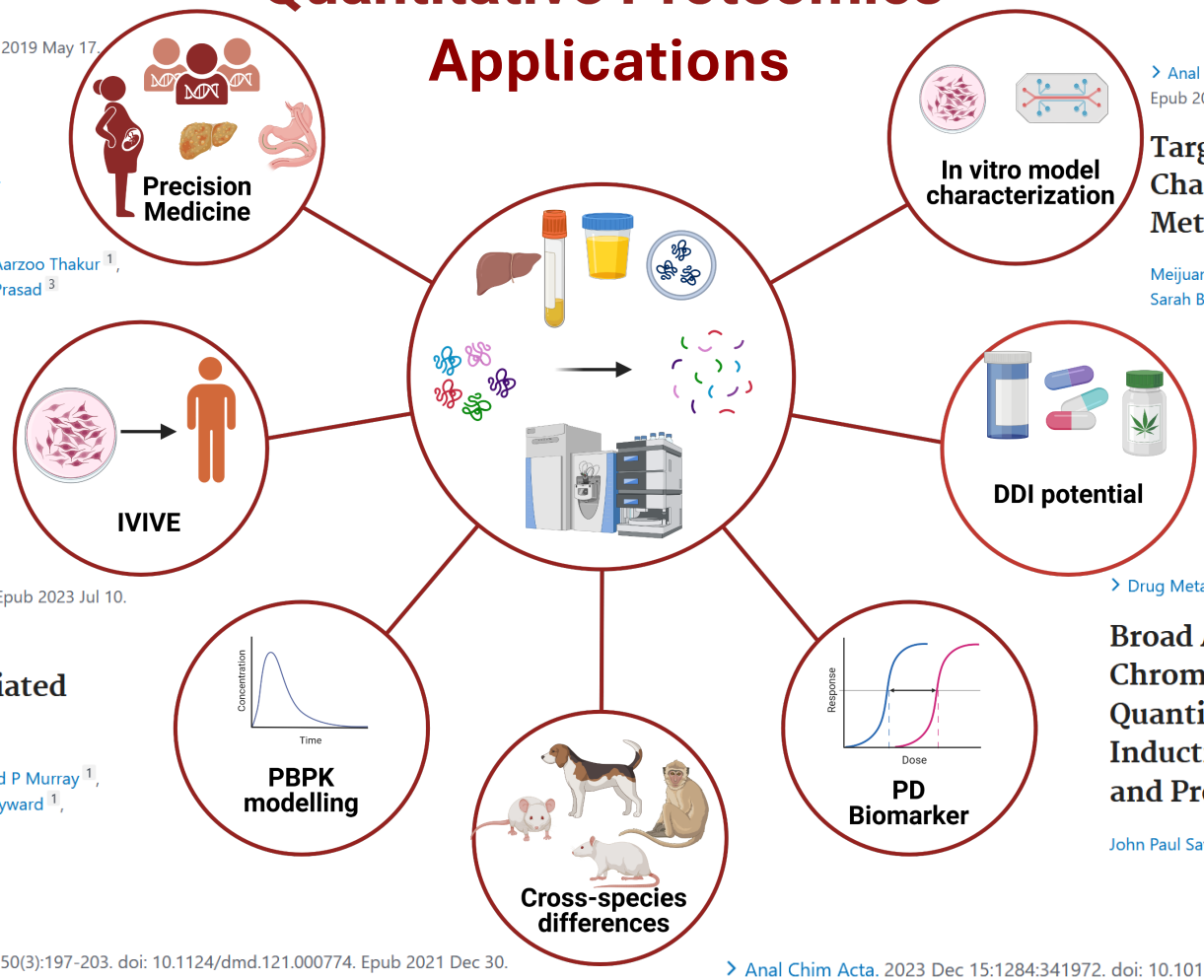


⑥ Liquid chromatography peptide separation ⑦ Electrospray ionization ⑧ Ion-peptide ⑨ Mass spectrometry ⑩ Data analysis

Advantages:

- Multiplex analysis
- Selective
- Precise
- Cost effective

Quantitative Proteomics Applications



Drug Metab Dispos. 2019 Aug;47(8):818-831. doi: 10.1124/dmd.119.086462. Epub 2019 May 17.

Ontogeny of Hepatic Sulfotransferases and Prediction of Age-Dependent Fractional Contribution of Sulfation in Acetaminophen Metabolism

Mayur K Ladumor [1], Deepak Kumar Bhatt [1], Andrea Gaedigk [1], Sheena Sharma [1], Aarzoo Thakur [1], Robin E Pearce [1], J Steven Leeder [1], Michael B Bolger [1], Saranjit Singh [2], Bhagwat Prasad [3]

Anal Chem. 2018 Oct 16;90(20):11873-11882. doi: 10.1021/acs.analchem.8b01913. Epub 2018 Sep 28.

Targeted LC-MS/MS Proteomics-Based Strategy To Characterize in Vitro Models Used in Drug Metabolism and Transport Studies

Meijuan Xu [1 2], Neha Saxena [1], Marc Vrana [1], Haeyoung Zhang [1], Vineet Kumar [1], Sarah Billington [1], Cyrus Khojasteh [3], Scott Heyward [4], Jashvant D Unadkat [1], Bhagwat Prasad [1]

Drug Metab Dispos. 2023 Oct;51(10):1362-1371. doi: 10.1124/dmd.123.001379. Epub 2023 Jul 10.

Dissecting Parameters Contributing to the Underprediction of Aldehyde Oxidase-Mediated Metabolic Clearance of Drugs

Sandhya Subash [1], Dilip K Singh [1], Deepak S Ahire [1], S Cyrus Khojasteh [1], Bernard P Murray [1], Michael A Zientek [1], Robert S Jones [1], Priyanka Kulkarni [1], Bill J Smith [1], Scott Heyward [1], Ciarán N Cronin [1], Bhagwat Prasad [2]

Drug Metab Dispos. 2022 Feb;50(2):105-113. doi: 10.1124/dmd.121.000638. Epub 2021 Dec 2.

Broad Application of CYP3A4 Liquid Chromatography-Mass Spectrometry Protein Quantification in Hepatocyte Cytochrome P450 Induction Assays Identifies Nonuniformity in mRNA and Protein Induction Responses

John Paul Savaryn [1], Jun Sun [1], Junli Ma [1], Gary J Jenkins [1], David M Stresser [2]

Drug Metab Dispos. 2022 Mar;50(3):197-203. doi: 10.1124/dmd.121.000774. Epub 2021 Dec 30.

Comparison of Tissue Abundance of Non-Cytochrome P450 Drug-Metabolizing Enzymes by Quantitative Proteomics between Humans and Laboratory Animal Species

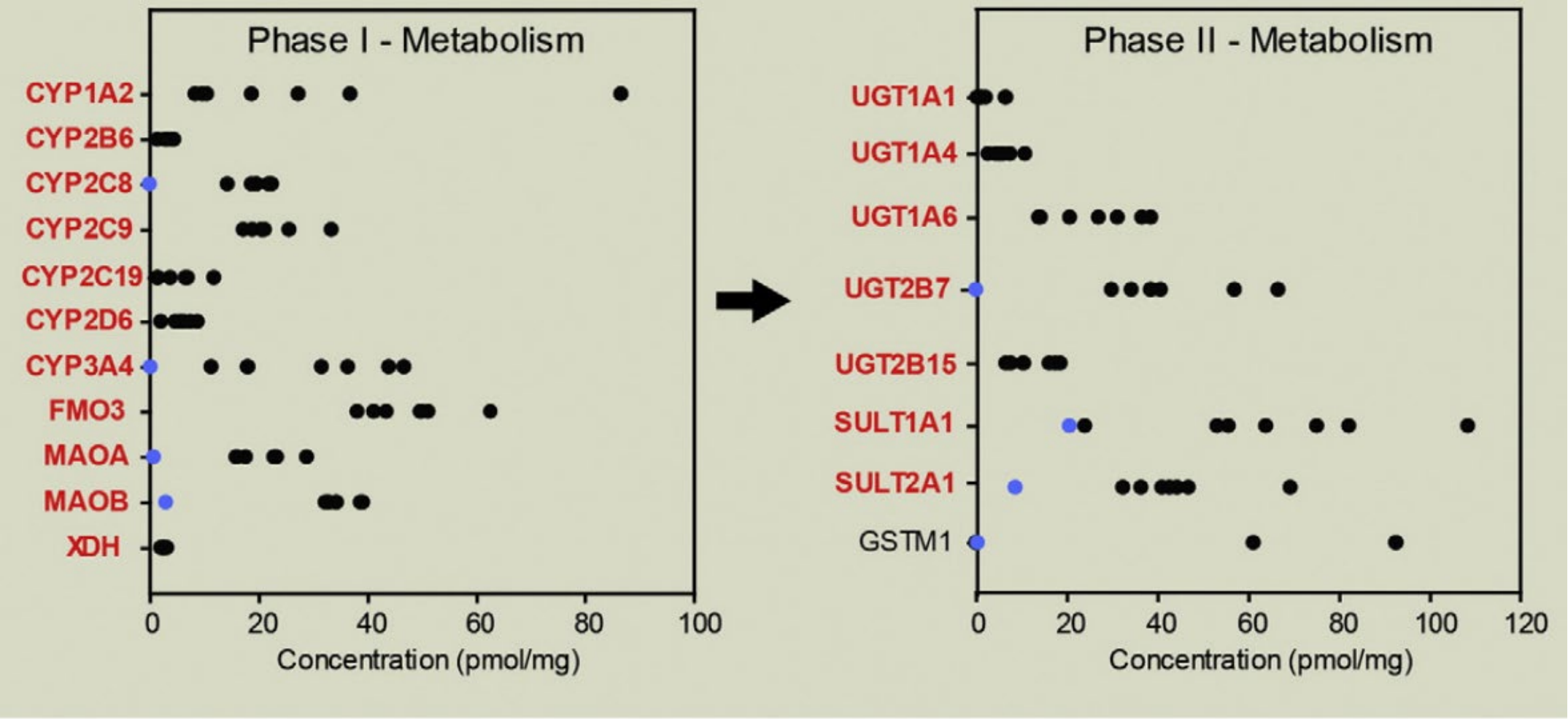
Abdul Basit [1], Peter W Fan [1], S Cyrus Khojasteh [1], Bernard P Murray [1], Bill J Smith [1], Scott Heyward [1], Bhagwat Prasad [2]

Anal Chim Acta. 2023 Dec 15:1284:341972. doi: 10.1016/j.aca.2023.341972. Epub 2023 Nov 5.

