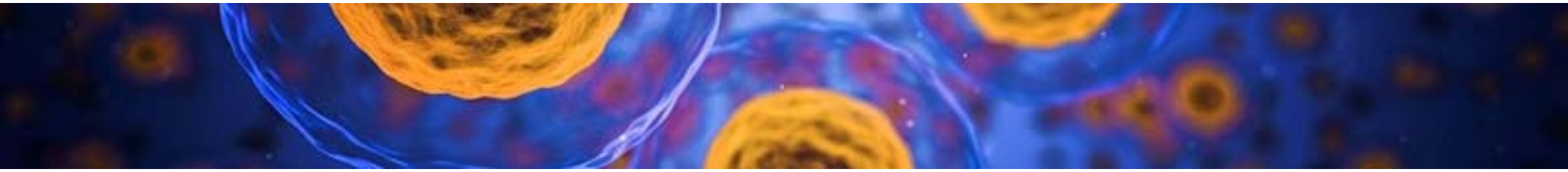


3D Cell Culture Models for Drug PK, Safety, and Efficacy Assessment

FDA and M-CERSI Collaborative Workshop

CERSI
University of Maryland
Center of Excellence in Regulatory Science and Innovation

Co-Organizer

Hongbing Wang Ph.D
Professor and Program Chair,
Dept. of Pharmaceutical Sciences
University of Maryland
School of Pharmacy
20 Penn Street, Baltimore MD
Tel: 410-706-1280
hongbing.wang@rx.umaryland.edu

Co-Organizer

Shiew-Mei Huang, PhD
Deputy Director
Office of Clinical Pharmacology
Office of Translational Sciences
CDER, FDA
301-796-1541
iPhoe 240-401-0739
shiewmei.huang@fda.hhs.gov

M-CERSI Director

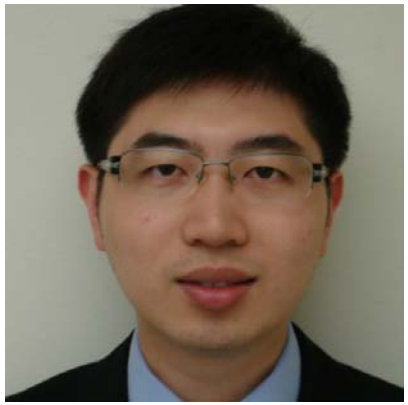
James E. Polli, Ph.D.
Professor and Ralph F. Shangraw/Noxell Endowed Chair in Industrial Pharmacy and Pharmaceutics
University of Maryland School of Pharmacy
20 Penn Street, Baltimore, MD 21201
410-706-8292
jpolli@rx.umaryland.edu

Logistics Checklist

- Panelists: Please keep your mic **muted** when you are not talking.
- Presenters: Please keep your presentation within **10 minutes** and say "**Next**" to advance slides.
- Attendees: Please use the "**Chat Box**" to ask questions, the Q&A will not be monitored. Questions will be addressed during panel discussion.
- All Participants: Please set your **video to off** during the meeting, including when you speak (to "save" bandwidth).

Session One:

3D Models in Drug Safety and Risk Assessment



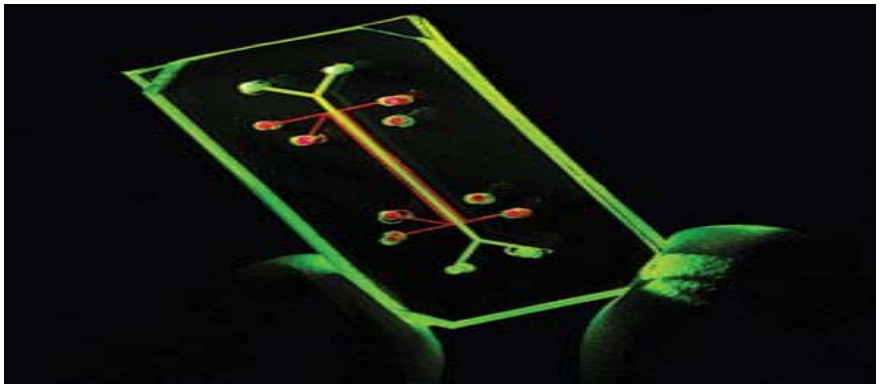
Session Chair: Dr. Edwin Chow FDA

U.S. FOOD & DRUG ADMINISTRATION

Clinical Pharmacology Reviewer
Office of Clinical Pharmacology | Office of Translational Sciences
Center for Drug Evaluation and Research | U.S. FDA
Edwin.Chow@fda.hhs.gov

Overview of 3D Cellular Model Research at FDA

Suzanne Fitzpatrick, PhD, DABT, ERT
US Food and Drug Administration



History of FDA's Involvement with MPS

- In 2010 FDA and NIH Common Fund awarded grant money to Wyss to develop a heart-lung micromachine.
- In 2011, DARPA approached the FDA Office of the Chief Scientist requesting to work together to develop a human body on a chip for medical countermeasures.
- In 2011 DARPA funded MPS research. DARPA involved the FDA from the beginning of the MPS program to help ensure that regulatory challenges of reviewing drug safety and efficacy are considered during development of the MPS platform
- In 2012 NCATS funded the Tissue Chip Development Program. FDA has been a partner throughout the program
- And the rest is MPS history!
- IMPORTANT LESSON-Critical to have regulators at the table from the beginning if aim is to use method for regulatory use

Alternative Methods Working Group (AMWG)

- Under Office of Chief Scientist, Office of Commissioner
  - Chaired by Drs. Fitzpatrick (CFSAN) and Mendrick (NCTR), members from each Center and OCS
- Strengthen FDA's long commitment to promoting the development and use of new technologies and to reduce animal testing
- Discuss new alternative *in vitro/in silico/in vivo methods* across FDA
- Interact with U.S. Federal partners and other global stakeholders to facilitate discussion and development of draft performance criteria for such assays.
- https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda

AMWG First Case Study – *In vitro* Micro physiological Systems

- Define agreed-upon terminology for MPS and research/regulatory gaps for which MPS may be useful.
- Identify partnerships to advance MPS technology.
- Develop draft performance criteria for MPS and discuss internally and then with stakeholders
- Develop mechanisms to request information from MPS developers and end users

FDA Draft Definition

Microphysiological System (MPS) is an in-vitro platform composed of cells, explants derived from tissues/organs, and/or organoid cell formations of human or animal origin in a micro-environment that provides and supports biochemical/electrical/mechanical responses to model a set of specific properties that define organ or tissue function.

Organ-on-a-chip (OoC) is a miniaturized physiological environment engineered to yield and/or analyze functional tissue units capable of modeling specified/targeted organ-level responses.
Comments- send to alternatives@fda.hhs.gov

FDA Internal Research- FDA User Group

- FDA scientists are developing in-house MPS and collaborating with several external partners

Start with a Regulatory Question-Context of use

- What question needs to be answered and for what purpose?
  - How much “validation/qualification” is needed for a particular assay will depend on the particular context of use.

  - Helps define acceptable applicability domain and limitations
  - Context could be expanded over time

Remember-Change Takes Time- But It will Happen If We All Work Together

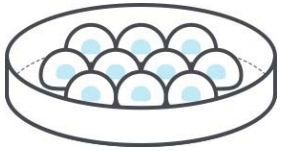
emulate

Organs-on-Chips Application in Drug PK, Safety and Efficacy Evaluation

Lorna Ewart, Ph.D.

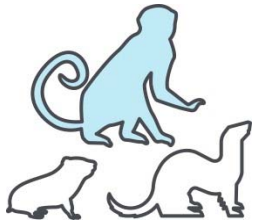
August 14, 2020

Challenges in Conventional Culture & Animal Models



Conventional Culture Models:

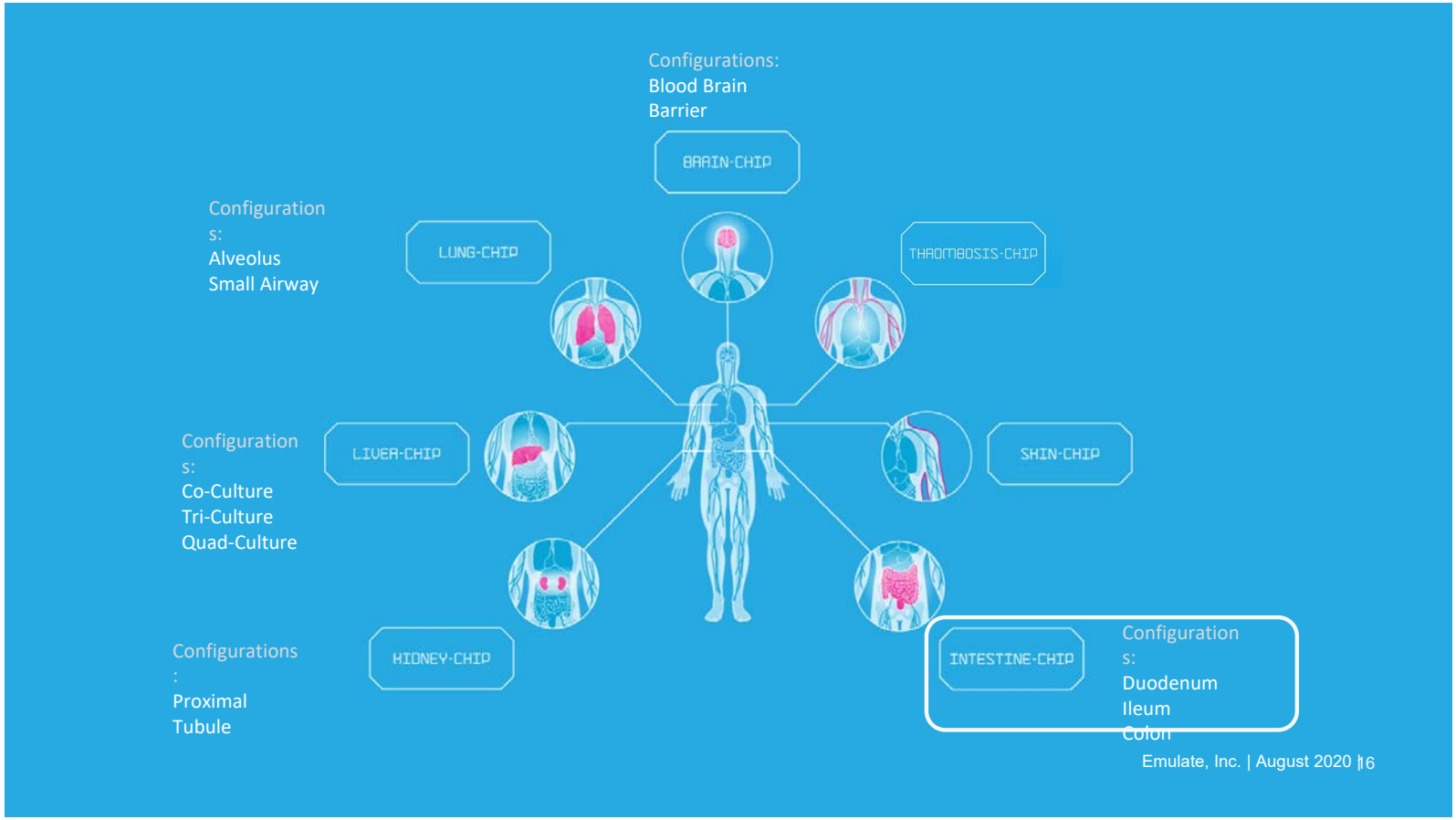
Limited biological functionality and relevance to *in vivo* physiology



Animal Models:

Species differences can preclude accurate extrapolation to clinic

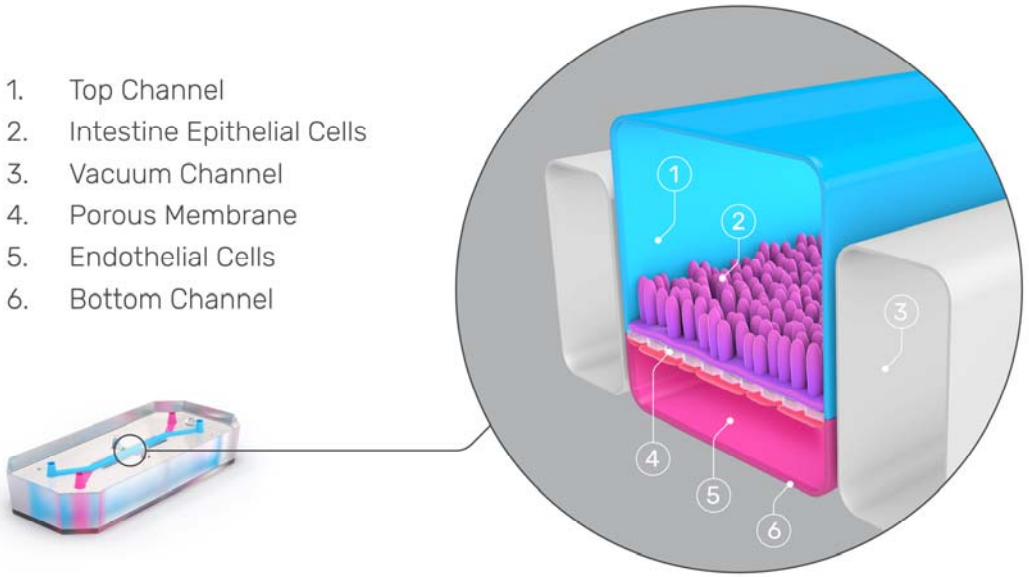

Organ-Chips:
A Window Into
Human Biology
Unprecedented level of biological function allowing transparency into complex human biology and disease mechanisms.
Emulate, Inc. | August 2020 | 15

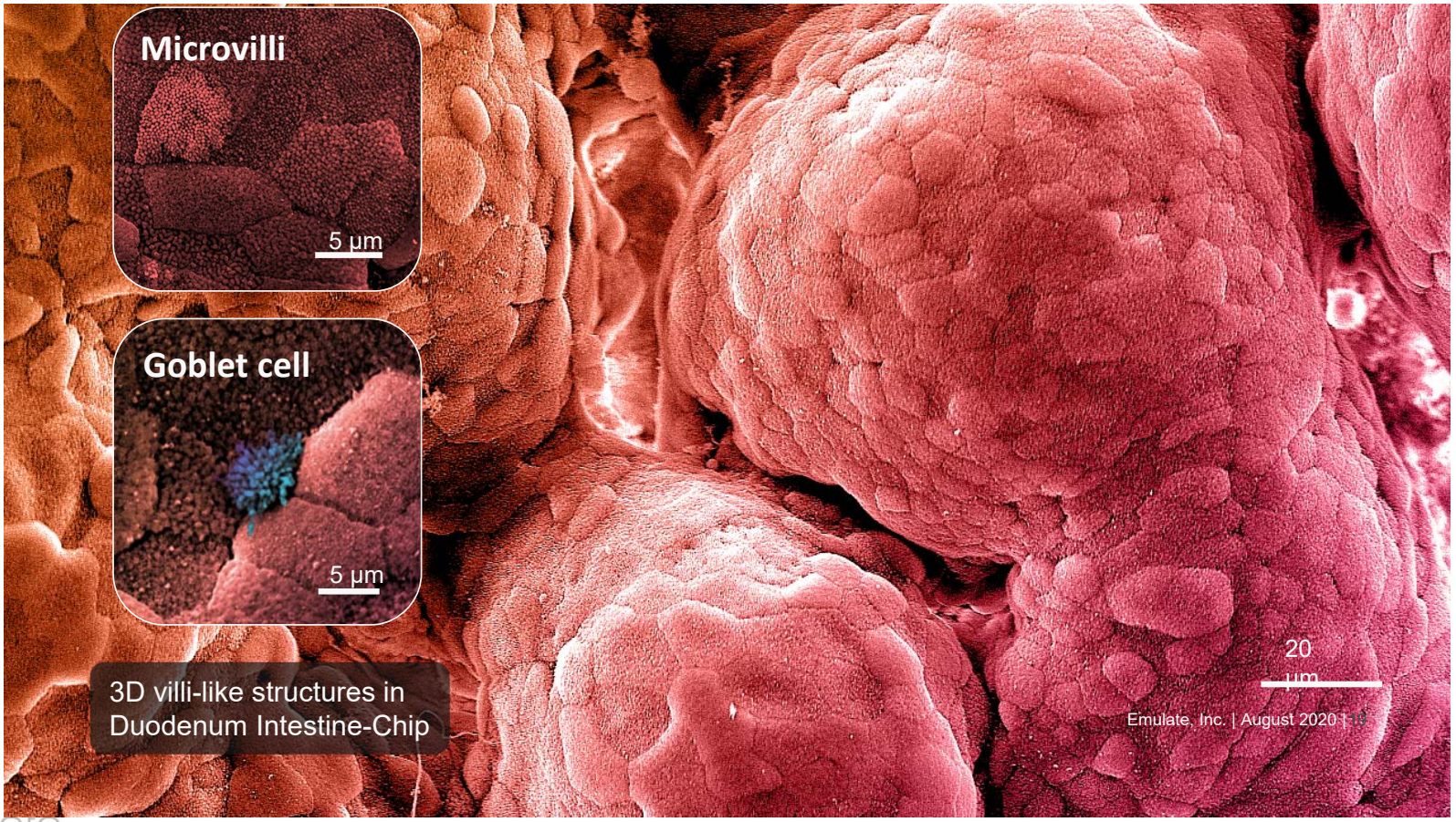

Configurations:
Blood Brain
Barrier
BRAIN-CHIP
Configuration
s:
Alveolus
Small Airway
LUNG-CHIP
THROMBOSIS-CHIP
Configuration
s:
Co-Culture
Tri-Culture
Quad-Culture
LIVER-CHIP
SKIN-CHIP
Configurations
:
Proximal
Tubule
KIDNEY-CHIP
INTESTINE-CHIP
Configuration
s:
Duodenum
Ileum
Colon

Characterization of Intestine-Chip

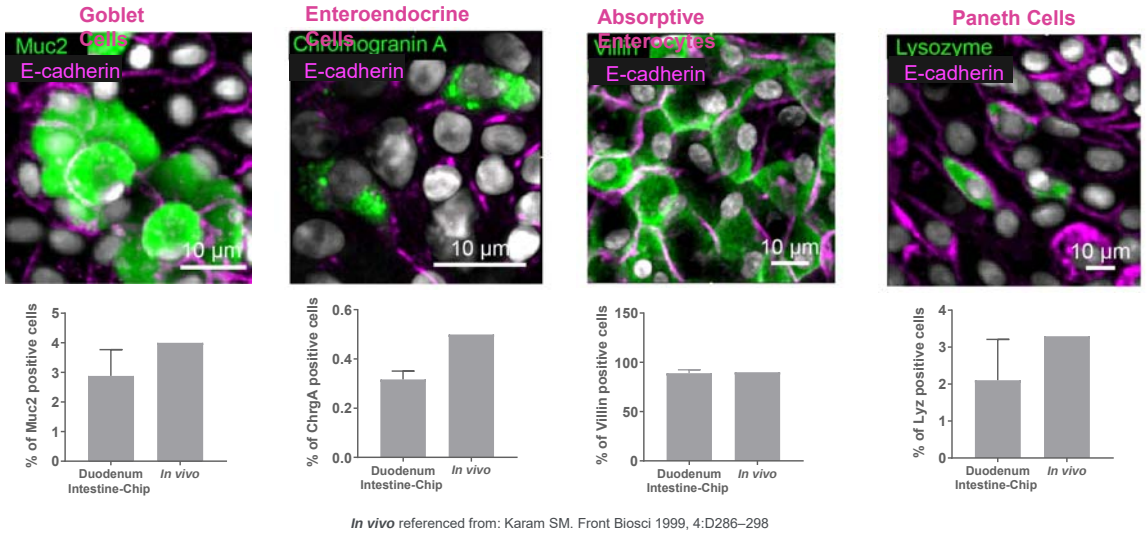
Intestine-Chip Configuration

1. Top Channel
2. Intestine Epithelial Cells
3. Vacuum Channel
4. Porous Membrane
5. Endothelial Cells
6. Bottom Channel




Microvilli
5 µm
Goblet cell
5 µm
3D villi-like structures in
Duodenum Intestine-Chip
20 µm
Emulate, Inc. | August 2020 | 19

