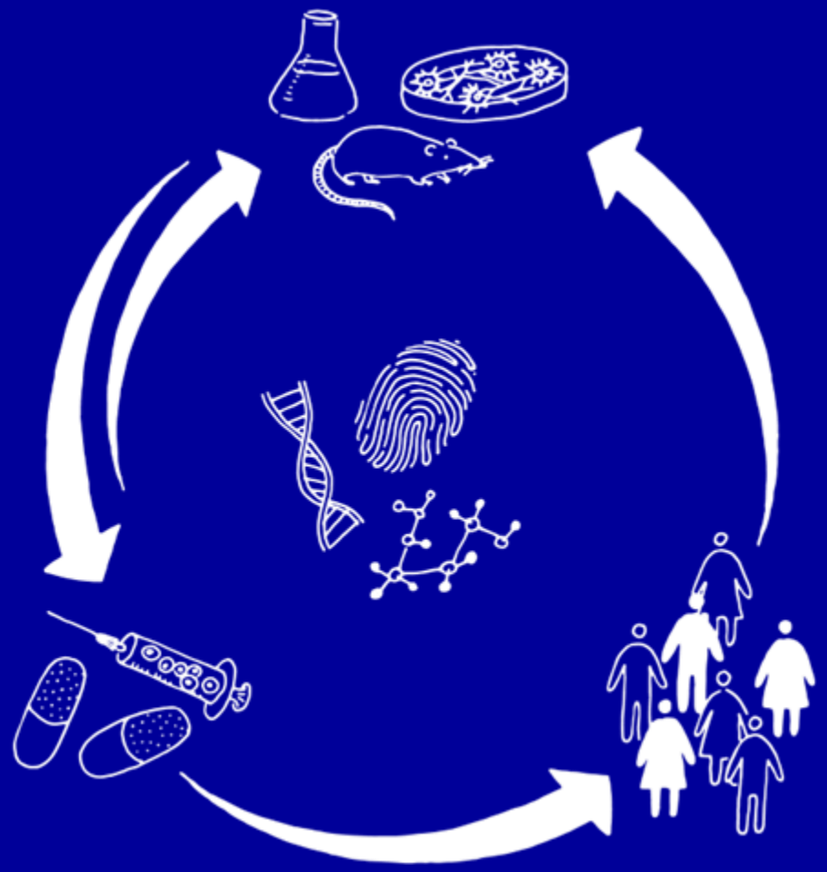
evotec

#RESEARCHNEVERSTOPS

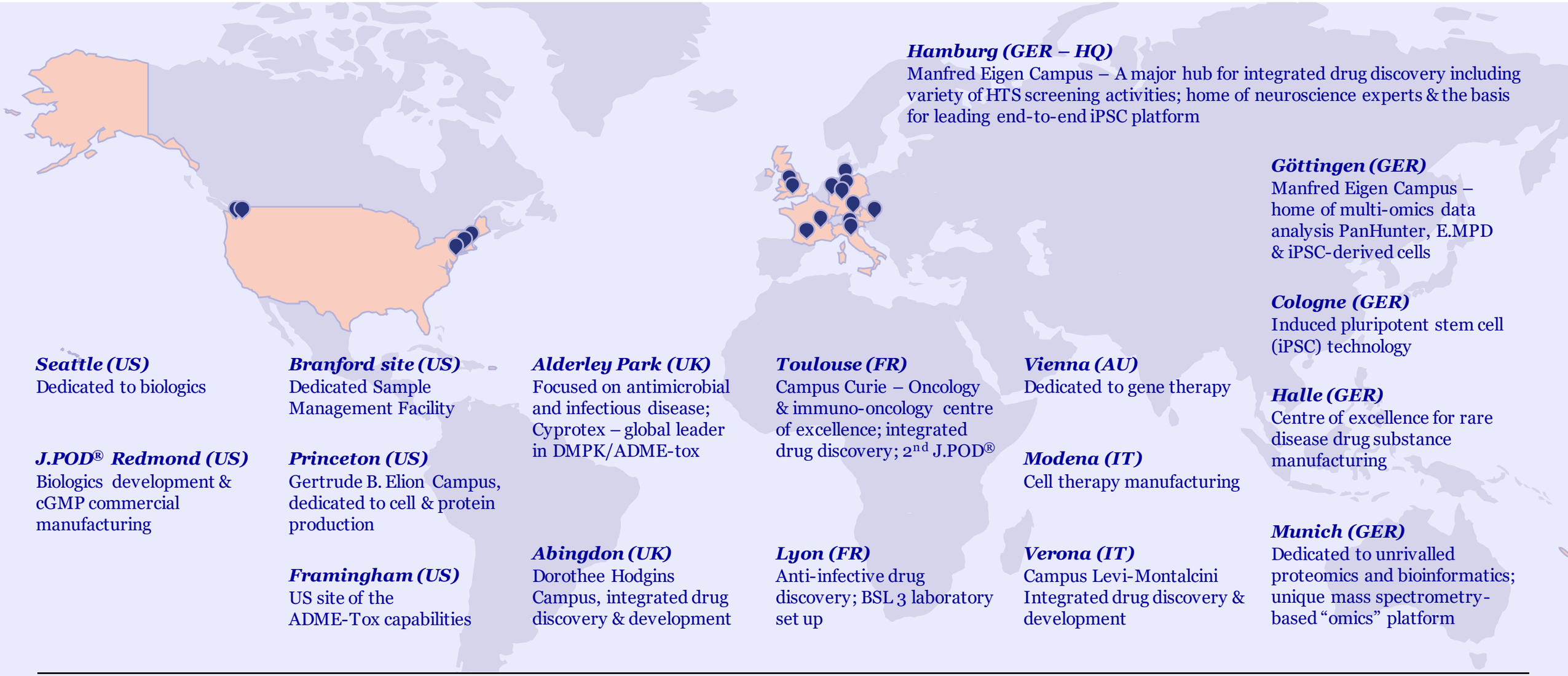
Immuno-Oncology Therapeutic Area

The Cancer Immunotherapy revolution has just begun



Centres of excellence to discover, develop & manufacture

Together for medicines that matter with ~5,000 people at 17 sites



Hamburg (GER – HQ)
Manfred Eigen Campus – A major hub for integrated drug discovery including variety of HTS screening activities; home of neuroscience experts & the basis for leading end-to-end iPSC platform

Göttingen (GER)
Manfred Eigen Campus – home of multi-omics data analysis PanHunter, E.MPD & iPSC-derived cells

Cologne (GER)
Induced pluripotent stem cell (iPSC) technology

Halle (GER)
Centre of excellence for rare disease drug substance manufacturing

Munich (GER)
Dedicated to unrivalled proteomics and bioinformatics; unique mass spectrometry-based "omics" platform

Seattle (US)
Dedicated to biologics

J.POD® Redmond (US)
Biologics development & cGMP commercial manufacturing

Branford site (US)
Dedicated Sample Management Facility

Princeton (US)
Gertrude B. Elion Campus, dedicated to cell & protein production

Framingham (US)
US site of the ADME-Tox capabilities

Alderley Park (UK)
Focused on antimicrobial and infectious disease; Cyprotex – global leader in DMPK/ADME-tox

Abingdon (UK)
Dorothee Hodgins Campus, integrated drug discovery & development

Toulouse (FR)
Campus Curie – Oncology & immuno-oncology centre of excellence; integrated drug discovery; 2nd J.POD®

Lyon (FR)
Anti-infective drug discovery; BSL 3 laboratory set up

Vienna (AU)
Dedicated to gene therapy

Modena (IT)
Cell therapy manufacturing

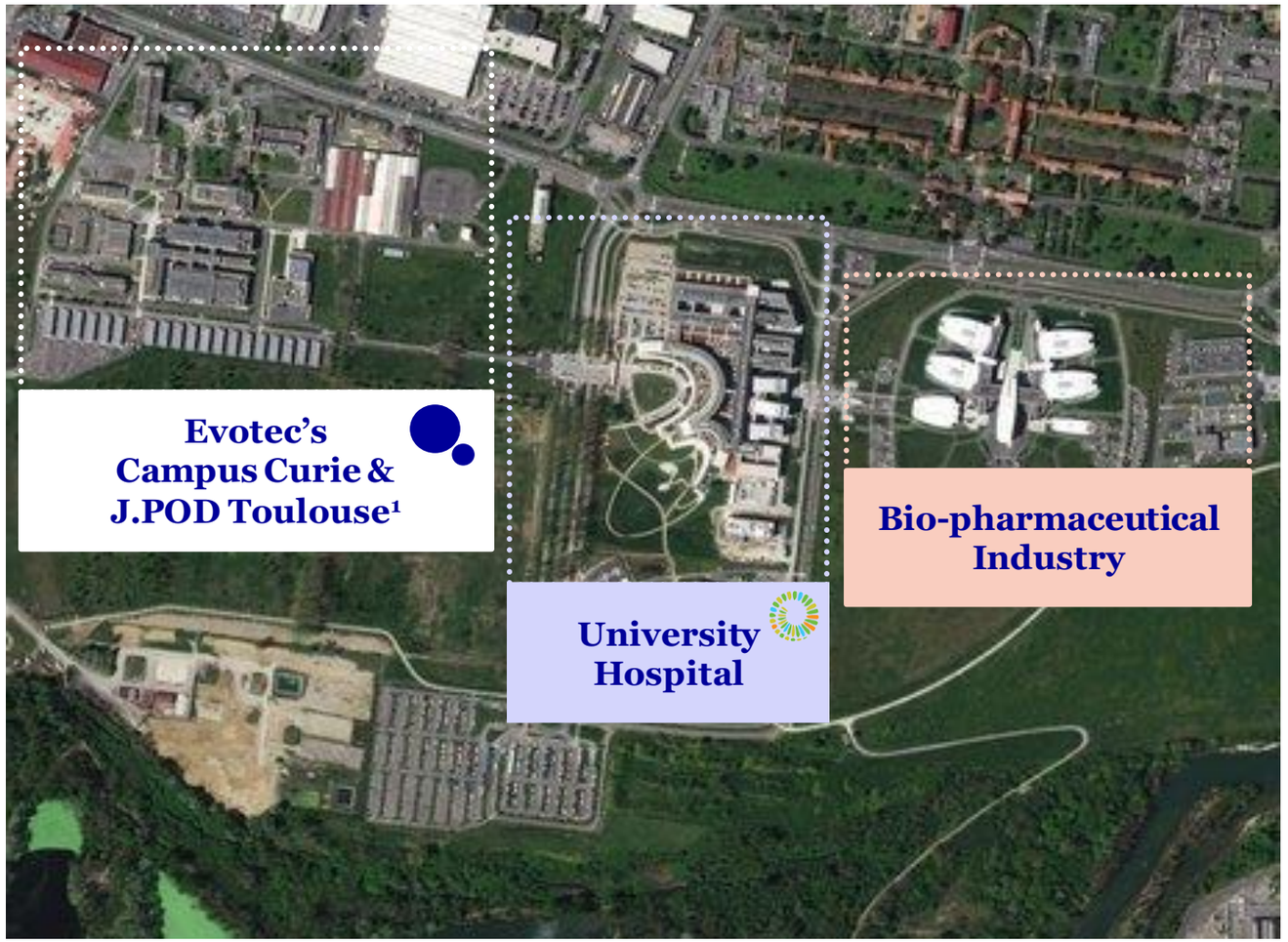
Verona (IT)
Campus Levi-Montalcini Integrated drug discovery & development