Characterization of Gla proteoforms and non-Gla peptides of gamma carboxylated proteins: Application to quantification of prothrombin proteoforms in human plasma

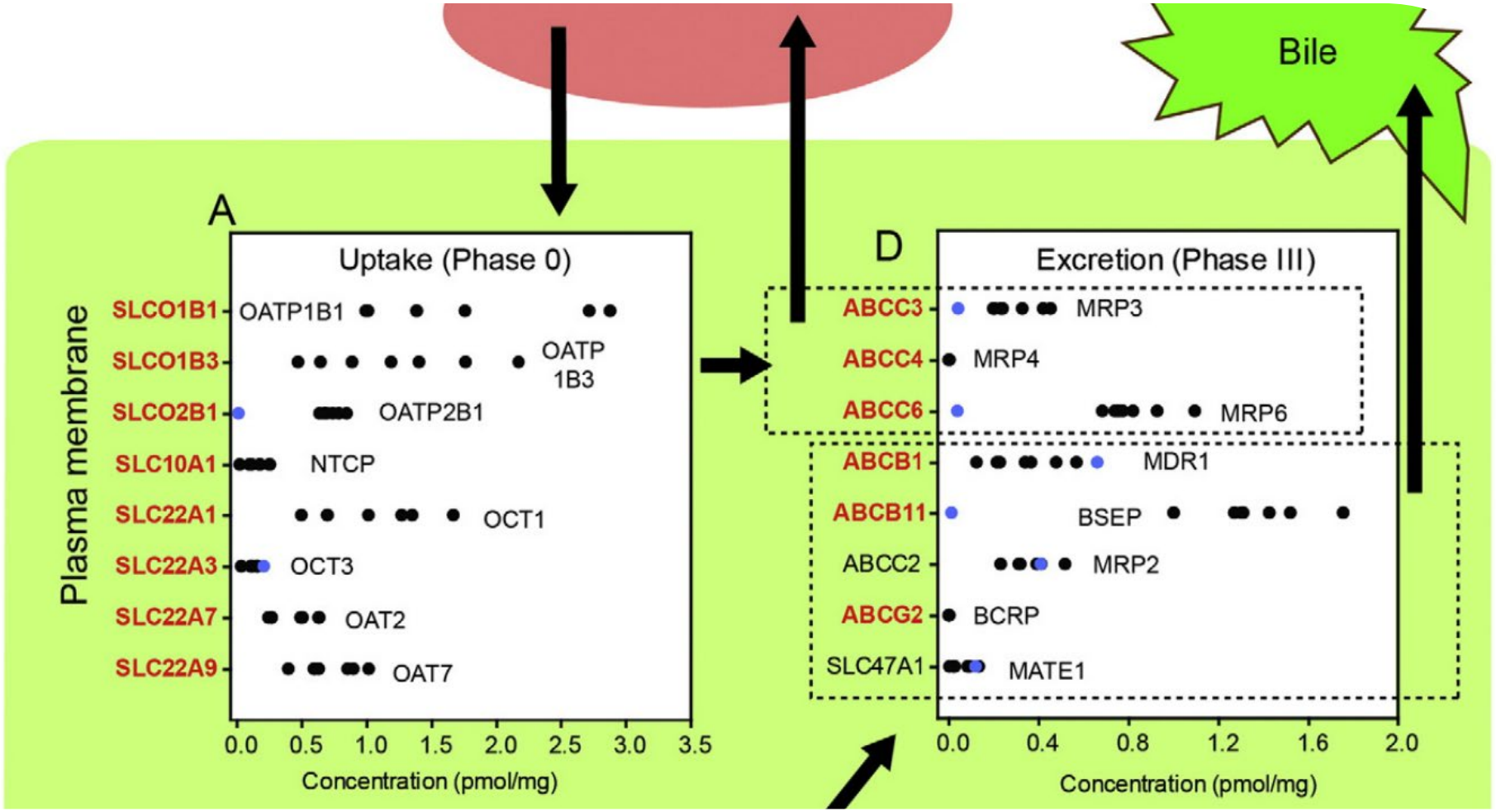
Dilip Kumar Singh [1], Abdul Basit [1], Allan E Rettie [2], Nathan Alade [3], Kenneth Thummel [3], Bhagwat Prasad [4]

Primary Human Hepatocytes vs. HepG2



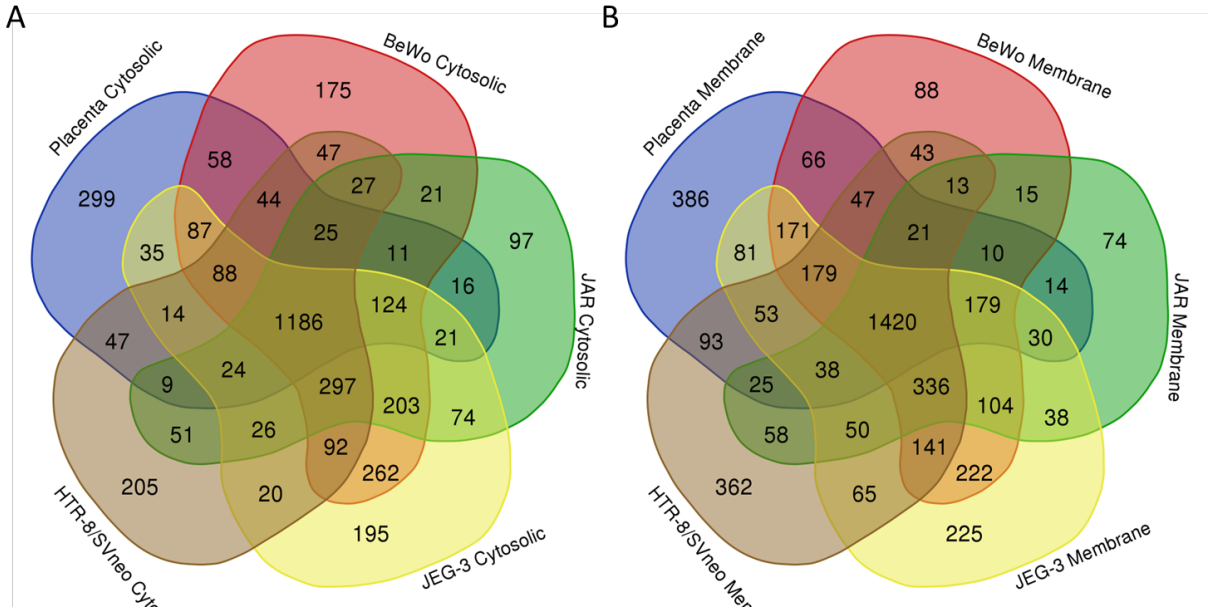
Wiśniewski et al., Journal of Proteomics, 2016.

Primary Human Hepatocytes vs. HepG2



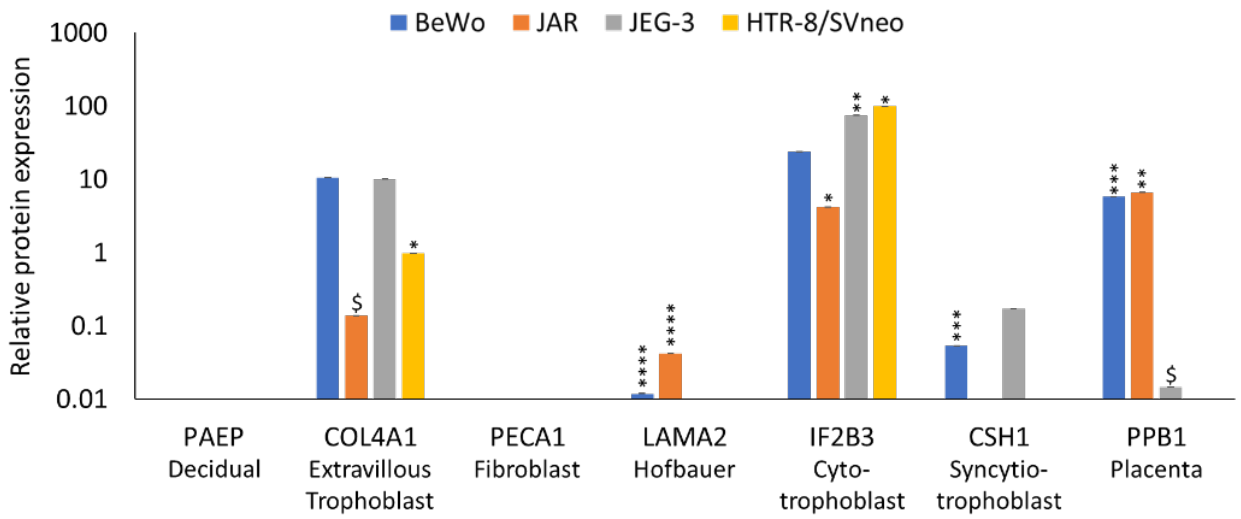
Wiśniewski et al., Journal of Proteomics, 2016.