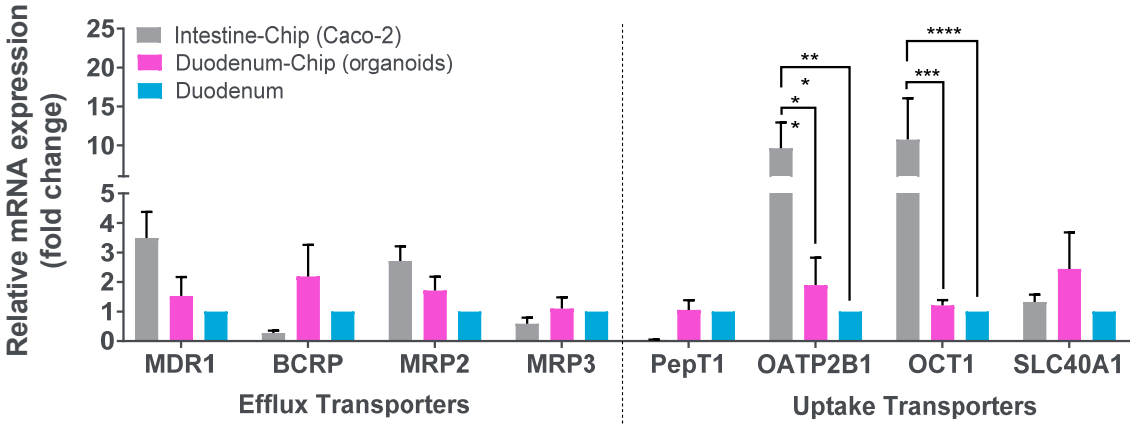
Cell Populations in the Duodenum Intestine-Chip



In vivo referenced from: Karam SM. Front Biosci 1999, 4:D286–298

Morphological markers demonstrate the Duodenum Intestine-Chip contains all key cell populations at *in vivo*-relevant ratios

Transporter Expression in the Duodenum Intestine-Chip



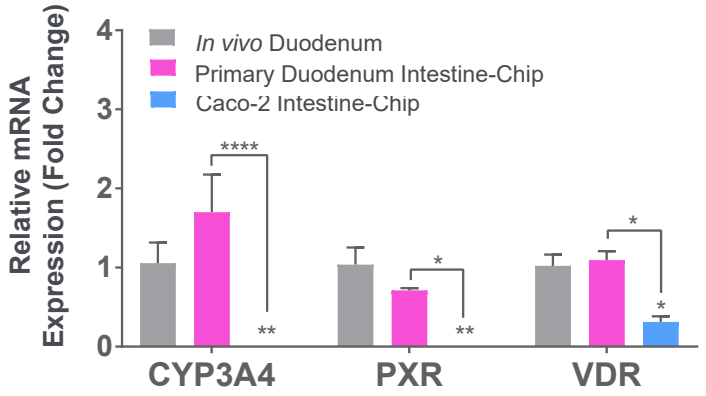
Data from Caco-2 Intestine-Chips, three donor-specific Duodenum Intestine-Chips and the duodenal tissue of three donors, n=3

Demonstrated expression of major intestinal drug transporters, with average expression of OATP2B1 and OCT1 closer to *in vivo* than observed in Caco-2 Intestine-Chips

emulate

Nuclear Receptors in the Duodenum Intestine-Chip

Expression of CYP3A4, PXR, and VDR in Duodenum Intestine-Chip is closer to *in vivo* versus Caco-2-Chip. Caco-2 cells lack PXR expression, limiting utility for drug metabolism, drug transport, and drug-drug interaction studies

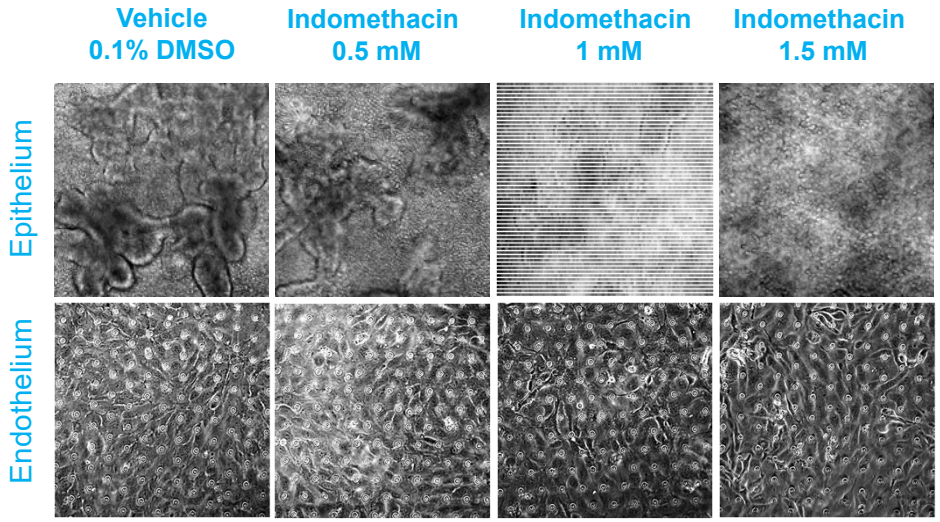


Data from Caco-2 Intestine-Chips, three donor-specific Duodenum Intestine-Chips and the duodenal tissue of three donors, n=3

emulate

Intestine-Chip for Safety Evaluation

Indomethacin Toxicity in Duodenum Intestine-Chip: Morphological Changes

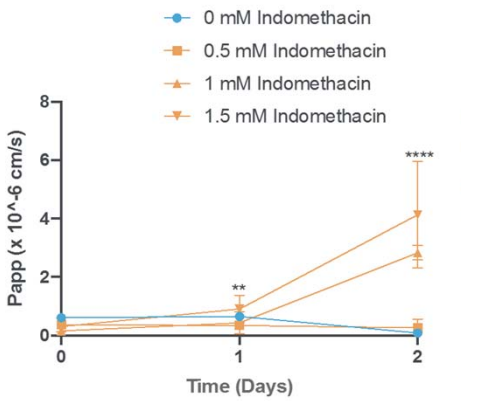


Indomethacin induced significant injury – blunting of villi-like structures and appearance of apoptotic cells at concentrations of 1 mM and higher

Emulate, Inc. | August 2020 | Unpublished Data 34

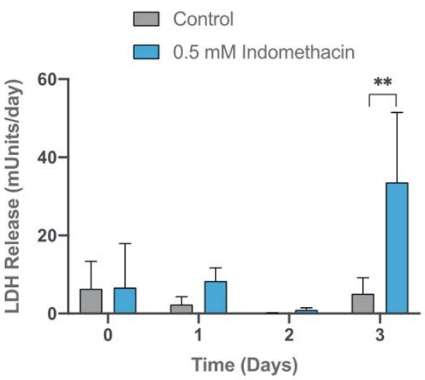
Proof-of Concept Data for Toxicity Testing Application: Indomethacin

Barrier Function



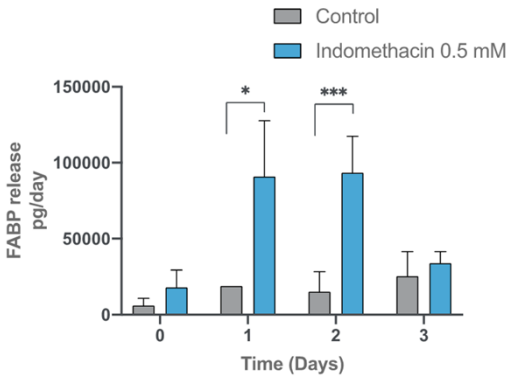
n = 3, Two Way ANOVA, ** p < 0.005, **** p < 0.0001

Viability



n=3, 2 way ANOVA, **p<0.005,
Compared to control on the day tested

Biomarker

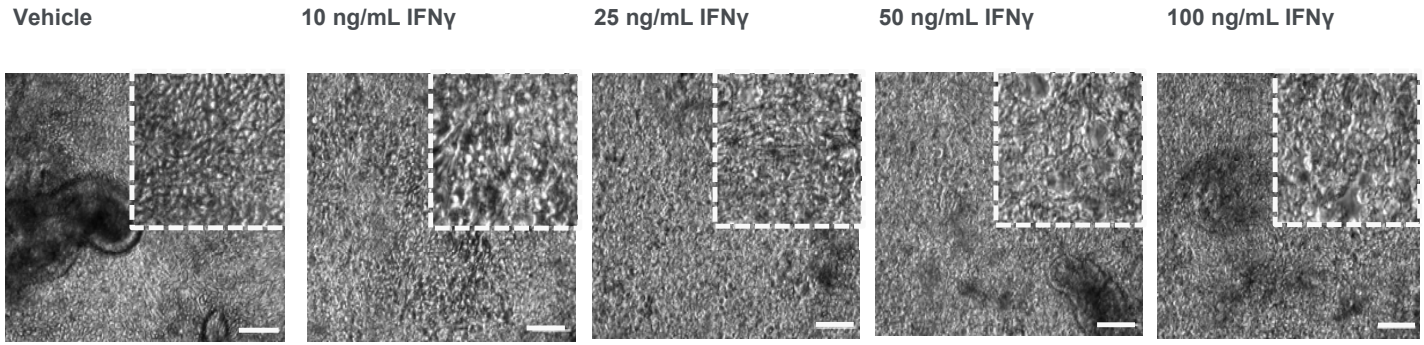


n=3, 2 Way ANOVA, *p<0.05, ***p<0.005,
Compared to control on the day tested

Indomethacin toxicity demonstrated loss in apparent permeability indicating loss of barrier function, increase in LDH release, and increase in I-FABP (intestinal fatty acid-binding protein) indicative of mucosal damage

Intestine-Chip for Efficacy Evaluation

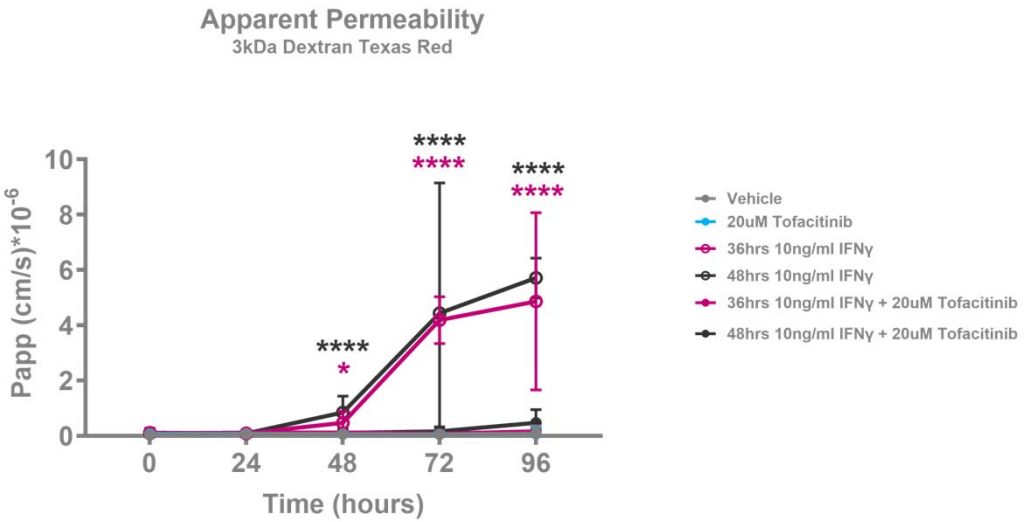
IFNγ Mediated Degeneration of Epithelial Cell Morphology



Scale bar: 100um

A compromised morphology of epithelial cells in the Colon Intestine-Chip is observed 48 h post-stimulation with IFNγ

Effect of Tofacitinib on Epithelial Barrier Integrity Upon Stimulation with IFNγ

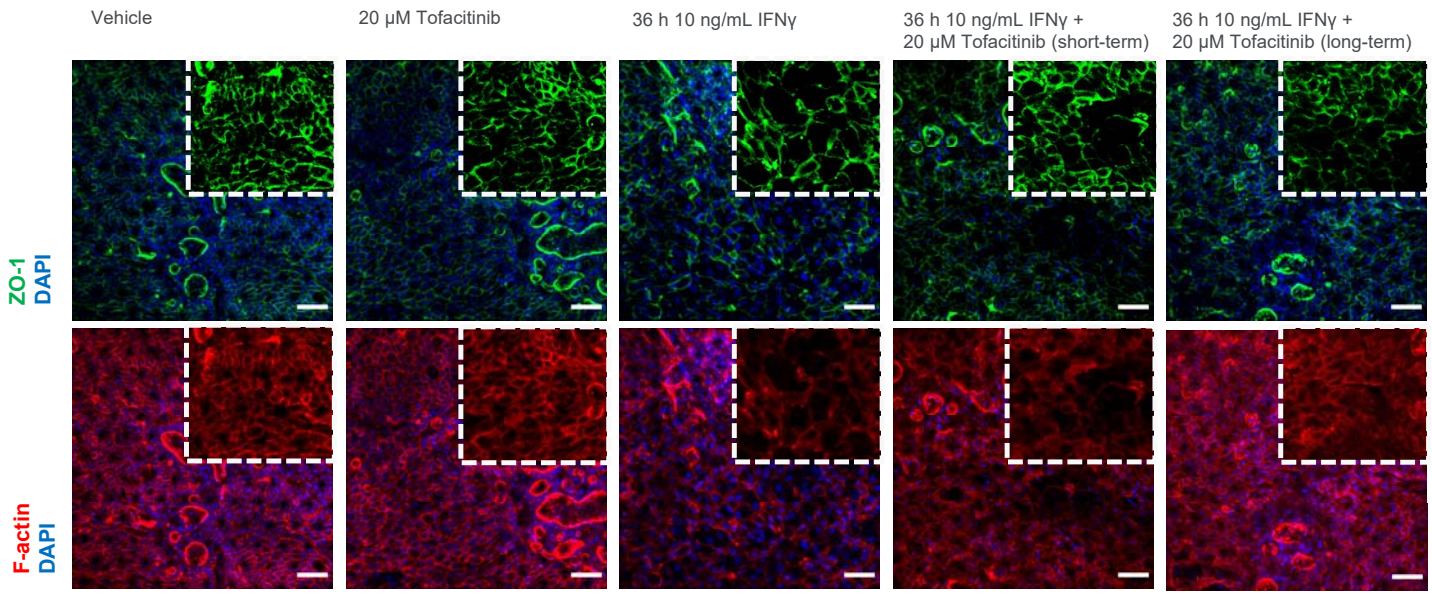


N=3 chips/group
Mean±95%CI
Two-way ANOVA, Tukey's post hoc test
Ns: p>0.05, *: p<0.05, **: p<0.01, ****: p<0.0001

A time dependent loss of the epithelial barrier integrity is observed upon treatment with 10 ng/mL IFNγ. Prophylactic treatment with 20 µM Tofacitinib prevented the increase in epithelial apparent permeability.

Emulate, Inc. | August 2020 | Unpublished Data

Effect of Tofacitinib on IFNγ Mediated Disruption of Epithelial Tight Junctions



Co-treatment with Tofacitinib and IFNγ abrogated the loss of the epithelial tight junction integrity, as indicated by staining against ZO-1 and F-actin at the end of the culture.

Emulate, Inc. | August 2020 | Unpublished Data

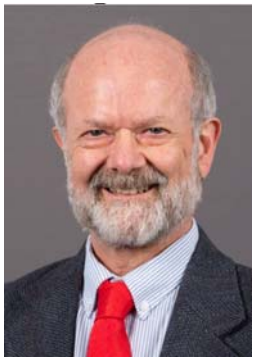
Summary

- Organ-Chips have a valuable place in drug discovery for evaluation of PK, Safety and Efficacy
- The Intestine-Chip models derived from organoids, display physiologically relevant characteristics that are analogous to in vivo settings
- Further mechanistic data, with multiple compounds, will illustrate the superiority of these models compared to more conventional approaches

Considerations About 3D Culture Models for Nonclinical Safety Evaluation

Ronald Wange and Paul C. Brown,
Associate Director for Pharmacology and Toxicology
Office of New Drugs
CDER/FDA

August 14, 2020



R Wange

P Brown

This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

Highlights

- Regulations allow alternatives
- Guidance allows alternatives
- Useful assays are those that meet a data need
- Data are needed to show an assay does what is claimed
- Multiple ways to talk to FDA

Regulations allow submission of alternative methods

IND regulations

21 CFR 312.23 (a)(8) *Pharmacology and Toxicology Information*

"Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or in vitro, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations."

NDA Regulations have similar wording.

Guidances allow submission of alternative methods

Example ICH guidance wording:

"…consideration should be given to use of new in vitro alternative methods for safety evaluation. These methods, if validated and accepted by all ICH regulatory authorities, can be used to replace current standard methods."

ICH M3(R2)

Some guidances explicitly describe alternative approaches

- ICH S3 Q&A - microsampling
- ICH S5(R3) - in vitro, ex vivo and nonmammalian embryofetal toxicity
- ICH S10 - in chemico and in vitro phototoxicity
- Draft Nonclinical Immunotoxicity guidance– in silico, in chemico and in vitro skin sensitization methods

Other alternatives routinely accepted

- Ocular irritation - OECD Guidelines 437, 438, 460, 491, 492 (Reconstructed human Cornea-like Epithelium), 494 (Vitrigel-Eye Irritancy Test Method)
- Skin irritation – OECD Guideline 439 (In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method)

CDER experience with complex in vitro models in regulatory applications

Searched CDER's electronic document room for study reports in section M4 of IND/NDA/BLAs

- ***Microphysiological*: 13 results – but all are just in literature references**
- ***Liver chip*: 2 results – but just in a discussion about possible follow up studies**
- ***Reconstructed human epidermis*: 115 – mostly skin corrosivity and irritation**
- ***Organoids*: 61 results – mostly pharmacology; examples: bronchial epithelium, intestinal (including from patients with disease), retinal**
- ***Spheroids*: 566 results – many are histopath results; 66 in "other toxicity studies" – mostly hepatocyte, also thyroid, and angiogenic assays**
- ***iPSC*: 145 results – mostly pharmacology; hepatocytes, neurons, cardiomyocytes**

- **No hits for organ chip, tissue chip, brain chip, kidney chip, microbrain, microphysiological systems**

Moving toward regulatory use

- Does an assay provide data that can be used to answer fundamental drug development questions?
- Is the assay mature enough?
  - Stable platform, cells
- What endpoints are being measured?
  - Are they predictive of in vivo effects?
  - Translatable to human?
- Has scientific validity been shown?
  - Is it reproducible?
  - What test compounds have been assessed?
    - Need compounds with in vivo data
    - Positives and negatives
- Applicability domain
  - Define compounds the assay can assess and not assess
- Criteria for success
  - What are sensitivity and specificity?

