Mechanistic Approaches Using 3D Microtissues to Evaluate Preclinical and Clinical Drug-Induced Liver Injury

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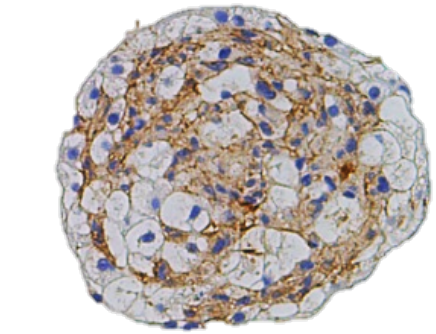
Chief Scientific Officer, InSphero

American College of Toxicology
Signature Webinar
October 21, 2020

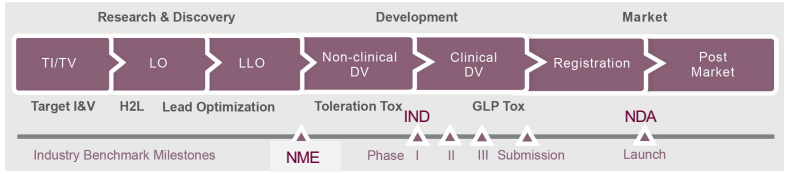
Presentation Overview

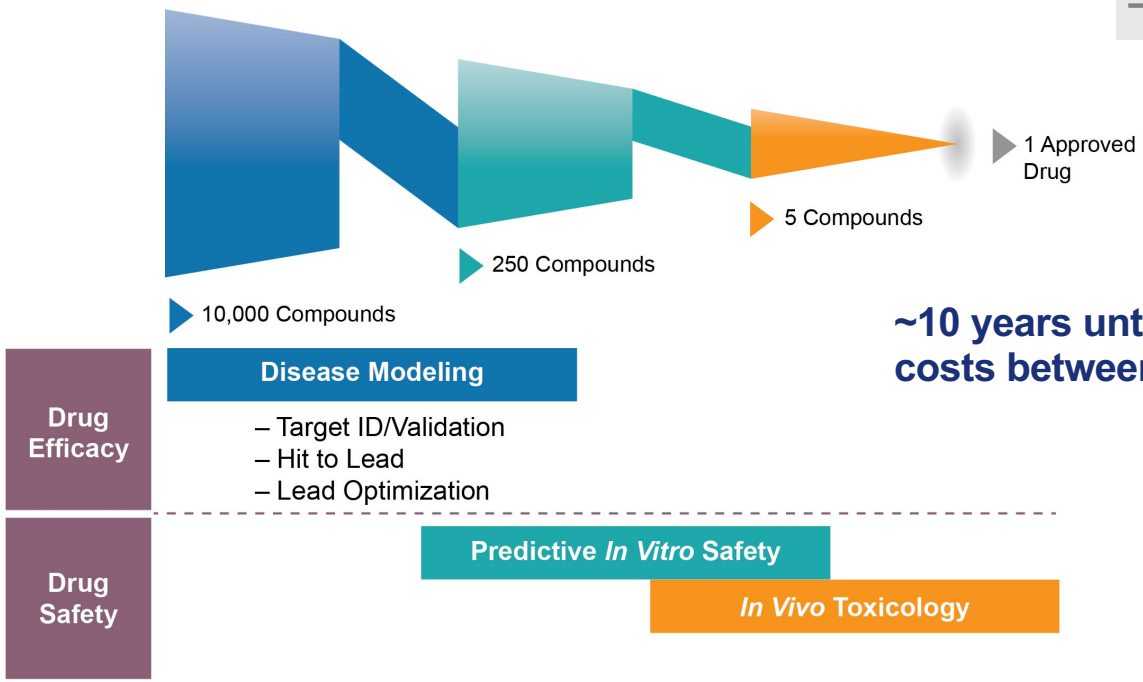
- Introduction: Drug discovery and translation to man
- ATP-based DILI hazard identification
- Stepping beyond ATP: from hazard identification to risk assessment
- Impact of 3D microtissues in drug development
- Outlook: challenges and opportunities



Drug Discovery and Development Workflow

The rocky road to success

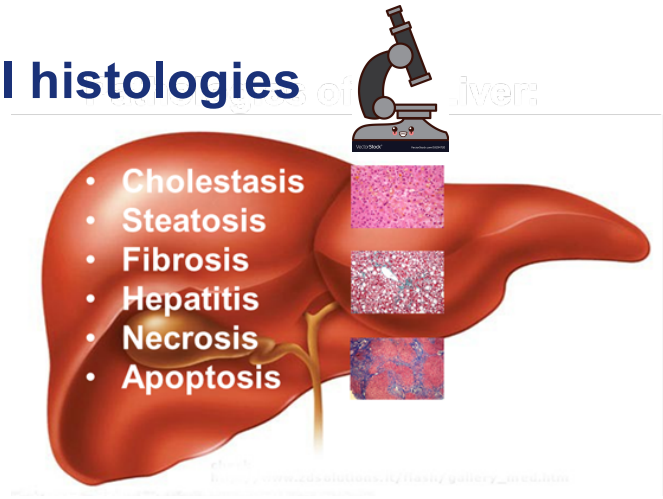


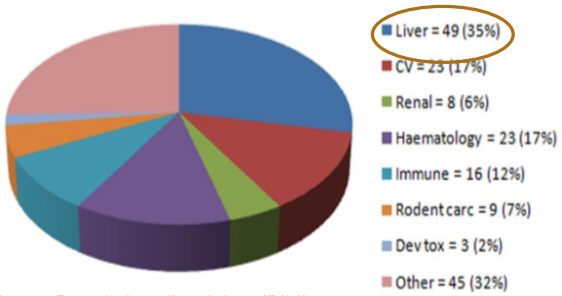


~10 years until launch and costs between $2-3 billion USD

Drug-induced Liver Injury (DILI): A Major Cause for Attrition

DILI histologies





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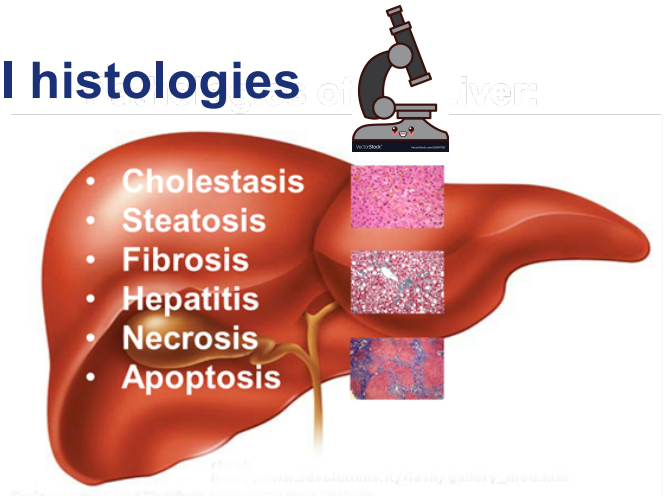

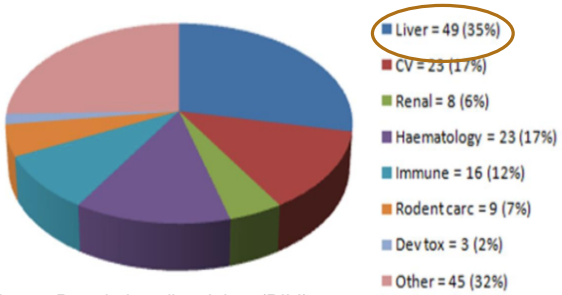

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Preclinical attrition

- After animal tox studies
- Pharma average nonclinical attrition rates due to DILI are between 30-45%

Clinical attrition

- Liver toxicity in clinical testing phases I, II, III after preclinical risk assessment

Post-launch market withdrawals

- Acute liver injury (idiosyncratic DILI) after successful clinical evaluations

Why is Drug Development So Challenging?

High failure rate suggests we're getting Lost in Translation

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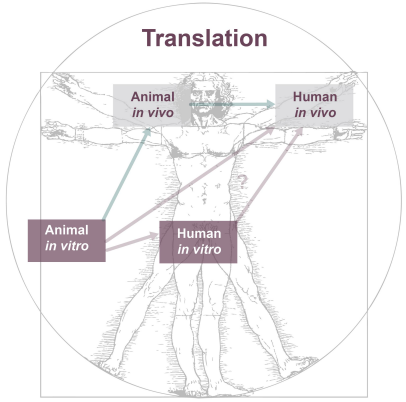
- Tremendous need for improved translation to human



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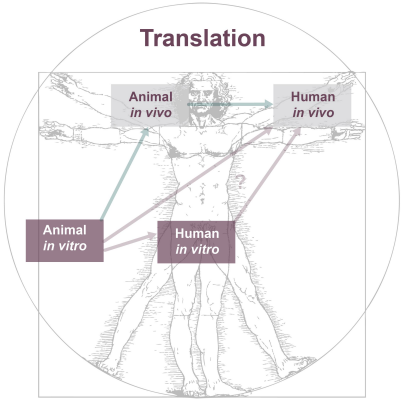


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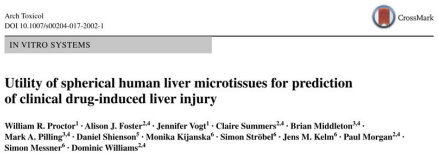
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IN VITRO SYSTEMS

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

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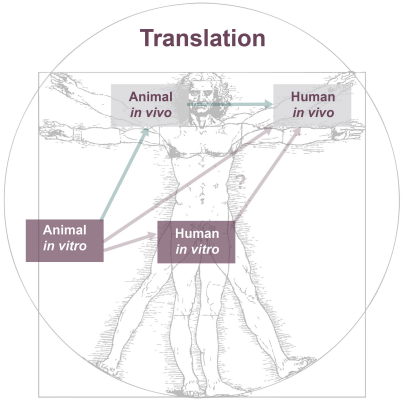


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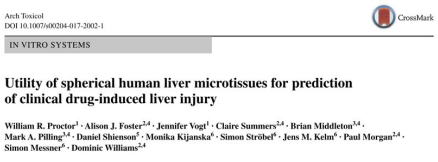
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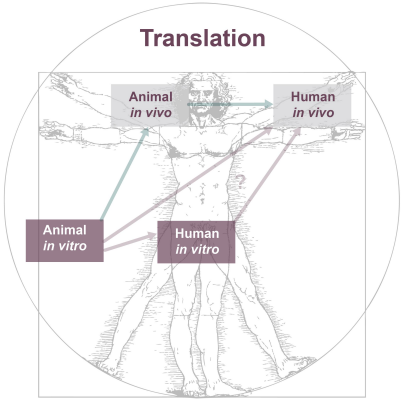

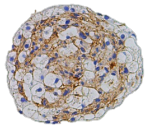

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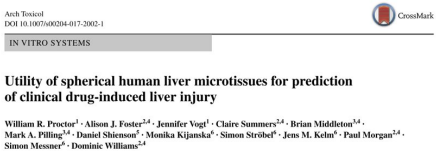

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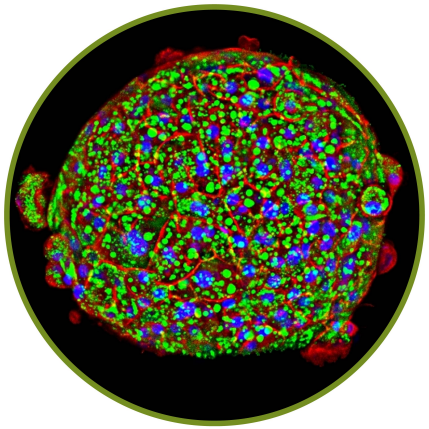
Physiologically Relevant Features of 3D Liver Models

Why 3D-liver microtissues/spheroids ?

Multi-cellular spheroid model

Smallest functional unit of the liver



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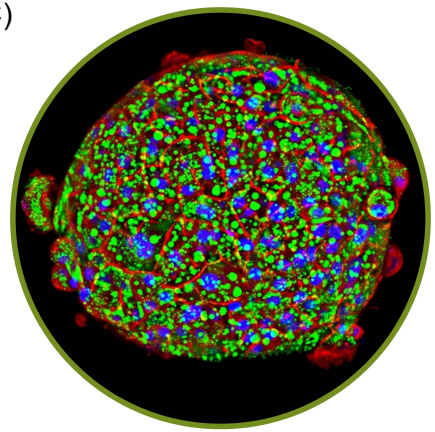
(PHH, LEC, KC, and SC)



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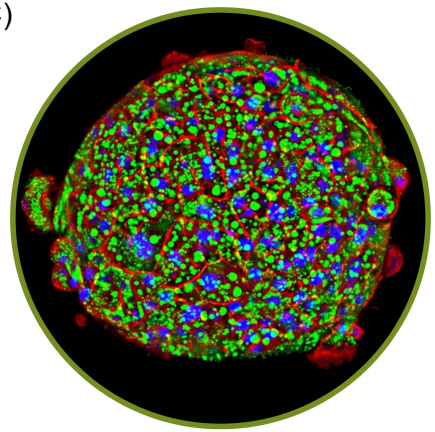

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Drug metabolism, transport, synthesis and secretion

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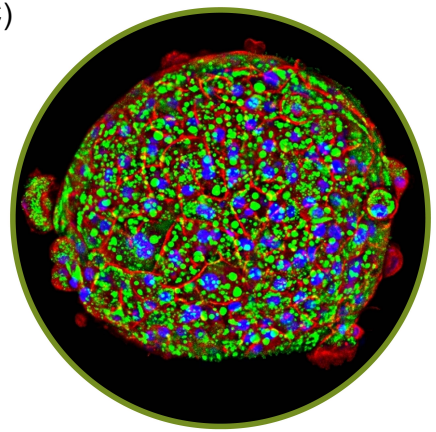

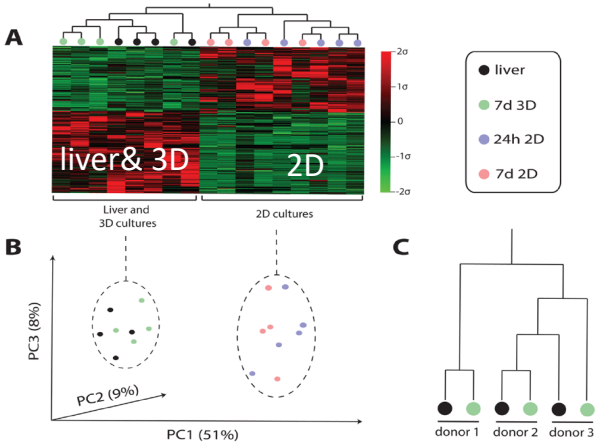

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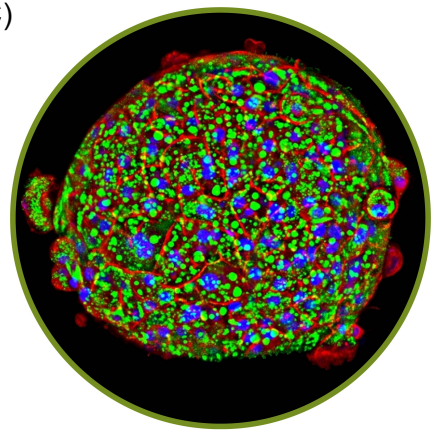

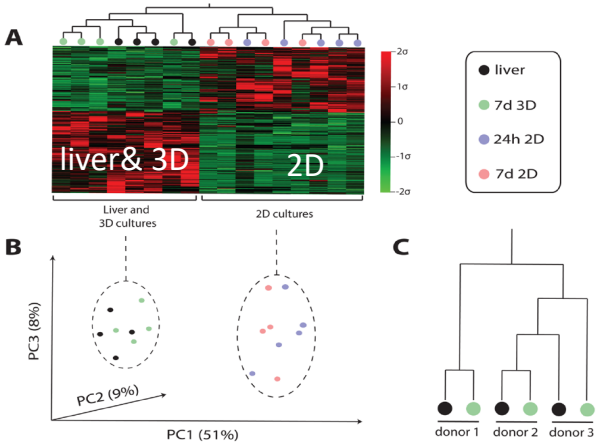

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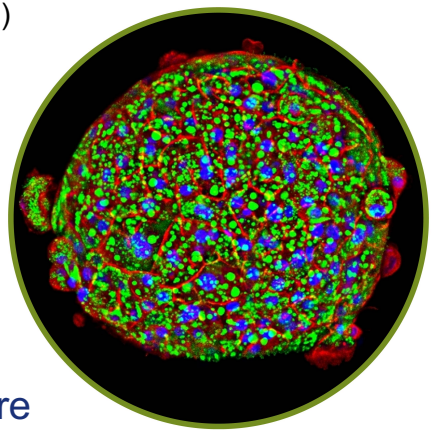

