# Evotec, an ideal partner in Immunology & Inflammation drug discovery

The different ways to work with us

## On your specific target or programme

Access to Evotec drug discovery expertise and capabilities to support your programme

## Starting from a phenotypic assay concept

Access to Evotec phenotypic screening expertise followed by target deconvolution leading into a drug discovery programme

## On an existing Evotec programme

Sponsor an established theme in the areas of immunology or inflammation

**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*

# Immunology & Inflammation at Evotec

A leading platform for rapid progress and increased success

**1** Proven track record in Immunology & Inflammation drug discovery: Contribution to the discovery and development of multiple pre-clinical and clinical candidates

**2** 8 Active Immunology & Inflammation projects from Target ID to PDC in the area of autoimmune disorders, pain, endometriosis, inflammation

**3** Cutting edge technologies and integrated drug discovery platform enable Evotec to go beyond single target classes or gene families

**4** Experienced Immunology & Inflammation team with **>40 FTEs**

**5** Fully integrated drug discovery platform and project management expertise accelerate our partners projects

PDC: Preclinical Drug Candidate

# Novel Immunology and inflammation targets

Pipeline overview

| Molecule(s) | Indication (mechanism) | Partner | Status | Next milestone | Commercials |
|---|---|---|---|---|---|
| **EVT401** | Inflammation (P2X7 inhibitor) | CONBA GROUP 康恩贝集团 | Phase I/II | Phase II start | Up to € 60 m milestones, royalties |
| **Various** | Endometriosis | BAYER | Pre-clinical | Pre-clinical candidate | € 12 m upfront, up to approx. € 580 m milestones, royalties |
| **Various** | Various | Boehringer Ingelheim | Pre-clinical | Phase I start | Undisclosed upfront, research payments, milestones, royalties |
| **Not disclosed** | Various | NOVARTIS | Pre-clinical | Successful PoC | Research payments, milestones, royalties |
| **Various** | Inflammation | ucb | Discovery | Pre-clinical | Research payments, up to € 183 m milestones/product, significant royalties |
| **Not disclosed** | Inflammatory pain | Convergence PHARMACEUTICALS | Discovery | Pre-clinical | Milestones, significant royalties |

# Experience with key target classes and mechanisms

*In vitro* biology: validated assays for screening & medicinal chemistry

**1** High quality screening libraries: HTS 400K, Fragments: 21K

**2** Extensive portfolio of biochemical and bio-physical assay systems

**3** State-of-the-art cellular assay systems with high content readouts

| Target class | Indication | Biology contribution |
|---|---|---|
| Ion channels | Immunomodulation | HTS: membrane potential, YOPRO, calcium flux<br>Automated & manual patch clamp<br>Translational assays, animal models |
| Kinases | RA, SLE, asthma, sepsis | Biochemical FP/FRET, Cellular: MSD<br>Translational assays: MSD, phenotypic, high content |
| Transporter | Anaemia in chronic inflammation | Phenotypic screen + SAR<br>Translational assays<br>Animal models |
| Cytokine PPI | RA, SLE | Biochemical FP/FRET<br>Biophysical SPR, NMR, thermal shift<br>Cellular reporter assay, translational assay |
| GPCRs | Pain & inflammation<br>IBD, asthma | Cellular assay: HTS, H2L, moa<br>Translational: migration assay, animal models |
| Enzyme | Neuroinflammation | Biochemical LC/MS<br>LC/MS + primary µGlia |



Effect of Ruxolitinib on IL-2 stimulated STAT5 phosphorylation in primary human PBMCs
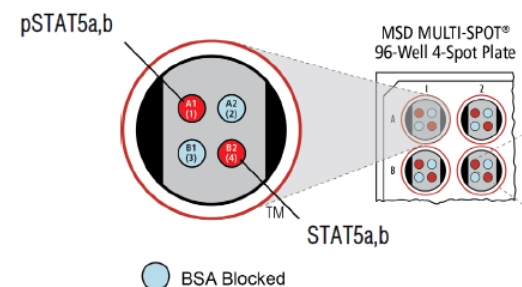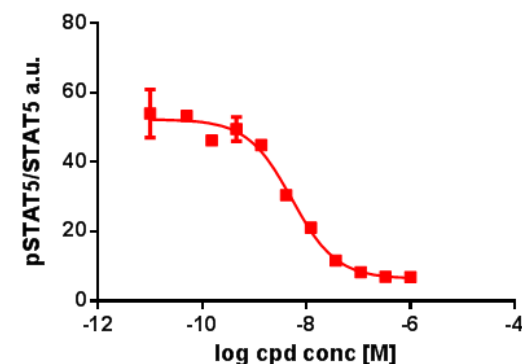
# Use of relevant primary cells in all stages of drug discovery

