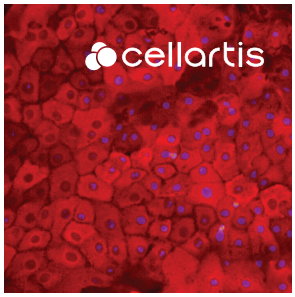

Stem Cell Products

Imaging based high content analysis (HCA) to study mechanistic hepatotoxicity in Cellartis® Enhanced hiPS-HEP

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Introduction

Cellartis Enhanced hiPS-HEP are homogenous hepatocytes derived from human induced pluripotent stem cells and display functional drug metabolizing enzymes similar to cryopreserved human hepatocytes. In the current application note it is shown that Cellartis Enhanced hiPS-HEP is well suited as a potential source of cells with hepatocellular characteristics for use in imaging based HCA.

Cell-culture

Cryopreserved Cellartis Enhanced hiPS-HEP were thawed and plated in 96-well imaging plates (Nunc 165305) according to the technical manual and with a cell density of 90000-95000 cells per well to obtain a confluent monolayer of cells. Cellartis Enhanced hiPS-HEP were maintained for five days with medium change every other day before the start of toxicity assay.

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Toxicity assay and HCA

Cellartis Enhanced hiPS-HEP were exposed to clinically relevant hepatotoxic (amiodarone, disulfiram, bosentan, nefazodone and perhexiline) and non-hepatotoxic compounds (metformin, pioglitazone) with increasing doses of 0.3, 0.5, 1, 3, 5, 10, 30, 50, 100 and 300 µM at a fixed concentration of 1% DMSO. The compounds were incubated for 24h prior to staining with fluorescent probes (Table 1), and fixation. They were then monitored by HCA using the ThermoFisher ArrayScan™ VTI high content imager and analyzed using the integrated ThermoFisher BioApplication V4 software. Six images per well, compound and dose were analyzed, Data is presented as percentage of vehicle control, mean±SEM, n=6.

TABLE 1 FLUORESCENT PROBES AND MONITORED READ-OUTS

Probe	Read-out
Hoechst	Nuclei counts
LysoTracker Green	Lysosomal activity
MitoTracker Orange	Mitochondrial membrane potential
TOTO-3	Plasma membrane integrity

Results

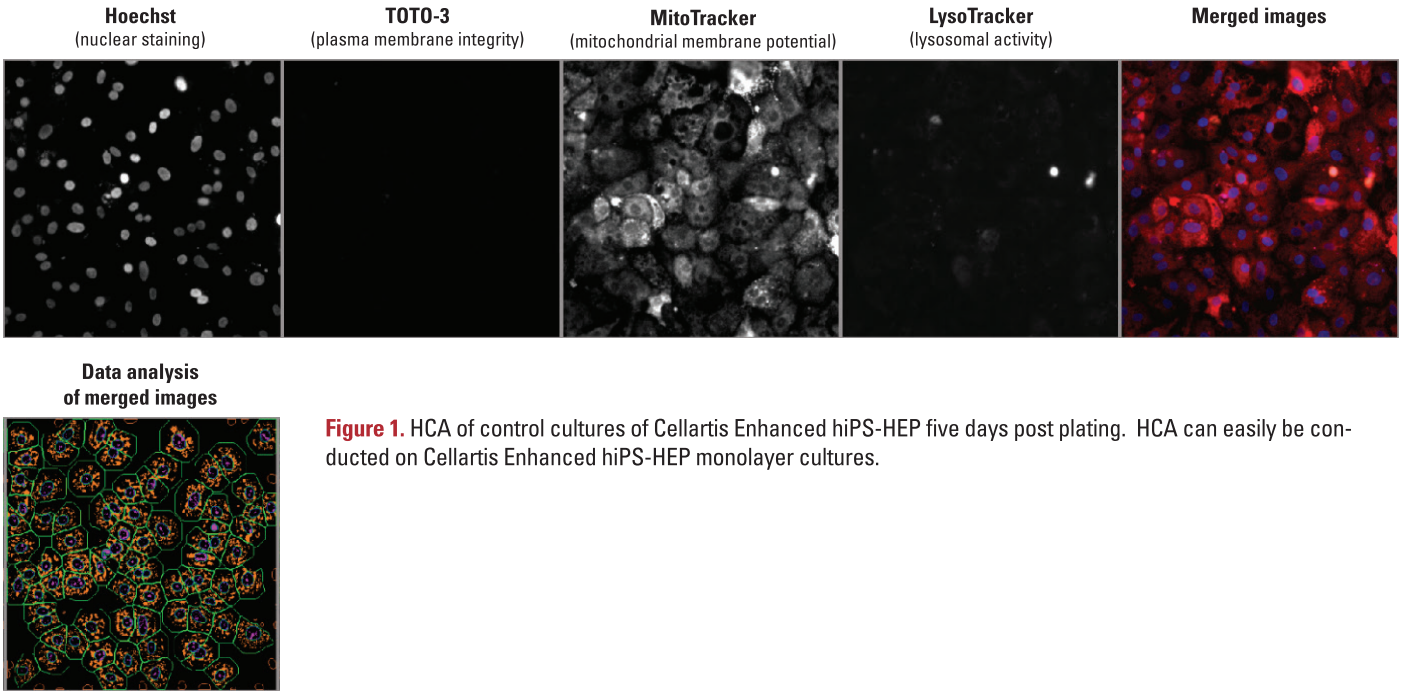

Figure 1. HCA of control cultures of Cellartis Enhanced hiPS-HEP five days post plating. HCA can easily be conducted on Cellartis Enhanced hiPS-HEP monolayer cultures.

