evotec

DRUG DISCOVERY UPDATES #08

DDup

iPSC-BASED DRUG DISCOVERY

A leading industrialised platform with unparalleled breadth

evotec — DDup

What's inside this issue?

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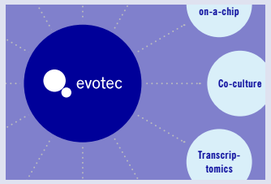

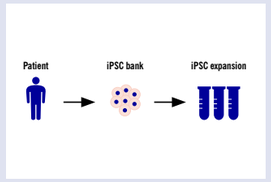

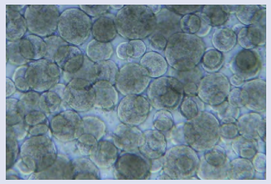

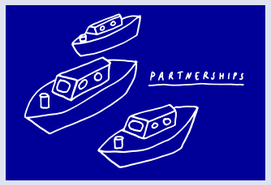

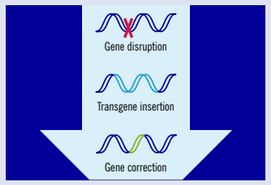

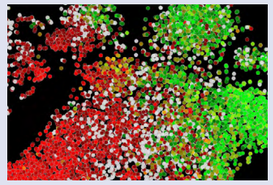

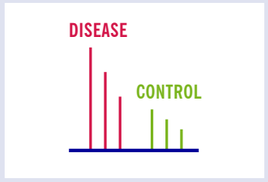

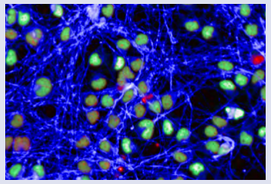

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DEAR FRIENDS
OF EVOTEC

A message from Evotec CEO
Dr Werner Lanthaler

Welcome to this seventh issue of DDup, an Evotec publication providing you with more insights into the company and its capabilities. This edition is dedicated to our highly innovative iPSC (induced pluripotent stem cell) platform, which we started developing more than six years ago with the goal to industrialise iPSC-based drug screening in terms of throughput, reproducibility and robustness to reach highest industrial standards. Today, it represents one of the largest and most sophisticated iPSC platforms in the industry and plays an essential role in iPSC alliances with strategic partners, such as Celgene and Sanofi.

I am by no means exaggerating when I say that I am absolutely convinced that patient-derived iPSCs have great potential for drug discovery in the coming years. Especially for disease areas with high unmet medical need like neurodegenerative diseases (e.g. Alzheimer's, ALS or Huntington's) or diabetes, the application of iPSC-based models represents a paradigm shift in developing desperately needed new drugs. Combining our iPSC-platform with leading expertise in high-throughput screening and Evotec's core competencies covering all stages up to IND, we can generate human models of disease that allow us to select safer drugs to take into clinical development.

Our team of experts has grown significantly in recent years and with a core iPSC team of more than 100 scientists, further growth is underway, Evotec is very well prepared to meet future challenges. We are continuously expanding our research activities to cover additional disease areas, such as lysosomal storage disorders and multiple sclerosis. For this, we are in the process of developing novel iPSC-derived cell types, more complex model systems and read-outs to set up new assays that hold great potential for identifying better drugs for such devastating diseases.

This edition marks the beginning of a series of DDups focussing on Evotec's iPSC efforts. Future editions will provide more insights on some of our drug discovery programmes, e.g. on lysosomal diseases.

Thank you for reading this issue of DDup, for your thoughts, input and hopefully also the cooperation in this field with us. I hope you find this latest edition of DDup of particular interest and as always please do not hesitate to contact us.

Yours sincerely,
for the management of Evotec
Werner Lanthaler,
CEO of Evotec SE

CHAPTER 01

iPSC PLATFORM

FROM iPSCS TO DRUG CANDIDATES AND BEYOND

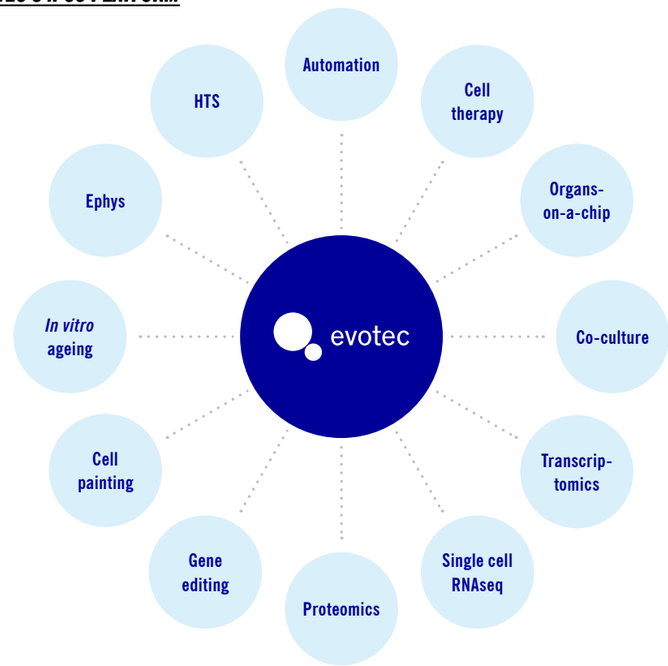
Dr Sandra Lubitz

Dr Sandra Lubitz received her PhD in Cell Biology from the International Max Planck Research School in Dresden, Germany, focusing on epigenetic regulation in embryonic stem cell self-renewal and differentiation. She continued this work in her postdoctoral studies at the Biotechnological Centre in Dresden before moving to Genea BIOCELLS, a stem cell company based in Sydney, Australia, to work with disease-specific human embryonic stem cells. In her role, she established neural differentiation and phenotypic high-content assays for human embryonic stem cells. Sandra then moved to Pfizer Neusentis in Cambridge, UK, where she was involved in projects using human stem cells for high-throughput screening and cell therapy. Joining Evotec in 2011, she played a key role in building the iPSC platform at Evotec and establishing iPSC-based model systems for neurodegenerative disease. Sandra is heading Evotec's stem cell team, overseeing activities for the Celgene collaboration and internal EVT Innovate activities.

Over the past decade, several breakthroughs in induced pluripotent stem cell (iPSC) research have revolutionised the way drug discovery is performed. With their potential to generate any human cell type, patient-derived iPSCs are clearly more physiologically relevant and better suited for modelling disease pathophysiology and for understanding a drug's mechanism of action than previous models. Hence, iPSC-based high-throughput screening approaches provide unique opportunities as a tool for disease modelling and predicting drug efficacy. However, technical challenges exist in culturing and differentiating these iPSC-derived cells in a reproducible manner at industry scale. At Evotec, we have overcome such hurdles through development of scalable and robust protocols that allow us to produce specific disease-relevant cell types in a consistent manner. This involves generation of a cell bank of fully validated iPSC lines (see page 6), upscaling of iPSC culture and differentiation protocols to industry standards (see page 8), as well as automation of iPSC-derived cultures (see page 10). Importantly, our large-scale production of iPSC-derived cells has been validated with reliable quality control assays at different stages along the manufacturing process, to reduce clone-to-clone or batch-to-batch variations. Applying strict quality control parameters throughout all steps of the process have enabled us to make a big step towards modelling human disease in a dish at an industrial scale.

