Induced pluripotent stem cell (iPSC)-derived hepatocytes demonstrate functional Phase I and Phase II drug metabolism and can accurately predict drug-induced liver injury (DILI)

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INTRODUCTION

Liver disease is a rising cause of mortality worldwide. Despite careful approaches taken during drug development, most therapeutics still fail to reach clinical trials due to the lack of translatability between pre-clinical models and the clinic. Primary human hepatocytes (PHH) and hepatocellular cancer cells are currently the predominant pre-clinical liver models in the industrial workflow. However, they come with limitations, including short life-span, rapid loss of function, and malignant origin. We have hypothesized that induced pluripotent stem cell (iPSC)-derived hepatocytes can overcome these limitations, offering a sustainable *in vitro* platform that is more relevant to human liver physiology, amenable, and expandable for large-scale drug screening and predictive toxicology studies.

MATERIALS AND METHODS

Healthy iPSCs were differentiated towards hepatocytes (Ulti-HEP) in 2D format for six weeks. Following iPSC differentiation, characterization of Ulti-HEP functionality was assessed, including liver maturity marker expression by immunocytochemistry, urea synthesis and secretion by western blotting and biochemical assays, and Phase I and Phase II drug metabolism-associated gene expression by qPCR, alongside that of hepatocellular carcinoma HepG2 cells and PHH. Suitability of Ulti-HEP to predict drug-induced liver injury (DILI) was finally evaluated by cell viability assays (ATP endpoint) following a 48 hour-incubation with drugs with known DILI liability, alongside metabolite analysis of Ulti-HEP treated with acetaminophen and diclophenac and analysed by Liquid Chromatography/ Mass spectrometry analysis.

Ulti-HEP DEMONSTRATE COMPARABLE HEPATOCYTE MATURITY MARKER EXPRESSION TO PRIMARY HUMAN HEPATOCYTES

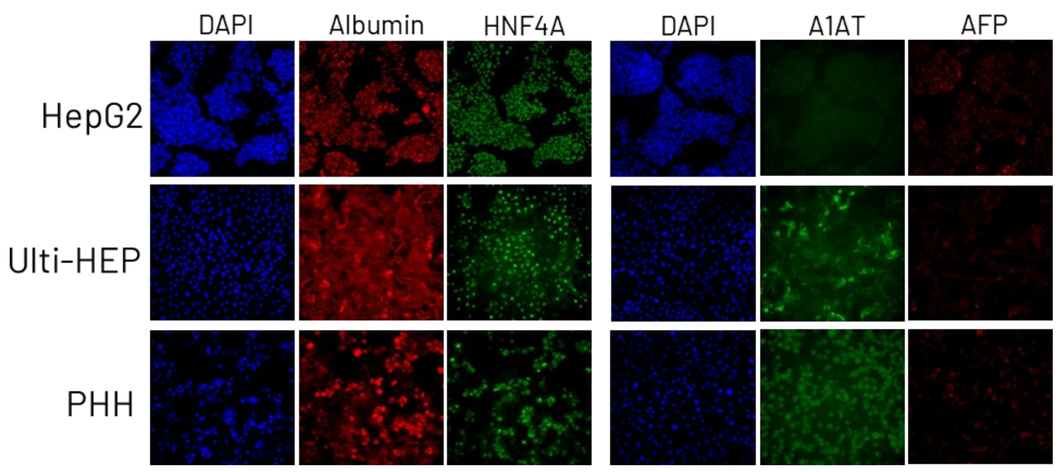

Figure 1: Representative immunocytochemistry pictures showing protein expression of the hepatocyte maturity markers albumin (red), alpha-1-antitrypsin (A1AT; green), HNF4A (green), and AFP (red) in liver carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and primary human hepatocytes (PHH). Nuclei were counterstained with DAPI. Data are presented as mean±SEM of n=3-4 independent experiments.

