# IN VITRO

## PHARMACOLOGY, MICROBIOLOGY & TRANSLATIONAL SCIENCE

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### OFFERING

**The cornerstone of Evotec's *in vitro* pharmacology function is disease and target biology expertise coupled with state-of-the-art technology platforms. A large team of scientists with extensive industrial experience supports the *in vitro* pharmacological characterisation of compounds as part of hit expansion, lead finding and lead optimisation projects. Our team routinely generates project-relevant high-quality data in short turnaround times. In addition, we support *in vivo* studies with PD read-outs and engage in early translational biology research.**

**In addition to its extensive capabilities in mammalian biological systems, Evotec has in depth *in vitro* microbiology expertise spanning a broad range of pathogens from bacteria to fungi and viruses.**

Typical activities include:

- Development of biochemical and functional assays
- Secondary and tertiary characterisation of screening hits
- Compound profiling as part of hit-to-lead and lead optimisation programmes:
  - — Design and implementation of target-relevant assays (screening cascades)
  - — Potency and selectivity testing
  - — Mode of action studies (e.g. competitive versus allosteric mechanisms, reversibility, use-dependent mechanisms for ion channel modulators)
  - — Translational cellular assays to bridge the gap between *in vitro* and *in vivo* studies: testing of compound potency and mechanism using disease-relevant primary cells from rodents, primates or human
  - — Translational biomarkers: building assays in the discovery phase that allow measuring target engagement in patients and thus de-risking of clinical projects

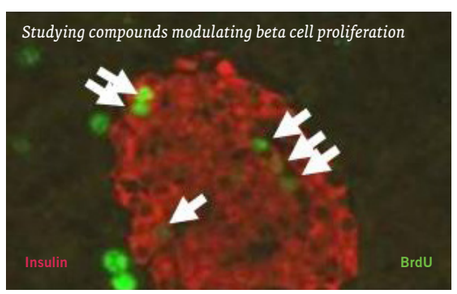
In more than 15 years of compound profiling at Evotec, >400 assays have been developed and executed. Such activities are part of Evotec's integrated lead finding and optimisation projects but are also frequently used to support medicinal chemistry projects that are executed in the labs of Evotec's partners.

### AVAILABLE READ-OUT AND ASSAY TECHNOLOGIES

Evotec has access to a wealth of assay technologies that can be utilised to assess compound activity. Appropriate technologies and expert teams are selected to answer key project questions and to ensure project advancement. Beyond standard and established read-out technologies for biochemical and cellular assays, Evotec has built key expertise in a number of technological areas that have shown to be drivers for the success of our partner's projects.
These include:

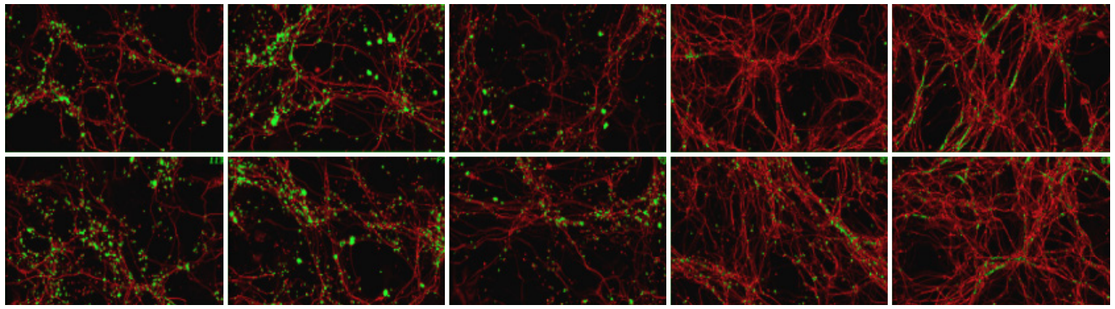
- The use of stem cells to derive neurons and primary neuronal cultures to build disease-relevant cellular models and to identify and characterise new compounds with disease-modifying properties

