

1 **Title**

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3 **Potential of human Induced Pluripotent Stem Cells in studies of liver disease**

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1 **Footnote Page**

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5 **List of abbreviations:**

6 hIPSC: Human Induced Pluripotent Stem Cells

7 HLC: hIPSC-derived hepatocyte-like cells

8 CLCs: hIPSC-derived cholangiocyte-like cells

9 A1AT: α 1-Antitrypsin

10 FTA: Familial Transthyretin Amyloidosis

11 TTR: Transthyretin

12 GSD1a: Glycogen Storage Disease type 1a

13 WD: Wilson's Disease

14 FH: Familial Hypercholesterolemia

15 HCV: Hepatitis C Virus

16

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24

1 **Abstract**

2 Liver disease is a leading cause of death in the Western World. However, our insight
3 into the underlying disease mechanisms and development of novel therapeutic
4 agents has been hindered by limited availability of primary tissue, intra-species
5 variability associated with the use of animal models and reduced long-term viability of
6 isolated and diseased liver cells. The emergence of hIPSCs (human Induced
7 Pluripotent Stem Cells) and differentiation protocols to generate hepatocyte-like cells
8 has opened the possibility of addressing these issues. Here we discuss the recent
9 progress and potential in the production of various cell types constituting the liver and
10 their applications to model liver diseases and test drug toxicity *in vitro*.

Liver disease constitutes a leading cause of death worldwide (1). However, depending on the etiology, treatment options can be limited and liver transplantation remains the only available therapy for end stage liver failure (2). Understanding the disease pathogenesis is crucial for developing new therapeutic agents and diagnostic modalities. Although existing models for the study of hepatic disorders have proven very useful, they also exhibit significant limitations. *In vivo* models are constrained by intra-species variability (3), while the use of primary tissue is limited by poor availability and technical challenges in culturing primary cells (4). Furthermore, established cell lines are restricted by their malignant background and the requirement for non-physiological manipulations, such as protein overexpression, to replicate the disease phenotype.

Human Induced Pluripotent Stem Cells (hiPSCs) derived from reprogrammed somatic cells (5) demonstrate great potential for overcoming such challenges. Their capacity for self-renewal and differentiation into almost any cell type provides a unique system for generating large quantities of autologous, disease-specific liver cells *in vitro*. Cells generated through this system constitute an optimal platform for modeling hepatic disorders, provided that they closely resemble their native counterparts, recapitulate key aspects of the disease in question and demonstrate measurable responses to therapeutic agents.

Here, we review how these requirements are addressed by existing culture systems for the generation of hiPSC-derived hepatocytes, describe their applications for modeling hepatic disorders and discuss future directions for the use of hiPSCs in the study of liver disease.

Current approaches for the generation of liver cells from hiPSCs

Given the potential of hiPSCs for modeling hepatic disease, the derivation of different liver cell populations has been the subject of intensive research. Most of these efforts have concentrated on the generation of hepatocytes, which occupy the largest

1 proportion of the organ volume and are responsible for the majority of its metabolic
2 functions including detoxification, glucose metabolism, synthesis of plasma proteins
3 and bile production (6). However, the metabolic activity of hepatocytes is supported
4 by multiple other cell types collectively known as non-parenchymal liver cells. This
5 heterogeneous population includes sinusoidal endothelial cells, hepatic stellate cells,
6 Kupffer cells and cholangiocytes (6). With the exception of cholangiocytes,
7 differentiation of hiPSCs to non-parenchymal cell types of the liver has not yet been
8 reported. In this section, we concentrate on current platforms for the generation of
9 hiPSC-derived hepatocytes and cholangiocytes, their advantages and limitations.

10 **Hepatocytes**

11 Primary hepatocytes remain the gold standard for *in vitro* modeling of liver diseases.
12 However, hiPSC-derived hepatocyte-like-cells (HLCs) could present an excellent
13 alternative for addressing availability issues, provided they recapitulate key features
14 of their native counterparts. Indeed, a minimum set of criteria need to be met prior to
15 characterizing hiPSC-derived cells as 'hepatocyte-like'. These include expression of
16 hepatic markers at gene and protein level, demonstration of specific ultrastructural
17 characteristics, production of albumin, urea and fibrinogen, induction of P450
18 enzymatic activity following treatment with specific inducers and the use of selected
19 substrates to confirm phase 1 and 2 metabolic enzyme activity (7-10).

