evotec

#RESEARCHNEVERSTOPS

Evotec

Translational biomarkers in Oncology

Agenda

Translational biomarkers expertise within Evotec

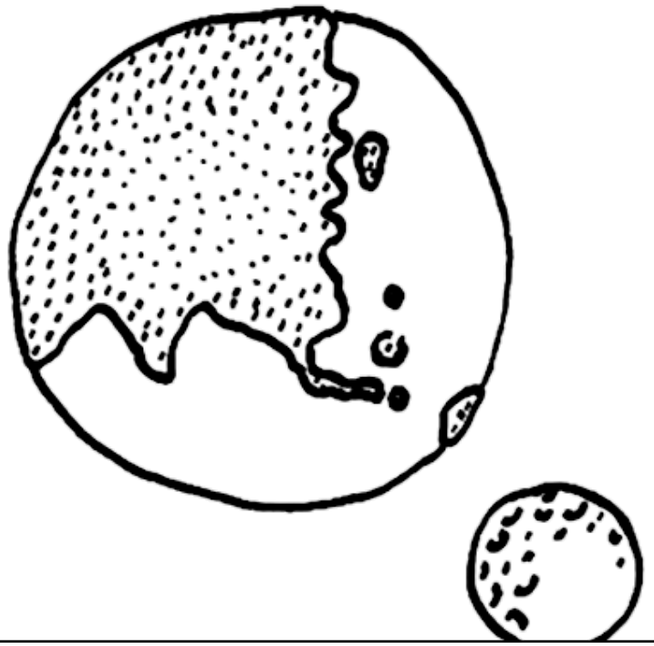
The Evotec-Oncopole Collaboration

Human Sample Access & Management

Biomarkers Platforms

Safety Biomarkers

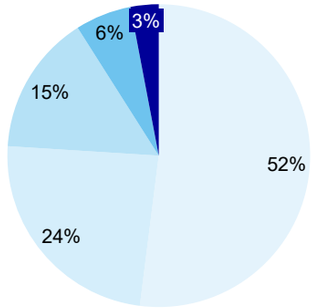
Clinical Trials and Biomarkers



Our mission

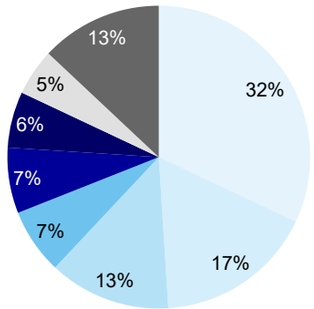
To reduce the number of failures in drug discovery and development

Many clinical trials do not succeed



Efficacy
Safety
Strategy
Commercial
Operational

Major causes for failures are safety and efficacy

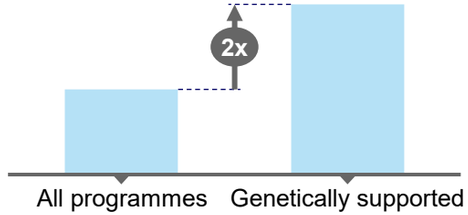


Oncology
Central nervous system
Musculoskeletal
Cardiovascular
Alimentary
Metabolic
Infectious disease
Other

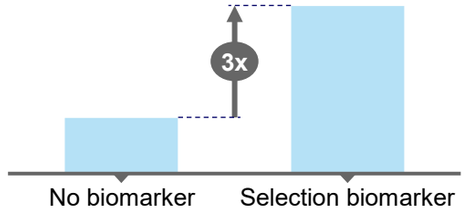
Highest impact TA is Oncology and CNS

Adding Precision Medicine improves Success

Human genetics supported targets[1)]
% success



Biomarker based stratification[2)]



Having **one or more translatable biomarkers** to measure safety, stratify patients (responders/ non-responders), and ensure target engagement (PD) and efficacy will:

1	Improve the chance of success (better target, better drug, better design)
2	Reduce the cost of (pre)clinical development
3	Improve the chance of approval
4	Significantly increase market uptake and acceptance
5	Add value to the asset (especially if combined with CDx[3)])

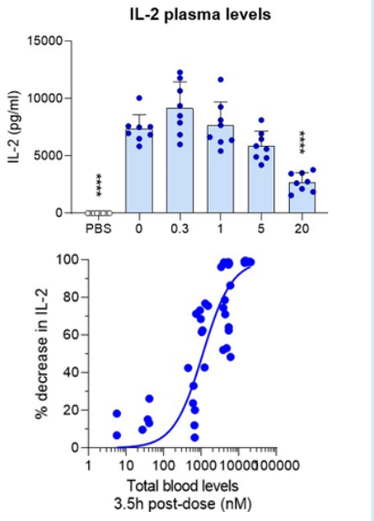
[1)] Margan, P. et al. Nature Rev Drug Discovery 2018 Mar 17 (3): 167-181
[2)] Evotec-Bayer report "Excelling Together for the Benefit of Women Suffering from Endometriosis"
[3)] CDx = companion diagnostic

The Type of Biomarkers we work on

From knowing we hit the target to finding the best patient and indication

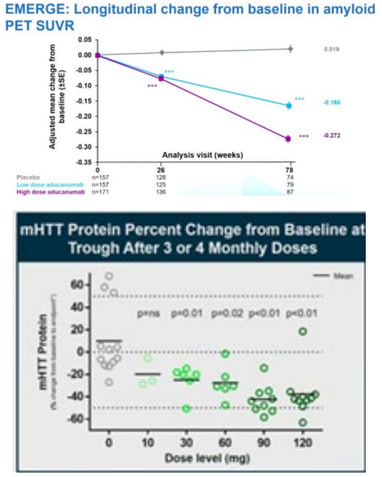
1

Target Engagement / Pharmacodynamic



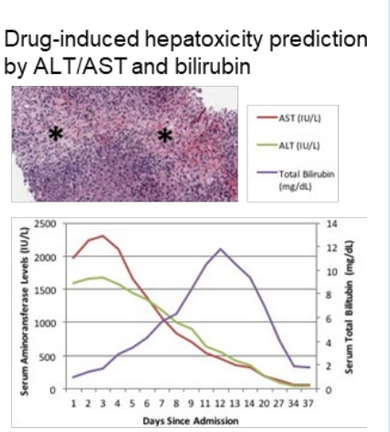
2

Surrogate End-point/Efficacy



3

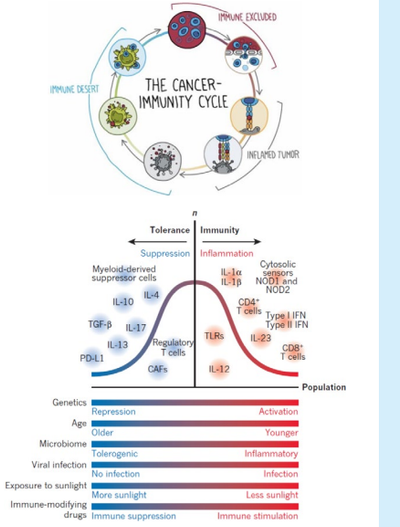
Safety / Toxicity marker



Data of a patient on Dasatinib that presented with acute hepatoxicity as confirmed by a biopsy and serum parameters

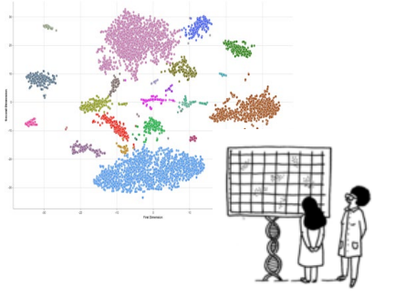
4

Predictive or Stratification marker



5

Diagnostic / prognostic



(A) Population-based approach

Pos. (e.g. resp.)

