Metabolic Disease drug discovery at Evotec
Evotec, an ideal partner in metabolic disease drug discovery

The different ways to work with us

<table>
<thead>
<tr>
<th>On your specific target or programme</th>
<th>Starting from a phenotypic assay concept</th>
<th>On an existing Evotec programme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access to Evotec drug discovery expertise and capabilities to support your programme</td>
<td>Access to Evotec phenotypic screening expertise followed by target deconvolution leading into a drug discovery programme</td>
<td>Sponsor an established theme in the metabolic disease space</td>
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</tbody>
</table>

Flexible commercial solutions: multiple business models available to suit our partners

Access to expert discovery platform as *stand-alone activities* or as part of *integrated drug discovery programmes*
A leading platform for rapid progress and increased success of your programme

Metabolic disease research platform

<table>
<thead>
<tr>
<th></th>
<th>1. Experienced metabolic disease drug discovery team with &gt;55 FTEs</th>
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<tbody>
<tr>
<td>2</td>
<td>Extensive expertise in metabolic disorders such as diabetes, obesity, metabolic syndrome, kidney and diabetic complications with particular attention placed on beta cell regenerative and insulin sensitivity approaches as well as energy dissipation in peripheral tissues</td>
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<tr>
<td>3</td>
<td><em>In vitro</em> pharmacology platform covering islet, kidney, muscle, hepatic, intestinal and adipose tissue-based mechanisms</td>
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</tbody>
</table>
| 4 | Extensive portfolio of drug discovery capabilities:  
- Phenotypic screening and cellular target deconvolution  
- HCS and HTS capabilities  
- Integrated medicinal chemistry, ADMET & DMPK support  
- Hit finding & library screening  
*In vitro*, *ex-vivo* & *in vivo* biology  
- Maximally automated histology, imaging and image analysis platforms |

15 years experience in metabolic diseases drug discovery, starting from target ID up to pre-clinical development to support our partners through innovative solutions
In-depth expertise across early phases of metabolic disease research

Overview on Evotec’s projects success in metabolic diseases

<table>
<thead>
<tr>
<th>Target/Partner</th>
<th>Target ID/validation</th>
<th>Hit ID</th>
<th>H2L/LO</th>
<th>Pre-clinical &amp; clinical</th>
</tr>
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<tbody>
<tr>
<td>DPP-IV/Biotech</td>
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<tr>
<td>Enzyme target/Biotech</td>
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<tr>
<td>5-HT2C/Boehringer</td>
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<tr>
<td>Enzyme target/Pharma</td>
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<tr>
<td>Enzyme target/Biotech</td>
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<td></td>
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<tr>
<td>β cell mass (MI)/AZ/MI</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Insulin sensitization/Boehringer</td>
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<td></td>
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<tr>
<td>Enzyme target/Biotech</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>β cell differentiation/Evotec R&amp;D</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Energy dissipation/Evotec R&amp;D</td>
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<tr>
<td>Target EEM/Harvard</td>
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</table>
Integrating technology and disease expertise for successful Target ID and validation

Target identification and validation

- Deep sequencing
- Phenotypic screening
- Customer or literature candidates or sequencing data
- Bioinformatics; pathway analysis
- Expression profiling
  - Human or rodent tissues
  - Disease vs. normal

- Cellular Target Profiling®

**In vitro validation**
- Gain and loss-of-function (RNAi)
- Lentiviral, plasmid...
- Stable or transient
- Pharmacological
- Disease relevant primary cells or cell lines

**In vivo validation**
- Gain- or loss-of-function via AAVs
- Adenovirus
- Analysis of genetic models

- Target-based drug discovery
- Human or in vivo disease model tissues and cells

Human or in vivo disease model tissues and cells

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Metabolic disease expertise combined with process excellence

In vitro Pharmacology

- High-content cellular analysis applied to phenotypic screens for new target classes in diabetes, obesity and chronic kidney disease
- Assay development both for screening and validation
- Cell line and primary cell models such as adipocytes, myocytes, hepatocytes and kidney established
- *Ex vivo* primary islet, kidney and muscle preparations from animal and human for compound profiling in physiologically most relevant environment
- Viral genetic modification for mechanism-of-action studies

1. Provide metabolic disease relevant data in relevant cellular models enabling earlier and better decision making

2. Translation from animal to human models for early target and hit selection
**Exploration of disease relevant phenotypes *in vitro***

Imaging capabilities at Evotec

Ten years of expertise with the OPERA HCS drug discovery

**Automated fluorescence microscopy** for high-quality immuno-cyto-chemistry combined with 3D deconvolution and customized script-based analysis