20 To fulfill these criteria, multiple groups have developed protocols of directed
21 differentiation towards HLCs (11-16), aimed at recapitulating key stages of natural
22 liver development including definitive endoderm, foregut, hepatic endoderm,
23 bipotential hepatoblasts and hepatocyte like cells (17) (Figure 1). All these
24 approaches converge on the modulation of pathways known to have a pivotal role in
25 hepatic embryogenesis, such as FGF, BMP4, Wnt, Activin and HGF signaling (17).
26 The resulting cells bear significant similarities to primary hepatocytes in terms of
27 transcriptional profile and functional properties, such as albumin and apolipoprotein
28 B100 (ApoB100) secretion, functional bile transport, LDL uptake, urea synthesis,

1 cytochrome P450 activity (Cyp3A4) and glycogen storage (16,18). However, HLCs
2 resemble more closely fetal rather than adult hepatocytes, with ongoing expression
3 of fetal markers (AFP) and embryonic P450 activity (Cyp3A7) (Table 1).

4 The fetal identity of HLCs has given rise to a plethora of studies focusing on
5 increasing their functionality. Among these approaches two main strategies emerge:

6 The first concentrates on increasing differentiation efficiency, promoting maturation
7 or enhancing P450 activity and drug metabolism. The use of small molecule
8 compounds (19-20), sequential overexpression of transcription factors controlling
9 various stages of hepatic development (17), direct reprogramming of fibroblasts to
10 induced Hepatic Stem Cells through forced expression of hepatic transcription
11 factors (21-22) and direct reprogramming of fibroblasts to endoderm progenitor cells
12 by combining overexpression of OCT4, SOX2 and Klf4 with small molecule treatment
13 (23) have all been reported to contribute towards achieving this goal. Therefore, it is
14 possible that such methodologies will increasingly complement growth factors driven
15 differentiation of hIPSCs in the future.

16 The second strategy focuses on accurately simulating the liver microenvironment
17 through 3D culture of HLCs (24), co-culture with endothelial or non-parenchymal
18 cells (25), the use of artificial scaffolds (26) or a combination of these techniques
19 (27). These approaches have also been reported to increase functionality, optimize
20 the transcriptional profile of HLCs and even allow the generation of functional liver
21 organoids with the capacity to engraft in the vasculature of immunodeficient mice
22 (27).

23 Considered collectively, these advances have contributed towards bridging the gap
24 between primary hepatocytes and HLCs (Figure 1). However, many challenges
25 remain prior to fully achieving this goal, such as the limited capacity of HLCs to
26 repopulate the liver of animal models of hepatic failure. Furthermore, considering the
27 notable differences between primary hepatocytes cultured *in vitro* and their native
28 counterparts, it is possible that there is a limit to how closely *in vitro* HLCs can

1 reproduce the phenotype and function *in vivo* hepatocytes. Indeed, culture systems
2 maintaining the full functional repertoire of primary hepatocytes are yet to be
3 developed and until then, generation of fully mature hepatocytes *in vitro* might remain
4 elusive.

5 **Cholangiocytes**

6 Cholangiocytes constitute the main cell type affected by diseases of the biliary
7 system, or cholangiopathies. Therefore, differentiation of hIPSCs to cholangiocyte-
8 like cells could provide a novel platform for the study of biliary disorders and the
9 development of new therapeutic agents. The generation of hIPSC-derived
10 cholangiocyte-like cells (CLCs) and organoids has been reported by various groups
11 (28-32). The common denominator in these approaches is the suspension of
12 hepatoblast- or cholangiocyte progenitor-like cells in Matrigel supplemented with
13 EGF, Wnt or HGF. The cells re-organize in 3D polarized structures expressing biliary
14 markers and lacking expression of hepatic markers. The resulting organoids exhibit
15 secretory functionality (28,29,32), alkaline phosphatase (30) and gamma-glutamyl
16 transpeptidase activity (31), as well as responses to hormonal stimuli including
17 somatostatin (32). Despite these advances, the relevance of these approaches to
18 natural development remains vague, which limits their applications for developmental
19 studies and drug screening. Therefore, further optimization of the current culture
20 systems remains a prerequisite for effectively modeling cholangiopathies using
21 hIPSCs.