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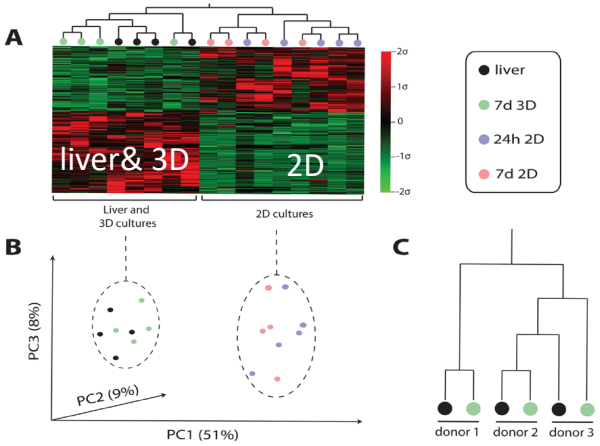

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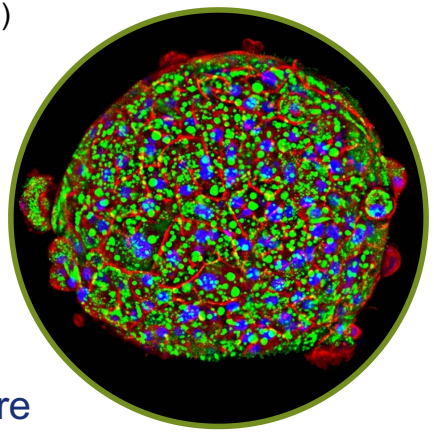

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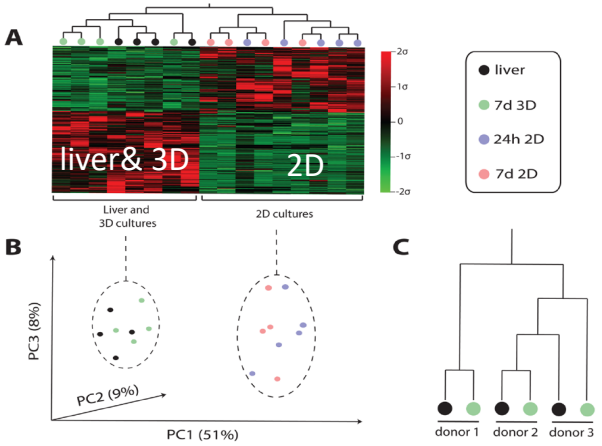
Rat, dog, monkey and human



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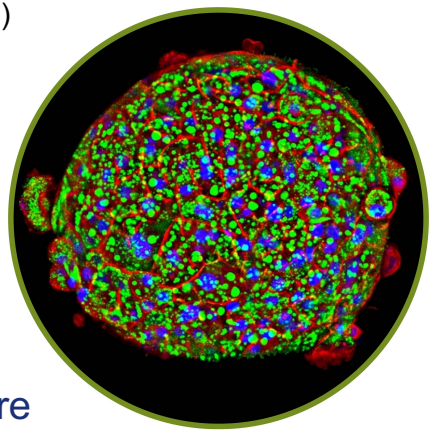

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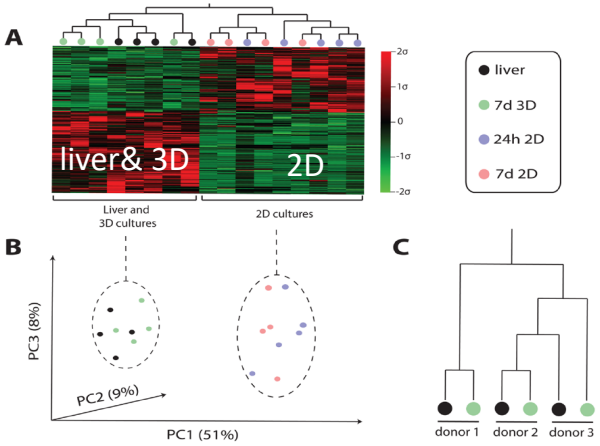

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1 microtissue per well in 70 µL supernatant

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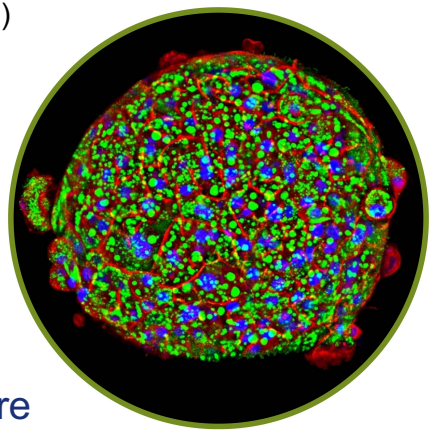

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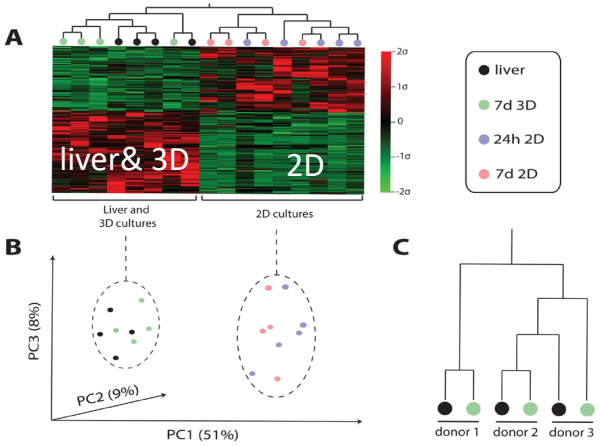

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Biochemical and cellular biomarkers, HCI, 'omics data

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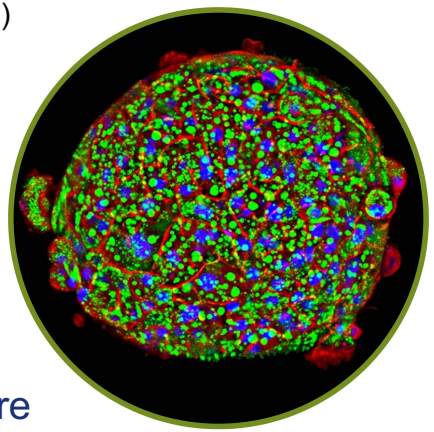

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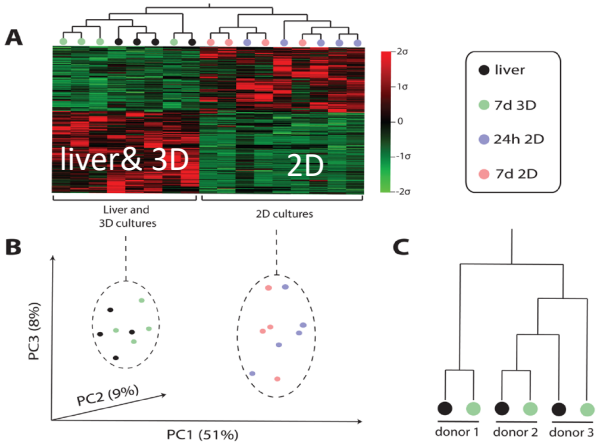

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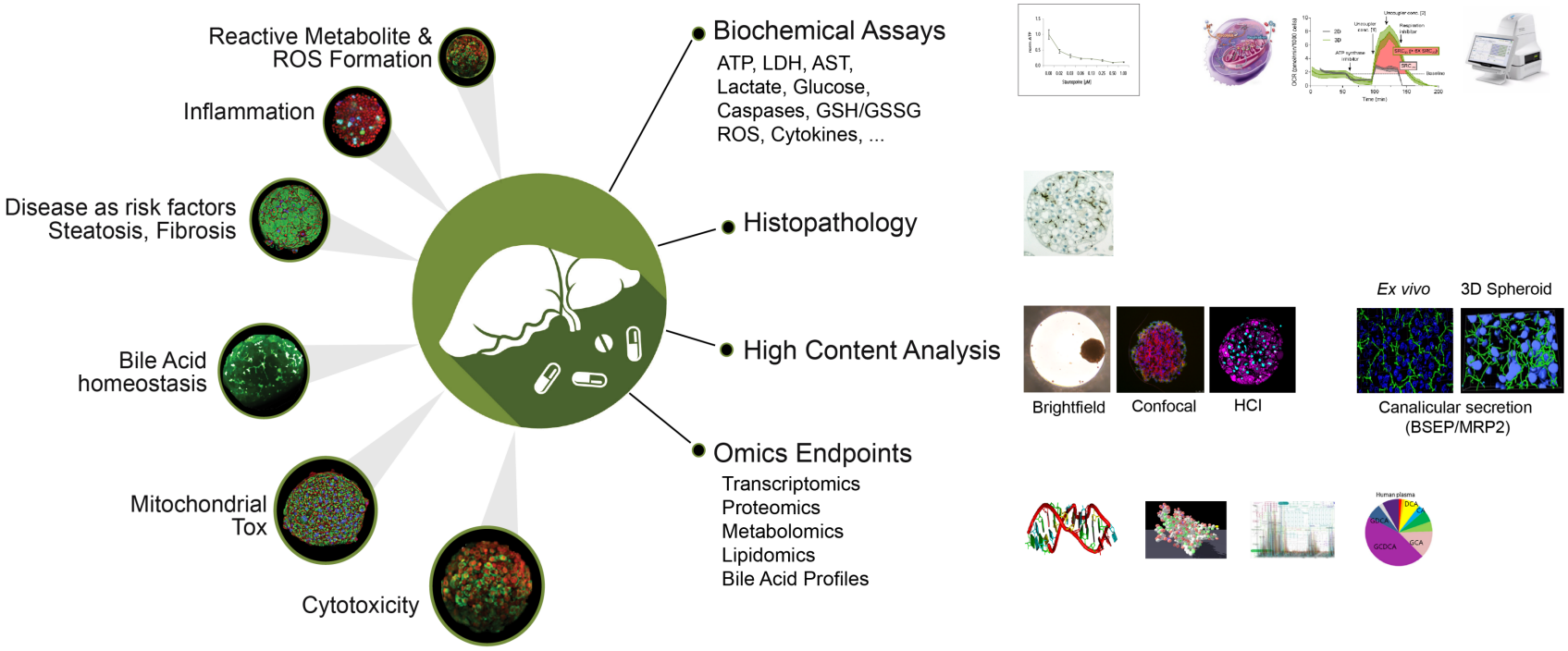
Scalable, robust, for reproducible data
Available in 96 and 384 plate formats

Biomarkers that can be measured in 3D InSight™ Liver Microtissues

Investigative Cellular DILI Key Events

Morphology → Histology → Cellular → Biochemical → Molecular



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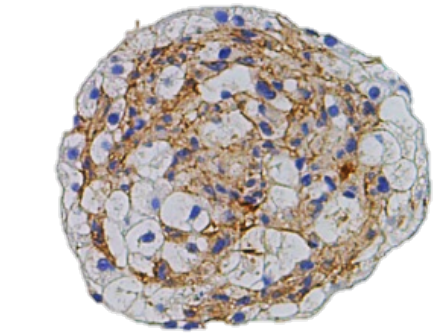
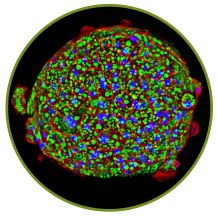


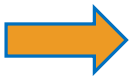
DILI Hazard Identification

DILI Screening

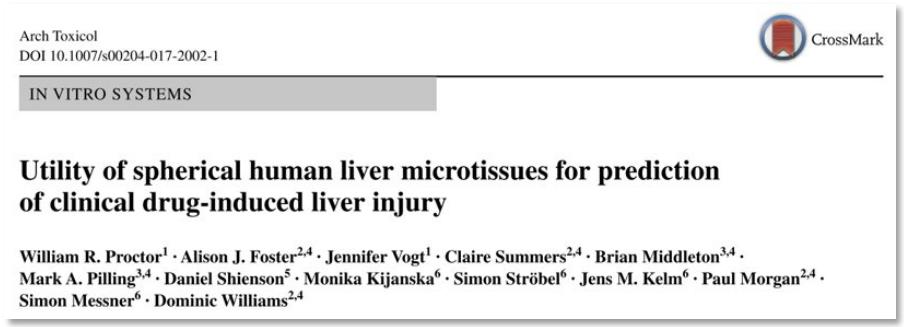


Predictive Toxicology

- Data science
- Correlations and statistics
- Specificity and sensitivity



- ATP based assay for hazard identification
- Screening for human DILI
- No mechanistical information needed



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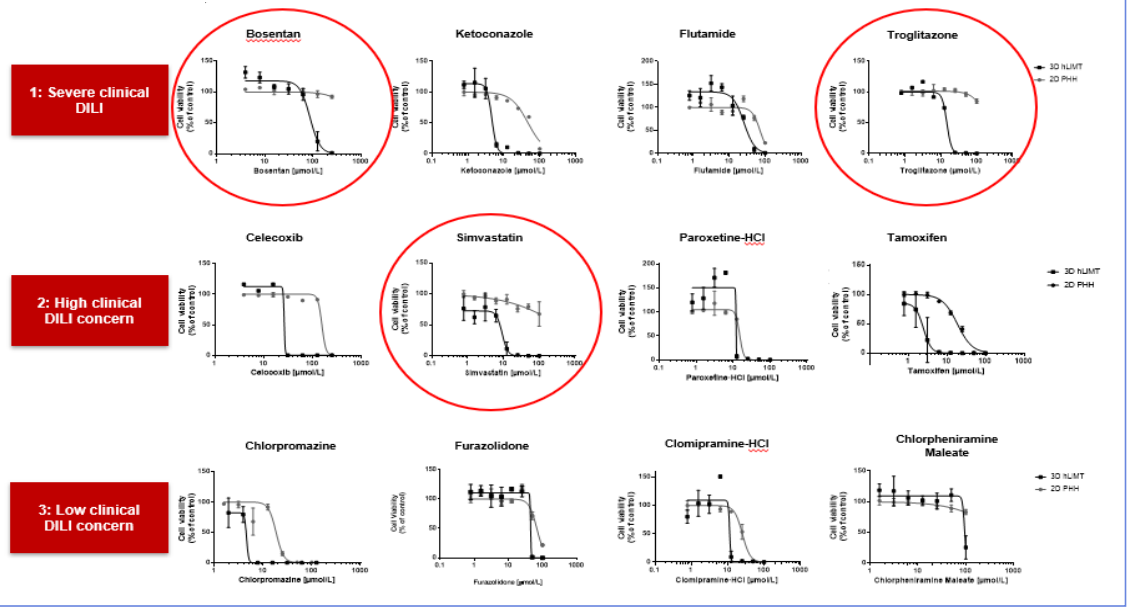
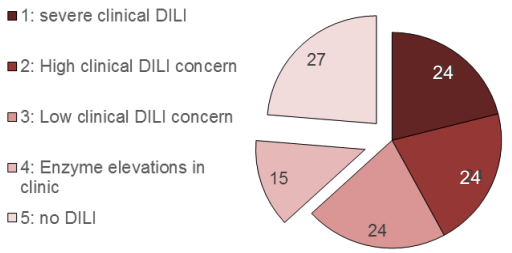
DILI Hazard Identification

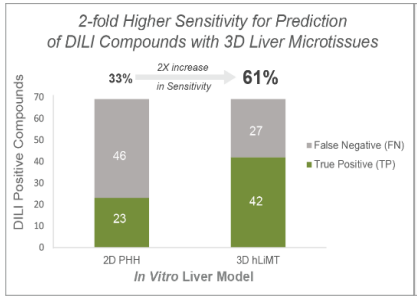
Comparison 2D (after 2 days) versus
3D MT (PH, KC, LEC) (after 14 days)

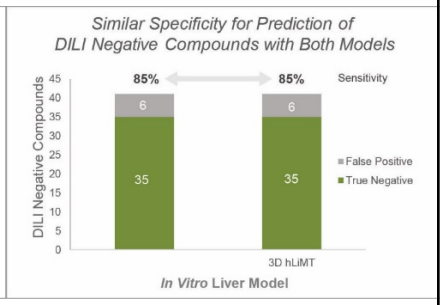
Example data:



108 Clinical Compounds



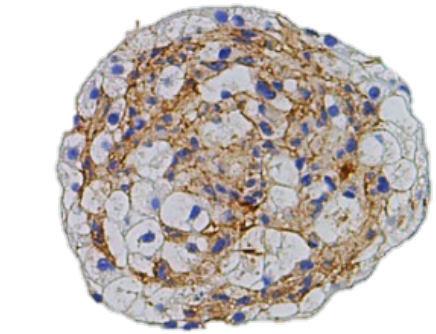




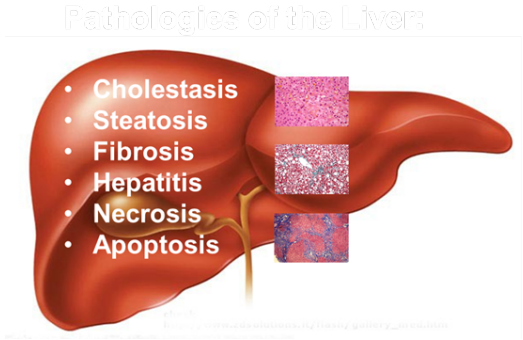
- 3D MT showed ~2-fold higher sensitivity compared to 2D PHH
- Similar specificity of 85% in 2D PHH and 3D MT