»At Evotec, we are convinced that iPSC modelling is a road to success for devastating diseases that remain poorly understood. I would like to personally thank all patients donating samples for iPSC research and our team of researchers who provide invaluable insights while building better therapeutic options for the patients who need them.«

UNPARALLED BREADTH OF EVOTEC'S iPSC PLATFORM



As of today, iPSC-derived cells in two-dimensional monolayers provide a simple and scalable tool for exploring disease pathogenesis and underlying mechanisms. At Evotec, we are routinely using iPSC-derived cell types for phenotypic screening activities in various disease areas. In addition, we develop more complex models, such as co-cultures of multiple cell types or microfluidic organs-on-a-chip approaches, which enable interaction between different cell types. These systems enable modelling of human diseases under conditions that more closely mimic the physiological environment. In Evotec's NEPLEX project we are using a 3D organ-on-chip approach with iPSC-derived podocytes (more details can be found in DDup #7).

Disease modelling with iPSC technology has enabled us to identify new genes and pathways, in particular for conditions that show a strong genetic component. However, some read-outs for adult onset diseases may require the use of mature neurons demonstrating key features of cellular aging for full manifestation of pathological features. Hence, parts of our research activities focus on maturation, aging and metabolism of disease-relevant cell types to better recapitulate pathological features in patients.

Combining Evotec's iPSC platform with modern techniques in genome editing (see page 16) and our PanOmics platform, we have been collecting large datasets from transcriptome & proteome analyses (see page 18 and 20) to define disease signatures, and causal relationships between genetic mutations and perturbation of specific molecular pathways. This creates powerful human translational models that will ultimately enable us to identify better targets and drugs.

Our long-term goal is to utilise patient-derived iPSCs to stratify patient populations for clinical trials. With the advent of our in-house iPSC bank, representative iPSC samples are available for comparative analyses in so called 'clinical trials in a dish' (see page 22) to enable patient selection and to pre-determine patients likely to respond to the drug in question. This may ultimately enable us to select better and safer drugs for clinical development in disease areas with high unmet clinical need.

CHAPTER 02

iPSC BANK

HIGHEST iPSC QUALITY STANDARDS

Dr Christian Kannemeier

Dr Christian Kannemeier received his PhD in Cell Biology/Biochemistry from the University of Gießen in 2003, working on the isolation and cellular characterisation of a newly identified plasma protease. After that, he received his postdoctoral training in the Department of Molecular Biology at the Scripps Research Institute in San Diego, CA working on transformation mechanisms of cancer cells. In 2007, he started working for a small biotech company in Irvine, CA before he moved to Madison, WI in 2009. There he worked for Cellular Dynamics International for 9 years as a Senior Scientist and Group Leader developing protocols for the production of iPS-derived cells. Christian joined Evotec in 2018 and is leading the iPSC Core team.

Human iPSCs generated by direct reprogramming of somatic cells can proliferate almost indefinitely *in vitro* while maintaining their capacity to differentiate into a broad diversity of cell types. They hold unique potential for clinical applications, because of their ability to produce infinite quantities of human patient-derived cells for disease modelling, drug screening and cell-based therapy. However, intrinsic variations between iPSC lines attributed to cell type of origin, donor, culture condition, or reprogramming method, need to be considered for large-scale analyses.

With the goal to minimise variation and to industrialise iPSC-based models in terms of throughput, reproducibility and robustness, Evotec has started to establish its own internal repository of hundreds of patient-derived iPSC lines covering multiple disease areas as well as controls with highest quality standards (Figure 1). For this, we are working closely with our partners Censo Biotechnologies and Fraunhofer IME-SP, both providing their expertise in sourcing and reprogramming technologies. All iPSC lines utilise a non-integrative approach to maintain genomic integrity.

Manufacturing of iPSC master cell banks requires detailed characterisation of the initial status of the iPSCs. This involves standard tests for sterility, pluripotency and differentiation potential of iPSC lines, complemented by batch testing of all media and supplements. Furthermore, every iPSC line is extensively genetically characterised and compared to the original patient cells. Evotec's iPSC platform has been developed so that quality control parameters are routinely checked for each line and those

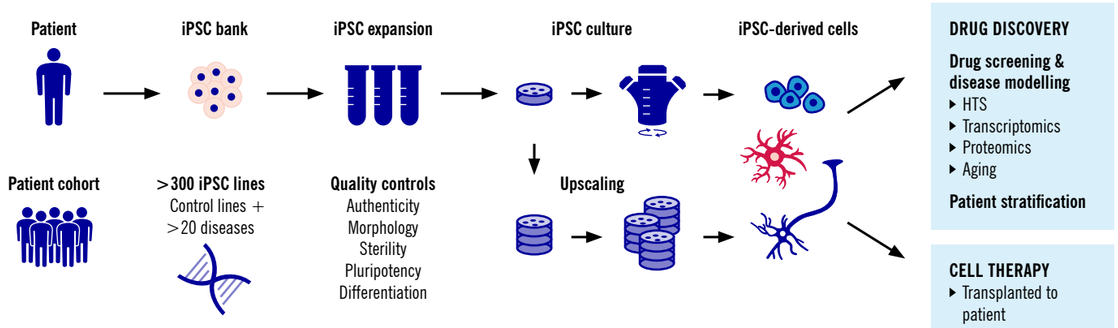
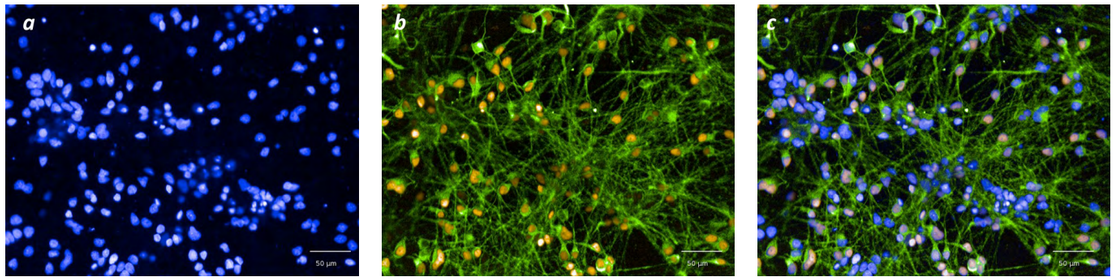


Figure 1

iPSC lines that fail to reach criteria will be discarded. All iPSCs that pass QC, are used to create master cell bank stocks, which will then be further expanded as working cell banks to support ongoing research activities. Once an iPSC line is banked, it is scaled up for manufacturing to larger formats.

The next important step is the definition of pass/fail criteria for batches of iPSC-derived differentiated cells. Variability in iPSC differentiation can result from multiple sources such as variations in culture conditions such as small molecule, growth factors or matrices but also well-to-well differences in cell densities. Therefore, distinct panels of markers have been set up at Evotec for each standard operating procedure (SOP) protocol to monitor quality of the iPSC-derived cells and we are convinced that such stringent QC parameters have enabled us to reach highest industrial standards for reproducibility and robustness. Standardisation is essential for assay development and the evaluation of cellular phenotypes arising from a specific mutation in iPSC-derived cells in drug screening. Moreover, it is also essential for bulk omics analyses (transcriptomics and proteomics) and single-cell RNA sequencing to assess cellular heterogeneity. Overall, we have established a step-by-step process to manufacture disease-relevant cell types from iPSCs for disease modelling and cell therapies and are routinely using these patient iPSC-derived cell types for a number of research activities in the field of neurodegenerative diseases, neurodevelopmental diseases, lysosomal storage diseases, retinopathies, multiple sclerosis, diabetes, and others.



iPS derived neurons from a patient with Fragile X Syndrome. (a) blue: DAPI, indicating the nucleus of the cells, (b) green: TuJ1, a neurite marker for beta III tubulin that serves as a general neuronal marker; red: TBR1, a specific cortical neuron marker, (c) overlay.

