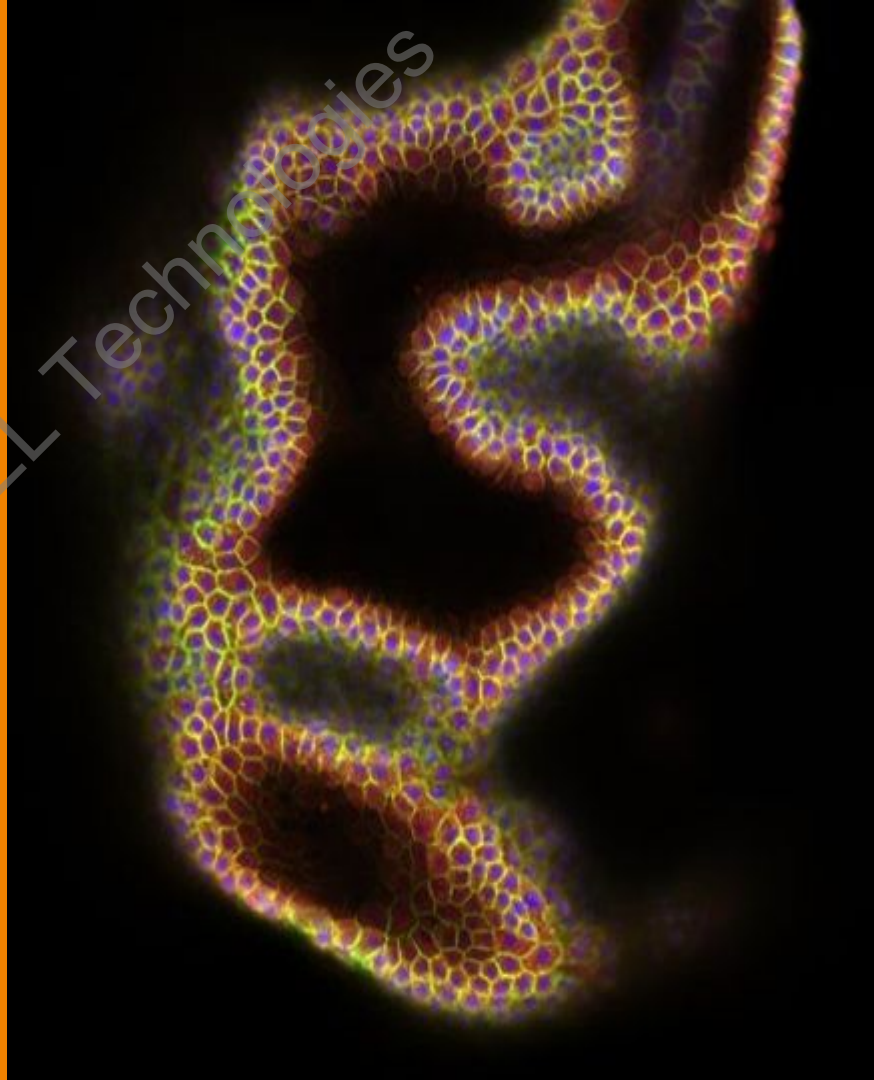
Applications of Hepatic Organoids

Lecture 4

Presenter

Kiran Bhullar

Scientist, Scientific Support, Epithelial Cell Biology



Learning Objectives

After this session, you should be able to:

- Understand the key research applications for human hepatic organoids and possible experimental endpoints

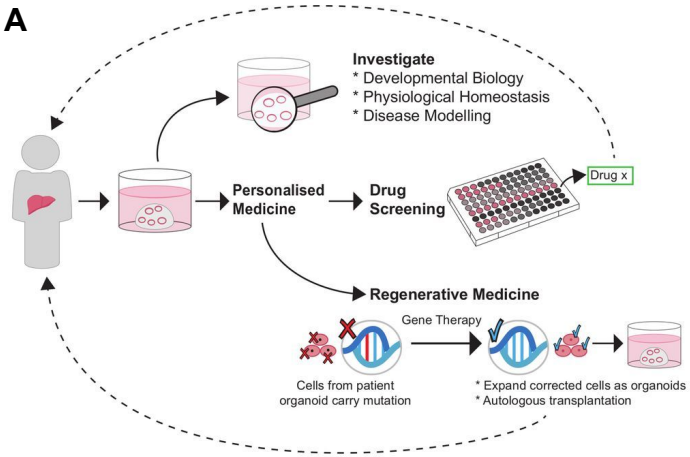
Outline

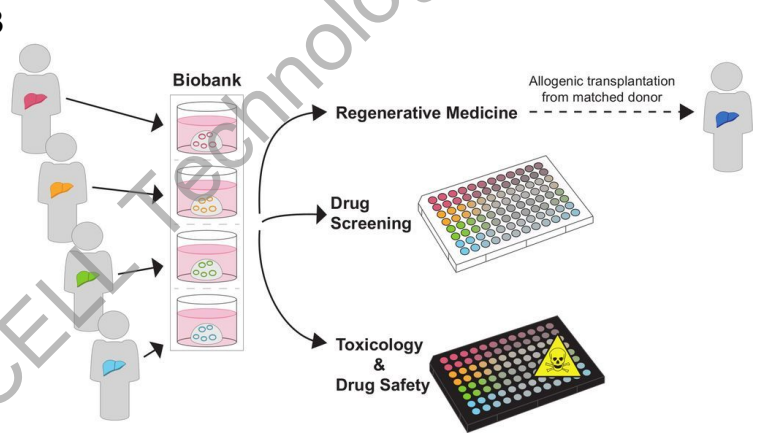
1. Key Downstream Applications
2. Application Highlights

- Hepatotoxicity
- Disease Modelling
- Gene Editing

Section 1 | Overview of Downstream Applications

Applications for Hepatic Organoids





Investigate

- Liver development and function in healthy conditions
- Study mechanisms of disease (disease modelling)

Personalised Medicine

- Patient-specific drug screening
- Gene therapy (gene editing)

Biobanking

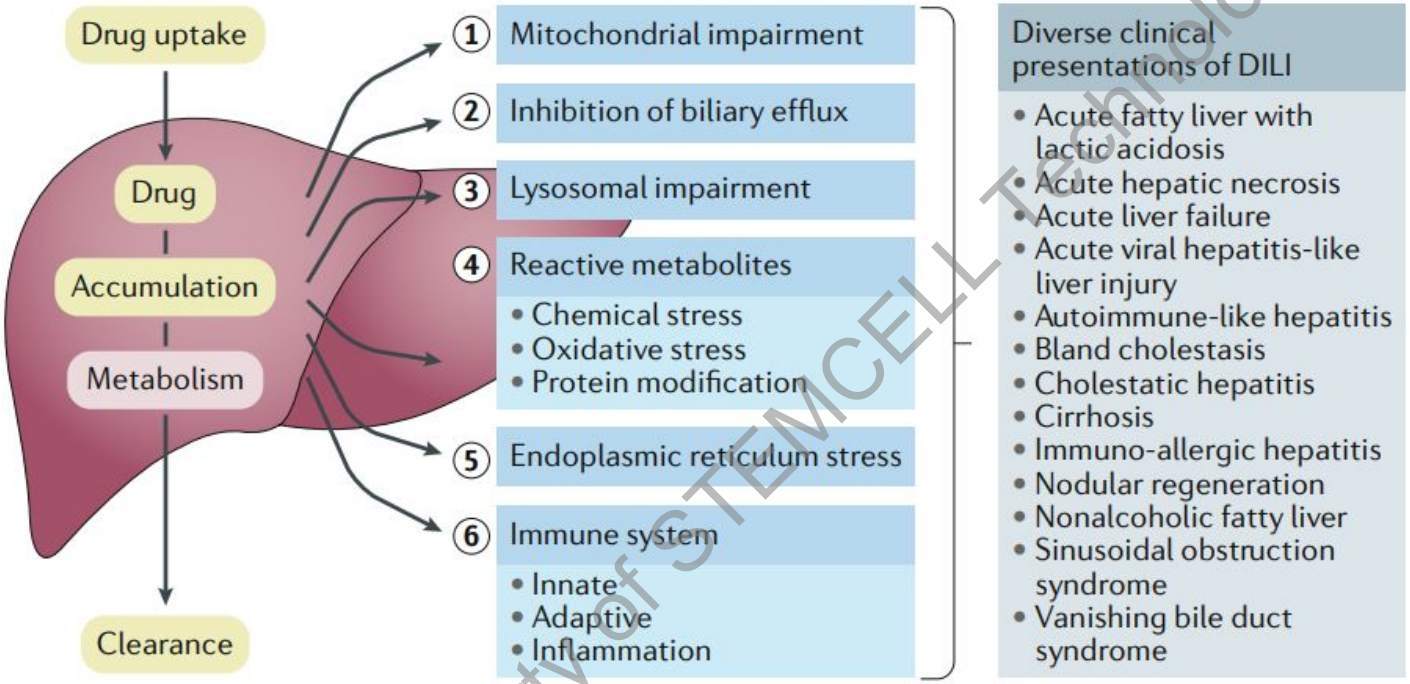
- Regenerative medicine (transplants)
- Drug screening (patient-derived organoids)
- Toxicology studies (drug-induced liver Injury/DILI)

