evotec

#RESEARCHNEVERSTOPS

Evotec

Oncology iR&D
Integrated Research and Development

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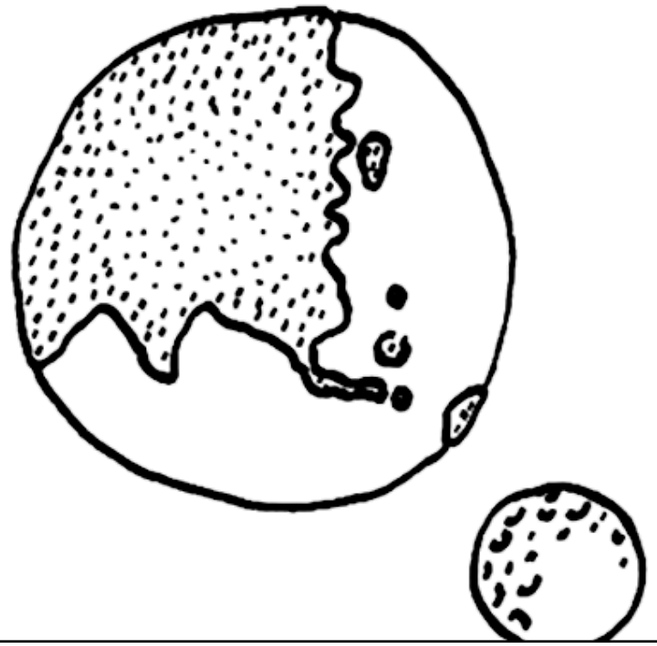


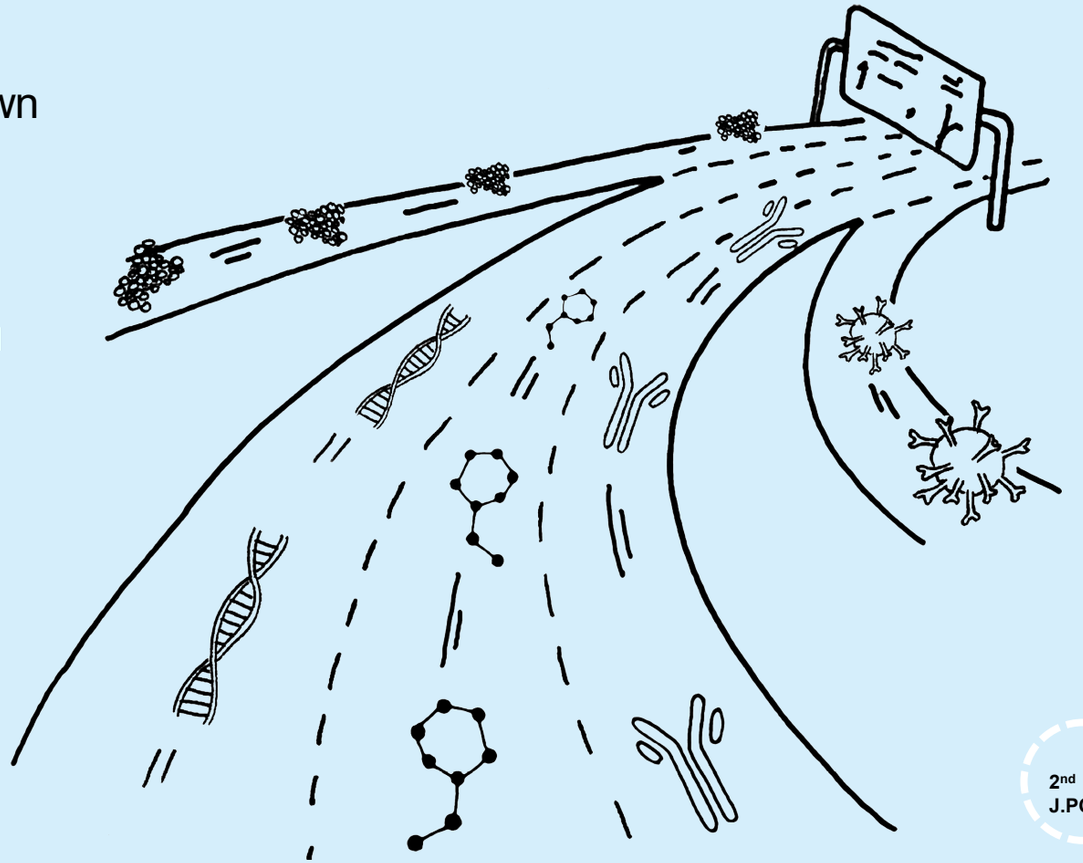
One platform – more efficiency, better precision, higher speed

Evotec footprint – 14 Sites & more than 4,000 employees

Princeton, Seattle, Branford, Watertown
~400 FTE

Orth an der Donau
~35 FTE

Verona (Campus Levi-Montalcini)
~700 FTE



Hamburg (HQ), Goettingen (Manfred Eigen Campus) Cologne, Munich,
~900 FTE

Abingdon (Dorothy Crowfoot Hodgkin), Alderley Park
~850 FTE

Lyon, Toulouse (Campus Curie)
~800 FTE

Our Innovation hub is highly integrated and synergistic

Capabilities & expertise overview

Industry needs

R&D efficiency platforms[1)]

Precision medicine platforms

Just – Evotec Biologics[1)]

Right modality drug design

Capabilities & expertise (illustrative)

Target ID & validation | Hit identification | Lead optimisation | Sample management | Chemistry | DMPK & ADME-Tox | Research informatics | Bio Reagents | *In vitro* biology | *In vivo* Pharmacology | Biomarker discovery | Antibody discovery | INDiGO | Integrated CMC | Integrated pre-clinical development | Clinical development solution

EVO*panOmics* | **EVO***panHunter* | **EVO***gnostic* | iPSC Drug Discovery

ScreenSeq – High-throughput transcriptomics | ScreenPep – High-throughput proteomics | J.HAL℠ – AI designed mAB library | E.MPD – Translational molecular patient database

J.DISCOVERY™ – Molecule discovery | J.HAL℠ – AI designed mAB library | J.MD™ – Molecule design | JP3® – Process & product design | J.POD® – Manufacturing design

EVO*cells* | **EVO***genes* | Antibodies & Bifunctionals | Small molecules | Antisense | Protein degradation | Exosomes | E.RNA – Targeting RNA space

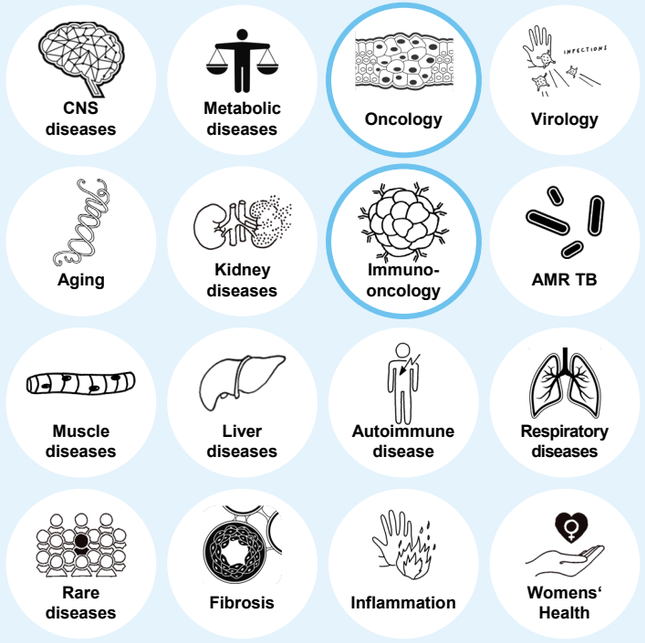
[1)] Also partly accessible as stand alone “Fee for Service” or FTE-rate based offerings

An essential combination: technology, disease-models and broad modality expertise

Focus areas to optimally deliver the next generation of patient medicines

Therapeutic area focus

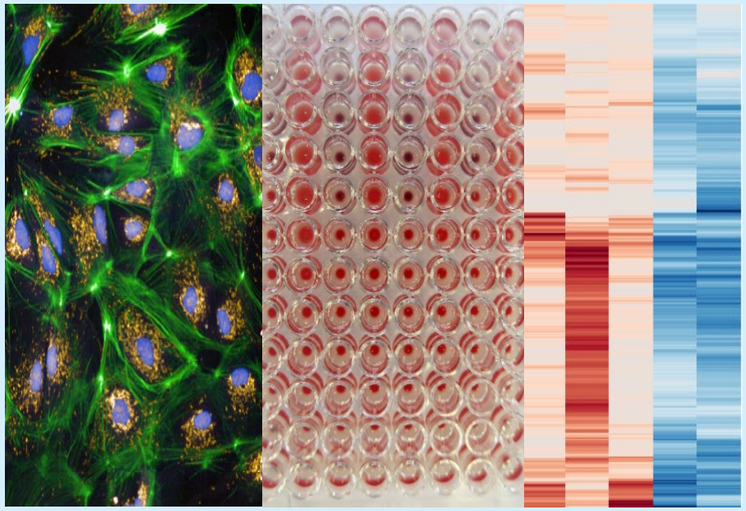
Translational cellular models & MoA studies



Technology agnostic

Leading technologies to address phenotype and molecular signals

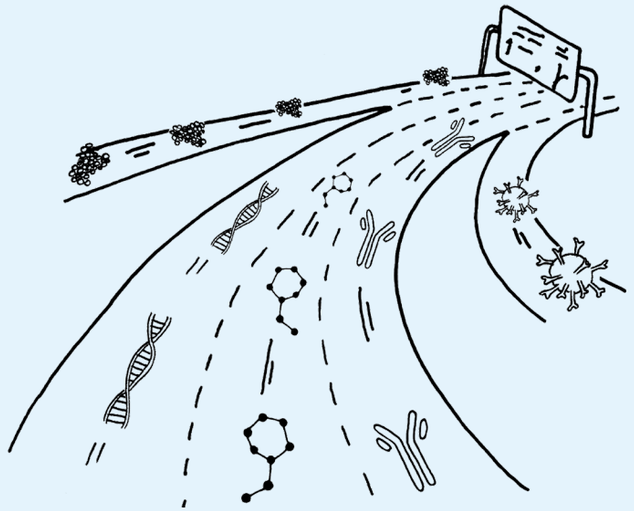
CRISPR, high content imaging, flow cytometry, Ephys etc. applied to screening, TV & MoA studies



Multi-modality

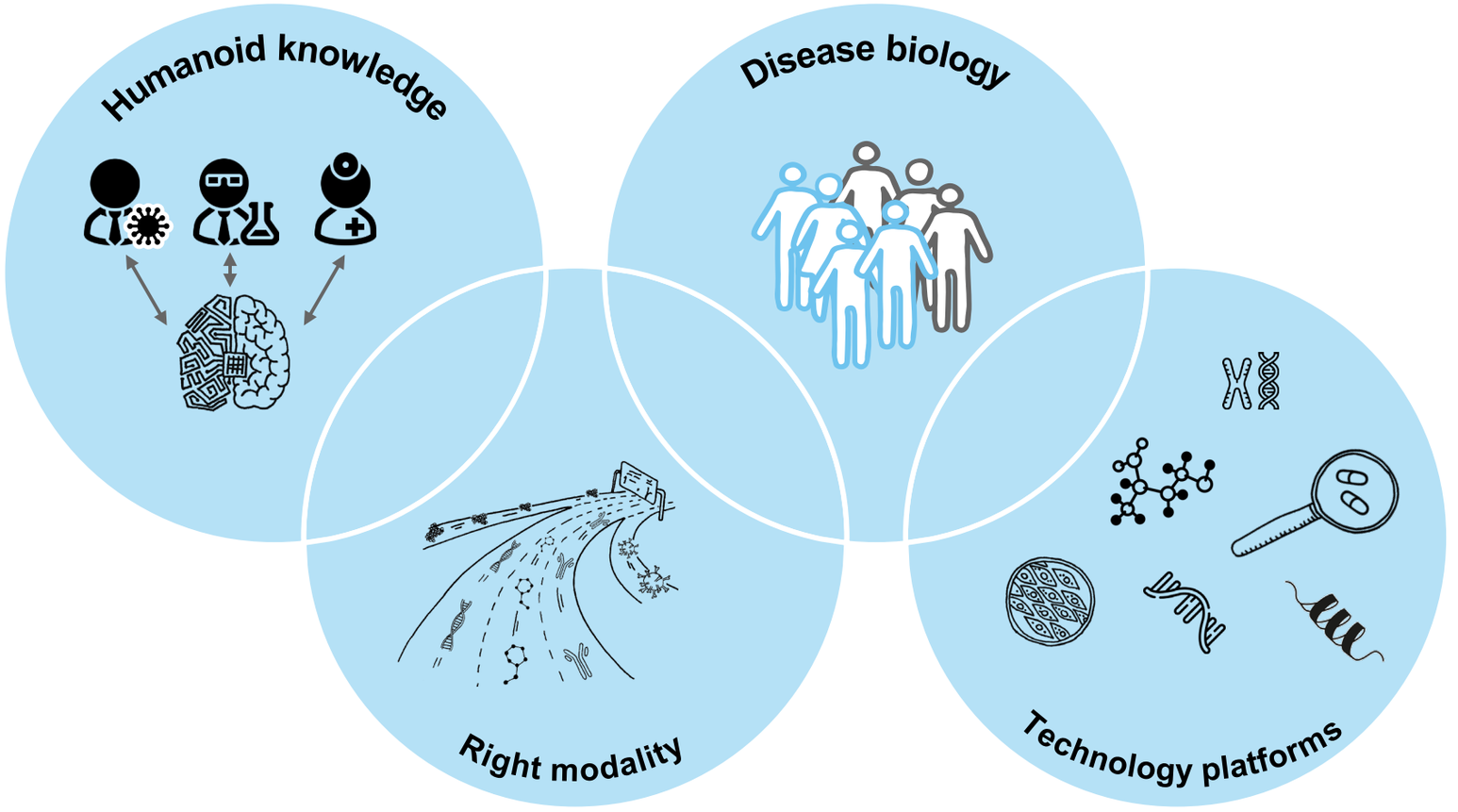
Modality-agnostic integrated drug discovery

Biomarker and drug discovery workflows for small molecules, antibodies, RNA, cell therapy etc.



Vision for iR&D

Optimised drug intervention strategies enabled with AI to rapidly deliver high quality NMEs



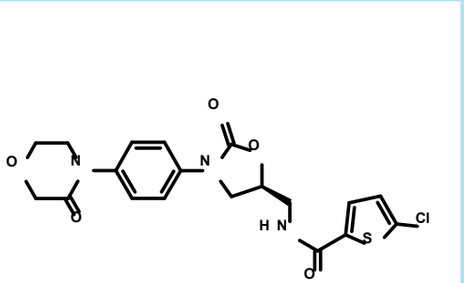
Creating success in precision medicine

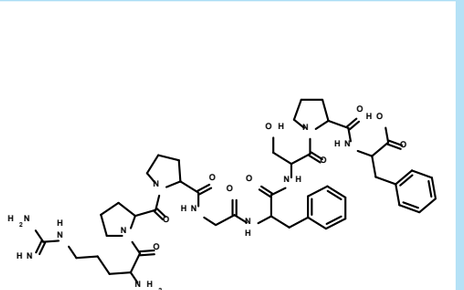
- Knowledge-driven decision-making
- Toolbox for unhindered problem-solving & invention
- Disease biology with translational focus
- Depth in drug-hunting knowledge and experience
- High-level intellectual engagement
- Rapid progression to the clinic

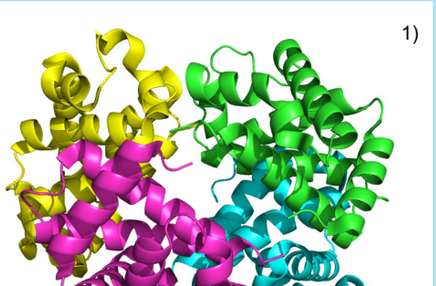
What are the options to address a biological target?

