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Stem cell-derived models to improve mechanistic understanding and prediction of human drug induced liver injury

Christopher Goldring (C.E.P.Goldring@liverpool.ac.uk) [1], Daniel J. Antoine (bs0u1156@liverpool.ac.uk) [1], Frank Bonner (frankbonner@focusbio.com) [2], Jonathan Crozier (jonathan.crozier@epaaind.eu) [3], Chris Denning (Chris.Denning@nottingham.ac.uk) [4], Robert J. Fontana (rfontana@med.umich.edu) [5] Neil A. Hanley (Neil.Hanley@manchester.ac.uk) [6], David C. Hay (davehay@talktalk.net) [7], Magnus Ingelman-Sundberg (Magnus.Ingelman-Sundberg@ki.se)[8], Satu Juhila (Satu.Juhila@orionpharma.com) [9], Neil Kitteringham (neilk@liverpool.ac.uk) [1], Beatriz Silva-Lima (beatrizlima@netcabo.pt) [10], Alan Norris (alan.norris@liverpool.ac.uk) [1], Chris Pridgeon (cpr@liverpool.ac.uk) [1], James A. Ross (J.A.Ross@ed.ac.uk) [7], Rowena Sison Young (Rowena.Sison-Young@liverpool.ac.uk) [1], Danilo Tagle (tagled@mail.nih.gov)[11], Belen Tornesi (m.tornesi@abbvie.com) [12], Bob van de Water (water_b@lacdr.leidenuniv.nl) [13], Richard J. Weaver (richard.weaver@servier.com) [14], Fang Zhang (Fang.Zhang@liverpool.ac.uk) [1], B. Kevin Park (B.K.Park@liverpool.ac.uk) [1]

[1] MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK

[2] Stem Cells for Safer Medicines, London, UK

[3] European Partnership for Alternative Approaches to Animal Testing (EPAA), Brussels, Belgium

[4] Department of Stem Cell Biology, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK

[5] Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

[6] Centre for Endocrinology & Diabetes, University of Manchester; Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre Manchester, UK

[7] MRC Centre for Regenerative Medicine, University of Edinburgh, UK

[8] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

[9] R&D, In Vitro Biology, Orion Pharma, Espoo, Finland

[10] Universidade de Lisboa, Faculty of Pharmacy, Lisbon, Portugal

[11] National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD, USA

[12] Abbvie Global Pharmaceutical Research and Development, North Chicago, IL, USA

[13] Faculty of Science, Leiden Academic Centre for Drug Research, Gorlaeus Laboratories, University of Leiden, Netherlands

[14] Institut de Recherches Internationales Servier (I.R.I.S), Suresnes, 92284, Cedex France

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Contact Information :

Christopher Goldring, PhD, MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, Sherrington Building, Ashton Street, University of Liverpool, Liverpool, L69 3GE, UK ; Tel +44 151 794 5979 ; Fax +44 151 794 5540 ; email C.E.P.Goldring@liverpool.ac.uk

List of Abbreviations :

DILI, drug-induced liver injury; ADR, adverse drug reaction; SC-HLC, stem-cell-derived hepatocyte-like cell; EPAA, European Partnership for Alternative Approaches to Animal Testing; NC3Rs, National Centre for the Replacement, Refinement and Reduction of Animals in Research; MRM, multiple-reaction-monitoring; CRISPR, clustered regularly-interspaced short palindromic repeats; iPSC, induced pluripotent stem cell

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Abstract

Current preclinical drug testing does not predict some forms of adverse drug reactions in humans. Efforts at improving predictability of drug-induced tissue injury in humans include using stem cell technology to generate human cells for screening for adverse effects of drugs in humans. The advent of induced pluripotent stem cells means that it may ultimately be possible to develop personalised toxicology to determine inter-individual susceptibility to adverse drug reactions. However, the complexity of idiosyncratic drug-induced liver injury (DILI) means that no current single cell model, whether of primary liver tissue origin, from liver cell lines, or derived from stem cells, adequately emulates what is believed to occur during human DILI. Nevertheless, a single cell model of a human hepatocyte which emulates key features of a hepatocyte is likely to be valuable in assessing potential chemical risk; furthermore understanding how to generate a relevant hepatocyte will also be critical to efforts to build complex multicellular models of the liver. Currently, hepatocyte-like cells differentiated from stem cells still fall short of recapitulating the full mature hepatocellular phenotype. Therefore, we convened a number of experts from the areas of preclinical and clinical hepatotoxicity and safety assessment, from industry, academia and regulatory bodies, to specifically explore the application of stem cells in hepatotoxicity safety assessment, and to make recommendations for the way forward. In this short review, we particularly discuss the importance of benchmarking stem cell-derived hepatocyte-like cells to their terminally-differentiated human counterparts using defined phenotyping, to make sure the cells are relevant and comparable between labs, and outline why this process is essential before the cells are introduced into chemical safety assessment.

Prediction of adverse drug reactions in the liver: why it is important, limitations of current in vitro models and how stem cells may prove useful in drug screening

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Adverse drug reactions (ADRs) are a significant clinical problem, resulting in considerable patient morbidity and mortality[1] and thus represent a major financial burden on healthcare systems. ADRs also represent a major challenge for the pharmaceutical industry leading to attrition of drugs in development and the withdrawal of drugs post-licensing[2]. Amongst different forms of ADRs, the liver is particularly susceptible to drug toxicity; drug-induced liver injury (DILI) is the second highest cause of attrition and accounts for more than 50% of cases of acute liver failure[3].

The principal cause of these high attrition rates is the failure of current preclinical drug testing procedures to effectively predict idiosyncratic DILI in patients[2]. This is true for *in vitro* models and even for *in vivo* models - a recent study that related the preclinical assessment of drugs with the occurrence of DILI in the clinic showed that between 38% (Medline database: 269 out of 710 compounds) and 51% (EMEA database: 70 out of 137 compounds) of drugs that subsequently caused liver injury in patients were not predicted from animal studies[4]. Concerted worldwide efforts are therefore required to improve the assessment of hepatotoxic risk for new compounds. In Europe, the SEURAT (http://www.seurat-1.eu/pages/cluster-projects/scrtox.php) and MIP-DILI (http://www.mip-dili.eu/) consortia, and in the US, DILIN (http://www.dilin.org/) and iSAEC (http://www.saeconsortium.org/) are attempting to address this issue. The clinical manifestation of DILI indicates that it is a multi-dimensional and multi-faceted disease[5]. Indeed, the diagnosis of DILI is largely based upon exclusion criteria[5]. Although the use of currently available cell lines and primary human hepatocyte models has been able to correctly classify a number of DILI compounds as hepatoxins[6-9], idiosyncratic DILI is inherently difficult to model in the laboratory, and therefore highly unlikely to be predicted by simplistic screening strategies, often based on single-cell models involving cell lines. Many approaches use liver-derived cancer cell lines, e.g. HepG2 and HepaRG, which may have value for identifying drugs lacking a propensity to cause idiosyncratic DILI (90-95% predictability), but perform less well for positive predictions (50-89%)[9-11]. Metabolically-competent freshly-isolated, or cryopreserved human primary adult hepatocytes are still considered to be the gold-standard single cell model of DILI. Nevertheless, human hepatocytes are difficult to source, they