Figure adapted from Prior et al. Gut 2019

Section 2 | Application Highlights

Application 1: Hepatotoxicity

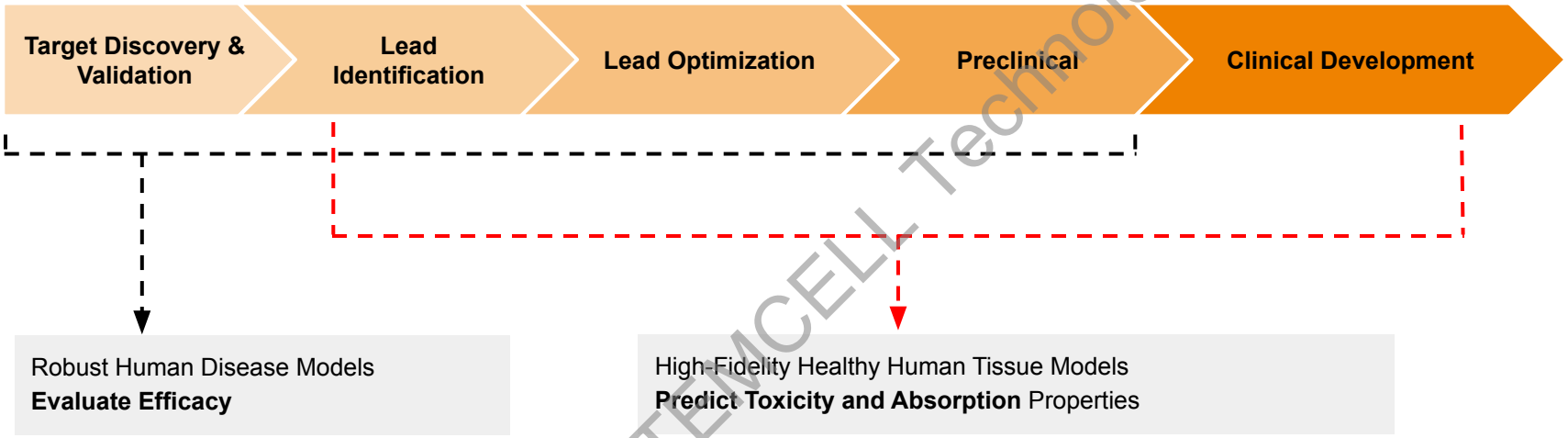
Drug-Induced Liver Injury (DILI)



Drug or its metabolites may be toxic to the liver

Weaver et al. Nature Reviews Drug Discovery 2016

Challenges in Drug Discovery



Fewer than 10% of compounds entering Phase I trials are successful in making it to market

75 - 80% of failures are due to problems with **efficacy and/or safety**

More predictive methods in discovery and preclinical phases stand to de-risk programs before they move to the clinic, including patient stratification to inform trial cohort selection

Hepatic Organoids as a Model for Hepatotoxicity

International Journal of *Molecular Sciences*

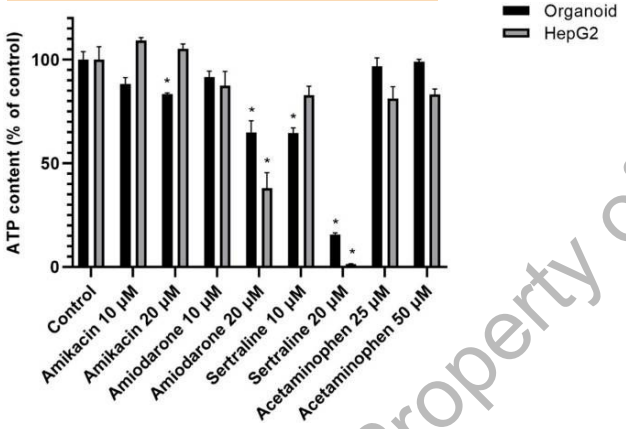
MDPI

Article

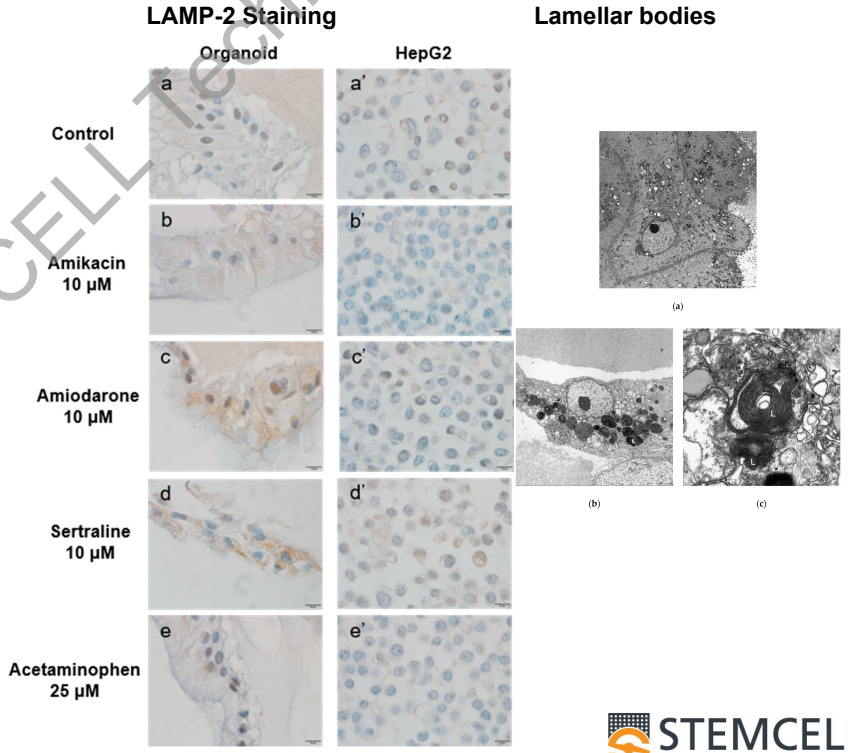
Use of 3D Human Liver Organoids to Predict Drug-Induced Phospholipidosis

Ji-Young Lee [1], Hyo-Jeong Han [1], Sang-Joon Lee [1], Eun-Ho Cho [1], Han-Byul Lee [1], Ju-Hyung Seok [1], Hee Seon Lim [1] and Woo-Chan Son [2,*]

Cell Viability Changes



Evaluation of Drug-Induced Phospholipidosis



Lee et al. Int J Mol Sci. 2020

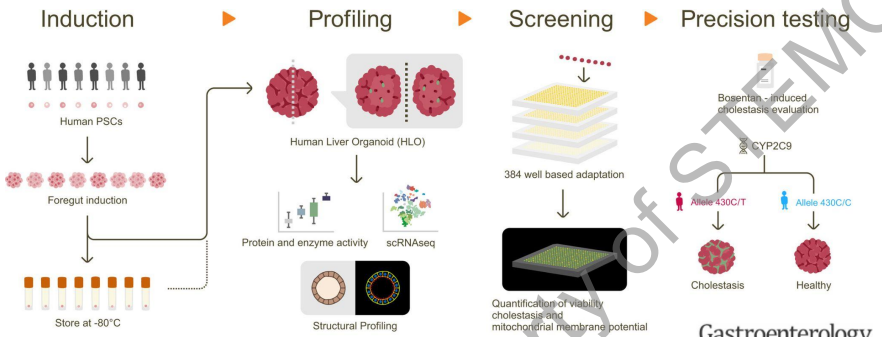
Hepatic Organoids as a Model for Hepatotoxicity

