evotec

DDup

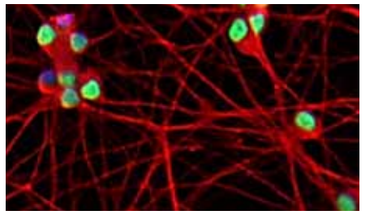
DRUG DISCOVERY UPDATES #6

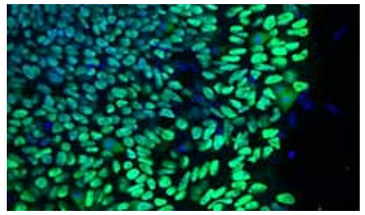
MOTOR NEURON DISEASE

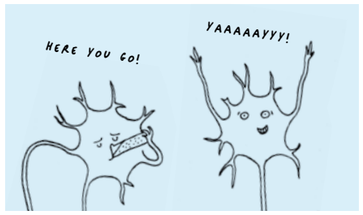
NEW OPPORTUNITIES FOR IPS CELLS IN DRUG DISCOVERY

AMYOTROPHIC LATERAL SCLEROSIS

A devastating disease without effective treatments







DR WERNER LANTHALER, CEO

Welcome to DD*up*!

DEAR FRIENDS OF EVOTEC,
the IceBucketChallenge initiated by the ALS Association generated a lot of media attention for ALS disease during the past year and world-wide awareness for ALS has clearly increased. However, sadly there is still no cure available for this devastating neurodegenerative disease. Patients and their families anxiously await much-needed effective treatments.

Evotec's scientists have accepted the ALS challenge in two ways: on the one hand through buckets of ice water and on the other hand by asking ourselves the question how we could contribute to identifying effective treatments for ALS. We are convinced that this challenging task requires a novel innovative drug discovery approach. In a strategic partnership with the Harvard Stem Cell Institute, our ambitious goal is to leverage patient-derived induced pluripotent stem cells with Evotec's drug discovery infrastructure and expertise to identify compounds that will have therapeutic value for this life threatening disease.

This 6th DDup issue highlights the details of Evotec's Cure*MotorNeuron* collaboration with Dr Lee Rubin and Dr Kevin Eggan from the Harvard Stem Cell Institute that aims to accelerate drug discovery for ALS. It is my great honour that we can include both of them as DDup interview guests.

Despite its great potential for more high throughput screenable disease-relevant models the use of pluripotent stem cell models in the drug discovery process has just begun. Together with Kevin and Lee, Evotec embarked on a systematic screening approach to identify and investigate novel mechanisms and targets for the treatment of ALS. Together with multi-scale predictive modelling, induced pluripotent stem cells will not only enable insights into disease mechanisms but provide drug screening paradigms that more faithfully recapitulate human disease biology.

Overall, we believe that induced pluripotent stem cell technology holds great promise for disease modelling and provides an invaluable tool for understanding the molecular events at the heart of ALS pathogenesis and other CNS diseases as well as many other diseases where relevant human disease models are in short supply.

I hope you enjoy browsing through this month's issue and let yourself be infected by our enthusiasm about induced pluripotent stem cell-based drug screening as a potential game changer for future medicine. Please do not hesitate to contact us should you have any questions.

Yours sincerely
Werner Lanthaler

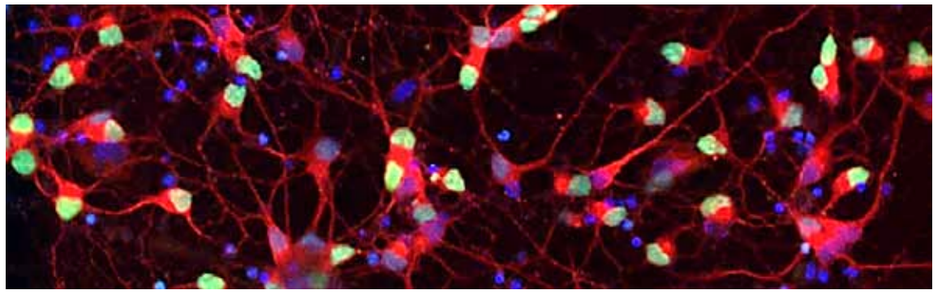
Motor Neuron Diseases

Motor neuron diseases result from the progressive degeneration and death of motor neurons. The two most studied motor neuron diseases are adult-onset Amyotrophic lateral sclerosis (ALS) and childhood-onset spinal muscular atrophy (SMA). Both diseases involve neuromuscular dysfunction and eventually result in fatal paralysis. ALS is the most prevalent late-onset motor-neuron degeneration disorder worldwide and SMA is the leading genetic cause of infant mortality. In contrast to SMA, which is characterised by loss of spinal motor neurons, ALS affects both cortical and spinal motor neurons. For both diseases, ALS and SMA there are no effective treatments available.

AMYOTROPHIC LATERAL SCLEROSIS

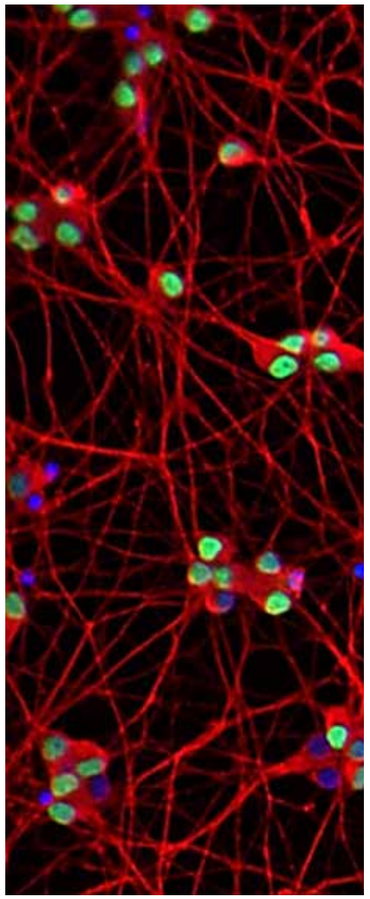
ALS, also known as Lou Gehrig's disease, is a rapidly progressive, neurodegenerative disorder that ultimately causes paralysis and premature death. The underlying causes remain uncertain, but appear to be multifactorial, including genetic and environmental causes. Approximately 10% of ALS cases are classified as familial, leaving the majority of cases to be considered sporadic in origin. Familial ALS cases are inherited in a dominant manner and involve mutations in about 20 genes of which superoxide dismutase 1 (SOD1), TAR DNA-binding protein-43 (TDP-43), fused-in-sarcoma (FUS), and C9orf72 are the most common. Importantly, all genes associated with familial ALS have also been found mutated in sporadic ALS. The first gene found to be associated with ALS, Cu/Zn SOD1, is responsible for 20% of familial ALS and has been the focus of much research, including the generation of the first genetically modified rodent models replicating the human disease.

The biology of ALS is very complex. It primarily affects upper and lower motor neurons that reach from the brain to the spinal cord and from the spinal cord to the muscles. The molecular mechanisms underlying the progressive loss of motor neurons are only partially known. Studies of post-mortem patient tissue and animal models have revealed the presence of intracellular aggregates in many cases of ALS associated with gene mutations, including SOD1, TDP43 and FUS. The contribution of these protein aggregates to disease pathology remains elusive, i.e. it is unclear whether protein aggregation is a cause or consequence of these molecular malfunctions in ALS.



Motor neurons derived from human induced pluripotent stem cells characterised by co-expression of Islet 1 (green) and the dendritic marker MAP2 (red). Nuclei were counterstained with DRAQ5 (blue). Source: Evotec

Motor neurons derived from human induced pluripotent stem cells characterised by co-expression of Islet 1 (green) and the neurite marker beta III tubulin (red). Nuclei were counterstained with DRAQ5 (blue). Source: Evotec



ALS remains one of the most devastating and incurable neurological diseases. Although appearing to be relatively rare, as most individuals survive from diagnosis to death by only two or three years, the incidence is around two per 100,000. Multiple pathogenic mechanisms have been proposed to contribute to the selective motor neuron degeneration, including alterations in RNA metabolism, mitochondrial dysfunction, abnormal protein aggregation, endoplasmatic reticulum stress, excitotoxicity, axonal transport defects and gliosis. However, despite extensive research, ALS remains incurable with limited therapeutic options. A number of drugs have been tested for this disease and several are in clinical trials. Rilutek is the only approved drug shown to have a positive impact in ALS patients, however its effects on ALS disease progression are limited. In the absence of any curative treatment, and a poor understanding of the disease pathogenesis, it is critical to establish predictive translational models and to enhance ALS drug development efforts.

