A shift in paradigm towards human biology-based systems for cholestatic-liver disease

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Systems Toxicology – *In Vitro* Metabolomics

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Saarland University, Germany

BioMed21 – A Human Pathways Approach to Disease Research

Brussels, 8-9th December 2015

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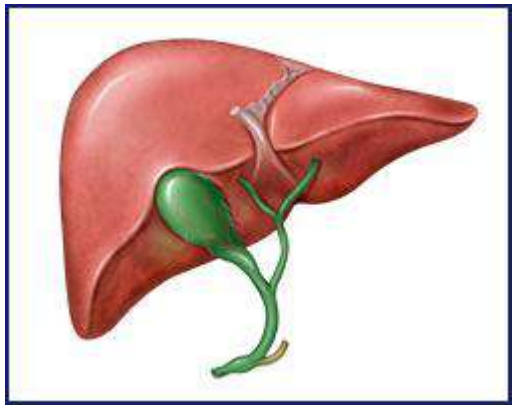
Cholestatic-Liver Diseases - Introduction



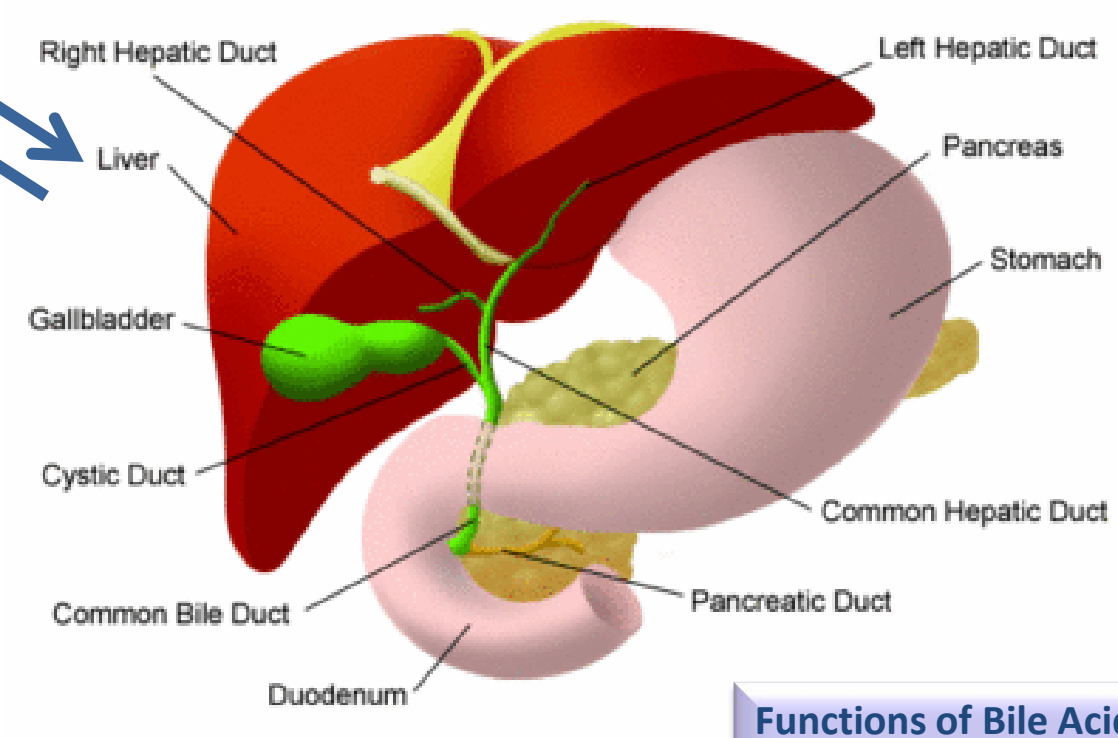
Hepatobiliary System

Disease of Civilization

- Obesity
- Diabetes
- Non-alcoholic fatty liver disease
- Chronic liver disease
- Heart disease
- Metabolic syndrome
- Other nutritional disorders

Cholestatic Liver Diseases

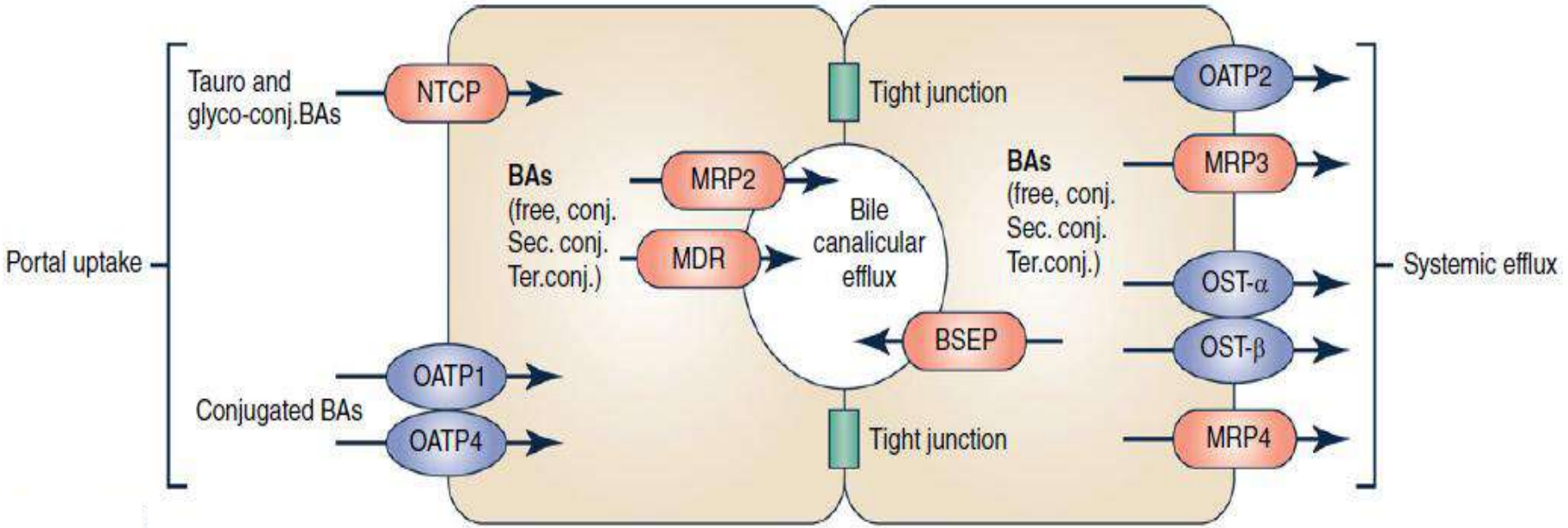
- Biliary atresia
- Progressive familial intrahepatic cholestasis
- Alagille syndrome
- Primary biliary cirrhosis
- Primary sclerosing cholangitis
- Cholangiocarcinoma
- Drug induced cholestasis
- …



Functions of Bile Acids

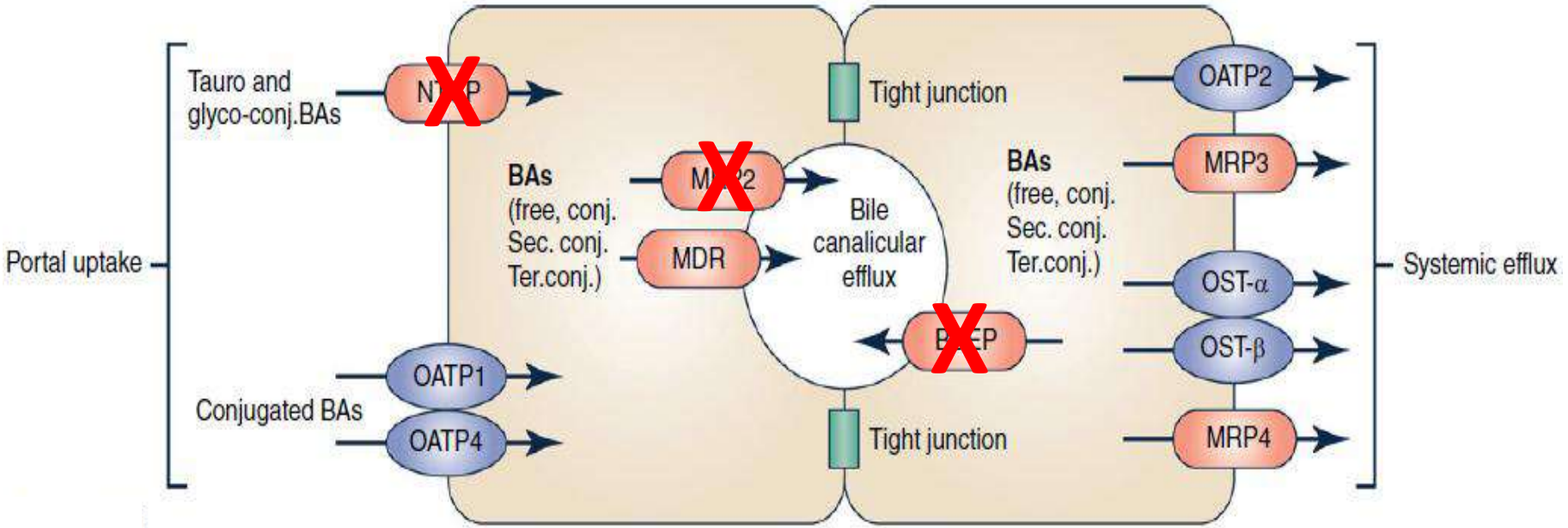
- Glucose metabolism
- Lipid metabolism
- Cholesterol metabolism
- Energy expenditure
- Control of gut microbiota
- Xenobiotic metabolism
- …

Bile acid transport system



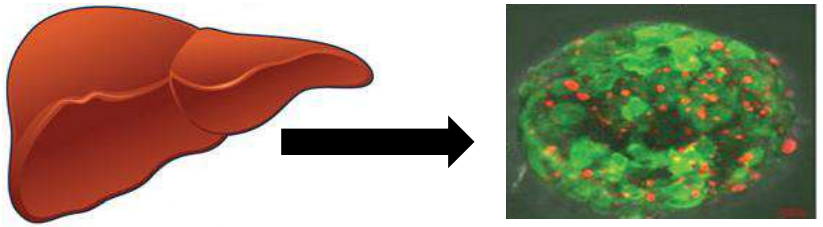
Noor, J Physiol., 2015

Bile acid transport system



Noor, J Physiol., 2015

Human specific cell models



Why human models ?

***In-vivo* animal models differ from humans in:**

- Bile acid composition
- Transporters activities
- Milder phenotypes
- Effect on nuclear receptors
- Immune and inflammatory response
- CYP 450 system for metabolism and clearance
- Gut microbiota
- Mechanisms of parenchyma injury (necrosis vs. apoptosis)

