evotec

DDup

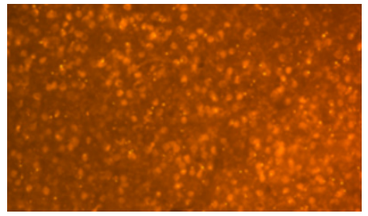
DRUG DISCOVERY UPDATES #2

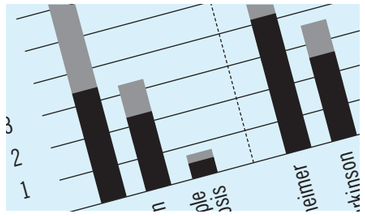
NEURO-DEGENERATIVE DISEASES

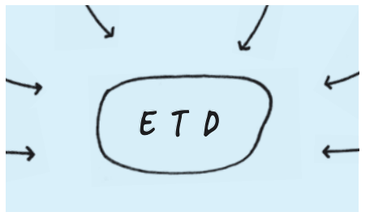
MAJOR DISORDERS WITH INADEQUATE STANDARDS OF CARE:

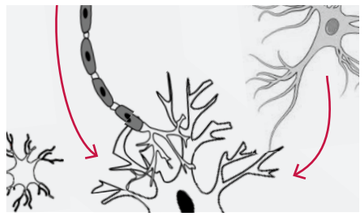
AD/PD/HD/ALS
No cure or effective treatments
New approaches urgently needed

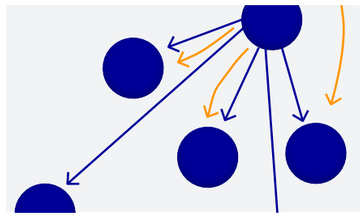
CONTENT & CONTACT











FOR YOUR FUTURE DD PROJECT PLEASE CONTACT:

Mario.Polywka@evotec.com

Dr Werner Lanthaler, CEO

WELCOME TO DDUP!

DEAR CUSTOMERS AND FRIENDS,

First of all let me please thank you for all the positive feedback regarding DDup. Great to see the scientific input and real life project experience that feeds back into this tool.

Our discovery alliances and technology infrastructures have only one goal, namely to do the best for our shared endproducts, and here starting with a shared learning process within our global academic and technological network is clearly the best way to improve endproducts for drug development.

MANY WAYS IN THE SAME DIRECTION

It is our vision to keep you further updated with what is going on within Evotec, to tell you more about new integrated technologies, but also deepen the information about also historically strong core disease areas of Evotec. One of these historically very strong disease areas, with deep in-house expertise and knowledge, is CNS – neurodegenerative diseases. Neurodegenerative diseases are globally wide spread and surely represent one of the major burdens for the healthcare systems around the world. Our goal is, in collaboration with you, to create and develop new and more effective drugs in this field.

This second edition will guide you through our recently established alliance in Alzheimer's Disease (AD) with Roche and will give you a closer look on our in-house AD drug discovery expertise.

Coupled with a selected overview about our technological capabilities, this edition should illustrate to and give you more visibility about the strength of Evotec in the area of neurodegenerative diseases.

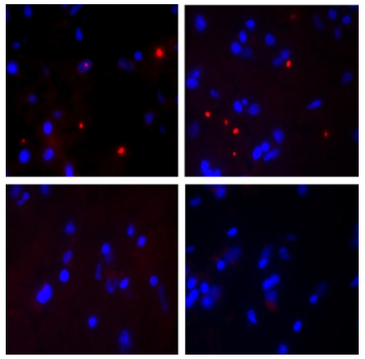
We will also highlight our long-term alliance with CHDI in the field of Huntington's Disease and it's therefore a big pleasure for me, that it was possible for us to win Robert Pacifici, PhD as our second interview guest, who is the CSO of CHDI. We have enjoyed a close cooperation with CHDI for now more than 5 years.

I hope you enjoy browsing through our second edition of DDup and let me please repeat, if you may have any further questions about DDup or Evotec, don't hesitate to contact us!

Yours sincerely
Werner Lanthaler
on behalf of the management team

DEDICATED TO FINDING THERAPEUTICS FOR HUNTINGTON'S DISEASE

Robert Pacifici was the Site Director and Chief Scientific Officer at the Research Triangle Park Laboratories of Eli Lilly and Company. There he oversaw the company's global screening and quantitative-biology efforts. Prior to joining Lilly, Robert was Vice President of Discovery Technologies at Xencor, a privately held biotechnology company that applied rational design principles to the development of protein therapeutics. At Amgen for nearly ten years, Pacifici's responsibilities increased. He led their automation, high throughput screening, and information technologies groups. In addition, he was instrumental in forging Amgen's relationships with Caliper Technologies and The Automation Partnership as well as the acquisition of Kinetix Pharmaceuticals.



The figure shows the staining of mutant huntingtin aggregates in two human brain samples from HD-patients (upper two pictures) and, as a control, two brain samples from age matched healthy controls (lower two pictures). The blue signal indicates the nuclei of cells and the red staining is indicative of the huntingtin aggregates stained with an antibody specifically recognizing these pathological hallmarks

Robert received a BS in Biochemistry from the University of Massachusetts, Amherst, and a PhD in Biochemistry from the University of Southern California. He holds an adjunct appointment at the University of Southern California's Department of Molecular Pharmacology and Toxicology. He is also Chair of the Spinal Muscular Atrophy Project's Scientific Steering Committee, which is part of the National Institute on Neurological Disorders and Stroke (NINDS). He currently sits on several additional external boards and advisory committees, including SMA Foundation, and the USC Board of Supervisors of the International Center for Regulatory Science. He joined CHDI in 2004.

5 MINUTES
WITH ROBERT PACIFICI ON CHDI AND HUNTINGTON'S DISEASE (HD)

CD: CHDI is dedicated to increasing the understanding of Huntington's disease (HD) in order to discover and develop therapies that slow the progression of the disease. CHDI's approach is a bit unconventional as it is unbiased in terms of therapeutic options and very comprehensive. Could you tell us a little more about CHDI's approach and your thinking behind it?

RP: CHDI is exclusively dedicated to finding therapeutics for HD. On the one hand, this gives us a laser-like focus and a continuity of mission that many other research organizations lack. On the other hand, with the constraint of focusing on a single disease, we really need to ensure that we explore every degree of freedom in other dimensions, so we try to make sure that our efforts are as diverse as possible to maximize our chances of success. This includes the exploration of both unprecedented targets and, as you correctly point out, novel therapeutic modalities. Perhaps the best example is our work on the huntingtin protein itself. Conventional wisdom would discourage even considering this large protein with no known catalytic activity as a target for small molecule drug discovery. However, since HD is a monogenic disease with 100 percent penetrance that is caused by the expression of this mutant protein, CHDI cannot dismiss this pharmacological target as "too difficult" because it is simply too well-validated. Instead our most likely to shape our drug-hunting campaigns and the profile of the resulting drug candidates. Back to the example of huntingtin lowering, a better understanding of the biology might help us determine what degree of huntingtin lowering is needed, where it needs to be lowered, and at what age it needs to be lowered to have the desired therapeutic effect.

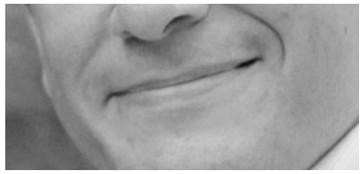
> "CHDI cannot dismiss this pharmacological target as *too difficult*"

approach has been to look at new and innovative ways of modulating huntingtin levels using platforms like siRNA, antisense oligonucleotides, and zinc-finger proteins. In other words, we are willing to take on the additional risk of these emerging cutting-edge options because we feel the challenges are both well-defined and tangible.

Our desire to further understand HD is solely driven by our needs in drug discovery. While there is certainly no shortage of "interesting" questions about HD whose answers would be eminently publishable, CHDI prioritizes its work based on the results that are Neurodegenerative diseases in general, and HD in particular, have proven to be very difficult areas for conventional drug discovery with precious few success stories so far. If our approach is unbiased or unconventional, it is because we believe that a different approach is going to be required to find therapies for these still unmet medical needs.

CD: What are the major differences between CHDI and typical biotechnology or pharma companies?