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are also costly and functionally variable (reflecting variation in the human population), they undergo severe stress during the isolation process and, critically, they rapidly lose key functions when cultured *in vitro*. Moreover, it is important to note that hepatocyte toxicity *per se* is not the sole cause of hepatotoxicity which, in the intact liver, may involve multiple different cell types including lymphocytes and macrophages. Yet it is reasonable to assume from the work of several groups, over many years, that a metabolically-competent hepatocyte will be an essential component of any model of hepatotoxicity *in vitro*. Thus, a robust and reproducible metabolically-competent hepatocyte-like cell derived from directly reprogrammed cells, or from pluripotent stem cells, would represent a major step forward for the development of a new generation of *in vitro* models.

The imperatives of industry and academia are driven by different model requirements. The priority for industry is a cost-effective and scalable high-throughput screening model that has direct input into 'go/no go' decision making during drug development, whilst academic scientists are driven by the need to understand hepatic physiology and the mechanistic basis of DILI. Hepatocytes derived from stem cells can, however, be central to both of these objectives. Whilst significant progress towards a functional hepatic phenotype has been made, it is clear that stem-cell-derived hepatocyte-like cells (SC-HLCs) still fall well short of recapitulating the full mature hepatocellular phenotype[12-15].

Because of the importance and likely impact of developments in this field, scientists with expertise in preclinical and clinical hepatotoxicityand complex and novel forms of *in vitro* cell culture, representing industry, academia and regulatory bodies, assembled at a workshop at the University of Liverpool, under the auspices of the European Partnership for Alternative Approaches to Animal Testing (EPAA) (http://ec.europa.eu/growth/sectors/chemicals/epaa/index_en.htm) and the MRC Centre for Drug Safety Science (https://www.liverpool.ac.uk/drug-safety/). The purpose of the workshop was to specifically explore the application of stem cells in hepatotoxicity safety assessment, and to make recommendations for the way forward. This workshop follows the

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EPAA/NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research) (https://www.nc3rs.org.uk/) "Stem Cells in Safety Testing Forum" workshop that took place in 2013, with a mandate to provide a platform for permanent dialogue between research groups, to share experiences, problems, successes and opportunities.

Current challenges in the use of stem cell-derived hepatocytes in the safety assessment of new chemical entities

It is clear from a large number of studies[13, 14, 16-47] (see Table 1) that hepatocytes generated from stem cells are not currently sufficiently mature to emulate an adult primary human hepatocyte, and that these cells are probably closer in phenotype to a fetal hepatocyte[12]. Many studies using SC-HLCs purport to demonstrate a hepatocyte-like phenotype but do not actually incorporate a physiologically-relevant benchmark (e.g. freshly-isolated human hepatocytes) and a non-physiologically-relevant benchmark (e.g. HepG2 cells); in addition, often very few markers of the hepatic phenotype are used and studies do not always employ quantitatively-relevant assays (e.g mass spectrometry). Thus, inadequate benchmarking has hampered the field and there is likely significant value in identifying a common framework that might allow end users to readily interpret cell phenotype.

Despite the challenges in generating mature hepatocytes, SC-HLCs have recently been shown to retain the cytochrome P450 (CYP) expression profile (specifically CYP2C9 and CYP2D6) of the donor hepatocyte[48, 49], yielding metabolism-specific toxicity for CYP2C9 (benzbromarone) and CYP2D6 (tamoxifen). This is highly relevant as the CYPs are key enzymes of Phase I drug metabolism, that play a key role in the chemical functionalization and eventual elimination of drugs from the body, but which also can yield significant intracellular concentrations of chemically reactive metabolites, leading to cellular and tissue damage of the liver, and therefore DILI (for a review of this area, see Park et al, 2011[50]).

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The recent studies outlined above [48, 49] are particularly important as they suggest that modelling some forms of DILI (such as that elicited by benzbromarone or tamoxifen) using stem cell-derived hepatocytes may be possible, and that ultimately the challenges to generating a fully mature HLC will not always be insurmountable.

We consider that there are at least three major challenges to producing mature, physiologically- and pharmacologically-relevant hepatocytes from stem cells:

- Stem cell-derived hepatocytes must mimic several years of development *in vivo*.
- Like primary hepatocytes, the stem cell-derived hepatocyte phenotype is unstable currently in culture[51].
- At the moment, it is difficult to emulate the complexity of the liver, with its unique blood supply and exposure to relevant concentrations of intestinal products and nutrients *in vitro.* Development of three-dimensional culture systems that employ co-cultivation of all cell types found in the liver acinus is likely to be required if we are to recapitulate the liver *in vitro*[51, 52]. Following on from this, it is important to remember that a hepatocyte is not a single entity but varies functionally according to the hepatic zone in which it is located. The consequence of this is that some hepatotoxins induce hepatocellular damage in a zone-specific manner and this has not yet begun to be addressed meaningfully in the stem cell field, as we focus our attempts on improving basic functional maturity of the SC-derived cells, but it will need to be considered.

Despite these challenges, there are many promising leads in development, e.g. the discovery of several small molecule inducers of the hepatic phenotype[53], and the finding that microbial-derived secondary metabolites to which immature hepatocytes are likely to be exposed to post-partum may induce a significant increase in maturity. A further paradigm comes from the exploitation of SC-HLCs for demonstration of efficacy; specifically, for the reversal of the hepatic alpha1-antitrypsin-deficient

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phenotype, shown by Yusa et al[54]. This study demonstrated restoration of alpha1-antitrypsin activity was possible on a "sufficiently" mature background, rather than one that was necessarily fully mature and identical to a freshly-isolated adult hepatocyte. Furthermore, a recent study by Ware et al[55] suggests that DILI detection is possible using SC-HLCs in micopatterned co-cultures, in which cells mature to significant levels. It is worth remembering that the hepatocyte exhibits more individual functions (>500) than any of the other ~200 terminally differentiated cell types in the human body. Therefore it is perhaps not surprising that this cell is amongst the most challenging to mature, and we should still continue to explore the utility of hepatocyte-like cells as prototypes rather than await the final "product".

