IN VIVO

PHARMACOLOGY

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OFFERING

Evotec has developed considerable *in vivo* pharmacology expertise in our key therapeutic areas of CNS, metabolic disease and complications, oncology and infectious disease. Our *in vivo* pharmacology team (> 70 staff), consisting of highly experienced scientists with extensive biopharmaceutical and drug hunting expertise, supports *in vivo* studies from early target validation through to candidate selection. Furthermore, we have the flexibility to support defined stand-alone studies in addition to *in vivo* work conducted as part of larger integrated drug discovery programmes. All experimental procedures involving animals are carried out to the highest standards of animal welfare in state-of-the-art facilities and approved according to European Union and national regulations.

Evotec will work with you to identify the most appropriate *in vivo* pharmacology solution for your drug discovery programme; whether that's an off-the-shelf standard protocol or developing bespoke assays / read-outs for your specific requirements. We utilise a plethora of acute, mechanistic, pharmacodynamic models relevant to the target biology to assess target engagement and establish PK / PD relationships. Our efficacy models are used to assess biological effects on pathophysiology's relevant to the disease of interest and are validated with appropriate standards of care and supported by rigorous statistical methods. At Evotec we are continually striving to develop *in vivo* models and endpoints with greater translational relevance to the clinic and all our models can be supported by relevant pharmacokinetic measurements to determine exposure to effect relationship. Importantly, Evotec's *in vivo* pharmacology functions are typically co-localised with our *in vitro* pharmacology units and this complementarity leads to disease biology platforms with a stronger disease focus and breadth of expertise.

CENTRAL NERVOUS SYSTEM (CNS) ACUTE / CHRONIC PAIN

Evotec offers a range of acute and chronic pain models in rodents with different read-outs depending on the model of interest. Importantly, our scientists are fully blinded to reduce the potential for operator bias.

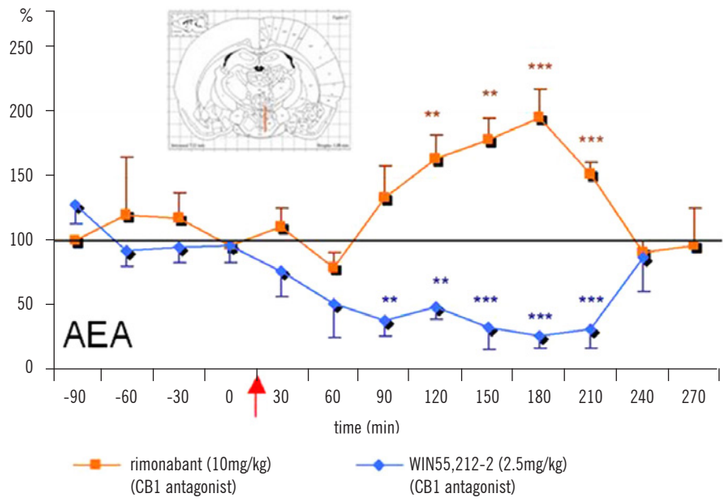
Pain Models

Spinal Nerve Ligation model (SNL); Visceral pain: colorectal / vaginal distension-induced visceromotor reflex; CFA-induced Inflammatory pain: Formalin induced nocifensive behavior, mustard-oil induced neurogenic inflammation

Key Read-outs

- Thermal sensitivity: Hargreaves Test, hot plate, tail flick
- Mechanical sensitivity: Von Frey test, pressure application measurement device, dynamic weight bearing
- Laboras automated recording for formalin paw and locomotor activity
- Visceral motor reflex-EMG activity

Microdialysis reveals differential kinetic profiles of endocannabinoid release in the hypothalamus upon treatment with a CB1 agonist. Red arrow indicates dosing of drug.



NEURODEGENERATIVE DISEASE

Evotec offers phenotypic screening in a variety of models of neurodegeneration, primarily in transgenic animals for Chorea Huntington and for Alzheimer's disease.

Key Read-outs

- Locomotor activity (rotarod)
- Emotion (Elevated Zero Maze, fear conditioning)
- Cognition (Novel Object Recognition, spatial memory)
- Irwin test
- Pre-Pulse Inhibition
- Gait analysis

ADENO-ASSOCIATED VIRUS (AAV) TARGET VALIDATION PLATFORM

Neonatal and adult (stereotaxic) injections into specific brain regions in rodents using AAV-vectors designed for knock down or over-expression of specific proteins to support target identification.