## Comprehensive platform with relevant readouts

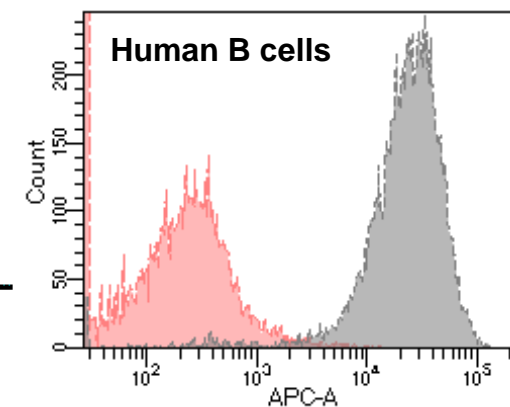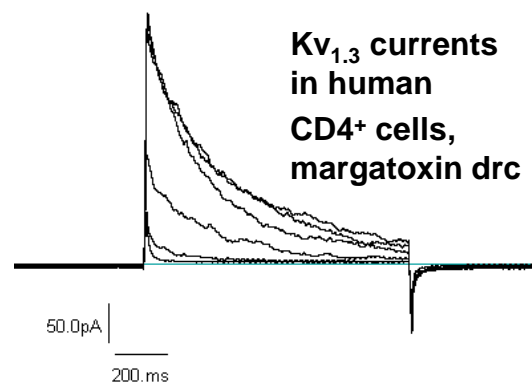- **Isolation, cultivation and manipulation of primary cells**
  - PBMCs, CD8, CD4, $T_H1/T_H2$ populations, B cells, neutrophils, spleenocytes, monocytes, macrophages, endothelial cells, whole blood assays, co-cultures
- **Assay read-outs**
  - Flow cytometry: Fortressa, Melody
  - High content imaging: Opera
  - ELISpot, cytokine profiling
  - $Ca^{2+}$-flux
  - Electrophysiology
  - Target oriented readouts
- **Applications**
  - Primary screening
  - Regular profiling
  - Mechanism of action studies



Effect of Ruxolitinib on IL-2 stimulated STAT5 phosphorylation in primary human PBMCs

Human neutrophils

100 μm

Kv$_{1.3}$ currents in human CD4$^+$ cells, margatoxin drc

50.0pA
200.ms

Human B cells
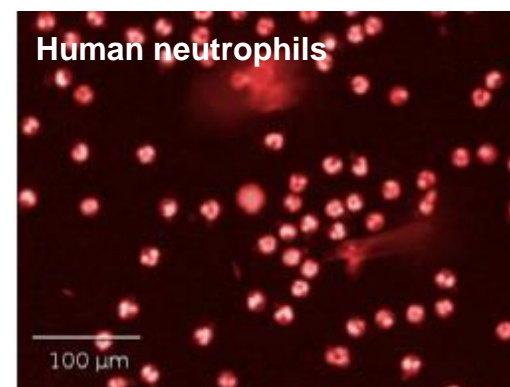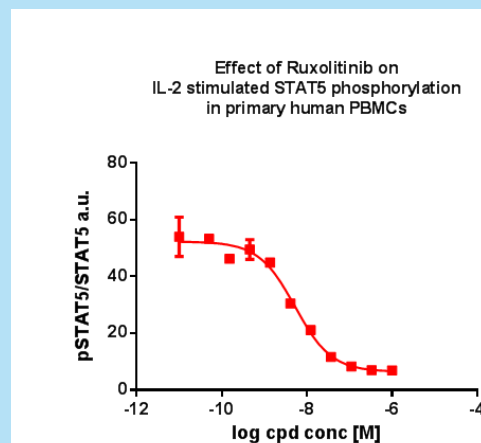
# Phenotypic characterisation of immune cells

