Differential Drug Responses Amongst 4 Idiosyncratic Drug-Induced Liver Injury Patient-derived Human Liver Organoids

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Introduction

Idiosyncratic DILI is a rare but important cause of liver injury. Pre-clinical liver models frequently fail to identify high risk medications and susceptible individuals largely due to inadequate *in vitro* systems and inability to sample population diversity. Our previous work demonstrates the utility of human liver organoids (HLOs) to model human liver toxicity of intrinsic DILI agents. To gather population diversity and capture idiosyncratic events *in vitro*, we have begun to generate HLOs on a per-patient basis.

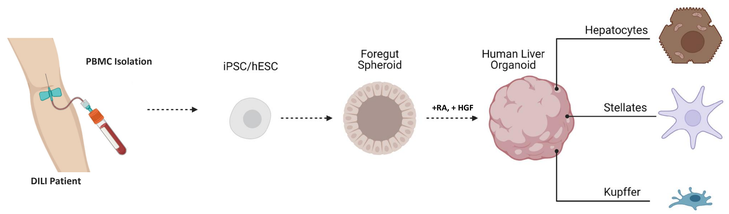
Aims

The aim of our study is to build and optimize a patient HLO screening model that 1) leverages interindividual response to more reliably identify DILI agents compared to current industry standards and 2) can recapitulate the idiosyncratic phenotype *in vitro* to identify toxic mechanisms and improve our understanding of pathogenesis.

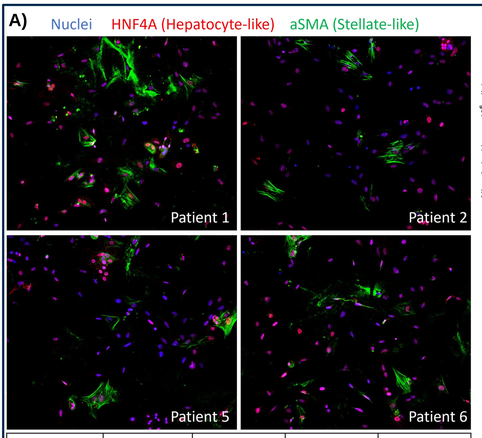
Selection of Patient Candidates

All selected patients were adults (> 18 yrs) with idiosyncratic DILI from a single drug or HDS product and previously enrolled in the DILIN prospective study at the University of Michigan (clinicaltrials.gov = NCT00345930). Enrollment was focused on the following DILI agents, amoxicillin/ clavulanate (**AC**), trimethoprim/ sulfamethoxazole (**TS**), and green tea extract (**EGCG**). Subjects with HIV, HCV, or HBV infection, prior organ or bone marrow transplant, or receiving immunosuppressive drugs were excluded. All subjects provided written informed consent and had 2 x 10 mL tubes of blood drawn for iPSC reprogramming and 1 x 10 mL blood drawn for PBMC preservation.

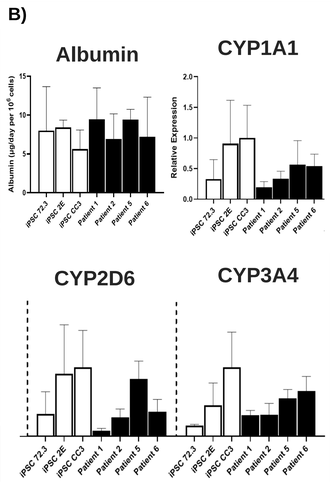
HLO Differentiation and Characterization



HLOs were differentiated from iPSCs based on a protocol by the Takebe lab at Cincinnati Children's Hospital. In brief, iPSCs are differentiated into definitive endoderm (foregut spheroids) followed by retinoic acid and hepatocyte growth factor treatment to form HLOs, consisting of a mixture of hepatocytes and non-parenchymal liver cells. We have previously adapted these HLOs to various cell culture platforms, including 384-well based assays and the Emulate Chip system.[2]