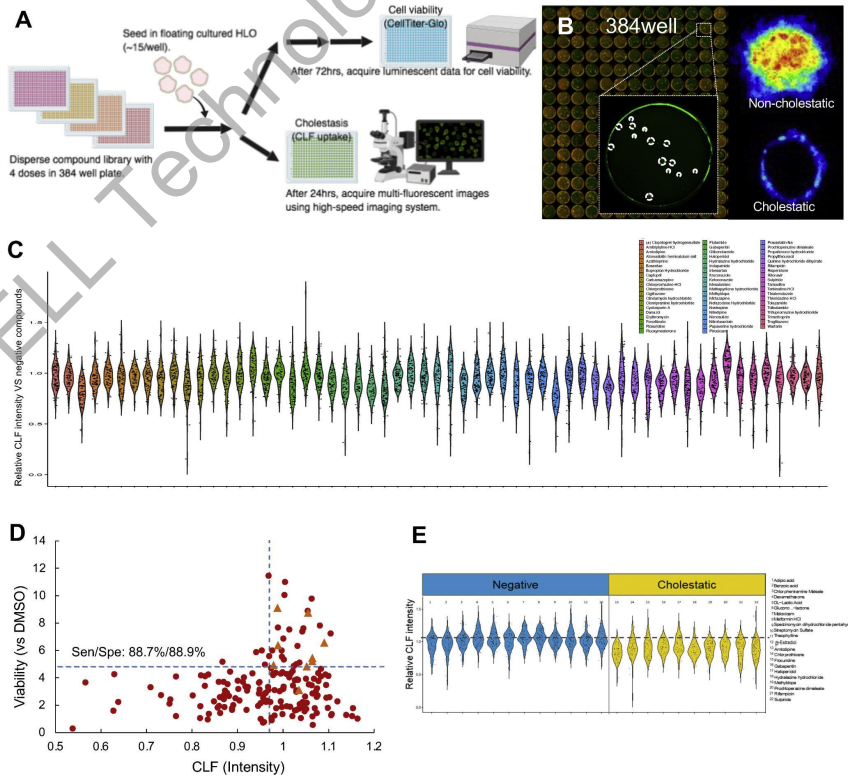
Gastroenterology 2021;160:831–846

BASIC AND TRANSLATIONAL—LIVER

High-Fidelity Drug-Induced Liver Injury Screen Using Human Pluripotent Stem Cell–Derived Organoids

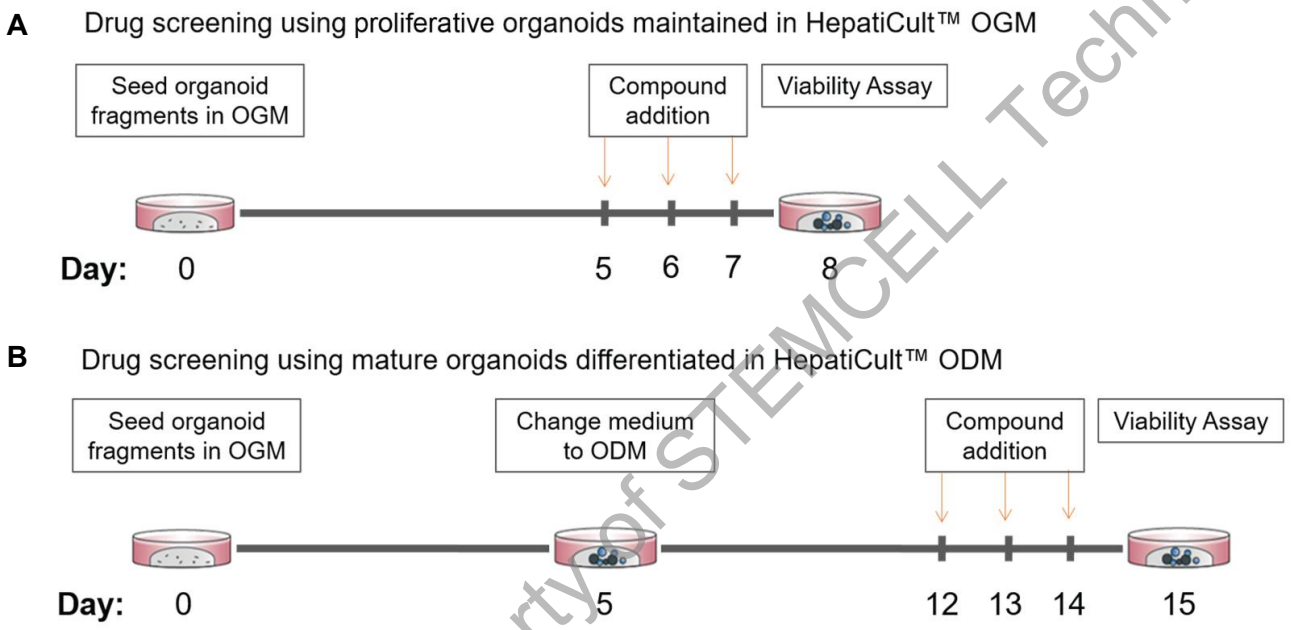
Tadahiro Shinozawa,[1,*] **Masaki Kimura**,[1,*] **Yuqi Cai**,[1,*] Norikazu Saiki,[2] Yosuke Yoneyama,[2] Rie Ouchi,[1] Hiroyuki Koike,[1] Mari Maezawa,[2] Ran-Ran Zhang,[1] Andrew Dunn,[1] Autumn Ferguson,[1] Shodai Togo,[1] Kyle Lewis,[1] Wendy L. Thompson,[1] Akihiro Asai,[1] and Takanori Takebe[1,2,3,4]





Shinozawa et al. Gastroenterology 2021

Cytotoxicity Screening Using Hepatic Organoids Cultured in HepatiCult™ (Human)

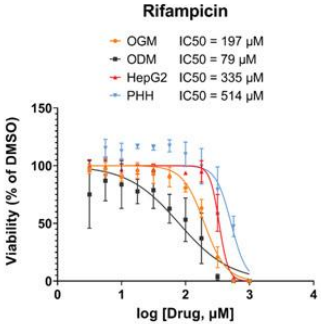


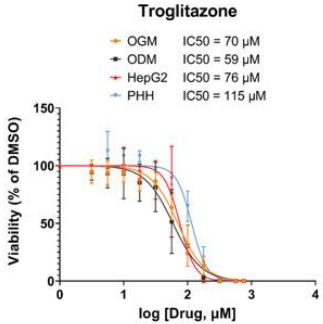
Schematic of drug screening and cell viability assay protocol

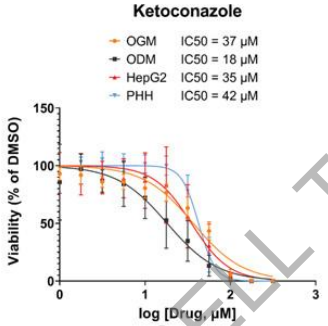
Scientific Poster
HepatiCult™ for Human Hepatic Organoids: A Validated Culture System for Drug Toxicity Screening

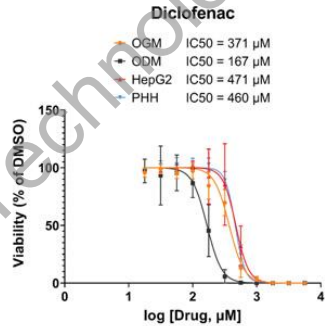
Protocol
How to Perform Cytotoxicity Screening Using Hepatic Organoids Cultured in HepatiCult™ (Human)

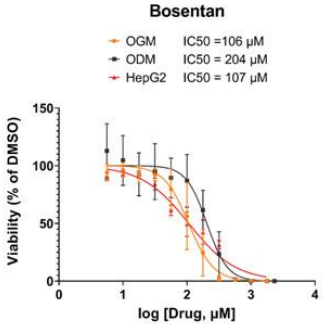
Cytotoxicity Screening Using Hepatic Organoids





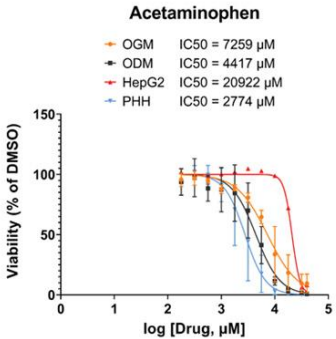


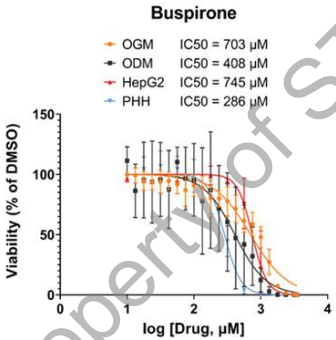




High sensitivity to toxic effects; IC50 values < 200 µM

Moderate sensitivity to toxic effects; IC50 values 100 - 400 µM





Low sensitivity to toxic effects; high IC50 values of > 400 µM

Scientific Poster

HepatiCult™ for Human Hepatic Organoids: A Validated Culture System for Drug Toxicity Screening

Cytotoxicity Screening Using Hepatic Organoids

	Ketoconazole	Troglitazone	Bosentan	Rifampicin	Diclofenac	Buspirone	Acetaminophen
OGM	37	70	106	197	371	703	7259
ODM	18	59	204	79	167	408	4417
HepG2	35	76	107	335	471	745	20922
PHH	42	115		514	460	286	2774

Summary of drug toxicity effects in human hepatic proliferative (OGM) and mature (ODM) organoids, HepG2 cells, and PHH.

