Proof of concept: Use of iPSC derived human liver organoids from patients with idiosyncratic DILI to assist in DILI diagnosis and mechanistic research

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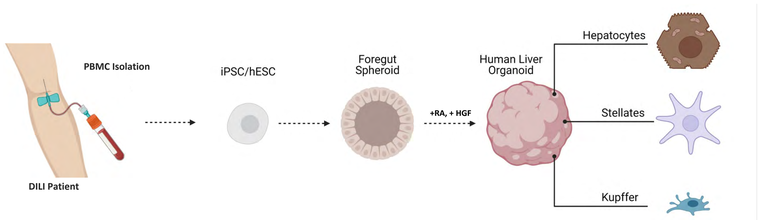
Introduction

Idiosyncratic DILI is a rare but important cause of liver injury. Pre-clinical in vitro and in-vivo animal models as well as large scale clinical trials frequently fail to identify high risk medications and susceptible individuals. Our previous research and literature demonstrate the feasibility of human liver organoids (HLO) for modelling drug-induced liver injury (DILI) in both traditional flat-bottom culture plates and a patient-derived liver-on-chip system (PaDLOC) using the Emulate Bio platform. As iPSCs can be reprogrammed from peripheral blood mononuclear cells (PBMC), HLOs can be developed on a per-patient basis.

Aims

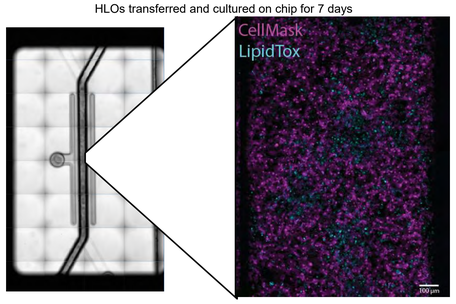
The aim of our study is to report on the creation of an in vitro platform of liver organoids from peripheral blood-derived iPSCs of well-phenotyped DILI patients and controls to improve understanding of human DILI pathogenesis and model idiosyncratic DILI.

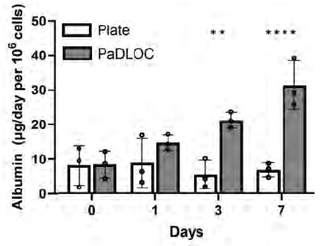
HLO Differentiation and Chip Culture



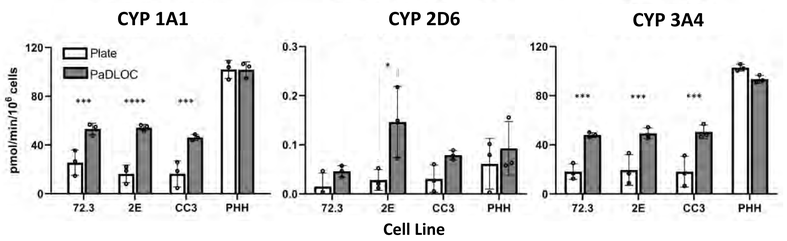
HLOs were differentiated from iPSCs based on a protocol by the Takebe lab at Cincinnati Children's Hospital. In brief, iPSCs are differentiated into definitive endoderm (foregut spheroids) followed by retinoic acid and hepatocyte growth factor treatment to form HLOs, consisting of a mixture of parenchymal 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]

HLOs transferred and cultured on chip for 7 days





After an initial 7 days of culture, PaDLOCs demonstrate increased albumin expression as compared to plate cultured HLOs nearing that of a primary human hepatocyte line (PHHs). Increased expression and activity of CYP450s were also detected across HLOs developed from multiple iPSC lines.