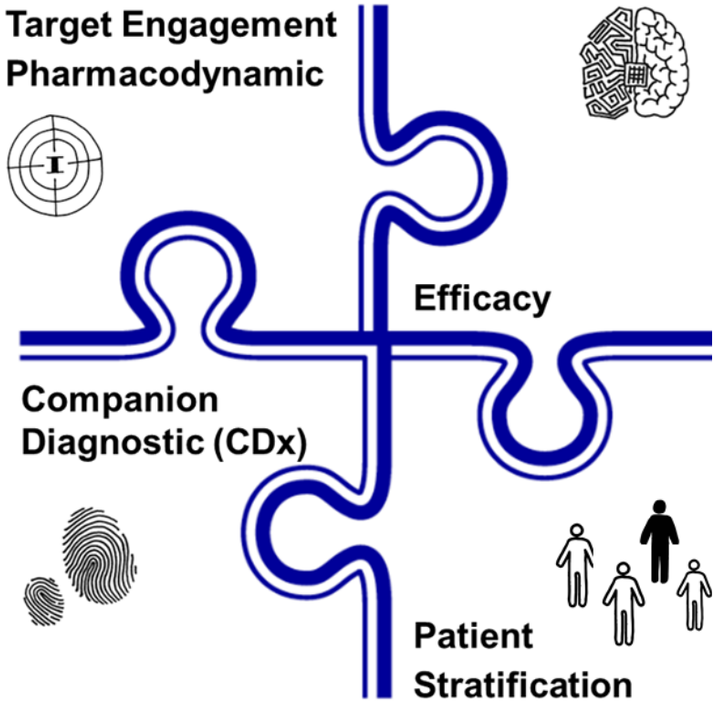
Neg. (e.g. non-resp.)

(B) Patient-based approach

Personalised therapy

How we do it

From knowing we hit the target to finding the best patient and indication



From Unbiased biomarker discovery and validation …

- Genomics, transcriptomics, proteomics and metabolomics
- Post-translational changes, Secretome analysis, and Immuno-phenotyping

… to Hypothesis-driven Validation

- *In vivo* and *in vitro* models with high translational value
- *Ex vivo* drug treatment and/or analysis of samples
- Exploration of prevalence in the context of pathology
- Evaluation of stratification, PD, toxicity, efficacy biomarkers

Together with state-of-the art, high quality human samples to have better translatability!

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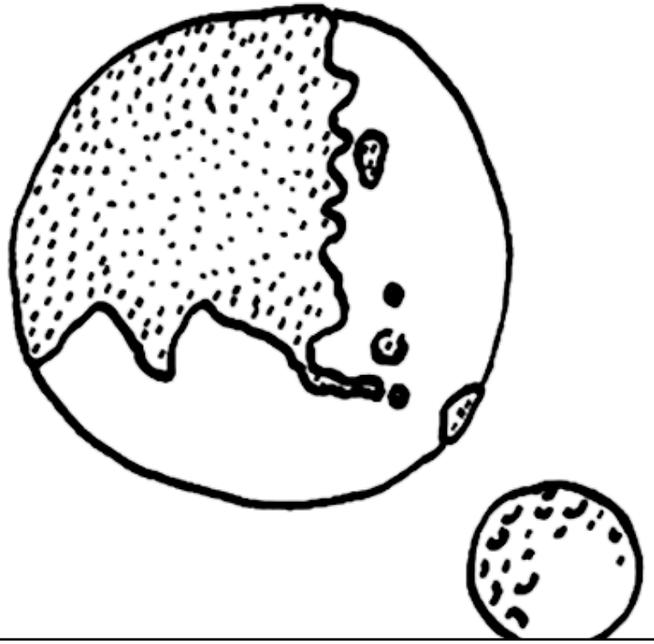
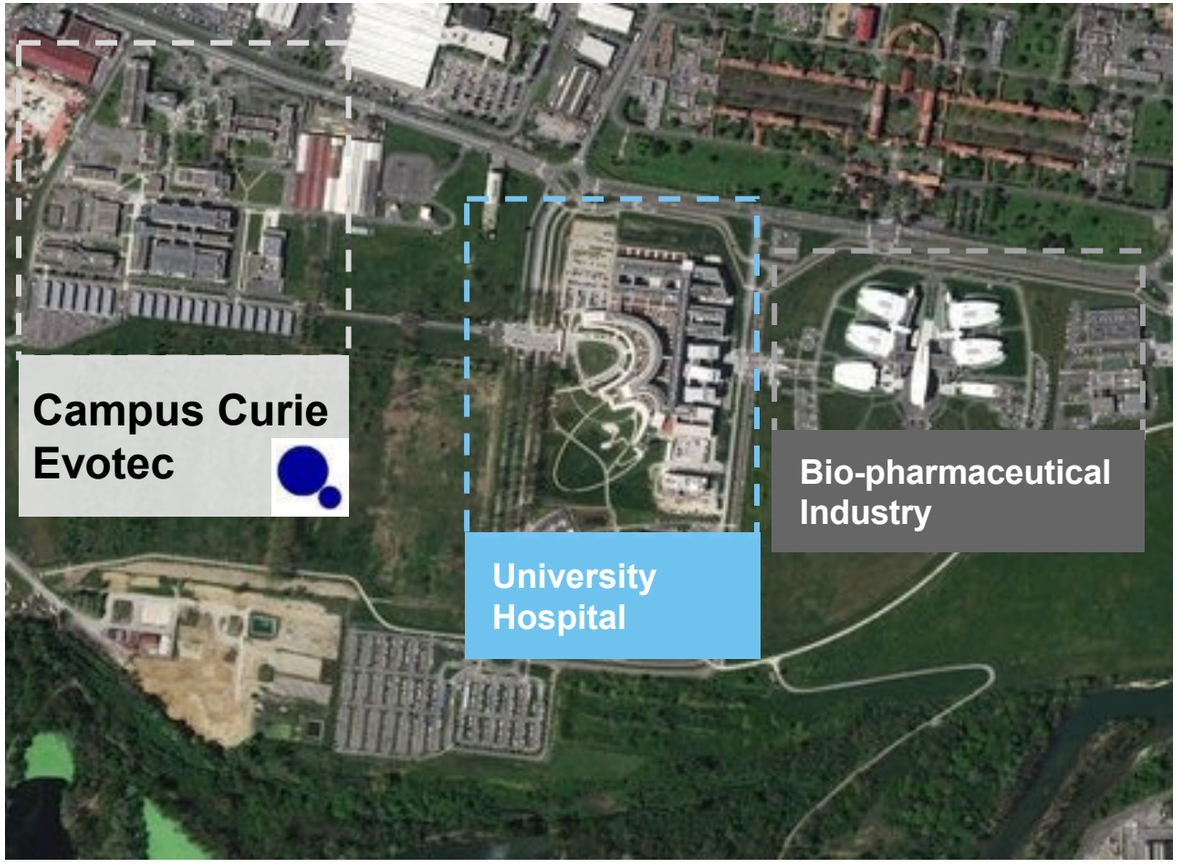


The Evotec-Oncopole Collaboration: Combining Medical and Research Excellence

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
- Example of working together:
  - Kazia (EVT801) supported by Evotec, has started to enroll oncology patients (Nov 2021) for a phase I at Oncopole (NCT05114668)
  - Exploratory biomarkers evaluation are performed by Evotec