SPINAL MUSCULAR ATROPHY

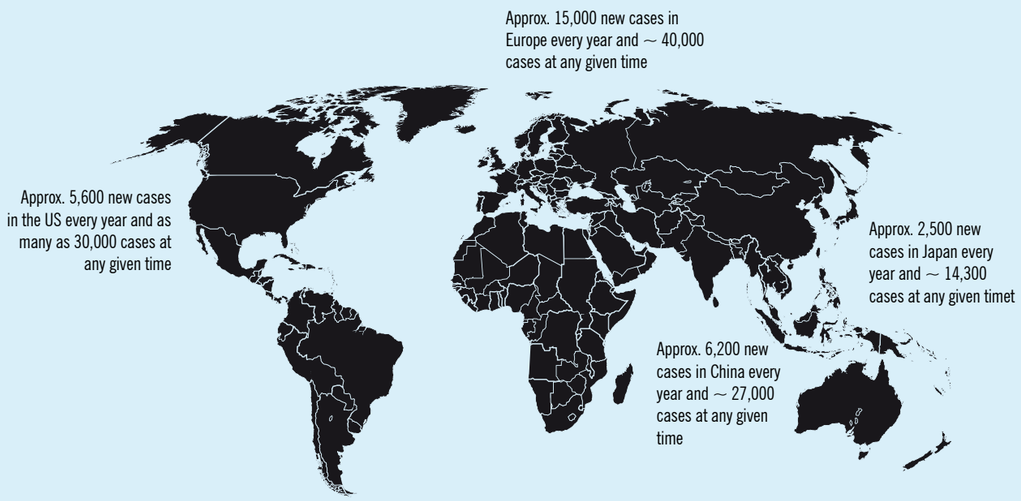
SMA is an autosomal recessive, monogenic disease characterised by the loss of the survival motor neuron (SMN) gene function. There are two SMN genes in humans, SMN1 and SMN2, and the vast majority of SMA cases are caused by loss-of-function mutations in the ubiquitously expressed SMN1 gene. While SMA patients lack a functional SMN1 gene, they do have an almost identical SMN2 gene. The SMN2 gene differs from SMN1 by a single nucleotide change causing a change in splicing of exon 7. As a consequence, only 5-10% of SMN2 RNA is correctly spliced and yields a functional full length SMN protein. Incorrectly, spliced SMN2 RNA produces a truncated, rapidly degraded form of SMN protein. Disease severity is strongly determined by SMN2 copy number. The more copies of SMN2 gene people with SMA carry, the more SMN protein they produce and the less severe the disease. Therapeutics capable of elevating SMN levels are currently being tested for efficacy in treating SMA in the clinic. It remains uncertain how deficiency in SMN, a ubiquitously expressed protein, causes selective loss of motor neurons.

FACTS & FIGURES

THE MOST PROMINENT MOTOR-NEURON DISEASE IS AMYOTROPHIC LATERAL SCLEROSIS (ALS), ALSO KNOWN AS LOU GEHRIG`S DISEASE. OTHERS ARE:

- Spinal muscular atrophy
- Primary lateral sclerosis
- Progressive muscular atrophy
- Progressive bulbar palsy
- Pseudobulbar palsy

ALS – GLOBAL INCIDENCE AND PREVALENCE RATES



The incidence rates of ALS range from 1.7 to 2.3 per 100,000 population over the world, and the prevalence rates between 4 to 6 per 100,000 population

- Most people who develop ALS are between the ages of 40 and 70, with an average age of 55 at the time of diagnosis. Disease can also occur at a younger age.
- ALS is 20% more common in men than in women. However with increasing age, the incidence of ALS is more equal between men and women.
- ALS occurs throughout the world with no racial, ethnic, or socioeconomic boundaries.

SPORADIC VS FAMILIAL

According to the US NIH, in 90 to 95% of all ALS cases, the disease occurs with no clearly associated risk factors. Individuals with this sporadic form of ALS do not have a family history of ALS, and their family members are not considered to be at increased risk for developing it.

Only 5 to 10% of all cases are inherited. This familial form of ALS requires just one parent to carry the gene responsible for the disease. Mutations in more than a dozen genes have been found to cause familial ALS. The most prominent gene that is known to cause ALS is commonly called SOD1. SOD1 causes about 20% of all familial cases, which means about 1-2% of all ALS cases.

PREVALENCE OF FAMILIAL ALS GENE MUTATIONS

C9orf72	30%-40% in USA and Europe
SOD1	20% worldwide
TDP43	5% worldwide
FUS	5% worldwide
ANG	1% worldwide

Mutations in several genes cause familial ALS, such as the hexanucleotide repeat expansion in C9orf72 (chromosome 9 open reading frame 72), or mutations in SOD1 (superoxide dismutase 1), TDP43 (transactive response DNA binding protein 43 kDa), FUS (RNA binding protein Fused in Sarcoma) and ANG (angiogenin). Prevalence of familial ALS mutations is different, with C9orf72 and SOD1 being the more most prominent.
Source: http://ghr.nlm.nih.gov/condition/amyotrophic-lateral-sclerosis
http://www.alsa.org/research/about-als-research/genetics-of-als.html

- **AVERAGE LIFE EXPECTANCY OF DIAGNOSED ALS PATIENTS IS BETWEEN 2-5 YEARS**
- **ONE MARKETED DRUG (RILUTEK) HAS LIMITED EFFICACY EXPANDING LIFE EXPECTANCY BY UP TO THREE MONTH**

UNMET MEDICAL NEED

- There is no cure for ALS, diagnosis is difficult, and the major problem of developing more effective drugs is the still unknown etiology of ALS
- There is only one approved and marketed drug (Rilutek) available, associated with very limited efficacy and less favourable safety profile
- The life expectancy of an ALS patient averages about two to five years from the time of diagnosis, but more than half of all patients live more than three years after diagnosis
- About 20% of people with ALS live five years or more and up to 10% will survive more than ten years and 5% will live 20 years

Induced pluripotent stem cells

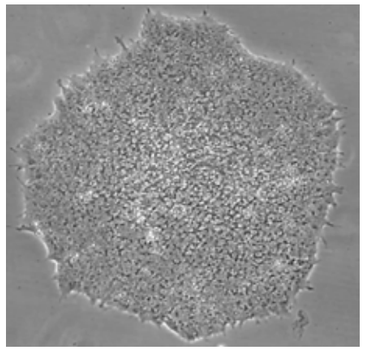
A PARADIGM SHIFT IN DRUG DISCOVERY

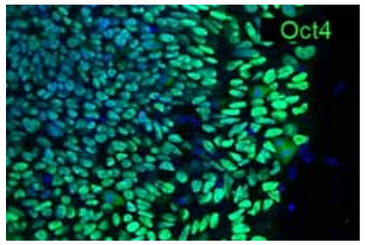
Considerable efforts and resources have been spent on drug discovery programmes for motor neuron disorders. However, most drug candidates were withdrawn at various stages of the discovery and development process for reasons such as poor ADME properties, safety issues and in particular lack of efficacy in the clinic.

For decades the drug discovery paradigm in neurodegenerative disease has relied on animal disease models. Meanwhile, mounting evidences suggest that many of these models are not predictive for human disease and have contributed to failure in clinical trials. Capturing the biological complexity of the disease state in a human model system might result in better clinical translation. However, availability of affected patient tissues is limited. Biopsy of live neurological tissue is highly invasive and presents considerable risk without ascertainable benefit to the patient. Owing to the poor expansion and survival of primary human cells their use in disease modelling and drug screening has been extremely limited.

The discovery of iPS cells by Nobel Prize laureate Shinya Yamanaka in 2007 provides a new source of patient derived cells for human disease modelling and has facilitated research on a wide range of disorders, including many rare diseases. Patient-specific human iPS cells are derived from postnatal somatic cells through transient ectopic expression of pluripotency associated transcription factors. These factors establish an epigenetic state able to maintain unlimited proliferation capacity in the undifferentiated stage while retaining the ability to differentiate into any somatic cell type under the appropriate culture conditions. iPS cells are scalable and make it possible to analyse cells from patients and to use them for high-volume screening of new candidate drugs.

Brightfield image of induced pluripotent stem cell colony grown under feederfree conditions. Source: Evotec





Undifferentiated induced pluripotent stem cells expressing pluripotency marker Oct4 (green). Nuclei were counterstained with DRAQ5 (blue). Source: Evotec

Disease modelling

USING PATIENT-DERIVED IPS CELLS

One of the major obstacles in studying neurodegenerative diseases is the difficulty in obtaining relevant cell types for analysis. The differentiation of neural cell populations from pluripotent stem cells presents an exciting opportunity to obtain large numbers of human neuronal cell types for disease modelling and drug screening. However, it is of prime importance that quality standards for disease modelling and drug screening efforts are being defined.

