MIP-DILI

Mechanism–based Integrated Systems for the Prediction of Drug-Induced Liver Injury

Innovative in vitro models of toxicology assessments

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London EUFEMED 2017

Drug-Induced Liver Injury

RESEARCH ARTICLE Open Access

Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature

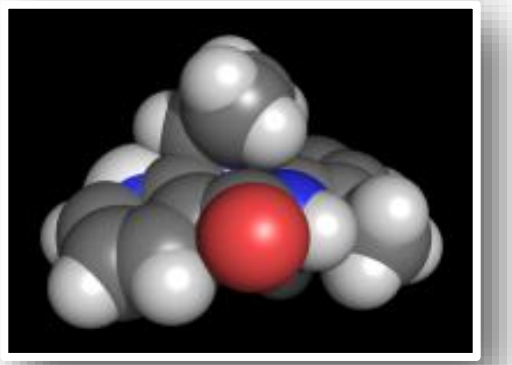
Igho J. Onakpoya*, Carl J. Heneghan and Jeffrey K. Aronson

BMC Med. 2016 Feb 4;14:10

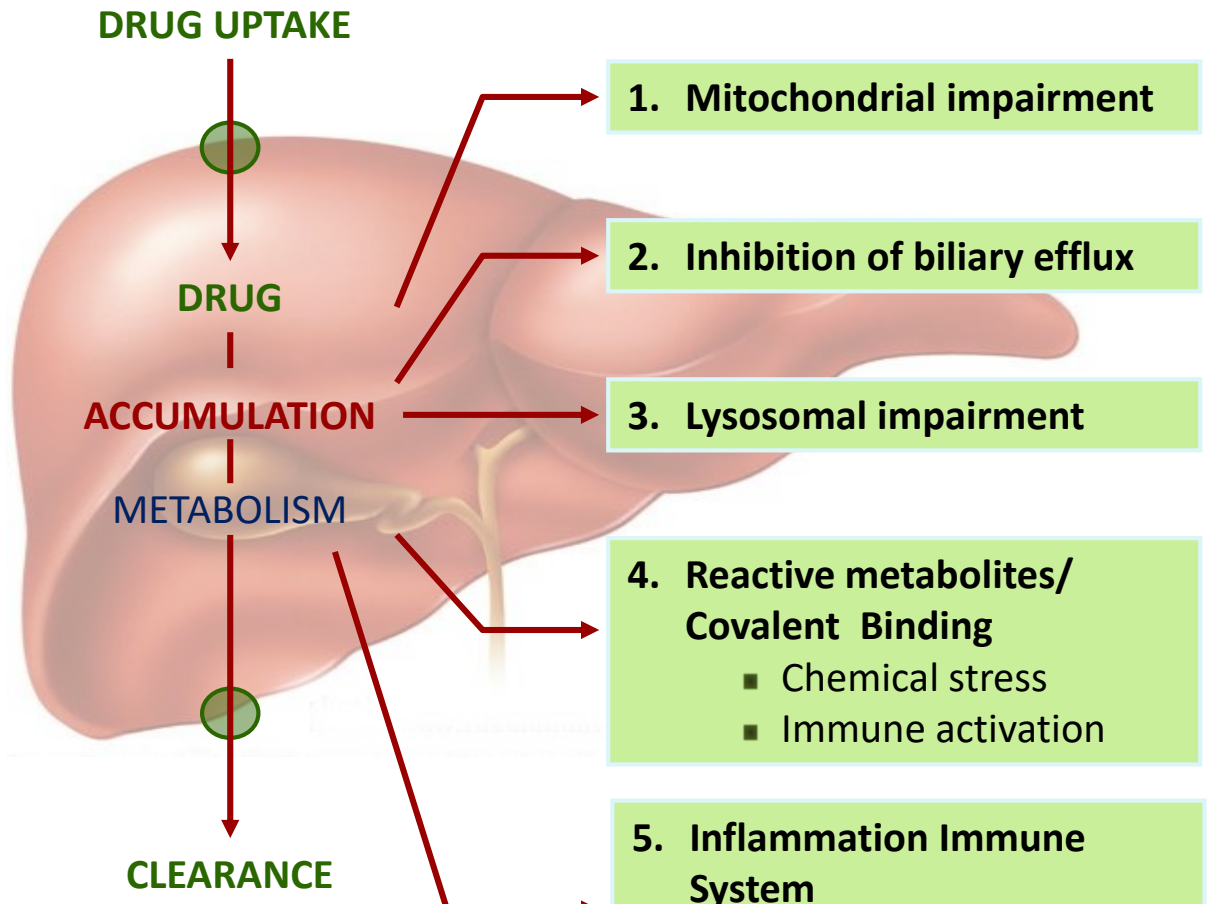
- From 1953-2013 462 drugs were withdrawn post-marketing due to ADRs.
  - Hepatotoxicity (81 cases; 18%)
  - Immune-related reactions (79 cases; 17%)
  - Cardiotoxicity (63 cases; 14%)

Drug-Induced Liver Injury

- Dose-dependent
- Species selective
- Selective individuals
- Idiosyncratic

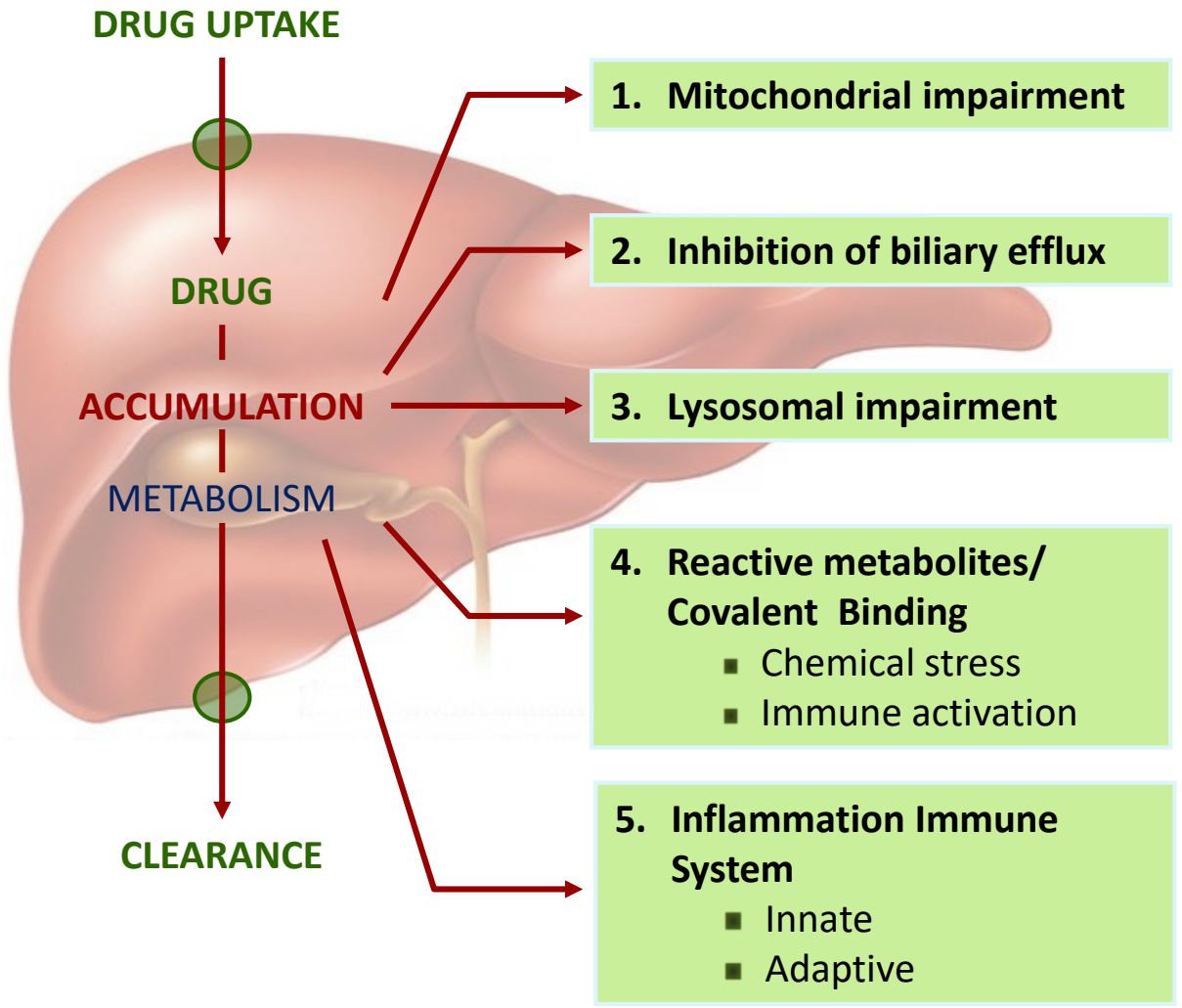


Chemical Insults and DILI



1. **Mitochondrial impairment**
2. **Inhibition of biliary efflux**
3. **Lysosomal impairment**
4. **Reactive metabolites/ Covalent Binding**
   - Chemical stress
   - Immune activation
5. **Inflammation Immune System**
   - Innate
   - Adaptive