*Studying compounds modulating beta cell proliferation*




- An extensive knowledge of high-content screening and a state-of-the-art hardware platform to run complex and disease-relevant imaging assays, e.g. using primary neuronal cultures, kidney cells, muscle cells as well as rodent and human beta cells
- A world-leading ion channel discovery platform including fluorescence-based assays, automated and manual patch clamp methods
- An excellent suite of biophysical methods including SPR and NMR that are utilised as part of our structure-based drug design projects but also LC-MS-based methods that are utilised to assess difficult-to-assay enzyme targets
- In the oncology area, the setup of integrated assays based on tumour cells, fibroblasts, macrophages, endothelial cells, or adipocytes in 2D, co-culture or 3D culture to recapitulate tumour and microenvironment conditions
- The use of extensive read-outs, from signalling to metabolic pathway engagement, to develop new ways to address pathologies and overcome resistance to treatments

*Neuroprotective compound*



**Read-out technologies**

**CELL-BASED ASSAY TECHNOLOGIES**

- Fluorescence read-outs
  - — HTRF, standard dyes, ligand binding
- Second messengers (Ca2+, cAMP, IP3)
- Membrane potential (GPCRs, ion channels and transporters)
- Manual and automated patch clamp
- Reporter gene assays
- ELISA (standard and Mesocale)
- Imaging (HCS)
  - — OPERA®, Operetta, Zeiss and ArrayScan
- Radioactive binding and uptake
- Flow cytometry and cell sorting
- Migration and invasion assays
- Whole cell blood assays
- Metabolic analysis (seahorse, metabolomics, mitochondrial OXPHOS function)
- Primary cell culture
- Stem cells
- Established cell lines
  - — Co-culture
  - — 3D culture

**BIOCHEMICAL ASSAY TECHNOLOGIES**

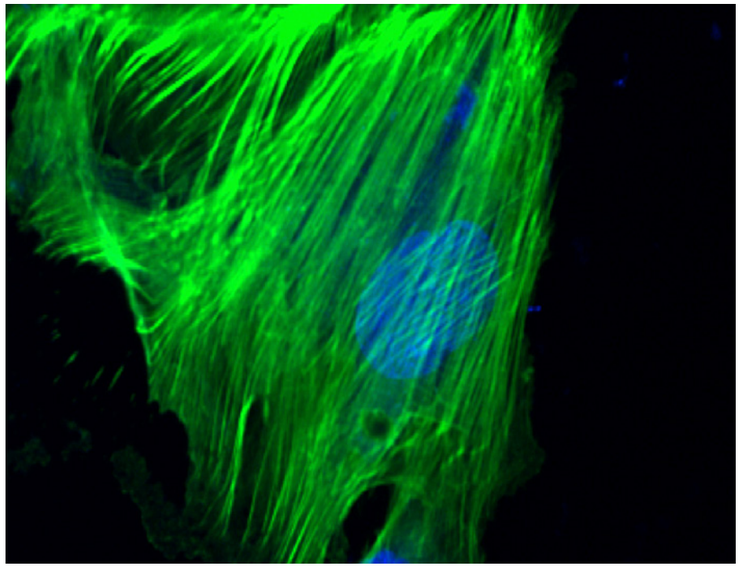
- FCS+plus
- Fluorescence polarisation
- Fluorescence intensity
- HTRF/Delfia
- AlphaScreen
- ELISA
- Mesoscale electrochemiluminescence
- Singulex single protein molecule counting
- Luminescence
- LC/MS

**BIOPHYSICAL READ-OUT TECHNOLOGIES**

- Surface Plasmon Resonance (SPR)
- Mass Spectrometry (LC-MS)
- Nuclear Magnetic Resonance (NMR)
- Radiometric
- Thermal Shift

## OVERVIEW TARGET CLASSES

Our in-depth experience in the biology and pharmacology of disease-relevant target classes is what our partners come to us for. This expertise is a key driver to the successful and rapid execution of lead finding and optimisation processes. Evotec's *in vitro* pharmacology team has gained expertise across a wide area of disease targets. Beyond a large number of projects that have successfully been run in the classical target areas such as GPCRs, ion channel and kinases, we have also worked on a large diversity of other target areas such as transporters, protein-protein interactions and multiple enzyme families, including novel target classes such as epigenetic regulators and immune and metabolic pathways modulators.