Key Read-outs

- Behavioral assays, histology and immuno-histochemistry (IHC), morphometric analysis
- High-content IHC analysis
- Biomarkers (e.g. cytokines using Mesoscale)

EX VIVO RADIOLIGAND BINDING AUTORADIOGRAPHY (ARG) PLATFORM

Beta Imager allows fast screening, Phosphoimager for high resolution images. Multiple applications:

- KD and Bmax determinations in specific tissue regions
- Receptor / protein localisation

Functional responses:

- GTPγS radioligand binding as a marker of G protein-coupled receptor (GPCR) receptor activation
- *Ex vivo* receptor occupancy to determine target engagement

ONCOLOGY

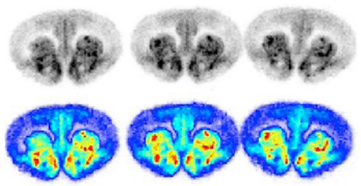
Evotec offers a range of rodent models, spanning xenograft, syngeneic and orthotopic models coupled with the possibility of a broad range of efficacy and biomarker read-outs. In addition, we have access to a range of relevant human tumour tissues via the Institut Universitaire du Cancer Toulouse Biobank which can be utilised for biomarker studies or *ex vivo* evaluations including compound treatment.

Oncology models

Subcutaneous human xenograft models. Syngeneic models suitable for the evaluation of immuno-oncology therapies Orthotopic models including: Bladder, brain, breast, colon, kidney, liver, lung ovary, skin. Suitable for metastasis studies. Angiogenesis models (in mice and rats), with Laser Doppler

Adjacent sections (20 µm), male Sprague Dawley rat, 8 weeks old

1 nM ^{3}H-naloxone



Key Read-outs

Efficacy evaluation by tumour volume and / or Bioluminescence (IVIS Spectrum) imaging for 3D tomography and metastasis.

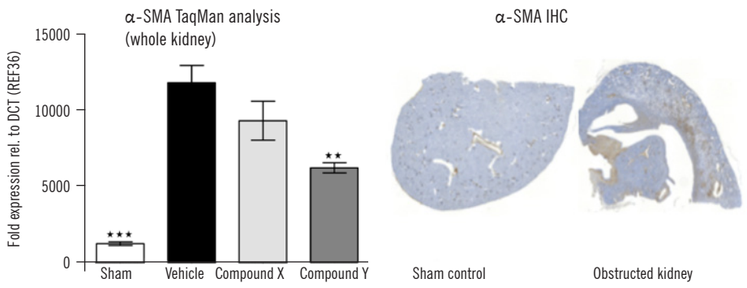
- Full Hematological analysis including FACS analysis
- Bioanalysis for associated pharmacokinetic studies (PK / PD)
- Tissue sampling for:
  - Genomics studies including: RT-PCR; Taqman; TLDA
  - Protein analysis (total and phosphorylated protein profiling)
  - ELISA, Western Blotting, dot-blotting, histology, immunohistochemistry
  - Quantitative image analysis (Definiens technology)
- Mitochondrial metabolism:
  - Cellular ATP generation: Glycolytic vs mitochondrial pathways
  - Oxygen Consumption Rate (OCR) and Extracellular acidification rate (ECAR)
  - Oxphography: a way to analyse the isolated mitochondrial function
  - Mitochondrial membrane potential, respiration rat, phosphorylation rate
  - Metabolite analysis for quantification of lactate, glucose, glutamine, glutamate
- Microdialysis
  - Detection of metabolities and/ or drug levels by *ex vivo* microdialysis in tumour, brain, adipose tissue or biological fluids

METABOLIC AND DIABETIC COMPLICATION

Metabolic diseases like Diabetes mellitus, obesity or the metabolic syndrome belong to the major chronic diseases in the world. Long term Diabetes mellitus results in a number of severe diabetic complications impairing kidney, eyes, heart and peripheral nerve function.

Animal models of metabolic diseases include genetic models (ie. ZDF rats, Ins2 Akita mice), diet-induced models using different diets, chemically-induced models with Streptozotocin (STZ) or Alloxan as well as a humanised beta-cell model where human islets are transplanted into STZ-treated diabetic NOD-SCID mice.