Chemical Insults and DILI



Diverse Clinical Presentations of DILI

- Acute fatty liver with lactic acidosis
- Acute hepatic necrosis
- Acute liver failure
- Acute viral hepatitis-like liver injury
- Autoimmune-like hepatitis
- Bland cholestasis
- Cholestatic hepatitis
- Cirrhosis
- Immuno-allergic hepatitis
- Nodular regeneration
- Nonalcoholic fatty liver
- Sinusoidal obstruction syndrome
- Vanishing bile duct syndrome

Genetic Restriction and Drug Hypersensitivity: Discovery of HLA Allele Associations

MRC Centre for Drug Safety Science

- Discovery of associations between HLA alleles and drug hypersensitivity represents an important advance
- Screening for HLA alleles during clinical practice effectively prevents reactions
  - Abacavir
  - Carbamazepine
- ***This is not the case for DILI***
- ***So we need biomarkers and models***

Pharmacogenetics and Clinical Syndromes

Abacavir hypersensitivity	HLA-B*5701	OR = 132
Flucloxacillin DILI	HLA-B*5701	OR = 72
Carbamazepine SYS/TEN	HLA-B*1502 (Chinese)	OR = 1000
Carbamazepine Hypersensitivity	HLA-A*3101 (Japanese)	OR = 11
Carbamazepine Hypersensitivity	HLA-A*3101 (Caucasians)	OR = 30
Lumiracoxib DILI	HLA-DRB1*1501 HLA-DQA1*0102	OR = 7
Ximelagatran **Lapatinib** DILI	HLA-DRB1*0701 HLA-BQA1*0201	OR = 4.4 OR = 9.0

UNIVERSITY OF LIVERPOOL

Mallal, 2008; Kindmark et al., 2008; Daly et al., 2009; Chung et al., 2004; McCormack et al., 2011; Singer et al., 2010; Spraggs et al., 2011

MIP-DILI Roadmap - Stratification of *In Vitro* systems

Physiology ⟷ **Pharmacology** → **Toxicology**

Tier 1

Single cell 2D systems

- PHH
- Cell-lines
- Reporter-signalling

Tier 2

Multi-dimensional 3D systems

- Multi-cell culture
- Spheroids
- Long term culture

Tier 3

Complex systems

Patient-dependent factors

- Viral infection
- HLA restriction
- T cells

MIP-DILI Roadmap - Stratification of *In Vitro* systems

Tier 1

Single cell 2D systems

Physiology	Pharmacology	Toxicology	
Expression phenotype	Drug Accumulation	Cytotoxicity	Mitotoxicity
Transcriptome Proteome	Drug Bioactivation/ Covalent Binding	Transporter inhibition	GSH depletion
Function	Drug Detoxication	Cholestasis	ER stress
Cell Signalling Albumin secretion Biomarker secretion		Lysosomal impairment	Oxidative stress

Tier 2

Multi-dimensional 3D systems

- Multi-cell culture
- Spheroids
- Long term culture

Tier 3

Complex systems

Patient-dependent factors

- Viral infection
- HLA restriction
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Databases

Chemoinformatics

Bioanalysis

Transcriptomics

Proteomics

Metabolic profiles

Drug distribution

Biomarkers

Functional measurements

Drug metabolizing enzyme activity

Transporter activity

High content analysis

Innate Signalling

Adaptive response

Mathematical modelling

Selection of Model Systems

Importance of the physiological and pharmacological phenotype for the application of a toxicological test

- Is a particular test system fit for purpose?
- What purpose is it fit for?

Importance of the physiological and pharmacological phenotype for the application of a toxicological test

TIER ONE

Tier one cell types

Primary Hepatocytes

Liver-derived cell lines

Fresh

Cryo-preserved

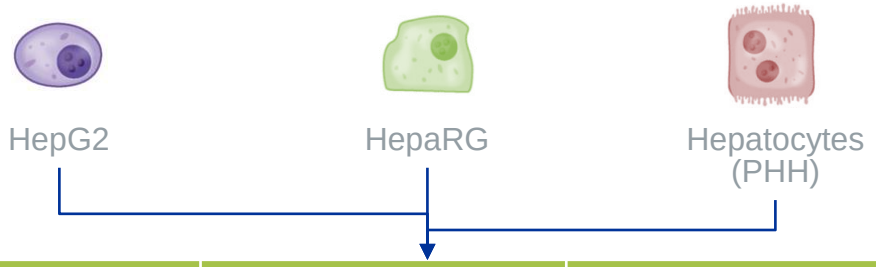
HepG2

HepaRG

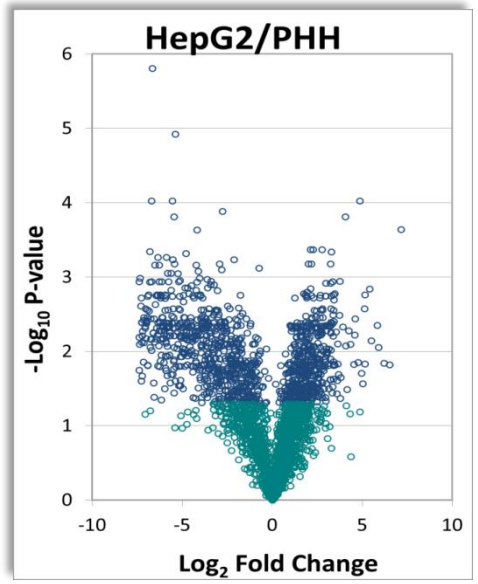
UpCytes

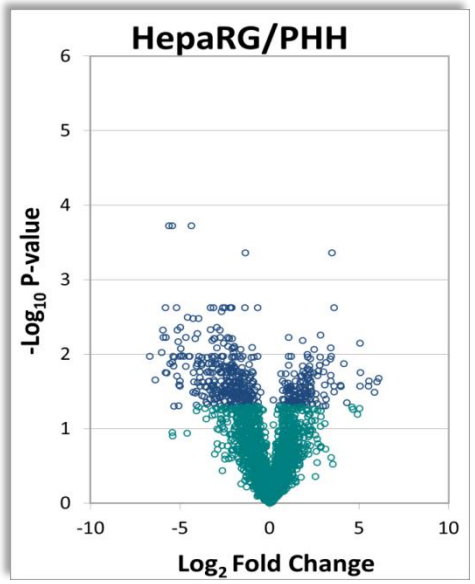
iTRAQ proteomic comparison

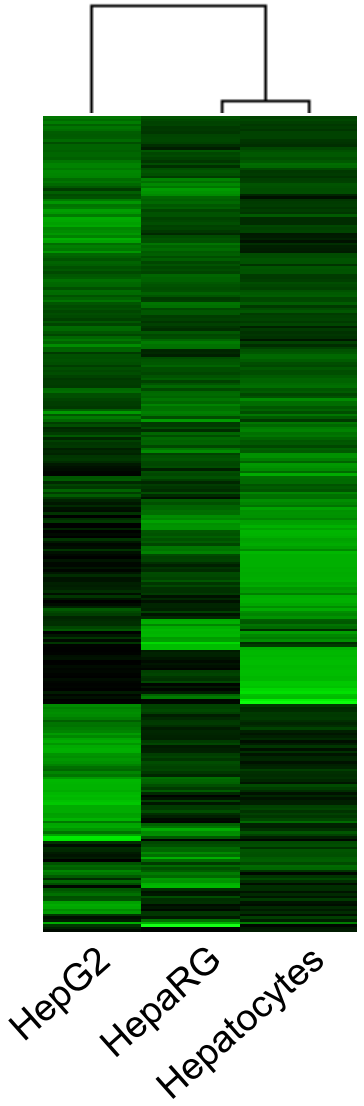
Proteomic phenotype



Biological Replicate	No. of proteins identified	Proteins quantified
1	4335	3197
2	4887	4397
3	4891	3794
Total (common to all replicates)		**2726**







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Advance Access Publication Date: July 8, 2015
Research Article

Comparative Proteomic Characterization of 4 Human Liver-Derived Single Cell Culture Models Reveals Significant Variation in the Capacity for Drug Disposition, Bioactivation, and Detoxication

