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Human iPSC derived hepatocytes: A novel tool in screening drug candidates for liver and metabolic disease

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1. Introduction

Liver disease is a significant and rising cause of mortality around the world[1]. Organ transplant remains the only available option for patients at risk of liver failure, but supply is far outweighed by demand. Therefore, there is a need to develop treatments capable of slowing or preventing disease progression. Despite careful stepwise approaches taken during the development of new treatments, many do not fail until final stage clinical trials or have to be withdrawn post-approval[2,3]. Withdrawal or late-stage failures represents a significant financial loss for the company or institution. Therefore, there is a need to de-risk the candidate selection process and optimise the triaging of treatments at an earlier stage of the development pipeline.

2. Current pre-clinical models used in candidate development pipelines

A considerable roadblock in the successful triaging of new therapeutics for liver disease is the lack of translatability between preclinical models and the clinic.

Table 1: Current problems and potential solutions in liver drug discovery

	Non-liver cell lines	HCC cell lines	Primary hepatocytes	hiPSC-derived hepatocyte-like cells
Expansion capacity	+++	++	-	++
Normal karyotype	No	No	Yes	Yes
Liver functionality	-	+	+++	++
Phenotype stability	+	+++	-	++

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2.1 Stable cell lines

Initial screening of putative treatments commonly utilises the highly proliferative Chinese Hamster Ovarian (CHO) or Human Embryonic Kidney (HEK) cell lines with the liver protein(s) of interest exogenously expressed[4]. This system allows researchers to investigate the effect of the treatment on the target protein, but the cellular environment bears little resemblance to that of the human liver – an impediment to reliable compound triaging.

Hepatocellular carcinoma-derived cell lines, such as HepG2s, are less proliferative than CHO or HEK cells but remain readily expandable and retain aspects of hepatocyte function, including robust albumin secretion[4,5]. Despite being of hepatic origin, hepatoma lines lack many of the key proteins essential for modeling liver disease[5]. In addition, stable cell lines derived from cancers represent a single and karyotypically abnormal genotype. Therefore, modeling conditions related to gene polymorphisms or differences across populations is restricted and the oncogenic phenotype limits physiological relevance.

2.2 Primary Human Hepatocytes

Primary hepatocytes represent the gold standard in vitro cell model for functionality. When isolated from liver tissue, primary cells maintain expression of key proteins for a short period, permitting acute testing of therapeutic agents[4]. Extending culture of primary hepatocytes beyond 24 hours results in rapid dedifferentiation, characterised by loss of function and apoptosis[6]. Significant efforts to improve hepatocyte culture methods have identified techniques such as spheroid and co-culture systems capable of maintaining function for longer periods[7,8].

Notwithstanding these breakthroughs, expansion of the cells remains difficult and each donor has a finite supply of hepatocytes, limiting screen size and repeat testing – a concern as hepatocytes can differ greatly between donors[6]. Moreover, accessing hepatocytes from patients with rare liver conditions is prohibitively difficult and gene editing approaches are stymied by their limited expansion capacity.

2.3 Human iPSC-derived hepatocyte-like cells (HLCs)

An ideal model for the study of liver disease would combine the expansion capacity and phenotypic stability of hepatoma cell lines with the functionality and normal karyotype of primary hepatocytes. Human induced pluripotent stem cell-derived hepatocyte-like cells (HLCs) have the potential to fulfil this niche.

Human induced pluripotent stem cells (iPSCs) were first described by Shinya Yamanaka in 2007[9]. Through the overexpression of embryonic stem cell associated factors in somatic cells, the Nobel Prize winning work demonstrated that adult human cells could be reprogrammed to induced pluripotent stem cells[9] – a cell type found in very early development capable of becoming any cell in the human body.

For the study of liver disease, iPSCs allow the reprogramming of somatic cells from patients diagnosed with hepatobiliary diseases[10]. Once the iPSC line has been generated, it can be expanded indefinitely and differentiated to HLCs using step-wise protocols that mimic known developmental stages[11].

DefiniGEN's proprietary differentiation protocol permits large-scale generation of HLCs with field leading purity and functionality. Importantly, the HLCs successfully recapitulate key aspects of disease pathophysiology across a wide- range of conditions that affect different aspects of liver function[10].

For rare forms of liver disease, identifying patients with a genomic mutation of interest is challenging. CRISPR/ Cas9 can be used with high editing efficiency in iPSCs when compared to primary cell types[12]. In practice, this means specific mutations associated with liver disease can be precisely introduced to iPSCs with relative ease, before expansion and subsequent differentiation to HLCs. This approach allows for the study of rare monogenic diseases and polymorphisms that increase susceptibility to types of liver disease previously inaccessible to researchers.

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3. Disease modeling and therapeutic screening for liver diseases using iPSC-derived HLCs

To date, the majority of research looking to identify new therapeutics with iPSC-derived HLCs have focused on monogenic liver diseases [10,13]. Monogenic diseases are a class of conditions caused by a single mutation in an essential gene. The liver performs a wide range of essential functions, so it follows that there is a catalogue of monogenic diseases with hepatic penetrance. Importantly, these conditions commonly affect younger populations, significantly impairing their quality of life.