RP: CHDI is different from typical biotechnology companies in several important ways, each of which has unique consequences for us. First

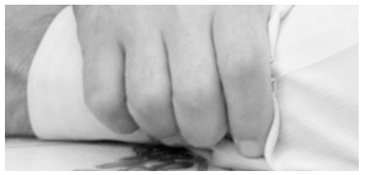


> "We base our decisions on the highest quality information and maintain the strongest standards of scientific rigor."

and foremost, as discussed above, we are exclusively dedicated to HD. There are many companies that say that they are committed to a particular disease or disease-area, but the reality is that most will waiver on their commitment if they find a financially or scientifically expedient alternative. CHDI is funded by private donors to whom we have a fiduciary responsibility to remain focused on this one disease. Unlike other organizations, CHDI cannot rescue sunk costs by "repurposing" drugs from one indication to the next – such as Viagra from cardiovascular disease to erectile dysfunction – and this has influenced our strategy to place a strong emphasis on early target validation. In other words, before we dedicate resources to a particular target, it is imperative for us to have a strong evidence-based hypothesis that modulating the target will have a beneficial effect on HD pathophysiology. Having a long-term commitment to a single disease has also allowed us to build up very deep domain knowledge in HD which we are happy to bring to bear on our own efforts or the efforts of others.

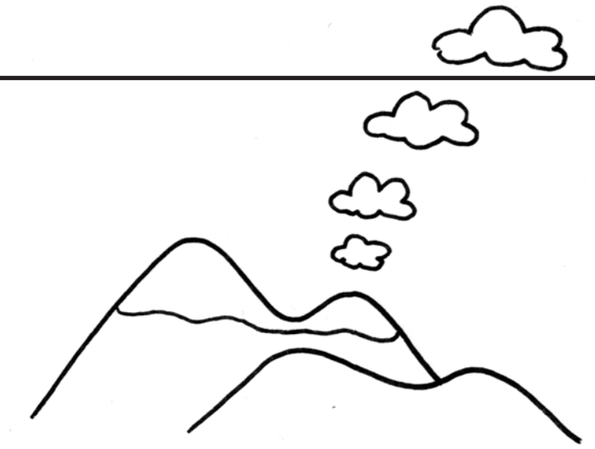
Which brings me to our second biggest difference: CHDI is not-for-profit. Unlike typical venture-backed biotechnology companies who are driven, ultimately, by the desire to make money, our bottom-line is time. CHDI wants to find treatments for HD as quickly as possible. That means that we don't have any competitors, only collaborators! CHDI's policy is to openly share tools, reagents, models, and know-how to "collaboratively enable" as many good ideas in high-quality laboratories as possible.

Thanks to the generous resources that our donors provide, we are not beholden to some of the artificial milestones that are all too common at traditional biotechnology companies: "A Phase I start by end of Q3 2012", "A multi-million dollar deal with big pharma X", "Closing round X of financing". Instead we have the luxury of focusing on the long-term and on ensuring that we base our decisions on the highest quality information and maintain the strongest standards of scientific rigor.



The final defining aspect of CHDI relative to other biotechs is that we do not have any of our own internal "wet labs." There are nearly 60 people who currently work within the "four-walls" of the foundation across our three sites in New York, Princeton, and Los Angeles, about half of whom are PhDs and/or MDs. These science directors are responsible for the design and interpretation of experiments, but all of the bench work is carried out using the "virtual" or "outsourcing" model.

CHDI is fortunate to have established a global network of nearly 600 investigators with whom we partner to carry out the laboratory work, including representation from government, academic and industrial sectors. A critical part of this portfolio is the fee-for-service contract research organizations (CROs) like Evotec. As you might imagine, orchestrating such a large and diverse effort represents serious challenges in tracking material and information flow. Having "anchor" CROs like Evotec that have integrated with many drug discovery core competencies under one roof is very attractive to CHDI. It is also important to establish long-term relationships with our key partners so that they can accumulate some



of the critical HD knowledge and share not just in the task but the passion of our mission.

CD: CHDI is collaborating with multiple partners worldwide. How do you identify these partners and how do you structure your collaborations?

RP: One of the advantages of the virtual model is that CHDI can fashion a workforce that is both high quality and flexible enough to meet our dynamic needs. The selection process is driven by science first; in other words who is the best partner to get a body of work done? We typically establish a set of metrics a priori that allow us to survey candidate organizations in a thorough and objective way. Once we've identified a few possibilities we typically complete the due diligence process with a site visit. CHDI typically undertakes small "pilot" projects at first to test the waters. It is our experience that it is best to build on initial scientific and operational successes rather than trying to do too much too early. We've learned over time that while cost, cycle-time, and throughput are all important metrics, ultimately quality, passion, and innovation are the best predictors of success.

"The selection process is driven by science first; in other words who is the best partner to get a body of work done?"

Another important aspect is to craft a contract that meets the needs of both the foundation and the new partner. For example, our academic contracts accommodate the investigator's sensitivity to the right to publish by giving them a period of exclusivity. Some biotechs are engaged as collaborators where we share the investment, the risk, and the potential downstream benefits from the partnership. In other cases, CHDI bears the entire economic burden by employing fee-for-service organizations and as such retains all of the rights to these programs. One of the important lessons that we have learned across all our contracts is that it is imperative to allow for some flexibility to accommodate the dynamic nature of the science.

CD: What aspects of CHDI's current R&D efforts are you most excited about?

RP: It would be unfair to single out one project over another since so much good work is going on! People often ask me if I'm optimistic about the future of HD therapeutic research. The answer is most definitely yes, and I'll tell you why. The first is the size of our portfolio. We all know that drug discovery suffers from very high rates of attrition at all stages of development, so to mitigate this CHDI maintains about 12 programs in parallel so that at any given moment we have a significant number of "shots on goal." The second reason for optimism is the diversity of the portfolio. Each of our projects has a risk profile that is different from the others based on the target, mechanism of action, and the therapeutic modality (antibodies, small molecules, nucleic acids, etc.). Lastly, each project is crafted to give an unambiguous outcome with regard to its therapeutic potential in HD; if a program "fails" we will know why so that we can either definitively walk away or redouble our efforts to resolve tangible problems. Overall, it is encouraging to see the maturation of the portfolio. There are now seven programs at a sufficiently mature stage of development to be ready for initiation of clinical trials within the next 18–24 months.

"Each of our projects has a risk profile that is different from the others based on the target, mechanism of action, and the therapeutic modality"

CD: What are the major challenges and how do you plan to address them?

RP: There is certainly no shortage of challenges at CHDI in finding treatments for HD! We believe that, while difficult, this is ultimately a problem whose solutions will unfold over time. By way of example I can cite two things that we struggle with on an ongoing basis, one is scientific, the other more operational.

Like many other late-onset, progressive, neurodegenerative diseases, there are several animal models of HD. However, it is unclear which, if any, of them are useful in predicting the human efficacy of drug candidates. A further confound is that the models which most closely resemble the human genetics develop phenotypes very slowly, resulting in long and costly preclinical animal studies. CHDI has abandoned the one-size-fits-all philosophy as we simply no longer believe that it is possible to recapitulate the whole of human pathosphysiology in any single animal model. Instead, we have now adopted a more customized approach where the species, perturbation, and outcomes for our animal studies are tailored to the individual needs of each program. This is accomplished by having a firm mechanistic hypothesis that allows us to design the appropriate pharmacokinetic/pharmacodynamic assays that will tell us if a compound is, at the very least, acutely able to exert its biochemical effects. Once that has been established, it is a lot easier to justify the time and expense required to achieve the more macroscopic phenotypic benefits, like improvement in survival.

"Models which most closely resemble the human genetics develop phenotypes very slowly, resulting in long and costly preclinical animal studies"

Together with its many partners, CHDI has generated a huge volume of scientific data. Over the past eight years, we have probably erred on the side of "doing" rather than "telling." As a consequence, there is a huge backlog of information that needs to be communicated to the broader community. With a dedicated Scientific Communications Director onboard and new informatics hires on the way, we hope to do a better job of publishing and sharing what we have learned to reduce redundancy and increase the chances that a new investigator can fully leverage the existing knowledge base. Articles like this are a good start and I think you'll be seeing much more in the way of news flow from CHDI using different media platforms.