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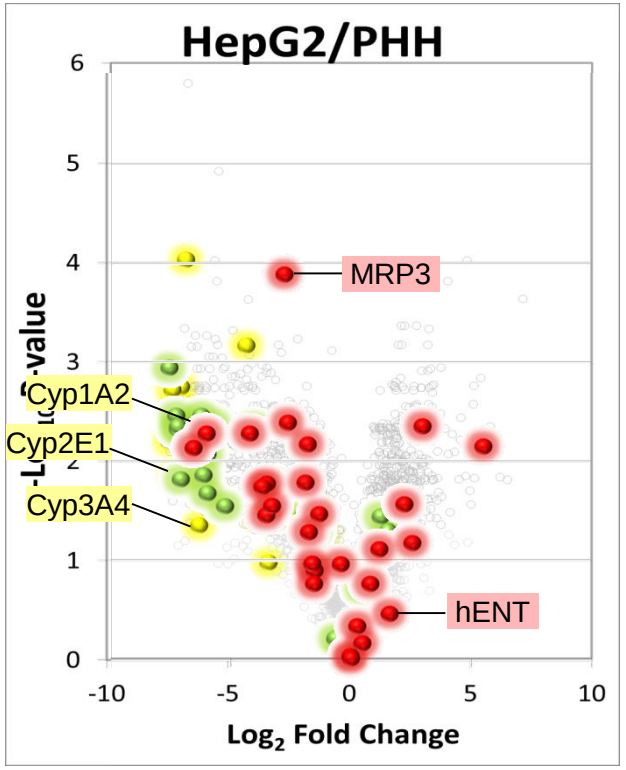
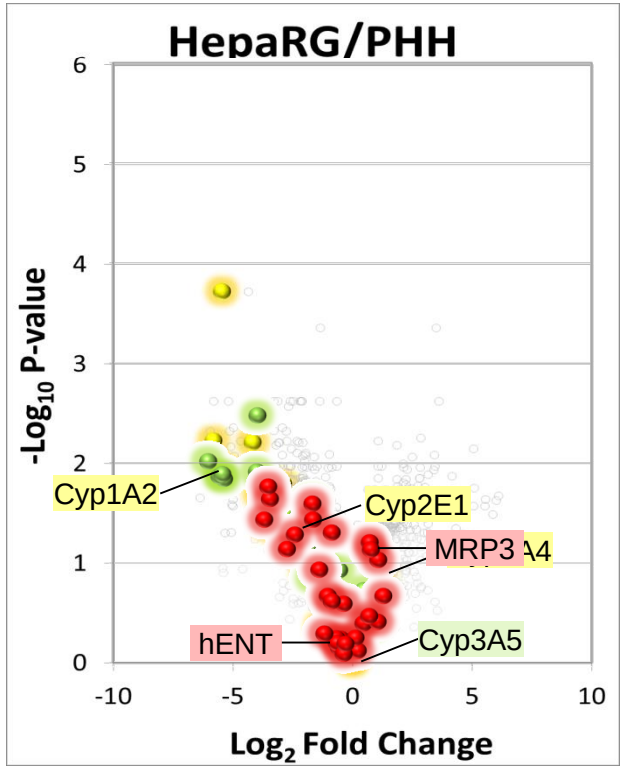
Rowena Sison

HLCs Proteomic Phenotype





Cytochrome P450s

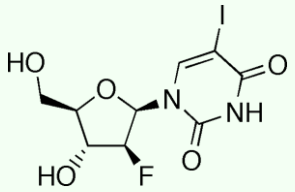
Phase II drug metabolising enzymes

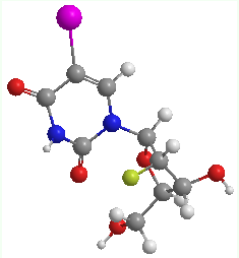
Transporters

MIP-DILI Refined Mitotox functional analysis conforms with Literature

	Compound	Mitochondrial Liability Literature	MIP-DILI Mitotox Analysis	Reference
MIP-DILI Training compounds	Paracetamol	**Yes** – Inhibits ATP synthase, MPTP opener, mitochondrial ROS,	Positive	Parmar et al, 1995, Kon et al., 2004
	Amiodarone	**Yes** – OXPHOS uncoupler, inhibits fatty acid oxidation via CPT1 inhibition	Positive	Fromenty et al., 1990 Kennedy et al., 2006
	Nefazodone	**Yes** - complex I > complex IV inhibition of ETC in HepG2 and isolated rat liver	Positive	Dykens et al., 2008
	Tolcapone	**Yes** - OXPHOS uncoupler, interacts with ETC complex proteins, FAO, bile acid synthesis. Forms MPT pores, decreases MMP	Positive	Korlipara, Cooper and Schapira, 2004
	Entacapone	**Yes** – mild OXPHOS uncoupler	Negative	Korlipara et al., 2004
	Bosentan	**No**	Negative	Clinical Pharmacology & Therapeutics (2001) 69, 223–231
	Buspirone	**Yes** - complex I inhibition of ETC in HepG2 and isolated rat liver	Positive	Dykens et al., 2008
	Diclofenac	**Yes** – MPTP opener, mild OXPHOS uncoupler, inhibits ATP synthase and adenine nucleoside translocase	Positive	Moreno-Sanchez et al., 1999
	Metformin	**Yes** – complex I inhibitor	Positive	Carvalho et al., 2008
	Pioglitazone	**Yes** –complex I inhibitor	Positive	Scatena et al., 2004 Garcia-Ruiz, et al. 2013
	Ximelagatran	**No**	Negative	Kenne et al., 2008
	Fialuridine	**Yes** – impairs mtDNA replication,	Negative (chronic toxicity)	Lewis et al., 1996
	Perhexiline	**Yes** – Inhibits carnitine uptake via CPT1, inhibits fatty acid oxidation	Negative (mechanistic factors)	Kennedy et al., 2006
	Troglitazone	**Yes** –reported inhibitor of complex I, II, III, IV, V inhibitor, MPTP opener, OXPHOS uncoupler	Positive	Nadanaciva, 2008 Scatena et al., 2004

Fialuridine – *fatal clinical trial*





- Developed as an antiviral for HIV – later considered as a treatment for hepatitis B
- Preclinical testing in mice, rats, dogs and monkeys

Clinical Testing

Patients	Duration	Comments
HIV⁺/CMV⁺ (12)	35 d	Duration prolonged to sustain antiviral (HBV⁺) No signs of toxicity
HIV⁺/HBV⁺ (43)	14 d	
HBV⁺ (24)	28 d	
HBV⁺ (15)	6 mth planned, terminated at wk 13	

Sudden Hepatotoxicity & Pancreatitis

5 patients died (ALF)

2 survived after emergency liver transplant

3 recovered

3 showed no adverse effects (lower doses)

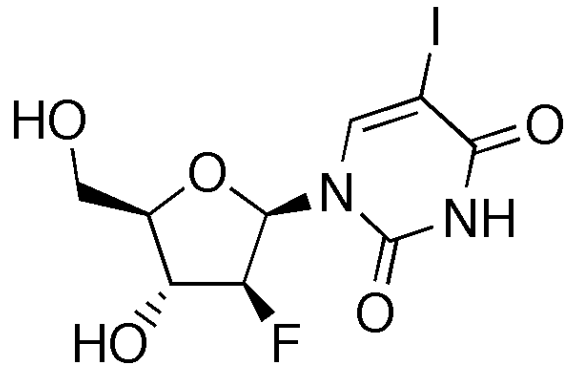
Clinical Features of Toxicity

- Delayed (from week 13)
- Lactic Acidosis
- Micro and macro vesicular hepatic steatosis
- Abnormal mitochondria

} ***Indicative of mitochondrial dysfunction***

McKenzie et al, 1995 (333)

Fialuridine *in vitro* requirements



Fialuridine

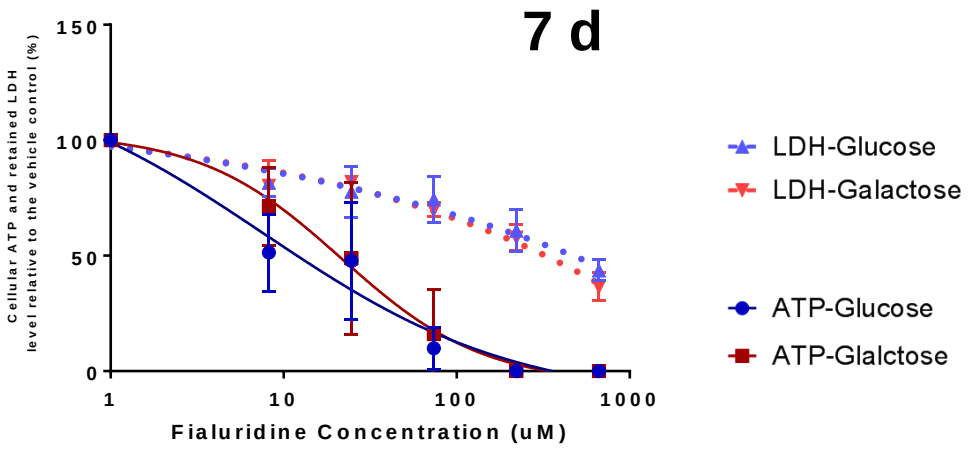
KEY FEATURES OF MECHANISM OF TOXICITY

- **Human specific (via hENT mitochondrial localisation)**
- **Bioactivation to triphosphate (mitochondrial thymidine kinases)**
- Delayed onset (wk 13 of clinical trial)
- Sudden and rapid acceleration of toxicity (threshold effect)
- Targets mitochondrial DNA replication
- Lactic acidosis and steatosis in humans

