Modernizing Drug Discovery Using Organ-on-a-Chip Technology

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10th February 2023

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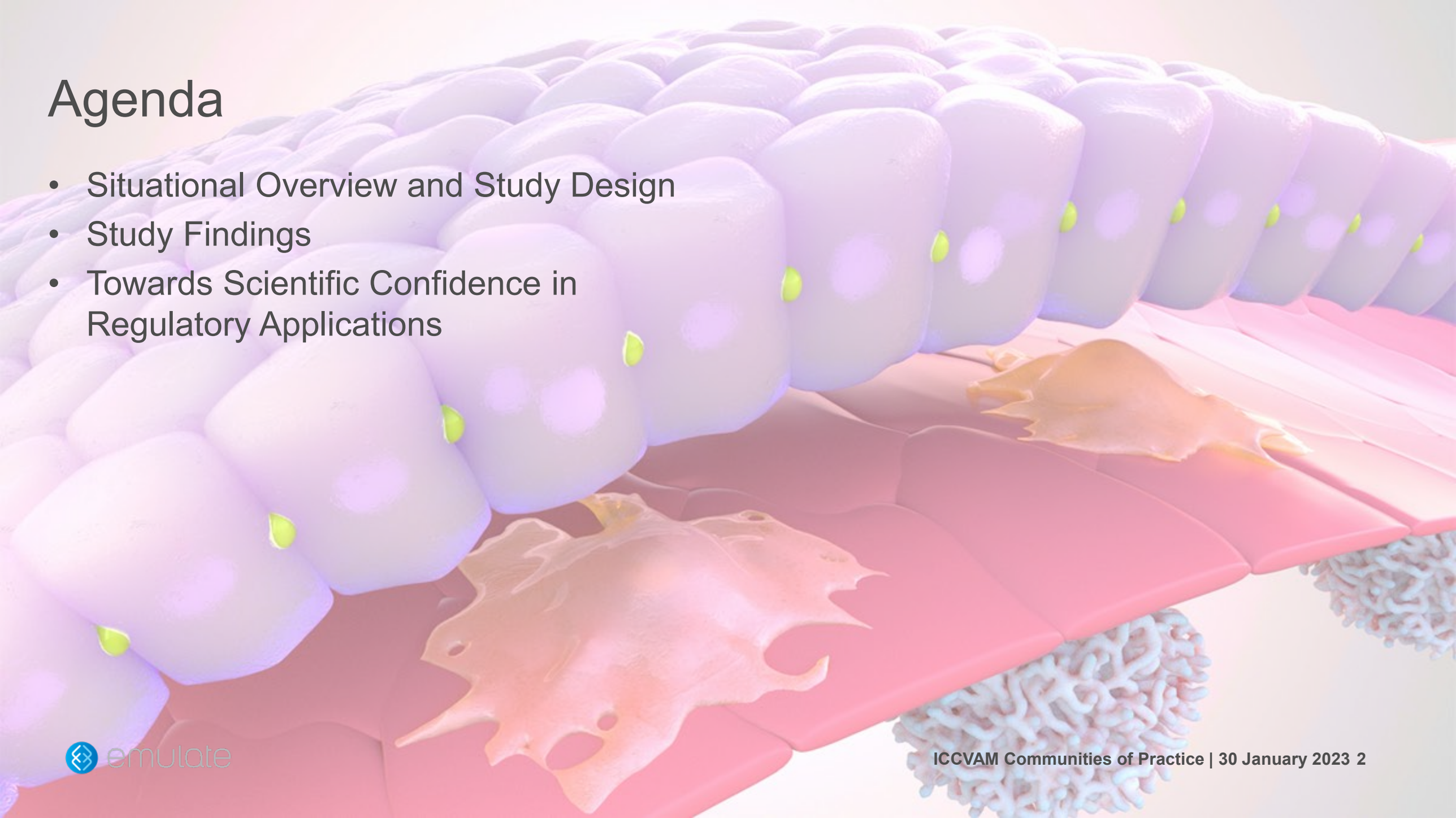
Agenda

- Situational Overview and Study Design
- Study Findings
- Towards Scientific Confidence in Regulatory Applications



Situational Overview and Study Design

Drug-Induced Liver Injury Continues to Cause Drug Attrition

Phase IIb stopped: Vupanorsen associated with dose-dependent elevations in transaminases and liver fat

January

Phase III partial hold: Tolebrutinib associated with DILI in patients with that may be predisposed to DILI

June

On market: 2 patient deaths resulting from acute liver failure following Zolgensma treatment

August

2022

Phase Ib stopped: ALG-020572 associated with elevations in transaminases and risk of DILI

March

Phase Ib raises flag: Lumakras in combination with Keytruda or Tecentriq resulted in 50% of patients with severe liver toxicity

August

Phase Ib paused: SZN-1326 associated with increased transaminases in healthy participants

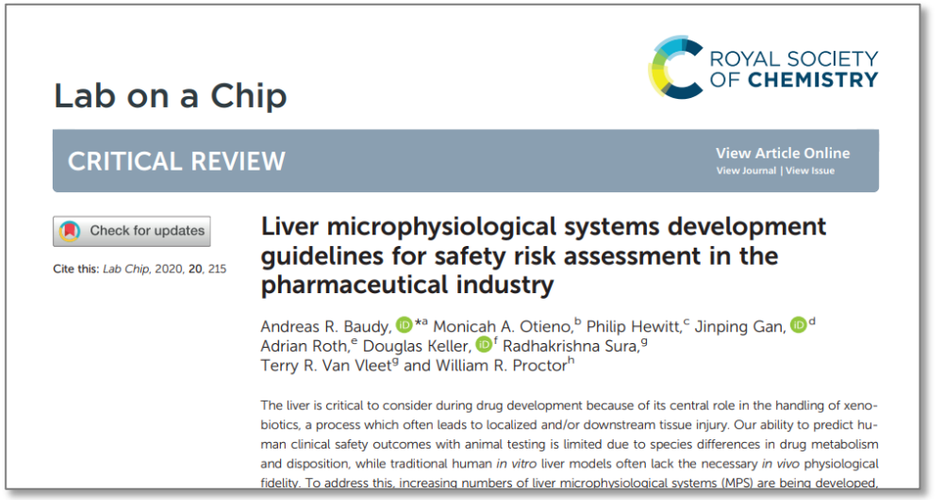
November

Towards a Rigorous Model Evaluation of the Liver-Chip

Study Design Guided by Pre-Specified Criteria Set by Expert 3rd party

1. **Biological recapitulation**: Does the model capture *relevant* aspects of the clinical state (e.g., known drug responses, histology, gene expression, etc.)
2. **Tests and endpoints**: Is testing and scoring of candidates relevant to the clinical state?
3. **Statistical and experimental hygiene**: Does the model minimize bias, noise, etc.
4. **Domains of validity**: Within what set of circumstances are the results of the model generalisable?

