Introduction
Isolated liver cells, such as hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer Cells and hepatic stellate cells, are frequently used to study hepatic metabolism, toxicity and disease pathogenesis. However, the current in vitro models exhibit several disadvantages, e.g. short culture longevity and artificial culture conditions focusing on a single cell type in 2D culture. The use of primary cells in vitro is compromised by the limited quantity of cells that can be isolated from one donor; a lack of or very restricted proliferation capacity (hepatocytes and LSECs) and/or the change from a quiescent to an activated state (hepatic stellate cells). To overcome this, we have developed a technique which causes primary human hepatocytes to proliferate up to 40 population doublings whilst retaining an adult and metabolically competent phenotype with phase I (Cytochrome P450) and phase II activities when cultured at confluence. The resulting cells are called “upcyte® hepatocytes” and combine proliferation with drug metabolizing activity, a feature which makes them uniquely applicable to metabolism and toxicity studies.

Results
Metabolism & Characteristics

<table>
<thead>
<tr>
<th>Substance</th>
<th>Specific Activity (pmol/min/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>#1 0.3 ± 0.4</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>#2 91.8 ± 5.5</td>
</tr>
<tr>
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<td>#3 21.4 ± 8.7</td>
</tr>
</tbody>
</table>

Self-assembly in Matrigel®-coated 24-well plates
After 72 h, cells showed formation of structures resembling a liver organoid. Hepatocytes were located in the center and formed the outer petals (HF-1a). LSECs formed an inner ring (C013), whereas HSCs revealed a typical, “star-shaped” morphology (a-SMCA).

Viral Infection: HCV

Upcyte® hepatocytes support HCV infection in vitro, but infectivity of primary human hepatocytes in vitro is minimal. To assess whether upcyte® hepatocytes support the full lifecycle of the HCV cell cycle variant (MCV), we exposed differentiated hepatocytes (donors 740 and 653-03) to culture medium containing infectious particles of the HCV genotype expressing an NSSA-RFP fusion protein. HCV-positive cells were confirmed by strong NSSA staining. More than 80% of the cells were infected in both cultures.

Cytotoxicity - acute and repeated-dose studies

Acute and repeated-dose toxicity using sub-cytotoxic concentrations
Exposure time length had dramatic effects on the toxicity profile of a compound. For APAP, no effect was observed after 24 h, whereas 1-week treatment significantly increased mitochondrial depolarization, ROS production and intracellular Ca²⁺ levels. Other tested compounds caused effects after 24 h, although a significant difference was detected between the two incubation periods at the lowest concentration. CIT (non hepatocyte control) did not produce any effects after 24 h or 1 week treatment.

Summary & Conclusion
In conclusion, upcyte® hepatocyte cultures are characterized by a differentiated phenotype and exhibit functional phase I, phase II and transporter activities. These data support the use of upcyte® hepatocytes for various applications, such as metabolism & toxicity screening assays, viral infection and 3D culture. Moreover, this technology allows for the generation of large batches of upcyte® hepatocytes (up to 12 x 10⁶ cells per donor), enabling a reproducible and standardized experimental setting.

Generation of expanded primary hepatocytes (upcyte® cells) in large quantities for for cell based toxicity and metabolism screenings
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upcyte technologies GmbH, Osterfeldstr. 12-14, D-22529 Hamburg, Germany

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