In-vivo

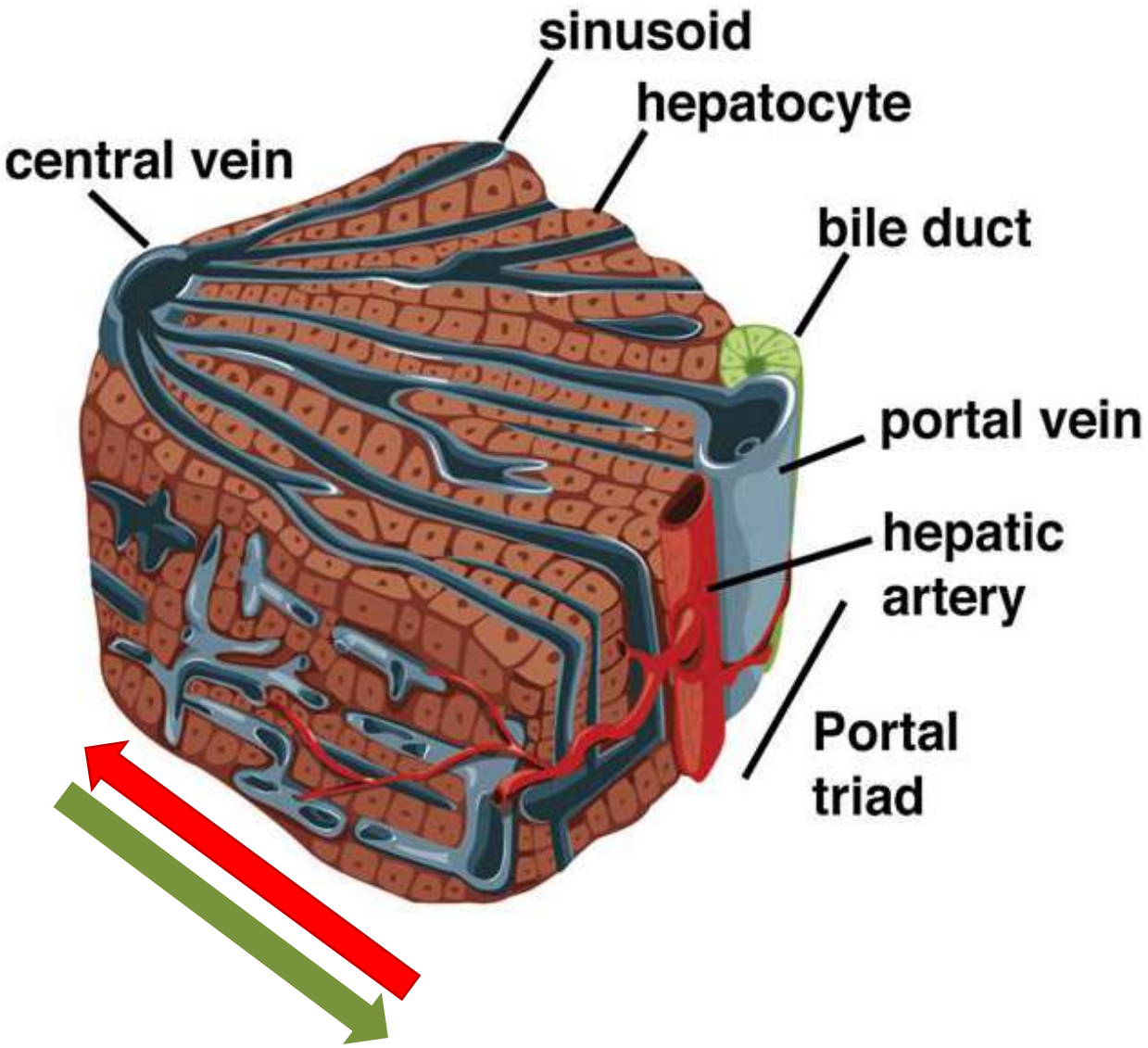
In-vitro

***Common in-vitro* models**

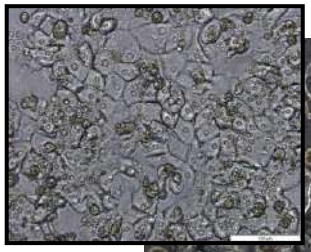
- usually 2D
- Monocultures
- Very often rodent primary cells
- Cell lines

Human in vivo-like models are needed !

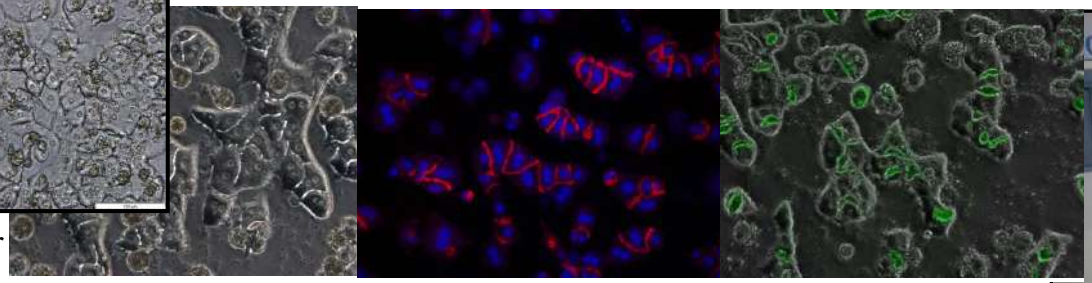
Liver


sinusoid
hepatocyte
central vein
bile duct
portal vein
hepatic artery
Portal triad

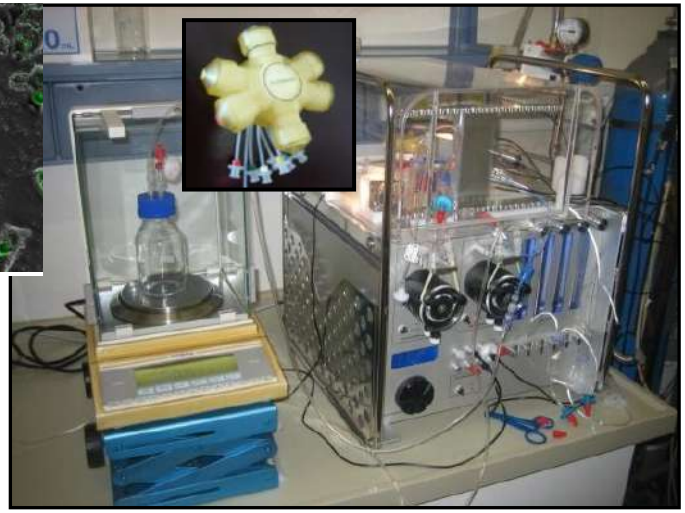
In vitro 3D cultivation systems for liver



Monolayer

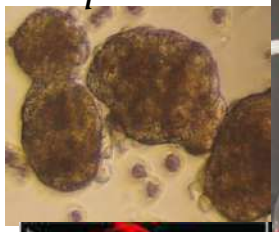


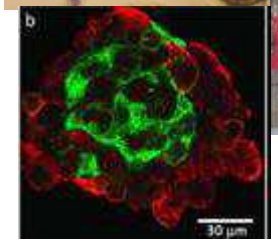
Collagen sandwich cultures of primary hepatocytes (Saskia Müller)



Bioreactor 3D cultivation of primary hepatocytes (Charité, Berlin; Gerlach et al., Mueller et al.)

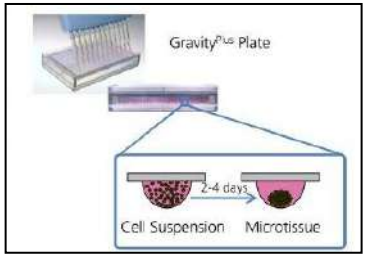
Single cells
Aggregates
Alginate
encapsulation etc



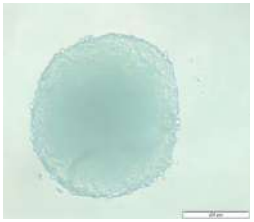


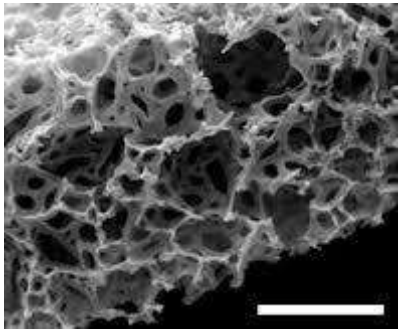
Hepatology, 2011

Spinner flask cultures (Carrondo and Alves, IBET, Portugal)



3D organotypic cultures of primary hepatocytes (Insphero technology, Zurich)

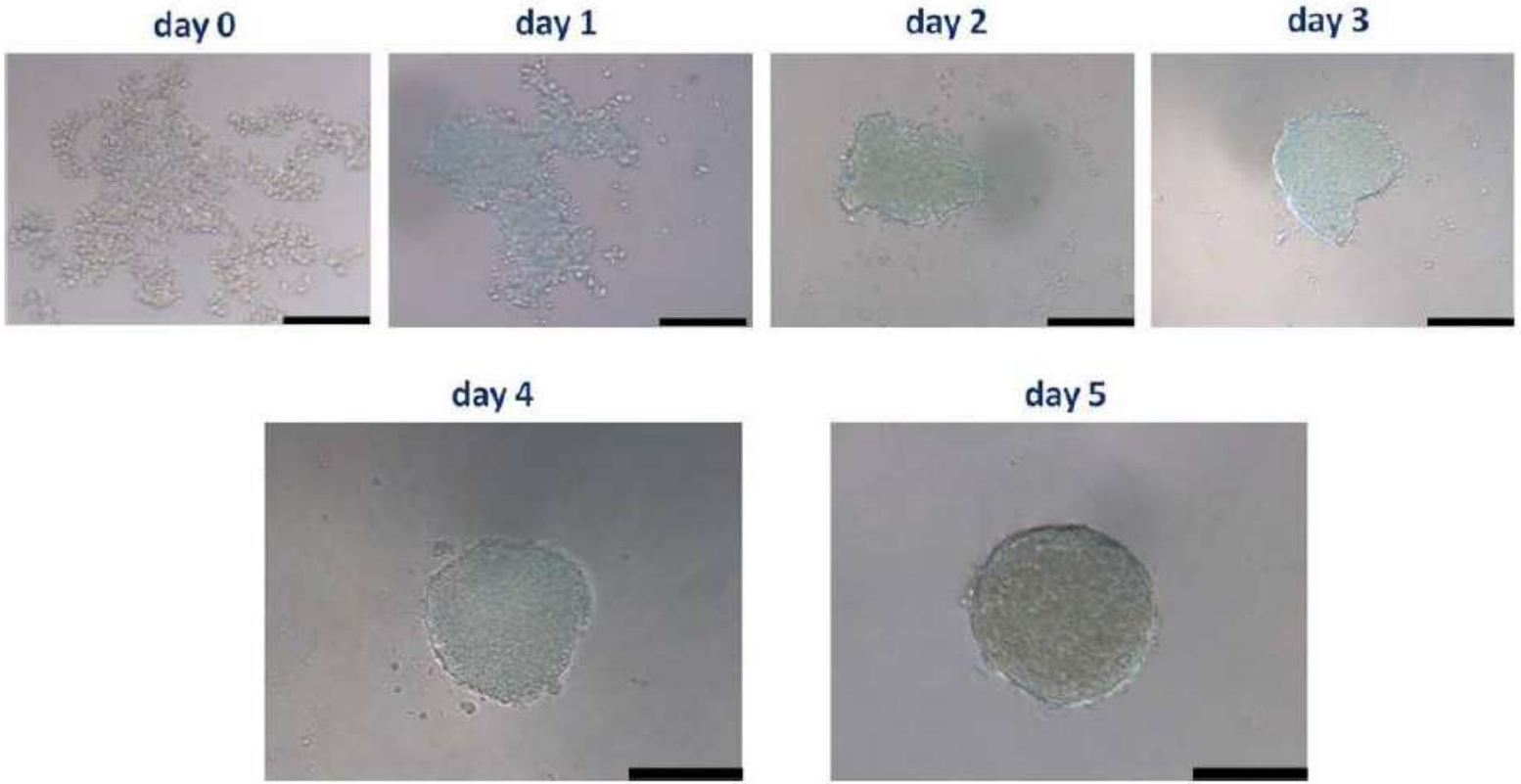




Alvetex scaffold

Mueller et al., Bioeng Biomed Sci 2011
Gunness et al, Tox Sci., 2013
Mueller et al., Tox in vitro, 2014