CD: From your experience in HD, what are the lessons you have learned and do some of these lessons apply to other neurodegenerative diseases?

RP: Each neurodegenerative disease has its own peculiarities and challenges, but I agree that there are some universal themes. Perhaps the most obvious are the need for central delivery and long-term safety. We believe that delivery and distribution of therapeutics to relevant regions of the brain is so critical that we have dedicated an internal program to evaluating and implementing several cutting-edge technologies. These span the gamut from the use of mechanical pumps and cannulae provided by Medtronic to deliver Alnylam's siRNAs to more molecular approaches like immunoliposome encapsulation. We are hoping to have a repertoire of clinically compatible methods to safely deliver any candidate therapeutic payload.

"… strive to develop methods for early detection and diagnosis"

Unlike many other sporadic neurodegenerative diseases, HD is inherited, which means that it is possible to use genetic testing to predict who will ultimately manifest HD long before they are symptomatic. Despite the fact that overt phenotypes, such as the motor symptoms, are not obvious until much later in life, we now know that the deleterious effects of mutant huntingtin are in play very early on. Therefore, we believe that early drug intervention to slow disease progression is critical. We believe that this is a lesson that will apply to other neurodegenerative diseases and that they should strive to develop methods for early detection and diagnosis.

CD: What is your view of stem cell-based approaches for HD? Where do you see the biggest opportunities for stem cell-based approaches in neurodegenerative diseases?

RP: Stem cells offer the hope of truly regenerative medicines and as such CHDI has invested heavily in establishing an HD-specific platform. However, we believe that in the near term, stem cells will likely be more valuable as tools and that their utility as therapeutics remains a future aspiration. We are working with both industrial and academic partners to establish high-quality banks of stem cells, standardized growth conditions, and robust differentiation protocols. We strongly believe that these stem cell-derived models will fit nicely into our translational projects by providing physiologically relevant assays in which to test compounds identified in biochemical screens. With the advent of iPSC technology it should now be possible to answer important questions about HD biology as well.

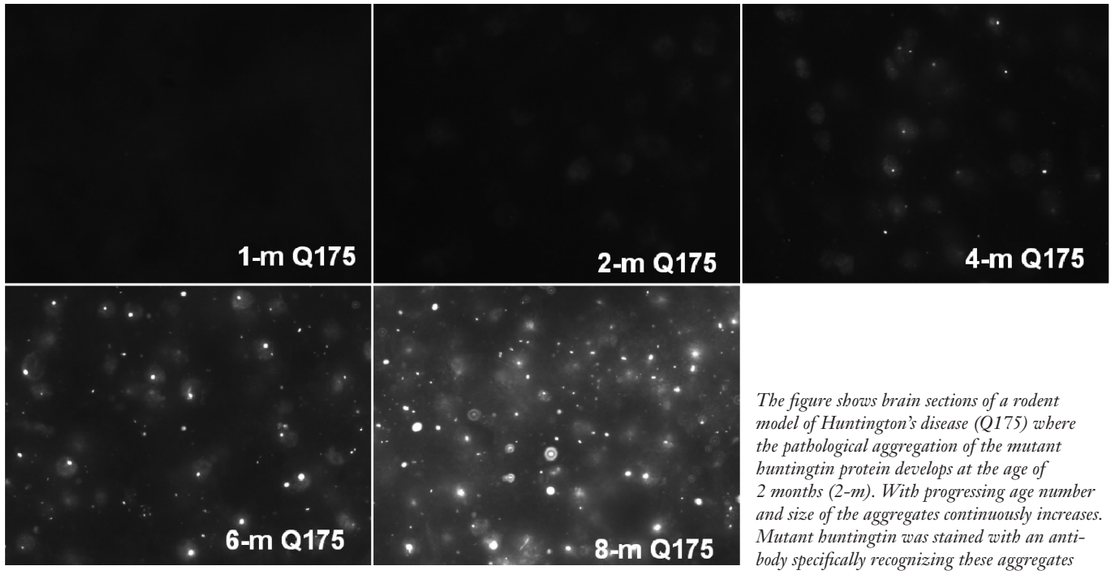
"We strongly believe that these stem cell-derived models will fit nicely into our translational projects by providing physiologically relevant assays"

CD: Monitoring disease progression is a challenge for many neurodegenerative diseases as there are few if any suitable biomarkers. What is the strategy in HD and is there a role for imaging techniques?

RP: Finding suitable biomakers to assess target engagement and compound pseudoefficacy are a huge challenge for neurodegenerative diseases, and HD is no exception. However, this is an area that is too important to ignore and CHDI has dedicated considerable resources that are now beginning to show real promise. Together with an impressive group of external clinical investigators, CHDI has funded an extensive observational study called TRACK-HD. The detailed findings from this study have been published elsewhere, but the take-home message is that there are numerous robust measures that can be made non-invasively in human subjects long before the traditional onset of motor symptoms that can be used to both stage and track disease progression. Given that HD is largely attributed to pathology that occurs centrally, imaging has certainly figured prominently in these and other studies. It is very obvious that both volumetric changes as well as more sophisticated functional changes are observable very early in the disease progression. It is our great hope that future trials involving pharmacological intervention will show reversal of these signals concomitant with the more traditional clinically meaningful outcomes used for drug registration. The utility of biomarkers in dose selection, safety, and efficacy is undeniable and so we must continue to find suitable measures to include in our future efforts.

CD: Thank you for your time.

Dr Cord Dohrmann (CD) joined Evotec AG as Chief Scientific Officer and Member of the Management Board in September 2010. Dr Dohrmann has spent over 20 years in biomedical research at leading academic institutions and in the biotech industry.



The figure shows brain sections of a rodent model of Huntington's disease (Q175) where the pathological aggregation of the mutant huntingtin protein develops at the age of 2 months (2-m). With progressing age number and size of the aggregates continuously increases. Mutant huntingtin was stained with an antibody specifically recognizing these aggregates

EVOTEC–CHDI RESEARCH COLLABORATION

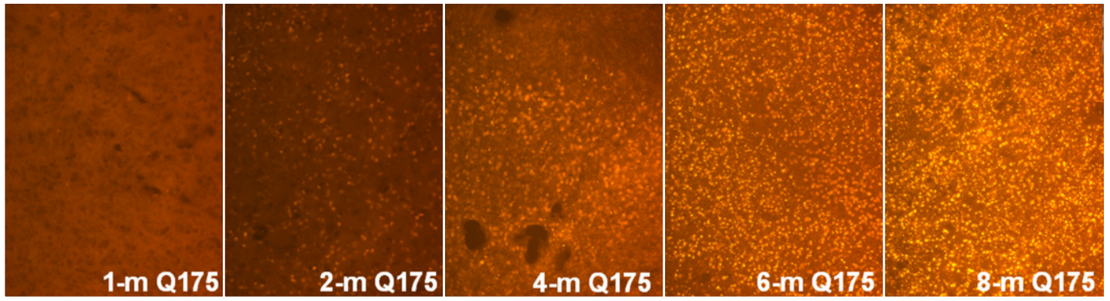
CHDI Foundation is a private, not-for-profit biomedical research organization that selected Evotec as one of its strategic drug discovery partners in the search for therapies that slow the progression of Huntington's disease (HD). As a largely virtual organization, CHDI relies on a network of academic and industrial partners to conduct its research and development activities. Evotec is one of the major providers of discovery research for CHDI based on its integrated suite of core competencies in drug discovery coupled with a profound expertise in CNS diseases and related disease biology know-how. Since the start of the collaboration in 2006, HD research has made significant progress and we are very much looking forward to continuing this highly-productive collaboration to further advance knowledge and understanding of the disease and, importantly, to explore mechanisms that could lead to future therapies.