22

23 **Disease models to date**

24 Since the first proof-of-principle that patient-specific HLCs can be used to model liver
25 diseases (33), the field has expanded rapidly to address a broad range of disorders.
26 Here we review the current hIPSCs-based models for liver disease and their
27 contribution to advancing our insight in the pathophysiology of hepatic disorders
28 (Table 2, Figure 2).

1

2 **Disease caused by accumulation of misfolded proteins**

3 **α 1-Antitrypsin (A1AT) Deficiency:** A1AT deficiency is an autosomal recessive
4 disorder caused by mutations in the A1AT-encoding *SERPINA1* gene (34). A1AT is
5 a protease inhibitor synthesized primarily by hepatocytes. *SERPINA1* mutations lead
6 to low levels of serum and alveolar A1AT, which is associated with panlobular
7 emphysema from over-active proteases such as pulmonary neutrophil elastase.
8 Retention of misfolded A1AT polymers within the hepatic endoplasmic reticulum (ER)
9 results in hepatic dysfunction and liver disease (34). A1AT deficiency was the first
10 hepatic disorder to be modelled *in vitro* using hiPSCs, by differentiating patient-
11 derived hiPSCs into A1AT polymer-retaining HLCs. The disease phenotype was
12 confirmed by ELISA and immunocytochemistry for the polymeric form of A1AT, as
13 well as ER-specific enzyme digests, which indicated entrapment of A1AT in the ER.
14 In a subsequent study, the same group reported rescue of the disease phenotype,
15 following correction of the *SERPINA1* mutation using zinc finger nucleases, thereby
16 demonstrating a direct association between the phenotype observed and the
17 underlying genetic defect (35). These results illustrate the potential of hiPSCs for
18 genetic correction, which represents a powerful tool for dissecting the mechanisms of
19 inherited disorders and generating autologous healthy tissue for personalised cell-
20 based therapy.

21 **Familial Transthyretin Amyloidosis (FTA):** FTA is a lethal autosomal dominant
22 disease, caused by mutations in the transthyretin-encoding *TTR* gene, leading to
23 secretion of monomeric misfolded TTR proteins by the liver and formation of
24 extracellular fibrils accumulating as amyloid in target organs, mainly the brain and
25 heart (36). Although the liver participates in the disease pathogenesis through the
26 production of pathological TTR, it does not sustain end organ damage (37).
27 Consequently, *in vitro* modeling of FTA has been limited by the requirement of
28 multiple cell types to capture the complexity of the disease. These include

hepatocytes for studying the mechanisms controlling TTR production and misfolding, as well as several target cell populations for interrogating the effects of the *TTR* mutation products.

The capacity of hiPSCs to differentiate towards almost any cell type renders them an ideal platform to overcome this challenge. Indeed, recently Leung et al. reported the generation of hepatocytes, cardiomyocytes and neurons from hiPSCs of patients with FTA (37). Neurons and cardiomyocytes exposed to hepatocyte-secreted mutant TTR, using conditioned media, exhibited oxidative stress and increased cell death. More importantly, the use of small molecules stabilizing TTR exerted a protective effect on the target cell populations (37). Considered collectively, these results illustrate the potential of hiPSCs for modeling diseases affecting several organs simultaneously.

Liver diseases caused by disruption of enzymatic activity

Glycogen Storage Disease type 1a (GSD1a): GSD1a is a rare autosomal recessive disorder caused by mutations in the *G6PC*-encoded glucose-6-phosphatase (G6PT) (38). G6PT is essential for the regulation of glucose homeostasis through gluconeogenesis and glycogenolysis (38). Consequently, G6PT deficiency manifests with hypoglycemia, lactic acidosis, hyperuricemia, and hyperlipidemia. Lipid and glycogen accumulation in liver and kidneys result in hepatorenomegaly, while growth retardation is also reported. Importantly, glucagon stimulation in these patients increases serum lactate but does not affect serum glucose levels (38).