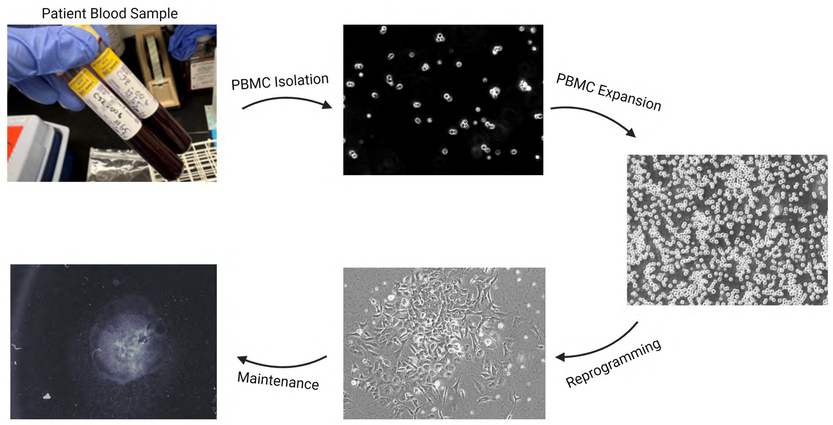


Activity of CYPS 1A1, 2D6, and 3A4 were measured by turnover assays with substrates acetaminophen, cyclophosphamide, and darunavir, respectively.

Selection of Patient Candidates

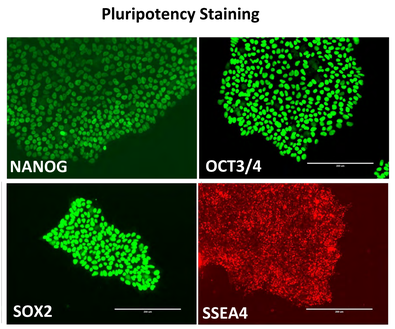
All selected patients were adults (> 18 yrs) with idiosyncratic DILI from a single drug or HDS product and previously enrolled in the DILIN prospective study at the University of Michigan (clinicaltrials.gov = NCT00345930). Subjects with HIV, HCV, or HBV infection, prior organ or bone marrow transplant, 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. Laboratory investigators remain blinded to the suspect drugs/HDS product.

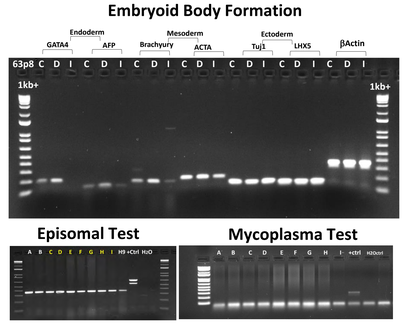
PBMC Reprogramming to iPSC



iPSC colonies are formed from patient PBMCs through a >5-week process with assistance from the University of Michigan Human Stem Cell and Gene Editing Core. Erythroid progenitors from PBMCs are expanded and reprogrammed by transduction with transcription factors. Transduced cells are then cultured on Geltrex coated 6-well dishes until colony formation. Individual colonies (clones) are then picked into fresh 6-well dishes for expansion and characterization.