Huntington's disease (HD) is a dominantly inherited neurodegenerative disease caused by a single mutation in the huntingtin *(HTT)* gene that leads to an expansion of the CAG tract encoding for a polyglutamine stretch in *HTT*. The mutation leads to intracellular aggregation in one of the most vulnerable areas of the brain, the striatum. The ultimate consequence is the shrinkage of the striatum and clinical manifestations are behavioral changes, cognitive deficits, and motor disturbances in patients. The length of the CAG tract strongly influences disease onset and is on average in between 30–50 years of age. Although discovered more than a decade ago, the function of *HTT* is still unclear. Currently there are only very limited treatment options for HD and out of many proposed targets only *HTT* is well validated.

It is estimated that 1 in 10,000 people is affected by HD. A prominent sufferer of the disease was Woody Guthrie, an American folk singer who inspired many artists, among them Bob Dylan. When he died in the late 1960s his wife helped establish a foundation to fight HD and support, which is now called the Huntington's Disease Society of America (HDSA). CHDI Foundation was set up in 2004 with a mission to discover and develop therapies that slow the progression of HD, adhering to the highest industry standards.

CHDI is now active as a private, not-for-profit research organization that has established an international network of collaborators within academia, biotech, and large pharmaceutical companies. The exclusive focus of all activities is towards HD research and extends from exploratory biology to the identification and validation of therapeutic targets, and from drug discovery and development to clinical studies and trials. For this purpose CHDI has assembled a team of renowned scientists located in Los Angeles, New York City, and Princeton, managing and financing a network of approximately 600 scientists in academic and industrial laboratories worldwide. The data and research tools generated by these multiple collaborations are being made accessible to the whole HD research community in order to accelerate the development of effective therapies for HD.

The figure shows brain sections of a rodent model of Huntington's disease (Q175) where the pathological aggregation of the mutant huntingtin protein develops at the age of 2 months (2-m). With progressing age number and size of the aggregates continuously increases. Mutant huntingtin was stained with an antibody specifically recognizing these aggregates



CHDI is currently pursuing several specific therapeutic strategies; the key focus is on lowering the expression of mutant *HTT*, thereby reducing the deleterious aggregate load in neurons. In addition, posttranslational modifications of *HTT* are being evaluated, as is the synaptic and metabolic function of neurons and the clearance of *HTT* aggregates. Evotec is playing an active role in most of these approaches and is currently involved in up to 10 different programs ranging from medicinal chemistry-based small molecule drug development programs to sophisticated projects in various rodent models of HD to identify and validate therapeutic targets, as well as stem cell approaches.

The most advanced programme within the Evotec/CHDI collaboration targets kynurenine monooxygenase (KMO) via selective small molecule inhibitors.

Evidence from animal models indicates that KMO activity may contribute to the progression of HD. Starting from early assay development and initial medicinal chemistry, joint efforts advanced the project to clinical candidate nomination by CHDI with front-running compounds now scheduled to enter formal toxicology. In addition, Evotec is significantly contributing to the KMO project through its *in vivo* biology/pharmacology expertise, for instance analyzing KMO activity in rodent models of HD dosed with small molecules. Furthermore, Evotec is subjecting brain sections from these animals to high-end imaging technologies (Opera™) for quantitative immunohistochemical analysis of inflammatory markers. In another more recently launched joint project, Evotec is developing small molecule tools to improve diagnosis and monitoring of disease progression in HD patients by means of positron emission tomography (PET) that could be critically important in future clinical trials. Specifically, Evotec is collecting and analyzing human brain tissue from HD patients and utilizing its medicinal chemistry expertise to identify PET ligands that allow quantitative determination of *HTT* aggregate formation in HD to accompany clinical studies. To support and improve the translatability of results from animal models to patients, Evotec is a member of the European Neuromodel Initiative, within which Evotec is establishing cognitive readouts in rodent models of HD that are expected to lead to more disease-relevant readouts and therefore should improve predictability in advancing projects to the clinic.

FACTS & FIGURES

5 SERIOUS DISEASES HAVE THREE THINGS IN COMMON:

- NO CURE
- NO DISEASE MODIFYING TREATMENTS
- NEW DRUGS ARE URGENTLY NEEDED

NEURODEGENERATIVE DISEASES (ND)

Major healthcare burden

Combined drug sales for AD, PD and MS in 2010 exceeded $ 16 bn. Currently used symptomatic therapeutics for AD treatment are either only short-term effective (acetylcholinesterase inhibitors) or of only minor potency (subtype-unspecific NMDA receptor antagonists).

Currently there are no approved drugs for HD, which could slow the deadly progression of the disease.

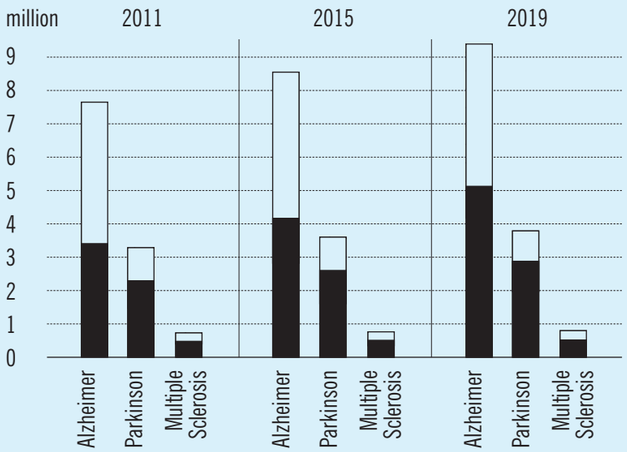
> No approved drug is able to stop disease progression and no one is tackling the root of the disease.

For ALS there is just one drug approved (Rizole/Sanofi-Aventis), which has demonstrated to give a 2–3 month survival benefit to ALS patients.

TOP 5 OF ND – PREVALENCE IN MAJOR MARKETS (G7)

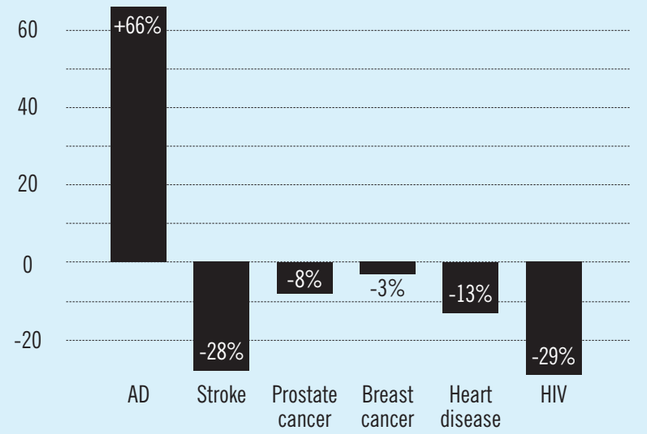
Alzheimer's Disease (AD)	**7.7 million**
Parkinsons's Disease (PD)	**3.3 million**
Multiple Sclerosis (MS)	**ca. 770,000**
Huntington's Disease (HD)	**ca. 100,000**
Amyotrophic lateral sclerosis (ALS)	**ca. 100,000**

STEADILY INCREASING PATIENT NUMBERS



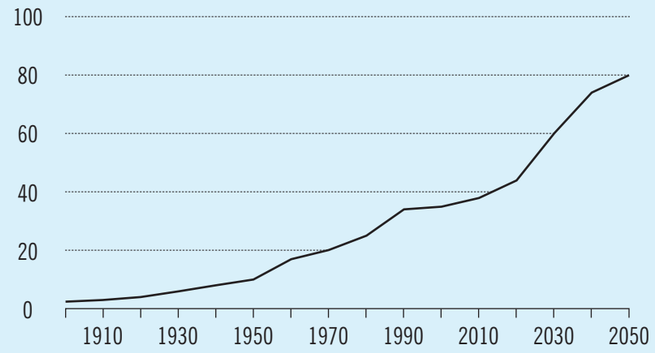
Especially sales in AD have enormous long-term growth potential, due to the lack of efficacy of currently approved drugs. To date less than 50% of AD patients are drug treated. Sales are estimated to grow by more than 200% from 2011–2019. In the list of the 15 leading causes of death in 2010 in the US, AD was ranked on the 6th place. The age-adjusted deaths between 2007 and 2008 increased significantly by 7.5% and from 2009 to 2010 by another 3.3%.