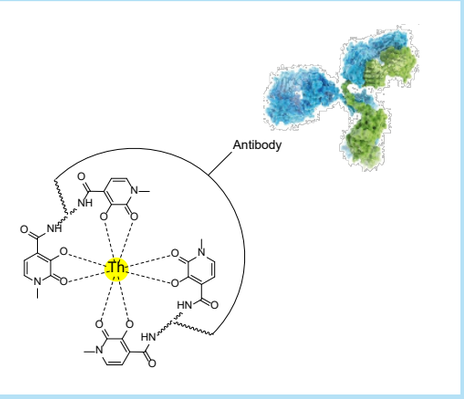
An optimal approach for targets and disease therapy should be selected

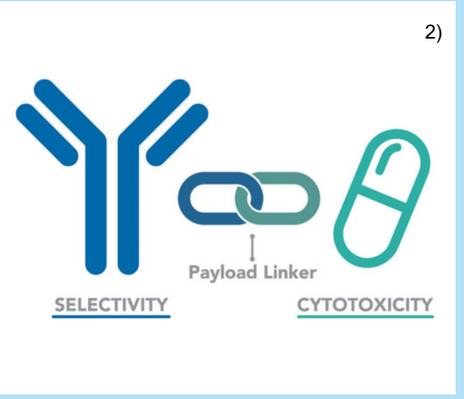
- **There are different ways to interact with a drug target (typically a protein, but also can be DNA, RNA, cell type, metabolite)**
  - Small molecules
  - Peptide therapeutics
  - Biologics (including conjugates)
  - RNA therapeutics
  - Gene and cell therapies
- **Other modalities have also been identified**
  - Targeted protein degradation (“PROTAC”, also “LYTAC”, “RIBOTAC”) – “beyond rule of five”
  - Novel allosteric modulators
  - Interference with microRNAs
  - Alteration of mRNA splicing

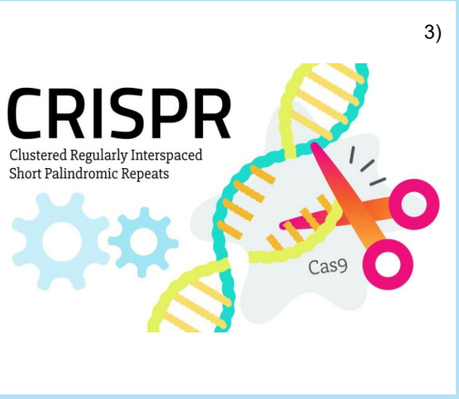












[1] https://nanosolutionsfp7.com/small-clumps-in-the-body-how-nanoparticles-react-to-proteins/
[2] https://www.genengnews.com/magazine/288/antibody-drug-conjugates-catalysts-for-chemistry/
[3] https://explorebiotech.com/crispr-gene-editing-tool/

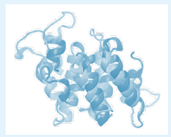
Discovery of novel chemical starting points for data-rich biological targets

Literature-to-lead using data mining and AI/ML

Common Start point

Lit to Lead / multiple targets

Data mining

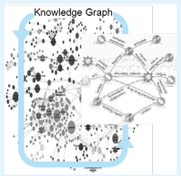


- Biological Pathway
- X validated biological targets
- Y known inhibitors per targets
- Z PDB structures
- etc.

Data Package

Creation of a curated database

Database + knowledge graph

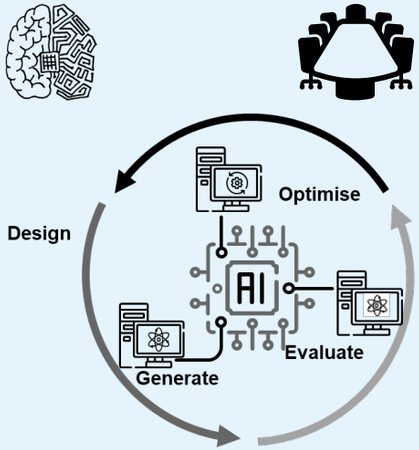


Knowledge graphs

- integrate heterogeneous data from across the entire discovery/development Continuum
- connect our data into a relationship network suitable for mining using AI/ML

AI/ML-Leveraging data

Design novel Hit series

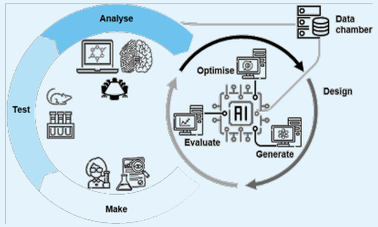


Scaffold Hopping

ML-based generative models

DMTA

Optimisation



Multi objective design engine

- AI driven generative design (2D / 3D / 4D)
- Structure-Based design
- Drug design expertise and cheminformatics
- Focus on properties and developability
- DMTA iterative cycles

Outcome

Novel starting points for LO

1-2 novel chemical series per target

- *In vivo* PK and PKPD in relevant pharmacological models
- IP: Evidence for freedom to operate in the chemical space

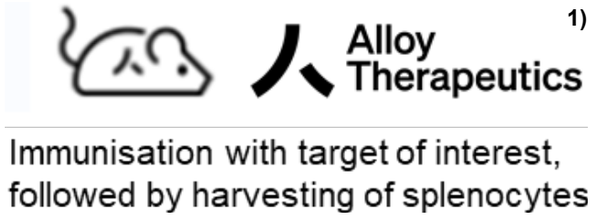
E.INVENT-AI
MOLECULAR DESIGN EXCELLENCE

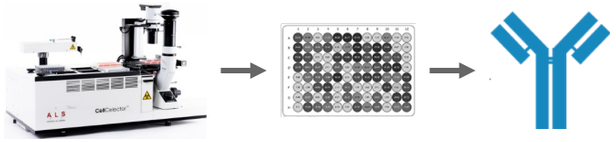
Evotec's own proprietary AI toolkit and appropriate integration of Talents and Drug Discovery expertise to deliver better medicines faster

Leading antibody discovery solutions

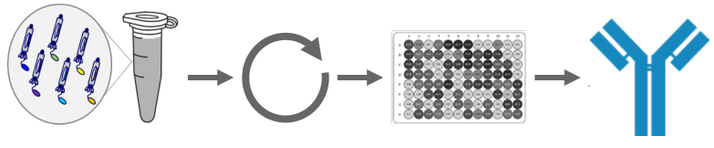
Immunisation and library-based approaches

In Vivo Antibody Production
Evolved for affinity



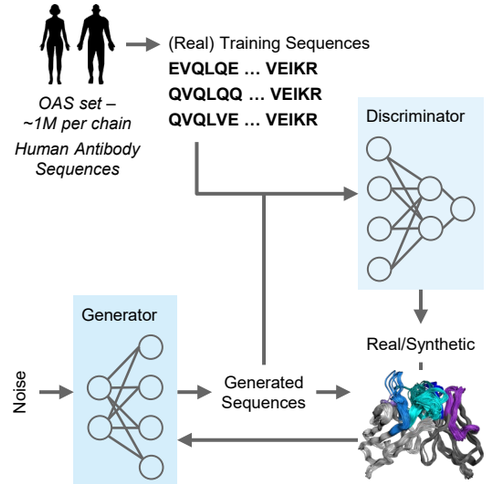


Hybridoma Technology
State-of-the-art hybridoma generation and high-throughput screening



Immune Antibody Libraries
Phage display lab for the exploration of natural immune repertoires

In Vitro Antibody Production
Designed for diversity, humanness & developability



Humanoid Antibody Library (J.HAL^{SM})[2)]
Large, diverse, manufacturable & developable discovery libraries with machine-learned biasing

[1)] https://www.evotec.com/en/invest/news--announcements/p/evotec-partners-with-alloy-therapeutics-to-expand-its-antibody-discovery-platform-6010
[2)] Designing Feature-Controlled Humanoid Antibody Discovery Libraries Using Generative Adversarial Networks: https://www.biorxiv.org/content/10.1101/2020.04.12.024844v2

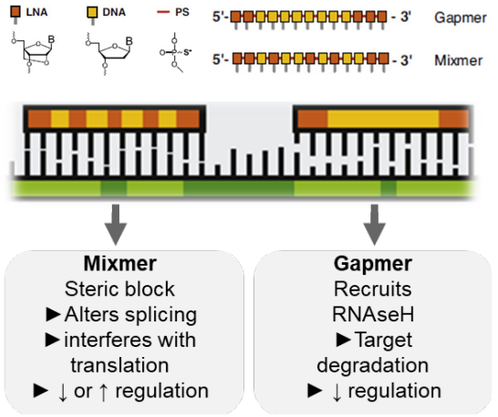
Targeting RNA to broaden modality options

Three RNA interference approaches offered for drug discovery

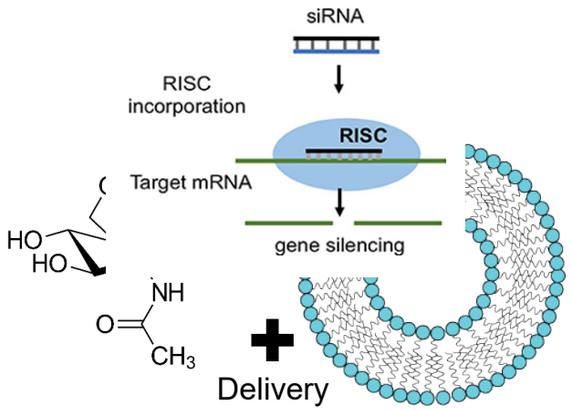
Antisense (ASO) and Interfering RNA (siRNA)
Oligonucleotide Therapeutics – sequence targets

Small Molecule
Structural targeting of tertiary structure

ASO



siRNA



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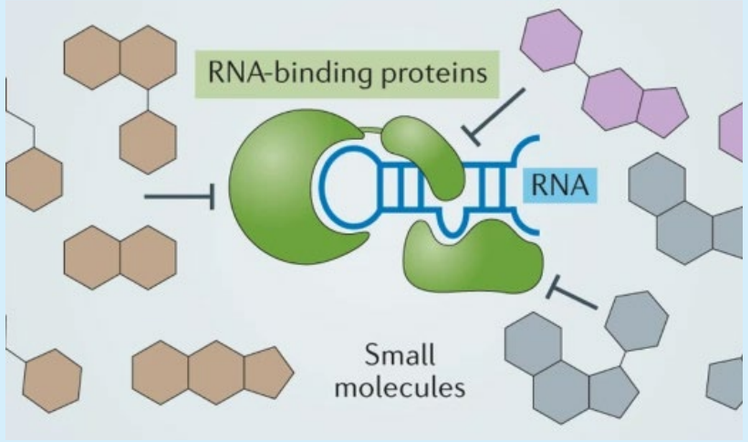

Up or Down regulation of RNA target

Down regulation of RNA target

Alter translation, location, degradation

SM image adapted from: *Nat Rev Chem* **4**, 441–458 (2020).

Unmatched integration in the industry!

Data-driven methods impact every stage from Idea to IND to Clinic

DISCOVER — **OPTIMIZE** — **SELECT** — **DEVELOP**

Data Surface linking Independent Data Chambers

Unique in the industry: high quality data generation & use at every stage in the value chain to de-risk projects, design modalities, create biomarkers, drive projects…

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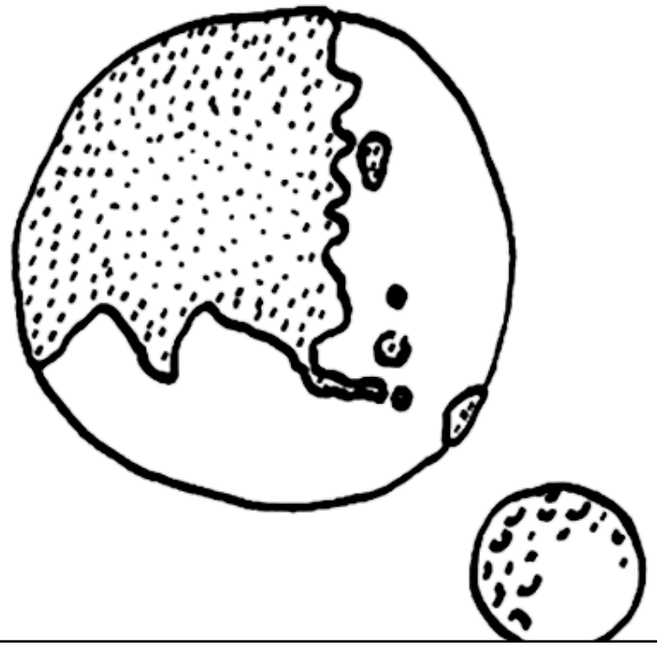


Oncology drug discovery at Evotec

A leading platform for rapid progress and increased success

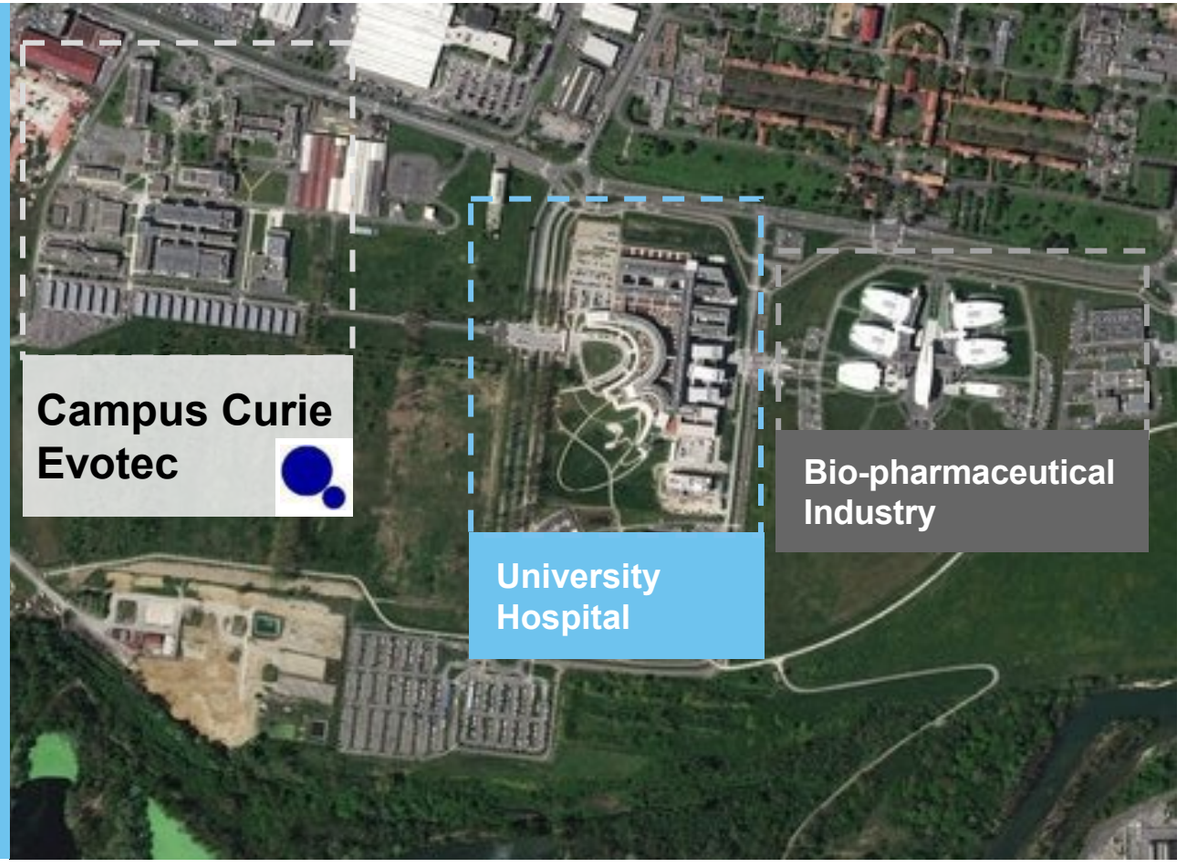
1	**Proven track record** in Oncology discovery: large number of Development Candidates (incl. back-ups); >15 compounds evaluated in humans; one marketed drug (vismodegib)
2	**Innovative platforms** to identify novel biological starting points and biomarkers: patient tissue + blood access, *ex vivo* assays, scRNAseq, CRISPR screening, in-house bioinformatics software
3	**Successful, longstanding oncology partnerships** with leading industry partners, as well as multiple academic bridge collaborations within the oncology space
4	**Dedicated Oncology Biology Research** team with **>150 FTEs**, accessing multiple technology platforms and having a **broad target class expertise**
5	**Fully integrated** drug discovery platform and project management expertise accelerate our partners projects

