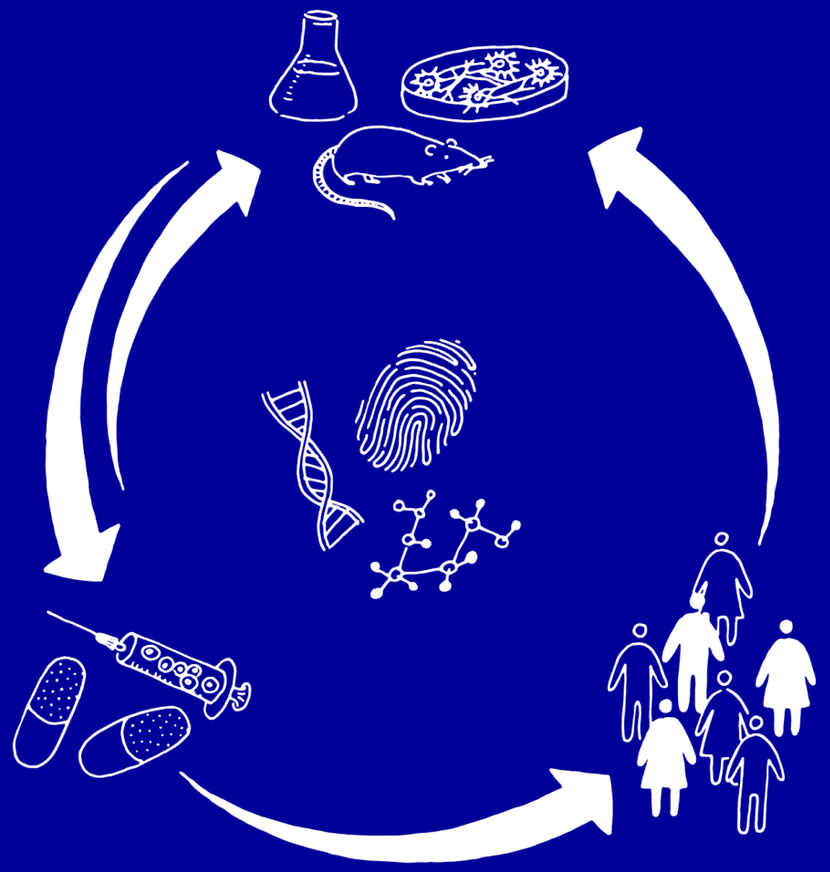
evotec

#RESEARCHNEVERSTOPS

Translation to clinical success by using state-of-the-art biomarker assays & multi-omics

Oncology Expertise



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Topics

1. Supporting the drug discovery continuum with biomarkers
2. Platforms related to biomarkers
3. From unbiased testing to targeted
4. Case studies
5. Evotec has oncology expertise
6. Patients derived organoids at Evotec

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Timing of Precision Medicine/Biomarker Discovery

A parallel process to R&D efforts

TV | HTS | HE | H2L | LO | Pre-IND/IND | Clinical development

The road of a biomarker

Discovery	Analytical Validation	Clinical Validation	Qualification
Identify • Proof of Concept (human or animal samples) • Pilot studies to show relevance	**Establish acceptance of performance** • Selectivity, Accuracy • Recovery, Sensitivity • Reproducibility, Stability	• Diagnostic accuracy • **Utility for clinical practice and drug development** • **Additional criteria** for assay performance	• FDA/EMA approval

- Finding and validating biomarkers is a **parallel process to R&D with a different scientific and regulatory focus**
- To ensure biomarker assays to be discovered, ready and validated before clinical trials, **activities should start mid/late H2L (exact timing dependent on availability of good references/tools), but latest in LO**

Finding the best Translatable Biomarkers

From hitting the target to finding the best patient and indication

Target engagement / pharmacodynamic

Surrogate endpoint / efficacy

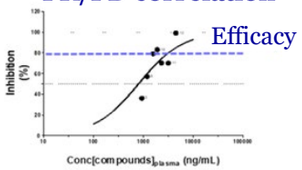
Safety / toxicity marker

Predictive or stratify-cation marker

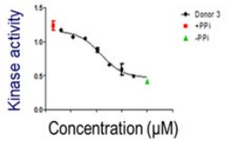
Diagnostic / prognostic

Finding the right dose

PK/PD correlation

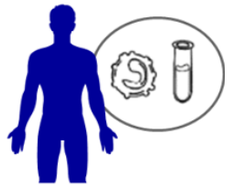


Human blood biomarker



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 material

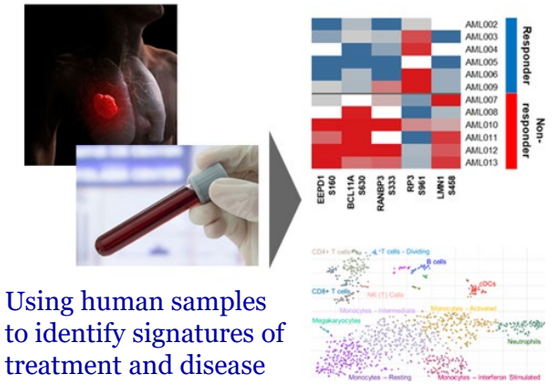


Pertinent high-quality human samples with associated clinical data (tissues and/or fluids)

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 human. Moreover, understanding the biomakers's behaviour in humans is essential to design the best clinical trial.

Identify the right indication & patient



Using human samples to identify signatures of treatment and disease

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

Translational biomarkers within Evotec

Expert support from Target validation to clinical trial

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

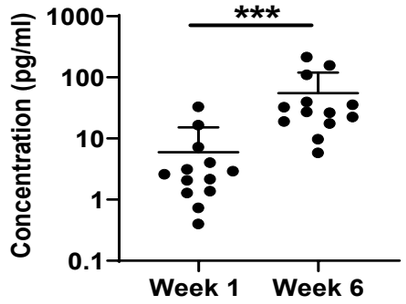
- Cell lysates & supernatant
- Body fluids e.g. blood, plasma, saliva, CSF
- Animal and human tissues
- *Ex-vivo* assays on patient samples

Platforms

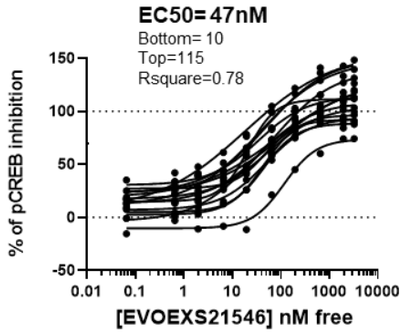
- Biomarkers strategy
- Human sample access and Human biorepository
- Immunoassays: MSD, TR-FRET, Luminex, Quanterix SMC, flow cytometry
- MS-based: Deep or single-shot proteome profiling; targeted MS using MRM, Metabolomics & LC-MS
- IHC/IF, ISH, histology, Ventana multi-colour staining
- RNAseq, Fluigdim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

Clinical Case studies

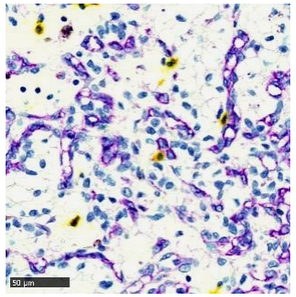
IFN-γ



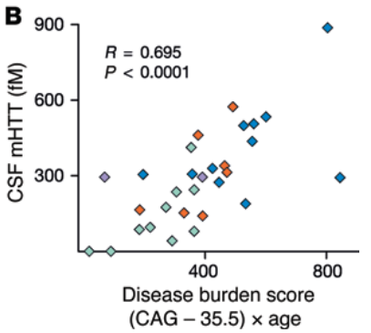
Cytokines quantification (by MSD) in *plasma from patients included in* clinical trial



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

Proteomics and Metabolomics Platforms

Global Leadership in Cutting-Edge Proteomics

> 50
MS instruments

> 100
Employees MUC & TLS

> 1.5 B
Datapoints generated p.a.

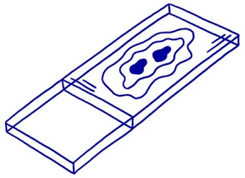
- Evotec operates two of the **largest proteomics & metabolomics** facilities world-wide
- Evotec has more than **20 years' experience** in mass spectrometry, chemical proteomics, metabolomics, and bioinformatics and their applications in **drug and biomarker discovery**
- Evotec drives the **paradigm shift** towards high-throughput and **high-coverage proteomics screening**
- Evotec is a proven partner of **global pharma, biotechnology, and crop science** companies such as

Boehringer Ingelheim · Bristol-Myers Squibb · Takeda Pharma · janssen · MSD · Bayer CropScience · The Mark Foundation for Cancer Research · CHDI FOUNDATION · Bayer HealthCare

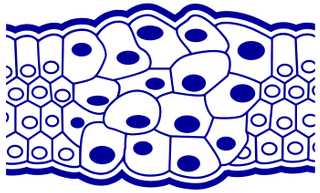
Translational Biomarkers strategy using pertinent samples

The need for translational thinking

Biological samples



FFPE
tissue



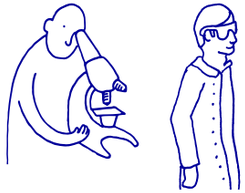
Fresh (*ex vivo*)
or frozen tissue

Fluid samples
(blood & urine)

Clinical data

Histological & gene
mutation reports

Patient
history



Clinical & histological
consultancy

1 Retrospective hypothesis testing

- **Target engagement / pharmacodynamic**
- **Surrogate endpoint / efficacy**
- **Safety / toxicity marker**

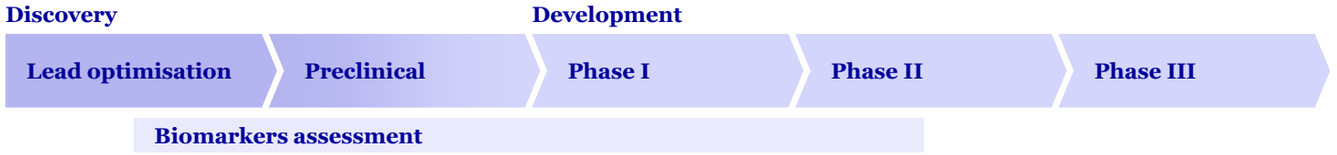
2 Prospective hypothesis testing

- **Predictive or stratify-cation marker**
- **Diagnostic / prognostic**

Ability to support GCP Validation of Biomarker Assays

Overview – GCP certified laboratories

- **Strong collaboration with DDD biomarker experts**
- **Integrated process & translational approach**



Clinical study support experience

- Bioanalysis of small molecules, peptides, oligonucleotides and biotherapeutics in human plasma / serum (both ELISA and LC/MS-MS based assays)
- Immunogenicity assessment (ADA analysis using MSD platform)
- mAb RO analysis by Flow Cytometry; associated target expression on lymphocyte subset and soluble target
- Molecular markers analysis by qPCR (gene expression and gene methylation) including genotyping analysis as enrolment criteria (e.g. TMTP assay)
- Phosphokinases analysis in whole blood from Phase I / Phase II studies
- Renal Injury Markers analysis by MSD assays
- Neurodegeneration biomarkers analysis by SMCxPRO and Quanterix assays (mHTT, total HTT, NfL)

Method qualification / validation performed in advance to sample analysis (GCP) depending on the relevance of the endpoint and on the use of data generated

Topics

1. Supporting the drug discovery continuum with biomarkers
2. Platforms related to biomarkers
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5. Evotec has oncology expertise
6. Patients derived organoids at Evotec

Translational biomarker strategy platform

Missions and deliverables

Target engagement / pharmacodynamic

Surrogate endpoint / efficacy

Safety / toxicity marker

Predictive or stratify-cation marker

Diagnostic / prognostic

Our missions

- Define the best biomarker strategy for your new drug at each stage of its development
- Set-up biomarker assays that could be translated to the clinic
- Define patient segment who may benefit from the drug and a predictive biomarker

Biomarker coordination

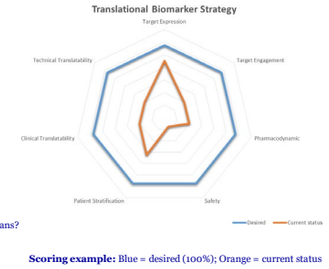
- Discovery
- Benchmarking with competitors
- Validation in PK/PD studies
- GCP assay set-up and validation
- Clinical biomarker assessment

Early **Translational Strategy Document** (TSD)

	Base case	Upside case
Primary indication	Base case tumour indication	2 upside case tumour indications
Patient population	Patient segment if possible (e.g., Target X positive, Target X/Y amplified, specific mutational status, …	
Patient characterisation	Definition of stratification biomarkers	*EXAMPLE*
Target engagement assay	Circulating biomarkers privileged	Non-circulating biomarkers
Efficacy endpoints biomarker	Exploratory biomarkers for Phase I/II	–
Safety biomarkers	On target safety biomarkers	Off-target safety biomarkers to be explored by toxicity studies
Competitive advantage	Competitors	–

IDD tool

1) **Target Expression**
   - Is the target similar between species?
2) **Target Engagement Markers**
   - Are there any markers/options to measure direct TE?
3) **Pharmacodynamic Markers**
   - Are there pathway markers/options?
   - How much understanding of PK-PD/behavior?
   - Translatability across species?
4) **Safety Markers**
   - Are there on- and off-target safety alerts?
   - Are they measurable across species?
5) **Patient Selection/Stratification**
   - Indication? Heterogeneous population?
6) **Clinical Translatability**
   - What is known of the markers in the human setting?
   - How easily can this be measured in a clinical trial?
   - What do we understand of the behavior of the markers in humans?
7) **Technical Translatability**
   - Can we design validated assays for human setting?