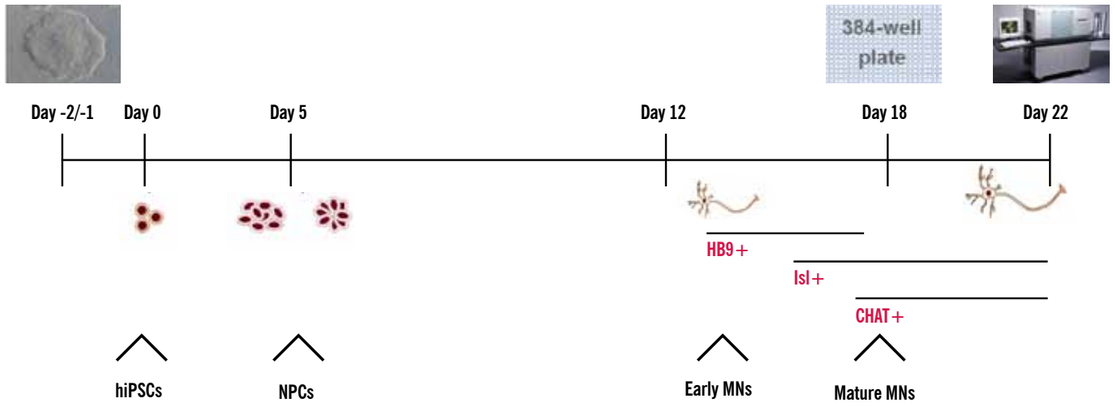
To realise disease modelling and drug discovery based on *in vitro* differentiated, patient-specific iPS cell-derived cells it is necessary to develop robust, reproducible, and relevant assays with reasonable throughput. While these characteristics are essential to all small molecule screening assays, the use of patient-specific, stem cell-derived cells presents unique challenges. For example, reports have suggested that the method of re-programming can affect the differentiation potential of iPS cells. As a result non-integrating re-programming methods (modified mRNA, Sendai virus, episomal vectors, small molecules, proteins, etc.) with higher efficiency at establishing a pluripotent state have been developed and have rapidly become the standard. Furthermore, thorough evaluation of iPS cells to ensure high quality before use is essential. The minimum characterisation of any patient-derived iPS cell line should include karyotype analysis, pluripotency marker expression, pathogen testing, evaluation of *in vitro* differentiation potential (i.e. Pluritest™, Scorecard™), comparison with original material (i.e. comparative genome hybridisation) and whole exome/genome sequencing. In addition to iPS cell validation, optimisation of *in vitro* differentiation protocols is required to achieve similar robustness and reproducibility as with traditional cell lines for screening campaigns. This includes use of defined media conditions using small molecule modulators of key developmental pathways to reduce heterogeneity and to improve yield of specific cell types. Furthermore, adaptation and scale up to HTS assay formats (96-, 384- or 1,536-well plates) is required to ensure cost efficient utilisation of cells, reagents and labour.

Evotec's stem cell scientist, Carolin Obieglo, working for the CureMotorNeuron team.

Protocol adaptation and optimisation is required for iPS cell-based HTS

Motor neuron differentiation protocols from induced pluripotent stem cells follow principles of normal embryonic development. It starts with derivation of early neuroectoderm by dual SMAD inhibition using small molecules to inhibit transforming growth factor β (TGFβ) and bone morphogenic protein (BMP) signalling.

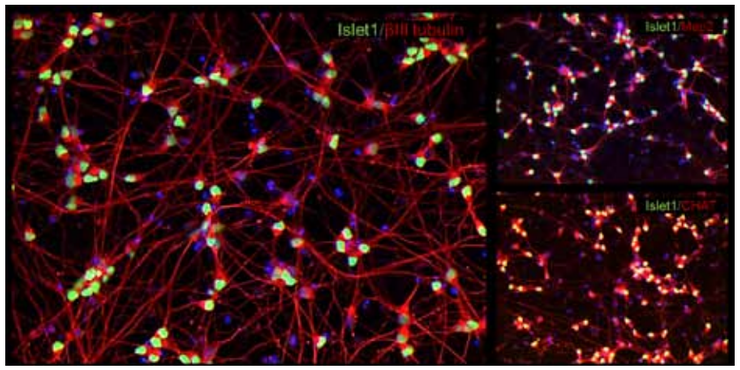
Addition of retinoic acid steers differentiation towards caudal cell types of the spinal cord, and activation of the sonic hedgehog pathway initiates development of the neural stem cells towards ventral motor lineages. For classification of *in vitro*-derived motor neurons, unipolar neuronal morphology as well as expression of at least a subset of motor neuron markers represents a minimal requirement. Neuronal morphology can be additionally assessed by immunostaining for cytoskeletal proteins, such as beta III tubulin and Map2. Whereas beta III tubulin is present in both axons and dendrites, Map2 expression is limited to dendrites. Motor neuron identity and maturation can be evaluated based on a number of parameters: 1. expression of specific markers, such as transcription factors homeobox gene HB9 or Islet1 (Isl1), and choline acetyltransferase (CHAT), 2. morphology of cell body and neurites, and 3. electrophysiological recording of neural excitability and firing patterns.



Timeline for directed differentiation of human iPS cells into motor neurons as applied by Evotec. Expression of motor neuron marker HB9 (homeobox 9) precedes later markers, Isl1 (Islet1) and CHAT (choline acetyltransferase); hiPSCs, human induced pluripotent stem cells, NPCs, neural progenitor cells; MNs, motor neurons

ALS disease modelling using patient-derived iPS cells

iPS cell disease modelling of late-onset neurological disorders is still in its infancy but is advancing at a rapid pace. The key enabling factors in cellular disease modelling are as follows: 1. efficient differentiation of iPS cells into cell types impacted by the disease, 2. detection of genotype-associated disease phenotypes, and 3. confirmation of disease phenotypes with genetic rescue experiments. Recent advances in iPS cell technology provide new opportunities which may overcome some of the challenges associated with disease modelling and drug screening. For example, genetic aberrations of iPS cells or lack of well-defined isogenic controls have been addressed through development of non-integrating re-programming methods or generation of isogenic control lines via gene editing technologies (such as Zinc Finger Nucleases (ZFN), Transcription Activator-Like Effector Nucleases (TALEN) or Clustered Regularly Interspaced Short Palindromic Repeat technology (CRISPR)), respectively.



Motor neuron networks in 384-well format: Induced pluripotent stem cell-derived motor neurons at day 22 of in vitro differentiation. Motor neurons are characterised by co-expression of characteristic markers, such as Islet1 or choline acetyltransferase (CHAT) with pan-neuronal markers, such as β-III tubulin or microtubule associated protein 2 (Map2). Nuclei are counterstained with DRAQ5 (in blue). Source: Evotec

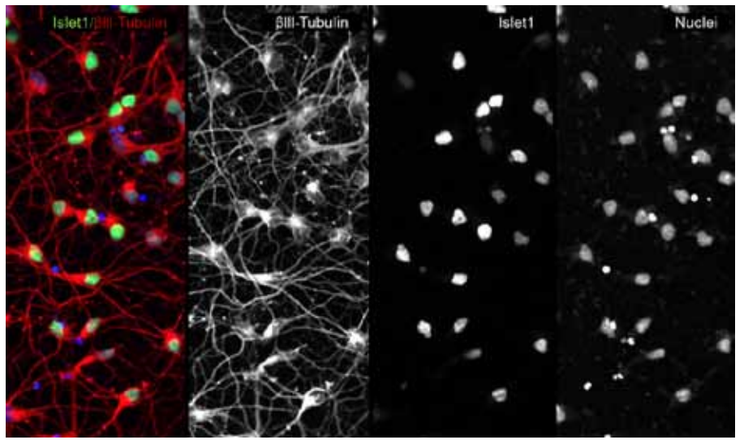
As directed differentiation protocols produce embryonic neuronal cell types that need to mature to become functional neurons, it can be expected that embryonic cells derived from patients with adult-onset neurodegenerative diseases lack clear phenotypic signs of neurodegeneration usually observed in aged neurons. Therefore, a lot of efforts are undertaken to enhance the pathophysiological processes in the culture systems using various stimuli, stressors or providing specific-cell-cell interactions.

First attempts of disease modelling utilising iPS cell-derived motor neuron from ALS patients carrying i.e. mutations in SOD1 and TDP43 have revealed encouraging results. Eggan and colleagues reported that human mutant SOD1 motor neurons showed increased oxidative stress levels, reduced mitochondrial function, altered subcellular transport, and activation of the ER stress and the unfolded protein response pathways as previously reported in ALS (Kiskinis et al, 2014). Moreover, mutant SOD1 motor neurons recapitulated a hyperexcitability phenotype that is detected by clinical neurophysiological studies in ALS patients. Importantly, motor neurons produced from a genetically corrected but otherwise isogenic SOD1 stem cell line did not display hyperexcitability (Waigner et al., 2014). In recent studies, Chen et al. linked the reduction in soma size and altered dendrites of iPS cell-derived SOD1 motor neurons to the deregulation and aggregation of neurofilaments, which ultimately led to neuronal apoptosis. It is important to note that although mutant SOD1 aggregation is thought to be a key molecular event driving neurotoxicity, studies by both Kiskinis and Chen et al. have shown that mutant SOD1 aggregation levels *in vitro* were extremely low and were only detected after inhibiting the proteasome or by extremely sensitive methods (electron microscopy). In contrast to SOD1, Burkhardt et al. developed a model of ALS using TDP43 iPS cell-derived motor neurons and showed distinct *de novo* TDP43 intra-nuclear inclusions on a subgroup of clones (Burkhardt et al, 2013). Overall, studies using iPS cell-derived motor neurons from patients harbouring distinct ALS mutations have recapitulated essential disease features making them a useful tool for *in vitro* drug screenings.