CHAPTER 03

3D CULTURE

iPSC CULTURES AT LARGE-SCALE

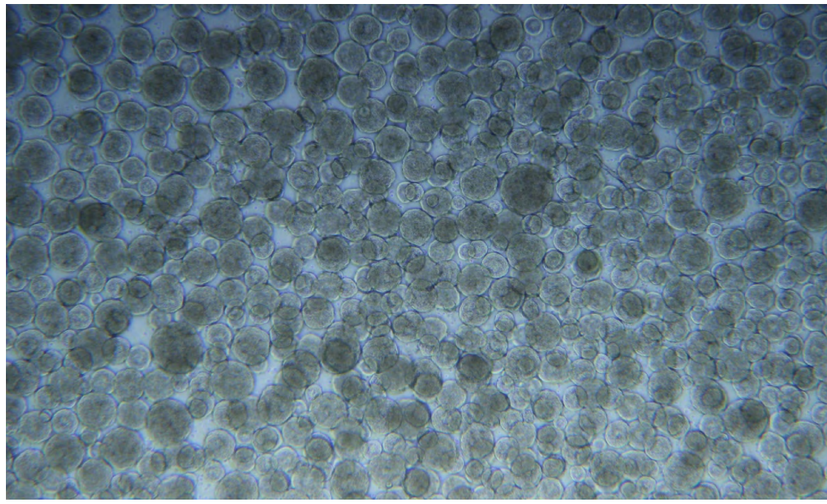
Simone Strauch

Dipl.-Ing. Simone Strauch worked for more than 5 years at Amgen's HTS centre focusing on automated cell culture systems and cell-based assays for high-throughput screening. There she established a fully automated 2D protocol for maintaining and expanding iPSCs and generating assay-ready plates for iPSC-based screening and assay development. Since joining Evotec Goettingen in 2015, she has been responsible for iPSC production in 2D and 3D, protocol optimisation for differentiation into human beta cells and upscaling of production and differentiation processes in bioreactors.

Traditional methods for maintenance and expansion of iPSCs rely on two-dimensional (2D) culturing techniques using plastic culture plates and xenogenic media. These methods provide limited expansion and iPSCs tend to loose clonal and differentiation capacity upon long-term passaging. To achieve high numbers and constant supply of high-quality iPSCs required for cell therapy applications and high-throughput screening, 3D culture systems for maintenance and expansion of iPSCs offer tremendous potential. Dynamic culture techniques involving spinner flasks and bioreactor systems support human iPSC proliferation to high density. Culturing iPSCs as cell-aggregates in suspension as 3D culture overcomes the limitations of surface-adherent 2D culture, as it allows scale-up, detailed online monitoring of key process parameters such as pH, temperature and dissolved oxygen, and automated feeding strategies. This results in a more homogeneous culture environment, and enables higher cell yields while at the same time reducing workload.

Dynamic bioreactors increase the reproducibility of iPSCs grown at large scale. One of the main advantages of 3D culture is the expansion and subsequent differentiation into specific cell lineages using differentiation media. Large screening campaigns require billion-fold expansion of high-quality pluripotent cells as starting material to generate sufficient yield of

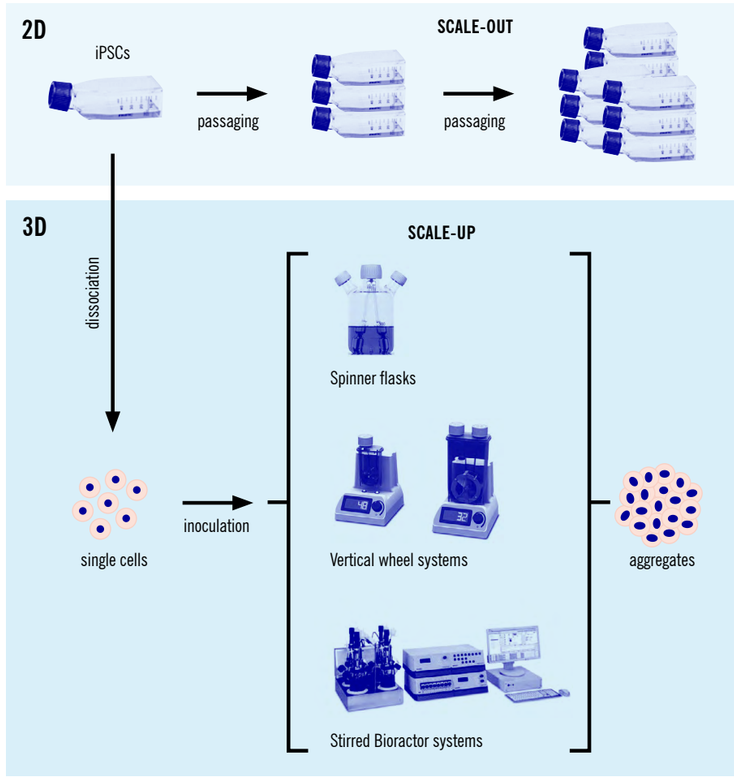
iPSC-derived differentiated cells for high-throughput screening. Likewise, 3D culture systems overcome limitations for cell replacement therapies by producing large quantities of high quality, GMP-grade iPSC-derived cells for various types of disorders, such as diabetes. In a strategic collaboration with Sanofi, Evotec has successfully applied 3D culture to achieve pre-clinical proof-of-concept for a beta cell replacement therapy based on functional human beta cells derived from human stem cells. With upscaling of the manufacturing process and suitability testing of the encapsulated beta cells in diabetes models, an important milestone was achieved in June 2018. Both teams are continuing to further validate the new encapsulated cell product that may revolutionise standard of care for diabetes patients.



Single cell-inoculated suspension culture forming cell aggregates 1 day after seeding into bioreactor system (4× magnification)

Following systems for 3D iPSC culture are used at Evotec:

- Various formats of **spinner flasks:** allow cultures between 100 ml to 1 L. The stirring mechanism of the flasks relies on two glass pendula, which guarantee low shear forces while maintaining optimal mixing. Especially suitable for sensitive cells and complex differentiation protocols
- **Vertical wheel culture systems:** allow cultures between 60 ml and 500 ml. Optimal for culturing shear-sensitive cell aggregates
- **Bioreactor systems:** offer controllable agitation, temperature, pH and O_2. Media change by perfusion is possible. Culture volumes vary between 250 ml and several litres



CHAPTER 04

iPSC AUTOMATION

HIGH-THROUGHPUT SYSTEM FOR iPSC-BASED DRUG SCREENING

Dr Anne Schlüter

Dr Anne Schlüter received her PhD in Biology from the University of Hamburg, working in the Centre of Molecular Neurobiology in the department of PD Dr Ingolf Bach. She worked in the field of developmental neurobiology on RNF6 an RLIM-like Ubiquitin ligase in vertebrates. After three more years as a postdoc in the same field she started working with CCS (Cell Culture Service GmbH) in Hamburg where she was leading the department of Cell Line Development & Molecular Biology. In 2013, when CCS was acquired by Evotec, Anne started to work on cell line development projects as well as on large-scale upscaling processes for HTS screening with many different cell lines. In January 2017, Anne started working in the Stem Cell Biology Department in Hamburg, where she is leading the Cell Production Core and supporting the upscaling process of iPSC-derived cell types at Evotec.

High-throughput screening activities in iPSC-derived cells are inversely correlated with a requirement for upscaling and automation to provide scalable and standardised assays that allow for screening millions of small molecules. Miniaturisation and automation contribute to cut reagent use, minimise labour-intensive steps and reduce variation that could normally occur upon manual handling.