“Pre-regulatory” Opportunities

- No FDA “acceptance” is required in drug discovery
- Increased understanding of disease processes and identifying promising interventions
- Early screening and derisking for toxicity
- Early use of such models can contribute to the 3Rs by reducing iterative cycles of drug candidate selection

Context of use

- What question needs to be answered and for what purpose?
  - How much “validation/qualification” is needed for a particular assay will depend on the particular context of use.

  - Helps define acceptable applicability domain and limitations
  - Context could be expanded over time

Submitting drug development data to the FDA

- There are no preset requirements for submitting in vitro data to a drug application.
- A method does not have to be formally validated before it is submitted.
- When assessing in vitro data submitted to the agency, reviewers consider how scientifically valid the information is for the particular purpose based on supporting information.

Advancing Alternative Methods at FDA

- FDA has a page on the FDA External Site (www.fda.gov) on Advancing Alternative Methods
- Includes information on
  - The Alternative Methods Working Group
  - FDA Webinar Series on Alternative Methods
  - Draft Definitions of MPS and tissue-on-a-chip
  - FDA publications
- Comments can be sent to alternatives@fda.hhs.gov

- Sponsors are encouraged to discuss with FDA the potential use of NAMs
  - AMWG webinars
  - Pre-IND meetings/written responses
  - Critical Path Innovation Meetings – outside of a regulatory application
  - CDER is exploring other possible pathways (stay tuned)

paul.brown@fda.hhs.gov


FDA
U.S. FOOD & DRUG
ADMINISTRATION

Session Two:

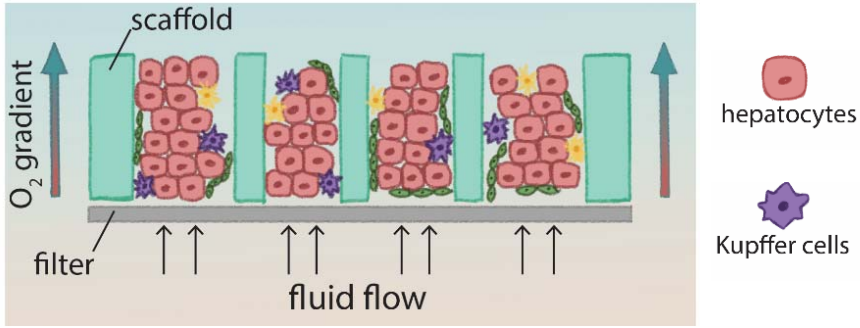
3D In vitro Liver Models for DILI

Session Chair: Dr. Qi Liu

Senior Science Advisor
Office of Clinical Pharmacology | Office of Translational Sciences
Center for Drug Evaluation and Research | U.S. FDA
Qi.Liu@fda.hhs.gov

U.S. FOOD & DRUG ADMINISTRATION

Liver-on-chip model for toxicity and PK



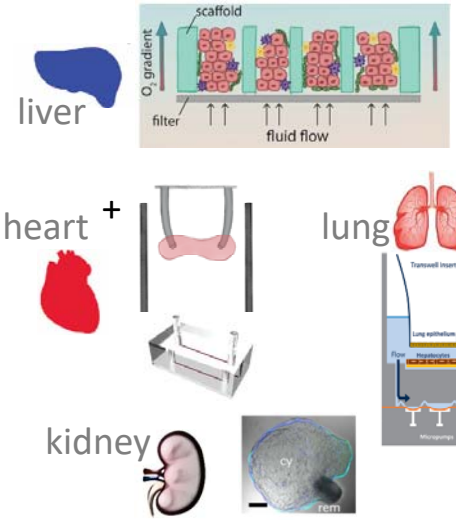
Ribeiro, AJS et al. Clin Pharmacol & Ther. 2019

Alexandre Ribeiro, PhD
Division of Applied Regulatory Science
Office of Clinical Pharmacology
Office of Translational Sciences
CDER, FDA

- Disclaimer: The opinions expressed in this presentation are mine and do not necessarily reflect the official views of the U.S. Food and Drug Administration (FDA)
- No conflicts of interest

Scientific Innovations → Drug Development Applications

Cellular Microsystems



Endpoints

- cell type specific function
- toxicity
- transport
- adsorption
- distribution
- metabolism
- cellular respiration
- toxicity
- biomarkers
- gene expression
- mechanism of action

human cells

iPSCs

Predict and detect human- specific drug effects

in vitro

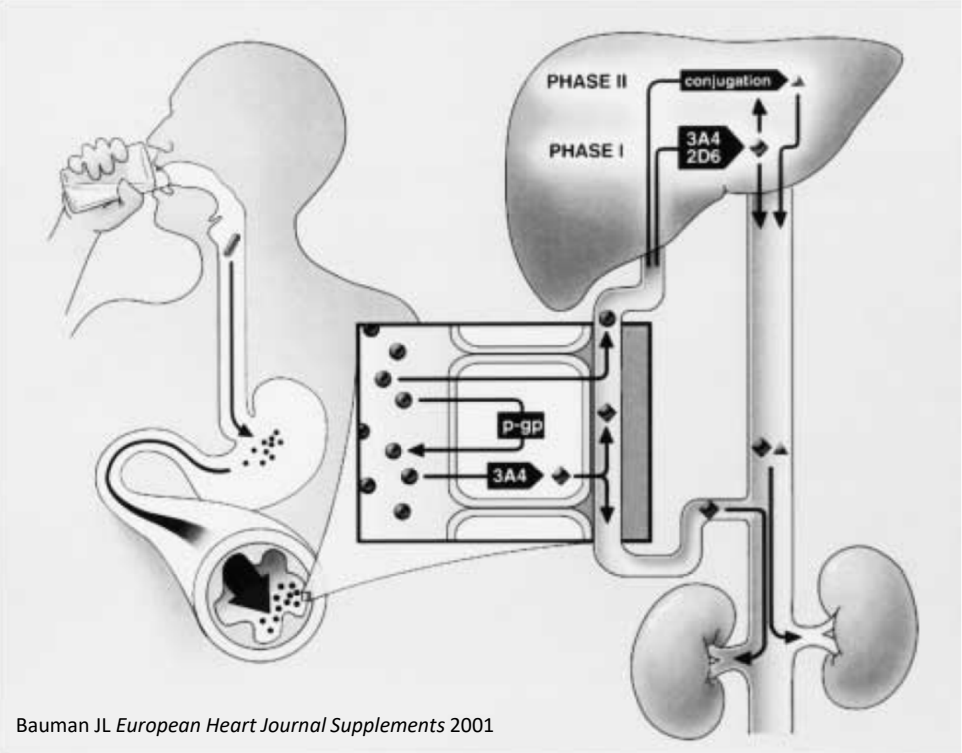
Organ-specific contexts of use:

- pharmacology
- toxicity
- mechanism of action
- efficacy
- safety
- new drugs
- generic drugs

In addition to toxicity, the liver is a key organ to model pharmacokinetics

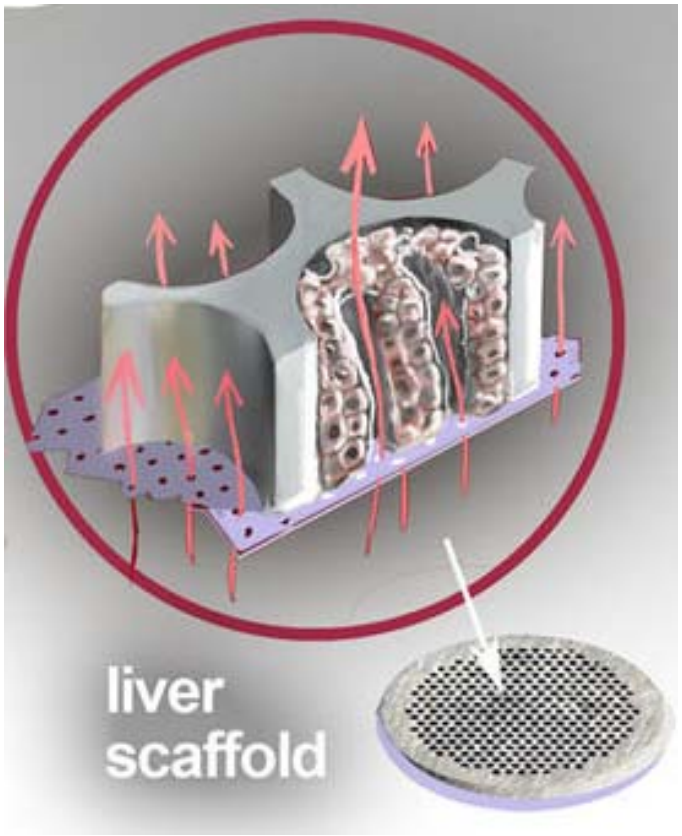
Liver:

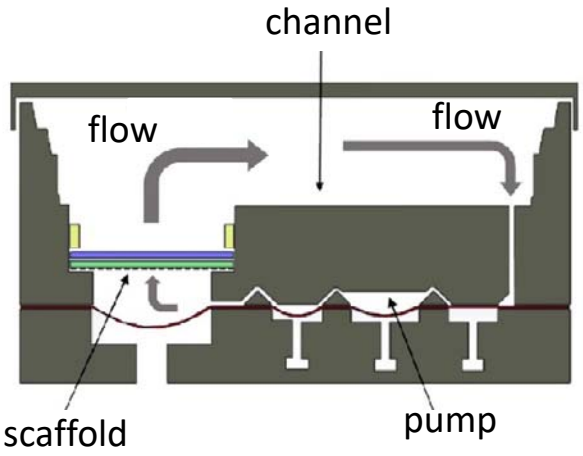
- drug metabolism
- generation of toxic or efficacious drug metabolites
- drug clearance
- drug-drug interactions
- data needed for PBPK models and for IVIVE

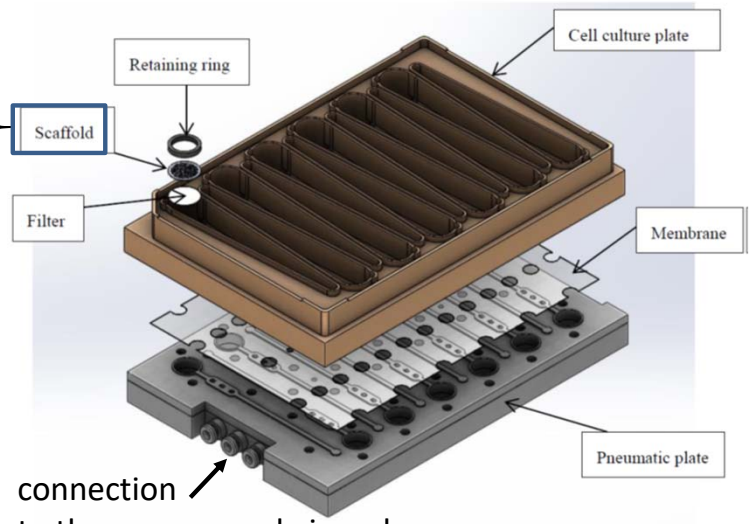


Bauman JL *European Heart Journal Supplements* 2001

Liver system cultures hepatic cells in 3D and under flow



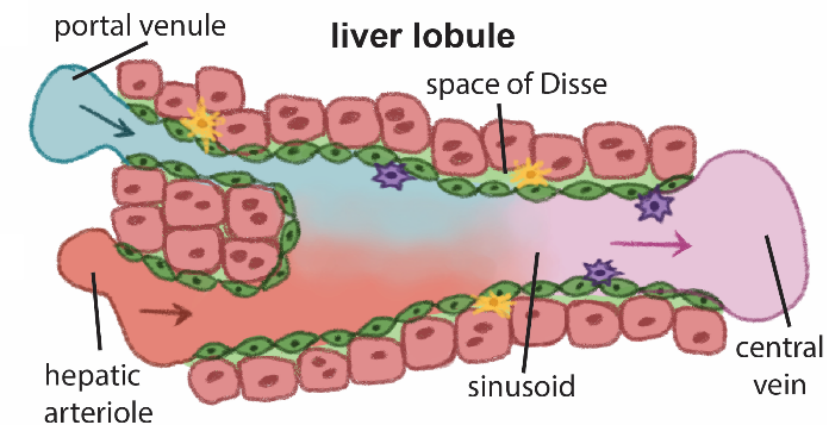




connection
to the compressed air and vacuum

Edington, CD et al., Sci Rep, 2018

The liver microenvironment is 3D, under fluid flow and multicellular



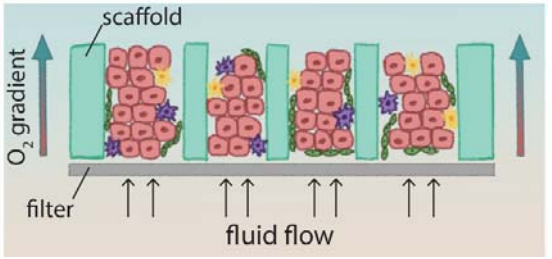
Ribeiro, AJS et al. Clin Pharmacol & Ther. 2019

Liver microphysiological systems:

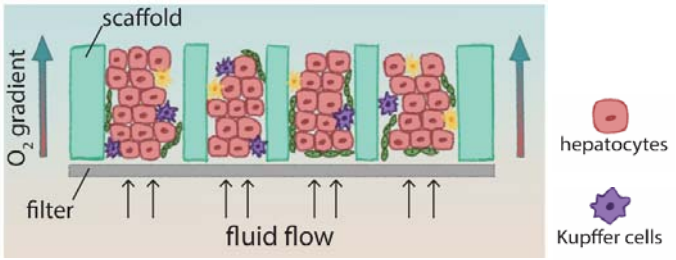
- long-lasting hepatic properties
- heterogeneity of cell types
- universally reliable and robust properties

Opportunities from prolonged function:

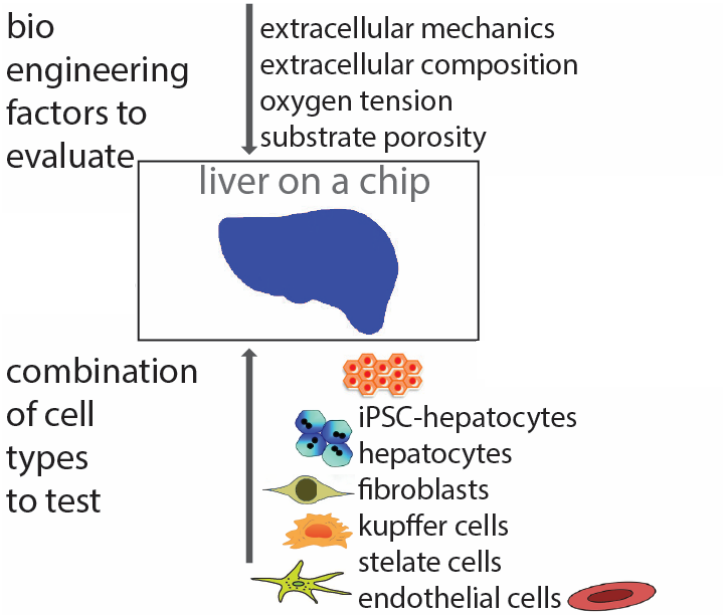
- predict toxic chronic effects
- long-term effects on metabolism
- model multiple or long-term dosing
- model slow drug clearance



Developers aim to establish handling procedures and culture protocols to optimize system use



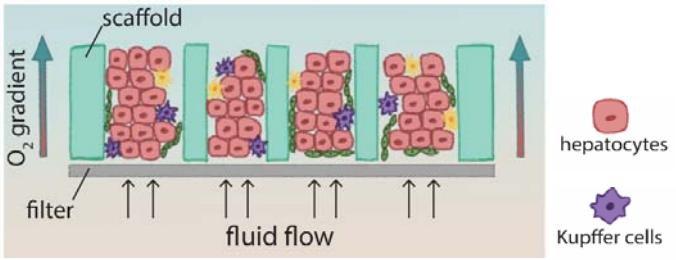
Ribeiro, AJS et al. Clin Pharmacol & Ther. 2019



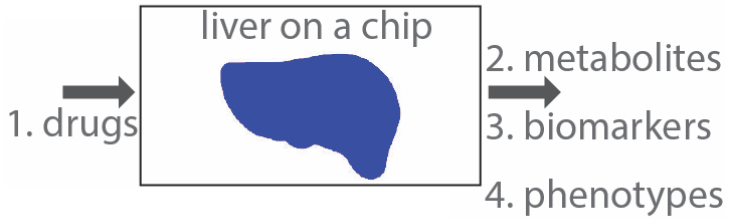
CDER-DARS laboratories focus on evaluating the potential of these systems for use in drug development

- toxicity
- transport

- metabolism
- accumulation



Ribeiro, AJS et al. Clin Pharmacol & Ther. 2019



- For regulatory use, systems must:
  - operate robustly
  - originate reproducible results
  - improve gold standard

} establish criteria for our initial evaluations

- Systems and cells to evaluate:
  - published systems and preferentially used in different laboratories
  - commercially available cells with quality control protocols

General criteria for our evaluation of cellular microsystems (liver system)

- Site-to-site variability: system developers repeat our experiments
- Chip-to-chip variability: how different chip batches and different cell donors affect results
- Focus on PTMS protocols: Preparation, Treatment and Measurement Schedules

