# A novel generation of human pluripotent stem cell-derived hepatocytes with substantially improved functionality and several adult characteristics

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## INTRODUCTION

Hepatocytes derived from human pluripotent stem cells (hPSC) have the potential to serve as predictive human in vitro model systems for toxicity testing and drug discovery studies, provided that they display relevant levels of hepatic functions. However, until recently, the functionality of stem cell-derived hepatocytes has been insufficient for applications requiring high expression of multiple drug metabolizing enzymes.

We have developed a novel 2D differentiation protocol in order to obtain more functional hepatocytes from human induced pluripotent stem cells (hiPSC). The resulting hiPSC-derived hepatocytes, Cellartis® Enhanced hiPS-HEP , have an substantially improved functionality and display several adult hepatic features. To the best of our knowledge, this is the first time such improved functionality is described for stem cell-derived hepatocytes in a 2D culture system. Interestingly, hepatocytes derived from several different hPSC lines using the new differentiation protocol reflect the inter-individual variation found in human population.

## RESULTS AND DISCUSSION

The Cellartis® Enhanced hiPS-HEP (derived from hiPSC line ChiPSC18):

- display high CYP1A, 3A, 2C9, and 2E1 activities and lower CYP2B6, 2C19, and 2D6 activities (Fig.1) with low batch-to-batch variation.
- have stable CYP activities between day 6 and 11 after thawing (data not shown).
- express substantial mRNA levels of the adult CYP enzymes CYP3A4 and 3A5, the phase II enzyme UGT1A1, and the transporters MRP2 and NTCP (Fig.2A,B,D-F).
- express low mRNA levels of the fetal genes CYP3A7 (Fig.2C) and a-fetoprotein (data not shown).
- are highly homogenous (> 90% hepatocytes) and have a typical hepatic morphology, e.g. a polygonal cell shape and the presence of binucleated cells (Fig.3A, arrowheads).
- express CYP1A2, 2C9, 3A4, Albumin, a1-Antitrypsin, Cytokeratin 18 and GSTA1-1 (Fig.3B-H). Interestingly, for all markers except for Cytokeratin 18, distinct subgroups of hepatocytes are immuno-positive reminiscent of the different hepatocyte phenotypes in the liver lobe.

Importantly, hepatocytes derived from 7 different hPSC-lines using the enhanced differentiation protocol have different CYP activity profiles reflecting the diversity of metabolic phenotypes observed in human primary hepatocytes from different donors (Fig.4).

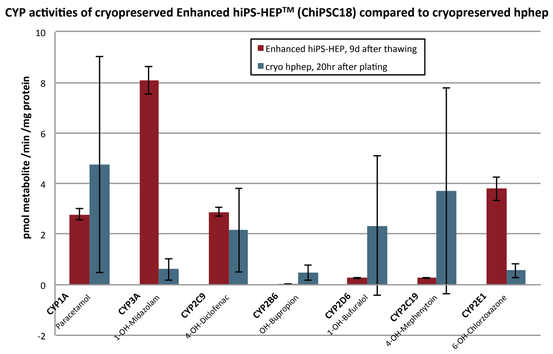


**Figure 1.** *CYP activities of Cellartis® Enhanced hiPS-HEP and cryopreserved human primary hepatocytes (hphep). Cryo-preserved Cellartis® Enhanced hiPS-HEP (derived from hiPSC line ChiPSC18; 9 days after thawing) have similar CYP1A, 3A, 2C9, and 2E1 activity levels compared to cryoplateable hphep (cultured for 20hr), whereas CYP2B6, 2D6 and 2C19 activities are lower in Cellartis® Enhanced hiPS-HEP than in hphep.*

*Results are presented as mean ± SEM. Cryo Cellartis® Enhanced hiPS-HEP: n=3 ; cryo hphep: n=4 (4 different donors). Metabolite concentration in the medium measured by LC/MS.*

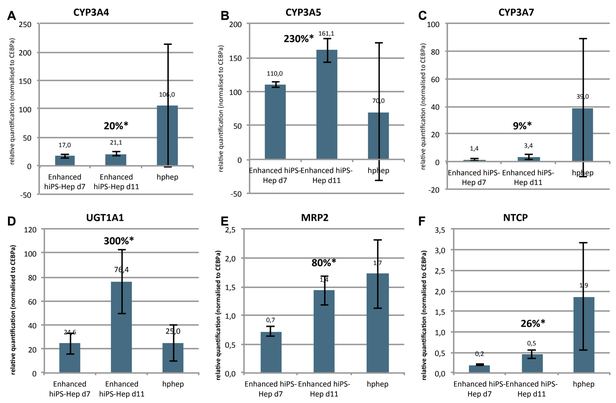


**Figure 2.** *qPCR analysis of cryopreserved Cellartis® Enhanced hiPS-HEP compared to cryopreserved human primary hepatocytes (hphep) directly after thawing.*

