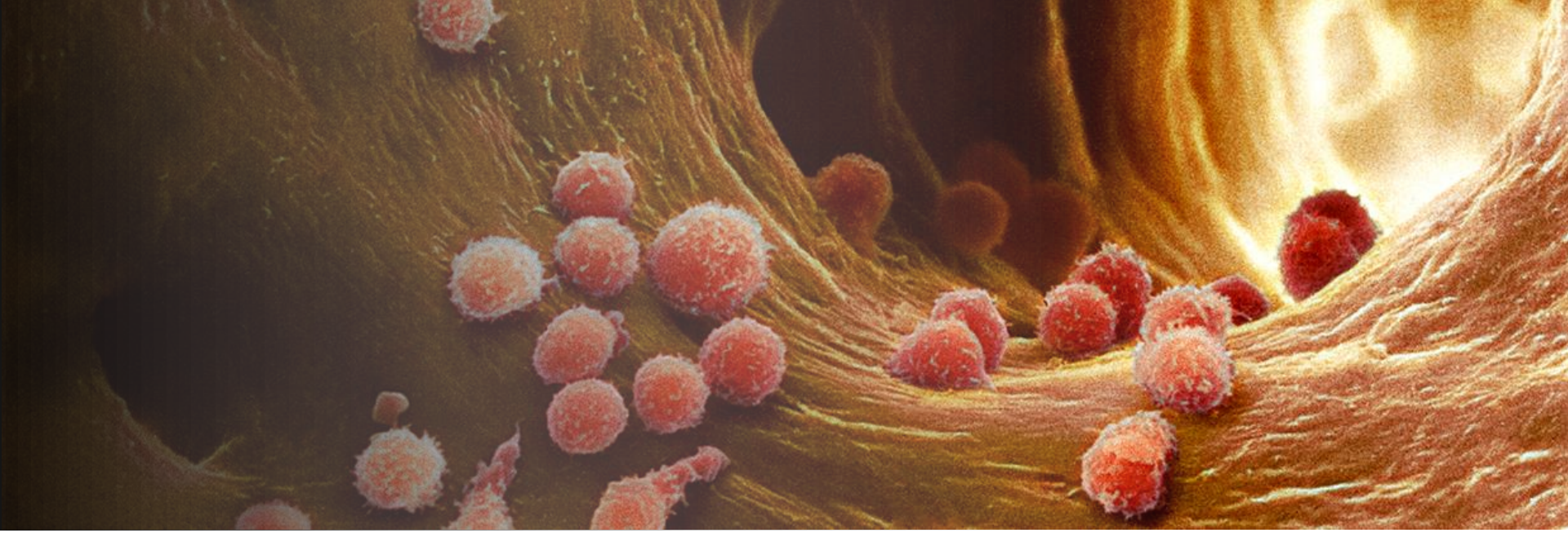


30th June 2016

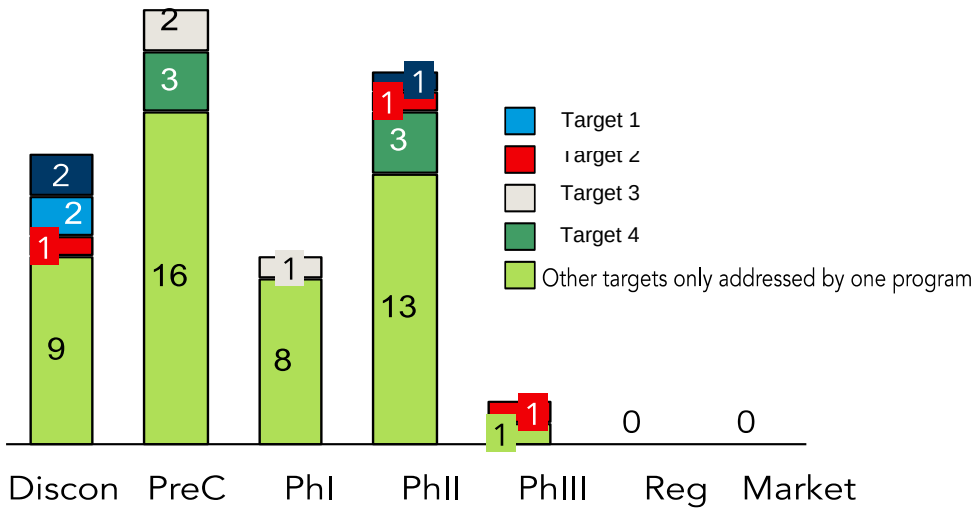
HEMOSHEAR THERAPEUTICS

In Vitro Liver Models And Their Applications For NASH

Ajit Dash, MD, PhD
Senior Scientific Director
HemoShear Therapeutics

Non Alcoholic Steatohepatitis: Disease Problem and Unmet Needs

- 1 in 3 adults in the U.S. has non-alcoholic fatty liver disease
- 75% of people with NASH also have type 2 diabetes
- Fastest growing disease in China and India.
- Approximately 50 active programs with 38 distinct therapeutic targets

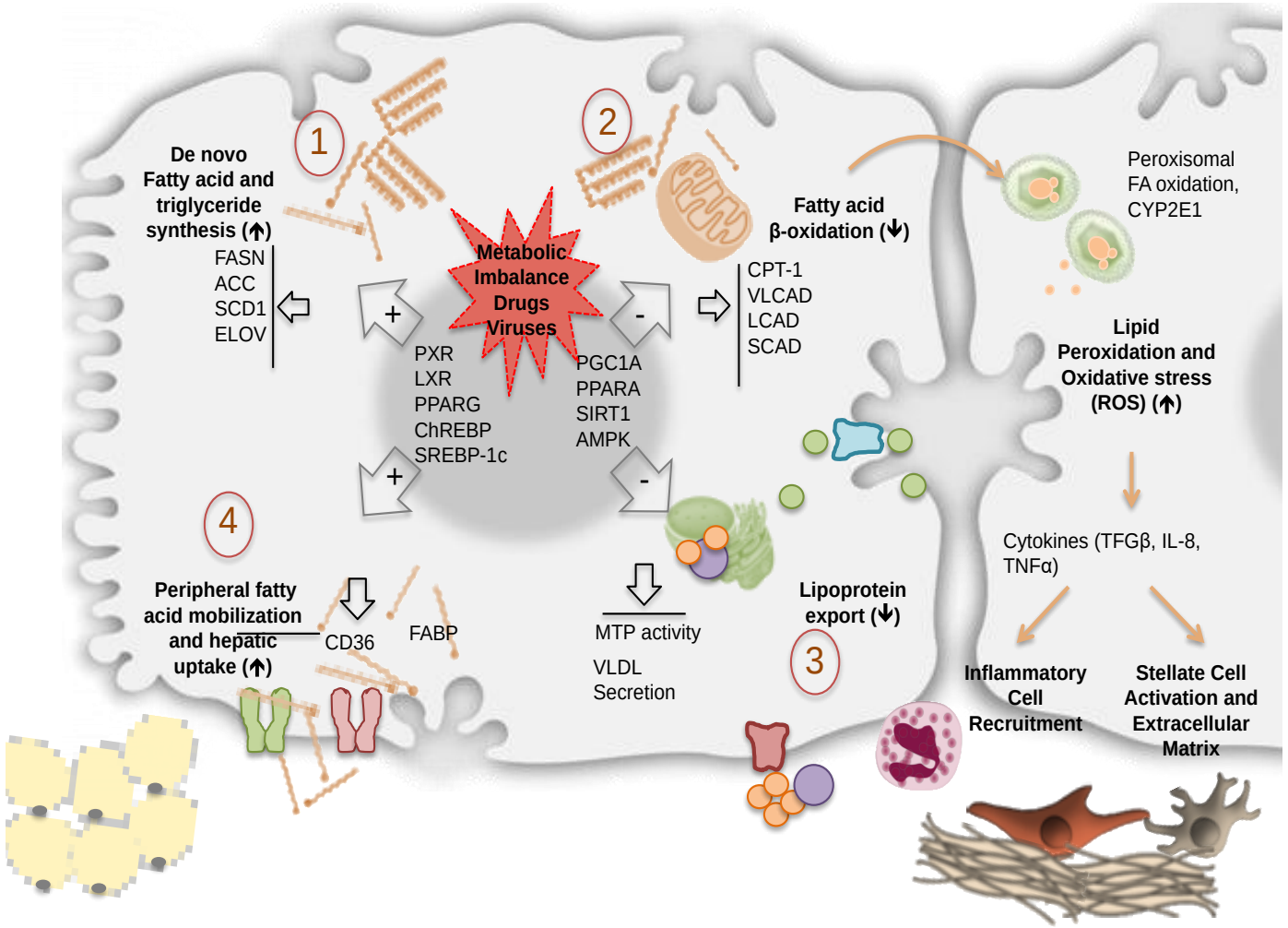


Source: Catenion analysis based on ADIS database, 2014

An ideal in vitro liver model would fulfill various unmet needs:

- Unbiased novel target discovery
- Development of non-invasive translational biomarkers for diagnosis and monitoring disease.
- Understanding/predicting efficacy differences, in stratified sub-populations (Personalized medicine).
- Safety assessment under disease-like conditions.

