DefiniGEN Ltd
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Redefining in vitro drug testing

UK-based DefiniGEN has developed iPSC-derived cell lines that can replace primary human hepatocytes for toxicity testing and accelerate the development of novel liver-targeted therapeutics.

Drugmakers and regulators need to reduce the complexity of drug development and improve success rates in the identification of safe therapies. Given the critical role of the liver in drug metabolism, cell models that can accurately recapitulate liver function 'in-a-dish' have become a crucial component of the drug development pipeline. In this regard, primary human hepatocytes (PHHs) are considered the gold standard for in vitro toxicity testing. However, PHHs are in limited supply and associated with significant donor-to-donor variability, rendering their incorporation into routine testing difficult.

A new predictive platform for hepatology

DefiniGEN has spent the last decade developing and characterizing a more consistent, scalable alternative to PHHs: optimized hepatocytes derived from induced pluripotent stem cells (iPSCs), Opti-HEP (Fig. 1). The spin-out from the University of Cambridge was founded by Ludovic Vallier, who is the company's CSO. Vallier and colleagues have developed a proprietary protocol to differentiate iPSCs into Opti-HEP while retaining many aspects of functionality observed in the human liver. DefiniGEN's work has largely been focused on optimizing this process, and the company is now generating iPSC-derived hepatocytes with comparable function to PHHs.

DefininGEN has demonstrated that Opti-HEP can match the ability of PHHs to measure hepatotoxicity through the major metabolic pathways, including phase 1 and phase 2 metabolism, and predict drug-induced liver injury (DILI) in vitro.

"Opti-HEP have several advantages over traditional models like PHHs," said Nikolaos Nikolaou, head of research and development (R&D) at DefiniGEN. "PHHs come from donated livers, a finite source with significant variability. They are ill-suited for long-term studies and lead to poor reproducibility. Massively scalable Opti-HEP match their functionality but with greater consistency and prolonged stability."

As a result, the cells can be deployed to study the mechanisms underpinning inherited diseases that are undertreated or lack disease-modifying therapies entirely. They can also be used to screen drug candidates and test leads for efficacy faster and at much lower cost than using animal models.

> "The newest addition to DefiniGEN's armamentarium is Opti-HSC—the first commercially available iPSC-derived HSC line model

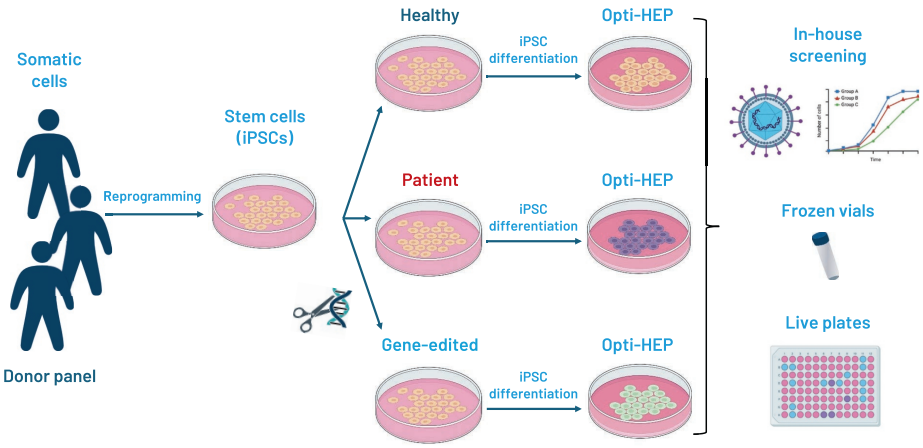

Fig. 1 | The DefiniGEN platform. IPSC, induced pluripotent stem cell; Opti-HEP, iPSC-derived hepatocytes.

Innovative cell-based models

By leveraging the opportunities that clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology offers in the field of disease modeling, the company has generated a large portfolio of mutant Opti-HEP that recapitulate the disease phenotypes of inherited metabolic disorders, and has used this approach to develop models for early-stage in vitro drug-efficacy screens. Opti-HEP models are now routinely used for the identification of novel therapies against rare monogenic liver diseases including alpha-1-antitrypsin deficiency (A1ATD) and Wilson's disease.

In addition, DefiniGEN has recently developed an in vitro model of a more complex disease, metabolic dysfunction-associated steatotic liver disease (MASLD), which affects about 100 million Americans and can progress to cirrhosis and eventually liver failure. "For the development of this in vitro model, cells are exposed to high-fat and carbohydrate treatments, resulting in phenotypes that reflect fat accumulation, inflammation, insulin resistance, and reduced drug metabolism. Importantly, we can additionally generate genetically modified Opti-HEP that include known genetic determinants of MASLD, such as in *PNPLA3* [patatin-like phospholipase domain-containing protein 3] and *TM6SF2* [transmembrane 6 superfamily, member 2] genes, aiming to further investigate how genetic predisposition drives disease severity," said Nikolaou.

Next-generation solutions

The newest addition to DefiniGEN's armamentarium is Opti-HSC—the first commercially available iPSC-derived hepatic stellate cell (HSC) line model. HSCs respond to liver injury and are closely linked to liver fibrosis. While primary human HSCs and immortalized SC lines are currently used for studying liver fibrosis in vitro, they are difficult to source, have significant heterogeneity, and lack key stellate-cell specific functions.

Scalable Opti-HSC can recapitulate the liver fibrosis phenotype seen in fatty liver diseases. "In addition, co-culturing Opti-HSC with Opti-HEP in vitro enhances collagen secretion and SC activation-driven steatosis to even more closely resemble the complex MASLD phenotype," said Nikolaou.

To expand the utility of its iPSC-derived hepatic-cell products, DefiniGEN offers its cells in cryopreserved vials as well as hydrogel-encapsulated 96-well live plates for in vitro assays. The company also performs screening in-house, functioning as a contract research organization (CRO).

For DefiniGEN, these offerings are just the beginning. "We are continuously developing complex models by adding new types of iPSC-derived cells that resemble human liver cells," said Nikolaou. "Our aim is to offer a novel, genetically homogeneous in vitro platform for liver diseases as well as additional Opti-HEP lines from a variety of iPSC donors aiming to closely reflect genetic diversity. Eventually, we want to reduce the need for animal testing through robust and tailored in vitro assays for efficacy, toxicity, and drug screening."

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