Evaluating different modalities of drug-induced liver injury using a sensitive and selective human liver microphysiological system and clinical biomarkers

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Drug-induced liver injury (DILI) is the most common cause for acute liver failure in the USA and Europe and a determining factor for drug attrition during development process with over 1000 FDA-approved drugs currently known to potentially cause different levels of DILI severity in humans. Over the recent years, focus has turned to human *in vitro* 3D liver models to provide solutions to standard *in vitro* cell culture models' limitations in detecting chronic drug exposure. Here we describe a human liver microphysiological system (MPS), comprised of human primary liver parenchymal and non-parenchymal cells cultured in 3D microtissues on an engineered scaffold, under perfusion. The methodology was validated with a broad set of fifteen severely and mildly hepatotoxic small molecules and two antisense oligonucleotides (ASOs).

Co-cultures of cryopreserved primary human hepatocytes (PHHs) and Kupffer cells (HKCs) were seeded on PhysioMimix™ OOC Multi-chip Liver plates to form 3D microtissues and were cultured for eight days. Each compound was repeatably dosed onto liver microtissues at seven test concentrations, for up to four days. A broad spectrum of functional liver-specific endpoints was analyzed, including clinical biomarkers – alanine aminotransferase (ALT), highlighting the MPS' advantage over standard *in vitro* models. Distinct "signature of hepatotoxicity" was created for each compound by analysing functional liver-specific endpoints from cell culture media and microtissues.

The *in vitro* model shows high sensitivity and specificity compared with classic 2D PHHs cultures, and even some standard non-MPS 3D models, in detecting DILI (sensitivity 70%, specificity 100%). The Liver MPS also identified compounds of high-clinical DILI concern (e.g., levofloxacin), that were not captured by standards 2D cultures, and mild toxicity in compounds of low-DILI concern (e.g., pioglitazone). Moreover, the liver MPS model distinguished between safe and toxic ASOs, mirroring published data from *in vivo* trials conducted on mouse and rat models.

Overall, we demonstrate that the liver MPS model is well-suited to explore the molecular mechanisms that underlie DILI and its association with hepatic toxicity. The model can additionally be used to assess novel compounds, in distinct patient subsets, and how toxicity profiles may be affected by liver disease state (e.g., viral hepatitis, fatty liver disease).