PERCENTAGE CHANGES IN SELECTED CAUSES OF DEATH (ALL AGES) BETWEEN 2000 AND 2008



Neurodegenerative diseases are accelerating within the aged population. Mitochondrial DNA mutations as well as oxidative stress both contribute to aging. One constant factor is that in each disease, neurons gradually lose function as the disease progresses with age.

NUMBER OF PEOPLE AGE 65 AND OVER



ALZHEIMER'S DISEASE

A KEY TOPIC AT EVOTEC

Alzheimer's disease (AD) is representing one of the biggest healthcare challenges as well as one of the most complex diseases in medical science. A hallmark of AD is the appearance of plaques in the brain and most approaches currently pursued in the clinic are focused on reducing beta amyloid plaque formation either by inhibition of a degrading enzyme (gamma secretase) or antibodies designed to bind beta amyloid directly. Despite disappointing clinical results for gamma secretase inhibitors much hope is still riding on the beta amyloid pathway.

Evotec is taking very different approaches. One of it is based on small molecule inhibitors for monoaminooxidase-B (MAO-B) which are currently in Phase II clinical studies in partnership with Roche. MAO-B is a well validated target that has been linked to oxidative stress, known to contribute to neurodegeneration and has been demonstrated to be highly upregulated in the brains of AD patients. Another approach is a systematic search for novel AD targets based on human brain tissues sampled from AD patients at various stages of disease.

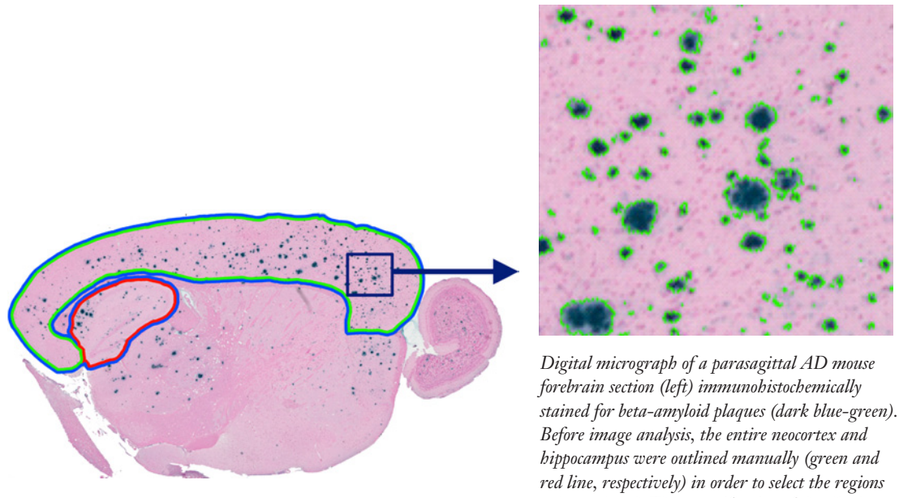
HUMAN TISSUE BASED APPROACH IN AD

Over the last few years Evotec has identified a large number of novel potential targets for AD and other neurodegenerative disorders. The key observations that relate the majority of these target candidates to CNS diseases stem from differential analyses of human brain tissue samples of both non-AD control subjects and individuals afflicted by AD, thereby considering human disease pathology but not a model system for target identification. Evotec has assembled a unique collection of more than 200 human brain tissue samples dissected from post-mortem specimens of different cerebral regions. A rapid autopsy procedure allowed for the recovery of high-quality brain specimens with very short post-mortem intervals thereby preserving disease specific features of the tissue samples as much as possible. The samples were obtained from age-matched donors that were well characterized with respect to clinical diagnosis, medical history, demoscopic data, and most importantly neuropathological confirmation including Braak staging.

A comprehensive gene expression analysis has been performed, allowing us to precisely monitor the chronology of events in the course of the disease, and thus to distinguish at the molecular level early and potentially causative events from late and symptomatic effects.

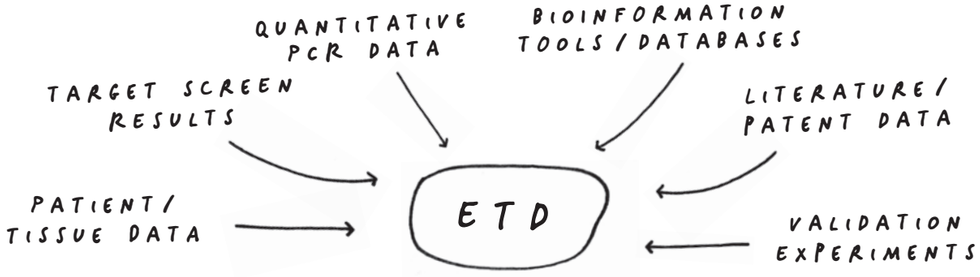
TARGET VALIDATION MODELS

Evotec has a highly comprehensive set of *in vitro* and *in vivo* models suitable for AD target validation and compound optimization. Many state-of-the-art methodologies have been established at Evotec and applied successfully for the characterization and validation of novel target candidates. Several cell lines are available as well as primary cells, for which differentiation protocols have been established allowing the analysis of neuritic functions and structures. Viral-based as well as inducible expression systems are used routinely in addition to the standard transient over-expression models. For the specific down-regulation of potential target the RNAi-technology has been established.



Digital micrograph of a parasagittal AD mouse forebrain section (left) immunohistochemically stained for beta-amyloid plaques (dark blue-green). Before image analysis, the entire neocortex and hippocampus were outlined manually (green and red line, respectively) in order to select the regions to be analyzed. The magnification of the clipping (right) shows plaques recognized and delineated by the proprietary Acapella™ image analysis software.

In addition to the more standard models Evotec has established a fully digitized proprietary technology for the quantitative evaluation of stained beta-amyloid plaques in microscopic slide high content images. A specialized object recognition algorithm, developed to work within the Acapella™ image analysis software environment, enables the fast, robust, reproducible and reliable recognition of plaques in high-resolution entire-view micrographs of the mouse brain. The procedure is suited for the use with either brightfield or fluorescence microscopic images. After regions of interest (e.g. cortex and hippocampus) have been defined (Fig.), the software evaluates the plaque load, number and size distribution for the selected regions with high speed and superior quality.



EVOTEC TARGET DATABASE (ETD)

Opportunities for collaborations based on novel targets for AD

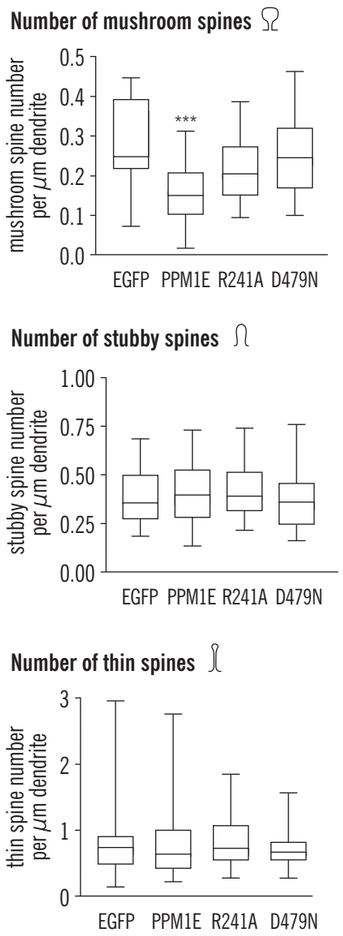
Evotec's AD target database is a customized system covering all aspects of data produced for each individual target candidate. Genes derived from the target identification efforts are annotated according to information available in public and commercial databases. Targets of interest out of the ETD have been pursued into target validation using the available repertoire of know-how and a full range of established cellular and animal models. This resulted in an interesting set of proprietary targets that are readily available for a joint program. We are strongly convinced that these novel target opportunities as well as the wide battery of techniques, tools and models successfully set up and applied during the last years provide an excellent basis for a collaborative target validation and compound discovery programe.