Multi-dimensional biology approach for understanding neurodegenerative disorders

The enormous amount of panomic data that have been generated to characterize human neurodegenerative diseases, such as ALS, can be integrated in order to build predictive network models of normal and disease states. This may help elucidate the key biological drivers of the disease state. Multimodal models cover genome, epigenome, transciptome and proteome data and can be used to organise disease signatures according to the subnetworks (and the biological processes that they define) which are associated with that disease. Multiscale models of disease can therefore be used to elucidate disease mechanism and stratify patient populations.

Towards more predictive models in ALS

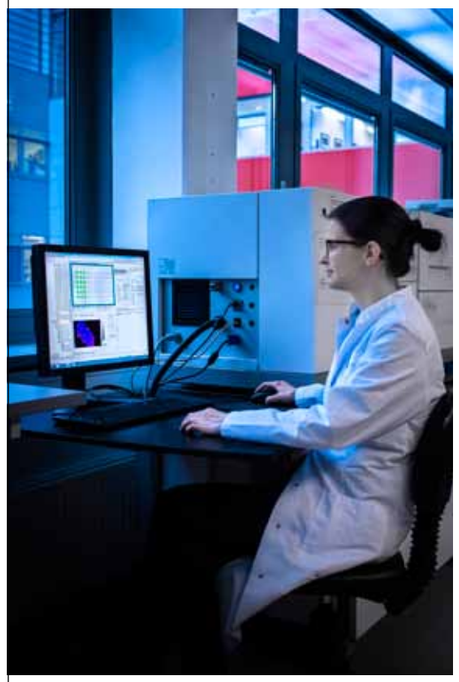


Induced pluripotent stem cell-derived motor neurons from SOD1 patient. Motor neurons are identified by co-expression of Islet 1 (green) and neurite marker βIII tubulin (red). Nuclei were counterstained with DRAQ5 (blue). Source: Evotec

High throughput screening in iPS cell-derived neurons with the intent of identifying novel therapeutic compounds has the potential to transform the way drug discovery is performed.

As an attempt for 'preclinical testing in a dish' Rubin and colleagues recently reported a screen of 5,000 small molecule compounds in stem cell-derived motor neurons from both wild type and mutant SOD1 mouse embryonic stem cells (Yang et al., 2013). Among other hits, they identified the non-selective kinase inhibitor Kenpaullone which strongly promoted survival from growth factor withdrawal in motor neurons derived from mouse stem cells with SOD1 mutation as well as motor neurons derived from patient-specific iPS cells harbouring SOD1 or TDP43 mutations.

By way of additional *in vitro* validation work the authors presented evidence that Kenpaullone's neuroprotective effects are possibly mediated via dual inhibition of GSK-3 alpha/beta and HPK1/GCK-like kinase (HGK; also known as MAP4K4), a kinase upstream in the phospho-c-Jun-mediated neuronal apoptosis pathway. While to our best knowledge there are no clinical data on motor neurons diseases available for Kenpaullone, Rubin and colleagues compared the neuroprotective actions with those of two non-related compounds from clinical trials on ALS: 1. olesoxime and 2. dexpramipexole. Both compounds showed efficacy in rodent models of ALS but subsequently failed in clinical trials for ALS. Dexpramipexole, a compound which had previously been shown to improve mitochondrial function and to confer significant cellular protection in neurons under stress, failed in an ALS phase III trial led by BiogenIdec. Olesoxime, the lead compound of Trophos' proprietary cholesterol-oxime compound family of mitochondrial pore modulators failed to demonstrate a significant increase in survival versus placebo in an ALS Phase III trial. Intriguingly, both olesoxime and dexpramipexole also failed to rescue the death of motor neurons carrying human SOD1 mutations in the stem cell-based model system developed by Rubin and colleagues. This finding provides an example of 'preclinical testing in a dish', suggesting that such preliminary screening steps could accelerate drug discovery by excluding ineffective compounds before they proceed into preclinical and clinical testing (Kim and Lee, 2013).



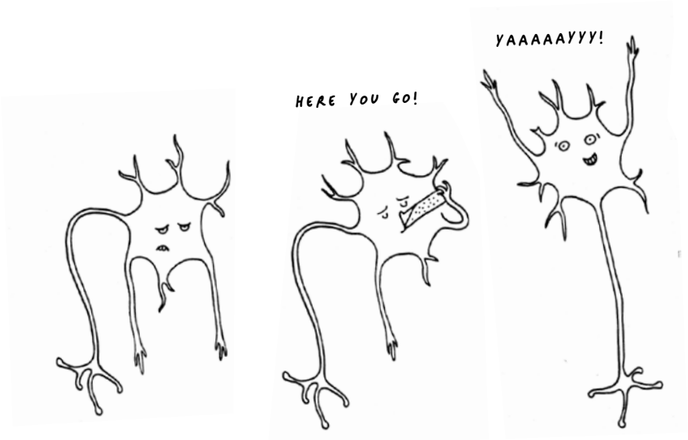
Evotec's stem cell scientist, Mareen Glausch, analysing iPS cell-derived motor neurons on the OPERA high-content screening system.

The great hope is that stem cell-based screening will allow for the identification of clinically relevant targets and mechanisms prior to moving into animal models of disease for proof of efficacy. Initial screens suggest that new mechanisms can actually be identified while excluding those that have proven unsuccessful in clinical trials despite efficacy in animal models. Compounds from recent drug screening efforts using patient-specific iPS cells have not yet been tested *in vivo*, so it will be interesting to see whether hit compounds identified via stem cell-based screening approaches exhibit positive results in animal models, as well as clinical trials, in the future.

Most trials that fail do so for two main reasons: 1. lack of efficacy in the selected patient cohort, and 2. adverse effects and safety concerns. A preclinical, disease-relevant, human pharmacology model that identifies, optimises and selects drug candidates could mitigate these risks. Human iPS cell models have the potential to enable patient stratification *in vitro* through so called 'clinical trials in a dish', which might allow us to predict how individual patients will respond to specific drugs.

EARLY PATIENT STRATIFICATION THROUGH 'CLINICAL TRIAL IN A DISH'

Studying an array of different familial and sporadic mutations simultaneously is often referred to as '*in vitro* clinical trial' or 'clinical trial in a dish'. Studying the disease in patient-derived cells offers a unique opportunity to obtain more insight into the underlying molecular pathomechanisms and to discover disease-modifying treatments for complex genetic diseases like ALS. Such *in vitro* trials with patient cells are expected to be more relevant and predictive than currently used cell models. Furthermore, studying a panel of familial and sporadic disease-specific mutations might enable to identify common mechanism treatment across a panel of familial and sporadic disease-specific mutations and could facilitate patient stratification in clinical trials. With this, stem cell-based human models could help bridging the gap between preclinical research and clinical development and facilitate understanding of human disease and the development of drugs for currently incurable diseases.



CureMotorNeuron

HARVARD AND EVOTEC COLLABORATE TO SYSTEMATICALLY SCREEN MOTOR NEURONS FROM ALS PATIENT IPS CELLS

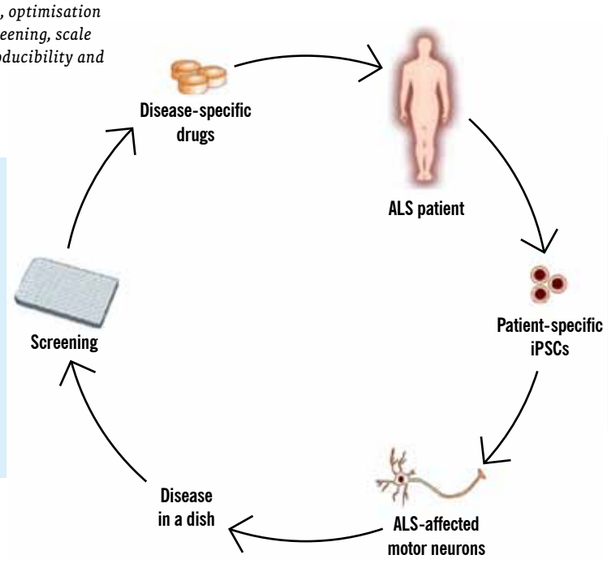
The Harvard Stem Cell Institute ("HSCI") is a unique collaborative organisation that brings together more than 1,000 scientists in its affiliated hospitals and institutes, dedicated to advancing stem cell biology and to develop new treatments and cures for disease. HSCI is home to one of the largest concentrations of stem cell scientists in the world, including many of the leaders in the field. Dr Kevin Eggan and Dr Lee Rubin, both Principal Faculty members and professors in the Department of Stem Cell and Regenerative Biology at HSCI, are leaders in ALS and SMA research and have been instrumental in developing motor neuron assays based on patient-derived induced pluripotent stem cells. With the Cure*MotorNeuron* collaboration, Evotec, HSCI and Harvard share a commitment to accelerating promising ALS research from the lab to the clinic.