Dr Jack Scannell Criteria for Predictive Validity Assessment



Lab on a Chip

ROYAL SOCIETY OF CHEMISTRY

CRITICAL REVIEW

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Liver microphysiological systems development guidelines for safety risk assessment in the pharmaceutical industry

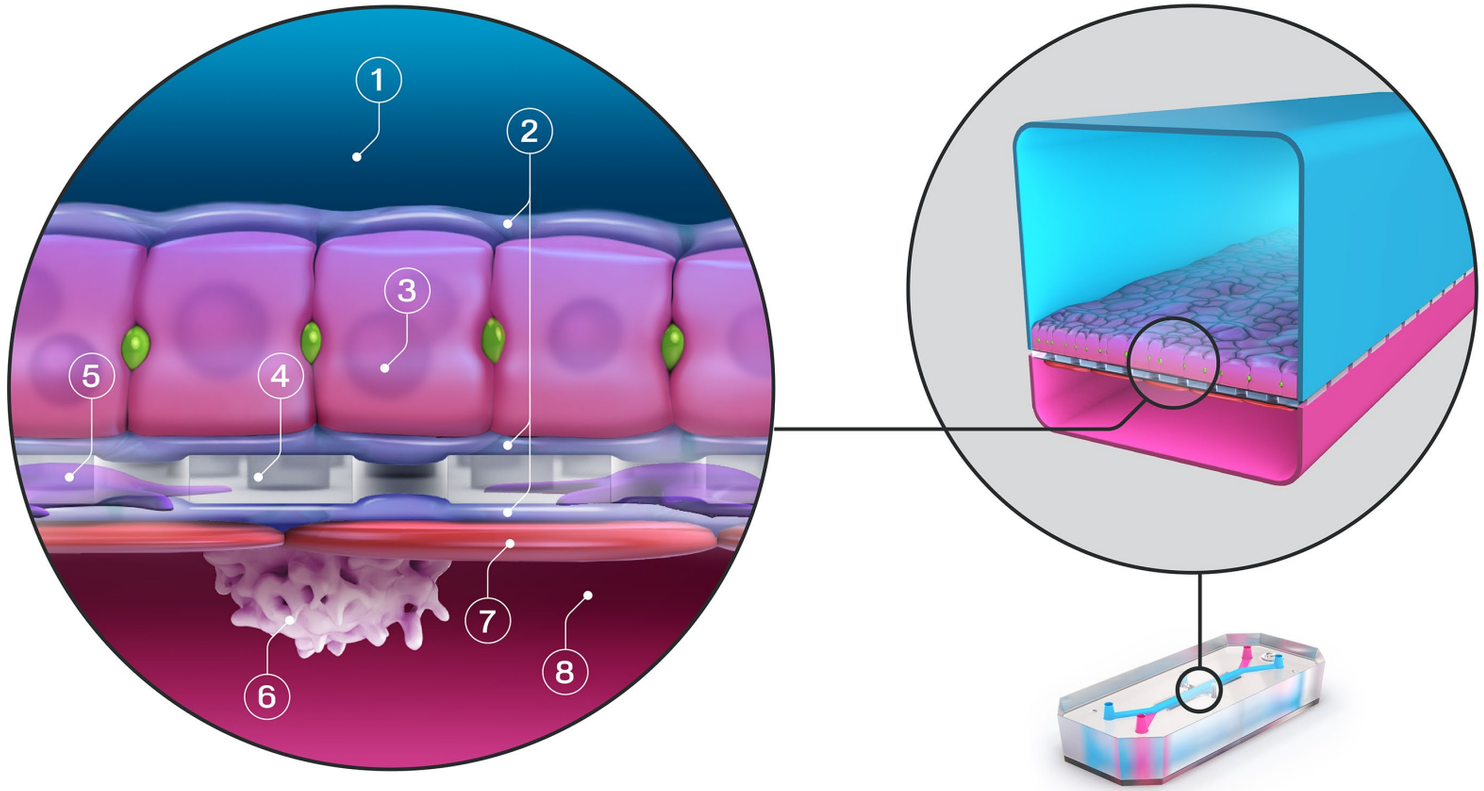
Andreas R. Baudy, *[a] Monicah A. Otieno,[b] Philip Hewitt,[c] Jinping Gan,[d] Adrian Roth,[e] Douglas Keller,[f] Radhakrishna Sura,[g] Terry R. Van Vleet[g] and William R. Proctor[h]

The liver is critical to consider during drug development because of its central role in the handling of xenobiotics, a process which often leads to localized and/or downstream tissue injury. Our ability to predict human clinical safety outcomes with animal testing is limited due to species differences in drug metabolism and disposition, while traditional human *in vitro* liver models often lack the necessary *in vivo* physiological fidelity. To address this, increasing numbers of liver microphysiological systems (MPS) are being developed,

Baudy et al., Lab Chip 2020

Human Quad-Culture Liver-Chip

1. Top Channel
2. Extracellular Matrix
3. Hepatocytes
4. Porous Membrane
5. Stellate Cells
6. Kupffer Cells
7. Endothelial Cells
8. Bottom Channel



