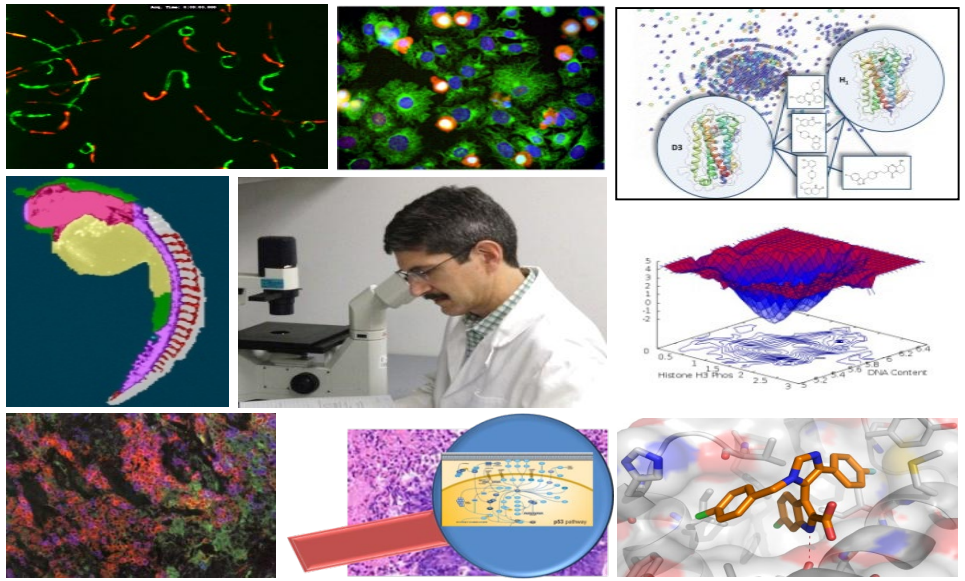
University of Pittsburgh Drug Discovery Institute

Harnessing Human Biomimetic Liver MPS Combined with QSP to Predict Drugs/Combinations for Treating MAFLD



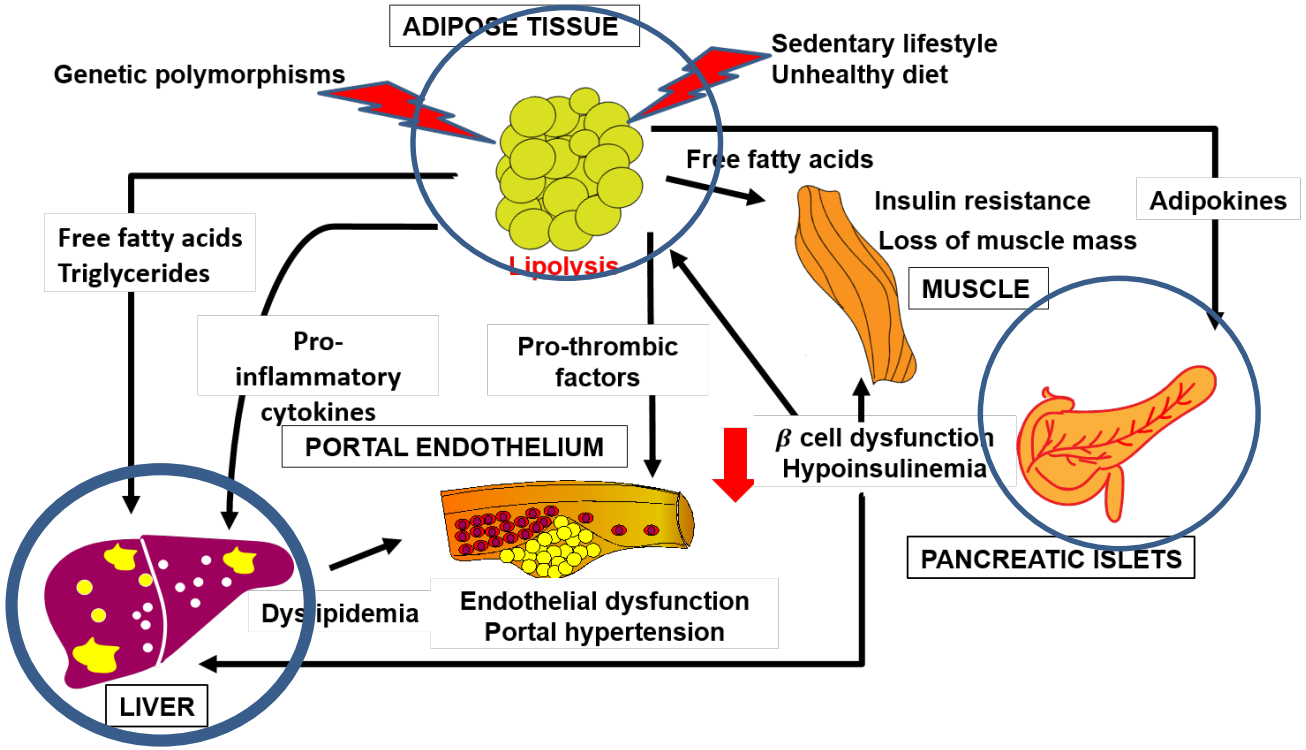
Microphysiological Systems (MPS): Bridging Human and Animal Research

A National Academy of Sciences Roundtable on Science and Welfare of Laboratory Animal Use
January 19-20, 2021

D. Lansing Taylor, Ph.D.
Director, Distinguished Professor and
Allegheny Foundation Professor of Computational and Systems Biology
University of Pittsburgh

www.upddi.pitt.edu

Metabolic Syndrome is a Complex Disease Involving Multiple Organs Comorbidities Include: NAFLD, Obesity, Type 2 Diabetes (T2D) & Hypertension



NAFLD

NAFLD is the traditional term for the heterogeneous disease in which fat accumulates in the liver in patients that drink little or no alcohol. It can progress from a fatty liver (NAFL or steatosis) to non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma.

MAFLD

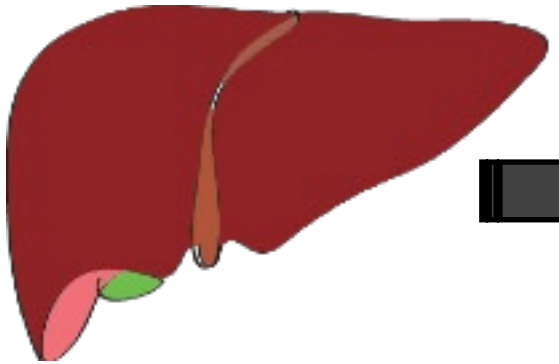
MAFLD, metabolic-dysfunction associated fatty liver disease, is a replacement term for NAFLD, suggested by a panel of experts since it better reflects the heterogeneity of patient-specific drivers and pathologies of metabolic fatty liver diseases. It includes steatosis and NASH associated with a variety of metabolic dysfunctions (e.g. T2D).