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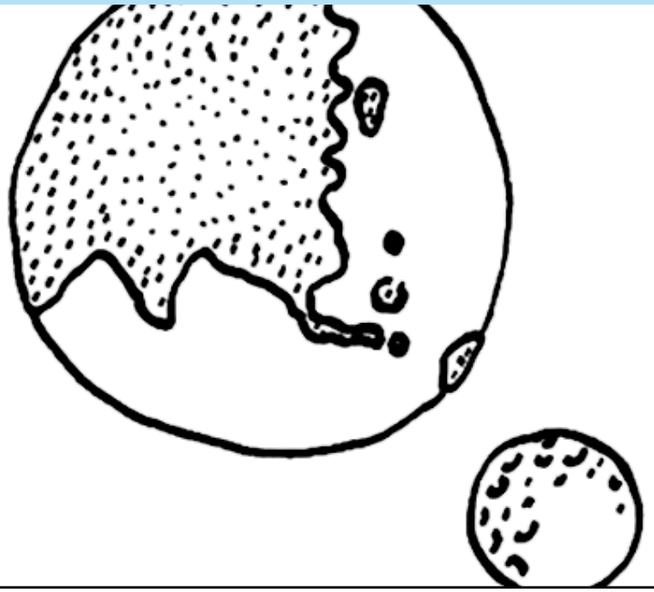
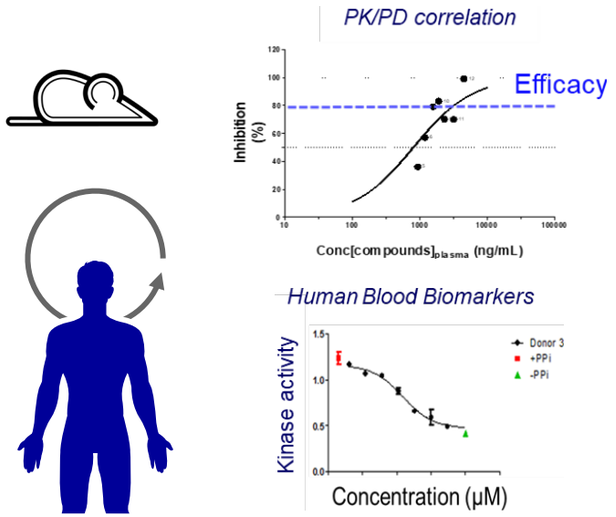


Translatability to Humans

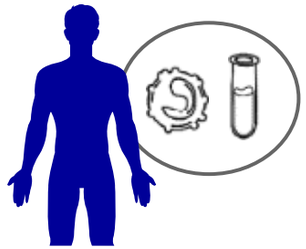
Ensuring translatability from bench to bedside and back

Finding the right dose



An effective translational strategy should focus on the human response which requires building a bridge between "*in vitro* and *in vivo*" PK and PK/PD insight across species

Using the right materials

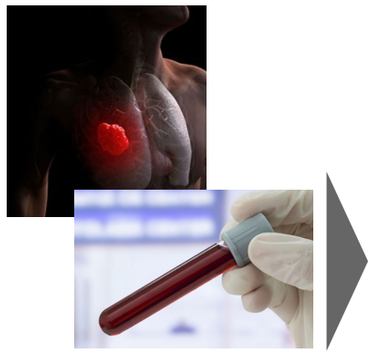


Pertinent High-quality Human Samples with Associated Clinical Data (tissues &/or fluids)

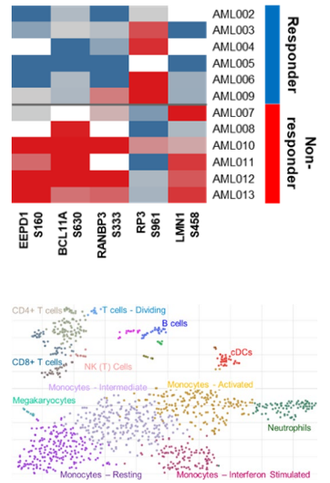
For example, Evotec has a strategic partnership with a clinical site dedicated to cancer patient healthcare, enabling sample access (retrospective/prospective)

A biomarker that works *in vitro*/*in vivo* is not useful if not translatable to humans. Moreover, understanding the biomarker's behaviour in humans is essential to design the best clinical trial

Identifying the right indication and patient



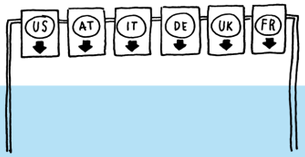
Using human samples to identify signatures of treatment and disease



Building a translational strategy to find the best responder to your drug can make or break a clinical trial and the success of your treatment: all-comers vs. targeted patients

Human Biosample Acquisition & Management Centralization within Translational Biomarkers department

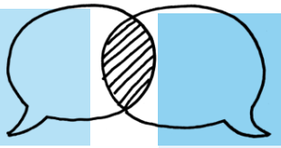
Two teams working closely together supporting the whole process



Sample and Clinical Data Acquisition

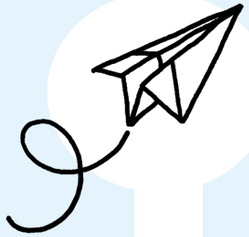
Sample and Clinical Data Management

Human Sample Acquisition (HSA) Team



Human Biosample Strategy and Operations (HBSO) Team

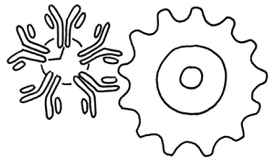
Works together with project leads, procurement, and legal to support the **sourcing of human samples.**



HBSO is responsible for the **safe storage, tracking and distribution of samples and the management of sample and clinical data.**

Human Sample Access

Incorporating patient samples early in the drug discovery process



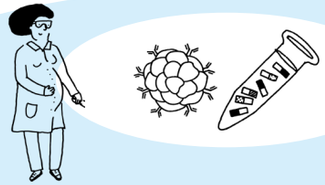
Evotec
Translational Biomarkers Team

- Biomarkers strategy
- Biomarkers validation
- State-of-the art technologies
- Clinical Samples storage

Human Samples Access Team

Translation from discovery to clinic

- Identify clinicians & key people
- Specification & guidelines



Pertinent High-quality Human Samples
(tissues &/or fluids)

- Regulatory dossier & Contract / Budget
- Database / Operational Procedures

Translation from clinic to discovery

Clinical team

Clinical expertise on disease and patients

- Patient selection
- Samples collection
- Associated clinical data

- Other sources such a bio-banks or commercial vendors[1)]

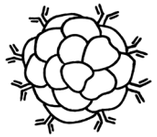
[1)] With full HIPAA or GDPR compliance; and ensuring access to high quality samples with disease-relevant information

Access to human samples and clinical data is key to accelerating translational work

Collaboration on access to human samples, clinical data and medical network

Human Samples

Blood
- Healthy donors
- Patients with solid tumors
- Patients with hematological cancer



- Fresh/frozen pathological resection
- Healthy tissue closed to the resection
- samples (non fresh) of FFPE blocks from multiple sources and multiple indications


Human fluid
- Urine, follicular fluid, broncho-alveolar lavage


Human healthy tissue
- Skin, adipose tissue, ovaries

Oncopole KOL network

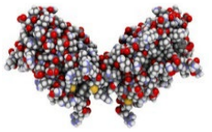
- **Prof Jean-Pierre Delord;** Head of clinical trial Phase I unit and Head of Oncopole
- **Prof Julien Mazieres;** Head of thoracic oncology and KOL in targeted therapies[1)]
- **Prof Rosine Gaimbaud,** Head of colorectal cancer and oncogenetic research
- **Prof Christian Recher,** Head of hematological cancer

Human Samples analysis

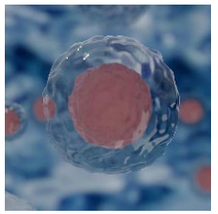
DNA
- Only dedicated mutations

RNA
- Total mRNA seq
- Nuclei mRNA seq
- scs-mRNA seq
- mRNA signature

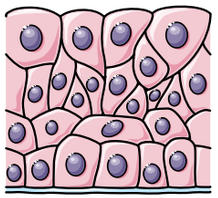
PROTEIN
- ELISA mutiplex
- MSD
- Proteomic
- Metabolomic
- Biomarker secretion



CELLS
- 3D culture
- CRISPR editing
- Cell sorting
- Surfaceome
- Flow cytometry
- *Ex vivo* assay
- Target engagement