Lessons learned from the use of stem cell-derived cardiomyocytes in detecting cardiotoxicity

A parallel example, from which lessons can be learned, comes from the use of stem cells in the assessment of drug-induced cardiotoxicity – a primary cause of drug attrition. Cardiotoxicity, specifically QT prolongation, has already been successfully modelled using such cells[56-58]. In comparison, there is only very recent evidence that SC-HLCs are able to recapitulate hepatotoxic events[49, 55]. The difference between successful application of cardiac models compared with hepatic models may reflect the relative specificity of some forms of drug-induced cardiotoxicity, in contrast with the rather pleiotropic and diverse manifestations of hepatotoxicity, at the molecular, cellular, and tissular level[59]. Cardiotoxicity often arises due to drug-induced electrical perturbation of the cell interfering with its contractile function[60]. Here, the stem cell-cardiomyocyte model provides advantages over recombinant tumour models. Thus, the impact of drugs that cause simple single ion channel or complex multi-channel perturbation can be related to cardiomyocyte arrhythmias and abnormalities in contractility[61]. In hepatotoxicity, however, there are myriad factors required to recapitulate toxicity, especially idiosyncratic toxicity where the immune system is

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also implicated. This is compounded by inter-individual variation in expression of xenobiotic metabolism and transporter proteins in addition to the chemistry of each drug.

Whilst protocols to differentiate stem cells towards cardiomyocytes generate cells that are not fully mature[61], these cells can recapitulate some facets of the cell phenotype required to produce specific forms of cardiotoxicity. This has prompted major international efforts to search for methods to further mature stem cell cardiomyocytes. Each incremental improvement made towards progressing the compliment of ion channels, regulatory pathways and structural proteins to the complete sets found in adult cells will dramatically increase the utility of stem cell cardiomyocytes. The demonstration that specific toxicological phenotypes can be mimicked by stem cell-derived cardiomyocytes allows the cell model to be considered "fit-for-purpose". This raises the notion of using stem cell-derived hepatocytes that may be sufficiently mature for a specific toxicological assessment even though the cells may lack the full hepatic functionality with respect to drug metabolism, transporter expression etc. For example, where one or two cytochrome P450s (P450s), some relevant phase II enzymes, such as the glutathione transferases and UDP-glucuronyl transferases, and some Phase III proteins (influx and efflux transporters) are expressed at a set and reproducible % of a "typical" human hepatocyte, this cell may in some cases represent a significant and useful model in understanding specifically drug metabolism and possible metabolism-dependent toxicity.

The Importance of Phenotypic Characterisation

For the field to continue to move forward and develop liver cell models that are useful in prediction and mechanistic understanding of DILI, it is essential that the SC-HLCs are properly benchmarked against currently used and relevant human cells, especially fresh primary human hepatocytes and HepG2 cells (see Table 1 and Figure 1). Moreover, the phenotype of the HLCs must be as reproducible as possible, and they should be fully characterised, particularly with reference to the

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pharmacological phenotype (using a defined panel of training compounds). It is also important that the cell model can provide a static point of reference that can be used to ascertain if real progress is being made. When assessing novel models of hepatotoxicity it is important to use functional assays employing quantitative mass spectrometry whenever possible, as this is now being routinely employed [48, 62, 63]in order to determine the true phenotype of the model. A global proteomic analysis however may be the most appropriate way to characterise the cells, as this would represent a broad visualisation of the physiological phenotype of the cells. Similarity to freshly-isolated hepatocytes/tissue can be established through proteomics and targeted multiple- reaction-monitoring (MRM)-based mass spectrometric analysis of key proteins, such as CYP450s, transporters and intracellular signalling molecules and metabolic and cellular uptake profiles determined. Developments in mass spectrometric technologies mean that it is now possible to analyse small panels of proteins (for example 10-20 transporters or P450s) using MRM, in order to quantify proteins per cell at an *absolute* level[64]. This would ensure valid comparisons between currently used models and cells, as well as cells that are developed in the future. Given the inherent deficiencies in a transcriptomic-only approach, which are well-illustrated in a recent landmark paper reporting only a 39% correlation between mRNA and protein at a global level[65], measuring mRNA levels is not recommended for cell characterisation purposes.

As part of a comprehensive assessment of HLC phenotype, recent developments in the field of hepatocyte-selective translatable biomarkers (e.g. miR122[66]) might allow us to translate the response to chemicals between humans, model organisms and cells including SC-HLCs and it is likely that additional novel and selective biomarkers will be identified in the future using models such as SC-HLCs. This is an important area for industry which requires selective and translatable biomarkers of liver injury to monitor potentially hepatotoxic compounds in the clinic.

The recently developed concepts of adverse outcome pathways and points of departure[67] in the field of systems toxicology should also be considered in the context of phenotyping the response to

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chemical exposure of hepatocyte-like cells that express relevant proteins and pathways. To this end, cells expressing genetic reporters for key adaptive pathways such as Nrf2, PXR andNF-κB will be useful as a means for understanding the earliest events in the biological response to a drug[68-70]. However, it is imperative that we develop ways to bridge our findings from these molecular investigations to what actually occurs in DILI in humans –the development of novel bridging biomarkers that allow extrapolation from *in vitro* test system to man will be invaluable in this endeavour. Another important development in relation to hepatocyte genotype and phenotype in DILI is the derivation of SC-HLCs with specific polymorphisms relevant to drug toxicology. Of particular interest in this regard is the developing use of CRISPR technology in SC-HLCs to edit, for example, genes relevant to drug metabolism and toxicity thereby providing a wild type cell and an almost identical cell with an alteration in drug metabolism and toxicological responses, respectively.

Finally, phenotypic characterisation may be assisted by a better understanding of the mechanisms contributing to de-differentiation or loss of phenotype. Consideration of the cellular complexity of the liver and the functional sophistication of a hepatocyte makes it unsurprising that the maintenance of a fully functional hepatocyte in culture is difficult to achieve[71]. The cells have been removed from their neighbouring hepatocytes, disrupting their gap junctions and tight junctions which are important for their phenotype, as well as their juxtaposed non-parenchymal cells, which may also be responsible for the differentiated hepatocyte phenotype[72, 73]. Dedifferentiation is not a unique process to the liver; when cardiomyocytes are cultured, they also lose some of their *in vivo* phenotype, e.g. the t-tubules are lost, glycogen is accumulated and chromatin becomes dispersed *in vitro*[74]. However, the key difference between hepatocytes and myocytes is the importance of the metabolic phenotype with respect to drug toxicity, and it is this function – particularly the phase I CYP450 capacity – that is most rapidly and profoundly depleted[71, 75] - and it is also this function, at a defined proportion of the activity present in human liver, that is essential in any *in vitro* model of a hepatocyte

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One area of research that could have a significant impact on attempts to re-establish a functional hepatocyte from stem cells, is the investigation of the precise cellular mechanisms underlying the de-differentiation process that occurs in hepatocytes once they have been removed from the liver. Whilst the factors driving de-differentiation may not be identical to those that drive differentiation, it is likely that one or more pathways and processes uncovered through research into de-differentiation will be amenable for testing in differentiation experiments. If it is not understood how to maintain the dynamic and sophisticated machinery of a fully mature hepatocyte in vitro, it is likely to be difficult to capture the same phenotype in a stem cell-derived cell grown under similar conditions.