- **All cells used are human primary cells**

Candidate Drug Selection

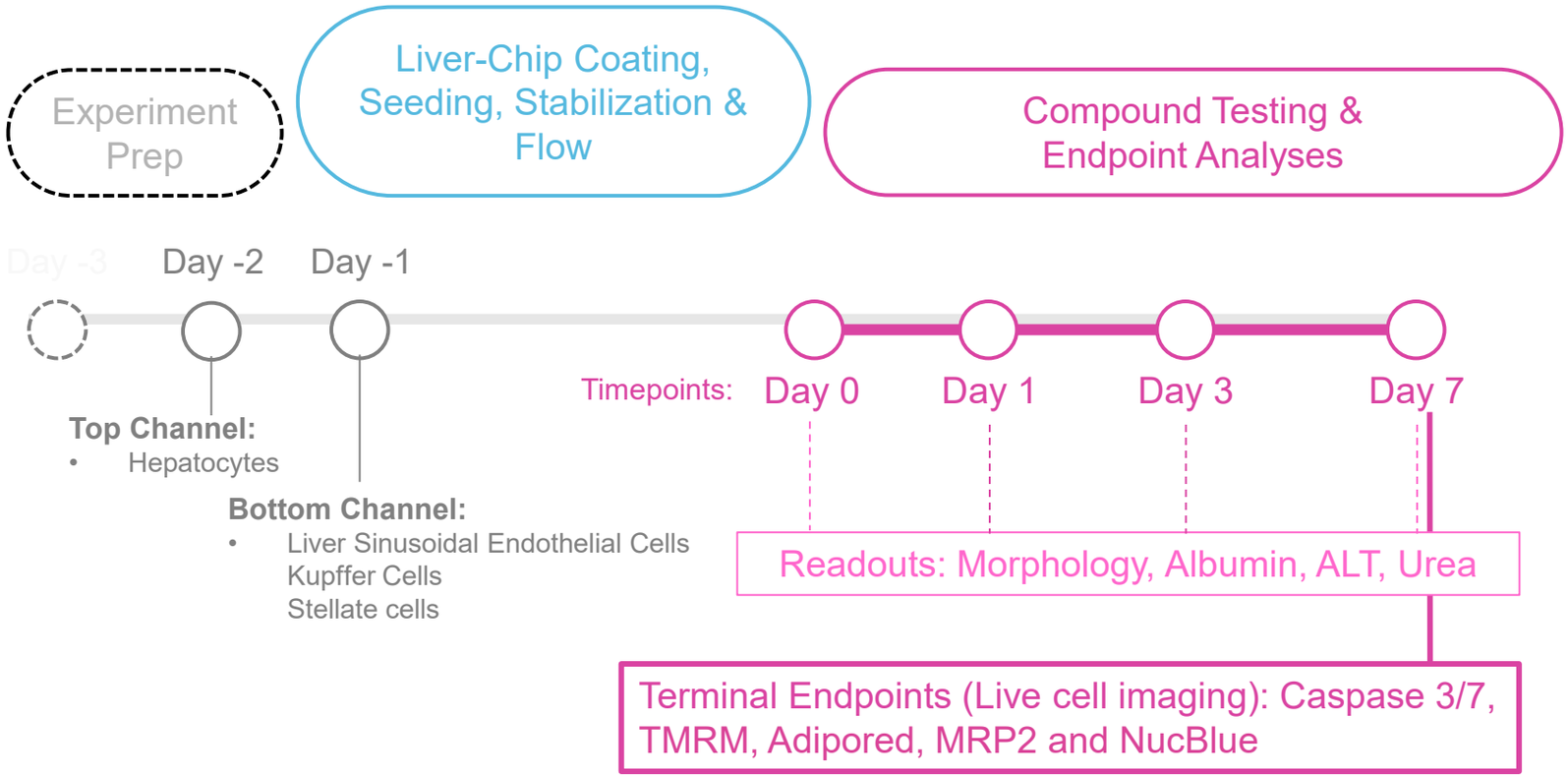
Drug	Proposed Major Mechanism of DILI	IQ MPS	Spheroid
Sitaxsentan	Inhibition of transporter	Yes	Yes
Clozapine	Metabolism leading to toxic intermediate	Yes	Yes
Diclofenac	Mitochondrial dysfunction	Yes	Yes
Zileuton	Reactive Oxygen Species production	Yes	Yes
Tolcapone	Reactive Oxygen Species production	Yes	Yes
Troglitazone	Mitochondrial dysfunction	Yes	Yes
Trovafloxacin	Mitochondrial dysfunction	Yes	Yes
Nefazodone	Metabolism leading to toxic intermediate	Yes	Yes
Ambrisentan	No reported DILI	Yes	Yes
Entacapone	No reported DILI	Yes	Yes
Pioglitazone	Mitochondrial dysfunction	Yes	Yes
Levofloxacin	Mitochondrial dysfunction	Yes	Yes
Fialuridine	Inhibition of transporter	Yes	Yes
Asunaprevir	Alteration of bile acids	Yes	No
Telithromycin	Alteration of bile acids	Yes	No
Olanzapine	No reported DILI	Yes	No
FIRU	No reported DILI	Yes	No
Buspirone	No reported DILI	Yes	Yes
Stavudine	Mitochondrial dysfunction	No	Yes
Benoxaprofen	Metabolism leading to toxic intermediate	No	Yes
Beta-Estradiol	Inhibition of transporter	No	Yes
Chlorpheniramine	Reactive Oxygen Species production	No	Yes
Labetalol	Metabolism leading to toxic intermediate	No	Yes
Simvastatin	Metabolism leading to toxic intermediate	No	Yes
Tacrine	Metabolism leading to toxic intermediate	No	Yes
Ximelagatran	Immune mediated	No	Yes
Lomitapide	Inhibition of triglyceride transport	No	No

27 small molecules tested

- 18 out of the proposed 20 drugs from IQ MPS Affiliate manuscript
- Enriched with drugs that were ‘false negatives’ in hepatic spheroids
- Major mechanisms of drug-induced liver injury (DILI) represented
- All drugs were tested blind in concentrations adjusted for protein in chip media
- Concentration range tested up to 300x calculated free Human C_{max} obtained from literature

Performance Assessment of Liver-Chip

870 Human Liver-Chips used in Five Experimental Cycles with Three Donors



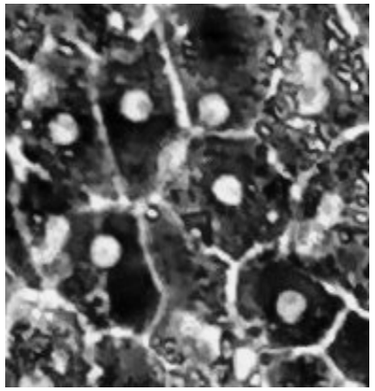
- Five experimental cycles were conducted:
  - Cycle one and two tested 25 drugs in donor one
  - Cycle three tested 14 drugs in donor two
  - Cycle four tested 6 drugs in donor one and 4 drugs in donor two
  - Cycle five tested 6 drugs in donor three
  - RNASeq was performed on freshly thawed hepatocytes and at Day 3 and 7 in donor 2 and 3

Study Findings

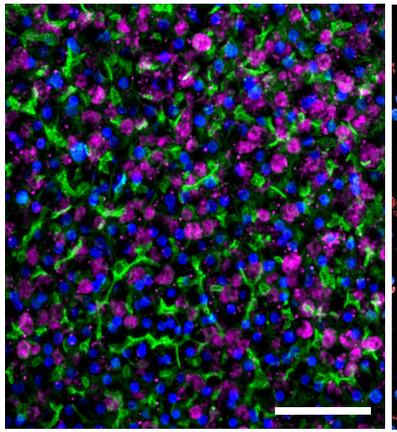
Liver-Chip Exhibits Typical Hepatic Phenotypic Markers