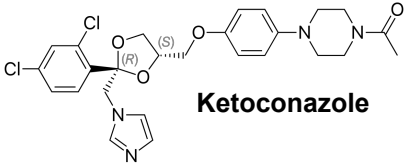
Scientific Poster
HepatiCult™ for Human Hepatic Organoids: A Validated Culture System for Drug Toxicity Screening

Applications Using hPSC-Derived Hepatic Cells

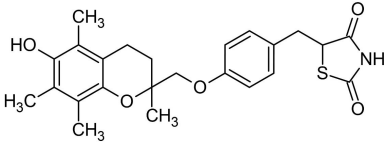
Hepatotoxicity testing using 2D and 3D hepatic models

Objective: Assess effects of compounds with known hepatotoxicity on 2D HLCs and differentiated HLC-derived organoids

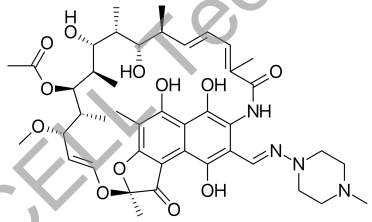
Experimental Setup:



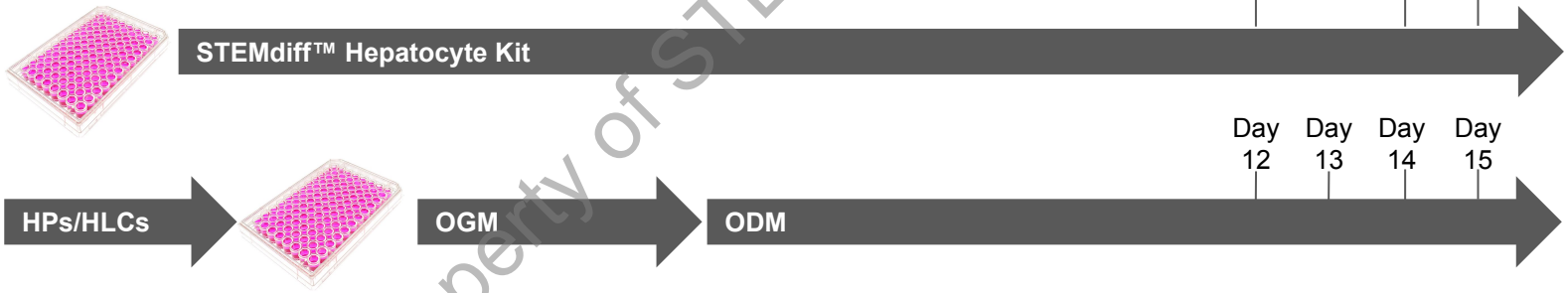
Ketoconazole



Troglitazone



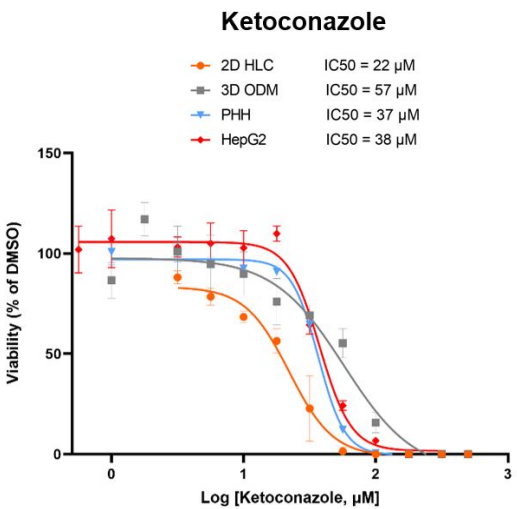
Rifampicin

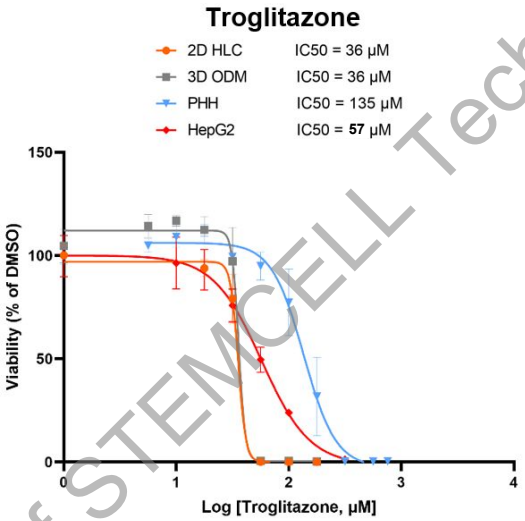


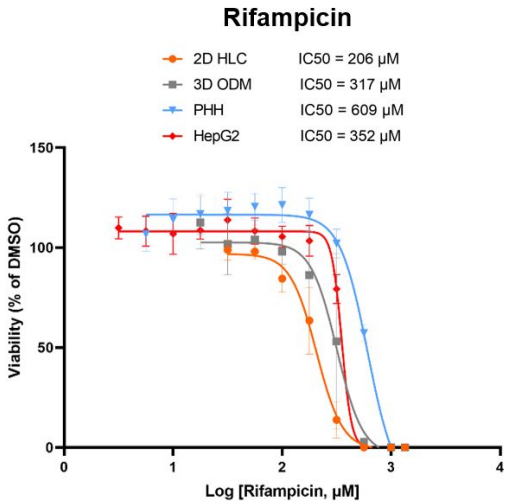
HPs = hepatic progenitor cells; HLCs = hepatocyte-like cells;
OGM = Organoid Growth Medium; ODM = Organoid Differentiation Medium

Applications Using hPSC-Derived Hepatic Cells

hPSC-derived hepatic cells are sensitive to compound-induced hepatotoxicity







(mean±SD)
n = 4-7 technical replicates per dose,
1-2 PSC lines and 3 PHH donors

MORE TOXIC LESS TOXIC

HLCs = hepatocyte-like cells; ODM = Organoid Differentiation Medium; PHHs = primary human hepatocytes

Hepatic Organoids as a Model for Hepatotoxicity

- Liver toxicity is a major cause of clinical drug attrition and acute liver failure
- All drugs (modality- and indication-agnostic) are tested for hepatotoxicity
- Compatibility of hepatic organoids (generated using HepatiCult™) with hepatotoxicity testing:
  - Hepatic organoids show amenability/compatibility with hepatotoxicity testing
  - Seven drugs were tested for IC50 values and compared to published IC50 values for HepG2 cell lines and primary human hepatocytes (PHH)
  - Differentiated (ODM) organoids showed higher sensitivity to tested drugs, suggesting an effect on more mature cell populations