Single cell analysis and sorting using **flow cytometry** and FACS

**IncuCyte live cell imaging** system for time lapse microscopy under cell culture conditions (TC-flask to 384-well microplate format)
Ideal tool to study human fat cell biology and insulin signalling

Human SGBS adipocytes: A primary-like pre-adipocyte cell strain ¹)

- Retained adipogenic potential for 50 generations
- Supply not limited
- Serum free adipogenesis protocol
- Pattern and time-course of gene expression during differentiation comparable to human primary adipocytes
- Glucose transport, lipogenesis and lipolysis are functionally not distinguishable from other primary human adipocytes
- Several insulin resistance models established ²)

“Differentiated SGBS cells behave biochemically and functionally like human adipocytes differentiated in primary culture. Therefore, this cell strain represents a new useful tool for the study of human fat cell development and metabolism in vitro.” ³)

¹) Fischer-Posovszky et al., Obesity Facts 2008; 1:184–189
²) insulin resistance induced by chronic insulin pre-incubation. TNFα, and THP-1 macrophage induced
³) Wabitsch et al., Int. J. of Obesity (2001) 25, 8-15
Evotec

**Beta cell biology – a key strength of the Evotec metabolics group**

Covering all mechanisms regulating functional beta cell mass

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**Beta cell replication**

*HCS for induction of primary beta cell replication*

- Beta cell replication (as well as alpha cell replication) quantification in dispersed rodent islets
- Beta cell replication in intact human islets (dispersion and staining after incubation)

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**Beta cell differentiation**

*De-differentiation T2D model in dispersed rat islet cells*

- Glucolipotoxicity induces progressive loss of key beta cell markers such as MafA, Pdx1, Nkx6.1, and insulin in T2D patients and diabetic animal models
- MafA transcription factor is a highly sensitive marker of glucolipotoxic stress in vitro

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**Beta cell apoptosis**

*Islet inflammation in dispersed rat islet cells*

- Primary rat islet cell apoptosis induced by cytokines or glucolipotoxicity
- Specific beta or alpha cells apoptosis quantification using TUNEL reaction (DNA-fragmentation) and cell count

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**Quantification of beta and alpha cell replication in human islets**

**Glucolipotoxic loss of MafA staining in rat beta-cells**

- 48 h 25 mM gluc. + vehicle
- 48 h 25 mM gluc. + 250 µM palmitate

**Script-based analysis of apoptotic beta cells**

- 48 h control
- 48 h cytokine treatment

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**In vivo Pharmacology for successful target validation and drug discovery**

Proprietary and validated assays

1. Adenovirus (AV), Adeno-Associated Virus (AAV) as *in vivo* validation tools
2. Establishment of bespoke assays to support PK/PD relationships for integrated drug discovery programmes
3. Comprehensive portfolio of application routes: po, iv, ip, sc, icb, im and infusion (ALZET pumps)
4. Acute and subchronic studies in a variety of animal models including biomarker approaches
5. From standard readouts to advanced metabolic phenotyping

**Determination of insulin sensitivity via hyperglycemic-euglycemic clamping in mice**

**Morphometric analysis of pancreas sections**

**ZDF rats: oGTT after three weeks treatment**
World-class histology work flow and imaging technology for *in vivo* studies

Bright field and fluorescence based morphometry

*In vivo* experiments
- Transgenic animals
- Drug treatments
- Disease models

Tissue preparation

Histology & Immunohistochemistry

Automised whole slide scanner and automated script analysis

**Characterise experiment by statistical analysis**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Compound</th>
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<tbody>
<tr>
<td>β-cell</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>non-β-cell</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Data quantification

Analyse cellular structures
Medicinal chemistry with strong expertise in addressing metabolic targets

Synthetic and medicinal chemistry

- Rapid synthetic execution & ability to address difficult chemistry
- Outstanding computational chemistry and structural biology
- Strong expertise in SBDD and optimisation of phenotypic screening hits
- Comprehensive in vitro ADME, in vivo PK and in vitro cardiac safety capabilities
- Largest chemistry group in the UK (>150 synthetic, medicinal and computational chemists), >35% of our scientists have >8 years prior experience at major Pharma and biotech companies

Effective delivery of clients’ objectives
Over 30 pre-clinical candidates nominated and 20 compounds approved for clinical trials across all therapeutic areas

Added value
Evotec medicinal chemists are named inventors on >275 client patents covering all major target and therapeutic areas
Case study: Metabolic disease integrated project