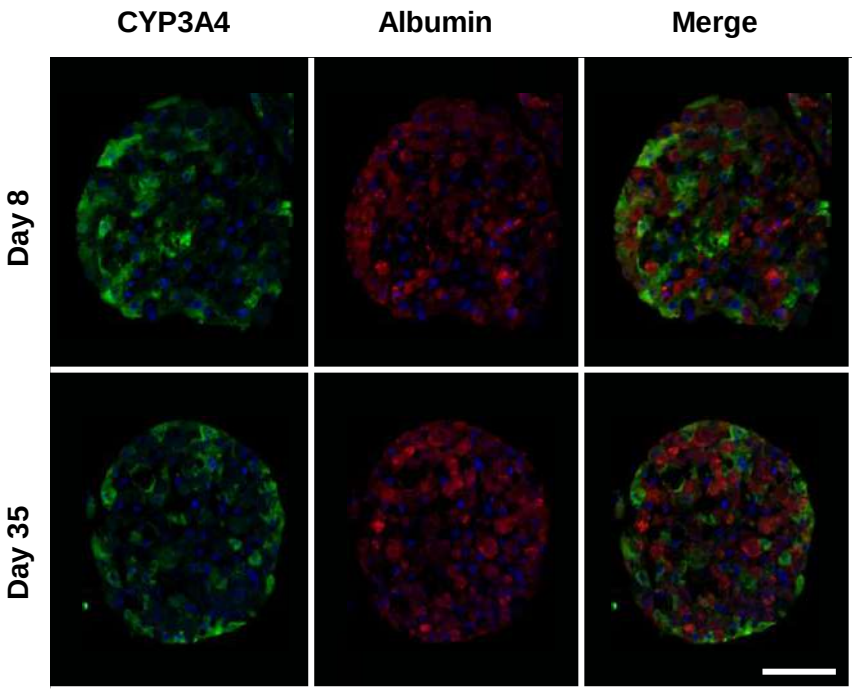
Human 3D *in-vitro* models



Mueller et al., 2014

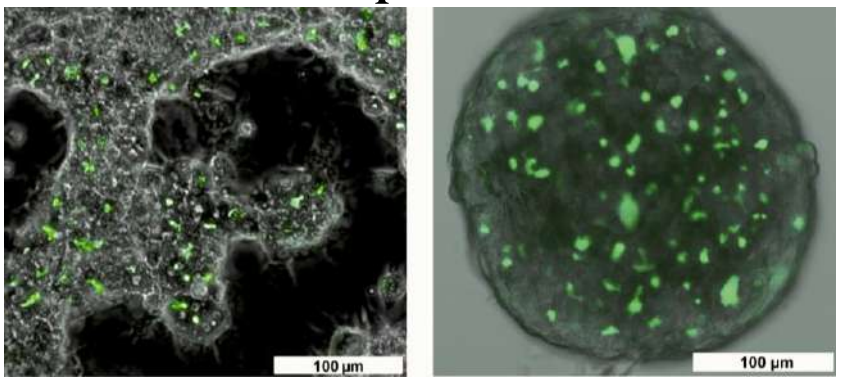
Human 3D *in-vitro* models

Primary human hepatocytes

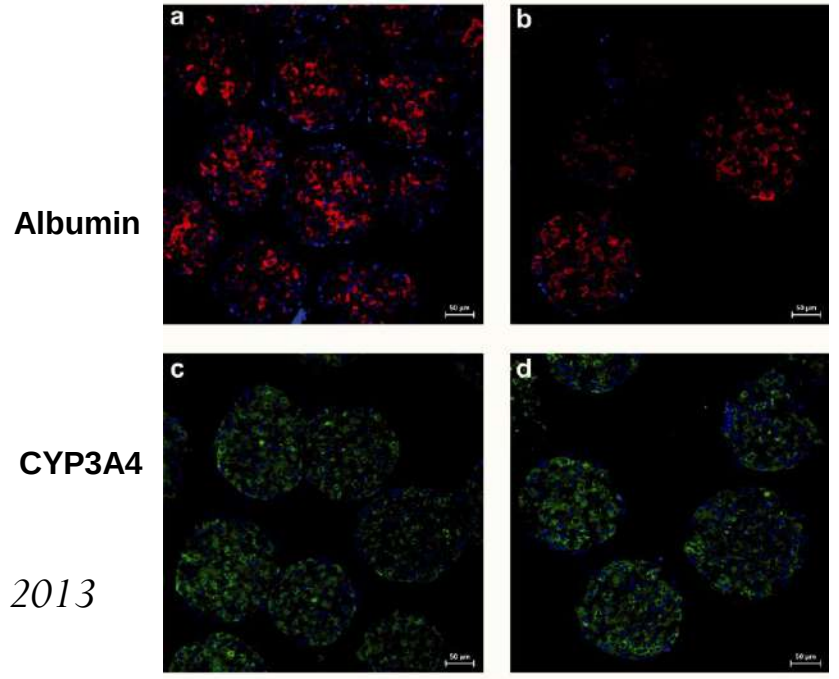


Fredriksson et al., 2015

Human HepaRG cell line



MRP2 activity



Gunness et al., 2013

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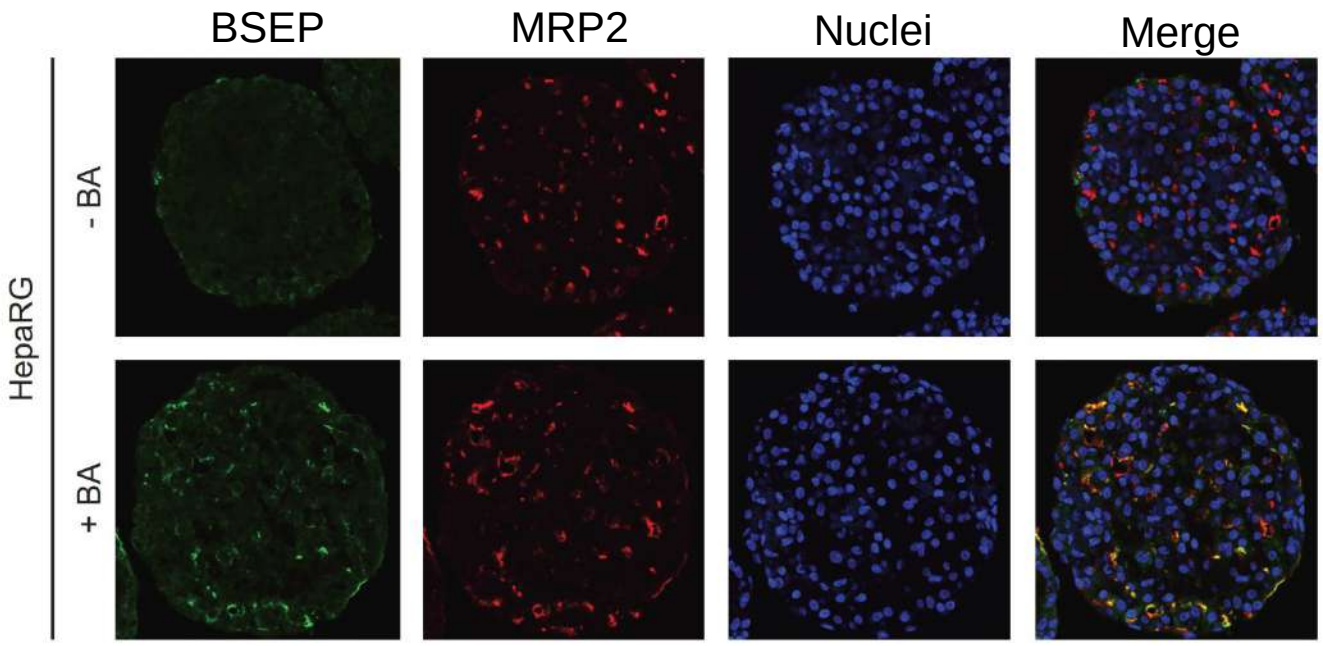
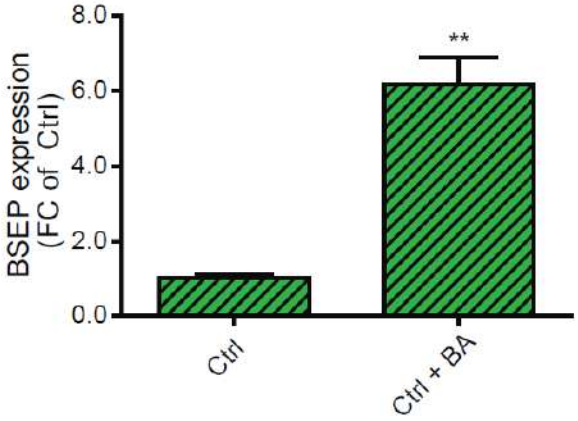
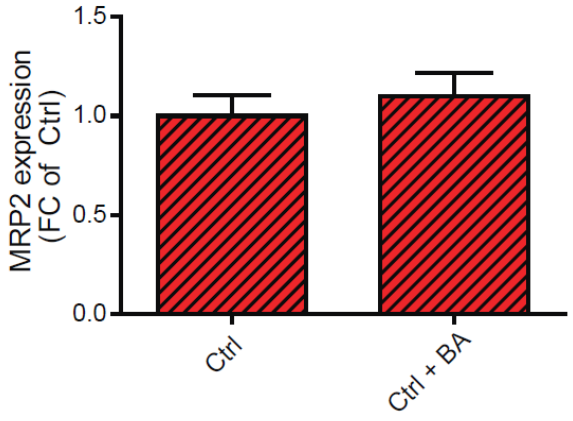
HepaRG spheroids express important bile acid transporters and they can be induced







Lisa Fredriksson Puigvert, Delilah Hendriks and Magnus Ingelman-Sundberg, submitted

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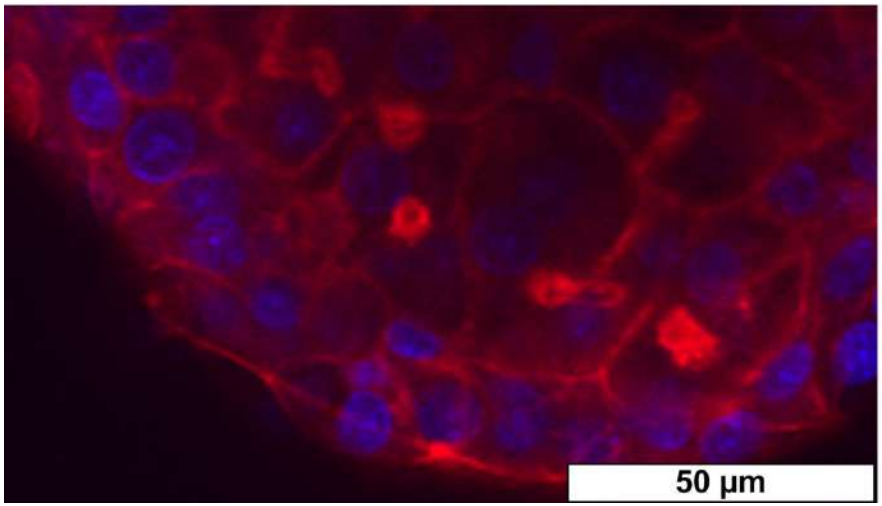
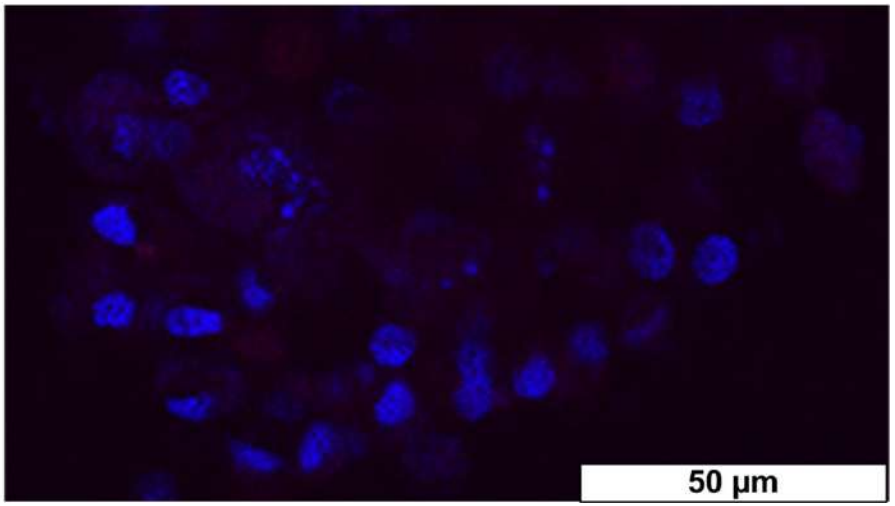
Disruption of bile canaliculi network