TISSUE
- FFPE
- Cryo
- IHC
- ISH

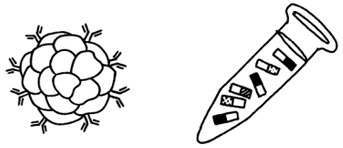


[1)] J.Mazieres & al. On target: Rational approaches to KRAS inhibition for treatment of non-small cell lung carcinoma; lung cancer 2021.
J. Mazieres & al. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: results from the European EUHER2 cohort; Ann. Oncol; 2016.
MSD: mesoscale discovery; FFPE: Formalin-Fixed Paraffin-Embedded; IHC: immunohistochemistry; ISH: in sity hybridization

Case study: access to fresh and pertinent samples of tumors & circulating blood from patients

Flow cytometry analysis and single cell mRNA sequencing

Access to healthy tissue, tumor resection & blood

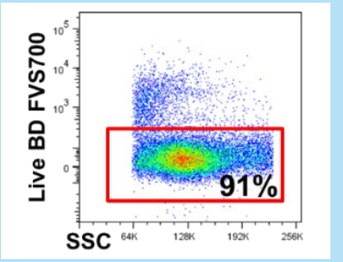


Transport to Evotec (<24h)

Tissue dis-sociation

Supernatants

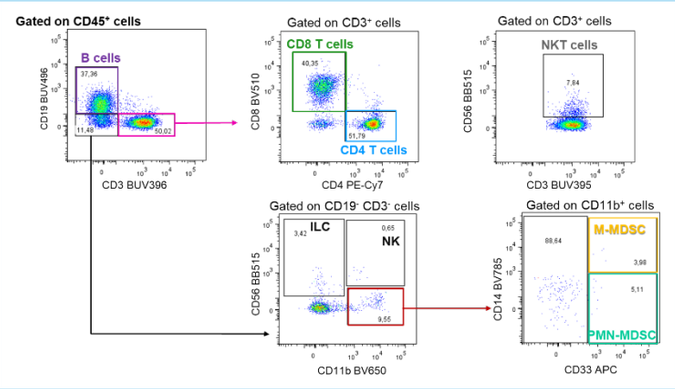
Living cells



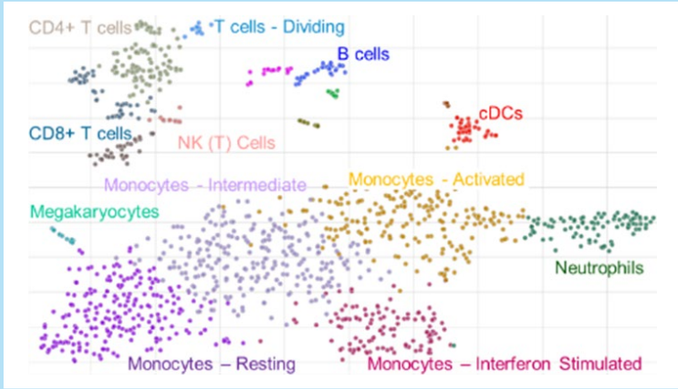
- **Flow cytometry**
- **scRNAseq**
- **3D culture**
- **etc.**

Proteomics

Flow cyto-metry



Single cell mRNA sequencing



- Well established protocols for analysis of cancer immune phenotypes in patients samples
- Possibility to study the tumour secretome (by ELISA, HTRF, proteomics)

Access to fresh and pertinent samples of blood from patients with hematological malignancies

Well-established workflow for blood samples and clinical associated data

Access to healthy & patient with haematological cancer blood

- Access to specific clinical data
- Follow-up of patients: in 2022
- Access to thematic biobank

Transport to Evotec (<24h)

- Fresh blood
- Serology at day 1 post sampling

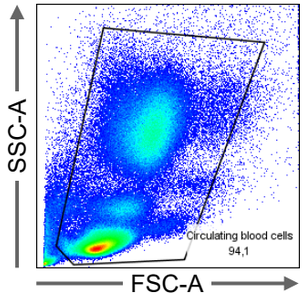
- **Whole blood**
- **Plasma**
- **PBMCs**

Starting material suitable for phenotypical & functional assays

- Flow cytometry (incl. whole blood staining)
- Live cell imaging (Incucyte)
- scRNAseq
- Cytokine dosage (MSD)
- Metabolomics
- Proteomics
- …

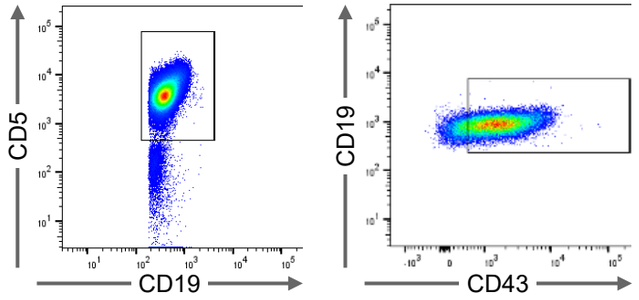
Sample quality
(high viability)

Whole blood staining by FACS



High number of tumor cells
(>50M/patient B-CLL)

B-CLL markers staining on patient-derived PBMCs by FACS

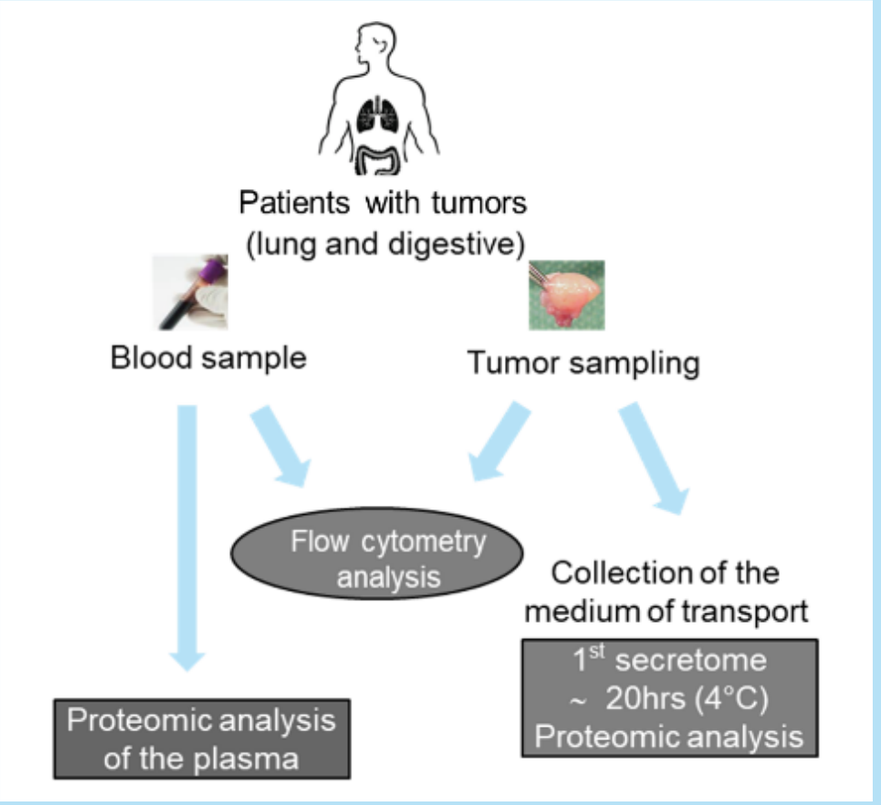


Case study: biomarkers identification in freshly isolated patients' samples

Identification of biomarker(s) associated to a tumor phenotype

Analysis of blood and tumor samples coming from the same patient

- Characterisation of the circulating immune cells and of the Tumor Micro Environment by flow cytometry
- Identification of the proteins of the plasma and secreted by the tumors (secreted in the medium of collection of the tumor) from these samples
- Analysis and comparison of the flow cytometry and proteomic data to identify circulating biomarker(s) produced by the tumors and correlate them to the TME phenotype