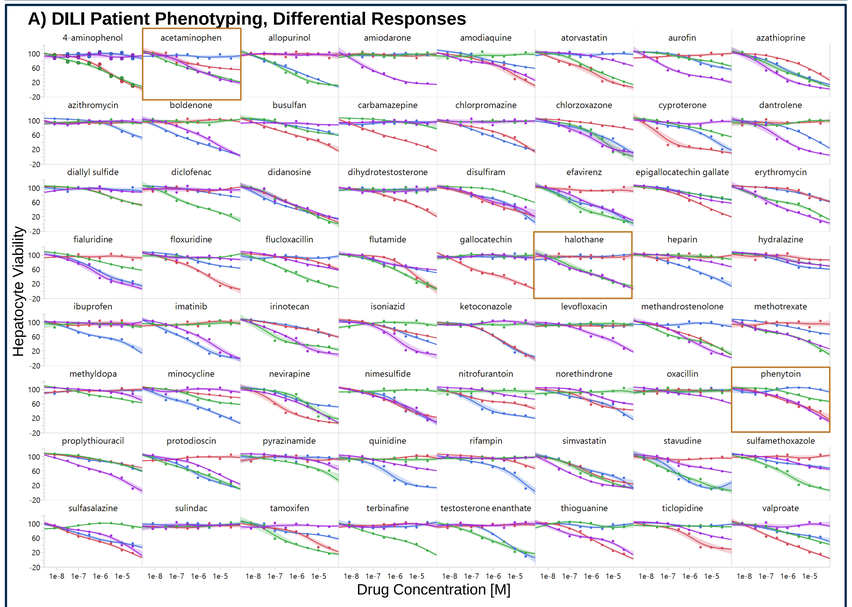


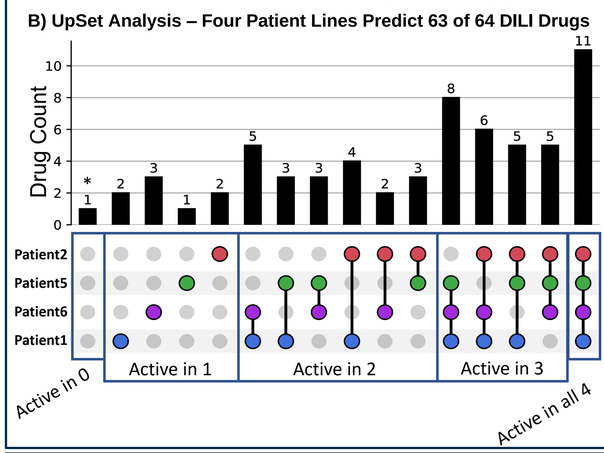
Cell Type %	Patient 1	Patient 2	Patient 5	Patient 6
Hepatocyte-like	62	74	70	66
Stellate-like	32	21	26	29



A) HLOs differentiated from patient PBMCs are dispersed and stained for hepatocyte and stellate markers. Based on immunostaining, we confirm that 60-70% hepatocyte-like cells and 20-30% stellate-like cells are produced for each patient, consistent with three control iPSC lines and published results.[1] **B)** Albumin, a guiding serum biomarker for liver health and function, is detected in comparable levels in all patient and previously characterized HLO lines. In addition, relative expression of CYPs 1A1, 2D6, and 3A4 suggest that drug metabolism may differ amongst our first four patient HLO lines. **C)**

4-Patient DILI Drug Screen – Heterogeneous Responses

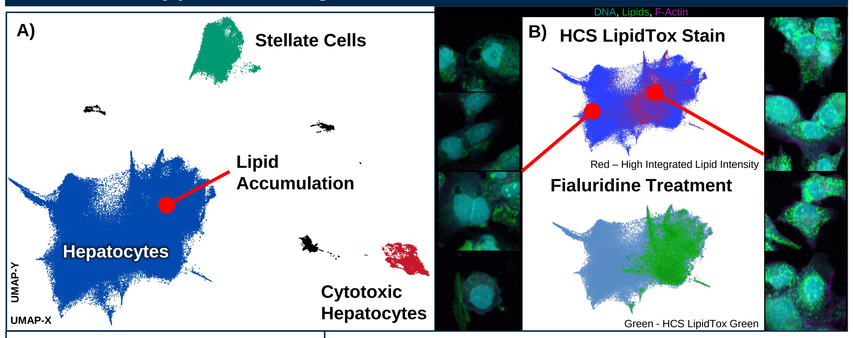


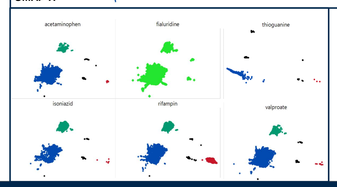


A) 64 drugs screened in 10-pt dose-response in four patient HLO lines showing interindividual variation in response. Select drugs that display interindividual differences in response are highlighted in gold. **B)** UpSet analysis shows that 11 drugs were active in all four patients, 24 drugs were active in three patients, 20 drugs were active in two patients, 8 drugs were active in one patient and only one drug was not hepatotoxic in any patient (Sulindac).

Colors: Patient 1, Patient 2, Patient 5, Patient 6

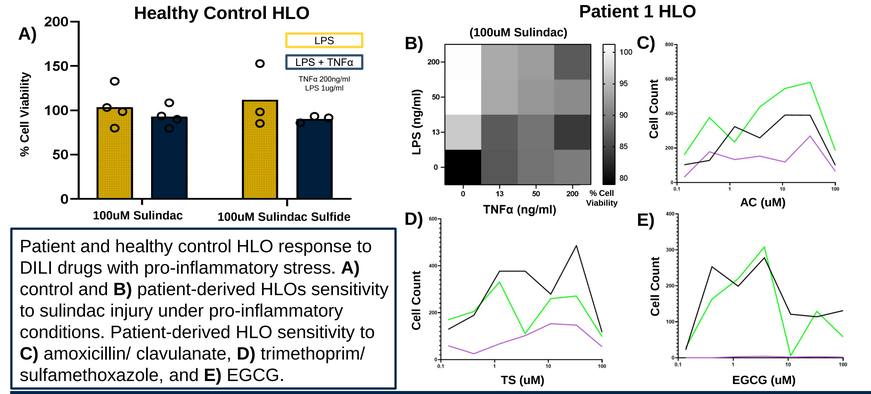
Phenotypic Profiling of DILI Patient HLO Cells - UMAP