Summary and recommendations

- DILI is a complex, multi-dimensional disease, with variable phenotype between individuals, even for a single drug. There is essentially no ideal *in vitro* or *in vivo* model that recapitulates all of the potential features of this injury.
- The aspiration of the field is a "perfect" mature hepatocyte as it exists in a liver - this has not yet been achieved. Until it is, hepatocyte-like cells with known, quantifiable and reproducible proportions of the function of two widely-used standards, i.e. primary fresh human hepatocytes, and HepG2, will be valuable biological models to explore the physiological, pharmacological and toxicological response of hepatocytes to drug exposure.
- These "immature" cells should be explored as models of chemical perturbation using genetic reporters and biomarkers, with continual effort to relate findings to human DILI.
- Global proteomic analysis aligned with biological pathway analysis may be the most appropriate way to characterise HLCs – a small targeted panel of proteins will also help to compare cells for key proteins and functions using absolute quantitation by mass spectrometry. Crucially, this will advance the field by avoiding over-reliance on a small panel

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of liver proteins, such as albumin, that may not be representative of a fully mature and functioning liver cell.

- It is likely that niche creation *in vitro*, deploying enhanced matrices[13] and even 3D bioprinting[76], and incorporating other cell types such as endothelial cells[76, 77] and Kupffer cells[78] *inter alia*, will mature and support hepatocyte function.
- A small panel of chemical benchmarks will be needed to probe the physiological, pharmacological and toxicological function of the cells, only once they have been properly phenotyped. There is little point in exposing HLCs to chemicals chosen as hepatotoxins in man unless we fully characterise the cells.

Bibliography

1. Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, Farrar K, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. BMJ 2004;329:15-19.
2. Waring MJ, Arrowsmith J, Leach AR, Leeson PD, Mandrell S, Owen RM, Pairaudeau G, et al. An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nat Rev Drug Discov 2015;14:475-486.
3. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, McCashland TM, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137:947-954.
4. Spanhaak S, Cook, D., Barnes, J. & Reynolds, J. Species Concordance for Liver Injury. BioWisdom Report 2008;http://bioblog.instem.com/downloads/SIP_Board_Species_Concordance.pdf.
5. Verma S, Kaplowitz N. Diagnosis, management and prevention of drug-induced liver injury. Gut 2009;58:1555-1564.
6. Tolosa L, Pinto S, Donato MT, Lahoz A, Castell JV, O'Connor JE, Gomez-Lechon MJ. Development of a multiparametric cell-based protocol to screen and classify the hepatotoxicity potential of drugs. Toxicol Sci 2012;127:187-198.
7. Xu JJ, Henstock PV, Dunn MC, Smith AR, Chabot JR, de Graaf D. Cellular imaging predictions of clinical drug-induced liver injury. Toxicol Sci 2008;105:97-105.
8. Tolosa L, Gomez-Lechon MJ, Lopez S, Guzman C, Castell JV, Donato MT, Jover R. Human Upcyte Hepatocytes: Characterization of the Hepatic Phenotype and Evaluation for Acute and Long-Term Hepatotoxicity Routine Testing. Toxicol Sci 2016.
9. Khetani SR, Kanchagar C, Ukairo O, Krzyzewski S, Moore A, Shi J, Aoyama S, et al. Use of micropatterned cocultures to detect compounds that cause drug-induced liver injury in humans. Toxicol Sci 2013;132:107-117.

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10. Gerets HH, Tilmant K, Gerin B, Chanteux H, Depelchin BO, Dhalluin S, Atienzar FA. Characterization of primary human hepatocytes, HepG2 cells, and HepaRG cells at the mRNA level and CYP activity in response to inducers and their predictivity for the detection of human hepatotoxins. Cell Biol Toxicol 2012;28:69-87.
11. Guillouzo A, Corlu A, Aninat C, Glaise D, Morel F, Guguen-Guillouzo C. The human hepatoma HepaRG cells: a highly differentiated model for studies of liver metabolism and toxicity of xenobiotics. Chem Biol Interact 2007;168:66-73.
12. Baxter M, Withey S, Harrison S, Segeritz CP, Zhang F, Atkinson-Dell R, Rowe C, et al. Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. J Hepatol 2015;62:581-589.
13. Cameron K, Tan R, Schmidt-Heck W, Campos G, Lyall MJ, Wang Y, Lucendo-Villarin B, et al. Recombinant Laminins Drive the Differentiation and Self-Organization of hESC-Derived Hepatocytes. Stem Cell Reports 2015;5:1250-1262.
14. Godoy P, Schmidt-Heck W, Natarajan K, Lucendo-Villarin B, Szkolnicka D, Asplund A, Bjorquist P, et al. Gene networks and transcription factor motifs defining the differentiation of stem cells into hepatocyte-like cells. J Hepatol 2015;63:934-942.
15. Liu J, Brzeszczynska J, Samuel K, Black J, Palakkan A, Anderson RA, Gallagher R, et al. Efficient episomal reprogramming of blood mononuclear cells and differentiation to hepatocytes with functional drug metabolism. Exp Cell Res 2015;338:203-213.
16. Kia R, Sison RL, Heslop J, Kitteringham NR, Hanley N, Mills JS, Park BK, et al. Stem cell-derived hepatocytes as a predictive model for drug-induced liver injury: are we there yet? Br J Clin Pharmacol 2013;75:885-896.
17. Cai J, Zhao Y, Liu Y, Ye F, Song Z, Qin H, Meng S, et al. Directed differentiation of human embryonic stem cells into functional hepatic cells. Hepatology 2007;45:1229-1239.
18. Ek M, Soderdahl T, Kuppers-Munther B, Edsbagge J, Andersson TB, Bjorquist P, Cotgreave I, et al. Expression of drug metabolizing enzymes in hepatocyte-like cells derived from human embryonic stem cells. Biochem Pharmacol 2007;74:496-503.
19. Soderdahl T, Kuppers-Munther B, Heins N, Edsbagge J, Bjorquist P, Cotgreave I, Jernstrom B. Glutathione transferases in hepatocyte-like cells derived from human embryonic stem cells. Toxicol In Vitro 2007;21:929-937.
20. Hay DC, Fletcher J, Payne C, Terrace JD, Gallagher RC, Snoeys J, Black JR, et al. Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. Proc Natl Acad Sci U S A 2008;105:12301-12306.
21. Shiraki N, Umeda K, Sakashita N, Takeya M, Kume K, Kume S. Differentiation of mouse and human embryonic stem cells into hepatic lineages. Genes Cells 2008;13:731-746.
22. Agarwal S, Holton KL, Lanza R. Efficient differentiation of functional hepatocytes from human embryonic stem cells. Stem Cells 2008;26:1117-1127.
23. Moore RN, Moghe PV. Expedited growth factor-mediated specification of human embryonic stem cells toward the hepatic lineage. Stem Cell Res 2009;3:51-62.
24. Basma H, Soto-Gutierrez A, Yannam GR, Liu L, Ito R, Yamamoto T, Ellis E, et al. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. Gastroenterology 2009;136:990-999.
25. Song Z, Cai J, Liu Y, Zhao D, Yong J, Duo S, Song X, et al. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. Cell Res 2009;19:1233-1242.
26. Duan Y, Ma X, Zou W, Wang C, Bahbahan IS, Ahuja TP, Tolstikov V, et al. Differentiation and characterization of metabolically functioning hepatocytes from human embryonic stem cells. Stem Cells 2010;28:674-686.
27. Synnergren J, Heins N, Brolen G, Eriksson G, Lindahl A, Hyllner J, Olsson B, et al. Transcriptional profiling of human embryonic stem cells differentiating to definitive and primitive endoderm and further toward the hepatic lineage. Stem Cells Dev 2010;19:961-978.