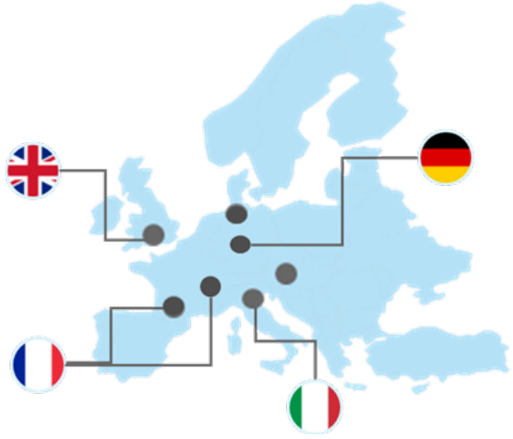


Scoring example: Blue = desired (100%); Orange = current status

Human samples platform: Access to Human Material

Acquisition and use of human samples has regulatory requirements & ethical considerations

- **Strategic collaborations** with clinical centers for **prospective** sampling
- **Retrospective samples** via commercial sources and dedicated biobanks
- **Blood from healthy volunteers**



Sample Management
Global human sample management; QC, registration

Storage Infrastructure
Storage infrastructure for human samples with disaster protection

Data protection
Inventory database

Resource optimization
Internally available sample stocks

Clinical data capture
Collection of data & data entry monitoring; clinical data QC

Global sample storage infrastructure

Global database and sample tracking

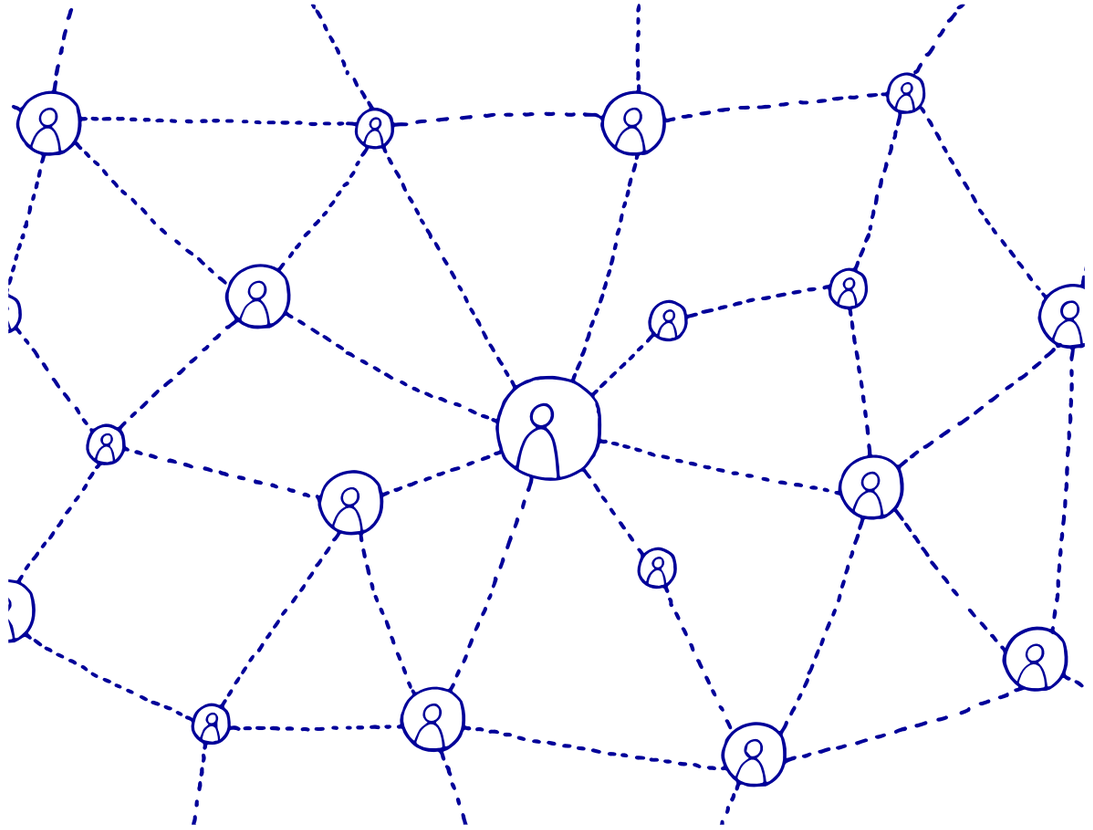
To ensure the best materials as well as compliance to all regulations, the Translational Biomarker department has both a team focused on human sample acquisition (HSA) and management (HBSO)

Access to High Quality Human Material

Global commercial provider network

Exemplary Indications

- Oncology (solid and liquid)
- Endometriosis
- Asthma
- Pulmonary hypertension
- Alzheimer
- Autoimmune Diseases (AIDs): SLE, RA, atopic dermatits, IBD
- Healthy volunteers



Exemplary type of samples

- (fresh) whole blood & derivatives
- FFPE blocks and slides
- Fresh healthy tissue
- Urine, feces, tears sperm
- Hair follicle
- Cerebrospinal fluid (CSF)

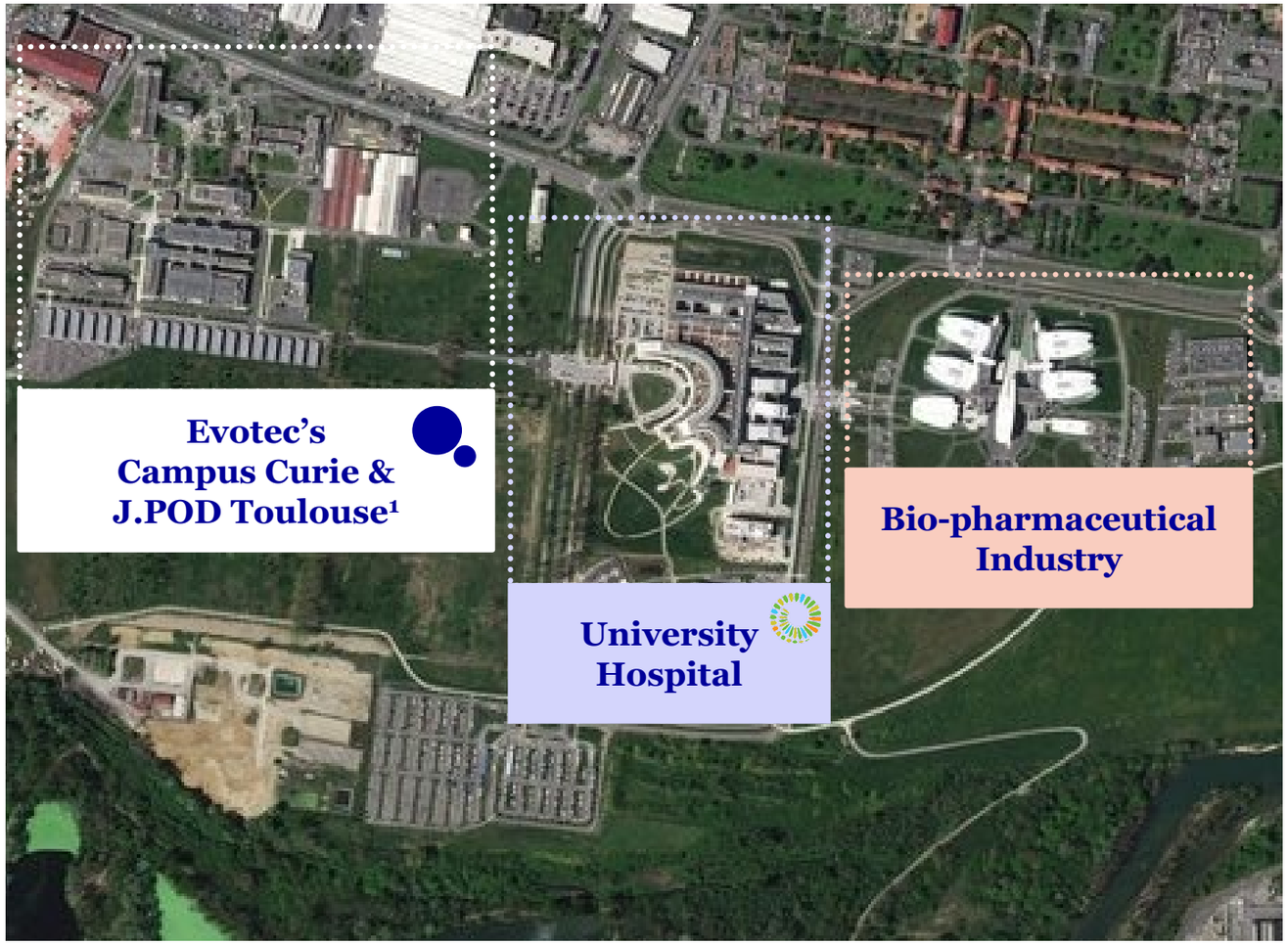
We will identify the best solution regarding quality, resources, timeline, and study constraints

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.

Identification of the Right Patient using pertinent samples

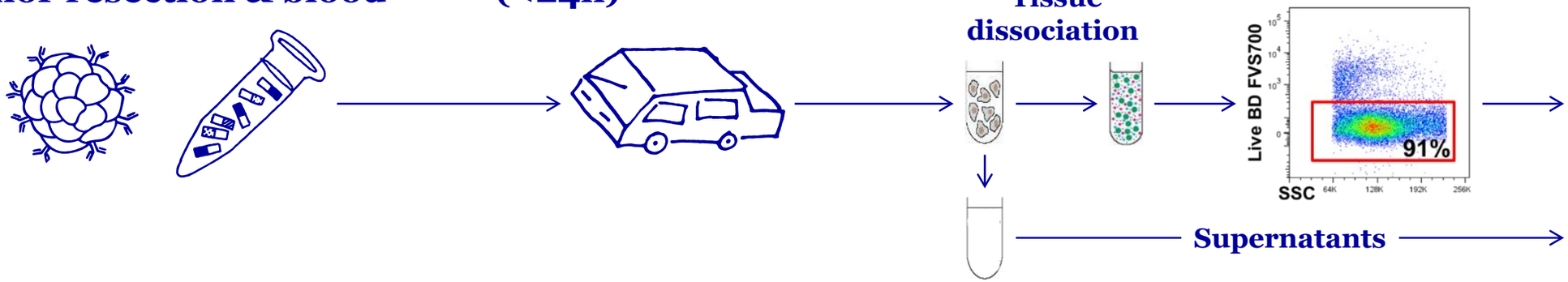
Supporting patient stratification and identification of new biomarkers

Access to healthy tissue, tumor resection & blood

Transport to Evotec (<24h)

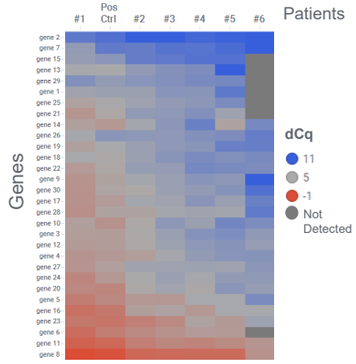
Tissue dissociation

Living cells

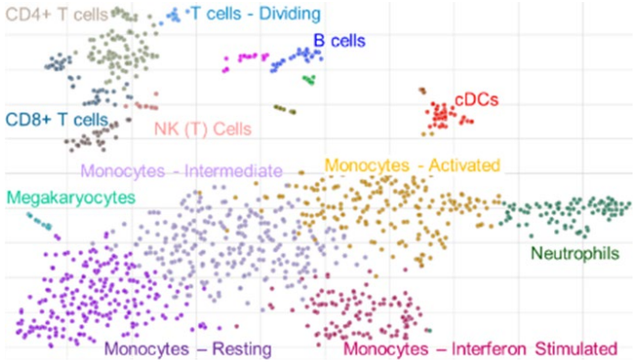


Access to fresh patient samples via established collaboration

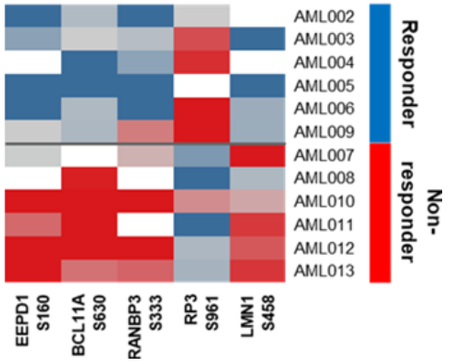
RNA signature



Single cell mRNA sequencing



Proteomics



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

Immunoassay and flow cytometry platforms for biomarker discovery

Multiple options for tailor-made approaches

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

Luminex MagPix
- Superior multiplex ability
- Large number of ready-to-use assay kits available
- bDNA and protein capability