Equipment and capabilities

- An extensive flow cytometry platform supports all activities
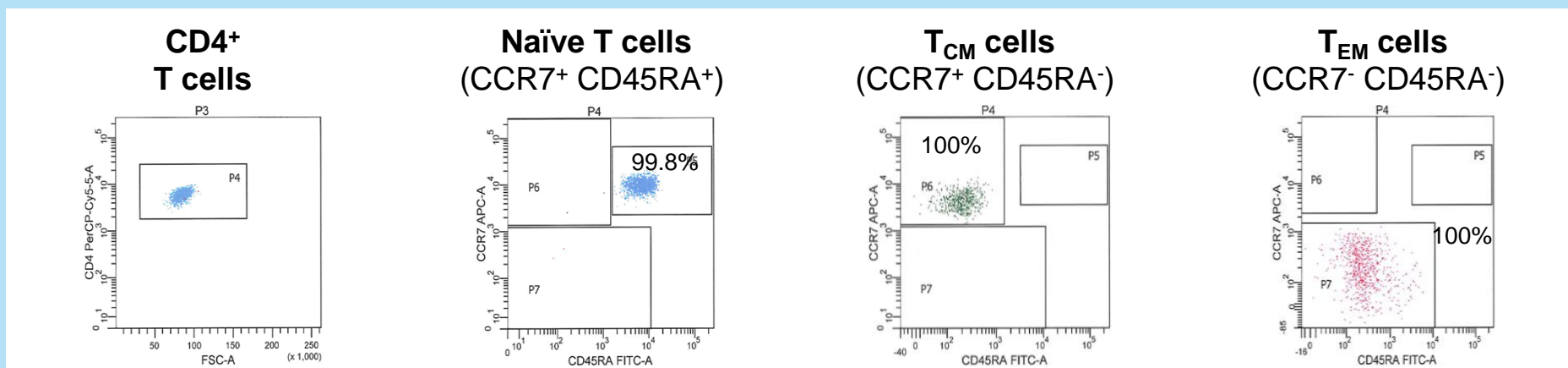  - FACS-Fortressa (BD) with 4 lasers and high throughput sampler
  - FACS-Melody (BD) for cell sorting
  - FACS-Canto II (BD) with 2 Lasers and high throughput sampler, 96- and 384-well
- Broad immuno-phenotyping expertise, combined with viability, Ca2+ influx etc.
- Functional evaluation of immune populations using ELISpot, confocal microscopy, incucyte etc.

**Cell sorting**



CD4$^+$ T cells

Naïve T cells (CCR7$^+$ CD45RA$^+$) — 99.8%

$T_{CM}$ cells (CCR7$^+$ CD45RA$^-$) — 100%

$T_{EM}$ cells (CCR7$^-$ CD45RA$^-$) — 100%

# Immunology & Immuno-Oncology capabilities

*In vivo* mouse models or PBMCs for *ex vivo* characterisation

## Source of immune cells

- **Therapeutic efficacy studies:** syngeneic tumour models
- **Immunization models:** therapeutic and prophylactic vaccinations
- **Transgenic mice:** TCR Tg mice (OT-I, OT-II), nude, SCID, highly immunodeficient BRGS mice designed for humanisation
- **PBMCs:** Healthy donors or patients; whole blood or buffy coat, cord blood (HSCs)
- **Tumours:** resection, tumour biopsies (FFPE, frozen or fresh)

## Analysis:
Functional & phenotypic characterisation

- **Flow cytometry:** multi-colour staining on spleen, blood, tumour, etc. → phenotypic characterisation of immune cells
  **Cell sorting:** isolation of particular immune cell subsets
- **Gene signature:** total mRNA gene signature (RNAseq), targeted signature (TLDA, Nanostring)
- **Metabolomics, lipidomics and proteomics:** e.g. analysis of IDO or adenosine suppressive pathways
- **Analysis of cytokines:** ELISA, CBA, Bioplex, MSD
  - On plasma: cytokines and also Ig for B-cells
  - After *ex vivo* restimulation/culture of immune cells
- **Confocal microscopy:** investigation of T-cell/APC or T-cell/ tumour cells interaction at the single cell level
- **IHC:** visualization of immune cells in tumour, angiogenesis, hypoxia, TME, etc.
- **Functional assays with immune cells**
  - T-cell proliferation, ELISpot
  - Ag-specific stimulation, polyclonal activation, irradiated tumour cells as APC