Effects of chlorpromazine on 3D structure

0 µM CPZ



100 µM CPZ



Mueller et al., 2014

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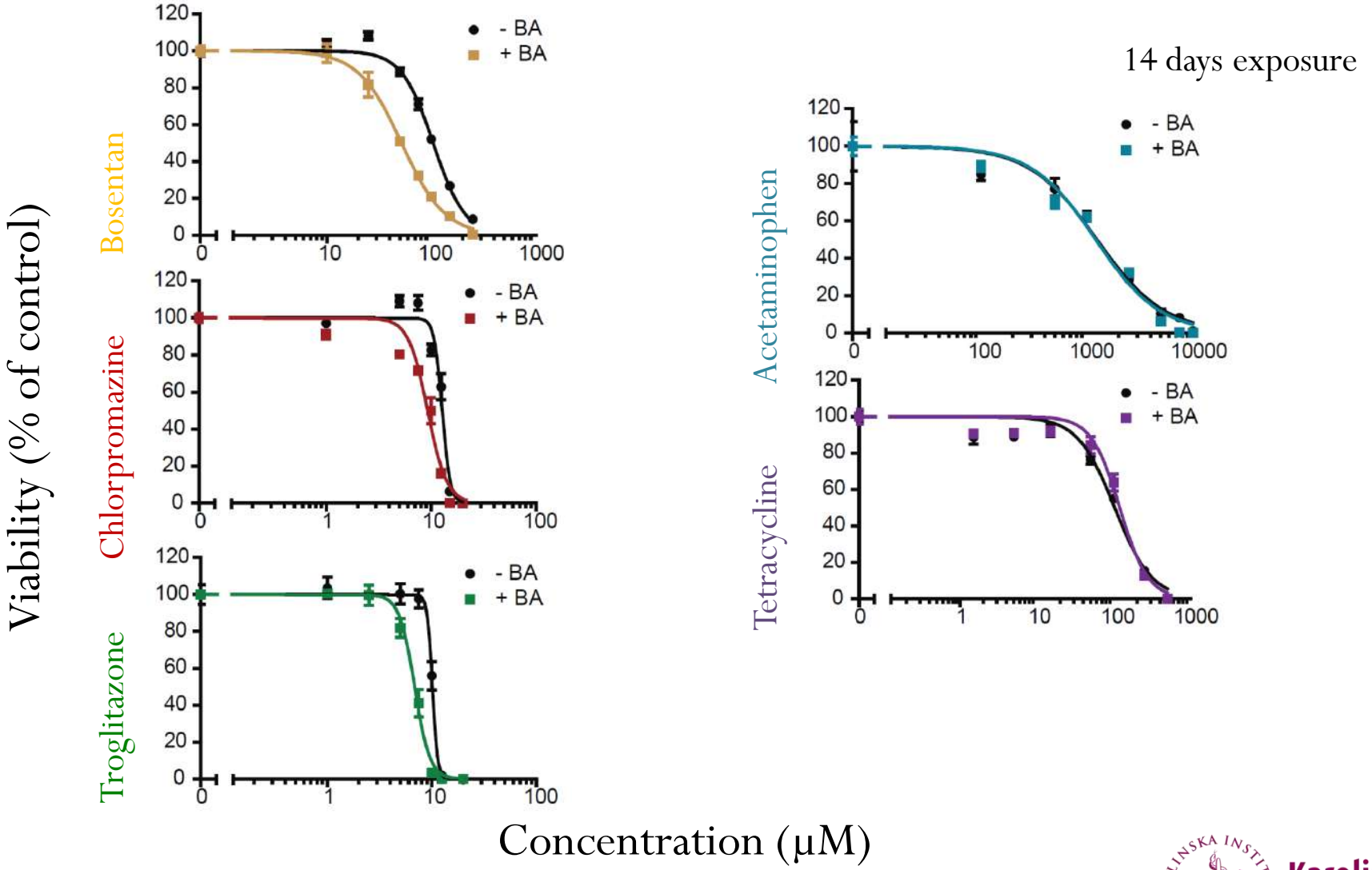
Drug induced cholestasis



Lisa Fredriksson Puigvert, Delilah Hendriks and Magnus Ingelman-Sundberg, submitted

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Drug induced cholestasis

	Compound	8 days			14 days		
		IC_{50} (-BA) (µM)	IC_{50} (+BA) (µM)	Cholestatic Index (IC50 (+BA) / IC50 (-BA))	IC_{50} (-BA) (µM)	IC_{50} (+BA) (µM)	Cholestatic Index (IC50 (+BA) / IC50 (-BA))
Positive	Chlorpromazine	17	15	0.88	14	8.8	0.63
		16	13	0.81	12	7.8	0.65
		15	14	0.93	13	9	0.69
	Troglitazone	9.5	5.3	0.56	7.4	1.9	0.26
		5	4.5	0.90	4.9	3.5	0.71
		15.1	10.3	0.68	10.14	6.8	0.67
	Bosentan	187	117	0.63	115	70	0.61
		150	81	0.54	104	52	0.50
		161	103	0.64	116	56	0.48
Negative	Tetracycline	242	220	0.91	121	115	0.95
		211	238	1.13	110	130	1.18
		199	222	1.12	103	119	1.16
	Acetaminophen	1600	1600	1.00	1000	930	0.93
		2200	2300	1.05	1200	1200	1.00
		1600	1600	1.00	960	860	0.90

Lisa Fredriksson Puigvert, Delilah Hendriks and Magnus Ingelman-Sundberg, submitted

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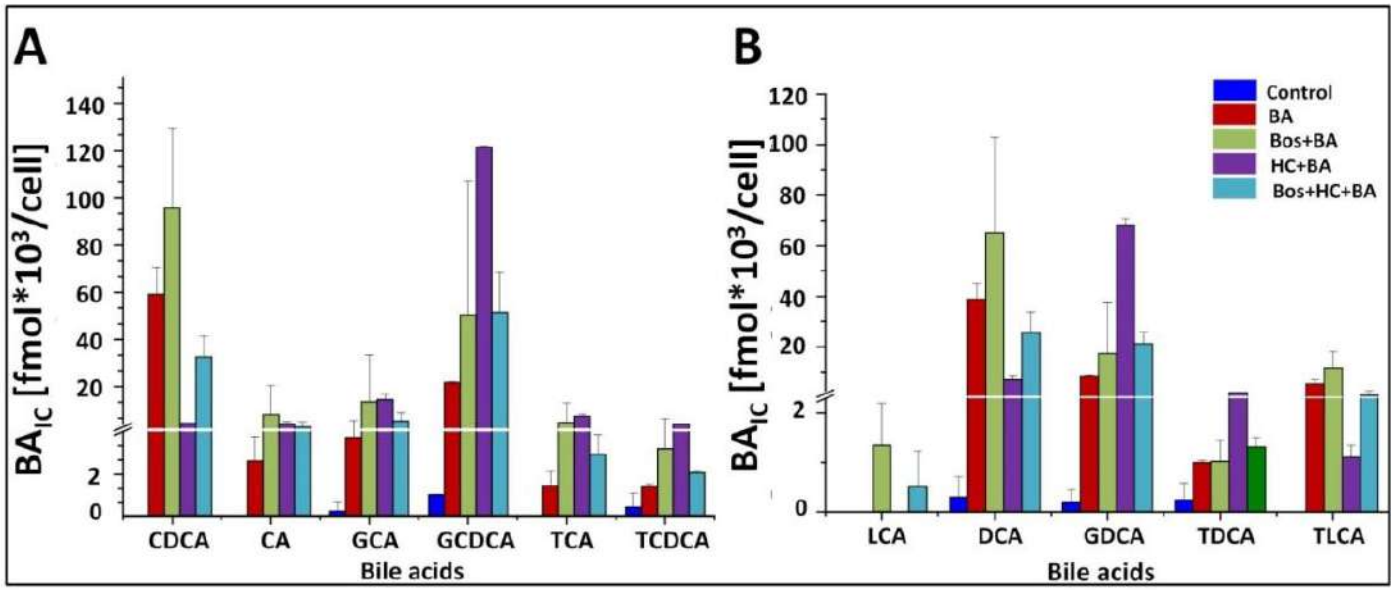
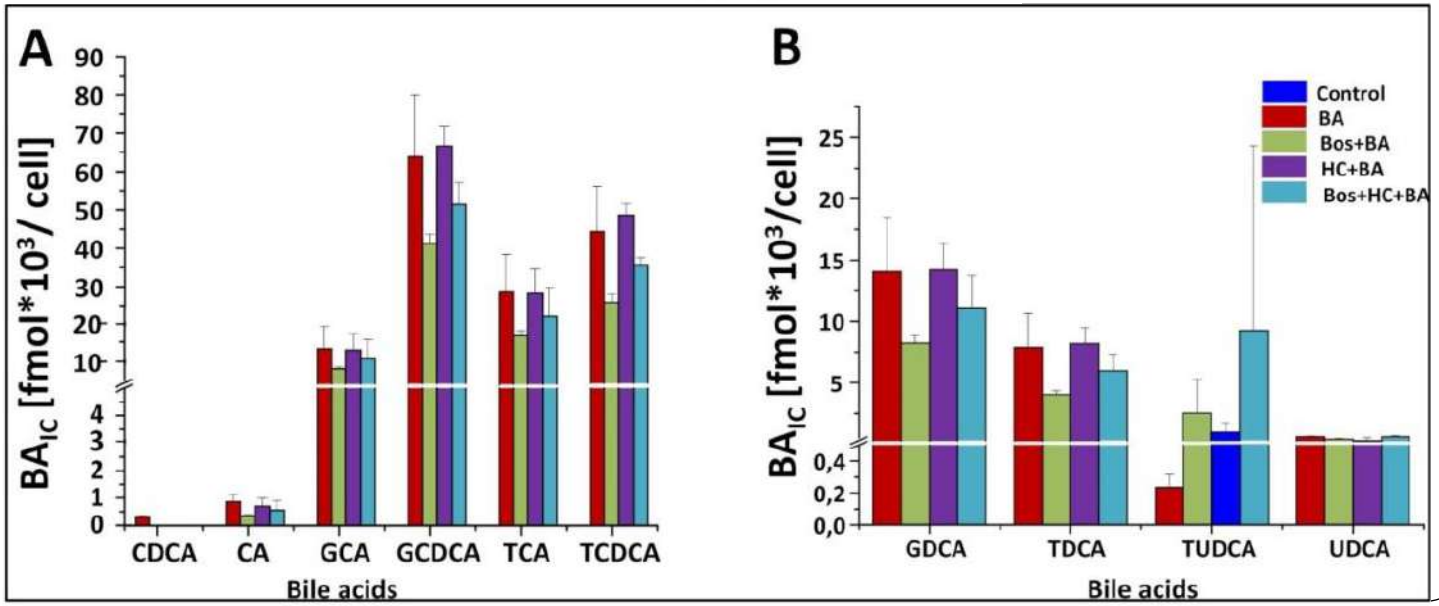
Accumulation of bile acids in HepaRG cells