GSD1a has been successfully modelled *in vitro* using patient-derived HLCs (33). Indeed, GSD1a-HLCs exhibited increased glycogen and lipid accumulation compared to wild-type controls, demonstrated through PAS and BODIPY staining respectively. Furthermore, GSD1a-HLCs showed similar sensitivity to glucagon stimulation compared to wild-type controls; however, lactate synthesis was significantly increased in GSD1a-HLCs. Considered together these experiments

1 demonstrate the potential of hIPSCs for recapitulating key features of GSD1a *in vitro*,
2 including glycogen accumulation, hyperlipidemia and lactic acidosis. More
3 importantly, the capacity of HLCs for mimicking the native regulation of glucose and
4 lipid homeostasis, renders them a unique system for studying more complex
5 metabolic liver disorders associated with type 2 diabetes and metabolic syndrome,
6 such as liver steatosis and Non Alcoholic Steatohepatitis (NASH).

7 **Wilson's Disease (WD):** WD is an autosomal recessive disorder caused by
8 mutations in the *ATP7B* gene, an ATPase expressed mainly in hepatocytes and
9 mediating hepatocellular excretion of copper in the bile and bloodstream. Hepatic
10 accumulation of copper results in liver damage leading to cirrhosis or in some cases
11 acute liver failure; while increased serum copper levels result in copper deposition
12 and toxicity, affecting multiple organs, mainly the brain, kidneys and cornea (39-40).
13 Current treatments with copper chelators are very effective in reducing copper serum
14 levels; however, liver transplantation remains the only treatment option for acute liver
15 failure and refractory disease (39). Concerning modeling of WD *in vitro*, 2 groups
16 have generated HLCs from WD patients, exhibiting abnormal cytoplasmic localization
17 of mutant ATP7B and defective copper transport (40-41). More importantly, Zhang et
18 al. reported rescue of the disease phenotype using the chaperone drug curcumin or
19 lentiviral overexpression of wild type *ATP7B*. These results provide proof-of-principle
20 for therapeutic approaches alternative to copper chelators in Wilson's disease and
21 validate the potential of hIPSC-derived hepatocytes as an appropriate platform for
22 drug validation in the context of this disorder (40).

23

24 **Diseases caused by receptor dysfunction**

25 **Familial hypercholesterolemia (FH):** FH represents the most common and severe
26 form of inherited hypercholesterolemia. The uptake and catabolism of LDL-C in the
27 liver is physiologically mediated by the low density lipoprotein receptor (*LDLR*) on the
28 membrane of hepatocytes. FH is caused by mutations in the LDLR inherited in an

1 autosomal codominant pattern, which result in poor hepatic uptake and high
2 circulating levels of plasma cholesterol levels (LDL-C) irrespective of diet,
3 medications or lifestyle, thereby leading to early onset of cardiovascular disease (42).
4 Furthermore, recent mouse studies suggest a role for LDLR in regulating plasma
5 levels of very low density lipoprotein (VLDL) and ApoB100 (42). However, human
6 data is extremely limited given the difficulties in obtaining primary tissue from FH
7 patients.

8 HLCs provide an excellent platform for addressing this challenge. Patient-derived
9 HLCs (33,43) exhibit reduced synthesis of LDLR and impaired receptor functionality
10 demonstrated by limited LDL-C uptake in FACS analysis and immunohistochemistry
11 assays (33). The capacity of this system to accurately reproduce the phenotype of
12 different *LDLR* mutations was demonstrated using a compound heterozygous patient
13 with a non-functional maternal allele and a paternal allele encoding a receptor with
14 physiological binding to LDL-C but defective internalization (43). The resulting HLCs
15 exhibited increased clustering of fluorescently-labelled LDL-C on the cell surface but
16 reduced uptake as a result of impaired endocytosis of LDL-C through the paternally-
17 encoded mutant LDLR. These observations underline the potential of HLCs for
18 dissecting the impact of genetic variations on the disease phenotype. Furthermore,
19 the application of FH-HLCs for drug screening in FH was demonstrated by
20 reproducing the effects of lovastatin, a lipid-lowering agent, known to be ineffective
21 for patients with FH (44). Indeed, FH-HLCs exhibited minimal change in LDL-C
22 uptake compared to wild-type controls, which increased cholesterol uptake
23 appropriately (43). These results provide proof-of-principle for the suitability of FH-
24 HLCs as a platform for testing the efficacy of lipid-lowering agents in the context of
25 FH. Finally, using this culture system, a role for LDLR in regulating ApoB100 levels in
26 human was identified. Indeed, FH-HLCs exhibited an 8-fold increase in secreted
27 ApoB100 compared to wild-type controls, in the absence of enhanced gene
28 expression, suggesting a mechanism involving post-translational degradation of

ApoB100 (43). These results demonstrate the potential of FH-HLCs as a model for studying the molecular pathogenesis of FH.