It is estimated that the prevalence of MAFLD is up to 25% of the global adult population

It is estimated that up to 70% of Type 2 Diabetes patients have coexisting "NAFLD"

MAFLD Represents a Key Liver Manifestation of the Metabolic Syndrome in Patients

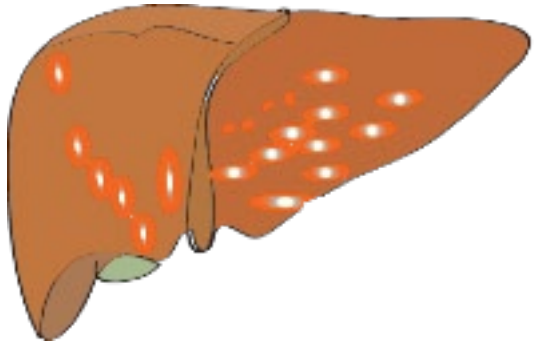
HEALTHY LIVER



Patient Heterogeneity: Genetic risk factors, environment, microbiome and metabolism

- Lipotoxicity (Lipid accumulation)
- Oxidative Stress
- Cell Stress (ER Stress)
- Cell death (apoptosis/autophagy)
- Insulin resistance
- Fibrosis

MAFLD LIVER



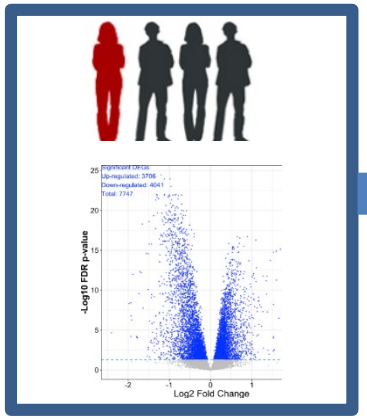
Hepatic steatosis
ROS production
Fibrosis
Inflammation
Insulin resistance
Glucose output

Challenge: There are no approved therapeutics for NAFLD/MAFLD although major drug discovery programs have applied target-centric methods and genetically engineered mouse models of disease for over a decade

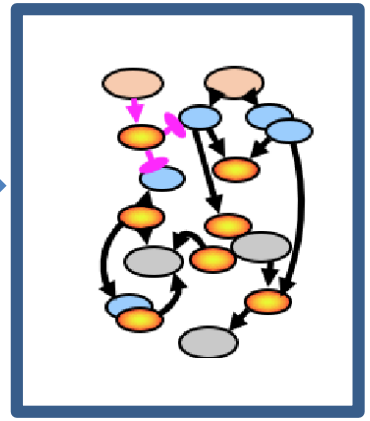
Our Solution:

Apply a QSP and Human MPS Platform for Drug Discovery and Development:
An Unbiased Approach to Complement Target-Centric Discovery & Animal Models of Disease

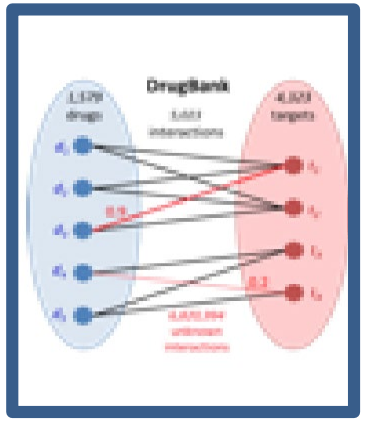
A. Patient Sample Clinical Gene Expression Data



B. Infer Pathways of Disease Progression

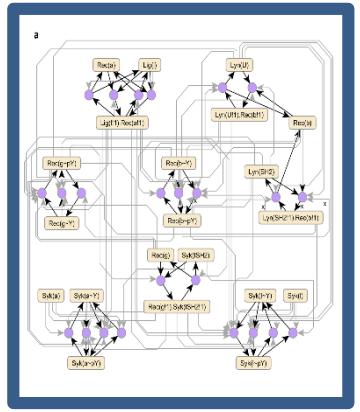


C. Predict Targets-Drugs by Machine Learning/ Systems Biology

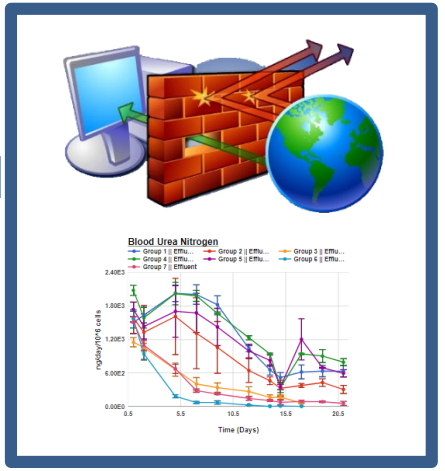


D. Test Drugs/Candidates in Human Biomimetic Liver-MPS

E. Computational Models of Disease & ADME-TOX



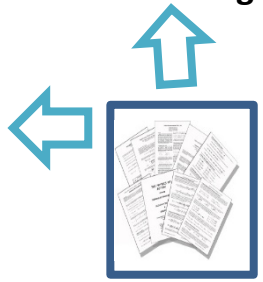
F. MPS-Database



G. Emergent Properties

- **Novel Targets**
- **Biomarkers**
  - Diagnostics
  - PD
- **Repurposed Drugs/Combinations**
- **Therapeutic Strategies**

Validated, Published Knowledge



Gough et. al. (2019). Handbook of Experimental Pharmacology

Lefever, Pei, Miedel, et. al. (2021). In preparation

Drug Discovery Institute
UNIVERSITY OF PITTSBURGH

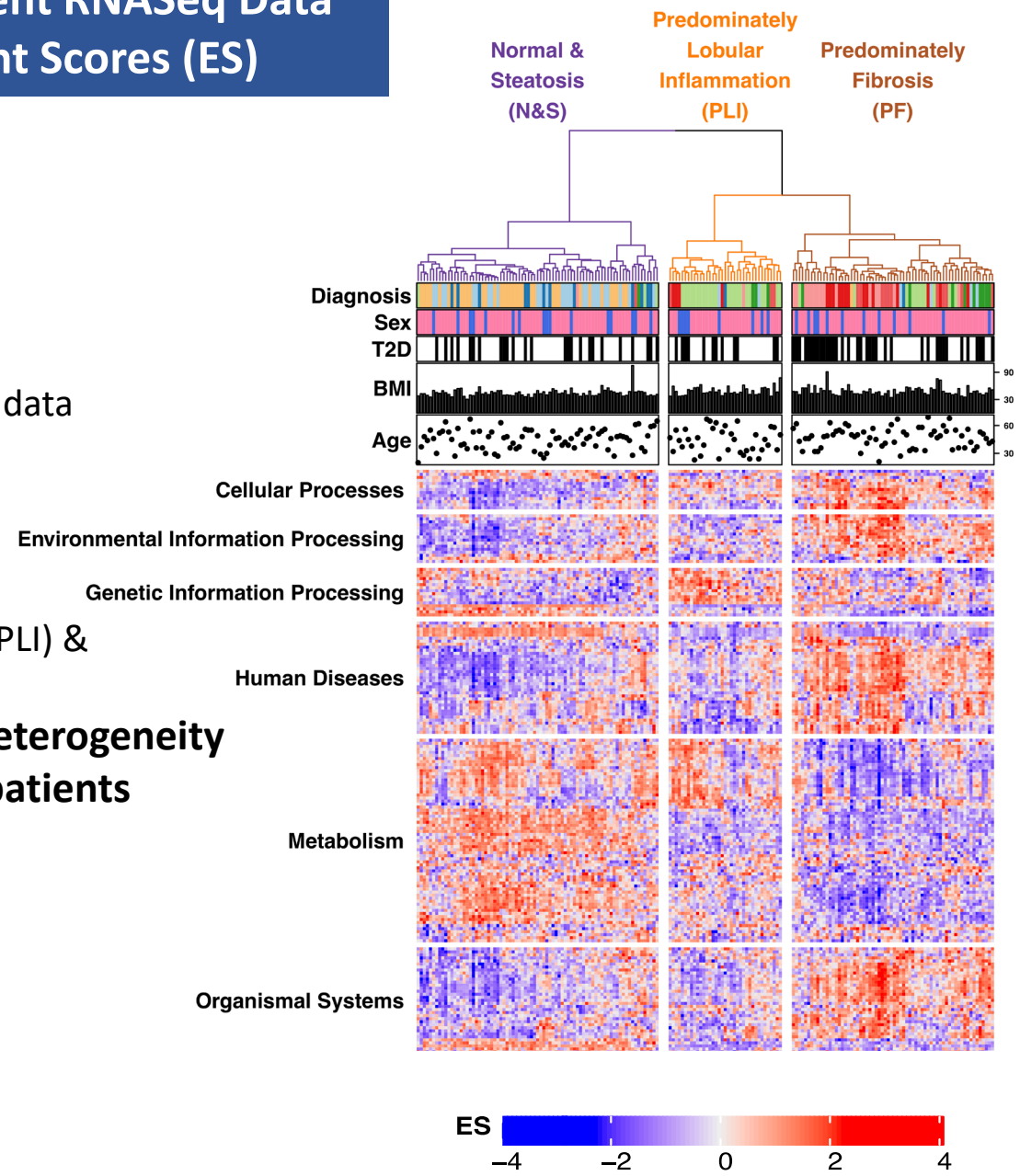
Harnessing Quantitative Systems Pharmacology to Deliver Personalized Medicine