Presentation Overview

- Introduction: Drug discovery and translation to man
- ATP-based DILI hazard identification
- **Stepping beyond ATP: from hazard identification to risk assessment**
- Impact of 3D microtissues in drug development
- Outlook: challenges and opportunities



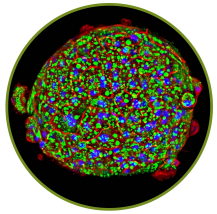
Moving from hazard identification to risk assessment



Bridging the gaps between *in vitro* and *in vivo*

- Starting by solid animal and human *in vivo* data
- Application of *in vitro* models with DILI specific mechanistic information
- Establish correlation between *in vivo* and *in vitro* data

Generation of mechanistic information



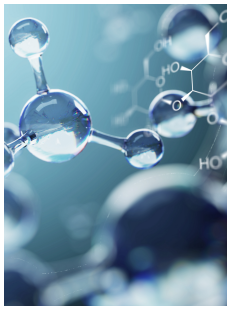
Causality Programs

- Evidence based science
- Individual cases
- Sequence of events
- Validated pathways

A Framework for Explaining and Exploring Mechanisms of DILI

Adverse Outcome DILI Pathway (AOP): sequence of key events by existing knowledge

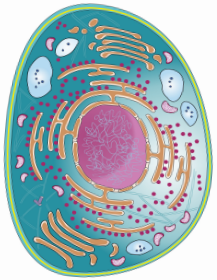
Molecular Initiation



- Compound physical-chemical properties
- Absorption, Distribution, Excretion
- Drug Metabolism

KE

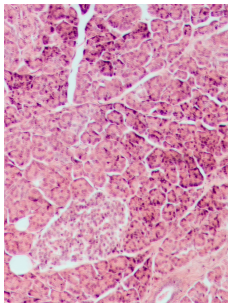
Cellular Response



- Mitochondrial tox
- ROS formation
- Biliary efflux inhibition
- Reactive metabolism
- Covalent binding
- Immune response

KE

Organ Response



- Cholestasis
- Steatosis
- Fibrosis
- Hepatitis
- Necrosis
- Apoptosis

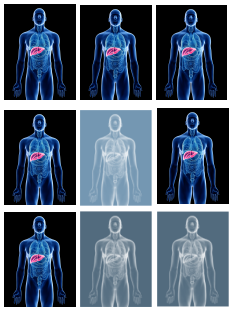
KE

Organism Response

- Hy's Law case
- Acute liver failure
- Idiosyncratic reaction

KE

Population Response



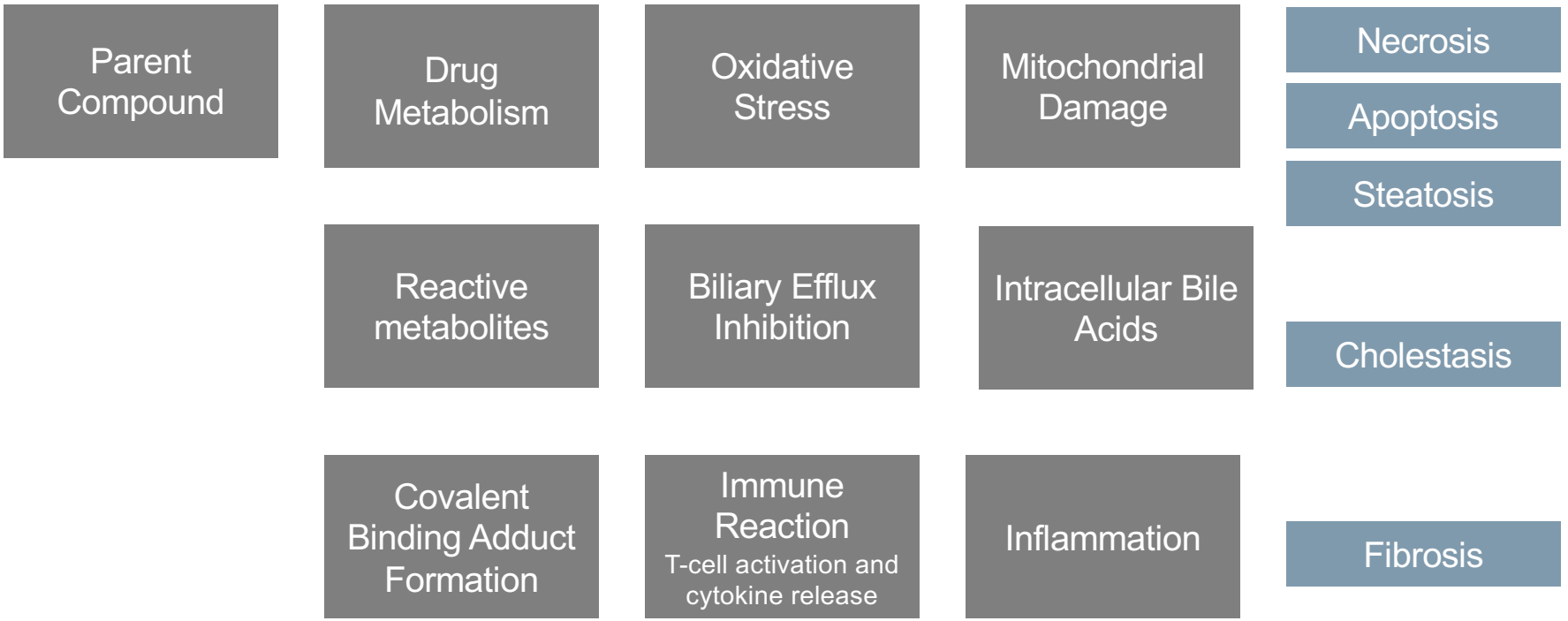
- Genetics
- Epigenetics
- Diseases
- Sex
- Obesity

KE

KE = Key Event

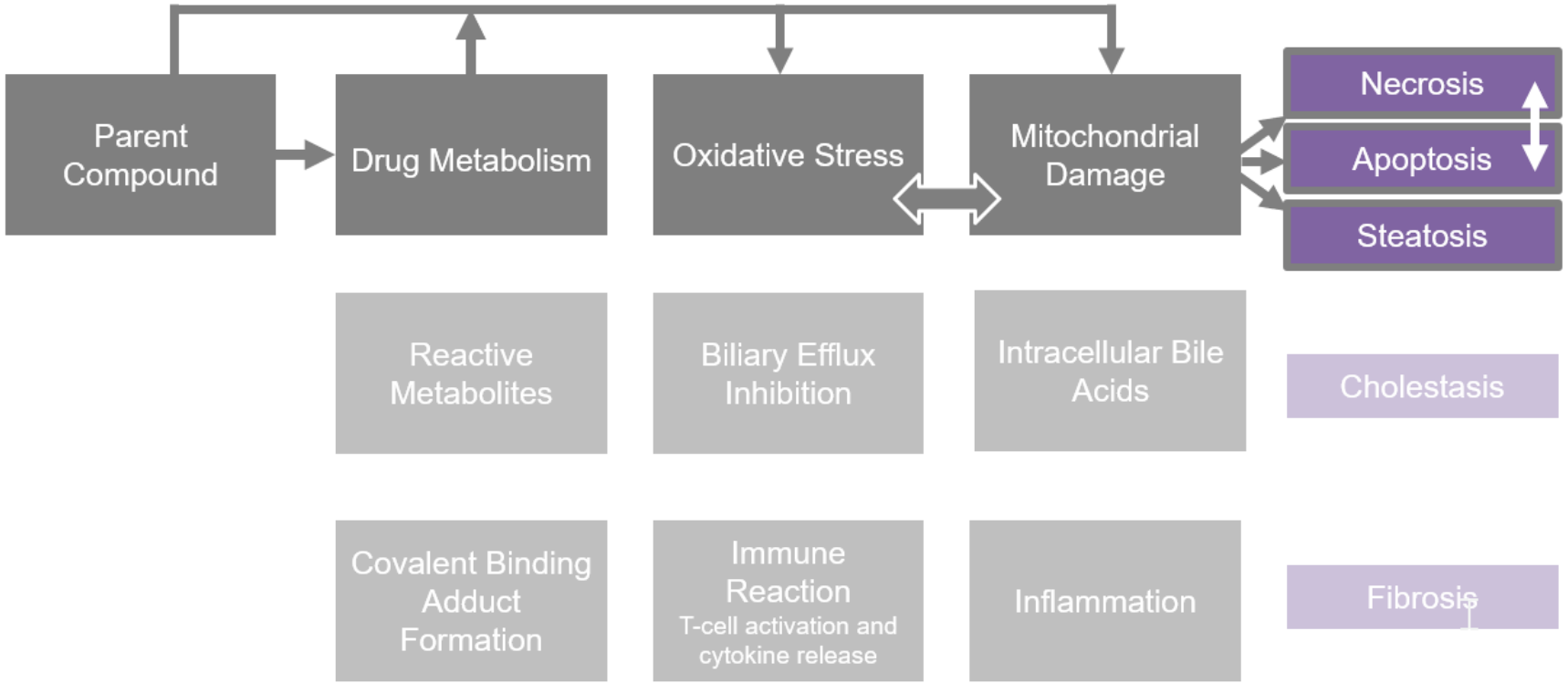
Building blocks of the cellular key event tool-box

With a focus on three major DILI Pathways



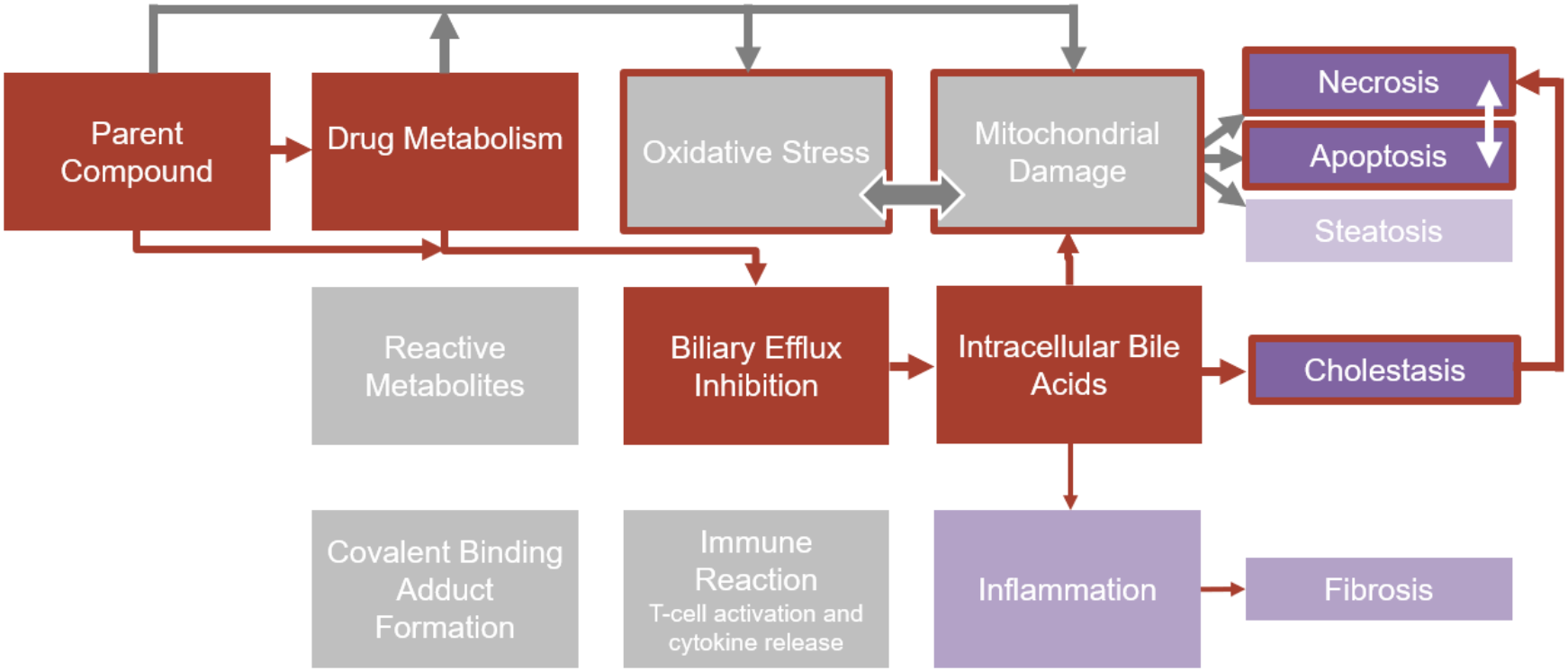
Hepatocyte Stress Hypothesis

Mitochondrial toxicity and ROS formation



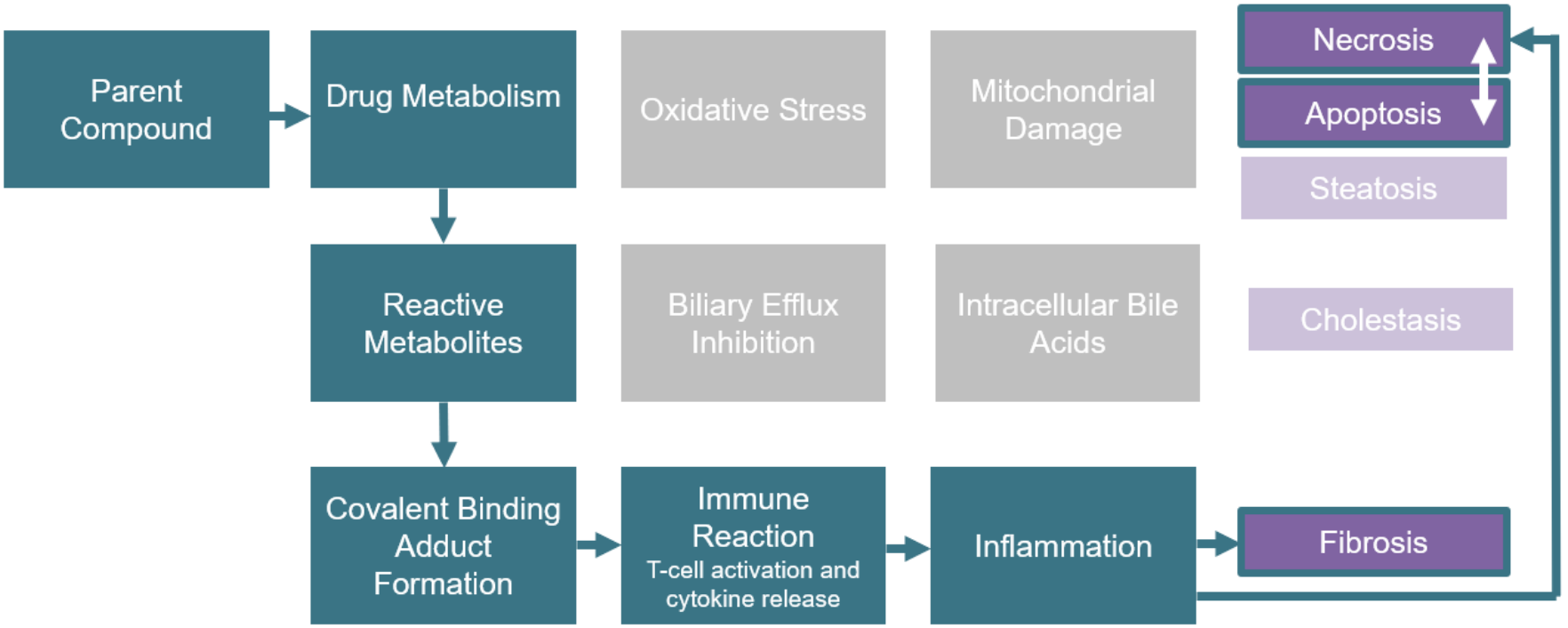
Hepatocyte Stress Hypothesis

Disturbed bile acid homeostasis



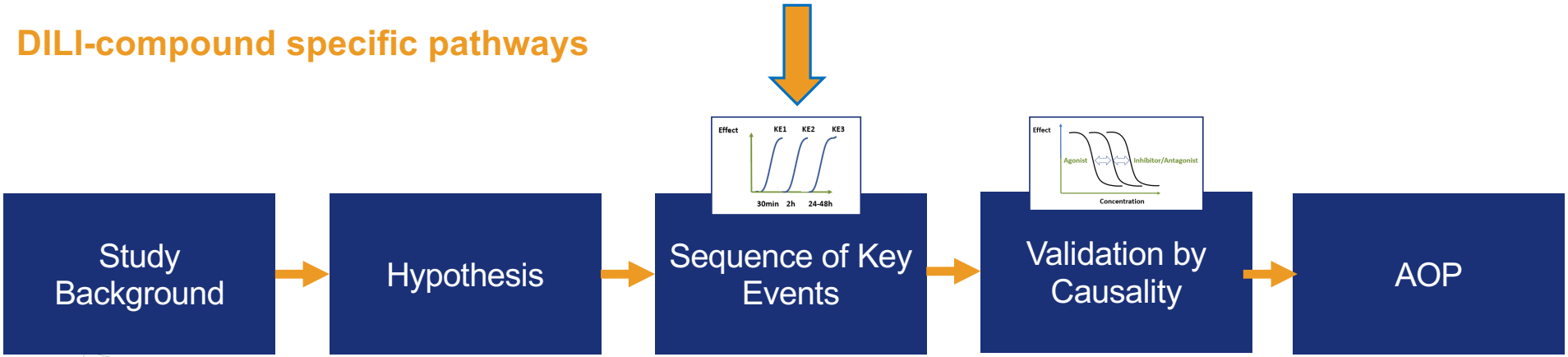
Hepatocyte Stress Hypothesis

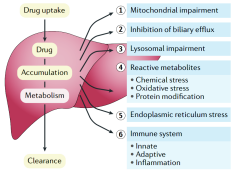
Neo-haptenization after covalent binding with the liver

