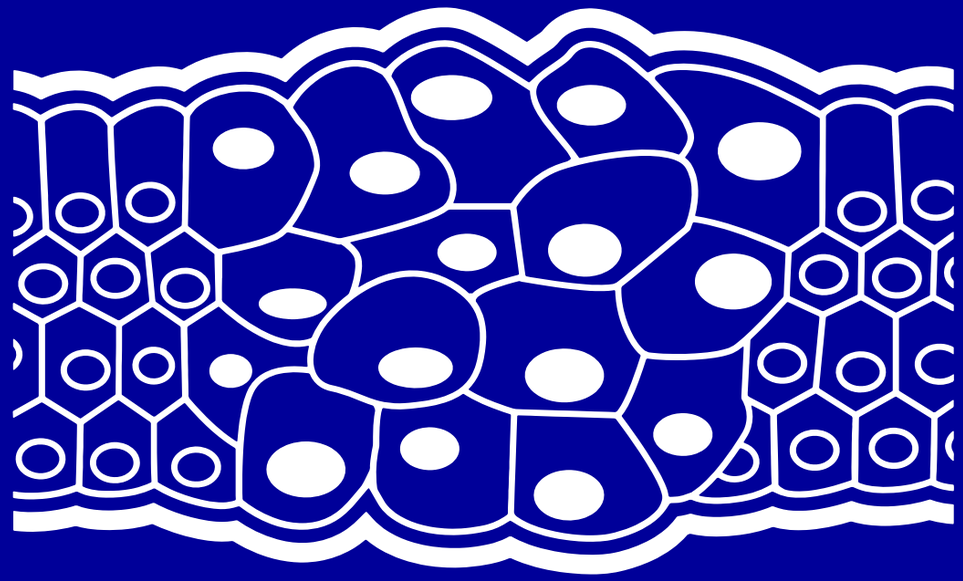
evotec

#RESEARCHNEVERSTOPS

Cancer Discovery at Evotec

Integrated Research and Development



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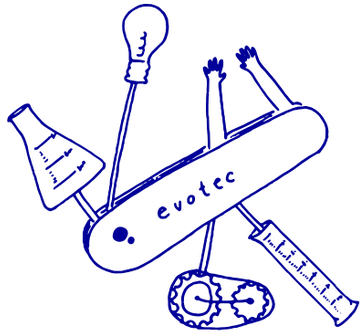
Evotec Cancer Discovery in a nutshell

Strong expertise and capabilities

Evotec's Cancer expertise

- Highly experienced team with deep disease knowledge and patient-focused thinking
- Knowledge of the competitive landscape in all approaches in cancer therapeutics
- Strong expertise in genomic/signalling drivers, immuno-oncology, protein degradation, DNA damage response, epigenetics and tumor metabolism



Capabilities dedicated to Cancer Discovery

- 160 staff *in vitro* team totally dedicated to cancer drug discovery and biomarkers, two thirds PhD/MSc level
- Over 30 staff *in vivo* oncology/immuno-oncology team
- Large dedicated facilities:
  - 1,400 sqm (15,000 sqft) *in vitro* labs
  - 1,000 sqm (10,000 sqft) cancer-focused animal facility

A team with a track record of achievement and delivery

Expert **cancer drug hunters** working collaboratively with our partners

Joanna Hergovich

EVP, Global In vitro Biology

>20 years Industry

Novartis

Protein degradation, Epigenetics

Francisco Cruzalegui

SVP, In vitro Biology, Oncology area lead

>20 years Pharma

AstraZeneca, Servier, Pierre Fabre

Cancer genomics, drug discovery, early clinical

Pascale Lejeune

SVP, In vivo Translational Biology

>20 years Industry

Sanofi, Bayer

Oncology preclinical models, ADCs

Michael Esquerré

VP, In vitro Biology, Immuno-Oncology

>16 years Biotech

Genticel

Immuno-therapies, Cell therapies

Steve Durant

VP, In vitro Biology, Oncology

>15 years Biotech/Pharma

AstraZeneca, Kudos

DNA damage response

Sandrine Delbary

VP, In vitro Biology, Oncology

>15 years Pharma

Sanofi

Targeted therapies

Frédérique Dol-Gleizes

VP, In vivo Pharmacology

>30 years Pharma

Sanofi

Drug Discovery Onco-logy & Inflammation

Pierre Fons

VP, Translational Biomarkers, Oncology

>15 years Pharma

Sanofi, Abtech

Angiogenesis, Clinical biomarkers

Thiérry Wurch

SVP, iR&D Biologics

>20 years Pharma

Pierre Fabre, Servier, Ipsen

Antibodies, ADCs, onco-logy, immuno-oncology

Jordi Gracia

VP, Medicinal Chemistry

>25 years Pharma

Almirall

Medicinal Chemistry, Drug Discovery

Christophe Boldron

VP, Molecular Architect

>20 years Pharma

Sanofi

AI/ML platform, Drug hunting, multi-modalities

Andrei Zinovyev

Principal Scientist, In silico R&D

>20 years Cancer Research

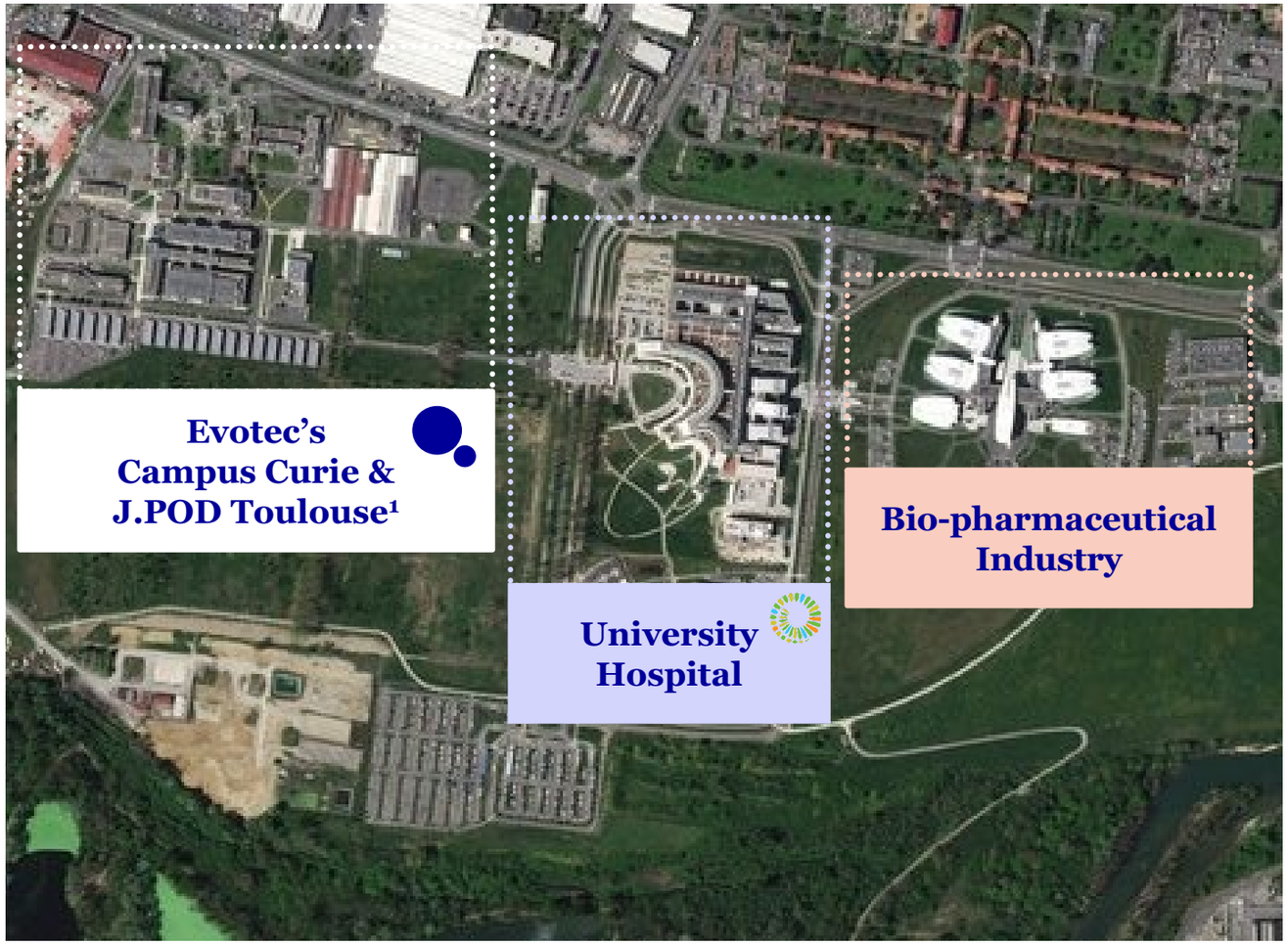
Systems biology, Oncology, AI/ML

Evotec Toulouse is embedded in a cancer biomedical campus

Combining medical and research excellence via proximity

IUCT-Oncopole is a highly recognised University hospital (~500M€ public funding)

- Combining medical and research excellence (IUCT, CRCT) in Oncology
- Incubator for midsize pharmaceutical and biotech companies
- **Example of working together:**
  - Kazia (EVT801) supported by Evotec, ongoing phase I trial at Oncopole
  - Exploratory biomarker assessments are performed by Evotec



ITAV Centre PIERRE POTIER

Pierre Fabre

CRCT Centre de Recherches en Cancérologie de Toulouse ONCOPOLE

INSTITUT UNIVERSITAIRE DU CANCER DE TOULOUSE Oncopole

EFS ÉTABLISSEMENT FRANÇAIS DU SANG

evotec

La Région Occitanie Pyrénées · Méditerranée

1 This project benefits from French Government funding as part of the investments for the future programme (programme d'investissements d'avenir in French) and is also supported economically by the Occitanie Region.

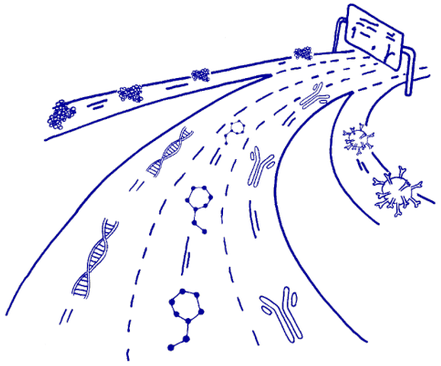
Fitting the pieces correctly together

Establishing a robust Integrated Drug Discovery portfolio of projects

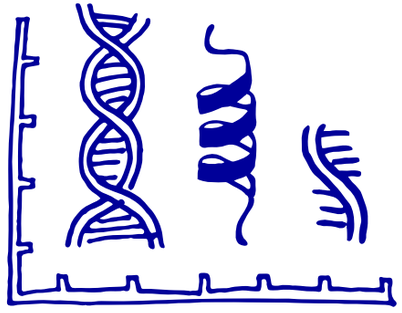
Humanoid knowledge

Disease biology

Multi-modality



Technology platforms



Creating success in precision medicine

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

Small molecules: reducing timelines to candidate

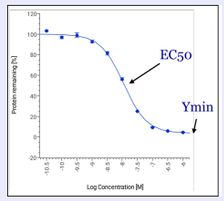
Powerful combination of AI/ML and efficient DMTA

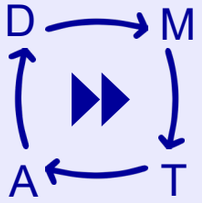
Advanced data curation and data-driven quality design

- Careful selection, cleaning and organisation of data for predictive modelling
- Data analysis and interpretation for project enablement and hypothesis generation
- Generative AI/ML & advanced computational design combined with drug hunting expertise

High-Speed Synthesis and efficient DMTA

- High speed synthesis (median TAT of 7 days) supported by access to state-of-the-art synthetic technologies
- Rapid DMTA cycles are enabled by full integration of Molecular Architects, Chemistry, DMPK and Biology
- Therapeutic area and development expertise, enables accelerated progression from LO to PDC and to IND





Evotec Biologics

Generation of fully human antibodies: from traditional platforms to A.I.-driven approaches

in vivo

Humanized mice-enabled hybridoma platform

Key distinguishing features

- Hybridoma generation merged with automated clone picking
- Screening of thousands of monoclonal candidates simultaneously

B cell technology

Key distinguishing features

- Direct screening of hundreds of thousands of B cells upon immunization or natural immune repertoire
- No species restriction

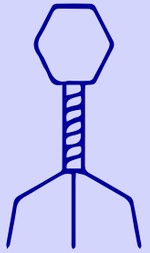
in vitro

Exploration of natural immune repertoire using phage display

Key distinguishing features

- Immune library generation upon immunization or natural infection
- *In vitro* selection of rare antibodies
- No species restriction