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28. Touboul T, Hannan NR, Corbineau S, Martinez A, Martinet C, Branchereau S, Mainot S, et al. Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology 2010;51:1754-1765.
29. Brolen G, Sivertsson L, Bjorquist P, Eriksson G, Ek M, Semb H, Johansson I, et al. Hepatocyte-like cells derived from human embryonic stem cells specifically via definitive endoderm and a progenitor stage. J Biotechnol 2010;145:284-294.
30. Ghodsizadeh A, Taei A, Totonchi M, Seifinejad A, Gourabi H, Pournasr B, Aghdami N, et al. Generation of liver disease-specific induced pluripotent stem cells along with efficient differentiation to functional hepatocyte-like cells. Stem Cell Rev 2010;6:622-632.
31. Liu H, Ye Z, Kim Y, Sharkis S, Jang YY. Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. Hepatology 2010;51:1810-1819.
32. Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, Duris C, North PE, et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 2010;51:297-305.
33. Sullivan GJ, Hay DC, Park IH, Fletcher J, Hannoun Z, Payne CM, Dalgetty D, et al. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology 2010;51:329-335.
34. Rashid ST, Corbineau S, Hannan N, Marciniak SJ, Miranda E, Alexander G, Huang-Doran I, et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Invest 2010;120:3127-3136.
35. Zhang S, Chen S, Li W, Guo X, Zhao P, Xu J, Chen Y, et al. Rescue of ATP7B function in hepatocyte-like cells from Wilson's disease induced pluripotent stem cells using gene therapy or the chaperone drug curcumin. Hum Mol Genet 2011;20:3176-3187.
36. Bone HK, Nelson AS, Goldring CE, Tosh D, Welham MJ. A novel chemically directed route for the generation of definitive endoderm from human embryonic stem cells based on inhibition of GSK-3. J Cell Sci 2011;124:1992-2000.
37. Yildirimman R, Brolen G, Vilardell M, Eriksson G, Synnergren J, Gmuender H, Kamburov A, et al. Human embryonic stem cell derived hepatocyte-like cells as a tool for in vitro hazard assessment of chemical carcinogenicity. Toxicol Sci 2011;124:278-290.
38. Chen YF, Tseng CY, Wang HW, Kuo HC, Yang VW, Lee OK. Rapid generation of mature hepatocyte-like cells from human induced pluripotent stem cells by an efficient three-step protocol. Hepatology 2012;55:1193-1203.
39. Cayo MA, Cai J, DeLaForest A, Noto FK, Nagaoka M, Clark BS, Collery RF, et al. JD induced pluripotent stem cell-derived hepatocytes faithfully recapitulate the pathophysiology of familial hypercholesterolemia. Hepatology 2012;56:2163-2171.
40. Schwartz RE, Trehan K, Andrus L, Sheahan TP, Ploss A, Duncan SA, Rice CM, et al. Modeling hepatitis C virus infection using human induced pluripotent stem cells. Proc Natl Acad Sci U S A 2012;109:2544-2548.
41. Takayama K, Inamura M, Kawabata K, Katayama K, Higuchi M, Tashiro K, Nonaka A, et al. Efficient generation of functional hepatocytes from human embryonic stem cells and induced pluripotent stem cells by HNF4alpha transduction. Mol Ther 2012;20:127-137.
42. Choi SM, Kim Y, Shim JS, Park JT, Wang RH, Leach SD, Liu JO, et al. Efficient drug screening and gene correction for treating liver disease using patient-specific stem cells. Hepatology 2013;57:2458-2468.
43. Ramasamy TS, Yu JS, Selden C, Hodgson H, Cui W. Application of three-dimensional culture conditions to human embryonic stem cell-derived definitive endoderm cells enhances hepatocyte differentiation and functionality. Tissue Eng Part A 2013;19:360-367.
44. Gieseck RL, 3rd, Hannan NR, Bort R, Hanley NA, Drake RA, Cameron GW, Wynn TA, et al. Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture. PLoS One 2014;9:e86372.