Evotec's automation platform "XLII cell::explorer system", developed in close collaboration between Perkin Elmer and Evotec, was designed with the specific goal to support fully automated handling of iPSC-derived cell types in 384-well format. It comprises two state-of-the-art pipetting robots, a 384-well head pipetting workstation and a multimode plate reader, all of which is contained in a sterile housing with laminar airflow to enable processing of several hundred plates according to strict quality control standards. Processes established on the platform include cell seeding in defined media conditions, media changes and compound treatment. Together with Evotec's Bioinformatics department, a novel, custom-made database has been created to operate this platform. With the "XLII cell::explorer system", we can now provide stable growth conditions for

XLII cell::explorer system

FACTS & FIGURES AT A GLANCE

- 2 robots for 360° operation
- 4 incubators for several hundred 384-well plates
- Real-time quality control checks
- Sterile long-term culture
- Custom-made database to operate platform
- Designed for future upscaling

long-term cultures of iPSC-derived cell types that need to mature further to present pathological features. Long-term cultivation can last up to several weeks or months with multiple media changes per week, which is very time-consuming if done manually. Long-term cultivation also increases the risk of contamination as well as of clustering of the cells that cannot be tolerated by most high-throughput screening assays. Real-time QC for sterility and clustering via the EnSight™ (multimode plate reader) enables smart go/no-go decisions managed by the database. In order to allow maximum flexibility, the platform is also equipped with four incubators, one of which is considered for quarantine. Cell plates excluded by the software could then be withdrawn or manually checked by an operator for false positives. Images of the multimode reader are monitored for QC purposes and tracked by the database enabling smart decisions at real time.

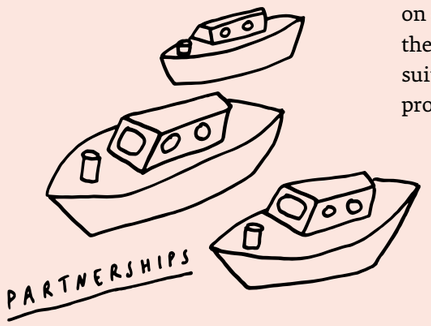
Overall, Evotec and Perkin Elmer jointly developed an automation platform that is tailor-made and allows generation of iPSC-derived cultures at industrial scale. It enables for long-term culture of iPSC-derived cell types in a sterile environment, which is a major prerequisite for disease modelling. We have very successfully used iPSC-derived cells generated on this platform for high-throughput screening campaigns in a variety of projects and we are looking forward to further expanding our efforts to screen for new mechanisms, targets and compounds in neurodegenerative disease and beyond.

CHAPTER 05

PHARMA PARTNERSHIPS

PROVIDING iPSC MODELS FOR DRUG DISCOVERY ALLIANCES

Evotec has built one of the industry's broadest iPSC platforms and is continuing to develop human disease models to identify new therapeutics for a variety of different disease areas. Through its partnering strategy, Evotec is building a pipeline without bearing the financial risk normally involved in such projects. Achievement of several milestones with our collaboration partners reveals the success story behind our iPSC platform, and with our constantly growing iPSC team we are open to discuss and develop novel long-term strategic partnerships to identify new therapeutics for other devastating diseases, such as retinopathies, kidney disease, lysosomal storage or neurodevelopmental diseases and beyond.



Two major alliances

iPSC ALLIANCE IN DIABETES WITH SANOFI (INITIATED IN AUGUST 2015)

Focused on development of beta cell replacement therapy and drug discovery based on functional human beta cells

Commercials: Upfront € 3 m, research payments, potential milestones > € 300 m, double-digit royalties

Achievements so far: In April 2017, Evotec has reached an important Milestone triggering a payment of EUR 3.0 m to Evotec for achieving pre-clinical proof-of-concept. Followed by a second EUR 3.0 m milestone in June 2018, for delivering on critical success criteria regarding the manufacturing process and suitability testing of the beta cell product.

iPSC ALLIANCE IN NEURODEGENERATION WITH CELGENE (INITIATED IN DECEMBER 2016)

Focused on iPSC-based drug screening to identify disease-modifying therapeutics for a broad range of neurodegenerative diseases, e.g. ALS, Alzheimer's, Huntington's or Parkinson's disease

Commercials: Upfront $ 45 m, potential milestones > $ 250 m per project, low double-digit royalties

Achievements so far: This collaboration has reached a first milestone triggering revenues of $ 5.0 m to Evotec, due to successful completion of a screening campaign using Evotec's iPSC-based screening platform. In May and October 2018, Evotec announced that Celgene decided to further extend the collaboration and include additional iPSC lines, which resulted in a $ 6.0 m milestone payment each. Following the advancement of the first iPSC programme into lead optimisation stage, Evotec announced that this scientific achievement resulted in a milestone payment of $ 14 m before year-end 2018.

CHAPTER 06

5 MINUTES

WITH DR RICHARD HARGREAVES ON iPSC DRUG DISCOVERY

Richard Hargreaves, Ph.D., FBPhS, *holds a BSc and Ph.D. from Kings College, London University, UK and is an Honorary Fellow of the British Pharmacological Society. He is currently Corporate VP, Head of the Neuroscience and Imaging Thematic Center of Excellence (TCoE) at Celgene. Previously VP, Head, New Indications Research Unit and Research & Early Development Centers of Excellence at Biogen and VP Global Imaging and VP Discovery Head for Neuroscience in Merck Research Laboratories. Richard has led teams that have advanced numerous neuroscience drug candidates and novel PET imaging agents to the clinic contributing to the successful development and registration of several NCEs. He is highly published with >200 journal articles and co-editor of 3 books. Richard has been recognised by an Innovation in Drug Development award from the ASPCT for his work on discovery imaging and the Sir James Black Award for Drug Discovery from the British Pharmacological Society.*

Cord Dohrmann, CSO of Evotec: Where do you see the advantage or even the need of iPSC-derived cells in the drug discovery process?

Richard: There are two main advantages for use of iPSC-derived cells that are particularly important for drug discovery in neurodegeneration.

First, neurons have many unique features and functions that simply are not represented in other common cellular models that are used in the high-throughput industrialised processes of early discovery. Neurons are physically large, electrically active cells that don't grow: they need to maintain incredibly high metabolic activity for very long periods of time. Many of the biological processes that maintain neuronal function and viability are supported by unique ensembles of genes specialised to neurons. Dysfunction in these pathways can lead to the development of neurodegenerative disease. Many of the genes that cause neurodegeneration are expressed in all cells but only affect neurons. Using iPSC-derived neuronal technology allows us to model neurodegenerative pathobiology in a system that has similar metabolic constraints and vulnerabilities as occurs in patient.

»Using iPSC-derived neuronal technology allows us to model neurodegenerative pathobiology in a system that has similar metabolic constraints and vulnerabilities as occurs in patient.«

»We believe the physiologic properties of iPSC-derived neurons, and their disease relevant genetics, will allow us to identify drugs more likely to work eventually in the clinic.«

Second, iPSC systems allow us to carry out drug discovery in systems that have relevant genetics very similar to the patients we ultimately treat. One hard-learned lesson in drug discovery is that your chance for success in the clinic goes up dramatically when you understand the relationship between your therapeutic approach and the genetic cause of disease. We hope to be successful in neurodegeneration, as we are focusing on diseases that are caused by specific genetic mutations, and we develop iPSC systems that contain those similar mutations, express similar disease genes, and develop therapies that affect the mutant proteins that drive disease. We believe the physiologic properties of iPSC-derived neurons, and their disease relevant genetics, will allow us to identify drugs more likely to work eventually in the clinic.

Cord: For you, what are the key aspects that can be achieved when working with iPSC-derived cellular models from healthy and diseased donors? Will we deliver better medicines faster with this approach?