Road map of liver system characterization

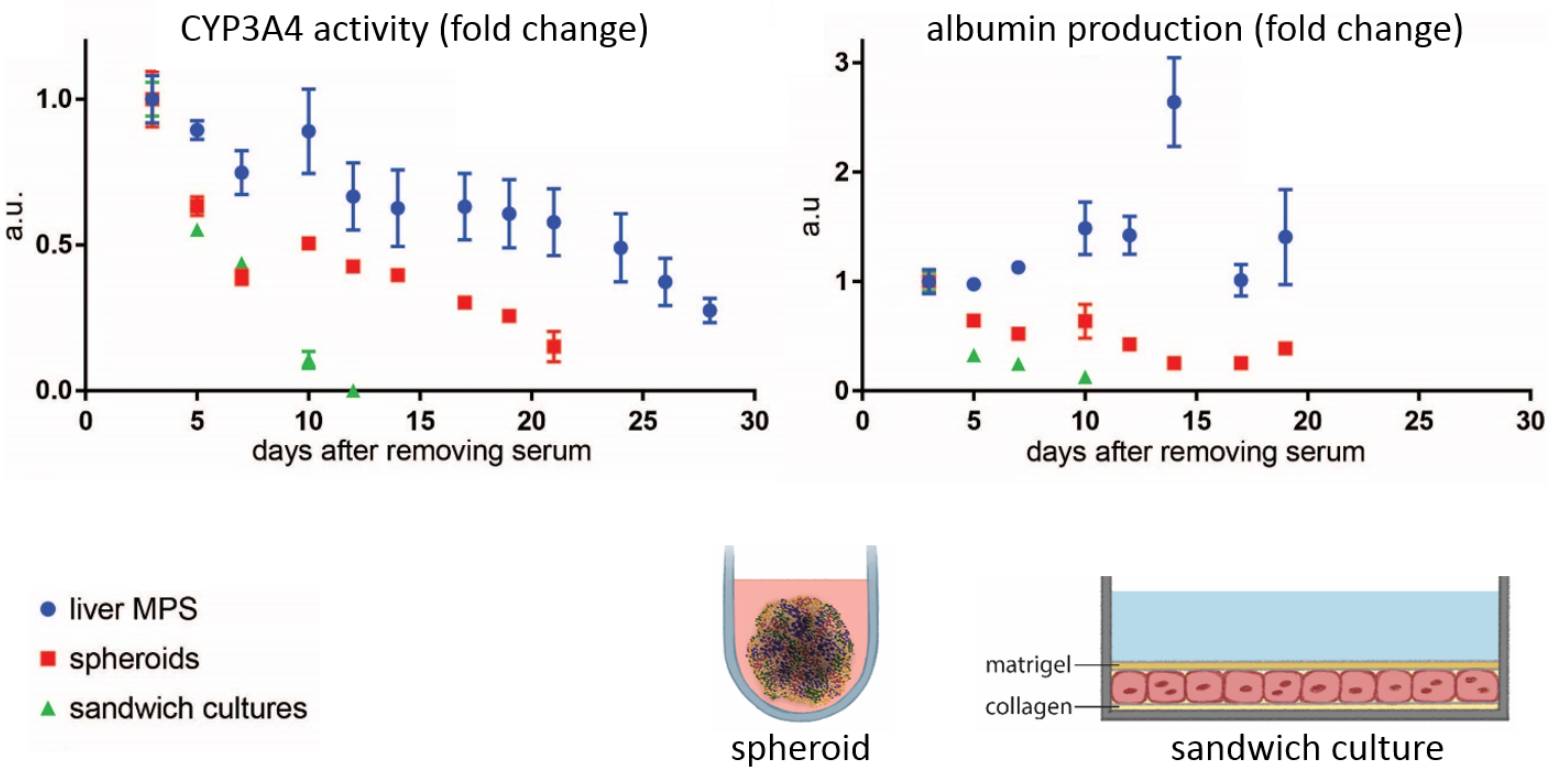
Milestone 1: Assemble, Operate, and Assay the Liver System

- **Variability**: chip-to-chip and well-to-well
- **Endpoints**: cell viability, cytochrome P4503A (CYP3A4) activity, gene expression, and structural organization
- **Set methodological standards**: cell seeding protocols, multiple cell types, drug toxicity studies

Milestone 2: Evaluate Different Applications for Drug Studies

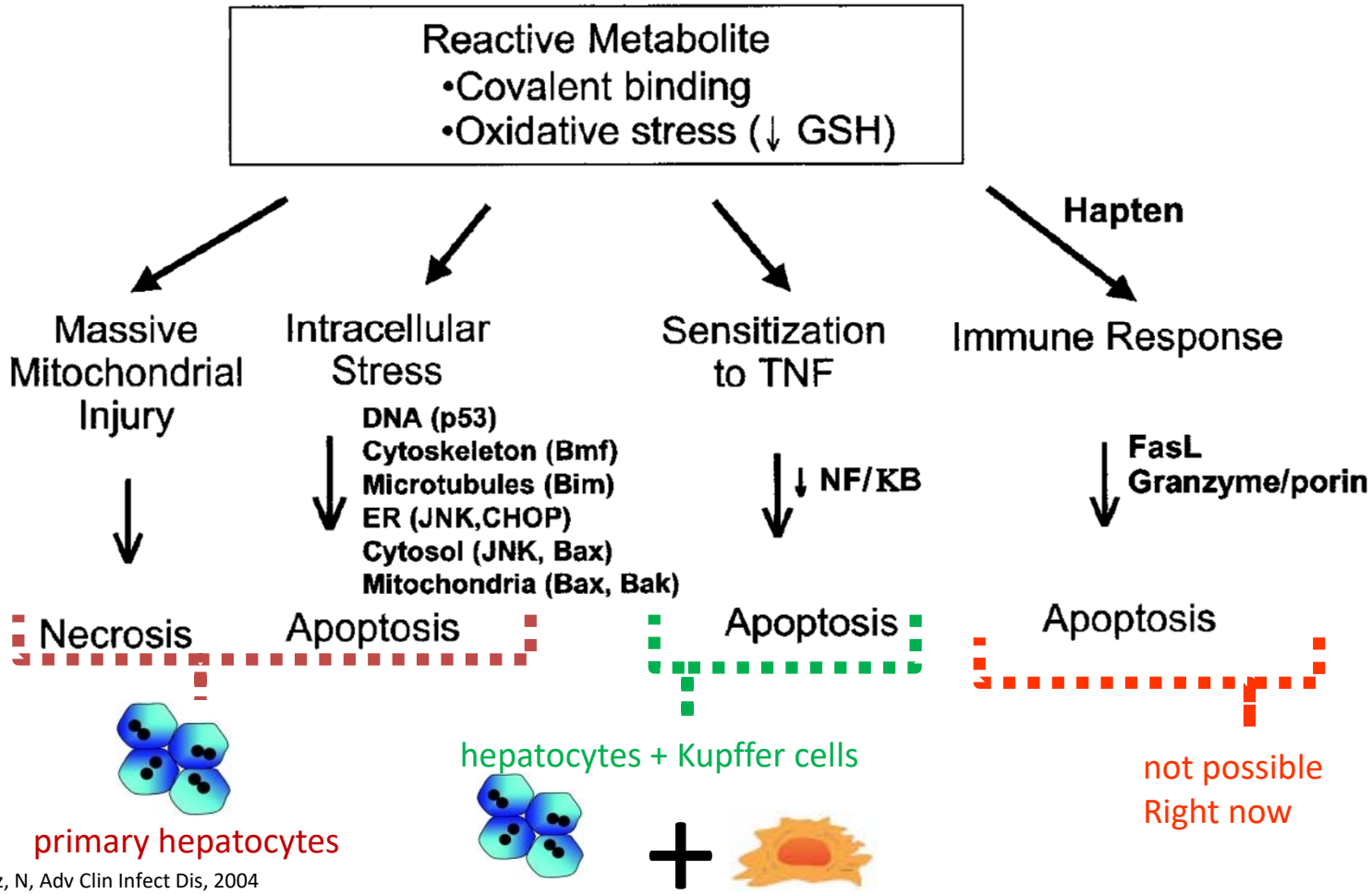
- **Phase I and phase II** metabolism: troglitazone
- Tissue drug **accumulation**: chloroquine
- **Toxicity** depending on **inflammation**: levofloxacin and trovafloxacin
- Sensitivity to toxicants **compared with other models**: troglitazone, tamoxifen and digoxin
- **Low adsorption** of compounds to the system materials: ibuprofen, propranolol, diclofenac, prednisolone, lidocaine and phenacetin
- Enable cellular hepatic properties to **last longer**: CYP3A4 activity and albumin

CYP3A4 activity and albumin production last longer in the liver system



Rubiano, A et al. Submitted
Ribeiro, AJS et al. Clin Pharmacol & Ther. 2019

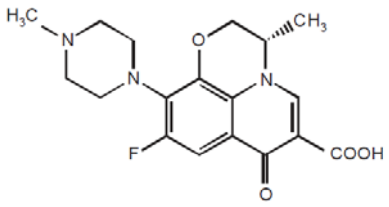
Multiple cell types improve the ability to predict different mechanisms



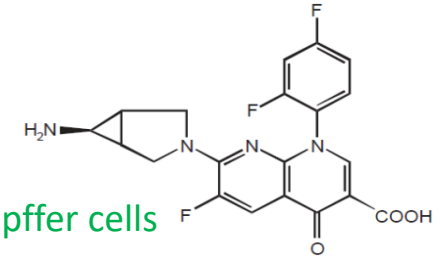
Kaplowitz, N, Adv Clin Infect Dis, 2004

We co-cultured hepatocytes with Kupffer cells to screen drug toxicity with known drugs

levofloxacin (FDA-approved)



trovafloxacin (restrictions for use due to hepatotoxicity)

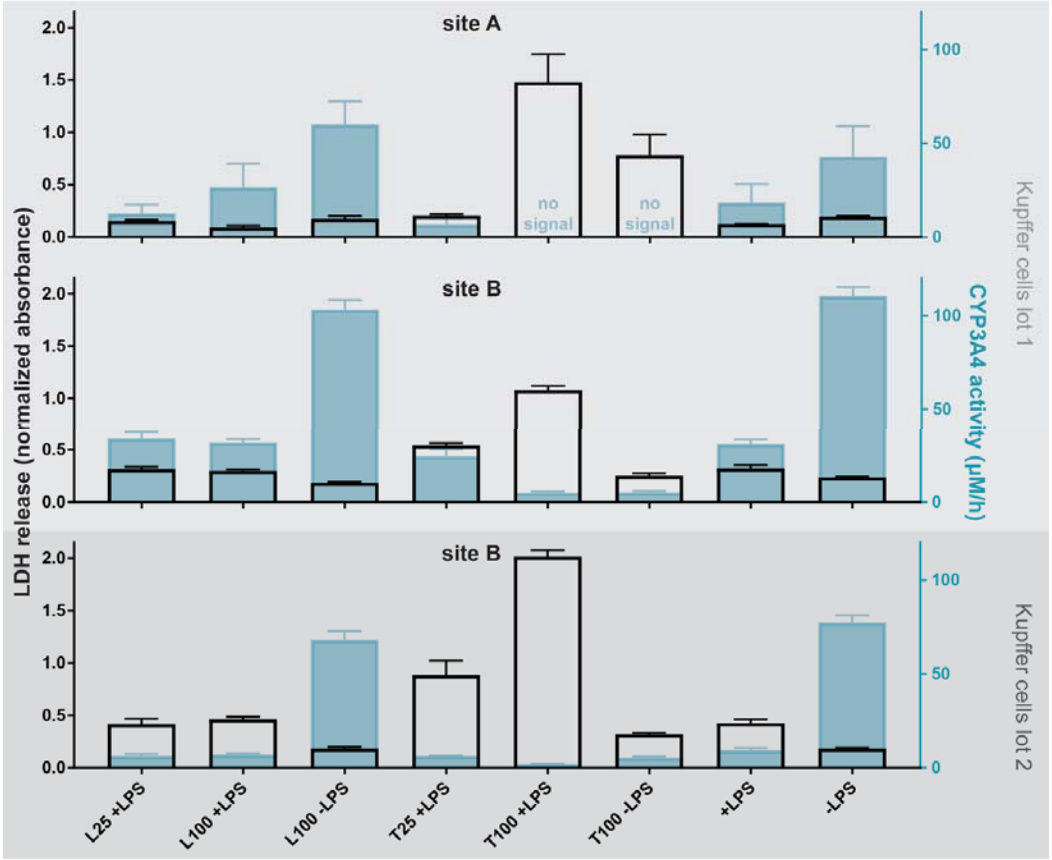


hepatocytes + Kupffer cells

We developed a protocol:

- Defined treatment schedules
- Co-dosing with (Lipopolysaccharides) LPS: induce inflammatory signaling
- Concentrations (0, 25, 100) µM

Trovafloxacin toxicity detected with liver system in different sites and batches of Kupffer cells

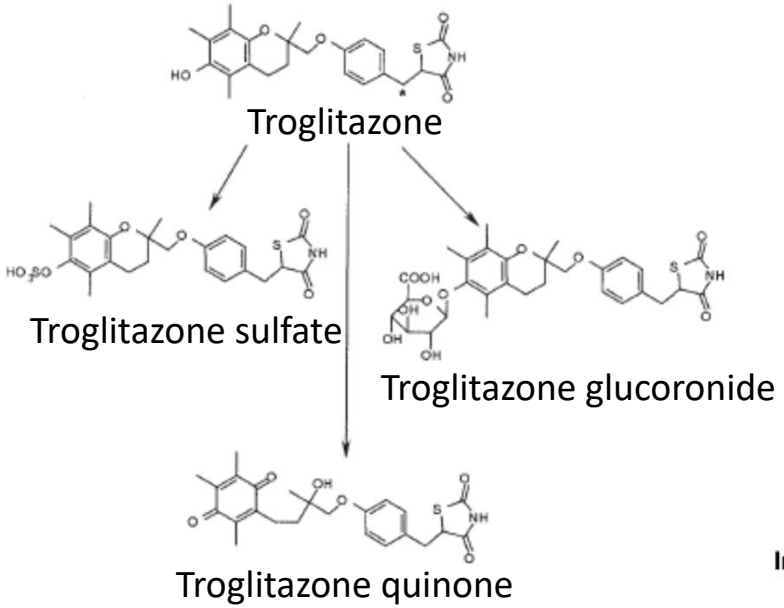


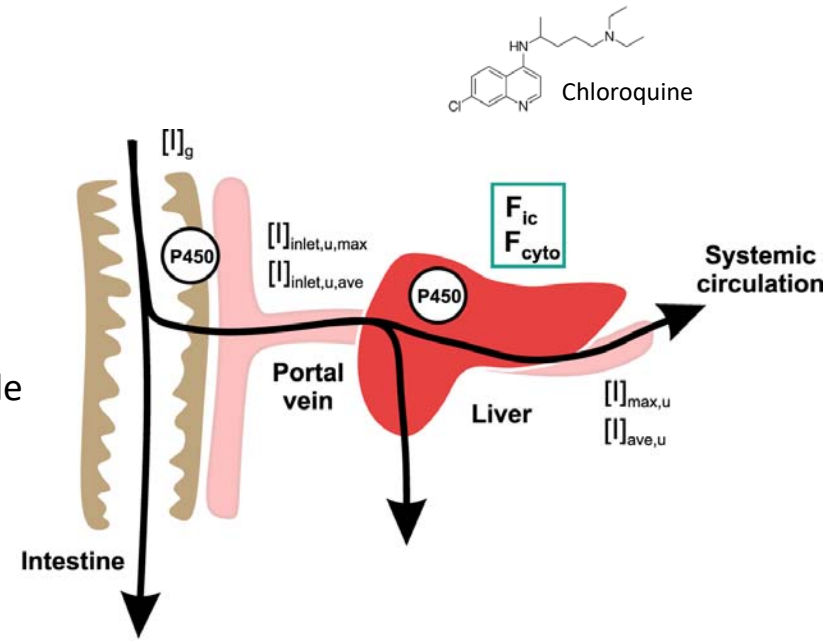
- Used the same:
  - batch of hepatocytes
  - drug catalog numbers
  - devices and instrumentation
- Used different analytical instruments:
  - plate reader
  - plate shaker
  - lot numbers of assays (CYP3A4, albumin, LDH)

Trovafloxacin (T) [uM]; Levofloxacin (L) [uM]; Lipopolysaccharide (LPS)

Rubiano, A et al. Submitted

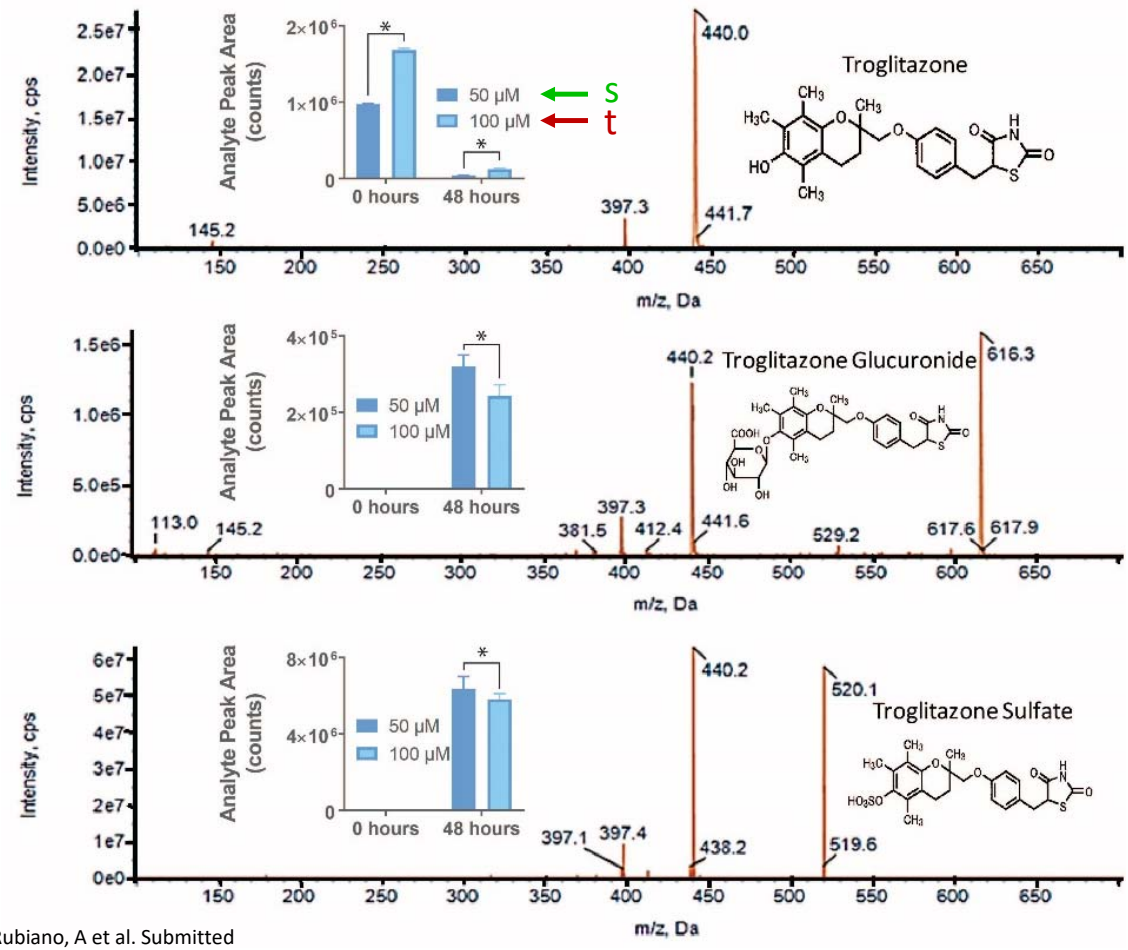
Troglitazone undergoes phase II metabolism and chloroquine accumulates in the liver