J.HAL®, A.I.-designed phage and yeast libraries

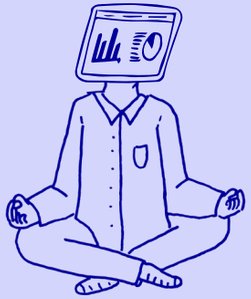


Key distinguishing features

- Highly diverse A.I. designed human library
- Time + cost savings for therapeutic development

in silico

In silico Ab design *(prototype stage)*

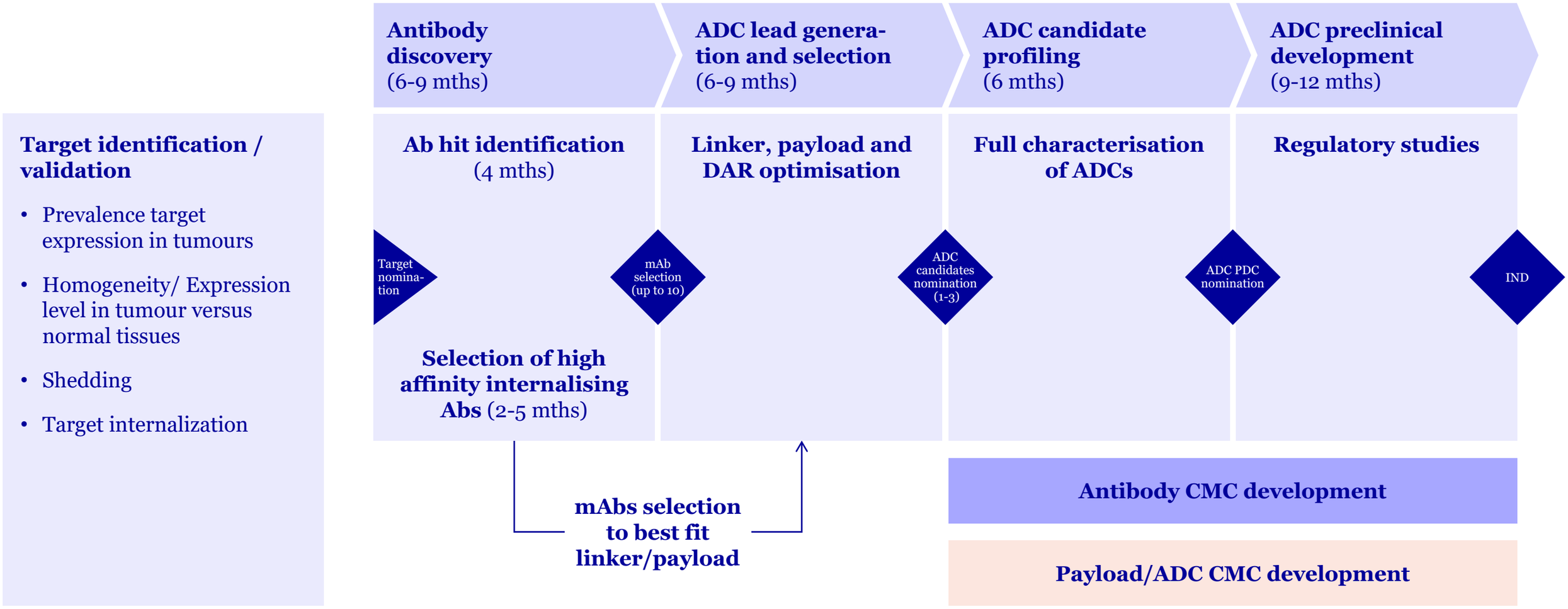


Key distinguishing features

- State-of-the-art platform to identify optimal binders *in silico*
- Fastest way to generate binders

Unique and integrated ADC drug discovery and development

One-stop shop: From target ID to IND (with multiple entry points)



See more details in the back up section

Targeting RNA

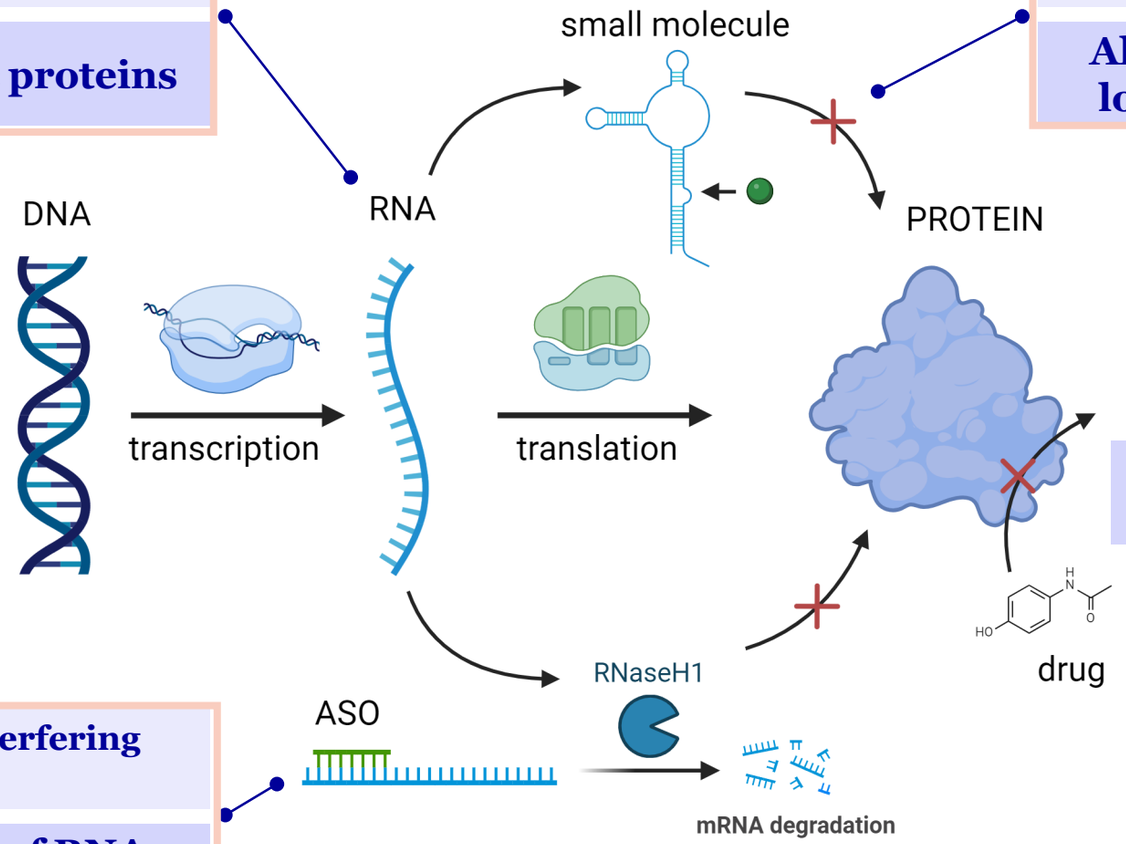
Applying multiple modalities with new target biology

mRNA therapeutics
capped and polyadenylated mRNA

Delivery of therapeutic proteins

Small molecule

Alter maturation, translation, location, degradation of RNA



Classic drug discovery inhibiting protein function

Antisense (ASO) and Interfering RNA (siRNA)

Up or Down regulation of RNA target, isoform selection

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

Case study: integrated drug discovery program for STORM Therapeutics

From target validation to candidate selection: first-in-class catalytic inhibitor of METTL3

HTS – Hit Identification → **Hit-to-Lead** → **Lead Optimization** → **IND-enabling Studies** → **IND submission**

HTS Hit

Biochemical (nM)	SPR (nM)	AMLCell IC_{50} (mM)
50,000	8,000	>25

Lead STM2457[1]

Biochemical (nM)	SPR (nM)	AMLCell IC_{50} (mM)
16	3	6

Candidate

Biochemical (nM)	SPR (nM)	AMLCell IC_{50} (mM)
<6	0.05	0.2

STC15[3] clinical candidate

Highly potent and selective METTL3 inhibitor in Phase I

STORM[2] THERAPEUTICS

Article

Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia

https://doi.org/10.1038/s41586-021-03536-w
Received: 18 December 2020
Accepted: 12 April 2021
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Check for updates

Eliza Yankova[1,2,3,13], Wesley Blackaby[3,13], Mark Albertella[3], Justyna Rak[2,4], Etienne De Braekeleer[2,4], Georgia Tsagkogeorga[1,3], Ewa S. Pilka[5], Demetrios Aspris[2,6], Dan Leggate[3], Alan G. Hendrick[3], Natalie A. Webster[3], Byron Andrews[3], Richard Fosbeary[3], Patrick Guest[3], Nerea Irigoyen[7], Maria Eleftheriou[1], Malgorzata Gozdecka[2], Joao M. L. Dias[8], Andrew J. Bannister[9], Binje Vick[10,11], Irmela Jeremias[10,11,12], George S. Vassiliou[2,4,6], Oliver Rausch[3]✉, Konstantinos Tzelepis[1,2,4,9]✉ & Tony Kouzarides[1,9]✉

1 Yankova et al Nature 593:597 2021
2 First-in-class catalytic inhibitor of METTL3
3 Giurguis et al Cancer Discovery 2023 https://doi.org/10.1158/2159-8290.CD-23-0007

Contents

Our expertise in key cancer biology areas

Covering important cancer hallmarks and therapeutic interventions

Targeted Therapies

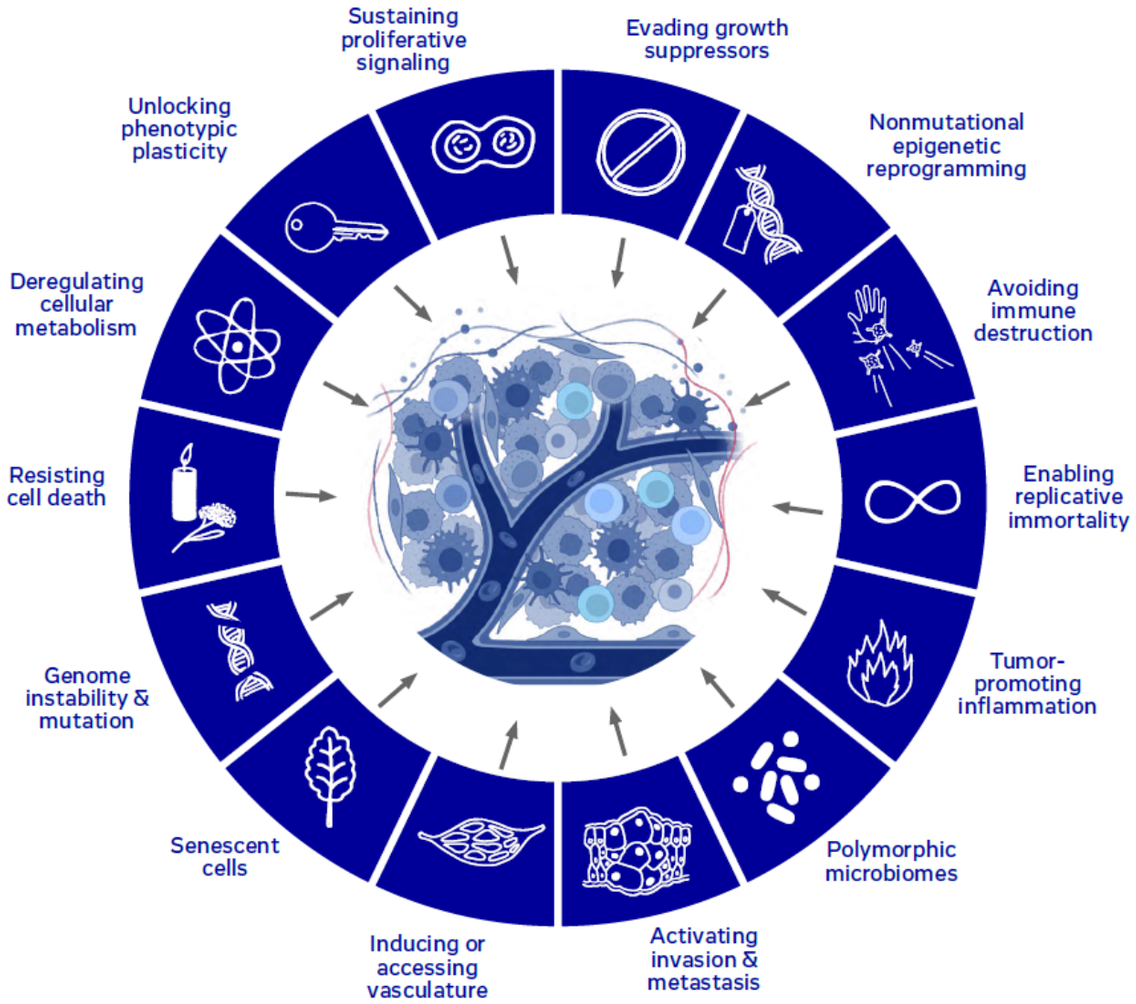
Targeting genomic drivers of tumour survival