*Identifying cpds that protect podocytes in chronic kidney disease*

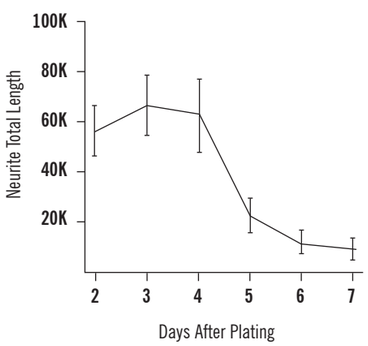
## DISEASE EXPERTISE, TRANSLATIONAL ASSAYS AND BIOMARKERS

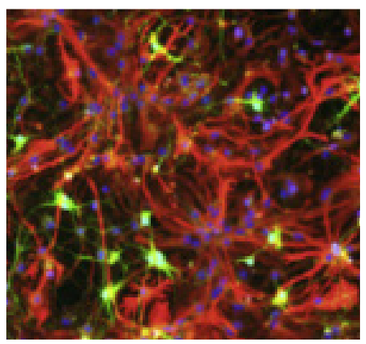
Evotec's core expertise includes areas such as CNS, neurodegeneration, pain, inflammation, metabolic disease, oncology and immunology. Our scientific expertise and understanding of disease mechanisms combined with our track record in setting up relevant *in vitro* models and translational biomarker assays for pre-clinical and clinical use are a key factor in our success when working with our partners.

Setting up complex *in vitro* assays utilising rodent or human primary cells to confirm the potency and mechanism of lead compounds is a prerequisite to build confidence in the translatability of any mechanism. We build these assays early in the drug discovery process to gather disease-relevant data before assessing compound efficacy *in vivo*. Such assays are also utilised to identify read-outs for *in vivo* target engagement. We routinely utilise various neuronal and stem cell cultures, pancreas and beta cells, kidney cells and various immune cells, tumour cells, endothelial cells, fibroblasts, adipocytes and muscle cells to assess bespoke read-outs and mechanisms, including cell health and survival, metabolism, apoptosis, differentiation, de-/regeneration; neurite outgrowth, retraction and synaptic density; cellular signalling and secretion.

A key challenge for bringing a new therapy to the market is to provide proof of efficacy in the clinic and to reduce the risk of a failed trial due to inadequate clinical read-outs. Robust and objective biomarker read-outs that are applicable to clinical samples are therefore needed to stratify patient populations, quantify disease progression and to demonstrate target engagement and dosing response. Together with our partners, we utilise state-of-the-art single molecule counting Singulex technology to routinely develop, validate and apply ultra-sensitive protein biomarker quantification read-outs for clinical samples such as human blood, plasma and CSF.