Underlying Mechanisms of Steatohepatitis are Complex



- Mechanisms of Steatosis
  - 1.↑ Synthesis of lipids/cholesterol
  - 2.↓ β-Oxidation of fatty acids
  - 3.↓ Export of lipoproteins
  - 4.↑ Uptake of fatty acids

Steatosis → Oxidative Stress → Inflammatory Cytokines → Macrophage/Stellate Activation → Extracellular Matrix Deposition

Existing in vitro Models: Challenges and Opportunities

Species	Cell Type(s)	Origin
Human	Hepatocytes	Primary (Healthy/Patient)
	Huh7	Hepatoma
	HepG2	Hepatocellular Ca
	Hepatic Stellate Cells	Healthy/Patient
	LX2 Stellate Cell line	Immortalized
	Hepatocytes + Adipose Cells	
	Huh7 + LX2	
Canine	Hepatocytes	Primary
Rat	Primary Hepatocytes	
	H4IE	Immortalized
	H4IEC3	Immortalized
	PAV-1	Immortalized
Mouse	RAW 264.7 Macrophages and AML-12 Cell co-cultures	Immortalized

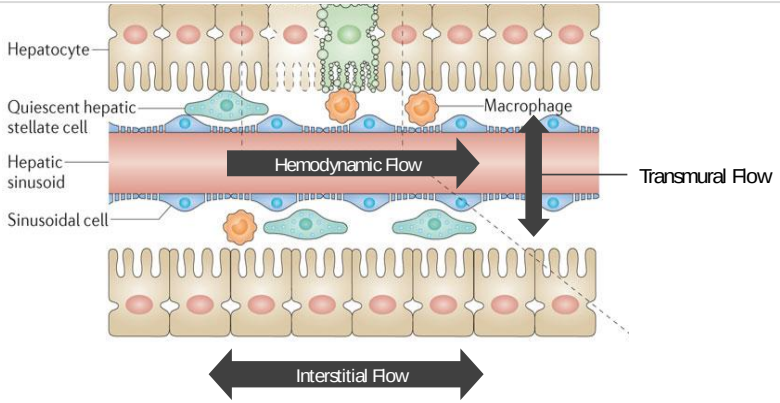
Challenges of existing models employing static flat-plate cell cultures:

- Dedifferentiation and loss of CYP activity.
- Non-physiological levels of glucose and insulin and loss of insulin sensitivity.
- Altered baseline inflammatory state.
- Hypoxia-reperfusion on media change.
- Non-relevant drug and metabolite concentration profiles

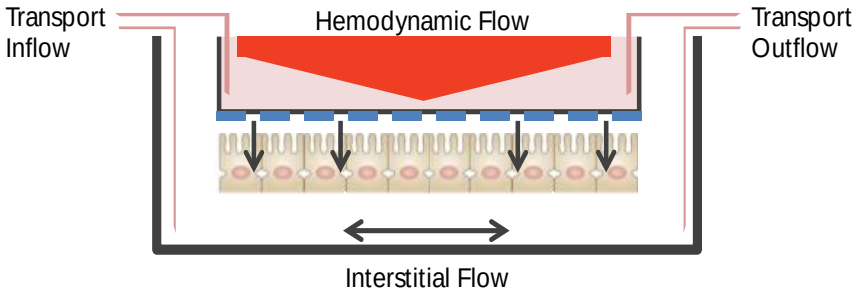
Opportunities for improvement:

- Organotypic approaches (3-D, heterotypic cell interactions, flow).
- Physiological media formulations and drug concentrations based on clinical pharmacokinetics
- Use of Translational biomarkers.
- Big data –omic approaches

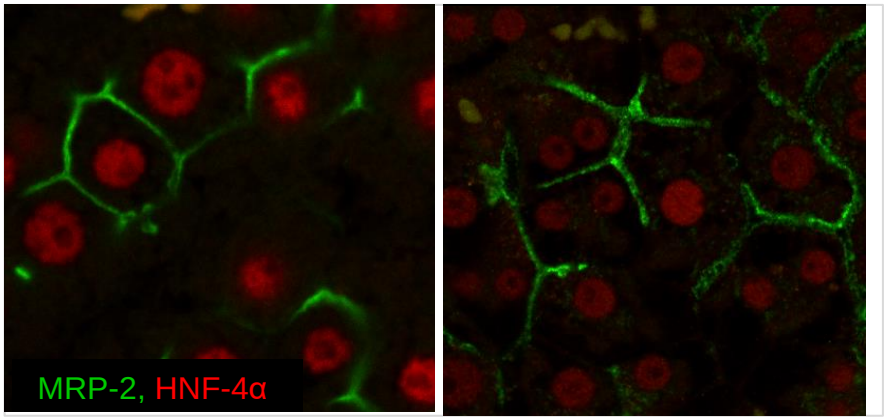
Recreating Physiological Milieu and Parameters in a 3D Culture Configuration



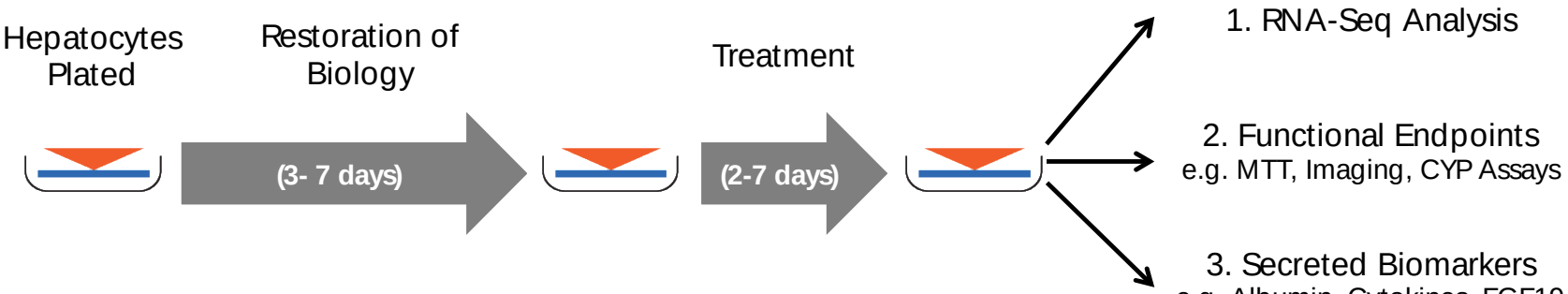
Adapted from: *Nature Reviews Immunology* **14**, 181–194 (2014)



Dash *et al*, AJP 2013, Terelius *et al* Chem Bio 2015, Chapman *et al* 2016

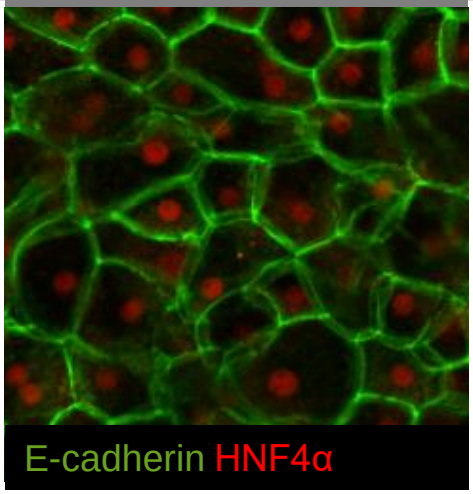


- 3D cell configuration - modeled on sinusoid with hepatocytes ± non-parenchymal cells.
- Simultaneous perfusion and hemodynamics - allows control of drug, nutrient and oxygen gradients
- Effluent and cells can be assessed from top and bottom separately.

