Benefits & Key Considerations of Using Human iPSC-Derived Disease Models in Drug Discovery

The process of drug discovery, development and commercialisation is long and associated with high costs. In addition, it is estimated that only 1% of the initially tested compounds make it to the market.[1] To decrease this high attrition rate, it is necessary to implement physiologically relevant disease models with higher predictability much earlier in of drug discovery. Disease models based on human induced pluripotent stem cell (iPSC) technology has the potential to revolutionise drug discovery. These models recapitulate, *in vitro*, many clinical features of human pathology and can be used for phenotypic screening with clinically relevant readouts. This article describes how human iPSC-derived disease models can improve drug discovery and what the main challenges and solutions are for their successful generation and application of these models.

Traditionally, target-based drug discovery has focused on biochemical assays or non-physiologically relevant cell-based assays. Biochemical assays are highly suitable for high-throughput screening (HTS), but it is difficult to predict the in vivo therapeutic potential of the hits found. Cell-based assays offer a more complex cellular environment and the possibility to evaluate the phenotypic effect of compounds. However, traditional cellular models, such as immortalised cells, present several limitations regarding disease modelling and translatability to the clinic. As an alternative, primary human cells provide more representative responses, although they have a significant donor-to-donor variability and are rarely available in large-enough quantities for HTS, especially for less accessible organs such as the heart or brain.

Animal models do offer an in vivo complex environment, but are more expensive, have serious scalability constraints and the substantial inter-species differences hamper modelling of certain human diseases, such as cardiac arrhythmias or neurodegenerative diseases.

In the past decade, iPSC technology has emerged as a powerful tool to bring the human biological context earlier into the drug discovery funnel. Human iPSCs are obtained from patients' or healthy donors' somatic cells and reprogrammed to pluripotent stages. They have the ability to self-renew, while maintaining the potential to differentiate to nearly any functional cell type in the body, closely mimicking the human (patho)physiology. Human iPSCs are relatively easy to obtain from adult tissues and they retain patient-specific genetic backgrounds, making them a preferred system for disease modelling (Figure 1).[2]

BENEFITS OF HUMAN IPSC-BASED DISEASE MODELS

The main benefits of using human iPSC technology for disease modelling are that iPSCs retain patient-specific genetic backgrounds and show clinically relevant phenotypes of many human diseases. These features facilitate the study of disease development and complex pathological mechanisms. In this section, we selected specific examples that highlight the advantages of human iPSC-based disease models.

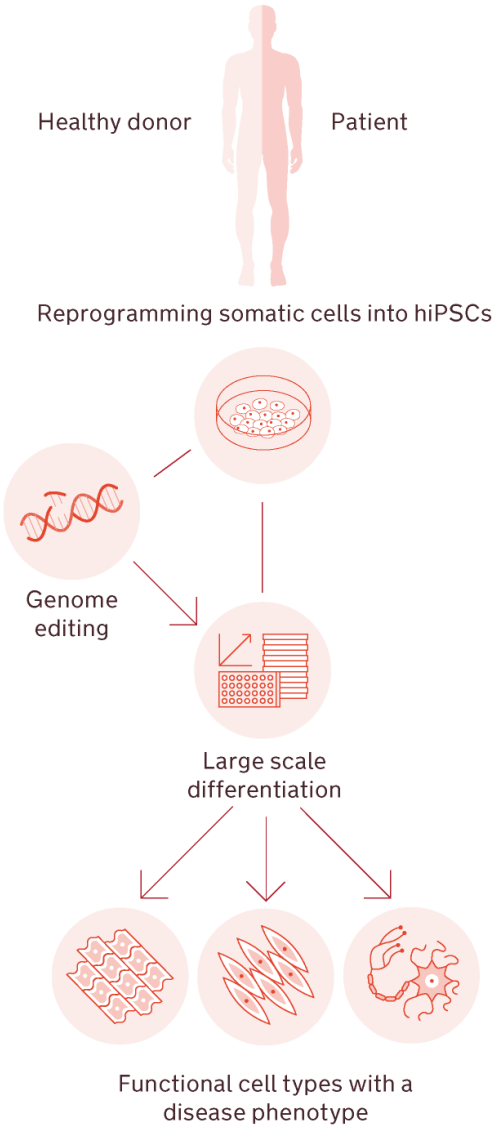

Figure 1 – The process of disease modelling based on human iPSCs. Human iPSCs can be obtained from somatic cells with non- or low-invasive techniques and used to generate physiologically relevant disease models that retain patient-specific genetic backgrounds.