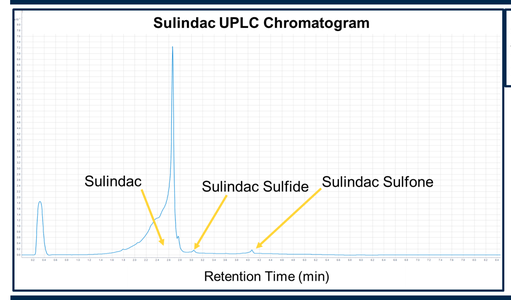
A) Single-cell phenotypic profiling & UMAP embedding for 300k HLO cells across 1109 cellular measurements show effective clustering by cell type and drug specific responses. **B)** UMAP colored by HCS LipidTox Green stain showing a gradient of lipid accumulation in the hepatocyte population. Fialuridine shifts the hepatocyte population to lipid enriched, consistent with its mitochondrial toxicity mechanism. **C)** UMAP colored by cell population for select compounds show cell type-specific effects.

Implications of Pro-inflammatory Stimulus on Drug-induced Injury



Patient and healthy control HLO response to DILI drugs with pro-inflammatory stress. **A)** control and **B)** patient-derived HLOs sensitivity to sulindac injury under pro-inflammatory conditions. Patient-derived HLO sensitivity to **C)** amoxicillin/ clavulanate, **D)** trimethoprim/ sulfamethoxazole, and **E)** EGCG.

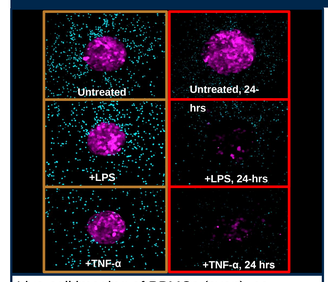
Metabolite Identification in Non-Responsive Interactions



A) Cell supernatant from Patient 1 HLO line treated with 100uM Sulindac in the presence of 200ng/mL TNFα and LPS.

In parallel with cell viability readouts, we are optimizing methods for toxic metabolite detection of parent drugs from our DILI patient screen. Acetaminophen and sulindac are known to produce toxic metabolites that are responsible for liver injury. Using UPLC and MS we will quantify the metabolic activity of patient lines with the goal of deconvoluting interindividual drug response.

Future Directions



Live-cell imaging of PBMCs (cyan) co-cultured with HLO (magenta) over 24 hours

As we proceed with optimizing assay conditions and metabolite identification methods, we plan to increase model complexity to recapitulate idiosyncratic phenotypes. We will continue to characterize sulindac response in a more physiologically-relevant liver-on-chip model which we have used previously to recapitulate difficult-to-model synergistic toxicity of tenofovir-inarigivir.[2] Since we suspect that a majority of idiosyncratic DILI is consequence of immune-mediated events, we plan to increase immune competency by including patient PBMCs as a cellular compartment in the liver-chip model. We have previously identified increased sensitivity in cell-death endpoints with proinflammatory stimulation of HLOs co-cultured with PBMCs and will use patient-matched PBMCs and HLO lines to recapitulate injury from patient-specific agents.

Conclusions

Patient-derived HLOs represent a potent approach for preclinical assessment of drug efficacy and safety in human livers. Our study, involving the evaluation of hepatotoxic drugs across **four distinct patient HLO lines, successfully identified toxicity in 63 out of 64 tested drugs**. Notably, Sulindac was the sole exception, as it exhibited no hepatotoxic indications in our screening. It is worth noting that previous reports have linked Sulindac to hepatotoxic responses in rodent models when exposed to pro-inflammatory factors TNFα and LPS. Interestingly, our static-culture HLO model, subjected to the same conditions, did not replicate such a response. These findings underscore the unique utility of patient-derived HLOs in assessing drug-induced hepatotoxicity and considerations for model optimization in identifying immune-mediated injury like that associated with Sulindac.

References and Acknowledgement

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1. Ouchi, R. et al. Modeling Steatohepatitis in Humans with Pluripotent Stem Cell Derived Organoids. Cell Metab. (2019) doi:10.1016/j.cmet.2019.05.007.
2. Zhang, C. et al. A Human Liver Organoid Screening Platform for DILI Risk Prediction. J. Hepatol. (2023) https://doi.org/10.1016/j.jhep.2023.01.019

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