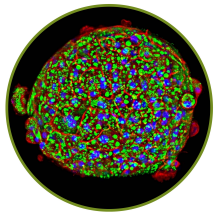


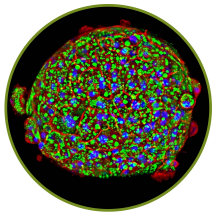
Program Design for Establishing Causal Links in AOPs

DILI-compound specific pathways







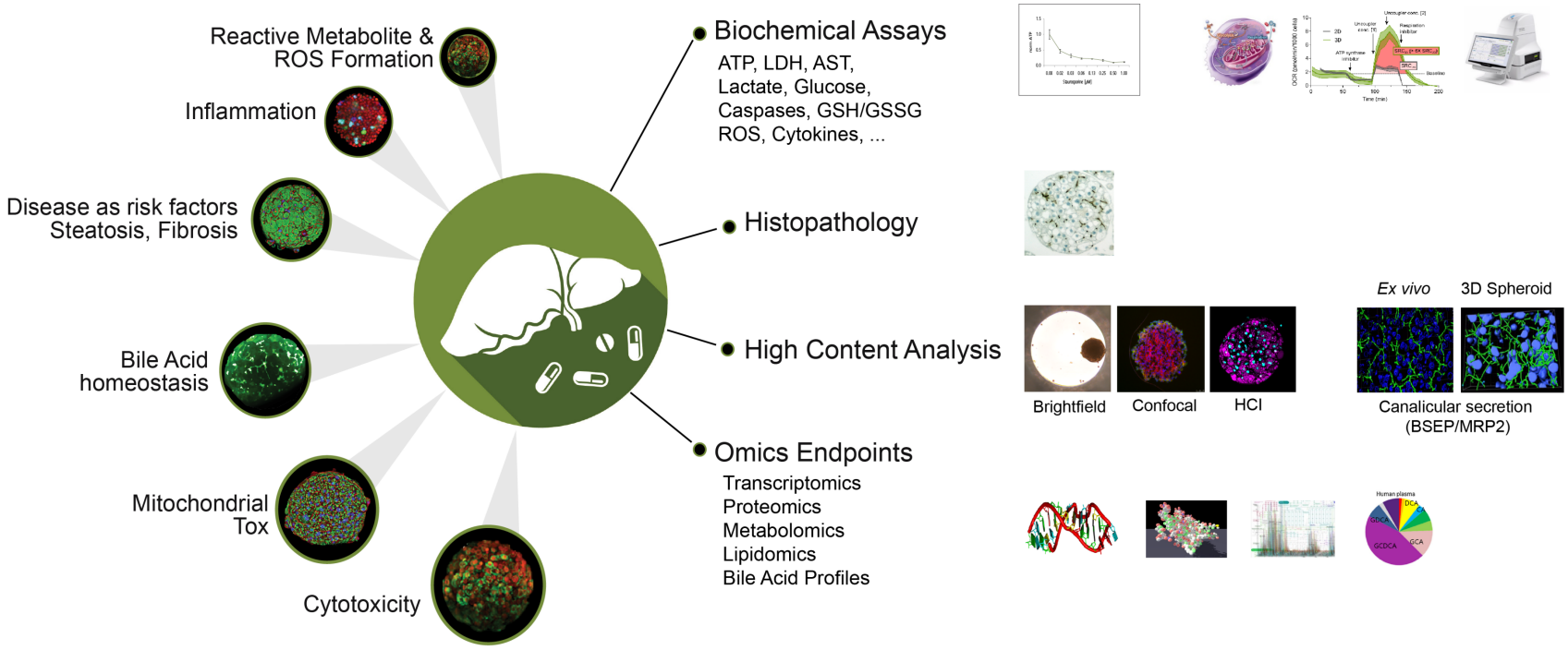


Functional validation by identifying the key event and pathway signature for each DILI compound

Biomarkers that can be measured in 3D InSight™ Liver Microtissues

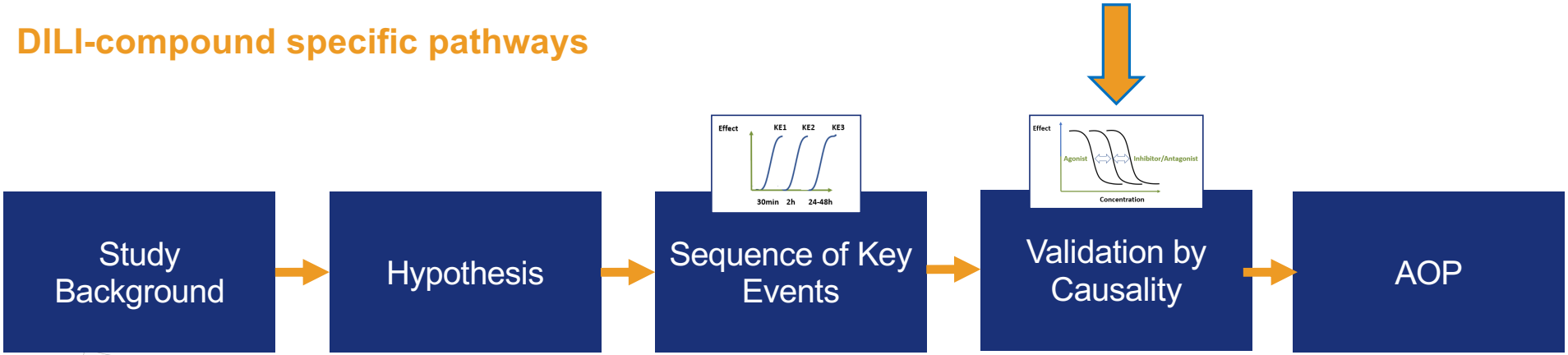
Investigative Cellular DILI Key Events

Morphology → Histology → Cellular → Biochemical → Molecular



Program Design for Establishing Causal Links in AOPs

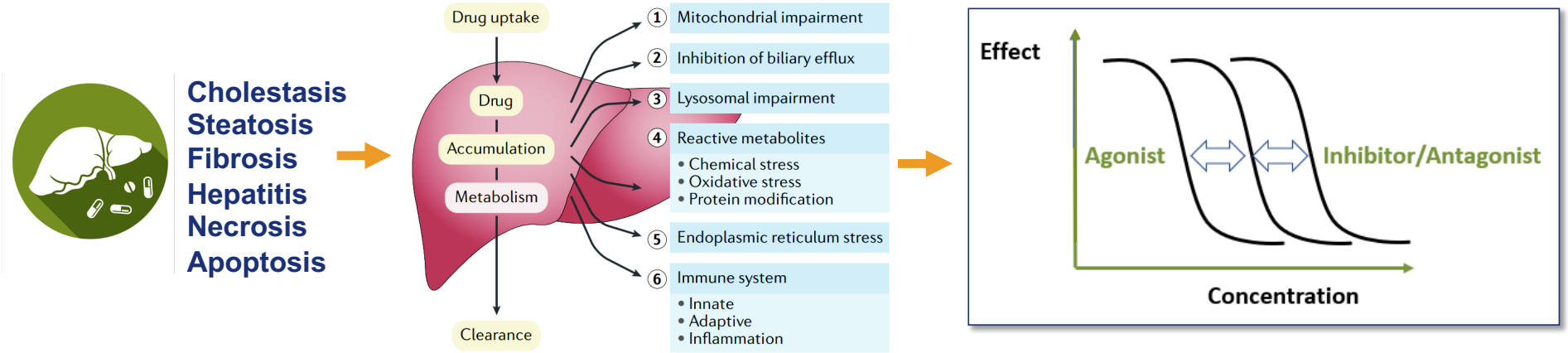
DILI-compound specific pathways



Functional validation by identifying the key event and pathway signature for each DILI compound

Causality Assay: Causal Links Between Pathways and Cellular Responses

Applying a suite of causality assays for functional validation of specific pathways



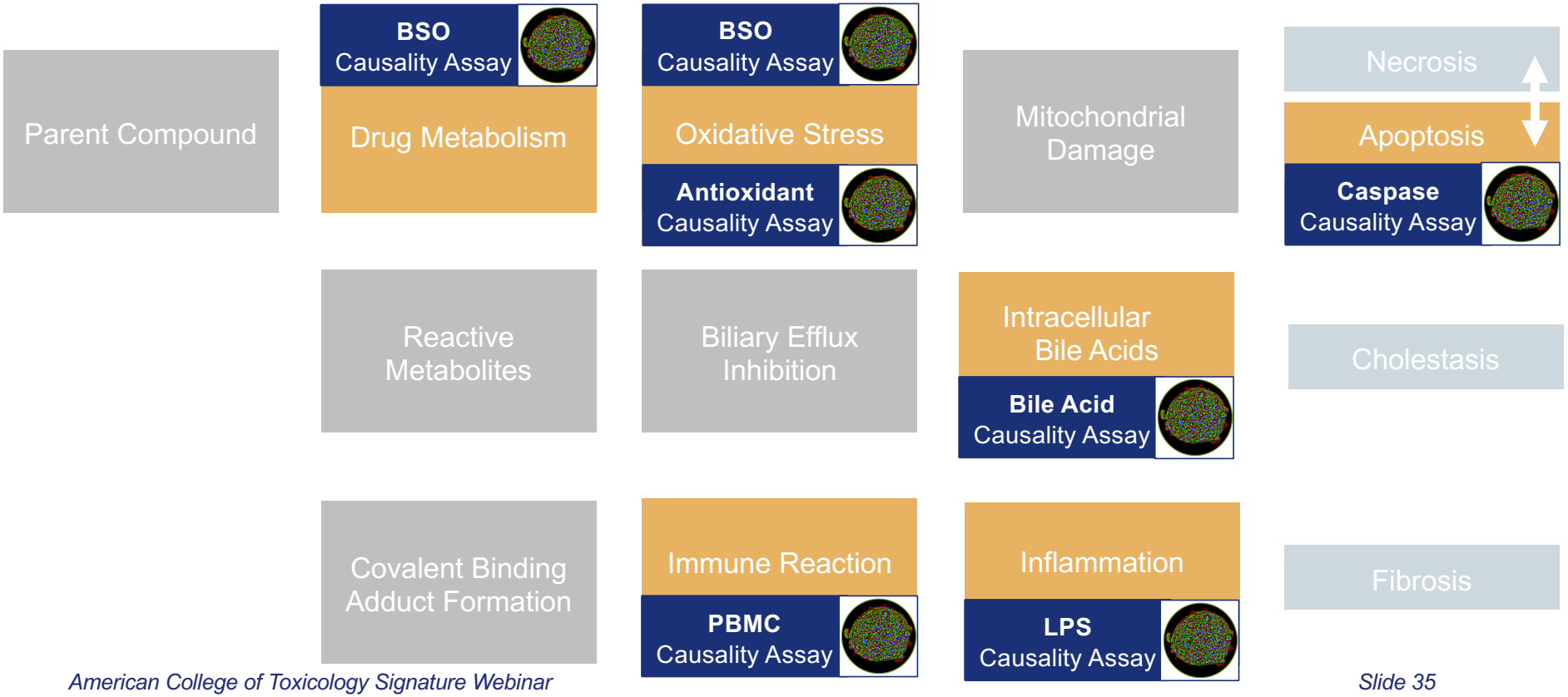
Richard Weaver et al, Nature Review Drug Discovery 19, 131-148 (2020)

Causality Assay principles:

1. IC_{50} of test compound (ATP/LDH)
2. IC_{50} in presence of specific modulator: Agonist (enhancer) and/or Antagonist (inhibitor)
3. IC_{50} curve shift indicates causality (Causality = cause & effect)

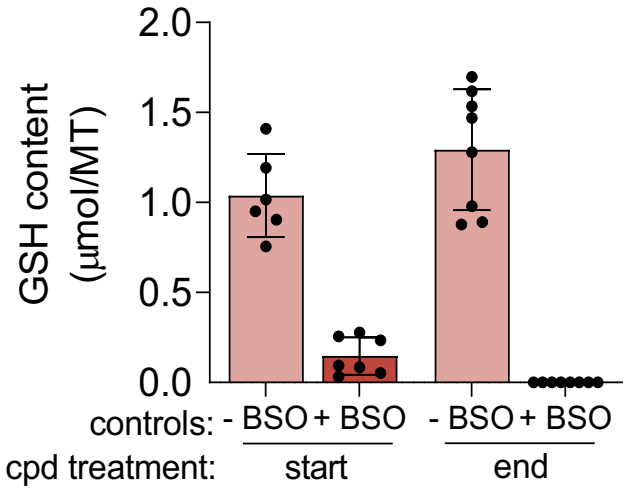
Building blocks for DILI AOPs with Causality Assays

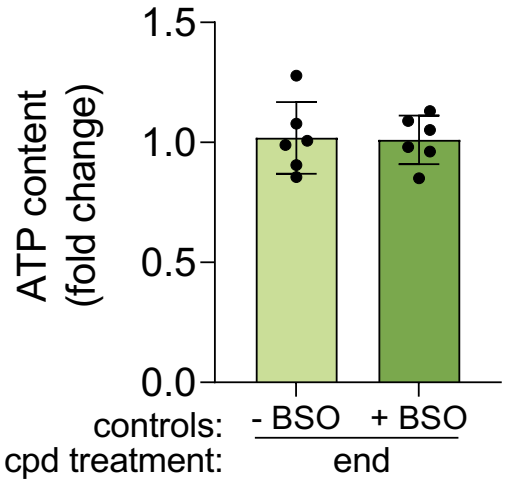
Application of 3D Human Liver Microtissues for Systemic Functional Validation

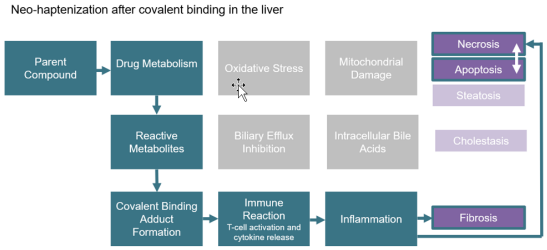


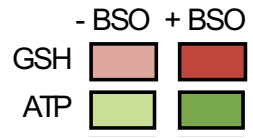
Case Study: Reactive Metabolite/Oxygen Species Causality Assay

Experimental Conditions





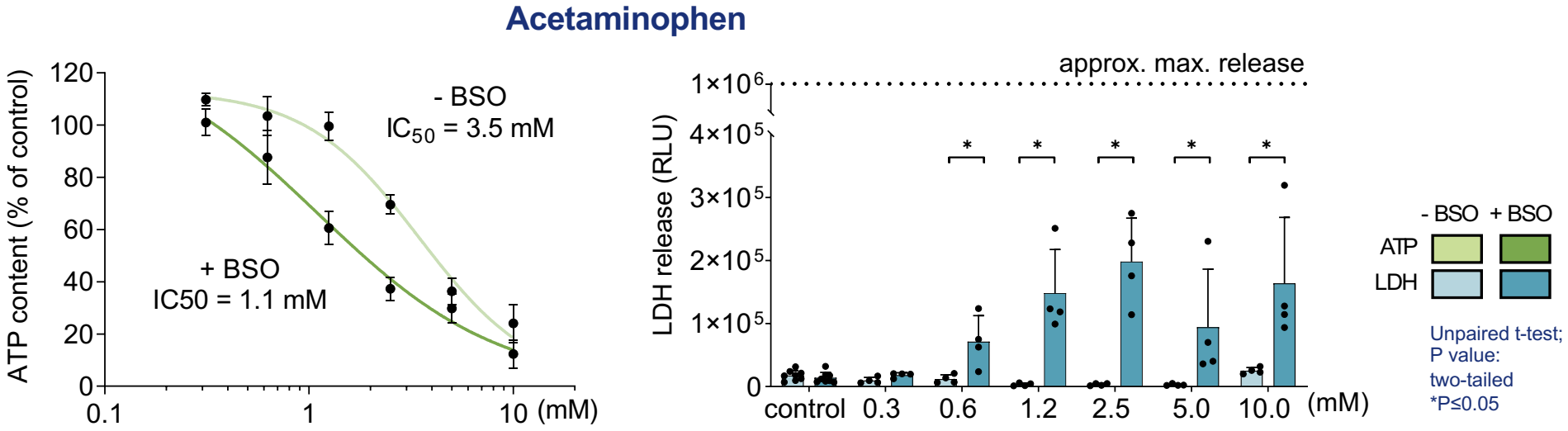




Reduced level of cellular GSH in microtissues maintained from start to end of compound exposure by the GSH synthesis inhibitor BSO without causing cytotoxicity