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45. Jia B, Chen S, Zhao Z, Liu P, Cai J, Qin D, Du J, et al. Modeling of hemophilia A using patient-specific induced pluripotent stem cells derived from urine cells. Life Sci 2014;108:22-29.
46. Avior Y, Levy G, Zimerman M, Kitsberg D, Schwartz R, Sadeh R, Moussaieff A, et al. Microbial-derived lithocholic acid and vitamin K2 drive the metabolic maturation of pluripotent stem cells-derived and fetal hepatocytes. Hepatology 2015;62:265-278.
47. Chien Y, Chang YL, Li HY, Larsson M, Wu WW, Chien CS, Wang CY, et al. Synergistic effects of carboxymethyl-hexanoyl chitosan, cationic polyurethane-short branch PEI in miR122 gene delivery: accelerated differentiation of iPSCs into mature hepatocyte-like cells and improved stem cell therapy in a hepatic failure model. Acta Biomater 2015;13:228-244.
48. Ulvestad M, Nordell P, Asplund A, Rehnstrom M, Jacobsson S, Holmgren G, Davidson L, et al. Drug metabolizing enzyme and transporter protein profiles of hepatocytes derived from human embryonic and induced pluripotent stem cells. Biochem Pharmacol 2013;86:691-702.
49. Takayama K, Morisaki Y, Kuno S, Nagamoto Y, Harada K, Furukawa N, Ohtaka M, et al. Prediction of interindividual differences in hepatic functions and drug sensitivity by using human iPS-derived hepatocytes. Proc Natl Acad Sci U S A 2014;111:16772-16777.
50. Park BK, Boobis A, Clarke S, Goldring CE, Jones D, Kenna JG, Lambert C, et al. Managing the challenge of chemically reactive metabolites in drug development. Nat Rev Drug Discov 2011;10:292-306.
51. Berger DR, Ware BR, Davidson MD, Allsup SR, Khetani SR. Enhancing the functional maturity of induced pluripotent stem cell-derived human hepatocytes by controlled presentation of cell-cell interactions in vitro. Hepatology 2015;61:1370-1381.
52. Davidson MD, Ware BR, Khetani SR. Stem cell-derived liver cells for drug testing and disease modeling. Discov Med 2015;19:349-358.
53. Shan J, Schwartz RE, Ross NT, Logan DJ, Thomas D, Duncan SA, North TE, et al. Identification of small molecules for human hepatocyte expansion and iPS differentiation. Nat Chem Biol 2013;9:514-520.
54. Yusa K, Rashid ST, Strick-Marchand H, Varela I, Liu PQ, Paschon DE, Miranda E, et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. Nature 2011;478:391-394.
55. Ware BR, Berger DR, Khetani SR. Prediction of Drug-Induced Liver Injury in Micropatterned Co-cultures Containing iPSC-Derived Human Hepatocytes. Toxicol Sci 2015;145:252-262.
56. Lahti AL, Kujala VJ, Chapman H, Koivisto AP, Pekkanen-Mattila M, Kerkela E, Hyttinen J, et al. Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. Dis Model Mech 2012;5:220-230.
57. Matsa E, Rajamohan D, Dick E, Young L, Mellor I, Staniforth A, Denning C. Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. Eur Heart J 2011;32:952-962.
58. Itzhaki I, Maizels L, Huber I, Gepstein A, Arbel G, Caspi O, Miller L, et al. Modeling of catecholaminergic polymorphic ventricular tachycardia with patient-specific human-induced pluripotent stem cells. J Am Coll Cardiol 2012;60:990-1000.
59. Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discov 2005;4:489-499.
60. Force T, Kolaja KL. Cardiotoxicity of kinase inhibitors: the prediction and translation of preclinical models to clinical outcomes. Nat Rev Drug Discov 2011;10:111-126.
61. Denning C, Borgdorff V, Crutchley J, Firth KS, George V, Kalra S, Kondrashov A, et al. Cardiomyocytes from human pluripotent stem cells: From laboratory curiosity to industrial biomedical platform. Biochim Biophys Acta 2015.
62. Sengupta S, Johnson BP, Swanson SA, Stewart R, Bradfield CA, Thomson JA. Aggregate culture of human embryonic stem cell-derived hepatocytes in suspension are an improved in vitro model for drug metabolism and toxicity testing. Toxicol Sci 2014;140:236-245.

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HEP-16-0938

63. Ma X, Duan Y, Tschudy-Seney B, Roll G, Behbahan IS, Ahuja TP, Tolstikov V, et al. Highly efficient differentiation of functional hepatocytes from human induced pluripotent stem cells. Stem Cells Transl Med 2013;2:409-419.
64. Kitteringham NR, Jenkins RE, Lane CS, Elliott VL, Park BK. Multiple reaction monitoring for quantitative biomarker analysis in proteomics and metabolomics. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:1229-1239.
65. Schwanhausser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, et al. Global quantification of mammalian gene expression control. Nature 2011;473:337-342.
66. Starkey Lewis PJ, Dear J, Platt V, Simpson KJ, Craig DG, Antoine DJ, French NS, et al. Circulating microRNAs as potential markers of human drug-induced liver injury. Hepatology 2011;54:1767-1776.
67. Willett C, Caverly Rae J, Goyak KO, Minsavage G, Westmoreland C, Andersen M, Avigan M, et al. Building shared experience to advance practical application of pathway-based toxicology: liver toxicity mode-of-action. ALTEX 2014;31:500-519.
68. Herpers B, Wink S, Fredriksson L, Di Z, Hendriks G, Vrieling H, de Bont H, et al. Activation of the Nrf2 response by intrinsic hepatotoxic drugs correlates with suppression of NF-kappaB activation and sensitizes toward TNFalpha-induced cytotoxicity. Arch Toxicol 2015.
69. Fredriksson L, Wink S, Herpers B, Benedetti G, Hadi M, de Bont H, Groothuis G, et al. Drug-induced endoplasmic reticulum and oxidative stress responses independently sensitize toward TNFalpha-mediated hepatotoxicity. Toxicol Sci 2014;140:144-159.
70. Wink S, Hiemstra S, Huppelschoten S, Danen E, Niemeijer M, Hendriks G, Vrieling H, et al. Quantitative high content imaging of cellular adaptive stress response pathways in toxicity for chemical safety assessment. Chem Res Toxicol 2014;27:338-355.
71. Elaut G, Henkens T, Papeleu P, Snykers S, Vinken M, Vanhaecke T, Rogiers V. Molecular mechanisms underlying the dedifferentiation process of isolated hepatocytes and their cultures. Curr Drug Metab 2006;7:629-660.
72. Bhatia SN, Balis UJ, Yarmush ML, Toner M. Probing heterotypic cell interactions: hepatocyte function in microfabricated co-cultures. J Biomater Sci Polym Ed 1998;9:1137-1160.
73. Zinchenko YS, Schrum LW, Clemens M, Coger RN. Hepatocyte and kupffer cells co-cultured on micropatterned surfaces to optimize hepatocyte function. Tissue Eng 2006;12:751-761.
74. Ausma J, Borgers M. Dedifferentiation of atrial cardiomyocytes: from in vivo to in vitro. Cardiovasc Res 2002;55:9-12.
75. Rowe C, Gerrard DT, Jenkins R, Berry A, Durkin K, Sundstrom L, Goldring CE, et al. Proteome-wide analyses of human hepatocytes during differentiation and dedifferentiation. Hepatology 2013;58:799-809.
76. Ma X, Qu X, Zhu W, Li YS, Yuan S, Zhang H, Liu J, et al. Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. Proc Natl Acad Sci U S A 2016.
77. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481-484.
78. Nguyen TV, Ukairo O, Khetani SR, McVay M, Kanchagar C, Seghezzi W, Ayanoglu G, et al. Establishment of a hepatocyte-kupffer cell coculture model for assessment of proinflammatory cytokine effects on metabolizing enzymes and drug transporters. Drug Metab Dispos 2015;43:774-785.

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Table 1. Summary of studies post-2007 of HLC-derivation from human pluripotent stem cells (adapted from Table 1 and Table 2, Kia *et al*[13] with modification). Note the limited number of Phase 1 and 2 phenotyping markers generally employed in the characterization of the HLCs.