SMCxPRO
- Ultra-high sensitivity
- Single molecule counting
- Reliable and suitable for clinically relevant matrices

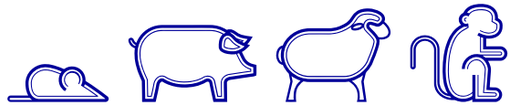
MSD S600
- High throughput and sensitivity
- ELISA-like, well customizable
- Very robust

JESS Digital Western Blot
- High sensitivity
- Reduced workflow complexity
- Low sample input & normalization options

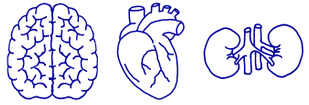
Quanterix HD-X/SR-X
- Ultra-high sensitivity
- Multiplexing and automation
- Reliable and suitable for clinically relevant matrices

Experience with a broad range of 'pre-clinical and clinical samples

HD animal models



Brain & peri-pheral tissue



Cellular models

Clinical samples, GxP and non-GxP

Measurements done for Human Samples
- Mutant, total, aggregated HTT
- Ataxin 1 and 3, mutant and total
- NFL
- Amyloid β 1-40, 1-42
- Tau and pTau
- Alpha-synuclein
- IL panel (multiplex or single)
- Cytokine panel (multiplex or single)
- TNF alpha, IFN
- Unbiased omics: proteomics and p-proteomics
- Unbiased omics: transcriptomics (various depth)
- Unbiased omics: metabolomics / lipidomics
- P180 Biocrates kit (metabolites)
- Autophagy readouts (p62/LC3)
- (any commercially available kit)
- bDNA panel
- qPCR, mRNA panels

Flow cytometry and cell isolation
- Isolation, purification and polarization of different immune cells
- FC[1] analysis for immunophenotyping, PD/TE assessment in blood and tissue (single cell suspension)

BD FACSVerse | BD FACSLyric | Thermo Attune NxT

Flow cytometry

Dasit XT 2000
Hematology analysis

MACS technology
Cell isolation

1 All assays can be run and have been run at Evotec on human samples; depending on the matrix offered, some matrix validation is required

Histology & IHC platforms: Processing and Analysis

Different workflows tailored to customer needs

Tissue sample preparation

FFPE and frozen blocks

Dehydration

Paraffin embedding

Tissue slicing

Vibratome

Microtome

Cryo-Microtome

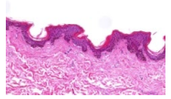
Cryostat

Classical Histology

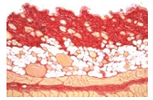
- Haematoxylin & Eosin (H&E)
- Masson's Trichrome
- Picrosirius Red
- Toluidin Blue
- Turnbull's Blue

Sakura Tissue Tek

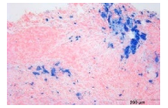
H&E



PSR

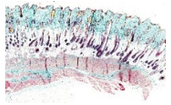


Turnbull's Blue

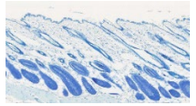


SLEE autostainer

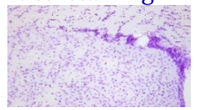
Masson's Trichrome



Toluidin Blue



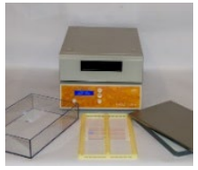
Nissl staining



Immunohistochemistry

- **Chromogenic immunohistochemistry (CIH):** up to 5 biomarkers
- **Immunofluorescence (IF):** up to 4 biomarkers
- **Tissue micro-array (TMA)**

ACD-Bio ISH system



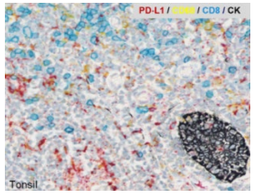
Leica BondRX

Ventana auto-stainersUltra/XT

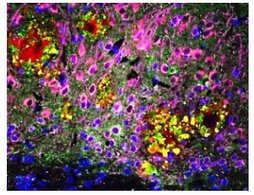
In situ hybridization

- **RNAscope:** up to 3 probes simultaneously
- Also possible in combination with IF
- **Basescope** and **miRNAscope**

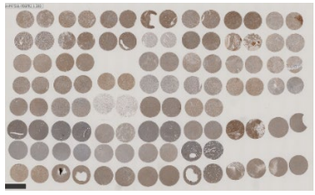
CIH



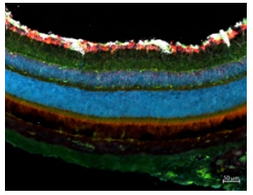
IF



TMA

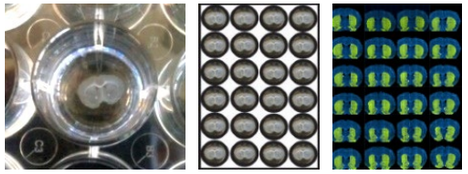


ISH/ IF



Scan & Digital Analysis

- **Mounting of samples:** multi-well plates or slides



- **Scan:** bright field or fluorescence; confocal image acquisition

OperaPhenix

Operetta

Slide Scanners

Quantification

- Automated High Content image analysis; annotation of ROIs using Deep Learning

Histopathology

- Veterinary pathologist expertise
- Histo-pathological image analysis (manual or automated)
- Clinical pathologist consultancy for clinical patients assessment

Molecular Biology Platforms

From single gene to full transcriptomics to support Biomarker discovery workflow

Small scale qPCR & direct qPCR

QuantStudio 7 + Orbitor

1-step & 2-step RT-qPCR

Digital qPCR

QIAcuity

Spatial

RNAscope

Fluidigm/qPCR Platform

- Flexible set up, larger throughput:
  - 24-192 samples analysed
  - 12-192 targets analysed
- Time saving
- More genes analysed in small amount of material

Olink

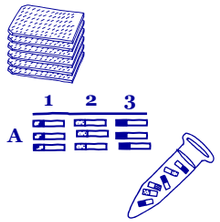
Protein detection & quantification (Olink technology)

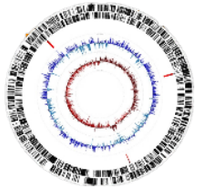
Juno system

Biomark™ HD

Transcriptomic / Genomics

ScreenSeq Compound screening	**Bulk RNAseq** Absolute quantification, differential expression splicing	**DNAseq** Whole genome Targeted enrichment & amplicon	**scRNAseq** Specific cell population differentiation (10X)	**Long reads** Nanopore (Min/Grid-IONO) RNA structure (SHAPE seq)





- Identify transcript biomarker that could be further validated by qPCR or Fluidigm in future experiment
- Identify transcript biomarker for further validation by IHC

Quantitative MS Supporting Drug Discovery

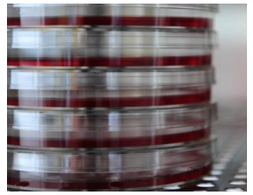
Chemical proteomics, global proteomics and metabolomics

Global Proteomics Platforms

- High-end quantitative mass spectrometry to monitor protein expression, phosphorylation, glycosylation, acetylation, ubiquitination or arginine methylation
- Targeted mass spectrometry assay development and deployment

1 *In vivo* mode-of-action analysis in cells, tissues or patients

2 Discovery and verification of biomarker candidates



Chemical proteomics

- Evotec Cellular Target Profiling™ technology to both identify and quantify interactions with cellular compound targets
- Drug photoaffinity labelling and activity-based protein profiling for covalent target capture

3 Cellular compound selectivity analysis in a native context

4 Target de-convolution of hit compounds from phenotypic screens

Metabolomics

- *In vitro* and *in vivo* quantification of metabolites in complex sample using SPE-LC–MS/MS

5 Targeted analysis in cells, tissues, body fluids or awake animals

Target candidates	Target deconvolution	Biological selectivity	Mode-of-action	Target engagement	Drug candidate prioritization	Biomarker candidate ID	Biomarker verification
Target ID	**Screening**		**Hit-to-lead**	**Lead Opt**	**Pre-clinic**		**Phase I/II**

Interactive and versatile bioinformatics software platform

PanHunter – a proven tool in many Evotec projects and partnerships


SC Browser

Comparison

Top Table


Signatures


Patient Data


Clustering


Cross Comparison

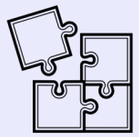

GO Terms


Pathways


Networks


Transcription Factor


Trend Analysis



Modular and extendable

Designed for collaborative work

Reproducible, trustworthy results

Machine learning algorithms

Visual and interactive data exploration

- Modern browser-based GUI with specialized apps using peer-reviewed algorithms
- Interactive setup of multivariate comparisons

Driven by research requirements

- All features implemented to cope for scientific need
- Powerful tool with the researching user in mind

Topics

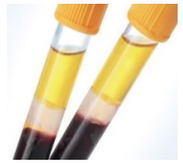
1. Supporting the drug discovery continuum with biomarkers
2. Platforms related to biomarkers
3. From unbiased testing to targeted
4. Case studies
5. Evotec has oncology expertise
6. Patients derived organoids at Evotec

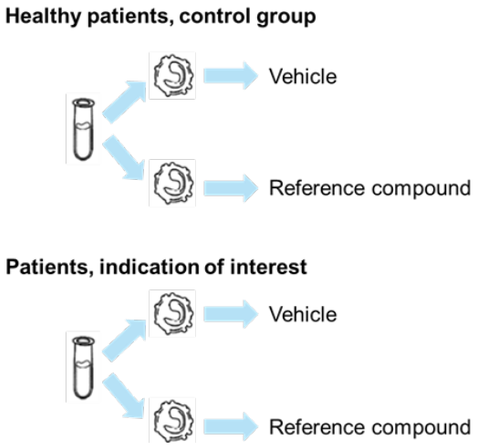
Data-driven Biomarker Discovery

From existing data to proving relevance for the selected targets and indications

Sample acquisition and generation

- Prospective sourcing
- Retrospective sourcing (biobanks)
- Sample generation

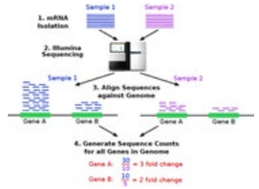




Sample evaluation

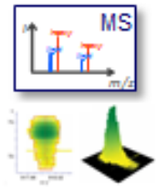
- Disease-specific biomarkers
- Pathway biomarkers
- Unbiased approaches:
  - Transcriptomics
  - (Phospho) Proteomics

Methodology



RNA-Seq

Proteomics

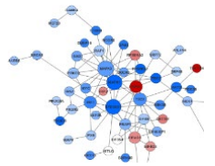


(Immuno) assays

Outcome

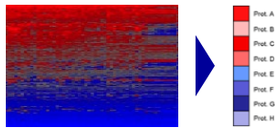
- New biomarkers (TE-PD)
- Validation of biomarkers
- Patient stratification markers
- Pathway relevance in the disease
- Target validation

Data analysis (PanHunter)

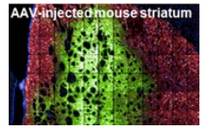


Functional/ Pathway analysis

Literature/patient data



Bioinformatic tools/databases



Validation experiments

Data generation

Translational Approaches/Technologies at Evotec

From targeted testing to unbiased to support the drug discovery and development project

Unbiased biomarker discovery

- Genomics
- Transcriptomics
- Mass spectrometry-based proteomics and metabolomics
- Post-translational modifications (methyl, acetyl, phosphate, ubiquitin, glycosylation)
- Secretome analysis
- Immuno-phenotyping

Hypothesis testing

- *In vivo* models with high translational value (orthotopic, syngeneic, PDX, humanized etc.)
- *Ex vivo* drug treatment and/or analysis of both animal and human samples
- Preclinical imaging in rodents
- Exploration of prevalence in the context of pathology
- Evaluation of stratification, PD, toxicity, efficacy biomarkers
- Proposal for Phase 1 clinical trial