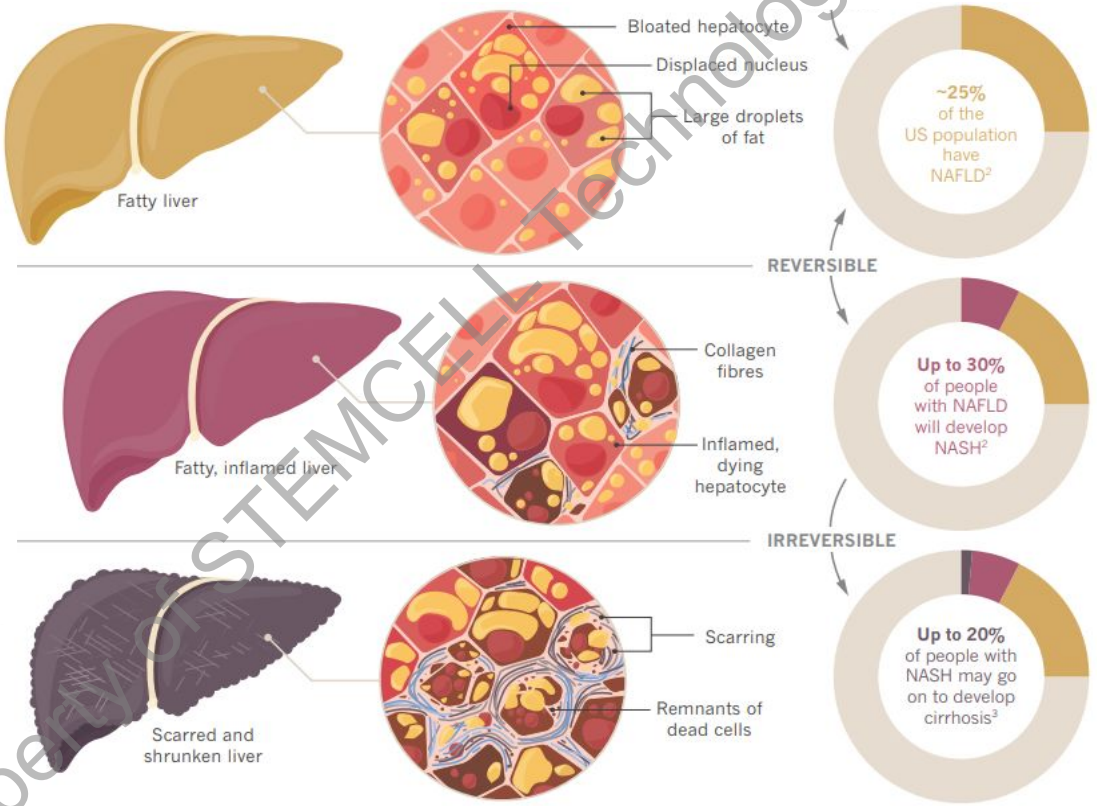
Application 2: Disease Modelling

Disease Modelling (NAFLD and NASH)

NAFLD- hepatocytes accumulate excess fat (diet, diabetes), a process known as steatosis

NASH develops if the accumulated fat cause stress and injury to hepatocytes. This results in hepatocytes swelling and eventual death causing inflammation

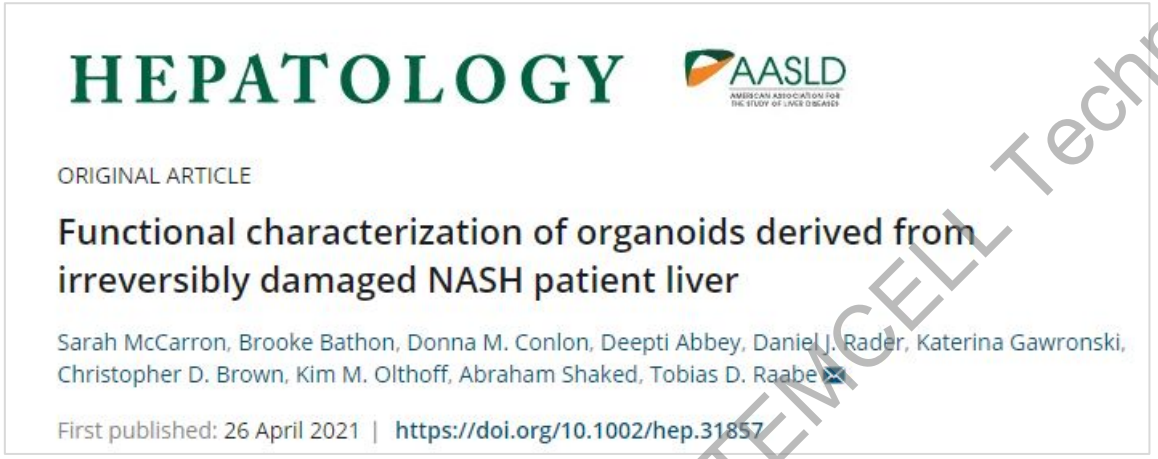
Dead hepatocytes are broken down resulting in accumulation of scar tissue → impairs liver function. This can lead to liver failure and increased risk of liver cancer



NAFLD: Nonalcoholic fatty liver disease
NASH: Nonalcoholic steatohepatitis

Image adapted from Nature Oct 2015 illustration and Inspero webinar

Hepatic Organoids for Disease Modelling (NASH) (NASH)



HEPATOLOGY

AASLD

ORIGINAL ARTICLE

Functional characterization of organoids derived from irreversibly damaged NASH patient liver

Sarah McCarron, Brooke Bathon, Donna M. Conlon, Deepti Abbey, Daniel J. Rader, Katerina Gawronski, Christopher D. Brown, Kim M. Olthoff, Abraham Shaked, Tobias D. Raabe

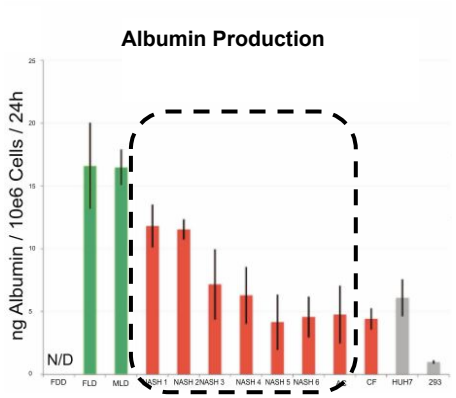
First published: 26 April 2021 | https://doi.org/10.1002/hep.31857

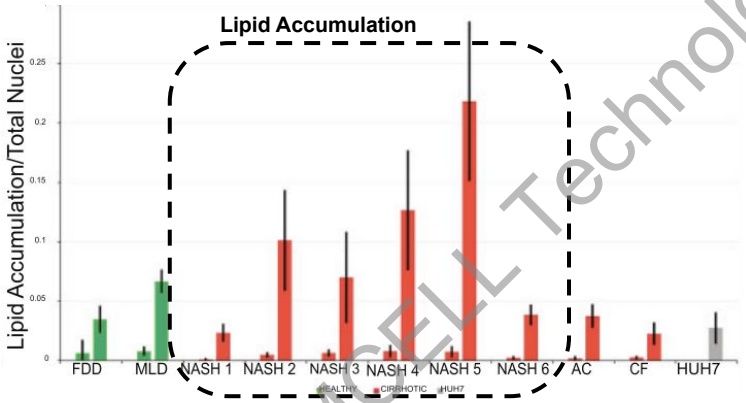
Researchers at the University of Pennsylvania have generated organoids from NASH patient liver tissue

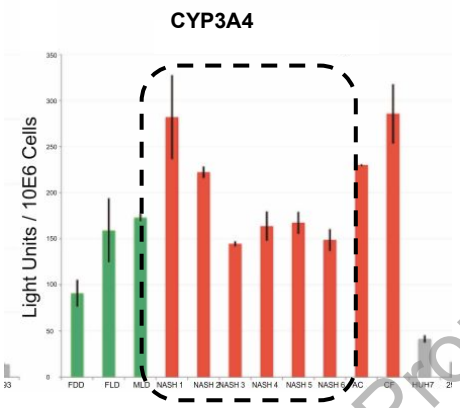
- Used protocol described in Huch et al., 2015 (HepatiCult™ is based on this technology)
- NASH patient derived liver organoids are able to replicate in vivo pathology better than mouse models

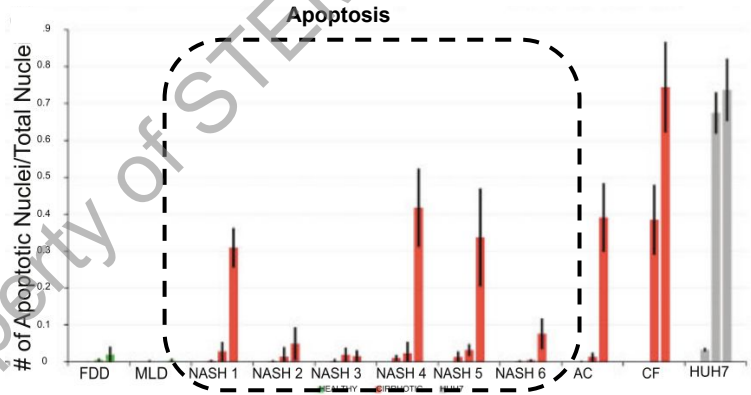
McCarron et al. Hepatology, 2021

Hepatic Organoids for Disease Modelling (NASH)









NASH Patient-Derived Liver Organoids Exhibited:

- Decreased albumin production
- Increased free fatty acid induced accumulation
- Increased CYP metabolism
- Increased apoptosis

Significance:

- NASH organoids recapitulated key diseases characteristics
- Avenues for drug/therapy development

Figures adapted/modified from McCarron et al. Hepatology 2021

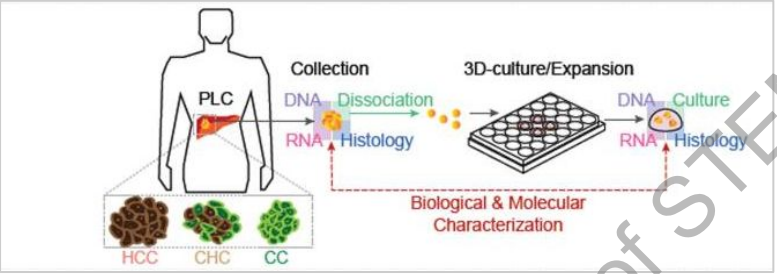
Hepatic Organoids for Cancer Modelling

Published: 13 November 2017

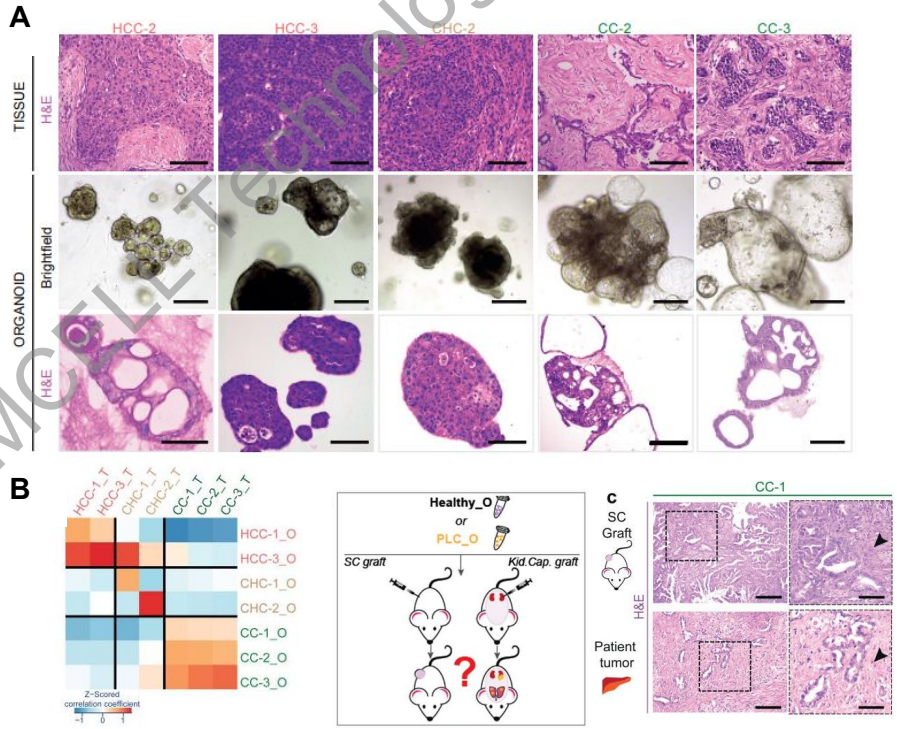
Human primary liver cancer–derived organoid cultures for disease modeling and drug screening

Laura Broutier, Gianmarco Mastrogiovanni, Monique MA Verstegen, Hayley E Francies, Lena Morrill Gavarró, Charles R Bradshaw, George E Allen, Robert Arnes-Benito, Olga Sidorova, Marcia P Gaspersz, Nikitas Georgakopoulos, Bon-Kyoung Koo, Sabine Dietmann, Susan E Davies, Raaj K Praseedom, Ruby Lieshout, Jan N M IJzermans, Stephen J Wigmore, Kourosh Saeb-Parsy, Mathew J Garnett, Luc JW van der Laan & Meritxell Huch

Nature Medicine **23**, 1424–1435 (2017) | Cite this article



hepatocellular carcinoma (HCC)
hepatocellular-cholangiocarcinoma (CHC)
cholangiocarcinoma samples (CC)



Broutier et al. Nature Medicine 2017

Hepatic Organoids for Cancer Modelling

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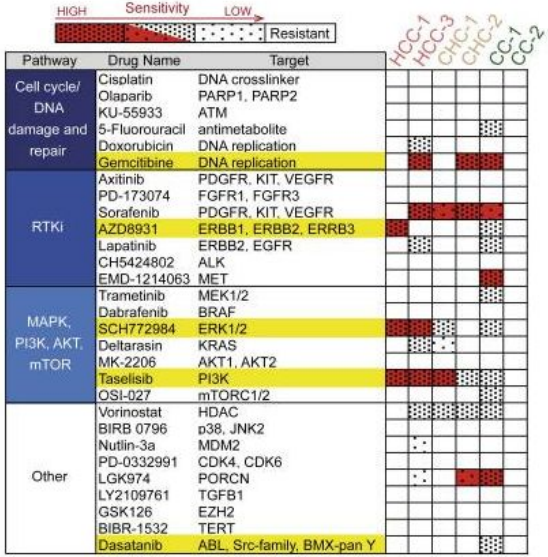
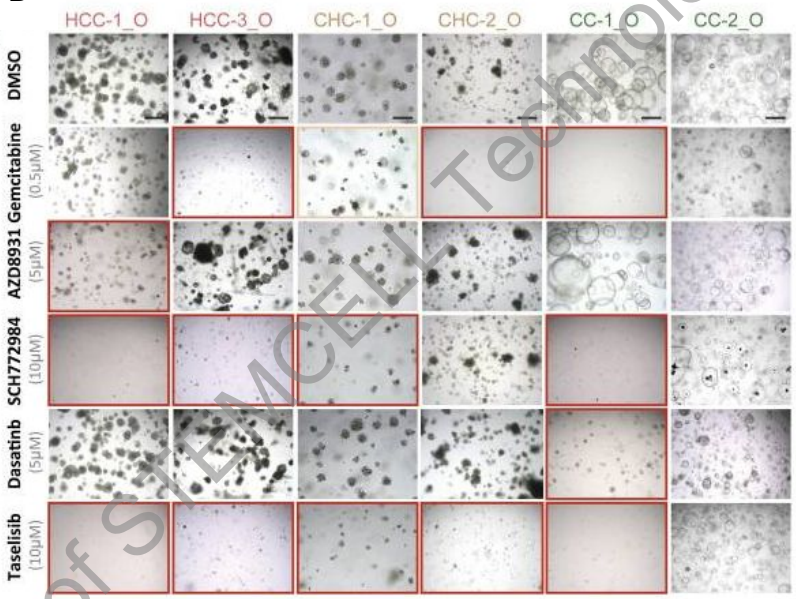


Pathway	Drug Name	Target
Cell cycle/ DNA damage and repair	Cisplatin	DNA crosslinker
	Olaparib	PARP1, PARP2
	KU-55933	ATM
	5-Fluorouracil	antimetabolite
	Doxorubicin	DNA replication
	Gemcitibine	DNA replication
RTKi	Axitinib	PDGFR, KIT, VEGFR
	PD-173074	FGFR1, FGFR3
	Sorafenib	PDGFR, KIT, VEGFR
	AZD8931	ERBB1, ERBB2, ERRB3
	Lapatinib	ERBB2, EGFR
	CH5424802	ALK
	EMD-1214063	MET
MAPK, PI3K, AKT, mTOR	Trametinib	MEK1/2
	Dabrafenib	BRAF
	SCH772984	ERK1/2
	Deltarasin	KRAS
	MK-2206	AKT1, AKT2
	Taselisib	PI3K
	OSI-027	mTORC1/2
Other	Vorinostat	HDAC
	BIRB 0796	p38, JNK2
	Nutlin-3a	MDM2
	PD-0332991	CDK4, CDK6
	LGK974	PORCN
	LY2109761	TGFB1
	GSK126	EZH2
	BIBR-1532	TERT
	Dasatanib	ABL, Src-family, BMX-pan Y

D



- Liver tumoroids allow the identification of patient-specific drug sensitivities and recapitulate multiple cancer subtypes
- Primary liver cancer (PLC) derived organoids present opportunities for drug testing, therapeutic development and advances in personalized medicine approaches