First Step: Unsupervised Clustering of Individual NAFLD Patient RNASeq Data Based on KEGG Pathway Normalized Gene Set Enrichment Scores (ES)

Bariatric patients (182)-wedge biopsies-RNASeq

Key Points

- Patients diagnosed by predominant liver pathology
- Other clinical data collected
- Columns of heat map (182)-Individual patient clinical and gene expression data
- Rows of heat map -specific KEGG pathways
- Blue-down regulated and red-up regulated genes (normalized enrichment scores)
- Unbiased, hierarchal clustering
- 3 clusters: Normal & steatosis (NS), predominantly lobular inflammation (PLI) & predominantly fibrosis (PF)
- **Unbiased clustering consistent with pathology diagnoses, with heterogeneity**
- **T2D is a comorbidity in ca. 50% of patients and maximally in PF patients**

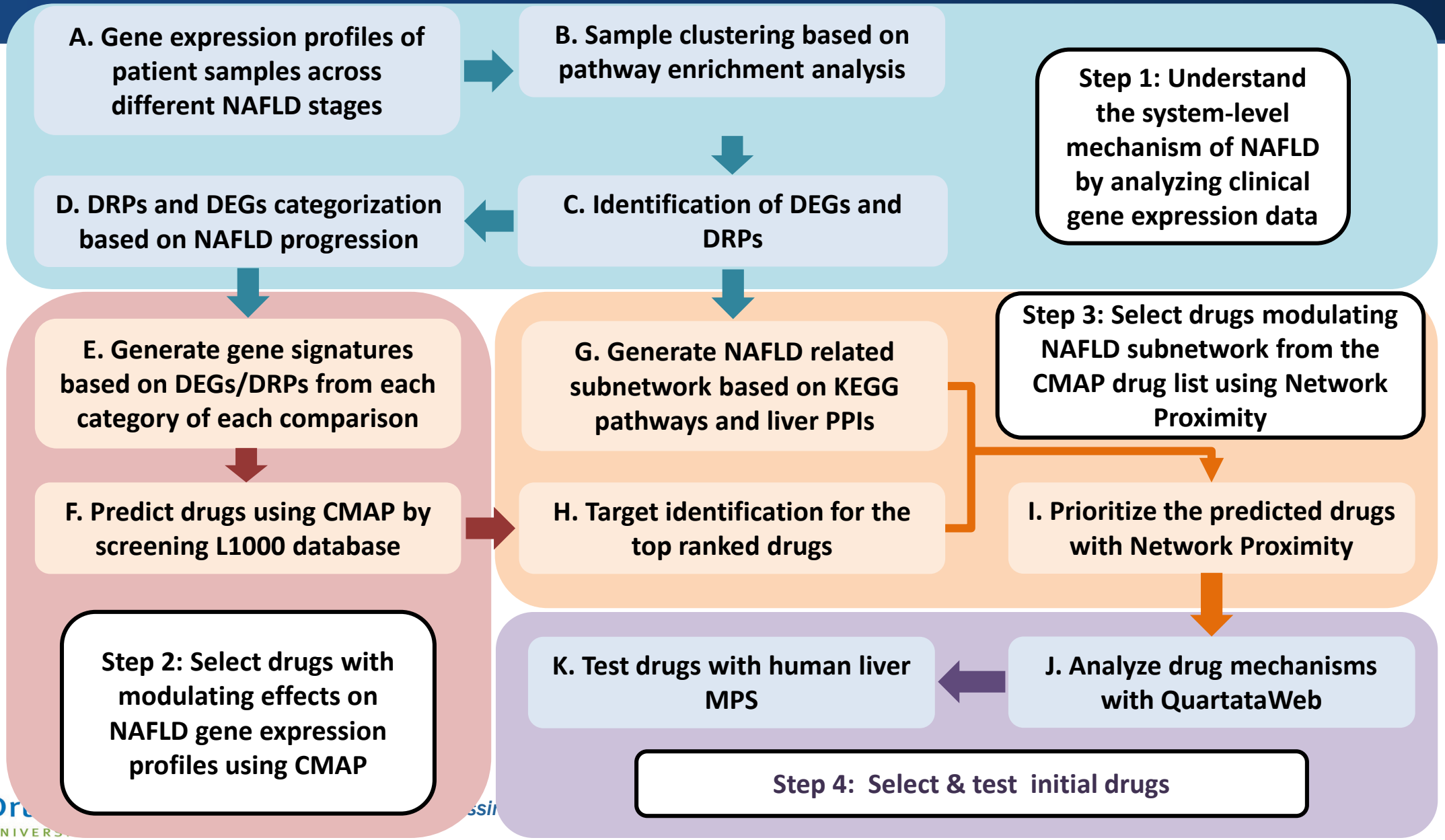


Diagnosis

- NORMAL (n=36)
- STEATOSIS_2 (n=32)
- STEATOSIS_3 (n=14)
- Lob_Inflam_1 (n=37)
- Lob_Inflam_2 (n=13)
- Fibrosis_3 (n=17)
- Fibrosis_3.5 (n=15)
- Fibrosis_4 (n=18)