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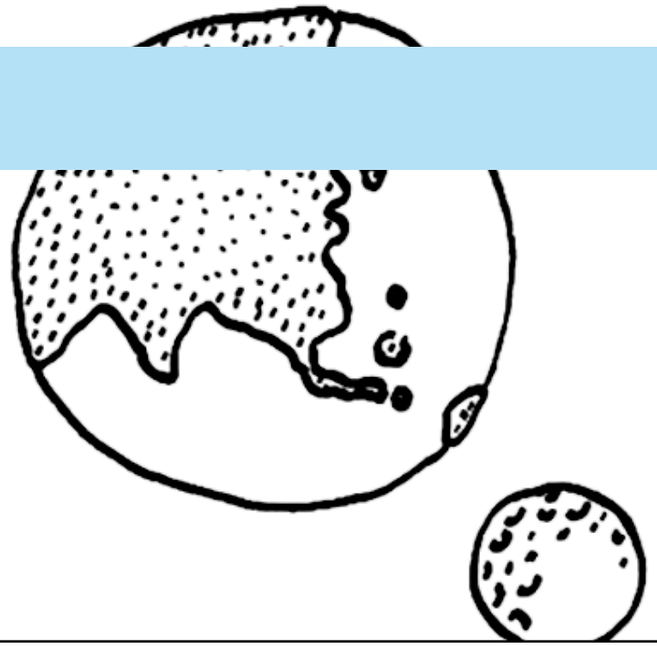
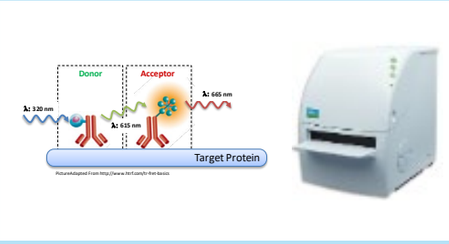


Large Immunoassay platform for hypothesis-driven biomarker research

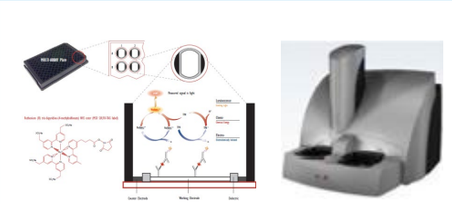
Multiple options for tailor-made approaches to the best immunoassay

TR-FRET
- Ultra high throughput
- Homogenous assay with simple SOP
- Conformation specific



Quanterix SR-X
- Ultra-high sensitivity (fM)
- Measurement of individual single molecules
- Multiplexing possibilities (up to 6 analytes)
- Extremely robust and reliable for clinical matrices

MSD
- Medium throughput
- Multiplex possibilities
- High sensitivity, custom-made ELISA



Luminex (MagPix)
- Medium throughput
- Multiplex possibilities (up to 50 analytes)
- bDNA and immunoassay capabilities

Single Molecule Counting (SMC)
- Ultra-high sensitivity (fM)
- Single protein molecule detection possible
- Extremely robust and reliable for clinical matrices

JESS (capillary based protein analysis)
- Medium throughput
- Higher sensitivity and reduced workflow complexity
- Robust results and low variability

Case study: Supporting Biomarkers During Clinical Trials

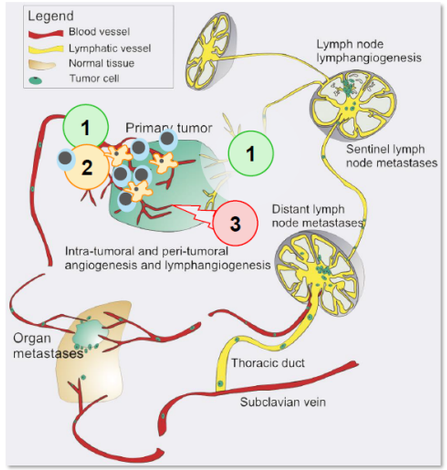
Further developing Efficacy and Patient Stratification markers

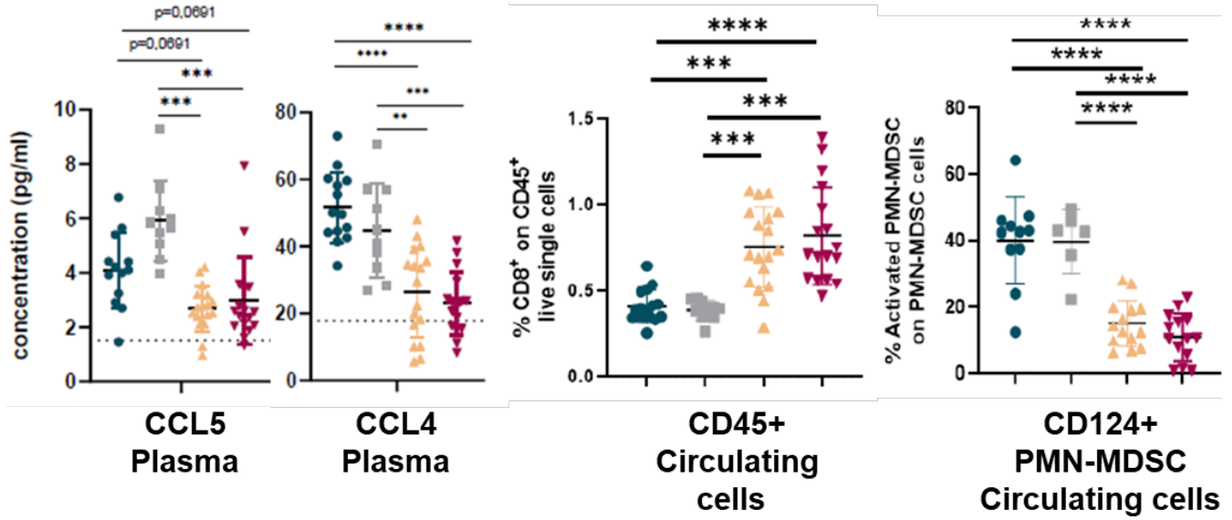
EVT801: a differentiating anti-tumor approach

Inhibition of tumour escape & metastasis
- Stabilisation of tumour vasculature
- Inhibition of lymphangiogensis
- Reduction of tumour hypoxia

Enhanced anti-tumour immunity*
- No impact on T-cell viability
- Decrease in immunosuppressive cells
- Enhanced effector cell infiltration

Tumour killing
- Direct effect on VEGFR-3⁺ tumour cells from endothelial origins
  e.g. soft-tissue sarcomas





EVT801 efficacy is associated with

- A decrease in circulating MDSCs and an increase in effector CD8⁺ T cells in blood
- A reduction of plasma CCL4 & CCL5 levels, the major cytokines involved in MDSC expansion resulting in a reduction on immune-suppressive cells and cytokines

● Vehicle + Iso ■ Vehicle + CTLA4 ▲ EVT801 + Iso ▼ EVT801 + CTLA4

[1] Iyer RV et al. Dose-Dependent Sorafenib-Induced Immunosuppression Is Associated with Aberrant NFAT Activation and Expression of PD-1 in T Cells. Cancers (Basel). 2019;11(5):681. doi:10.3390/cancers11050681

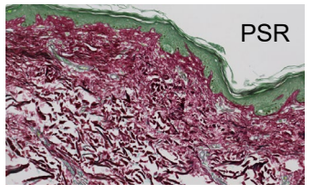
Evotec has a strong expertise in histology

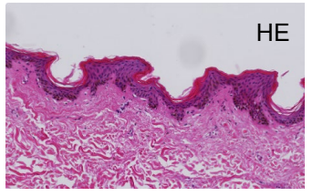
Histology platform capabilities

Classical Histology

- Hematoxyline -eosine
- Trichrome de Masson
- Red Picrosirius
- Alcian Blue …

Sakura Tissue Tek Film x1





Slides preparation from FFPE and frozen blocks

Parafine inclusion (x2)

Paraffin embedding (x2)

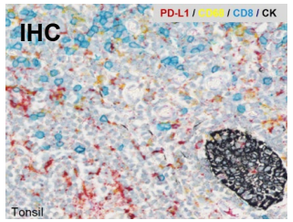
Microm Microtom HM 385S (x2)