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

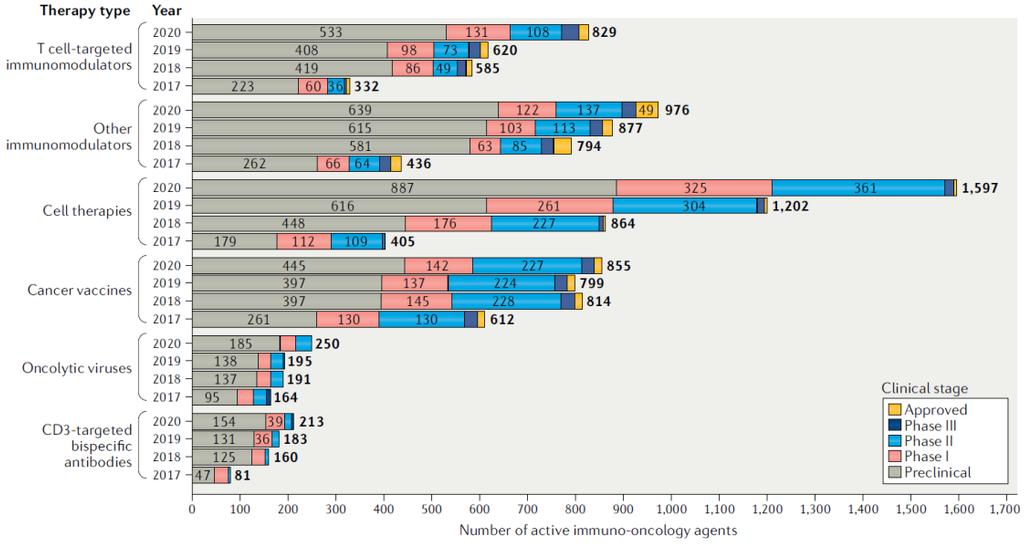
1 This project benefits from French Government funding as part of the investments for the future programme (programme d'investissements d'avenir in French) and is also supported economically by the Occitanie Region.

Introduction to Immuno-Oncology (IO) therapeutic area at Evotec

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

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

Cancer Immunotherapy is the fastest growing area within Oncology



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

Building on two key pillars for Immuno-Oncology drug discovery

Immunology understanding & versatility in therapeutic modalities

1

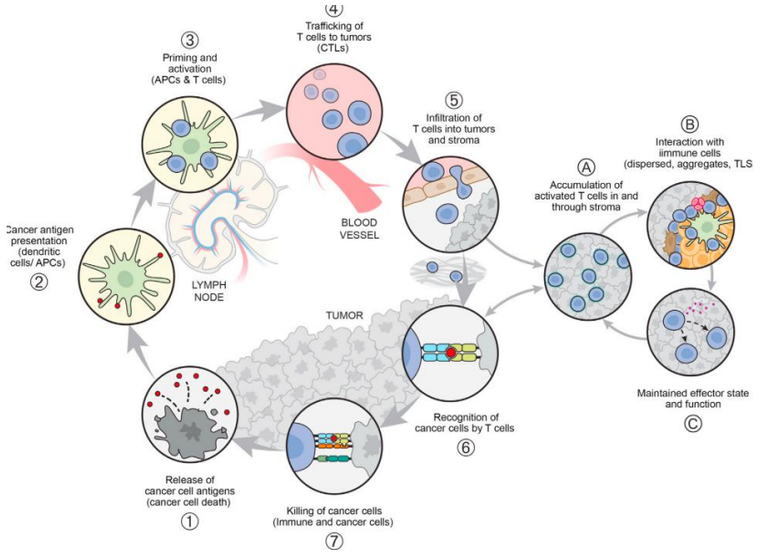
In-depth Immunology knowledge on:

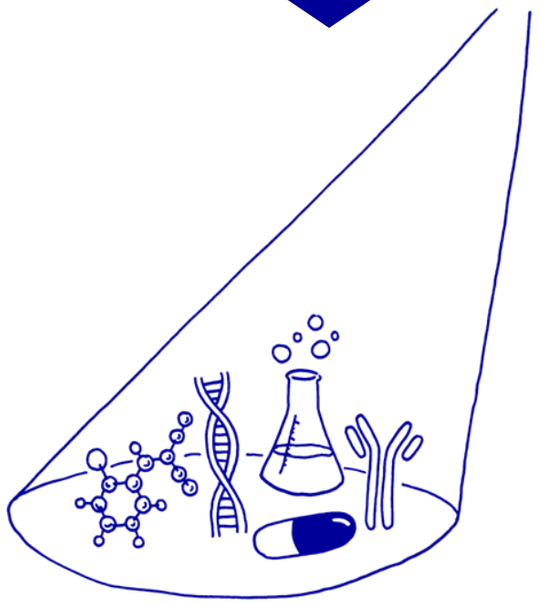
- Broad range of immune cell types
- Various targets
- On both the liquid and solid tumour space

2

Broad experience from Small Molecules to Cell Therapy:

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





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

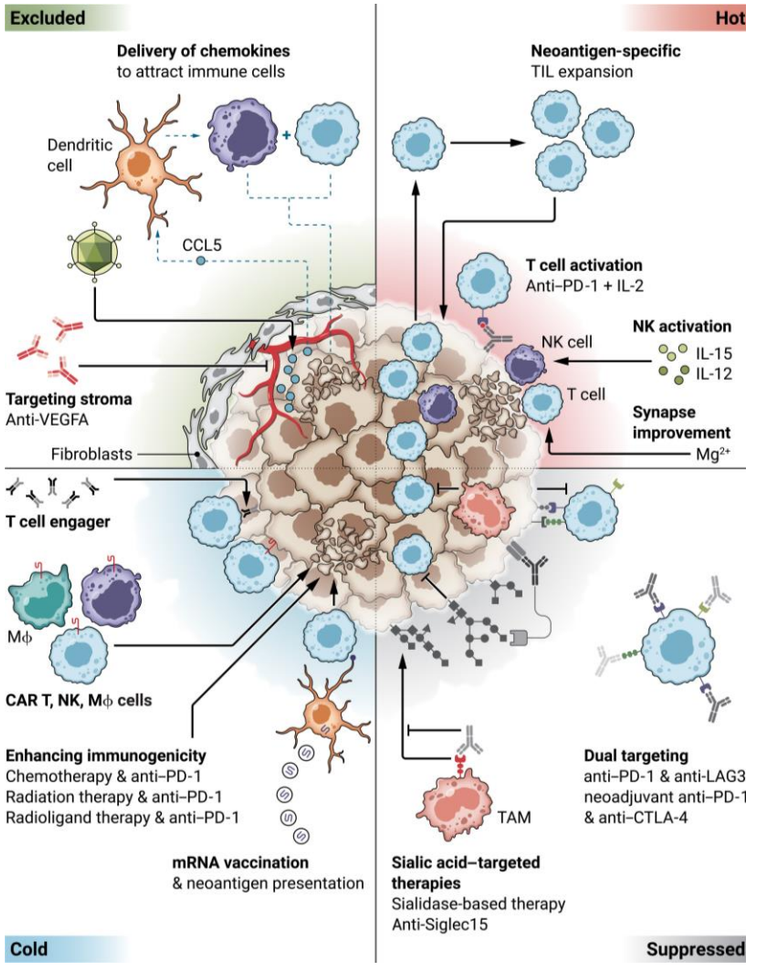
IO is a multi-modality therapeutic area: the combination thinking

Checkpoints inhibition is only the tip of the Cancer Immunotherapy iceberg

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

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

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



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

How Evotec IO Scientists are supporting Drug Discovery programs

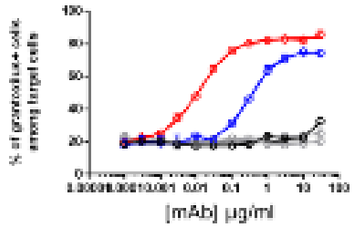
Of mice and men: a drug discovery continuum including cancer patient' samples

A broad expertise from the bench to the bedside

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

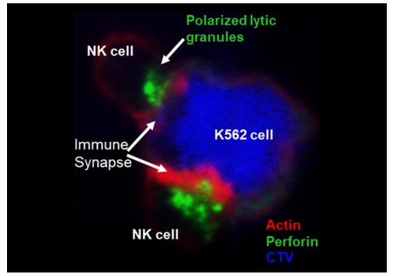
Immuno-Oncology Therapeutic Aera in a nutshell

Building together tailored approaches for successful drug discovery programs



Functional *in vitro* Immunological assays

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

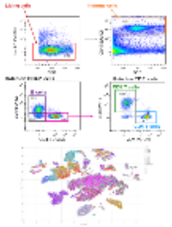


Visualising Immune cells "in action" at the contact of tumour cells

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

Preclinical *in vivo* rodent models in Immuno-Oncology

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



Filling the gap in drug discovery by accessing cancer patient samples

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

Note: TME (tumour MicroEnvironment)

Flow Cytometry platform in Campus Curie: core expertise for Immunology

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

Analyzing immune cells

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

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

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

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

Isolating immune cells

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

autoMACS Pro separator
Automated cell isolation
Magnetic cell separation

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

A dynamic team in the field of flow cytometry

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

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

Diving into Evotec Immuno-Oncology

1. **Priming anti-tumour immune response:**
   cancer vaccines, manipulating innate immunity
2. **Unleashing tumour-specific T-cell immunity:**
   checkpoints inhibition & T-cell targets
3. **Re-directing immune cells killing towards tumour cells:**
   bispecific Ab, ADCC, ADCP, cell therapy
4. **Paving the way to the clinic:**
   translational Immunological assays with cancer patient samples

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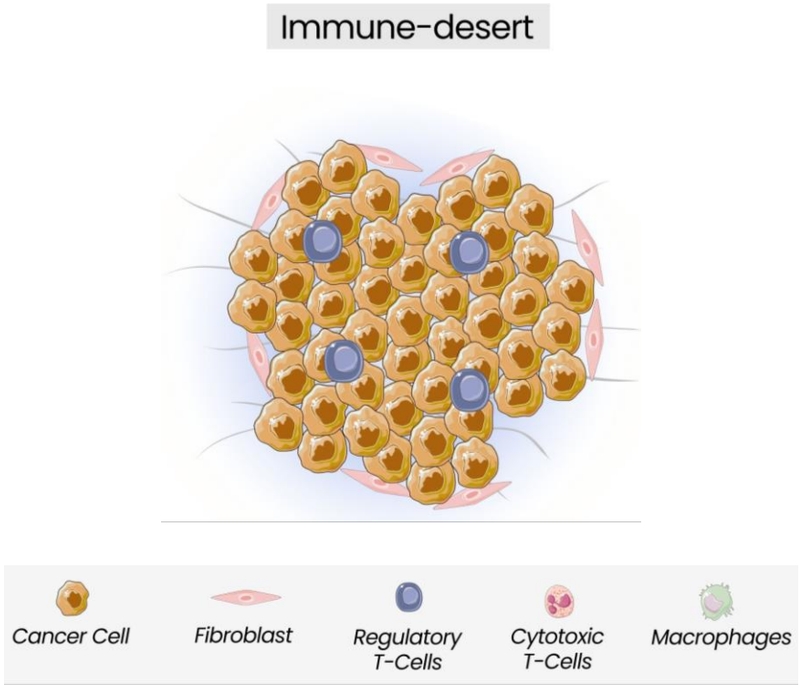
Tumours with immune-desert phenotype are not responding to ICIs

Boosting tumour immunogenicity and overcoming tolerance mechanisms

Targeting 3 pillars:

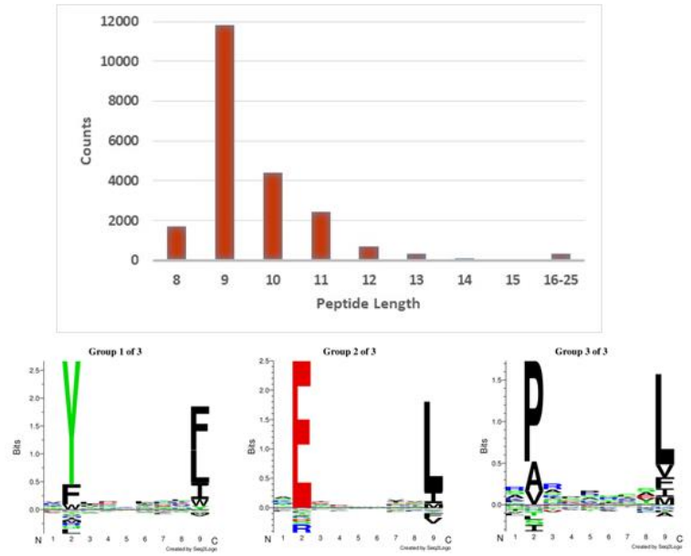
- No priming
- Tolerance
- Immunologic ignorance



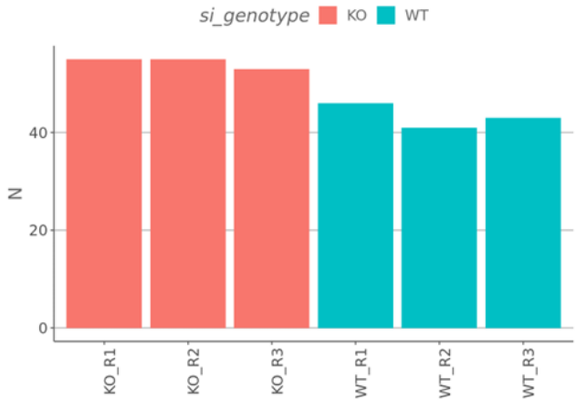
Case study: validation of target X as modulator of tumour neo-antigens presentation on tumour cells using LC-MS/MS approach

Generation of Target X-deficient tumour cell line	Affinity purification of MHC class I molecules / purification and enrichment of HLA class-I bound peptides from WT and Target X KO cells	LC-MS/MS analysis	MS war data analysis for peptide ID and quantification / Statistics and Bioinformatics

HLA class I-bound peptide counts following enrichment and GibbsClusters



Number of pSTY peptides between WT and Target X KO tumor cells



WT and KO cells were assessed as 3 independent samples each

20 pSTY peptides exclusively found in KO cells *vs* 3 pSTY peptides in WT cells

Note: adapted from Mendes AD et al. Frontiers 2022

Therapeutic cancer vaccines: the beginning of a new era

Educating and priming T-cells to eradicate malignant cells in cold & immune desert tumours

- So far, the concept of therapeutic vaccines has been pursued for decades with little success in the clinic
- **Only few cancer vaccines were approved by the FDA** (e.g. Provenge, cell-based vaccine for advanced prostate cancer, oncolytic virus vaccine for metastatic melanoma)
- **Major hurdles** to overcome are low immunogenicity and immunosuppression within the TME

MERCK moderna

OSE IMMUNO THERAPEUTICS

Genentech
A Member of the Roche Group

BIONTECH

Arising of RNA-based vaccine platforms or new personalized approaches with selected peptides epitope result in significant progress in the clinic and are leading to optimism

- **Major Phase II results** for mRNA-4157 are leading to a pivotal Phase III trial in resected melanoma with high risk of recurrence in combination with PD-1 inhibitor Keytruda[1]
- **Positive Phase III results** for Tedopi® T-cell epitope-based cancer vaccine in HLA-A2+ lung cancer patients who developed previous resistance to immunotherapies (increased OS)[2]
- Individualized Neoantigen-Specific Immunotherapy (iNeST) platform developed by Genentech in collaboration with Biontech based on patient's particular tumour mutations (neoantigens): **positive Phase I results** in patients with resected pancreatic cancer; sequential combination with atezoluzimab[3]

1 start of the Phase III in August 2023
2 results of the Phase III published in September 2023 Besse B et al.. Annals of Oncology 2023
3 presentation at ASCO 2022

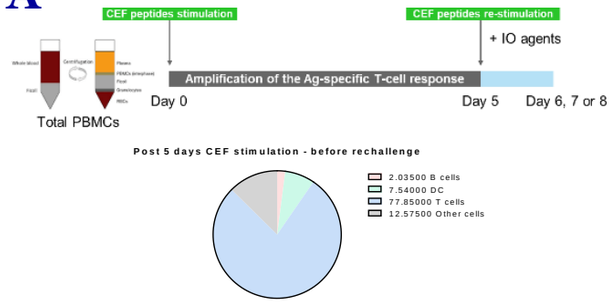
Therapeutic cancer vaccines: the beginning of a new era

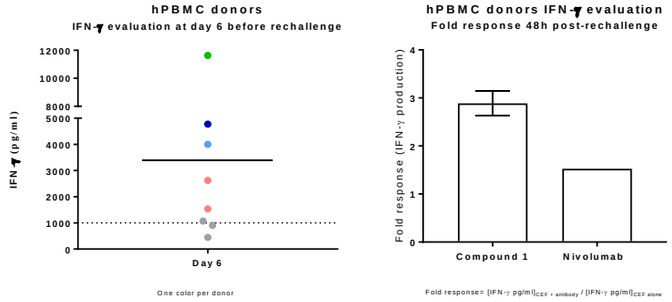
Expertise & experience in supporting the discovery of therapeutic vaccines

- **Assessing *in vitro* immunogenicity** assays using primary human immune cells (e.g. recall assay, etc.)
  - Validation of peptides Immunogenicity
  - Possibility to use blood samples from healthy donors or from patients with the selected indications
- **Assessing *in vivo* immunogenicity** with preclinical mouse models including the possibility to use humanized mice
  - Definition of the immunization scheme, route of administration
  - Definition of the best adjuvant for peptides or protein-based vaccines (licensing of DCs and APC)
- **Therapeutic efficacy** using preclinical mouse models and the selected route and scheme of vaccine administration
- **Translation to the clinic** with exploratory immunomonitoring approaches (e.g. ELISpot, flow cytometry, etc.)

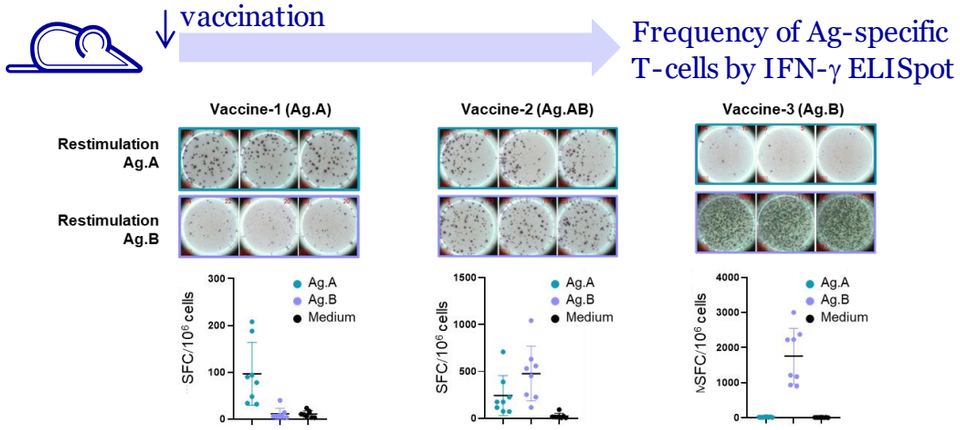
In vitro (A) and *in vivo* (B) immunization approaches are instrumental for characterizing vaccine candidates immunogenicity

A





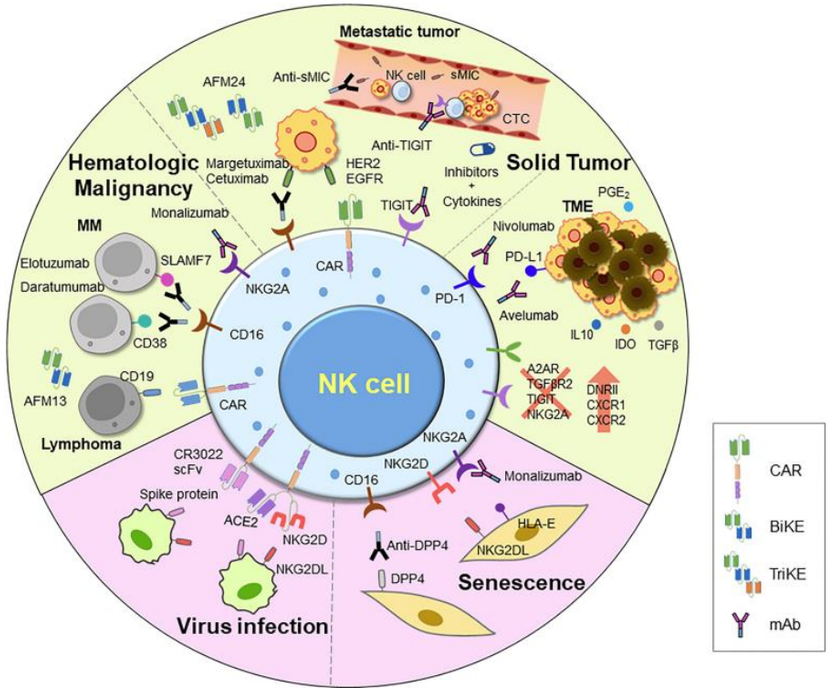
B



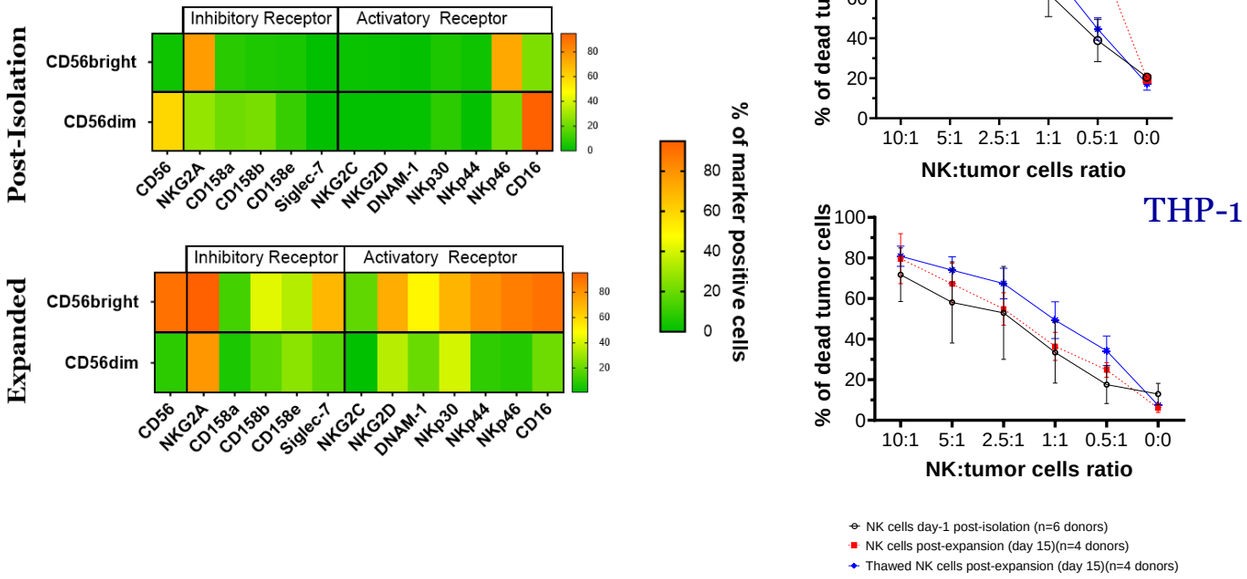
NK cells: a unique innate immune cell for cancer immunosurveillance