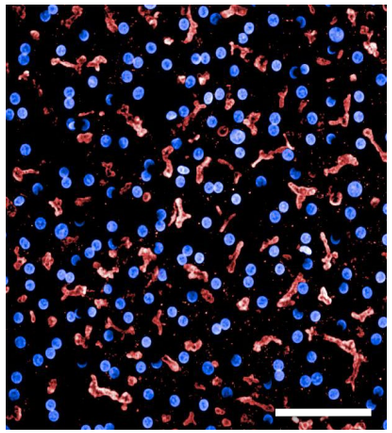
Immunofluorescent Imaging of both Channels in Human Liver-Chips

Hepatocytes



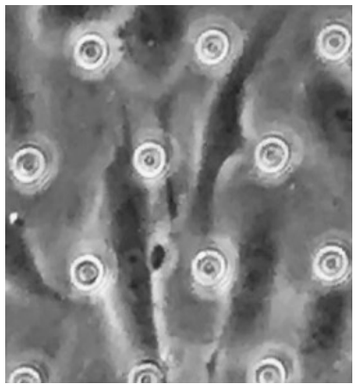
Cytoskeleton
Mitochondria
Nucleus



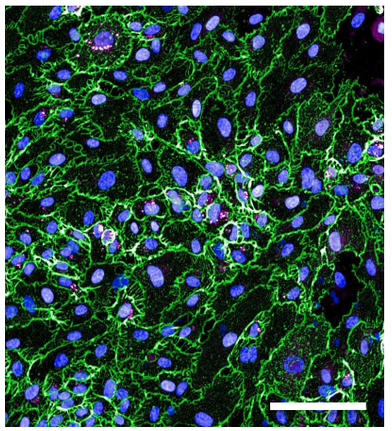


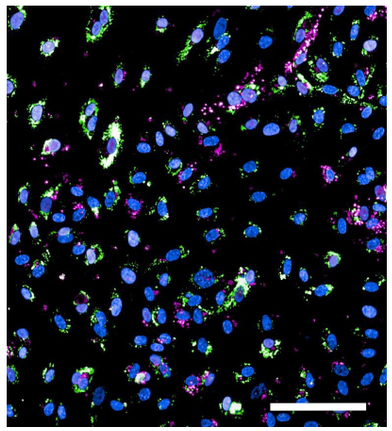
MRP2
Nucleus

Non-Parenchymal Cells



Endothelial Cells
Stellate Cells
Nucleus





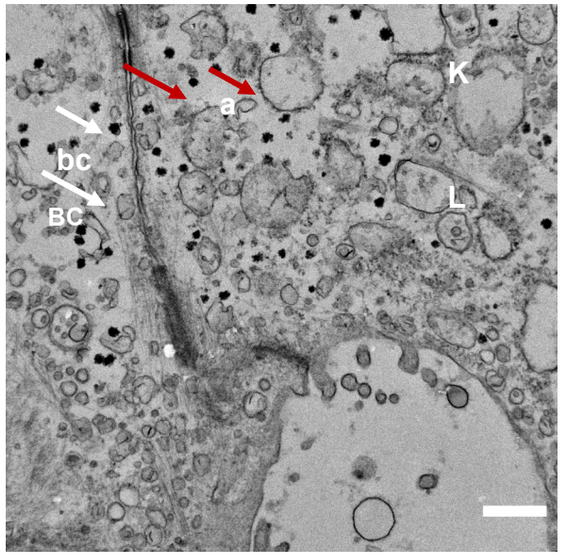
Kupffer Cells
Stellate Cells
Nucleus

Scale bar = 100 microns

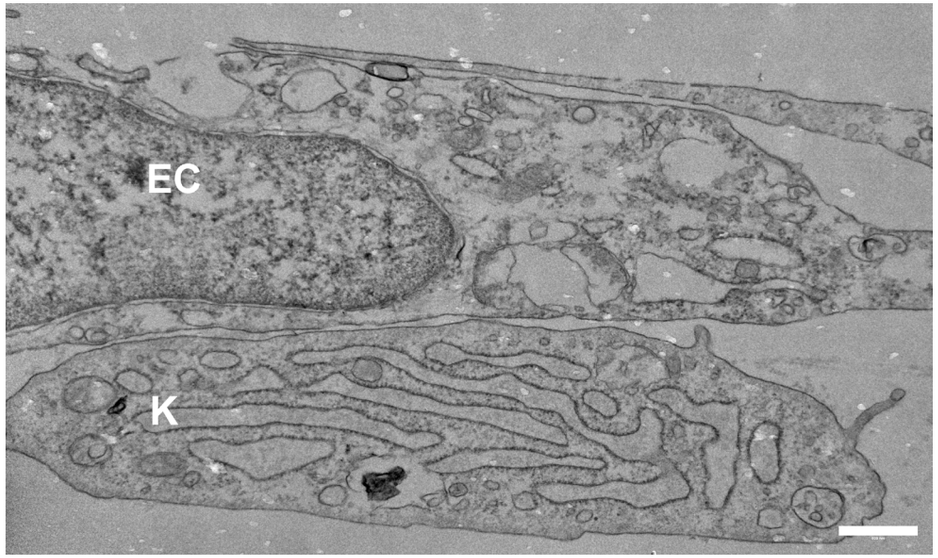
Transmission Electron Microscopy of Liver-Chip

Subcellular Structures Visualized in Both Channels of Liver-Chip

Bile Canaliculus (BC)