PPM1E – A PROMISING NEW AD TARGET

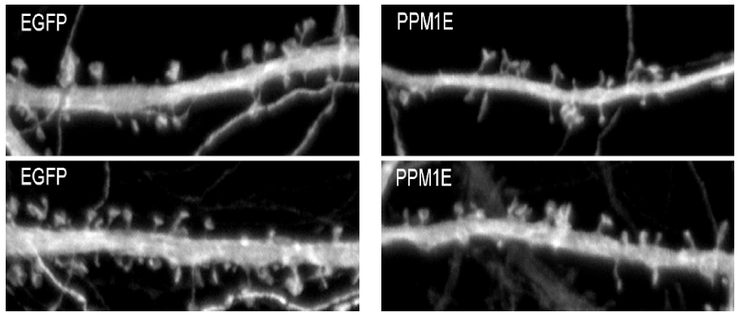
As an example for one of the identified target candidate has been applied to target validation approach using the implemented technologies at Evotec. The protein phosphatase 1E (PPM1E) was identified being significantly up-regulated with an early-onset already at Braak stage 1. Through overexpression in primary neuronal culture we showed that PPM1E has a neurodegenerative effect: dendritic spine density and morphology are considerably changed (Fig.).

Knock-down experiment of endogenous PPM1E meanwhile suggest a positive influence on dendritic spine morphogenesis or homeostasis. The early-onset dysregulation of PPM1E in AD could negatively affect the dendritic spine morphogenesis. Therefore inhibiting PPM1E in an early stage of disease may delay or at best even restore the progression of cognitive decline, hence PPM1E provides a promising new drug target for neurodegenerative diseases and especially for AD.

NUMBER OF SPINES/µM DENDRITE; OVEREXPRESSION OF PPM1E



FUNCTIONAL PPM1E MUTANTS DO NOT INFLUENCE NUMBER OF SPINES



*Human PPM1E expressing neurons show a significant and concentration dependent decrease in the number of mushroom-shaped spines per micrometer of dendrite compared to EGFP control neurons whereas it has no influence on other types of spines in differentiated hippocampal neurons in vitro. Overexpression of functionally inactive PPM1E mutants (R241A, D479N) does not affect the numbers of dendritic spines. Example microscopic pictures are from dendrites transfected with 0.2 µg pAAV/EGFP or PPM1E per 7.5*10⁴ neurons.*

CHINESE CHECKER: MAO-B BACK AND FORTH

- **The membrane-bound enzyme monoaminooxidase B, located predominantly in astrocytes in the central nervous system, catalyses the degradation of catecholamines (dopamine and histamine):**
- **Monoamine + H_2O + O_2 → Aldehyde + NH_3 + H_2O_2**
- **Dopamine is a "messenger of good mood", and the inhibition of its catabolism has been used to treat depression.**

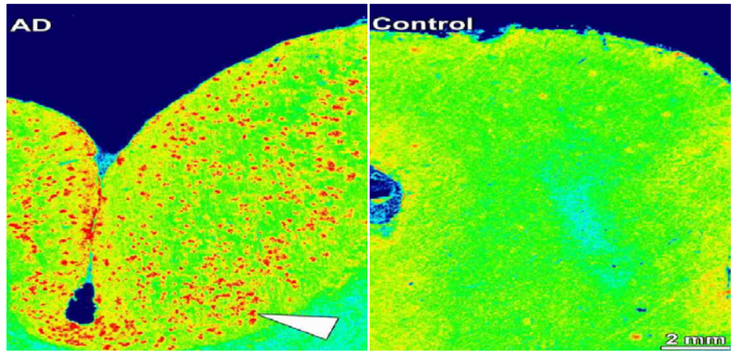
Alzheimer's disease (AD) represents a huge market opportunity for any new therapy driven by the growing patient population and increasing diagnosis rates. In only the seven major markets, excluding China and India, the number of prevalent cases will increase from currently approx. 7,4 m to about 9,5 m in 2019. Nowadays just 45% of all AD patients are drug treated, this is primarily due to unsatisfactory treatment options such as acetylcholine esterase inhibitors that only show a short term symptomatic effect.

Most other late-stage clinical development programmes target the beta-amyloid pathway, a concept that is still lacking clinical proof-of-concept. The number of drug treated patients is expected to grow significantly especially if new and improved treatments reach the market. Currently AD is the only cause of death among the top 10 in America without any effective treatment option that would prevent or cure the disease or just slow its progression.

In AD patients, it is well established that oxidative stress is contributing to neurodegeneration. Due to highly increased levels of MAO-B activity in AD patients (fig.), oxygen radical formation can be correlated to this enzymatic activity. Thus inhibition of MAO-B has the potential to slow down disease progression and to thereby improve disease symptoms.

Compelling preclinical and initial clinical results indicating robust efficacy and an excellent safety profile of Evotec's compound EVT302, convinced Roche to in-license Evotec's MAO-B inhibitor program. The program actually originated in Roche's laboratories was licensed by Evotec in 2005 when it was still at preclinical stages and encompassed a number of compound series. Evotec was able to select and develop a highly efficacious compound that demonstrated great selectivity, safety and tolerability. Early clinical development confirmed a superior safety profile over competing MAO inhibitors including the absence of potential potentially adverse food interactions (tyramine liability) preparing the basis for further development in AD patients.

Current clinical development plans indicate that the development of EVT302 will constitute one of the largest clinical efforts in AD targeting a substantial number of patients in parallel Phase II respectively Phase III trials. Financial cornerstones of the collaboration include an upfront payment of USD 10 m, development milestones up to USD 170 m and commercial milestones up to USD 650 m as well as tiered, double digit royalties.



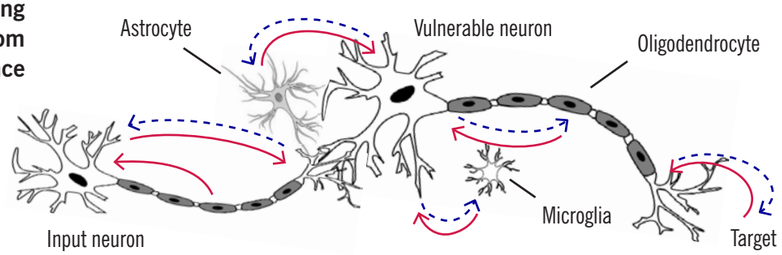
MAO-B expression in AD patient: Alzheimer's Disease patient vs. age-matched healthy individual

AMYOTROPHIC LATERAL SCLEROSIS AND PHENOTYPIC SCREENING

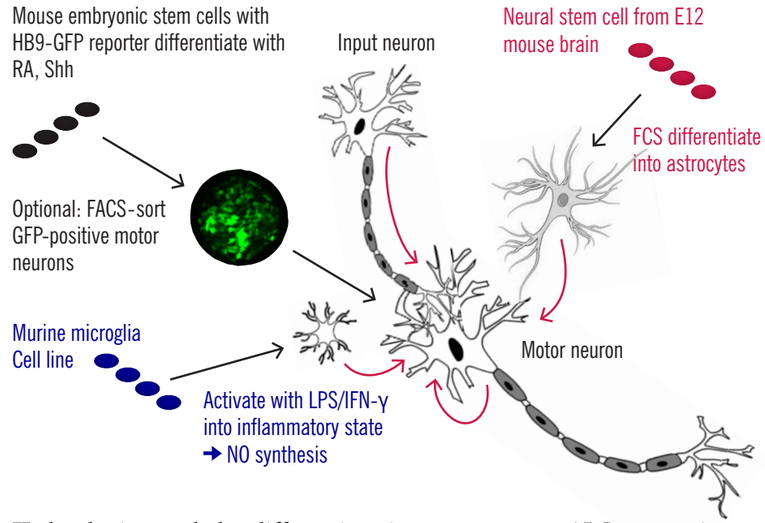
Amyotrophic Lateral Sclerosis (ALS) is a common type of motor neuron disease (MND). MND are progressive neurological disorders that primarily destroy the motor neurons in the spinal cord which control essential muscle activity such as breathing, speaking, swallowing, and also walking. As a result of motor neuron loss the connecting cells degenerate, i.e. the muscles in the periphery as well as the cortical neurons in the brain that normally control the motor neurons. In other words, peripheral target muscles become unable to function, weaken and finally atrophy. At the same time as muscle control is lost cognitive functions are largely spared. Thus, in contrast to prominent neurodegenerative diseases ALS will lead to death arising from breathing complications within 5 years from sympton onset, all in the presence of normal brain function.