Diversity of NK-based cell therapies for cancer and beyond

Expanding the scope of therapeutics arsenal to harness NK cells in cancer: from biologics to cell therapy



Deciphering NK cells biology to better tailor drug discovery programs



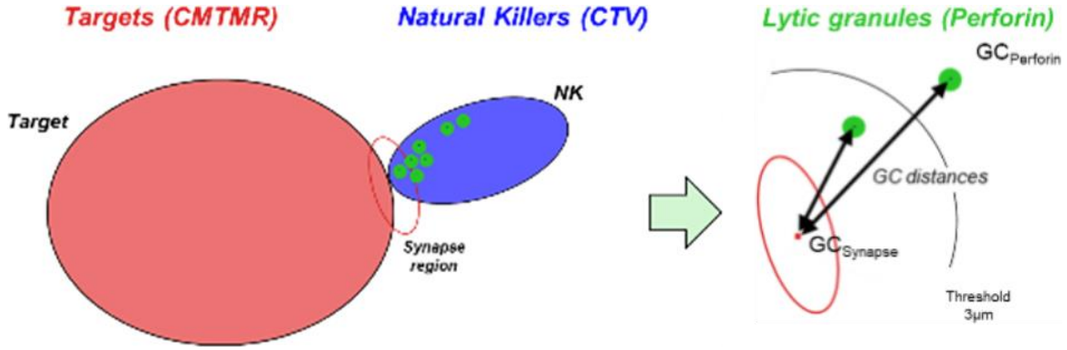
Strong experience in supporting NK-based therapeutic discovery projects from Ab to cell therapy

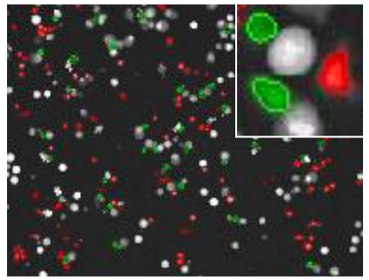
Source: Shin E *et al.* Frontiers in Immunology 2023

NK cells: a unique innate immune cell for cancer immunosurveillance

Measuring NK cells cytotoxicity at the Immunological Synapse level with high throughput imaging

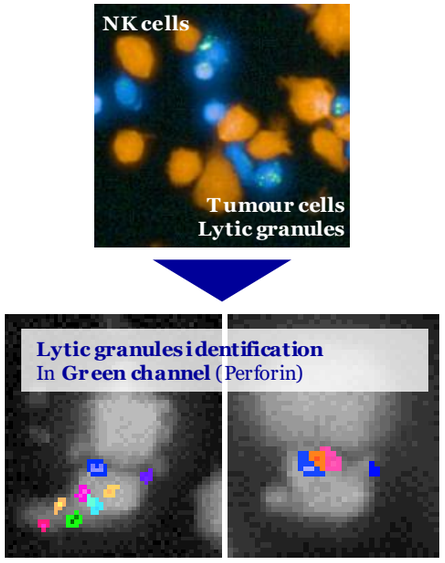
Evaluation of lytic granules (Perforin$^+$) polarization based on their mean distance to the IS using the Operetta (high-content confocal imaging system)



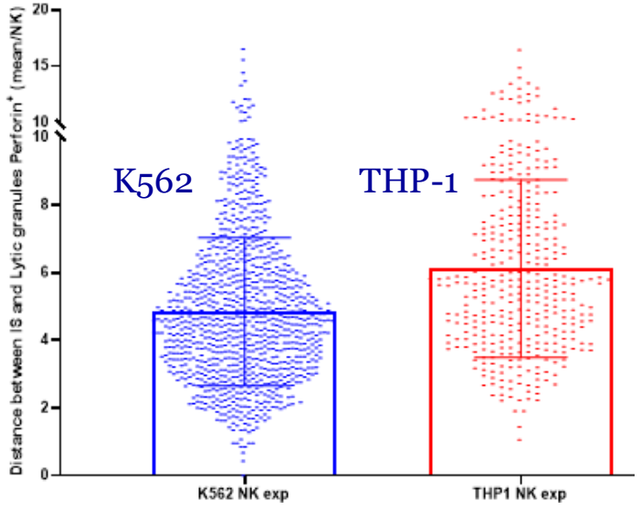


Interacting NK identification
Distance NK-target = 0
NK in interaction vs NK alone

Measuring tumour cell resistance to NK-mediated killing analyzing on perforin polarization to IS



Distance (µm) between IS and lytic granules (mean/NK cell)



Target cell killing efficiency:
% of apoptotic cells after 4h of co-culture
K562: 50%; THP-1: 10%

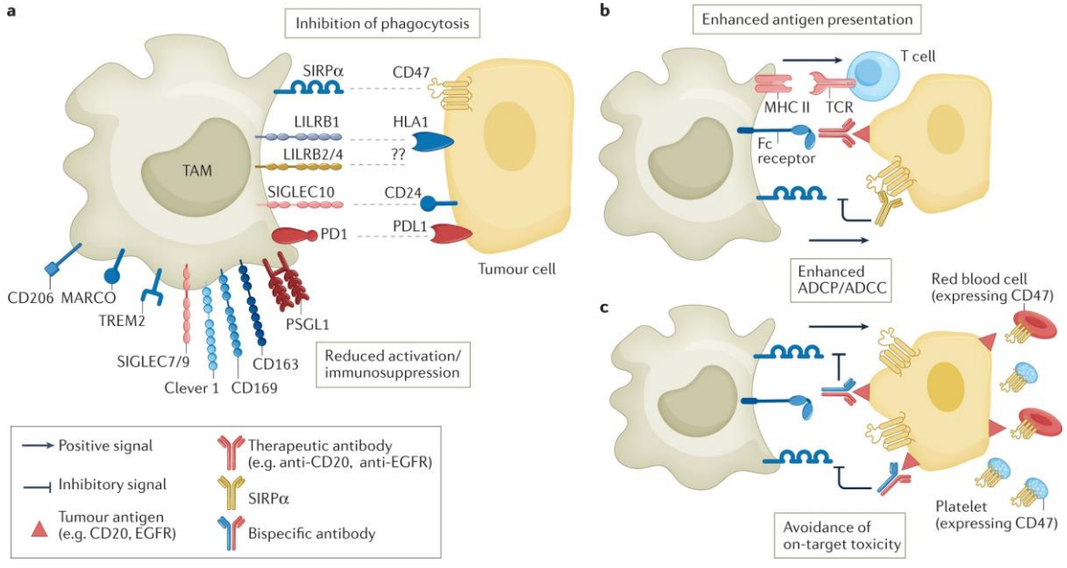
Possibility to rank lead ICEs or lead Ab candidates at the single NK cell level

Source: Shin E *et al.* Frontiers in Immunology 2023

Plasticity of macrophages in cancer: innate immune cells to target

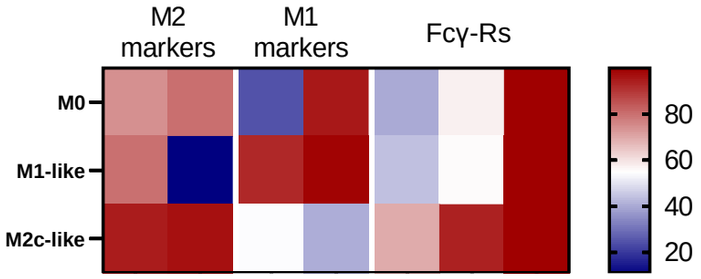
Exploring the potency of therapeutic mAbs in triggering tumour cells phagocytosis

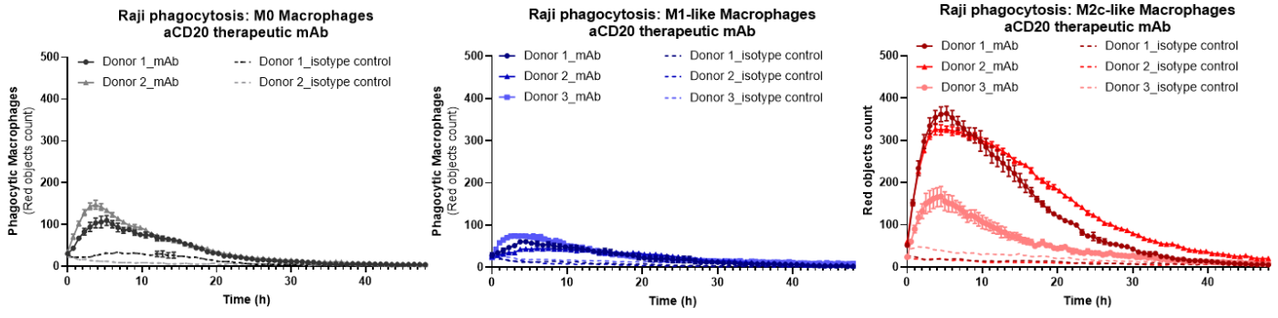
Overview of myeloid checkpoints and inhibitory receptors expressed by tumour-associated macrophages (TAMs)



Incucyte-based ADCP Assay Principle

- Polarized macrophages cocultured with tumour target cells +/- anti CD20 mAb
- Kinetic traces plots represent the number of phagocytic macrophage per condition; format is 384 well plate





A large of functional assays have been set up to better understand MoA underlying macrophages targeted therapies

Source: Mantovani A *et al. Nature Reviews Drug Discovery* 2022

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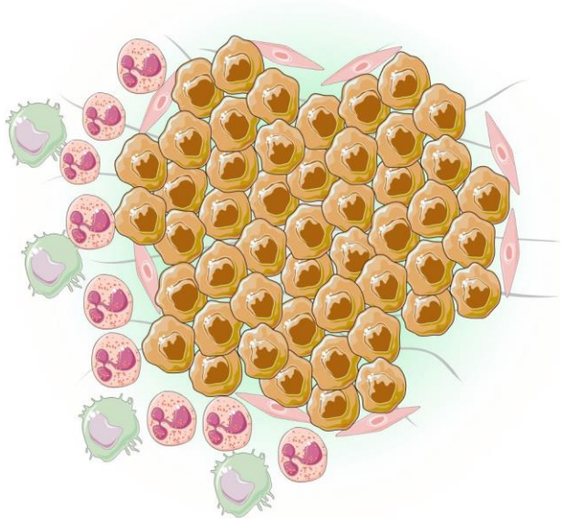
Tumours with immune-excluded phenotype are not responding to ICIs

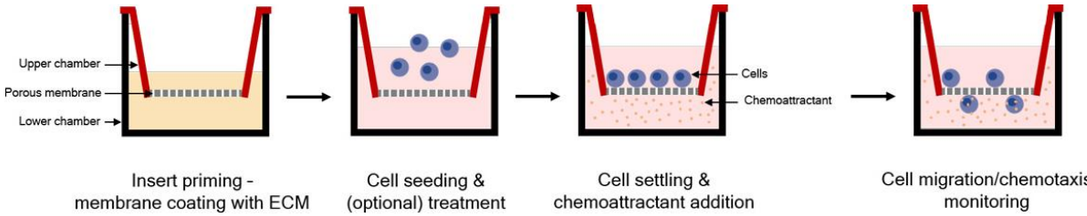
Development of drugs breaking the barriers and/or optimizing T-cell migration