*Cellartis® Enhanced hiPS-HEP (derived from hiPSC line ChiPSC18) on day 7 and 11 after thawing, respectively, show substantial mRNA levels of the adult phase I enzymes CYP3A4 and 3A5 (A,B), the phase II enzyme UGT1A1 (D), and the transporters MRP2 (E) and NTCP (F), and low expression of the fetal enzyme CYP3A7 (C) compared to cryo hphep directly after thawing.*

*Results are presented as mean ± SEM. Expression data are presented as relative quantification normalized to CEBPa. Cryo Cellartis® Enhanced hiPS-HEP: n=3; cryo hphep: n=5 (5 different donors); *= % of hphep.*

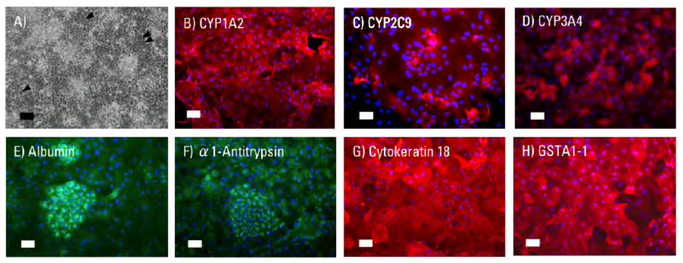


**Figure 3.** *Morphology and immunocytochemical stainings of cryo-preserved Cellartis® Enhanced hiPS-HEP.The Cellartis® Enhanced hiPS-HEP (derived from hiPS cell-line ChiPSC18; 7 days after thawing) display a typical hepatic morphology (A) with polygonal cell shape and binucleated cells (arrow heads).*

*Subgroups of the Cellartis® Enhanced hiPS-HEP are immuno-positive for CYP1A2, 2C9, 3A4, Albumin, a1-Antitrypsin, Cytokeratin 18 and GSTA1-1 (B-H; all merged with nuclear DAPI counterstaining). Scale bar: 100um in A and 50um in B-H.*

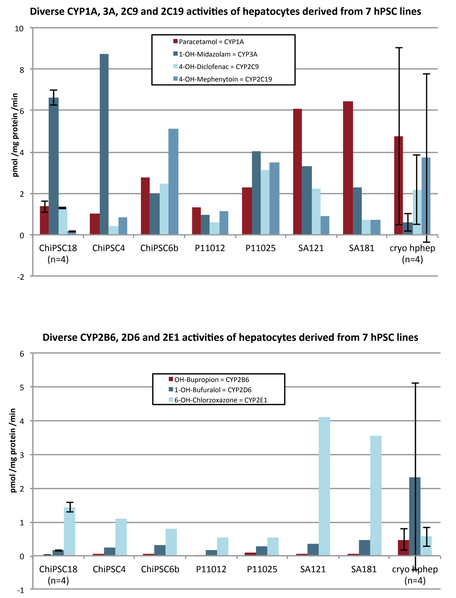


**Figure 4.** *Diverse CYP activity profiles of hepatocytes derived from 7 hPSC lines.*

*Hepatocytes derived from 5 hiPSC lines and 2 hESC lines with the enhanced differentiation protocol (after 29 days of differentiation) have different CYP activity profiles reflecting metabolic diversity found in human population (see error bars for hphep cultured for 20hr).*

*Results are presented as mean ± SEM.*

*Cellartis® Enhanced hiPS-HEP from ChiPSC18: n=4;*

*Cellartis® Enhanced hiPS-HEP from hiPSC lines ChiPSC4, ChiPSC6b, P11012 and P11025: n=1;*

*Cellartis® Enhanced hES-HEP from hESC lines SA121 and SA181: n=1; cryo hphep: n=4 (4 donors).*

## CONCLUSIONS

Cellartis® Enhanced hiPS-HEP :

- have high CYP activity levels relevant for toxicity testing and drug metabolism studies.
- have several adult features, e.g. substantial expression of the adult enzymes CYP2C9, 2C19, 2E1, and 3A4, and low expression of fetal genes like CYP3A7.
- can serve as an inexhaustible source of functional human hepatocytes.
- are ideal for applications that demand a highly reproducible platform and continuous supply of material from the same genetic background.
- derived from the hiPSC line ChiPSC18 are available from Cellartis by Takara Bio Europe AB (www.cellartis.com).

The novel differentiation protocol can be used to derive enhanced hepatocytes from several hPSC lines which show diverse CYP profiles mirroring interindividual variation found in the human population.

Within short, functionally diverse hPSC-derived hepatocytes from different genetic backgrounds using the improved differentiation protocol will be available from Cellartis by Takara Bio Europe AB. This will open up the possibility to compile panels of hepatocytes with different metabolic phenotypes for drug discovery and toxicity studies.

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**Selected references:**
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