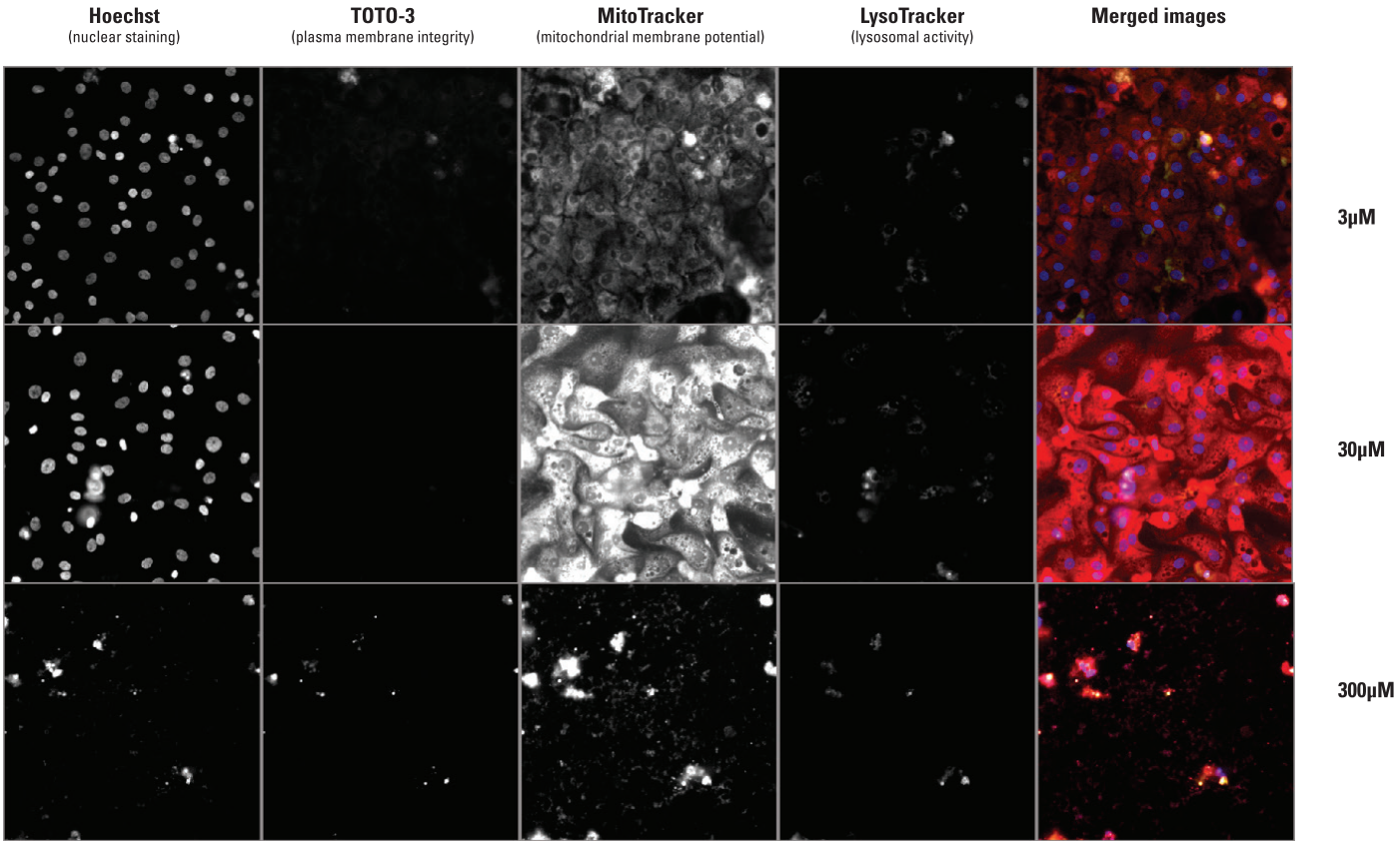

Figure 2. HCA of Cellartis Enhanced hiPS-HEP 24h post exposure to amiodarone with increasing doses of 3, 30 and 300µM. Amiodarone is known to impair mitochondrial function and hyperpolarizes the mitochondrial membrane potential of Cellartis Enhanced hiPS-HEP with increasing doses (30µM). At the maximum dose (300µM) there is a loss of cells due to cytotoxicity.