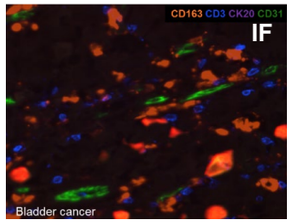
Cryostat

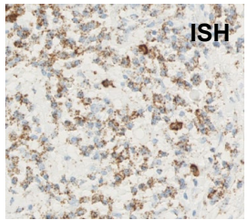
Molecular Histology

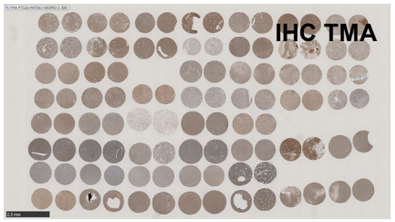
- **Protein detection:** IHC & IF: up to 5 biomarkers
- **Protein-Protein interaction** (Duolink® technology)
- **mRNA detection** (*in situ* hybridization)

Ventana autostainers







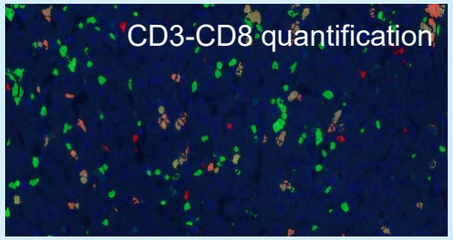


Scan & Digital Analysis

- **Scan** (brightfield, fluorescence)
- **Quantification** (machine learning)

VISIOPHARM®

Hamamatsu Nanozoomer



Transcript biomarkers platform

Hypothesis driven biomarkers vs unbiased discovery

Qiacube

TapeStation

QuantStudio 7
with Orbitor

NGS[1)]

1

One-step RT-qPCR

- Hypothesis driven qPCR → selected targets, know mechanism
  - Cell Plating, Treatment, Lysis → direct qPCR
  - qPCR with robotic arm → 20 plates hotel, up to 20 targets

2

Two-step RT-qPCR

- Hypothesis driven qPCR → selected targets, know mechanism
  - Cell pellet (2D, 3D) or Tissues (frozen, FFPE), blood
  - RNA extraction / Quantification and quality
  - cDNA synthesis and qPCR with robotic arm → up to 20 targets

3

Next Generation Sequencing (NGS)

- Unbiased biomarker discovery → Compound Mode Of Action, Target Engagement, off target effects
  - Absolute quantification, Differential expression, Alternative splicing
  - Sequencing / library currently outsourced via CRO

4

ctDNA

- Monitoring patient response / relapse throughout treatment
- Evaluation of genetic alterations acquired during selective therapeutic pressure
- Identified a CRO with ctDNA and gene fusion analysis expertise

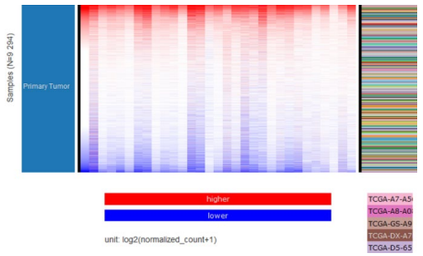
[1)] Ousourced to CRO for sequencing, possibility to generate NGS libraries internally or again via CRO

Case study:
mRNA signature helps with Patient Identification and Selection

mRNA analysis from FFPE blocks

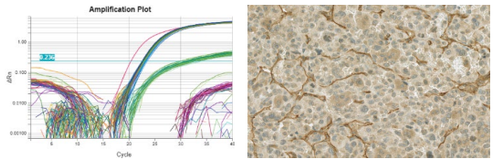
Protocol

List of genes directly correlated to target



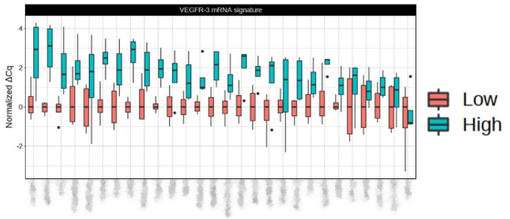
- Bioinformatic analysis of large databases (i.e. TCGA, …)
- mRNA signature identification

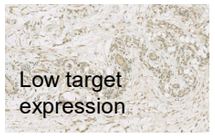
Archival tissues from multiples cancer indications

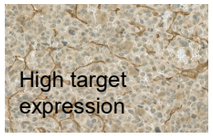


- Target expression at protein level
- mRNA signature refinement and validation

Results







- Correlation between target mRNA and target protein expression by IHC is confirmed
- Validation of a mRNA signature correlated with target expression allow us to develop strategies for:
  - Patient selection
  - Efficacy endpoint biomarkers

Conclusion

- mRNA signature on FFPE blocks is well established
- It helps to refine mRNA signatures derived from publicly available dataset on patient samples coming from tumor resection or biopsies
- Generate readouts at mRNA & protein levels with special indication in order to help for:
  - patient stratification biomarkers
  - Identify Efficacy endpoint biomarkers with on-treatment biopsies protocol