Ulti-HEP DEMONSTRATE A LONG-TERM FUNCTIONAL UREA CYCLE

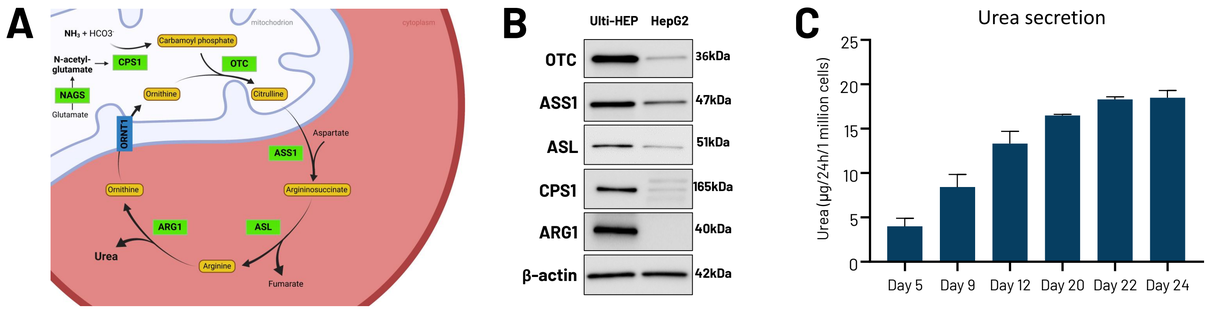

Figure 2: A) The urea cycle which metabolises ammonia to urea, highlighting the enzymes involved in green boxes. **B)** Protein expression levels of the urea cycle enzymes OTC, ASS1, ASL, CPS1, and ARG1 in liver carcinoma HepG2 cells and DefiniGEN Ulti-HEP. **C)** Urea secretion in DefiniGEN Ulti-HEP over a period of three weeks. Data are presented as mean±SEM of n=3-4 independent experiments.

Ulti-HEP SHOW COMPARABLE PHASE I AND PHASE II EXPRESSION OF DRUG METABOLISM ENZYMES TO PRIMARY HUMAN HEPATOCYTES

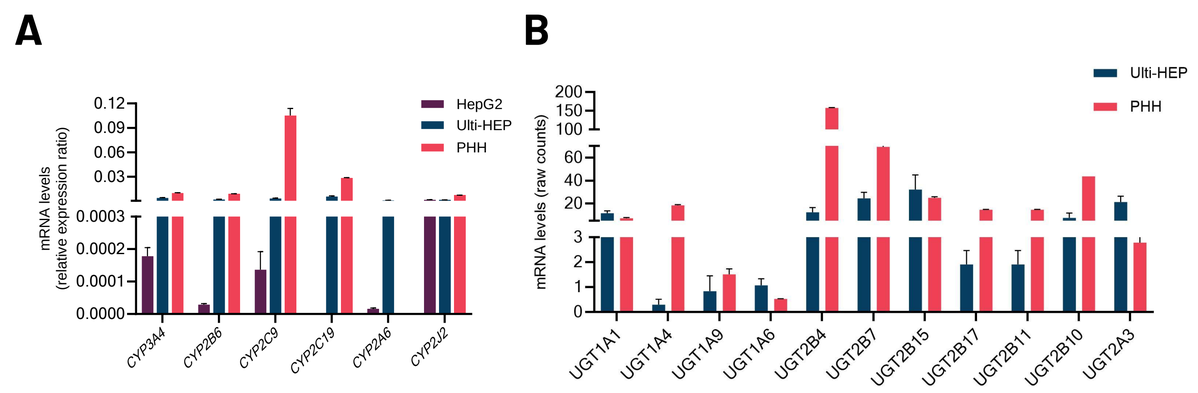

Figure 3: A) mRNA expression levels of Phase I CYP450 genes in hepatocellular carcinoma HepG2 cells, DefiniGEN Ulti-HEP, and primary human hepatocytes (PHH). mRNA data were normalized to the housekeeping gene 18S rRNA and are presented as mean±SEM of 3-4 independent experiments. **B)** mRNA expression levels of UDP-glucoronosyltransferases in Ulti-HEP and PHH. mRNA data are presented as raw counts following bulk RNA-sequencing analysis. For PHH data, cells from 1-3 independent donors were used.