Noonan syndrome with multiple lentigines, formerly known as LEOPARD syndrome, was one of the first diseases successfully modelled using iPSCs. Patients with LEOPARD syndrome develop cardiac hypertrophy in their late 40s. However, iPSC-derived cardiomyocytes (CMs) from patients with this syndrome showed hypertrophic phenotype within 30 days of cell culture. This allowed elucidation of molecular signatures associated with the disease, in a relatively short timeframe.[3]

Another advantage of using iPSCs in disease modelling was reflected by the derivation of CMs from patients' iPSCs with Long QT syndrome (LQTS). LQTS is characterised by delayed repolarisation of the heart that can lead to a severe ventricular arrhythmia called Torsades de Pointes. Mouse models failed to mimic the disease because of the different electrophysiological properties. However, iPSC-derived CMs from LQTS patients manifested the electrophysiological signature of LQTS and proved to be a powerful system for pathogenesis studies and therapeutic compound testin.[4]

Schizophrenia is a multifactorial disease and patients can show a range of symptoms and responses to treatment. The availability of iPSC-derived schizophrenia models from different patients can facilitate *in vitro* prediction of treatment responses and open the door for patient stratification and precision medicine.[5,6] In a research study, iPSC-derived neurons from patients with Schizophrenia exhibited diminished neuronal connectivity and decreased neurite numbers, which are characteristic features of this disease, and responded to treatment with a clinically approved antipsychotic.

During the differentiation process of iPSCs, the stages of organ development are replicated *in vitro*, which is a great benefit for modelling congenital and developmental diseases. Rett syndrome (RTT) is a genetic neurodevelopmental disorder that can manifest early after birth. RTT patient-specific iPSC lines have been used to investigate the phenotypic consequences of each specific mutation.[7] At the other end of the spectrum, iPSCs can recapitulate disease progression, even for late onset disorders, which enables modelling Alzheimer's disease (AD), Parkinson's disease and other neurodegenerative diseases. A study with iPSC-derived neurons from different AD patients showed good data correlation with patients' regimen-data, indicating the clinical translational power of these models.[8] Moreover, more complex environments can be mimicked by deriving multiple cell lineages from the same iPSC line. For instance, iPSC-derived microglia and astrocytes from the same donor can be co-cultured with neurons for the study of cell-cell interactions.

An additional advantage of using iPSC-based models was brought by the breakthrough discovery of CRISPR. This genome editing technology is cost-effective, relatively fast, and efficient in iPSC. Using CRISPR, disease models can be derived from healthy human iPSC lines via knock-in, knock-out, or introduction of point mutations described in patients. For instance, an iPSC-derived model for hypochondrogenesis was generated by introducing a patient mutation (COL2A1 p.G1113C) in the collagen type II gene (COL2A1) with CRISPR/Cas9.[9] In addition, genome editing enables the generation of isogenic controls to minimise genetic-background related variability and identify the true impact of the genetic variants on cellular phenotypes.

HOW CAN IPSC-DERIVED DISEASE MODELS IMPROVE DRUG DISCOVERY?

It has been demonstrated that human iPSC-derived disease models can successfully recapitulate many disease phenotypes that are clinically relevant and that cannot always be elucidated by traditional models. This feature makes iPSC technology an excellent tool to improve decision-making steps throughout the early phases of drug discovery (Figure 2).