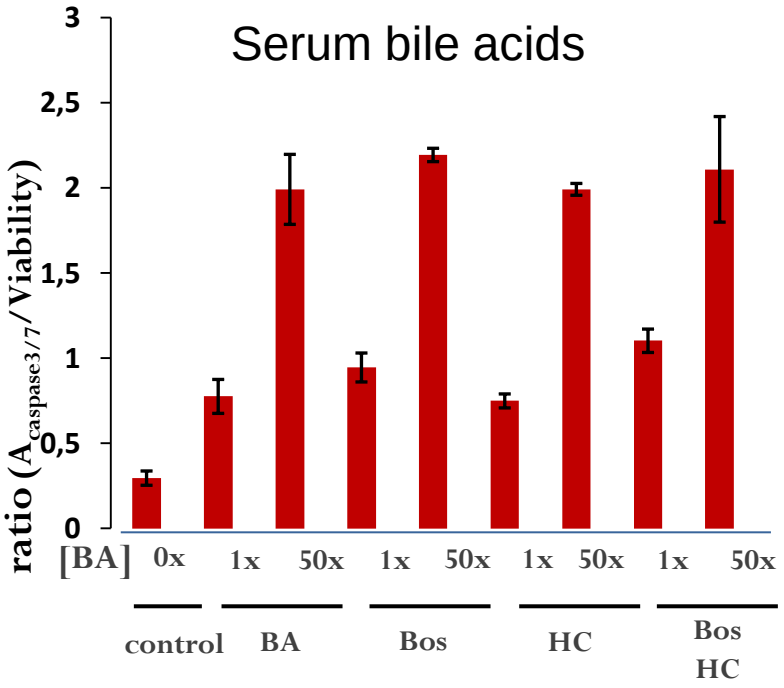


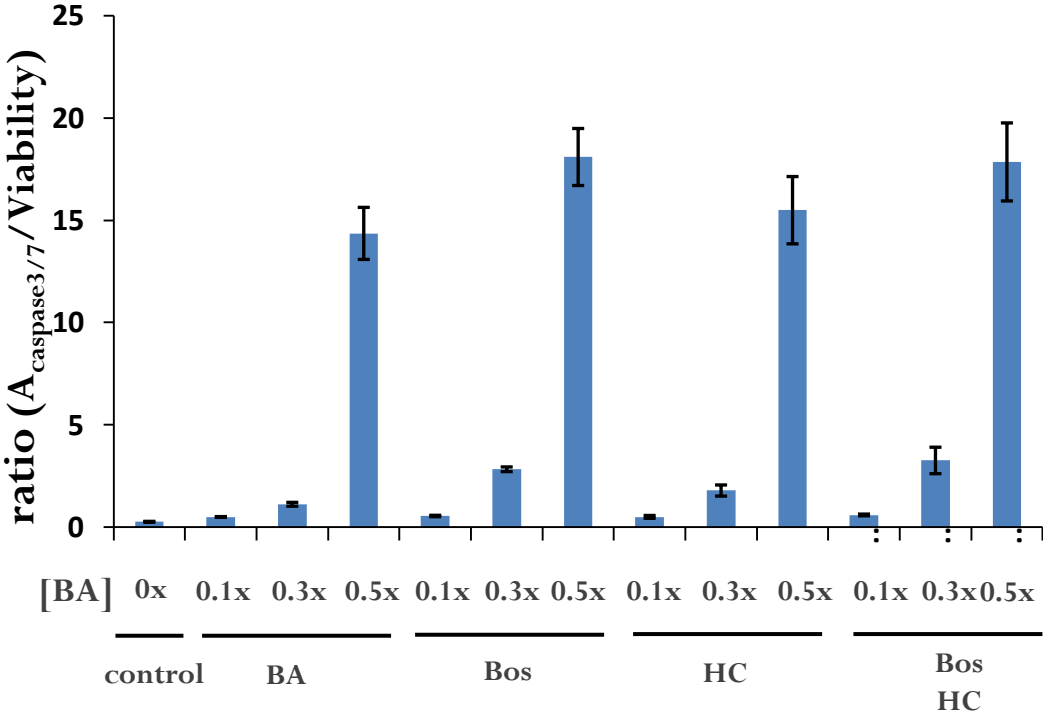
Upon exposure to serum concentrations in cholestatic patients

Upon exposure to biliary concentrations in cholestatic patients



Mechanism of cell death: apoptosis

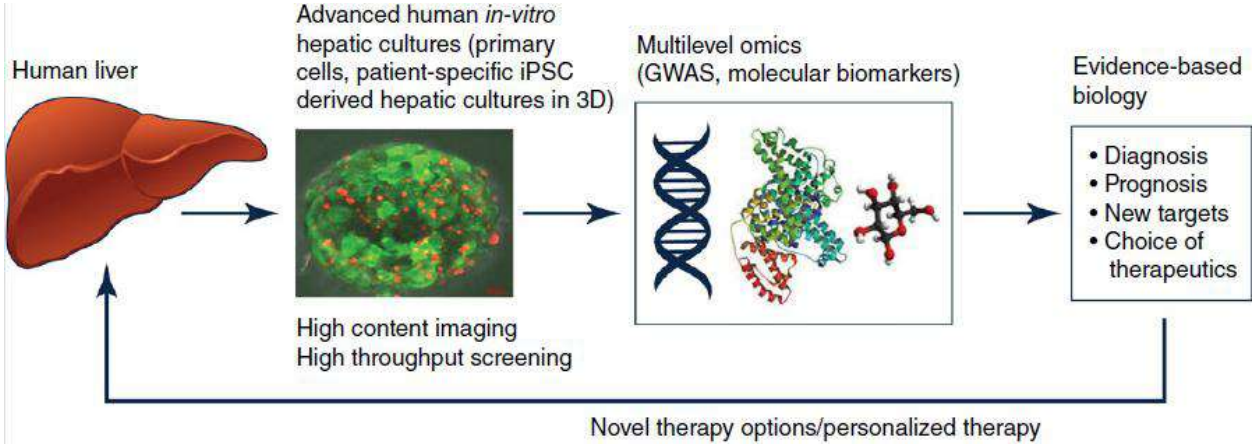




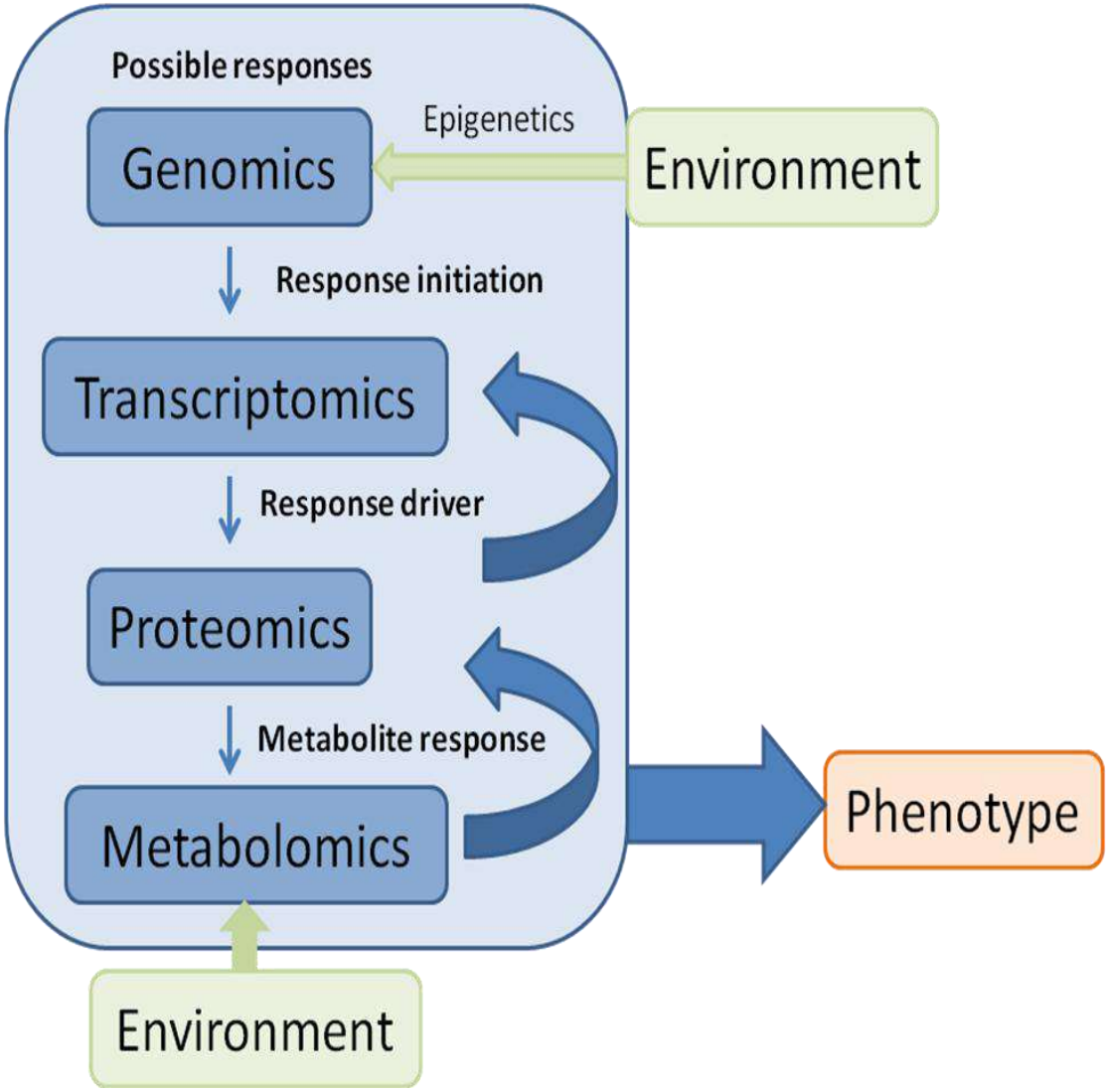
Hepatic parenchyma death in rodents is *via* necrosis !