The Evotec-Oncopole Collaboration: Combining Medical and Research Excellence

Accelerated R&D in Oncology through our close working relationship

Oncopole is a highly recognized University hospital (~500M€ public funding)

- Combining medical and research excellence (IUCT, CRCT) in Oncology
- Incubator for midsize pharmaceutical and biotech companies
- **Examples of working together:**
  - Kazia (EVT801) supported by Evotec, has started to enroll oncology patients (Nov 2021) for a phase I at Oncopole
  - Exploratory biomarker evaluation are performed by Evotec



A winning team of drug hunters

An experienced Oncology drug discovery leadership team

										
Joanna Lisztwan	**Francisco Cruzalegui**	**Pascale Lejeune**	**Michael Esquerré**	**Sandrine Delbary**	**Steve Durant**	**Pierre Fons**	**Anne-Sophie Casagrande**	**Eberhard Krauss**	**Gilbert Lassalle**	**Christophe Boldron**
EVP Head of Global *In vitro* biology	**SVP *In vitro* biology**	**VP Translational biology**	**VP Immuno-Oncology**	**VP *In vitro* biology**	**VP Scientific Director**	**VP Translational Biomarkers**	**Group Leader *In vitro* biology**	**Group Leader *In vitro* biology**	**SVP Medicinal Chemistry**	**VP Molecular Discovery**
>20 years Industry experience	>20 years Pharma experience	>20 years Pharma experience	>12 years Biotech experience	>15 years Pharma experience	>15 years Biotech and Pharma	>15 years Pharma experience	>15 years Industry experience	>20 years Industry experience	>25 years Pharma experience	>15 years Pharma experience
• Novartis	• Servier • AZ • Pierre Fabre	• Bayer • Sanofi	• GenTcell	• Sanofi	• Kudos • AZ	• Sanofi • Abtech	• Diaxonhit • Sanofi	• Janssen • Cenix • Cyclacel	• Sanofi • Synthelabo	• Sanofi

Expertise in key cancer biology areas

Covering important cancer hallmarks and therapeutic interventions

Targeted Therapies

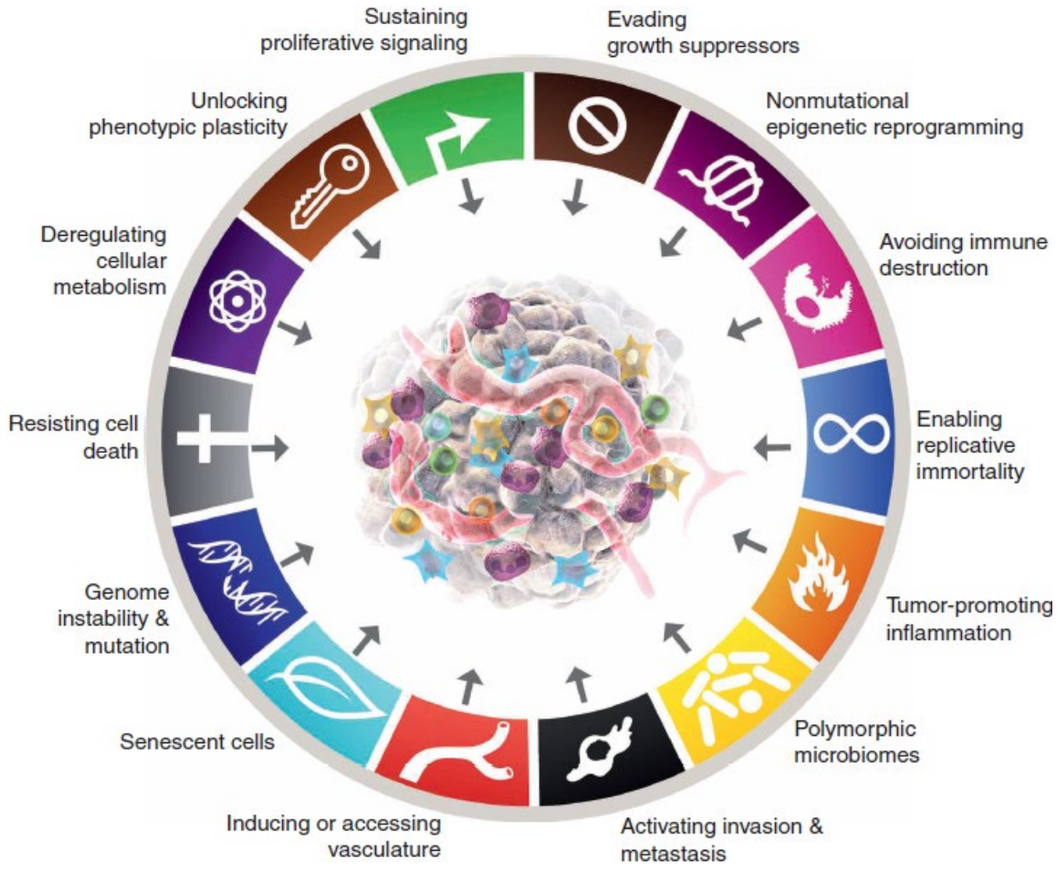
Targeting drivers underpinning tumour survival

Cancer Metabolism

Targeting mechanisms of metabolic adaptation

DNA Damage Response

Utilising genomic vulnerabilities and synthetic lethality



Epigenetics

Exploiting transcriptional regulation and post-translational modifications

Immune suppression

Targeting mechanisms of immune exclusion in tumours

Tumour Microenvironment

Hitting stroma-driven barriers and other tumour defenses

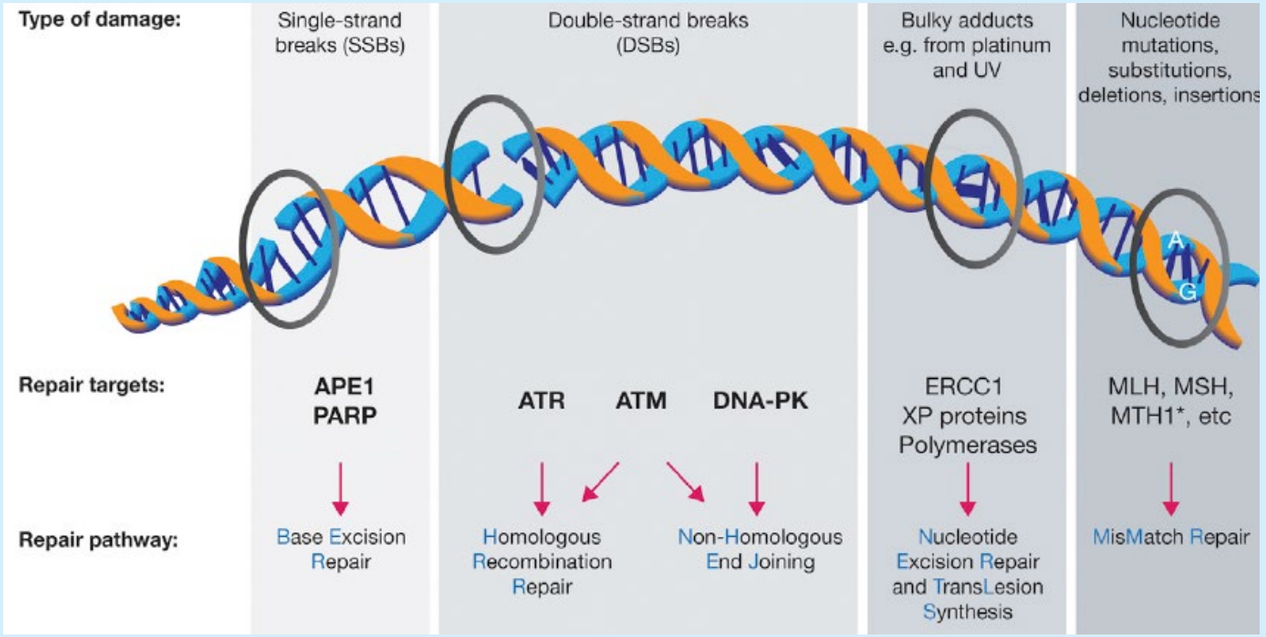
Hanahan D. Hallmarks of cancer: New Dimensions. Cancer Discovery (2022)

Expertise in DNA damage response (DDR) drug discovery

A broad range of *in vitro* assays for screening and medicinal chemistry support

- **DSB repair by homologous recombination, non-homologous and micro-homology end-joining:**
  - Kinases and ATPases (ATPase Glo)
  - Polymerases (primer extension)
  - Helicases (strand displacement)
- **DDR targets and cell cycle checkpoints:**
  - High content analysis: RAD51, 53BP1/γH2AX foci, pCHK1, pRAD50
  - Cell cycle analysis
  - Phenotypic assays (viability, proliferation, apoptosis) in isogenic cell models:
    - NHEJ-deficient (XRCC4-/-) vs wt
    - HR-deficient (BRCA2-/-) vs wt
- **Translation for *in vivo* studies:**
  - Detection of γH2AX by ELISA or WB

Experimental approaches boosted by deep bioinformatics analysis for best cell models reflecting the target patient population and pathway networks analytics

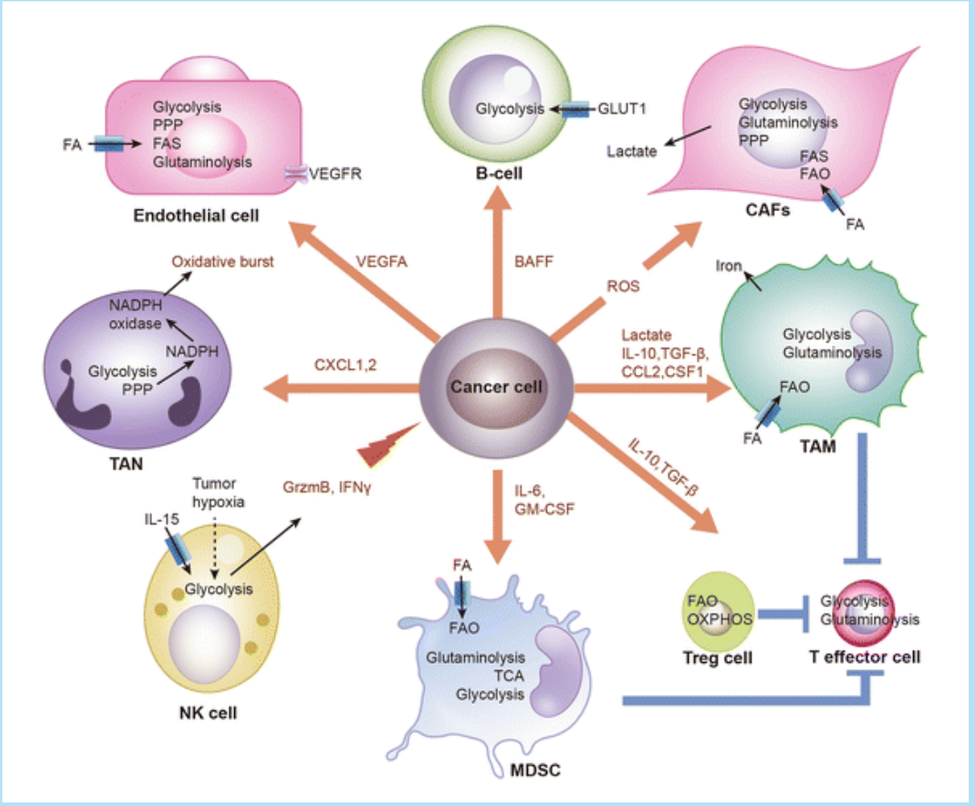


O'Connor (2015) Targeting the DNA damage response in cancer Molecular Cell

Understanding the Tumour Microenvironment as a strategy for Therapeutic Discovery

Cancer cells and TME cells share metabolic dependencies

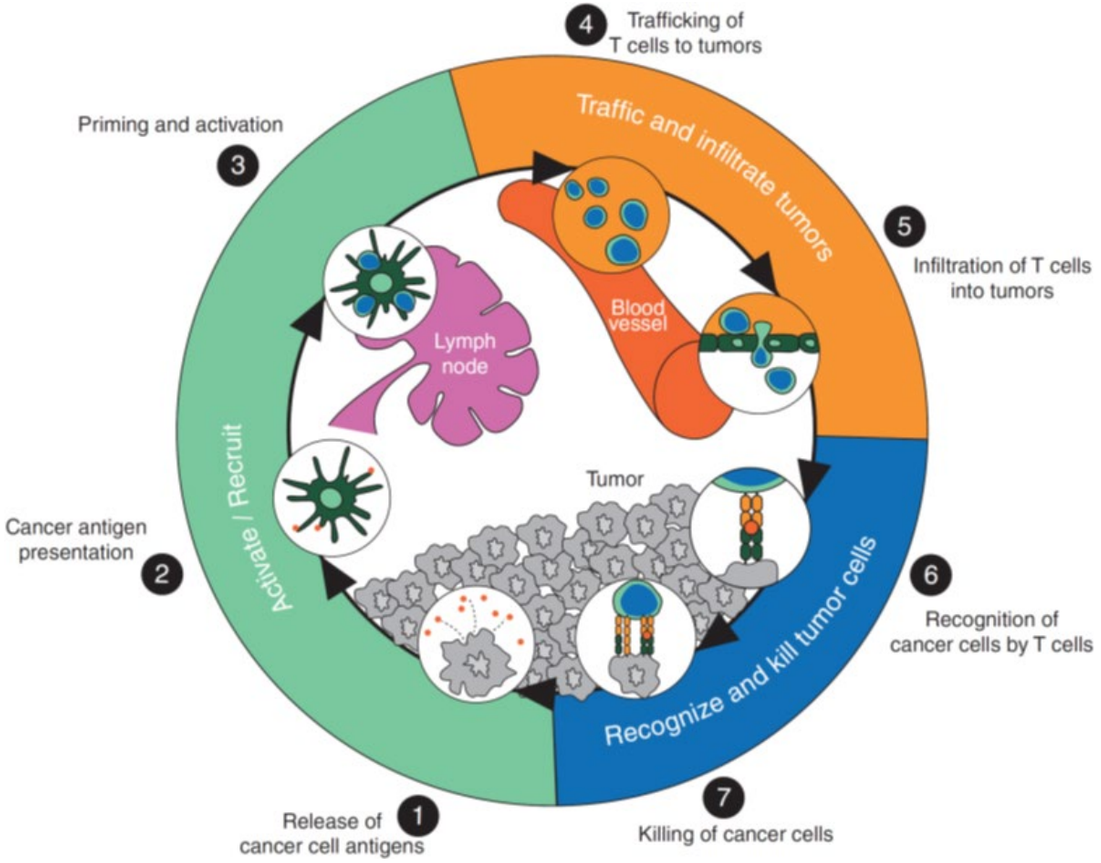
- Tumours are hubs for metabolically active cells comprising cancer cells, immune cells, stromal cells and blood and lymph vessel cells, all which are involved in cancer growth
- This tumour microenvironment (TME) is often characterised by nutrient competition, low pH, limited oxygen, and accumulation of metabolites
- Such conditions, in general, result in immunosuppressive or tolerogenic phenotypes of immune cells
- **Targeting mechanisms that alter cancer metabolism to improve the TME nutrient availability or that modulate immune metabolism will help maximise the efficacy of other cancer therapies**



Advances in Experimental Medicine and Biology 1011, DOI 10.1007/978-94-024-1170-6_5

Targeting tumour immune suppression

Generation of immunity to cancer is a cyclic process



Evotec has developed

- Preclinical mouse models (syngeneic tumour models, humanised mice)
- Assays to decipher the MoA of compounds for each stage of the cancer-immunity cycle
- Assays that can be translated into the clinic for biomarker development & follow-up

Immune phenotype per stage	Assays to evaluate
Immune-desert tumour Generate immunity	Flow cytometry, IHC, ISH, ELISpot, proteomics, metabolomics, gene signature, cytokine measurements (*ex vivo* analysis)
Immune excluded tumour recruit/infiltrate	IHC (angiogenesis), Flow cytometry for biomarkers (blood and secondary lymphoid organs), chemokine measurements
Immune Inflamed tumour Invigorate T-cells	Flow cytometry, IHC, *ex vivo* assays

Source: Kim JM & Chen DS Annals of Oncology 2016

Evotec partnerships in Oncology

iR&D success stories in Oncology

Evotec and Exscientia announce start of human clinical trials of novel immuno-oncology drug

Hamburg, Germany, April 9, 2021

Evotec SE today announced the most advanced asset arising from their joint venture with Exscientia has entered human clinical trials. The A2a receptor antagonist, which is in development for adult patients with advanced solid tumours, was co-invented and developed between Exscientia and Evotec, including application of Exscientia's next generation 3-D evolutionary AI-design platform, Centaur Chemist®. The drug candidate has potential for best-in-class characteristics, with high selectivity for the target receptor, bringing together potential benefits of reduced systemic side effects as well as minimal brain exposure to avoid potential undesired centrally-mediated side effects.