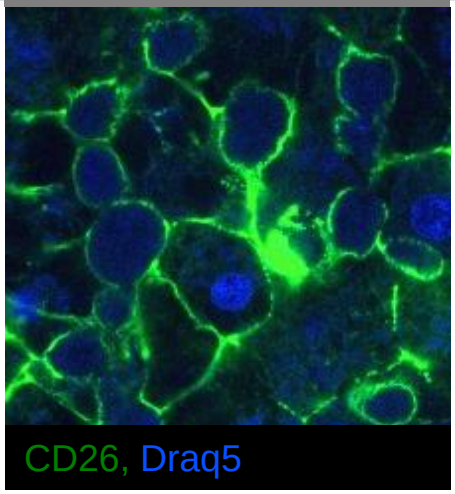


Liver-like Polarized Morphology and Function Maintained Over Time

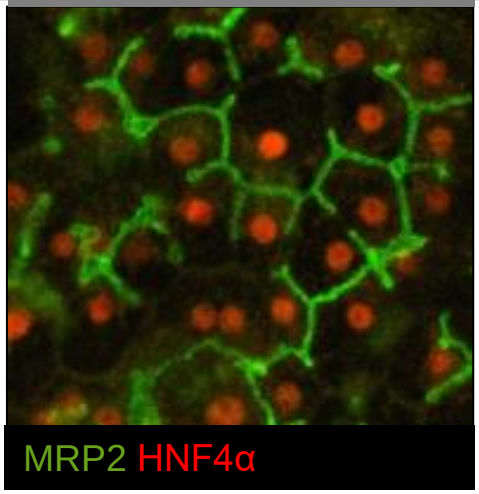
TIGHT JUNCTIONAL PROTEIN



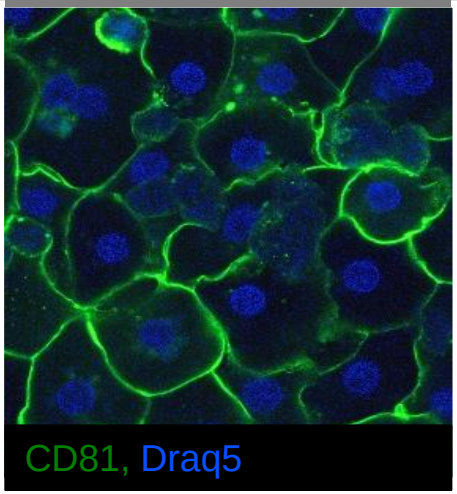
SURFACE ANTIGENIC ENZYME



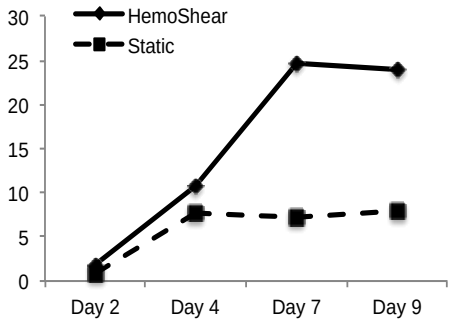
BILIARY EFFLUX TRANSPORTER



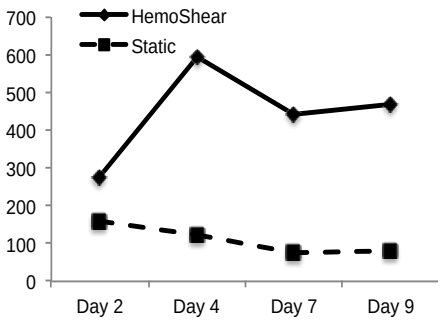
SURFACE GLYCOPROTEIN



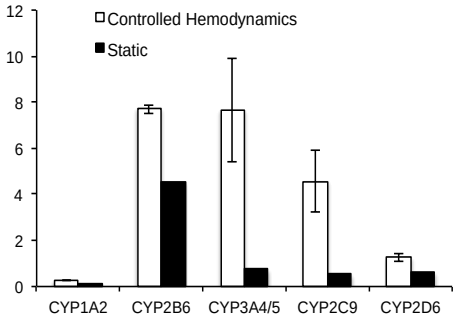
ALBUMIN SECRETION (ANABOLIC)



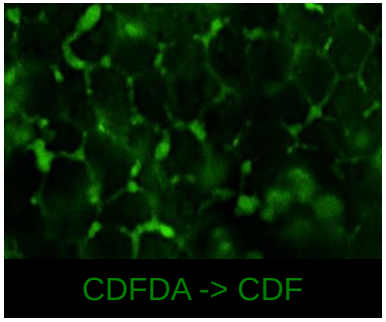
UREA SECRETION (CATABOLIC)



CYTOCHROME P450 ACTIVITY

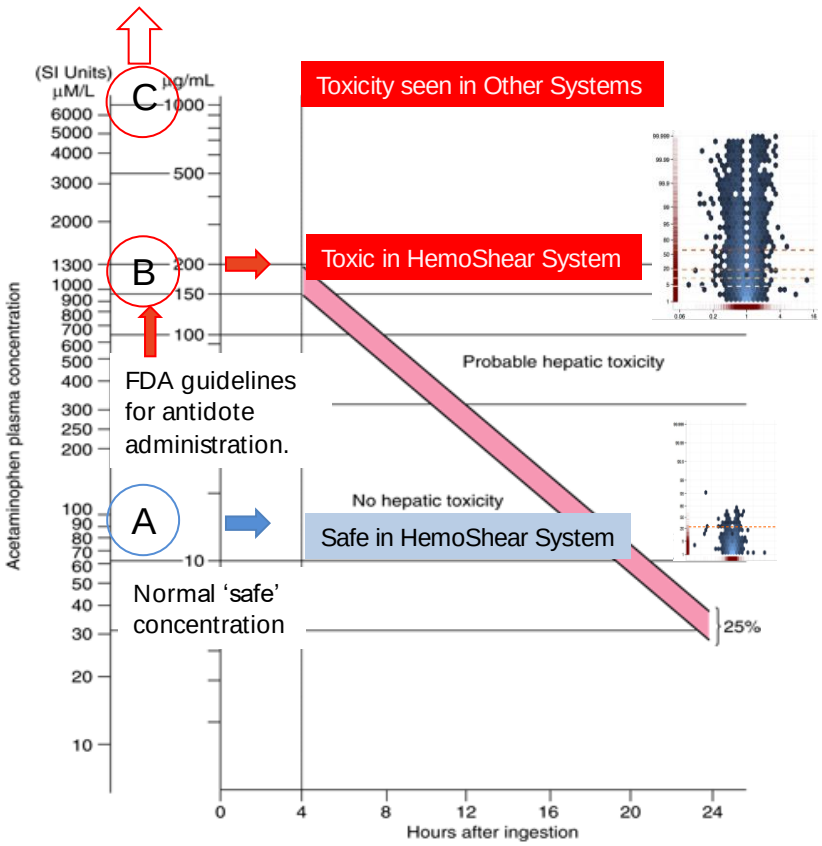


TRANSPORTER ACTIVITY



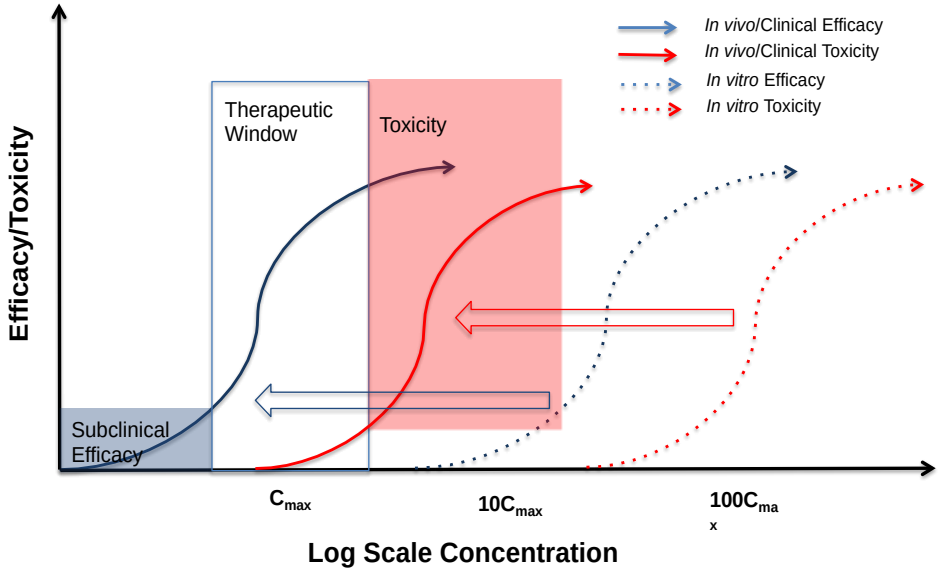
Dash et al SOT 2013, Marukian et al AASLD 2013

Drug Responses Exhibited at Clinically Relevant Concentrations



Rumack-Matthews nomogram for serum concentration thresholds for clinical treatment of Acetaminophen poisoning.