Lefever, Pei, Miedel, et. al. (2021). In preparation

Summary of Workflow to Predict & Test Drugs from DrugBank/STITCH for NAFLD/MAFLD

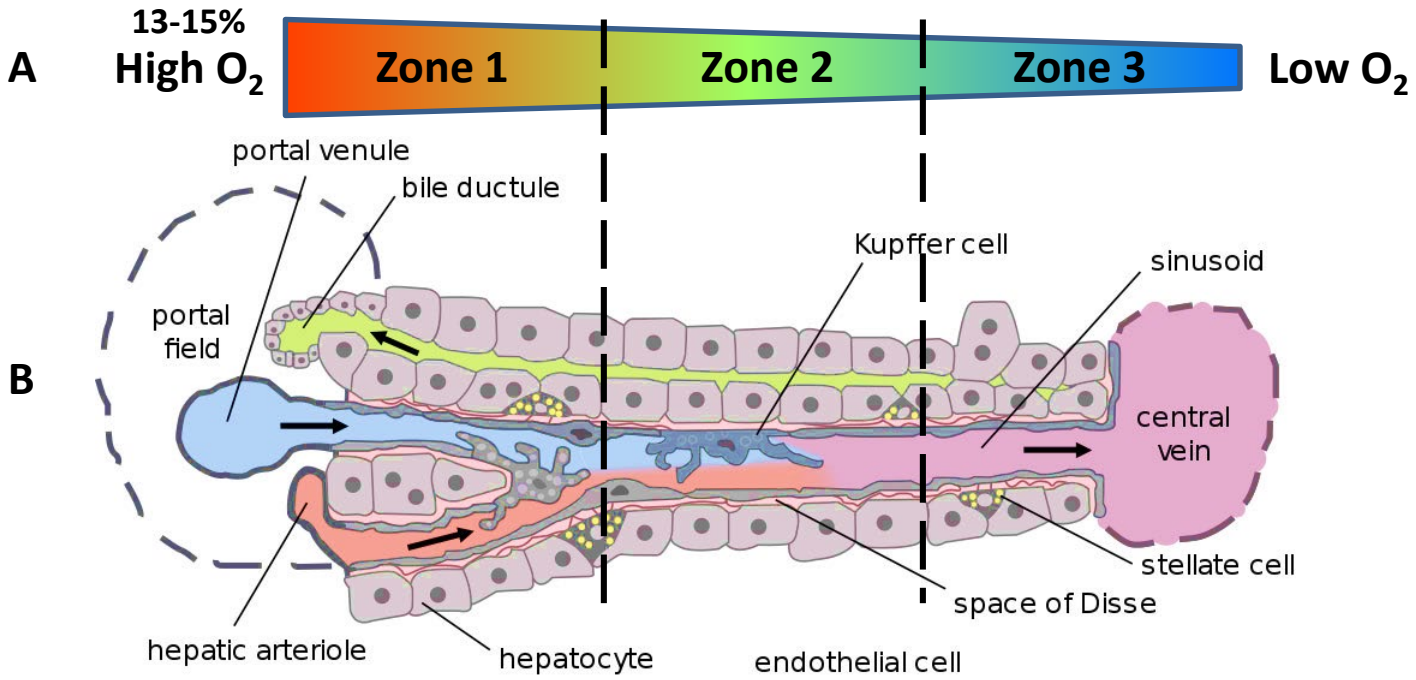


Graph Showing 8 Prioritized Drugs and Canonical Targets out of 70 in Relation to the NAFLD Associated Pathways Subnetwork.

8 Prioritized drugs, their canonical targets and KEGG pathways have been blurred/blocked since a disclosure has been made on the drugs and combinations and a provisional patent is being drafted

Lefever, Pei, Miedel, et. al. (2021). In preparation

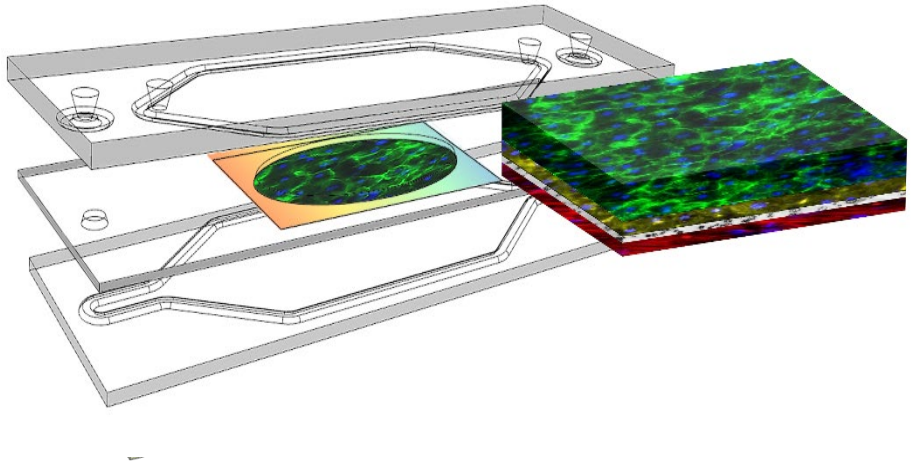
Design and Construct Human Biomimetic Liver-MPS (HBL-MPS) to Test Drugs: Goal is to Recapitulate the Human Liver Acinus Structure and Functions



Adapted from Frevert et. al .2005. PLoS Biol. 3

Vascularized Human Liver Acinus MPS (vLAMPS)

A

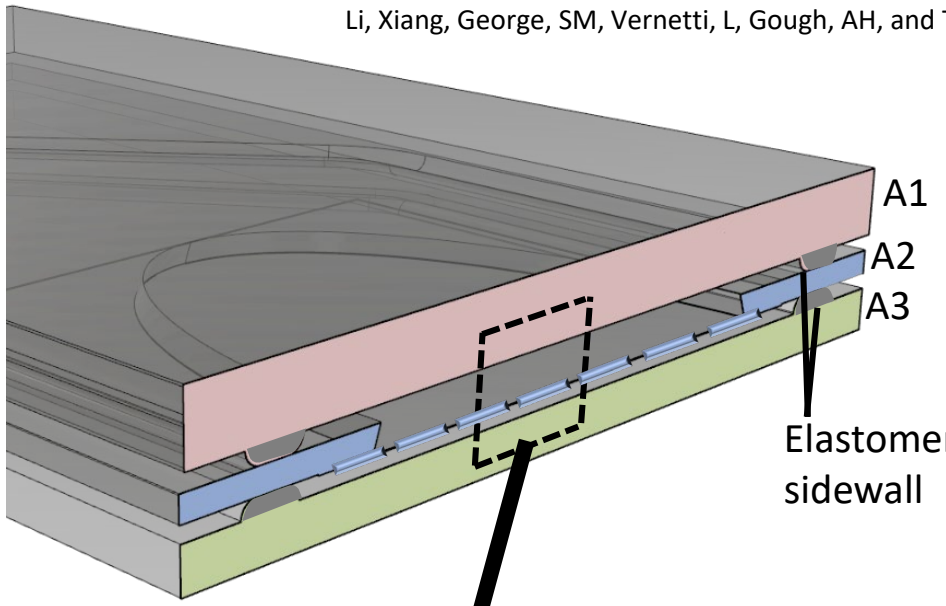


B

	Cell type	Seeding Number
Hepatic chamber	Hepatocytes	100,000
	Stellate	15,000
Vascular chamber	LSEC	100,000
	Kupffer	36,000

C

Li, Xiang, George, SM, Vernetti, L, Gough, AH, and Taylor, DL (2018). Lab on a Chip 18: 2614-2631