Targeting 3 pillars:

- Angiogenesis
- Extracellular matrix
- Chemokines



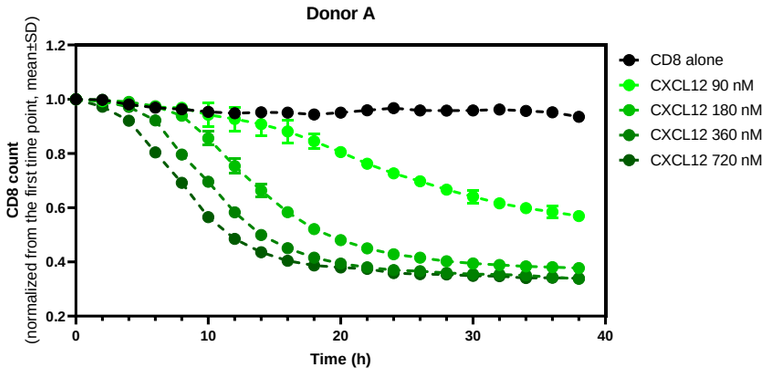
Human primary CD8⁺ T-cell chemotaxis assay with real time imaging

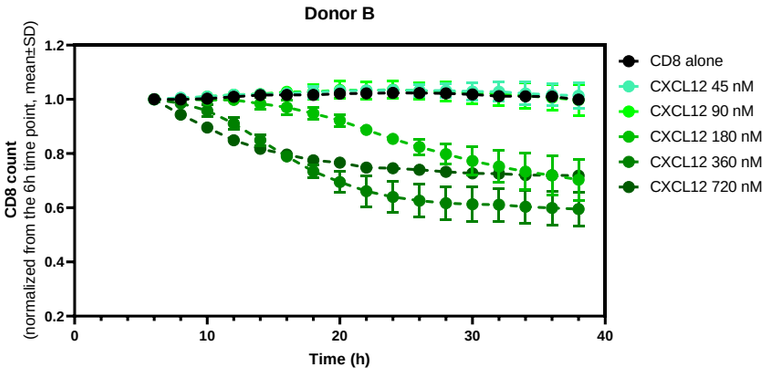


Incucyte S3 (Sartorius)

Cells migrate in response to chemoattractant +/- drug, the number of CD8⁺ T-cells decreases overtime in the upper chamber (kinetic up to 40 hours)

CD8⁺ T-cells migrate towards CXCL12-containing medium in a time and dose-dependent manner





Note: adapted from Mendes AD et al. Frontiers 2022

Targeting tumour Angiogenesis with the VEGFR-3 Inhibitor EVT801

An opportunity to address immune exclusion in solid tumours and combine with ICIs

Background on EVT801

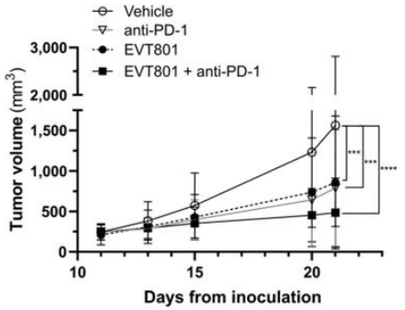
- VEGFR-3 plays a crucial role in cancer-induced angiogenesis and lymphangiogenesis
- **EVT801** is highly selective with a better safety profile than other VEGFRs inhibitor
- **EVT801:** an optimal drug partner for ICIs in VEGFR3^{+} tumours or TMEs:
  - Induces normalization of tumour blood vessels and reduce hypoxia
  - Reduce immunosuppressive chemokines (CCL4 and CLL5) as well as MDSCs
  - Favor the induction of tumour-specific T-cell responses

EVT801: a Phase I stage compound partnered with Kazia Therapeutics

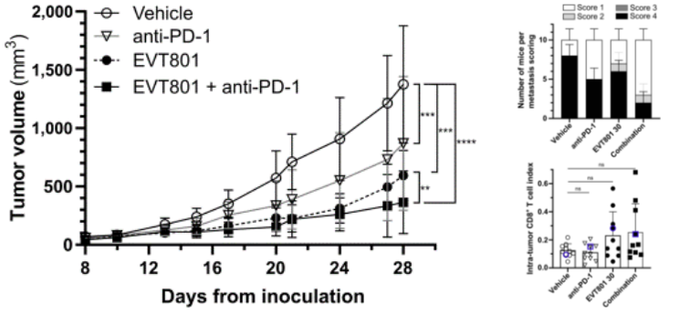
- Clinical trial initiated at the IUCT-Oncopole in Q4 2021
- Biomarkers strategy including immunomonitoring is overseen by Evotec in partnership with Kazia Tx and the hospitals (Toulouse and Lyon, France)

Preclinical learnings regarding EVT801 MoA and its synergy with immunotherapy

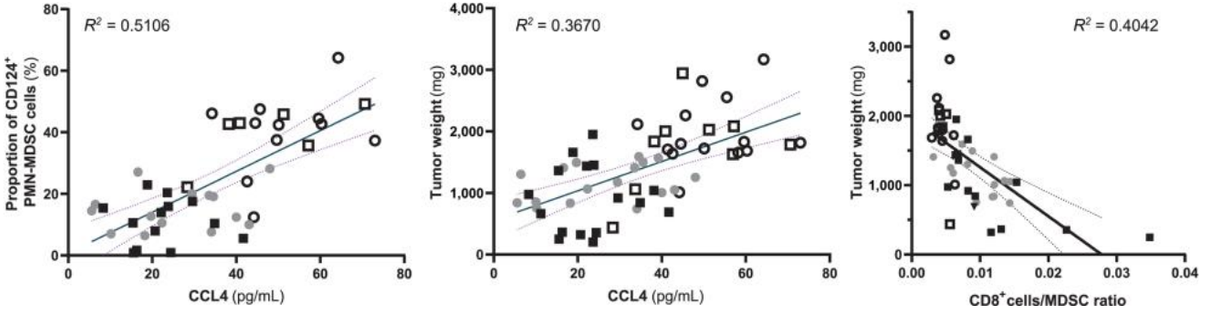
CT26 colon carcinoma



4T1 mammary carcinoma



4T1-tumour bearing mice treated with EVT801 alone or EVT801 + aCTLA-4 mAb



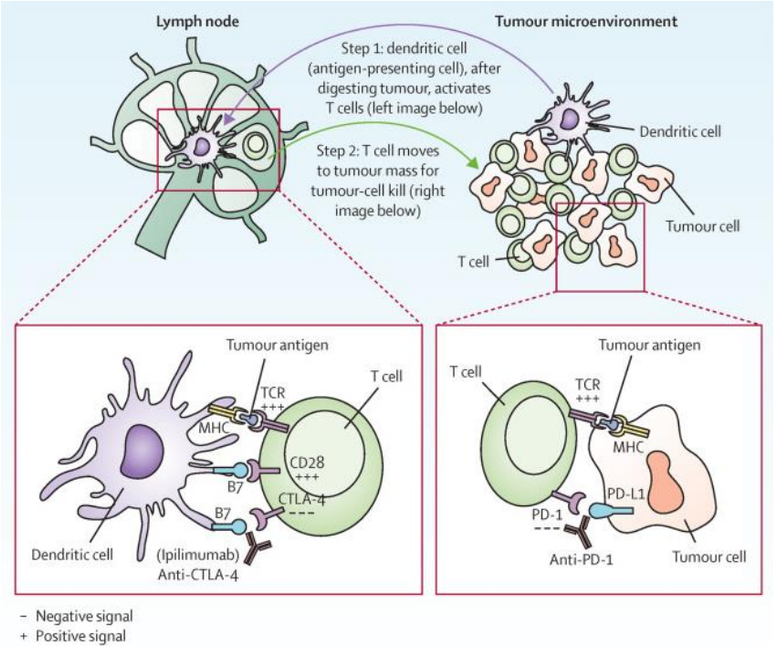
Preclinical data published in Cancer Research Communications in 2022 (Paillasse MR *et al.*)

Note: ICI (Immune Checkpoint Inhibitor)

Dual inhibition of checkpoint targets to improve clinical efficacy

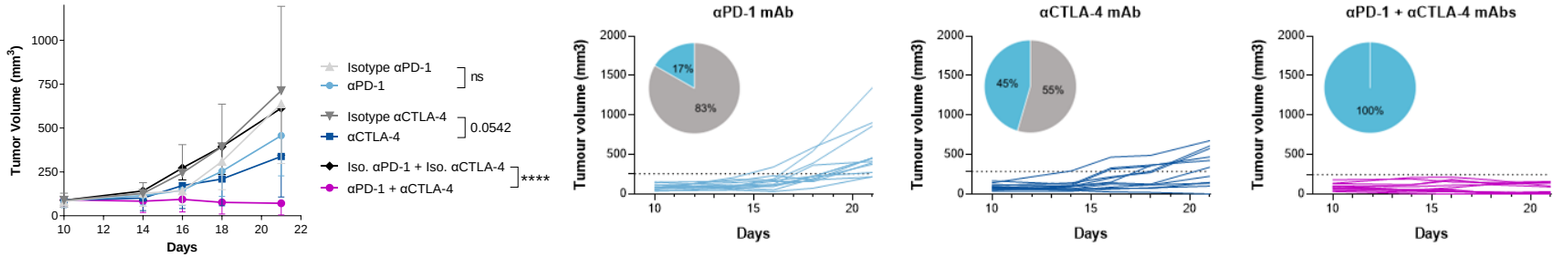
Using preclinical mouse models for testing combination hypotheses in drug discovery programs

Combination of Ipilimumab and Nivolumab approved by FDA for: Metastatic melanoma, metastatic renal cell carcinoma, colorectal cancer with MSI-H and MMR aberrations

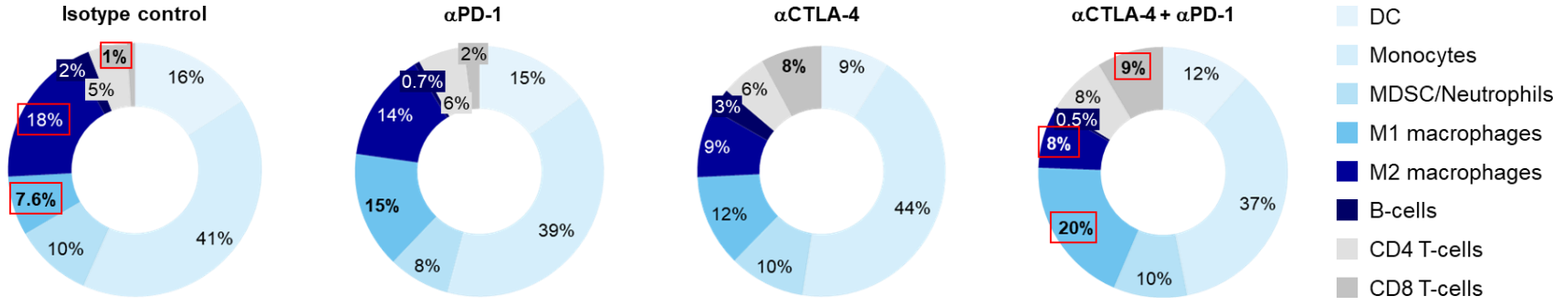


Combining anti-CTLA4 & anti-PD-1 antibodies in a MC38 colon tumour model

Co-administration of aCTLA-4 and anti-PD-1 mAbs increase therapeutic efficacy



Ex vivo analysis of immune cells infiltrate within the TME using multi-parametric flow cytometry