Cancer Metabolism

Targeting mechanisms of metabolic adaptation

DNA Damage Response

Genomic vulnerabilities and synthetic lethality



Epigenetics

Transcriptional regulation and DNA/histone modifications

Immuno-Oncology

Harnessing the immune system against cancer

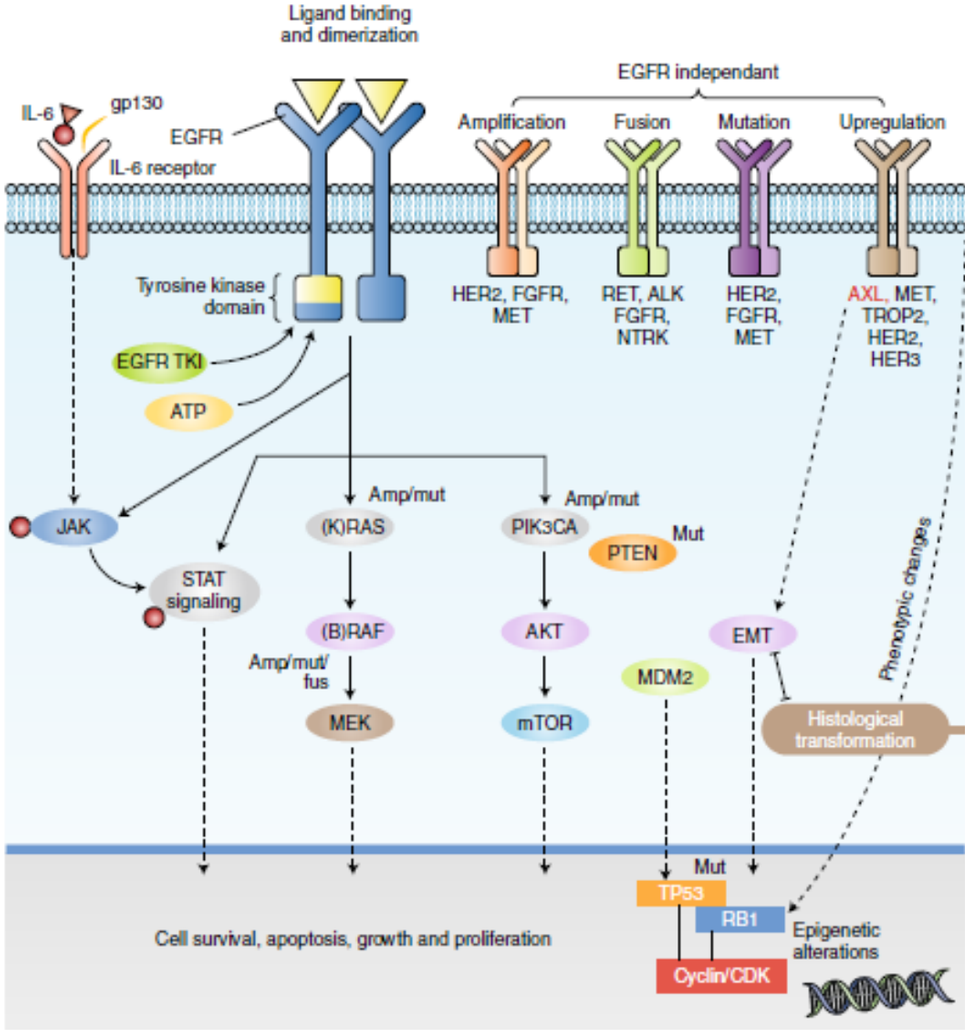
Tumour Microenvironment

Hitting stroma-driven barriers and other tumour defenses

Modified from Hanahan D. Hallmarks of cancer: New Dimensions. Cancer Discovery (2022)
Created with BioRender.com

Genomic and signalling drivers

Expertise in GTPases, kinases and phosphorylation readouts



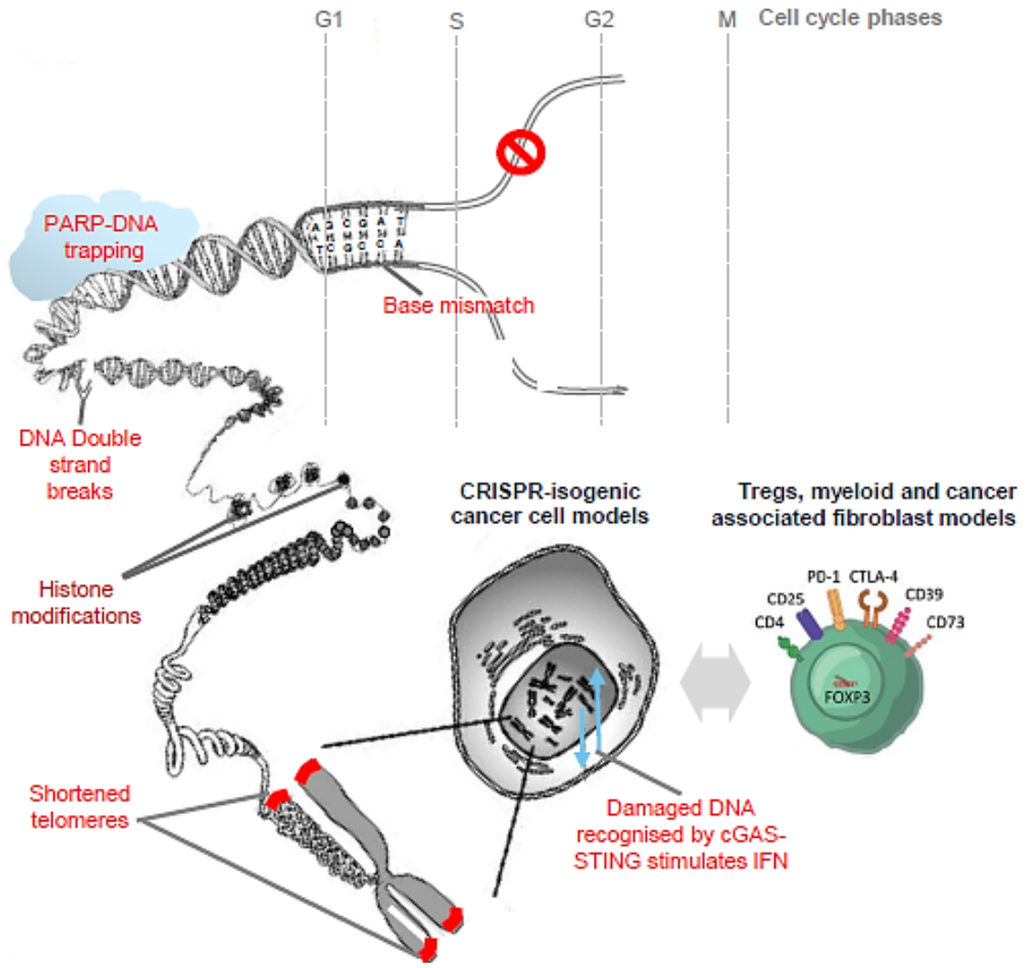
- **Cancer signalling drivers:**
  - Model generation for clinically relevant genomic alterations
  - Mutant receptor and non-receptor Tyr kinases (e.g. EGFR, FGFR, ALK fusions)
  - Ser/Thr kinases (RAF, CDKs)
  - Signalling switches (GTPase e.g. KRAS)
- **New binding modes and modalities explored to increase selectivity:**
  - Allosteric inhibition
  - Protein degradation
  - Protein-protein interactions
  - RNA binders
- **Transcription factors and chromatin regulators:**
  - Reporter assays and gene expression readouts
  - Epigenetic signatures

Modified from Passaro et al (2021). Nature Cancer

World-class capabilities in measuring the DNA Damage Response

Biochemical, cellular assays, *in vivo* models, and biomarkers for DDR drug discovery

- Cell cycle analysis combined with DNA damage readouts
- Measuring PARP trapping on chromatin
- Multiplexed DNA DSB repair reporters of NHEJ, MMEJ/ HR
- Histone and DDR protein modifications at DNA damaged regions (e.g. γH2AX, pRAD50)



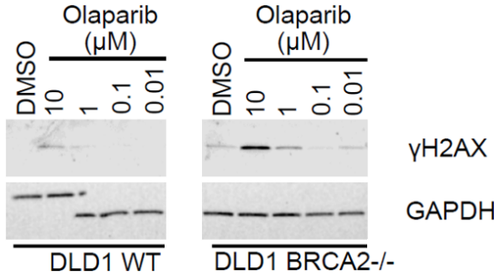
- Replication stress, fork stalling and collapse
- Microsatellite instable models
- Tumour microenviron-ment biomarkers
- Innate IFN response biomarkers (e.g. STING)

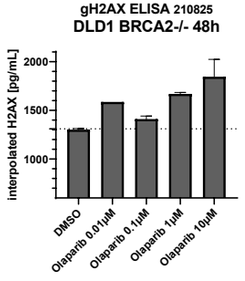
Adapted from: REVIEW article Front. Immunol., 03 December 2019 Sec. T Cell Biology Volume 10 – 2019 | and from https://www.physio-pedia.com/Genetic_Conditions_and_Inheritance

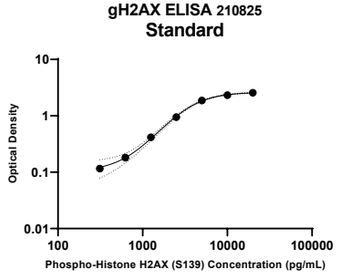
Case study: DNA Double Strand Break detection

Quantifying γH2AX foci and Rad51 as a proxy for DSB induction using high-content imaging

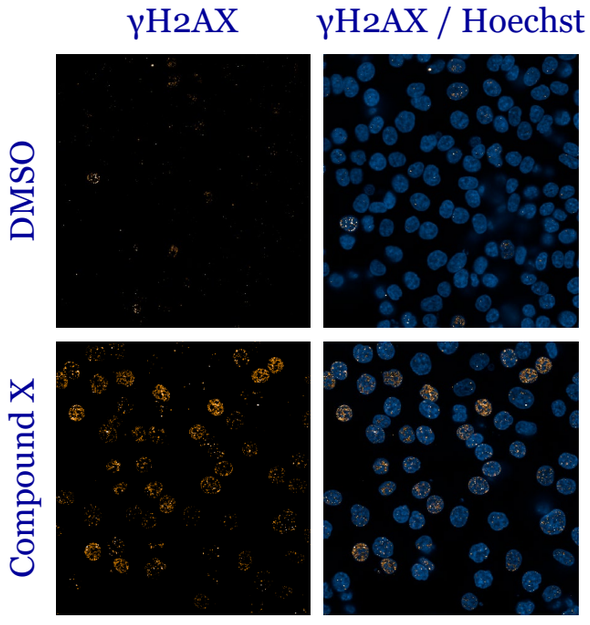
- **Rationale:**
  - Double-Strand breaks induce γH2AX foci formation
  - Phosphorylation on Serine 139 is mediated by the kinases ATM, ATR and DNA-PK and is an early cellular response to DSBs
- **Throughput:**
  - 27 compounds in dose-response
  - Suitable for Tier1 assay
- **Possibility of multiplexing** (Up to 4 colours): with other biomarkers (ex Rad51 for HR) or with cell cycle marker (ex EdU, H3S10-P)
- Alternative technologies to be considered for *in vivo* studies:
  - Detection of γH2AX by Western Blot and ELISA

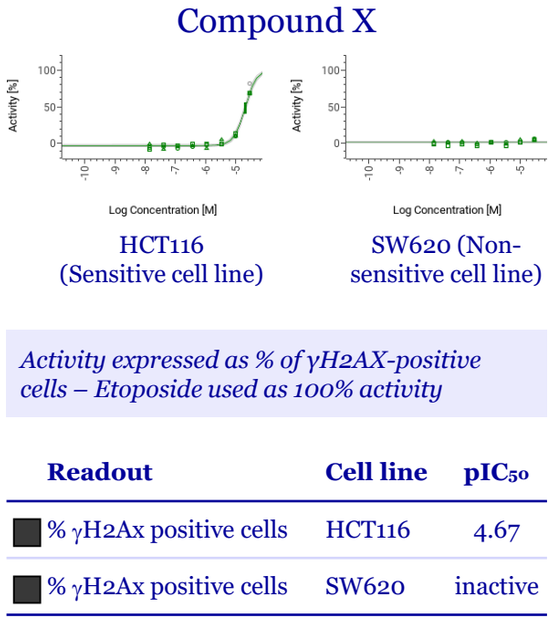






Case study: screening in HCT116 using Operetta® technology





Activity expressed as % of γH2AX-positive cells – Etoposide used as 100% activity

Readout	Cell line	pIC_{50}
% γH2Ax positive cells	HCT116	4.67
% γH2Ax positive cells	SW620	inactive

γH2AX detection also developed in SW480, RKO, DLD1, Kuramochi, U2OS and HT1299 cell lines

A broad range of Oncology *in vitro* assays

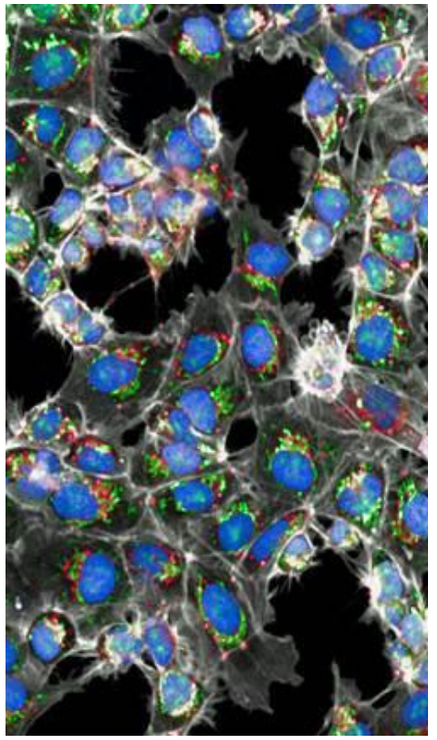
Validated assays for modality-agnostic drug discovery support

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	HTRF, ATPGlo, MSD, ELISA, Dot blot/Western, proteomics etc.
Tumour metabolism	Seahorse, Oxography, ATP, metabolomics etc.
Immuno-oncology	Flow cytometry and sorting, Incucyte, IHC, ELISpot, MLR, MSD etc.
Tumour metastasis and vascularisation	*In vitro* angiogenesis, hypoxic chambers, transwell etc.
Apoptosis	Incucyte, IF/IHC, flow cytometry, Western etc.
Epigenetics	RF/MS, SPR, HTRF, ChIP, TLDA/RT-qPCR, proteomics etc.
DNA damage response	Reporter assays, Operetta, synthetic lethality, replication stress etc.
Protein homeostasis	HiBit assays, nanoBRET, ubiquitination, Operetta, proteomics etc.
Imaging and phenotypic assays	Operetta, Incucyte, confocal microscopy, qPCR etc.