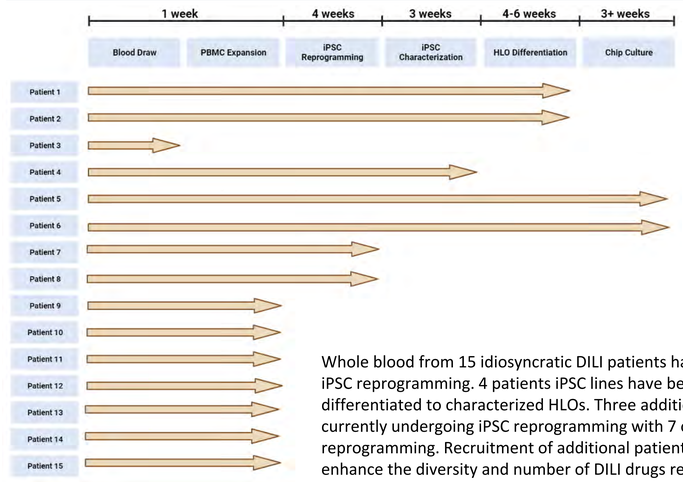
iPSC Characterization





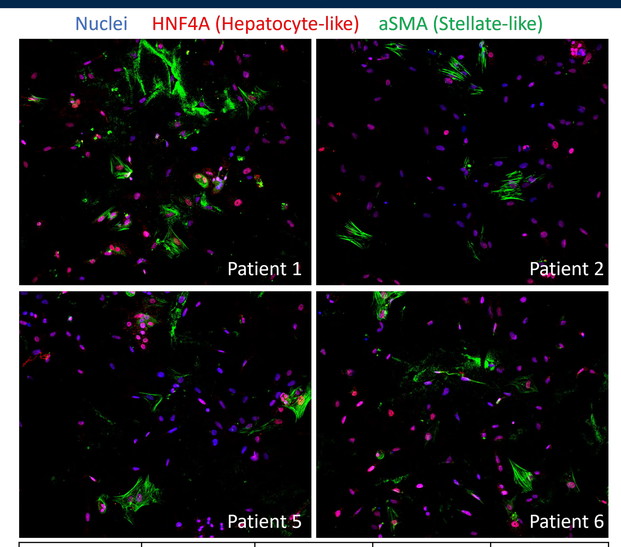
iPSC colonies are stained for pluripotency markers NANOG, OCT3/4, SOX2, and SSEA4. In addition, iPSCs undergo a 7-day embryoid body differentiation followed by PCR testing for endoderm, mesoderm, and ectoderm markers. Lastly, all colonies are confirmed to be both episomal and mycoplasma negative. After characterization is completed, iPSC colonies are transferred to Matrigel-coated 6-well dishes for passaging and HLO differentiation.

Patient Sample Progress



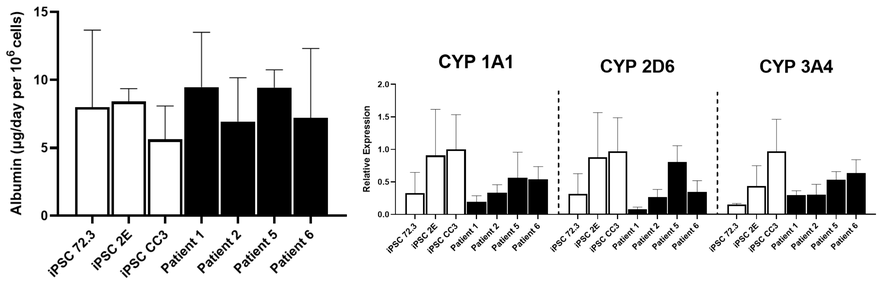
Whole blood from 15 idiosyncratic DILI patients has been collected for iPSC reprogramming. 4 patients iPSC lines have been successfully differentiated to characterized HLOs. Three additional lines are currently undergoing iPSC reprogramming with 7 other lines awaiting reprogramming. Recruitment of additional patients is ongoing to enhance the diversity and number of DILI drugs represented.

Patient Human Liver Organoid Characterization



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

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]



Albumin, a guiding serum biomarker for liver health and function, is produced by all HLOs thus far. Compared to HLOs from previously defined iPSC lines, those differentiated from reprogrammed patient PBMCs show similar albumin secretion. In addition, expression of CYPs 1A1, 2D6, and 3A4 can be detected across all lines.

Future Directions

Future directions include benchmark of generated organoids in ability to model DILI with focus on the original idiosyncratic DILI culprit drugs/products. This will be done with adaptation to advanced chip culture systems and complex multicellular cultures including respective patient PBMCs and/or other iPSC-derived cell types.

Conclusions

- iPSC-derived HLOs have shown promise in mimicry of liver biology in the context of DILI
- Whole blood from idiosyncratic DILI patients were obtained to isolate PBMCs followed by reprogramming to iPSCs
- Patient-derived iPSCs are continuously being differentiated to human liver organoids and characterized
- Future studies will attempt to model organoid hepatotoxicity response to culprit DILI drugs

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. bioRxiv (2021) doi:10.1101/2021.08.26.457824

ANOVA with multiple comparison was done to determine significance with *, **, ***, *** denoting P < 0.05, 0.01, 0.01, and 0.001 respectively.

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