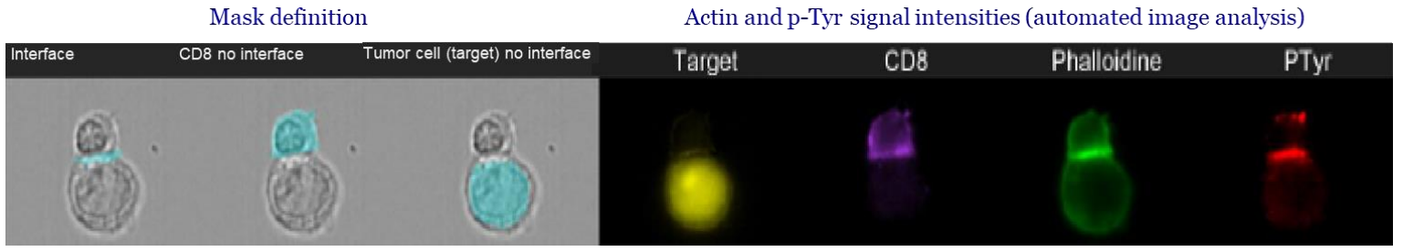


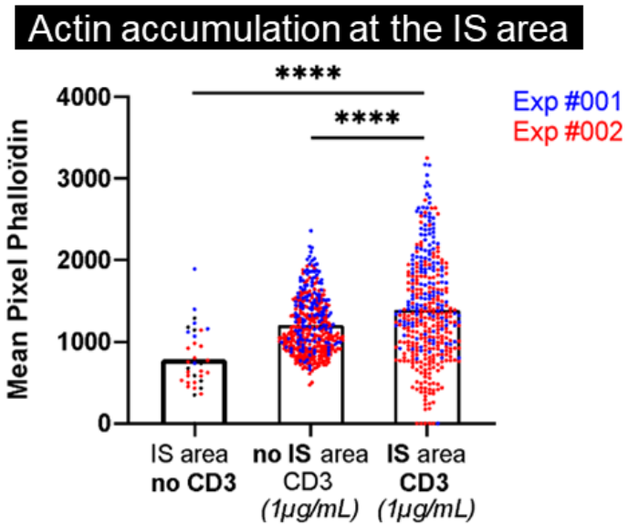
Source: Carlino MS et al.The Lancet 2021 (Immune checkpoint inhibitors in melanoma)

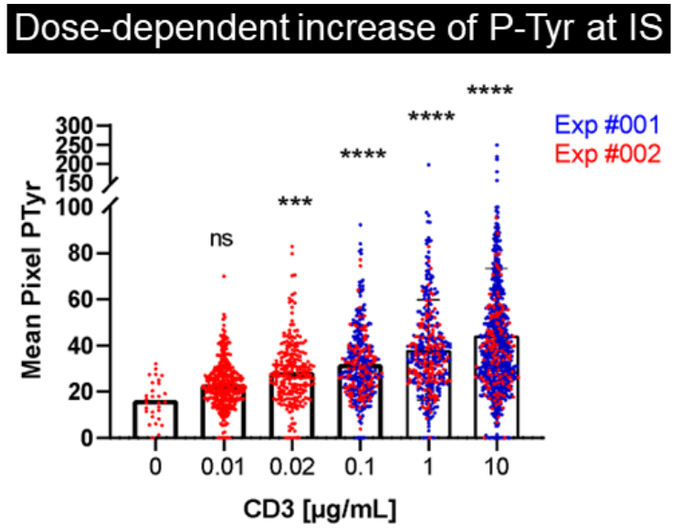
Deciphering T-cell activation features at the single cell level

High-speed imaging of the Immunological Synapse by Multispectral imaging flow cytometry

- Assess a high-speed method for quantitative and qualitative analysis of the Immunological Synapse (IS) between effector cells (NK, CTL) and tumour cells
- Use of the Image Stream X technology
- More data points per condition and robust statistical analysis of the IS for testing small molecule compounds, ICEs or cell therapies (e.g. CAR-T cells constructs)
- Demonstration of the sensitivity of the system and its robustness using a comprehensive dose response of aCD3 mAb to induce different level of IS productivity and stability







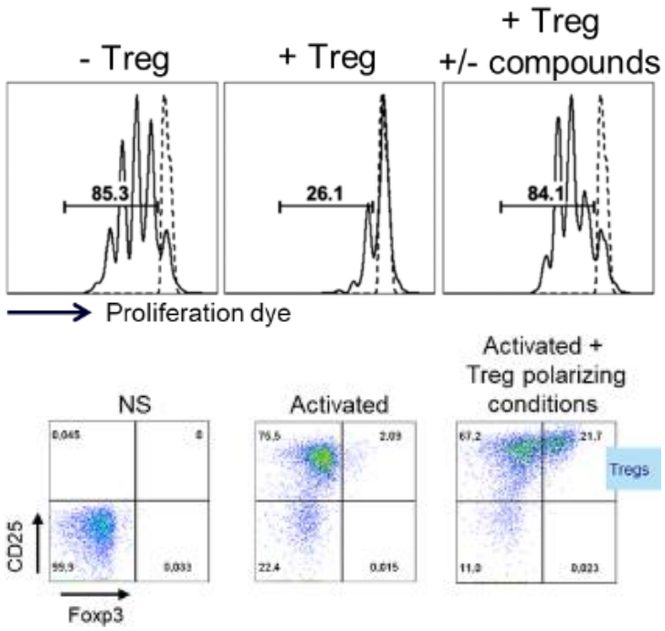
A cutting-edge approach to evaluate T-cell modulating therapies and better understand their MoA

Note: IS (Immunological Synapse)

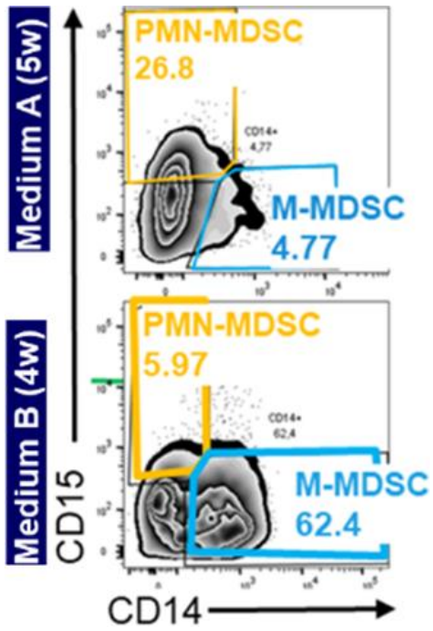
Enhancing T-cell activation by blocking immunosuppressive cells

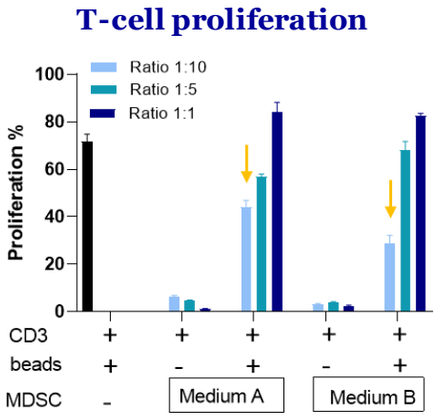
Targeting regulatory T-cells (Treg) & Myeloid-Derived Suppressor Cells (MDSCs)

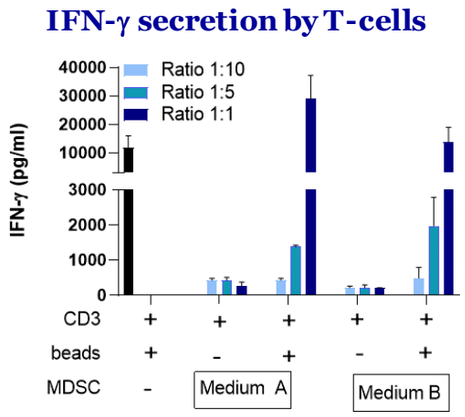
Assessing Treg immunosuppression with either nTregs or *in vitro* induced Tregs



CD34⁺ HSC-differentiated MDSC allow evaluation of drugs modulating their biology in a 100% human *in vitro* model







Using immunotherapy for modulating immunosuppressive cells within solid tumours participate to reinforce anti-tumour immunity in immune-inflamed phenotypes

Note: IS (Immunological Synapse)

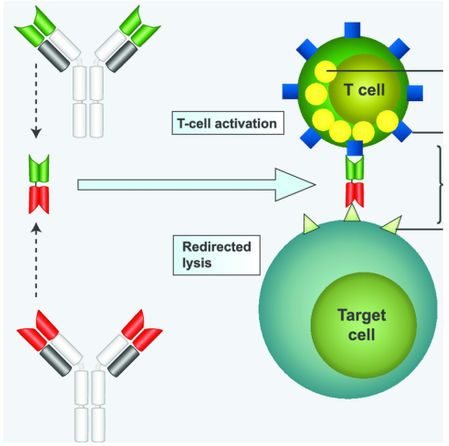
Diving into Evotec Immuno-Oncology

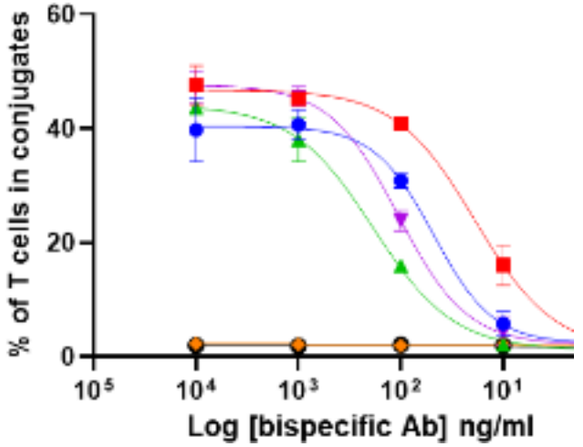
1. **Priming anti-tumour immune response:**
   cancer vaccines, manipulating innate immunity
2. **Unleashing tumour-specific T-cell immunity:**
   checkpoints inhibition & T-cell targets
3. **Re-directing immune cells killing towards tumour cells:**
   bispecific Ab, ADCC, ADCP, cell therapy
4. **Paving the way to the clinic:**
   translational Immunological assays with cancer patient samples

Evaluating bispecific antibodies – T-cell engagers

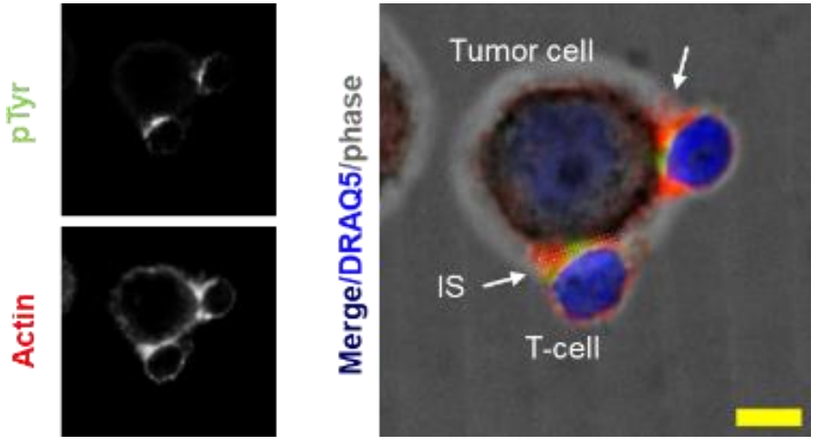
Enabling CD8-mediated killing of tumour cells using bispecific Abs / ICEs