The high unmet medical need for ALS disease is compounded by clinical heterogeneity, lack of robustly predictive *in vitro/in vivo* disease models and limited understanding of the molecular mechanisms of disease pathogenesis. CureMotorNeuron aims to identify compounds that can prevent or slow the loss of motor neurons that occurs with the progression of ALS.

The collaboration leverages human motor neuron assays based on ALS patient-derived induced pluripotent stem (iPS) cells that were developed by Dr Lee Rubin and Dr Kevin Eggan at Harvard, as well as Evotec's leading drug discovery infrastructure and expertise to identify compounds that will have therapeutic value against this life-threatening disease. This novel phenotypic screening approach involves a panel of well characterised human induced pluripotent stem cell lines both from familial and sporadic ALS patients as basic models of disease.

CureMotorNeuron - a collaboration between Evotec and Harvard. Harvard provides iPS cells from ALS patients, motor neuron differentiation protocols and readouts for phenotypic screening. Evotec covers banking of iPS cells according to internal quality control standards, optimisation of differentiation protocols for screening, scale up to 384-well format, assay reproducibility and robustness, and screening.

EVOTEC
- Cell banking & quality control
- Optimisation of differentiation protocols
- Scale up for screening
- Optimisation of readouts for high-content imaging
- Drug discovery



HARVARD
- Generation of iPS cell lines from ALS patients
- Motor neuron differentiation protocols
- Development of phenotypic readouts for screening

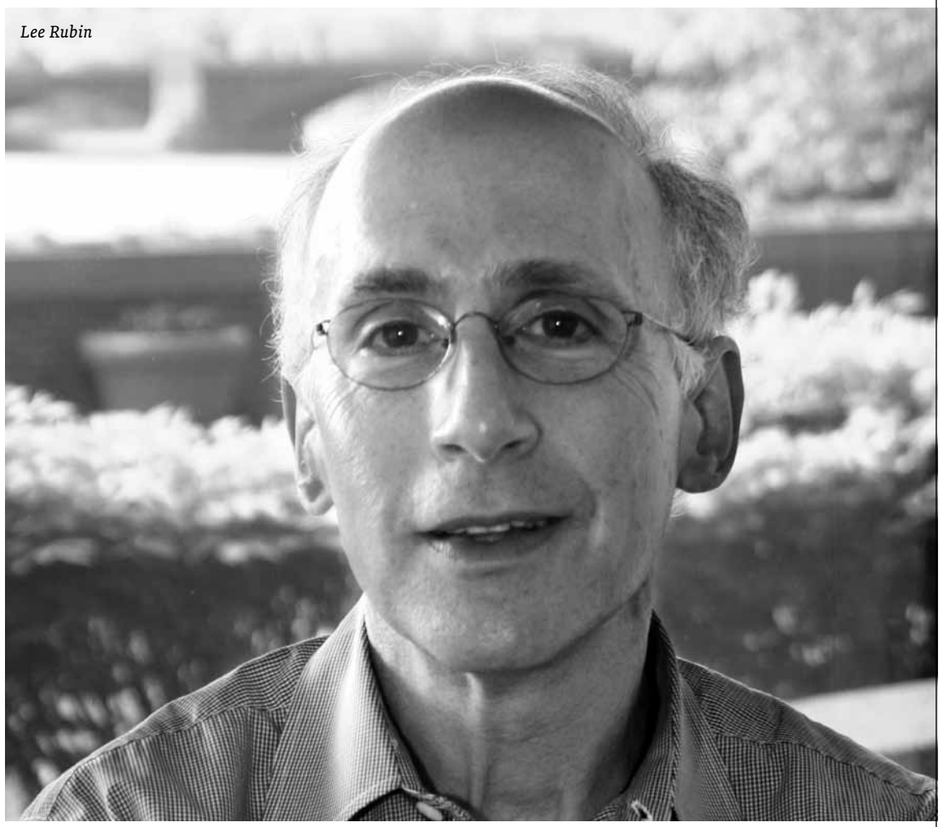
Scale up to 384-well format is essential for cost effective screening

Complex screening protocols that involve extended culturing and differentiation procedures as well as high-content readouts often prove very costly and require adaptation to higher throughput formats in order to stay cost effective. For this, we have scaled up the motor neuron differentiation protocol to 384-well format and optimised it for robustness and reproducibility. As an initial parameter for screening, phenotypic analysis will focus on an ER stress-related phenotypic readout in iPS cell-derived motor neurons that was established in collaboration with Kevin Eggan and Lee Rubin at HSCI. Post mortem data from ALS patients has revealed elevated levels of ER stress markers. Protein misfolding in mutant SOD1 was reported to cause ER stress in various *in vitro* and *in vivo* models. ER stress leads to up-regulation of targets of the unfolded protein response and disturbance of ER proteostasis and has been described for many neurodegenerative disorders such as Amyotrophic lateral sclerosis, but also Alzheimer's disease, Parkinson's disease and Huntington's disease. Thus, targeting the unfolded protein response is an attractive strategy to identify ALS disease-relevant mechanisms and compounds. In addition, we are progressing towards further disease modelling focusing on disease-relevant mechanisms. Our immediate goal is to systematically screen for new mechanisms, targets and compounds that have therapeutic value for ALS and potentially other motor neuron diseases.

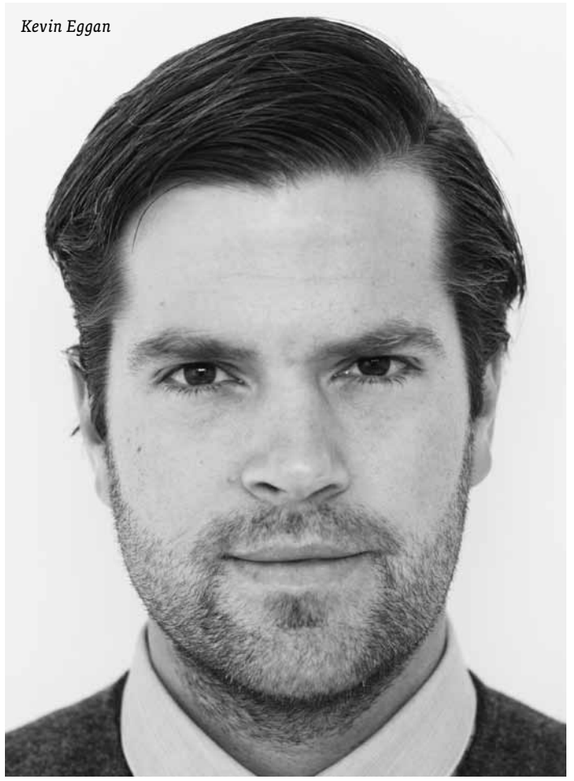
Evotec's motor neuron research team helped raise awareness for ALS disease. In August 2014, Evotec scientists and members of the management board took on the ALS IceBucketChallenge and collected donations for the ALS Association.

IN PURSUIT OF A

new drug discovery paradigm



Lee Rubin



Kevin Eggan

Dr Lee Rubin and Dr Kevin Eggan are both professors in the Department of Stem Cell & Regenerative Biology at Harvard University.

Dr Lee Rubin received his Ph.D. in Neuroscience from The Rockefeller University and completed postdoctoral fellowships in Pharmacology from Harvard Medical School and in Neurobiology from Stanford University School of Medicine. He has a broad experience in both academia and industry, particularly in the realms of cell-based assays and drug discovery. Prior to coming to Harvard, he was Chief Scientific Officer of Curis, Inc., a Cambridge-based biotechnology company, where his group identified the first small molecule regulators of the hedgehog signalling pathway. At Harvard, much of his work is focused on finding key molecular mediators of different neurodegenerative diseases and on searching for effective preclinical therapeutic candidates. His group's research takes advantage of their ability to produce large numbers of patient-derived induced pluripotent stem cell lines and of effective means of deriving large numbers of differentiated neurons from them. They have set up an array of techniques that allow them to identify early cellular and physiological changes in neurons as they become diseased. For example, they have identified new targets for the treatment of the motor neuron disorders Spinal Muscular Atrophy and Amyotrophic lateral sclerosis.

Dr Kevin Eggan received his Ph.D. in Biology from the Massachusetts Institute of Technology in February of 2003. In September 2003, Dr Eggan came to Harvard University as a Junior Fellow in the Harvard Society of Fellows. In 2012, he became a tenured Professor of Stem Cell and Regenerative Biology. As a young investigator in the burgeoning field of stem cell biology, Dr Eggan has garnered international recognition for his seminal work and a number of high profile awards for his creativity and productivity, including the MacArthur Foundation "Genius Grant" in 2006. He has made fundamental contributions to the fields of stem cell biology and cellular re-programming which in turn led his group to pioneer an entirely new strategy for studying human disease. While training, Dr Eggan performed nuclear transfer studies that challenged preconceived notions concerning the limits of cellular plasticity. His lab then became the first to demonstrate that human somatic cells could be reprogrammed to an embryonic stem (ES) cell state. This demonstration that human ES cells harboured re-programming activities has been cited as an inspiration for the discovery of factors used to generate induced pluripotent stem cells (iPSCs). Through persistent re-programming attempts his lab became the first to generate patient-specific iPSCs and use them to produce the cell type that degenerated in that individual.