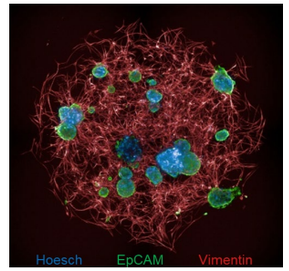


3D cell assays developed at Evotec Oncology

Models and read-outs

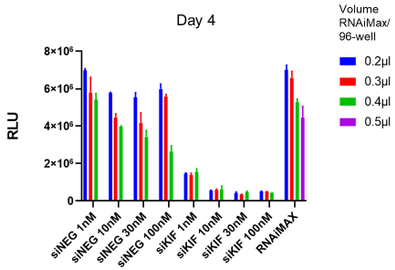
3D models

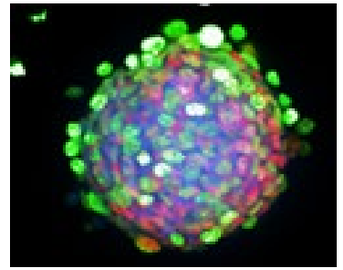
- Cancer cell growth in 3D
  (suspension or matrix-embedded spheroids & colonies)
- Migration/invasion in a 3D matrix
- Assays with co-culture of tumor cells with primary fibroblasts
- Patient derived organoids (PDOs)

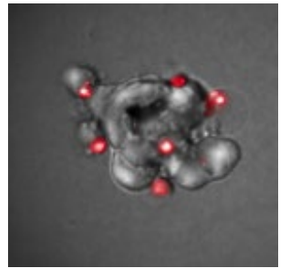


Proliferation / Viability / cell death measurements

- Colony or spheroid number, size, roundness
- CellTiter Glo (lytic system) or
  RealTime Glo (non-lytic)
- Dye-based apoptosis/necrosis assay,
  immunostaining

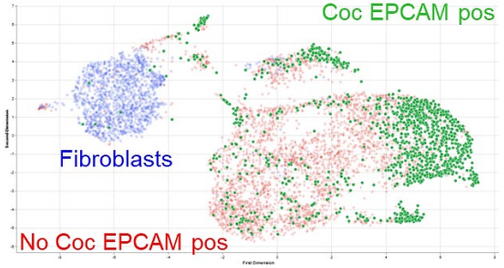






Target expression (coupled to RealTime Glo)

- mRNA levels (RT-qPCR) or protein levels
  (Western blot/JESS)
- Single cell mRNA sequencing
- Confocal imaging



From Human Primary Samples to 3D Cultures

Overview of Evotec Capabilities for Patient-derived Organoid (PDO) culture

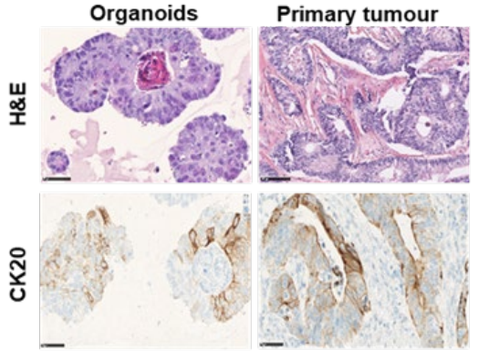
Establishment and characterization of PDO culture:

- Strong network with Toulouse hospital allowing access to healthy tissue, tumor resection & blood
- Setup high quality protocols for tissue dissociation suitable with:
  - Cancer cell and TME characterization (single cell RNA sequencing and Flow Cytometry)
  - *Ex vivo* 3D culture of PDO in Matrigel domes

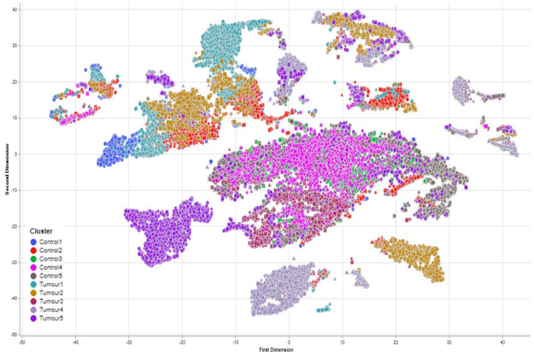
Ex vivo platform (PDO)

- Gene signature & biomarker secretion analysis
- 3D confocal imaging, IHC, Tumour metabolism
- Compound testing and TV study using siPOOLs (Lipofection)
- Viability (RealTime-GLO)/Apoptosis/Cell death plus secondary assay:
  - qPCR on 20-30 genes
  - protein detection via JESS / Immunofluorescence

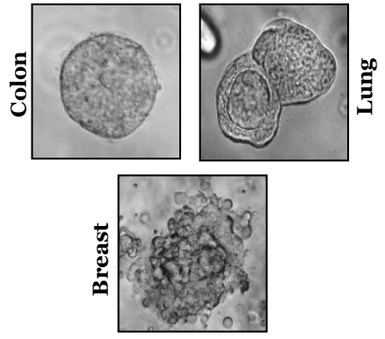
Immunohistochemistry



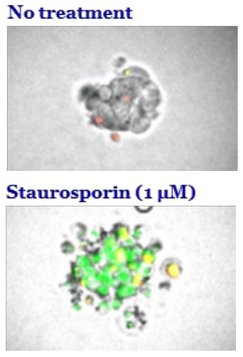
Single cell RNA sequencing



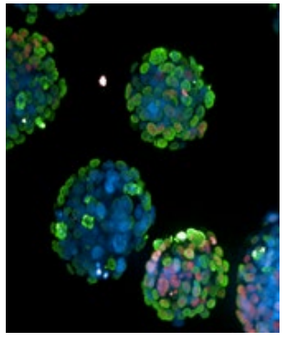
Brightfield analysis



Apoptosis/Cytotoxicity



Immunofluorescence



Access to PDO models:

- ❑ Evotec holds a MSA with a partner providing access to a biobank of >500 PDO models from various cancer indications.
- ❑ Additionally, EVT's partner can establish new PDO lines from fresh patient material, either sourced by the partner, Evotec or our clients

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

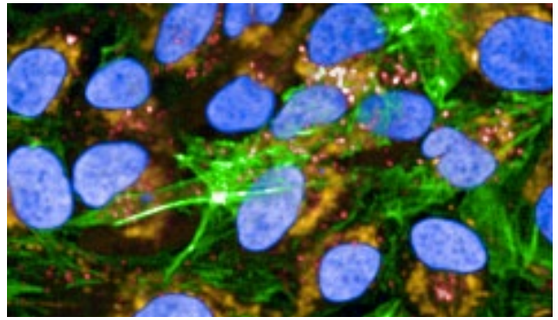
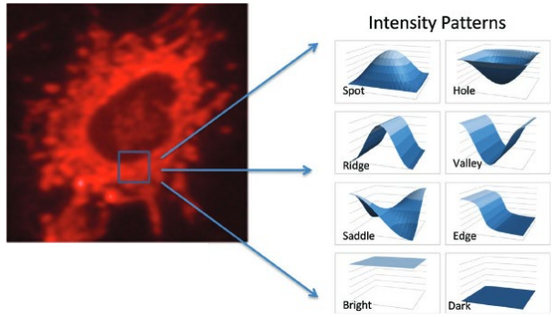


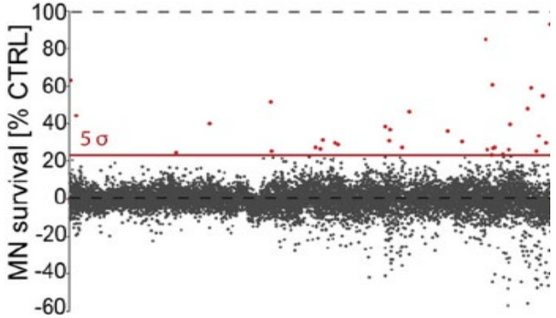
Image Analysis

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



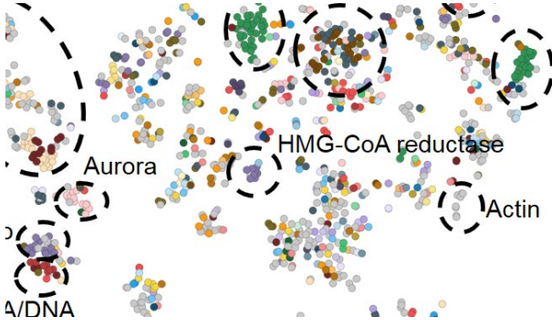
Data Analysis

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



Data Science

Proven track record of success-fully 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

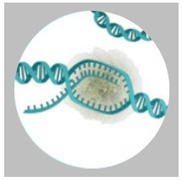
Professional and flexible development

Target Identification @ Evotec

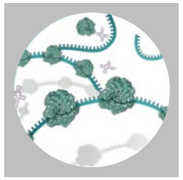
Integrating multiple data streams for hypothesis generation

Multiple data streams for Target Identification

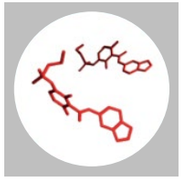
- CRISPR technology for target identification & validation
- Genomics



- RNAi approaches for target identification and validation
- Transcriptomics

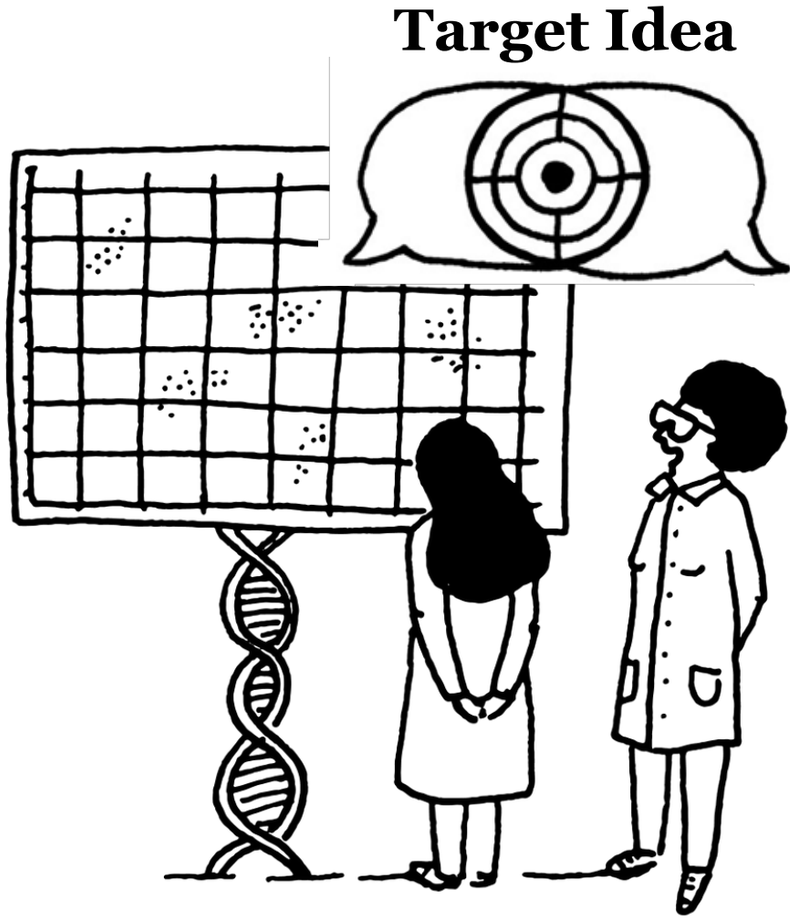


- Proteomics for target identification



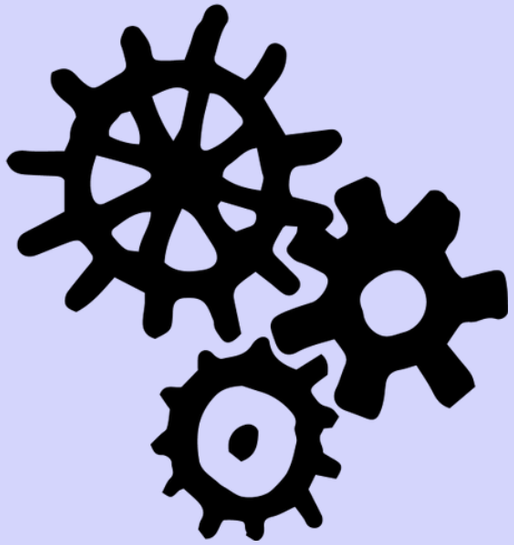
- Metabolomics for target identification