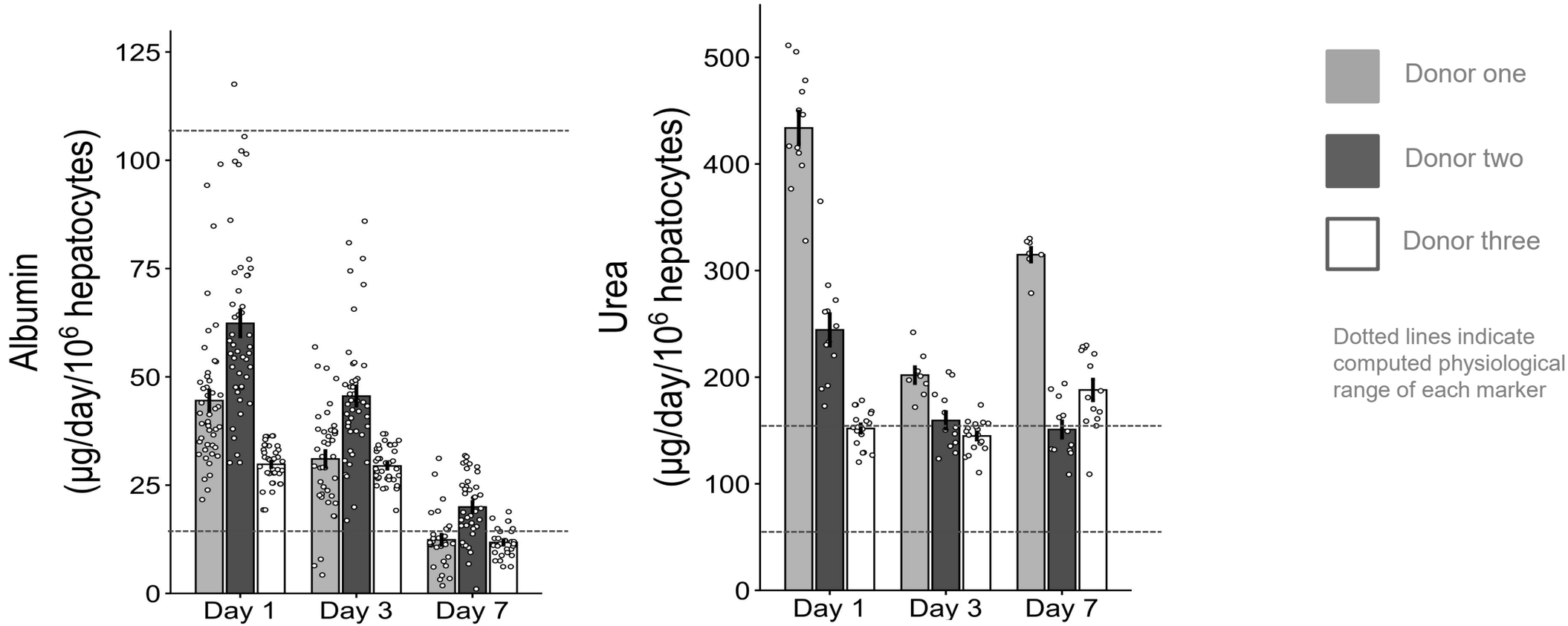
Kupffer Cell (K) and Endothelial Cell (EC)



Red arrow shows albumin deposition

Baseline Fidelity of Liver-Chip Measured using Biomarkers

Effluent from the Hepatocyte Channel was Measured

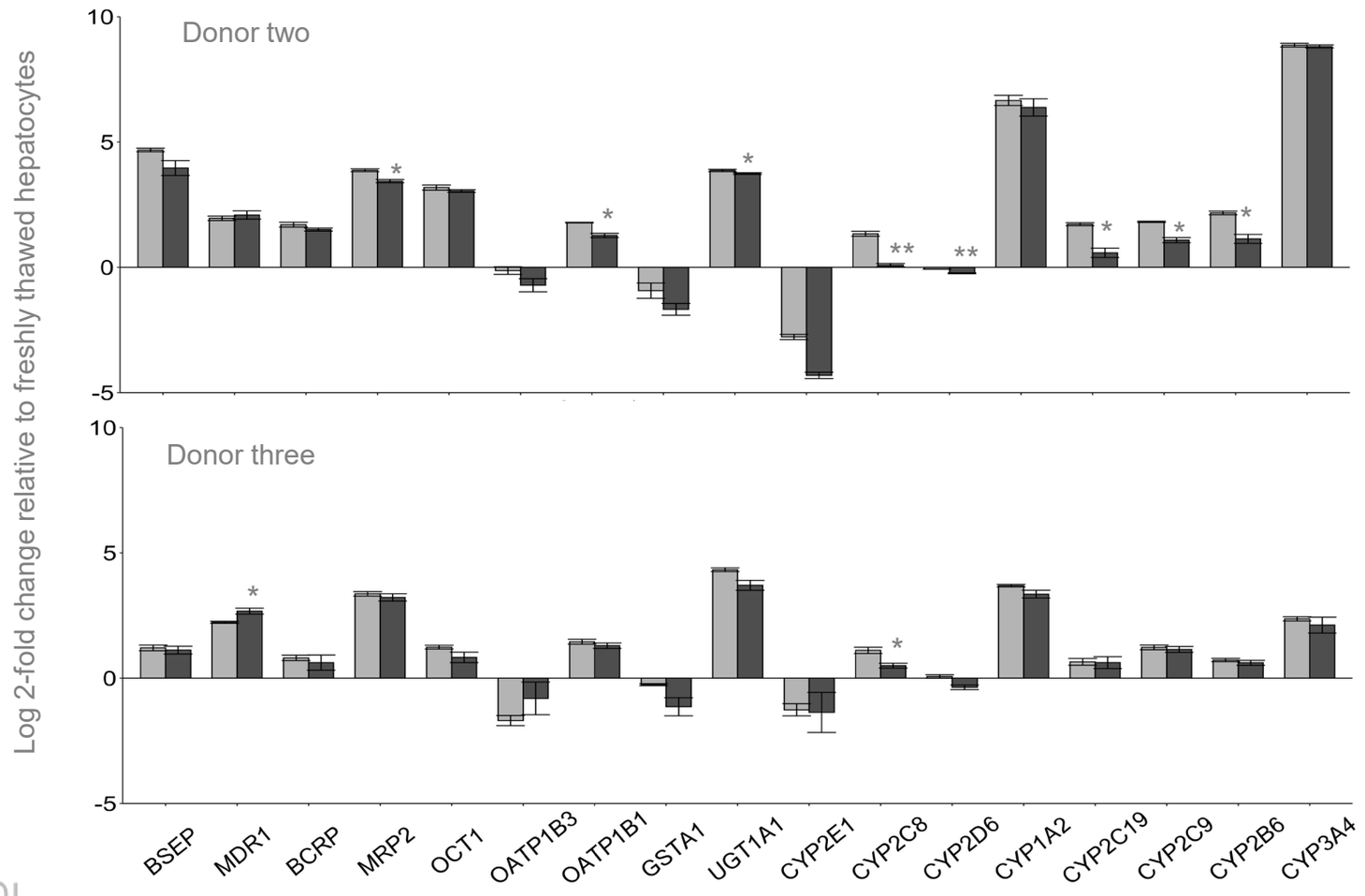


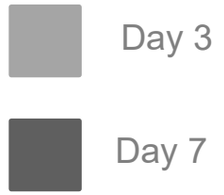
Healthy hepatocellular functionality was demonstrated by measurement of albumin and urea production