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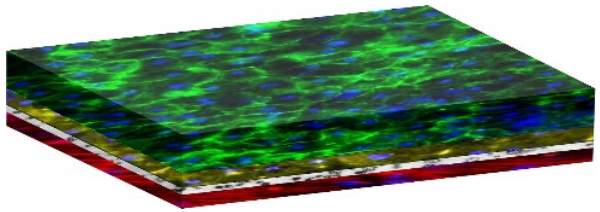
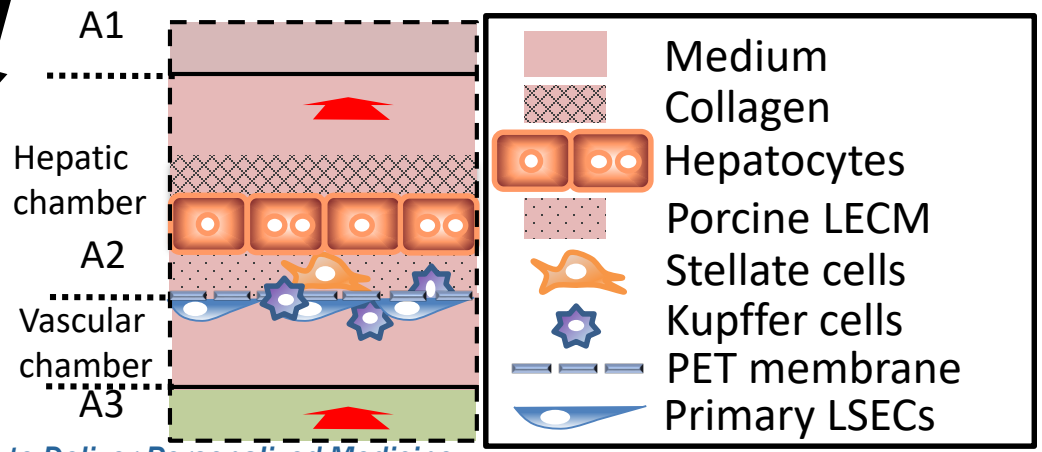
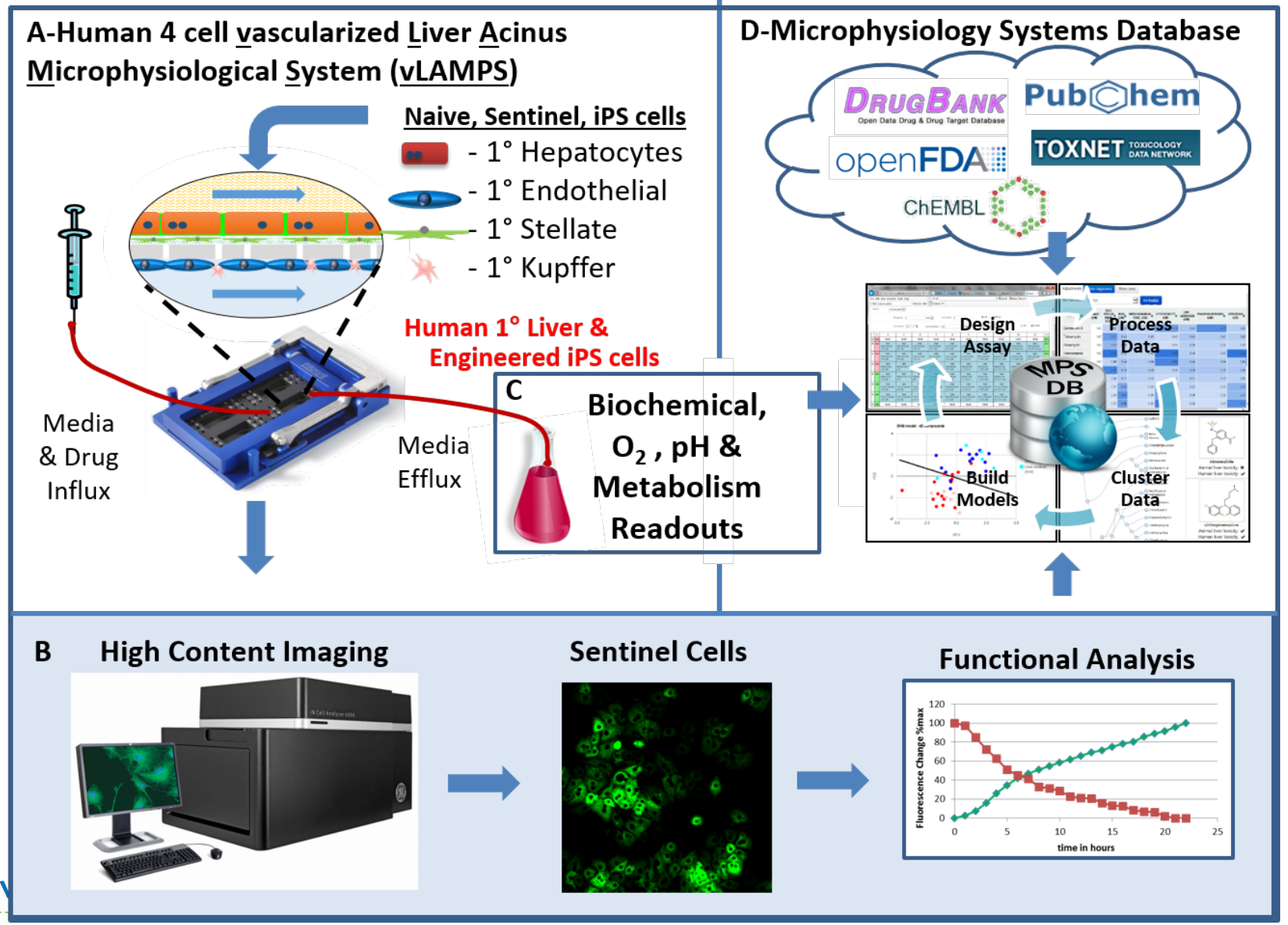




Platform for Investigating Experimental Human Disease Models & ADME/TOX



Goal to Generate a Progressing MAFLD Disease Model Starting with Normal/NAFL Patient Cells by Changing the Media Contents Over Two Weeks

Component	NF (Normal Fasting)	EMS (Early Metabolic Syndrome; MAFLD)	LMS (Late Metabolic Syndrome; MAFLD, T2D)
Glucose	5.5 mM	11.5 mM	20 mM
Insulin	10 pM	10 nM	10 nM
Glucagon	100 pM	10 pM	10 pM
Oleic acid	-	200 uM	200 uM
Palmitic acid	-	100 uM	100 uM
LPS	-	-	1 ug/mL
TGF-β	-	-	10 ng/mL
Glutamine (glutaMAX)	2mM	2 mM	2mM

Media formulations were designed to mimic disease progression from Normal Fasting (NF) to early metabolic syndrome (EMS; MAFLD) and late metabolic syndrome (LMS; T2D). These medias were developed with glucose-free Williams E media supplemented with physiologically relevant levels of glucose, insulin, glucagon, oleic acid, palmitic acid and molecular drivers of disease including TGF-β and LPS. We then adjusted these components to reflect the pathophysiological conditions

Phenotypic, Functional and Genomic Metrics of the HBL-MPS to Characterize Liver MAFLD Disease Progression and to Test Responses to Therapeutic Treatments

Readout	MPS Live Measurements	Clinical Relevance
Secretome		
Albumin secretion	ELISA	Metabolic competence, overall hepatocyte function
Urea secretion	Colorimetric	Metabolic competence, overall hepatocyte function
LDH release*	Colorimetric	Hepatocellular injury corresponding with ALT and AST
Glucose secretion	AmplexRed Glucose Assay	Glucose regulation
β-hydroxybutyrate	Colorimetric	Fatty acid oxidation
Lactate secretion	Colorimetric	Glycolysis
Cytokine release	Human Cytokine Panel	Liver stress/injury response
Fatty acid secretion	Mass spectroscopy	Fatty acid metabolism
TNFα secretion	ELISA	Kupffer activation, innate inflammatory response
Bile acids (BA)	BA profile by Mass spectroscopy	NAFLD, NASH altered plasma BA profile corresponds
Exosomes (proteins/RNA/microRNA)	antibody (α-SK1), qRT-PCR	Circulating extracellular vesicles containing SK1, miRNA-122 & -21 as markers for NAFLD, NASH, HCC
Fluorescent Protein Biosensors (FPBs)		
Hepatocyte Apoptosis	Biosensor	Hepatocellular injury/death
ROS (Hepatocytes & Kupffer)	Biosensor	Hepatocellular injury, Kupffer cell activation
Insulin resistance (Hepatocytes)	Biosensor	Lost suppression of glucose production
Cell Tracking (stellate & Kupffer)	Biosensor	Morphology of early liver injury, proliferation
Additional Imaging		
Steatosis	Brightfield & LipidTox	Normal and/or pathological fat storage in hepatocytes
PMN Infiltration	Imaging labeled PMNs	Adaptive inflammatory response
Mitochondrial Function	TMRE	Hepatocellular health and function
Glucose Uptake	6-NBDG	Hepatocellular insulin resistance marker
Endpoint Measurements		
Hepatocyte Ballooning	Cytokeratin, anti-CK8/18	A key indication of NASH
LSEC Activation	ICAM antibody – IF	Regulates fibrosis and inflammation responses
Fibrosis (Stellate Activation & Collagen Synthesis)	α-SMA, COL1A2 IF	Early fibrosis indicator and direct measure of fibrosis
RNAseq	RNA sequencing, multiple methods	Comparison to patient results
*Based on known clinical data; α-SK1 = sphingosine kinase 1 ; α-SMA = alpha smooth muscle actin; COL1A2 = collagen type 1A2		

Summary of Selected Results in Creating the Experimental MAFLD Disease Model

A.