TARGET IDENTIFICATION AND VALIDATION

Target identification and validation stages benefit from the use of iPSC-based disease models because of their accurate representation of the disease and human biology, especially when the mechanistic landscape is not completely understood. These models can make hypothesis generation more precise through the study of disease physiology and open the door for target validation based on phenotype rescue assays. As an example, human iPSCs derived from patients with a high ratio of mutant mitochondrial DNA were used to identify a potential therapeutic target for mitochondrial diseases. The patient-derived iPSC line exhibited defective differentiation into neuronal cells, and it was found that the compound tryptolinamide (TLAM) was able to rescue the phenotype. Based on this approach, the protein inhibited by TLAM was identified, and could therefore be classified as potential therapeutic target.[10]

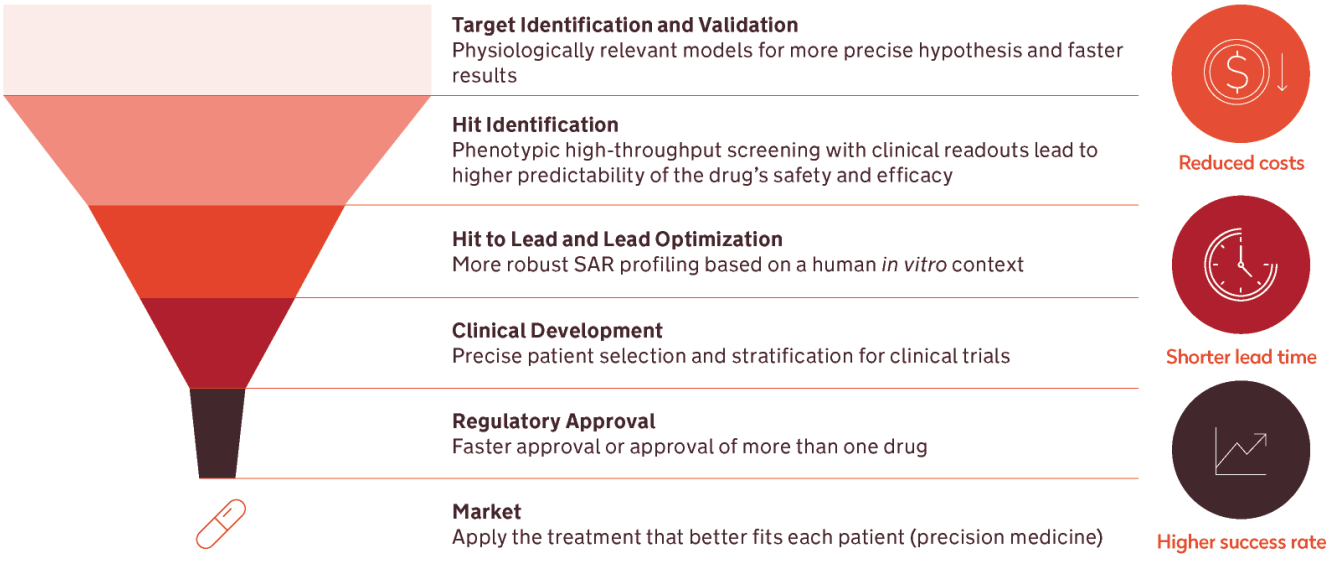

Figure 2 – Benefits of human iPSC-derived disease models along the different phases of drug discovery. The implementation of human iPSC-derived disease models in drug discovery can reduce costs, shorten the process, and decreases the attrition rate by bringing the true human biology earlier into the drug discovery and development process.

