# Recapitulating Immune-Driven Hepatotoxicity Using a Liver Microphysiological Platform

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## Introduction

Drug-induced liver injury (DILI) is a major cause of drug development failure, with idiosyncratic, immune-mediated forms posing the greatest challenge.[1] Immune DILI (iDILI) arises when reactive metabolites or drug–protein complexes trigger antigen presentation, T-cell activation, and amplification of inflammatory pathways, ultimately causing hepatocellular injury.[2] Genetic factors—particularly HLA polymorphisms—further influence susceptibility.[3]

Traditional preclinical systems lack the immune complexity needed to capture these human-specific mechanisms, motivating the use of liver microphysiological systems (MPS). Incorporating PBMCs into these platforms enables mechanistic evaluation of immune-driven hepatotoxicity. In line with the FDA's 2025 roadmap supporting New Approach Methodologies (NAMs), we describe using the PhysioMimix® DILI assay in combination with circulating PBMCs to offer improved translational relevance for assessing monoclonal antibodies (mAbs) and other modalities.[4]

## Materials & Methods

Cryopreserved Primary Human Hepatocytes (PHHs) and PBMCs were obtained from LifeNet Health and STEMCELL Technologies, respectively. Cells were seeded at a 1:10 ratio, with PHHs seeded at 400,000 cells per chip in the PhysioMimix Multi-chip Liver-12 plates and cultured under perfusion for 8 days using the PhysioMimix® Core System (Figure 1).

At day four of culture, PBMCs were co-cultured with the liver microtissues and dosed with 2 concentrations of the mAbs Ipilimumab or Infliximab, which have documented DILI risks with one acting intrinsically and one idiosyncratically. Daily dosing was performed with each condition tested in triplicate.

Albumin production was measured by ELISA (AssayPro), LDH release using the Cytotox96 assay (Promega), ALT activity was measured with the human ALT Assay kit (Abcam) and CRP, IL-6, IL-10, TNF-α and IFN-γ by ELISA (R&D Systems).

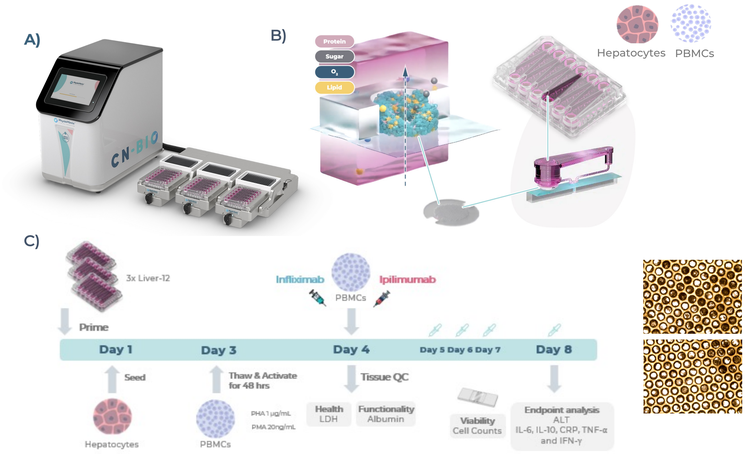


Figure 1 – Experimental set up of Liver MPS for toxicity DILI testing including human PHHs and PBMCs. A) The PhysioMimix Core System B) Schematic of the Liver-12 plate, including the perfusion path, scaffold and cross-section of the liver microtissue within the scaffold. C) Timeline and cells used for toxicity experiments: in-house validated PHH and HLA-matched PBMCs (coculture). Endpoint assays for cell health (LDH and ALT) and cytokine expression (IL-6, IL-2, IL-10 TNF-α, IFN-γ and CRP), cell counts and viability assessment of PBMCs, representative light microscopy images of the scaffolds.