Ulti-HEP SHOW DEMONSTRATE PHASE I AND PHASE II DRUG METABOLISM

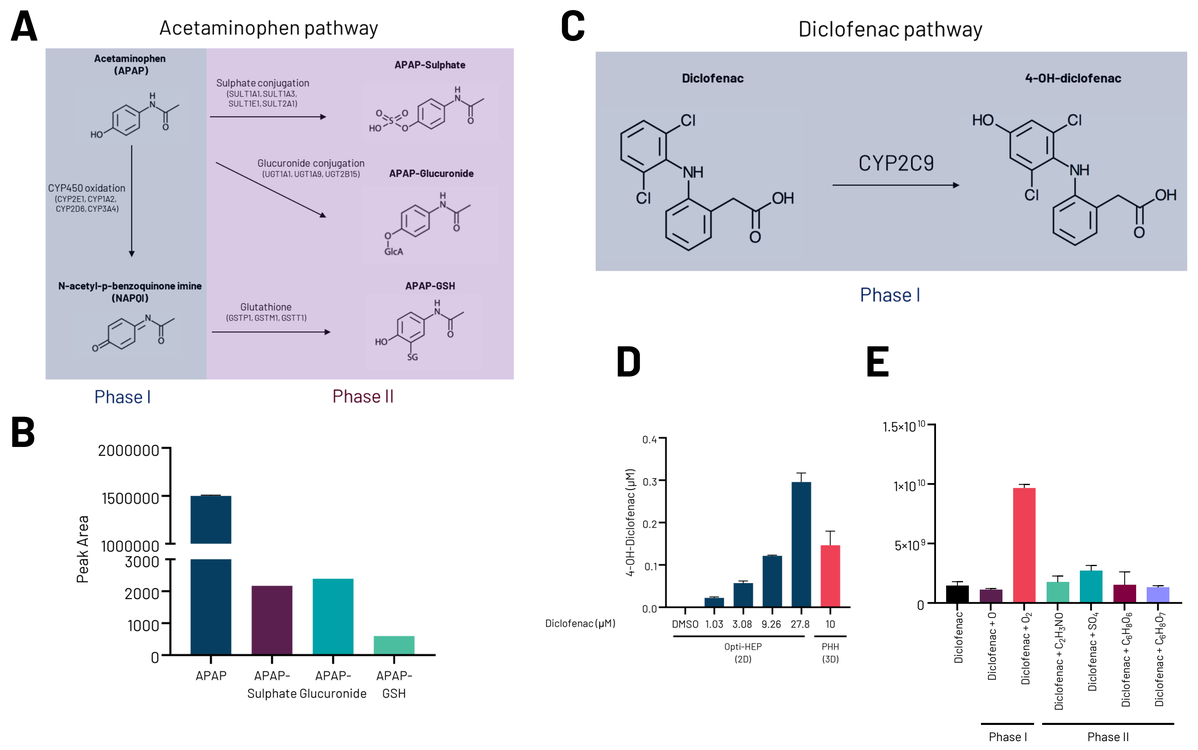

Figure 4: A, C) Simplified schematics demonstrating acetaminophen and diclofenac metabolism within human liver. **B, D, E)** Wild-type Ulti-HEP media metabolite analysis following treatment with acetaminophen or diclofenac, as measured by Liquid-Chromatography/ Mass spectrometry analysis. Ulti-HEP in **(D)** were treated with increasing diclofenac concentrations and compared against a 3D PHH model treated with 10 µM diclofenac. Data are presented as mean±SD of n=3 technical replicates.

Ulti-HEP CAN ACCURATELY PREDICT DRUG-INDUCED LIVER INJURY (DILI) *IN VITRO*

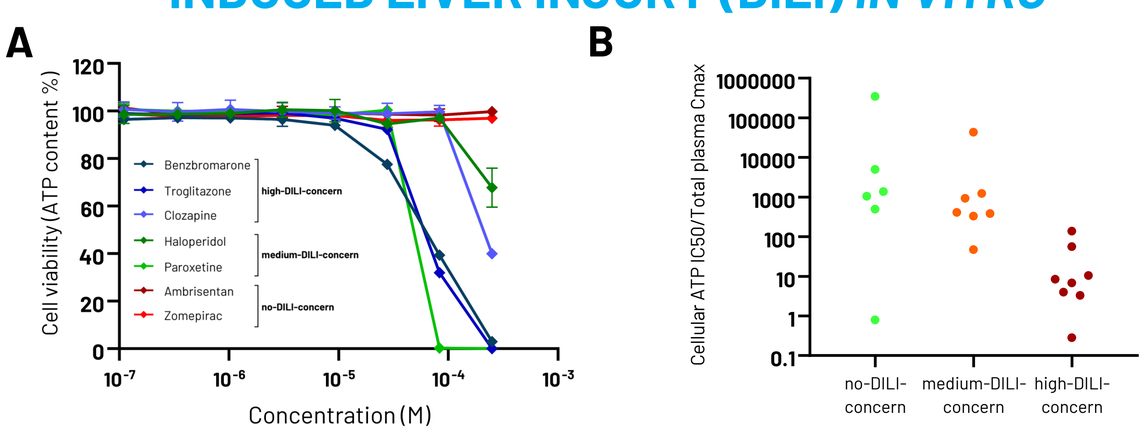

Figure 5: A) Cell viability (ATP content) in DefiniGEN Ulti-HEP following 48 hours of treatment with increasing concentrations (0-250 µM) of 7 compounds with known DILI liability. Cell viability data were normalized to positive (250 µM chlorpromazine) and negative controls (0.2% DMSO) and are presented as mean±SD of n=3 technical replicates. **B)** The ratio of cellular ATP IC50 to total plasma Cmax (RA/C) was calculated and plotted; the RA/C values of the "high-DILI-concern" drugs cluster below 100, whereas the RA/C values of the "no-DILI-concern" drugs cluster above 100. The RA/C values of the "medium-DILI-concern" drugs cluster between the two other groups.

DISCUSSION

To conclude, we have shown that Ulti-HEP offer a superior *in vitro* liver model compared to HepG2 cells, with greater liver functionality that is comparable to primary human hepatocytes. These data alongside the expansion capacity and amenability of Ulti-HEP using CRISPR-based gene editing showcase the spectrum of opportunities iPSC-derived hepatocytes can offer in the fields of disease modelling, large-scale drug screening, genotoxicity, and hepatic safety.