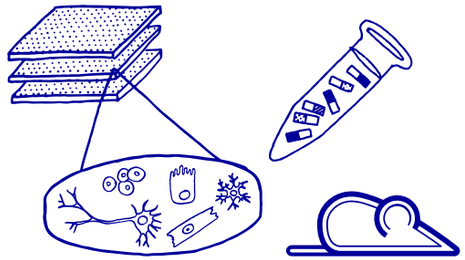
Clinical translation

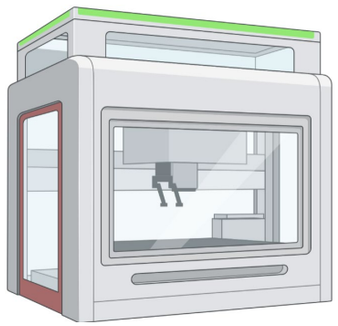
Integration of different approaches to biomarker discovery best supports translational biology activities

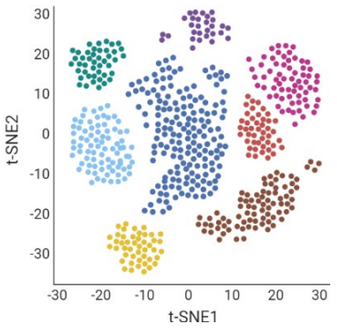
PDX = patient derived xenograft

NGS/Transcriptomics for Biomarker discovery

Evotec capabilities







Sample: cells, tissues, FFPE, biofluids

- **Cells:**
  - 2D, 3D, organoid, Primary cells & isolated cells from blood
- **Animal & human tissues:**
  - Many species & tissue type
  - Fresh, frozen, FFPE
  - Biofluids: blood, CSF, faeces

DNA/RNA isolation

- **Tissue homogenizer, FFPE slicing**
- **Small to high through put RNA/DNA extraction:**
  - 96 (KingFisher, Qiagen) / 12 QiacubeConnect
- **Samples QC:** Tapestation, Fragment analyser

Library preparation

- **PolyA: 3'UTR or whole transcript**
- **Ribo or globin depletion**

Sequencing

From high to small scale sequencing power

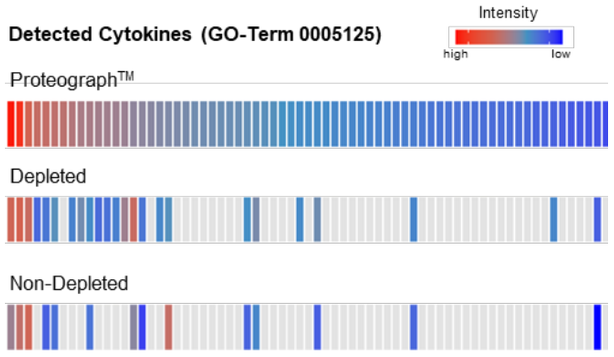
- **NovaSeq6000:** up to 384 transcriptomes or 48 human genomes
- **NextSeq 550:** optimal for medium throughput projects
- **MiSeq, iSeq:** highly flexible conditions, longest readlength in short read sequencing

Data analysis and interpretation

Clinical Proteomics for biomarker discovery

Use of mass spectrometry based proteomics in combination with nanoparticle enrichment

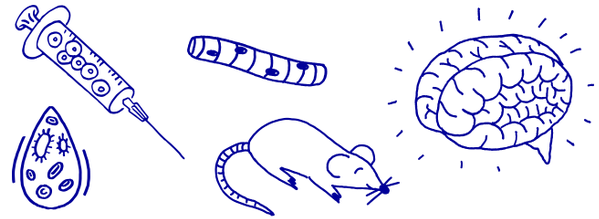
Strategic Alliance with Seer for deep proteomics using nanoparticle based Proteograph™ technology



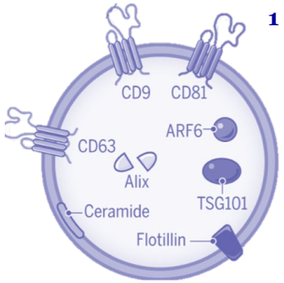
- Alliance with Seer to drive deep proteomics
- Detection of >3,000 proteins in plasma/serum
- Other applications, e.g. Secretomics from tumour samples or cell culture
- Webinar on demand with results from biofluid benchmark study: https://news.evotec.com/joint-evotec-seer-webinar-ondemand

Analysis of samples from human cohorts and animal preclinical studies

- Analysis of body fluids (e.g. plasma, serum, cerebrospinal, urine, sputum, synovial fluid…)
- Tissue proteomics (e.g. liver, brain, skin, gut..) and analysis of post-translational modifications like phosphorylation; fresh-frozen or FFPE
- Screening of large human patient sample cohort
- Support of pre-clinical studies (e.g. mouse, rat, NHP, mini pigs, cat, dog)

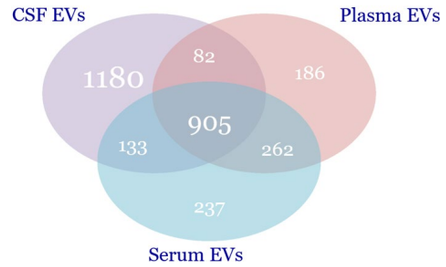


Proteomics of extracellular vesicles as novel source for biomarkers



[1]

- Detection of ~1,000 different proteins from plasma or serum EVs and ~2,000 from CSF EVs
- Important markers for EVs were detected



1 Figure adapted and modified from: The biology, function, and biomedical applications of exosomes; Science. 2020, Feb, 7, 367(6478)

Translational Approaches/Technologies at Evotec

From targeted testing to unbiased to support the drug discovery and development project

Unbiased biomarker discovery

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- Preclinical imaging in rodents
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- Evaluation of stratification, PD, toxicity, efficacy biomarkers
- Proposal for Phase 1 clinical trial

Clinical translation

Integration of different approaches to biomarker discovery best supports translational biology activities

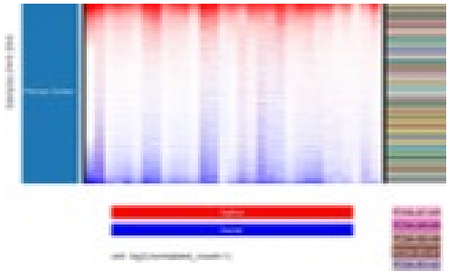
PDX = patient derived xenograft

Patient characterization from FFPE archival tissues

Established link between clusters of genes and tumor micro-environment

Data mining

- Describe predictive signature to treatment
- Target co-regulated genes
- Validation of the signature using Fluidigm
- Transfer to Nanostring technology during clinical trials

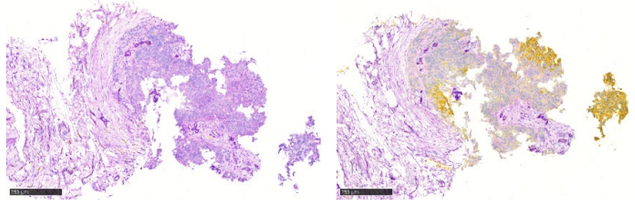


Identification of gene signature from the literature of from publicly available dataset

FFPE Archival tissues to provide mRNA and protein expression data

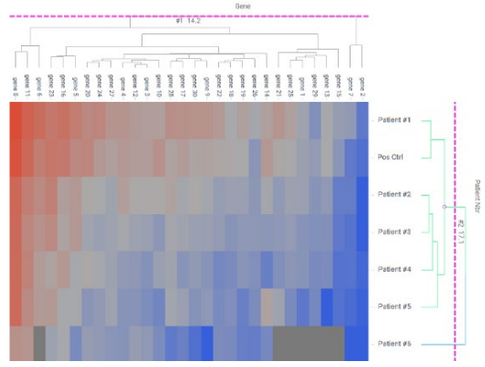
Histology labeling

- tumor architecture to identifier the regions to collect RNA
- Labelling of tumor micro-environment:
  - Immune infiltration
  - Vascularisation

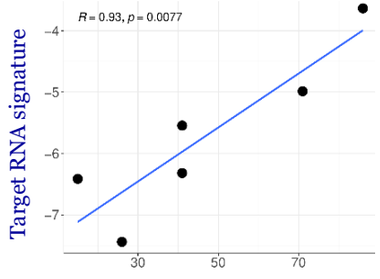


RNA signature from limited quantities of material including needle biopsies

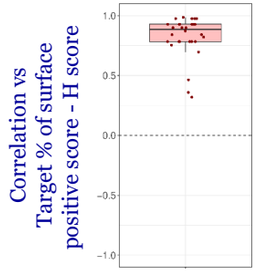
- RNA signature developed by Evotec bio-informatic department



Correlation of RNA signature and protein expression (p=0.0077)

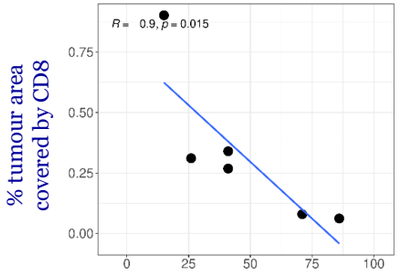


Target IHC % of surface positive score - H score

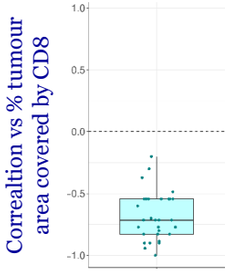


Target RNA signature

Establishment of an anti-correlation between target expression and immune infiltration



Target % of surface positive score - H score



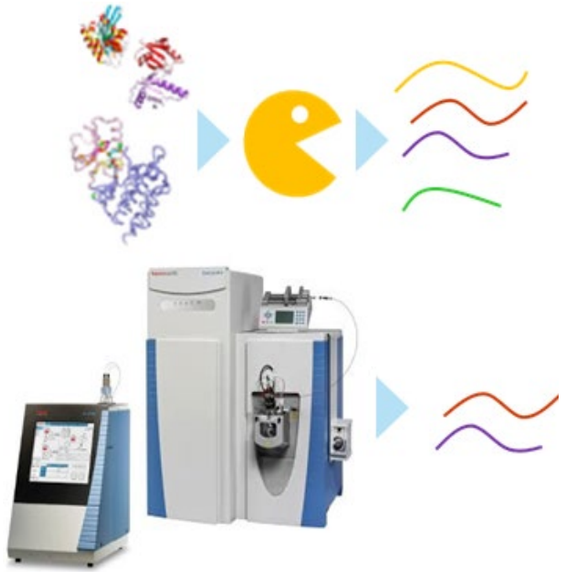
Target RNA signature

MS-Based Absolute Quantification of Proteins

General Workflow in Research Use Only (RUO)

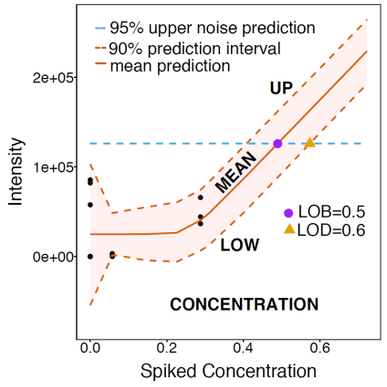
Sample Preparation Optimization

- Optimisation of sample preparation
- Peptides selection for up to **50 proteins in parallel**



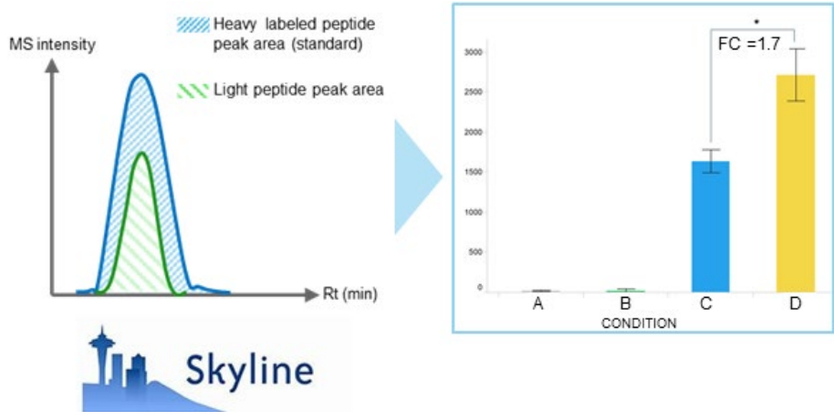
Standards synthesis & Targeted Method Optimization

- Targeted PRM or MRM assay development
- **Use of stable isotope labeled peptides (SIL)**
- Evaluation of **LOD and LLOQ** for each protein (up to 50)



Absolute Quantitative analysis

- Peak detection and integration using Skyline
- Calculation of absolute protein concentration **(up to 50 in parallel per sample)**