- Bispecific antibodies are redirecting CD8⁺ T-cells towards tumour cells expressing the target antigen and inducing activation of the CTL which results in tumour cell killing
- Several type of assays can be used to evaluate the potency of bispecific antibodies:
  - **CD8-mediated killing of tumour cells**
    - Killing assay, GranToxiLux® assay
    - Upregulation of CD107a on CD8⁺ T-cells
  - **T-cell activation features**
    - Cytokines production
    - Activation markers
    - Percentages of T-cells: tumour cells conjugates
    - icCa2⁺ fluxes in T-cells
  - **Visualizing bispecific Abs effect at the Immunological Synapse level**
    - Quantification of the data & signaling pathways





T-cell activation with ICEs



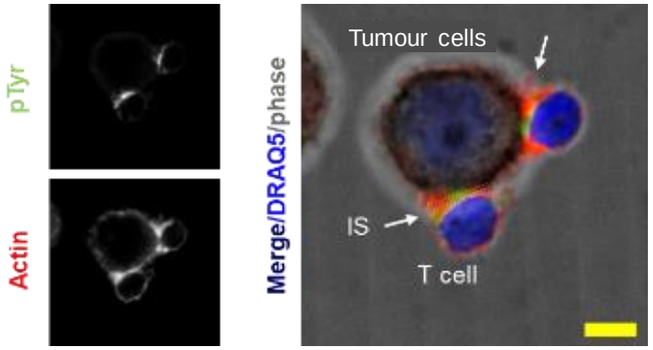
Note: IS (Immunological Synapse), CTL (Cytotoxic T Lymphocytes), ICEs (Immune Cell Engagers)
Source: scheme from Baeuerle PA and Reinhardt Cancer Research 2009

Evaluation of Immune Cell Engagers with T-cells and NK cells

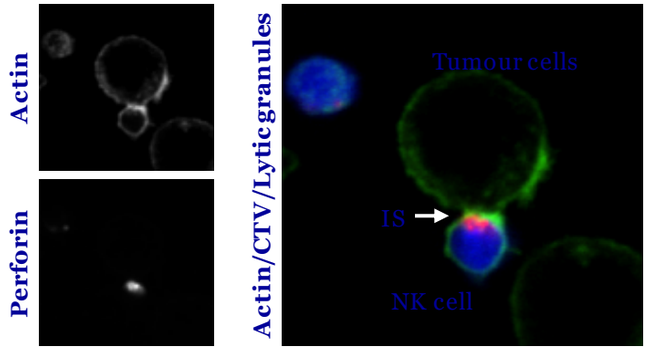
Combining functional flow cytometry-based assays & Immunological Synapse for optimal triagging

Confocal imaging

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

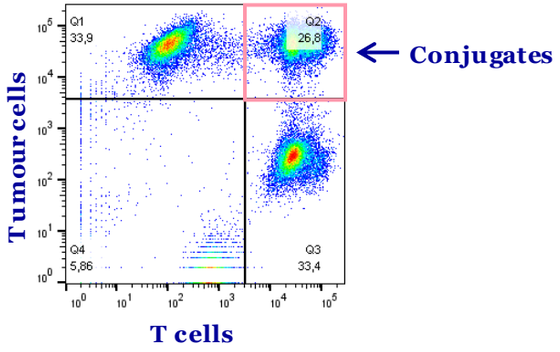


IS lytic function

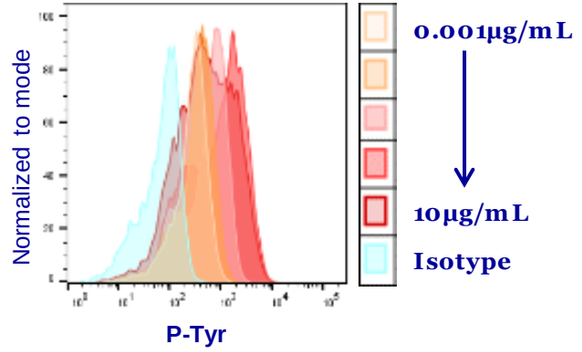


FACS

IS stability % of T cells in conjugate



IS productivity P-Tyr staining in CD8⁺ T cells

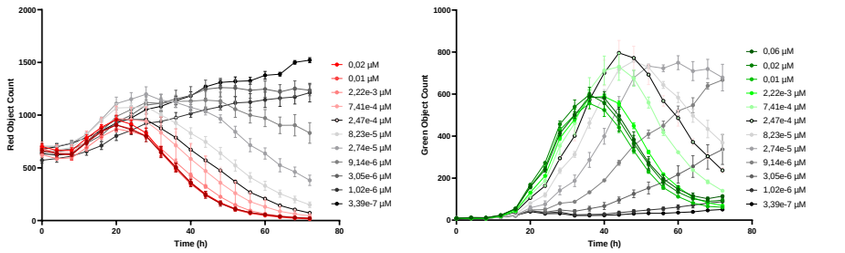


Exploring the efficiency of ICEs to trigger T-cell based cell killing

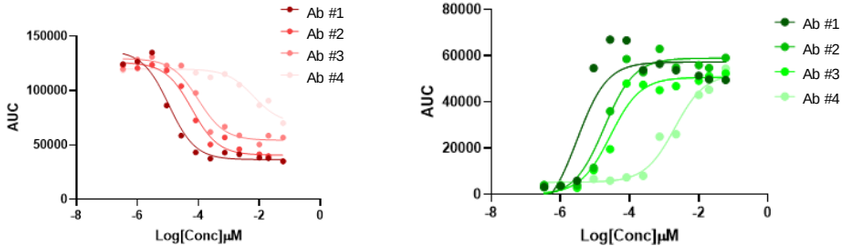
Development of a streamlined platform to evaluate ICEs in a high throughput fashion (384w)

- Bispecific antibodies are redirecting $CD3^+$ T-cells towards tumour cells expressing the target antigen and inducing activation of the CTL which results in tumour cell killing
- A platform is up and running to explore the impact of Abs in triggering the tumour cells killing:
  - **$CD3^+$-mediated killing of tumour cells:**
    - Incucyte detection of fluorescent tumour cells kinetic traces
    - Complementary analysis of caspase 3/7 substrate cleavage upon treatment
  - **T-cell activation features:**
    - Cytokines release assessed by Luminex or MSD
    - High throughput multiplex assay (up to 10 cytokines per run, 384 well plate)
    - Flow cytometry based immuno-phenotyping of effector cells to correlate the response to the CD3 activation state

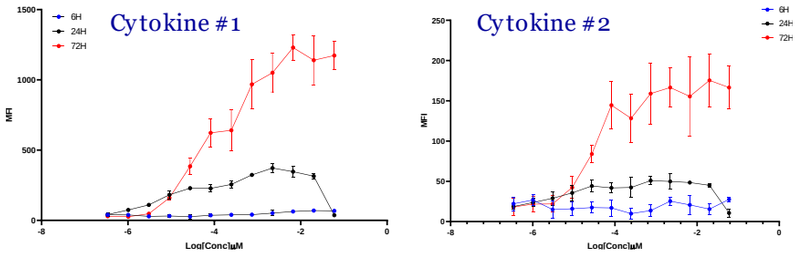
Kinetic traces from Incucyte Run



AUC and IC_{50} calculation



Time course cytokine quantitation



Note: ICEs (Immune Cell Engagers)

Evaluation of IO therapeutics in a 3D-model of solid tumour

ADCC activity against HER2⁺ cancer cells in 3D using referent clinical grade mAb

Aim

- Evaluation of IO therapeutic strategies validated in 2D cell culture models in a more elaborate 3D model that better mimic the growth and the heterogeneity of solid tumour micro-environment[1]

Experimental setup

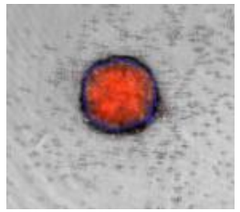
- TAA⁺ red fluorescent solid tumour cells
- Blood-derived NK cells (frozen cells)
- Fluorescence overtime by Incucyte

Outcome

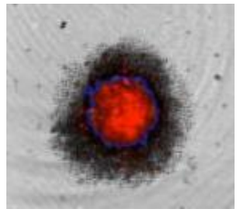
- Validation of ADCC of blood-derived NK cells against HER2⁺ tumour cells in a 3D-organization using the clinical-grade Trastuzumab as referent mAb
- *3D-ADCC model validated using HER2⁺ breast cancer line[2]*

Trastuzumab-mediated ADCC overtime against HER2⁺ breast cancer cells in 3D

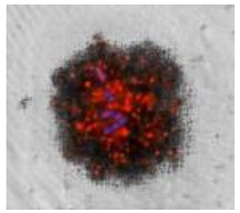
Generation of fluorescent-tumour cell line → **Day -3: Spheroïd formation** → **Day 0: Addition of blood-derived NK cells** → **Day -3 to Day +5 Follow-up of spheroid diameter overtime by Incucyte (red area)**



Day 3 (no NK)

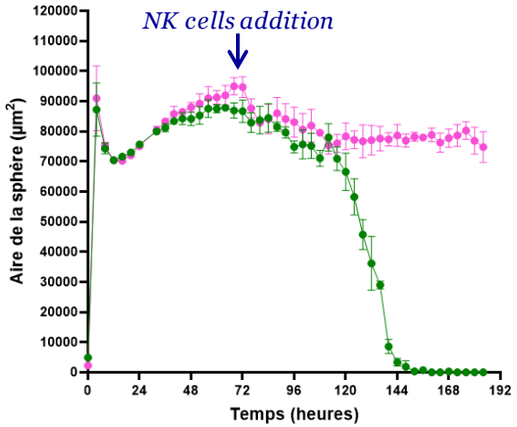


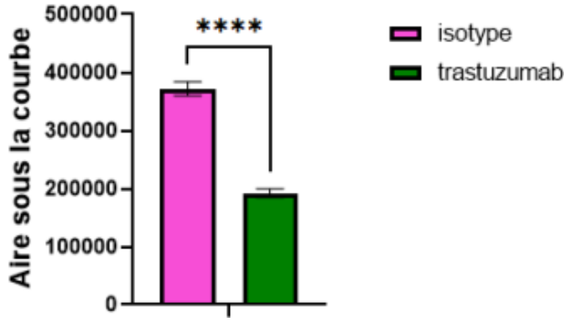
Day 6 NK + Isotype



Day 6 NK + Trastuzumab

NK cell:HCC-1954 co-culture 2:1 E:T ratio[3]





Representative of 3 independent experiments and blood-derived NK cells from 3 healthy donors

1 Hypoxic cores, nutrient/O1 gradients, biologics/cells penetrance
2 Assay development to be done for other lines: validation of spheroid formation (medium, kinetic, cell number…), Effector to Target ratio…
3 E:T Effector to Target, Statistical analysis: unpaired T test, $p<0.0001$

Diving into Evotec Immuno-Oncology

1. **Priming anti-tumour immune response:**
   cancer vaccines, manipulating innate immunity
2. **Unleashing tumour-specific T-cell immunity:**
   checkpoints inhibition & T-cell targets
3. **Re-directing immune cells killing towards tumour cells:**
   bispecific Ab, ADCC, ADCP, cell therapy
4. **Paving the way to the clinic:**
   translational Immunological assays with cancer patient samples