Evotec and Rappta Therapeutics enter discovery and development partnership focused on oncology target

Hamburg, Germany, November 24, 2020

Evotec SE today announced a new multi-year drug development partnership with Rappta Therapeutics, a Finland-based biopharmaceutical company, focused on an innovative oncology target.

Evotec partner Kazia Therapeutics announces full regulatory approval for Phase I study of EVT801

Sydney, Australia, 02 September 2021:
Evotec partner Kazia Therapeutics Limited ("Kazia", ASX: KZA; NASDAQ: KZIA) today announced that the planned phase I study for EVT801 has received full approval from L'Agence Nationale de Sécurité du Médicament et des Produits de Santé ("ANSM"), the French regulatory agency. The study is expected to open to recruitment by the end of CY2021.

Bristol Myers Squibb exercises option to extend targeted protein degradation partnership with Evotec

- **BRISTOL MYERS SQUIBB EXTENDS COLLABORATION WITH EVOTEC IN THE FIELD OF TARGETED PROTEIN DEGRADATION**
- **EXTENSION TRIGGERS AN ADDITIONAL COLLABORATION PAYMENT TO EVOTEC'S FAST-GROWING PANOMICS PLATFORM**
- **PARTNERSHIP AIMS TO FURTHER EXPAND THE PROTEIN DEGRADATION PIPELINE**

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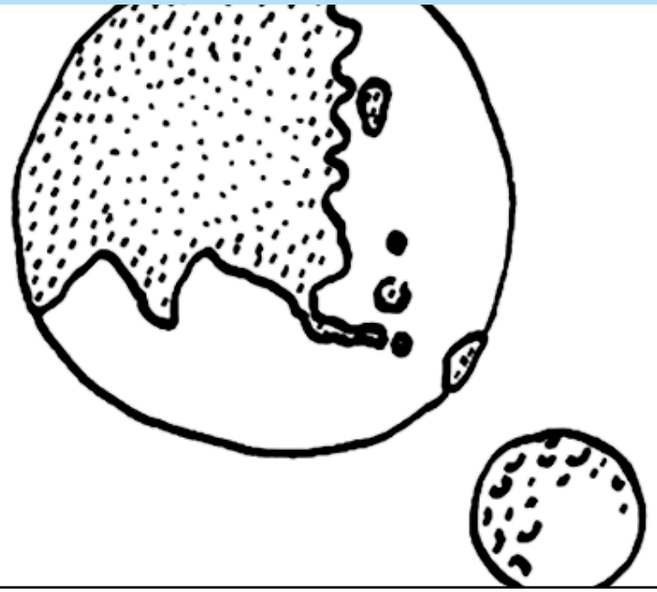


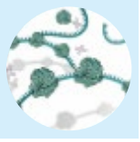
Unlocking target-based drug discovery

A comprehensive target ID and validation technologies portfolio

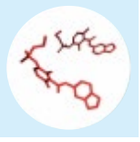
Addressing Target ID and Validation with different means

- CRISPR technology for target identification and validation
- Generation of cellular models using genome engineering

- RNAi approaches for target identification and validation
- Transcriptomics



- Proteomics for target identification
- Cellular target profiling and PALMs for target deconvolution



- Metabolomics for target identification and validation
- Biomarker

- Phenotypic screening for novel Hit ID
- Phenotypic characterisation following target manipulation

- Tool box of approaches and formats for target manipulation
  - CRISPR, RNAi, cDNA, tool compounds
- Extensive experience in cell models across disease areas
  - Cell models, primary cells, iPSC derived
- Vast array of technologies to detect and characterise effects of target manipulation
- In-house AAV and cell line engineering platforms
- Access to disease and xenograft models across different disease areas

Target Validation Capabilities at Evotec

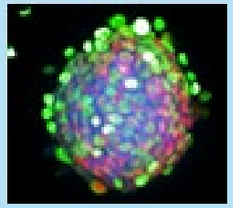
RNAi and/or CRISPR

***siPOOLs:* Defined pools of 30 selected siRNAs**

- Reduce off-target effects
- A second independent pool is rarely needed

- Optimise transfection
- Develop JESS (Simple Western), and/or qPCR
- Confirm target expression
- Validate siRNAs for efficient knock-down

- Functional cell-based assays (2D & 3D)
- Always monitor KD in parallel
- Option: Proof for SL or drug sensitisation



Option: Verify results by a rescue experiment

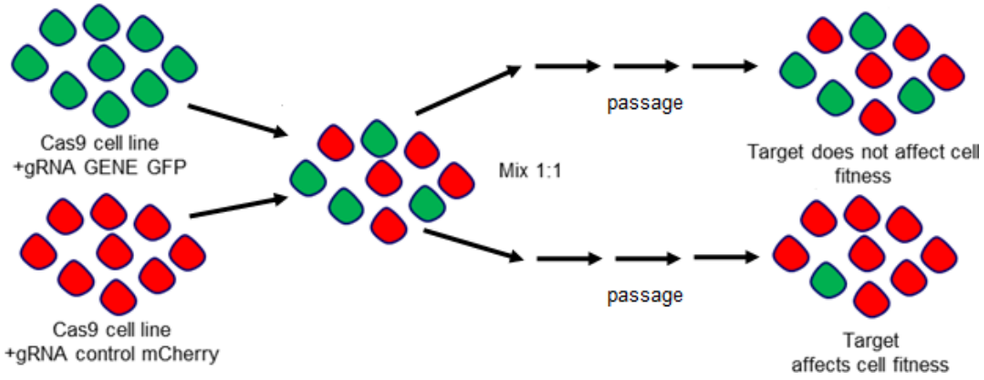
Option: Confirm with an independent technology

***CRISPR:* Gene editing, generate stable KO**

- Cas9/gRNA complex (RNP) delivery
- Jump-start with stable Cas-9 lines
- Generate constitutive or inducible Cas-9 lines

Fluorescent Competition Assay

- Monitor growth disadvantage/lethality or SL

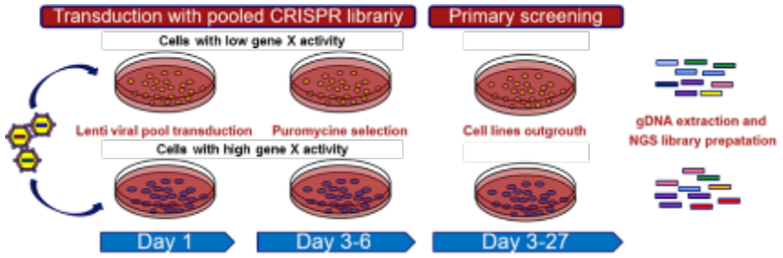


SL: Synthetic Lethality; KD: Knock Down; KO: Knock Out; RNP: Ribonucleoprotein

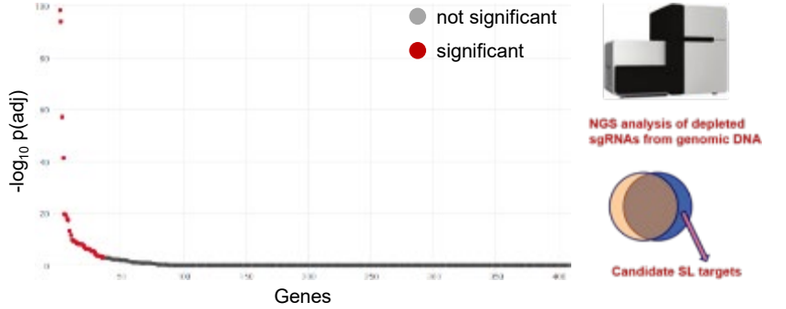
Case study: Identification of novel vulnerabilities for cancers expressing non-druggable targets

Synthetic lethality CRISPR screening for novel target ID and validation

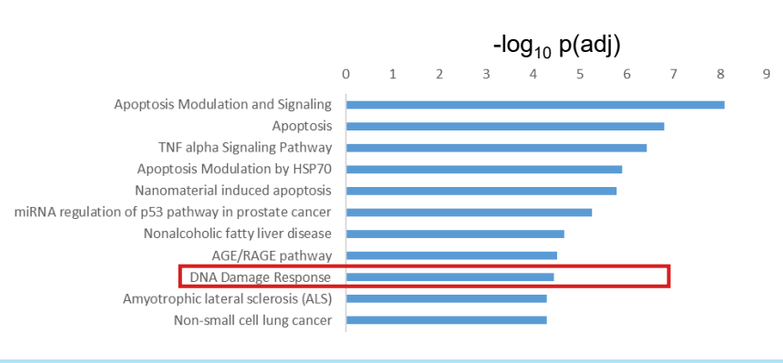
Druggable genome drop-out CRISPR screen overview



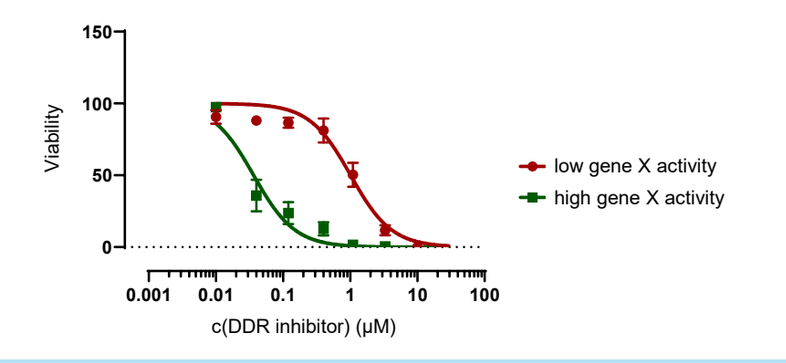
Identification of dropout genes



Pathway analysis identifies vulner-abilities associated with Gene X activation



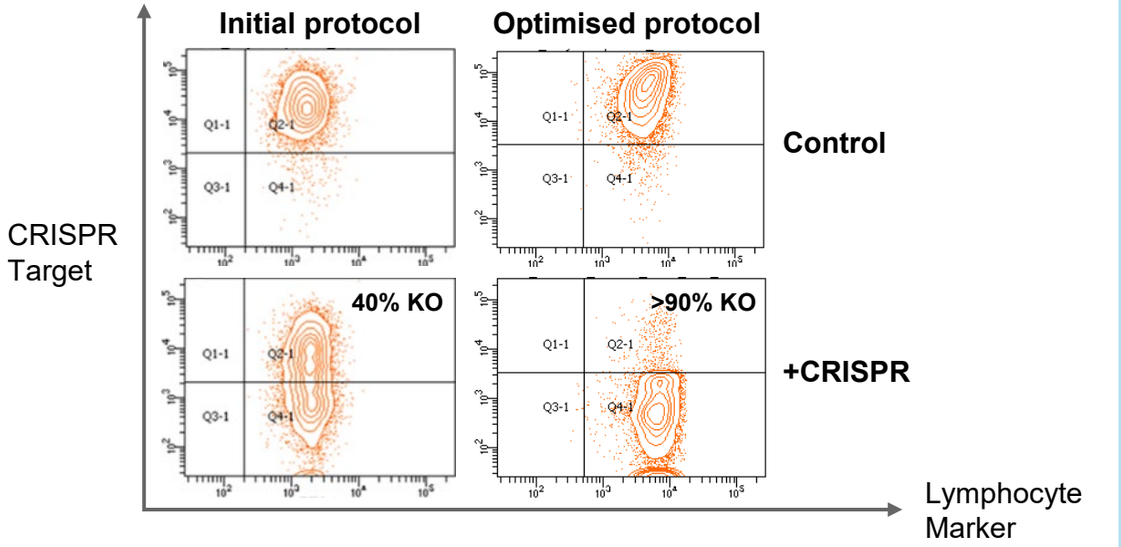
Confirmation of gene X induced sensitivity to DNA damage inhibitors



Case study: Validating targets and their MoA in immune cell populations

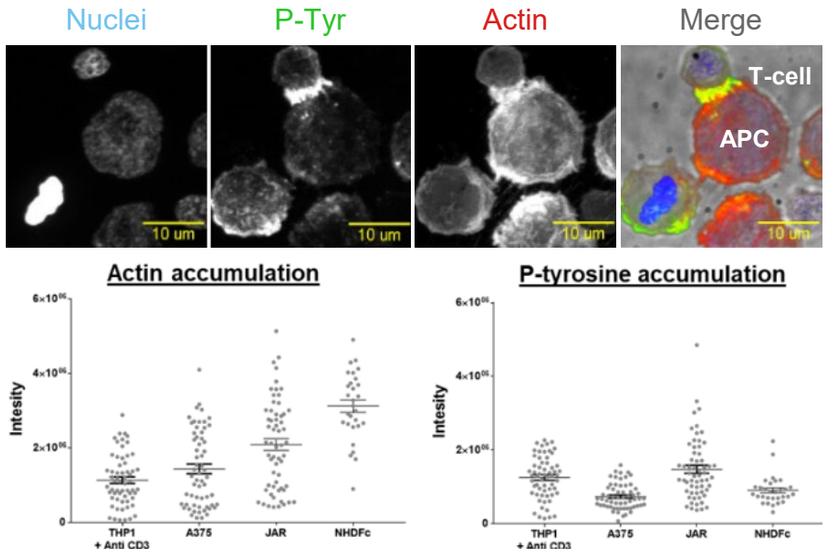
CRISPR and immunofluorescence applied in disease relevant systems

CRISPR in primary human lymphocytes



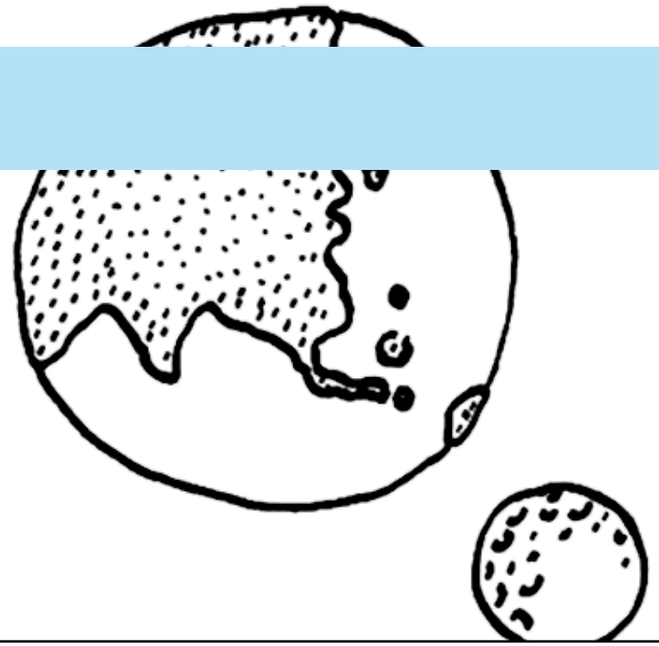
Optimised workflows for isolation and manipulation of primary cells for Target Identification and Validation