Infectious diseases of the liver

Hepatitis C Virus: Hepatocytes are susceptible to infection from multiple pathogens including viruses (Hepatitis A–E, CMV, EBV) and parasites (*Plasmodium*, *Schistosoma*). However, the most common infective cause for liver transplantation is viral infection with Hepatitis C (HCV) (2). Current treatment options for HCV offer only a 50% viral clearance rate, while liver transplantation is complicated by disease recurrence in the graft (2,45). Primary hepatocytes represent the gold standard for studying the pathophysiology of hepatitis C viral infection; however, their limited availability has led to the use of hepatoma lines such as Huh 7.5 (46-47). Although these systems have proven useful for studying the disease, they are limited by inherent differences from primary hepatocytes associated with their cancerous nature, reduced infection efficiency and defects in interferon production. More importantly, cell lines can only be infected by specific HCV genotypes and are resistant to infection with human sera (47). HLCs provide an alternative platform for the study of HCV that addresses some of these challenges. In particular, they express the necessary receptors for the entry of HCV in the cells (47-49), support the full life cycle of the virus (50) and reproduce key inflammatory responses to infection, including TNF α and interleukin 28 secretion (47,50). In contrast to hepatoma lines, HLCs can be infected using infected patient sera (47,49). Interestingly, using HLCs, Wu et al. have identified key factors regulating permissiveness to viral infection including the upregulation of micro-RNA 122 and suppression of antiviral genes such as interferon-induced transmembrane protein-1 (47). Most importantly, the same group generated genetically modified hepatocytes resistant to HCV infection, thereby addressing a major challenge for liver transplantation and autologous cell therapy in HCV patients; preventing recurrence of the disease in the transplanted tissue (2,47).

1 Considered collectively, these studies demonstrate the potential of hiPSCs for
2 modeling host-pathogen interactions in infectious hepatic disorders and constitute
3 the first example of HLCs used in the study of a non-genetic liver disease.
4 Importantly, a similar approach could be applied for the study of other common liver
5 pathogens, including HBV (51) or parasitic infections (52).

6

7 **The potential of hiPSCs for modeling complex liver disease**

8 With the exception of HCV, all liver diseases modeled to date using hiPSCs
9 constitute rare monogenic metabolic disorders that collectively account for less than
10 10% of the total number of liver transplantations performed (2) (Table 2). Therefore,
11 a major challenge for regenerative medicine in hepatology is evolving from proof-of-
12 principle models to modeling more complex disorders that constitute leading causes
13 for chronic liver disease and transplantation.

14 **Modeling cirrhosis:** Hepatic failure secondary to cirrhosis constitutes the endpoint
15 in the natural history of the majority of chronic liver disorders and one of the most
16 common causes of death in the Western world (2). It is caused by disruption of the
17 liver architecture due to excessive deposition of connective tissue by hepatic stellate
18 cells (53). However multiple other cell types contribute to the disease through
19 activating stellate cells or triggering inflammatory responses. These include
20 hepatocytes, Kupffer cells and sinusoidal endothelial cells (53). One of the major
21 difficulties in modeling cirrhosis *in vitro* is accurately reproducing these intricate
22 cellular interactions controlling the disease pathogenesis. To overcome this
23 challenge various approaches have been used. *In vivo* models based on
24 administration of toxic compounds, *Schistosoma* infection or bile duct ligation to
25 induce cirrhosis have proven useful; however they are not specific to the underlying
26 liver disease and they are limited by intra-species variability (54). *In vitro* models
27 based on 3D platforms enabling co-culture of primary hepatocytes and stellate cells
28 (53) address some of these issues; however, they are also restricted by poor access

1 to primary tissue. The use of hiPSCs-based platforms simulating the liver
2 microenvironment could contribute towards overcoming these limitations. Indeed,
3 introduction of stellate and Kupffer cells in existing organoid culture systems
4 combining HLCs, endothelial and stromal cell populations (25,27) could provide an
5 excellent model for studying the cellular interactions controlling the pathogenesis of
6 cirrhosis. In the absence of protocols for the generation of hiPSC-derived stellate
7 cells, stellate cell lines could be used, while macrophages acting as Kupffer cells
8 could be derived from hiPSCs or isolated from peripheral blood.