From target ID to *in vivo* efficacy proof-of-concept

<table>
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<tr>
<th>Partners</th>
<th>Programme</th>
<th>Target</th>
<th>Starting point</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Evotec-DeveloGen joint-venture</td>
<td>Integrated project</td>
<td>EVT244 lipid mediator synthetic enzyme</td>
<td>Target ID</td>
<td>Orally available compounds with potent glucose lowering effects</td>
</tr>
</tbody>
</table>

- Target ID in genetic screen and validation *in vitro* & *in vivo*
- HTS, structure guided optimisation, PK, *in vitro* & *in vivo* pharmacology

**EVT244 protein expression in WAT**

**EVT244 enzyme co-structure with inhibitor molecule**

**Random fed blood glucose**

**Relative Bodyweight**
Collaboration focused on the treatment of metabolic disease
Potent and selective 5-HT$_{2C}$ agonists were developed with the assistance of in silico methods
The reduction of hERG activity was a key optimisation criteria for the project
Activity in an in vivo weight loss model was demonstrated for compounds from the lead series (e.g. compound A)

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<td>Boehringer Ingelheim</td>
<td>Hit to lead optimisation</td>
<td>5-HT$_{2C}$</td>
<td>HTS</td>
<td>Advanced leads</td>
</tr>
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• Collaboration focused on the treatment of metabolic disease
• Potent and selective 5-HT$_{2C}$ agonists were developed with the assistance of in silico methods
• The reduction of hERG activity was a key optimisation criteria for the project
• Activity in an in vivo weight loss model was demonstrated for compounds from the lead series (e.g. compound A)

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<tr>
<th>Compound #</th>
<th>A</th>
<th>B</th>
</tr>
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<tbody>
<tr>
<td>5-HT$<em>{2C}$ EC$</em>{50}$ (nM) / Emax (%)</td>
<td>95 / 76</td>
<td>8.4 / 99</td>
</tr>
<tr>
<td>Selectivity 5-HT$<em>{2A}$ / 5-HT$</em>{2B}$ (fold) &gt;30* / &gt;5*</td>
<td>90 / 9</td>
<td></td>
</tr>
<tr>
<td>hERG (% inhib @ 10 µM)</td>
<td>93</td>
<td>11</td>
</tr>
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1) Weak partial agonist

**Case study: Morphometric analysis of the pancreas**

Evaluation of drug candidate inducing beta cell replication

- One week treatment by QD sc injection
- Pancreas fixation and paraffin sectioning
- Automated staining, image acquisition, and image analysis of tissue sections
- Robust quantification of
  - islet size
  - relative islet area
  - replication rate of beta & non beta cells

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<tr>
<td>Undisclosed</td>
<td>Target validation and compound optimisation</td>
<td>Receptor</td>
<td>Compound with good tolerability</td>
<td>Confirmation of selective beta cell pro-replicative effect</td>
</tr>
</tbody>
</table>

**Image analysis of a mouse pancreas section stained against insulin (green) and BrdU (red) as replication marker**
- Red line: Pancreas tissue
- Yellow line: Insulin positive area
- Yellow dots: Beta cell nuclei
- White dots: Non beta cell nuclei
- White squares: Replicating beta cell nuclei
- Blue squares: Replication non beta cells
Case study: Natural extracts as insulin sensitizers

Phenotypic in vitro screen in SGBS adipocytes

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<tbody>
<tr>
<td>Undisclosed</td>
<td>Phenotypic screen</td>
<td>Insulin resistance</td>
<td>Assay feasibility</td>
<td>Hit ID</td>
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</tbody>
</table>

- Screening for rescue of insulin sensitivity in partially insulin-resistant human SGBS adipocytes
- Scope: Assay development, screen, hit validation
- Partial insulin-resistance induced by chronic insulin treatment
- Assay: Radiometric insulin stimulated glucose uptake (ISGU)
- 160 natural extracts tested in 2 concentrations
- Treatment with 21 of 160 extracts resulted in significantly increased glucose uptake

![Graph showing insulin resistance through chronic insulin exposure](image)
Why us?

Evotec – The right partner in metabolic disease drug discovery

| A track record of success means that we consistently deliver on our clients’ needs |
| State-of-the-art capabilities and scientific excellence will maximise your chances of success |
| Fully integrated drug discovery platform and project management expertise will accelerate your drug discovery programme |
| Evotec is a low-risk outsourcing partner who is continually investing in its platform to the benefit of the customer |

Flexible commercial solutions: multiple business models available to suit our partners