About 90% of cases of ALS are "sporadic", meaning that the case appears to have occurred with no known cause and that the patient has no family history of ALS. In addition to environmental factors however, mutations in a number of genes have been found important in determining an individual's susceptibility to ALS: Cu/Zn superoxide dismutase SOD1, ALS2, NEFH (a small number of cases), senataxin (SETX) and vesicle associated protein B (VAPB).

Although contributing to only 10% of ALS cases the study of these target genes has led to the important learning that ALS as well as other inherited forms of neurodegenerative disease is not mediated solely by damage from the mutant protein within the target neurons but also within non-neuronal cells that shape the neuron's environmental condition. More specifically, conditional gene targeting studies of SOD1 which is linked to 20% of familiar ALS cases, have revealed that expression of mutant SOD1 in either of the implicated cell types, i.e. motor neurons, astrocytes, and microglia is not sufficient to cause ALS. In other words, ALS is an example for a non-cell autonomous disease that involves the expression of mutant protein in neuronal as well non-neuronal glial cells, altogether producing the disease-causing toxic events that drive disease progression.



Motor neuron disease mechanisms are non-cell-autonomous, requiring the convergence of damage within the vulnerable neurons and their neighboring glial cells.
Concept: Lobsiger & Cleveland, 2007



Given the above findings the drug discovery community has become increasingly aware that screening systems are needed that are a better image of the physiological situation. In the case of ALS such physiologically relevant assays then would be cell-based, they would be phenotypic and they would involve the identified key players of the disease, namely motor neurons, astrocytes and microglia. While astrocytes and microglia are dividing cells and could therefore theoretically be isolated from primary tissues of mouse or rat origin in sufficient amount, primary motor neurons are tedious to come by from primary tissue in the numbers needed for screening assays and they are impossible to amplify in the culture dish (neurons do not divide).

Two technological developments of the last 10 years now allow realization of complex but physiologically revelant screenable assays, (i) High Content Imaging and (ii) stem cell isolation, stable culture and directed differentiation into desired cell types. The Opera™ High Content imaging platform and Acapella™ script based image evaluation, both originally developed by Evotec Technologies, and the differentiation of mouse embryonic stem cells into motor neurons, developed by Thomas Jessell and colleagues at the Columbia University, exemplify these advances.

Evotec has teamed up with Prof. Hans Schöler, Dr. Jared Sterneckert and colleagues from the Max Planck Institute for Molecular Biomedicine and the Center for Advanced Regenerative Medicine (CARE), Münster, to combine leading stem cell biology expertise with leading drug discovery technologies. While Evotec is contributing compound libraries and compound management, as well as High Content Screening expertise exemplified by the Opera™ High Content Screening platform (HCS), MPI Münster is contributing ALS associated assay principles and biology for adaption at Evotec. The HCS assay involves mouse embryonic stem cell derived motor neurons, mouse neural progenitor cell derived astrocytes and activated mouse microglia. Since microglia have been known to contribute in important ways to ALS progression the chosen stress paradigm is biased to model the neuroinflammatory aspects of ALS. More than 11,000 small molecules including a subset of known drugs were screened.

As a first result an intriguingly low hit rate of 0.3% of screened compounds produced the rare but compelling neuroprotective outcome. These small molecule hits have been screened in a number of orthogonal assays probing relevant signaling pathways in neurons and glia, for example the stress response pathway, the JNK cell death execution pathway and the Nitric Oxide pathway in microglia. Intriguingly, evidence for compounds hitting multiple effector pathways in diverse cell types has been gathered, thus reproducing the non-cell autonomous nature of ALS and other neurodegenerative diseases. Finally, the modular nature of the assay will allow variation of cell types and stress paradigms to address multiple ALS relevant settings.

STEM CELL BASED ASSAYS FOR NEURODEGENERATIVE DISEASES

The contributions of phenotypic screening to the discovery of first-in-class small molecule drugs exceeded that of target based approaches between 1999 and 2008 (Swinney and Anthony, 2011). Although primary cells in principle are ideal for phenotypic screening, the isolation of primary cells is extremely cumbersome, giving both low yields and heterogeneous results, which makes a high throughput screening campaign almost impossible.
In contrast, properties of stem cells are uniquely suited to provide large numbers of homogeneous cells with a defined stage of differentiation and maturation.

Stem cells have the unique ability to both continually self-renew as well as to differentiate into specialized cells. Pluripotent stem cells have the largest developmental potential and are able to differentiate into every somatic cell lineage as well as germ cells. Therefore, using stem cell technology, it is theoretically possible not only to construct disease models *in vitro*, but also to use these models to discover new drug candidates, which represents a new paradigm for drug discovery.

While the HCS aspect of complex phenotypic screening assays such as the one described in the previous section has been developed to sufficient maturity during the last ten years, relevant stem cell based technologies that are prerequisite for successful and efficient assay development, are still in a comparably early developmental stage. In fact, the case of stem cell derived motor neurons may appear as a relatively straight forward and reproducible procedure, yet it is on its own already quite resource intensive and time consuming.

Specifically, setting up a motor neuron based HCS assay required (i) the generation of a transgenic mouse strain carrying a green fluorescent motor neuron reporter gene, (ii) the derivation of embryonic stem cells from this strain, (iii) the establishment of a neuronal differentiation protocol enriching for motor neurons by combinations of specific morphogenic factors, (iv) the purification of green fluorescent motor neurons by fluorescence assisted cell sorting, (v) the isolation of neural progenitor cells from embryonic mouse brain, (vi) the differentiation of the latter into astrocytes, (vii) the generation of an immortalized microglial cell line, and finally (viii) multi-step assay development including the optimization of the densities of three different cell types and of the stress paradigm, not to mention the various growth media and factors needed for the various protocols.

Despite this inherent complexity, stem cell technologies are maturing at a fast pace, applications in regenerative medicine are reaching the clinical development phase, and drug discovery has been seeing stem cell based assays marrying phenotypic screening approaches. Significant milestones have been achieved that are relevant to drug discovery in particular:

- Embryonic stem cell derivation, both human and rodent (however limited by ethical and legal concerns and restrictions)
- Induced pluripotent stem cell (iPS) generation, by various means of forced transcription factor expression
- iPS cell derivation from carriers of genetic disease (e.g. spinal muscular atrophy (SMA), HD, ALS)

And most importantly:

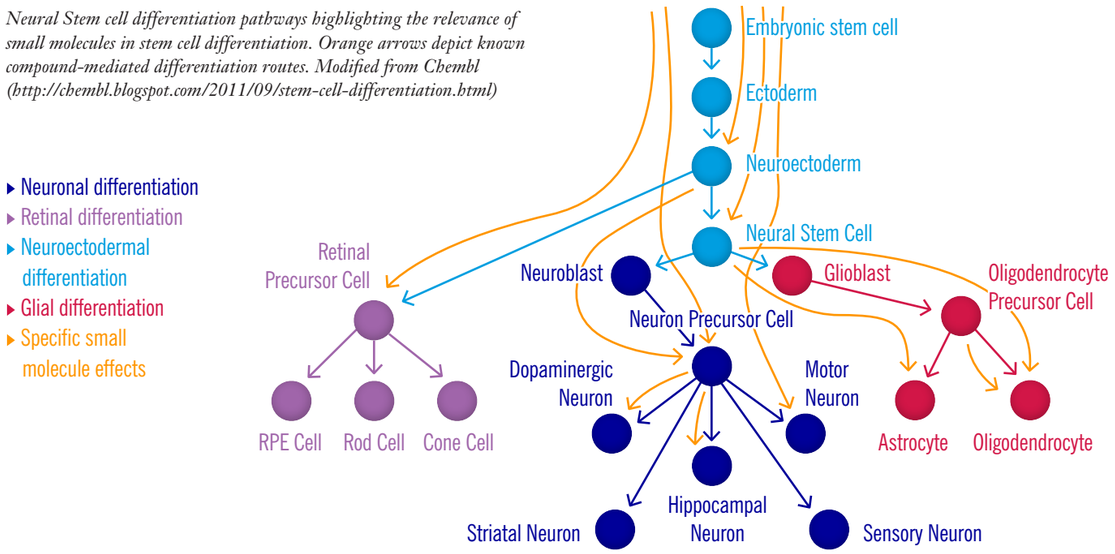
- Directed differentiation and purification of specific cell types from stem cells for drug discovery and toxicity screening (cardiomyocytes, hepatocytes, motor neurons, neural progenitor cells)
- Identification of small molecule compounds directing or enhancing the differentiation of stem cells into specific cell types (e.g. Purmorphamine as Shh mimic, gamma secretase inhibitors as neural differentiation enhancer [Notch pathway blockade], …)

In conclusion, multiple stem cell based screening scenarios are becoming applicable to neurodegenerative diseases.