## Results

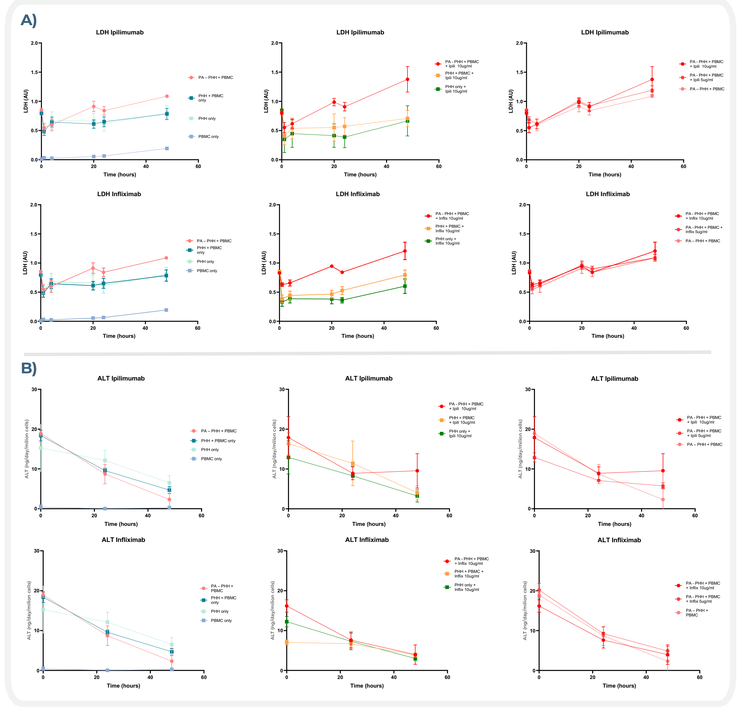


Figure 2 – Health and functional biomarker profiles for PHHs co-cultured with PBMCs in MPS. A) PHHs show signs of cell stress by increased production of LDH in the presence of preactivated PBMCs and mAbs (Ipilimumab and Infliximab), compared to co-cultured PHH and PBMC conditions. B) The clinically relevant PHH health biomarker, ALT, indicates towards cell stress when co-cultured with preactivated PBMCs and mAb.

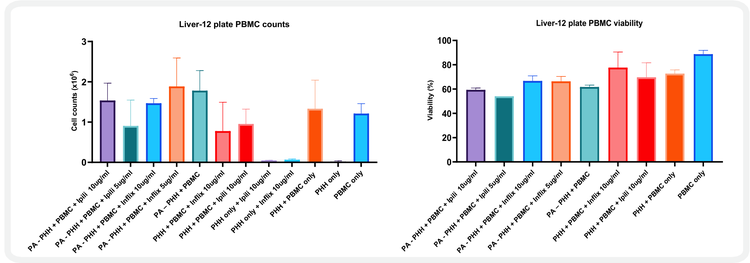


Figure 3 – PBMC recovery and viability. From a seeding of 4 x $10^6$ roughly 40% of the cells were recovered from the Liver-12 plate (left) while viability remained high over the 48h co-culture (right).

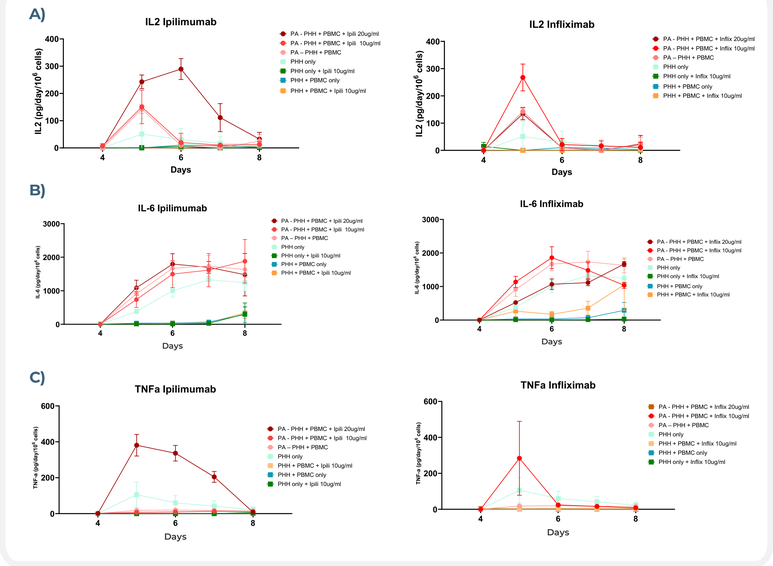


Figure 4 – IL-2., IL-6 and TNF- α cytokine expression for PHHs co-cultured with PBMCs in MPS in the presence of Ipilimumab (left) or Infliximab (right) A) IL-2 expression increased and spiked day 5 with both drugs in the presence of preactivated PBMCs, however Ipilimumab induced consistent expression over time. B) IL-6 expression increased over time for all conditions with preactivated PBMCs in the presence of mAbs showing highest expression for Ipilimumab. C) TNF-α expression showed an increase in Ipilimumab at higher concentration of mAb and consistent expression over time whereas Infliximab spiked at day 5 at the lower concentration only.

## Conclusion

Integrating human HLA-matched PBMCs into the PHH Liver MPS provides a promising, human-relevant approach to evaluating the iDILI potential of mAbs. The PhysioMimix® Core platform enables continuous perfusion of nutrients, oxygen, and immune cells through 3D liver microtissues, maintaining physiological flow, supporting long-term hepatocyte function, and facilitating dynamic immune–hepatic interactions that more closely mirror *in vivo* human liver physiology. Evidence of mAb-specific iDILI effects can be seen via increases in PHH stress markers (ALT) and increases in cytokine expression only in the presence of preactivated PBMCs and mAbs, with higher and more consistent levels found over time in the presence of Ipilimumab. Importantly, the potential to recover immune cells for downstream phenotyping will allow further in-depth analyses to be made. Overall, the data highlight the power of Liver MPS as a means of modelling immune-related DILI to support risk assessment and drive the development and regulatory acceptance of novel antibody therapeutics.

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## References

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