Figler et al AASLD 2015.

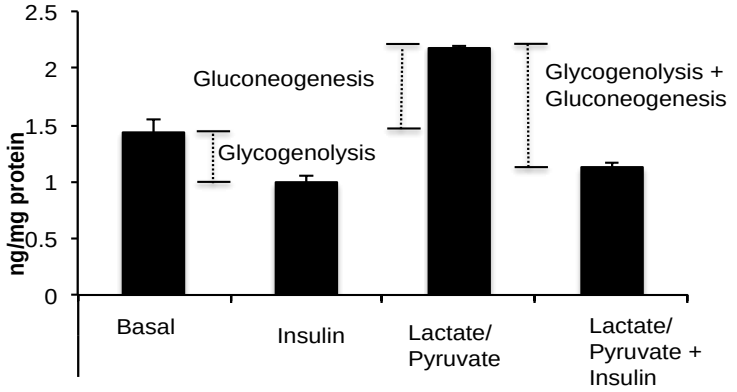


Dash et al Expert Opinion 2012

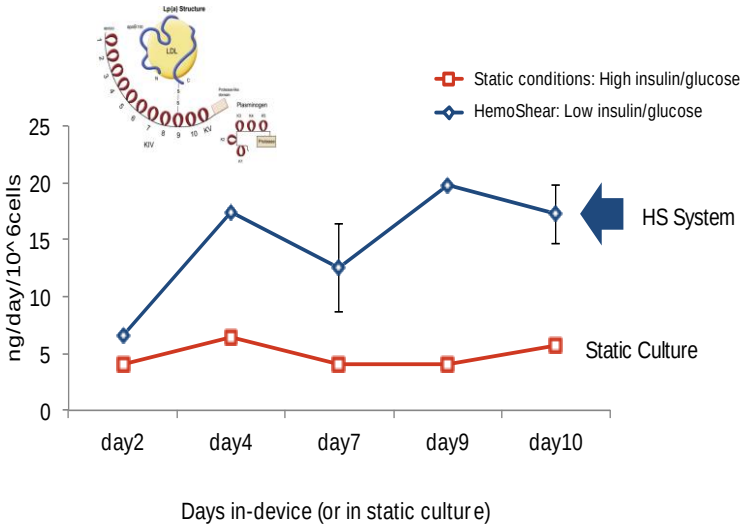
- Efficacy and toxicity responses seen at concentrations that match clinical therapeutic exposures.
- Over 30 drugs assessed for mechanistic differences using transcriptomics. **(NIH SBIR Award R44 DK091104-02)**

Insulin Sensitivity And Lipogenic Responses Maintained

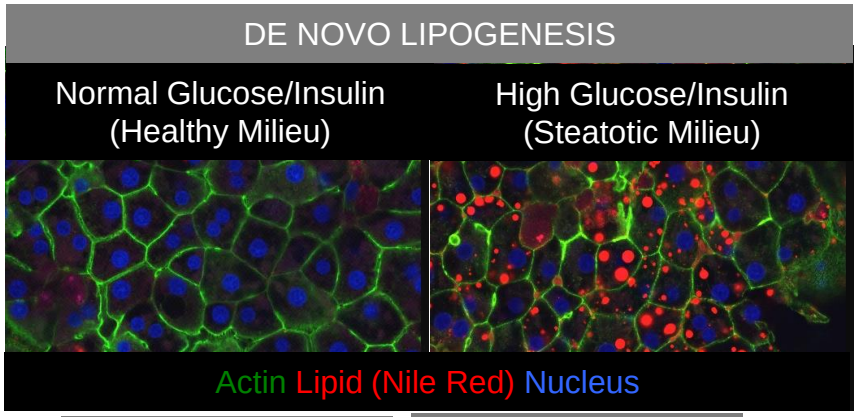
GLUCONEOGENESIS



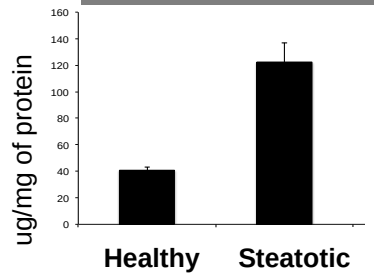
LIPOPROTEIN (a) SECRETION



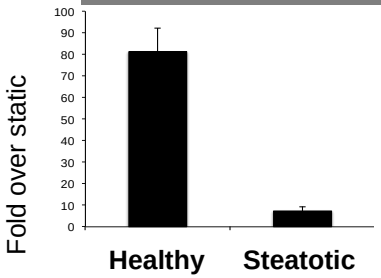
DE NOVO LIPOGENESIS



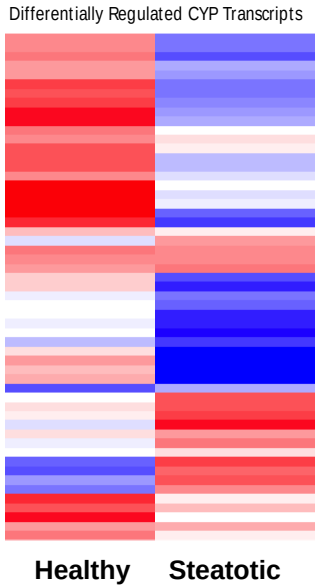
TRIGLYCERIDES



CYP3A2



CYP GENES



CYPs involved in drug metabolism

CYPs involved in cholesterol/ lipid synthesis

- Insulin sensitivity allows culture in a close to physiologic milieu and altered disease-like steatotic phenotype under hyperglycemic, hyperinsulinemic conditions.

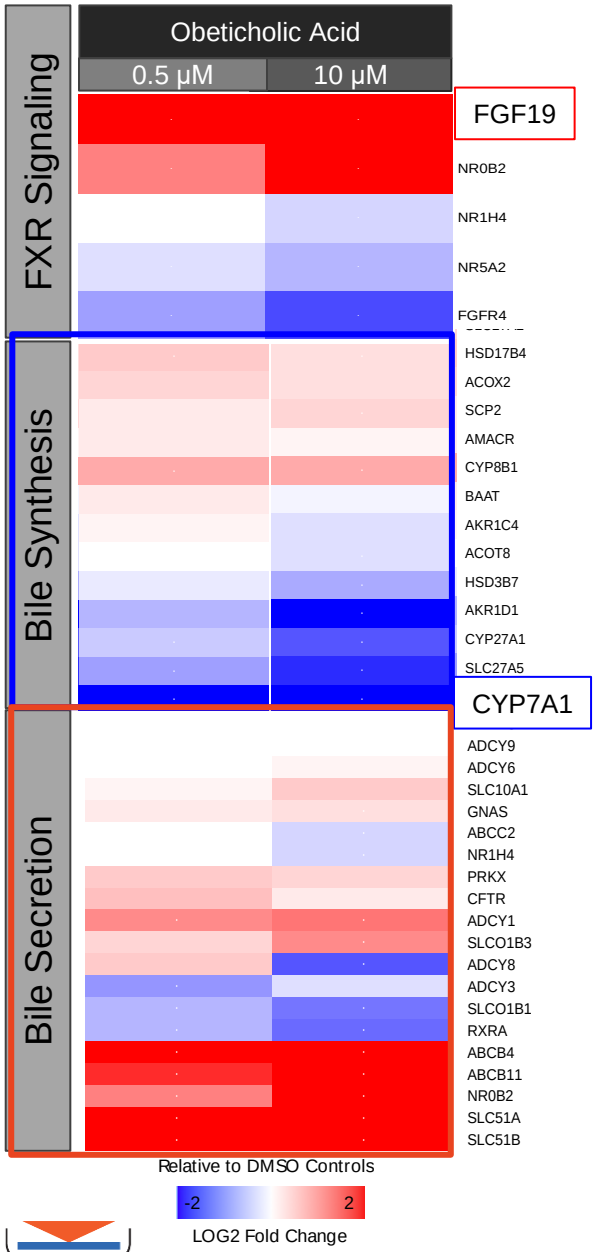
Deering et al AASLD, 2012, Dash et al ADA 2013, Cole et al AASLD 2015