IF imaging of immune-specific mechanisms



Established protocols for imaging of the immunological synapse as an example of Mechanism of Action exploration

Contents

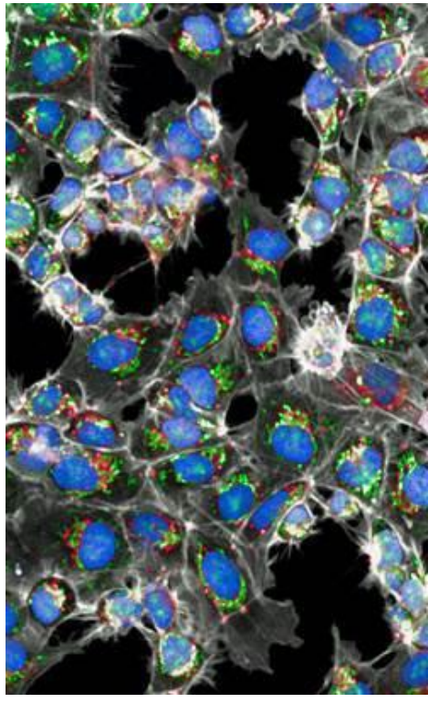


A broad range of Oncology *in vitro* assays

Validated assays for modality-agnostic drug discovery support

1	**Target validation and deconvolution approaches** RNAi/CRISPR, PALMS/CTP
2	**Extensive portfolio of biochemical, biophysical and cell assay systems**
3	**2D/3D assay formats and patient-derived material** PBMCs, tumour, cell lines

Signal transduction	CDK, HSP90, Shh, Akt, Alk, Aurora, Plk2, FGFR, b-Raf, c-Met, Erk, mTORC1 etc. supported by HTRF, ATPGlo, MSD, ELISA, Dot blot/Western, proteomics etc.
Tumour metabolism	PKM1 and PKM2, HK1 and HK2, LDH, IDH, MC1 etc. supported by Seahorse, Oxography, ATP, metabolomics etc.
Immuno-oncology	Adenosine pathway, VEGFR3 etc. supported by flow cytometry and sorting, Incucyte, IHC, ELISpot, MLR, MSD etc.
Tumour metastasis and vascularisation	VEGFR2, Tie2, MMPs, methionine aminopeptidase etc. supported by *in vitro* angiogenesis, hypoxic chambers, transwell etc.
Apoptosis	Bcl-2 family, PARPs, TRPM8 etc. supported by Incucyte, IF/IHC, flow cytometry, Western etc.
Epigenetics	HDACs (all 3 families), KDMs, HMTs, HATs, NHRs, RNA methylases etc. supported by RF/MS, SPR, HTRF, ChIP, TLDA/RT-qPCR, proteomics etc.
DNA damage response	Nucleases, polymerases, helicases, etc. supported by reporter assays, Operetta, synthetic lethality, replication stress etc.
Protein homeostasis	Targets supported by HiBit assays, nanoBRET, ubiquitination, Operetta, proteomics etc.
Imaging and phenotypic assays	Cell proliferation / death / apoptosis, mitotic index, response biomarkers, differentiation, migration etc. supported by Operetta, Incucyte, confocal microscopy, qPCR etc.



Technology toolbox to address all project needs

Evotec selects the best read-outs with the best models

Incucyte

Meso-scale

Operetta

Simple

FACS Fortressa

Sea-horse

Cell-based assay technologies

- Fluorescence readouts including HTRF, FP and many others
- Reporter gene assays
- ELISA (standard, Mesoscale, AlphaLISA)
- Imaging (HCS)
- Flow cytometry and ELISpot
- Proliferation, survival, anchorage independent growth, spheroids
- Migration, invasion assays
- Whole blood assays
- Metabolic analysis (Seahorse, glycolytic and OXPHOS ATP, metabolites)
- Automated Western blotting
- Gene expression (RT-qPCR, RNAseq)
- BRET, nanoluc, Hi/LoBit

Biochemical assay technologies

- FCS+plus
- Fluorescence Polarisation
- Fluorescence Intensity
- HTRF/Delfia
- AlphaScreen
- AlphaLISA
- Luminescence
- RF/MS

Biophysical read-out technologies

- Surface Plasmon Resonance/SPR
- Mass Spectrometry / LC/MS
- Nuclear Magnetic Resonance/NMR
- Thermal Shift, Thermophoresis

Envision Pherastar

Rapid-Fire

Via7

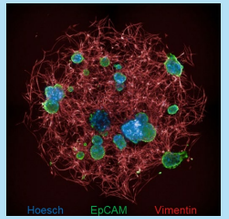
Biacore

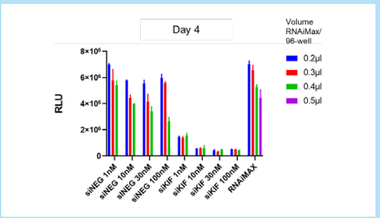
Nano-temper

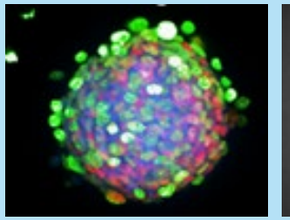
3D cell assays developed at Evotec Oncology

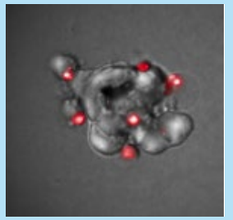
Summary of models and read-outs

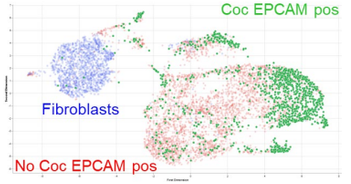
3D models	• Cancer cell growth in 3D (suspension or matrix-embedded spheroids & colonies) • Migration/invasion in a 3D matrix • Assays with co-culture of tumor cells with primary fibroblasts
Proliferation / Viability / cell death measurements	• Colony or spheroid number, size, roundness • CellTiter Glo (lytic system) or RealTime Glo (non-lytic) • Dye-based apoptosis/necrosis assay, immunostaining
Target expression (coupled to RealTime Glo)	• mRNA levels (RT-qPCR) or protein levels (Western blot/JESS) • Single cell mRNA sequencing • Confocal imaging











High content imaging and analysis at Evotec

A dedicated team of specialists & best-in-class imaging hardware

Instrumentation
Best in class imaging devices for HCI and HCS acquisition, globally operated according to professional industry standards

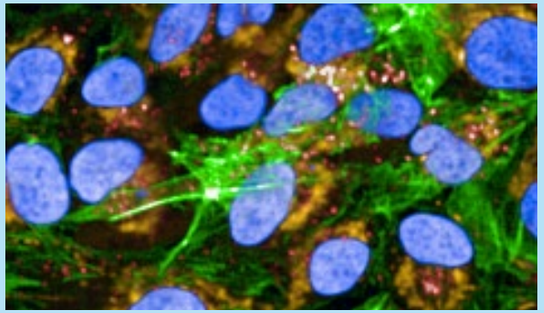
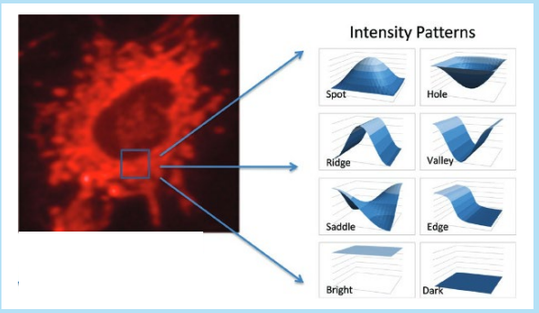
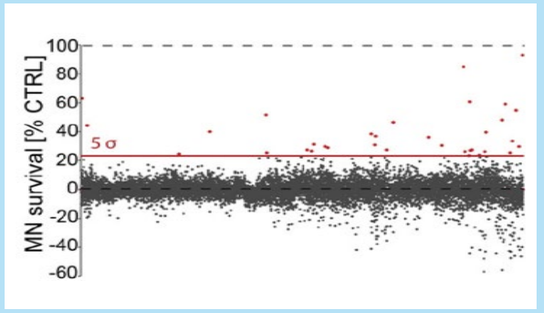


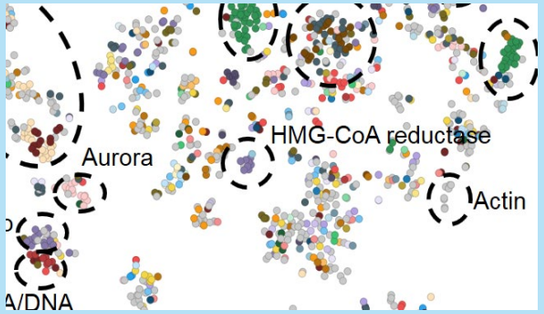
Image Analysis
Cutting edge image analysis capabilities and broad portfolio of efficient and robust readouts



Data Analysis
State of the art data analysis and machine learning/artificial intelligence based algorithms



Data Science
Proven track record of successfully completed projects based on applied cellular and tissue image analysis



Workflows
Experienced team of scientists working in close cooperation with biological application experts

Data Management
Applied big data handling

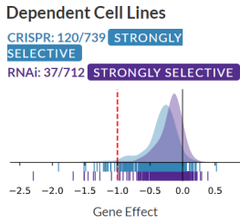
High Performance Computing
Fast and reliable data processing

New Solutions
Professional and flexible development

Bioinformatics approaches for target validation

A team of bioinformaticians focused on oncology data analytics

Perturbation screen



DepMap: CRISPR KO, RNAi KD;
PRISM, GDSC: drug inhibition
Other data: e.g. IC50

Comparison between sensitive & non-sensitive cell lines

e.g. CCLE

Expression data (RNAseq, proteomics)
WSG (Mutation, struct. variants, CNV)

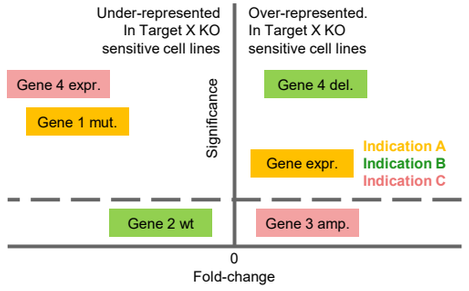
Sensitive

Resistant

vs

- Preferential indications
- Differential gene expression
- Differential gene alterations

Signature & markers of sensitivity



Patient sample scoring

Patient cohorts[1)]

TCGA cBioPortal FOR CANCER GENOMICS

Patient tumor RNA-seq

Patient tumor WGS

Signature

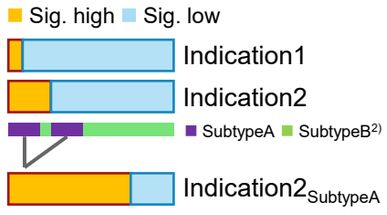
Scoring & Classification

Patient signature score

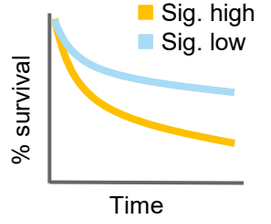
Patient biomarker incidence

Search for relevant indication & patient population

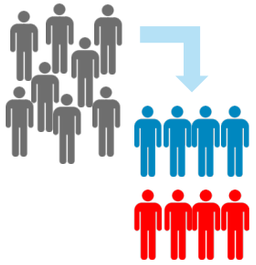
Incidence per indication/subtype



Link with patient survival



Stratification & responder prediction

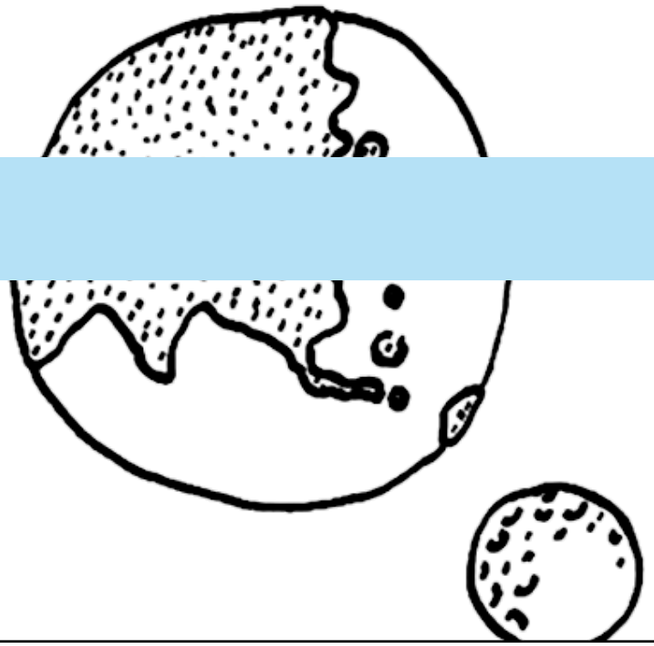


Responder

Non-responder

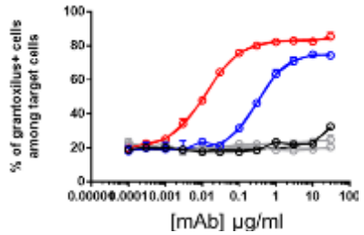
[1)] Public or proprietary cohorts
[2)] clinical, treatment, genetic

Contents



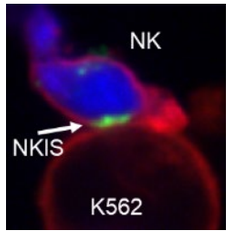
Immuno-Oncology: *in vitro* & *ex vivo* focus

A cutting edge technology platform to make a deep dive into MoA



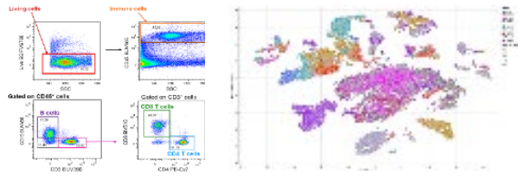
Functional *in vitro* and *ex vivo* immunological assays

- Supporting small molecules, biologics and cell therapy programs
- T-cells (αβ & γδ), Tregs, NK cells, B-cells, Neutrophils, M1/M2, Dendritic Cells, MDSCs
- Proliferation, cytokines production, killing, tracking of surface markers, suppression assay



Visualising immune cells "in action" at the contact of tumour cells

- Evaluation of IO products at the single-cell level monitoring Immunological Synapse (IS)
- Quantification of the data using Metamorph software



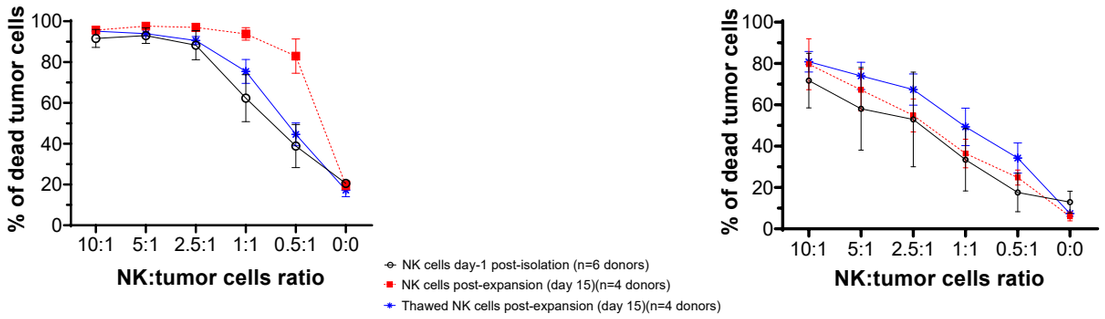
Filling the gap in drug discovery by accessing cancer patient samples

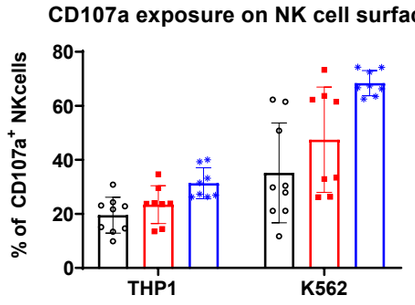
- Complex flow-cytometry based analyses on fresh human tumour resections
- Functional assays on the blood for target engagement validation, etc.
- Additional technologies for biomarkers identification: scRNAseq, proteomics, metabolomics, etc.