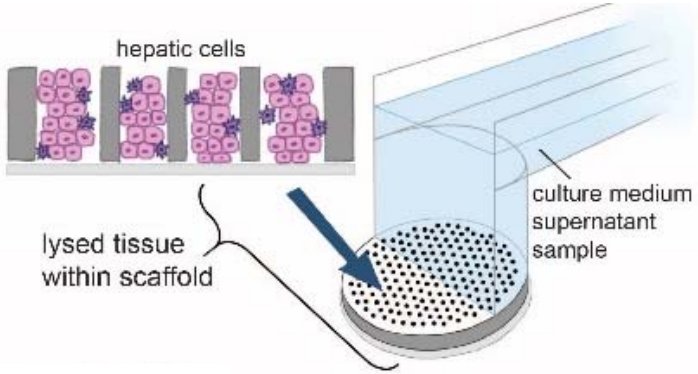
He, K et al. Drug Metabolism and Disposition 2004
Flippula, AM et al. Scientific Reports 2019

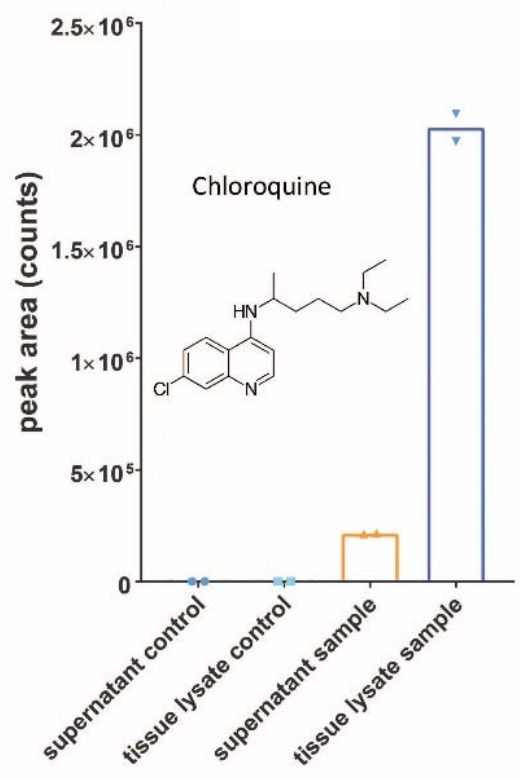
Formation of phase II metabolites: glucuronide and sulfate



Rubiano, A et al. Submitted

Accumulation of chloroquine detected in microtissues of liver system





Rubiano, A et al. Submitted

Key takeaways

- 3D culture and media flow enhance and prolong activity of hepatocytes

- Liver system can be used for:
  - mechanistic evaluation of drugs: role of inflammatory factors
  - drug metabolism studies
  - tissue accumulation of drugs relative to perfusate

- Observed reproducibility of results: different cells, published and performed in different site

Future:

- Establish quality control and performance criteria for systems and cells (primary and iPSC-derived)
- Develop specific contexts of use in toxicity and pharmacokinetics

Acknowledgements

Paul Brown
Suzanne Fitzpatrick
Karen Boyd
Donna Mendrick
Tracy MacGill
Kyung Sung
Johnny Lam
Barry Rosenzweig
Donna Volpe
Neil Hartman
Keri Dame
Ayesha Arefin
Melissa Mendoza
Madhu Lal-Nag

David Strauss
Rodney Rouse
Vikram Patel
Jim Weaver
Kristina Howard
Shiew-Mei Huang
Raj Madabushi
Xinning Yang
Anu Ramamoorthy

Alexandre.Ribeiro@fda.hhs.gov

DARPA (IAA):

Bradley Ringeisen
Gina Kost
Rebekah Cecil
David Krizman

CN Bio Innovations:

Tom Kostrzewski
David Hughes

University of California:

Kevin Healy
(Berkeley)
Ed Hsiao
(San Francisco)

Researchers in the ~~field~~ lab

Postdoctoral Fellows:

Keri Dame

Ayesha Arefin

Iveth Garcia

Biological Scientist:

Barry Rosenzweig

Researcher:

Ryosuke Yokosawa

Summer Fellows:

Chloe Moulin

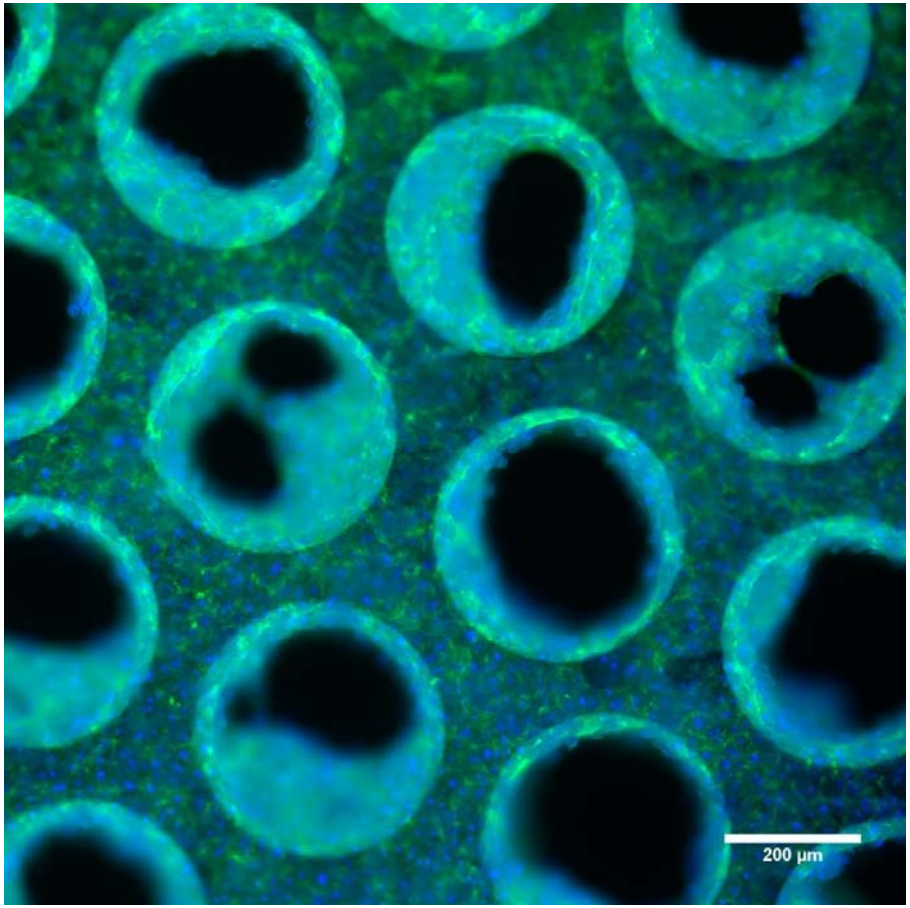
Melissa Mendoza

Former Trainees:

Andrés Rubiano

Moran Choe

Thank You and Stay Safe!



NTP
National Toxicology Program

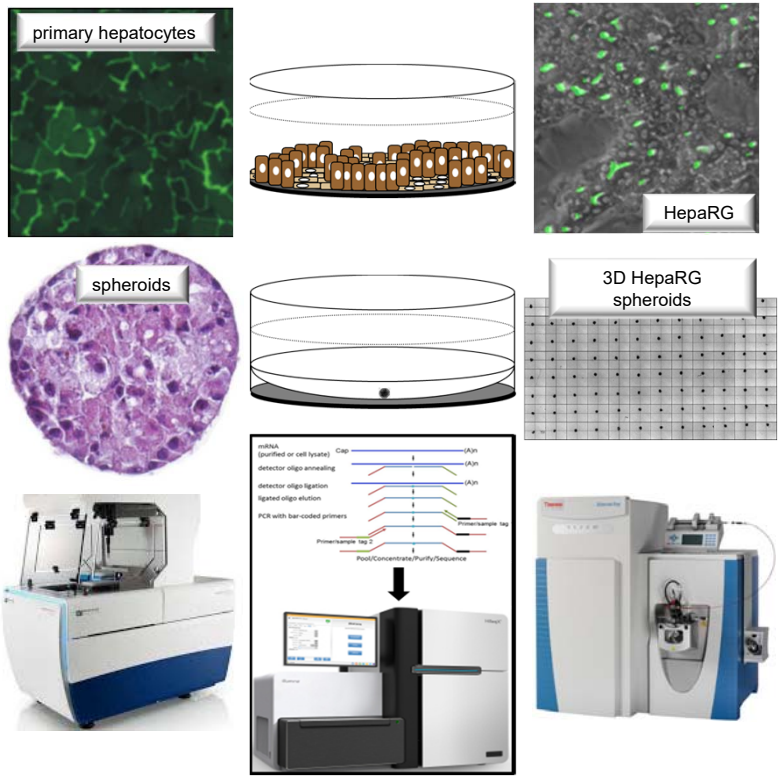
HepaRG 3D Spheroids in Comparison to 2D Models

Stephen S. Ferguson, Ph.D.

- I have no financial relationships to disclose.
- The statements, opinions or conclusions contained therein do not necessarily represent the statements, opinions or conclusions of NIEHS, NIH or the US government.

Tox21 Evolution: Predictive Toxicology Screening

- Physiologically-relevant in vitro screening models
  - improved cellular differentiation/functionality
  - **xenobiotic metabolism & bioactivation/detoxification**
  - longevity to model progressions towards apical outcomes
  - cross-species parallelogram comparisons
- Multi-dimensional assay platforms (time/concentration)
  - high throughput transcriptomics
  - high content imaging
  - metabolomics
- Quantitative translation to humans
  - C_{max}/BMC ratios
  - Pathway Analyses
  - IVIVE
- Extend approach to:
  - Extrahepatic tissues: kidney & intestine
  - Susceptibility models: developmental, disease, population



HepaRG Cells

Liver Progenitor Cell Line (INSERM/BioPredic)

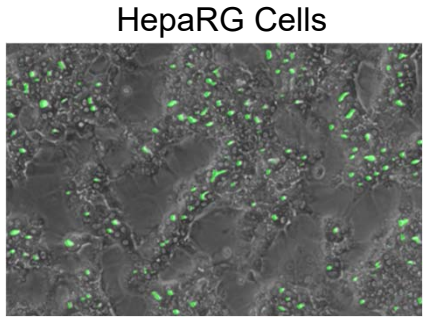
- Derived from female patient with hepatocellular carcinoma & hepatitis C
- Differentiate to two distinct cell populations
  - **hepatocyte-like cells**
  - **cholangiocyte-like cells**

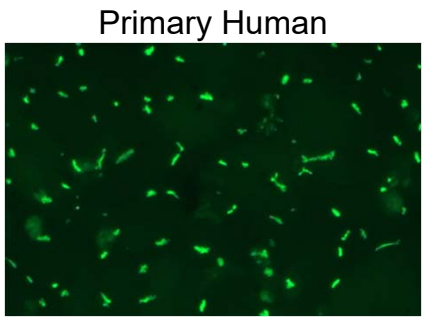
Differentiated Hepatocyte Functionality

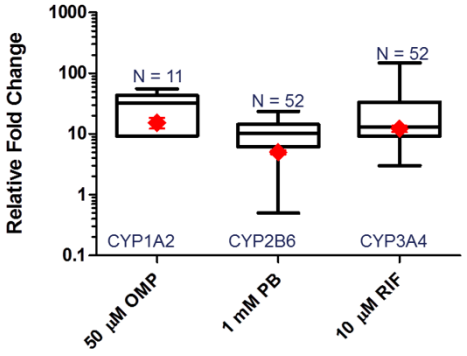
- Transporters
  - uptake (e.g. OATP, NTCP)
  - efflux (e.g. MRPs, MDR)
- DMEs
  - Phase I (e.g. P450, FMO)
  - Phase II (e.g. UGTs, SULT)
- Receptor Pathways
  - functional CAR, PXR, AhR
  - induction of DMEs and Transporters

Advantages over PHHs

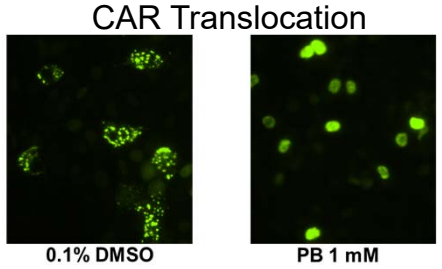
- Year-over-year availability
- Markedly reduced lot-to-lot variability
- Ability to transdifferentiate & proliferate

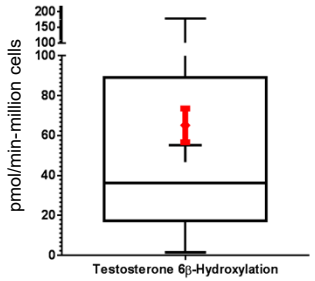


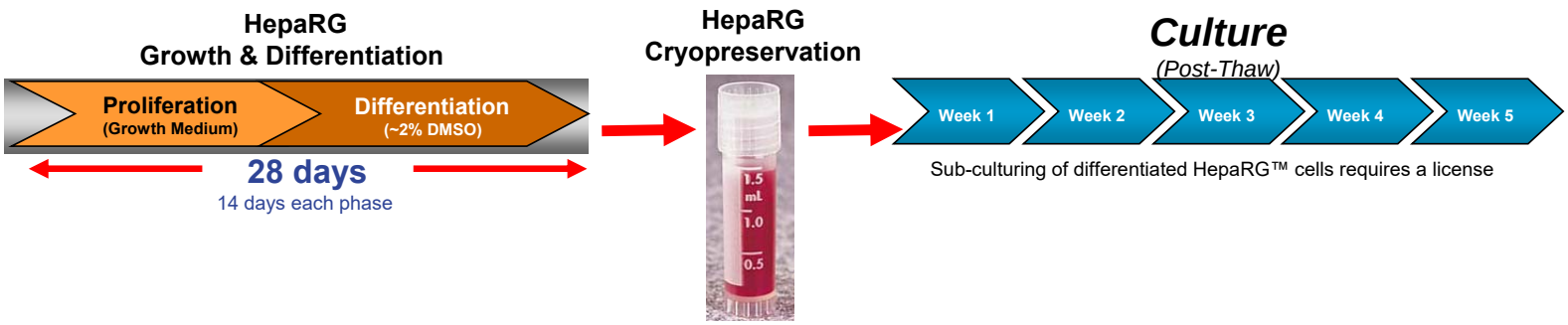


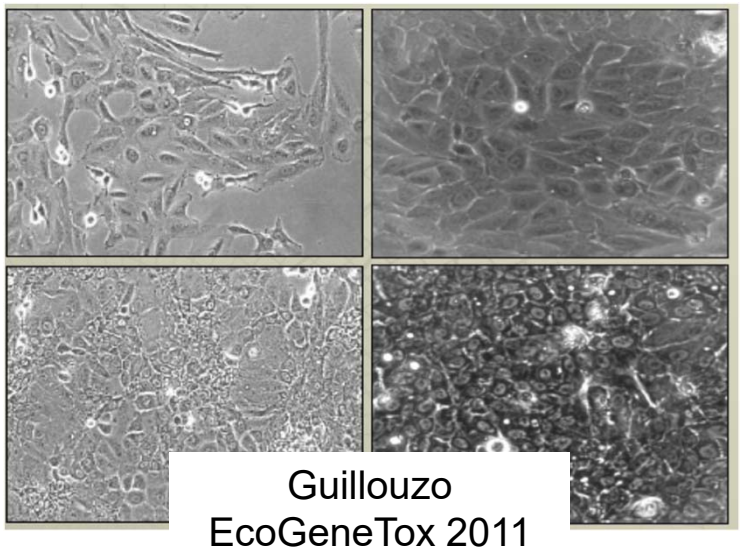


Jackson et. al, DMD, (2016) v.44(9): 1463-79.

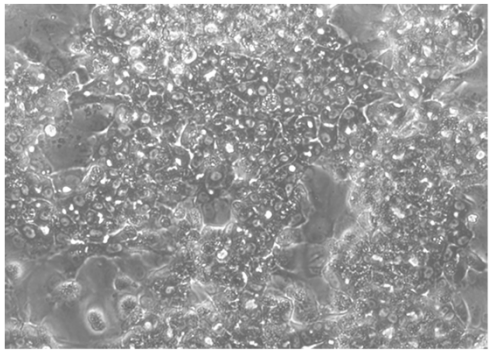




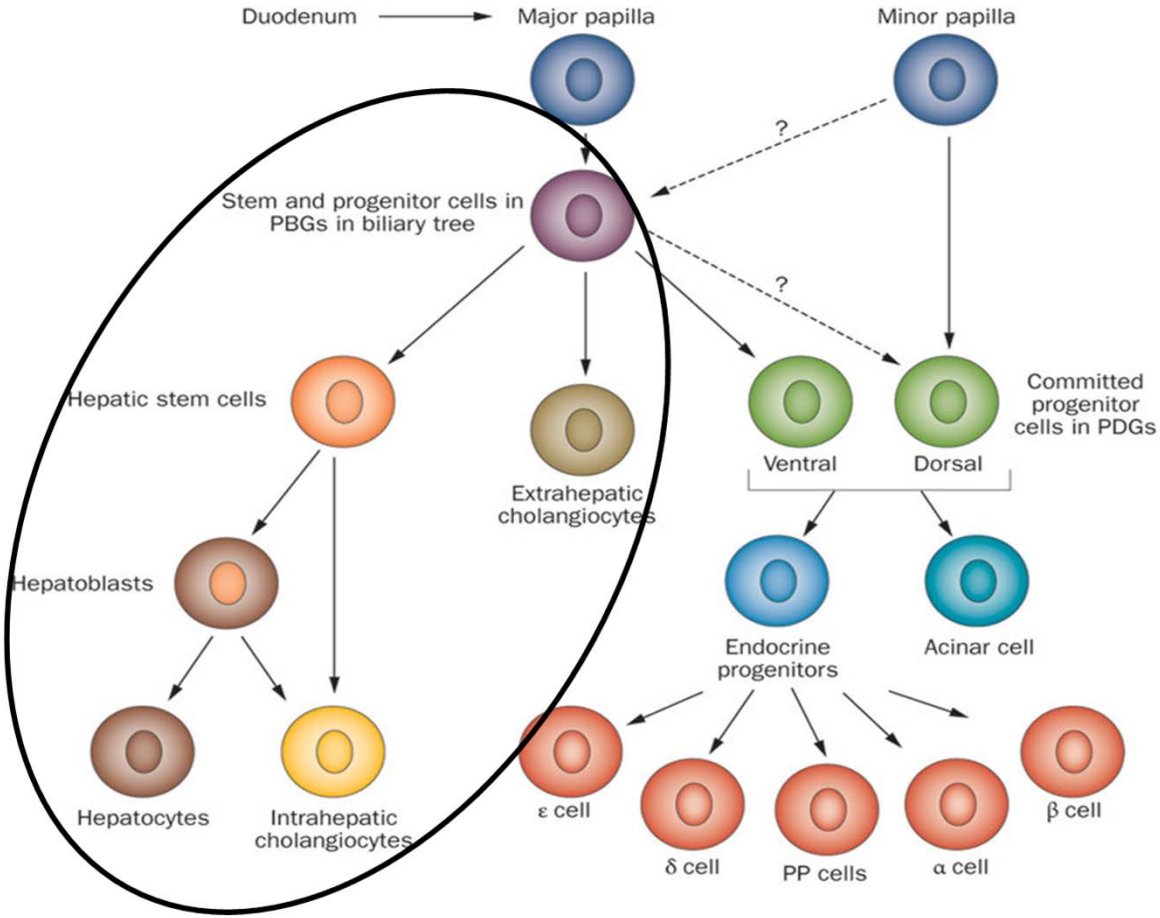




Guillouzo
EcoGeneTox 2011

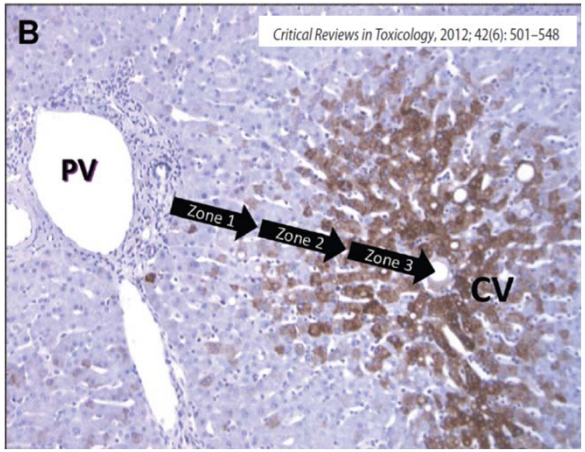


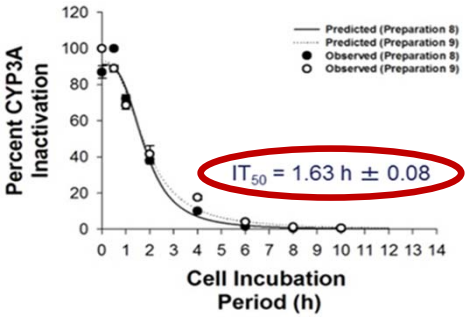
HepaRG Cells (Post-thaw)