9 Finally, the most challenging aspect might not be to source all the cells necessary to
10 mimic the liver environment but to reproduce chronic hepatocyte injury *in vitro*.
11 Indeed, cirrhosis/fibrosis secondary to certain etiologies, such as congestive cardiac
12 failure can prove challenging to model especially in a culture system with a short
13 half-life (10). Furthermore, the impact of the fetal phenotype of HLCs for such
14 platforms remains unclear. To conclude, multicellular organoid platforms based on
15 HLCs could provide one of the most accurate systems to date for reproducing the
16 complexity of cirrhotic tissue *in vitro*. However, several challenges remain to be
17 addressed, which will require major advances in 3D co-culture systems.

18
19 **Modeling biliary disease:** Bile duct disorders constitute an important cause for
20 chronic liver disease and transplantation (2). However, treatment options are
21 extremely limited (2) and our understanding of the disease pathogenesis is restricted
22 by lack of appropriate disease models. Indeed, *in vivo* models fail to fully recapitulate
23 the disease phenotype, while primary cholangiocytes are extremely difficult to culture
24 *in vitro* (55). CLCs could contribute towards addressing this challenge. Culture
25 systems recapitulating key stages of native biliary differentiation and tubulogenesis
26 could increase our insight into the mechanisms of bile duct specification and provide
27 an ideal platform for modeling infantile cholangiopathies caused by defects in biliary
28 tree development (56). Furthermore, patient-derived CLCs represent an excellent

1 system for interrogating the impact of genetic modifiers in the pathogenesis of
2 complex biliary disorders, such as Primary Biliary Cirrhosis (PBC). Recent genome-
3 wide association studies have identified multiple loci associated with PBC (57), but
4 their expression and function in cholangiocytes remains largely unknown. CLCs
5 could address this challenge by delivering an *in vitro* platform with the capacity to
6 characterize which of these loci are expressed in biliary tissue and enable further
7 studies on their function and mechanism of action.

8

9 **Modeling hepatotoxicity and Drug Induced Liver Injury**

10 Hepatotoxicity constitutes the second most common cause for drug failure in human
11 (58), while Drug Induced Liver Injury (DILI) accounts for 50% of acute liver failure
12 cases (59). The mechanisms controlling drug sensitivity and idiosyncratic reactions
13 remain largely unknown. Genetic factors are considered to play a major role;
14 however limited access to primary tissue precludes the development of large scale *in*
15 *vitro* platforms capturing the genetic diversity of a population and allowing in-depth
16 genomic studies. HLCs could overcome this limitation by providing large numbers of
17 isogenic cells capable of reproducing the effects of various therapeutic compounds *in*
18 *vitro*. However, the differences in drug metabolism between primary hepatocytes and
19 HLCs remain a significant limitation that needs to be assessed and addressed in
20 order to maximize the potential of HLC-based platforms for modeling hepatotoxicity
21 and DILI. The development of new protocols (16,20,24) could provide novel
22 perspectives towards achieving this goal. Furthermore, the recent development of
23 extensive hPSC libraries such as the HipSci project (<http://www.hipsci.org/>),
24 combined with advances in automated cell culture and high throughput techniques
25 (60-61), provide an excellent infrastructure for deriving substantial cohorts of HLCs
26 reproducing the genetic variability and polymorphisms accounting of drug toxicity.
27 High-throughput sequencing and phenotyping platforms could enable direct
28 comparison of the genetic profiles between HLC cohorts derived from healthy

1 individuals and patients with DILI or hypersensitivity reactions in order to identify
2 novel genetic variants associated with hepatotoxicity. HLCs expressing these
3 variants could subsequently be used to interrogate the mechanisms controlling drug
4 toxicity and provide more accurate screening platforms for early identification of toxic
5 compounds.

6

7 **Conclusion**

8 In conclusion, recent technical advances in differentiation protocols combined with
9 the development of complex organoid culture systems have significantly improved
10 the quality and spectrum of hPSC-derived liver cell types. As a result hPSC-based
11 liver disease modeling is rapidly expanding from reproducing key phenotypic aspects
12 of rare monogenic disorders to modeling diseases representing major causes for
13 chronic liver disease and transplantation, such as HCV. In addition, novel
14 technologies becoming rapidly available in the field will enable studies at the deepest
15 molecular level, linking genetics, epigenetics and phenotype in the context of
16 complex liver disorders; thereby pushing the boundaries of regenerative hepatology
17 even further in the near future.