Placental Cell Lines vs. Human Placenta

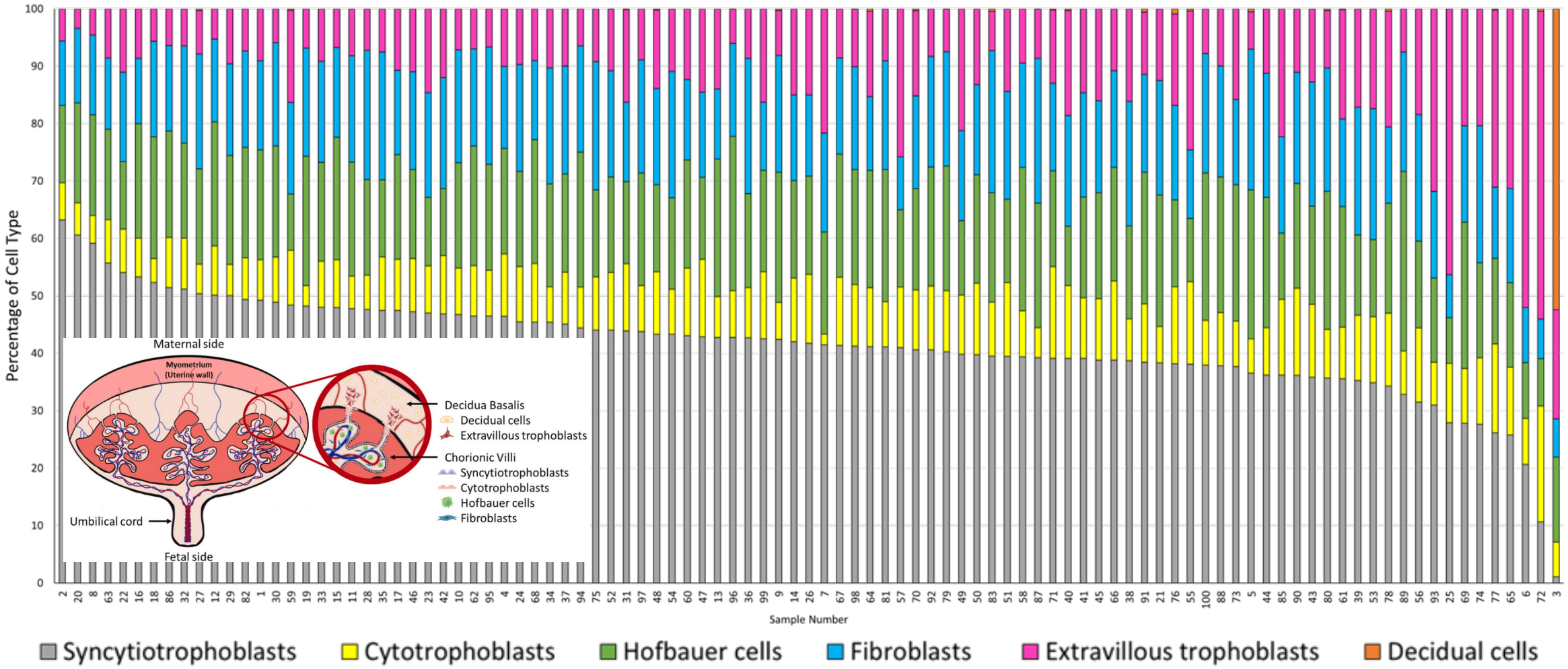


Kruger, et al., J Steroid Biochem Mol Bio, 2022

Placental cell type markers

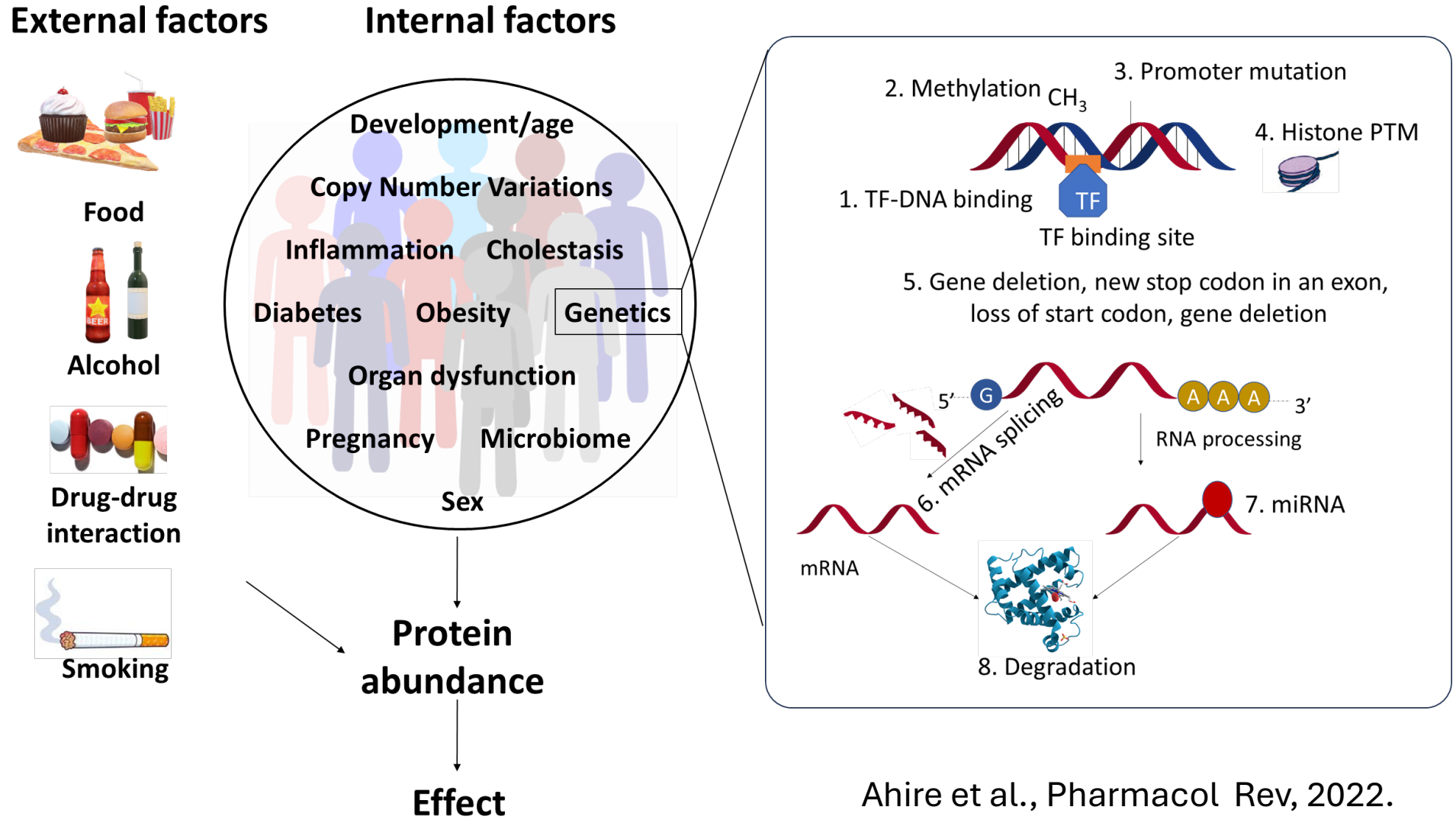


Characterization of Cellular Heterogeneity



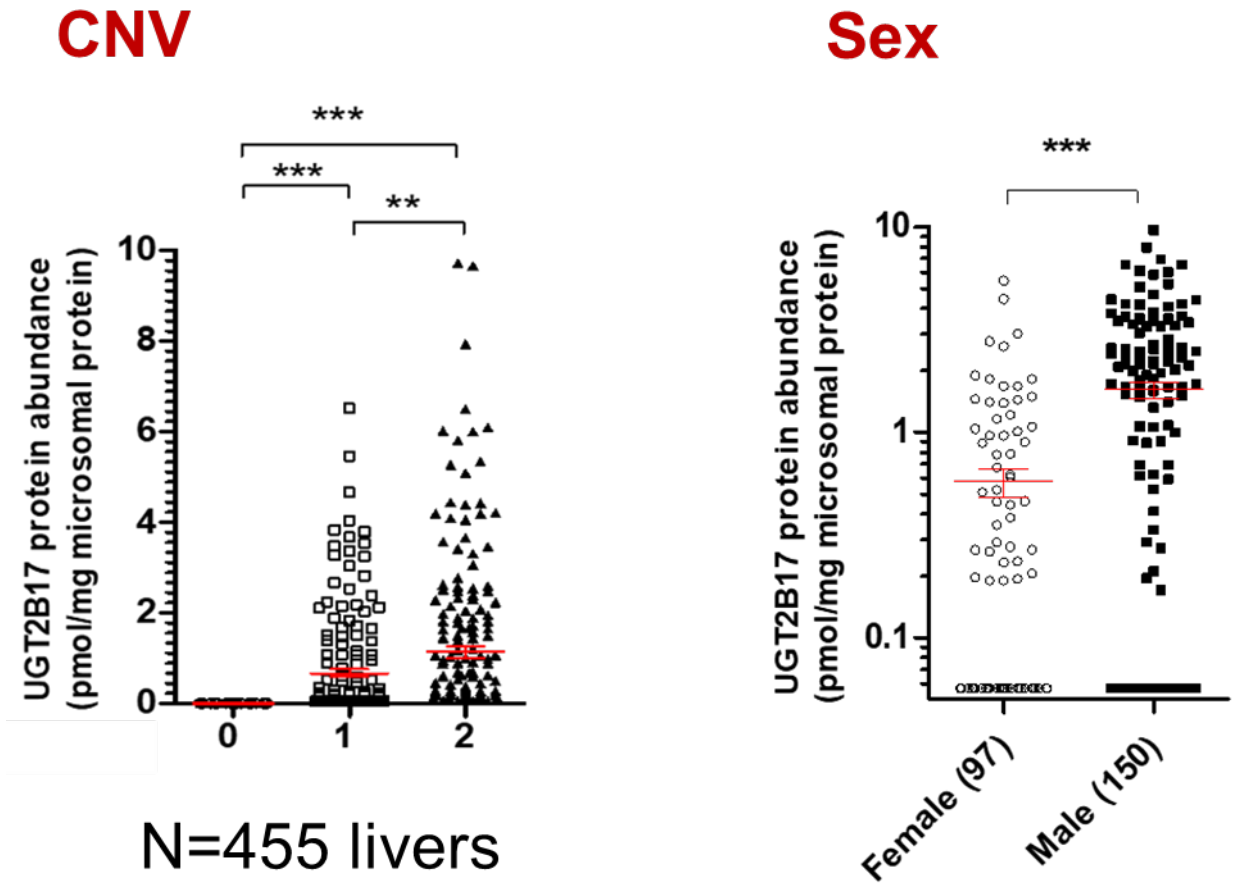
Kruger et al., Placenta 2023.

Quantitative Proteomics for Predicting Interindividual Variability and PBPK Modeling



Ahire et al., Pharmacol Rev, 2022.

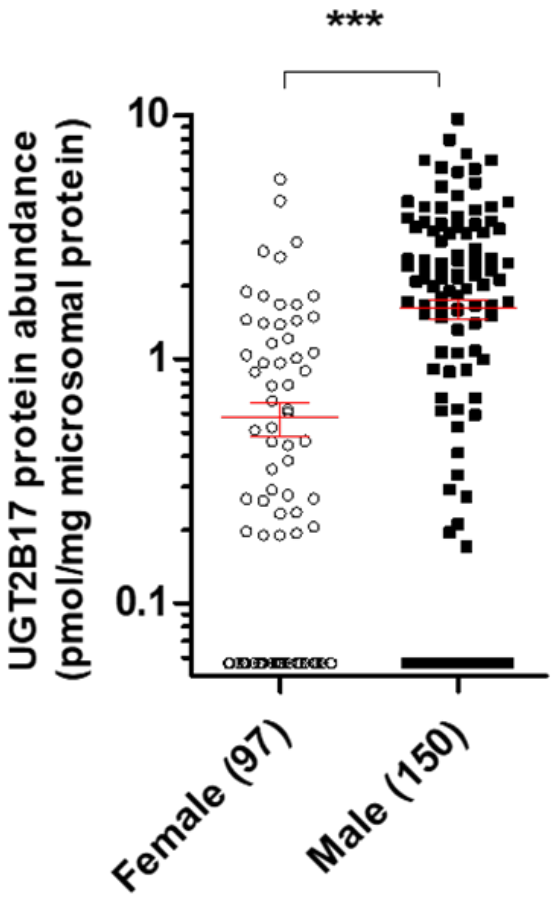
Interindividual Variability (e.g., UGT2B17)



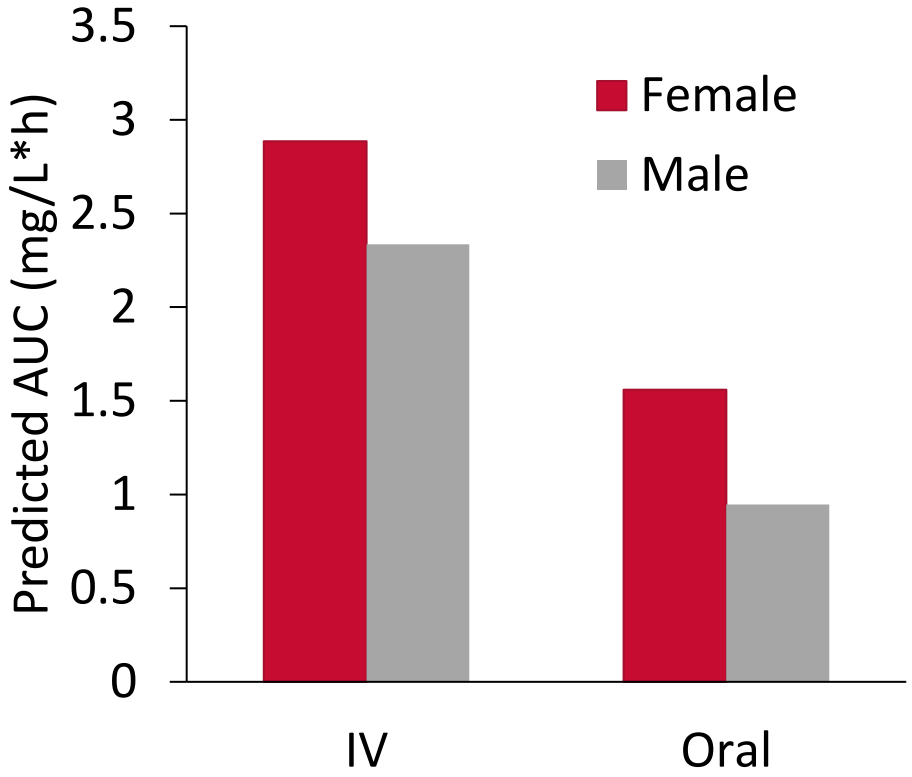
Protein abundance data correlate with mRNA expression and activity

Bhatt et al., Drug Metab Dispos, 2018.