Fialuridine *in vitro* models: HepG2 vs HepaRG

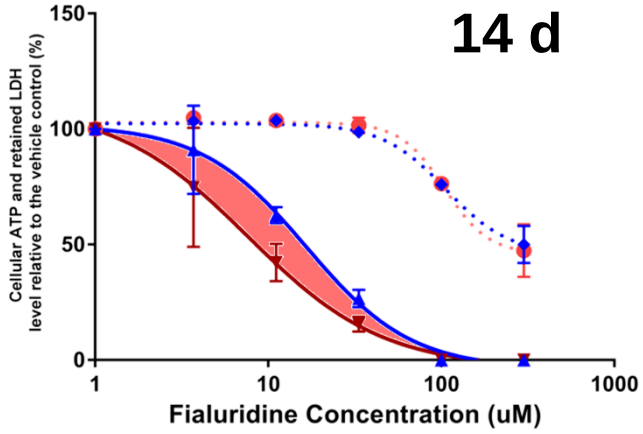
Cmax	
Healthy volunteers (5 mg dose)	0.6 µM (measured)
Patients with DILI (0.1 – 0.25 mg/kg/day)	~ 2.0 – 2.5 µM (estimated from volunteers)

HepG2: Max time course 7 days



Negative mitotoxin

HepaRG: Max time course 5 weeks



Positive mitotoxin

Cytotoxicity in HepG2 at high concentrations not related to the clinical mitochondrial mechanism of action

Extended dosing in HepaRG allows mitochondrial toxicity to develop.

Evidence for Mitoxicological Mechanism seen in Man

FIAU

↓

Mitochondrial uptake

↓

Phosphorylation

↓

Incorporation/inhibition of Pol γ

↓

↓ mtDNA replication

↓

↓ mito-encoded respiratory complexes

↓

↓ OXPHOS and ↑ Glycolysis

↑ Lactate

↓ ATP

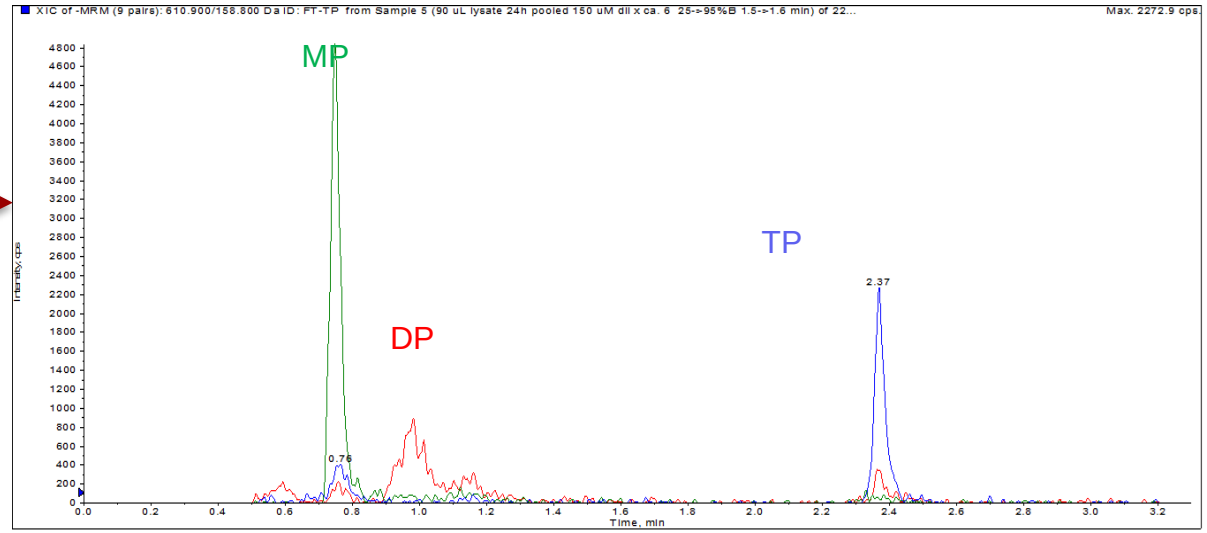
↓

Cell stress

↓

Cell death

BIOACTIVATION



Intracellular presence of FIAU-MP, -DP and –TP (LC-MS)

Evidence for Mitoxicological Mechanism seen in Man

FIAU

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Mitochondrial uptake

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Incorporation/inhibition of Pol γ

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↓

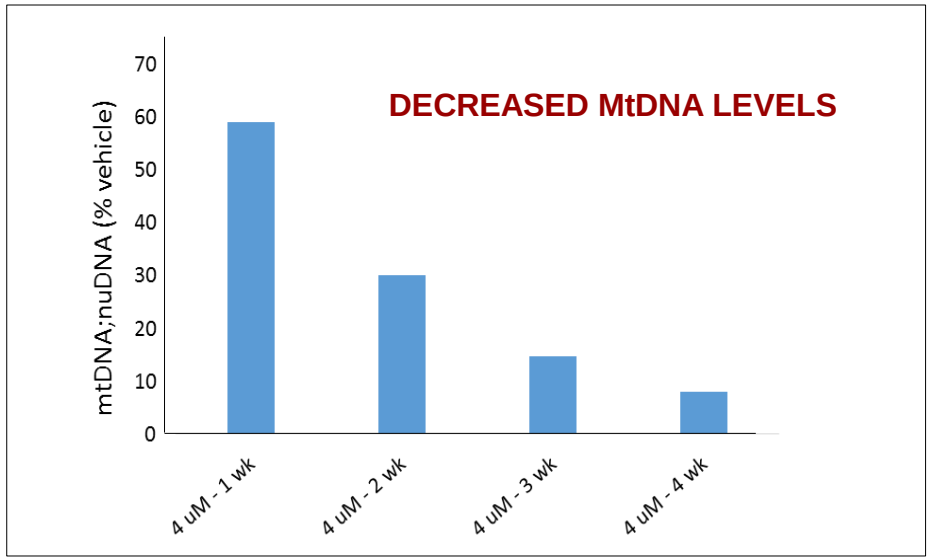
↑ Lactate | ↓ ATP

↓

Cell stress

↓

Cell death



Quantified using PCR

(mtDNA and nuDNA quantified)

Evidence for Mitoxicological Mechanism seen in Man

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Mitochondrial uptake

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Cell stress

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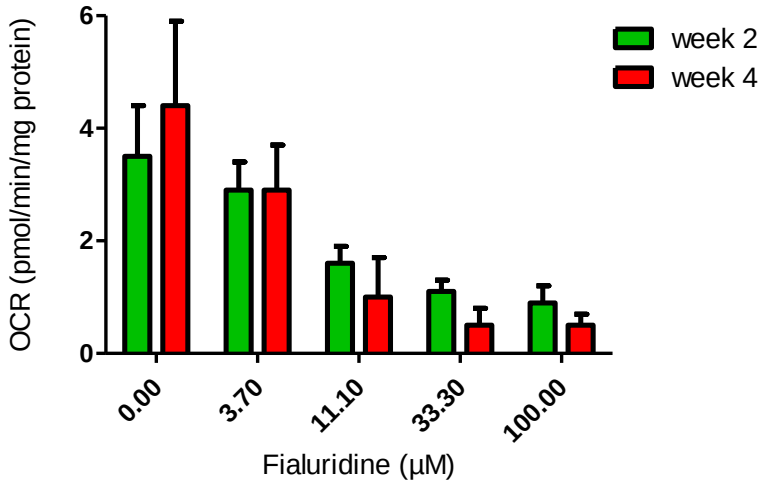
Cell death

Decreased oxidative phosphorylation

(using Seahorse Technology to measure oxygen consumption)