- Public domain and literature mining
- Bioinformatics



The 3 R‘s for Target Validation

Integrating relevant disease model, target manipulation and readout



The right model

- Relevant *in vitro* and *in vivo* models
- Focus on primary and iPS derived cells
- In-depth disease understanding

The right tool

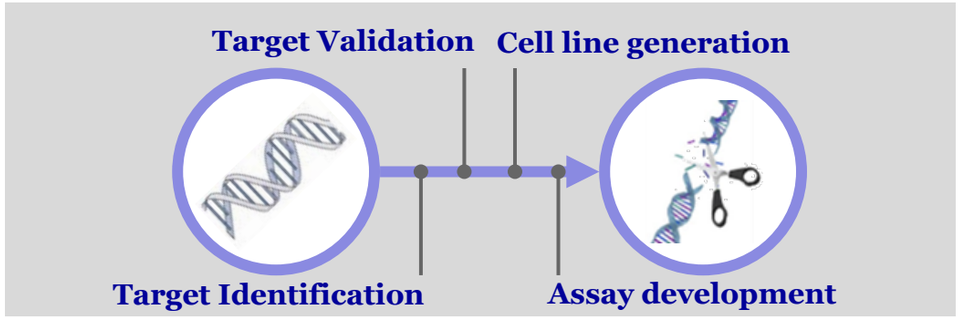
- Cutting edge genetic toolbox
- Engineering models to mimic disease
- Long term experience in target manipulation

The right readout

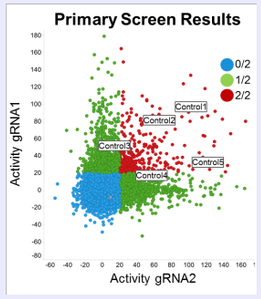
- Target specific assays
- In depth omics and information rich readouts
- Cutting edge bioinformatic tools for analysis

A CRISPR Toolbox for Target Identification and Validation

Different solutions based on project needs

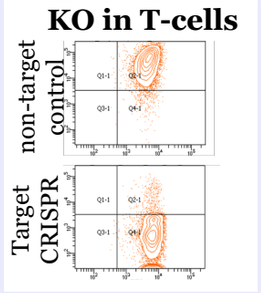


- Application of CRISPR and other genetic approaches at different stages of drug discovery process
- Dedicated team of scientists with broad experience in different disease areas
- Plug and play integration into existing Evotec platforms
- Close interaction with project teams ensures broad applicability and high success rates



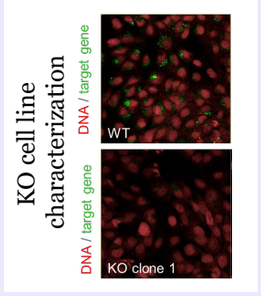
Target identification:

- Whole genome and genome subset screening
- Flexible screening formats and approaches



Target Validation:

- Validation of individual targets in disease relevant models
- Dedicated workflows for knock-out validation



Cell line generation:

- Workflows for CRISPR mediated genome engineering
- Genetic and phenotypic cell line characterization

1 Footnote
Source

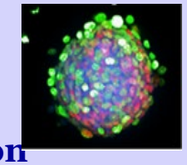
Target Validation Capabilities by CRISPR and RNAi

The ideal modular RNAi – CRISPR combined approach

siPOOLs: Defined pools of 30 selected siRNAs
- Reduce off-target effects while keeping potency
- However, 2 independent pools is good practice

- **Characterise cell growth & 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**



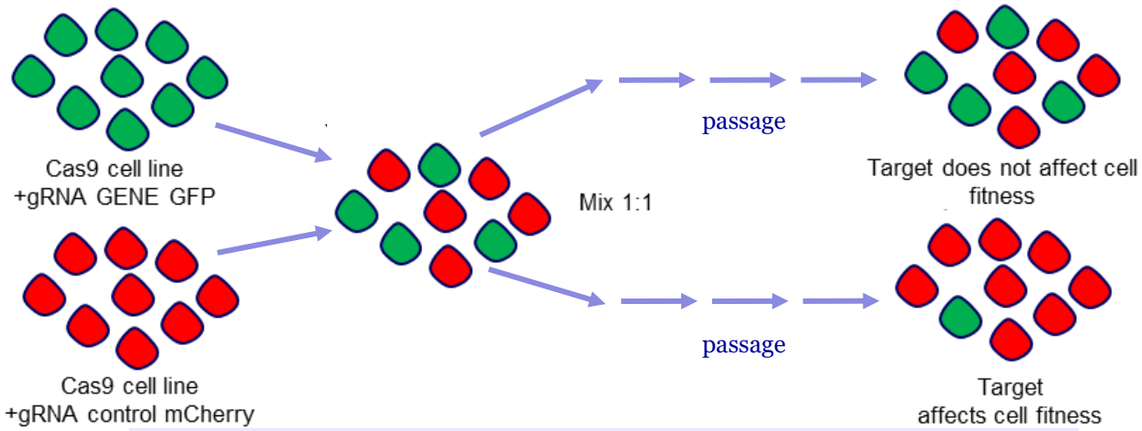
Rescue experiments possible but limits siP design

Option: Confirm with an independent technology

CRISPR: Gene editing, generate stable KO

- **Cas9/gRNA complex (RNP) delivery**
- **Generate Cas-9 lines (clonal lines or pools)**

Fluorescent Competition Assay
- **Monitor growth disadvantage/lethality or SL**



Ultimately, verify results by rescue experiments

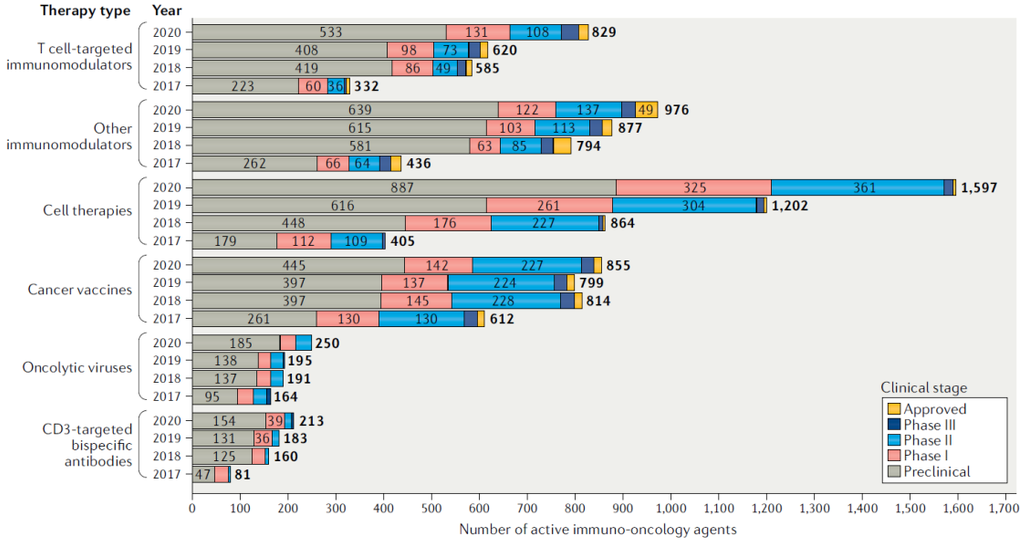
* For more details on siPOOLs, please refer to the back-up slide

Immuno-Oncology (IO) at Evotec

Campus Curie in Toulouse, France: the core location for Cancer Immunotherapy

- **Over 50 highly skilled and experienced Immunologists** working in the IO space (*in vitro*, *ex vivo* & *in vivo*)
- **Since 2015, Evotec has successfully developed partnerships in the IO field** as illustrated by many **press releases**[1]
- **Since 2021**, two IO drugs have been moved to **human clinical trials** in collaboration with: Exscientia ($A_{2A}R$ antagonist) and Kazia Therapeutics (EVT801)
- **Collaboration with Translational Biomarkers** to develop relevant translational evaluation of cancer immunotherapeutics on patient samples
- Bringing Immunology expertise to projects in the **I&I Therapeutic Area**
- **Supporting our Evotec's Oncology R&D portfolio highly focused on IO:**
  - **Biologics:** Immune Cell Engagers
  - Next generation **Cell Therapies** in Oncology with various **iPSCs-derived immune cell types** (e.g. iNK cells): presented at AACR23, AACR24 and SITC23

Cancer Immunotherapy is the fastest growing area within Oncology



1 e.g. Sanofi/Apeiron, Tesaro/GSK, The Mark Foundation for Cancer Research, Exscientia, OxVax, etc.
Source, Upadhaya S. et al. Nature Rev. Drug Discovery 2020

Building on two key pillars for Immuno-Oncology drug discovery

Immunology understanding & versatility in therapeutic modalities

1

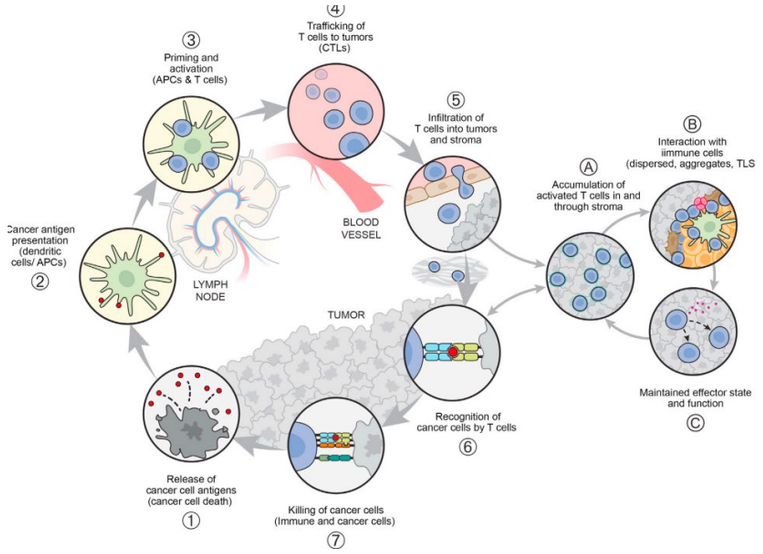
In-depth Immunology knowledge on:

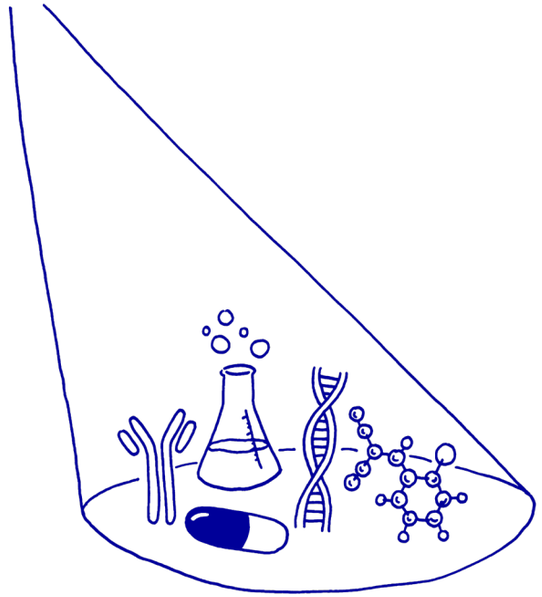
- Broad range of immune cell types
- Various targets
- On both the liquid and solid tumor space

2

Broad experience from Small Molecules to Cell Therapy:

- Small molecules
- **Biologics:** antibodies, bispecific, cancer vaccines, peptides
- **Oligonucleotides:** ASO, RNA
- Cell Therapy





Source: Ira Mellman, Daniel S. Chen, Thomas Powles and Shannon J. Turley, Immunity 2023

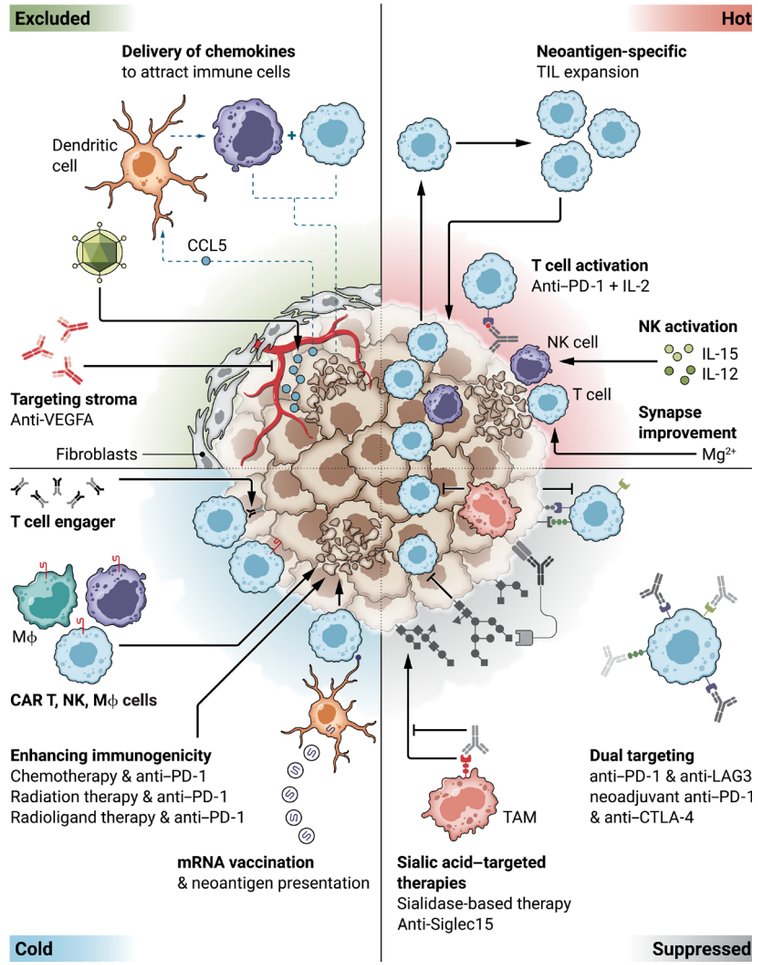
IO is a multi-modality therapeutic area: the combination thinking