Case Study: Reactive Metabolite/Oxygen Species Causality Assay

Example: Acetaminophen as a model reference compound[1]



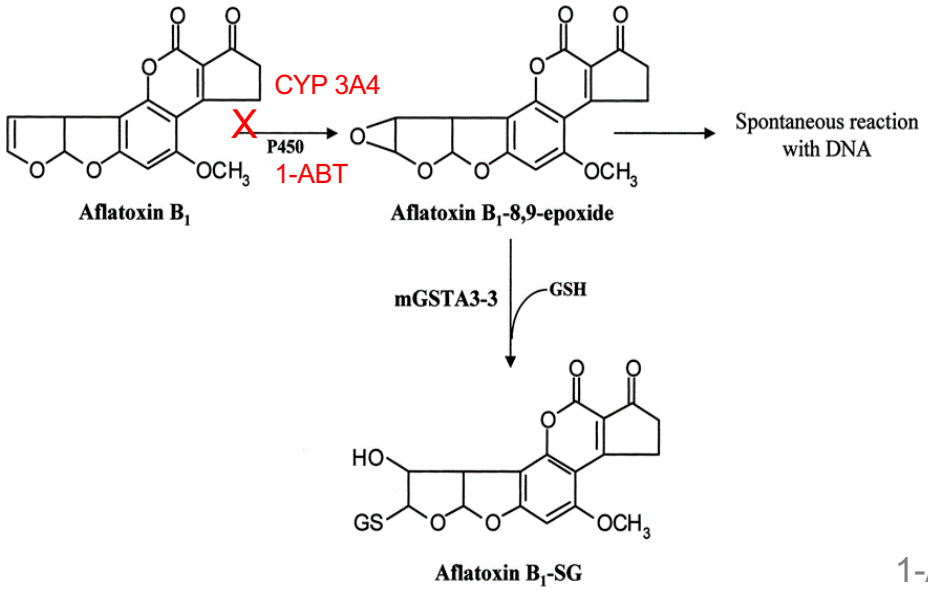
GSH depletion potentiates cytotoxicity of acetaminophen

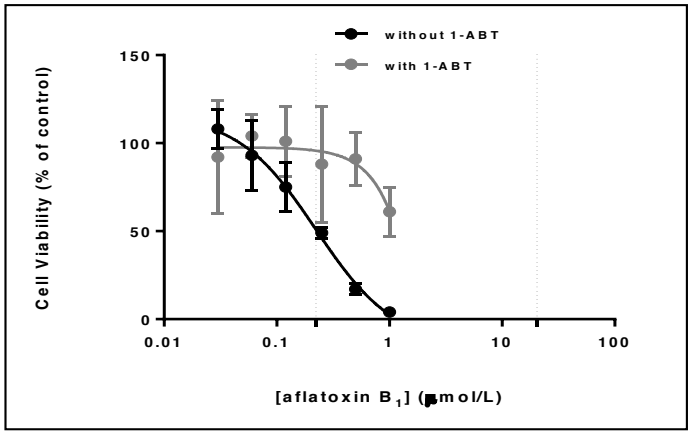
Left shift of ATP IC_{50} and ATP dose-response curve, and LDH release signal indicating membrane damage under GSH depletion conditions (+BSO).

1. James, LP, et al. Acetaminophen-induced hepatotoxicity. Drug Metab Dispos. 2003 Dec;31(12):1499-506

Case Study: Reactive Metabolite Causality Assay

Example: Modulation of the AFB1 cytotoxicity by 1-ABT in human 3D MT



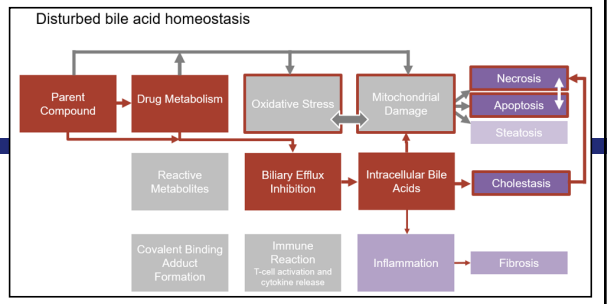


IC_{50} (µmol/L)	
without 1-ABT	with 1-ABT
0.22 (0.11-0.47)	N/A

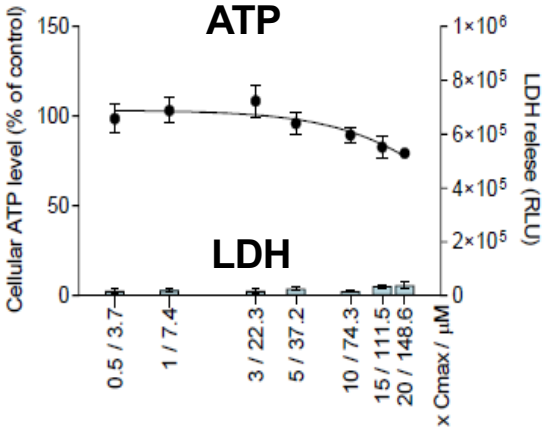
1-ABT (1-aminobenzotriazole) = pan cytochrome P450 inhibitor

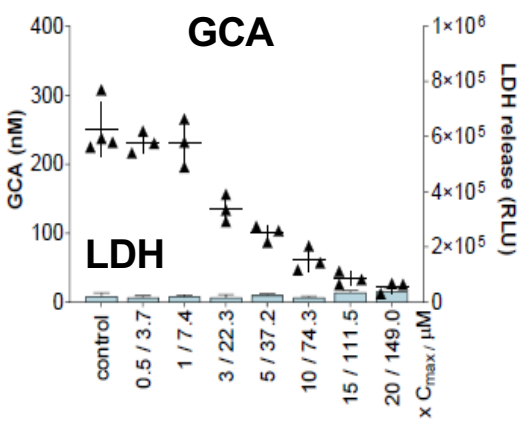
Results suggest reactive epoxide formation

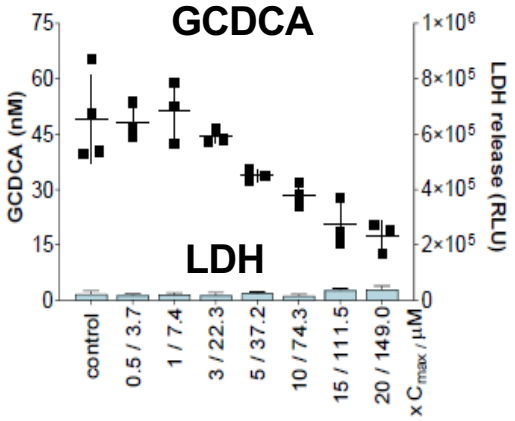
Case Study: Bile Acid Causality Assay



Example: Effect of the ET_A-Antagonist Bosentan on Bile Acid secretion



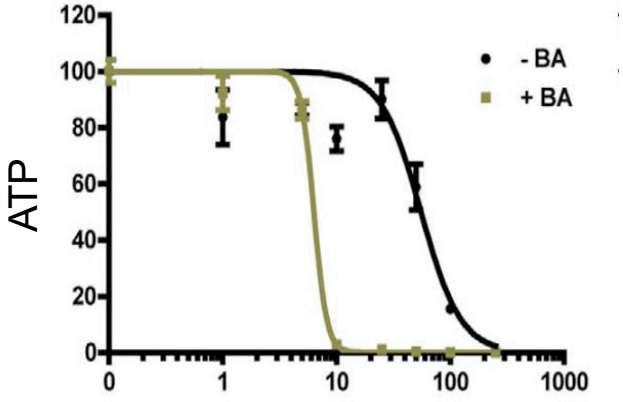




- **Bosentan (DILI positive) decreased the secretion of the endogenous Bile Acid metabolites GCA and GCDCA at non-cytotoxic concentrations.**
- **The structural DILI negative analog ET_A-inhibitor Ambrisentane had no effect.**

Case Study: Bile Acid Causality Assay

Example: Bosentan +/- Bile Acids



Hendriks et al. Nature, Scientific reports, October 2016,

Bile acid composition as in human plasma

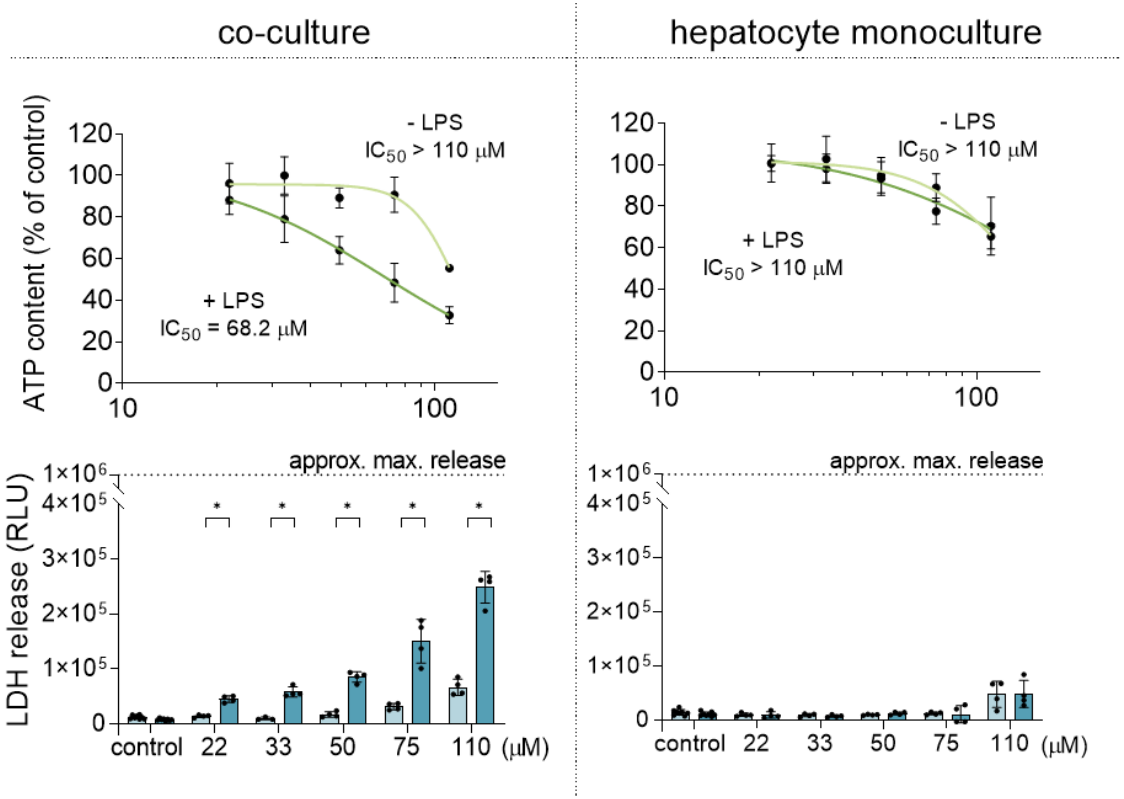
Bile acid	Concentration in human plasma (μM)[48]
Cholic acid	0.41
Chenodeoxycholic acid	0.64
Deoxycholic acid	0.48
Lithocholic acid	0.008
Ursodeoxycholic acid	0.14
Glycochenodeoxycholic acid	0.80
Sum	2.478

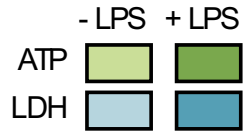
Increased bosentan cytotoxicity by non-cytotoxic bile acid concentration

Case Study: Inflammation Causality Assay

Inflammation

Example: LPS-enhanced cytotoxicity of Trovafloxacin

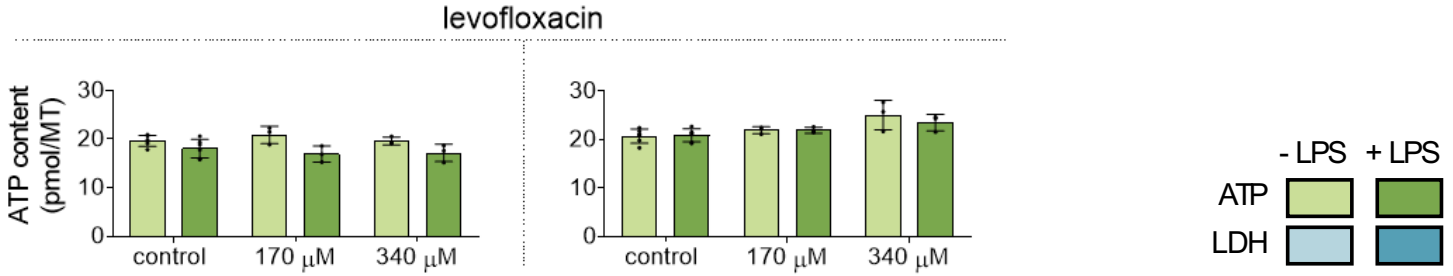




Unpaired t-test; P value: two-tailed *P≤0.05

Case Study: Inflammation Causality Assay

Example: LPS-enhanced cytotoxicity of Trovafloxacin

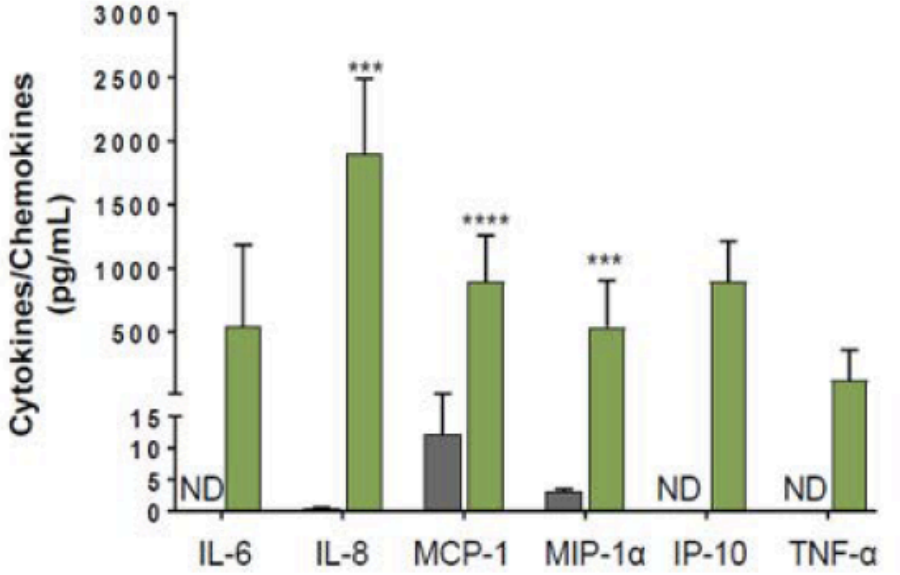


Summary:

- **LPS-enhanced trovafloxacin cytotoxicity in 3D liver MT with NPCs (PHH, KC, LSEC)**
- **No effect in PHH monoculture 3D MT**
- **No effect of LPS by levofloxacin (negative control, no liver toxicity *in vivo*).**

Case Study: Inflammation Causality Assay

Example: LPS-enhanced cytokine/chemokine panel in the InSphero NASH disease model at day 5



Secreted cytokines/chemokines
KCs: IL-6, MIP-1α, TNF-α
HSCs: IP-10, MCP-1, IL-8
PHH: IL-8, MCP-1

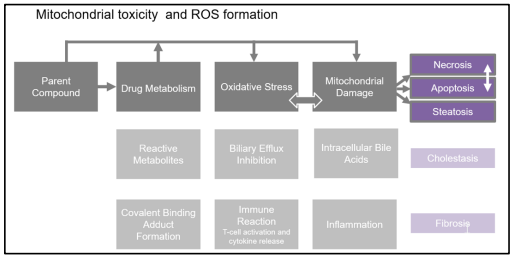
Mean +/-SD, n= 4 microtissues
***p≤0.001, ****p≤0.0001, NASH vs control
1 exp. out of n=3

Biomarker toolbox to monitor specifically inflammation in cell culture supernatants

Case Study: Steatosis Causality Assay

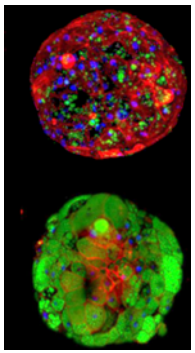
Steatosis

Example: Steatosis-enhanced Chlorpromazine cytotoxicity

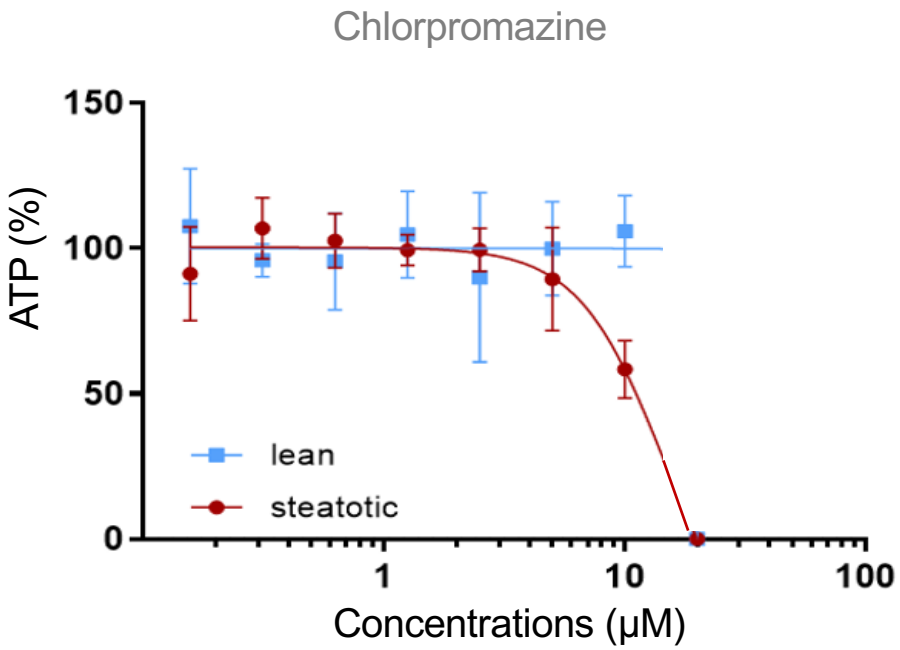


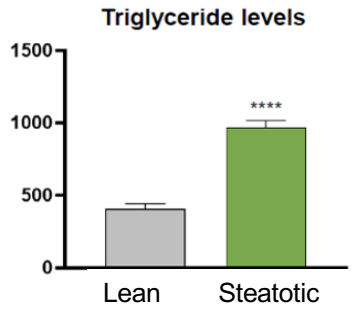
Lean

Steatotic



Nile Red O (green),
Nuclei by Hoechst (blue),
Plasma membrane by CellMask (red)



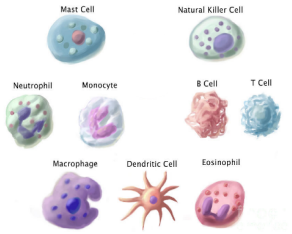


Steatosis is a possible risk factor for DILI. Testing of new compounds under lean and steatosis conditions might help patient stratification.