Richard: An important aspect of using iPSCs is that we hope to fail less in the clinic. One observation we have made, is that when we test drugs that according to the literature should have positive effects, we find they are ineffective in functional human neurons made from iPSCs even though they may be active in other common cellular systems. Human iPSC systems are not perfect, but we hope they may be more predictive than mouse systems or using non-neuronal cell systems that are easier to use in the lab.

In addition to genetic and physiologic relevance, iPSC systems have a number of other key attributes. While you can use iPSCs to make specialised non-dividing cells that act a lot like a neuron does when it's in the brain, or even in a slice of a brain that can be monitored in the lab, we can make as many of the iPSC-derived cells as we need, if you take the time to engineer processes required to grow and turn them into the specialised cells we want to study. Additionally, since iPSCs are theoretically immortal while in the undifferentiated stem cell state, we have time to edit their genes, to make them better models of a disease, or to adapt them for highly industrialised approaches.

The question of why you would want iPSC models from healthy and diseased donors is a good one. Ultimately, we want to make patient relevant models of disease in a dish that we can easily study and use to discover curative therapies. Being able to compare the properties of a neuron derived from healthy versus diseased donors allows us to identify, with some confidence, properties of those cells that are truly abnormal. We can build even more confidence that we are studying a disease-specific process by using gene-editing technologies to convert a disease-causing gene in a patient's iPSCs to a normal gene, and thereby prove that the characteristic we are studying in that cell is directly caused by a disease-linked mutation.

Many patients have neurodegenerative diseases without a family history or obvious genetic risk factor. Using neurons derived from healthy patients, we can ask what factors may drive disease in that population. This is critical as, unfortunately, the largest population of patients with neurodegenerative diseases are idiopathic, meaning the disease arises spontaneously without a known cause. Finally, and perhaps most interestingly, we can also begin to look at what makes a patient who has lived a very long, disease-free life, resistant to the development of age-related degenerative conditions.

»Ultimately, we want to make patient relevant models of disease in a dish that we can easily study and use to discover curative therapies.«

Cord: What makes Evotec a good partner for you and where do you see synergism between Celgene and Evotec?

Richard: Our skill sets, capabilities and technologies are extremely complementary. Perhaps the most attractive combination of tools was that Evotec developed the technical capabilities to produce and screen iPSC-derived neurons from a variety of diseases that are caused by build-up of toxic proteins, but had no specialised chemistry or drugs designed for the study of protein handling or protein-homeostasis. On the other hand, Celgene had developed an entirely new way of targeting proteins for degradation through our CELMoD technology and built large chemical libraries based on the CELMoD concepts. Currently, a significant part of our collaboration is testing our proprietary CELMoD chemical collection for the ability to ameliorate the effects of toxic protein accumulation in neurons or enhancing the clearance of those toxic proteins that we know underlie most neurodegenerative diseases.

We have formed a cadre of scientists, led for Celgene by Mark Labow, with diverse biology, chemistry, and technological expertise into an extraordinarily high-performing, collaborative and interactive team that thinks about drug discovery through different lenses, with experts in neuroscience drug discovery, cell biology, engineering, neuroimaging, neurology, small molecule screening, chemistry, protein structural biology and animal model engineering contributing to almost every discussion. Our working meetings are goal-oriented and intense, enabling us to synthesise new hypotheses and test concepts in the lab rapidly.

Cord: Looking ahead, where do you expect iPSC-based drug discovery to contribute to in the future?

Richard: First of all, we are still in the early stage of learning and optimising how to model human disease *in vitro*. The field will move toward making more complex models with multiple cell types that develop, interact and even age in ways relevant for disease.

On that note, an area of keen interest for us is how we can model the effect of aging, which is the biggest risk factor in the development of neurodegeneration, and many other diseases as well. Once we understand how to model aging in a reliable and practical way – as culturing neurons for years in the lab, for example, will not work for large-scale drug discovery – we can begin to think about striving to reduce the effect of aging on disease risk.

Finally, the field of cell and tissue replacement with iPSC-derived cells is in its infancy. During development, cells have remarkable abilities to self-assemble into tissues and organs, to migrate to their needed locations, to function in or to repair tissue. We are confident the genetic programming for these capabilities reside in the iPSC, and the field will slowly figure out how to use those innate capabilities, and iPSC-derived cells could eventually become therapeutics.

Cord: Thank you for your time!

Dr Cord Dohrmann is Chief Scientific Officer and Member of the Management Board at Evotec. Dr Dohrmann has spent over 25 years in biomedical research at leading academic institutions and in the biotech industry.

CRISPR-BASED PLATFORM

CHAPTER 07

FOR GENE EDITING AND GENETIC SCREENING

Dr Hauke Cornils

Dr Hauke Cornils received his PhD in biochemistry/cell biology from the Friedrich Miescher Institute for Biomedical Research and the University of Basel focusing on the roles of NDR/LATS kinases in cell and cancer biology. In 2011 Hauke joined David Pellman's group at the Dana-Farber Cancer Institute and Harvard Medical School as a postdoc to study the consequences of mitotic errors on genome stability. During his stay at Dana-Farber Hauke made significant contributions to the understanding of the effects of tetraplodisation and micronucleisation on cancer biology. He gained in-depth understanding of genetic screening and single-cell sequencing approaches for biomedical research. His research was published in high-ranking journals including Cell, Nature and Nature Communications. Hauke joined Evotec 2014 as a Research Scientist in the *In Vitro* Pharmacology department in Hamburg and building on his experience in RNAi screening has started to implement CRISPR technology at Evotec starting 2015. Since then he is leading the CRISPR platform to use the technology alongside other genetic approaches for target identification, validation and cell model generation.

Since its discovery, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has revolutionised basic and applied biomedical research. At its core, CRISPR consists of a complex between a guide RNA (gRNA) and a Cas9 protein, which form a DNA endonuclease. While DNA endonucleases are established tools in molecular biology the revolutionary aspect of CRISPR is that the endonuclease can be easily programmed to cut at basically any site within the target DNA sequence by simply changing the sequence of the RNA moiety. Once introduced into the target cell, the Cas9 complex will search the genome for its target sequence and generate a double strand break at this site. Based on the experimental design we can harness the repair process of the DNA break to give rise to loss of function mutations (Knock-out; KO) or precise manipulation of the target site (knock-in mutations). As compared to earlier methods, CRISPR allows this genetic manipulation at unpreceded efficiency and effectivity, thereby giving us a new set of tools for target validation, target identification and cell model generation.

The CRISPR toolbox is most powerful when applied to disease-relevant systems and cell types. At Evotec we have successfully applied CRISPR technology to primary cells and are combining CRISPR technology with Evotec's world leading iPSC platform to create unique opportunities to further enhance the impact of the iPSC technology on drug discovery (Figure 1). One of these opportunities is to generate cell models in iPSCs using precise knock-in mutations. An obvious use of this approach is to introduce disease mutations into iPSCs and use these cells to derive disease models for further studying disease mechanisms or for hit identification in high-throughput screening. Furthermore, the approach can be used to introduce tags or reporter into target genes thus creating reporter cell lines, which can then be used at various stages in the drug discovery process.

Furthermore, CRISPR technology can be applied in large-scale to introduce KO mutations, which allows genome-wide genetic screening to identify genes implemented in a disease phenotype or mediating the activity of a compound coming from phenotypic screening. In neurodegenerative disease, specific neurons are selectively vulnerable and transcriptomics or proteomics analysis can be applied to determine the differences. However, a functional approach (Figure 2) is required to determine causality between the expression of specific genes and their role in selective vulnerability. For this we developed a genetic screening platform that exploits CRISPR technology to systematically assess the contribution of these gene to the disease phenotype. This approach enables genome-wide screening in iPSC derived human cells and can be performed in a pooled or an arrayed format.