Checkpoint inhibition is only the tip of the Cancer Immunotherapy iceberg

- **Checkpoint inhibitors are blockbusters and have transformed cancer care since a decade (first one approved in 2011[1]):**
  - Now used as **1st line treatment** in many indications (>65 FDA approvals in 20 different indications[2])
  - **Low response rate (around 20%[2]) but strong and long-term clinical efficacy** and reduced side effects as compared to conventional chemo (outside of inflammatory / auto-immunity AE)
- **Many challenges** are associated such as low response rate, toxicity, additional immune escape mechanisms (opportunity for combination therapy)

Evotec Immuno-Oncology team perspective: *"the main challenge for the next decade will be to unravel why some patients respond and the others don't"* – by:

- **Evaluating combination** with new ICTs in development and other immunotherapies (e.g. vaccines, cell therapy, bispecific Ab, etc.) / chemotherapies / radiotherapy
- **Integrating knowledge about biomarkers** into patient selection in trials



Source: Kirchhammer N et al. Science Translational Medicine 2022; Shi Y et al. Frontiers in Pharmacology 2021
Note: AE (Adverse Event)
1 Yervoy Ipilimumab label (fda.gov); 2 Nature Review Biotechnology, vol 40, 981 2022

How Evotec IO Scientists are supporting Drug Discovery programs

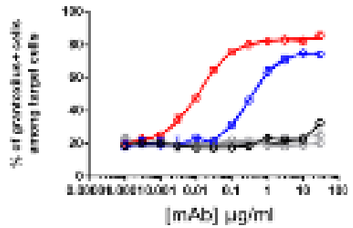
Of mice and men: a drug discovery continuum including cancer patient samples

Broad expertise from bench to bedside

1	**Target validation**	• Genetic editing of primary immune cells by **CRISPR** technology
2	**From H2L to PDC**	• Tailored ***in vitro*** **functional immunological assays with primary immune cells** • Broad range of ***in vivo*** **preclinical mouse models** • Evaluation of **immune-related toxicity** with different animal models
3	**From PDC to Phase I**	• **Whole blood functional assays** for on-target biomarker strategy • Translational assays using samples from **cancer patients accessed thanks to the collaboration with the IUCT-Oncopole**

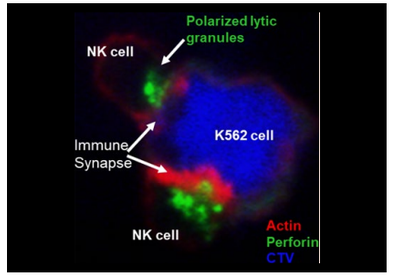
Immuno-Oncology Therapeutic Aera in a nutshell

Building tailored approaches for successful drug discovery programs



Functional *in vitro* Immunological assays

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

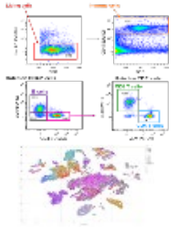


Visualising Immune cells “in action” at the contact of tumour cells

- Evaluation of IO products at the single-cell level monitoring Immunological Synapse
- Quantification of the data using Metamorph software
- High-speed imaging of the Immunological Synapse (ImageStream X)
- 384w plate assays with High-throughput confocal imager: Operetta

Preclinical *in vivo* rodents models in Immuno-Oncology

- Syngeneic tumour models and human xenograft models with humanized mice
- Therapeutic efficacy, PK/PD, analyse of the TME, *ex vivo* functional assays, etc.



Filling the gap in drug discovery by accessing cancer patient samples

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

TME: Tumor MicroEnvironment

Flow Cytometry platform: core expertise for Immunology

A dynamic flow cytometry facility with a dedicate expert team & powerful instruments

Analyzing immune cells

BD FACS Canto II
10 parameters (3 lasers)
Plate sampler (96 & 384)

BD Fortessa X20
20 parameters (4 lasers)
Plate sampler (96 & 384)

Bio-Rad ZE5
30 parameters (5 lasers)
Plate sampler (96 & 384) and tube loader

BD Symphony
30 parameters (5 lasers)
Plate sampler (96 & 384)

Isolating immune cells

FACSAria-Fusion (4 lasers) in a dedicated BSL2+ lab: optimal for patient samples

autoMACS Pro separator
Automated cell isolation
Magnetic cell separation

- **Strong knowledge in Immunology**
  - Human
  - Mouse
  - Rat
- **Complex phenotypic and functional analyses**
  - FlowJo software
  - Diva software
  - ImageStreamX
- **Development of AI tools in panel design**
- **Data analysis automation**

A dynamic team in the field of flow cytometry

- **Presentations to external events**
  - AFC conferences 2022 & 2023
  - Bio-Rad seminar
  - Genedata virtual presentation 2023
  - ELRIG flow cytometry event GSK 2023
- **Local flow cytometry workshops & events** organized with partners at the Evotec Campus Curie

BIO-RAD Genedata AFC Association Française de Cytométrie Miltenyi Biotec BD

Contents

State-of-the-art animal facility and *in vivo* expertise in Toulouse

In vivo team of ~90 staff

- **Drug discovery and research services (non-GLP) include**
  - **PK studies** supported by formulation assay/screening
  - **PK/PD studies** in accordance with *in vitro* assays and identification of PD biomarkers
  - **Efficacy studies**
  - **Early discovery toxicology:** type/ severity of injury, MTD, NOAEL, dose-exposure relationship, therapeutic index …
  - **Biomarker discovery** and hypothesis testing/validation
- **Disease area expertise**
  - ***Oncology and immuno-oncology***
  - ***Immunology and inflammation***
  - ***BSL3 infectious disease*** *(tuberculosis, SARS-Cov-2 …)*

- **AAALAC** accredited animal facility
- **Area:** >4,000 m² animal facility with dedicated procedure & surgery rooms, drug preparation rooms, cell culture room
- **Animal capacity:** 46,440 mice, 5,400 rats, 1,080 gerbils and hamster, 540 guinea pigs and 540 rabbits
- **3 in-house veterinarians**

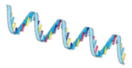
Over 30 scientists dedicated to Oncology including 13 scientists specialized in Immuno-Oncology

- *In vivo/ex vivo* support from **early target validation to candidate selection**
- **Activity fully integrated within drug discovery programs**

Building a tailored *in vivo* approach for the project

In vivo models adapted to the treatment modality

Cancer therapies

- Small molecules 
- Antibodies (Abs)[1]
  Ab-conjugates, BITES 
- Oligonucleotides
  (ASO, siRNA ...) 
- Vaccines 
- Adoptive cell therapies:
  TIL, chimeric antigen receptor
  (CAR) or engineered TCR 
- Oncolytic viruses 
- Cytokines therapies 
- Radiation 

In vivo models

Xenograft tumour models

- s.c. or othotopic in Immunodeficient mice
- Working with human cancers with the relevance of an *in vivo* host of an *in vivo* host

Humanized tumour models

- Immunocompromised mice with a human immune system
- Opportunity to assess immunotherapy efficacy and pharmacodynamics in a human immune-tumour context

Syngeneic tumour models

- s.c. or orthotopic in immunocompetent mice
- Featuring full murine immunity and comprehensive stroma

Specific models

- *In vivo* T cell proliferation
- GvHD model
- Immunogenicity/ELISpot
- PDX models outsourced on demand

- **General evaluation and clinical pathology:** clinical signs, body weight, food consumption, hematology (RBC and WBC counts)
- **Tumour growth:** digital caliper system, *in vivo* imaging (bioluminescence, fluorescence)
- **Target engagement- PD/Biomarkers** modulation in relation to **compound exposure or biodistribution**
- **Survival/Relapse efficacy models:** Therapeutic index-Driver of efficacy

Adapted from Fang Chen et al. (https://doi.org/10.1016/j.eng.2018.11.010)
1 Monoclonal antibody binding to cancer cell surface target and leading to cancer cell death (e.g. trastuzumab)
2 Checkpoint inhibitors (e.g. pembrolizumab)
BiTEs: Bi-specific T-cell Engagers, TIL: Tumour-Infiltrating Lymphocyte, CAR: Chimeric Antigen Receptor, TCR: T-Cell Receptor

Extensive expertise in preclinical tumour models

Associated to a broad range of assays/read-outs to fit the therapeutic target

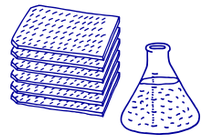
- **MODEL set up based on project need**
  - Based on therapeutic indication, molecular profile, *in vitro* work…
  - In immunocompetent, immunocompromised mice or rats or humanized mice
- **Orthotopic implantation if tumour environment is required**
  - Skills in breast, lung, liver, bladder, ovary, leukemic cells implantation …
  - Luciferase-engineered cells for time-course follow up of tumour growth
- **PDX models outsourced on demand (established partnership with a number of providers)**
  - Model identification based on target indication
  - Study design: dose and schedule, sampling time points
  - Study follow up with the CRO
  - *Ex vivo*, PK analysis of the samples

Examples of tumour indications for which models have been set up

Tissue	Indication / Characteristic features	Site of inoculation	Species
Bladder	Transitional cell carcinoma	s.c. / Intravesical instillation	Murine
Brain	Glioblastoma	s.c.	Human
Breast	TNBC, Her2+, ER+/PR+	s.c. and orthotopic	Human and mouse
Colon	Wild type or BRAC2 deficient, Ras mut ….	s.c.	Human and mouse
Fibrosarcoma	–	s.c.	Mouse
Kidney	Cell carcimoma or cortical adenocarcinoma	s.c./renal capsule	Human and mouse
Leukemia	AML	s.c.	Human
Liver	p53 wt, null p53, p53 mut	Liver	Human
Lung	NSCLC, SCLC, squamous cell carcinoma	s.c. / intratracheal, transpleural/ Intracranial	Human and mouse
Lymphoma	DLBCL, NH, MCL, T lymphoblasts	s.c.	Human and mouse
Oesophagus	Esophageal squamous cell carcinoma	s.c.	Human
Ovary	High grade serous, clear cell, surface epithelial ovarian cancer	s.c./i.p.	Human and mouse
Pancreas	Ductal adenocarcinoma	s.c.	Human
Skin	Melanoma	s.c. /intradermal	Human and Mouse

KEY *ex vivo* READ OUTS:

- **Tumour micro-environment:** flow cytometry, IHC, **Tumour angio/lymphogenesis:** anti-CD31/anti-LYVE IHC, **Metastasis on xenograft models:** Alu ISH, human CK19 IHC
- **Cancer metabolism:** leading mass spectrometry-based proteomics, metabolomics and lipidomics
- **Gene signature and signal transduction:** qRT-PCR, RNA-Seq and single cell RNA-Seq transcriptomics supported by proprietary bioinformatics tools for data mining and pathways analysis
- **Functional assays with immune cells:** proliferation assay, ELISpot, flow cytometry, **Cytokines release:** MSD & HTRF, ELISA
- **Analysis of proteins and phosphoproteins:** MSD & HTRF technology, western blot, ELISA, enzyme activity assay
- **Discovery & Translational biomarkers**
- **Compound exposure:** bioanalysis; mass spectrometry, ELISA

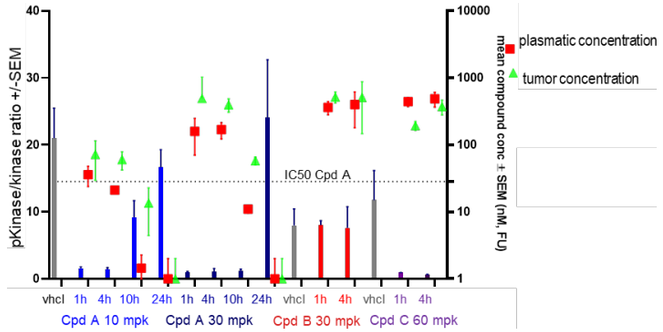


License may be required for some cell lines

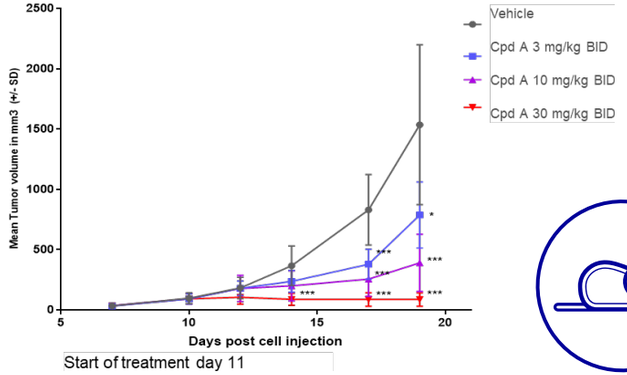
Focusing on developing efficacious and safe drugs in patients