Broutier et al. Nature Medicine 2017

Hepatic Organoids for Modelling Disease Progression

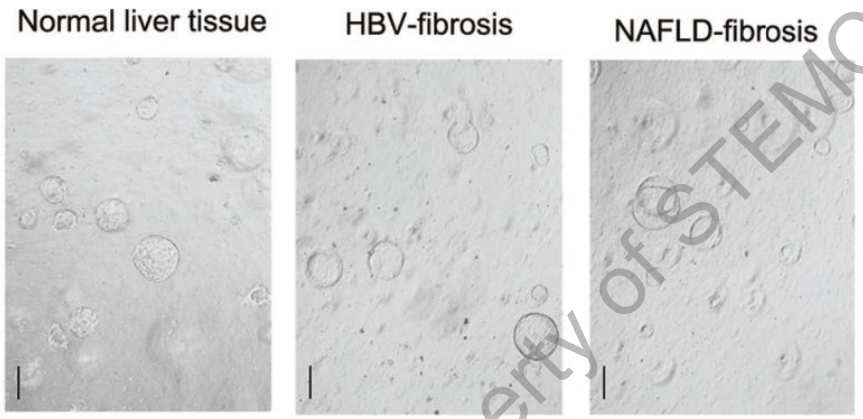
Acta Pharmacologica Sinica

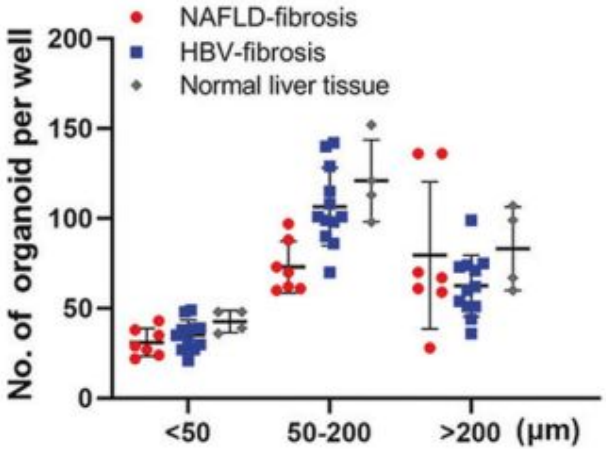
www.nature.com/aps

ARTICLE

Hepatocyte-derived VEGFA accelerates the progression of non-alcoholic fatty liver disease to hepatocellular carcinoma via activating hepatic stellate cells

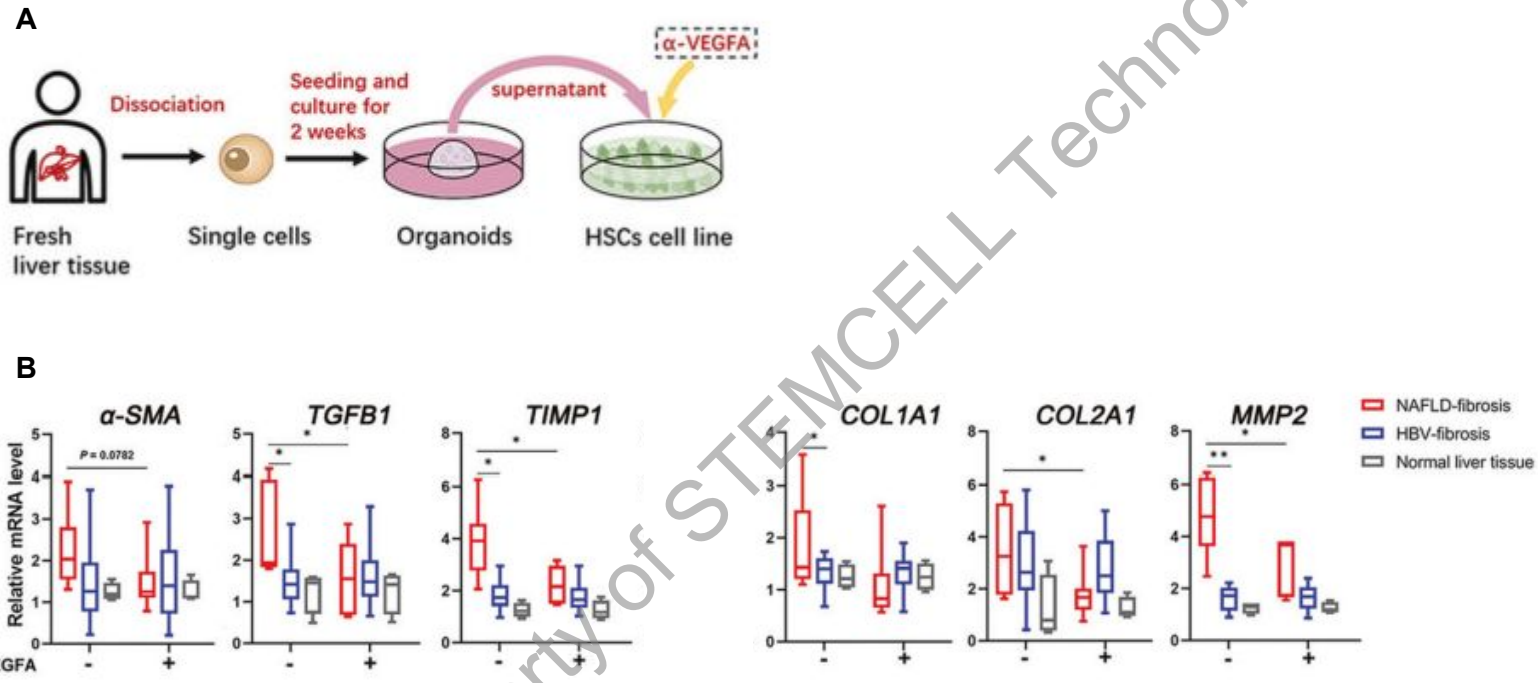
Hao Shen[1], Han Yu[1], Qian-yu Li[2], Ya-ting Wei[1,3], Jing Fu[1], Hui Dong[1], Dan Cao[1], Lin-na Guo[1], Lei Chen[1], Yuan Yang[4], Ying Xu[1], Meng-chao Wu[1], Hong-yang Wang[1,3] and Yao Chen[1]





Shen et al. Acta Pharmacologica 2022

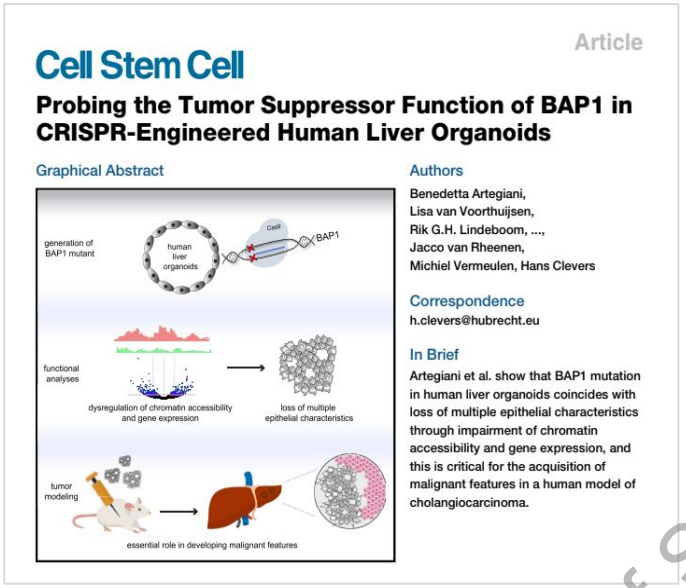
Hepatic Organoids for Modelling Disease Progression



Shen et al. Acta Pharmacologica 2022

Application 3: Gene Editing

Hepatic Organoids for Gene Editing Applications


Article

Cell Stem Cell

Probing the Tumor Suppressor Function of BAP1 in CRISPR-Engineered Human Liver Organoids