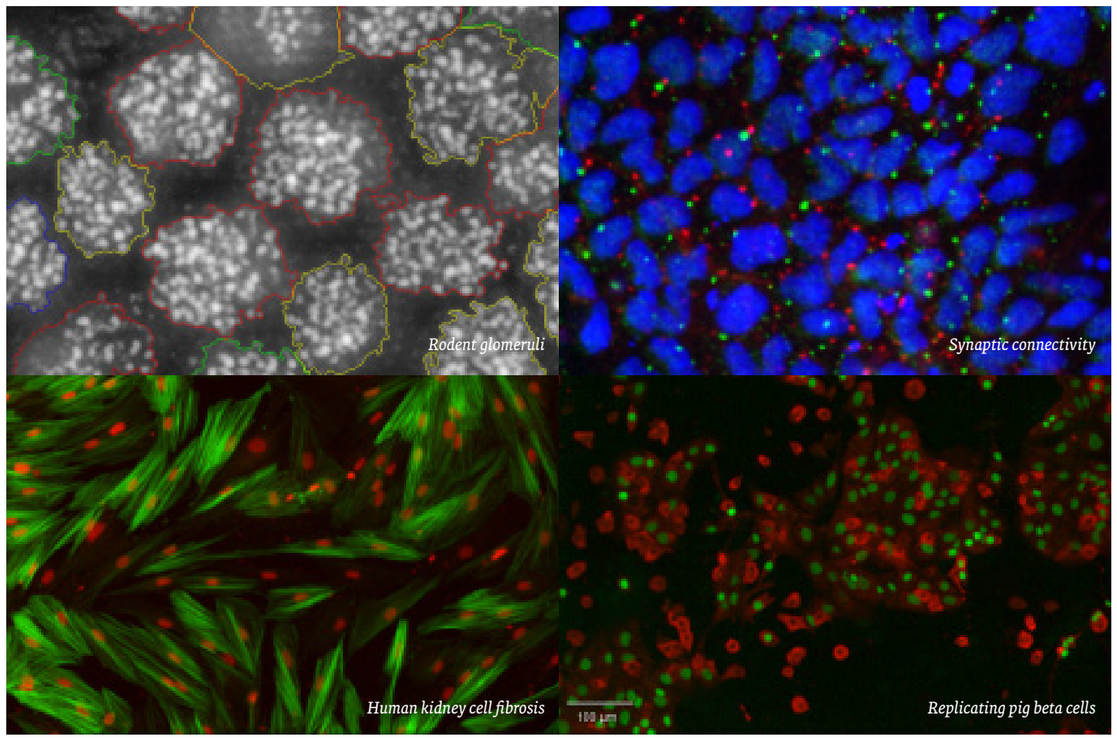
*Co-culture system of stem cell derived motoneurons and astrocytes together with microglia to identify cpds for the treatment ALS*



D3 After Plating GFAP/GFP/DNA

**Extensive expertise with translational cellular assays using primary cells and tissues to investigate compound potency and mechanism**




### ISOLATION, CULTIVATION AND MANIPULATION OF PRIMARY CELLS

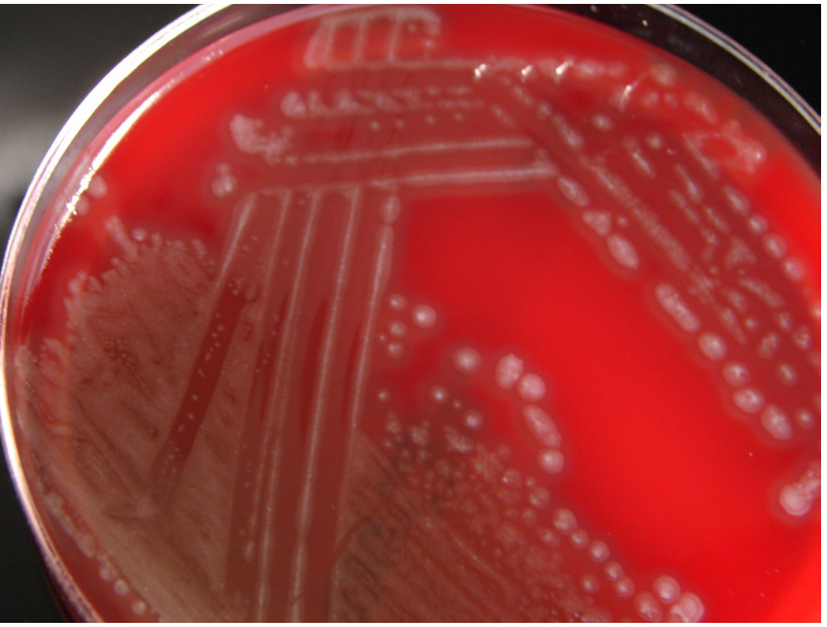
- Neurobiology: neurons (CNS, DRGs, motoneurons), astrocytes, microglia including co-cultures
- Blood: PBMC, TH1/TH2 populations, B cells
- Pancreas: islets, beta cells
- Kidney: podocytes, glomeruli

### ASSAY READ-OUTS

- Cell density, degeneration, regeneration, survival
- Cytokine secretion
- Transcrptional activity
- Protein phosphorylation