MS-Based Absolute Quantification of Proteins

Absolute Protein Quantification Methods: from Preclinical to Clinical Studies

Quantitative LC/MS method (RUO) *Preclinical Studies*

- Cell/Tissues/Biofluid
- Protein digestion
- Addition of SIL peptitdes[1]
- Peptides desalting
- nLC-PRM or µLC-MRM
- Data analysis with Skyline

Quantitative LC/ MS method (GCP)

- Cell/Tissues/Biofluid
- Addition of SIL proteins[2]
- Protein digestion
- Peptides desalting
- µLC-MRM
- Data analysis with Analyst & LIMS

GCP LC/MS method Validation *Clinical Studies*

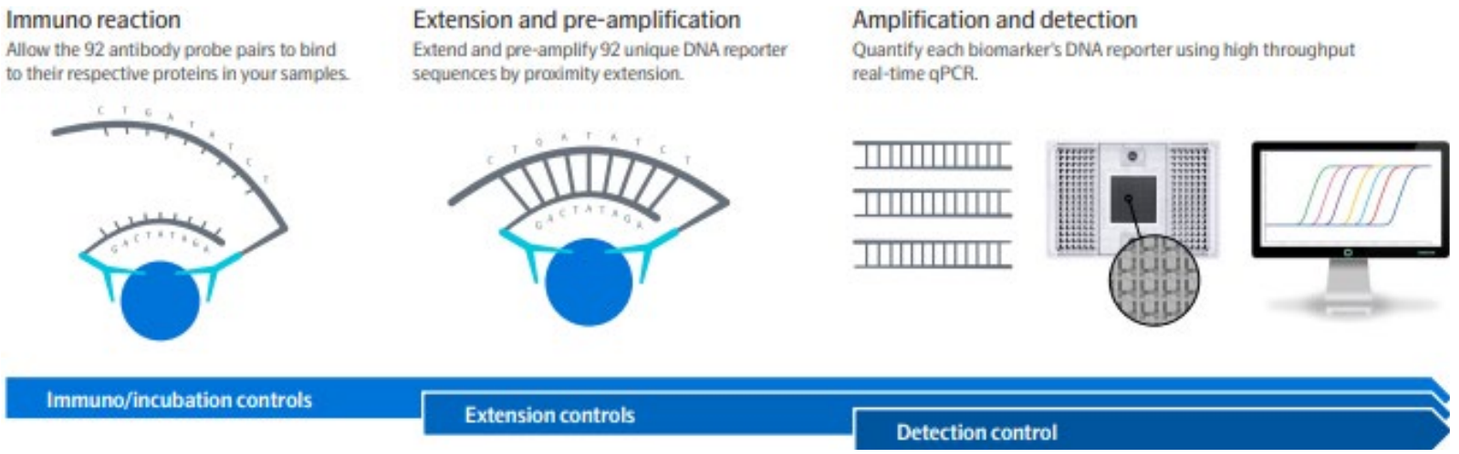
- Method validation according to the ICH M10 guideline
- Measurement of clinical samples (including primary and secondary endpoints)

1 External provider
2 Evotec internal capability
RUO: Research Use Only; GCP: Good Clinical Practice
ICH M10 guideline: Guideline describing the validation of bioanalytical methods and study sample analysis that are expected to support regulatory decisions

High-multiplex immunoassays targeted protein panel analysis

Olink: Technology based on Proximity Extension Assay (PEA) run on Fluidigm chips

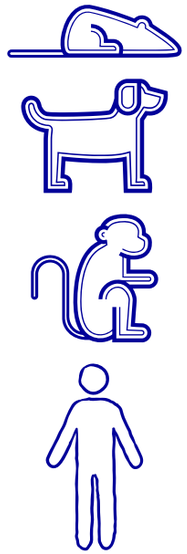
- **Proximity Extension Assay (PEA) technology performed on Fluidigm Biomark:**
  - oligonucleotide labeled antibody probe pairs bind to their respective target proteins
- **Minimal sample input is 1 µL**
  - Simultaneous analysis of 44 to 96 protein biomarkers
  - Provide a qPCR readout with high sensitivity, specificity & precision
  - Sample type analysed serum/plasma[1] or cell/tissue protein lysate[2]
- **Olink has established 15 targeted panels to analyze specific diseases/functions[3]**
  - Cardiometabolic
  - Oncology
  - Immuno-oncology
  - Neurology
  - Inflammation
  - Biological process
  - Custom panels



1 serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples
2 https://pubmed.ncbi.nlm.nih.gov/31779705/; https://pubmed.ncbi.nlm.nih.gov/32668248/
3 https://olink.com/products-services/target/#relative

Drug related biomarkers in surrogate tissues

From preclinical species to human



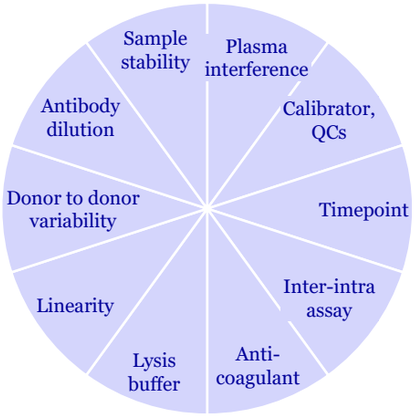
Whole blood

Ex vivo treatment with compound or challenge

PBMCs

Multiple Biomarker assessments

Immunoassay set up and validation for clinical setting

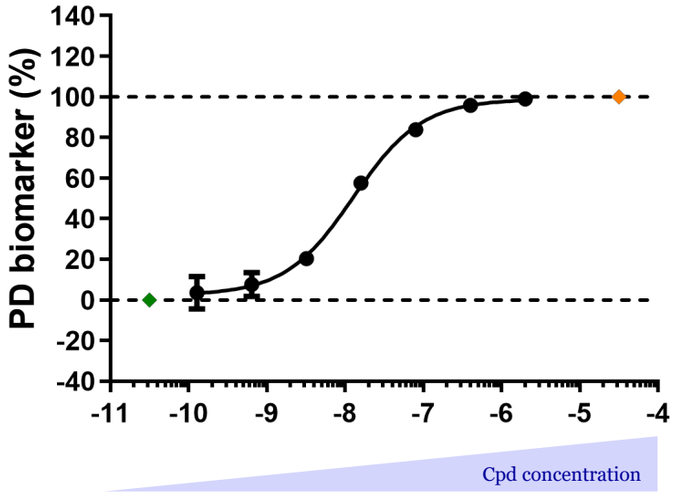


- Daily availability of fresh blood from different donors collected at Evotec
- Possibility to retest same donor
- Established protocols for PBMCs isolation, cell culture and *ex vivo* treatment
- Unbiased biomarkers assays on different subset of PBMCs has been already established
- Same workflow can be applied to naïve or study samples (PKPD, disease models, clinical trials)

Development of PD biomarkers assay for clinical application

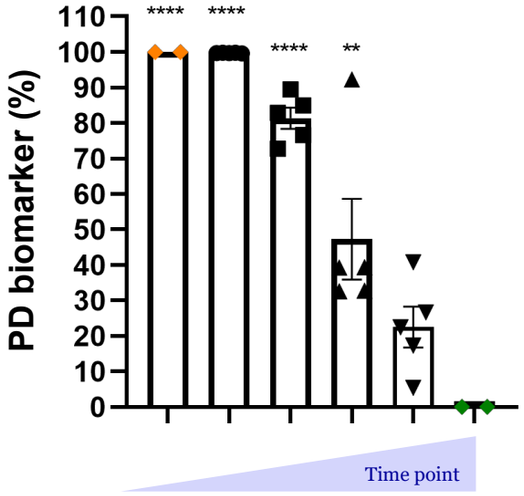
Example of Flow Cytometry application for assessment of target engagement

Primary cells obtained from dissociated mouse tissue



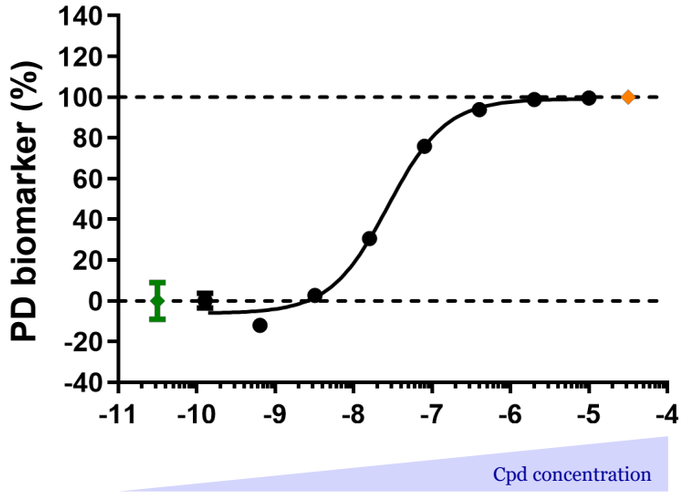
- Assay set up
- Pharmacological characterization of compounds

Mouse blood in PK/PD study



- Assay transfer
- Analysis of pharmacodynamics
- PK/PD correlation

Human blood



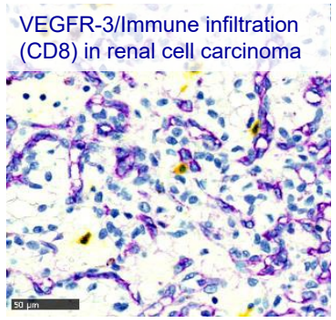
- Assay transfer
- Pharmacological characterization of compounds
- PD biomarker for clinical application

Example of exploratory biomarkers during clinical trial Phase 1 study

EVT801 is pursuing dose escalation stage of Phase I study

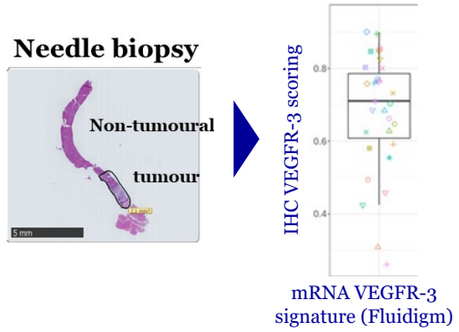
Patient stratification based on VEGFR-3 expression on archival tissues and/or biopsies

- VEGFR-3 signature by IHC:
  - VEGFR-3/CA9/CD8/CD31/ PD-L1



VEGFR-3 & Resistance to immune checkpoint therapies mRNA signature by Fluigdim

- VEGFR-3 mRNA signature
- PD-1 mRNA signature on archival tissues and/or biopsies



Circulating drug related biomarkers

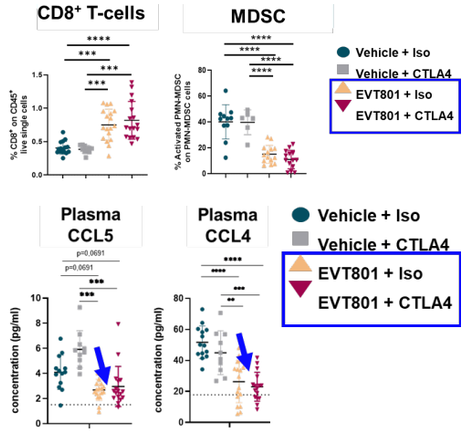
- Total RNA signature on blood cells at C1D1 vs CD2D1 (Paxgene tube)

Safety biomarkers to control hypertension

- Blood pressure measurement to control that EVT801 dose not induce hypertension (as demonstrated in preclinical model)

Circulating endpoint biomarkers

- **Immunomonitoring** based on CD8⁺ T-cells/MDSC ratio
- **Proteins signature** based on chemokines involved in inflammation & angiogenesis



Resting samples will include

- Frozen plasma
- Frozen PBMCs
- FFPE biopsies

Topics

1. Supporting the drug discovery continuum with biomarkers
2. Platforms related to biomarkers
3. From unbiased testing to targeted
4. Case studies
5. Evotec has oncology expertise
6. Patients derived organoids at Evotec

From whole blood assays to Clinical Trial Support

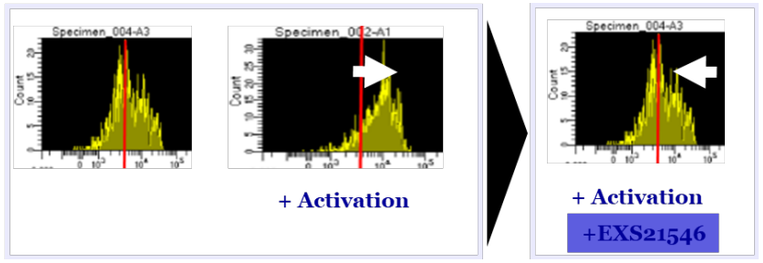
Ensuring Pharmacodynamic biomarkers in the clinic