Agenda

Translational biomarkers expertise within Evotec

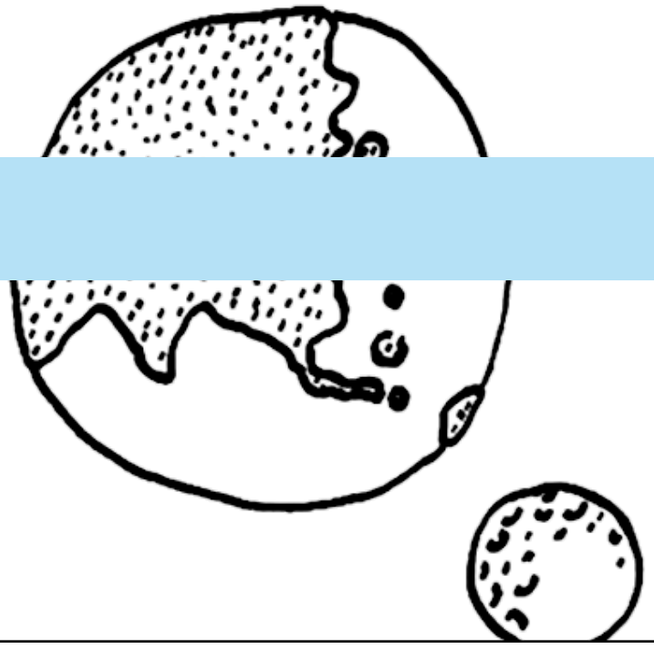
The Evotec-Oncopole Collaboration

Human Sample Access & Management

Biomarkers Platforms

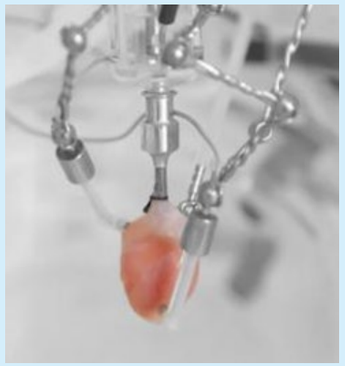
Safety Biomarkers

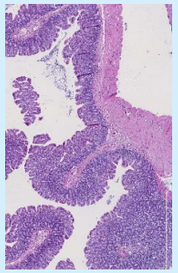
Clinical Trials and Biomarkers

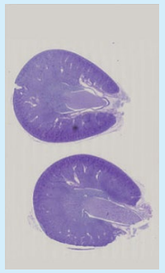


Early Safety biomarkers on targeted organs

Cellular assays; *ex vivo* or *in vivo* assays





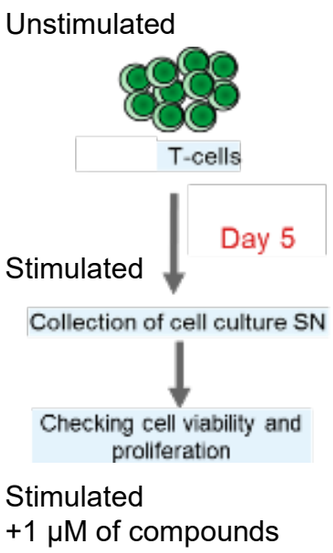


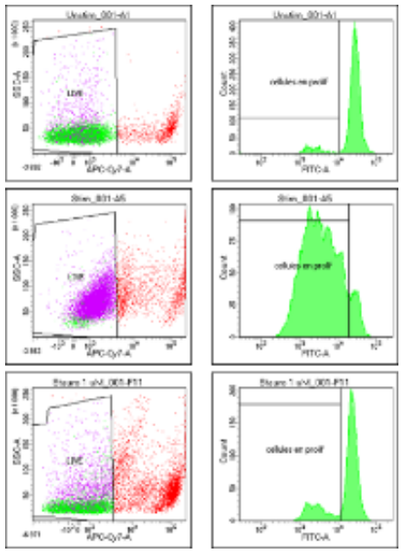
Liver Toxicity	Drug-induced hepatotoxicity is a major cause of safety-related failures	• Clinical chemistry tests: transaminases, bilirubin… • Early safety markers: RNAs • Histology
Renal toxicity	• Clinical chemistry tests: creatinin, urea, total protein, albumin… • Specific biomarker KIM-1 • Histology	
Toxicity towards immune system	• Evaluation of changes in leukocytes counts & phenotypes (haematology analyzer; flow cytometry) • Cytokine release assays (cytokine storm, sepsis) (Mesoscale) • *In vitro* viability of PBMC	
Cardiotoxicity	• Ion channels • Isolated perfused heart, Purkinje fibers[1)] • Early biomarkers (ex Atrial Natriuretic Peptide RNAs)	
Neurotoxicity	• Intra cerebro ventricular or intrathecal administration • Irwin test • Evaluation of distribution in the different part of the brain by dissection (cortex, hippocampus, striatum, brainstem, cerebellum…)	

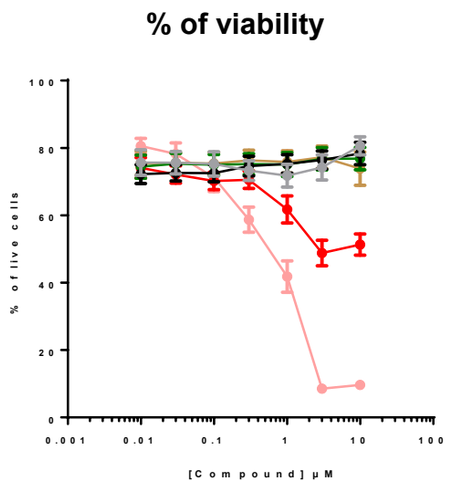
[1)] Ousourced to Physiostim, a CRO specialized in cardiac electrophysiology and preclinical regulatory cardiac safety studies

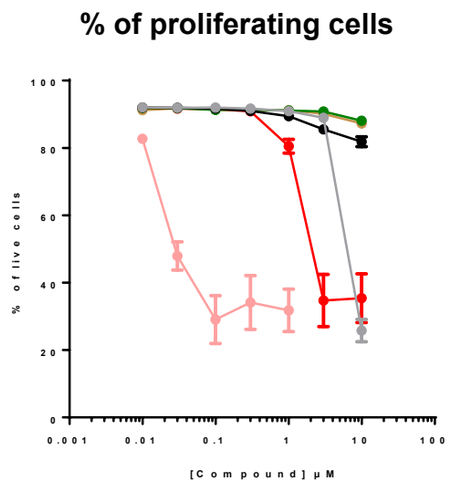
Ex vivo evaluation of advanced compounds on CD3+ T-cell activation in comparison to pan-TK inhibitors

Compounds activity on primary human T-cell (proliferation & viability)









Results are mean of T-cells isolated from 4 healthy donors

- Staurosporin has been used as positive control
- Sorafenib and axitinib inhibit T-cell proliferation
- Agents have no negative impact on T-cell viability and proliferation

Agenda

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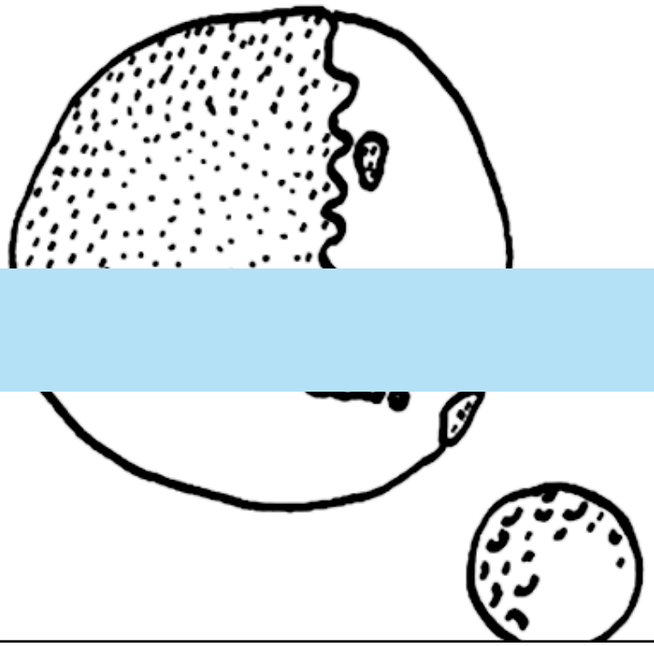
Clinical Trials and Biomarkers



EXS21546 & EVT801: Two agents developed by Evotec have entered clinical trials in 2021

Translational Biomarkers will be/are evaluated in clinic

EXS21546 is a peripherally-restricted and selective antagonist of the Adenosine A2a Receptor, designed for anti-cancer immunotherapy

Target ID	exs21546 PDC	Clinical trial phase I
April 2016	Sept 2018	Dec 2020

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

Hamburg, Germany, April 9, 2021

Exscientia

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.

EXS21546 Ph1 healthy volunteers completion in Q1 22

- EXS21546 co-invented by Evotec and Exscientia
- Biomarkers assay used was developed by Evotec

EVT801 is a novel selective VEGFR-3 inhibitor targeting tumor angiogenesis

EVT801 PDC	Clinical trial phase I
2016	Nov 2021

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

KAZIA THERAPEUTICS

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.

First patient treated: Nov 3rd at Oncopole, Toulouse

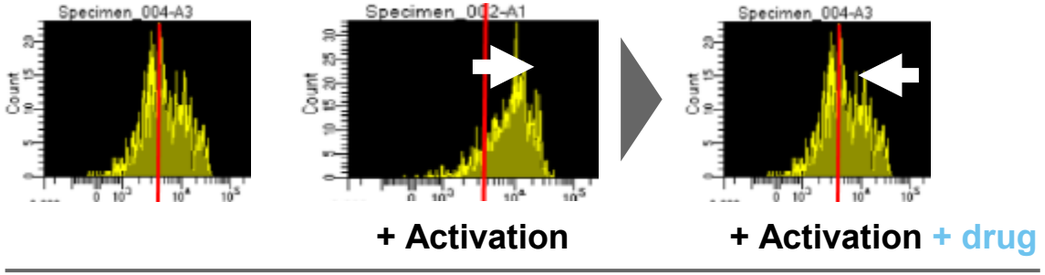
- Evotec support the clinical development
- Exploratory biomarkers evaluation performed by Evotec

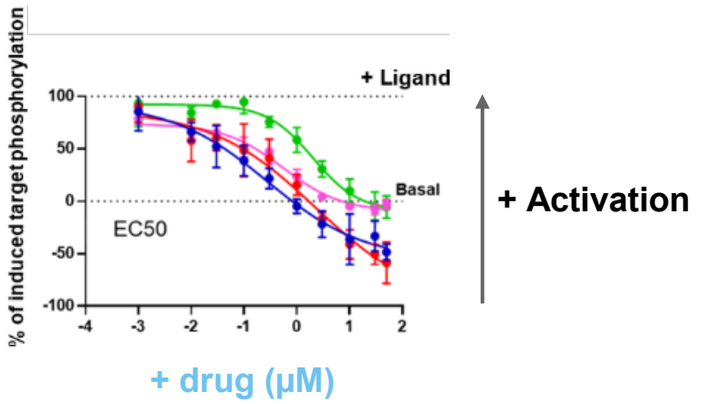
Target Engagement Biomarker assay developed by Evotec and used during EXS21546 clinical trial