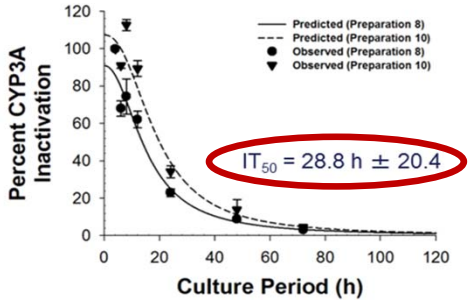


Nature Reviews Gastroenterology & Hepatology **9**, 231-240 (April 2012)

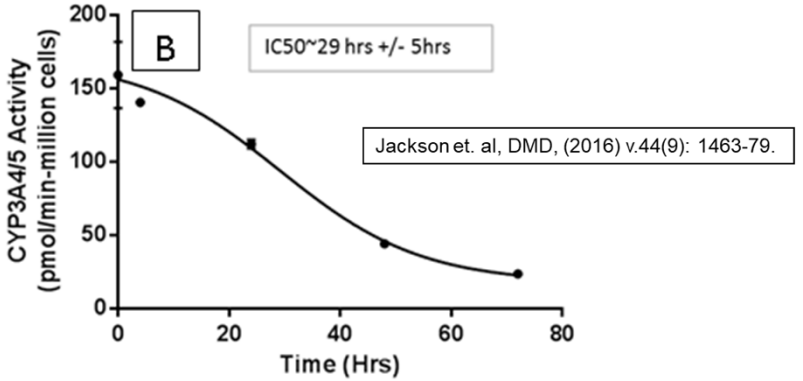
Isolated Primary Liver Cells Rapidly De-differentiate Once Removed from Liver Tissue

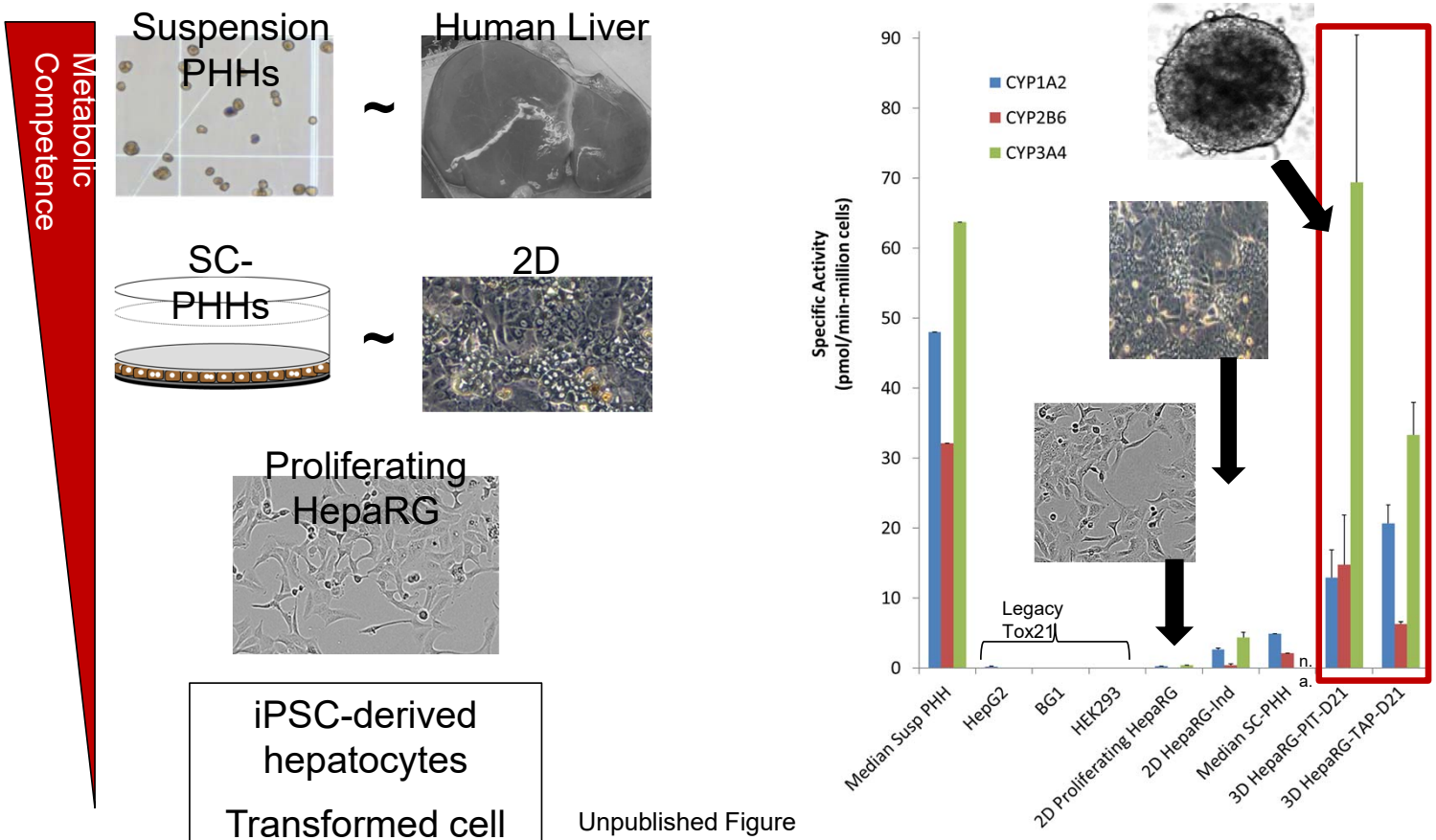






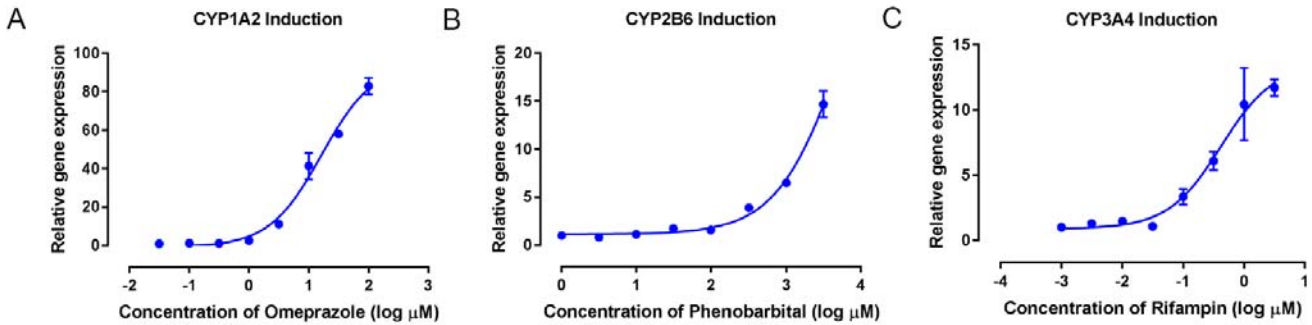
Smith et al. J. Pharm. Sci. 2012. v.101(10):3898.

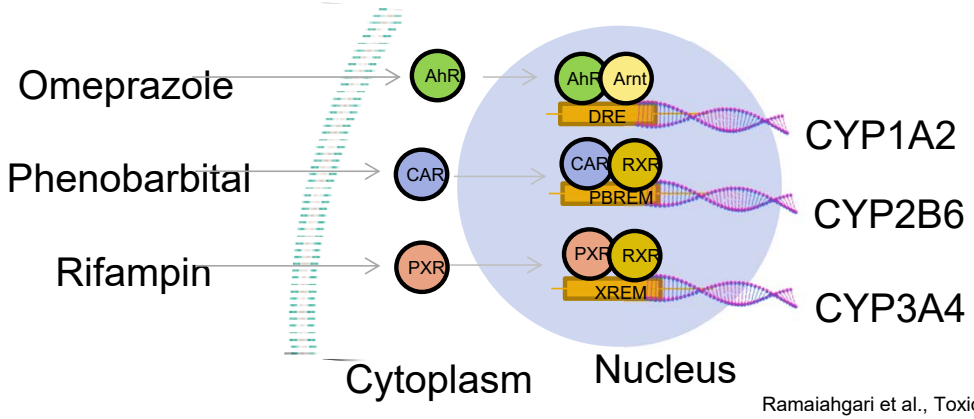




Unpublished Figure

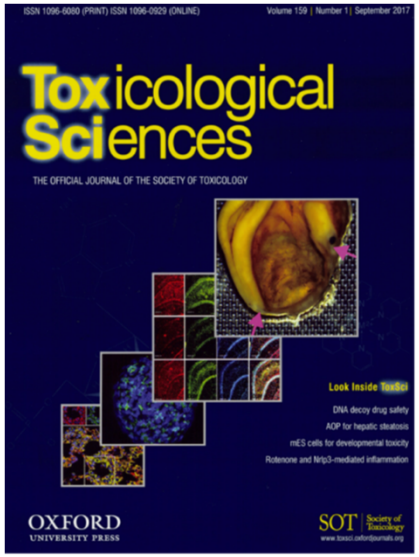
AhR-, CAR-, & PXR-Mediated Liver Enzyme Induction



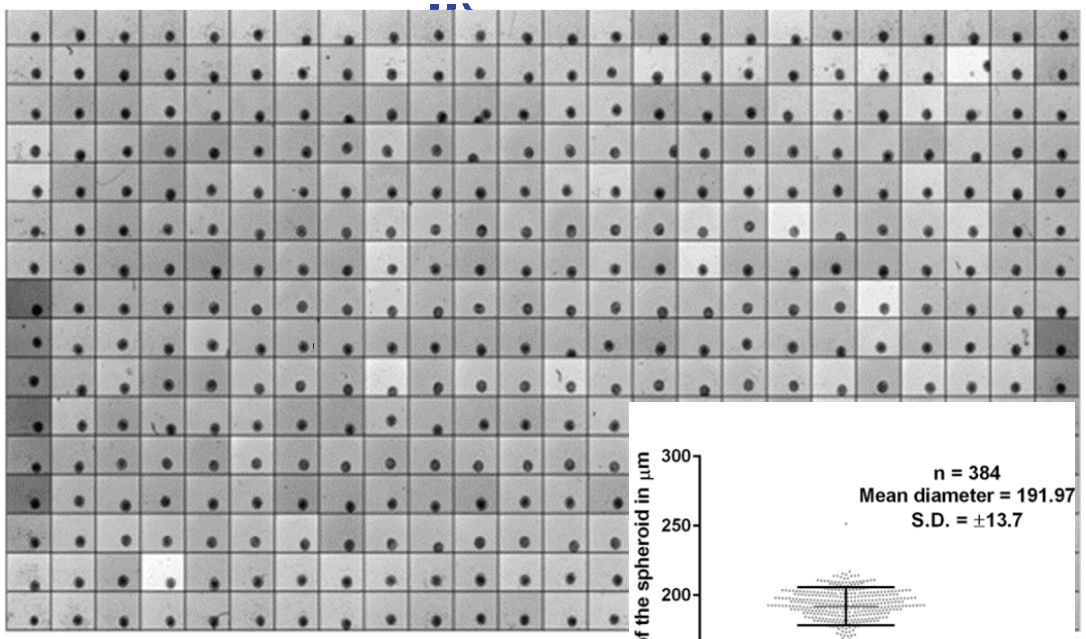


Ramaiahgari et al., Toxicol Sci (2017) v.159 (1): 124-136

3D HepaRG Spheroids (384-



Dr. Sreenivasa Ramaiahgari

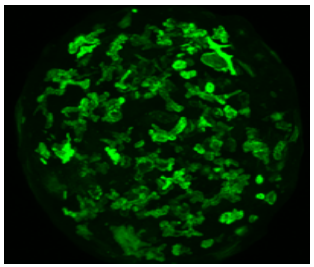


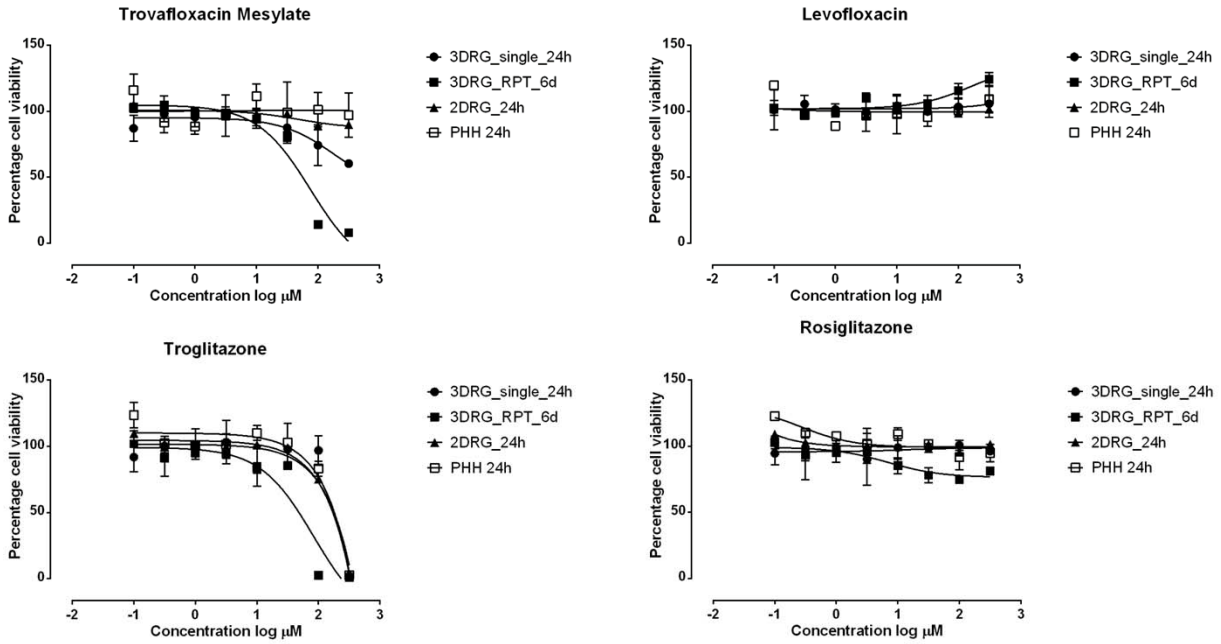
1 vial (10 million cells) @ ~1-2k cells/spheroid
2D: 1 X 384-well plate
3D: 12-25 X 384-well plates

From the Cover: Ramaiahgari et al., Toxicol. Sci (2017) v.159 (1): 124-136

3D HepaRG Spheroid Responses to Drug Analogues

MRP2



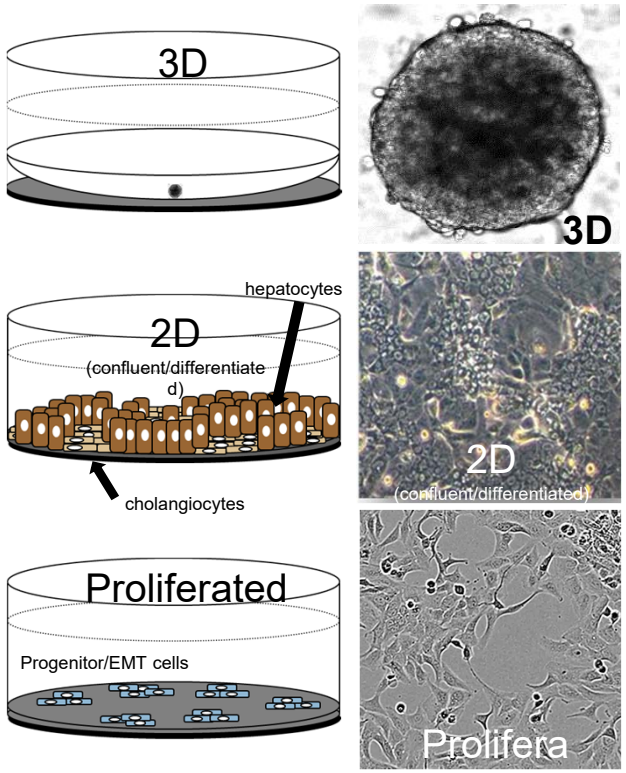


Ramaiahgari et al., Toxicol Sci (2017) v.159 (1): 124-136

High Throughput Transcriptomics (HTT) Paired with HepaRG Cultures

- 3 Culture Configurations of HepaRG Cells (384-well formats)
- 24 Compounds
  - Liver injury/metabolically-activated toxicity
  - Hepatic receptor activators
  - Drug analogue comparisons
  - 'Negatives' for liver injury

acetaminophen	caffeine	diphenhydramine	DMN	rifampicin	tamoxifen
aflatoxin B1	CDCA	fenofibric acid	omeprazole	ritonavir	troglitazone
aspirin	chlorpromazine	levofloxacin	phenobarbital	rosiglitazone	trovafloxacin
benzo(a)pyrene	cyclophosphamide	menadione	KCl	sucrose	valproic acid