Hit Identification

Phenotypic screening with physiologically relevant iPSC-based disease models facilitates the selection of relevant hits during drug efficacy testing. This type of screening also enables the description of novel therapeutic drug mechanisms of action and increases predictability. Since human iPSC-derived models can be used to study disease phenotypes, for example measured by changes in morphology, biomarker expression, metabolism or cellular function, they provide a more complete understanding of drug efficacy, compared to other screening platforms. Furthermore, the results of phenotypic screening with iPSC-based disease models are easier to extrapolate to the clinical situation because, in many cases, the selected readouts are equivalent to the clinical markers used for diagnosis. Recently, Ncardia developed a human iPSC-derived model of cardiac hypertrophy for the phenotypic screening of 3600 compounds. Human iPSC-derived cardiomyocytes were exposed to Endothelin-1 (ET-1) to induce hypertrophy and an AlphaLISA assay was developed to measure secretion of NT-proBNP, a clinical marker of hypertrophic cardiomyopathy. 341 hits were identified following this strategy and 192 confirmed with additional repetitions of AlphaLISA, high-content imaging of BNP protein expression and exclusion of false-positive hits.[11]

Hit to lead & lead Optimisation

iPSC-derived disease models can be used to establish a structure-activity relationship (SAR) and measure the potency of newly synthesised compounds by looking at phenotypic changes, such as cellular functions, protein expression or metabolism. To facilitate a lead validation study with compounds that rescue Parkinson's disease (PD) phenotype, Ncardia developed a PD model using Ncyte CNS Neurons. This co-culture of human iPSC-derived neurons and astrocytes was exposed to alpha-synuclein preformed fibrils (PFF) to induce neurodegeneration mimicking PD. Multi-electrode Array (MEA) analysis of the iPSC-derived PD model showed decreased neuronal firing rate in response to PFF treatment. The same assay was used to calculate the PFF-induced toxicity and to successfully validate compounds that rescued PD's phenotype.[12]

KEY CONSIDERATIONS

Disease modelling based on human iPSC-derived cells has the potential to revolutionise drug discovery. Nonetheless, significant expertise is required to overcome some technical and conceptual challenges for the widespread implementation of these models in the pharmaceutical and biotechnology industry (Figure 3). Outsourcing can be a solution to achieve meaningful and actionable results in the shortest timeline possible. Collaboration with experts avoids common pitfalls, enables selection of risk mitigation strategies, enhances productivity, and ultimately reduces costs.

hiPSC sourcing & reprogramming

The first step to building a human iPSC-derived cell model is to obtain the most suitable iPSC line, taking into consideration patient genotype, tissue selection, age, gender, and availability of matched controls. This can be difficult due to informed consents not optimised for use in drug discovery. In order to make iPSC sourcing less complex and save time with procurement, having a broad network of contacts and agreements with multiple biobanks is beneficial. It is also essential to choose a reprogramming method that is efficient, has been validated in multiple somatic cell types, and has no genomic footprint.

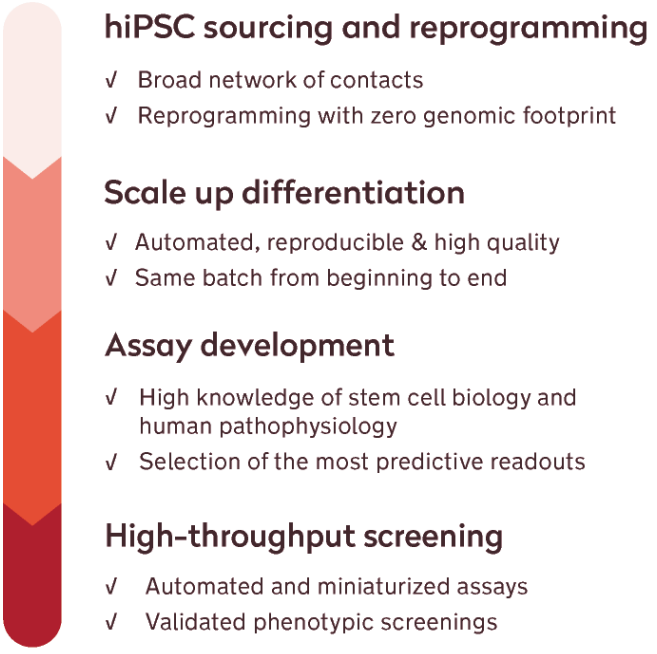

Figure 3 – Main considerations for the widespread use of human iPSC-derived cells in drug discovery. Significant expertise, in-house equipment and biological knowledge are required for the successful generation of disease models based on human iPSC technology.