With iPSC and CRISPR we are joining two revolutionary technologies at Evotec creating opportunities in drug discovery in which "the sum is greater than it's parts".

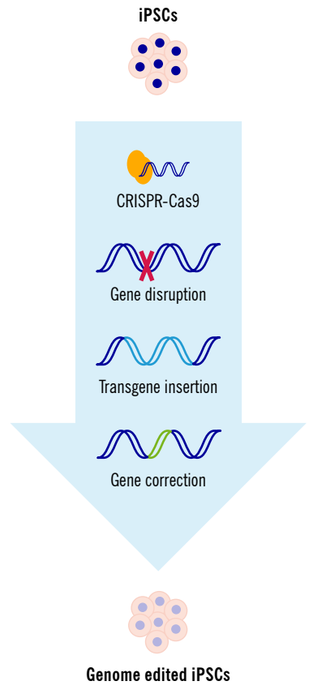


Figure 1: CRISPR/CAS9 RNA-guided nuclease technology can be applied in human iPSCs for gene editing via specific gene disruption, gene addition, or gene correction.

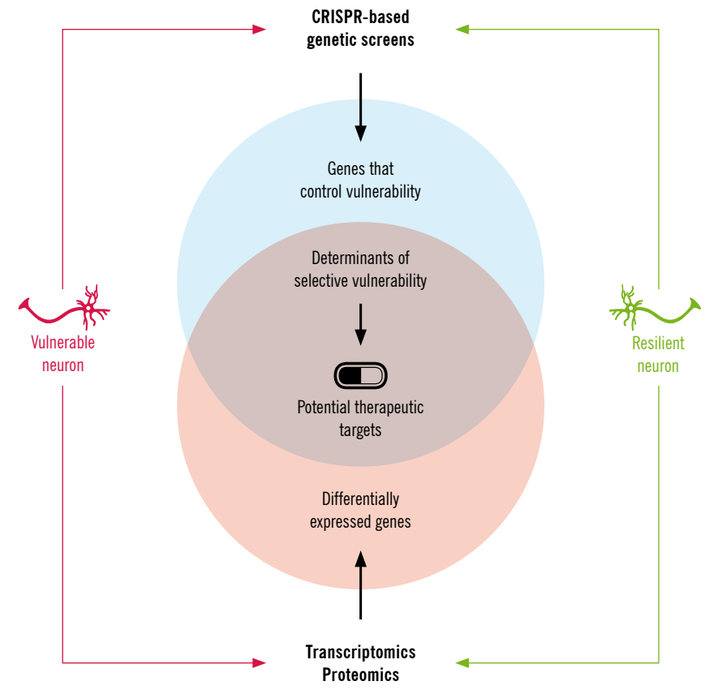


Figure 2: Functional differences relevant for selective vulnerability of neurons can be determined through a combination of transcriptomics/proteomics and CRISPR. With transcriptomics/proteomics analysis differentially expressed genes can be determined in vulnerable and resilient neurons, CRISPR-based genetic screening can then reveal specific genes that control vulnerability. A combination of both allows to identify determinants of selective vulnerability and potential therapeutic targets (modified from DOI: 10.1016/j.molmed.2017.04.003).

CHAPTER 08

TRANSCRIPTOMICS

TRANSCRIPTOME PROFILING ON A SINGLE CELL LEVEL

Dr Thomas Siegmund

Dr Thomas Siegmund received his PhD in biology from the Free University of Berlin, working on developmental biology and neuroscience in Drosophila. As a postdoc Thomas moved into bioinformatics – at a time when there was no formal bioinformatics training yet, but a strong need to analyse the first animal genome data sets. He soon provided some of the first bioinformatics seminars at the FU Berlin. In 2002, he joined the former DeveloGen, now Evotec International, in Goettingen. Since this time, he has supported target ID and screening projects with bioinformatics expertise and tools. Since January 2019, Thomas is responsible for bioinformatics and biostatistics globally within Evotec.

Whereas the cells in a human being in principle all have the same set of genes, the activity of these genes is regulated very dynamically. A gene can be completely off, or it can be activated gradually, depending on the cell type, on environmental conditions, on nutrition, or on disease state. A number of technologies summarised under the term *transcriptomics* allow for a very precise measurement and analysis of gene activity. At Evotec, we use deep sequencing of RNAs (RNA-Seq) as a state-of-the-art method for transcriptomics.

There are more than 20,000 protein coding genes in human cells, and RNA-Seq can quantify all their RNAs with high precision in parallel. This results in very rich, biologically interpretable data. RNA-Seq can be used to characterise cell lines: Do they properly model normal human cells, or a given disease phenotype? Do they function as expected and do they react properly to external stimuli? We use RNA-Seq routinely alongside genome sequencing and other techniques to characterise new iPSC lines and their differentiation protocols.

An exciting new method in transcriptomics is single cell RNA-Seq (scRNA-Seq). As the name implies, it generates detailed transcriptome data from thousands of individual cells in one sample. As a result, biologists may gain fundamentally new insights how genes regulate the interaction of a variety of different cell types in a tissue.

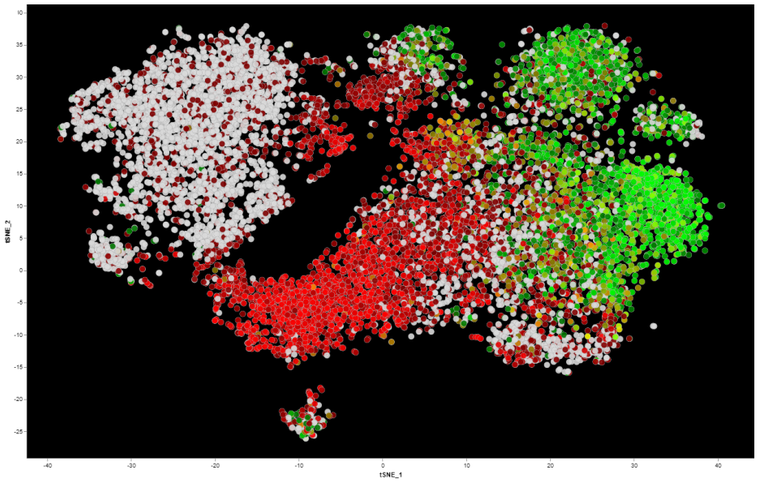
One key step in iPSC research is the optimisation of a differentiation protocol for an industrial application. Producing a homogenous population of functional, differentiated cells from a batch of stem cells is a complex procedure. Over a period of multiple weeks, cells need to reach various differentiation steps in processes that aim to recapitulate the differentiation cascades found during embryonic development. The *in vitro* process may fail in different ways: cells may differentiate too slowly and miss steps, or they may differentiate into an unwanted direction.

Observing problems and identifying their cause in a differentiating cell population can be challenging. Here, scRNA-Seq is an ideal method: it can interrogate thousands of genes in as many cells in parallel, giving completely unbiased insights into a cell population. Different cell types and differentiation stages can be identified based on the active genes in each cell. Once an unwanted subpopulation has been identified, signalling mechanisms within these cells may be analysed. Was a certain differentiation signal not strong enough to move them over a threshold or did another signal send them onto an alternative path? Based on these insights, Evotec researchers make informed decisions how to optimise the differentiation protocol.

Obviously, data analysis and interpretation is crucial in this field of research. RNA-Seq, and especially single cell RNA-Seq, generate a huge amount and complex data sets. A single study may easily produce millions of data points. At Evotec, we have built an automated data analysis pipeline to process RNA-Seq data on an industrial scale. This also includes tools and standards for a rigorous quality control.