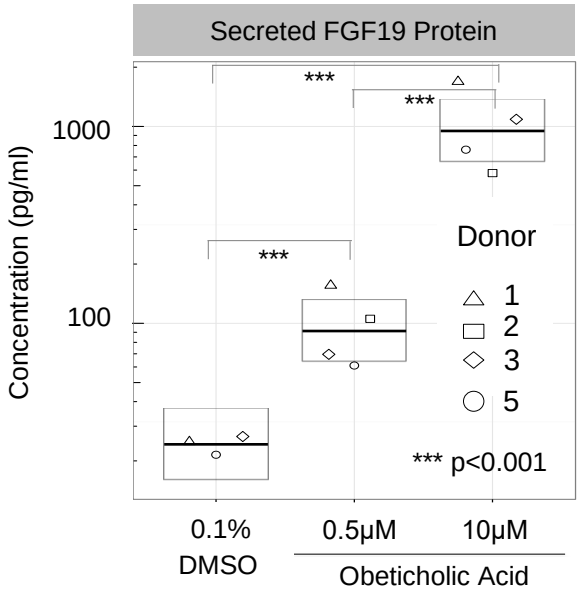
Applications of a Physiologically Responsive Liver Model

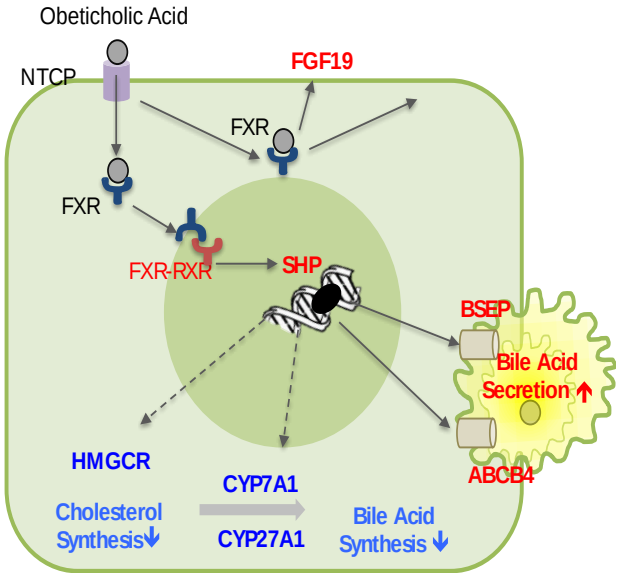
After demonstrating that the system maintained differentiated liver phenotype as evidenced by polarized morphology, liver specific functions, drug metabolizing enzyme and transporter activity and responsiveness to insulin, we tested the model for the following applications:

1. Assessing on-target and off-target pharmaco-toxicology of drugs at clinically relevant concentrations.
2. Distinguishing transcriptomic signatures of various phenotypes of drug induced liver injury (DILI).
3. Studying underlying mechanisms of drug induced steatohepatitis that could help understand potential NASH targets.
4. Developing a lipotoxic model with milieu mimicking metabolic disease.