Sex, UGT2B17 vs. Diclofenac PK Prediction



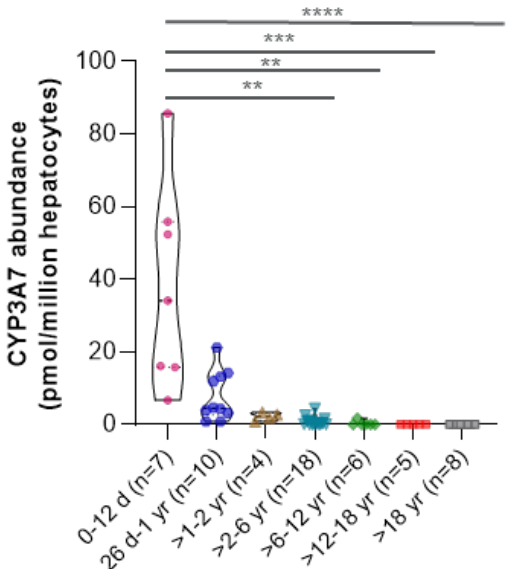
PBPK modeling (Simcyp)

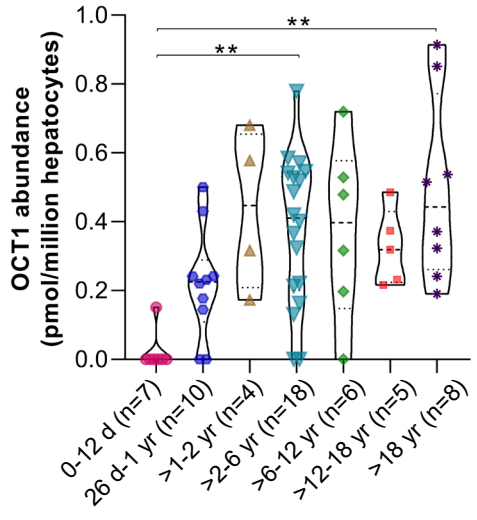


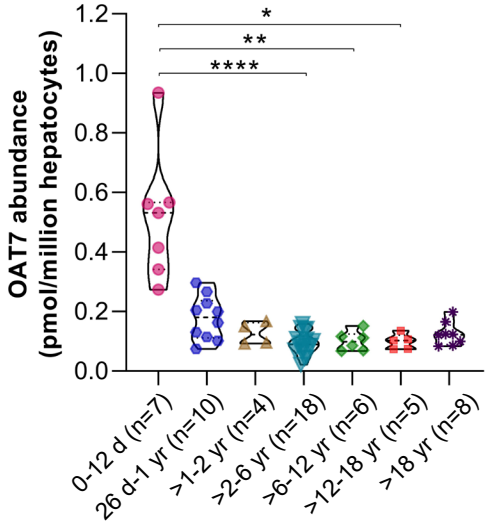
Ahire et al., Clin Pharmacol Ther, 2023.

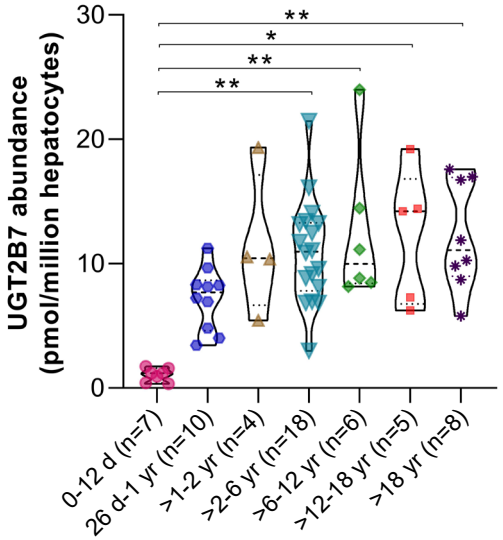
- **Higher risk of liver and cardiac injury in women than in men**
  - **Banks et al., *Hepatol.* (1995); Schmidt et al., *British Med. J.* (2018)**

Proteomics-Informed Dose Prediction

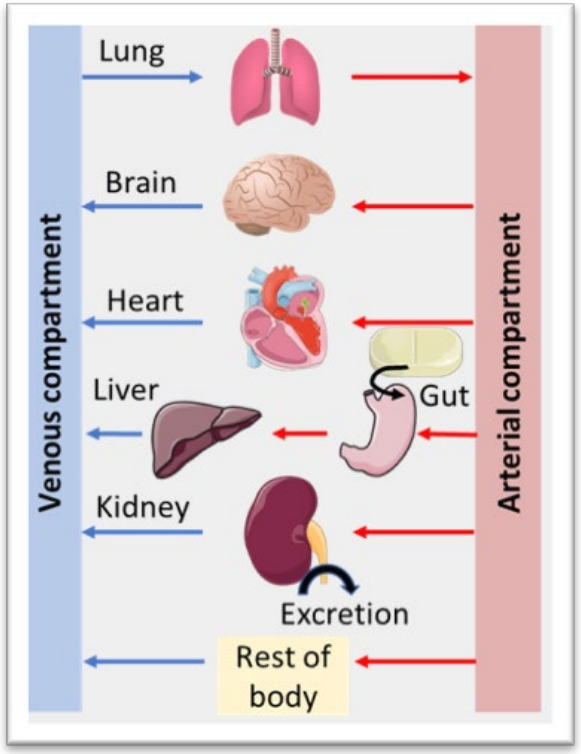




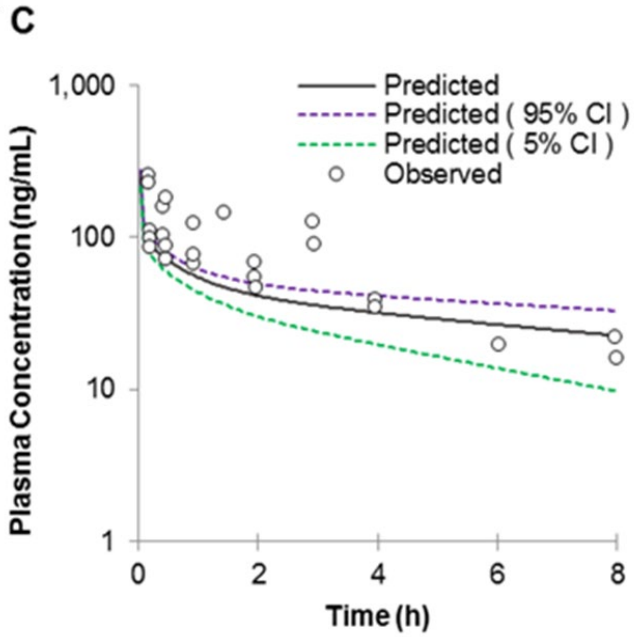




Physiologically-based pharmacokinetic model



Morphine dose prediction in newborns



Thakur et al., Clin Pharmacol Ther. 2024.
Bhatt et al., Clin Pharmacol Ther. 2018.

Summary

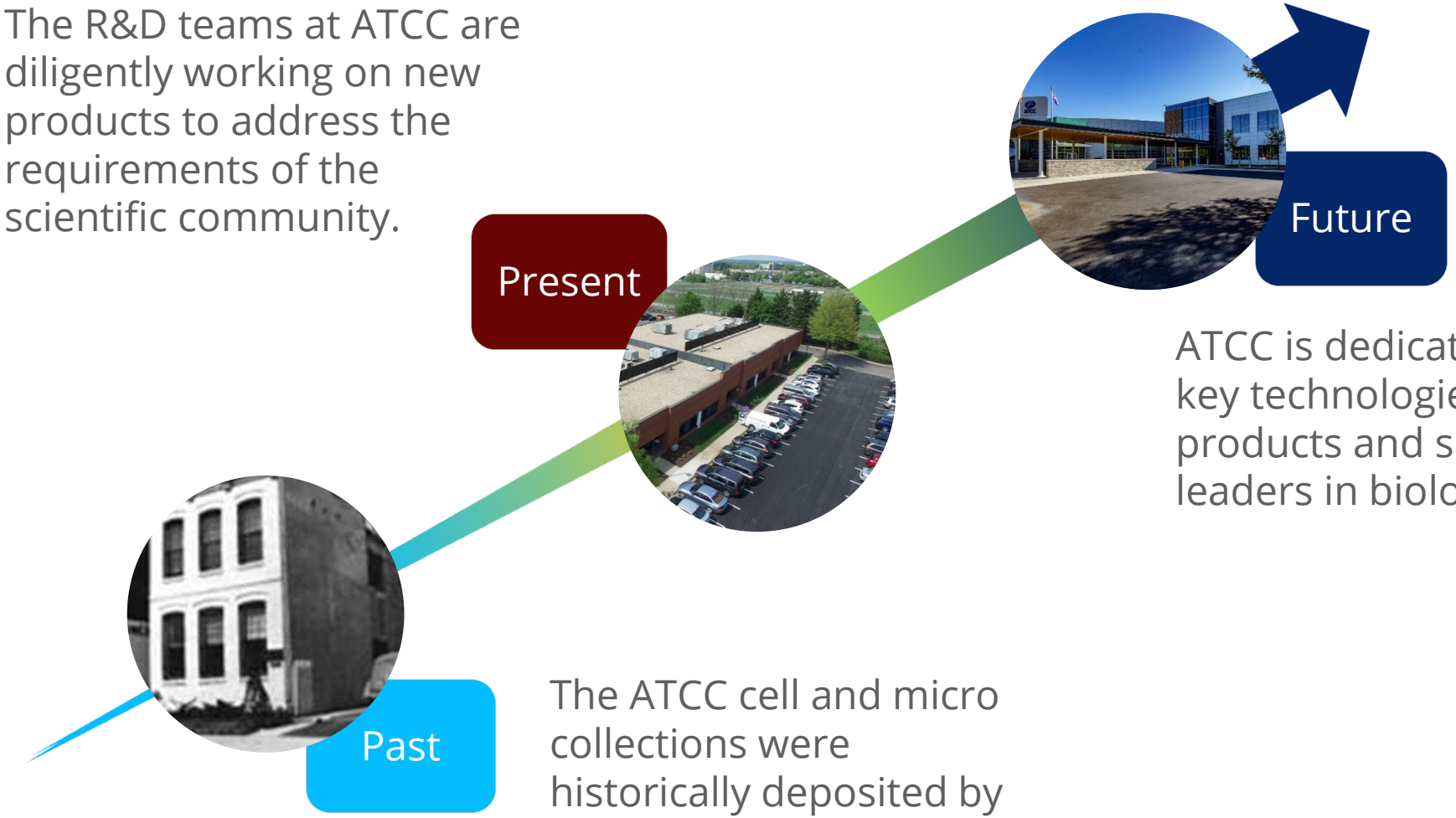
- **Use of authenticated biological reagents** is essential for successful IVIVE and more reliable translation of data for improved drug development outcomes.
- **Quantitative proteomics:**
  - Enables precise, multiplexed measurement of ADMET-relevant proteins.
  - Authenticates biological reagents by confirming identity, purity, and functional protein expression, reducing risk of misleading in vitro findings.
  - uncovers donor-dependent variability, aiding in lot selection and improving translational reliability.
  - Improves PBPK modeling and dose prediction, supporting precision medicine.
- **Measurement defines truth. Quantitation defines trust.**

The Case Example of HepatoXcell™

Ajeet Singh, PhD
Senior Scientist,
Sequencing and Bioinformatics Center, ATCC

Modernization of the ATCC® Portfolio

The R&D teams at ATCC are diligently working on new products to address the requirements of the scientific community.