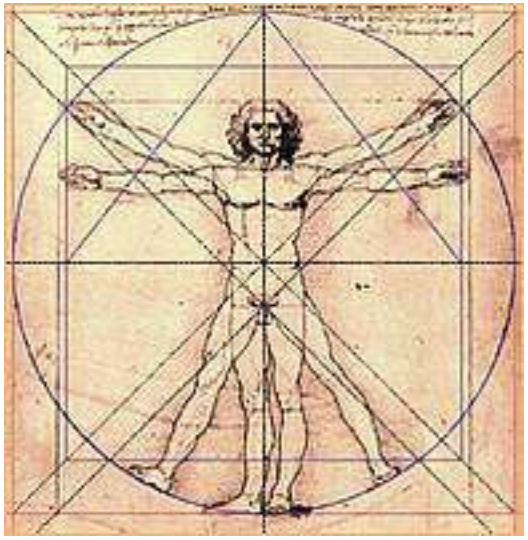
Biliary bile acids

Enabling Technologies &
Human disease pathways-based approaches



Metabolome: the Rosetta Stone



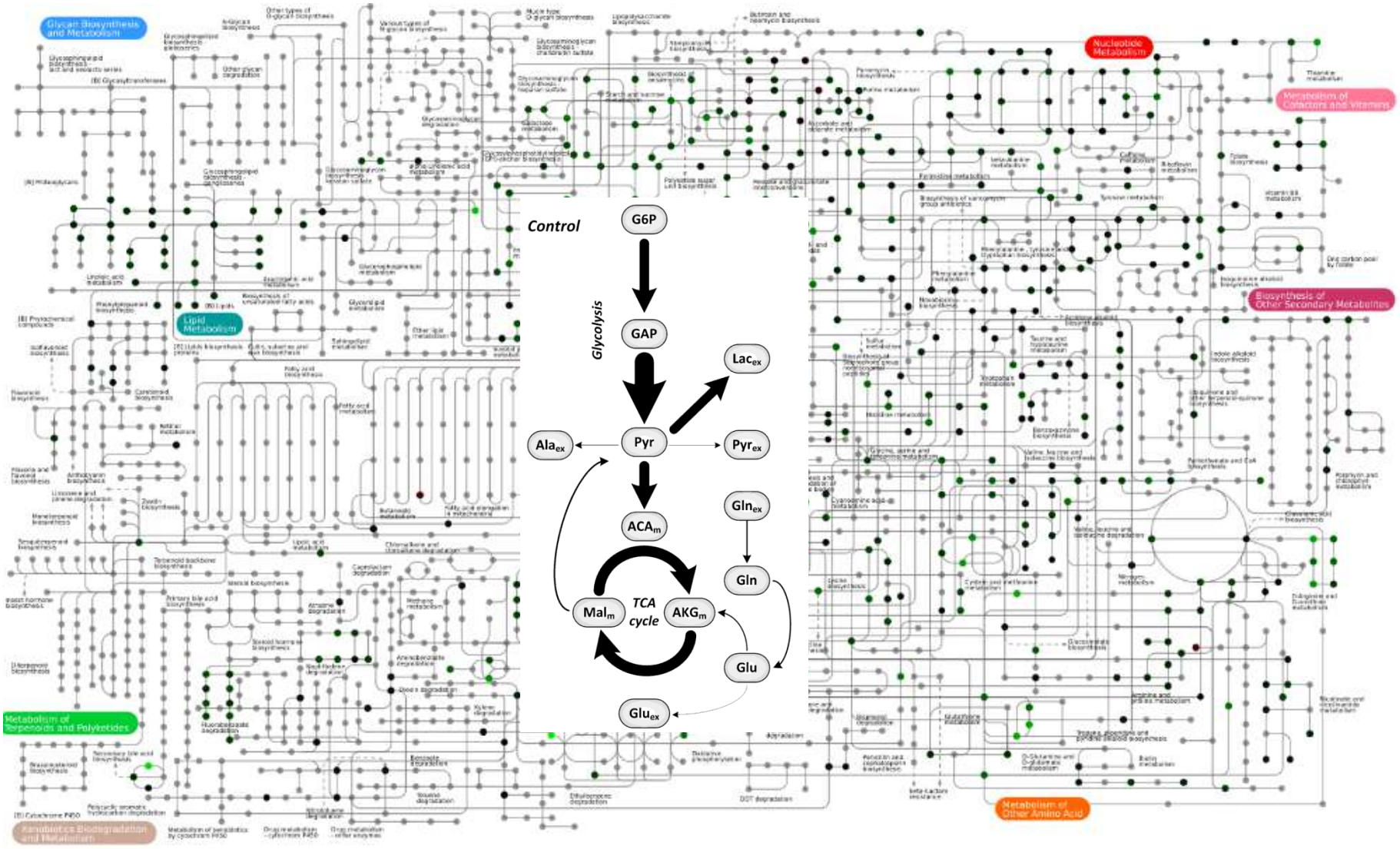


In vitro Metabolomics in pathway research

Application domains

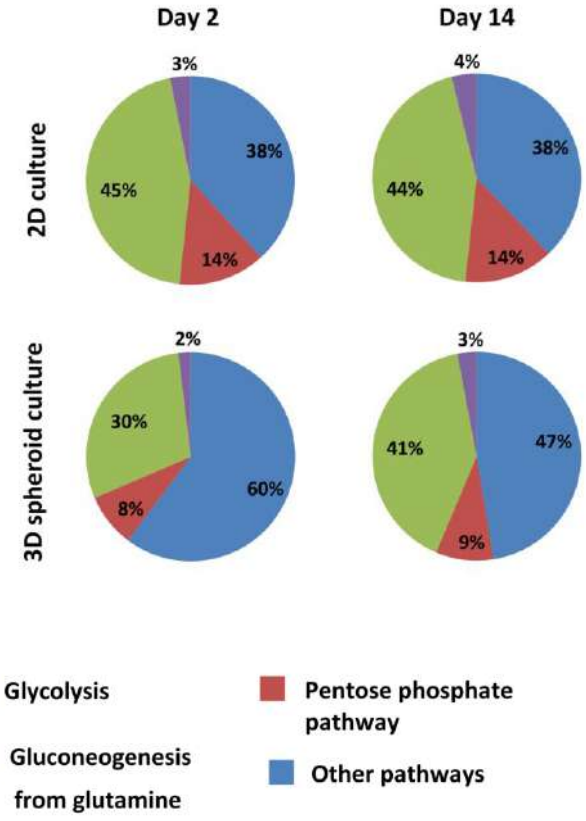
- Screening
- Mode of action (MoA)
- Biomarker identification

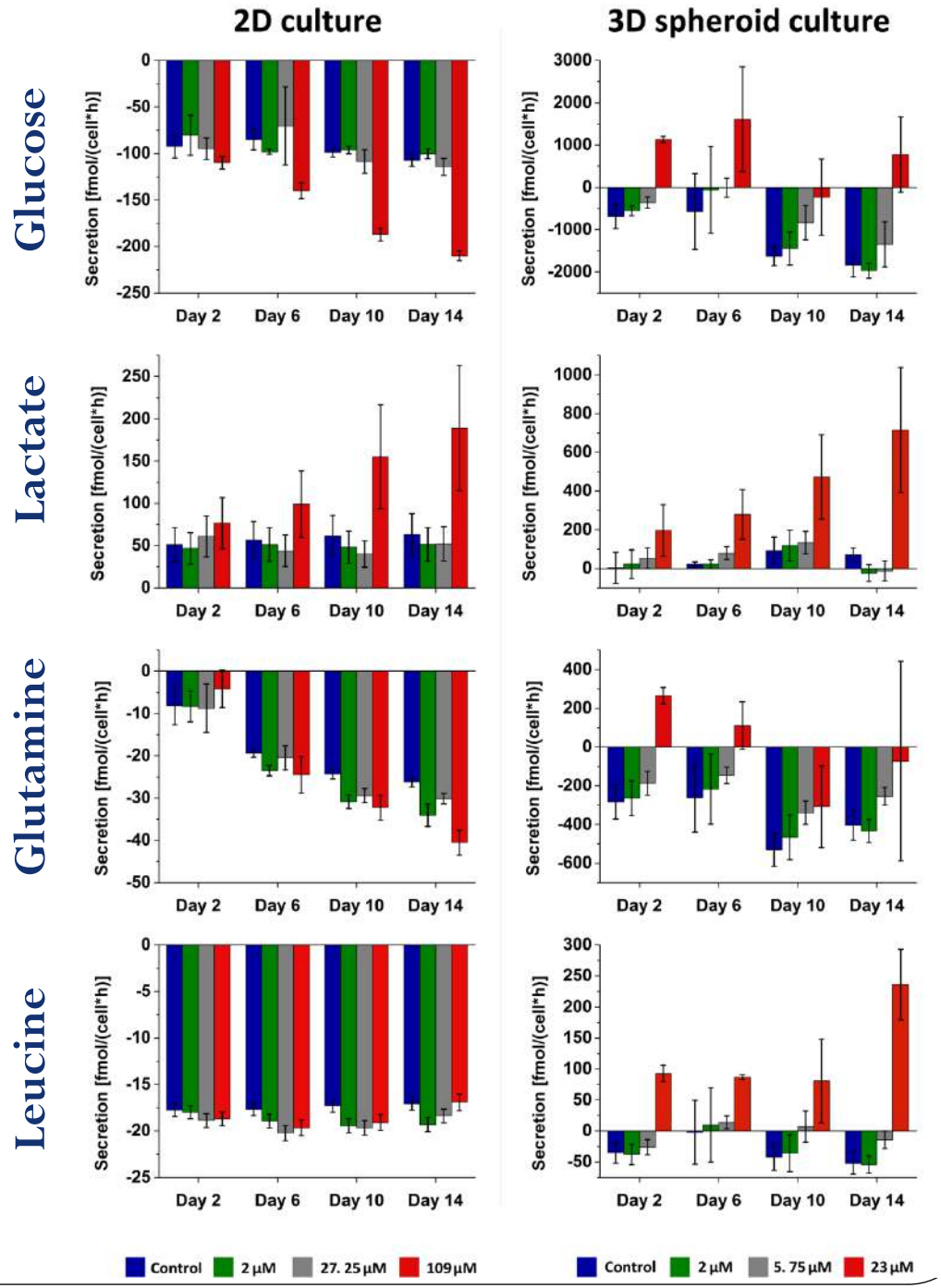
Metabolome analysis–biochemical networks



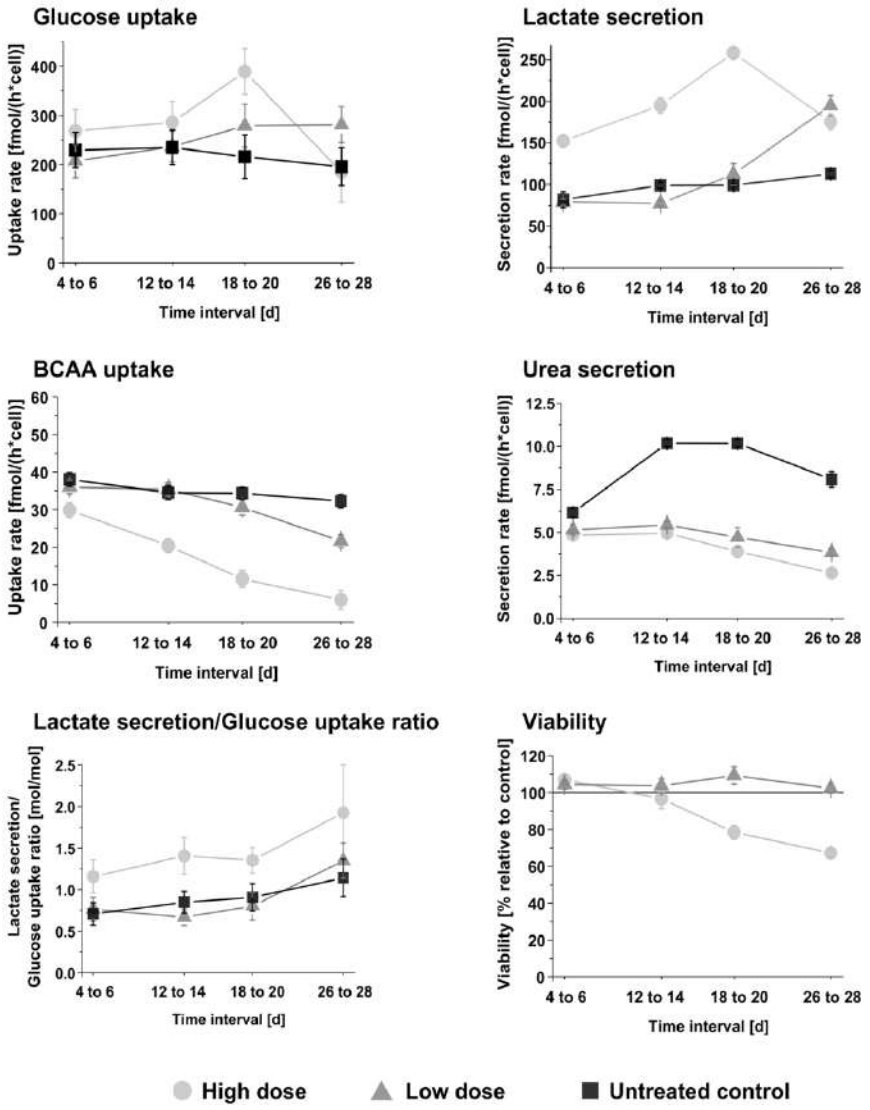
Metabolome

Metabolic alterations in 2D and 3D cultures upon repeated dose exposure to bosentan