Assessing On-target Pharmacology of Obeticholic Acid



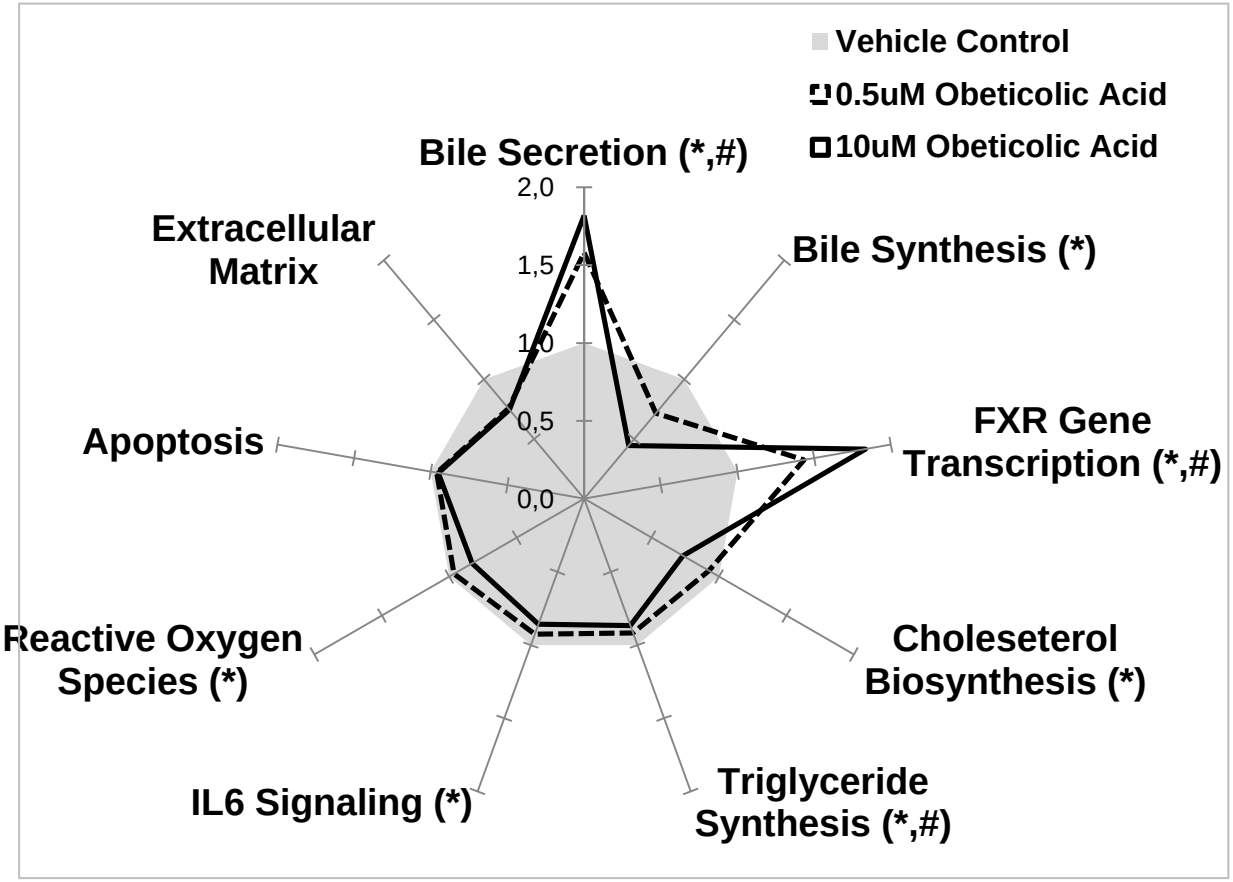


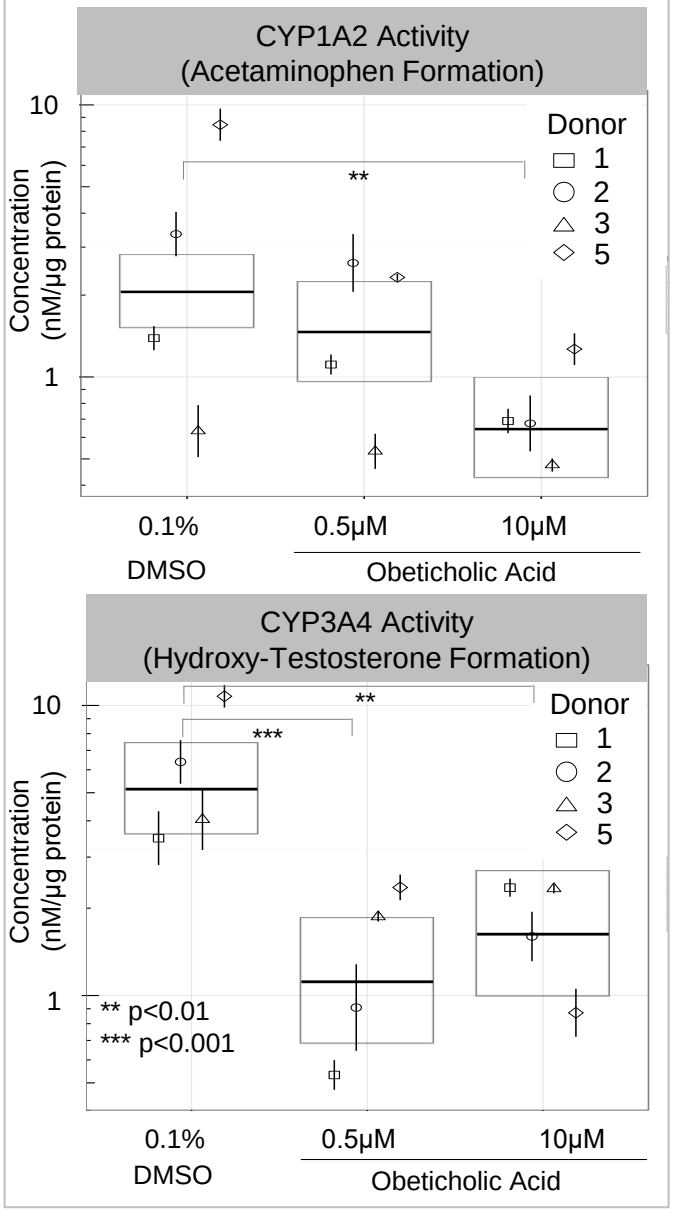


- Strongly induced FGF19 in hepatocytes, both at a gene and protein level, confirming a direct hepatic effect in addition to the widely appreciated FGF19 loop through the gut.
- CYP7A1 was the most down-regulated differentially expressed gene in the transcriptome, with simultaneous down-regulation of the bile synthesis pathway genes.

Sanyal, Oral presentation AASLD 2015

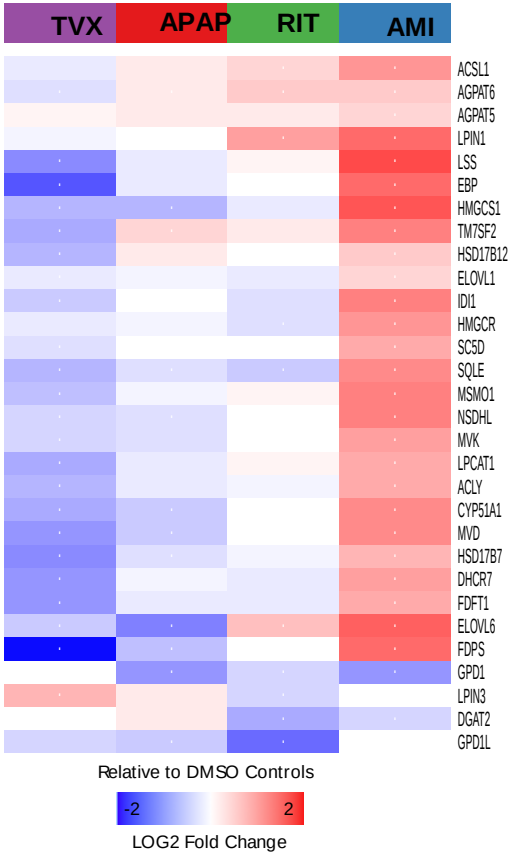
Pharmaco-toxicological Signature of Obeticholic Acid and Impact of CYP Activity

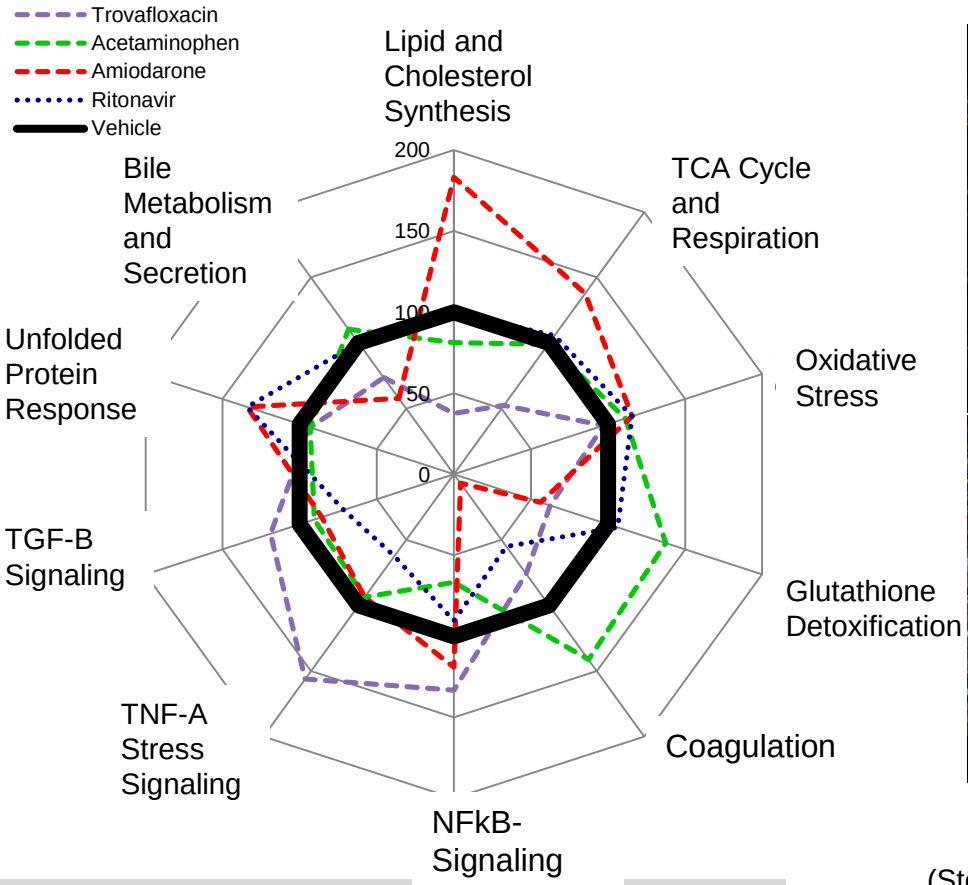


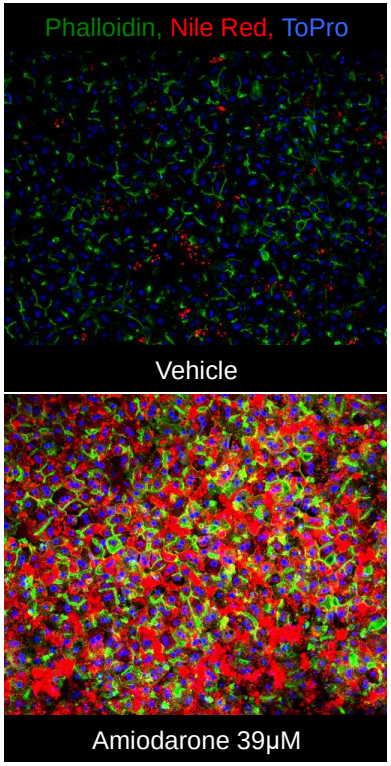


- Pathway analysis and scoring confirmed beneficial effects of obeticholic acid on reducing steatotic indices and inflammatory signaling.
- Functional CYP assays revealed that obeticholic acid suppressed CYP1A2 and CYP3A4 activity.

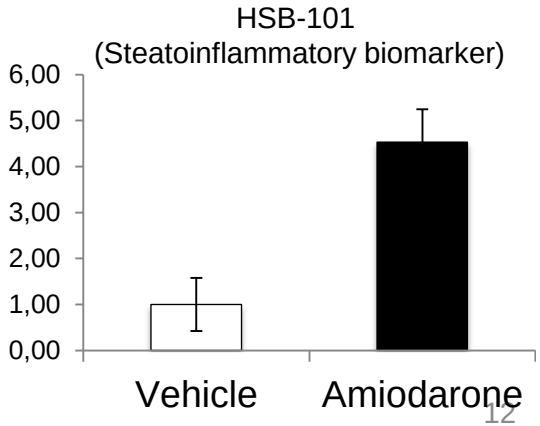
Distinguishing Drug Induced Steatohepatitis Signatures From Other Forms of Drug Induced Liver Injury