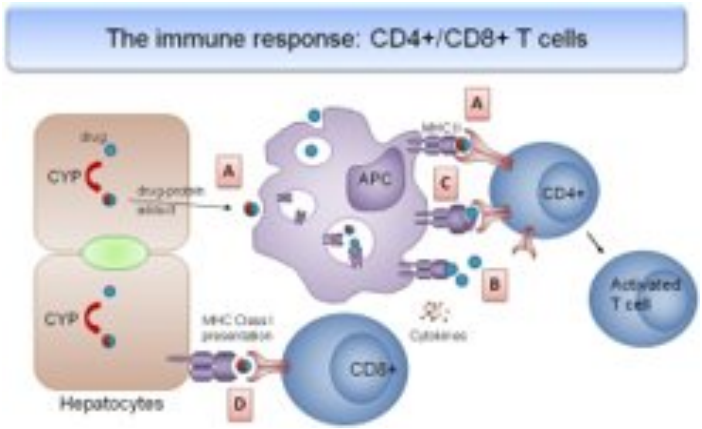
Immunology on the Chip: 3D liver MT – PBMC coculture

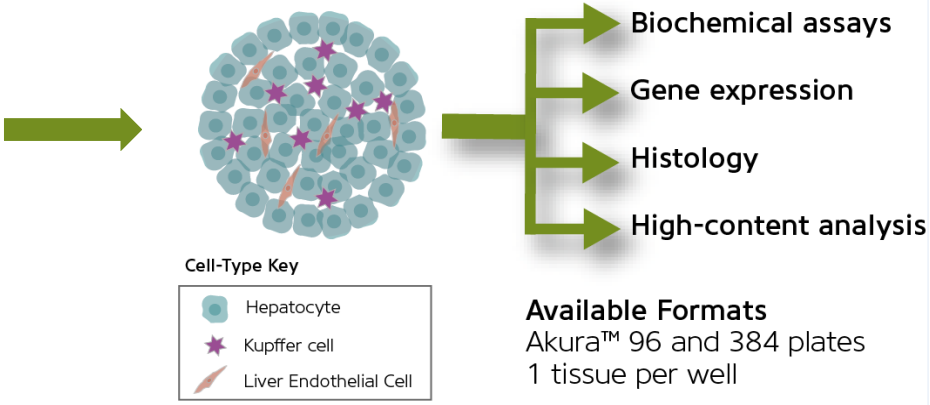
Interaction between drugs and human leucocyte antigen (HLA) molecule leading to an adaptive immune response

Gerd A Kullak-Ublick et al. Gut 2017;66:1154-1164

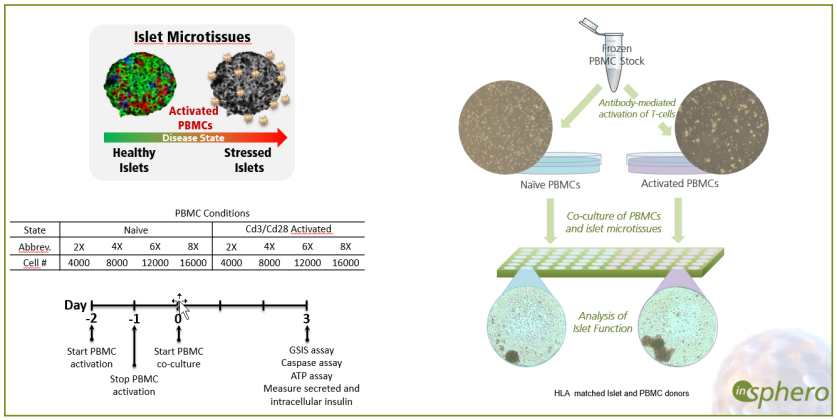


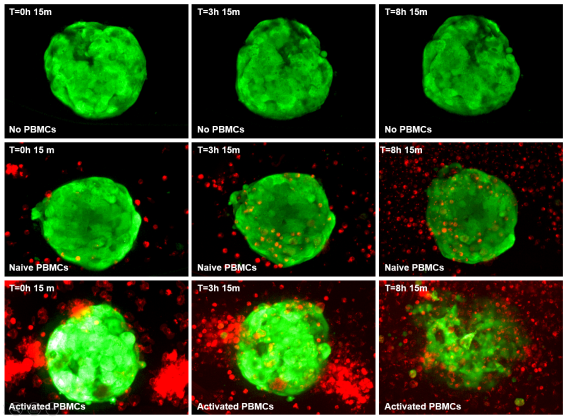
Human PBMCs	Frequency (%)
Monocytes	10-30%
Lymphocytes	70-90%
Total T cells (CD3+)	45-70%
CD4+ T cells	25-60% of total CD3
CD8+ T cells	5-30% of total CD3
Total B cells	5-15%
NK cells	5-10%
Dendritic cells	1-2%
Stem cells	0.1-0.2%

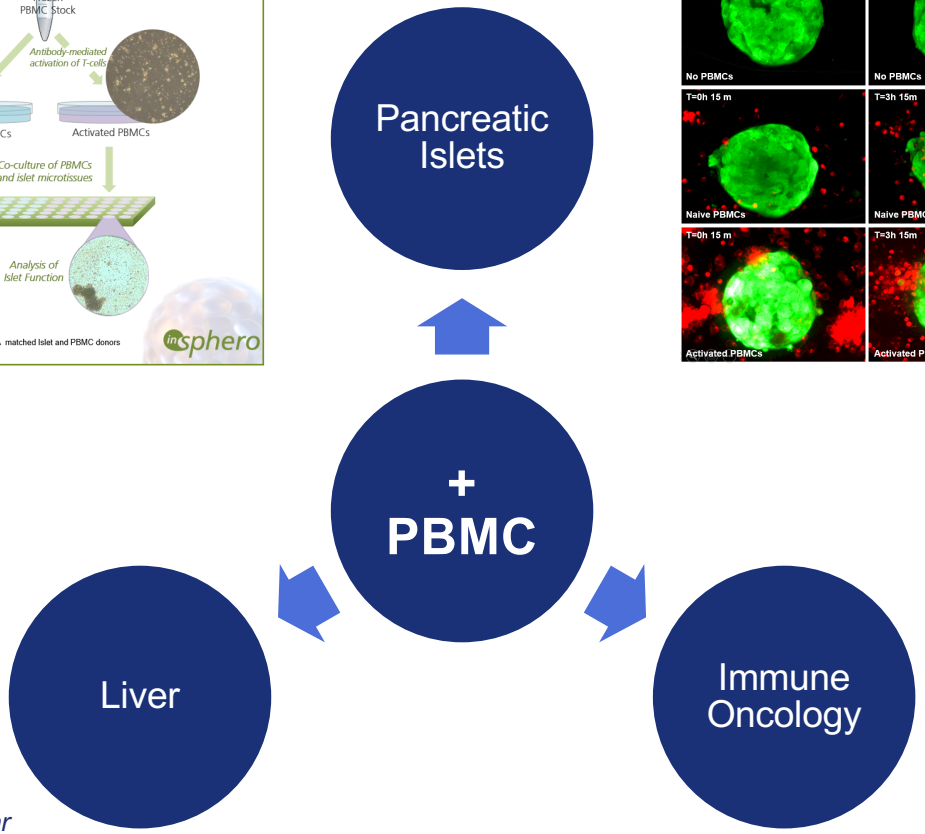




Status of 3D Human Microtissues + PBMC Developments

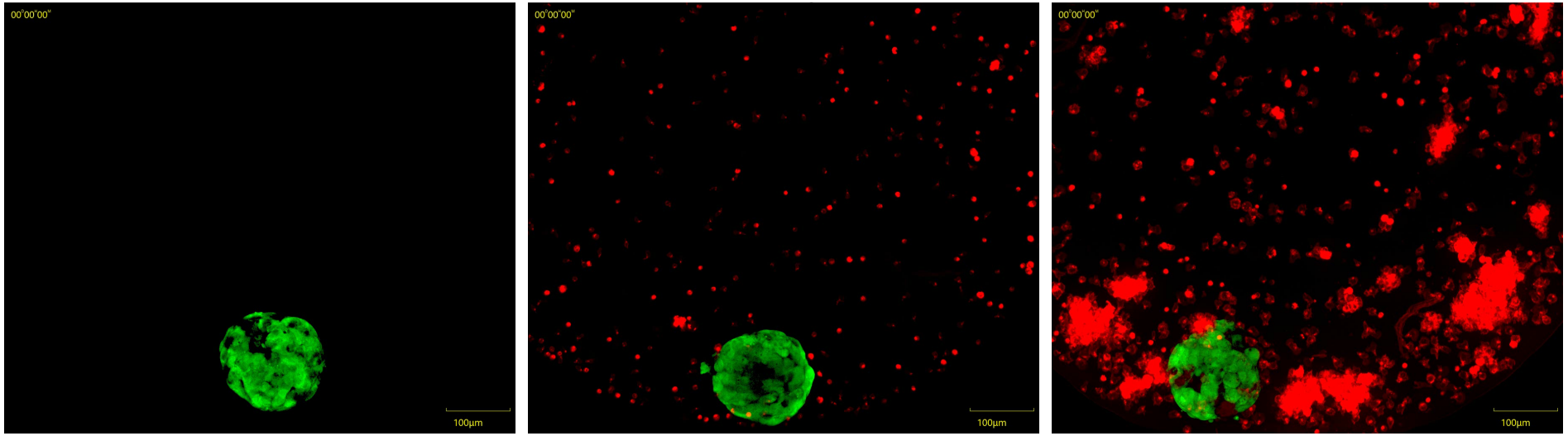






PBMC-Induced Islet Injury Assay

No PBMCs | **Native** | **CD3/CD28 activated PBMCs**



InSphero

Islets: Green CMFdA **PBMC: Deep Red Dye** **t = 15 – 38 min**

The PBMC – 3D microtissue coculture is a new tool to investigate immune-mediated mechanisms of toxicity in iDILI

In a Nutshell

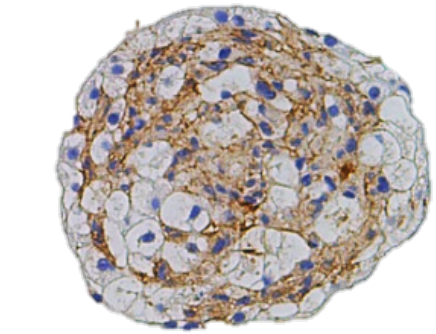
3D-MT causality assays can be adapted to a wide range of pathways by specific pathway modulator.

Possible applications of 3D-MT causality assays are:

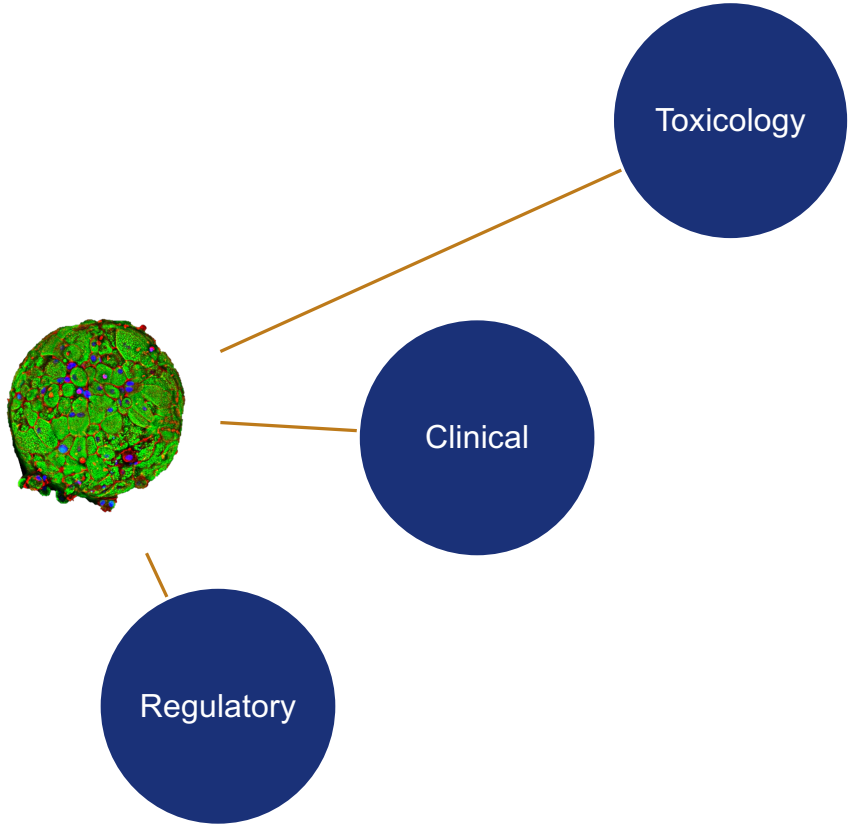
- DILI specific mechanistic investigations
- Mechanism-based screening

Presentation Overview

- Introduction: Drug discovery and translation to man
- ATP-based DILI hazard identification
- Stepping beyond ATP: from hazard identification to risk assessment
- **Impact of 3D microtissues in drug development**
- Outlook: challenges and opportunities



High Impact of 3D Microtissues in Drug Development



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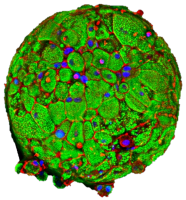


Toxicology

Clinical

Regulatory

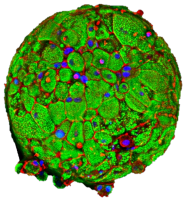
Enabling fast decision making in the drug developmental process

Predictive Toxicology → Hazard identification; Front loading screen

Mechanistic studies → Risk assessment; Mode of action; Translation to man; Clinical Biomarker

3Rs → Back-up support

High Impact of 3D Microtissues in Drug Development



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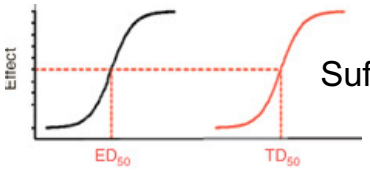
High Impact of *In Vitro* X-Species DILI Studies

X-Species DILI Testing

Therapeutic Index determined by *in vivo* animal data



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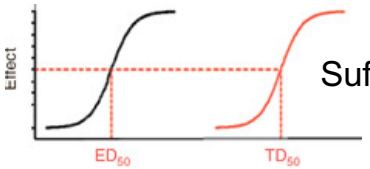

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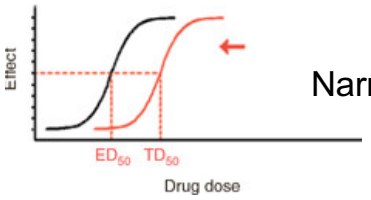

