Ncardia
Stem cell experts

White Paper

Benefits & Key Considerations for the Use of Human iPSC-Derived Disease Models in Drug Discovery

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Highlights

- Disease models based on iPSC-derived cells recapitulate many clinical features of human pathology, facilitating decision-making during the early stages of drug discovery
- Human iPSC-derived disease models can be used for phenotypic screening with clinically relevant readouts leading to highly translational results
- The challenges of iPSC-derived disease model generation and application in drug discovery can be overcome by partnering with expert organizations

Background

The process of drug discovery, development and commercialization typically takes 13-15 years and is associated with high costs. It is estimated that the cost per drug approved is $1.5 to 3.0 billion and that **only 1% of the compounds initially tested make it to the market** [1]. The main reasons for this high attrition rate are the lack of understanding of pathological mechanisms and the scant predictability of the pre-clinical models used.

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. Cellular models offer a more complex environment and the possibility to evaluate the phenotypic effects of compounds. However, platforms based on immortalized cells present several limitations regarding disease modeling 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. Animal models do offer an *in vivo* complex environment, but are more expensive, have serious scalability constraints and the substantial inter-species differences hamper modeling certain human diseases, such as cardiac arrhythmias or neurodegenerative diseases.

In the past decade, **induced pluripotent stem cell (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 modeling (Fig. 1) [2].
Here we discuss the advantages and main considerations for the use of human iPSC-derived disease models during the early stages of drug discovery.

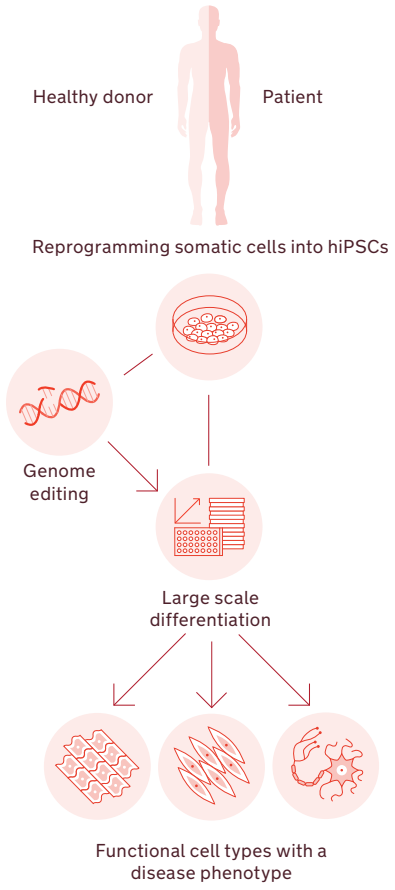

Figure 1. The process of disease modeling based on human iPSCs.
Human iPSCs can be obtained from somatic cells with non- or low-invasive techniques and used to generate physiologically relevant disease models that retain patient-specific genetic backgrounds.

Benefits of Human iPSC-based Disease Models

The main benefits of using human iPSC technology for disease modeling 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.

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. 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 for the use of iPSCs in disease modeling was reflected by the derivation of CMs from patients' iPSCs with Long QT syndrome (LQTS). LQTS is characterized by delayed repolarization 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 testing [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 modeling 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 modeling 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 for the use of iPSC-based models was brought by the breakthrough discovery of CRISPR. This genome editing technology is cost-effective, relatively fast and efficient in most cell types. 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 minimize genetic-background related variability and identify the true impact of the genetic variants on cellular phenotypes.

Human iPSC-based disease models retain patient-specific genetic background and show clinically relevant 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 (Fig. 2).

Target identification & 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,

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 decrease the attrition rate by bringing the true human biology earlier into the drug discovery and development process.

and could therefore be classified as potential therapeutic target [10].

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].

Human iPSC technology is an excellent tool to improve decision-making steps throughout the early phases of drug discovery

Hit to Lead & Lead optimization

iPSC-derived disease models can be used to establish a structure-activity relationship (SAR) and measure the potency of newly synthesized 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 modeling based on human iPSC-derived cells has the potential to revolutionize 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 (Fig. 3).