5 MINUTES WITH

Lee Rubin & Kevin Eggan

ON IPS CELL TECHNOLOGY AND ALS DISEASE

1 Cord Dohrmann: The use of iPS cell technology in drug discovery holds great promise. Could you summarise where you see the greatest potential but also the greatest challenges?

LR: iPS cells may provide unique insights into the development of human disease under controlled conditions and the ability to match drugs to individual patients (i.e. personalised medicine). The biggest challenge is to understand how to generate differentiated cells that behave as if they are in true human diseased tissue.

> "The availability of iPS cells from patients with ALS may be the start of a new generation of more effective treatments."

KE: Moreover, the ability to make limitless quantities of any cell type of the body is really transformative. Given that cell signalling pathways display dizzying variation from cell type to cell type, being able to screen on drug targets as they act in these pathways in disease affected cell types will likely improve outcomes as compounds move forward from screening towards animal models and into the clinic. The challenge remains making large scale populations of cells for screening, but this is improving as progress in the Harvard/Evotec collaboration demonstrates.

2 CD: Currently treatment options for ALS are very limited. In your opinion where do we stand today and what are the most promising new ideas, options and strategies for developing therapies that will hold significant benefits for patients afflicted by this devastating disease?

KE: Today, we are standing on the cusp of enormous opportunity for ALS drug discovery created by really remarkable advances in genetic understanding of the condition. This landslide of genetic information piling up is providing a host of new exciting drug targets. The stem cell-derived motor neurons we are producing provide a rapid way to parse through these many targets.

LR: Much of the disappointment in ALS drug development may be attributed to the lack of human testing systems. The availability of iPS cells from patients with ALS may be the start of a new generation of more effective treatments.

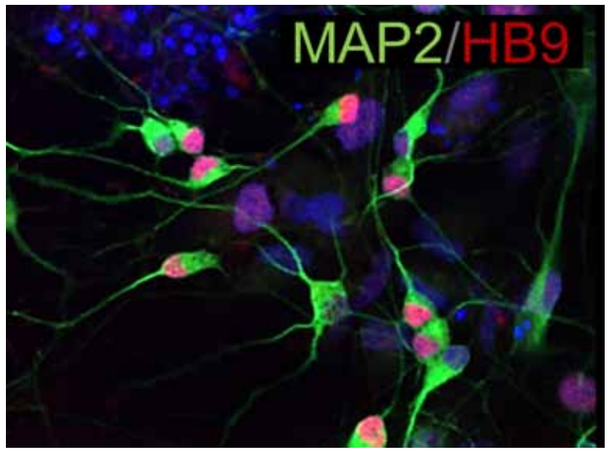
3 CD: Both of your labs have made significant contributions to our understanding of the underlying pathology of motor neuron diseases. Where do you see the biggest advantage of such a 'disease in a dish' model?

KE: To me the main advantage is to be able to test drug candidates across many genetic forms of ALS, this will allow us to eliminate the concern

> "The challenge remains making large scale populations of cells for screening"

that sub-types of patients won't respond to the drug in clinical trials.

LR: I agree. Such an approach will allow us to divide ALS into therapeutic categories: how many types of ALS are there? How can patients best be grouped: By mutation? By age of onset? Or by rate of progression? This will allow for the development of less expensive and more effective treatments. A disease-in-dish model provides insights into a disease from its earliest stages, when cells are still alive and healthy, this may allow us to discover better drug targets.



Motor neurons derived from human induced pluripotent stem cells characterised by co-expression of homeobox gene HB9 (green) and the dendritic marker MAP2 (red). Nuclei were counterstained with DRAQ5 (blue). Source: Evotec

4 CD: Talking about *in vitro* models, human disease is often a combination of inherited genetics and the environment, which manifests through epigenetics. To what extent could this be replicated in a dish?

KE: Stem cells allow us to replay development over and over again with a single genotype and therefore are a perfect way to study the interactions between genes and the environment. It allows one to test how chemicals that might be environmental components to disease are involved in pathogenesis.

LR: I must admit that to me this is not clear at the moment. However, I do believe it will be possible to mimic environmental factors that may act epigenetically.

5 CD: How important are currently available ALS mouse models? What will change, if human neuron based disease-in-a-dish models will deliver novel mechanisms, targets and compounds?

LR: The standard ALS mouse model appears to be poor at predicting human response to individual therapeutics, at least when considered from the perspective of the entire set of ALS patients. Perhaps it's good at predicting the response of patients with SOD mutations. New models are appearing that may also be better, but, in any case, it is vital to know if potential drugs are active on human target cells.

KE: I think, excitingly, that stem cell-derived motor neurons are already emerging as a credible source of drug targets. I think that over the coming years, new mouse models and stem cell models will go hand in hand.

> "Stem cell-derived motor neurons are already emerging as a credible source of drug targets. "

6 CD: Industrialisation of iPS cell culture could lead to widespread use of this technology in a personalised medicine context. This could include the use of differentiated cells from individual patients for testing of drug toxicity and efficacy. From your perspective what is the ultimate potential of the concept of conducting 'clinical trials in a dish'?

LR: Designing clinical trials around the idea of enrolling only patients who are most likely to respond to drugs will change the entire drug discovery system.

KE: I think the sky is the limit. We could see a real improvement in how drug discovery performs and efficacy in drug pipelines.

CD: Thank you for your time.

Dr Cord Dohrmann (CD) is Chief Scientific Officer and Member of the Management Board at Evotec. Dr Dohrmann has spent over 20 years in biomedical research at leading academic institutions and in the biotech industry.

PatientsLikeMe

PATIENT NETWORKS AND THE LATEST GENOMIC SCIENCE CAN LEAD TO BETTER MEDICINE

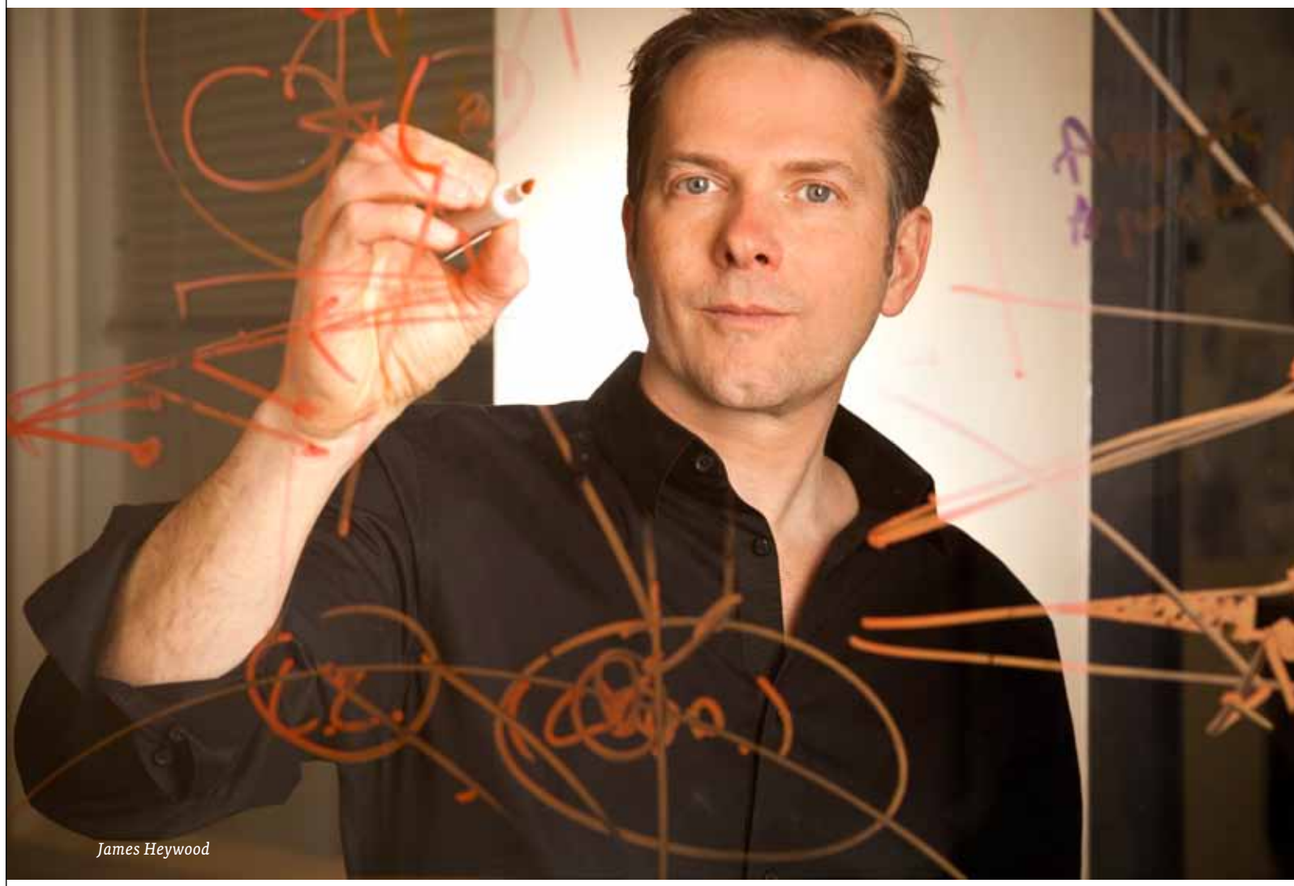
James Heywood is the Co-Founder and Chairman of the patient network PatientsLikeMe. He entered the field of translational medicine when his 29 year old brother Stephen was diagnosed with ALS.