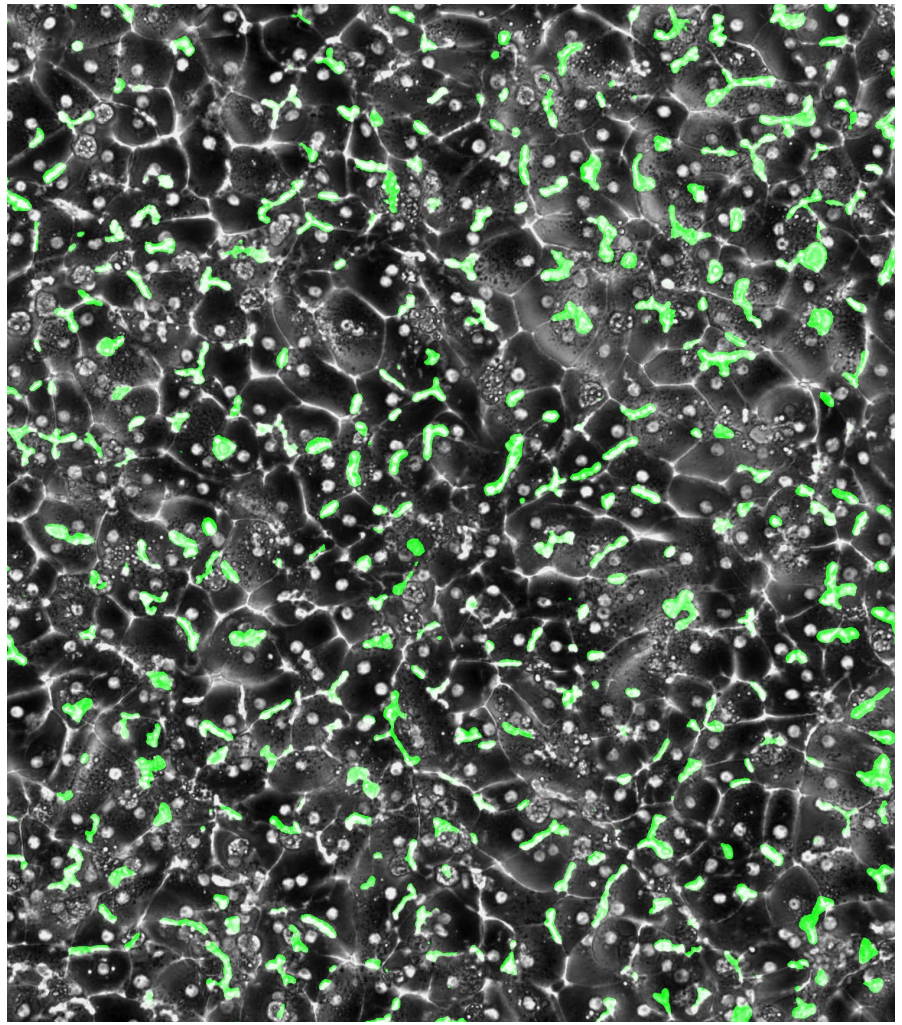


ATCC is dedicating resources to key technologies to ensure its products and services remain leaders in biological research.

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

Challenges in ADME-Tox Testing

- Limited in vitro models for ADME-Tox
- Heavy reliance on primary human hepatocytes (PHH)
- Limited success with immortalized or iPSC-derived hepatocytes
- Industry-wide PHH shortage due to increased liver transplants
- Difficulty sourcing healthy liver tissue
- Complex lot selection process (donor info, vial count, characterization)
- Lack of prequalified lots and genomic data
- High cost of PHH

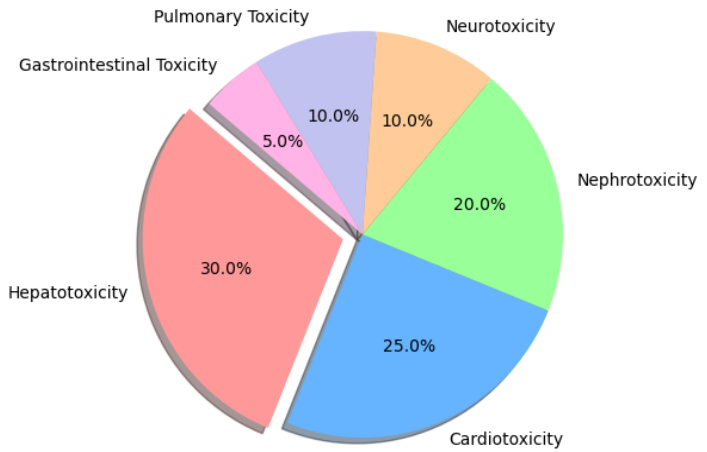


CDFDA accumulation in bile canaliculi of PHH culture

Primary human hepatocytes: why they matter?

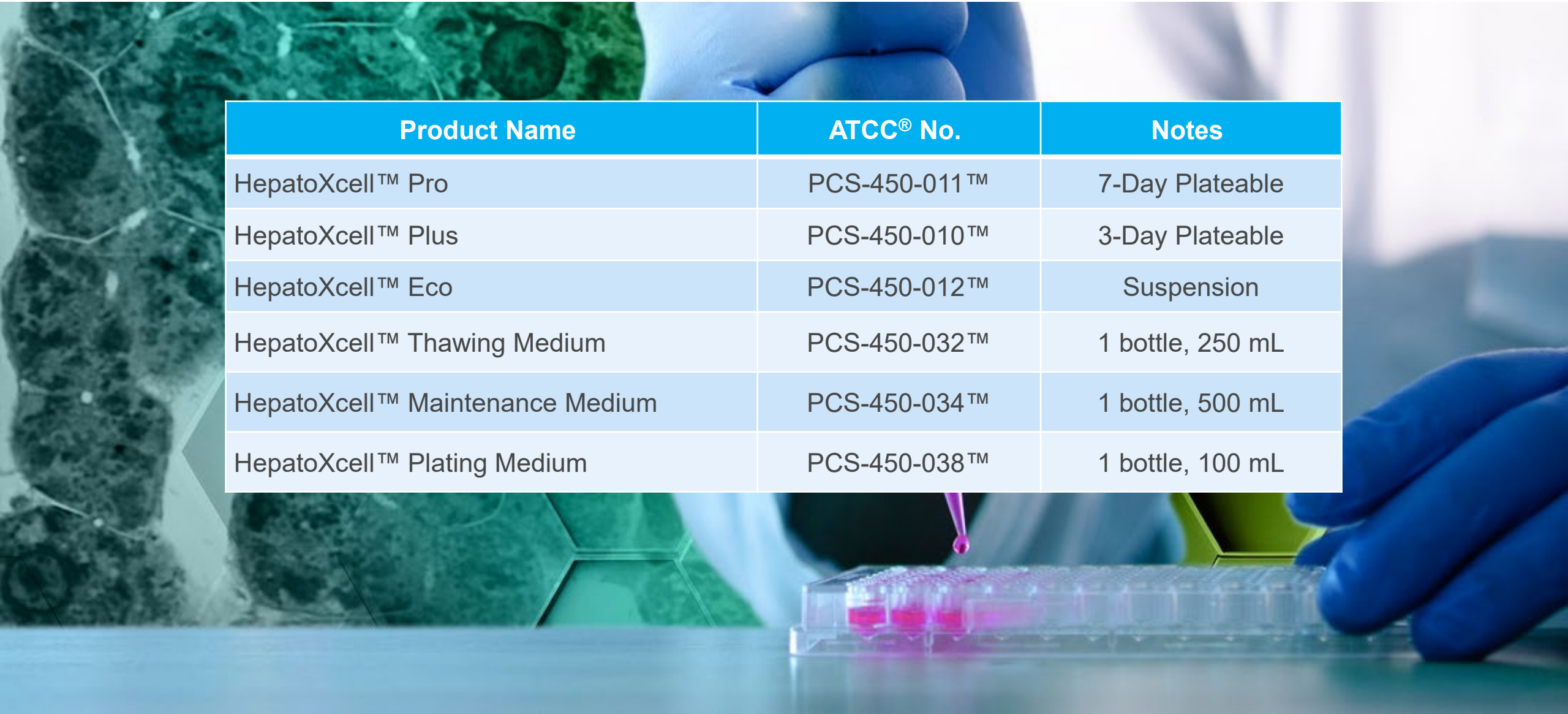
1. Primary Human Hepatocytes are the gold standard for in vitro liver models due to high physiological relevance and predictive accuracy
2. Liver toxicity causes ~20–30% of drug failures; the liver is the main site of drug metabolism and a key focus in preclinical safety
3. FDA recommends primary hepatocytes for IND submissions, including:
   - Drug metabolism & pharmacokinetics (DMPK)
   - ADME studies
   - In vitro liver toxicity & DILI assessments
4. Supports the 3Rs (Replacement, Reduction, Refinement) by reducing animal testing
5. Crucial for modeling liver diseases such as FLD, NASH, fibrosis, and cirrhosis, which impact one-third of the global population
   - Only one FDA-approved drug for NASH/fibrosis to date, with limited success
6. It was projected that human liver model market at $2.3B by 2025, growing at 10.8% CAGR

Organ-Specific Toxicity Testing in Preclinical Studies



HepatoXcell™

Primary human hepatocytes and media

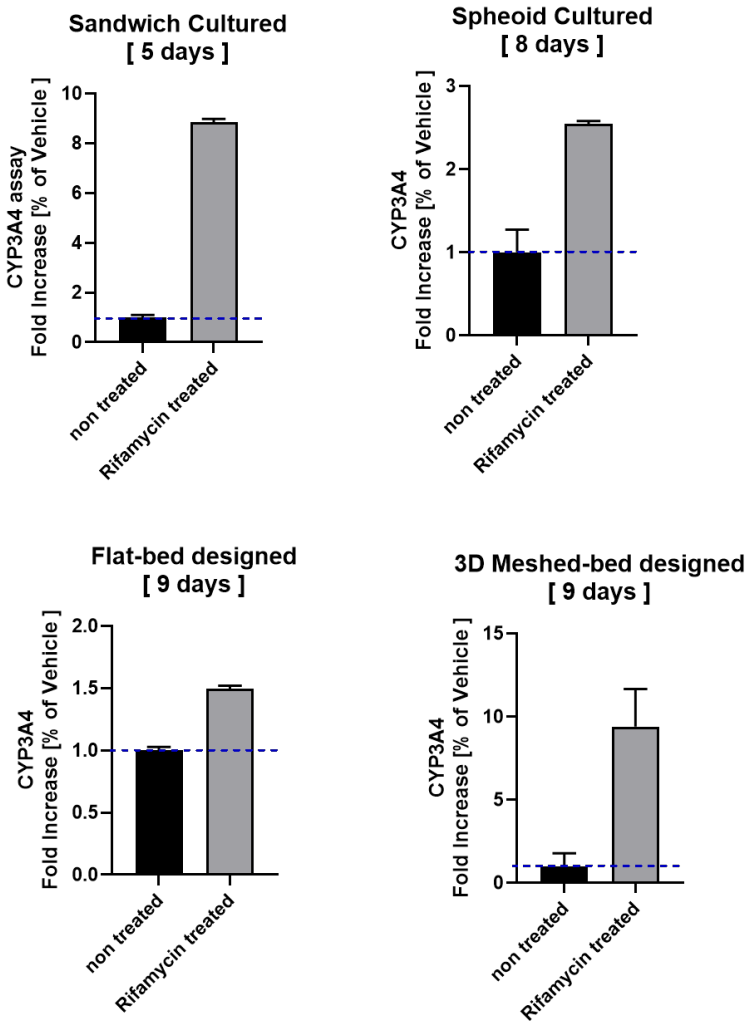


Product Name	ATCC® No.	Notes
HepatoXcell™ Pro	PCS-450-011™	7-Day Plateable
HepatoXcell™ Plus	PCS-450-010™	3-Day Plateable
HepatoXcell™ Eco	PCS-450-012™	Suspension
HepatoXcell™ Thawing Medium	PCS-450-032™	1 bottle, 250 mL
HepatoXcell™ Maintenance Medium	PCS-450-034™	1 bottle, 500 mL
HepatoXcell™ Plating Medium	PCS-450-038™	1 bottle, 100 mL