Background

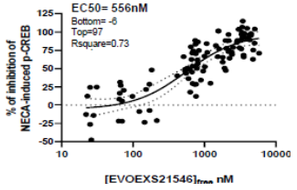
Develop a target engagement assay to demonstrate that EXS21546 is mechanistically active at the right dose

Whole Blood Assay Set-up

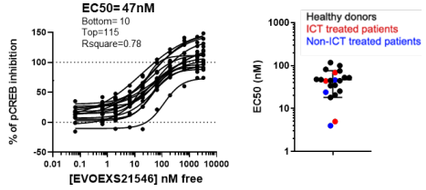
pCREB staining on CD8⁺ T-cells for one healthy donor



Mice whole blood



Human whole blood
(healthy donors and patients)



Clinical Trial (HV)

Clinical trial design in healthy adult males

Part 1:
Single ascending dose (SAD)

SAD Cohort
- Healthy adult males
- 4 dose levels
- Target engagement

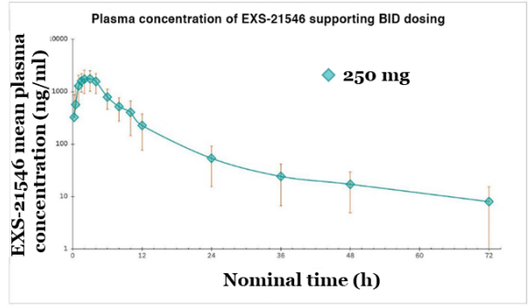
SAD dose level 1
SAD dose level 2
SAD dose level 3
SAD dose level 4

Part 2:
Multiple ascending dose (MAD)

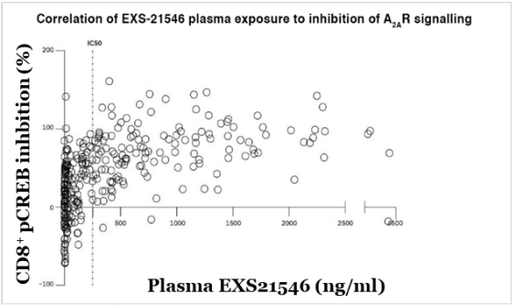
MAD Cohort
- Healthy adult males 2 dose levels for 14 days + confirmatory cohort
- Target engagement

MAD dose level X
MAD dose level Y

- Observed human PK for EXS-21546 was in line with what had been designed for and predicted in preclinical modelling
  - It supports a twice-daily (BID) dose for continuous A2A receptor inhibition over a dosing interval
- EXS-21546 showed dose-dependent inhibition of pCREB in CD8⁺ T-cells, with the PD profile mirroring plasma exposure
- Inhibition of $A_{2A}R$ signaling was sustained over the BID dosing period, demonstrating a level of lasting target engagement



Error bars= SD; n= up to 11



Data points include 90 mg, 250 mg and 400 mg cohorts

Outcome

- Pharmacodynamic biomarker was confirmed in Healthy Volunteers subject
- Exscientia initiated a Phase 1/2 study with high adenosine signature cancers in 2022 (NCT05920408)

EVT801 pursue enrollment for its phase I first-in-human study

Clinical operation & biomarkers evaluation performed at Evotec

Background

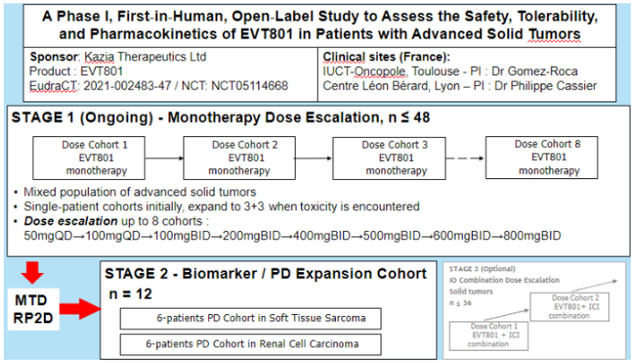
EVT801 is a novel selective VEGFR-3 inhibitor targeting tumor angiogenesis designed for single agent activity or in combination with standard of care (e.g. immune checkpoint therapies).

Rescue ambitious projects from big pharma

- **Re-evaluation of the drug**
  - PK/PD
  - Deciphering mode-of action
  - Clinical trial design with KOL consultancies
  - IB/IMPD dossier & IND
  - Support from clinical operation department within Evotec
  - Clinical trial phase 1 with French hospitals
- **Development of a Biomarkers plan**
  - IHC/IF
  - mRNA signature of tumour tissue
  - Immunomonitoring and bulk mRNA on blood cells
  - Proteins signature in plasma
  - Control of hypertension

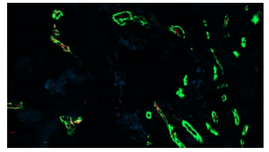
EVT801 in phase I clinical Trial KZA-081-101

Clinical trial design

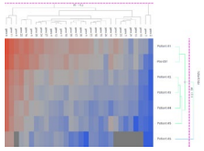


Biomarkers evaluation on patient samples enrolled in the CT

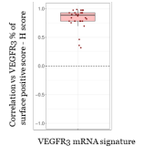
Histology



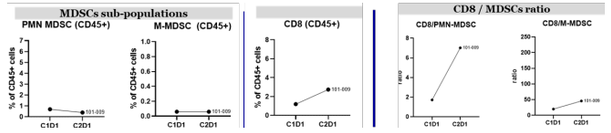
mRNA signature



Correlations



Immunomonitoring C1D1 vs C2D1



Outcome

- **Clinical trial**
  - Approvals from regulatory bodies obtained 09/2021 ((NCT05114668)
  - First-Patient-In in 10/2021 in 2 clinical sites in France (Toulouse and Lyon)
  - It is currently being evaluated as a single agent in a Phase I trial
- **Biomarkers**
  - To help to select the most responsive patients
  - To provide early indications of clinical efficacy as a monotherapy or in combination with standard of care (e.g. immune checkpoint therapies)

Multiplex labelling by immunohistochemistry

Development on FFPE tissues coming from patients

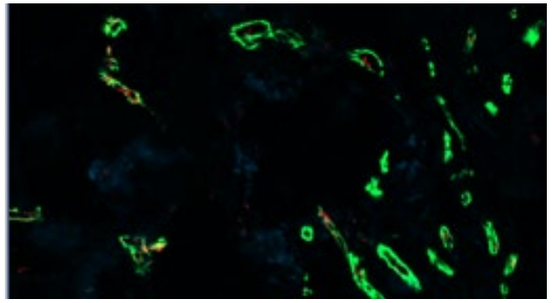
Background

- In order to perform patient characterization for a drug under clinical trial it was required to:
  - Perform the target expression in the tumor microenvironment
  - Correlate target expression on endothelial cells and with the level of intratumoral $CD8^{pos}$ T-cells

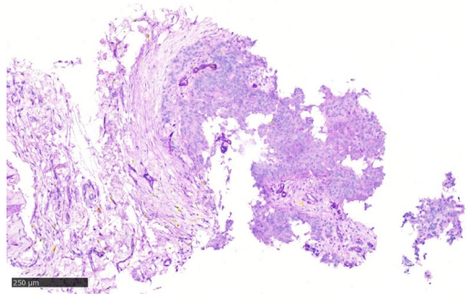
Materiel and methods

- FFPE staining of Human healthy and malignant tissues
- Brightfield and fluorescent multiplexing of target expression with endothelial marker (CD31), with $CD8^{pos}$ T cells (CD8)
- Image quantification
  - H-score by a clinical histopathologist
  - Absolute quantification using Visiopharm artificial intelligence

CD31/target



CD8/target



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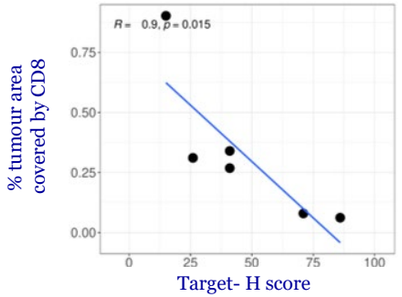


Outcome

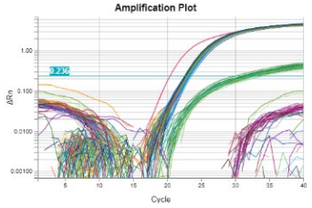
- Characterization of the tumor compartment expressing the target
- High level of target expression in tumor blood vessels is strongly correlated with a low CD8 infiltration in the tumor

mRNA signature & histology set-up on patient samples

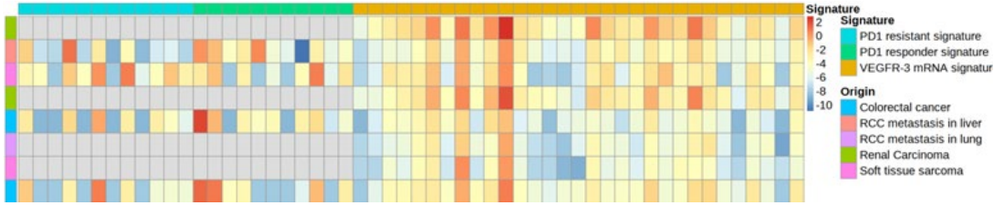
Pipeline is validated to support the biomarker identification during the in-life phase

- soft tissue sarcoma x3
- colorectal cancer x2
- renal cell carcinoma x3
- renal cell carcinoma metastase in liver
- renal cell carcinoma metastasis in the lung

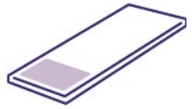
mRNA signature using Fluigdim platform



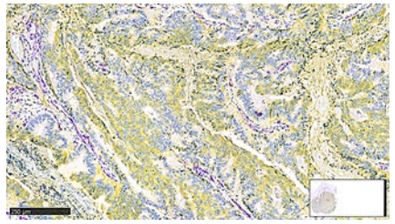
RNA extraction from FFPE blocks is working well and allows the quantification of both VEGFR3 and PD-L1 signatures



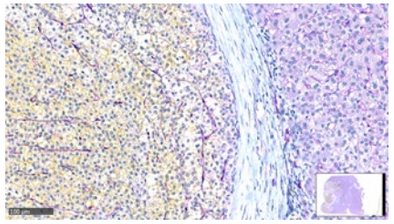
Histology



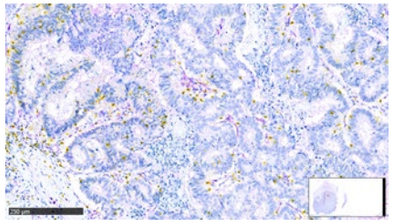
CAIXyellow-VEGFR3purple

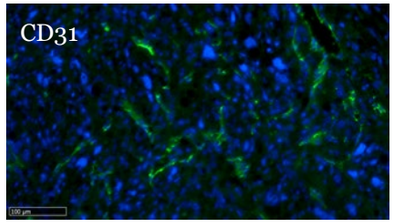


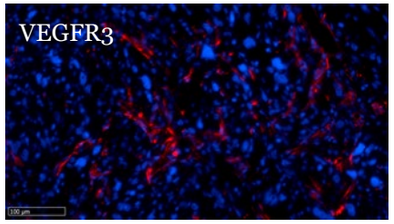
VEGFR3purple- PD-L1 yellow

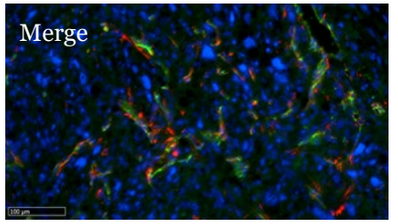


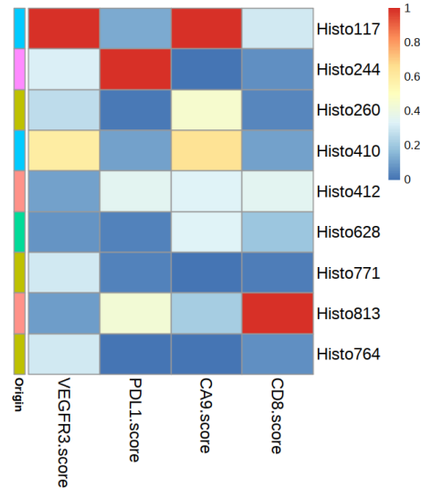
VEGFR3purple- CD8 yellow











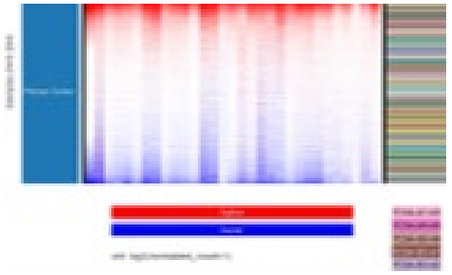
IHC: 9 patients, 4 scores, normalized by maximum (score between 0 & 1)
Gene signature: Minus deltaCq value, 1 patient missing (total 8 patients)