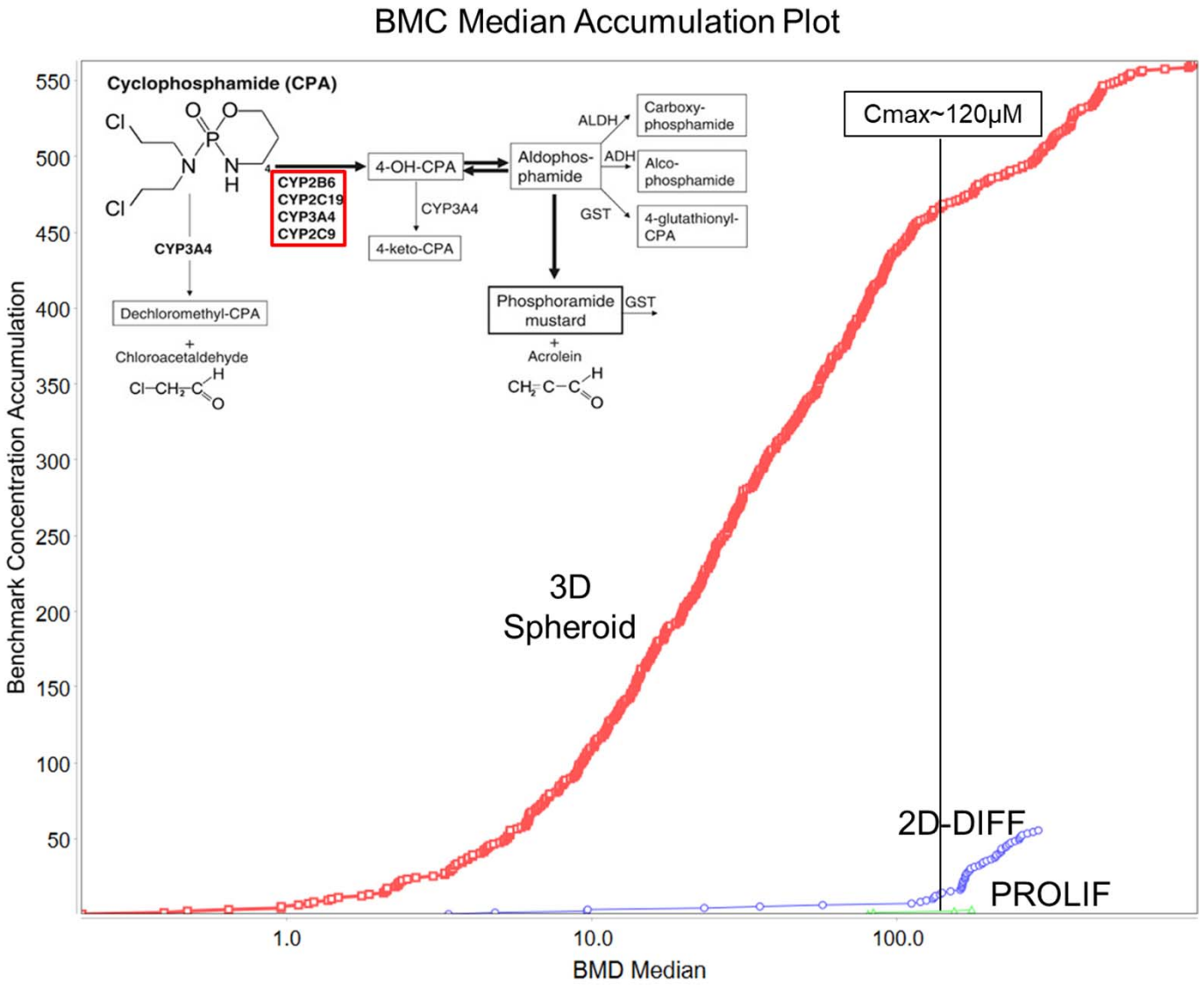
- Assays:
  - cell morphology (Incucyte, daily for each culture well)
    - Image classifications, quantitative masking of confluence
  - cytotoxicity (LDH leakage)
  - high throughput transcriptomics (HTT with S1500+, TempO-Seq)

Cyclophosphamide

(2-fold filter)

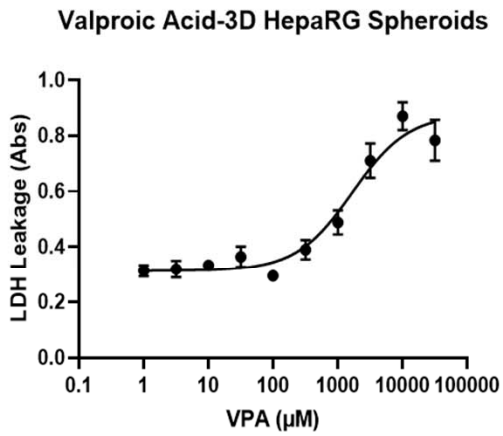
- 3D HepaRG Spheroids
- 2D-DIFF HepaRG
- PROLIF HepaRG

- Notable identified pathways:
  - Lipid hydroxylation
  - P450 metabolism
  - Cell cycle
  - ROS
  - DNA damage
  - Hypoxia

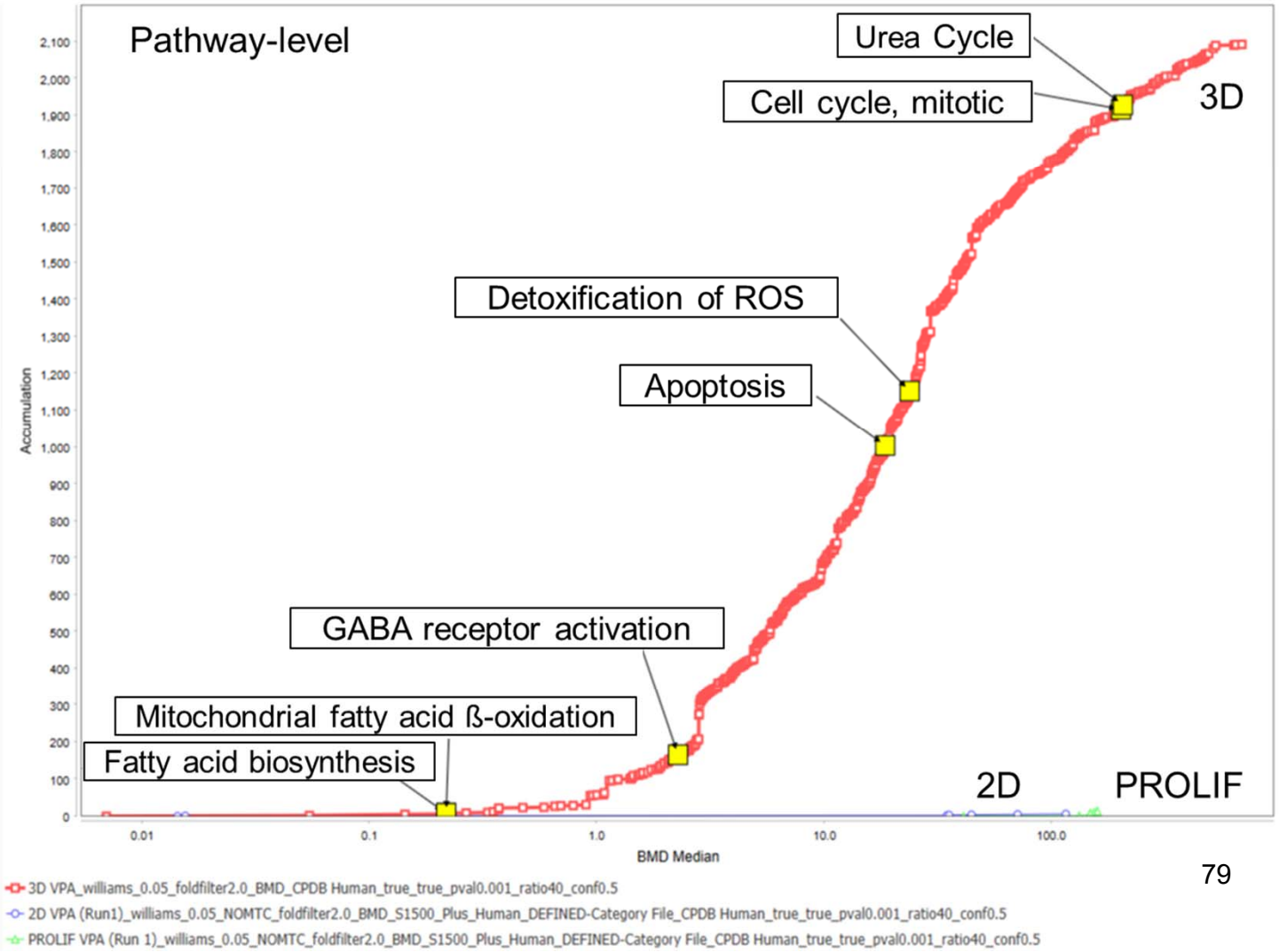


Unpublished Data

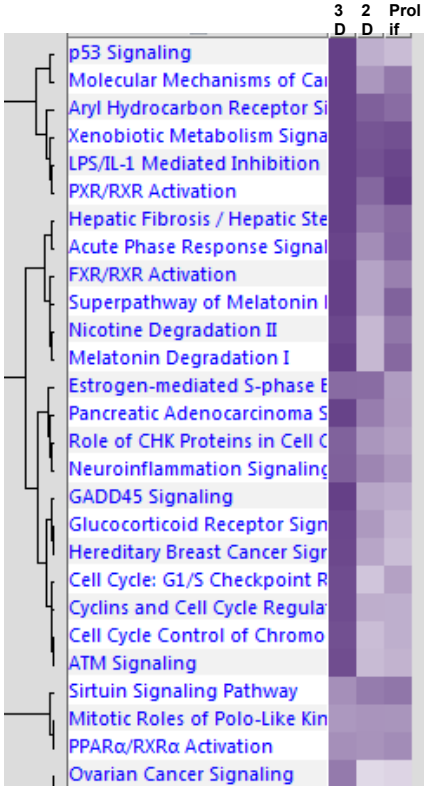
Valproic Acid HTT in 3D HepaRG Spheroids



- C_{max} ~240 µM (human plasma)
- Extensively metabolized (P450s)
- Cytotoxicity @ 1000 µM (3D only)
- Alters lipid & fatty acids levels
- Therapeutic target GABAergic receptor
- Hepatic mitochondrial toxicity & hyperammonemia
- Idiosyncratic liver injury compound



3D Spheroids & Biological Pathway Enrichment



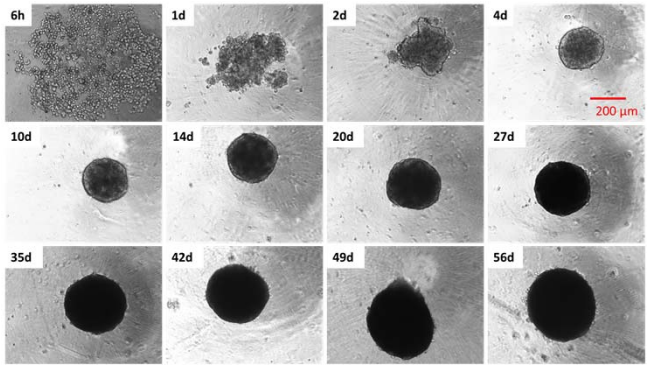
Benzo(a)pyrene (Group 1 carcinogen (IARC)) exposure on HepaRG cell culture models

	Significantly changed genes		
Canonical Pathways	3D_3 µM	2D_DIFF _3 µM	2D_PROLIF _3 µM
P53 Signaling	39	14	14
Molecular Mechanisms of Cancer	71	32	40
AhR Signaling	38	23	23
Xenobiotic Metabolism Signaling	52	35	39
PXR/RXR Activation	27	16	20
Hepatic Fibrosis	38	24	28
Acute Phase Response Signaling	36	22	28
Pancreatic Adenocarcinoma	30	19	18
GADD45 Signaling	12	7	7
ATM Signaling	26	12	14

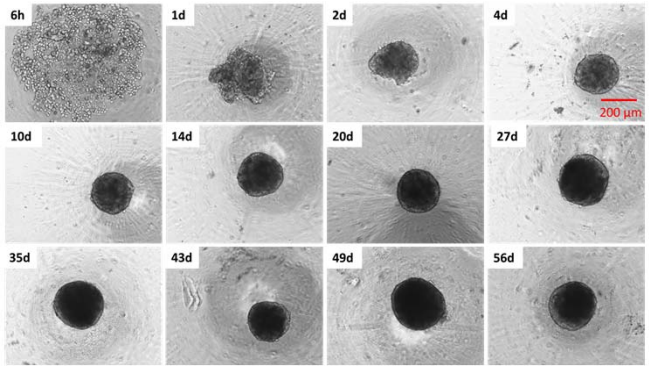
3D HepaRG Grow Over Time Absent DMSO

Dr. Katelyn Lavrich
Postdoctoral Fellow

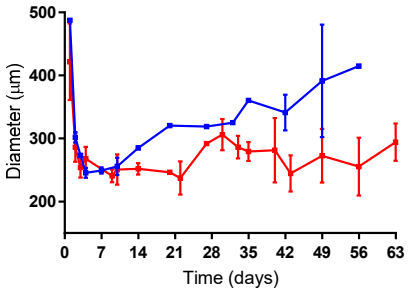
No DMSO

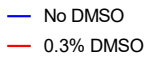


0.3% DMSO



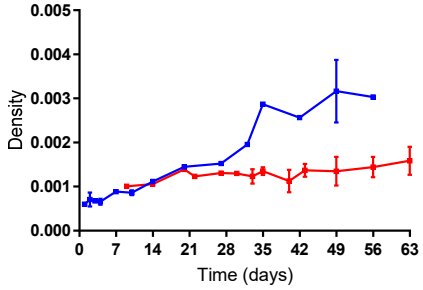
Diameter Size

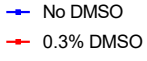




- 2,000 cells/sphere
- 96-well, Ultra Low Attachment Spheroid plates (Corning)
- Shown as mean ± SD

Density

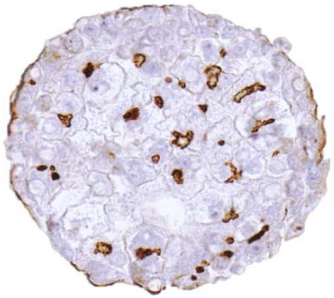




Opportunities & Challenges with 3D Hepatocyte Spheroids

Opportunities

- Easy, simple-to use model system
- Enhanced hepatocyte functionality
- Enriched transcriptomic pathway responses with reference drugs
- Long-term differentiation for repeated exposures, time-course, & reversibility
- Model longer-term complex phenotypes and histopathology (e.g., fibrosis, cholestasis, hepatomegaly, DNA damage)
- Emerging evidence for enhanced aerobic metabolism with free-floating spheroids
- Efficient use of hepatocytes ($)



Biliary Efflux Transporter MRP-2 Immunostaining of HepaRG Spheroids (21d)

Challenges

- Recent plate coating issues (HepaRG)
- Changing culture media without liquid handling
- Insufficient knowledge of spheroid maturation & stability dynamics
- Limited understanding for toxicological translation
- Allometric scaling & biomass challenges (e.g., metabolite profiling dynamics)
- Inadequate optimization of cell culture media, largely adopted from 2D (e.g., DMSO, hydrocortisone)

NIEHS/NTP Colleagues & Collaborators

Biomolecular Screening Branch





Warren Casey (Branch Chief)
Rick Paules
Scott Auerbach
Trey Saddler
Alison Harrill
Jui-Hua Hsieh
Fred Parham
Kristine Witt
Stephanie Smith-Roe
Alex Merrick
Stephen Ferguson
Sreenivasa Ramaiahgari
Katelyn Lavrich
Nisha Sipes
Julie Foley
Pierre Bushel

NIEHS
Georgia Roberts
Jennifer Fostel
Brad Collins
Suramya Waidyanatha
Windy Boyd

Tox21


LifeNet
Ed LeCluyse

Numerous colleagues
CellzDirect &
Life Technologies



Sciome
Ruchir Shah
Deepak Mav
Dhiral Padke
Jason Phillips

NTP Labs




Alex Merrick (Branch Chief)
Paul Dunlap
Julie Rice
David Crizer
Wei Qu
Will Gwinn
Nancy Urbano
Janice Harvey
Sreenivasa Ramaiahgari
Stephen Ferguson

US EPA
Josh Harrill
Rusty Thomas
John Wambaugh

BioSpyder
Jo Yeakley
Harper VanSteenhouse
Bruce Seligman
Jason Downing

US FDA
Weida Tong

Battelle
Barney Sparrow
Jenni Gorospe

ICF
Joanne Trogovich

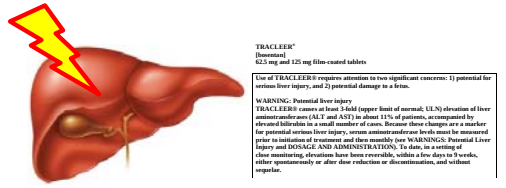
Predicting DILI Risk Using Hepatic Spheroid Co-Culture Models

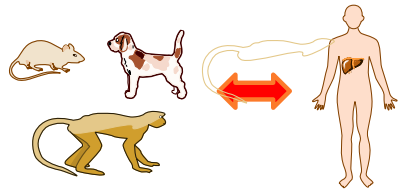
Genentech
A Member of the Roche Group

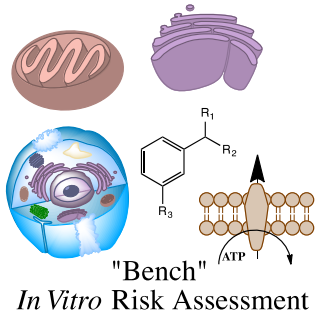
Will Proctor, PhD, DABT
Director, Predictive Toxicology
Safety Assessment
August 14, 2020

Drug Induced liver Injury (DILI)

- DILI is a major source of clinical attrition and black box warnings
- Poor concordance of non-clinical species to identify human-relevant hepatotoxicants
- DILI is often considered idiosyncratic but host factors or intrinsic molecule properties often implicated retrospectively
- DILI is comprised many different etiologies, it is unlikely that a single assay will cover them all



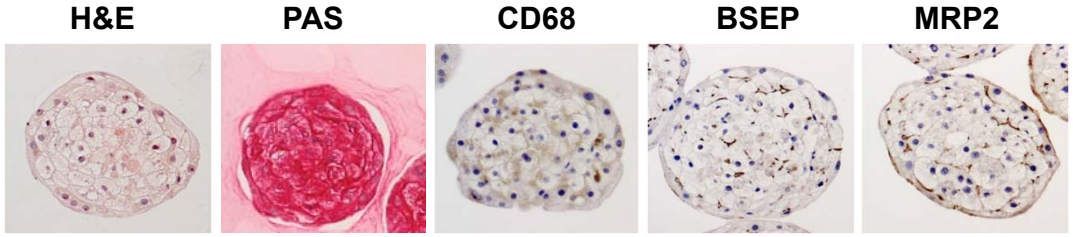




"Bench"
In Vitro Risk Assessment

Genentech
A Member of the Roche Group

3D Spheroid Hepatic Cultures: Human Liver Microtissues (hLiMT)



www.insphero.com

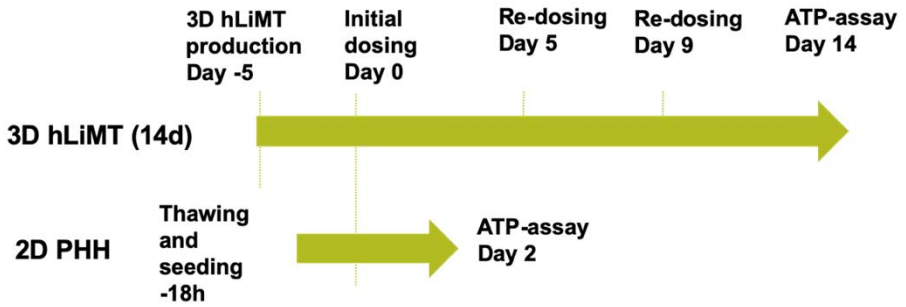
Utility of spherical human liver microtissues for prediction of clinical drug-induced liver injury

Genentech

AstraZeneca
IMED Biotech Unit

William R. Proctor[1] · Alison J. Foster[2,4] · Jennifer Vogt[1] · Claire Summers[2,4] · Brian Middleton[3,4] · Mark A. Pilling[3,4] · Daniel Shienson[5] · Monika Kijanska[6] · Simon Ströbel[6] · Jens M. Kelm[6] · Paul Morgan[2,4] · Simon Messner[6] · Dominic Williams[2,4]

Arch Toxicol (2017) 91: 2849-2863

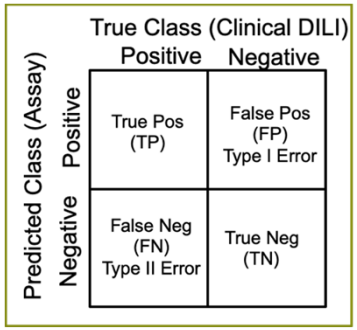


Comprehensive qualification (110 compounds (60% DILI+ve) evaluating cytotoxicity using an 8-point IC_{50} curves w/ top concentrations tested **≥100 x C_{max}** or limit of solubility vehicle 1% DMSO) for **hLiMT (14d)** and **PHH (2d)**.