HepatoXcell™ Primary Human Hepatocytes

Key advantages

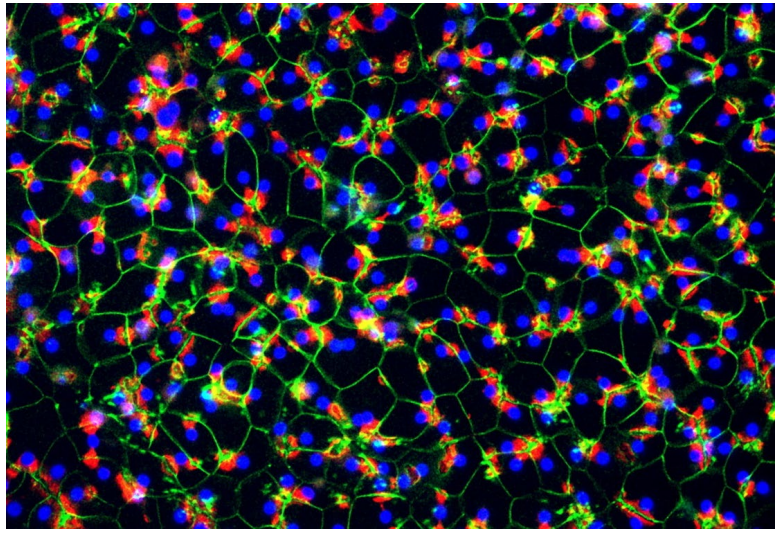
- Prequalified for plate-ability, suspension viability, or **spheroid formation**
- Certificate of analysis includes viability, metabolism, induction, and uptake data
- Easy lot selection with donor and lot data access
- Backed by ATCC quality assurance
- ATCC is the only supplier that performs **Mycoplasma testing**
- **Transcriptome and exome data** is available per lot

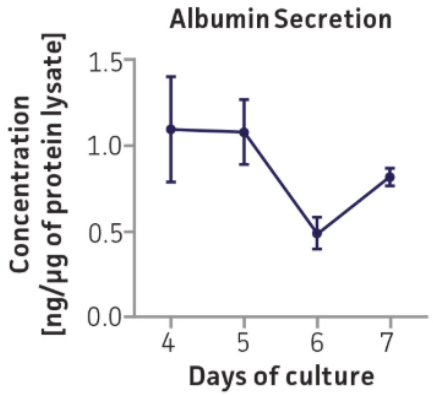


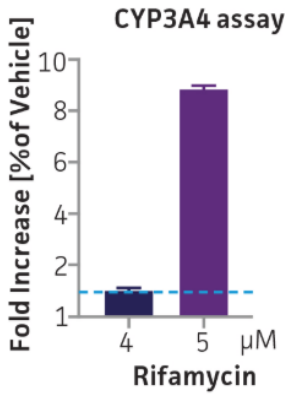
HepatoXcell™ Primary Human Hepatocytes

Key features

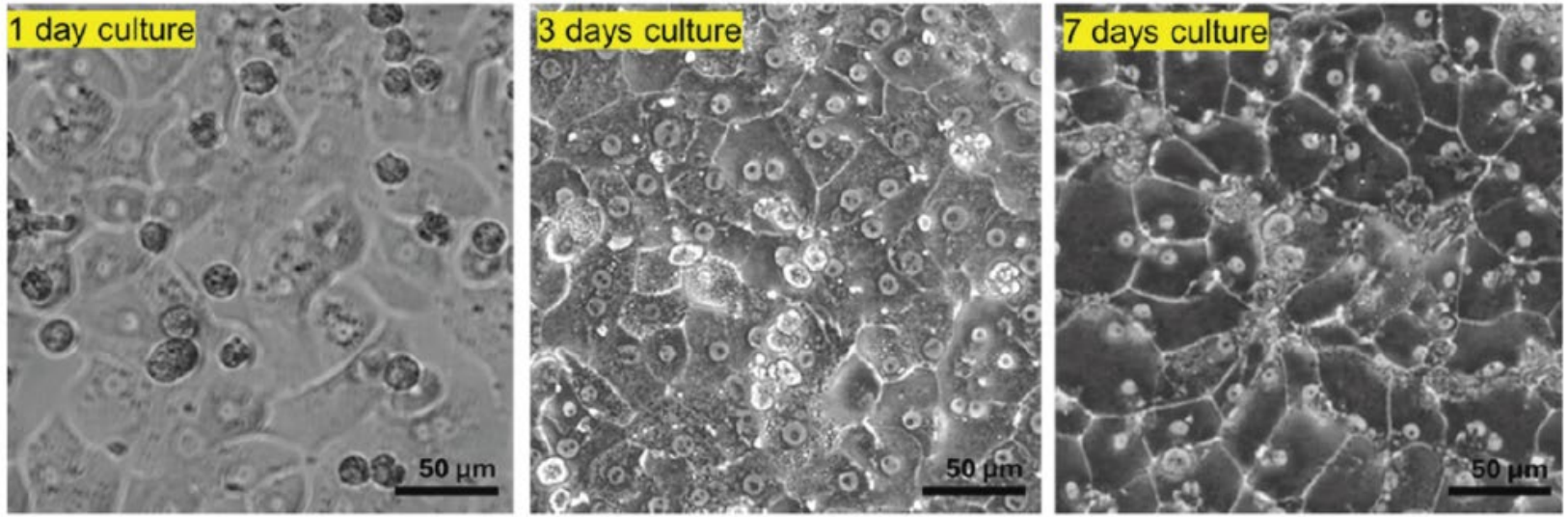
- **High viability and functionality:**
  - Excellent viability and retain key liver functions
  - Ideal for drug metabolism, toxicity studies, and liver disease research
- **Comprehensive characterization:**
  - Each batch undergoes rigorous testing to ensure consistency and reliability
  - Testing includes assessments of enzyme activity, protein expression, and metabolic function
- **Genetic diversity:**
  - Sourced from multiple donors, reflecting the genetic variability found in the human population
  - Allows for comprehensive studies on how different genetic backgrounds can influence liver function and drug response





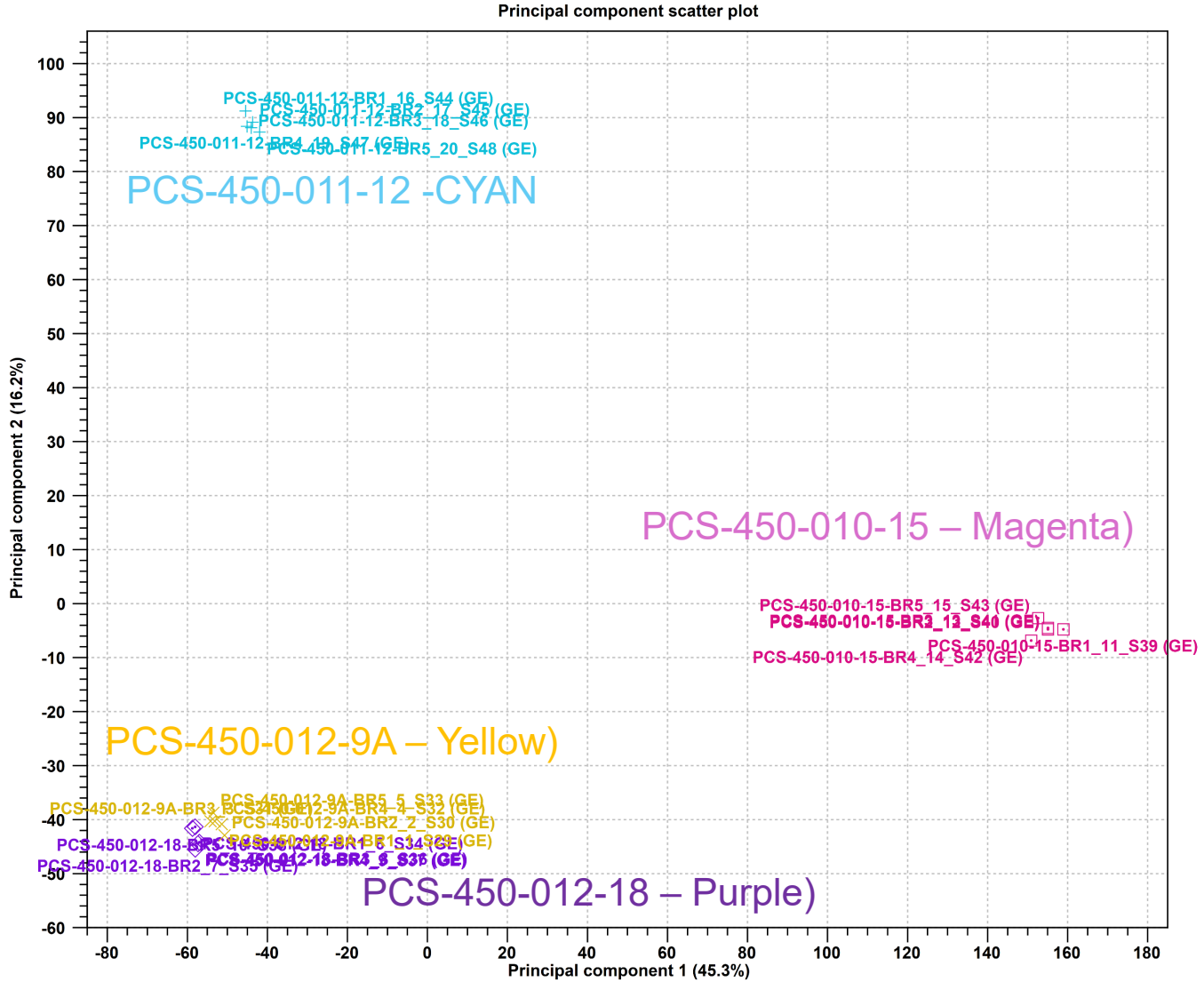


Hepatocytes Used in Omics Experiments



Cell Model	**HepatoXcell™ Pro**: 7-day plateable hepatocytes (ATCC® PCS-450-011™)
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