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 optimized for use in drug discovery. In order to make iPSC

hiPSC sourcing & reprogramming

- √ Broad network of contacts
- √ Reprogramming with zero genomic footprint

Scale up differentiation

- √ Automated, reproducible & high quality
- √ Same batch from beginning to end

Assay development

- √ High knowledge of stem cell biology and human pathophysiology
- √ Selection of the most predictive readouts

High-throughput screening

- √ Automated & miniaturized assays
- √ Validated phenotypic screenings

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

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.

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.

Partnering with iPSC experts is a common choice to overcome the challenges and achieve optimal results in the shortest possible timeframe

Assay development

Having the capabilities to measure phenotypic changes is essential for disease modeling 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 miniaturized 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.

Partnering with Experts

Outsourcing projects is becoming increasingly common in pharmaceutical research, to **achieve meaningful and actionable results in the shortest possible timeline**. When it comes to new technologies such as screening on human iPSC-based disease models, outsourcing is often the preferred option. Collaboration with experts avoids common pitfalls, enables selection of risk mitigation strategies, enhances productivity and ultimately reduces costs.

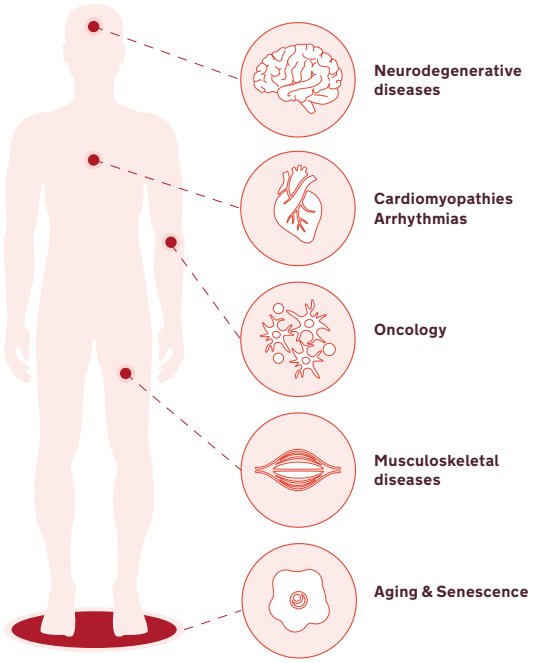

Figure 4. Ncardia's disease modeling expertise is in multiple therapeutic areas.
The long experience and deep knowledge of cellular biology of Ncardia's team enable the company to continuously expand into additional therapeutic areas.

Ncardia has more than 10 years of experience with human iPSCs and can guide and assist pharmaceutical and biotechnology companies in the process of disease modeling (Fig. 4), assay development and phenotypic screening. It has a broad network of contacts to facilitate iPSC sourcing and can execute the best reprogramming methods. The company has established large-scale differentiation protocols for multiple cell lineages with

reproducible and high-quality standards and has the flexibility to customize production according to the customer's project needs. Working with stirred-tank bioreactors ensures a high differentiation efficiency and enables the production of several billions of iPSC-derived cells for the screening of large libraries against the same batch from beginning to end.

In addition, Ncardia is equipped with in-house readout systems to develop physiologically relevant human iPSC-based phenotypic assays in multiple therapeutic areas. The expert team has developed high quality and reproducible assays to evaluate a wide range of phenotypes, from cell viability to electrophysiology, contractility, sub-cellular organization, biomarker expression, metabolism and more. Ncardia's scientists ensure the selection of optimal assay conditions and appropriate readouts for each project enabling screening of any modality whether it is a small molecule, RNA therapeutic, monoclonal antibody or gene therapy.

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Contact us for questions

At Ncardia we would like to hear all your ideas, challenges and questions, and bring those to a solution that fits your goals.

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