18

19 **Conflict of interest**

20 LV is a founder and shareholder of Definigen. The rest of the authors have nothing to
21 disclose.

22

23

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1 **Figure Legends**

2

3 **Figure 1**

4 Strategies the generation of Hepatocyte-Like-Cells (HLCs) from human Induced
5 Pluripotent Stem Cells (hIPSCs). Key stages of *in vivo* liver development are
6 compared to *in vitro* differentiation of HLCs and the growth factors controlling each
7 stage are mentioned. Approaches for improving HLC maturation are also
8 summarized.

9

10 **Figure 2**

11 Liver disease pathways modeled with patient-specific hIPSC-derived
12 hepatocytes. The underlying pathophysiology of several hepatic disorders modeled
13 in hIPSC-derived hepatocytes is outlined (red) and shown to deviate from the WT
14 phenotype (green). **(1) α 1-Antitrypsin Deficiency:** Accumulation of misfolded α 1-
15 antitrypsin in the ER. **(2) Familial Transthyretin Amyloidosis:** Secretion of
16 misfolded protein and formation of extracellular fibrils. **(3) Glycogen Storage**
17 **Disease Type 1a:** Disruption of enzymatic activity. **(4) Familial**
18 **Hypercholesterolemia:** Cell membrane receptor dysfunction. **(5) Hepatitis C Virus:**
19 Permissiveness of viral infection and replication.

20

1 **Table Legends**

2

3 **Table 1**

4 Key characteristics of HLCs generated *in vitro* including expression of liver specific
5 markers, *in vivo* engraftment potential and functionality in comparison to primary
6 hepatocytes.

7

8 **Table 2**

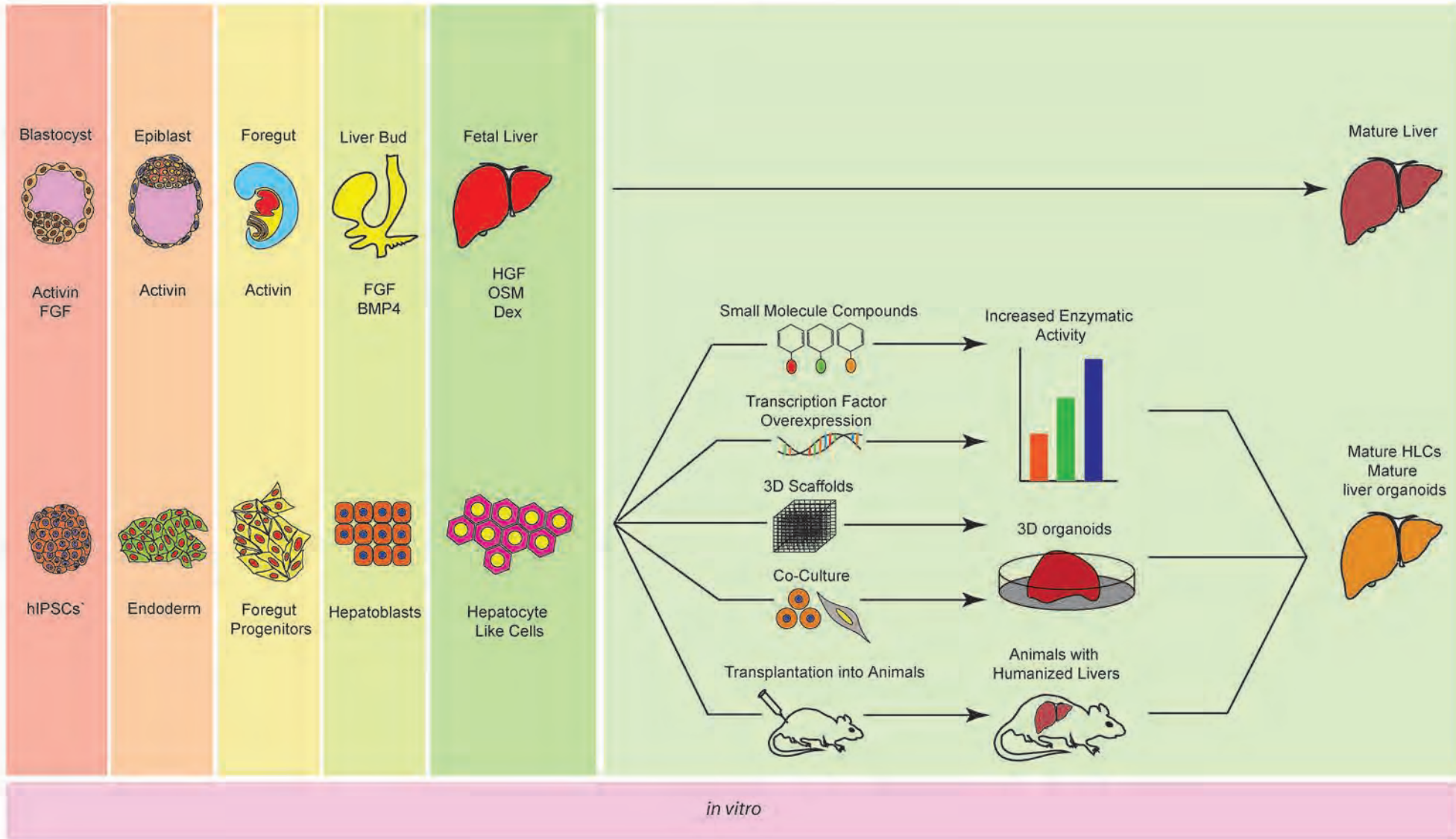
9 Contribution of hIPSCs to modeling liver disease. Key hepatic disorders leading to
10 liver transplantation and relevant hIPSC-based models currently available.