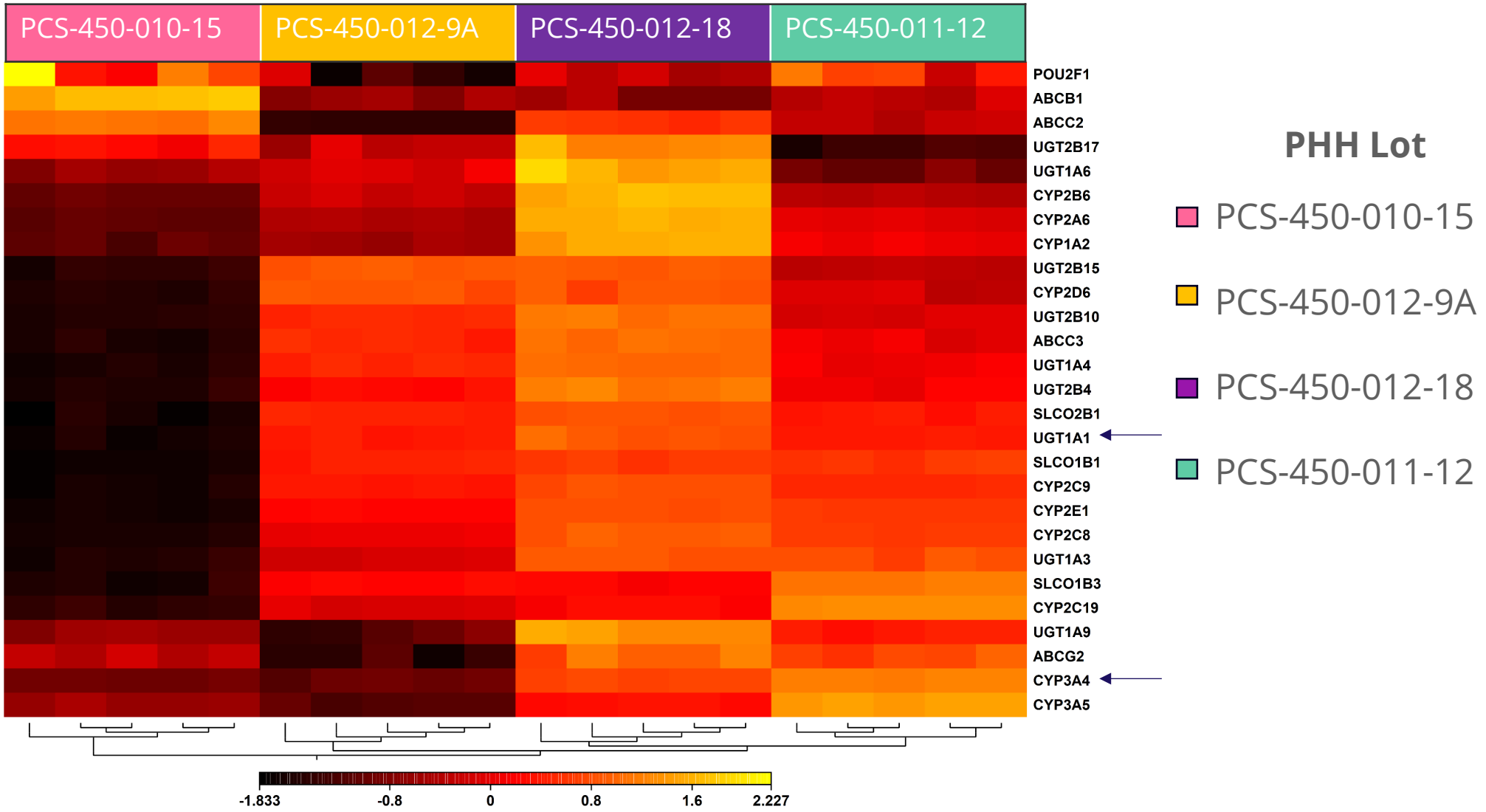
Donor-Specific Transcriptomic Signature in PHH



