

Human Liver Organoids to Assess Idiosyncratic Drug-Induced Liver Injury

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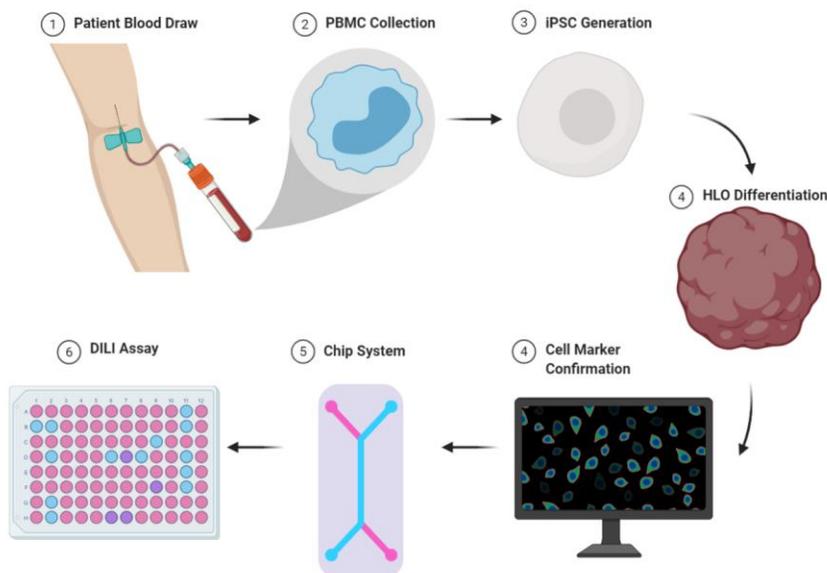
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The inability to reliably identify drugs that can lead to idiosyncratic drug-induced liver injury (DILI) in preclinical test systems results in large burden for drug developers, patients, and regulatory authorities. Current cellular and animal liver models fail to recapitulate human specific liver function and DILI is often only detected in late-stage clinical trials. We predict earlier, and more accurate prediction of DILI will reduce the time and cost of drug development while increasing patient safety. Encouragingly, human liver organoids (HLOs) from iPSCs have recently been developed to better model DILI pre-clinically. HLOs are composed of hepatocytes with CYP450 expression, hepatic stellate cells, macrophages (or Kupffer cells), and biliary tree-like cells from the same genetic lineage. When 3D HLOs are transferred to 2D cultures, they respond to known hepatotoxic compounds in dose-responsive manner while immortalized hepatocyte lines (Huh7, PH5CH8) do not. In our study, 2D HLOs were used to screen for potentially hepatotoxic agents from a panel of 32 drugs that most commonly lead to idiosyncratic DILI in humans.

HLOs, however, retain fetal markers suggesting they incompletely model liver function. In our studies, we attempted to further mature HLOs and assess their ability for DILI prediction using an Emulate Bio organ-on-chip system. This system allows for compartmentalization of cells and administration of media flow through with sustained cellular viability for up to 28 days that provides a more physiological but still controllable environment. Further, HLOs dissociated and cultured in this system produce human serum albumin and release ALT and AST in response to known hepatotoxic compounds acetaminophen and fialuridine. RT-PCR also shows increased expression of CYP450s 1A1, 2D6, and 3A4 as compared to whole HLOs.

A recent clinical trial of inarigivir soropoxil in combination with tenofovir alafenamide for treatment of chronic hepatitis B resulted in elevated ALT and hyperbilirubinemia in 30% of patients after 13-17 weeks of treatment. One patient died of multi-organ failure after suffering with lactic acidosis, necrotizing pancreatitis, and progressive liver failure. This level of hepatotoxicity was not identified in *in vitro* or animal studies and terminated this late-stage drug trial. However, 2D HLOs showed synergistic cytotoxicity with the combination of tenofovir alafenamide and inarigivir soropoxil compared to monotherapy with each drug. In addition, HLOs on chip released 4-fold greater ALT when treated with the drug combination as compared to individual treatments. Furthermore, synergistic cytotoxicity on chip was noted at drug concentrations that were 100-fold lower than those required for the 2D HLO's. Morphological cell profiling along with transcriptomics through scRNA sequencing of cells treated were also completed to identify the mechanism of hepatotoxicity. Future directions will involve the use of multiple patient-derived iPSC lines to accommodate for genetic diversity from actual patients who have previously experienced DILI from a multitude of drugs.



Graphical Abstract. iPSCs are differentiated into human liver organoids with confirmation of cell type markers and further cultured on an organ-on-a chip system for assessment of DILI (3 to 6). Future directions will involve inclusion of more patient-derived cell lines (1 to 2).