Whole blood assay

- **Background:** Develop a target engagement assay to demonstrate that EXS21546 is mechanistically active at the right dose
- **Experimental settings: Flow cytometry analysis**
  - Human whole blood
  - Identify drug efficacy on *ex vivo* activated T-cells
- **Outcome:** dose-dependent inhibition of activated T-cells by drug validated in:
  - Blood from healthy subjects
  - Blood from patients with high grade cancer

p-target staining on CD8⁺ T-cells for one healthy donor

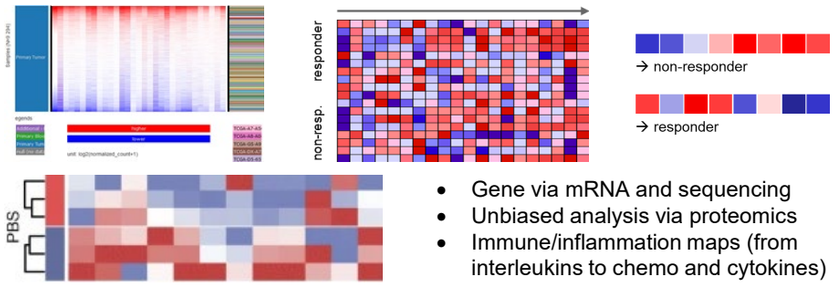




Case study: Supporting Biomarkers During Clinical Trials

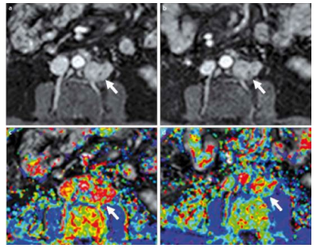
Further developing Efficacy and Patient Stratification markers

Signatures

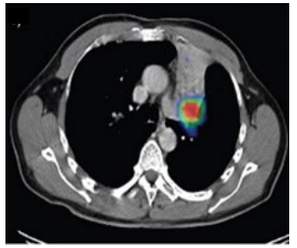


- Gene via mRNA and sequencing
- Unbiased analysis via proteomics
- Immune/inflammation maps (from interleukins to chemo and cytokines)

Imaging

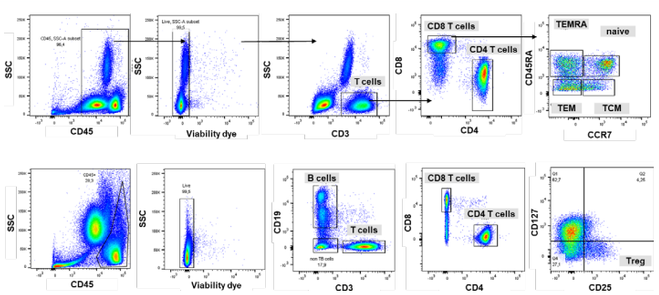


DCE-MRI



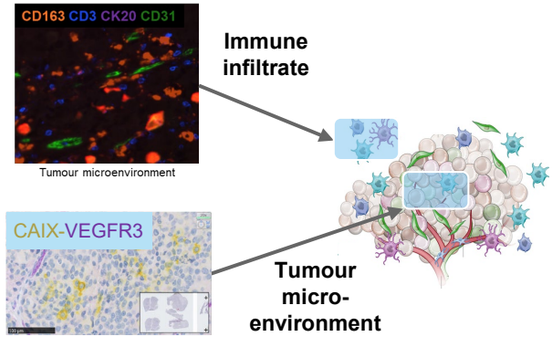
PET-scan

Immunomonitoring



Characterization of circulating immune cells

IHC



- EVT801 is in clinical trial Phase 1 with Evotec support, including biomarker validation and expansion towards additional markers for efficacy, patient stratification, and diagnosis
- Evotec has investigated in:
  - Gene, protein, and immune profile signatures
  - Immunomonitoring
  - Imaging parameters
  - Expression markers via IHC and mRNA signature of tumour tissue

Imaging figures taken from Journal of Radiation Research, 2013, 54, 1078–1084 and Frontiers in Oncology, 2020, 10, 972
DCE-MRI: Dynamic contrast-enhanced magnetic resonance imaging
CEUS: Contrast-Enhanced Ultrasound

Case study: Monitoring treatment effects on underlying pathology

Efficacy endpoint biomarkers: cytokines quantification

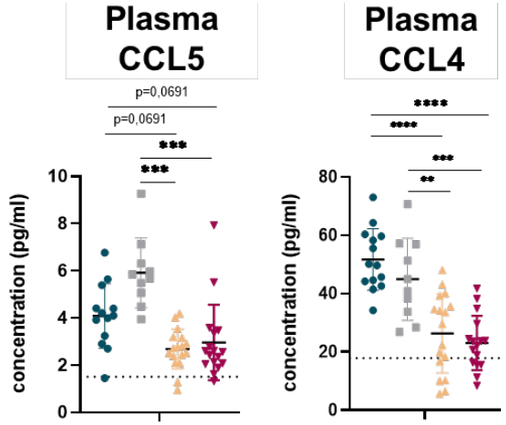
- **Background**
  - Cytokines are easily quantified and characterized and concentration can be modified by drug treatment
- **Main experimental settings**
  - in pre-clinical assays drug, leads to reduction of circulating cytokines
- **Outcome**
  - Panels of inflammatory and angiogenic cytokines will be evaluated as an efficacy endpoint biomarkers

Sector S600

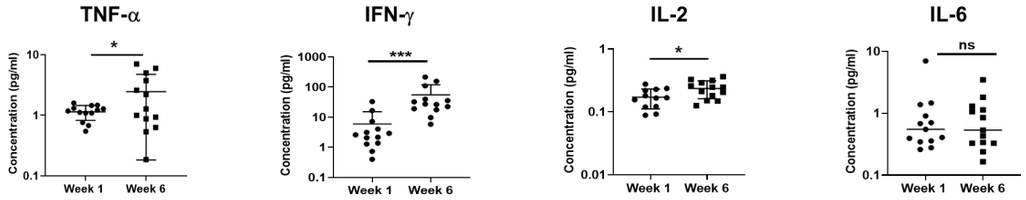
V-PLEX Proinflammatory Panel 1 Human Kit

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

Circulating chemokines



Chemokines evaluation in patients



● Vehicle + Iso ■ Vehicle + CTLA4 ▲ Drug + Iso ▼ Drug + CTLA4

Evotec – IO department Toulouse for Montfort A *et al. Clinical Cancer Research* 2020

The Evotec-Oncopole Collaboration journey to develop research and medical excellence

A 6 years collaboration to turn ideas into drugs

Therapeutic areas	2015 … 2018 2019 2021 →
Cancer (INSTITUT UNIVERSITAIRE DU CANCER DE TOULOUSE Oncopole; Hôpitaux de Toulouse)	Development of collaboration with KOL of Toulouse hospital →
	FFPE slides of human tumors →
	Participation to immunopredict **(NCT02827344)** clinical trial in lung cancer
	Frozen human tumors →
	Fresh human tumors →
	Fresh blood samples paired with solid tumors resection →
	Fresh blood samples from patients with hematological cancer →
	EVT801 Clinical trial phase I & biomarkers **(NCT05114668)** →
Others (Hôpitaux de Toulouse)	Fresh tissue, blood & urine samples →
	Clinical data & follow-up →
	THINK BIGGER !!! →

FFPE: Formalin-Fixed Paraffin-Embedded

evotec

#RESEARCHNEVERSTOPS

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