Many monogenic diseases impacting liver function lack efficacious treatments. Therefore, monogenic liver diseases represent an attractive target for corrective therapies.

It is important to note that while many hepatic diseases have been modelled using iPSC- derived HLCs, few screening platforms have been published. Outlined below are the studies that utilised iPSC-derived HLCs as a platform to identify novel treatments for conditions with unmet clinical need.

3.1 Alpha-1 antitrypsin deficiency

Alpha-1-antitrypsin deficiency (A1ATD) is the most prevalent monogenic disease affecting the liver. It is mainly caused due to mutations in the SERPINA1 gene, and results in a misfolded alpha-1-antitrypsin protein that forms polymers in hepatocytes[14]. Polymer accumulation is hepatotoxic with the most severe cases associated with liver cirrhosis and hepatocellular carcinoma development[14].

Numerous treatments have been developed aiming to reduce polymer accumulation, and their efficacy has been displayed in pre-clinical studies[14]. As yet, all have failed at various stages during subsequent clinical assessment. In part, these failings are due to sub-optimal pre-clinical models. Many studies utilise CHO cells overexpressing the polymer forming version of the A1AT protein for screening purposes during early triaging[14–16]. While this is a resilient model for large scale cultures, its non-human origin and lack of additional hepatic-enriched proteins compromise screening reliability in downstream clinical success. Primary hepatocytes from patients with A1ATD are hard to source and culture, limiting their use for screening libraries of small molecules until the final stages of compound assessment. In addition, the lack of a stable cell model that expresses the polymer-forming A1AT protein is further exacerbated by mouse models that fail to display the disease phenotype, raising the need for the development of complex humanised mice to successfully model the disease in vivo[17].

Therefore, researchers have turned to iPSC-derived HLCs. When patient-derived iPSCs carrying defined mutations in the SERPINA1 gene were differentiated to HLCs, increased accumulation of polymeric A1AT could be detected compared to wild-type controls[10]. Importantly, when TALEN-based gene editing was used to correct the mutation, polymer accumulation was ablated[18]. Concurrent work described an immunofluorescence-based screen to identify new treatments for A1ATD[19].

iPSC-derived HLCs were differentiated and small molecules from a repurposed drug library applied in a high-throughput format. A1AT accumulation was measured by plate reader assay which identified carbamazepine and other autophagy inducing small molecules as candidate treatments for A1ATD.[19]

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3.2 Wilson's disease

Wilson's disease is an autosomal-recessive disorder of hepatocellular copper accumulation caused by mutations in the gene encoding for the copper-transporter, ATP7B. Wilson's disease commonly manifests in liver failure; however, it is often associated with non-liver manifestations, including neurological, psychiatric, and ophthalmological symptoms, highlighting the detrimental effects of copper deposition within human body.

A variety of treatments have been developed aiming to "decopper" patients by increasing urinary copper excretion. Chelating agents, including penicillamine, trientine and zinc, are currently used in clinic, and act by inhibiting intestinal copper absorption, however, they usually come with adverse effects, including paradoxical neurological worsening.

Given the limitations of the currently available therapies, targeted molecular therapies aiming to restore localisation and/or function of ATP7B are being developed. Both Atp7b-/- and mutant e.g., H1069Q, R778L) rodent models are now available, aiming to serve as disease models for protein replacement and gene therapy (e.g., adeno-associated viral vector [AAV]) studies.

The data are so far promising, and indeed, a replication-deficient rAAV containing a shortened version of the ATP7B gene is now under a phase I/II trial in adults. However, animal studies also come with limitations, including the lack of neurological phenotype in the diseased animals as well as fundamental differences in the metabolic pathways between rodents and humans. Similar to A1ATD, primary human hepatocytes cannot offer a good in vitro alternative for disease modeling, due to their lack of proliferation, rapid apoptotic rate, and already compromised status at the movement of liver biopsy execution.

iPSCs-derived HLCS have the potential to bridge this gap, and offer a physiologically relevant in vitro model that recapitulates the Wilson's disease phenotype. Indeed, both patient-derived and CRISPR-derived hepatocytes carrying missense mutations on ATP7B are now developed, successfully demonstrating disease phenotype in-a-dish, and can offer an unlimited source of human hepatocytes as well as a unique platform for translational research and large-scale drug screening.

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4. Identifying new treatments for liver diseases using DefiniGEN's screening platform

The outlined examples demonstrate the power of screening for new therapeutics using iPSC-derived HLCs. The highly expandable nature of iPSCs and the physiological relevance of differentiated HLCs dovetail to produce a model ideally suited to therapeutic screening assays. This cell platform permits investigation of diseases where there is a paucity of animal models, and can be used to study aspects of rare disease states that are often disrupted in cancer cell lines.