Today Jamie is a chief scientist and architect for PatientsLikeMe. Described by CNNMoney as one of the 15 companies that will change the world, Jamie co-founded PatientsLikeMe to ensure patient outcomes become the primary driver of the medical care and discovery process. Jamie is also the founder and past CEO of the ALS Therapy Development Institute (ALS TDI), the world's first non-profit biotechnology company. During his tenure at ALS TDI Jamie helped pioneer an open research model and industrialised therapeutic validation process that made ALS TDI the world's largest and most comprehensive ALS research programme. Jamie and his brother were the subject of Pulitzer Prize-winning author Jonathan Weiner's biography His Brother's Keeper and the documentary So Much So Fast.

Inspired by the life experiences of Stephen Heywood, PatientsLikeMe was founded in 2004 by his brothers Jamie and Ben Heywood and long-time family friend Jeff Cole. Stephen was diagnosed in 1998 at the age of 29 with ALS. As his condition progressed, Stephen's family made many attempts to slow his disease and treat his symptoms, but the trial-and-error approach was time-consuming and repetitive. They believed there had to be a better way. Stephen's experience is like that of millions of people around the world who live with life-changing and chronic diseases. They often have specific questions about their treatment options, and about what to expect. They wonder - "Is what I'm experiencing normal?" or "Is there is anyone out there like me?"

An online patient network, PatientsLikeMe is where people find the answers to those questions, and connect with others who know first-hand what they are going through. Today, members have reported their real-world experiences on more than 2,300 diseases, everything from rare diseases like ALS to more prevalent diseases like depression, fibromyalgia, multiple sclerosis, and psoriasis. Through health profiles, members monitor how they're doing between doctor or hospital visits, document the severity of their symptoms, identify triggers, note how they are responding to new treatments, and track side effects. They learn from the aggregated data of others with the same experiences and see, often for the first time, just how they are really doing. They also get and give support from others that will help them live better day to day.

Together, members are also helping to fundamentally transform the world's understanding of disease by sharing their real-world experiences. A for-profit founded on a philosophy of "openness", PatientsLikeMe aggregates the data, analyses them and shares the results with health care and life science companies to accelerate research and develop more effective treatments. The value of this open, community-driven approach to health care research was first demonstrated in 2011, when



James Heywood

PatientsLikeMe revealed the results of a patient-initiated observational study refuting a 2008 publication that claimed lithium carbonate could slow the progression of ALS. The study, published in the scientific journal, Nature Biotechnology, marked the first time a peer-to-peer network was used to evaluate a treatment in a patient population in real time. It was the first of a number of patient-reported outcome studies that have increased our understanding of diseases.

Our patient focus is helping to drive a new era of health care in which people can benefit in real time from the information they share while contributing to a new way of measuring health. A "learning health system," PatientsLikeMe invites everyone to share their disease experiences in a way that can continuously improve care and dramatically accelerate our understanding of human health.

Evotec has reached an agreement in principal with PatientsLikeMe (www.patientslikeme.com) on approaches to rapidly evaluate any patient testable theories about progression or pathways that might express themselves in ALS patients.

TECHNOLOGY OVERVIEW

WHAT WE CAN DELIVER

TARGET MOTOR NEURON DISEASES WITH A BROAD PLATFORM OF TECHNOLOGIES

The integration of high-content imaging and analysis solutions with state-of-the-art target and pathway deconvolution technologies, as well as the implementation of bioinformatics driven data mining tools has produced a renaissance of phenotypic drug discovery approaches offering real chances for uncovering known as well as novel targets. Evotec's phenotypic drug discovery and target deconvolution solutions appear best-suited to tackle complex diseases with largely diverse unknown causes, such as motor neuron diseases.

1 STEM CELL PLATFORM

- Dedicated stem cell facilities
- 15 stem cell scientists
- Human induced pluripotent and embryonic stem cells
- Mouse embryonic stem cells harbouring HB9-GFP motor neuron reporter
- Various differentiation protocols in place, including generation of motor neurons, cortical neurons and astrocytes

2 CELL BASED ASSAY PLATFORM FOR MODELLING ASPECTS OF MOTOR NEURON DISEASES

- Opera® high-content screening system
- Flexible plate formats
- HCS customised Acapella™ script writing
- Multi-year experience in building assays from various classes including target based, mechanism-informed and purely phenotypic
- Range of neural phenotypic readouts e.g. quantification of neurites and synapses
- Neuro-inflammation paradigm: High-content neuroprotection assay combining microglia, astrocytes and motor neurons
- Trophic factor withdrawal paradigm: High-content neuroprotection assay combining astrocytes and motor neurons
- Unfolded stress response paradigm

3 RANGE OF SMALL MOLECULE LIBRARIES COVERING BIOLOGICAL AS WELL AS CHEMICAL DIVERSITY

- > 5,000 biologically diverse annotated compounds allowing quick target and pathway hypothesis building
- > 400,000 chemically diverse compounds directed towards identification of novel targets and novel chemical matter
- Building of focused libraries, target-oriented libraries and phenotypic-oriented libraries

4 HIT VALIDATION, H2L, LO

- Strong background in building assays for hit validation, H2L and LO
- Compound triaging using range of biophysical, biochemical and cell-based assay technologies
- Proteome and transcriptome profiling yielding insight on compound mechanism of action

5 STATE OF THE ART CHEMI-INFORMATICS PLATFORM

- Structural clustering and similarity searching for hit expansion by structure and by target
- Enriching compound libraries with annotations on signalling pathways, interaction networks and systems biology
- Predictive pharmacology for building and filtering possible ligand-target associations
- Network pharmacology tools for integrating data on gene regulation from disease models and from compound annotations

6 TARGET IDENTIFICATION WITH INDUSTRY LEADING PROTEOMICS PLATFORM

- Cellular Target Profiling™ for identifying the drug's target and for determining their binding affinities
- Deep proteome and phosphoproteome profiling to analyse the drug's Mode of Action

7 TARGET VALIDATION PLATFORM

- Knockdown technologies in place ranging from siRNA to AAV-mediated shRNA
- Multi-year experience in building target based assays
- Integration of mouse gene targeting models for *ex-vivo* and *in vivo* validation
- *In vivo* AAV-toolbox for target interrogation

8 IN VIVO MODELLING AND PRECLINICAL DEVELOPMENT

- Integration of mouse gene targeting models for preclinical studies, e.g.SOD1 G93A
- Pharmacokinetics and pharmacodynamics suite
- Expert neuropharmacology department dedicated to building relevant *in vivo* efficacy models
- Biomarker candidate discovery (proteomic and post-translational marks)
- MRM (Multiple Reaction Monitoring) for targeted quantification of protein biomarkers.
- Biomarker analysis and discovery in pharmacodynamic and disease-specific models.

COMPOUND SELECTION	PHENOTYPIC SCREEN	BUILD SAR PREDICT TARGETS	HIT VALIDATION H2L, LO	TARGET IDENTIFICATION AND VALIDATION	IN VIVO STUDIES
▸ Chemically diverse compounds ▸ Biologically diverse (annotated) compounds	▸ Structurally diverse hits ▸ Hits with target and pathway annotations	▸ Structural clustering ▸ Hit expansion by structure ▸ Similarity searching ▸ Predictive Pharmacology ▸ Hit expansion by target	▸ Orthogonal assays ▸ Secondary assays ▸ Selectivity assays (Cerep, Kinaffinity) ▸ Proteome / Transcriptome analaysis	▸ Mechanism-informed phenotypic assay ▸ Knockdown studies ▸ Cellular Target Profiling (CTP) ▸ Target-specific assays ▸ Gene targeting mouse model	▸ Disease models in vivo efficacy ▸ PK/PD ▸ Biomarkers

5 QUESTIONS TO

Sandra Lubitz & Rainer Kuhn

Dr Sandra Lubitz received her PhD in Cell Biology from the International Max Planck Research School in Dresden, Germany, focusing on epigenetic regulation in murine embryonic stem cell self-renewal and differentiation. During her postdoctoral studies at the Biotechnological Center in Dresden she extended her studies to human cell types and was among the first who studied human embryonic stem cell differentiation in Germany. She then moved to Genea BIOCELLS, a stem cell company based in Sydney, Australia, in order to work with disease-specific human embryonic stem cells. In her role she established neural *in vitro* differentiation and phenotypic high-content assays for human embryonic stem cells. Sandra then moved to Pfizer Regenerative Medicine (now: Neusentis) in Cambridge, UK, where she was involved in two projects using stem cells for high throughput screening and drug discovery and for cellular therapy. Joining Evotec in 2011, she played a key role in building the stem cell platform at Evotec and establishing human induced pluripotent stem (iPS) cell-based model systems for phenotypic screening and drug discovery for ALS and Huntington's disease. Sandra is heading Evotec's stem cell team and is leading the Cure*MotorNeuron* collaboration with Kevin Eggan and Lee Rubin at Harvard.