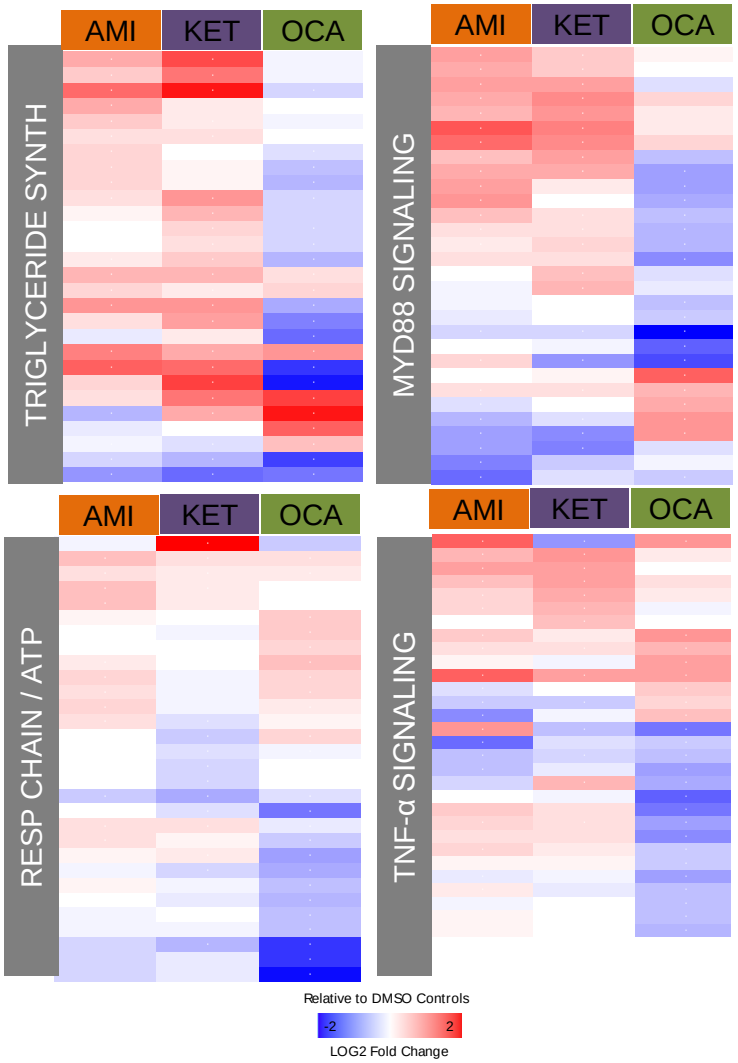


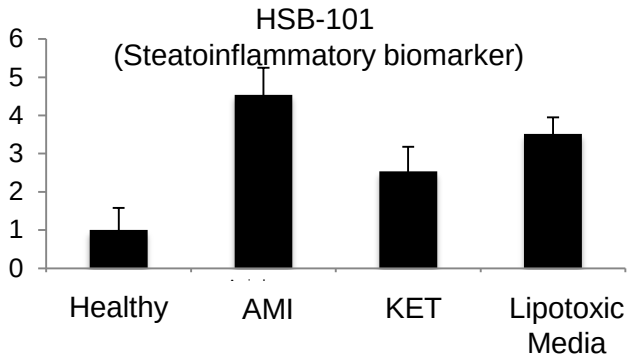
- Transcriptomic analysis allowed us to characterize distinct signatures for different drugs having different DILI phenotypes.
- Assays like Nile Red (neutral lipid) and secreted protein biomarkers in effluent media were confirmatory functional endpoints that defined the steatohepatitic phenotype.

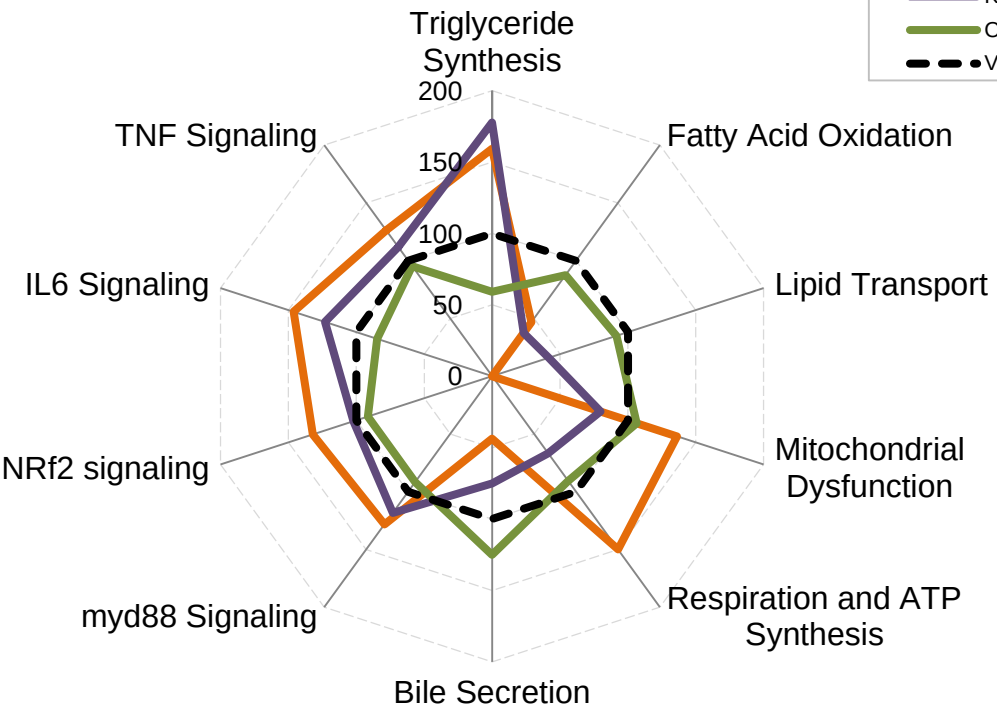


Figler et al AASLD 2015.

Understanding Mechanisms and Potential Targets of Steatohepatitis



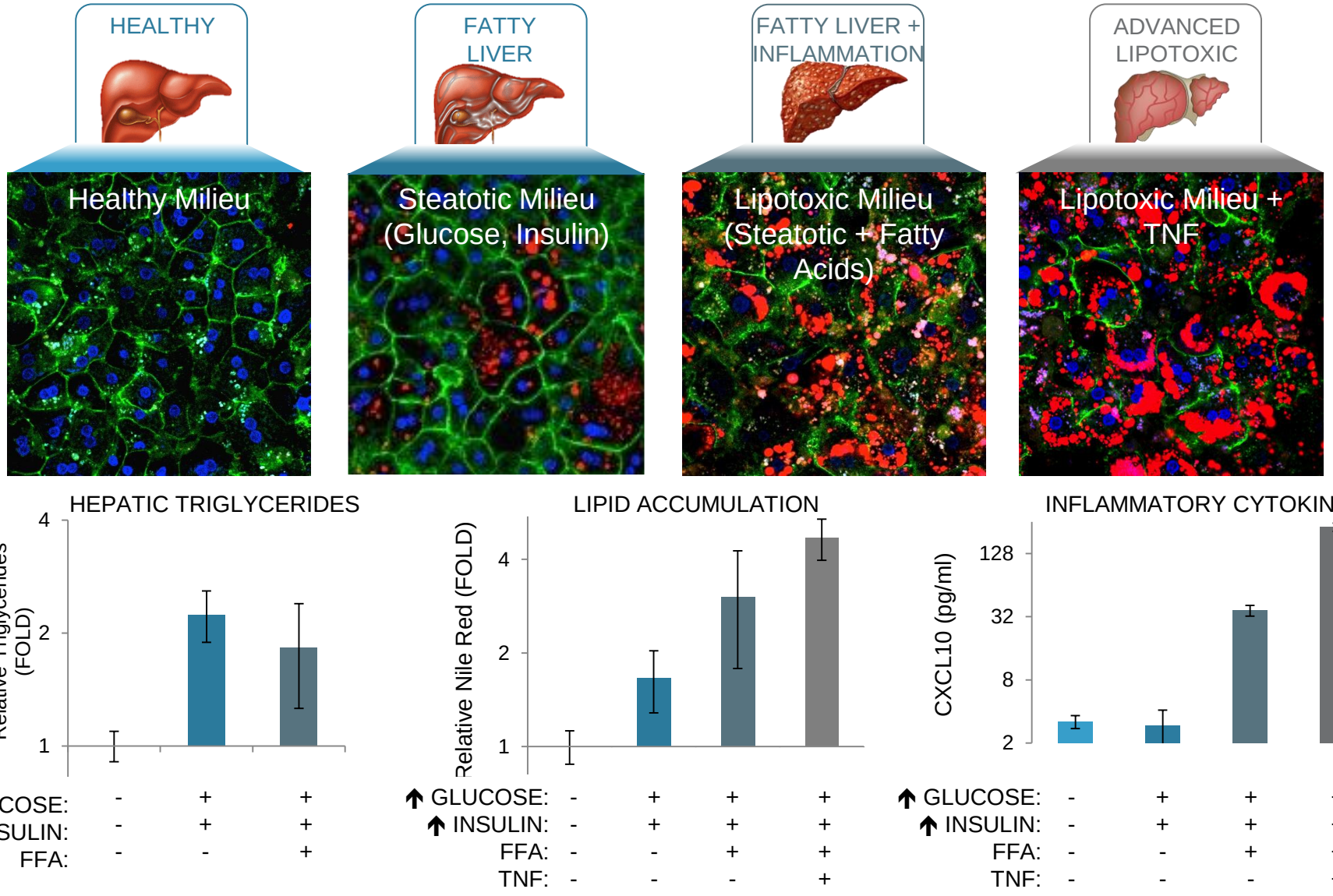




- Differential analysis of transcriptomic signatures for different drugs causing steatohepatitis versus those causing NASH may offer insights into mechanisms and targets.