UUO results in the upregulation of the fibrosis marker α-Smooth Muscle Actin
UUO as a model for interstitial fibrosis



Chronic kidney disease (CKD) is a progressive loss in renal function over a period of months or years resulting from e.g. Diabetes mellitus or genetic predisposition. In order to identify new therapeutic options clinically relevant animal models for acute and chronic kidney disease have been developed which include surgery models (e.g. Acute and Chronic Ischemia Reperfusion Injury models (IRI, CRI), Unilateral Ureter Obstruction model (UUO), genetic models of Diabetes mellitus (ie. BTBRob / ob mice on different diets, Ins2-Akita mice) and models of chemically induced Diabetes mellitus with STZ and combinations thereof.

Relevant animal models of eye disease for proliferative and diabetic retinopathy have been developed and validated. These include models of chemically induced diabetes (ie. STZ) as well as a model of Oxygen Induced Retinopathy (OIR).
All metabolic and diabetic complication animal models are run under standardised conditions and profiled with a variety of supporting **read-outs:**

Standard Read-outs

Standard clinical and metabolic read-outs including functional glucose/insulin tolerance test; ex vivo read-outs including RT-PCR, biochemical assays, hormonal status and histology.

Advanced Read-outs

Hyperinsulinemic euglycemic clamp, food intake profiles, pairfeeding, body composition analysis by Nuclear Magnetic Resonance (NMR) spectroscopy, morphometric analysis with customised algorithms e.g. with pancreas and adipose tissue, blood pressure analysis, assessment of Glomerular Filtration Rate (GFR).

Biomarker approaches

Plasma and tissue analysis (e.g. ELISA, quantitative RT-PCR), high-content tissue imaging for bright as well as immune histochemistry, single and multiplexing on the Mesoscale platform, e.g.cytokines, mass spectroscopy (ie. LC-MS/MS).

INFECTIOUS DISEASE

Evotec specialises in assessing the efficacy of lead and candidate compounds in highly relevant and validated models of infection. Our extensive range of established models are well-suited to the development of multiple classes of agent including small molecules, natural products, peptides, antibodies, other biologics and vaccines. We provide a highly bespoke service, customising studies to meet the exact requirements based on programme needs and parameters / endpoints of interest.

Models of Infection

For efficacy assessment against bacterial and fungal infections, our models address different sites of infection (localised and systemic) and pathogens by Gram-positive (including Staphylococcus aureus, Streptococcus pneumoniae, and others), Gram-negative (including Pseudomonas aeruginosa, Escherichiacoli, Acinetobacter baumanii and others), anaerobes (including Clostridium difficile) or fungal species (including Candida sp., Aspergillus sp., Mucorales, Malassezia sp.).
In particular, Evotec specialises in PK/PD profiling and modelling of antimicrobial agents in multiple disease models in order to understand the key drivers for efficacy and translation of data into clinical trial design.

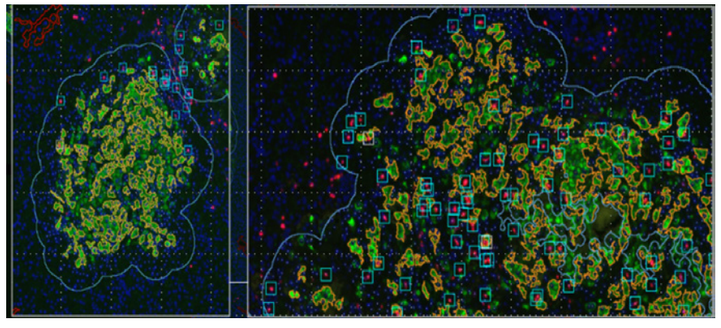
Key Read-outs

Efficacy evaluation by:

- Burden at site of infection and in multiple biological matrices including quantitative culture, QPCR and biomarkers
- Microbiome analysis and sequencing
- Bioluminescence (IVIS Spectrum) imaging.
- Host response endpoints including ELISA, Western Blotting, dot-blotting, histology, immunohistochemistry, cytokine profiling and cytometry
- Bioanalysis for associated pharmacokinetic studies (PK/PD) and pathogen-associated biomarkers

HISTOLOGY, IMMUNOHISTOCHEMISTRY AND MORPHOMETRIC ANALYSIS PLATFORM

The combination of *in vivo* pharmacology with histology techniques offers a powerful tool to further analyse disease relevant biomarkers. Whereas histology and IHC helps to identify disease and target relevant changes, automated morphometric analysis can yield unbiased quantitative information amenable to statistical analysis, which adds significant value to the customers drug discovery effort. Furthermore, histopathology on critical organ systems will allow early risk assessment.



Immunohistochemistry and morphometric analysis of male ZDF rat pancreas: Islet of Langerhans fragmentation in male ZDF rats