Genentech
A Member of the Roche Group

Increased Predictive Value of hLiMT over 2D Primary Hepatocytes

Assay	TP	TN	FP	FN	Threshold	Sens. (%)	Spec. (%)	PLR	NLR
2D PHH [IC_{50} / C_{max}]	14	40	1	55	10x	20.3	97.6	8.32	0.82
	23	35	6	46	50x	33.3	85.4	2.28	0.78
3D hLiMT [IC_{50} / C_{max}]	25	40	1	44	10x	36.2	97.6	14.86	0.65
	36	35	6	33	50x	52.2	85.4	3.57	0.56



Sens. = $\frac{TP}{TP+FN}$ Spec. = $\frac{TN}{FP+TN}$

PLR = $\frac{Sens.}{100-Spec.}$ NLR = $\frac{100-Sens.}{Spec.}$

Assay Predictivity: **PLR (Positive Likelihood Ratio)**
NLR (Negative Likelihood Ratio)

	Minimum Diagnostic	Moderate	
PLR :	1-3	3-10	>10
NLR :	1-0.75	0.75-0.1	<0.1

- **hLiMT outperformed PHH in regards to sensitivity at all IC_{50}/C_{max} thresholds**
- **PLR changes > NLR changes (confidence in positive prediction > than negative prediction)**

Genentech
A Member of the Roche Group

Conclusions Regarding Qualification of Hepatic Spheroids

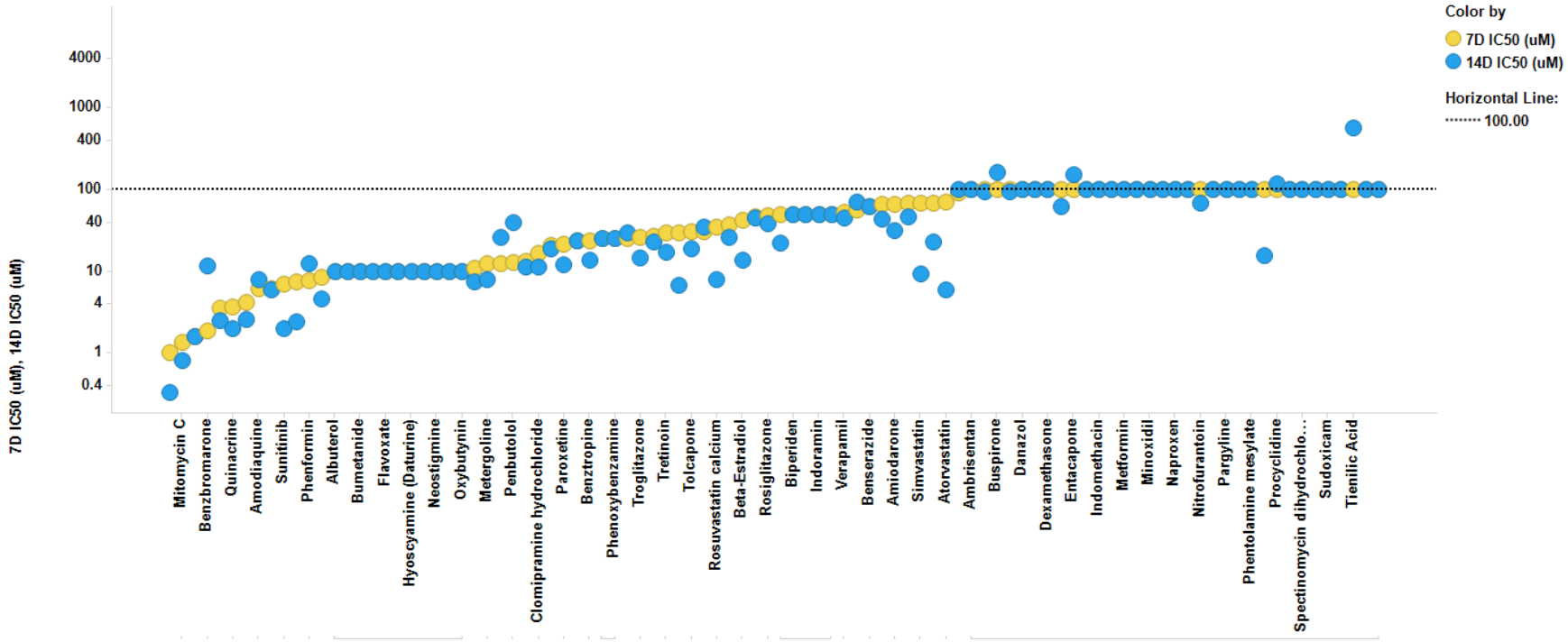
- Spheroid hepatic models exhibited increased sensitivity to detect hepatoxic compounds versus traditional 2D primary hepatocytes
- Spheroid models are promising *in vitro* tools for identifying hepatotoxicity risk and for mechanistic studies and issue mitigation

Challenges

- Difficulty in turning data around quickly to project teams with 14-day repeat dose studies
- Compound test set not representing contemporary chemical space
- Difficult to convince teams to make actionable decisions on in vitro data in isolation

Genentech
A Member of the Roche Group

Effect of Treatment Duration on Cytotoxicity Values (7d vs. 14d)

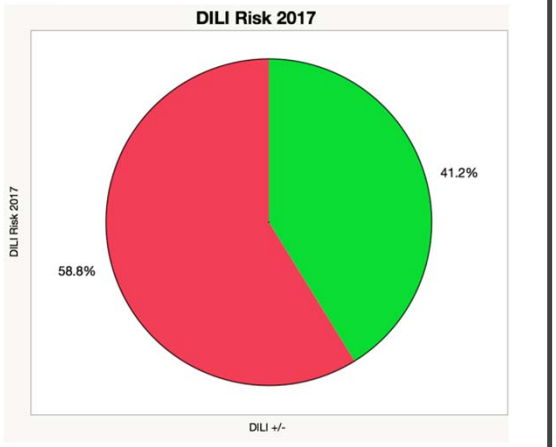


Cytotoxicity IC_{50} values for 110 compound screening of hLiMT for 7d and 14d treatment.

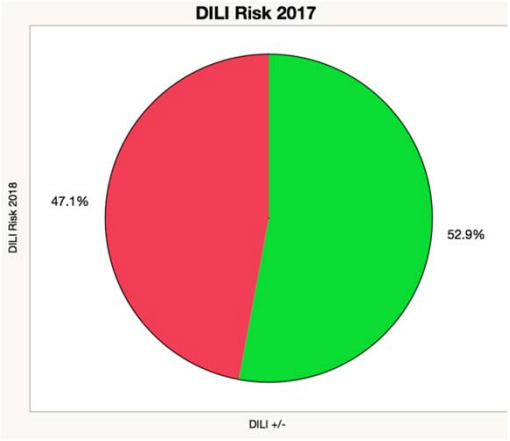
Genentech
A Member of the Roche Group

Expanded (N=175) Test Set and Refined DILI Categorization

Previous Classification



Updated Classification



Risk Level:	Description:
DILI Positive (High)	Severe or acute or evidence of immune involvement. Drugs withdrawn from the market, Clear incidence of hepatocellular injury/fulminant liver failure, Black-box warning
DILI Positive (Medium)	Incidence of liver enzyme increases (no Hy's Law violation), case reports of significant liver injury, but unclear causation due to comorbidities
DILI Negative	No evidence/incidence of liver enzyme increases or liver injury

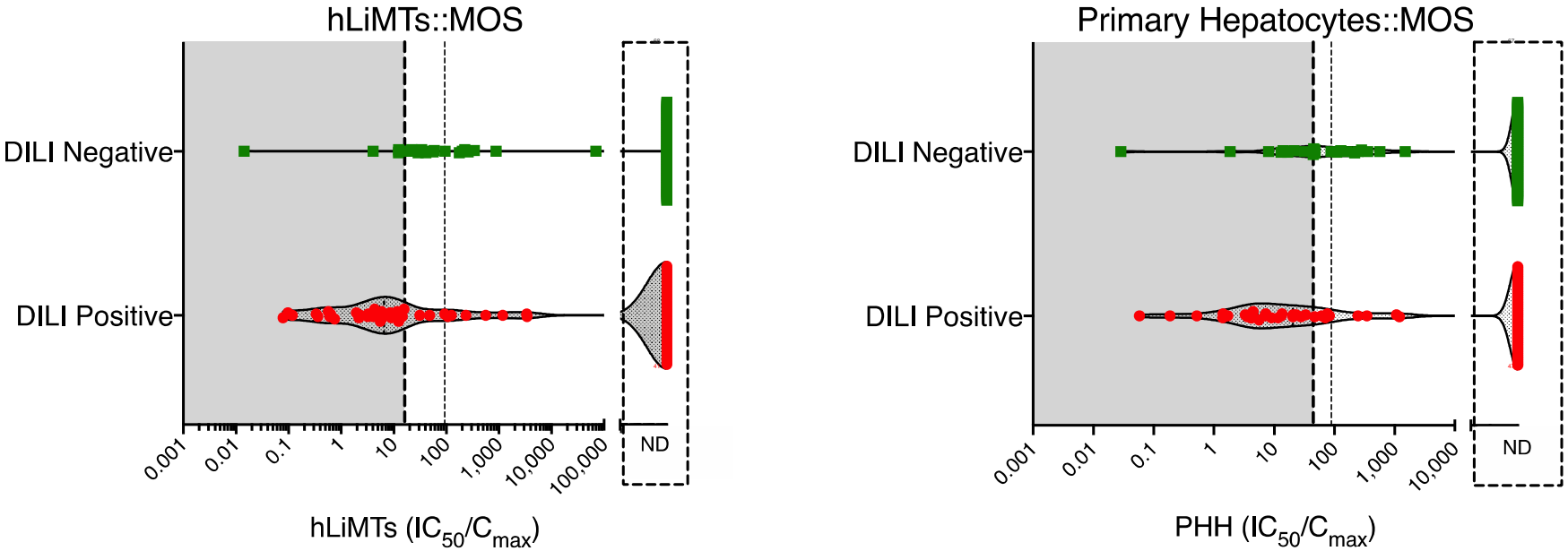
Examples:	Reclassified:	Reason:
Naproxen	High --> Low	OTC
Levofloxacin	Medium --> Low	Guilt by drug class association

Category Shift:	# of Compounds:
High ⇒ Low	2
High ⇒ Medium	7
Medium ⇒ Low	5
Low ⇒ Medium	5
Medium ⇒ High	1

New test set included additional compounds that better representing contemporary chemical space

Genentech
A Member of the Roche Group

hLiMT Assay Retained Superiority to PHH for Predicting DILI



		Threshold	Sensitivity	Specifcity	PLR	NLR
hLiMTs (MOS)	Minimum Distance (……)	93	0.40	0.83	2.40	0.72
	90% Specificity (- - - - - - -)	16	0.36	0.93	**5.44**	**0.68**
PHH (MOS)	Minimum Distance (……)	86	0.36	0.84	2.33	0.75
	90% Specificity (- - - - - - -)	45	0.30	0.90	**3.00**	0.78

Margin of safety (MOS) values for hLiMT cytotoxicity at 7d (IC_{50}/C_{max}). IC_{50} values alone had minimal predictivity.

Genentech
A Member of the Roche Group

hLiMT Assay Was the Most Predictive Parameter Assessed

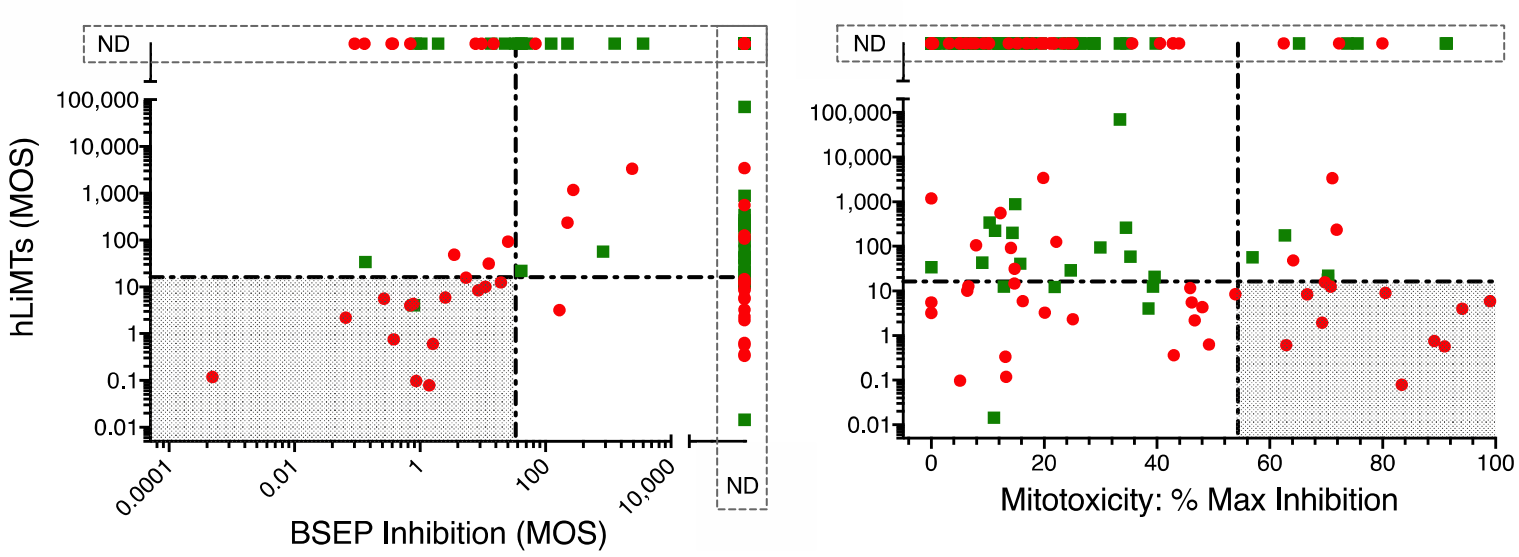
Parameter	Threshold	Sensitivity	Specificity	PLR	NLR
hLiMT MOS [IC_{50}/C_{max}]	16	**0.36**	0.93	**5.44**	0.68
BSEP MOS [IC_{50}/C_{max}]	16	0.29	0.93	**4.31**	0.76
PHH MOS [IC_{50}/C_{max}]	45	**0.30**	0.90	**3.00**	0.78
Mitotox Basal [%Inh Basal OCR]	20	0.31	0.87	**2.56**	0.78
Mitotox Max [%Inh Max OCR]	43	0.34	0.90	**3.38**	0.74
Exposure [C_{max}, uM]	3.0	**0.69**	0.68	2.13	**0.46**
BDDCS Classification	Class 2	0.63	0.70	2.08	**0.54**
$clogD_{7.4}$	2.3	0.43	0.64	1.2	0.89
GSH Adduct Ratio [Compound mZ/DCF mZ]	1.1	0.36	0.59	0.88	1.08

	Minimum	Moderate	Diagnostic
PLR :	1-3	3-10	>10
NLR :	1-0.75	0.75-0.1	<0.1

Tony Pourmohamad, Aaron Fullerton

Genentech
A Member of the Roche Group

Parameter Intersections with hLiMT Enrich DILI+ve Compounds



Bivariate parameters:	Sensitivity:	Specificity:	PLR:	NLR:
hLiMTs MOS x BSEP MOS	0.18	0.99	15.75	0.83
hLiMTs MOS x Mitotox: % Max Inh	0.14	1.00	>20	0.86

Consistent with previous reports in the literature, intersection of assay results can create a gate with ~100% Specificity for DILI+ve compounds

Genentech
A Member of the Roche Group

Overall Conclusions and Next Steps

- To date, 3D hepatic spheroids are currently the most predictive single assay of all DILI parameters/assays evaluated at Genentech
- Evaluations underway to determine which mechanisms can be address in this model (e.g. mitochondrial stress, bile-acid homeostasis)
- hLiMT, in combination with other DILI assays, can identify patterns of risk that can be decisional in the absence of other data
- 3D hepatic spheroid models are positioned as long-term cytotoxicity screen to support all small molecule discovery programs during lead optimization

Acknowledgements

Safety Assessment

Aaron Fullerton
Tomo Kiyota
Catrin Hasselgren
Chris Lawson
Min Tseng
Jennifer Vogt
Leah Norona
Leslie Leung
Jon Maher
Zoe Zhong
Lennart Anger
Orlando Zuniga
Gabriel Olivera

AstraZeneca
IMED Biotech Unit

Alison Foster
Dominic Williams
Mark Pilling

Drug Metabolism and Pharmacokinetics

Cyrus Khojasteh
Kevin Johnson
Zhengyin Yan
Donglu Zhang
Jane Kenny
Eugene Chen
Fabio Broccatelli
Emile Plise
Jonathan Cheong
Jamie Jorski
Katherine Gaffney
Mika Kosaka
Hadil Shalan
Peter Vuong
Suzanne Tay

insphero

Monika Kijanska
Johannes Haugstetter
Simon Messner
Jens Kelm
Jan Lichtenberg

Miscellaneous

Justin Feigelman
Tony Pourmohamad
Bing Chen
Mike Rothenberg
BinQing Wei
Kevin P Clarke

Roche

Simone Schadt
Christoph Funk
Stefan Kusterman
Adrian Roth

Genentech
A Member of the Roche Group

Five minutes Break!

Panel Discussion I

Chair: Shiew-Mei Huang (FDA)

Panelists:


S. Huang


S. Fitzpatrick


R. Wange


Q. Liu


A. Ribeiro


P. Brown


L. Ewart


S. Ferguson


E. Chow


W. Proctor


S. Mumenthaler