Patient characterization from FFPE archival tissues

Established link between clusters of genes and tumor micro-environment

Data mining

- Describe predictive signature to treatment
- Target co-regulated genes
- Validation of the signature using Fluidigm
- Transfer to Nanostring technology during clinical trials

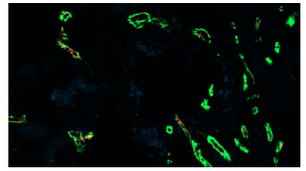


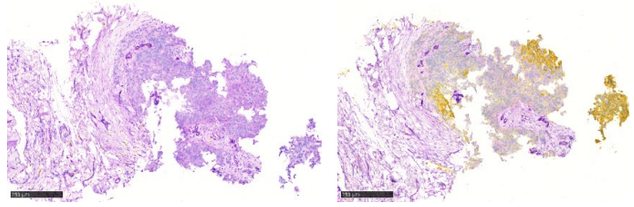
Identification of gene signature from the literature of from publicly available dataset

FFPE Archival tissues to provide mRNA and protein expression data

Histology labeling

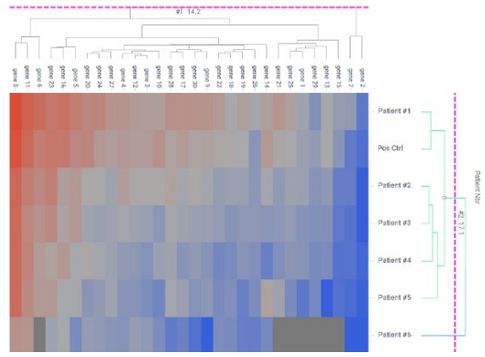
- tumor architecture to identifier the regions to collect RNA
- Labelling of tumor micro-environment:
  - Immune infiltration
  - Vascularisation



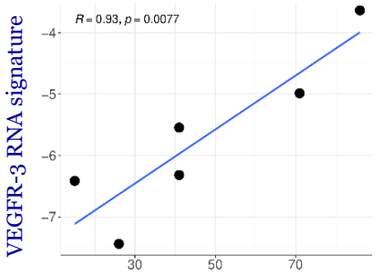


RNA signature from limited quantities of material including needle biopsies

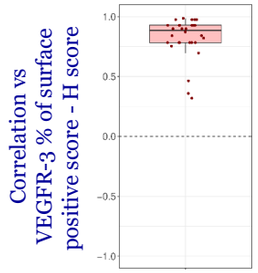
- RNA signature developed by Evotec bio-informatic department



Correlation of RNA signature and VEGFR-3 expression (p=0.0077)

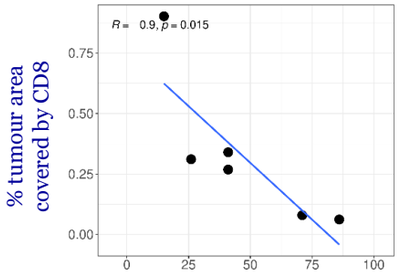


VEGFR-3 IHC % of surface positive score - H score

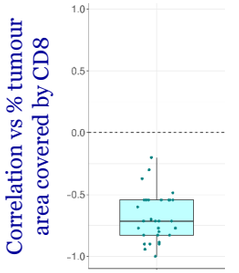


VEGFR-3 RNA signature

Establishment of an anti-correlation between VEGFR-3 expression and immune infiltration



VEGFR-3 % of surface positive score - H score



VEGFR-3 RNA signature

Patient characterization by IHC

Consultancy with clinical histopathologist from Toulouse cancer hospital

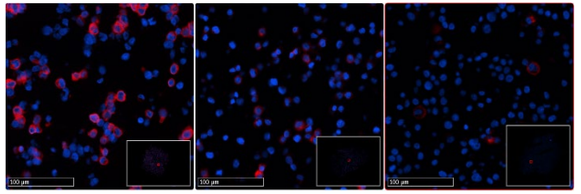
Background

- Identify heathy tissues in human exhibiting high target expression to highlight organs where safety issues
- Identify the patients that should benefit from the treatments:
  - Identification of indications exhibiting high level of target expression
  - Correlation of target expression with clinical or NGS data to fully characterize the pool of patients to target during clinical trial

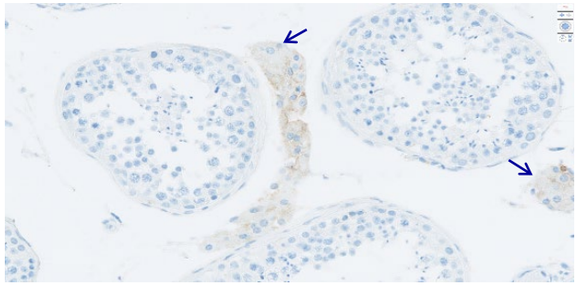
Materiel and methods

- FFPE staining of Human healthy and malignant tissues
- H-score by clinician pathologist from the Toulouse cancer hospital (H-score)
- Correlation with associated clinical and NGS data

Technical validation

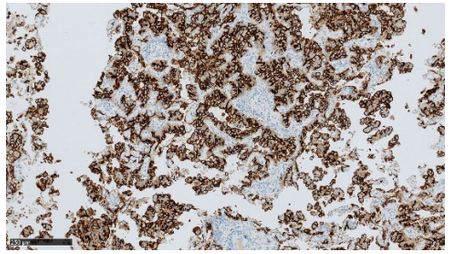


Tissue cross-reaction

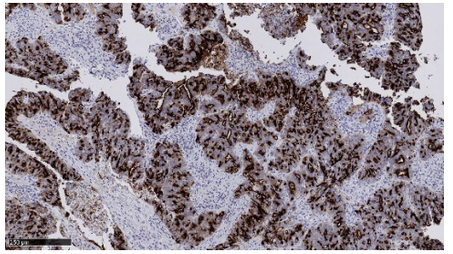


Clinician expertise, scoring

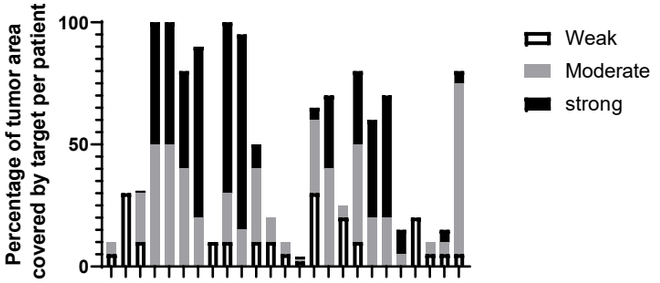
Indication 1 (n=50)



Indication 2 (n=50)



H-score component per patient for one indication (25 patients)



Outcome

- List of tissues where safety issues has been raised requiring specific attention during pre clinical and clinical phases
- Patient stratification based on target expression and associated clinical data has been validated in 4 indications

Target engagement assay by IHC

Development on FFPE tissues coming from mice

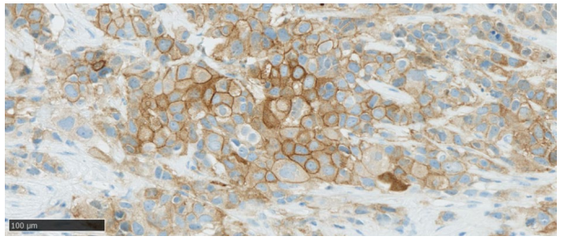
Background

- Impact of degraders drug on the target expressed in tumors cells were required to develop a target engagement assay
- Immunohistochemistry was selected to evaluate the impact of the degraders drug on its target

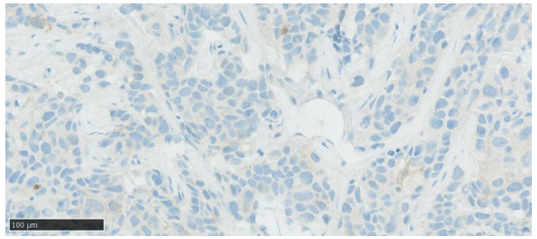
Materiel and methods

- Target engagement assay using Ventana autostainers
- FFPE staining of murine xenograft models
- Target labelling
- Evaluation after 3 hours of treatment

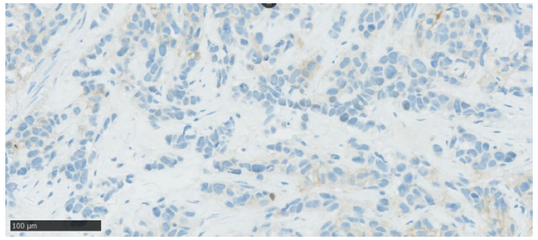
Vehicle 3 hours post treatment



Degrader 3 hours post Treatment (30 mpk)



Degrader 3 hours post Treatment (5 mpk)



Outcome

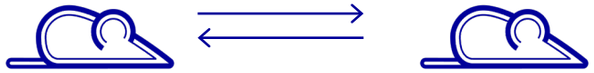
- At 5mpk and at 30 mpk; 3 hours of treatment is sufficient to observe a complete loss of target expression in xenograft model in 100% of the tumor
- This model validate Mode-of-Action of the degraders drug and can be used as target engagement assay

Use Case: Predictive Biomarker Development

Preclinical biomarker identification and validation in tumour xenografts

Preclinical discovery & validation

Xenograft models



10 responders *20 Non-responders*

- Discovery of proteomic signature based on 4,000+ proteins quantified in 30 xenograft models
- Preclinical validation with PPV of 91% in new xenograft models
- MRM assay development: Correct classification of 20 out of 22 validation samples

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NEWS RELEASE

24 April 2014

'RESEARCH NEVER STOPS' info@evotec.com | www.evotec.com

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Evotec receives first milestone in Roche biomarker collaboration

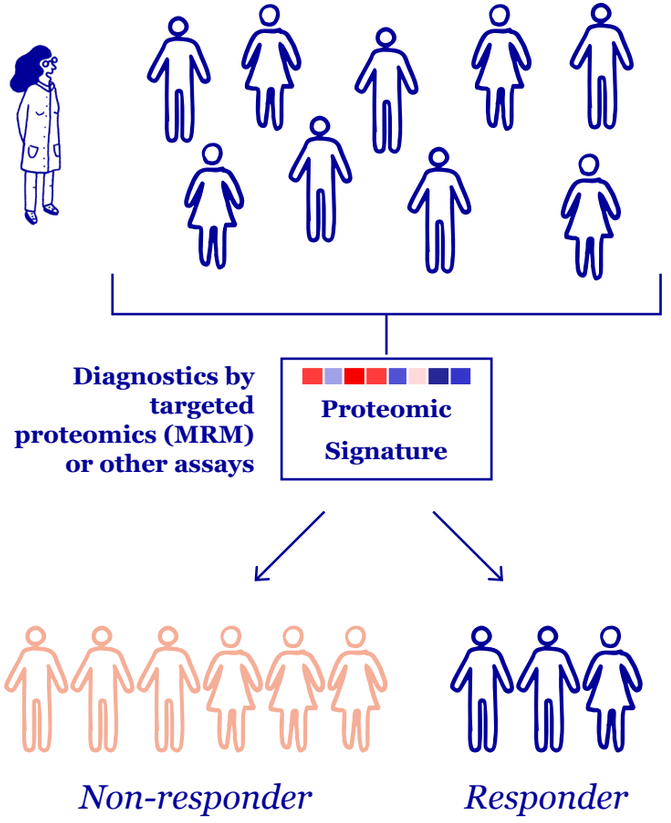
Hamburg, Germany – 24 April 2014: Evotec AG (Frankfurt Stock Exchange: EVT, TecDAX, ISIN: DE0005664809) today announced the successful achievement of a milestone in its biomarker alliance with Roche. The milestone was achieved on the decision by Roche to use a response prediction marker, identified using Evotec's Proteome Profiling platform, in an extended phase I oncology trial.

This is the first minor milestone achieved under the collaboration and licence agreement between Evotec and Roche, signed in 2011, which is part of the *m⁴ Munich Biotech Cluster Personalized Medicine and Targeted Therapies* initiative funded by the German Federal Ministry of Education and Research. Under the initial three-year term, Evotec and Roche conduct biomarker discovery and validation programmes for patient stratification in targeted cancer therapy. Evotec is eligible for further success-based payments upon clinical companion diagnostics development.

Proteomic signature

Protein A Protein B Protein C Protein D Protein E

Clinical validation phase



TE Biomarker Discovery and Development

Proteomics Platforms to Support Project Progress through Preclinical and Clinical Development

Aim: A new monoclonal antibody (mAb) targeting a protein of interest was developed by Bayer as a treatment for Alport syndrome, a rare genetic kidney disease. To support the preclinical and clinical development of this therapeutic antibody, **a new target engagement (TE) biomarker was identified and validated by Evotec.**

Biomarker Discovery

- **ScreenPep™:** Large-scale shotgun quantitative proteomics to profile global proteome from biofluids (plasma, urines) of murine models of kidney disease
- High-end data independent acquisition mass spectrometry for the detection of up to 3,000 proteins from serum/urine
- In-depth data analysis identified **one plasma biomarker**, that correlated with antibody treatment in several models and across species.