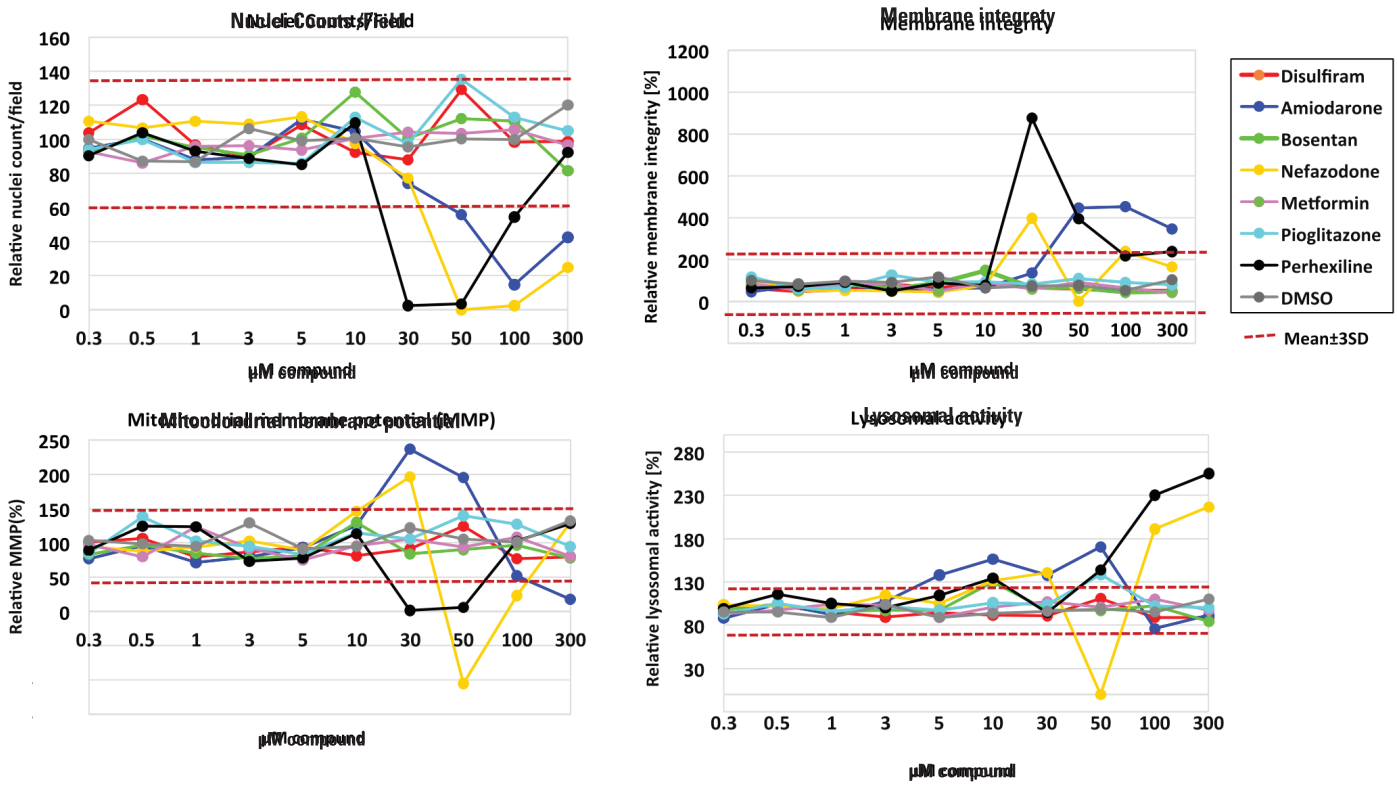

Figure 3. HCA of Cellartis Enhanced hiPS-HEP 24h post exposure to seven compounds known to be non-hepatotoxic or hepatotoxic. The non-hepatotoxic compounds for the monitored read-outs: metformin,pioglitazone and bosentan remained non-toxic in Cellartis Enhanced hiPS-HEP while changes in mitochondrial membrane potential was seen for amiodarone, nefazodone and perhexiline as expected. Perhexiline induced increased lysosomal activity in Cellartis Enhanced hiPS-HEP with increasing doses. NB. Visible precipitation of nefazodone was found at 50µM and higher which likely explains the values returning towards normal in the curves at the higher doses. Disulfiram, a drug showing low cytotoxicity in PHH and high cytotoxicity in HepG2 cells, is comparable to PHH in Cellartis Enhanced hiPS-HEP.

Conclusions

In conclusion, it has been demonstrated that Cellartis Enhanced hiPS-HEP are well suited for image based HCA and respond to known hepatotoxic compounds and endpoint measurements frequently employed in early *in vitro*-based cell screens.

Acknowledgement

Data generated in this application note were supported, in part, by the MIP-DILI project which is funded by the European Community under the Innovative Medicines Initiative (IMI) Programme through Grant Agreement number 115336.

These data are part of ongoing activities in MIP-DILI to compare the quantitative evaluation of all test systems for drug safety evaluation in line with its primary objective (http://www.mip-dili.eu/).