Graphical Abstract

Authors

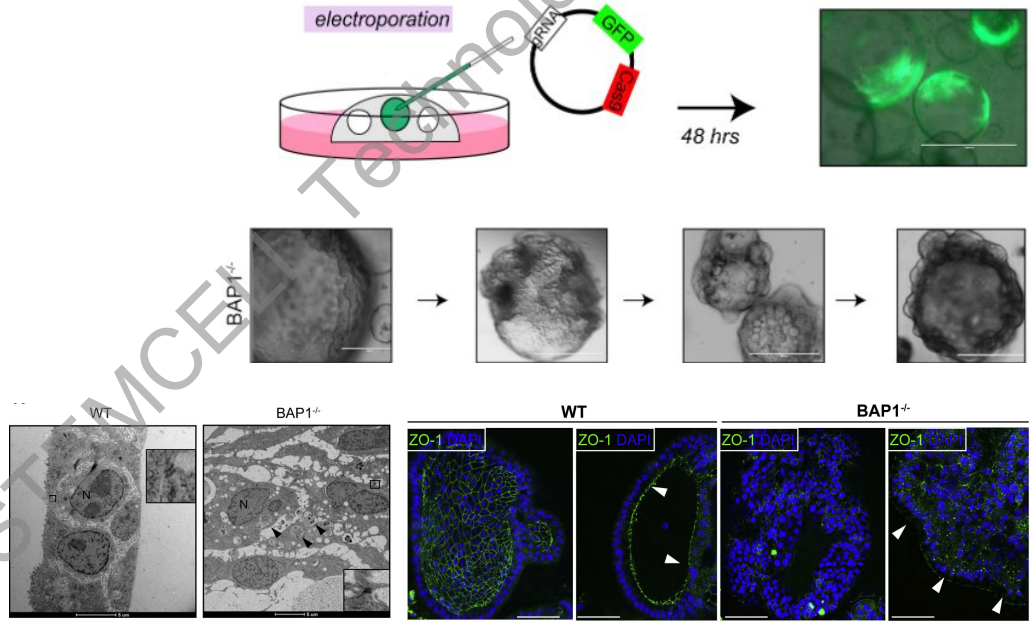
Benedetta Artegiani, Lisa van Voorthuijsen, Rik G.H. Lindeboom, ..., Jacco van Rheenen, Michiel Vermeulen, Hans Clevers

Correspondence

h.clevers@hubrecht.eu

In Brief

Artegiani et al. show that BAP1 mutation in human liver organoids coincides with loss of multiple epithelial characteristics through impairment of chromatin accessibility and gene expression, and this is critical for the acquisition of malignant features in a human model of cholangiocarcinoma.



Significance:

Model liver oncogenic process in specific human tissues using organoids and CRISPR-Cas9 gene editing approach.

Artegiani B et al. Cell Stem Cell 2019

Hepatic Organoids for Gene Editing Applications

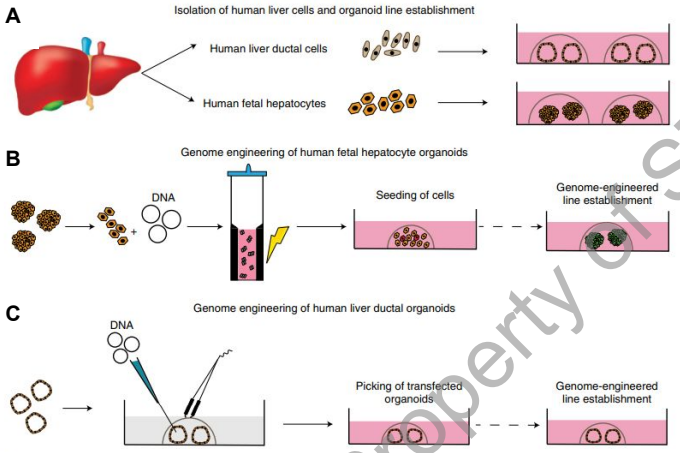
Protocol | Published: 27 November 2020

Establishment of human fetal hepatocyte organoids and CRISPR–Cas9-based gene knockin and knockout in organoid cultures from human liver

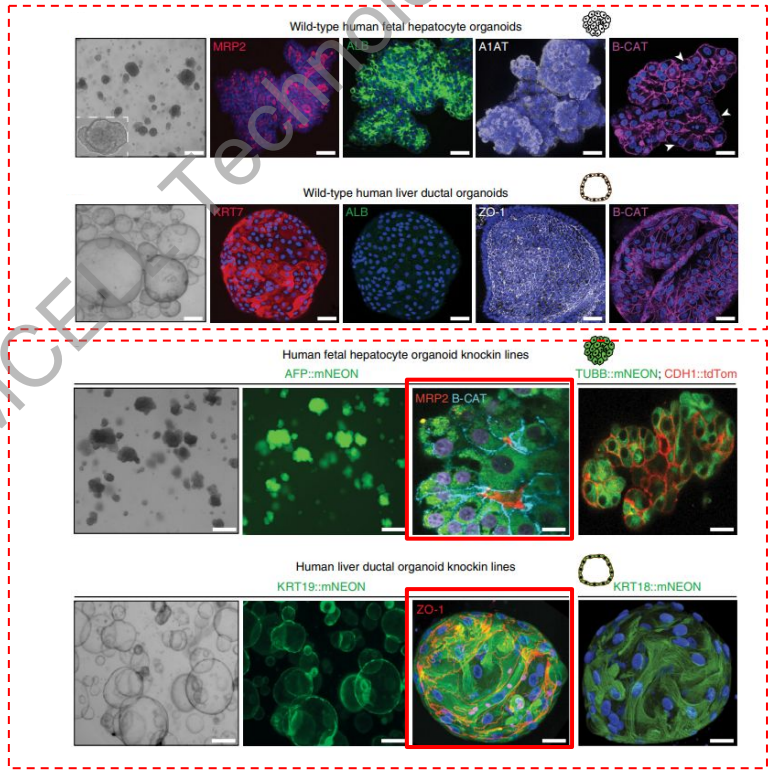
Delilah Hendriks, Benedetta Artegiani, Huili Hu, Susana Chuva de Sousa Lopes & Hans Clevers

Nature Protocols **16**, 182–217 (2021) | Cite this article

Gene editing workflow

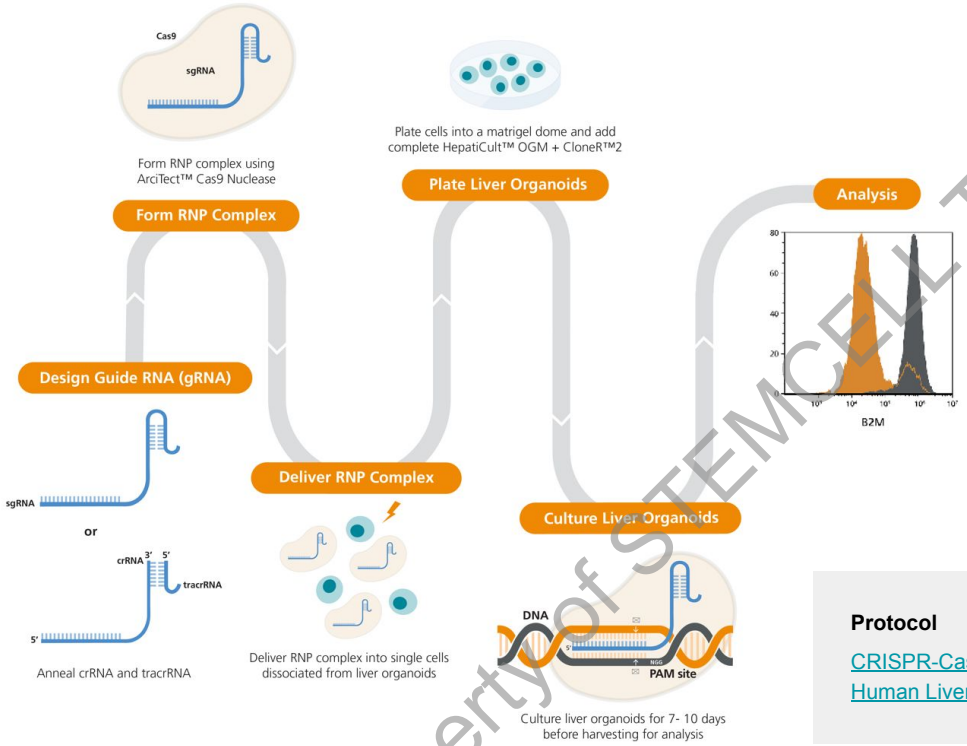


Hendriks et al. Nat Protoc 2021



Characterization of WT and knockin lines

CRISPR-Cas9 Editing of Human Hepatic Organoids



Protocol

[CRISPR-Cas9 Genome Editing of Human Liver Organoids]

Experimental Workflow for Hepatic Organoid Genome Editing

Summary

- Hepatic organoids can be used for a wide variety of applications, including hepatotoxicity/cytotoxicity, disease modelling, and gene editing applications

- Hepatic organoids can be derived from patients with inherited liver disease:
  - Opportunity to establish drug-screening platforms and toxicity assays for personalized medicine
  - Understand the signaling pathways involved in the underlying disease

- Genome editing technologies coupled with the hepatic organoid culture platform:
  - Reintroduction of genetically corrected stem cells into affected patients
  - Provides experimental platform for mechanistic studies of cancer gene function in a human context

Thank You

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