Scale up differentiation

The process of drug discovery involves testing thousands of compounds to identify the best hits that will potentially become a beneficial therapeutic. Ideally, the same batch of iPSC-derived target cell is used for the whole process to avoid additional variables impacting the screening campaign. Therefore, the production of iPSC-derived cells must be of high quality, reproducible and in a large-enough scale for HTS applications. Automation, regular in-process monitoring, and multiple controls are needed to successfully scale-up the production of iPSCs to the required levels. Differentiation protocols in the public domain are typically established in 2D, with standard culture equipment and for a low number of cells. High understanding of cellular biology and high-level technology equipment are needed to set-up all the conditions and steps for an efficient iPSC differentiation in a large scale.

Assay Development

Having the capabilities to measure phenotypic changes is essential for disease modelling and drug discovery. The assays used for phenotypic screening must be predictive, validated, and easy to perform in both high- and low-throughput. Finding the optimal assay conditions, in terms of cell density, number of replicates, type of cell culture media and coating matrices, volumes, washing steps, etc., requires time, experience and high knowledge of iPSCs and cell biology. During development, the assays are miniaturised and automated to avoid operator-related variability. However, the degree of compromise between throughput and assay complexity continues to be

a challenge. Another key aspect is the selection of the most suitable readouts for HTS, which must provide objective and clinically relevant data while being cost-effective.

CONCLUSIONS

iPSC technology is a powerful tool to bring the human biological context earlier into the drug discovery funnel and mitigate late-stage failures due to safety or efficacy concerns. Human iPSC-derived disease models are of great advantage because they retain patient-specific genetic backgrounds and recapitulate many clinical features of human pathology. Nonetheless, significant expertise is required to overcome some technical and conceptual challenges for the widespread implementation of these models in the pharmaceutical industry. Working together with stem cell expert companies can a cost-effective and time-saving solution toeffectively implement the use of iPSC-derived disease models in pre-clinical testing and increase the success rate of drug discovery campaigns.

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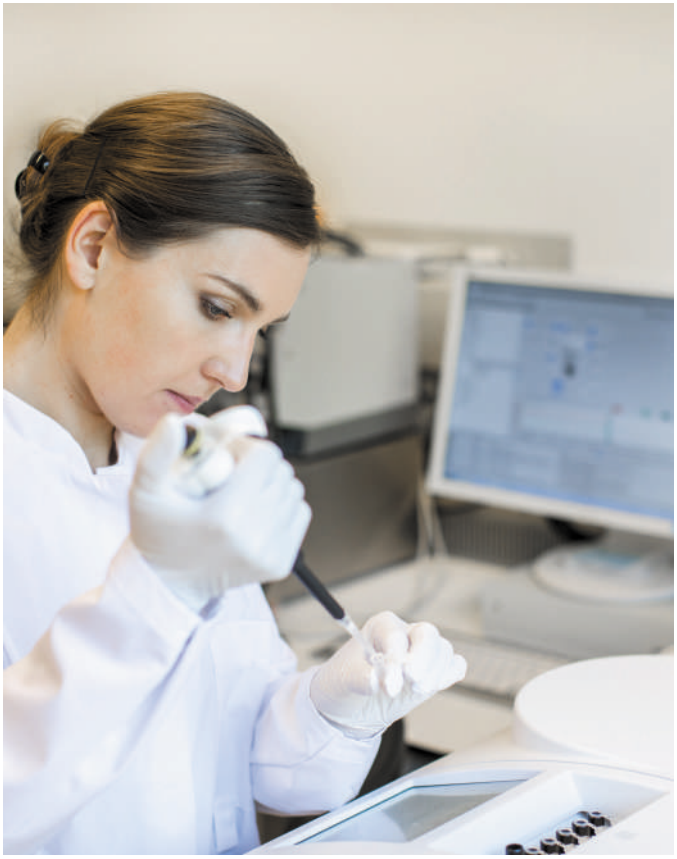

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