Case study: Deciphering NK cell biology

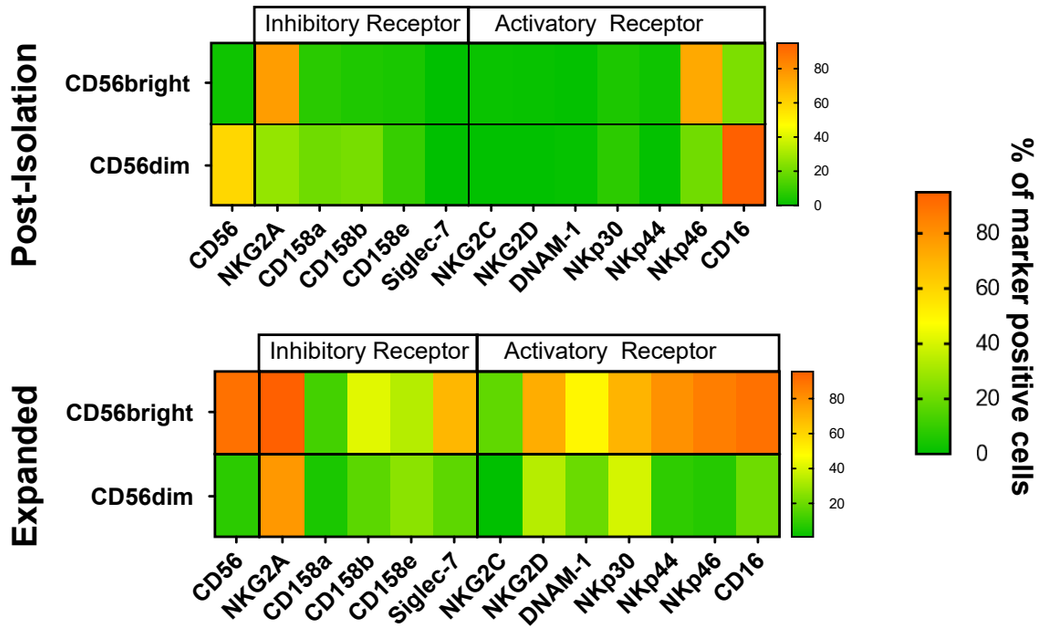
Functional & phenotypical characterisation of blood-derived NK cells

Cytotoxic assay:
NK cells post isolation versus post-expansion



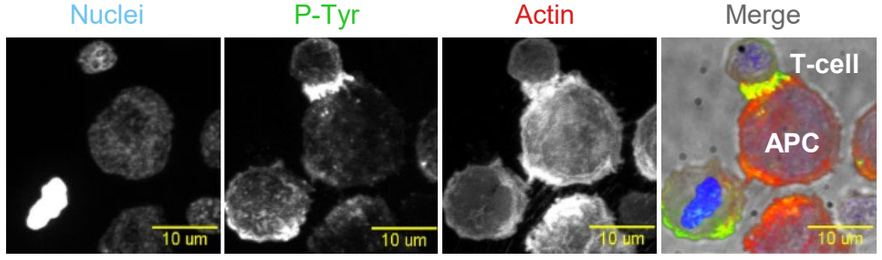


Phenotyping activating / inhibiting receptor balance
using flow cytometry: post isolation versus post-expansion



Immunological Synapse: a pivotal structure for characterisation across therapeutic modalities

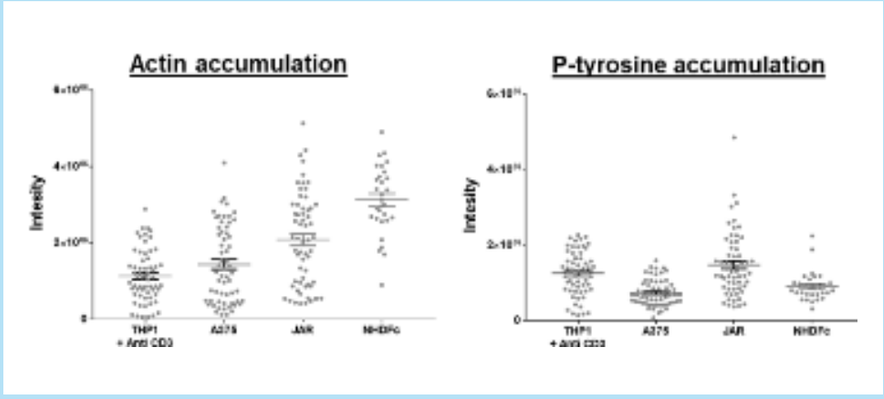
Visualisation of the immunological synapse at the single cell level by confocal microscopy



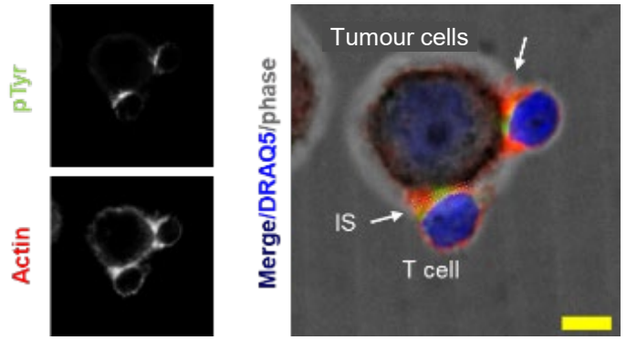
The Immunological Synapse (IS): a specialized interaction between **T-cells & APC** (Antigen Presenting Cells), **T-cells and tumor cells** and **NK cells and tumour cells**

- **Quantification** of the morphological data with the Metamorph software and associated statistical analysis
- **Evaluation of compounds/Ab** in the IO area modulating activation of T-cells when interacting with either APC or tumour cells

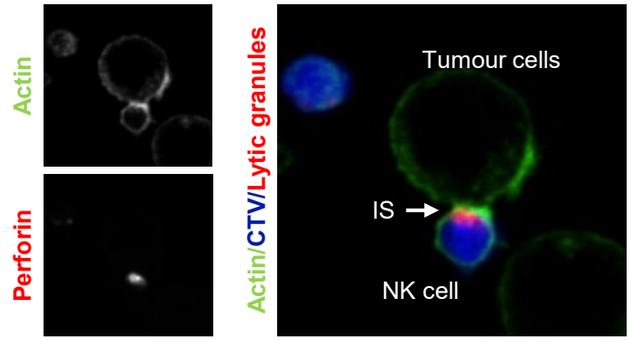
Quantification of T-cell interaction with tumour cells



IS stability (actin) and productivity (p-Tyr)

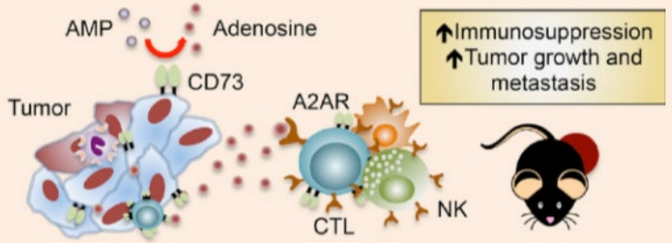


IS lytic function

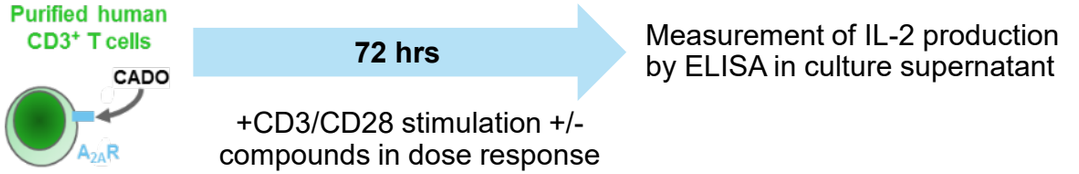


Case study: Targeting adenosine immuno-suppression

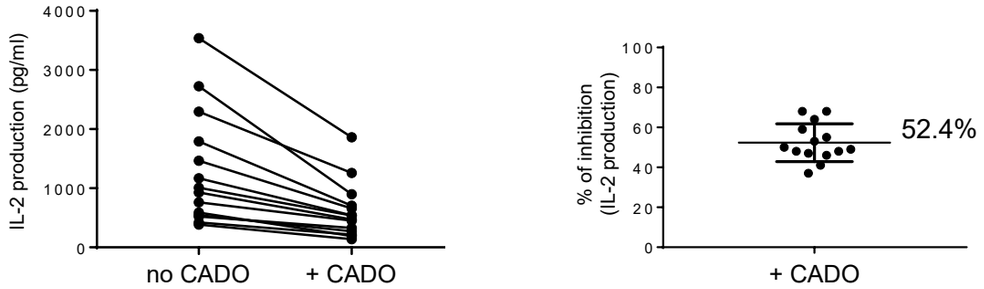
In vitro evaluation of compound antagonist of the A2AR on primary human T-cells



Recovery of IL-2 production by T cells

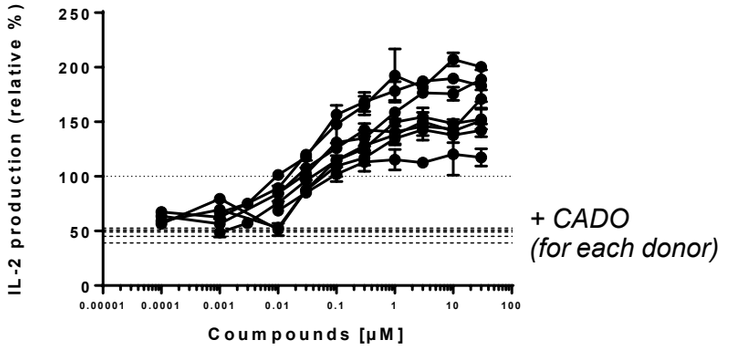


Basal effect of CADO on IL-2 production by T-cells



Results obtained for **16 tested donors** indicate good and robust level of CADO-mediated inhibition, CADO is a stabilized form of Adenosine

Recovery with a reference compound (Preladenant) on 8 donors



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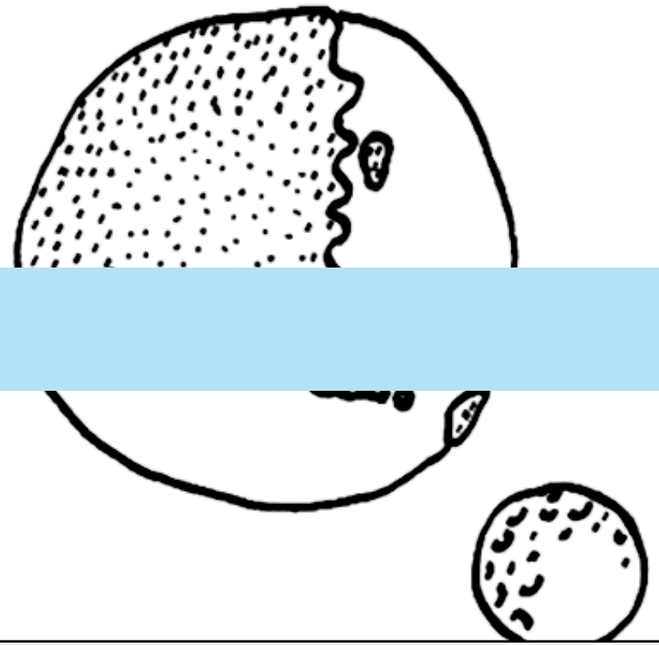
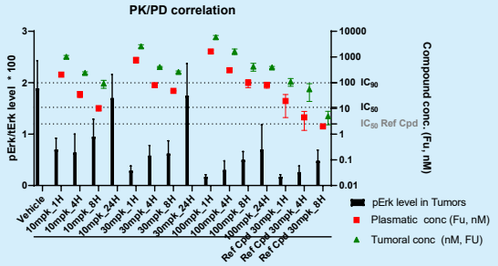
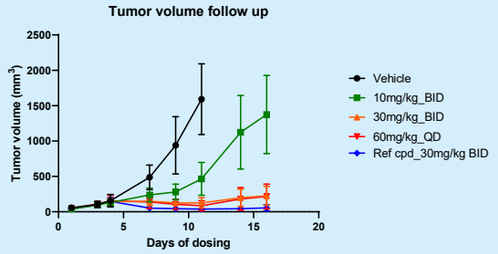


Oncology and immuno-oncology *in vivo*

A mix of proprietary assets and validated assays



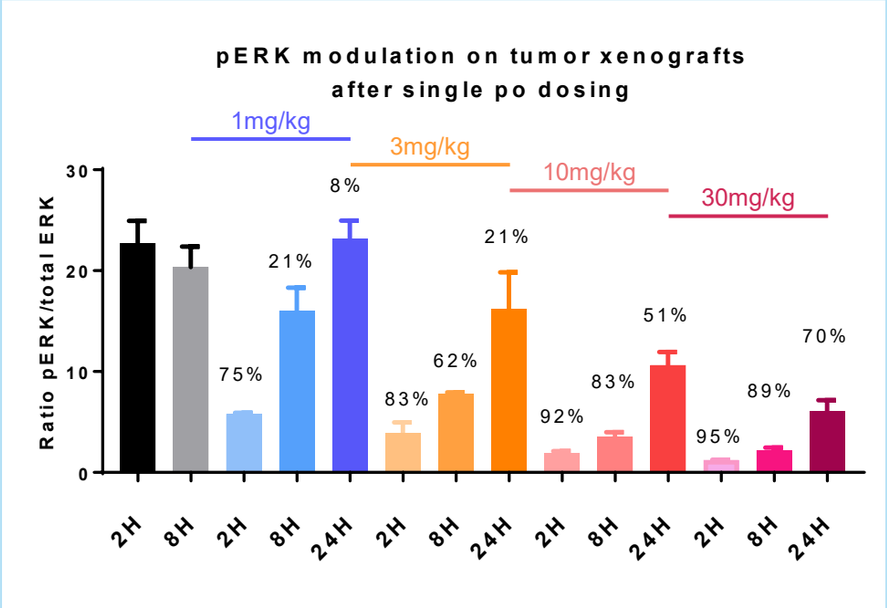


Mouse or rat tumour models	• **Human xenograft models:** s.c. & orthotopic models in **immunodeficient and humanised** mice • **Syngeneic models:** s.c. & orthotopic implantation of tumour cells in immunocompetent mice. suited for **immuno therapy & combination studies**
	• Tailored models with **PK and PD readouts** with biostatistical support – **Target engagement, biomarker or mechanism of action** studies – **Efficacy**
Specialised models	• ***in vivo* T-cell proliferation assay** • **Adoptive cell transfer** (in development) • **GvHD model** • **Chemo-induced alopecia** (hair loss)
Readouts	• **Sampling:** tumour, blood, urine, organs • **End points:** tumour size, haematology, clinical chemistry, phosphoprotein analysis (MSD Technology, Western blot, ELISA), mRNA analysis, flow cytometry, histology, immunohistochemistry • **Tumour imaging:** *in vivo* imaging (bioluminescence, fluorescence), X-ray imaging, Laser doppler, quantitative image analysis • **Compound blood exposure:** Bioanalysis (Mass spectrometry; ELISA for Biologics) • **Early toxicology:** blood biochemistry, haematology, organ specific & target specific safety biomarkers