ATP-linked respiration

Indicates the oxygen consumption which is linked to ATP-production by OXPHOS

Evidence for Mitoxicological Mechanism seen in Man

FIAU

↓

Mitochondrial uptake

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Phosphorylation

↓

Incorporation/inhibition of Pol γ

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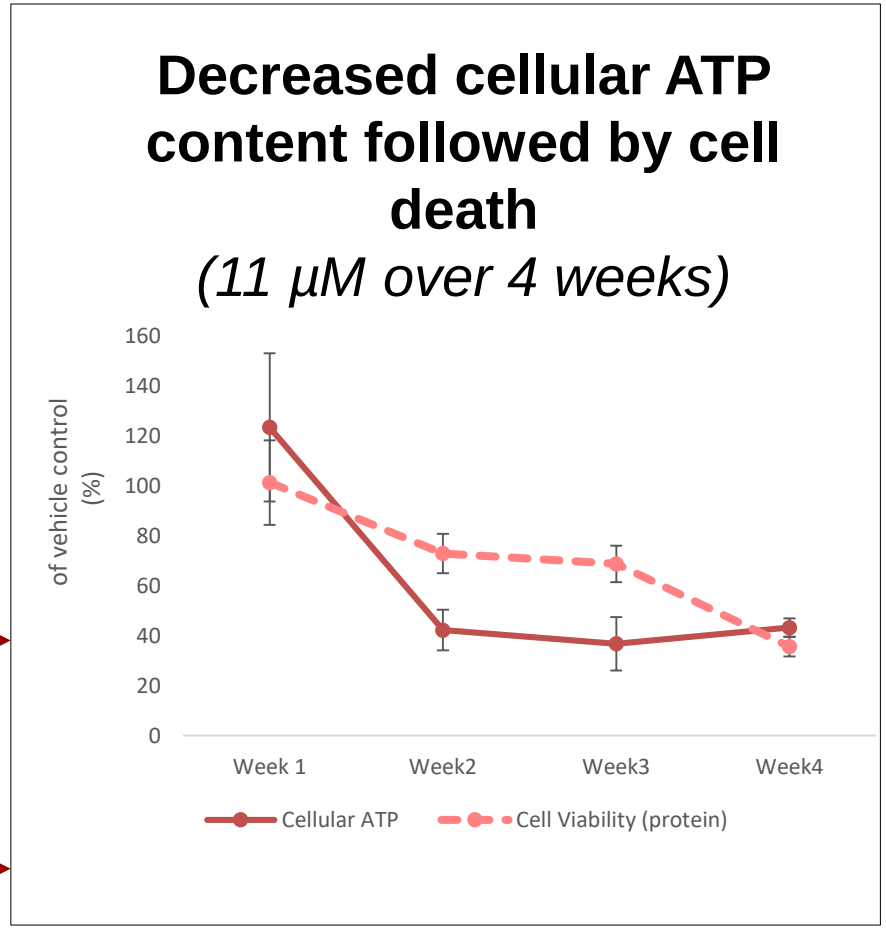
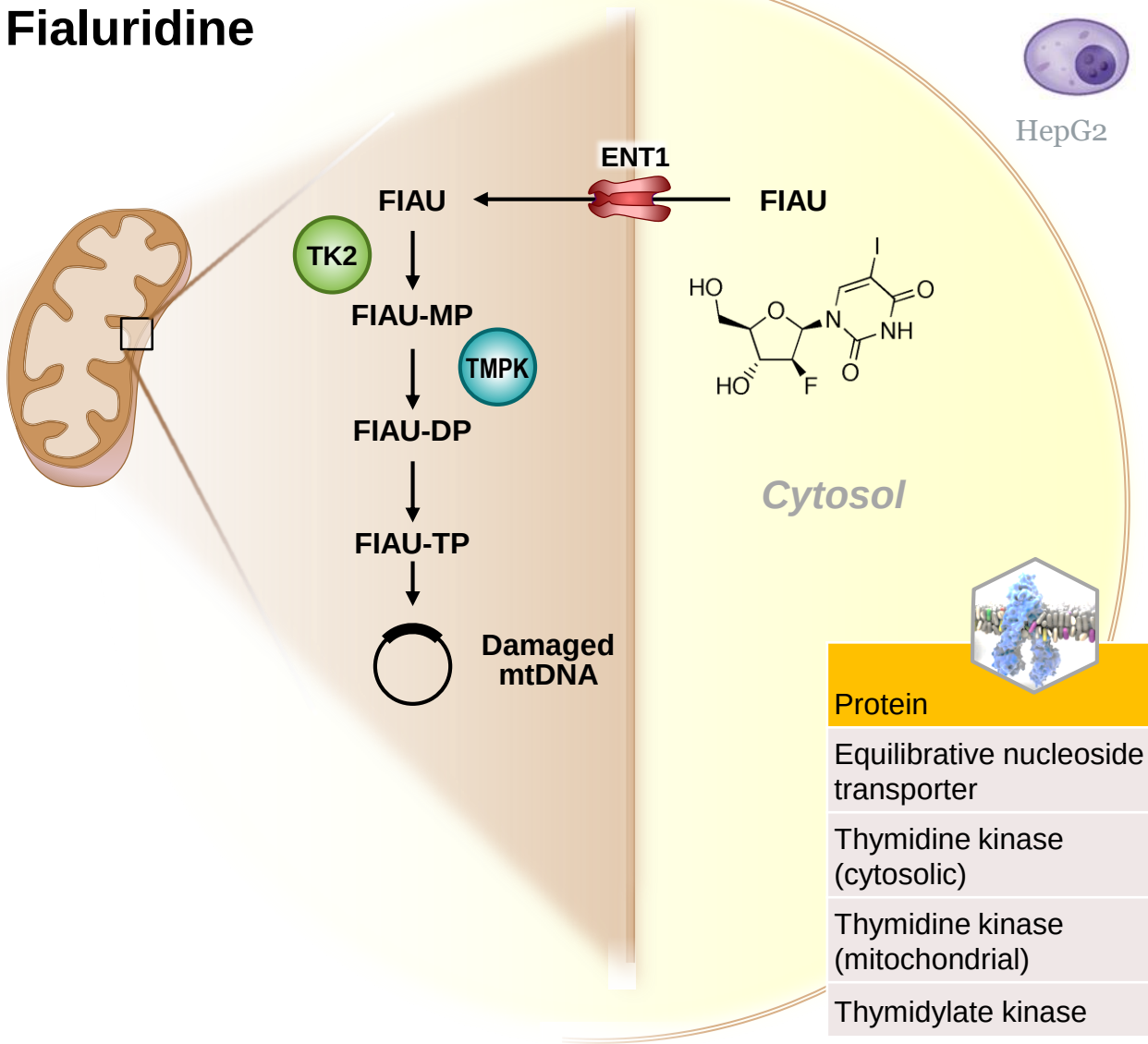
↓ ATP

Cell stress

Cell death



Importance of the physiological and pharmacological phenotype for the application of a toxicological test



Protein		HepG2 %	HepaRG %	Hepatocytes %
Equilibrative nucleoside transporter	ENT1	174.5	63.3	100
Thymidine kinase (cytosolic)	TK1	726.8	286.1	100
Thymidine kinase (mitochondrial)	TK2	51.5	73.9	100
Thymidylate kinase	TMPK	177.6	120.0	100

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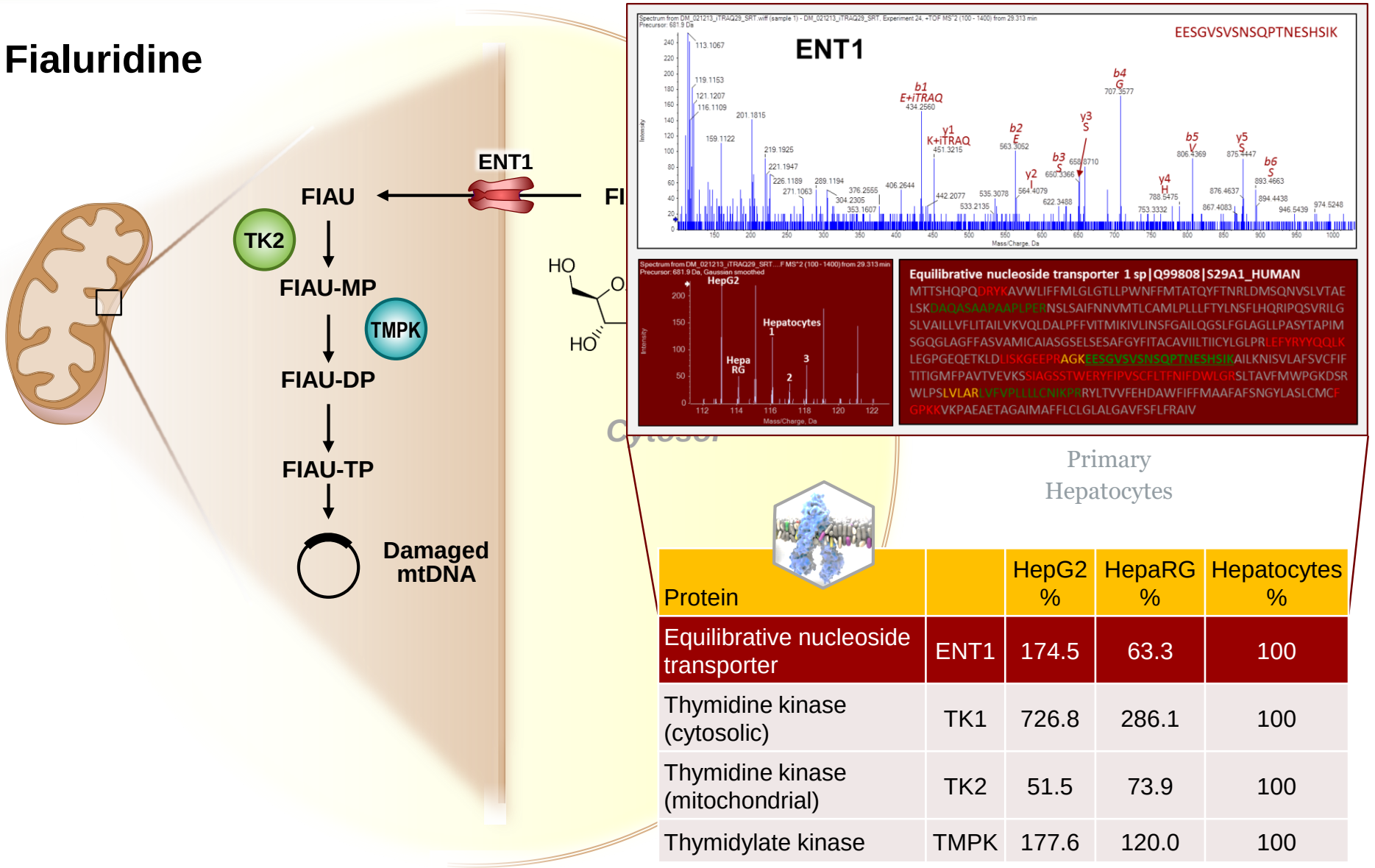