Ewart et al., 2022 Communications Medicine volume 2, Article number: 154

Gene Expression Remained Stable During Culture

RNASeq Data of Key Phase I and II Enzymes and Drug Transporters





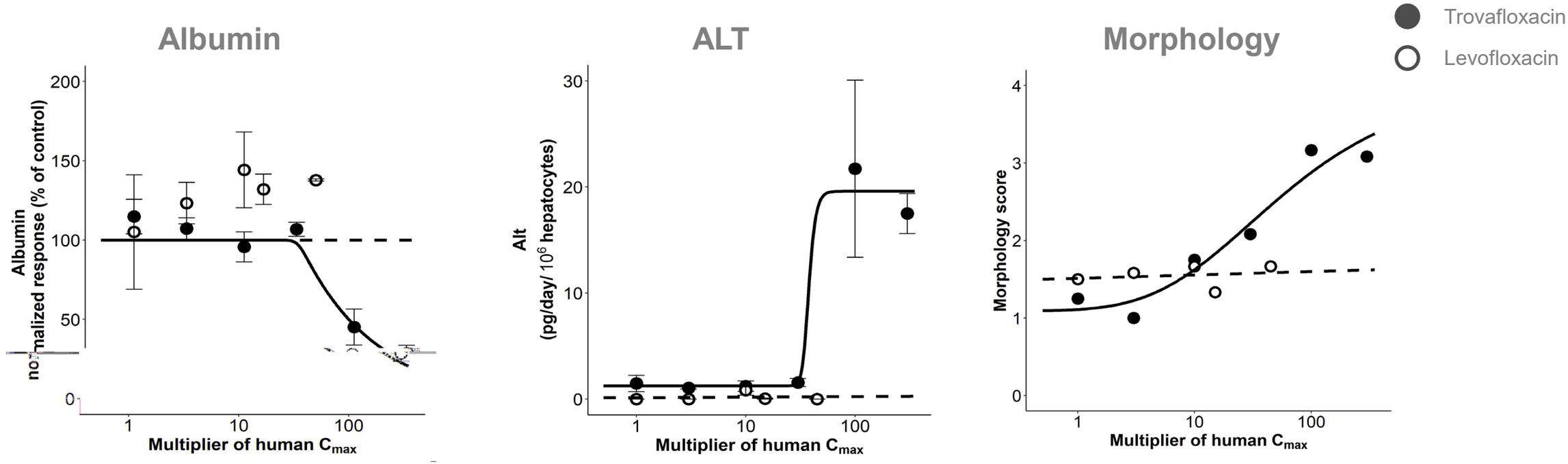
N = 4 chips; statistical significance of values between day 3 and 7 was determined using a paired t-test
**, p<0.05, **, p <0.01.*

- Data expressed relative to freshly thawed hepatocytes
- Stable expression of the majority of genes maintained in vehicle-treated Liver-Chips

Ewart et al., 2022 Communications Medicine volume 2, Article number: 154

Multiple Measures used to Determine Toxicity

Liver-Chip can Distinguish Between Toxic and Lesser Toxic Structural Analogs

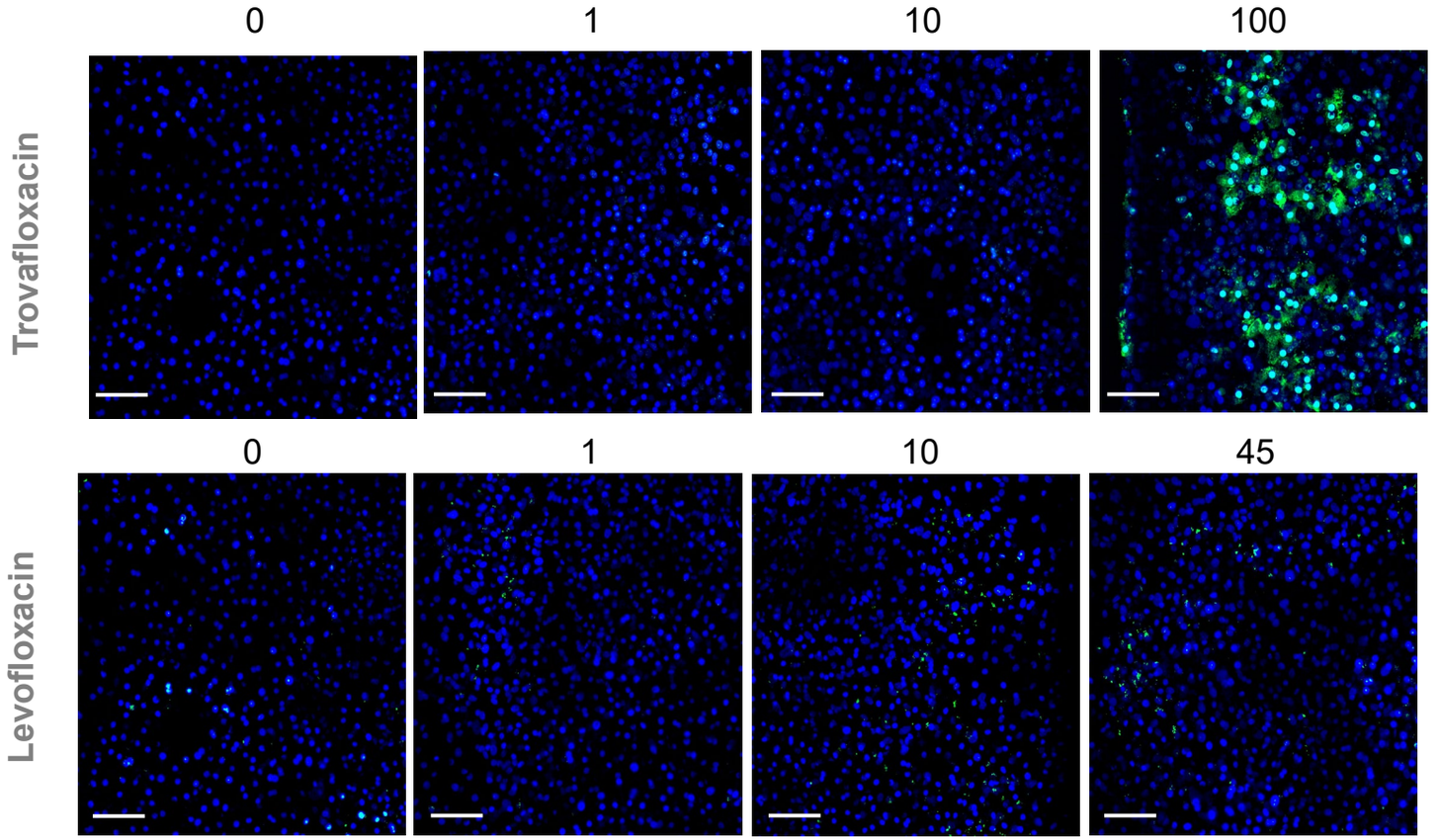


Trovafloxacin causes a reduction in albumin production and a concomitant increase in ALT and cell damage as indicated by morphology. The lesser toxic drug Levofloxacin is without effect on these parameters.