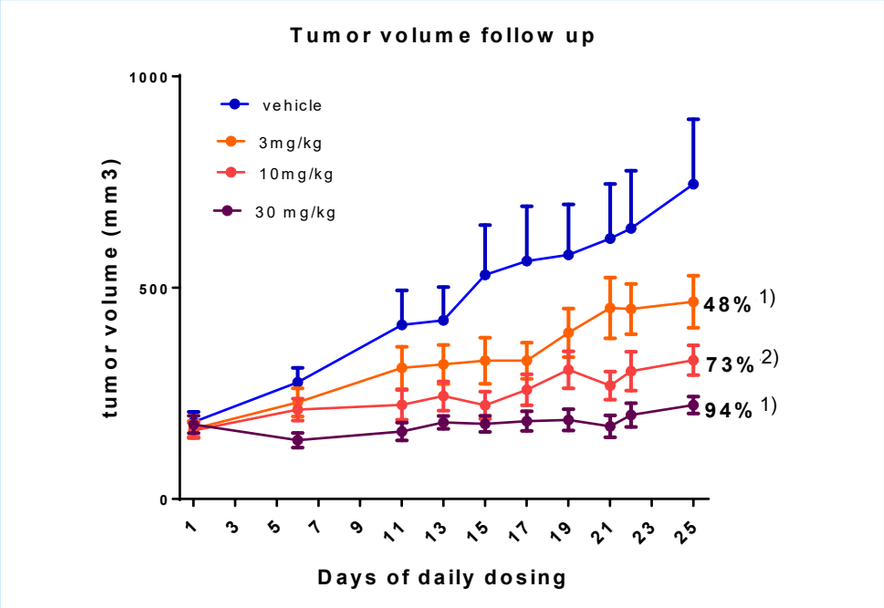
Dose-dependent PD response in tumours predicts efficacious dose

Case study: Routine use of PK/PD to predict efficacy in a pre-clinical model

Pharma-codynamic study



Efficacy study



- Single oral administration at 30 mg/kg leads to sustained pErk inhibition (>50%) up to 24 hours
- PK/PD data was predictive for dose and treatment schedule required to reach tumour stasis

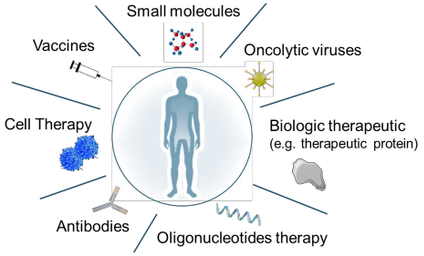
[1] p≤0.01

[2] p ≤ 0.001: ANOVA & Dunnett's multiple comparisons test

Immuno-oncology

Build together a tailored approach for the projects

Your therapeutic strategy

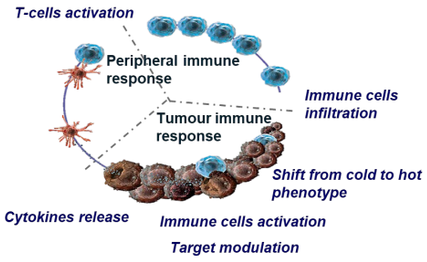


Model adapted to your need

- **Syngeneic mouse tumour models**
  - Featuring **full murine immunity** and comprehensive stroma
  - **Reduced cost / Rapid** (suitable when large group numbers is required)
- Humanized mouse tumour models
  - Opportunity to assess immunotherapy efficacy and pharmacodynamics in a **human immune-tumor context**
- **General evaluation & Clinical pathology:** clinical signs, body weight, food consumption, hematology (RBC & WBC counts)
- **Tumour growth:** digital caliper system, *in vivo* imaging (bioluminescence, fluorescence)
- **Survival**

Broad range of sample analysis (blood, urine, organs and tumours)

MoA of your candidate



Tailored *ex vivo* assays

- **Analysis of the tumour micro-environment:** flow cytometry, IHC
- **Functional assays with immune cells:** proliferation assay, ELISpot, flow cytometry
- **Analysis of cytokines release:** MSD & HTRF technology, ELISA
- **Analysis of phosphoproteins:** MSD & HTRF technology, western blot, ELISA
- **Analysis of gene signature:** qRT-PCR, RNAseq
- **Custom assay development**

IHC: Immunochemistry
MSD & HTRF: Meso Scale Discovery & Homogeneous Time Resolved Fluorescence

Case study: the CT26 colon carcinoma mouse model

Benchmarking single ICT response to select optimal immunotherapy combination

Study design

CT26 cancer cells (s.c.) in Balb/c (n=10-15/group)

Mice randomization based on tumour volume

Treatment with ICTs (i.p., 10 mg/kg, Q10Dx2-3)

D0 — D10 — D25

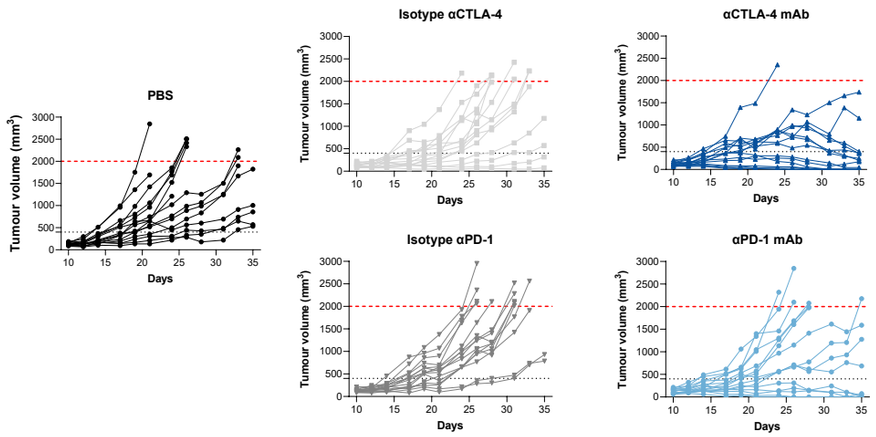
Time-course follow up of mice body weight and tumour volume (3x/week)

Endpoint tumour:

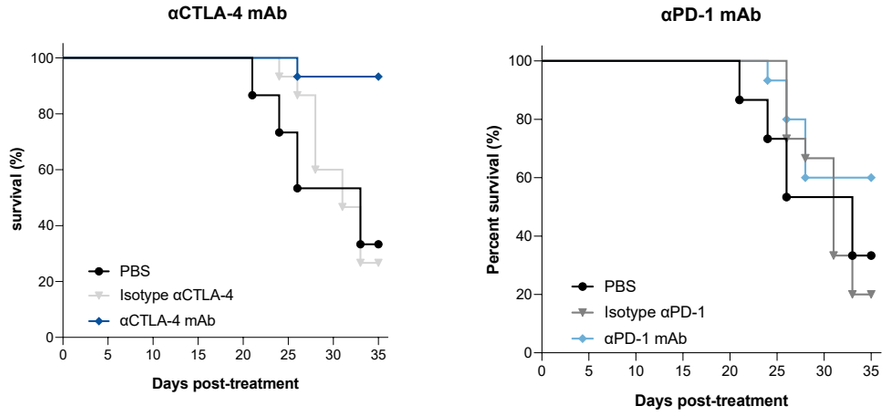
- Weight
- Immune cells infiltration (flow cytometry)

αCTLA-4 monotherapy markedly improves survival whereas αPD-1 mediates low anti-tumour efficacy

Time course of tumour growth (individual data)



Survival



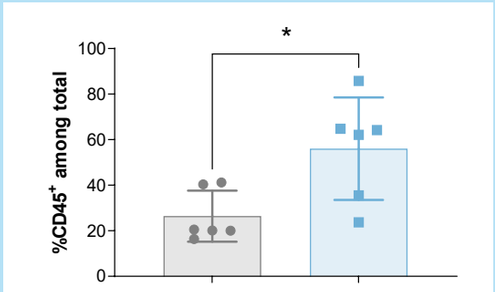
Statistical analysis: Log Rank Test Mantel Cox (ns p>0.05 and *** p<0.001)

Case study: the CT26 colon carcinoma mouse model

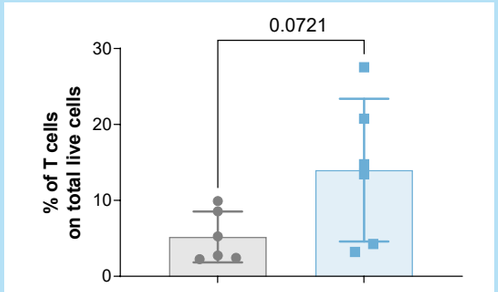
Increase in T-cells infiltration elicited by αPD-1 ICT is related to antitumor effect

αPD-1 elicits an increase in immune cells infiltration (particularly CD8⁺ T-cells) which is correlated to tumour weight

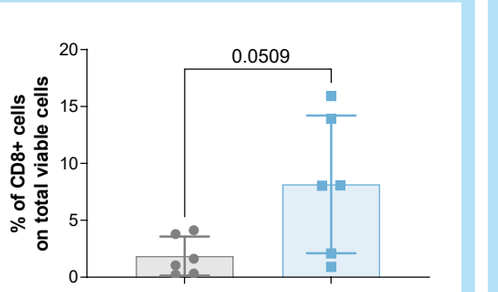
Hematopoïetic cells (among total viable cells)



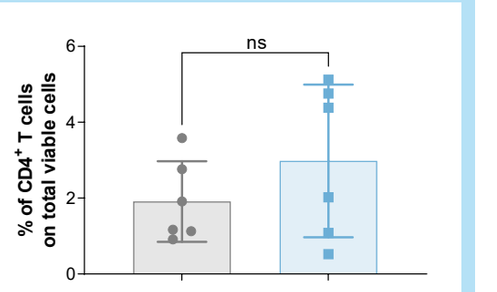
T lymphocytes (among total viable cells)



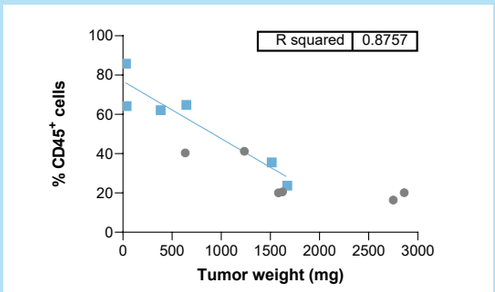
CD8⁺ T lymphocytes (among total viable cells)



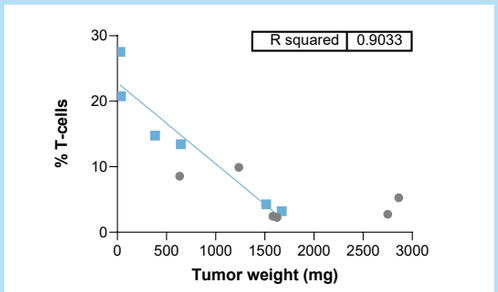
CD4⁺ T lymphocytes (among total viable cells)



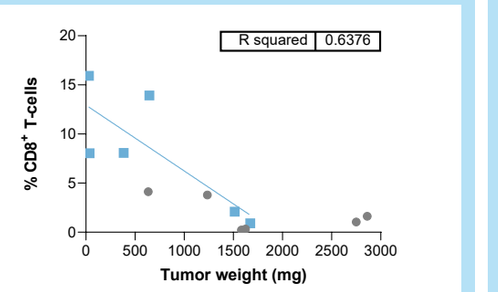
Correlation % CD45⁺ cells and tumour weight



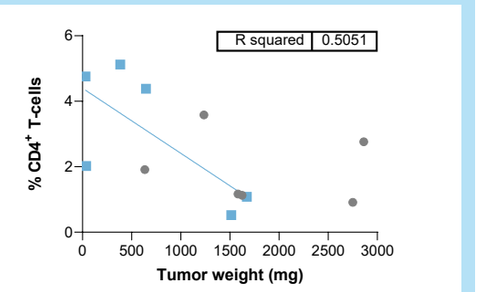
Correlation % T-cells and tumour weight



Correlation % CD8⁺ T-cells and tumour weight



Correlation % CD4⁺ T-cells and tumour weight



Isotype control αPD-1

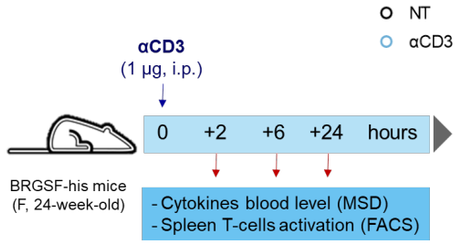
Results are expressed as mean ± SD.
Statistical analysis: Mann-Whitney, Unpaired t test w/ or w/o Welch's correction (ns $p>0.05$ and * $p<0.05$). Correlations were calculated using Pearson test.

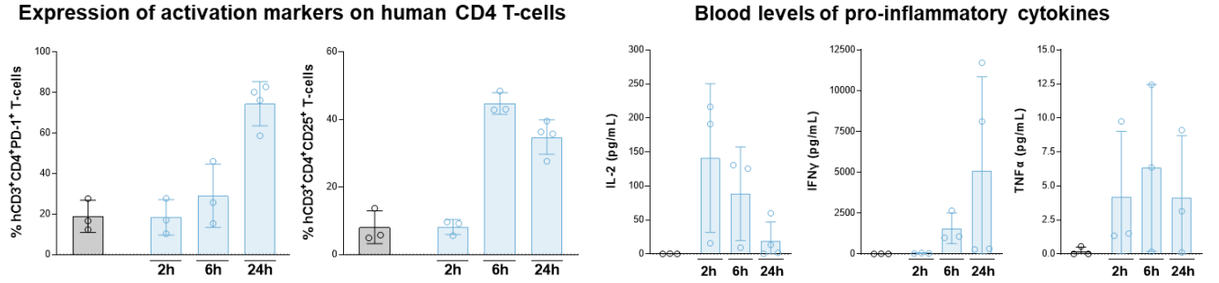
Case study: human immune-tumour context in BRGSF-His humanized mice

Opportunity to assess PD and immunotherapy efficacy in a human immune-tumour context

Human immune cells can be activated *in vivo*

αCD3 PD model of *in vivo* T-cells activation in humanized mice (n=3/time point):

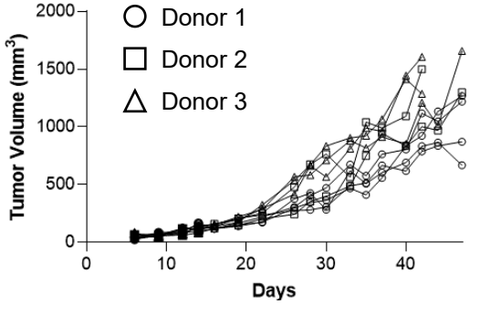




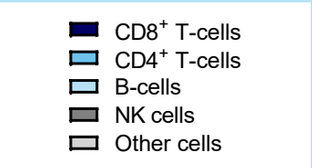
Full tumour take and homogeneous tumour growth

MDA-MB-231 TNBC model

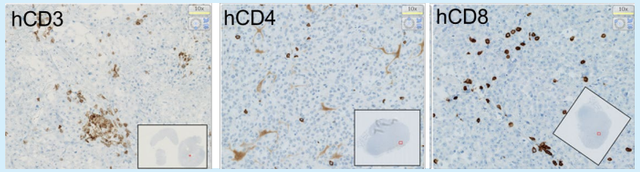
Similar results were obtained with A375 melanoma cells



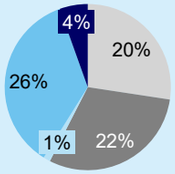
Human immune cells infiltrate human tumours (IHC and FACS)



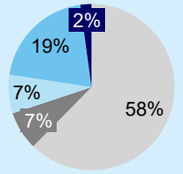
A375 melanoma model



MDA-MB-231

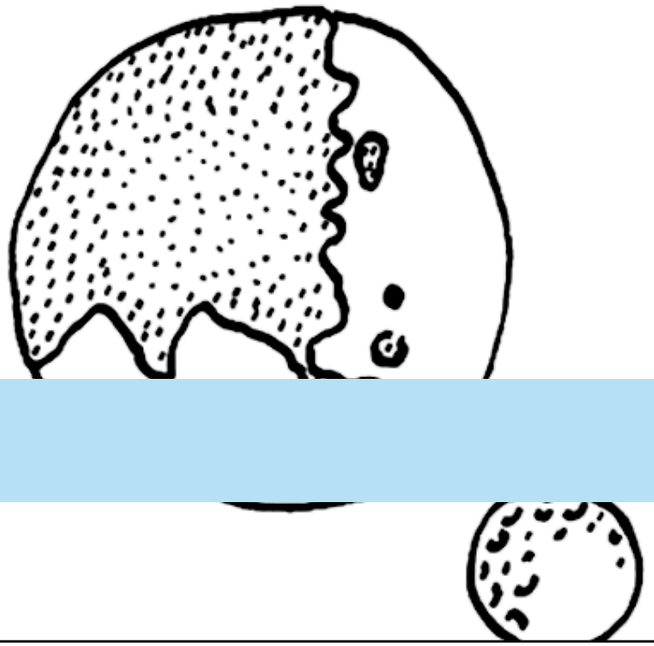


A375



PD = pharmacodynamic, MSD = Meso Scale Discovery, FACS = fluorescence-activated single cell sorting (flow cytometry), IHC = immunochemistry and TNBC = Triple Negative Breast cancer
Results are expressed as mean ± SD or individual data.