- Phenotypic screening for compounds that enhance the generation of a desired specific neural cell type from ES cells, iPS cells or neural progenitor cells
- Screening for upregulators of neuroprotective proteins in stem cell derived neural cells, e.g. up-regulation of SMN (survival of motor neuron protein) relevant to SMA in motor neurons, or up-regulation of Hsp27 relevant to neuropathy conditions in sensory neurons and as general neuroprotector.
- Differential phenotypic screening with ES or iPS derived wild-type and mutant neural cells, e.g. striatal neurons carrying HD causing polyQ lengths vs. wildtype control.

Taken together, stem cell derived neural assays hold great promises for developing multiple neurodegeneration relevant assay scenarios, in particular when combining phenotypic approaches with modern tools for informed segregation of involved pathways. However, phenotypic screening is only the first step in the drug discovery process. Subsequent elucidation of the molecular mechanism of effective compounds is becoming mandatory. Evotec is a leading provider of quantitative chemoproteomics and the profiling of targets for pharmacologically active compounds. This, together with its expertise in medicinal chemistry ensures that the molecular targets of screening hits can be identified and optimized at Evotec.

Neural Stem cell differentiation pathways highlighting the relevance of small molecules in stem cell differentiation. Orange arrows depict known compound-mediated differentiation routes. Modified from Chembl (http://chembl.blogspot.com/2011/09/stem-cell-differentiation.html)



DR ANDREAS EBNETH

SHORT SUMMARY OF SCIENTIFIC CAREER

Dr Andreas Ebneth received his PhD in biochemistry from the University in Hannover, Germany, focusing on the biophysical characterization of oncogenic transcription factors. He conducted his postdoctoral studies at the Max Planck Unit for Structural Molecular Biology in Hamburg studying the role of the microtubule associated protein tau and its role in Alzheimer's disease. In 1998, Andreas joined Genion, a newly founded start-up CRO focusing on ion channel research where he was responsible for assay development and screening, and supported electrophysiology. He then joined Evotec Neurosciences and was involved in a collaboration with Takeda focusing on target identification and validation in Alzheimer's disease before taking over the position as a group leader supporting internal R&D activities. Since 2010 Andreas is VP Neurobiology at Evotec and responsible for the collaboration with CHDI in the field of Huntington's disease.

1. One key field of Evotec is neurodegenerative diseases/CNS. What is the expertise and history of Evotec in this area?

Evotec has been actively involved in drug discovery and development in neuronal diseases and in particular neurodegenerative diseases for close to a decade. In neurodegenerative disease the primary focus was Alzheimer's disease and then adding projects in HD through a collaboration with CHDI, Parkinson disease through a collaboration with the Michael J Fox Foundation and more recently also in MS as key member of the NEU^2 consortium. In AD, Evotec's main focus was on the development of a small molecule MAO-B inhibitor as well as the identification of new AD targets through one of the most comprehensive screening efforts conducted based on well characterized diseased patient derived tissue samples.

Based on these internal and collaborative efforts Evotec has built a highly sophisticated and integrated drug discovery platform for neurodegenerative diseases covering essentially all biological and chemical aspects from target identification/validation to lead identification/optimization as well as formal preclinical and clinical development.

2. How can you and Evotec contribute to finding new drugs and new targets in this field?

My major contribution is my scientific interest not only in neurodegenerative diseases but in particular in HD. HD is the major topic I spent most of my time with for the past 2–3 years. During this time I benefitted tremendously from the enormous knowledge and expertise from my colleagues at CHDI who are clearly the leaders when it comes to translating new insights from basic science into possible drug discovery approaches for HD. I am extremely excited about being part of their team and hope to contribute to their efforts by efficiently moving experiments from the drawing board into the hands of expert scientists and technicians in our laboratories. This managerial role coordinating many projects in parallel always ensuring that they get the attention they need and thereby achieve results in a very timely and cost effective manner is my other major contribution. Beyond this, it is my ambition to try and push the boundaries of what is technically feasible in order to overcome major obstacles associated with a very challenging disease.

3. In which project(s) are you currently involved?

Currently, I am overseeing the Biology part of about 10 individual projects within our collaboration with CHDI. These projects reach from target validation to advanced small molecule inhibitor projects. It would be beyond the scope of this interview to go into more detail. At Evotec I work closely together with my colleagues in Abingdon, Daryl Walter and Steve Courtney, responsible for the medicinal chemistry part of the projects. One of the most exciting projects I have been involved in is the high resolution and high throughput imaging of animal models of HD where promising targets are being validated with regard to their role in the aggregation of huntingtin in different brain regions. In this project Evotec on behalf of CHDI launched new and cutting-edge virus-based target validation technologies. Another exciting and more advanced program is targeting the tryptophan metabolism: here Evotec supports CHDI since a couple of years already and the project currently triggers quite some hope and enthusiasm based on recent promising results obtained in animal models of HD.

4. How will your collaboration with CHDI look like?

In my opinion our collaboration with CHDI is definitely one of the most exciting major collaborations currently actively pursued by Evotec. Due to its scope and enormous continuity over many years we have built exceptional teams that are highly integrated and extremely constructive and project oriented. Such a very intimate working relationship is instrumental to answer or address questions and problems in a very pragmatic and non bureaucratic fashion. Secondly, as CHDI is not running their own laboratories Evotec is solely responsible for the hands-on laboratory work and maintenance of an infrastructure to ensure not only proper support of ongoing projects but also to keep up a certain infrastructure, e.g. animal models even if they are not immediately needed. In summary, it is a great privilege to be working with a highly professional group of people who are leaders in their fields in such a focused fashion on HD.

evotec

1) INTEGRATED SERVICES

- Target-to-IND integrated platform
- Hit identification
- Medicinal chemistry
- Structural biology and computational chemistry
- *In vitro* and *in vivo* biology
- ADMET

2) ASSAY DEVELOPMENT AND SCREENING

- Assay development
- High throughput screening
- High content screening
- NMR and label-free screening
- Secondary screening and profiling
- Screening library
- Ion Channel drug discovery
- GPCR drug discovery

3) FRAGMENT-BASED DRUG DISCOVERY

- Proprietary high throughput fragment screening platform
- Biochemical, NMR and SPR screening technologies
- Fragment library
- Structural biology
- Computational chemistry
- Fragment optimisation

4) MEDICINAL CHEMISTRY AND EARLY DEVELOPMENT

- Medicinal chemistry
- Computational chemistry
- Structural biology
- Compound library synthesis
- Chemistry and early development support

5) ADMET

- *In vitro, in vivo* and *in silico*
- Safety pharmacology
- Metabolite detection

6) CELLULAR TARGET PROFILING AND PHOSPHOPROTEOMIC

- Cellular target profiling
- KinAffinity
- PhosphoScout
- Epigenetics target profiling
- Epigenomics analyses
- Quantitative proteomics analyses

7) COMPOUND MANAGEMENT

- Compound identification, selection, procurement
- High-throughput compound analysis
- Multi-format plating and reformatting
- Storage and processing
- Disaster recovery and business continuity

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Mario.Polywka@evotec.com
COO of Evotec

Imprint
Editor: *Evotec AG;*
Chief Editor: *Michael Bayer*
Content: *CHDI, Cord Dohrmann, Werner Lanthaler, Klaus Maleck, Andreas Ebneth, Heinz von der Kammer, York Rudhard, Michael Bayer*
Design: *alessandridesign*
Programming: *bgcc*

For any further questions regarding DDup or Evotec, please feel free to contact us at: Werner.Lanthaler@evotec.com