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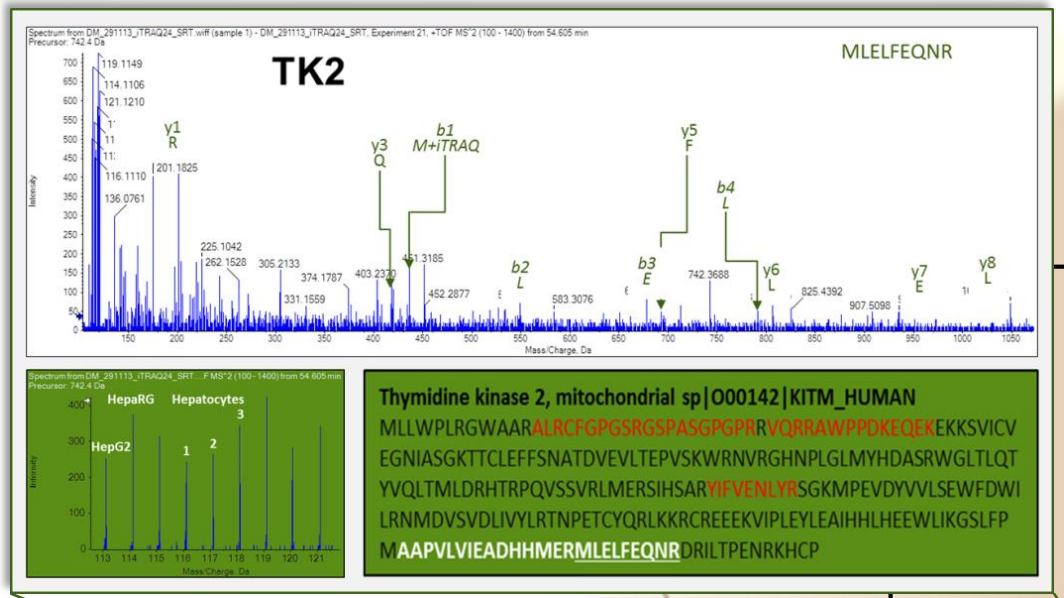

FIAU-TP

Damaged mtDNA

Protein		HepG2 %	HepaRG %	Hepatocytes %
Equilibrative nucleoside transporter	ENT1	174.5	63.3	100
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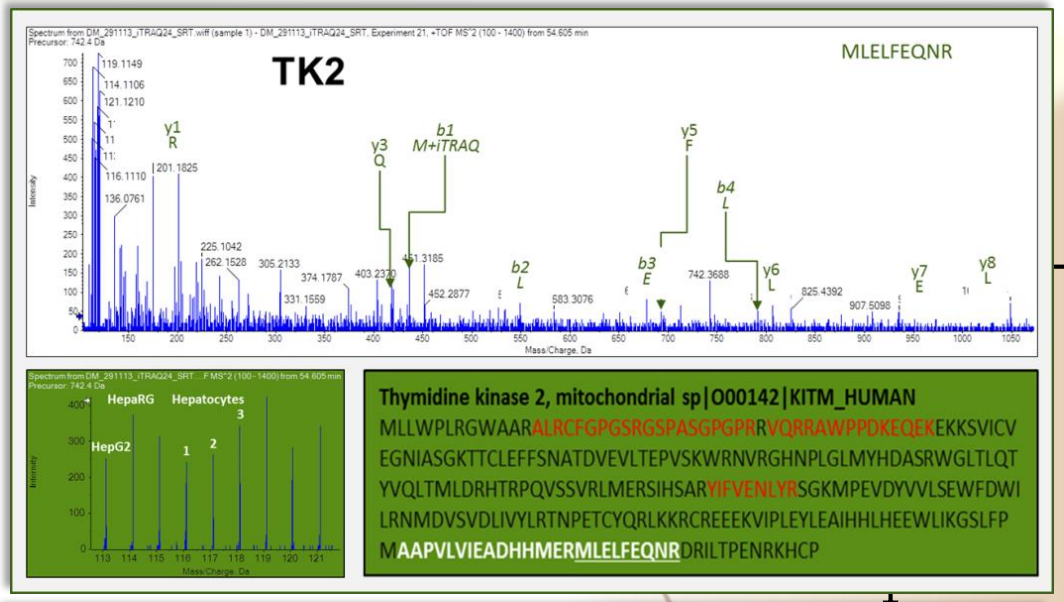
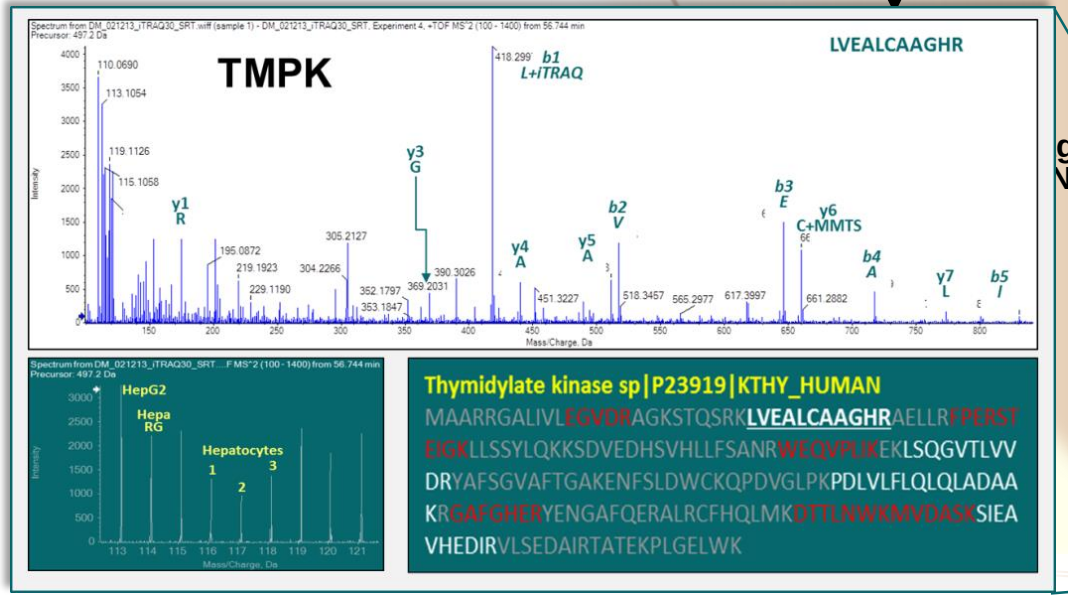
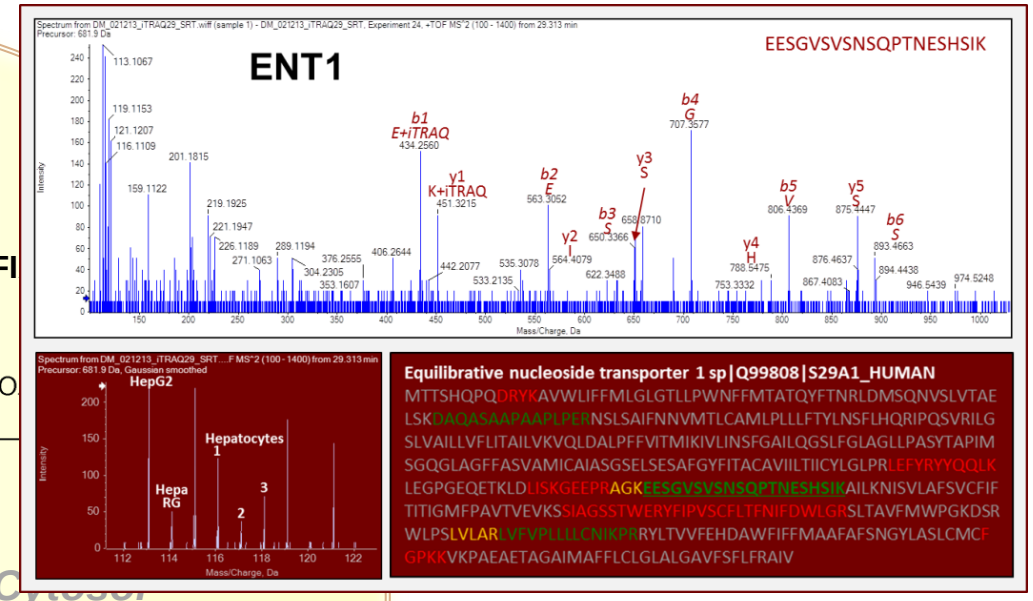
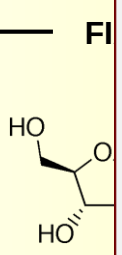
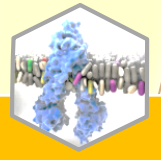