Ewart et al., 2022 Communications Medicine volume 2, Article number: 154

Day 7 Immunofluorescent Imaging of Hepatocyte Channel

Imaging Indicates Potential Mechanism of Toxicity



Nucleus
Caspase 3/7
Concentrations fold over C_{max}

Scale bar = 100µm

Levofloxacin treatment is not associated with an increase in Caspase 3/7 whereas Trovafloxacin treatment does increase Caspase 3/7 indicating potential of apoptotic cell death.

Ewart et al., 2022 Communications Medicine volume 2, Article number: 154

Study Findings

Liver-Chip Correctly Differentiates all Toxic Drugs as Proposed by IQ MPS[1]

More toxic Drug	Albumin	ALT	Morphology	IF Imaging		Less toxic Drug	Albumin	ALT	Morphology	IF Imaging	Outcome
Sitaxsentan	↓	↑	↑	Apoptosis Mitotoxicity	vs.	Ambrisentan	—	—	—	No change	✓
Clozapine	↓	↑	↑	Apoptosis	vs.	Olanzapine	—	—	—	No change	✓
Troglitazone	↓	↑	↑	Apoptosis	vs.	Piogliatzone	↓	—	—	No change	✓
Trovafloxacin	↓	↑	↑	Apoptosis	vs.	Levofloxacin	—	—	—	No change	✓
Fialuridine	↓	—	—	Minimal Steatosis	vs.	FIRU	—	—	—	Minimal Steatosis	✓
Nefazodone	↓	↑	↑	No change	vs.	Buspirone	—	—	—	No change	✓
Tolcapone	↓	↑	↑	Mitotoxicity	vs.	Entacapone	—	—	—	No change	✓

The lesser toxic drug of the pair typically showed no change across multiple parameters measured longitudinally across the study

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Ewart et al., 2022 Communications Medicine volume 2, Article number: 154
[1]Baudy et al., 2020 Lab Chip 20, 215

Quantitative Assessment of Liver-Chip Performance

Liver-Chip also has a Spearman Correlation Coefficient of 0.78

	DILI positive	DILI negative
Model positive	True Positive	False Positive
Model negative	False Negative	True Negative

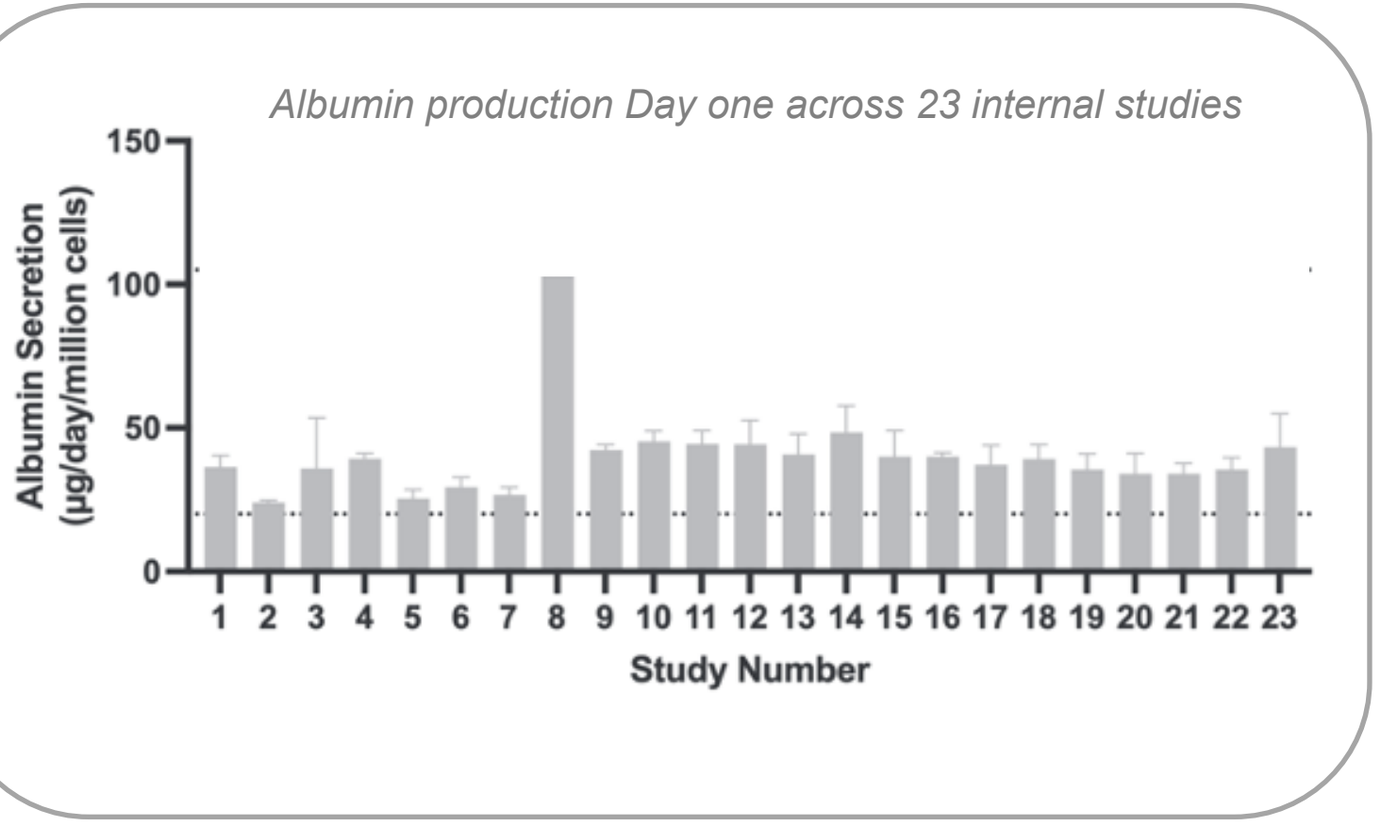
Analysis based on MOS threshold 375 & corrected for protein binding

	DILI +ve	DILI -ve	
Animal models (27 drugs)	0	0	**0%** sensitivity
	22	5	**100%** specificity
Spheroids (22 drugs)	9	0	**47%** sensitivity
	10	3	**100%** specificity
Chips – 1 donor (27 drugs)	17	0	**77%** sensitivity
	5	5	**100%** specificity
Chips – 2 donor (18 drugs)	11	0	**73%** sensitivity
	4	3	**100%** specificity
Chips – both donors (18 drugs)	13	0	**87%** sensitivity
	2	3	**100%** specificity