Narrow TI

NO GO

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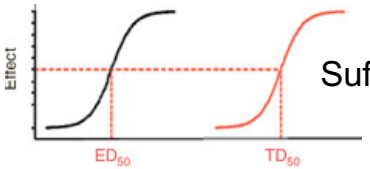

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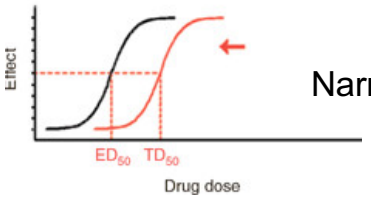

Narrow TI

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Case 1

Recapitulate the *in vivo* effects *in vitro*

Compound A	Rat	Dog	Human
ClinPath/HistPath Observation	ALT, centrilobular necrosis		
In vivo	-	+	?
*In vitro**	-	+	-

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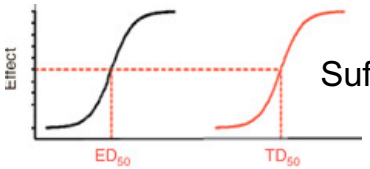

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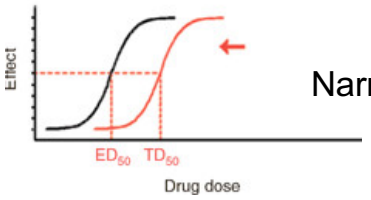

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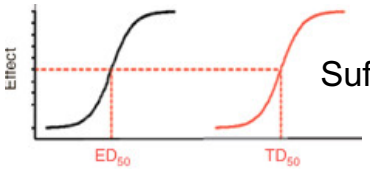

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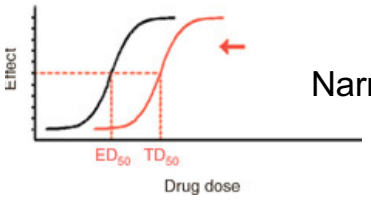

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Effect of Compound A in dogs is not translatable to man

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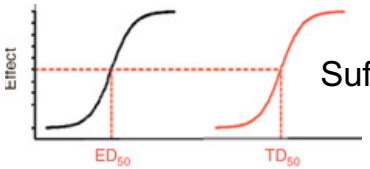

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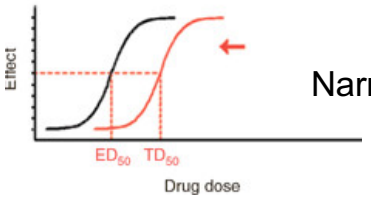

Narrow TI

NO GO

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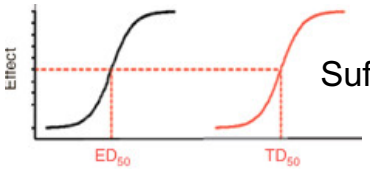

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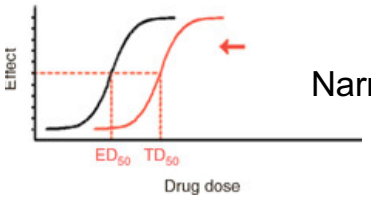

Narrow TI

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Case 2

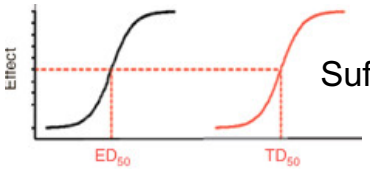
Recapitulate the *in vivo* effects *in vitro*

Compound B	Rat	Dog	Human
ClinPath/HistPath Observation	Micro vesicular steatosis		
In vivo	-	+	?
*In vitro**	-	+	+

High Impact of *In Vitro* X-Species DILI Studies

X-Species DILI Testing

Therapeutic Index determined by *in vivo* animal data



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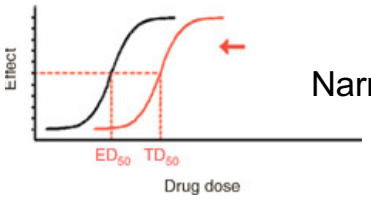

Narrow TI

NO GO

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X-Species DILI Testing

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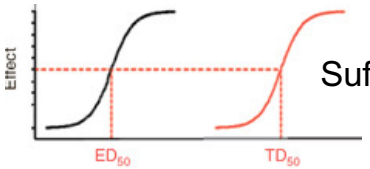

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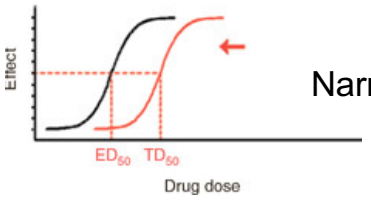

Narrow TI

NO GO

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ClinPath/HistPath Observation	Micro vesicular steatosis		
In vivo	-	+	?
*In vitro**	-	+	+

Compound A validates the *in vitro* model for species specificity

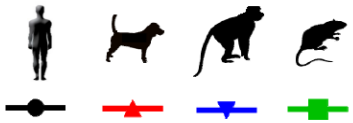
Effect of Compound B in dogs is translatable to man

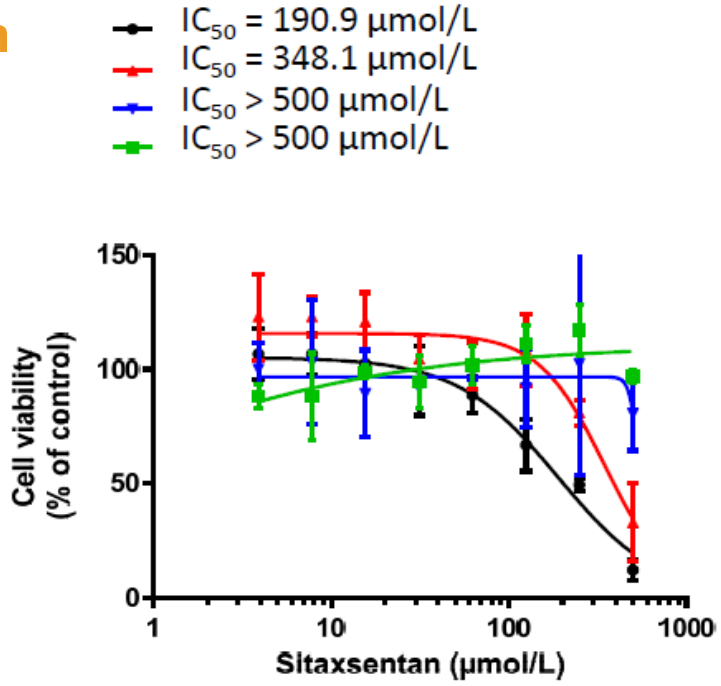
NO GO → Mechanistic Studies

High Impact of *In Vitro* X-Species DILI Studies

Example: Cross Species Evaluation of Sitaxentan

- Endothelin antagonist (ET_A)
- Clinical indication: Pulmonary Hypertension
- Withdrawn in 2010 due to acute liver injury
- Mechanism of Toxicity unknown
- Preclinical studies did not reveal hepatoxicity
- Not cytotoxic in 2D-models





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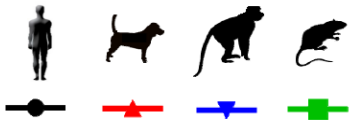

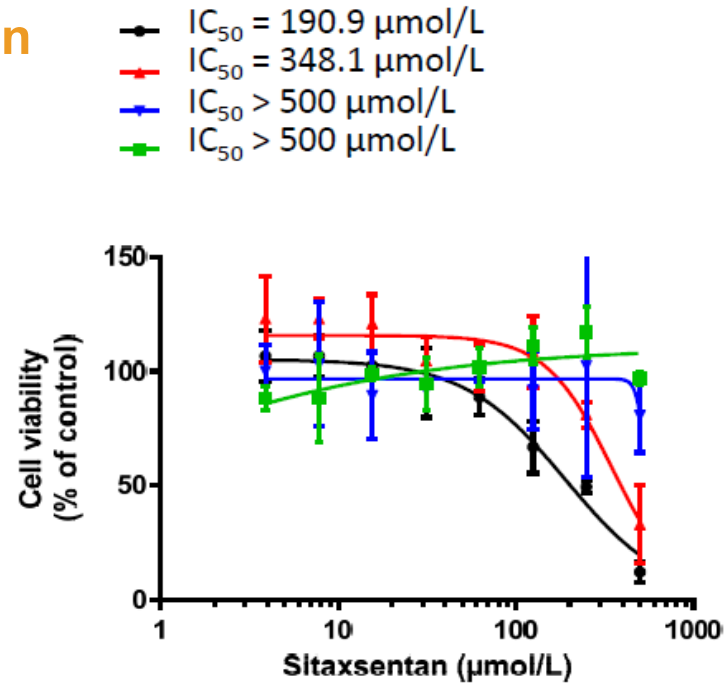

In vivo species-specific effects of Sitaxentan was recapitulated in 3D-Liver microtissues

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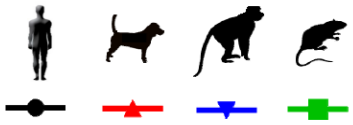
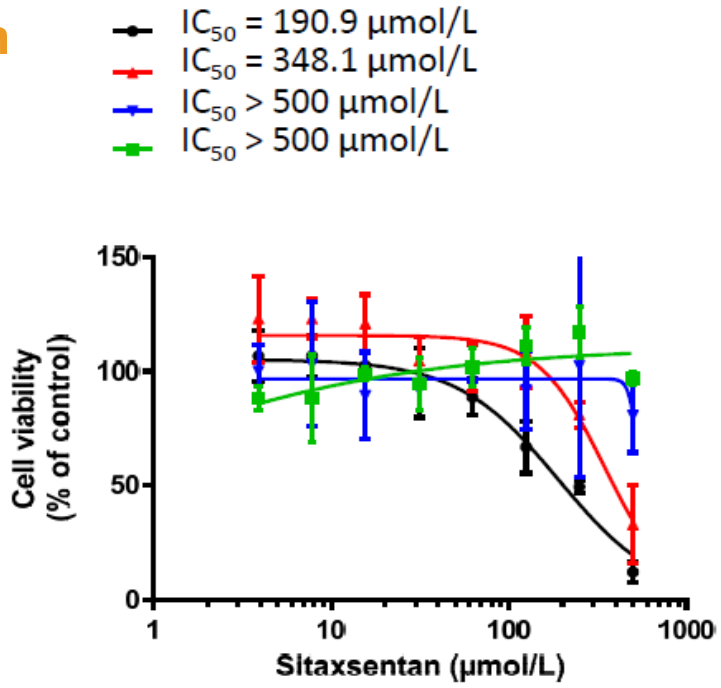




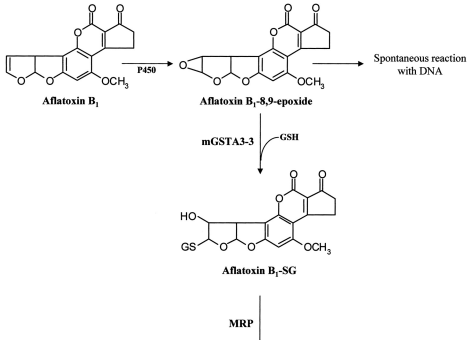
In vivo species-specific effects of Sitaxentan was recapitulated in 3D-Liver microtissues

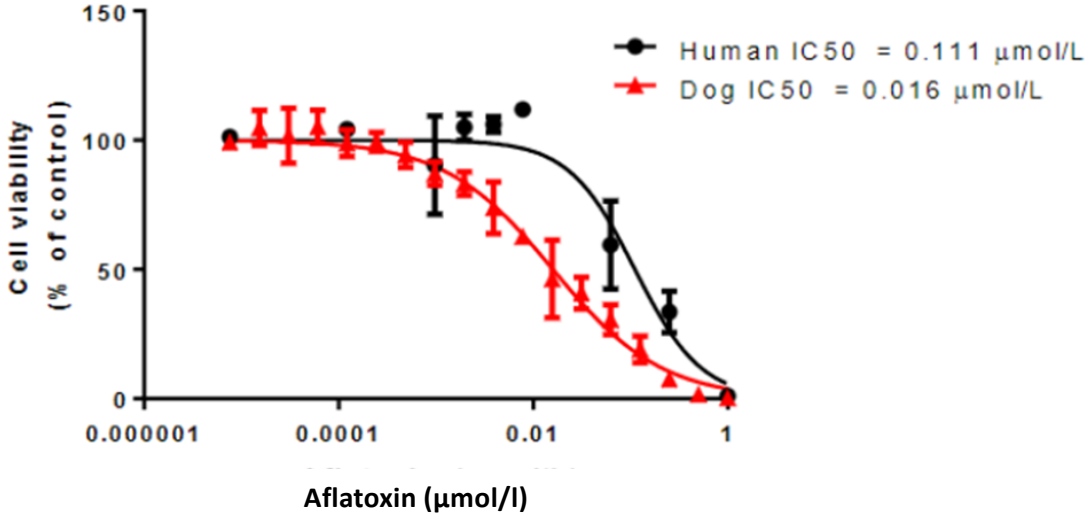
Higher sensitivity for Cytotoxicity of Sitaxentan in human 3D-liver Micro Tissues

High Impact of *In Vitro* X-Species DILI Studies

Example: Cross Species Evaluation of Aflatoxin B1

- Liver toxicity in dogs due to food contamination by AFB1
- Metabolic activation of AFB1 to reactive epoxide
- Epoxide involved in liver toxicity





Ian R. Jowsey, Qing Jiang, Ken Itoh, Masayuki Yamamoto and John D. Hayes
Molecular Pharmacology November 2003, 64 (5) 1018-1028

High Impact of *In Vitro* X-Species DILI Studies

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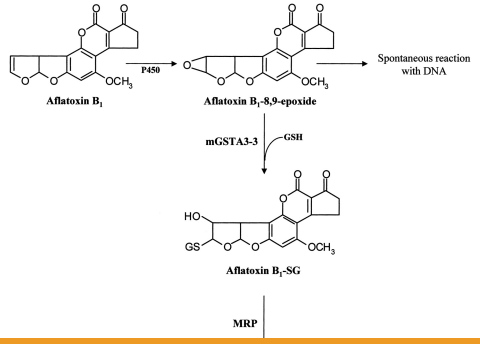
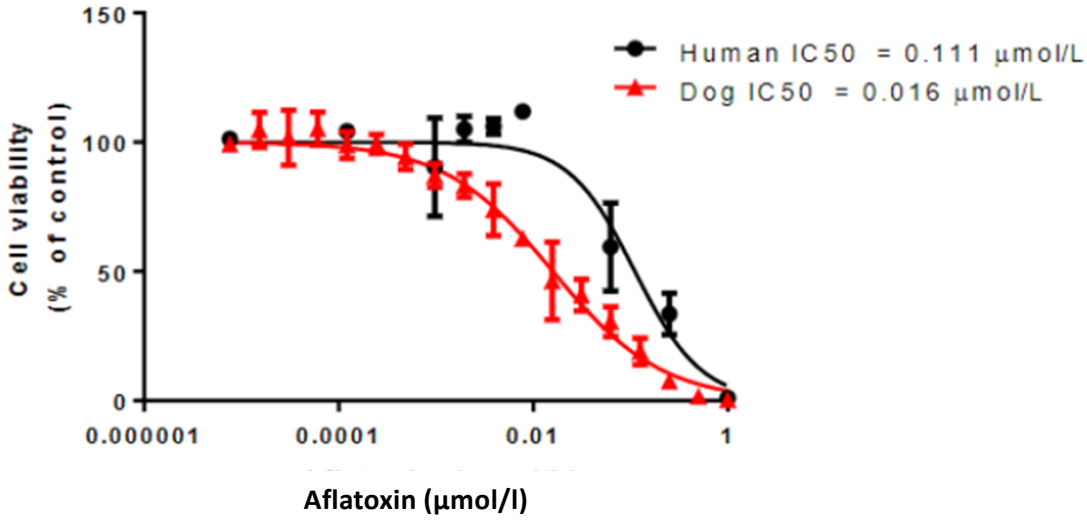
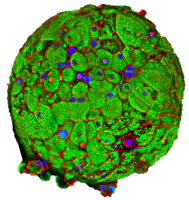




High sensitivity of AFB1 in dogs compared to man recapitulated in 3D MTs

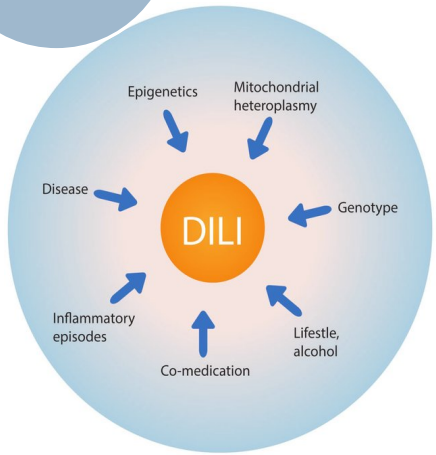
High Impact of 3D Microtissue Results influencing Clinical Studies