Primary Hepatocytes



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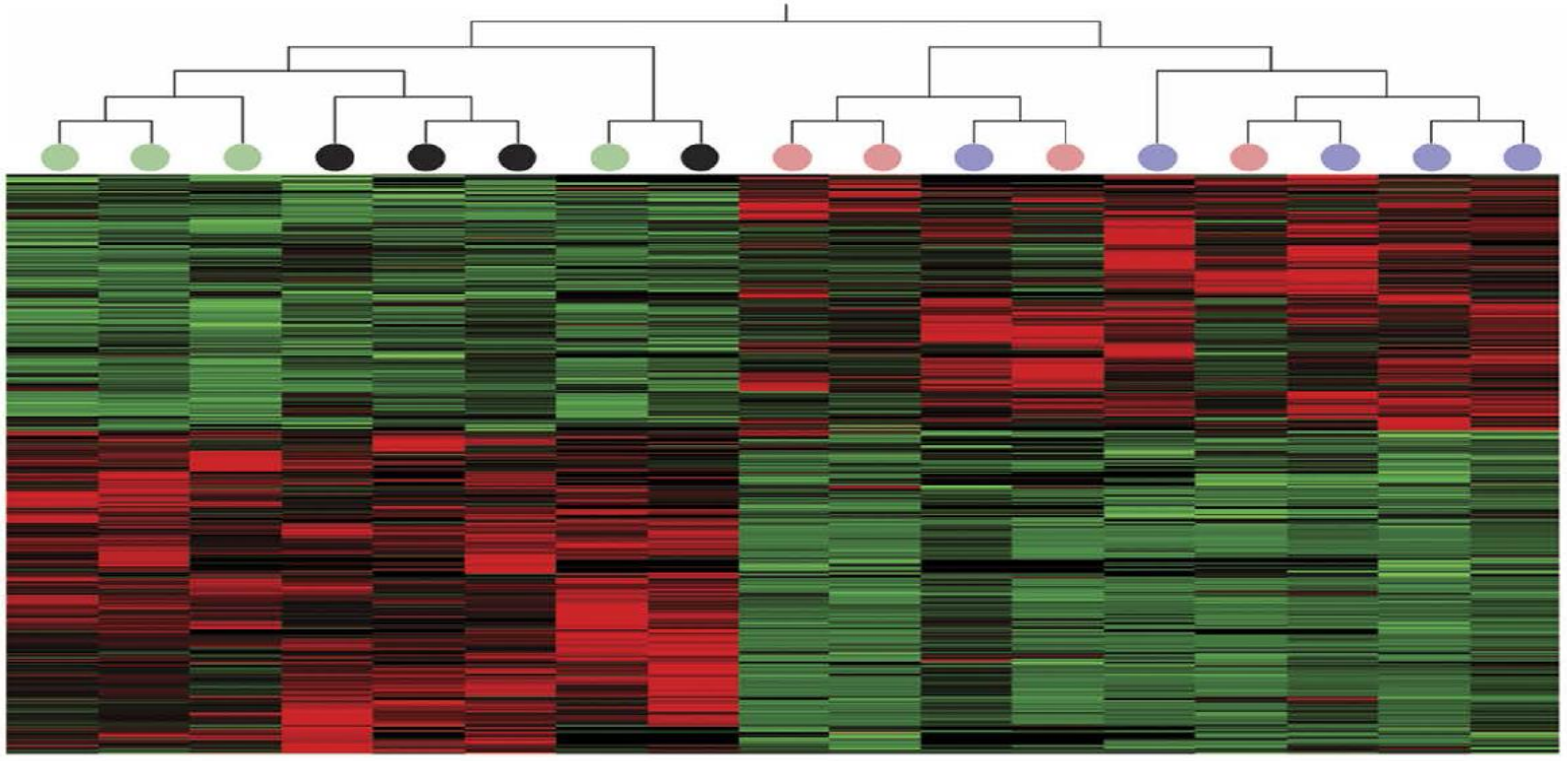
Beyond 2D liver cell culture
Phenotype of Spheroids: KI - CDSS

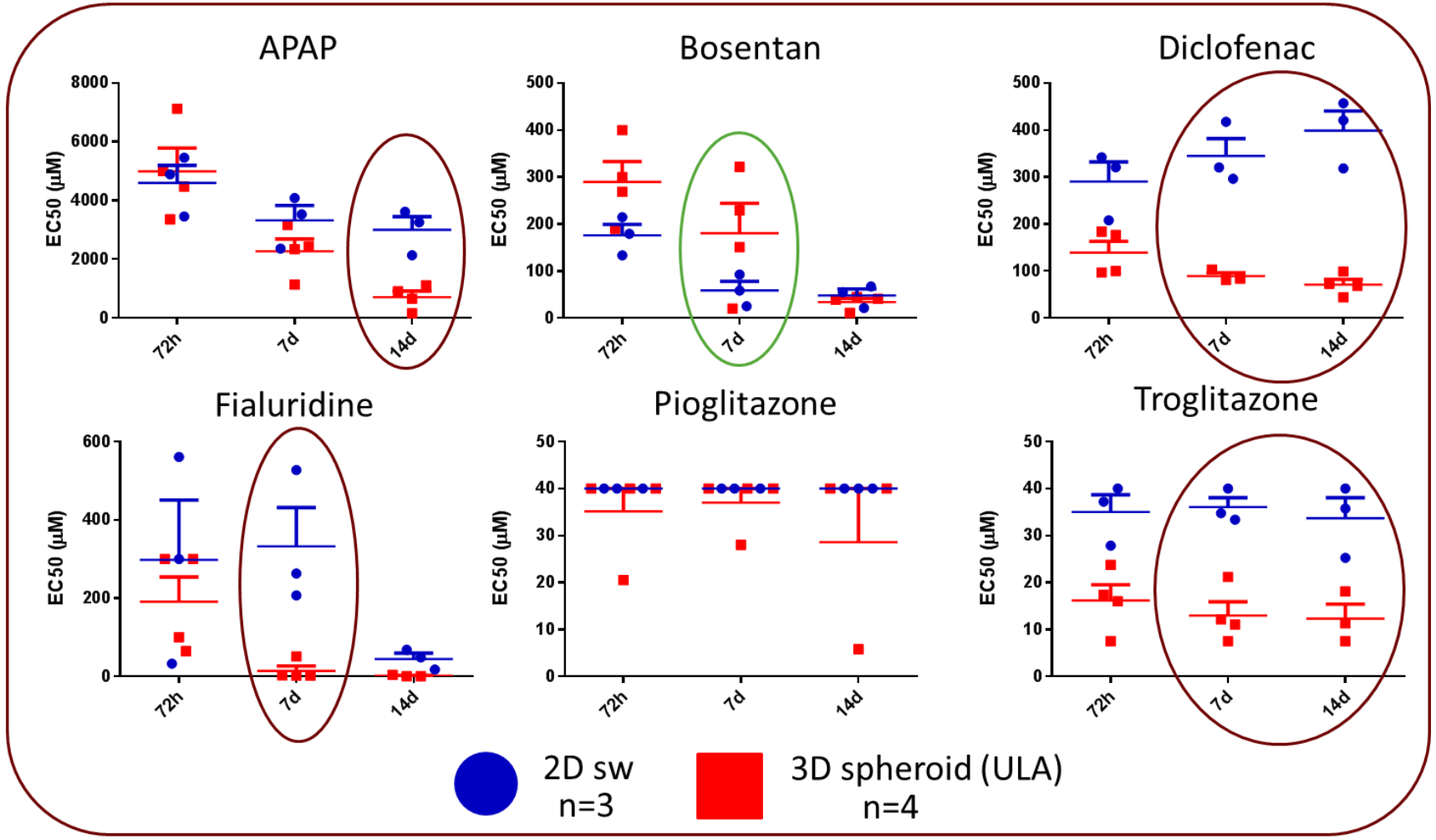
- **Whole proteome: 2D, spheroid, fresh primary hepatocytes and liver from several human donors**
- ***Data expressed relative to freshly-isolated hepatocytes from corresponding donor***