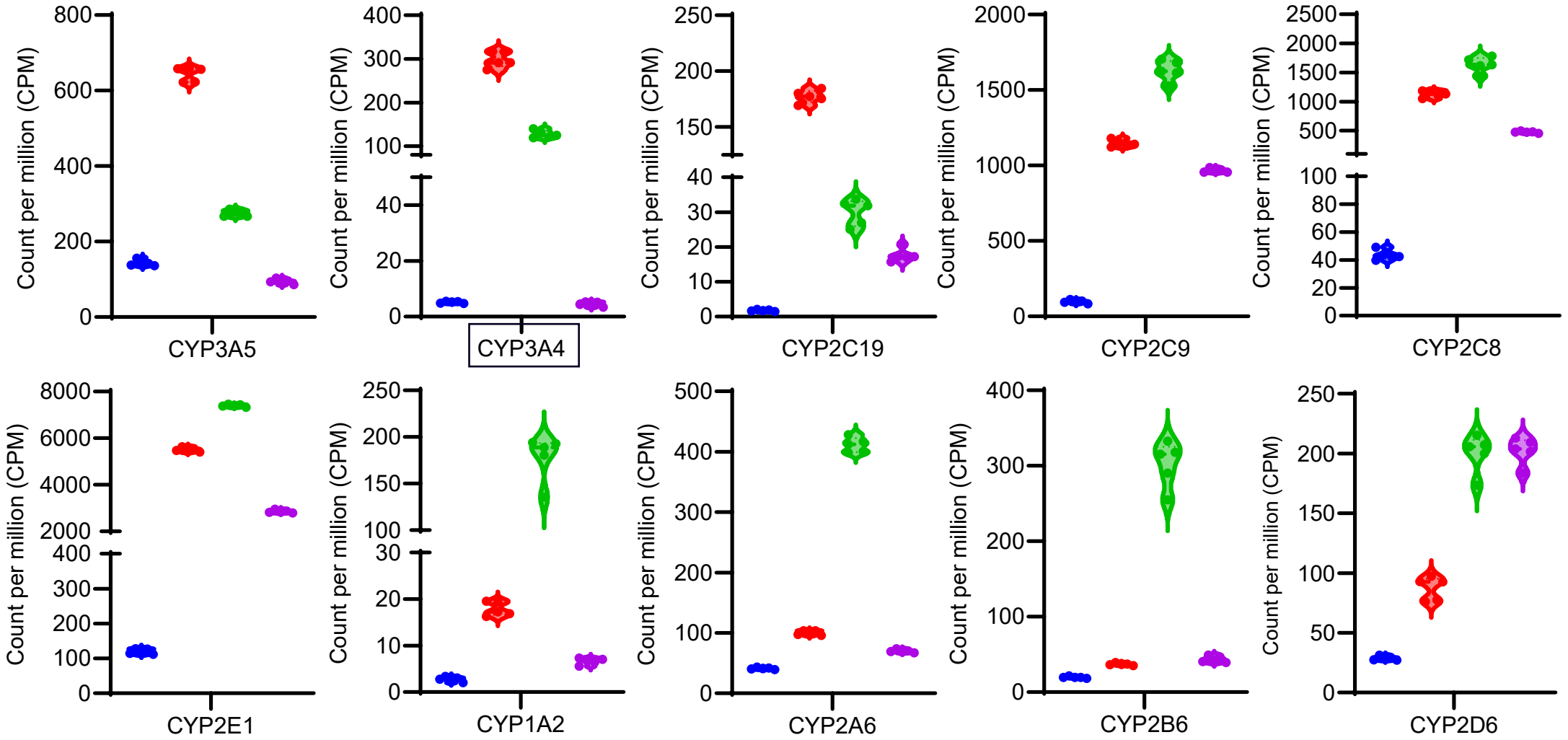
PHH Lot

- PCS-450-010-15
- PCS-450-012-9A
- PCS-450-012-18
- PCS-450-011-12

Cross-Donor Comparison of Key Gene Expression

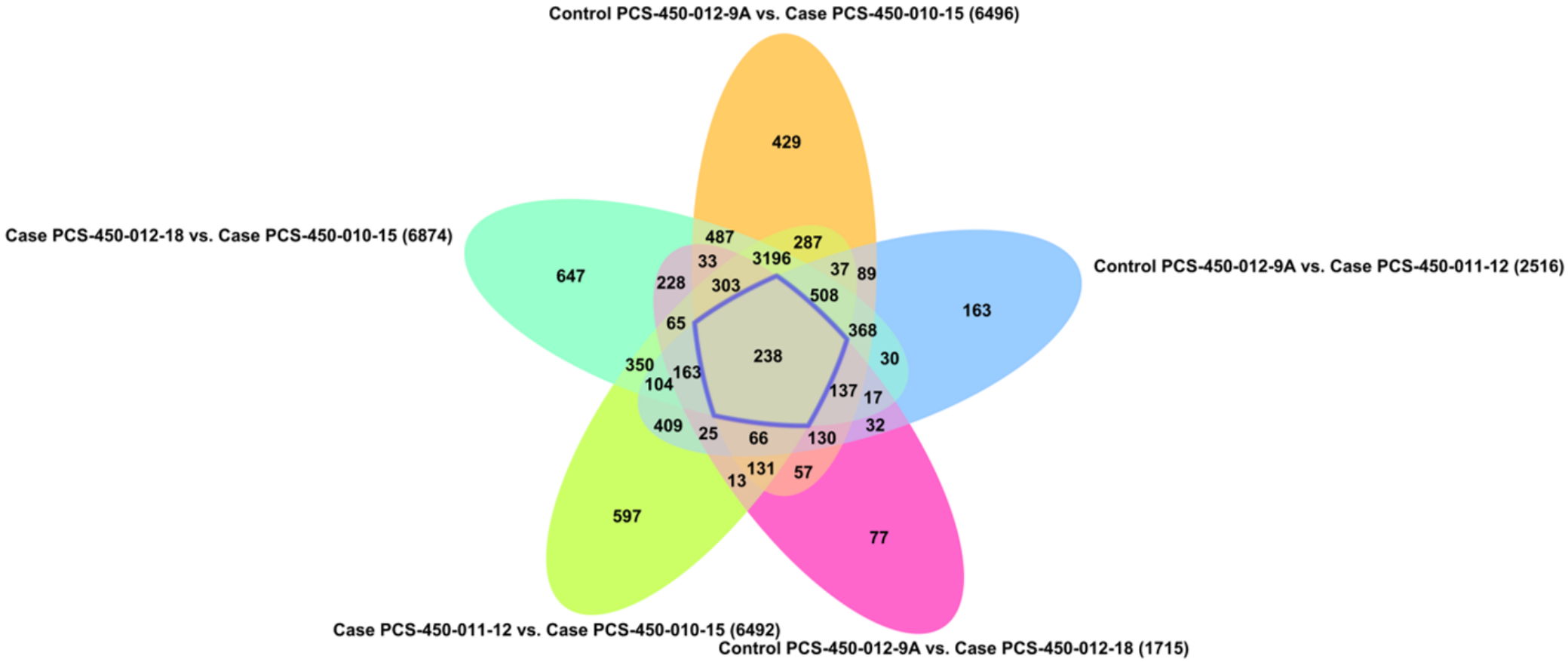


Donor-Dependent Shifts in CYP Gene Expression



Shared and Donor-Specific Gene Signature

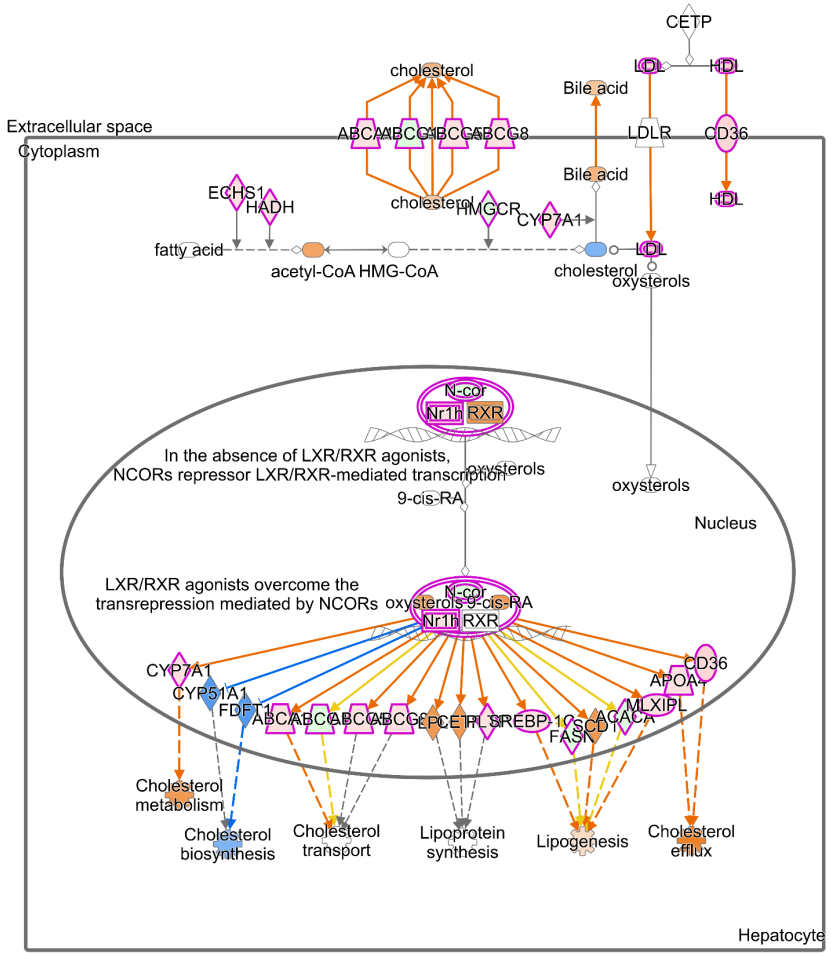
The Venn diagram pinpoints which gene expression changes are shared across donors versus uniquely donor-specific, enabling identification of stable biomarkers while revealing biological variability that influences PHH-based toxicity and metabolism studies.



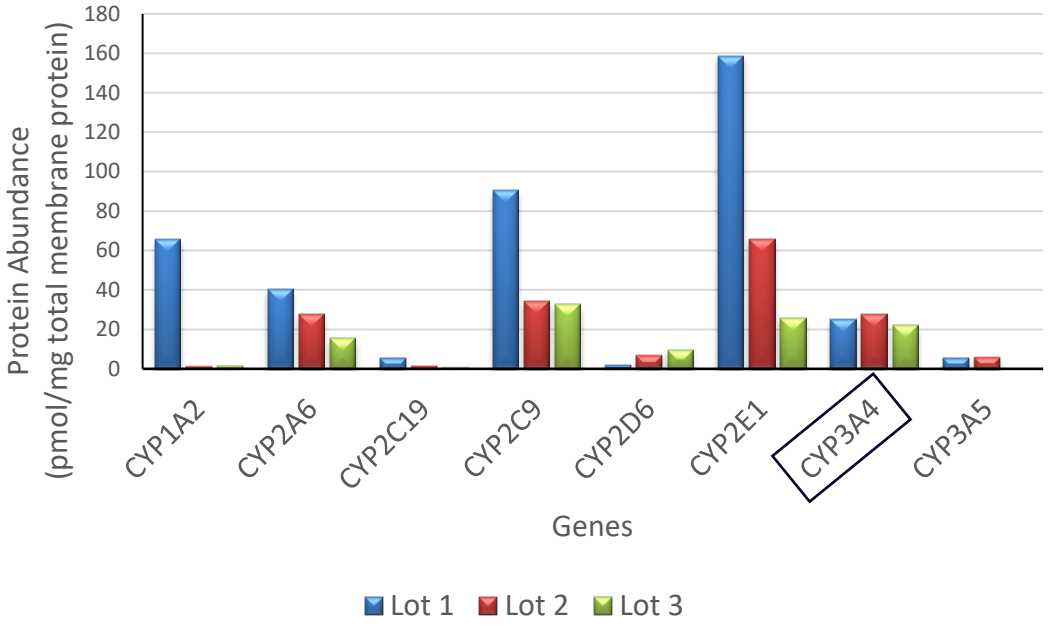
Enriched Canonical Pathways in PHH

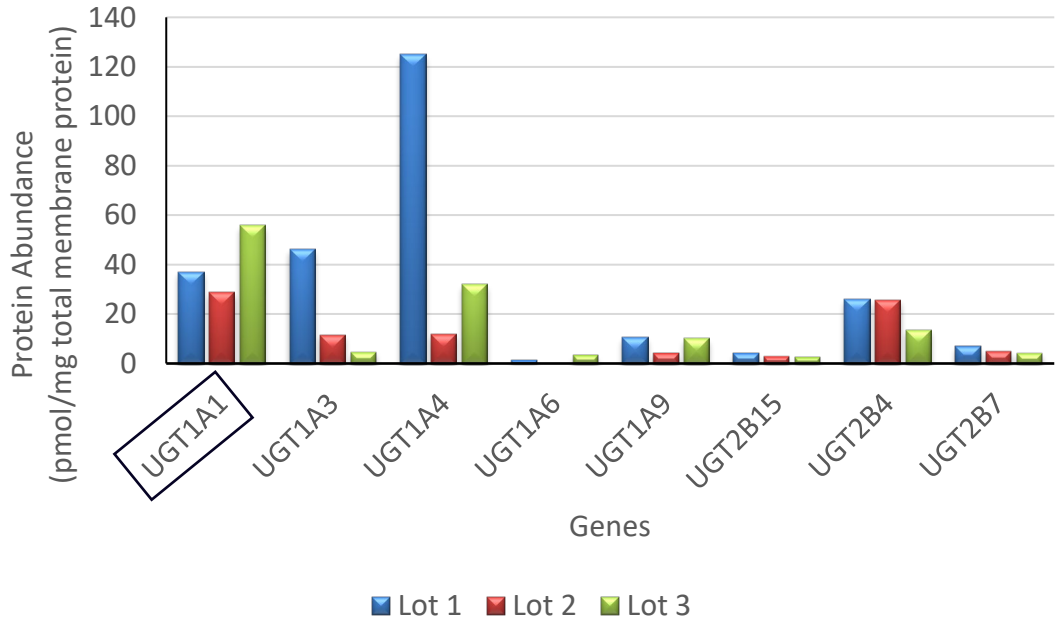
These pathways are enriched because they contain a disproportionately high number of differentially expressed genes, indicating that donor biology strongly influences key metabolic and regulatory networks in primary human hepatocytes.

Ingenuity Canonical Pathways	-log(p-value)	Ratio	z-score
LXR/RXR Activation	24.1	0.654	4.826
DHCR24 Signaling Pathway	21.5	0.608	4.249
Acute Phase Response Signaling	20.9	0.55	0.391
Neutrophil degranulation	20.5	0.423	2.97
Response to elevated platelet cytosolic Ca2+	19.6	0.606	3.85
Sirtuin Signaling Pathway	17.3	0.456	-0.606
Regulation of Insulin-like Growth Factor (IGF) transport and uptake by IGFBPs	17	0.589	4.154
Post-translational protein phosphorylation	16.2	0.607	4.158
Aspirin ADME	15.6	0.818	5.333
Phase I - Functionalization of compounds	14.1	0.571	5.939
Atherosclerosis Signaling	14	0.537	2.945
RHO GTPases Activate Formins	13.7	0.529	0.819
Warburg Effect Signaling Pathway	13.6	0.538	-1.477



Donor-Specific Differences in Metabolizing Enzyme Abundance



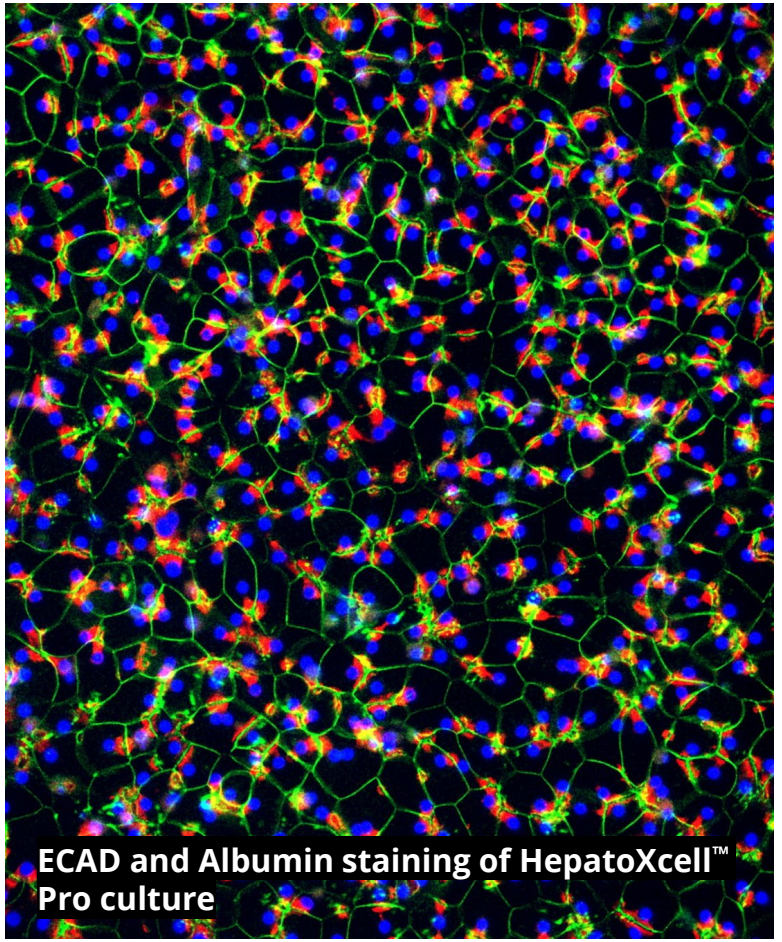


HepatoXcell™ Catalog #	Lot #
PCS-450-011-0012	1
PCS-450-011-0027	2
PCS-450-011-0037	3

CYP3A4 and UGT1A1 are ubiquitously expressed at both the mRNA and protein levels and serve as major drug-metabolizing enzymes: CYP3A4 mediates oxidative metabolism of ~50% of clinical drugs, while UGT1A1 catalyzes glucuronidation pathways essential for detoxification and clearance, together influencing drug exposure, efficacy, and risk of adverse effects.

Summary

- HepatoXcell™ demonstrates
  - High-viability, liver-relevant performance
  - End-to-end, batch-level quality validation
  - Multi-donor biology for real-world relevance
  - Amenable to many formats including 3-D
- Key genes involved in hepatic function and metabolism show donor dependent expression shift
- Protein abundance corresponds with the interdonor differences seen in mRNA expression



ECAD and Albumin staining of HepatoXcell™ Pro culture

ATCC®

CREDIBLE LEADS TO INCREDIBLE

Questions