# *In vivo* pharmacology: Immunology & Inflammation

A mix of proprietary assets and validated assays

| | |
|---|---|
| ***In vitro* ADME *In vivo* PK** | • A comprehensive portfolio of *in vitro* ADMET assays<br>• Distribution in tissues and fluids<br>• Bioavailability study (p.o., s.c., i.v., i.m.; i.p.; intra cerebrospinal)<br>• Bioanalytics (WinNonlin®) |
| **Pharmaco-dynamic assays – PK/PD** | • IL-1$\beta$/desArg9 bradykinin-induced paw oedema<br>• TLR agonist-induced cytokines<br>• (R)- $\alpha$-methylhistamine induced dipsogenia<br>• Anti CD3-induced T-cell activation |
| **Animal (disease) models** | • **Inflammation/(Pain):** Visceral pain (colorectal distension), Collagen Antibody-Induced Arthritis; Inflammation induced by Peptidoglycan-Polysaccharid and Complete Freund`s Adjuvants, vascular inflammation, Imiquimod-induced psoriasis<br>• **Neuroinflammation/Huntington:** Q175 (mouse); BACHD (mouse and rat)<br>• **Anemia of inflammation:** Peptidoglycan-Polysaccharide-induced Anemia, Adenine-kidney insufficiency |
| ***Ex vivo* character-isation** | • Histology/IHC including quantification via Definiens software<br>• Flow cytometry, biomarker quantification |

# Immunology & Inflammation

Covering a broad area of auto-immune & inflammatory diseases

**Immunology areas**

- **Inflammation:**
  MIA-induced arthritis

- **Auto-immunity:**
  Psoriasis

- **Modulation of immune responses:** T-cells

- **Development of bespoke PD models depending on target**
  - Biomarkers validation
  - PK/PD relationship

- **Development of disease models mimicking pathological conditions**
  - Deciphering MoA of compounds
  - Developing *ex vivo* readout for further clinical translation

- **Supporting vaccine & immunomodulatory compounds development**
  - Validation of antigenicity/immunogenicity of a vaccine platform
  - Evaluation of adjuvant

# Endometriosis

HTS to candidate, risk share drug discovery collaboration

| Partner | Evotec contribution | Therapeutic area | Starting Points | Outcome |
|---|---|---|---|---|
| BAYER | Hit finding to candidate (integrated) | Inflammatory and neuronal interactions | HTS, rational design, Evotec assets | 8 milestones 4 clinical candidates 2 FiM |

- Evotec have a 5 year collaboration with Bayer to develop novel, non hormonal-based approaches for the treatment of endometriosis
- >50 FTE collaboration across all disciplines
  - Evotec contributed late stage LO assets
- Multiple targets addressing inflammatory pain initiation and propogation
  - All target classes with emphasis on ion channels
- Evotec have developed key rodent endometriosis models to provide proof of concept
- New targets and identified and validated
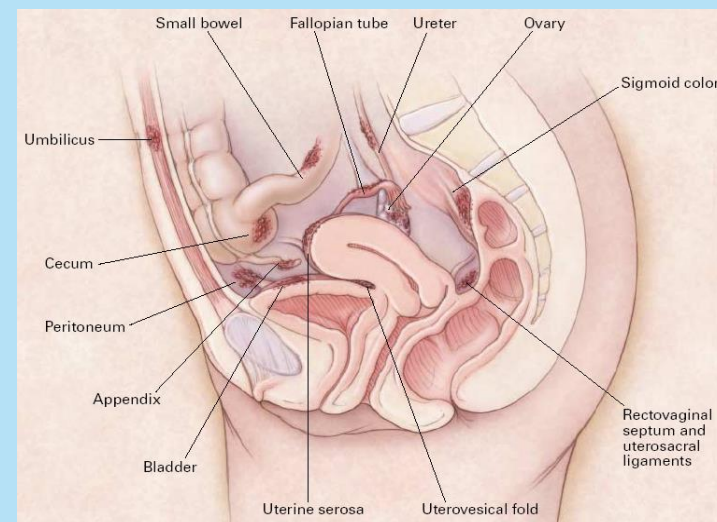
# Multi-project collaboration

FTE-based, multi-year drug discovery collaboration

| Partner | Program | Therapeutic area | Starting Points | Outcome |
|---|---|---|---|---|
| Major US biotech | Hit finding to candidate (biology) | Autoimmune disorders, CNS/PNS | Assays & hits | Approval for FIM study |