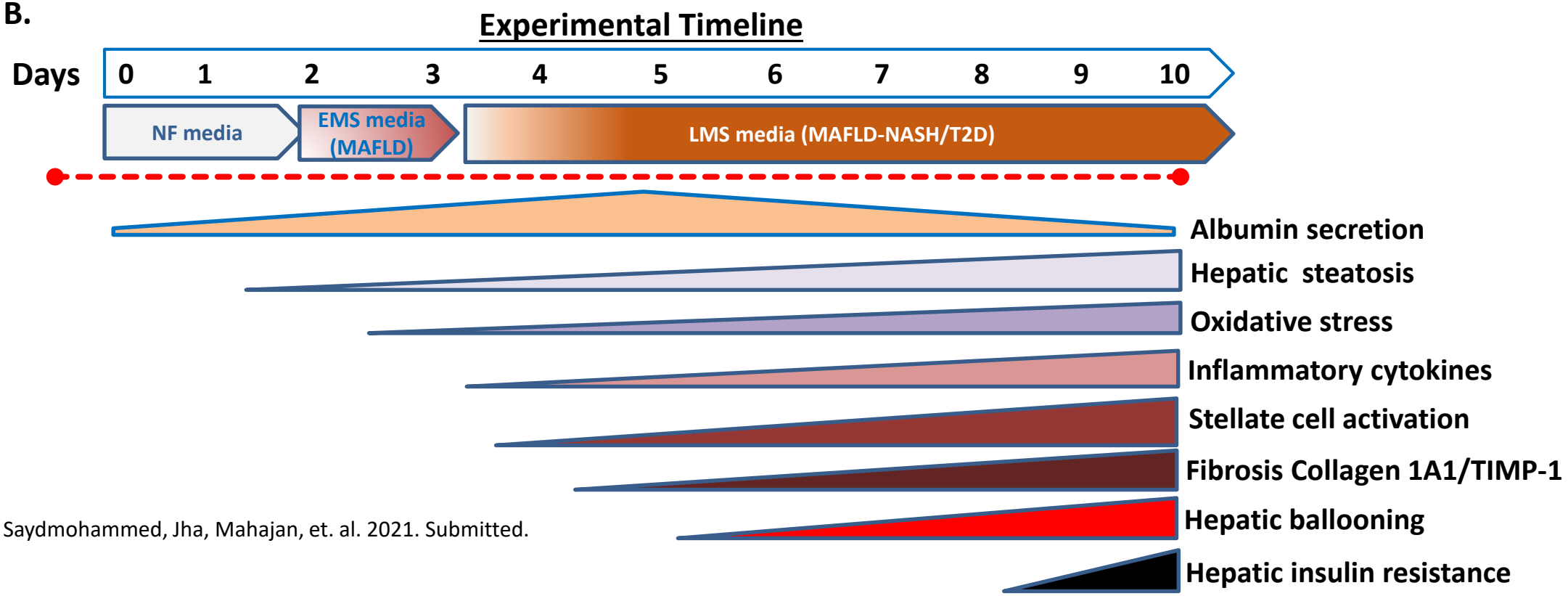
Healthy liver → **MAFLD** → **MAFLD-NASH/T2D**

Normal Fasting (NF) Media

Early Metabolic Syndrome (EMS) Media

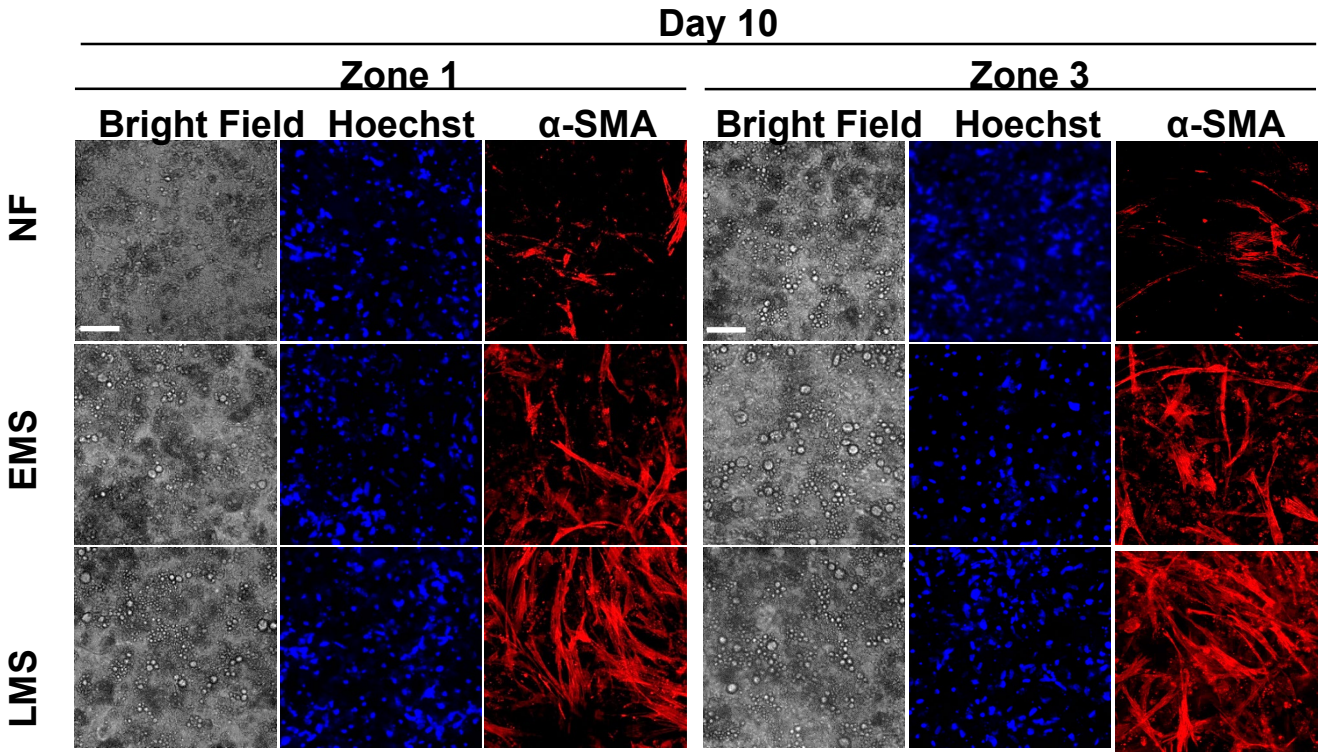
Late Metabolic Syndrome (LMS) Media

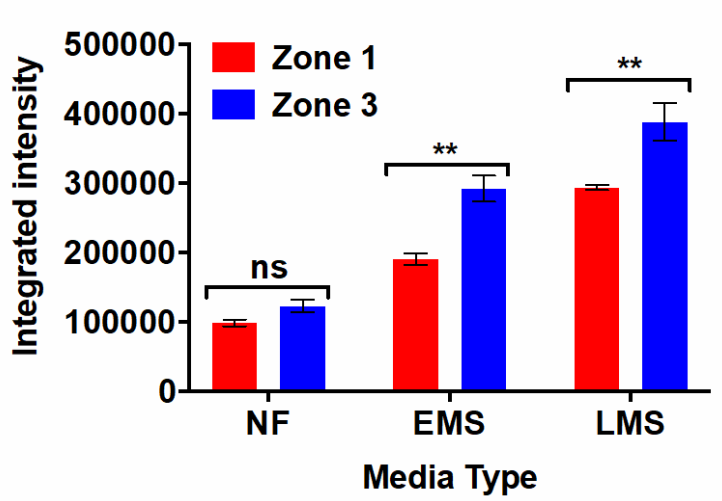
B.