Dash et al EASL 2016.

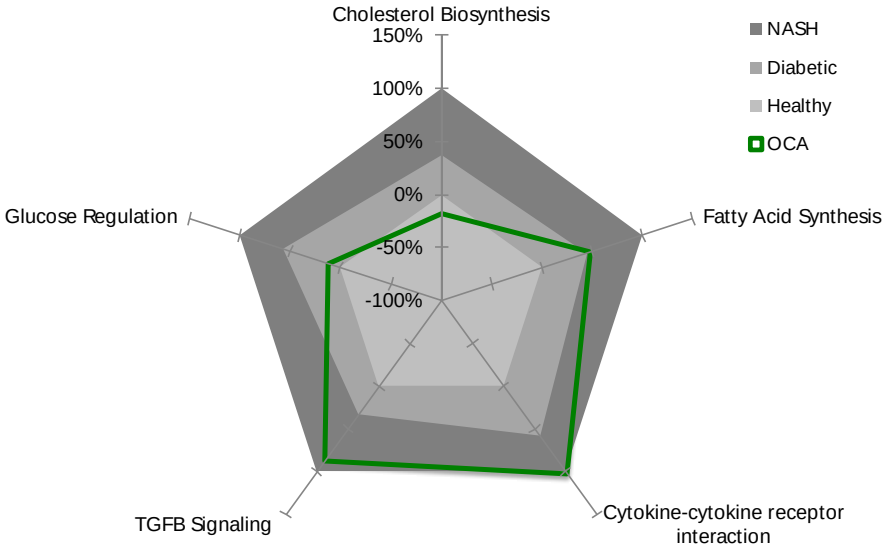
Liver Models to Recapitulate Metabolic Disease Spectrum

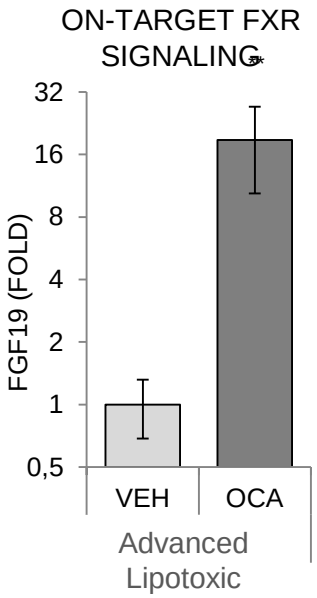


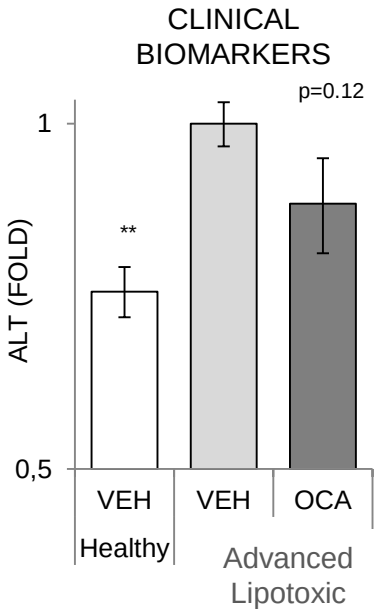
- Lipotoxic metabolic disease model has Kupffer cells and stellate cells added on opposite side of the membrane

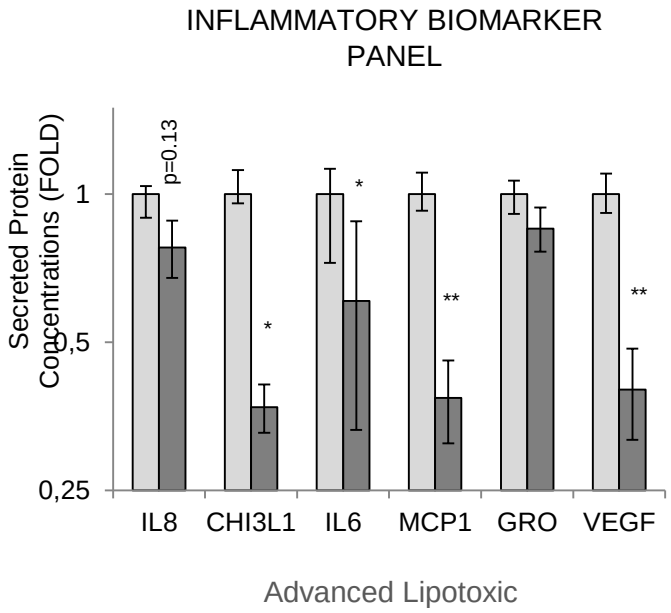
Ongoing Validation of Drug Responses in the Advanced Lipotoxic Liver System

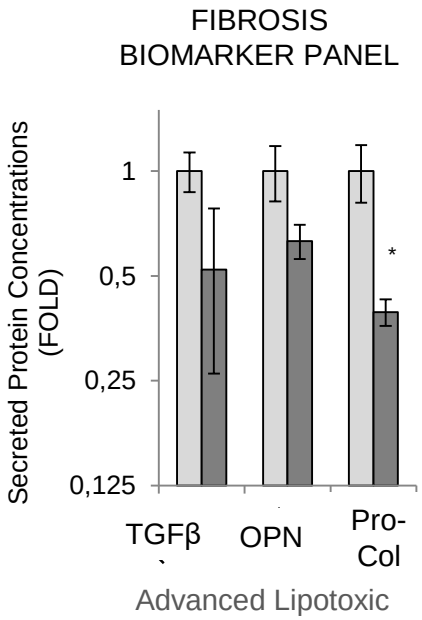
- In the advanced lipotoxic liver system, OCA
  - Reduced ALT levels (a clinical biomarker for NASH)
  - Promoted a robust increase in downstream targets of FXR signaling, including FGF19
  - Reduced several markers of inflammation
  - Reduced markers of fibrosis











**p<0.005,* p<0.05 relative to VEH

Conclusions and Future Directions

- **The physiologically responsive liver model allows assessment of on-target and off-target pharmaco-toxicology of drugs at clinically relevant concentrations.**
- **Comparative analysis of transcriptomic responses of drug induced steatohepatitis, lipotoxic NASH-like conditions and drugs that impact NASH could help identify and understand potential NASH targets.**
- **Ongoing/Future activities include:**
  - **Benchmarking signatures against clinical samples.**
  - **Analysis of non-parenchymal cell response within system.**
  - **Lipidomic analysis to gain a better understanding of lipid fractions under lipotoxic milieu and how they correlate with transcriptomics.**
  - **Characterization of translatable functional responses such as histology and extracellular matrix composition measurements.**
  - **Comparative analysis of drug response under healthy versus lipotoxic conditions and stratified patient derived hepatocytes versus human hepatocytes could provide additional insights about useful applications of this system.**

Acknowledgements and Funding

Brian Wamhoff, PhD
Head of Innovation

Arun Sanyal, MD
Consultant & Collaborator

Robert Figler PhD
Senior Director

Ryan Feaver, PhD
Program Leader, NASH

Banu Cole, PhD
Director

Entire HemoShear Scientific Team

NIH SBIR Grants:

• R43/R44 DK100136: Development of an in vitro system of human hepatic steatosis.

• R44 DK104456-01: Development of a human physiological multi-cellular liver platform for drug-induced liver injury and disease.

• R43/R44DK091104: Development of a human hepatocyte predictive pharmacology and toxicology system.

• R44GM109539: Development of an iPSC-derived human hepatocyte platform for drug development.