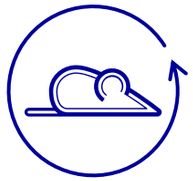
Key elements for an effective *in vivo* translational strategy

Target engagement vs cpd concentrations



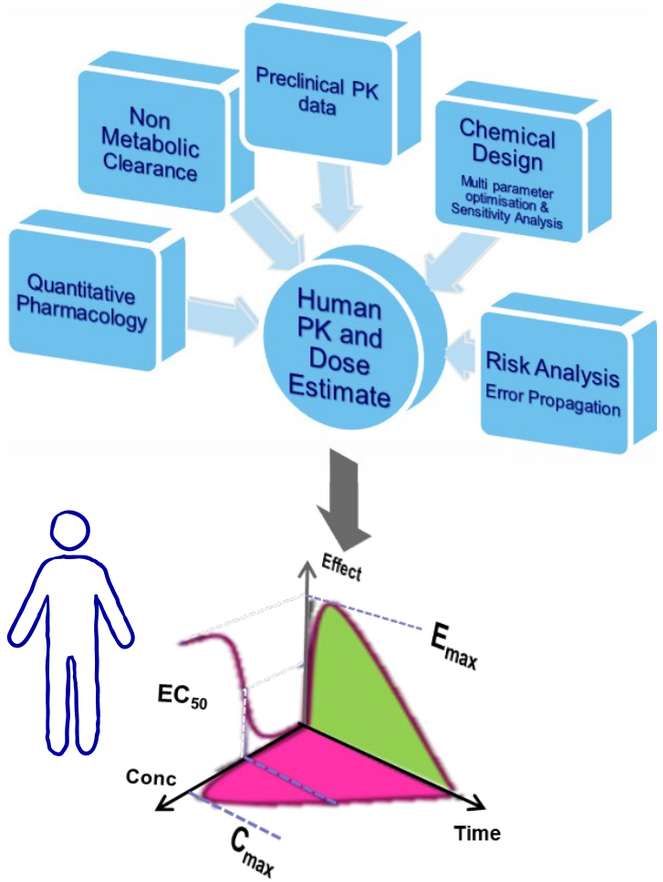
Efficacy in relevant disease model





Elucidate PK/PD and PK/efficacy/tox relationship

- Thorough understanding of the biology of the target and appropriate biomarkers
- Confidence in preclinical models: PK, PK/PD, PK/Efficacy and tox understanding
- Mechanistic *ex vivo* & *in vitro* investigations with human and animal cells and tissues (PK and PD)
- Understanding of disposition and clearance mechanisms
- Predict human PK and dose



Drive towards low human dose / exposure to mitigate potential toxicity

Contents

Translational biomarkers at Evotec

Translational biomarkers from Target validation to clinical trials

Evotec's translational biomarker department is applied to develop biomarkers strategy for integrated drug discovery projects and to support translational biomarker readouts that are applicable to clinical samples

The team

- Global team of >80 scientists
- Strong expertise in biomarkers strategy
- Omics experts from conception to analysis
- GCP capabilities

Sample analysis

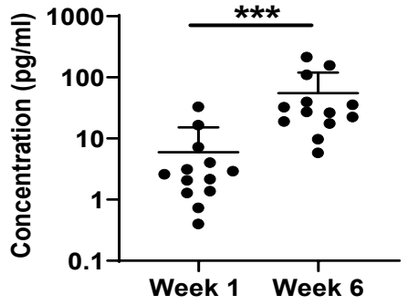
- Cell lysates & supernatant
- Body fluids e.g. blood, plasma, saliva, CSF
- Animal and human tissues
- *Ex-vivo* assays on patient samples

Platforms

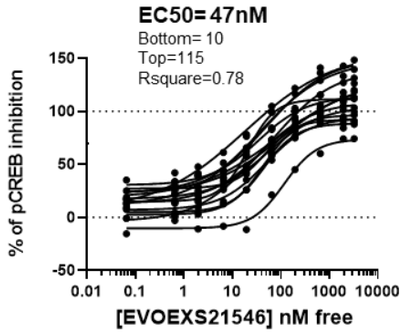
- Biomarkers strategy
- Human sample access and Human biorepository
- Immunoassays: MSD, TR-FRET, Luminex, Quanterix SMC, flow cytometry
- MS-based: Deep or single-shot proteome profiling; targeted MS using MRM, Metabolomics & LC-MS
- IHC/IF, ISH, histology, Ventana multi-colour staining
- RNAseq, Fluigdim platform for mRNA signature

Clinical Case studies

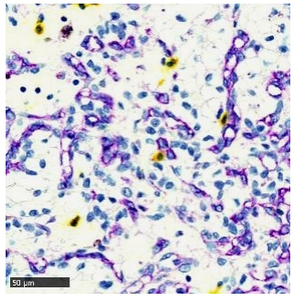
IFN-γ



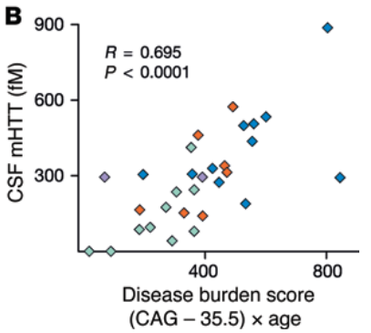
Cytokines quantification (by MSD) in *plasma from patients included in* clinical trial



Phosphomarker in CD8⁺ T-cells in whole blood (by Flow cytometry). Target engagement assay used in a clinical trial.



Target (in blue)/Immune infiltration (CD8 in yellow) in a patient with renal cell carcinoma under clinical trial (by multiplex IHC histology[2])



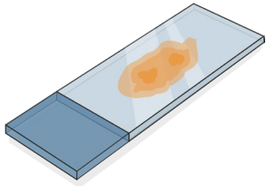
Ultra-sensitive mutant huntingtin protein quantitation in *clinical CSF samples* from patients by Singulex[2]

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
GCP: Good clinical practice, CSF: Cerebrospinal fluid, IHC: immunohistochemistry, IF: immunofluorescence, ISH: *in situ* hybridization

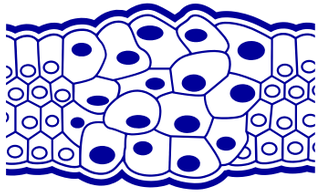
Translational Biomarkers strategy using pertinent samples

The need for translational thinking

Biological samples



FFPE tissue



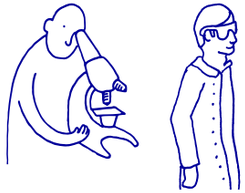
Fresh (*ex vivo*) or frozen tissue

Fluid samples (blood & urine)

Clinical data

Histological & gene mutation reports

Patient history



Clinical & histological consultancy

1 Retrospective hypothesis testing

2 Prospective hypothesis testing

Target engagement / pharmacodynamic

Surrogate endpoint / efficacy

Safety / toxicity marker

Predictive or stratify-cation marker

Diagnostic / prognostic

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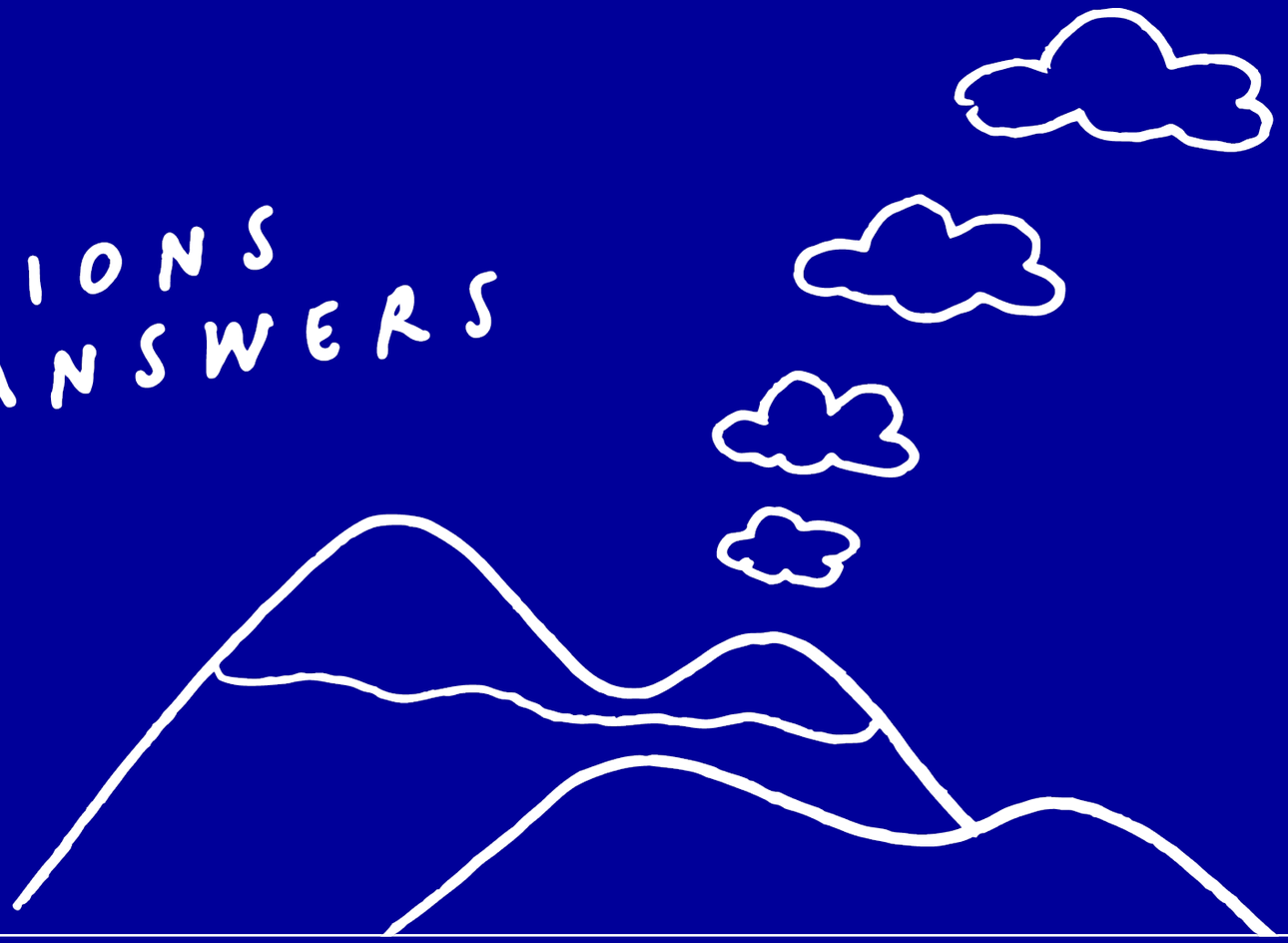
#RESEARCHNEVERSTOPS

QUESTIONS AND ANSWERS



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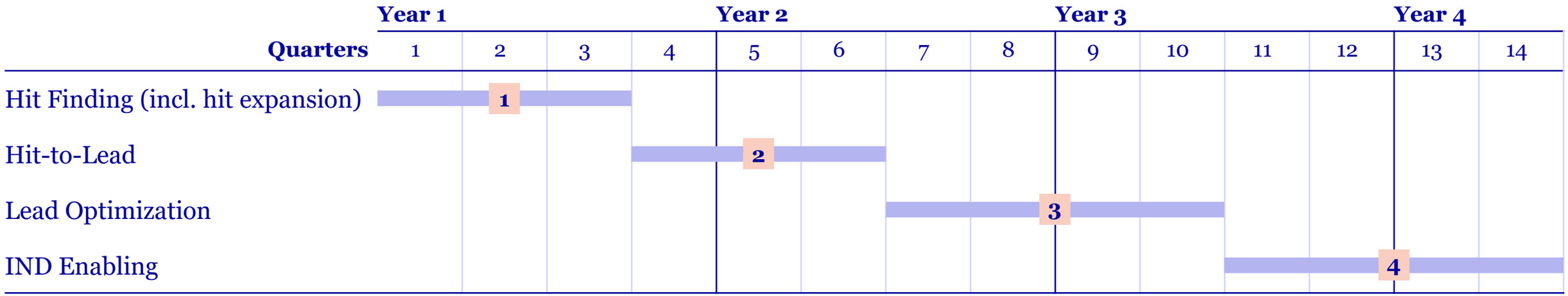
Business Development
114 Innovation Drive, Milton Park, Abingdon
Oxfordshire OX14 4RZ, UK

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

Accelerated plan for small molecules

Estimated timelines from hit ID to IND-enabling studies



1 2-3 Hit Series ready for Hit-to-Lead; transition criteria to be agreed by partner and Evotec

2 At least 1 Lead Series ready for Lead Optimisation; transition criteria to be agreed by partner and Evotec

3 **Pre-clinical development candidate (PDC) selection**; nomination criteria to be agreed by partner and Evotec