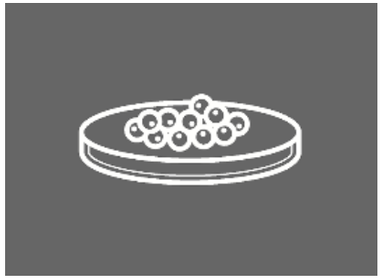
Contents



The need for translational thinking

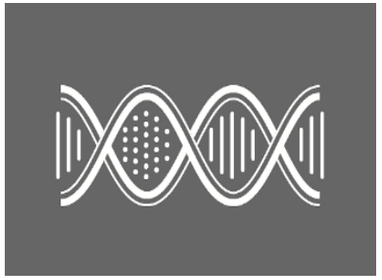
Incorporating patient samples early in the drug discovery process

FFPE tissue



Fresh (*ex vivo*) or frozen tissue

Fluid samples (blood & urine)



Whole genome and exome sequencing

Patient history

1 **Retrospective hypothesis testing**

2 **Prospective hypothesis testing**

3 ***De novo* clinical trial design**

Biomarker validation
Target MoA – Target engagement, PD, efficacy, toxicity biomarkers
Cancer indication – Stratification biomarkers (responder vs non-responder)

Technology-agnostic discovery and development of biomarkers

Translational biomarkers focused team

Evotec's translational biomarker expertise is applied to demonstrate target modulation in preclinical efficacy studies and to support translational biomarker readouts that are applicable to clinical samples

The team

- Global team of >50 scientists
- Strong expertise in immuno-assays
- Omics data analysts
- GCP capabilities

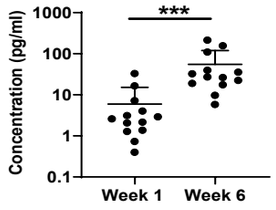
Sample analysis

- Cell lysates & supernatant
- Animal and human tissue biopsies
- Body fluids e.g. blood, plasma, saliva, CSF

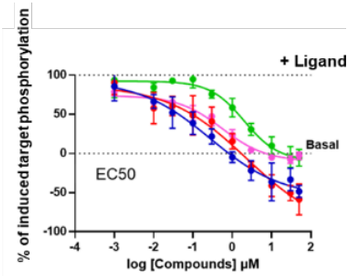
Technology platforms

- Immunoassays: MSD, TR-FRET, Luminex, Quanterix SMC, flow cytometry
- MS-based: Deep or single-shot proteome profiling; targeted MS using MRM, Metabolomics and LC-MS
- IHC, ISH, histology, Ventana multi-colour staining
- Others: colorimetric, Simple Western, qPCR, RNAseq, flow cytometry

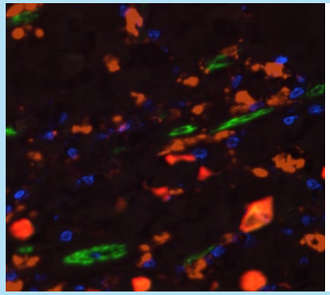
Case studies



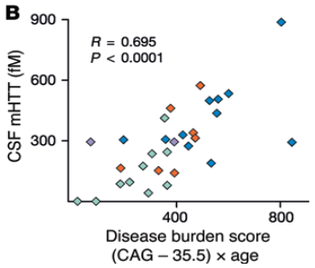
Cytokines (IFN-γ) in *patient plasma* by MSD



Phosphomarker in CD8+ T-cells in *whole blood* by Flow cytometry



TME[1] evaluation in *patient bladder cancer* by multiplex IF histology[2]



Ultra-sensitive mutant huntingtin protein quantitation in *clinical CSF samples* from patients by Singulex[2]

[1] TME = tumour microenvironment
[2] Performed on Roche Ventana BenchMark Ultra platform
[3] Singulex or SMCxPro (Single Molecule Counting); Wild et al. "Quantification of mutant huntingtin protein in cerebrospinal fluid from Huntington's disease patients", Journal of Clinical Investigation, April 2015

Strong clinical network already developed with IUCT

Network with the clinicians and the histopathologists

First-In-Human clinicians
IUCT, others

Head&Neck cancer:
IUCT, Hospital G. Pompidou

Hepatocarcinoma/iCCA:
IUCT, Berne university, Hospital of Bordeaux, Hospital of Lyon

Pancreas: IUCT

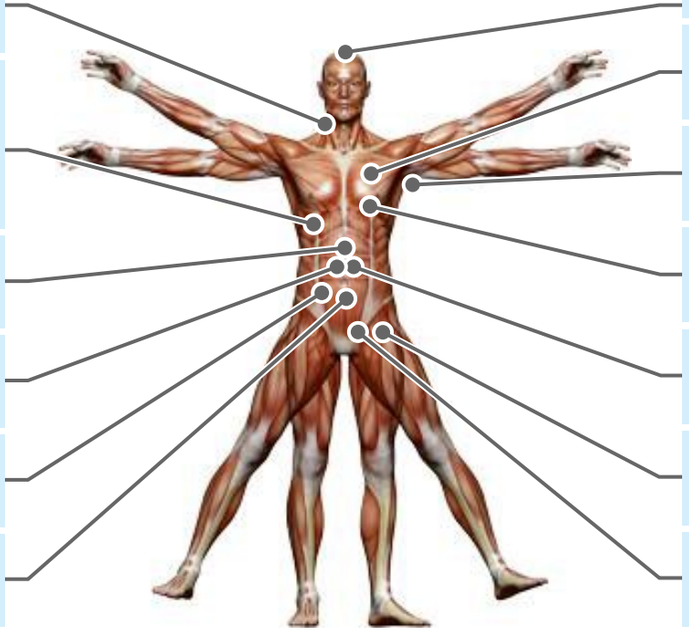
Ovary/Cervix: IUCT

Kidney: IUCT

Bladder: IUCT

Clinical trial office
IUCT

Pediatric oncology: IUC-T



Human Biological resources
IUCT

Glioblastoma: IUCT

Lung: IUCT and imaging

DLBCL: IUCT, Hospital Saint-Louis

Breast: IUCT

Onco-hematology: IUCT

Colon: IUCT

Prostate: IUCT

IUCT: Institut Universitaire du Cancer de Toulouse (Toulouse University Cancer Institute) is a major regional cancer hospital located at close proximity to Evotec (Oncopole site)

High viability of cells post-tissue digestion ensures high quality results

Evotec has validated a workflow for manipulating fresh patient samples

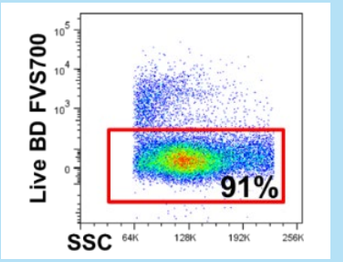
Access to healthy tissue, tumour resection & blood

Toulouse hospital

Transport to Evotec (<24h)

Tissue dis-sociation

Living cells

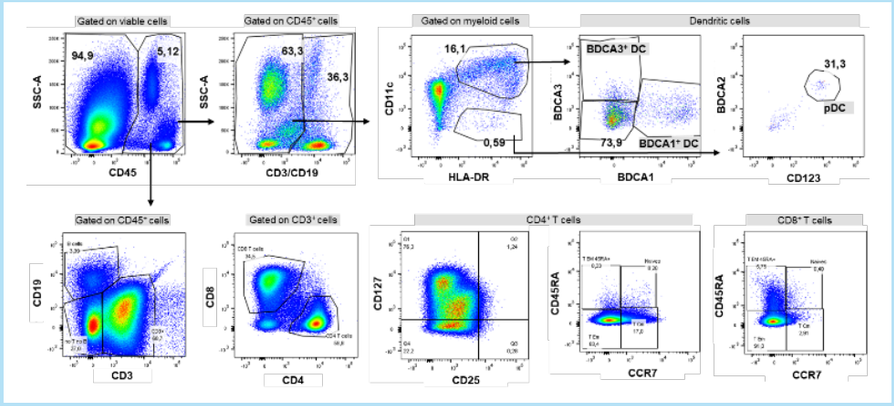


- **Flow cytometry**
- **scRNAseq**
- **3D culture**
- **etc.**

Proteomics

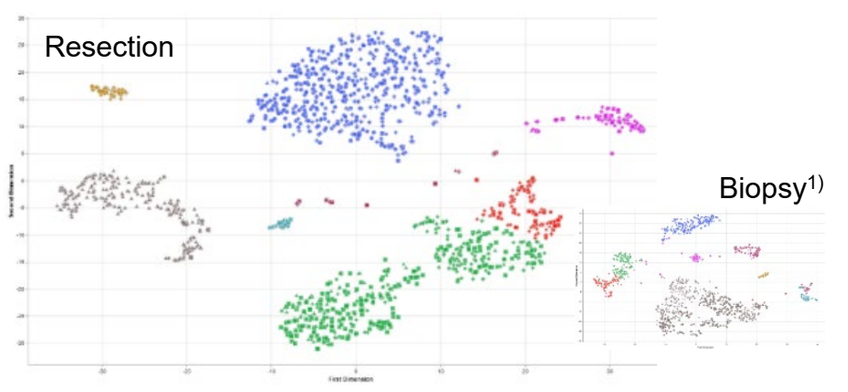
Supernatants

Immuno-phenotyping of tissue



Single Cell RNAseq of tissue

- B Cells
- CD4+ T cells
- CD8+ T cells
- Dendritic Cells
- Epithelial Cells
- Fibroblasts
- Mesenchymal
- Stem Cells
- Stromal Cells



1) Two punches were performed to increase heterogeneity; ~80% viability
MTA: Material Transfer Agreement
RNA SCS: RNA single cell sequencing

Case study: Pathway investigation in human frozen tumours

Analysis of targets / pathways

Fresh or frozen human tumour sample

Medical history of the patient

Metabolite quantification (200 mg)	**Total RNA sequencing** (50 mg)	**Proteome analysis** (50 mg)	**Western blot analysis** (50 mg)	**IHC ISH** (slides)

Characterisation of cancer immunosuppressive pathways correlated with the medical history of the patient

- **Objective:** Evaluate target / pathway markers to establish correlations and validate biomarkers
- **Requirement:** Prospective collection of larger tumour amounts (500mg) from specific cancers

Cytokine and chemokine characterisation in blood from cancer patient samples

Exploratory immuno-monitoring within a clinical trial

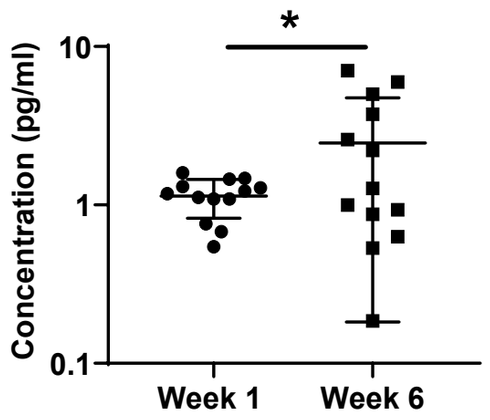
Objective:
MSD evaluation of cytokine levels in plasma samples from patients included in clinical trials

Sector S600

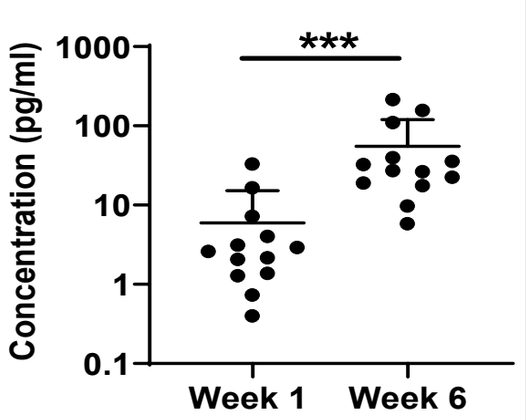
V-PLEX Proinflammatory Panel 1 Human Kit

IFN-γ	IL-2
IL-10	IL-4
IL-12p70	IL-6
IL-13	IL-8
IL-1β	TNF-α

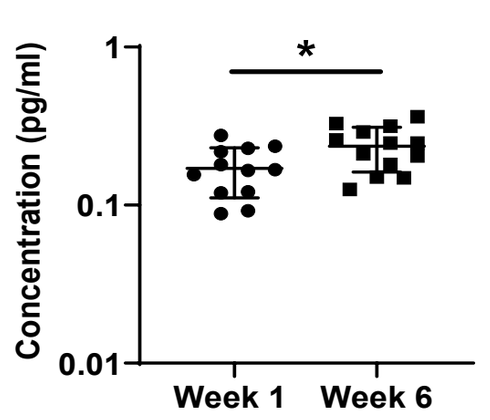
TNF-α



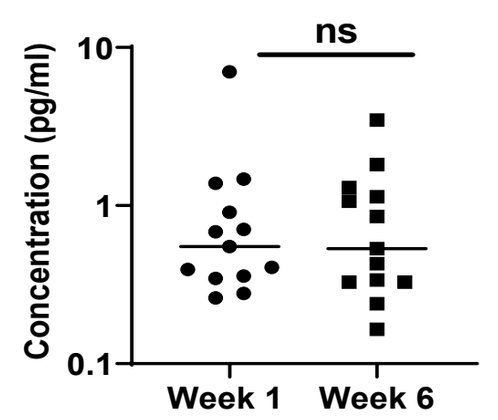
IFN-γ



IL-2



IL-6



Driving oncology projects through to the clinic

Evotec's integrated oncology platform

- An experienced team of scientists and drug hunters contributing to project success
- A track record in delivery on different target classes and disease mechanisms
- A leading technology platform and continual investment in CAPEX
- Early hypothesis testing for targets, biomarkers and chemical series
- Fully integrated drug discovery and biomarker discovery solutions
- Access to patient material at all stages of the drug discovery value chain
- Integration of feedback from clinicians and pathologists to help guide drug development and biomarker evaluation

evotec

#RESEARCHNEVERSTOPS

Your contact:

Business Development
114 Innovation Drive, Milton Park, Abingdon
Oxfordshire OX14 4RZ, UK

T: +44.(0)1235.86 15 61
F: +44.(0)1235.86 31 39
info@evotec.com