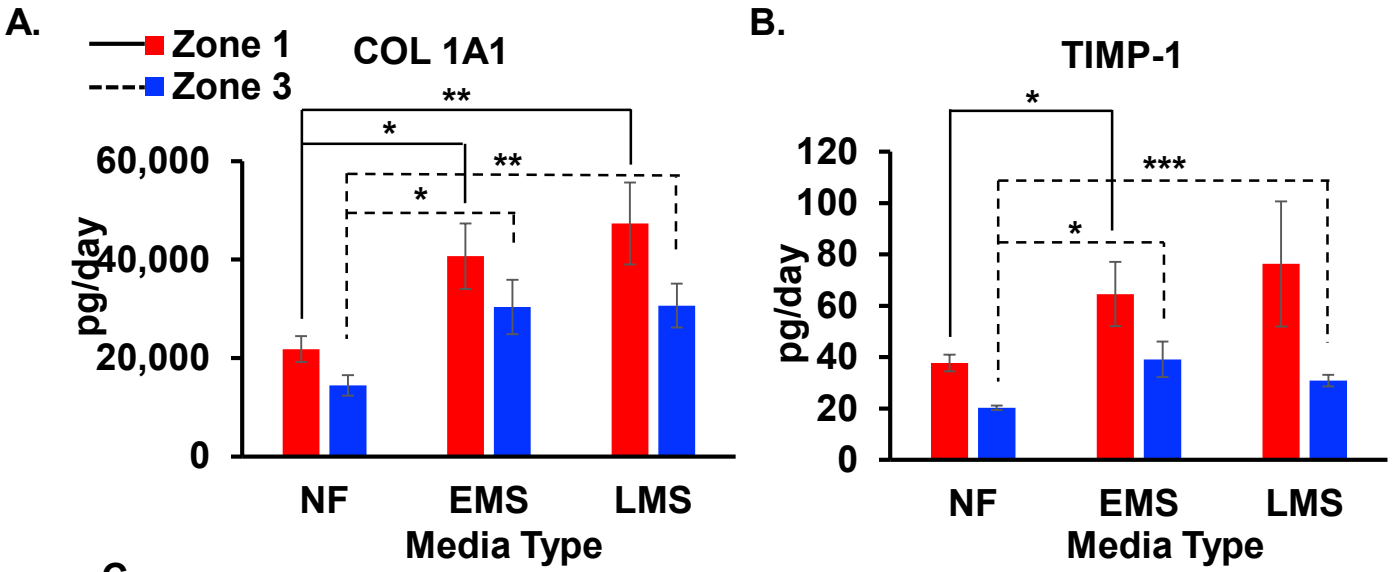
Saydmohammed, Jha, Mahajan, et. al. 2021. Submitted.

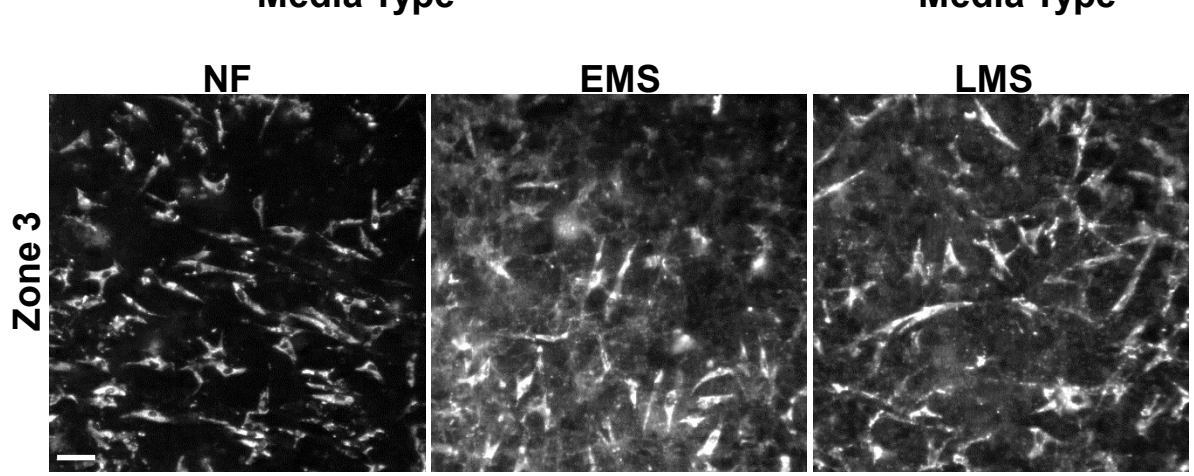
EMS and LMS Media Treatment Results in Increased α-SMA Expression in Stellate Cells Compared to NF Media





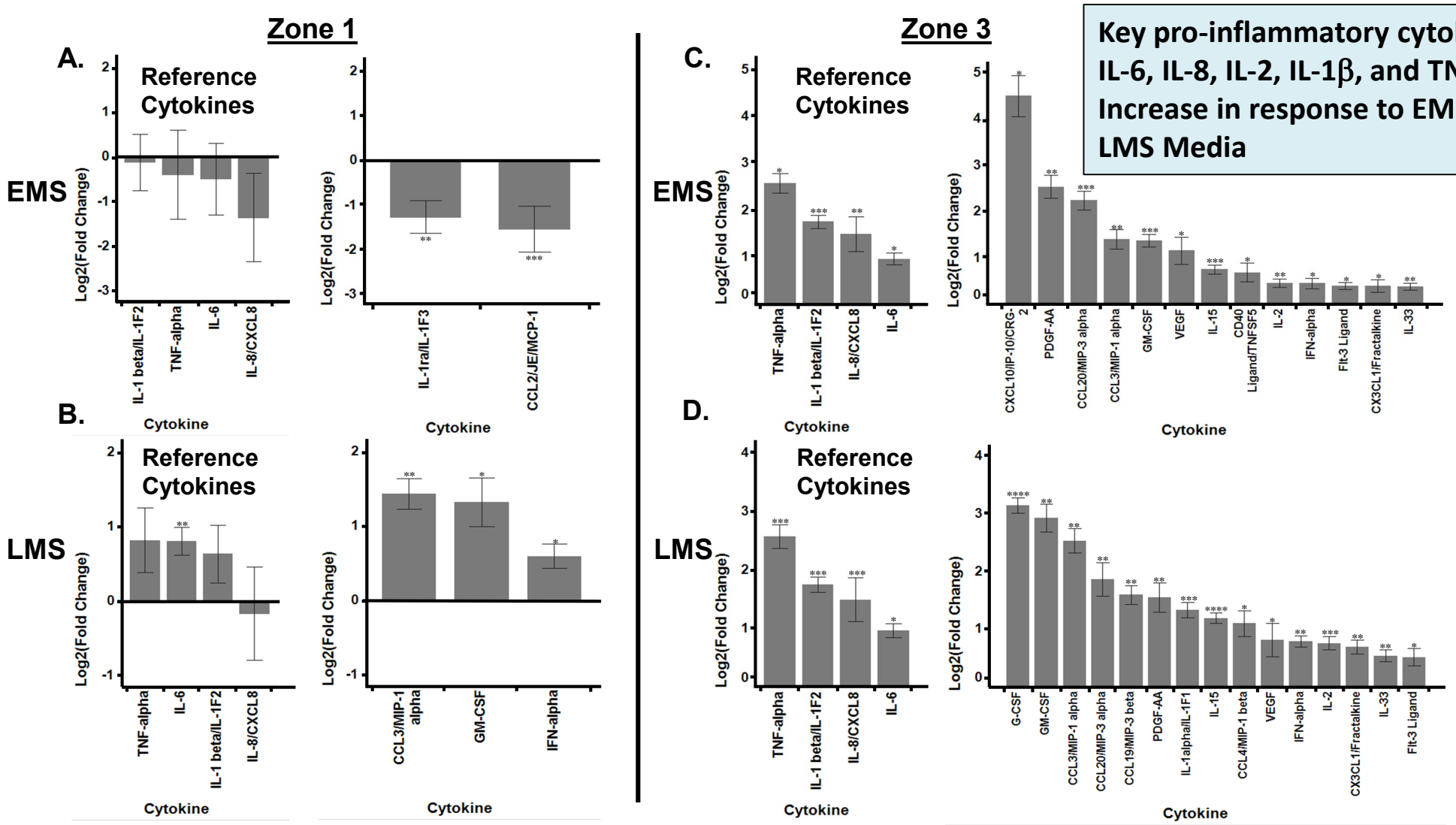
EMS and LMS Media Result in Increased Secretion of the Pro-Fibrotic Markers Collagen 1A1 and TIMP-1 Compared to NF Media at Day 10