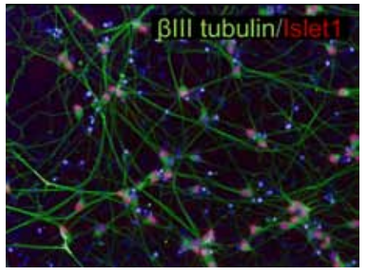
Dr Rainer Kuhn received his PhD in 1989 from the Institute of Genetics at the University of Düsseldorf (Germany) studying molecular mechanisms of germ cell development in Drosophila melanogaster. Following a postdoctoral training in molecular neurobiology at the Salk Institute (USA) he joined Ciba-Geigy/Novartis Neuroscience in Basel (Switzerland). He initiated research on metabotropic glutamate receptors, and identified with his team the first allosteric mGluR compounds and the mGluR5 antagonist AFQ056 (Mavoglurant). Over the years he served as Project leader, Unit Head and Executive Director for Neurodegeneration and -regeneration, where he managed large research efforts in psychiatric indications, Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, spinal cord injury, biomarkers and novel stem cell-based neuronal assay systems. In 2012, he co-founded the biotech startup Promidis (Italy) focusing on biomarker and therapeutics discovery for Huntington's disease. Since 2014, he is EVP Neuroscience at Evotec AG in Hamburg (Germany).

1 What are the current challenges for developing drugs for ALS?

SL: Key challenges for drug development in ALS and other neurodegenerative diseases are the lack of predictive *in vitro* and *in vivo* disease models and the lack of biomarkers that can inform about target engagement of a candidate drug, its effect on a molecular pathway and ultimately on disease. Furthermore, as ALS is quite heterogeneous, and its familial forms are caused by mutations in more than 20 different genes, it is important to be able to select the right patient population for novel drug candidates based on a better understanding of the underlying pathomechanism.

2 How can iPS cells impact on drug development for ALS and what lies ahead?

RK: To me human induced pluripotent stem cells derived from patients offer a novel basic model of disease enabling us to interrogate the underlying specific genetic factors and biology, and to perform drug screening for candidate drugs in patient cells. The field is rapidly advancing and many encouraging results have been obtained but it is still early days. We need to learn a lot more about how to model a "disease in a dish" to make fully use of the potential of this novel technology.



Motor neurons derived from human induced pluripotent stem cells characterised by co-expression of Islet1 (red) and the neurite marker beta III tubulin (green). Nuclei counterstained with DRAQ5 (blue). Source: Evotec

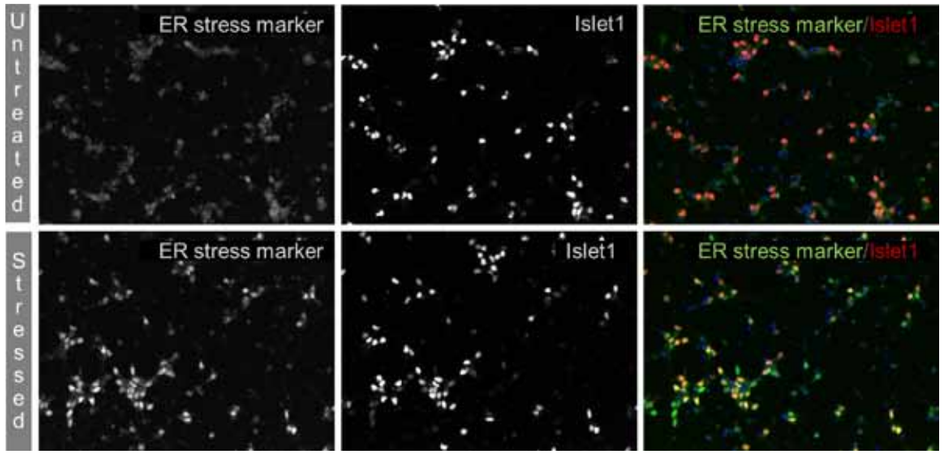
3 What is the expertise and history of Evotec in human disease modeling using induced pluripotent stem cells?

SL: The stem cell team at Evotec consists of 15 dedicated scientists with many years of practical experience. We have been operating for many years, expanding and characterising human and murine stem cell lines and optimising stem cell differentiation protocols for industrialisation (scale up). In recent years, identification of phenotypic readouts for screening has become a major focus. A variety of small molecule screens using human induced pluripotent stem (iPS) cells have already been described in the literature and collectively these experiments have helped to lay the conceptual groundwork for small molecule screens in iPS cell-derived cells. However, in order to overcome the scientific hurdles related to stem cell-based applications, it is essential to develop robust, reproducible, and disease-relevant assays with reasonable throughput that have been rigorously validated. Evotec's proven expertise in high-content screening and track record in ALS research facilitates the development of disease-relevant assays that better capture the complexity of human biology, so that safer, more efficacious medicines make their way to the patient.

> "At Evotec, we are developing an industrialised process for the manufacture of iPS cell-derived human motor neurons."

4 How can Evotec contribute to finding new drugs for ALS using iPS cells?

RK: The significant challenge for commercialisation of iPS cell technology is consistency in producing both starting material iPS cells and the differentiated cells in the quantity, quality, and purity required by the pharmaceutical industry. At Evotec, we are developing an industrialised process for the manufacture of iPS cell-derived human motor neurons. Another important point is disease-relevance. The team will develop screening approaches that have an impact on disease mechanisms in ALS and potentially other motor neurons diseases. It is important to gain an in-depth understanding of human ALS pathologies, therefore an array of familial and sporadic ALS-iPS cells will be used to model the disease *in vitro*. Isogenic control lines could be used to create genetically defined conditions in patient-specific iPS cells. With Cure*MotorNeuron*, our goal is to develop a human model system using these patient-derived iPS cells to identify compounds that can prevent or slow the loss of motor neurons that occurs with the progression of ALS.

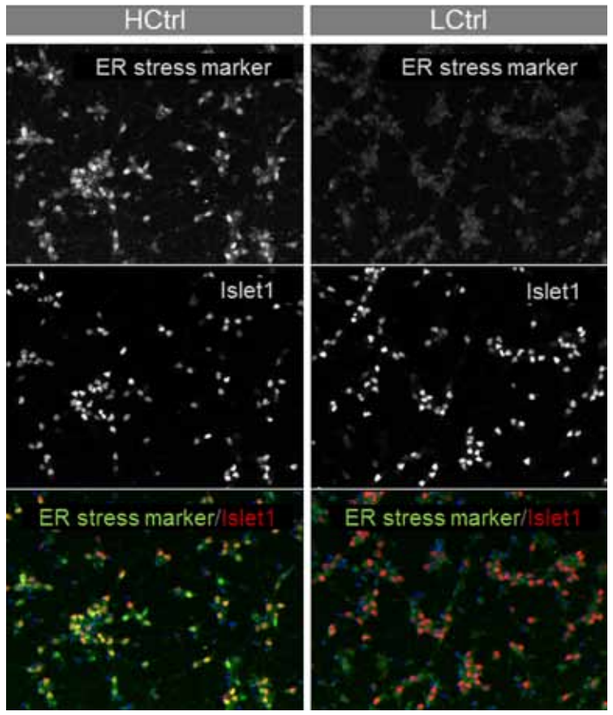


ER stress assay with iPS cell-derived motor neurons at day 22 in vitro (384-well format): unstressed cells do not show ER stress response (top panel) whereas stressed cells reveal strong ER stress response (bottom panel). Motor neurons are identified by Islet1 co-staining. Nuclei are counterstained with DRAQ5 (in blue). Source: Evotec

5 How does your collaboration with the HSCI look like?

SL: Motor neuron assays developed by Lee Rubin and Kevin Eggan at the Harvard Stem cell Institute have led the way to developing stem cell-based disease models to study motor neuron diseases, such as ALS. Our strategic partnership CureMotorNeuron leverages those assays and Evotec's leading drug discovery infrastructure and expertise to identify compounds that will have therapeutic value against ALS disease. With the experts at Harvard we have found the perfect partners to accomplish our ambitious goal to be amongst the first to develop and execute a successful and comprehensive stem cell-derived, drug efficacy screen for this life-threatening disease.

Selective small molecule inhibitor protects iPS cell-derived motor neurons from ER stress (384-well format): High control (HCtrl) and low control (LCtrl) conditions for ER stress assay have successfully been determined. For both conditions, a stress stimulus was applied which results in upregulation of an ER stress marker (in green). However, addition of a small molecule inhibitor for LCtrl was able to rescue cells from ER stress response. Motor neurons stained for Islet1 (red), nuclei counterstained with DRAQ5 (in blue). Source: Evotec



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