- Initiated in 2010, ca. 15 FTEs, flexible allocation
- Supporting integrated drug discovery projects with *in vitro* profiling: potency, selectivity, mechanism, *ex vivo* assays
- Targets/indication:
  - Kinase: SLE
  - Kinase: asthma
  - Ion channel: psychiatric
  - Ion channel: pain
  - Kinase: ALS, neurodegeneration
- Activities:
  - Routine compound profiling
  - High-content screening
  - Electrophysiology
  - Stem cell research
  - Proteomics
  - Structural biology
  - Cytotox profiling
  - Assay development
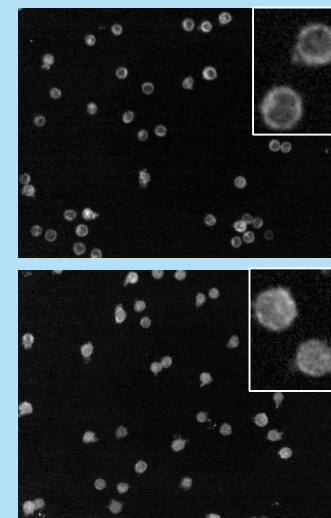
p52 translocation in stimulated human B cells

![evotec]

# Target*ImmuniT*

## HTS to Candidate

| Partner | Program | Therapeutic area | Starting Points | Outcome |
|---|---|---|---|---|
| SANOFI | HTS – LO | Cancer Immunotherapy | Assay development | Advanced lead |

- Phenotypic drug discovery for cancer immunotherapy

- Screening of 500k compounds on primary human PBMCs (increase of IL-2 production via HTRF)

- Optimization of chemical series, DMPK, *in vitro* pharmacology, *in vitro/vivo* phenotyping

- Target deconvolution and validation completed
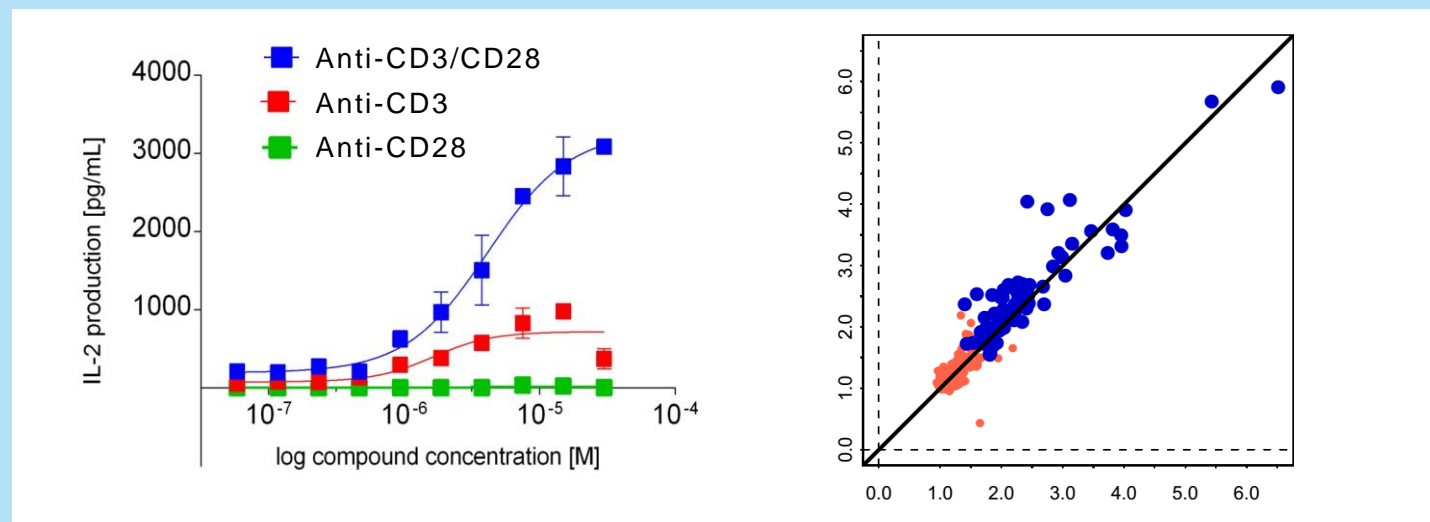
# Why us?

Evotec – The right partner in Immunology & Inflammation 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 man-agement 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**

evotec

**Your contact:**

info@evotec.com