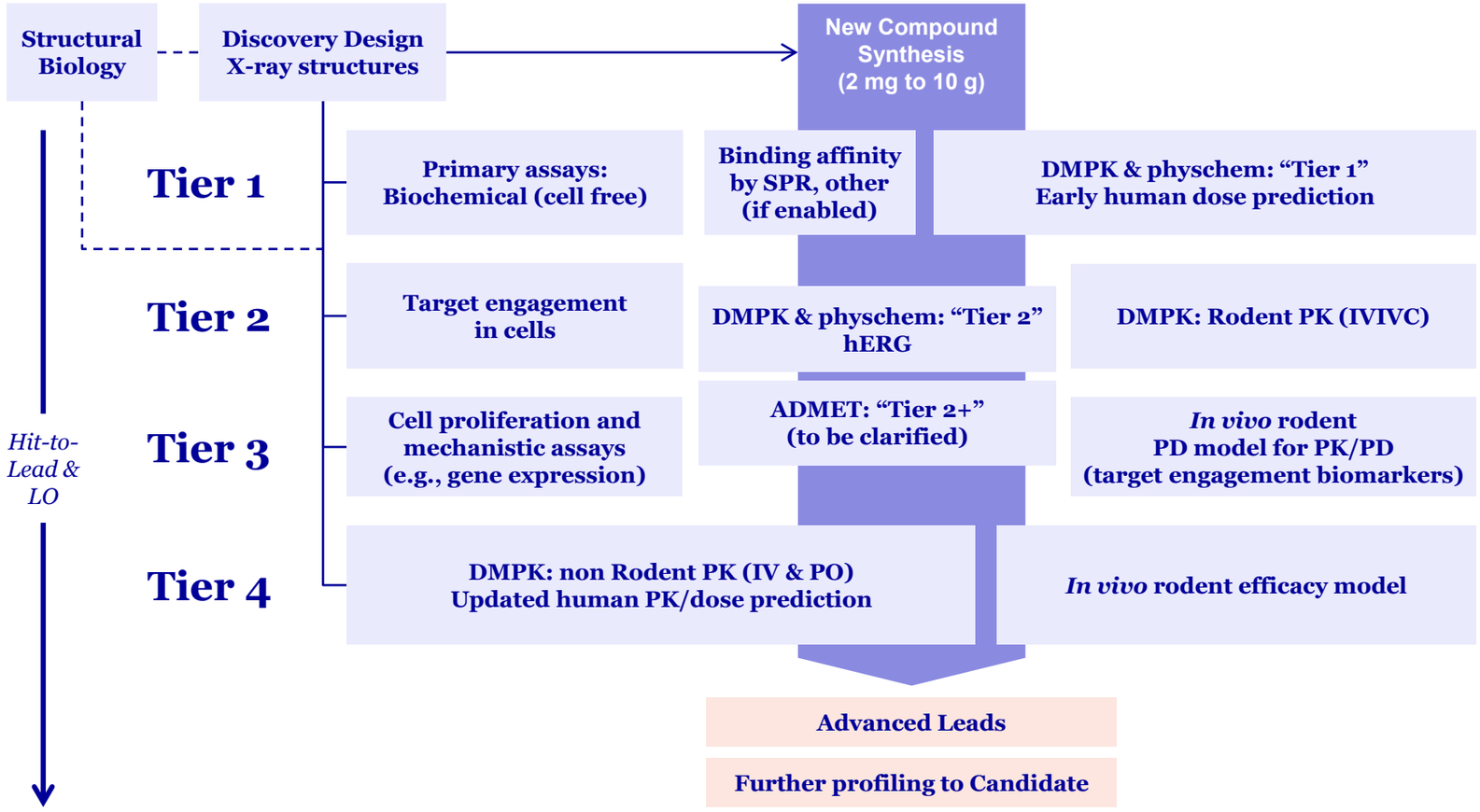
4 IND filing

- Evotec's E2E R&D engine to rapidly progress a structurally-enabled program to deliver a high-quality PDC in 2.5 years
- Project plan assumes target structural information is available early in the project to drive Hit Expansion and Hit-to-Lead phases
- Proposed (accelerated) Lead Optimisation timelines are based on suitably de-risked leads entering this phase

Evotec's Hit-to-Lead & Lead Optimisation Screening Cascade

Typical cascade from hits to advanced leads

Critical path activities



Evotec activities | Outsourced activities | Partner activities

Parallel de-risking activities and/or bespoke studies

- **Met ID**
- **CYP reaction phenotyping**
- **CYP inhibition / induction**
- **Transporter phenotyping** (uptake / efflux)
- **Liver toxicity** (cellular DILI)
- **Cardiotoxicity** (wider ion channel profiling; cardiomyocytes)
- **Ames, micronucleus**
- **DDI** (advanced profiling and modelling)
- **Biomarker program**

- **If enabled/required**

- **Extended selectivity**
- **Off-target panels (CEREP), Kinome panels**

Cascade can be supported by *in silico* global and/or local models

Achieving assay excellence with rapid turnaround

Case study for DMTA[1] *in vitro* biology cycle for a two-target project

Assay format	Number of assays	Average development time (weeks)	Cmpds per week	Assay Format	Data delivery turnaround	Z' Average
Biochemical	4	4	up to 28	384	1 week N=2	0.6-0.85
Target engagement	3	5-8[2]	up to 28	384	2 weeks N=2	0.5-0.75
Phenotypic (proliferation/survival)	6	5	up to 28	384	2 weeks N=2	0.5-0.75

- Partner requested set up of >10 assays to support the profiling of two targets
- Rapid delivery of data to support future design was a key criteria
- Partner opted to expand collaboration based on experience in first 12 months

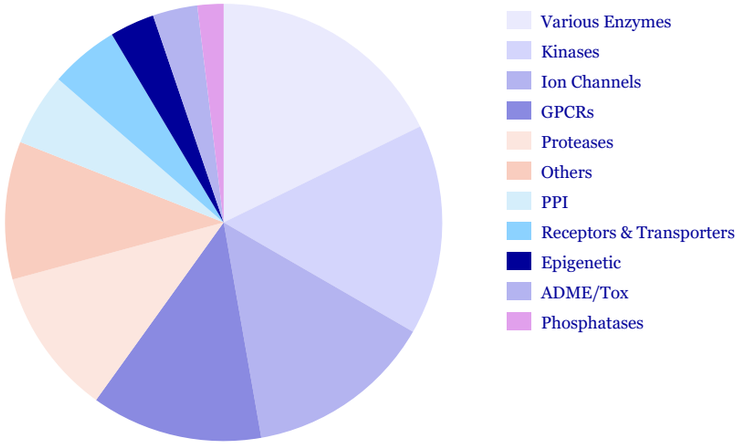
1 DESIGN – MAKE – TEST – ANALYSE
2 Depending on the complexity

Evotec's Track record in assay development and screening

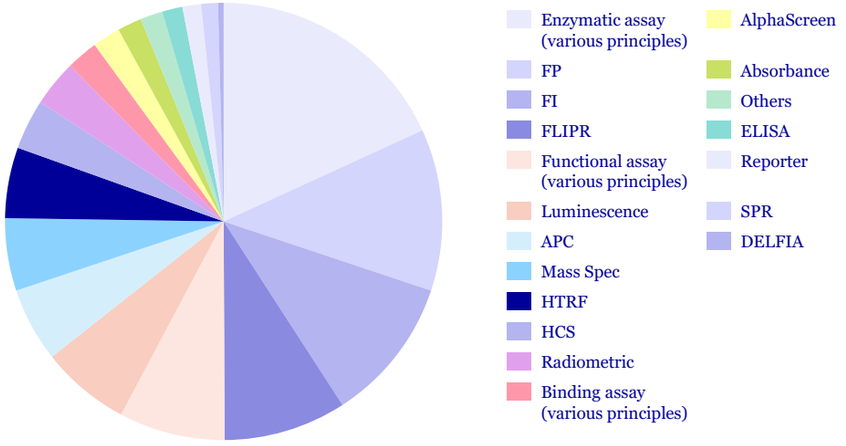
Expertise and know-how developed through 15+ years of collaborative research

- Vast amount of expertise in assay development, validation and automation
  - More than 1,000 assays developed
  - 60% biochemical, 40% cell based assays
  - More than 700 HTS projects completed
- Approximately 50% of our hit identification projects continue with hit expansion, hit-to-lead and lead optimisation projects at Evotec
- Track record in addressing challenging targets
  - PAM/NAM for GPCRs and Ion Channels
  - Allosteric modulators of enzymes, PPIs
  - Complex assay systems (stem cells, mitochondria, co-cultures, microorganisms, primary cells, blood cells, isolated proteins)
- Deep integration of alternative hit identification routes
  - Multiple assay modalities per target
  - High Throughput early liability assessment (eADMET panel)
  - Virtual screening

Target classes

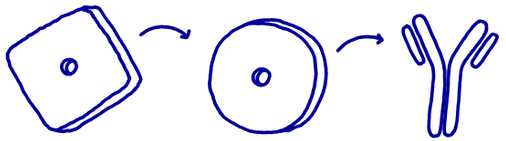


Assay principles



Generation of fully human antibodies

From traditional platforms to A.I.-driven approaches



In vivo

Hybridoma platform

Key distinguishing features

- Hybridoma generation merged with automated clone picking
- Screening of thousands of monoclonal candidates simultaneously

Exploration of natural immune repertoire using phage display

Key distinguishing features

- Construction of naïve and immune libraries
- Species-independent
- *In vitro* selection of rare antibodies

B cell cloning

Key distinguishing features

- Direct screening of hundreds of thousands of B cells upon immunization or natural immune repertoire
- No species restriction

In vitro

J.HAL®, A.I.-designed phage and yeast libraries

Key distinguishing features

- Synthetic and developable A.I.-designed Fab and VHH human libraries
- Allow to generate multi-specific formats

In silico

De novo mAb design *(prototype stage)*

Key distinguishing features

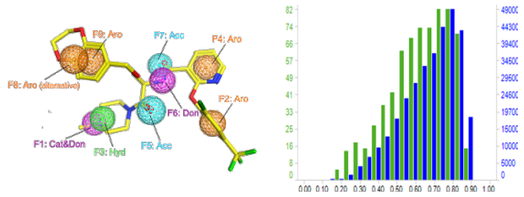
- State-of-the-art platform to design optimal binders *in silico*
- Fastest way to generate binders
- Rational design of binding mode

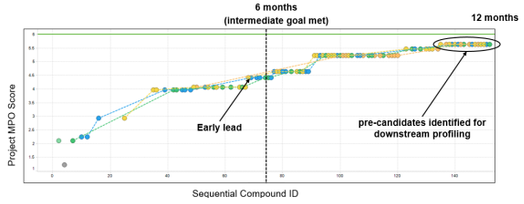
Evotec Hamburg/Toulouse Just – Evotec Biologics (J.Discovery™)

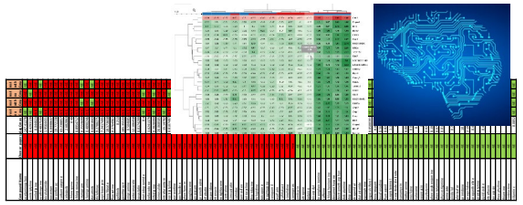
Unmatched Integration in the Industry

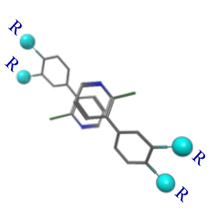
Computational methods and deep expertise impact every stage **from Idea to IND to Clinic**

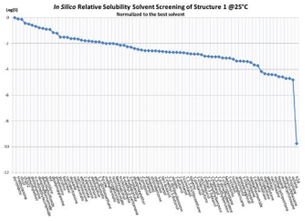
DISCOVER	OPTIMISE	SELECT	DEVELOP
(100,000s)	(10,000s)	(100s)	(10s)
Drug intervention point Modality HT Omics Biomarker(s)	DMTA cycles Biomarker validation	Drug Candidate(s) TPP	Safety API Formulation/Drug Product
			GMP











Data Surface linking Independent Data Chambers

Unique in the industry: high quality data at every stage in the value chain to de-risk projects, design modalities, create biomarkers, drive projects, … (e.g. tox prediction)

1. A complete offering to solve even the hardest discovery campaign

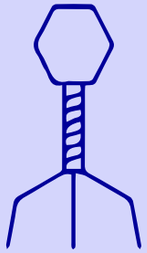
Generation of fully human antibodies: from traditional platforms to A.I.-driven approaches

Exploration of natural immune repertoire using phage display

Key distinguishing features

- Immune library generation upon immunization or natural infection
- *In vitro* selection of rare yeasts
- No species restriction

J.HAL®, A.I.-designed phage and yeast libraries



Key distinguishing features

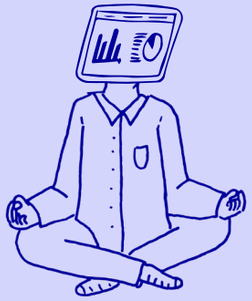
- Highly diverse A.I. designed human library
- Time + cost savings for therapeutic development

B cell technology

Key distinguishing features

- Direct screening of millions of B cells upon immunization or natural infection
- No species restriction

In silico mAB design *(coming soon)*



Key distinguishing features

- State-of-the-art platform to identify optimal binders *in silico*
- Fastest way to generate binders
- Initial client projects started

Agility ▮▮▮ Risk ▮▮▮ Cost ▮▯▯ Just – Evotec Biologics (J.Discovery™) Evotec Hamburg/Toulouse