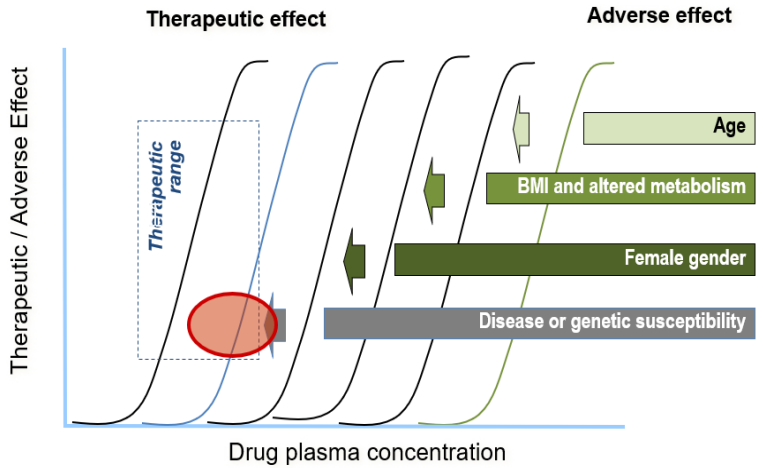
Toxicology

Clinical

Regulatory

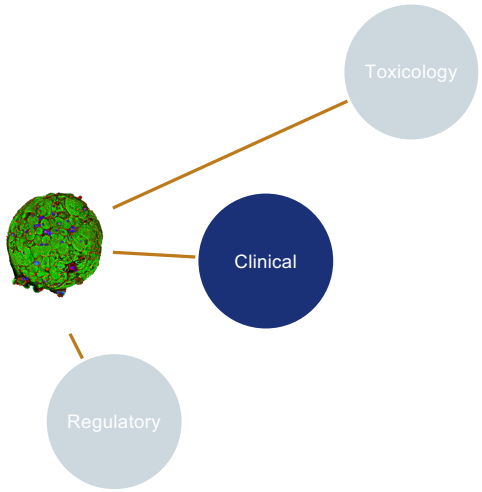


Changed therapeutic window in patients



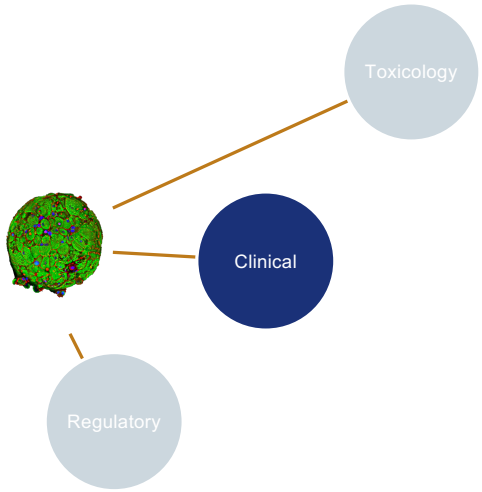
Clinical impact by toxicity testing in customized patient- and disease-specific 3D-MT production

High Impact of 3D Microtissue Results Influence Clinical Studies



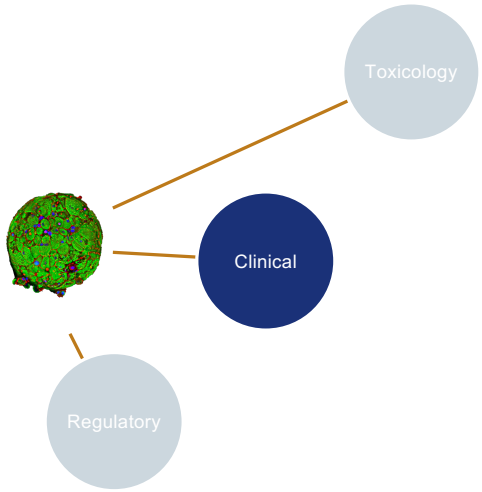
Clinical need/application	Customized 3D-MT production
Disease-specific DILI risk assessment, considering patient fragility	Toxicity testing in MTs from diseased or patient tissues

High Impact of 3D Microtissue Results Influence Clinical Studies



Clinical need/application	Customized 3D-MT production
Disease-specific DILI risk assessment, considering patient fragility	Toxicity testing in MTs from diseased or patient tissues
Support of patient stratification strategies	Tissues from respective population • Toxicity and efficacy testing from Ethnicity or underlying Polymorphism • Drug responder versus non-responder • Functional validation of specific genotype after GWAS

High Impact of 3D Microtissue Results Influence Clinical Studies



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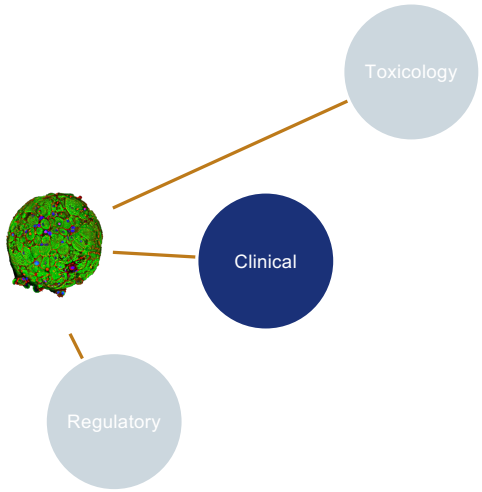

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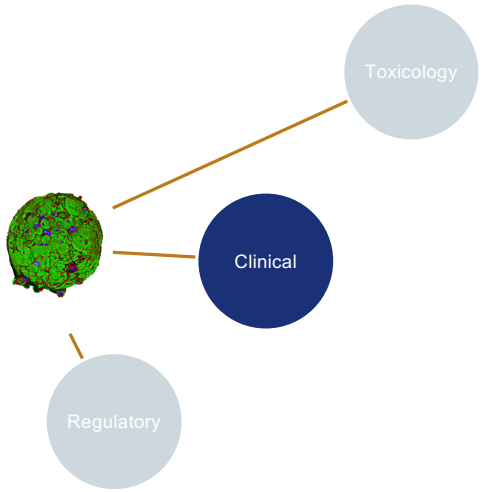
Individualized patient medicine	Patient-derived iPS MTs
Idiosyncratic DILI	Adaptive innate immune responses by patient-derived PBMC-MT coculture • PBMC and HLA-matched 3D-MT donor • PBMC and 3D-MT from same donor • PBMC from DILI responder

High Impact of 3D Microtissue Results Influence Clinical Studies



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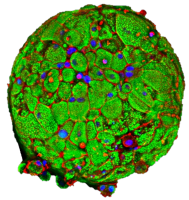
Support of patient stratification strategies	Tissues from respective population • Toxicity and efficacy testing from Ethnicity or underlying Polymorphism • Drug responder versus non-responder • Functional validation of specific genotype after GWAS
Individualized patient medicine	Patient-derived iPS MTs
Idiosyncratic DILI	Adaptive innate immune responses by patient-derived PBMC-MT coculture • PBMC and HLA-matched 3D-MT donor • PBMC and 3D-MT from same donor • PBMC from DILI responder
Clinical mitigation strategy	Mechanistic investigations • De-risking Hy's Law cases • Biomarker discovery • Antidote development

High Impact of 3D Microtissue Results Influence Clinical Studies



Toxicology

Clinical

Regulatory

Providing input for regulatory submissions

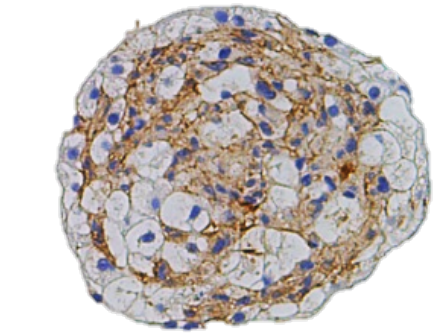
FDA

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Mechanistic explanations for Risk Assessment

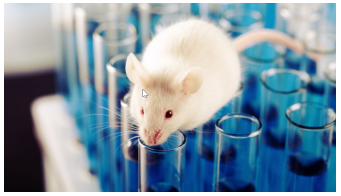
Presentation Overview

- Introduction: Drug discovery and translation to man
- ATP-based DILI hazard identification
- Stepping beyond ATP: from hazard identification to risk assessment
- Impact of 3D microtissues in drug development
- **Outlook: challenges and opportunities**



New Therapeutic Modalities Require New Strategies

Current Toxicity Testing Paradigm



Animals

Translation

Patients

New Therapeutic Modalities Require New Strategies

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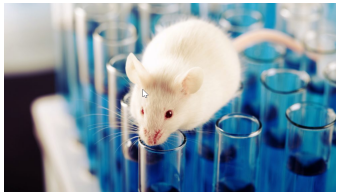


Animals

Translation

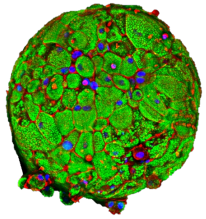
Patients

NEW Toxicity Testing Paradigm

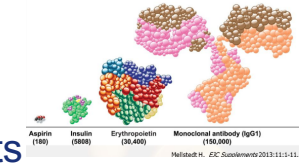
Human *in vitro* 3D-test systems

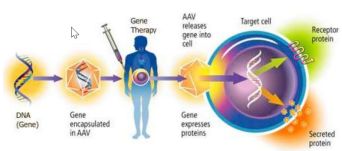
Back-Translation

Patient tissue



- New therapeutic modalities (Biologics, gene therapy, etc)
- Safety and efficacy testing customized for individual patients with specific diseases
- 3Rs

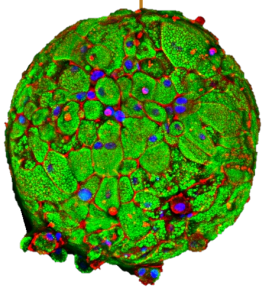




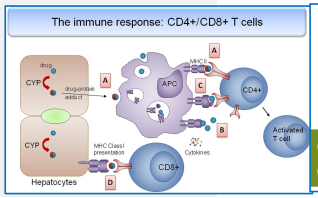
Translational Safety and Drug Discovery

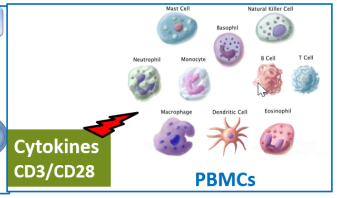
Challenges and Opportunities

New therapeutic modalities



- Monoclonal antibodies
- Recombinant proteins
- Gene therapy
- Stem cell therapy
- Immune-mediated DILI





Translational Safety and Drug Discovery

Challenges and Opportunities

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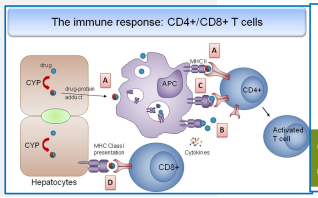
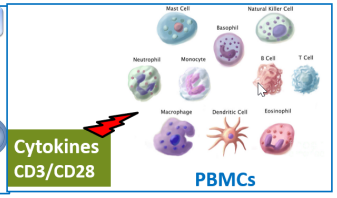
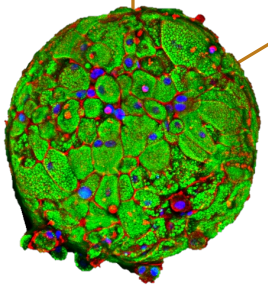




Predictive Toxicology

- DILI prediction by transcriptomics
- AI and Deep Learning



Translational Safety and Drug Discovery

Challenges and Opportunities

New therapeutic modalities

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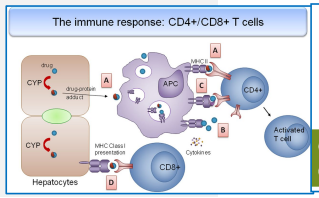

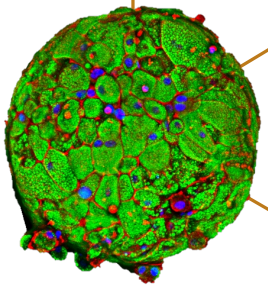

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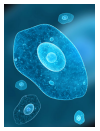
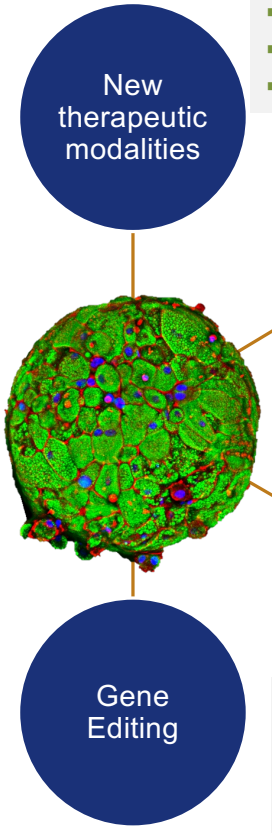
Single Cell Genomics

- Isolation
- Separation



Translational Safety and Drug Discovery

Challenges and Opportunities



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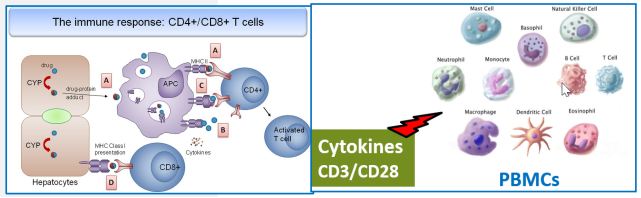

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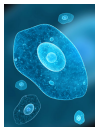

- AV, AAV, LV transduction
- CRISP/CAS9 Knock-down
- siRNA, microRNA, ASOs
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Translational Safety and Drug Discovery

Challenges and Opportunities

- Monoclonal antibodies
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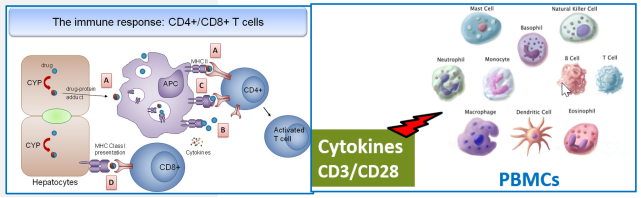

New therapeutic modalities

Predictive Toxicology

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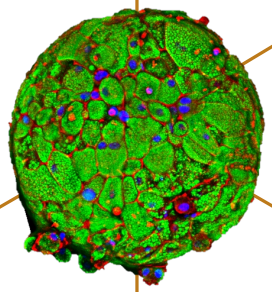
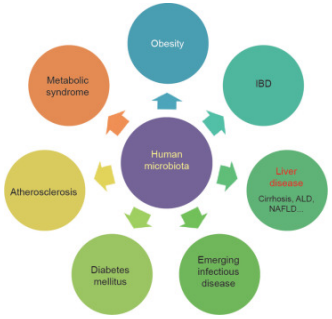


Microbiome



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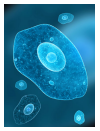

Gene Editing

- AV, AAV, LV transduction
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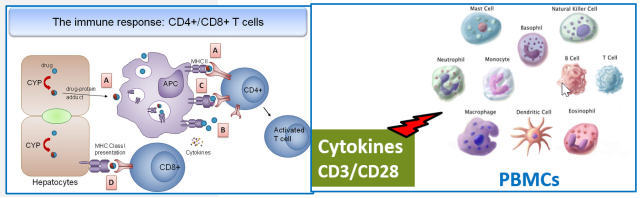
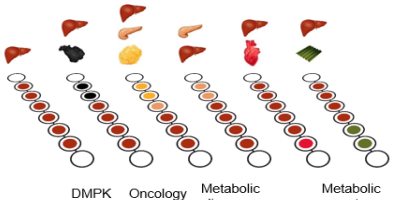
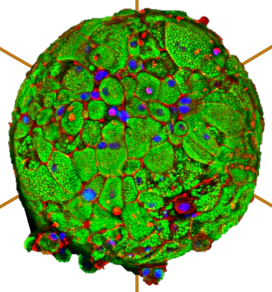




New therapeutic modalities

Organ on Chip (OoC)

Predictive Toxicology



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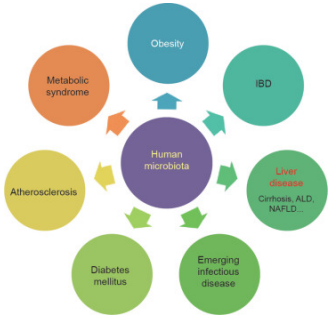

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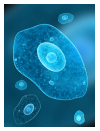

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Summary

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  - Models fulfill the biological requirements in terms of translatability to humans and building the bridge to patients.
  - Pragmatic and flexible adaptation of 3D MT to specific scientific questions and industrial needs.
  - 3D human liver microtissues are complex *in vitro* models amenable to high-throughput applications (quality, robustness and scalability of results).

Summary

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  - Models fulfill the biological requirements in terms of translatability to humans and building the bridge to patients.
  - Pragmatic and flexible adaptation of 3D MT to specific scientific questions and industrial needs.
  - 3D human liver microtissues are complex *in vitro* models amenable to high-throughput applications (quality, robustness and scalability of results).
- The 3D microtissue technology is accessible as investigative tool for challenges of the next generation of new therapeutic modalities.

Acknowledgements

- Radina Kostadinova
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