### ASSAY TECHNOLOGIES

- Imaging
- MSD
- LC-MS
- Standard methods



*Streptococcus pyogenes cultured on Columbia Blood Agar*

## MICROBIOLOGY

**Evotec's specialist group in the infectious disease therapeutic area boasts state-of-the-art microbiology facilities including a unique and highly characterised strain bank, EVOStrAIn™. Our team provides bespoke anti-infective drug discovery and development services to a growing number of global partners and has an established track record in collaborating to discover and develop new therapies and vaccines to treat and prevent serious and life-threatening infections resulting from multi-drug resistant pathogens including Gram positive and Gram negative bacteria, including the ESKAPE organisms, fungi, and viruses.**

The group's offering is fully integrated with the wider discovery platform at Evotec enabling either a standalone microbiology service or a fully integrated anti-infective drug discovery capability. Core strenghts of Evotec's microbiology group fall into the four areas of EVOStrAIn™, Microbiology, Pharmacology and ADME/PK/PD as follows:

EVOStrAIn™ is a highly valuable collection of clinical isolates that can be used to establish the activity profile of lead compounds and candidates. A key feature is that the isolates are highly characterised and, in many cases, mechanisms of resistance defined. EVOStrAIn™ contains an extensive range of geographically diverse human bacterial and fungal pathogens that cover isolates susceptible and resistant to current antimicrobial drugs.

We employ industry-standard methods such as CLSI, EUCAST and BSAC to test compounds for antimicrobial activity against strains and clinical isolates from EVOStrAIn™, or strains provided by our collaborators. This includes the ability to conduct whole-cell screening for antimicrobial activity for hit identification in an HTS format, MIC, MBC/MFC, time-kill and PAE studies using single or combinations of agents, hollow fibre PK/PD or bioreactor human cell systems for detailed profiling for characterisation of novel anti-infective agents, and compound/drug combination studies for assessment of synergistic, antagonistic and additive effects. Bespoke methods for susceptibility profiling can be developed for testing novel agents where standardised methods may not be appropriate. In parallel, mechanism of action determination studies and resistance frequency assays can be performed.

**EvostrAIn™: A collection of highly characterised clinical isolates available to establish a detailed activity profile of lead compounds, both *in vitro* and *in vivo* models of infection**

| BACTERIA: Gram positive pathogens | BACTERIA: Gram negative pathogens | FUNGI |
|---|---|---|
| *Staphylococcus aureus* including MRSA, VISA and VRSA strains | *E. coli* including Extended Beta lactamase producing strains | *Aspergillus* spp. (including strains resistant to azoles, polyenes and echinocandins with known mechanisms of resistance) |
| β-Haemolytic *streptococci* groups A, B, C and G | *Klebsiella pneumoniae* Carbapenemase producing strains (KPCs & MDR) | *Candida* spp. (including strains resistant to azoles, polyenes and echinocandins with known mechanisms of resistance) |
| *Streptococcus pneumoniae* (including penicillin, macrolide, fluoroquinolone, cephalosporin and MDRSP resistant strains) | *Acinetobacter baumannii* including MDRAB | *Mucorales* |
| Vancomycin Resistant *Enterococci* (VRE) | *Pseudomonas* spp. including multi-resistant strains | *Cryptococcus* |
| *Bacillus* species | *Haemophilus influenzae* | Dermatophytes including *Malassezia* spp and *Trichophyton* spp |
| *Listeria* species | *Bacteroides* spp. | Fusarium |
| *Corynebacterium* and *Propionibacterium* species | *Neisseria gonorrhoeae* and *N. meningitidis* | **Protozoa** *Acanthamoeba* spp |
| *Clostridium difficile* (multiple ribotypes including 012, 027 and 078) | Intestinal pathogens: *Vibrio* spp, *Campylobacter* spp, *Salmonella* spp, *Shigella* spp, *Yersinia* spp. | |
| Other *Clostridia* (including *C. perfringens*) | *Legionella* spp. | |
| | Mycobacterium | |