Protocol / Publication	Positive Control	Hepatic Markers (RT-PCR, ICC, FC, IHC)	Hepatic Functional Assays					Murine Engraftment <i>in vivo</i>	Disease Modeling
			CYP 3A4 Metabolic Assay (percentage relative to primary hepatocytes)	Albumin Secretion (percentage relative to primary hepatocytes)	Glycogen Synthesis / Storage	Cholesterol Uptake	Indocyanine Green Uptake		
Du et al. Cell Stem Cell 2014	freshly isolated human primary hepatocytes	ALB AAT AFP negative HNF4A CYP3A4	~ 80-270%	~ 60%	yes	yes	yes	yes	n/a
Toubul et al. Hepatology 2010, Hannan et al. Nature Protocols 2013, Gieseck et al. PLOS ONE 2014	freshly isolated human primary hepatocytes	ALB AAT AFP HNF4A CYP3A4	~ 10% (unpublished)	~ 75%	yes	yes	yes	yes	yes
Ogawa et al. Development 2013	freshly isolated, cryo-preserved human primary hepatocytes	ALB AAT AFP HNF4A CYP3A4	~ 900% *	~ 1400% *	n/a	n/a	yes	n/a	n/a
Si-Tayeb et al. Hepatology 2010	cadaveric human liver samples	ALB AFP HNF4A CYP3A4	n/a	n/a	yes	yes	yes	yes	yes
Hay et al. PNAS 2008	primary adult human hepatocytes	ALB AFP HNF4A CYP3A4	n/a	n/a	yes	n/a	n/a	yes	yes

* CYP3A4 activity and albumin secretion in HLCs were compared to those of cryopreserved plated hepatocytes, explaining the level observed.

Etiology		% of liver transplantations	hiPSC-based models
Cirrhosis	Viral (HBV, HCV)	24%	1. Wu X, et al. (40)
			2. Roelandt P, et al. (41)
			3. Yoshida T, et al. (42)
			4. Schwartz RE, et al. (43)
			5. Zhou XL, et al (44)
	Alcohol	19%	None available
	Alcohol + virus	3%	None available
	Primary Biliary Cirrhosis (PBC)	6%	None available
	Autoimmune	2%	None available
	Secondary Biliary Cirrhosis	1%	None available
Cancer		14%	None available
Cholestatic diseases		10%	None available
Acute Hepatic Failure		8%	None available
Metabolic diseases		6%	Rashid ST, et al. (26)
			Yusa K, et al. (28)
			Rake JP, et al. (31)
			Zhang S, et al. (33)
			Yi F, et al. (34)
			Cayo MA et al. (36)
Other (Budd Chiari, benign liver tumors, polycystic disease)		3%	Leung A, et al. (30)
			Klotz C et al. (45)

in vivo



in vitro