More interesting, though, is the question how to interpret these data in a biologically meaningful way. One answer is solid statistics to calculate differential gene expression, pathway and network analyses. Yet at least equally important are the knowledge and insights of project scientists. Our data analysis platform PanHunter brings algorithms and brains together and is available in a user-friendly web application that can visualise genes and their transcript features across iPSC differentiation. We anticipate that these data will ultimately facilitate the identification and experimental interrogation of transcriptional regulators of disease.

From the bioinformatics point of view, single cell RNA-Seq is an interesting challenge. Since the technology is very new and rapidly evolving, many analysis tools, too, have to be invented from scratch. At Evotec, we have built our own high-performance tools to process the massive amount of raw data. We have also developed graphical tools for the interactive analysis of scRNA-Seq data sets. In these visualisations, cells with a similar expression pattern show up as a distinct cluster. Based on gene expression profiles and marker genes, cell types can be identified and labelled. Once cell populations have been analysed in one sample, machine learning helps to identify similar cell populations in other samples. For the bioinformatics team at Evotec it is exciting to see these tools applied by the stem cell teams, driving biological projects forward and enhancing sparse cell-level insights towards understanding cellular populations and disease.



Single cell RNA-Seq visualisation of an early stage of a differentiating iPSC population. Each of these more than 12,000 dots represents the transcriptome of a single cell. Thousands of transcripts of each cell have been quantified. Based on similarity of the transcriptome profiles, a specific algorithm ("tSNE") clusters cells in a two-dimensional view.
The predominantly gray cluster on the upper left contains undifferentiated precursor cells.
The red colour in the central part indicates the expression of a transient differentiation marker gene.
Green finally represents the expression of a functional marker, indicating successful differentiation.

PROTEOMICS & METABOLOMICS

CHAPTER 09

QUANTITATIVE PROFILES OF iPSCS FOR DISEASE MODELLING

Dr Christoph Schaab

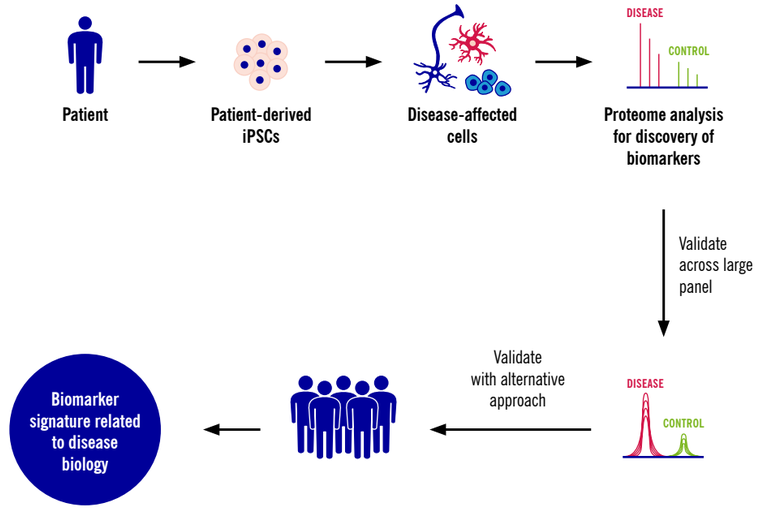
Dr Christoph Schaab joined Kinaxo Biotechnolgies, which now is part of Evotec, in 2008. He started as Head of Bioinformatics and supervised projects in the area of proteomics, biomarker discovery, and data management. Since 2018, he is globally heading the proteomics unit at Evotec and operations at the Munich site. Christoph has many years of experience in proteomics, bioinformatics, machine learning, and software and database development. From 2008 to 2017, he was affiliated with the group of Prof. Matthias Mann at the Max Planck Institute of Biochemistry. Before joining Kinaxo and Evotec, he worked as group leader at GPC Biotech, where he was responsible for statistical analysis of gene expression and screening data as well as software and database development. Christoph studied physics at the Ludwig-Maximilians University in Munich, where he received a doctoral degree in theoretical physics.

IPSC technology enables the generation of comprehensive proteomic maps representing healthy and disease cohorts. Proteomic profiles can be used for comparative analyses on how differentially expressed proteins involved in specific pathways, such as cell cycle, metabolism and DNA repair may contribute to disease. Evotec offers industry-leading technologies for unbiased protein expression and modification analyses of iPSC-derived disease models, which offer the potential to understand pathogenic mechanisms and to identify targets for future therapeutics.

The identification of suitable biomarkers plays an essential role in shifting new discoveries from bench to bedside as they provide a measurable indicator with which to evaluate pharmacological and toxicological effects in both pre-clinical and clinical settings. In general, during the pre-clinical stage of the drug discovery process, *in vitro* models are established to recapitulate human diseases by using a validated set of biomarkers. With their capability to differentiate into disease-relevant cell types, patient-derived iPSCs with either known or unknown genetic backgrounds, represent a unique human model for disease modelling. However, without highly relevant biomarkers that can be used to assess the pharmacological effects of drug candidates first *in vitro* and later on in the patient, the evaluation of a drug's proof-of-mechanism is hampered.

Proteomics analysis enables discovery of biomarkers in iPSC-based models that can be used for large-scale assessment of iPSC lines for pre-clinical drug testing and evaluation of responder and non-responder populations *in vitro*.

Same biomarkers could then be used clinically, in easily accessible body fluids, such as plasma, urine or cerebrospinal fluid, for assessing efficacy of the particular drug in clinical studies and for diagnosis or patient recruitment. Evotec has established a platform of innovative proteomics and metabolomics technologies to address key issues in translational biomarker discovery and validation in various therapeutic areas. Together with expert knowledge and data processing infrastructure for accurate quantification across many different samples, this platform defines a new benchmark enabling integrated, large-scale projects to discover and validate diagnostic, pharmacodynamic or predictive biomarkers based on patient iPSC data.



Next-generation proteomics holds great potential to unravel the unknowns in multifactorial disorders, where pathogenesis is due to altered splicing and protein modifications and further amplified by modified interconnectivity of protein complexes and signalling networks (modified from DOI: 10.1038/nrg3356).

GLOBAL PROTEOME EXPRESSION AND MODIFICATION ANALYSIS

For unbiased protein expression and modification analyses, Evotec's proteomics platform supports target discovery on the functional protein level. Depending on the application and required throughput, tailored proteome analysis workflows are available:

- High-end proteomics workflows monitor 10,000 distinct proteins, more than 20,000 phosphorylation sites, more than 8,000 ubiquitination sites, or more than 1,000 acetylation or methylation sites
- Leading expertise in quantitative mass spectrometry utilising metabolic (SILAC), chemical isotope labelling (TMT) or label-free quantification
- Bio-orthogonal chemical reporter strategies for specific profiling of newly synthesised proteins or various glycoproteins, including N- and O-linked glycoproteins, O-GlcNAc modified proteins, and sialic acid modified proteins

CHEMICAL PROTEOMICS AND TARGET IDENTIFICATION

Evotec's chemical proteomics platform employs quantitative mass spectrometry for compound selectivity analysis in the context of native proteomes and sub-proteomes. Selectivity data about cellular on- and off-target liabilities is particularly useful to inform decisions at various stages of drug development, for example lead optimisation or selection of pre-clinical candidate compounds:

- Evotec Cellular Target Profiling™ for unbiased, proteome-wide selectivity profiling to identify and quantify compound interactions with cellular on- and off-targets
- KinAffinity® as Evotec's hit-to-lead compatible approach for rapid target profiling of kinase inhibitors
- Activity-based protein profiling (ABPP) of a wide range of enzyme classes

METABOLOMICS

The team at Evotec has established state-of-the-art methodologies in targeted metabolomics to address key issues in biomarker discovery:

- Pre-clinical biomarker identification
- Disease mechanisms
- Discovery of biomarkers for patient stratification and clinical safety & efficacy assessment

Our targeted metabolomics approach focusses on the analysis of specific groups of metabolites related to certain metabolic pathways or a class of compounds including lipids (phospholipids, lysophospholipids, sphingolipids, and neutral lipids), endocannabinoids, eicosanoids, corticosteroids, neurosteroids, oxysterols, nucleotides free fatty acids, and nicotinamide metabolome. Due to its sensitivity and specificity, our approach allows absolute quantitation of metabolites using selected reaction monitoring (SRM), with detection limit of ng/mL, in sample matrices (e.g. biofluids, cells, organs).