Segregation of spheroid culture hepatocytes with fresh liver



Bell et al., 2016 Sci Rep

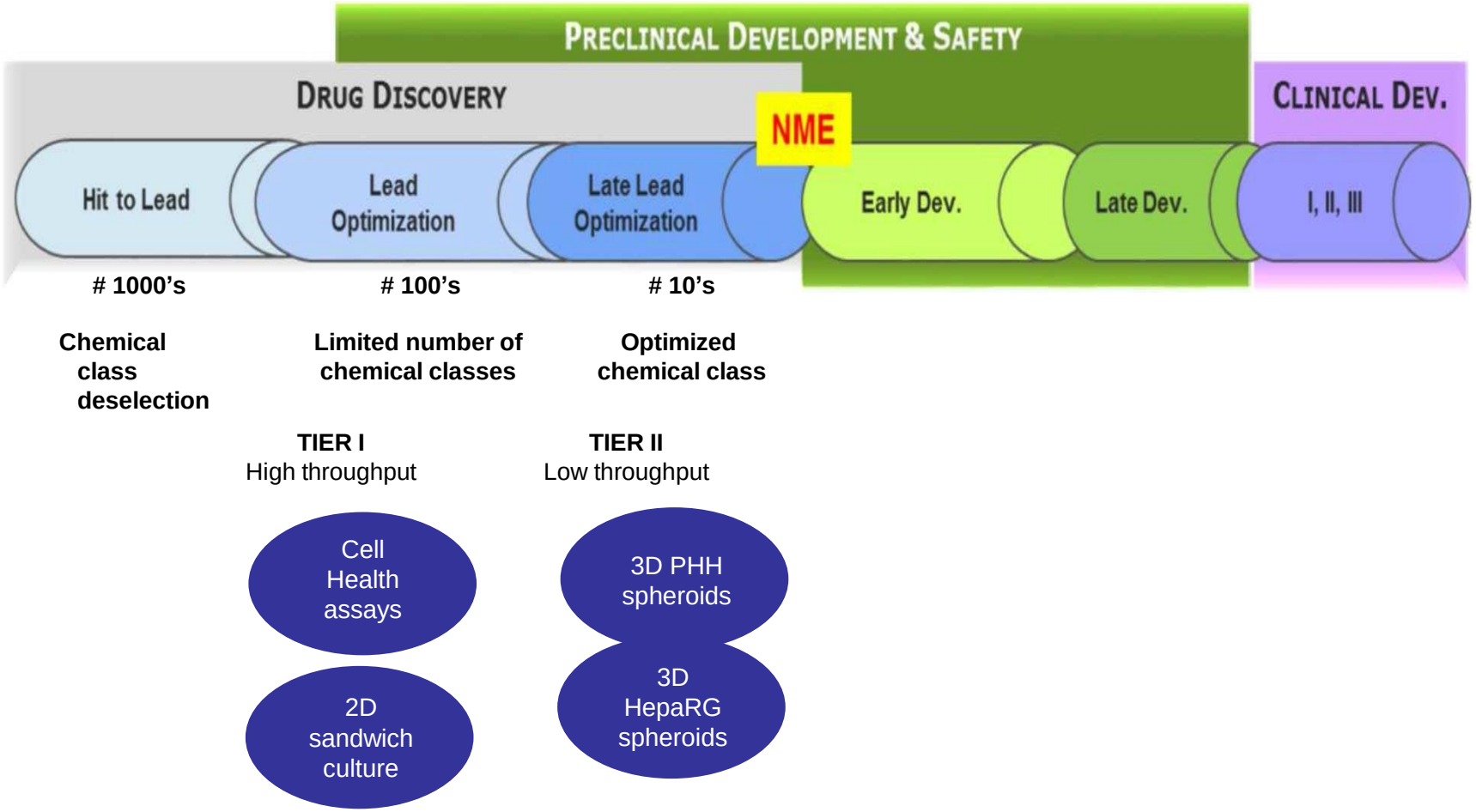
Cross-centre assessment of 3D spheroids



Compounds negative in a HCA analyses (n=35) were evaluated using the 3D spheroid system and overall 65% of compounds missed in the HCA screening were detected in the 3D spheroids as being hepatotoxic

3D spheroids within Industry

The application of 3D spheroids would be more focused towards lower compound throughput and/or mechanistic studies



Regeneration and homeostasis of the liver mass: Human In vitro

Article

Cell

Long-Term Culture of Genome-Stable Bipotent Stem Cells from Adult Human Liver

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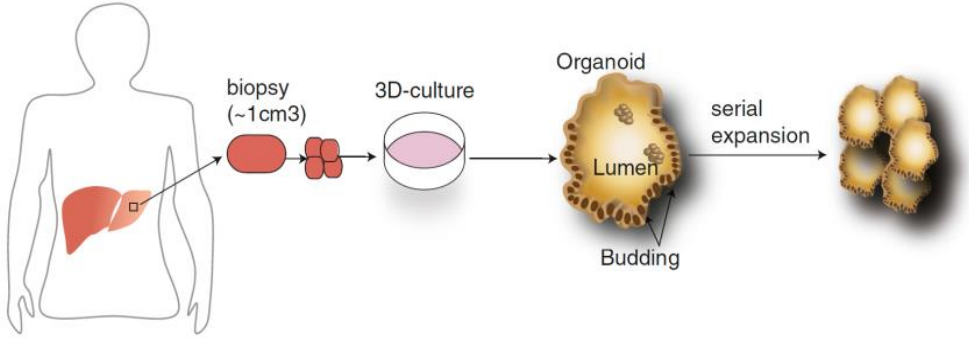

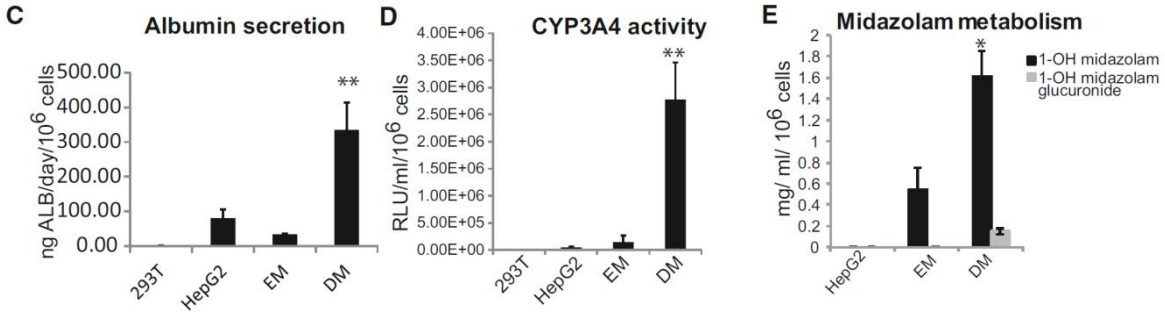

Generation of long-lived human liver-like organoids from biliary ductal epithelial cells

- Evidence for a role for these cells in ***human*** liver regeneration at least in vitro
- Alternative route for generation of liver models (ie use human liver tissue bipotential stem cells)

MIP-DILI Roadmap - Stratification of *In Vitro* systems

Tier 1

Single cell 2D systems

Physiology

Expression phenotype

Transcriptome
Proteome

Function

Cell Signalling
Albumin secretion
Biomarker secretion

Pharmacology

Drug Accumulation

Drug Bioactivation/ Covalent Binding

Drug Detoxication

Toxicology

Cytotoxicity

Transporter inhibition

Cholestasis

Lysosomal impairment

Mitotoxicity

GSH depletion

ER stress

Oxidative stress

Tier 2

Multi-dimensional 3D systems

Multi-cell culture

Spheroids

Long term culture

Tier 3

Complex systems

Patient Factors

Patient-dependent factors

Viral infection

HLA restriction

T cells

Idiosyncratic

Conclusions

- We can develop a battery test system based on current science which is fit for purpose - **refinement and benchmarking**.
- The implementation of novel model systems with respect to industrial application is being conducted - **EFPIA workshops.**
- Multidimensional *in vitro* systems, which have a relevant physiological and pharmacological phenotype and are therefore fit for toxicological application(s) are being progressed - **definitive qualitative and quantitative mass spectrometry**
- Current status of novel model systems for idiosyncratic DILI with respect to human relevance - **aspirational**

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