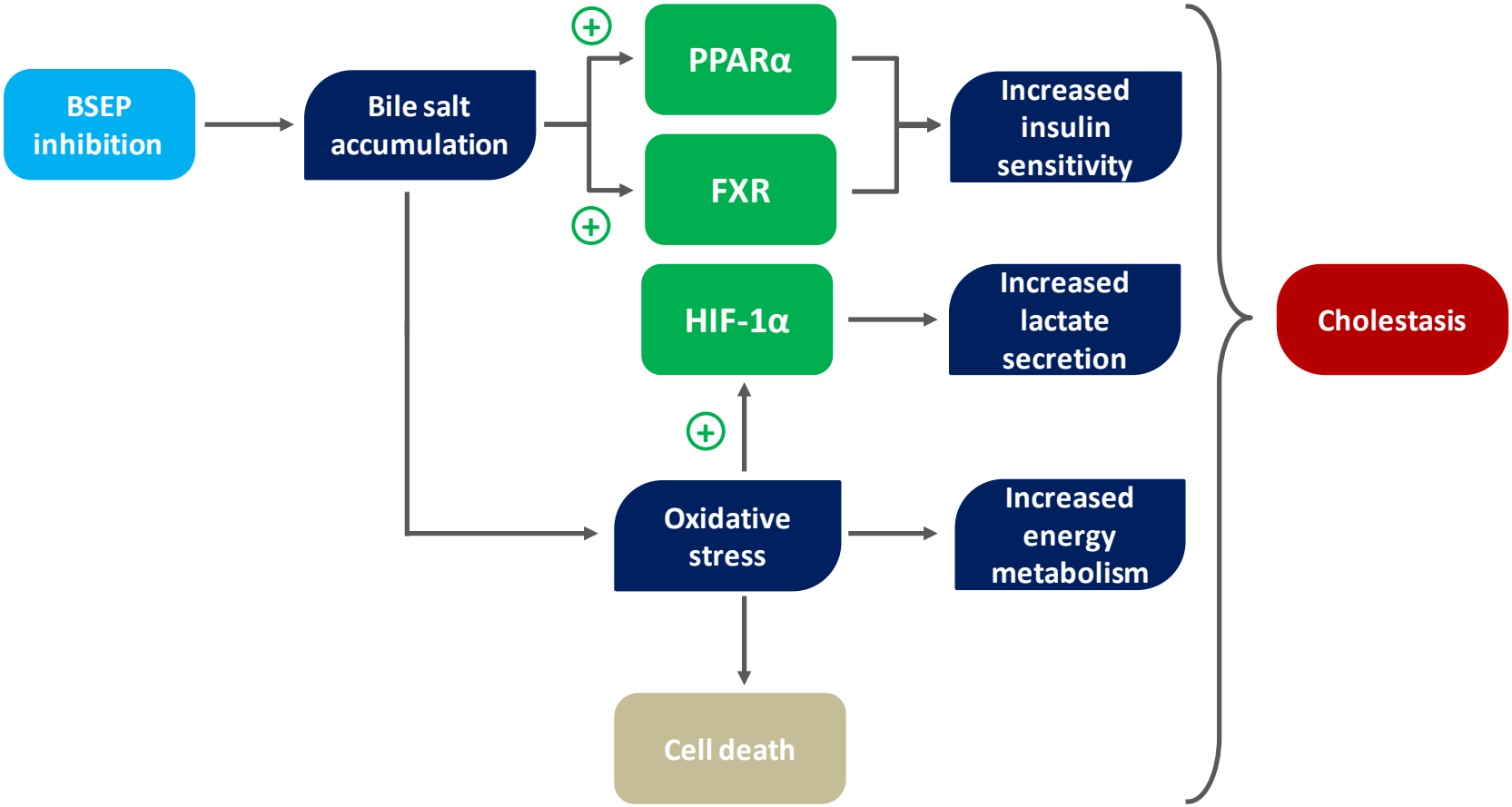


Metabolite profiles over time

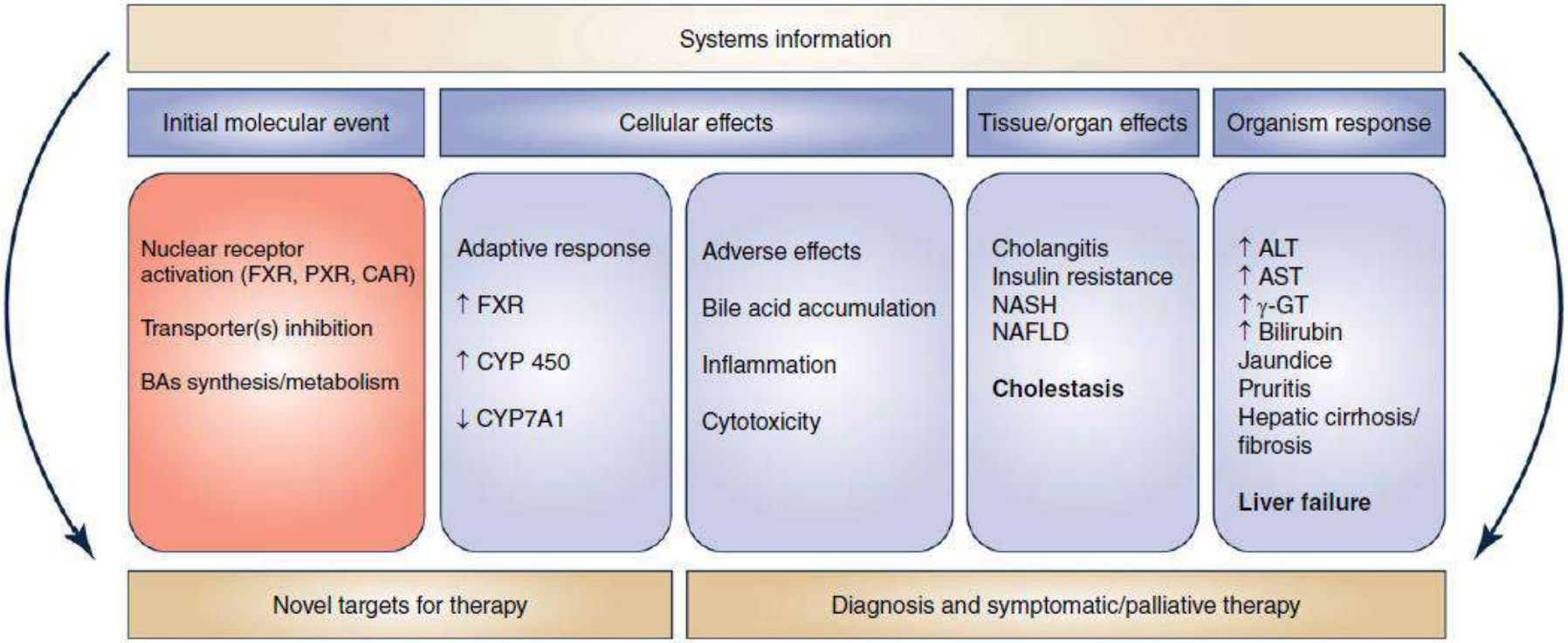


Adverse outcome pathway

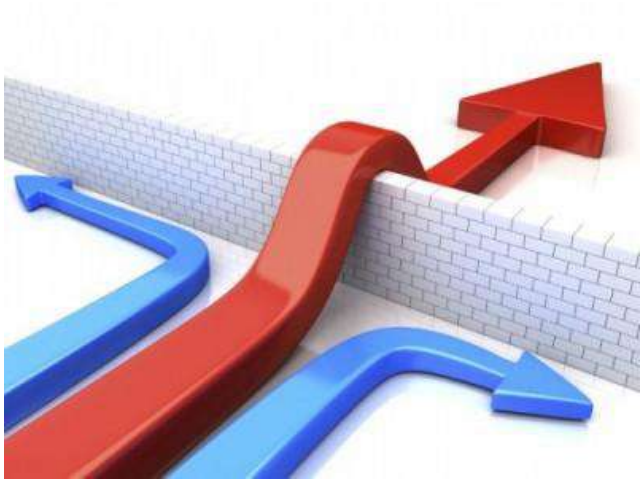
Drug induced cholestasis



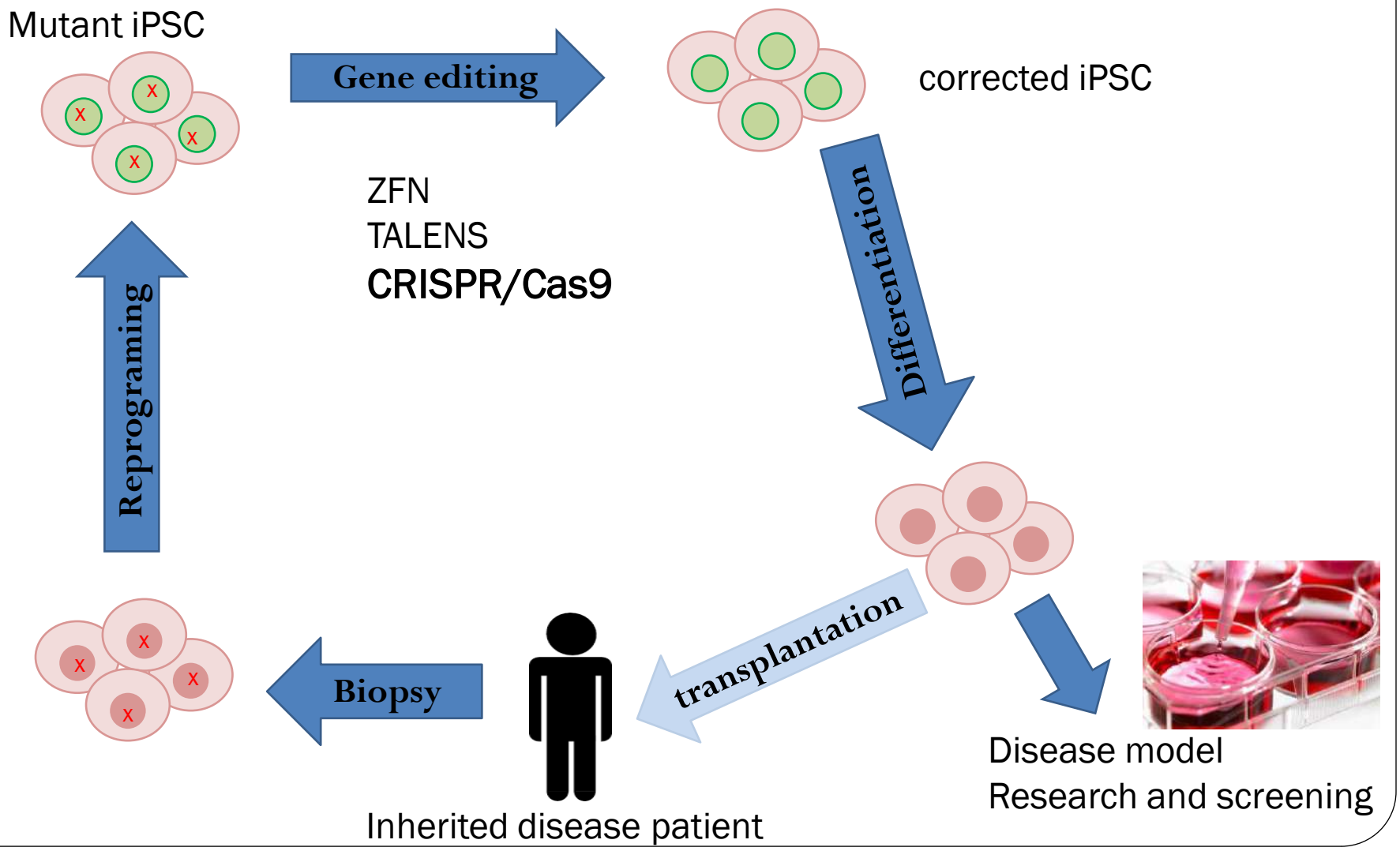
Systems approach within a disease-pathway framework