DefiniGEN have established an iPSC-derived HLC platform capable of screening candidate therapeutics with high accuracy and reproducibility. As a proof-of-principle, we have developed a platform that directly assesses the abundance of polymeric A1AT formation in A1ATD iPSC-derived HLCs. Using carbamazepine as an exemplar compound, intercellular polymer accumulation is reduced in a dose-dependent manner. Furthermore, emerging therapies, such as siRNA-based approaches, have been successfully validated using the same platform, and other technologies, such as base editing and gene therapies, can also be applied to the model.

Published studies from academic groups have focused on monogenic conditions with strong hepatic penetrance. Many more mono- and polygenic diseases have been modeled using iPSC-derived HLCs, but developing platforms that can either directly or indirectly measure disease phenotypes in a high-throughput format is challenging. DefiniGEN is at the forefront of efforts to increase the disease types amenable to therapeutic screens. Recent work by the company has expanded the number of monogenic liver diseases with predictive screening assays to include Wilson's Disease, Progressive familial intrahepatic cholestasis (PFIC2), Familial Hypercholesterolemia (FH) and Urea Cycle Disorders (e.g., OTC deficiency, Citrullinemia). Furthermore, similar experimental principles can be used to identify treatments for polygenic liver diseases, such as NAFLD.

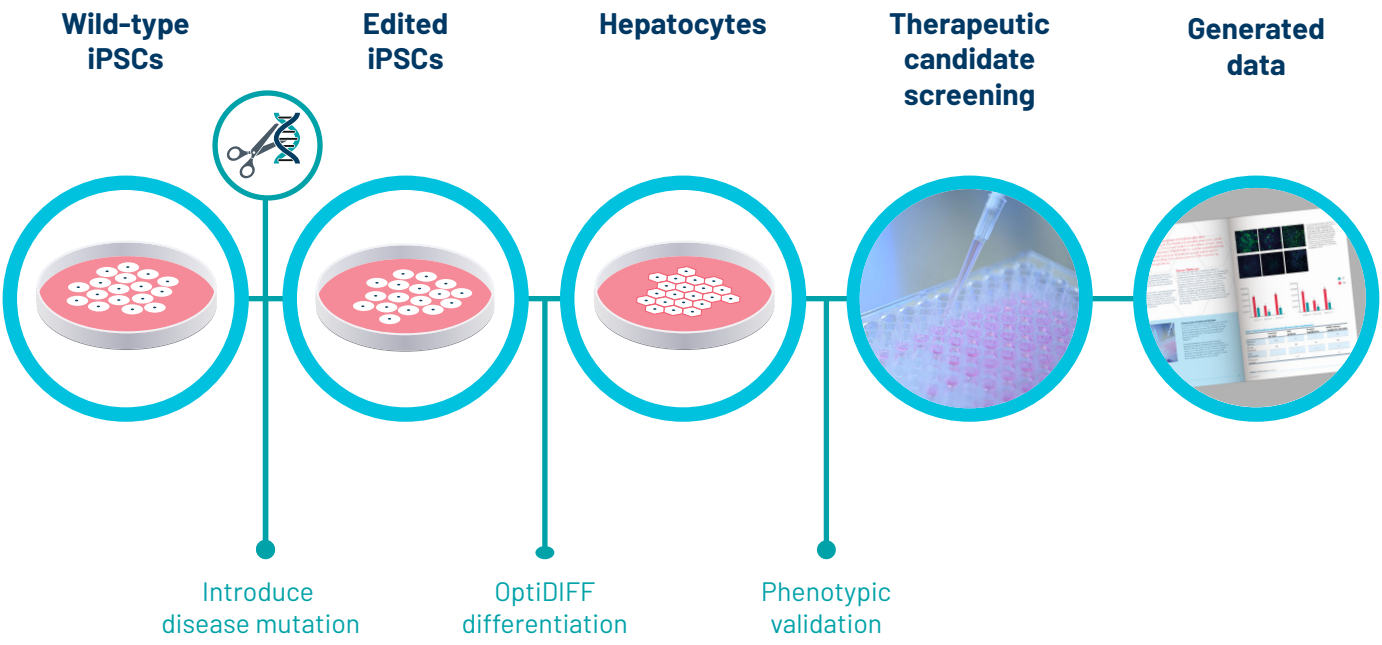

Figure 1. Schematic overview of DefiniGEN's disease modeling platform: introduction of relevant disease mutation, iPSC differentiation into hepatocytes, phenotypic validation with bespoke bioassay development

5. Using iPSC-derived hepatocyte-like cells in therapeutic developmental pipelines

Changes in FDA regulations mean that animal studies are no longer a pre-requisite to enter the clinical trial phase of development[20]. In this new legislative environment, iPSC-derived HLCs have the flexibility to be used as model at all stages of preclinical development, from initial screens to lead candidate optimisation. The capacity to include the same cell type at all stages of development will significantly increase the consistency of findings and minimise the loss of efficacious treatments from the development process.

We hope that by developing platforms that are highly relevant to human liver physiology and amenable to large-scale screening panels, we can introduce greater predictivity throughout preclinical development pipelines with our service offering. It is anticipated that by using models with enhanced predictivity at earlier stages of development, resources can be deployed with greater efficiency and reduce the number of treatments suffering damaging late-stage clinical trial failures and post-approval market withdrawals.

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