Reference	Method of stem cell differentiation			Differentiation efficiency % ALB +ve HLCs	PHENOTYPING: Phase I and II enzyme activity		
	Stem cell (cell line)	Culture format	Differentiation factors	% ALB +ve HLCs (assay method)	Enzyme (assay method)	% hPH comparator	Other comparators
Cai *et al.*, 2007[14]	hESC (H1, H9)	Monolayer, EB formation	AF V, AA, ITS, BMP2, FGF4, HGF, OSM, DEX	70 (ICC)	CYP2B6 (Fluorescence)	ND	hESC
Ek *et al.*, 2007[15]	hESC (SA002, SA002.5, SA167)	Monolayer	Proprietary differentiation medium, FGF2	ND	CYP1A1 (Fluorescence) CYP3A4 (Fluorescence)	0 0	– –
Söderdahl *et al.*, 2007[16]	hESC (SA001, SA002, SA002.5, AS034, SA121, and SA167)	Monolayer	Proprietary differentiation medium, bFGF	ND	GST (Fluorescence)	80	HepG2
Hay *et al.*, 2008[17]; **Godoy et al., 2015**[11]; **Cameron et al., 2015**[10]	hESC (H1, H9)	Monolayer	AA, Wnt3a	90 (ICC)	CYP 1A2 (LC-MS-MS) CYP1A2 (Luminescence) 100 CYP3A4 (Luminescence) 100	24	hESC
Shiraki *et al.*, 2008[18]	hESC (Khes-1)	Co-culture with M15 cell line	AA, BMP4, bFGF, HGF, DMSO, DEX, Ly294002	9 (ICC)	ND	–	–
Agarwal *et al.*,2008[19]	hESC (WA01, WA09)	Monolayer	AA, FGF4, HGF, BSA, OSM, DEX	67.4 (ICC)	ND	–	–
Moore *et al.*, 2009[20]	hESC (H1)	Monolayer, EB formation	AA, Wnt3a, HGF, OSM, DEX	72.8 (ICC)	CYP 1A2 (Fluorescence)	ND	hESC-derived HLCs in culture media of different components
Basma *et al.*, 2009[21]	hESC (H1)	Monolayer, EB formation	AA, FGF2, HGF, DMSO, DEX	55.5 (ICC)	CYP1A (Fluorescence) CYP3A (LC-MS-MS)	30 90	– –
Song *et al.*, 2009[22]	hESC (H1), hiPSC (hFb-derived 3U1, 3U2)	Monolayer	AF V, AA, ITS, BMP2, FGF4, OSM, DEX, KGF, B27	60 (ICC)	CYP2B6 (Fluorescence)	ND	hiPSC-derived versus hESC-derived HLCs
Duan *et al.*, 2010[23]	hESC (H9)	Monolayer	AA, sodium butyrate, BMP2, BMP4, FGF4, HGF DMSO, B27	75-90 (ICC, FACS)	CYP1A2 (LC-MS-MS) CYP2C9 (LC-MS-MS) CYP2D6 (LC-MS-MS) CYP3A4 (LC-MS-MS)	100 60 95 90	– – – –
Synnergren *et al.*, 2010[24]	hESC (SA002, SA167, SA461)	Monolayer	AA, ITS, FGF1, FGF2, BMP2, BMP4, HGF, OSM, DEX	ND	ND	–	–
Touboul *et al.*, 2010[25]	hESC (H9)	Monolayer	AA, BMP4,FGF2, FGF4, FGF10, HGF, EGF, retinoic acid, SB431542, Ly294002	ND	CYP3A (Bioluminescence)	ND	–
Brolén *et al.*, 2010[26]	hESC (SA001, SA002, SA002.5, SA167)	Monolayer	AA, BMP2, BMP4, FGF1, FGF2, HGF, OSM, DEX, Wnt3A	ND	CYP1A (LC-MS-MS) CYP2C (LC-MS-MS) CYP2A (LC-MS-MS)	ND	Spontaneously differentiated hESC-derived HLCs, HepG2
Ghodsizadeh *et al.*, 2010[27]	hiPSC (hFb-derived)	EB formation	AA, FGF2, HGF, DMSO, DEX	50 (FACS)	CYP2B6 (Fluorescence)	ND	hiPSC
Liu *et al.*, 2010[28]	hESC (WA01, WA09), hiPSC (hPH-derived)	Monolayer	AA, FGF4, HGF, OSM, DEX	ND	CYP1A2 (Bioluminescence) CYP3A4 (Bioluminescence)	ND	–
Si-Tayeb *et al.*, 2010[29]	hESC (H9), hiPSC (hFb-derived)	Monolayer	AA, BMP4, FGF2, OSM, B27	80 (FACS)	ND	–	–
Sullivan *et al.*, 2010[30]	hiPSC (hFb-derived)	Monolayer	AA, HGF, Wnt3A, DMSO, OSM, hydrocortisone, tryptose phosphate broth, B27	70-90 (ICC)	CYP1A2 (Bioluminescence) CYP3A4 (Bioluminescence)	ND	–