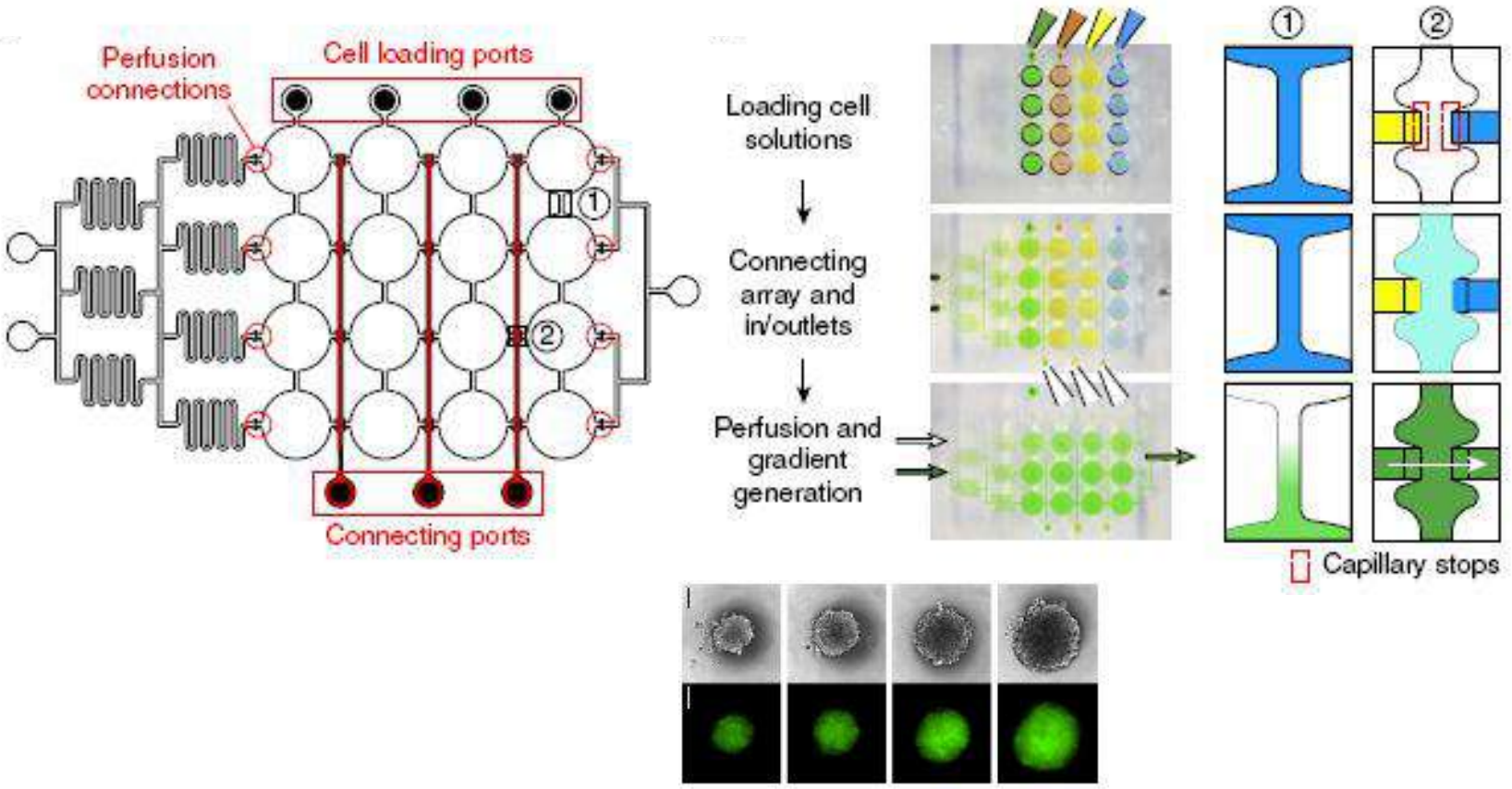
Opportunities and Challenges



Gene Editing technology



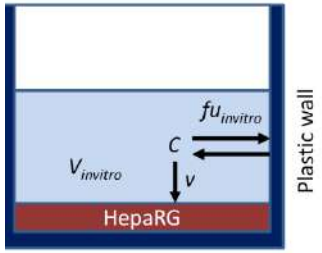
Microfluidic Systems

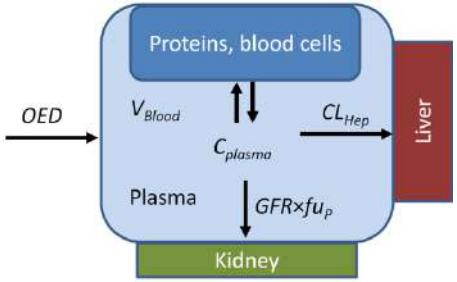


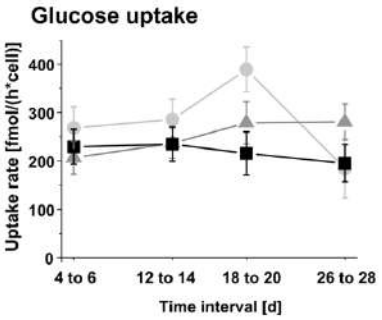
Frey et al., Nat Comm, 2014

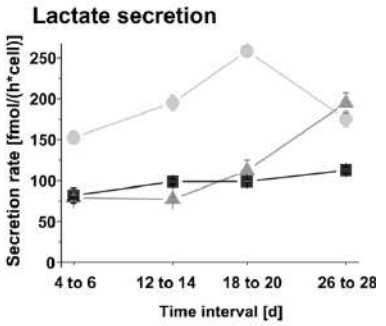
Prediction of dose and pathway related effects in humans

Reverse dosimetry and metabolome analysis

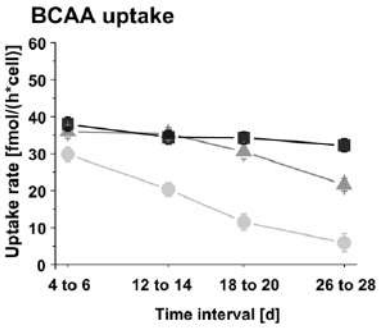


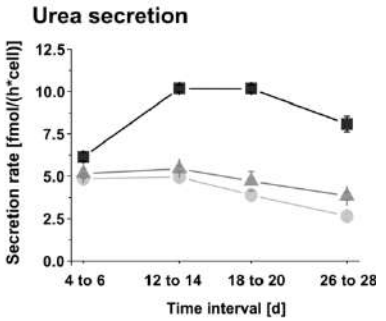


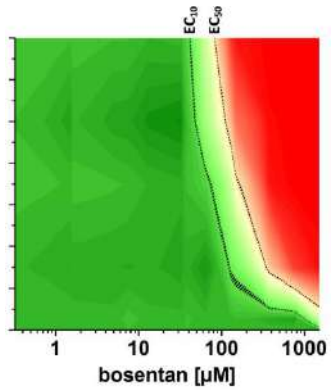


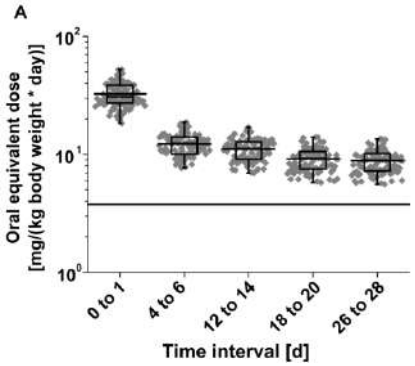


Control
Low exposure
High exposure







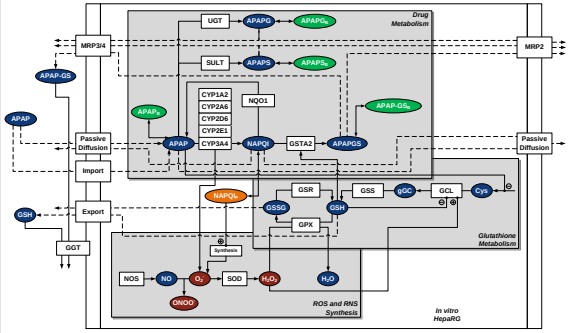


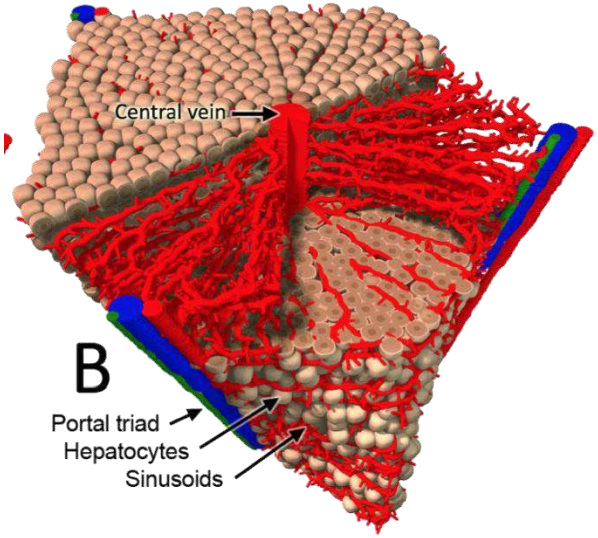
In vitro long term data

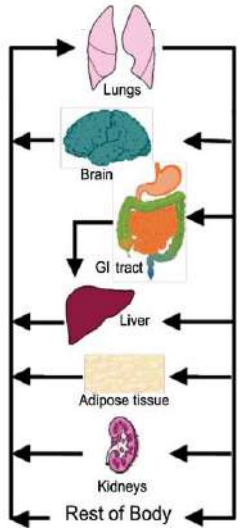
Klein et al., Tox Sci., 2015

Towards human *in vivo computational* models

Intracellular Model (ODEs) + Multicellular Model + Diffusion/Transport of drugs (PDEs) + PBPK model (rest of the body)





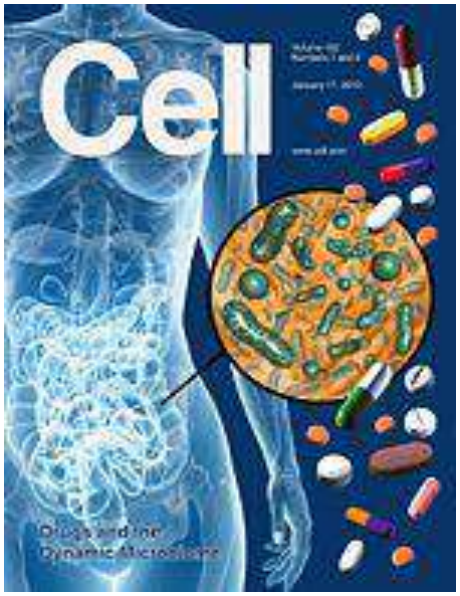


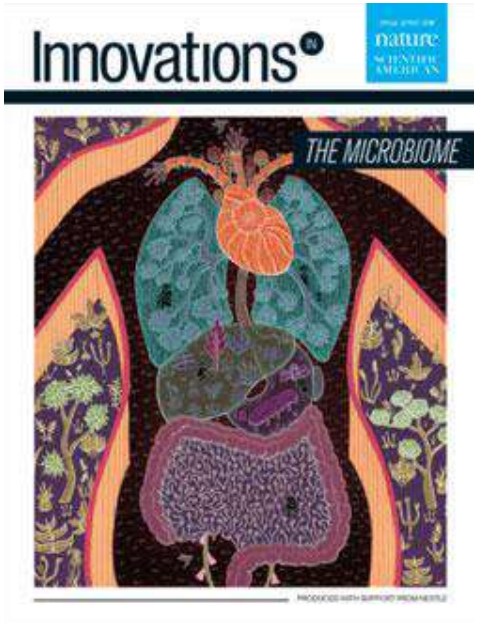
- ✓ One ODE system in each cell
- ✓ Cell to cell variability

- ✓ Agent-based mechanical model
- ✓ Blood flow
- ✓ Diffusion of drugs to the cells

- ✓ Absorption, distribution, metabolism and excretion by the rest of the body

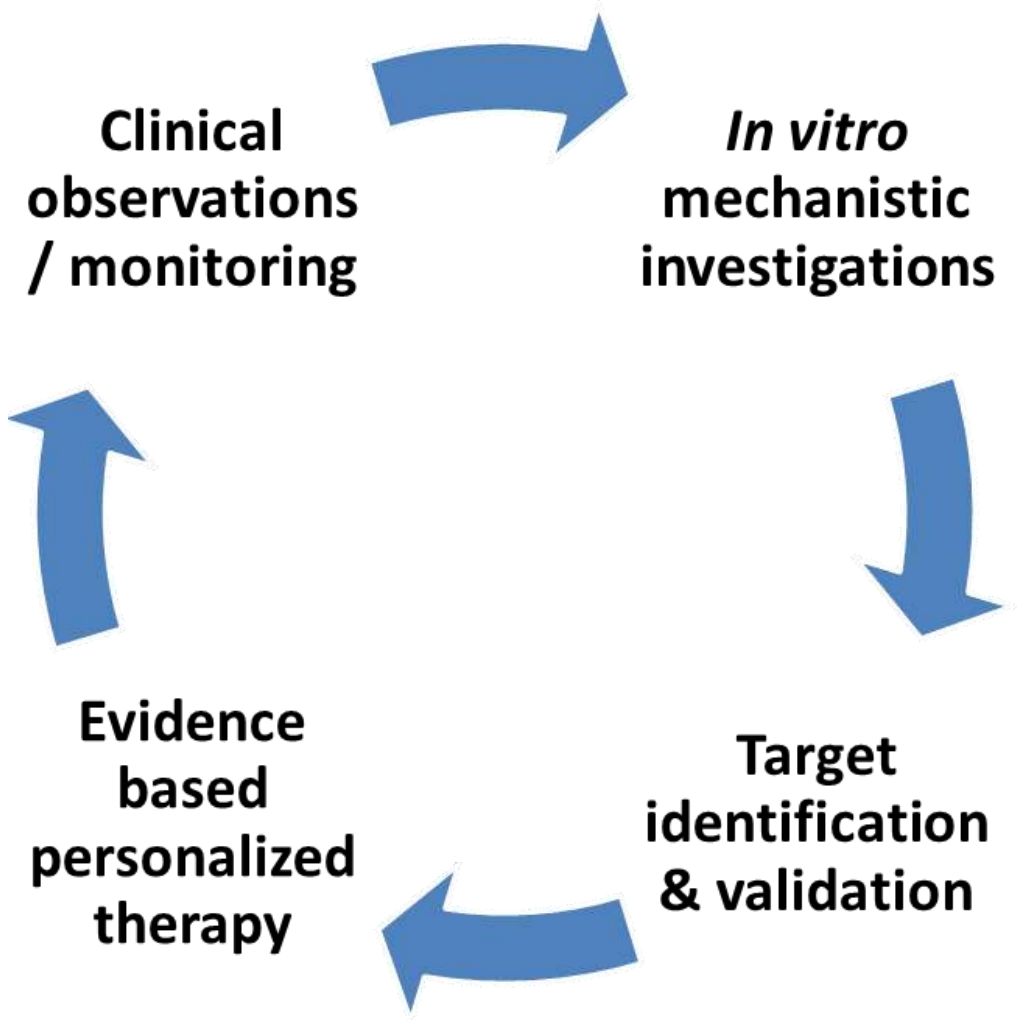
Microbiome





- The gut microbiome → the forgotten organ !
- Microbiome modifies the epigenome
- Bile acid signalling plays a role in health and disease
- Bile acids as therapeutic agents

Perspectives: towards evidence based science and personalized medicine



•Omics
•Biomarkers
•Human specific mechanisms
•Diagnosis and prognosis

Advanced 3D Models
•PHH
•iPSC derived cells from patients

•Huge clinical data available
•Omics
•Biomarkers

•Based on genetic background
•Therapy monitoring

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