Collagen 1A1 Immunofluorescence

Cytokine Panel Secretion Profiles on Day 10 Demonstrate Unique Zone-Specific Signatures for EMS and LMS Media Compared to NF Media

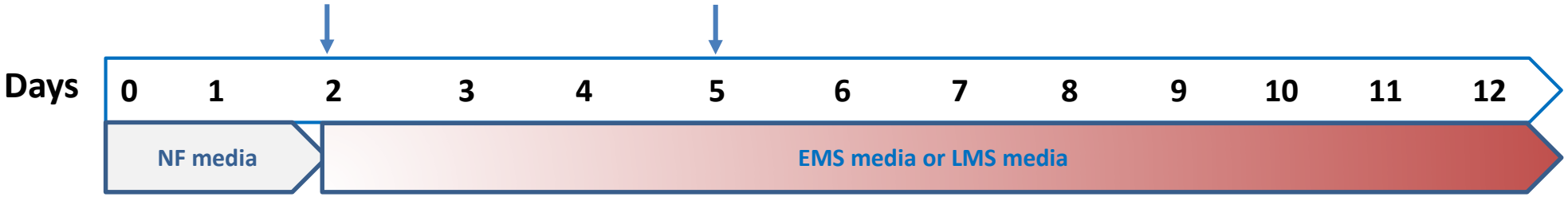


Key pro-inflammatory cytokines e.g. IL-6, IL-8, IL-2, IL-1β, and TNF-α Increase in response to EMS and LMS Media

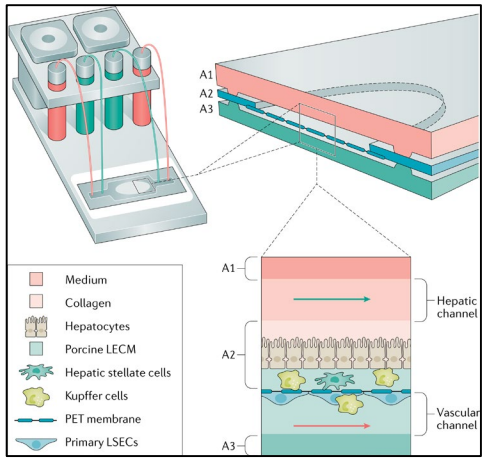
We Have a MAFLD Liver MPS Model That Can Be Progressed:

Now Testing the Predicted Drugs/Combinations to Halt and/or Reverse Disease Progression

Drug Testing Timeline



HBL-MPS MAFLD Progression w/ Drugs



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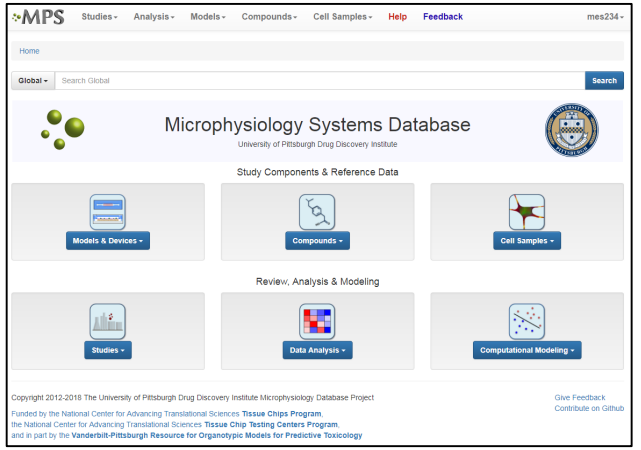
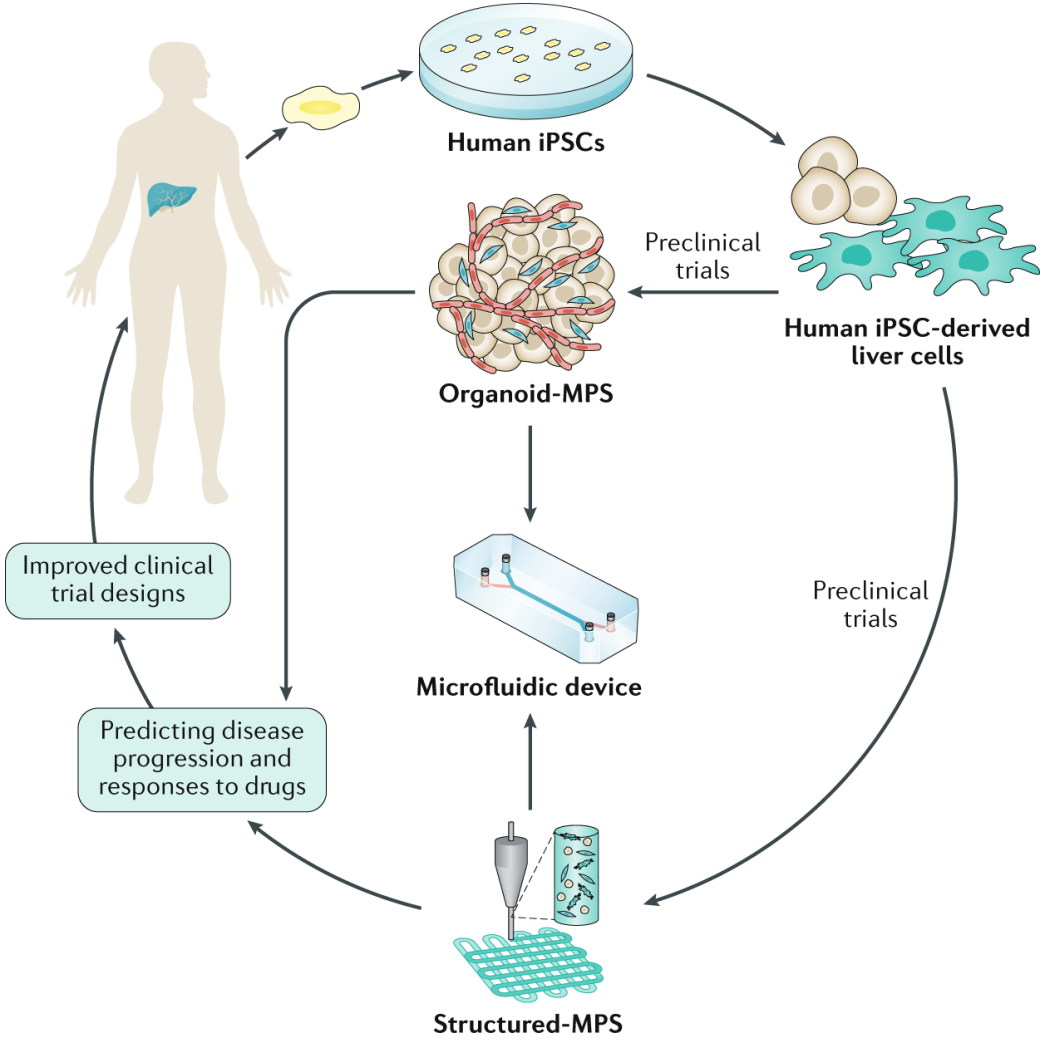
MPS Database



Future: Precision Medicine Will Include the Incorporation of Patient iPSC-Derived Cells into Advanced Human Liver MPS Platforms



Disclosures

- Advisor: **Von Baer Wolff**. A liver cell iPSC/Regenerative Medicine company.
- Co-founder and Chairman: **SpIntellx**. A computational and systems pathology company.
- Co-founder: **Cernostics**. A cancer diagnostics company.

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Dillon Gavlock-Staff Researcher
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