CHAPTER 10

DISEASE MODELLING

USING iPSC-DERIVED CELLULAR MODELS FOR PATIENT STRATIFICATION

Dr Bastian Zimmer

Dr Bastian Zimmer received his PhD in cell biology and toxicology from the University of Konstanz, Germany, where he developed the first toxicological test system to detect neurodevelopmental toxicants based on human iPSCs with a functional endpoint. Bastian joined the Lorenz Studer lab at the Sloan Kettering Cancer Center in New York City, USA as a postdoc in 2013, where he developed novel differentiation protocols for sensory neurons as well as hormone producing cells of the pituitary gland. He also was one of the lead authors of a study describing cGMP protocols to derive all four major ectodermal lineages from human pluripotent stem cells. His track record in the iPSC field includes research papers in Nature, Cell Stem Cell, Nature Biotechnology, Nature Medicine and PNAS. In 2013, he was awarded a NYSTEM postdoctoral training grant from the State of New York, allowing him to work on an iPSC-based cell therapy for hypopituitarism. Bastian joined Evotec Hamburg in 2017, where he leads the Stem Cell Protocol Development Team and serves as a project leader for a drug discovery project within the Celgene neurodegeneration collaboration.

iPSCs provide new diagnostic and therapeutic tools to model diseases. However, many iPSC-derived cells represent a more immature developmental stage as compared to mature, somatic cells. This is due to the reprogramming process for iPSC generation, which involves a reset of the epigenetic landscape to an embryonic stage irrespective of the age of the original donor and creates a challenge for modelling late-onset diseases with iPSCs. Aging is an important factor towards faithfully recreating late-onset diseases *in vitro*. Attempts to simulate aspects of aging in iPSC-derived cells include prolonging time in culture or introducing agents of cellular stress by chemical or genetic means. These studies have shown age-associated changes mimicking those characterising pathological and physiological aging. In addition, studies using direct reprogramming from aged adult fibroblasts have shown that induced neurons maintain their mature epigenetic stage through the transition process and could therefore serve as alternate models to study cellular and molecular aspects of disease pathology. Evotec's research activities focus on diverse approaches to study age-related disease-features, creating 'disease-in-a-dish' models with patient-derived cells.

Such iPSC-based disease models provide patient-specific efficacy in drug screening and thus have major advantages over immortalised cell or animal-based screening methods. Currently, more than 90% of compounds fail during clinical development. During this extremely costly phase of drug development, only one third of potential new drugs make it from Phase 2 to Phase 3. In particular, the field of neurodegeneration has experienced numerous setbacks in recent years. With rigorous controls and proper study designs, patient stratification studies with iPSCs have the potential to provide unique insights into cellular phenotypes inaccessible by other means. This requires thoroughly characterised iPSC banks and standardisation of iPSC differentiation protocols to ensure inter-line consistency. With the advent of Evotec's large, validated in-house iPSC bank covering multiple disease areas and genotypes and its industrialised platform for manufacturing iPSC-derived cell-types, we have all tools established to prospectively stratify patients in so called, 'clinical trials in a dish' (CTiD). CTiD offer tremendous potential to identify responders and non-responders already relatively early in drug development. In CTiD, iPSCs from different patients within a disease group are differentiated into the cell type of interest, e.g. cortical neurons (Figure 1), and used to study the pharmacological response of a novel drug.

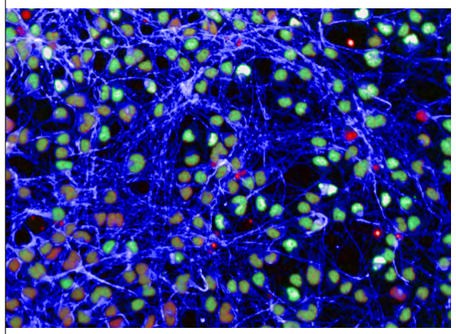


Figure 1: Cortical neurons derived from human iPSCs. Neurons are characterised by co-expression of the cortical marker TBR1 (green) and the neurite marker beta III tubulin (TUJ1, blue). Nuclei were counter-stained with DAPI (red).

The larger the number of iPSC lines included during this phase, the higher the chance of identifying disease subgroups and thereby distinguishing responders and non-responders. After completion of the iPSC-based trial, the responder cells can be used to identify responder markers (biomarkers), which can then be used for clinical study design (Figure 2). CTiD provide broad insights that cannot be obtained that early by any other model approach, as it allows testing of medical therapies for efficacy in a variety of representative human samples before moving into clinical trials.

With CTiD, Evotec's iPSC platform provides a missing link in drug discovery that will help us to identify safer compounds and reduce compound attrition rates in clinical trials. iPSC-based trials open up new avenues through access to iPSC-derived cells of numerous affected individuals that would otherwise be inaccessible, thereby revolutionising the way drug discovery is performed.

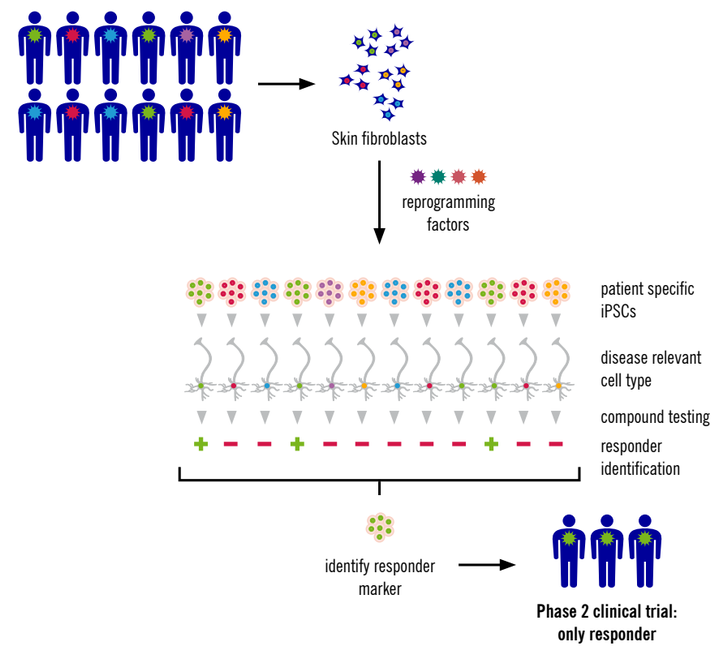


Figure 2: iPSCs generated from a diverse disease group are differentiated into disease-relevant cell types. These cell types are used to test efficacy of compounds using a disease-relevant endpoint. Based on the results, donors are stratified into responders (green +) and non-responders (red -). Based on responders, responder markers are identified. These responder markers guide patient stratification for clinical trial phase 2 recruitment.

For any further questions on Evotec's iPSC platform, please contact:

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