Towards Scientific Confidence in Regulatory Applications

Liver-Chip is Reproducible Internally and Externally

Intra-Laboratory



Inter-Laboratory

Food and Chemical Toxicology — 2020

Utilization of a model hepatotoxic compound, diglycolic acid, to evaluate liver Organ-Chip performance and in vitro to in vivo concordance

CURRENT PROTOCOLS — 2022

Co-Culture of Human Primary Hepatocytes and Non-parenchymal Liver Cells in the Emulate Liver-Chip for the Study of Drug-induced Liver Injury

FDA

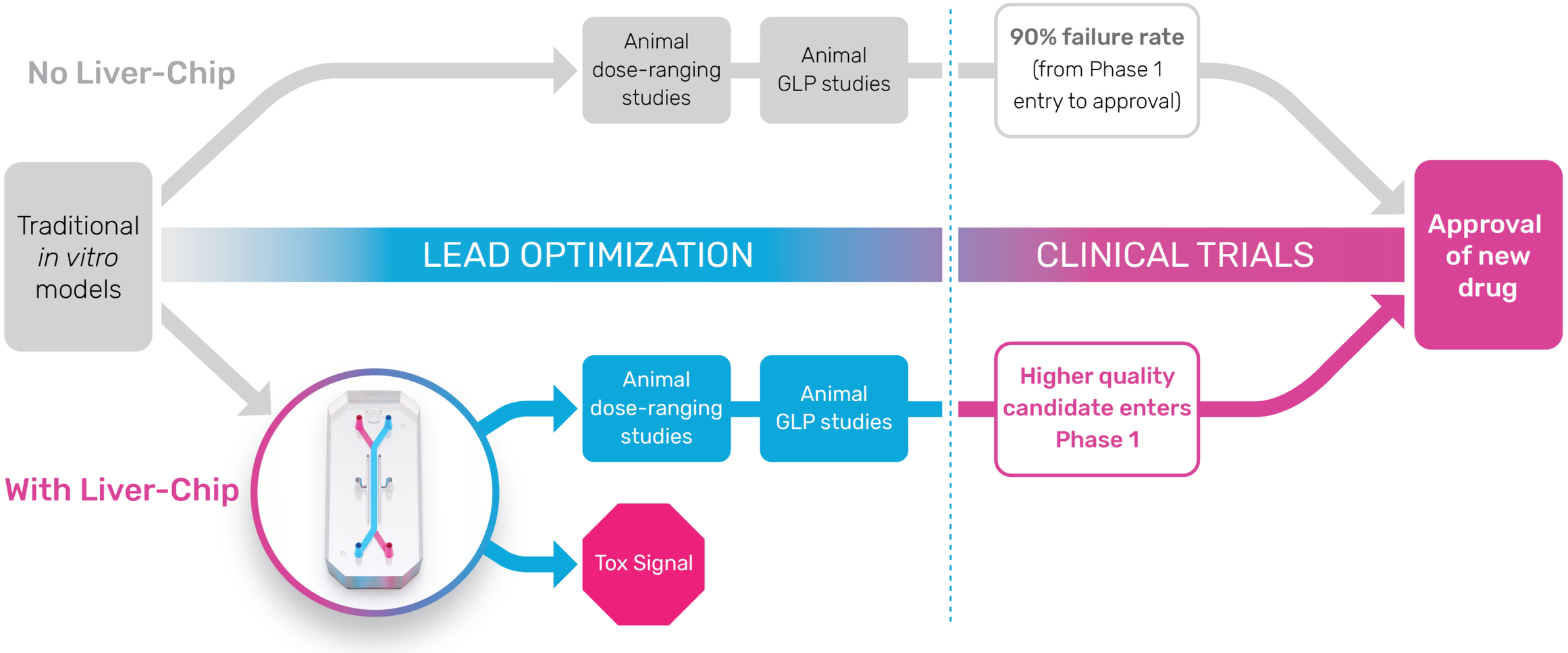
Both of these papers were conducted by FDA scientists in their laboratories under a CRADA

Translational Relevance: Implications on Patient Safety

Drug	Manufacturer	Market Status	Deaths	Liver-Chip Detected
Benoxaprofen	Lilly	Withdrawn 1983	139	Yes
Fialuridine	Lilly	Clinical trial terminated	5	Yes
Labetalol	Pfizer	On market with warnings	1	Yes
Nefazodone	BMS	On market black box label	20	Yes
Sitaxsentan	Pfizer	Withdrawn 2010	4	Yes
Stavudine	BMS	Withdrawn 2020	1	Yes
Telithromycin	Sanofi-Aventis	Withdrawn 2016	4	Yes
Tolcapone	Bausch Health Companies	On market black box label	1	Yes
Troglitazone	Parke Davis/Warner Lambert	Withdrawn 2000	61	Yes
Trovafloxacin	Pfizer	Withdrawn 1999	5	Yes
Ximelagatran	AstraZeneca	Withdrawn 2006	1	Yes

Tested drugs caused 242 patient deaths and 10 liver transplants

Implementing the Liver-Chip in Preclinical Workflows



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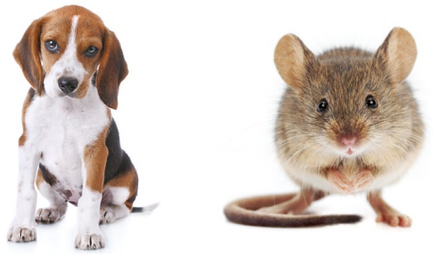
Relevant, Reproducible Organ-Chips in a Regulatory Setting

Regular Practice



Candidates Tested: +
Donors: +
Organ-Chip Models:+

Coexistence

Candidates Tested: ++
Donors: ++
Organ-Chip Models: ++

Reduce & Refine

Candidates Tested: +++
Donors: +++
Organ-Chip Models: +++

Replace

Candidates Tested: ++++
Donors: ++++
Organ-Chip Models: ++++

Time Qualification

Summary

- Liver-Chip predicts small molecule DILI risk
  - Qualified according to IQ MPS affiliate publication (Baudy et al., 2020)
  - Distinguished all toxic drugs from their non or lesser toxic structural analogs
  - Sensitivity was 87%, specificity was 100%
- Liver-Chip is reliable and has human relevance
  - Albumin production in vehicle groups show a high degree of consistency in house and in external labs
  - Liver-Chip detected drugs that subsequently caused death or liver transplantation
- Biological relevance can drive regulatory confidence
  - Liver-Chips are reproducible within and across laboratories
  - Well characterized models can “co-exist” with animals to continue driving confidence in the approach through more data acquisition

THANK YOU

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