Quantitative LC/MS method *Preclinical Studies*

- Set-up and validation of a targeted MS assay applied for a **TE biomarker absolute quantification** in ~1,500 plasma samples
- In plasma (human, rat, monkey, mouse), we showed that **the biomarker increased with antibody dose** and after antibody treatment in healthy mice and could be used as a **TE biomarker in healthy volunteers**
- This LC/MS method was applied to **predict human dose** from PK/PD and TK/PD studies

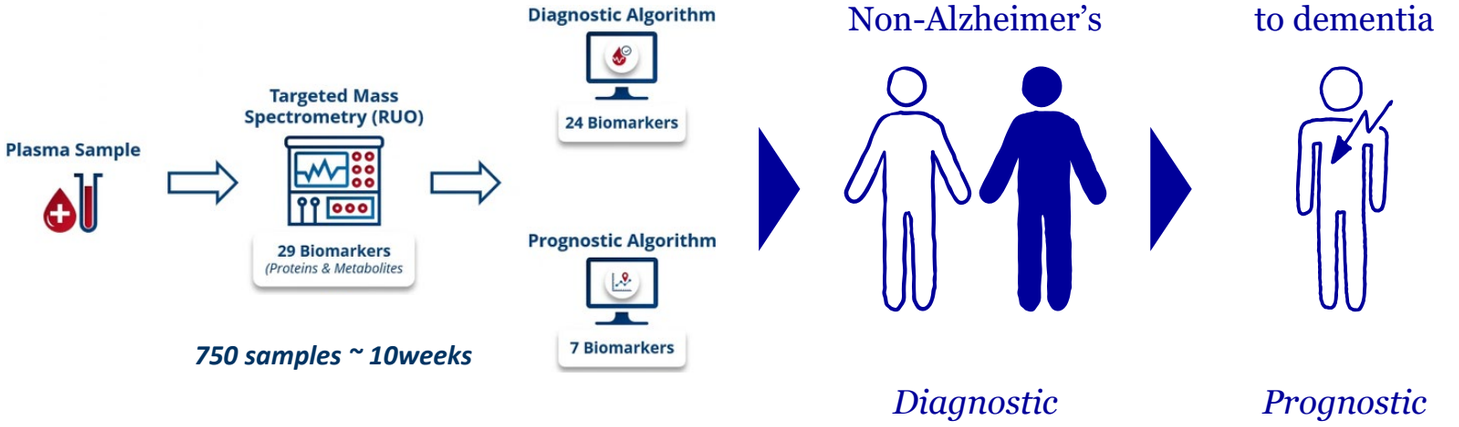
GCP LC/MS method validation *Clinical Studies*

- Method transfer to our GCP compliant environment in Verona
- Heavy labelled recombinant protein produced at Evotec to be compliant with FDA guidelines
- **FDA-compliant method validation** and fit to measure clinical samples (in-cluding primary and secondary endpoints)
- The method is currently used to **support clinical Phase 1 clinical trials**

Protein Biomarkers Quantification in Human Plasma Samples

Background, Goal, Challenges

- Aim is to develop a diagnostic test for **early-stage detection of Alzheimer** (up to 20 years before the onset of irreversible symptoms) with the aim of finding an effective treatment for the disease.
- **100 biomarkers** of Alzheimer disease have been previously identified in blood of animal models using global mass spectrometry
  - 50 proteins
  - 50 metabolites



To quantify 100 biomarkers in **750 human plasma samples**

Final selection of 29 biomarkers (metabo/proteo) based on **artificial intelligence**

optional

Algorithm refinement: quantification of the selected biomarkers in a second cohort.

Validation of quantitative methods under «**G**ood **C**linical **P**ractice» on 250 human plasma samples

Commercial diagnostic test for early-stage Alzheimer detection

Digital measure of H-Score in tumoral and non-tumoral tissues

Image analysis for histology

Background

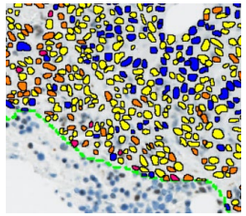
- H-score allows to determine effects of treatments on tumoral and non-tumoral tissues
- H-Score is calculated based on nuclear staining in the tumor area
- H-Score can be developed for any kind of cancer or tumors, in PDX, humanized tumors, human biopsies slides, or cell lines

Materiel and methods

A digital measure of the H-Score for each tumor compartment has been established: epithelial, non-tumoral or necrotic area (excluded to avoid artefacts).

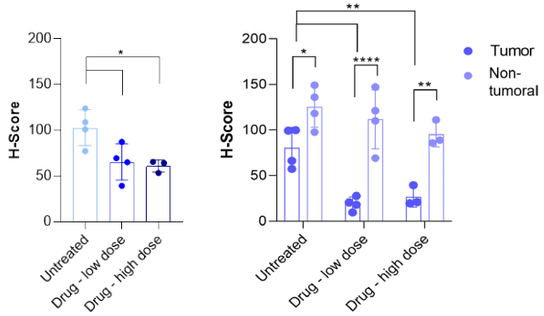
Software workflow

1) Delimitation of each area
2) Measure the cells percentage of each intensity in each area:
   - Negatives
   - Weak signal (+1)
   - Moderate signal (+2)
   - Strong signal (+3)



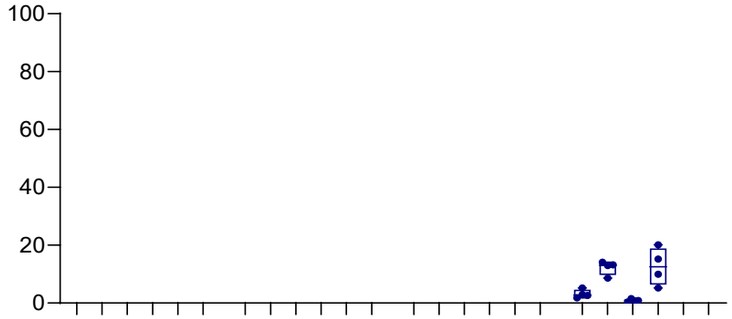
H-Score in whole tumor, tumoral and non-tumoral tissues

2



Percentage of each cell type in H-Score

1



Measurement of H-Score digitally in whole tumour, but also in tumoral and non-tumoral tissues independently to better appreciate target expression upon treatments.

- **Digital analysis of H-Score allows to measure the immunoreactivity of tumors after treatments**
- **This method also measures the immunoreactivity of each area, cell by cell, in response to treatments**

H-score: Histological score

TICIMEL (NTC03293784): Monitoring Treatment Effects

Efficacy 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

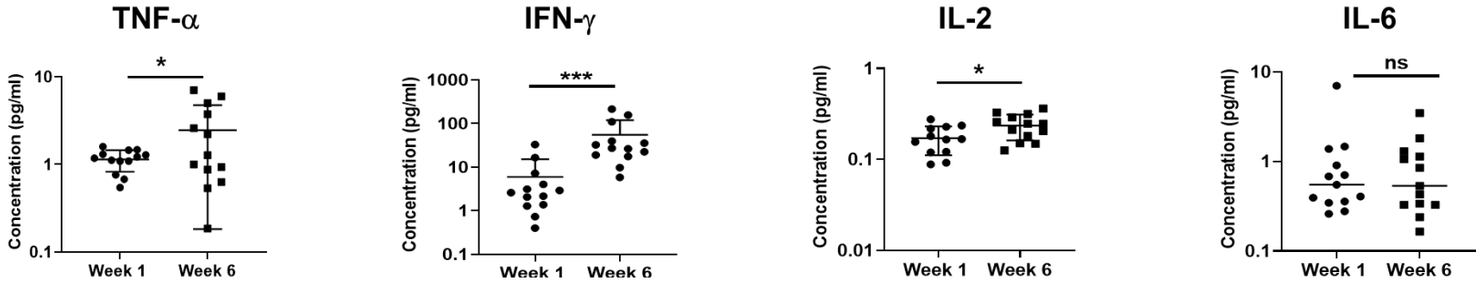
Panels of inflammatory cytokines has been evaluated in patients as an efficacy endpoint biomarkers

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

Chemokines evaluation in patients



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

Biomarkers WPs to support decision making

Biomarkers strategy to fulfill gaps and secure drug development

	Available data	Gaps	Biomarkers strategy
Indication	Indication = Melanoma post chemo or targeted therapy (ICT, MAPKi or MEKi) • CDx expressed in melanoma and most skin cells • CDx expression increased in TKI/chemotherapy resistant cells • CDx may act as co-receptor for the TrkR	• Target expressed in melanoma cells but quid of expression of co-receptors that have antagonist effects? • What about effect on TME?	• ICH assays for expression of co-receptors in melanoma (in duplex with CDx) • Target expression in Tumor Infiltrated Lymphocytes?
Target engagement assay	• Binding assay done with Sxx001 peptide • DD cleavage triggered by CDx activation	• No specific studies to define the binding pocket of Sxx001 on CDx performed yet • Binding assays difficult to translate to patients	• Assay to detect cleaved ICD domain of CDx and/or its binding to DD
PD biomarker	Caspase 3 assays and PARP cleavage activation • Phospho Jnk assay	CDx pathway activation PD marker?	Upstream signalling pathway activation: • Phosphoproteomics assays with detection of P-ERK and P-MEK • *In vitro* and *in vivo* during tox/PK studies
Safety assessment	Not disclosed	Target expressed in healthy tissues including pancreas and CNS: 1) Oxidative stress 2) neuronal toxicity to be assessed 3) insulin secretion decrease and glucose dysregulation 4) Immunogenicity	On and Off target safety biomarkers to be explored by *in vivo* toxicity studies with attention to: • ROS levels (ex: lipid peroxidation) • Brain damages • Glucose homeostasis (insuline and glucose levels) • ADA generation
Predictive biomarker – Patient selection	Target^{+} tumor cells for patient selection?	• Anti-CDx Ab available for patient selection? • CDx shedding: Ability of circulating extracellular Domain (ECD) of CDx to trap Sxx001	Possibility to quantify circulating extracellular domain of CDx? • PDX models CDxneg or pos to confirm CDx as predictive BM of drug efficacy • Clinical translation: Melanoma explants CDx neg or pos treated with various levels of Sxx001
Predictive biomarker – Efficacy	• Viability test on Mock or target overexpressing cell lines	• *In vivo* efficacy?	• PDX models target $^{-}$ or $^{+}$ to confirm target as predictive BM of drug efficacy • Melanoma explants?

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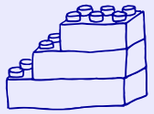
The Clinical Development & Translational Medicines at Evotec

Supporting all therapeutic areas

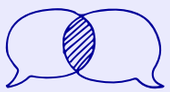
Clinical Development and Translational Medicines (CDTM) **supports Evotec's clients in the design, management, and execution of Phase 1 and early Phase 2 studies.**

CDTM is a fully integrated team comprising of Clinical Operations (incl. Medical Writing), Translational Medicine, Pharmacometrics, and Regulatory Affairs.



CDTM works closely with all other relevant functions, such as Bioanalytical Analysis, Translational Biomarkers, Quality Assurance, Legal, Clinical Supply to ensure that the right support is provided for each project at the right time. This includes, where needed, to use specific validated Vendors.

Examples of studies supported: FIH study execution at CRC Verona (2020-2021), oncology Ph1 study in France (ongoing), NIH sponsored study in US for a mAb in CHIKV infection (ongoing), submitted a CTA in Germany for a mAb FIH, and a cell-therapy FIH oncology study has been approved by AIFA.



For Oncology clinical studies in France leverage from the government support and allow a facilitated study initiation and access to patients. **EVT Clinical Operations has established a relationship with Oncopole Toulouse and has recorded experience of Phase 1 FIH study in oncology patients.**

FIH: First-in-Human

Clinical Development & Translational Medicines

Client's Sponsorship model

Sponsor
Client

IP
Client

Engage, contract & transfer responsibilities to Evotec

Evotec expertises

- **Clinical trial management – Clinical Monitoring oncology; Medical writing**
- **Medical monitoring, Clinical Dev. & Translational support**
- **Pharmacometrics (Data management, eCRF, Statistics)**
- **Bioanalysis (PK and PD samples)**
- **Regulatory affairs**
- **Quality**
- **IMP Supply**
- **Biomarker (exploratory and GCP)**

Third Parties

- **Pharmacovigilance (Arriello)**
- **Clinical monitoring (CRA) if needed**
- **eTMF provider (if necessary)**

Clinical Sites coordination