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Rashid *et al.*, 2010[31]	hiPSC (hFb-derived)	Monolayer	AA, BMP4, FGF2, HGF, OSM, Ly294002, CHIR99021 (GSK-3 inhibitor)	83 (FACS)	CYP3A4 (Bioluminescence)	ND	hiPSC
Zhang *et al.*, 2011[32]	hESC (H9), hiPSC (hFb-derived)	Monolayer, EB formation	AA, BMP2, FGF4, HGF, KGF, OSM, DEX	60-80 (ICC, FACS)	CYP3A4 (Bioluminescence)	0.32	hESC-derived HLCs
Bone *et al.*, 2011[33]	hESC (Shef1, Shef3)	Monolayer	FGF4, HGF, OSM, DEX, 1 m (GSK-3 inhibitor)	ND	ND	–	–
Yildirimman *et al.*, 2011[34]	hESC (SA002)	Monolayer	Proprietary differentiation medium	ND	CYP1A2 (LC-MS-MS) CYP3A4 (LC-MS-MS) CYP2B6 (LC-MS-MS) CYP2C9 (LC-MS-MS) CYP2C19 (LC-MS-MS)	50 50 10 50 50	– – – – –
Chen *et al.*, 2012[35]	hESC (H9), hiPSC (hFb-derived, CFB46)	Monolayer	AA, ITS, HGF, Wnt3A, OSM, DMSO, DEX	ND	CYP3A4 (Bioluminescence)	100	hiPSC
Cayo *et al.*, 2012[36]	hiPSC (FH patient JD fibroblast-derived)	Monolayer	OCT4, SOX2, NANOG, LIN28	ND	ND	–	–
Schwartz *et al.*, 2012[37]	hiPSC (hFb-derived)	Monolayer	AA, BMP4, FGF2, HGF, OSM	80 (ICC)	ND	–	–
Takayama *et al.*, 2012[38]	hES (H9), hiPSC (hFb-derived, MCR5 & 201B7)	Monolayer	AA, SOX17, HEX, BMP4, FGF4, LacZ, HNF4α, HGF, OSM, DEX	ND	CYP3A4 (Fluorescence) CYP2C9 (Fluorescence) CYP1A2 (Fluorescence)	100 > 10 < 1	– – –
Choi *et al.*, 2013[39]	hiPSC (derived from AAT deficient patients)	Monolayer	B27, AA, FGF4, HGF, OSM, DEX	ND	CYP3A4 (Bioluminescence) CYP2D6 (Bioluminescence) CYP2C19 (Bioluminescence) CYP1A2 (Bioluminescence)	80 70 90 90	– – – –
Ramasamy *et al.*, 2013[40]	hESC (H1)	Monolayer & 3D culture in Algimatrix plate	AA, DMSO, HGF, OSM	ND	CYP3A4 (Bioluminescence)	ND	HepG2
Gieseck *et al.*, 2014[41]	hiPSC (hFb-derived)	Monolayer, 3D-single cell or Clump culture in RAFT system	AA, FGF2,BMP4, LY-294002, Hepatozyme-SFM	ND	CYP3A4 for 2D Day 35 (HPLC-MS) CYP3A4 for 3D Day 45 (Bioluminescence)	4 25	–
Jia *et al.*, 2014[42]	hiPSC (from urine cells of HA patient)	Monolayer, EB formation	AA, FGF4, BMP2, HGF, KGF, OSM, DEX	64 (FACS)	ND	–	–
Avior *et al.*, 2015[43]	hESC (I3)	Monolayer	AA, B27, Wnt3A, HGF, DMSO, DEX, OSM, FGF2, LCA, MK4	83 (FACS)	CYP3A4, 1A2 (Fluorescence) CYP2E1, 2C9 (Fluorescence)	30 8	HepG2, hESC without LCA/MK4
Chien et al., 2015[44]	hiPSC (from dental pulp stromal cells)	Co-culture with MEF, EB formation	AF V, AA, FGF4, BMP2, HGF, KFG, OSM, DEX, B27, miR122 (delivered by PU-PEI in CHC)	ND	ND	–	–

Abbreviations: AA, activin A; AF V, albumin fraction V; bFGF, human recombinant basic FGF; BMP, bone morphogenic protein; BSA, bovine serum albumin; CHC, carboxymethyl-hexanoyl chitosan; DEX, dexamethasone; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; FACS, fluorescence-activated cell sorting; FGF, fibroblast growth factor; GFP, green fluorescent protein; GSK, glycogen synthase kinase; Hepatozyme-SMF, hepatozyme serum free medium; HEX, hematopoietically-expressed homeobox protein; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; HLCs, hepatocyte-like cells; HGF, hepatocyte growth factor; HNF4alpha, hepatocyte nuclear factor 4 alpha; ICC, immunocytochemistry; ITS, insulin-transferrin-selenium; KGF,

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keratinocyte growth factor; LacZ, beta-D-glactosidase; Ly294002, phosphoinositide 3-kinase inhibitor; miR122, microRNA 122; ND, not determined; OCT, octamer-binding transcription factor; OSM, oncostatin M; PU-PEI, biodegradable polyurethane-graft-short-branch polyethylenimine; qRT-PCR, quantitative real time polymerase chain reaction; SB431542, inhibitor for activin receptor-like kinase receptors ALK5, ALK4 and ALK7; SOX, sex determining region Y-box; Wnt3a, wingless-type MMTV integration site family, member 3a

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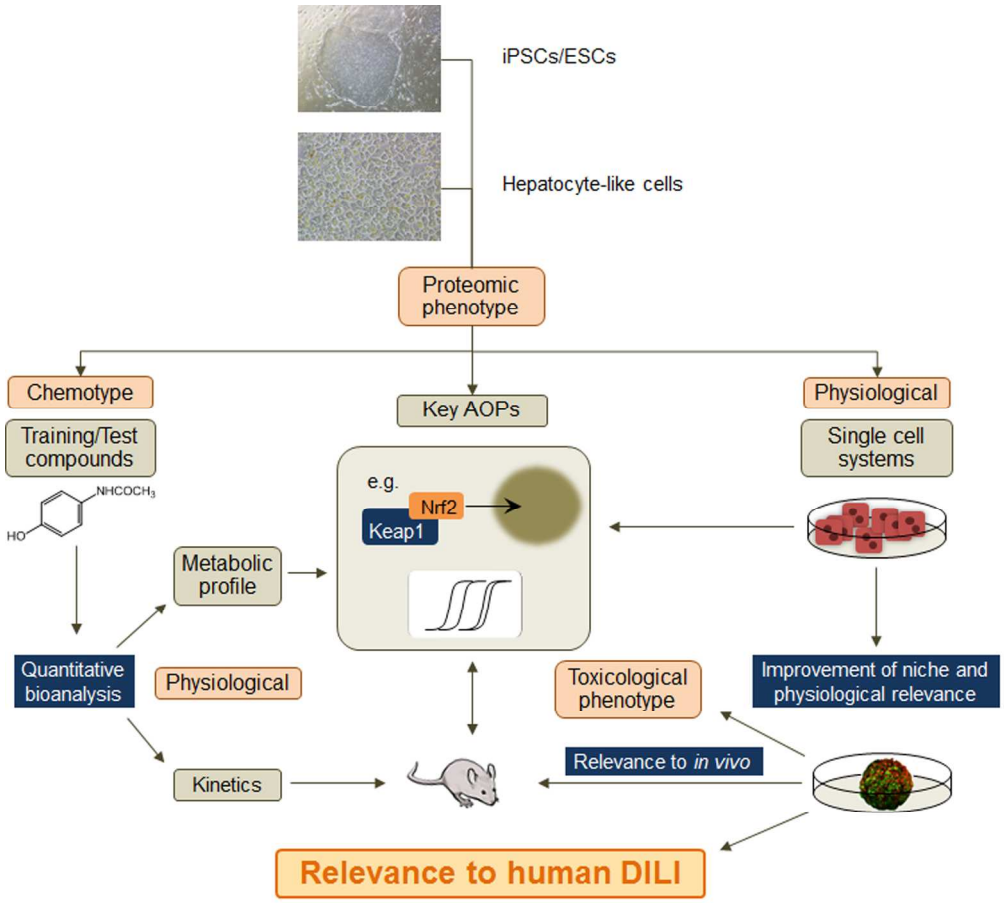

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