Identification of the Right Patient using pertinent samples

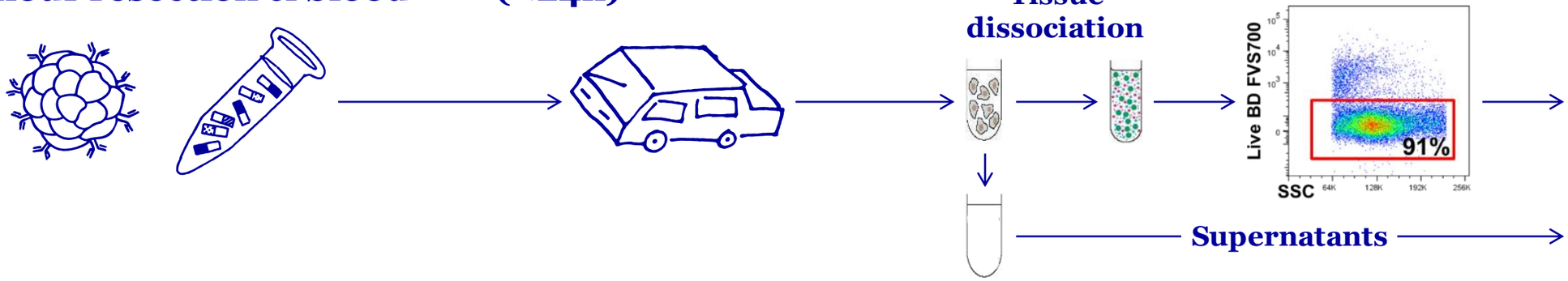
Supporting patient stratification and identification of new biomarkers

Access to healthy tissue, tumour resection & blood

Transport to Evotec (<24h)

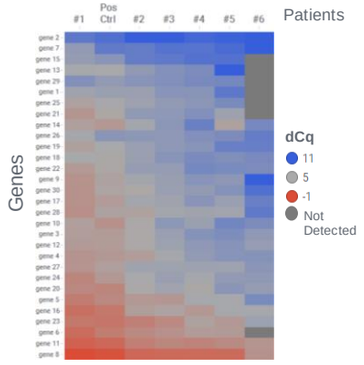
Tissue dissociation

Living cells

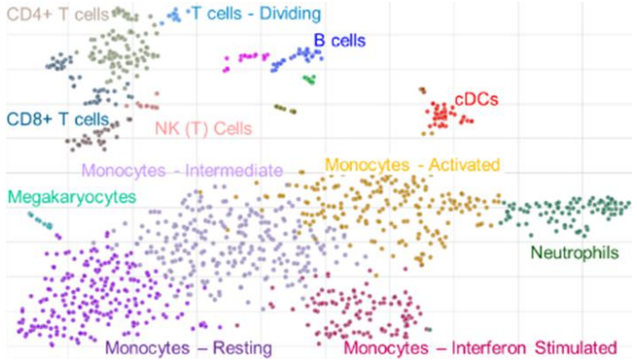


Access to fresh patient samples via established collaboration

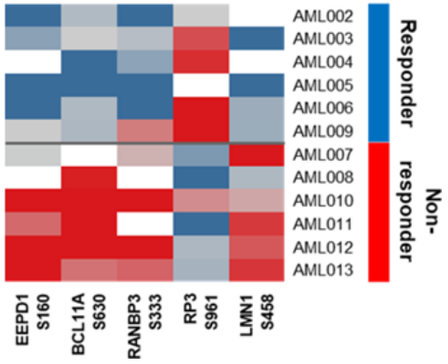
RNA signature



Single cell mRNA sequencing



Proteomics

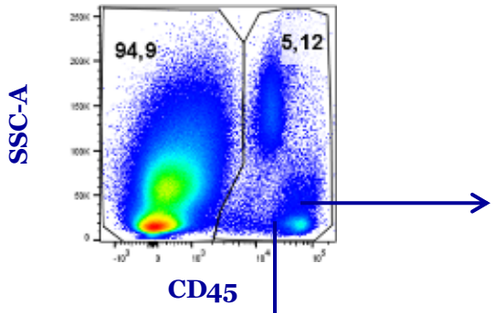


- Well established protocols for analysis of cancer phenotypes in patients samples and the possibility to study the tumour secretome
- Possibility to study the tumour secretome (by ELISA, HTRF, proteomics)

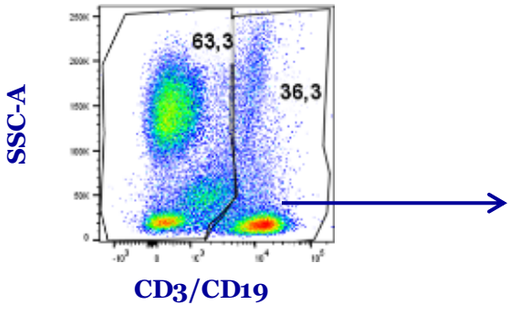
Flow cytometry analysis of patient freshly resected lung tumour

Multi-parametric analysis of immune cells infiltrating the tumour

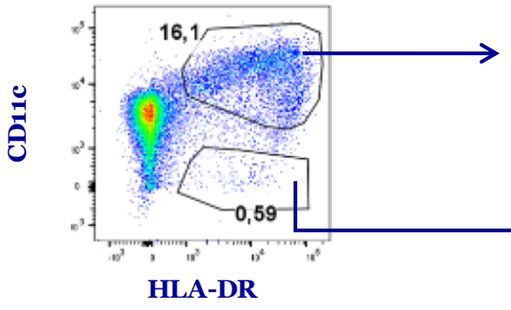
Gated on viable cells



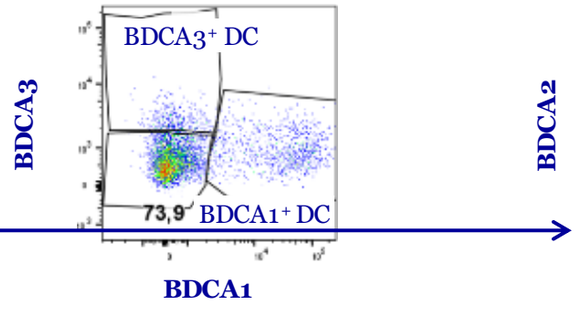
Gated on CD45⁺ cells

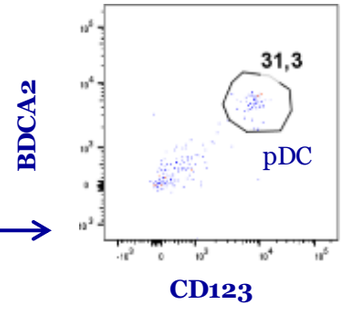


Gated on myeloid cells

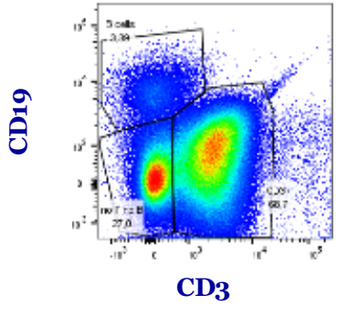


Dendritic cells

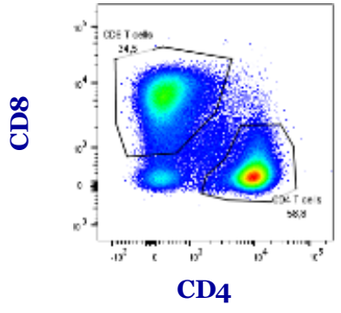




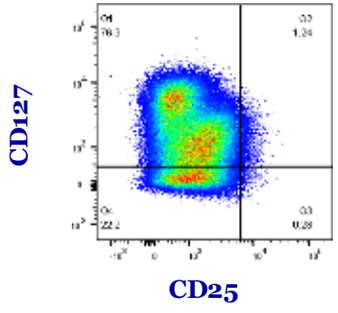
Gated on CD45⁺ cells

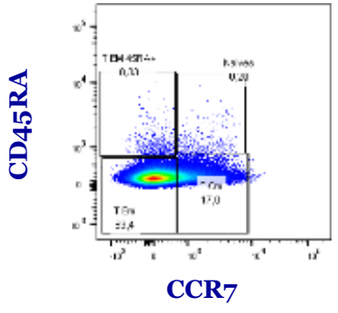


Gated on CD3⁺ cells

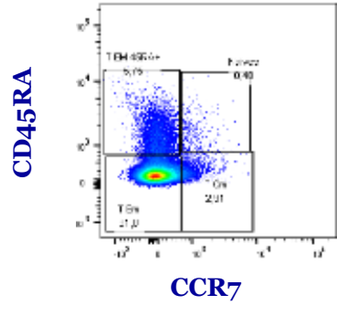


CD4⁺ T cells



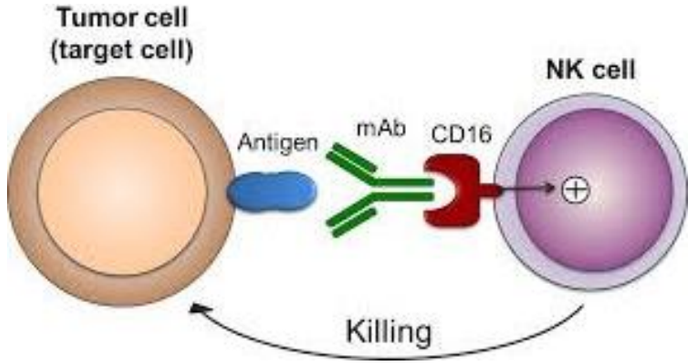


CD8⁺ T cells

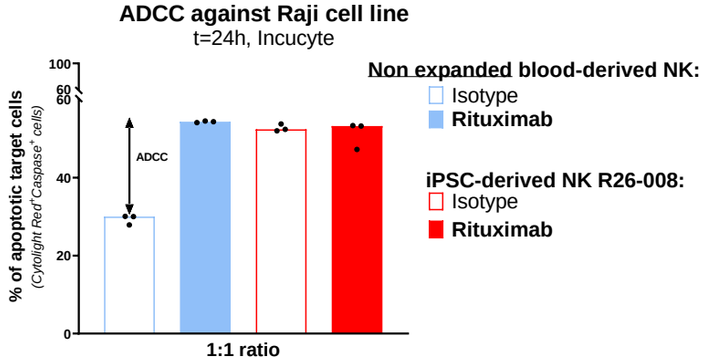


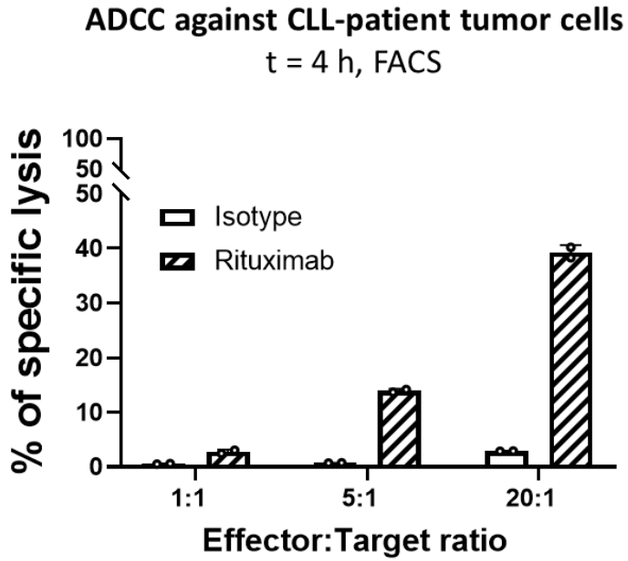
Translational validation of NK-based allogeneic cell therapy

Performing ADCC experiments with primary blasts obtained from CLL patients

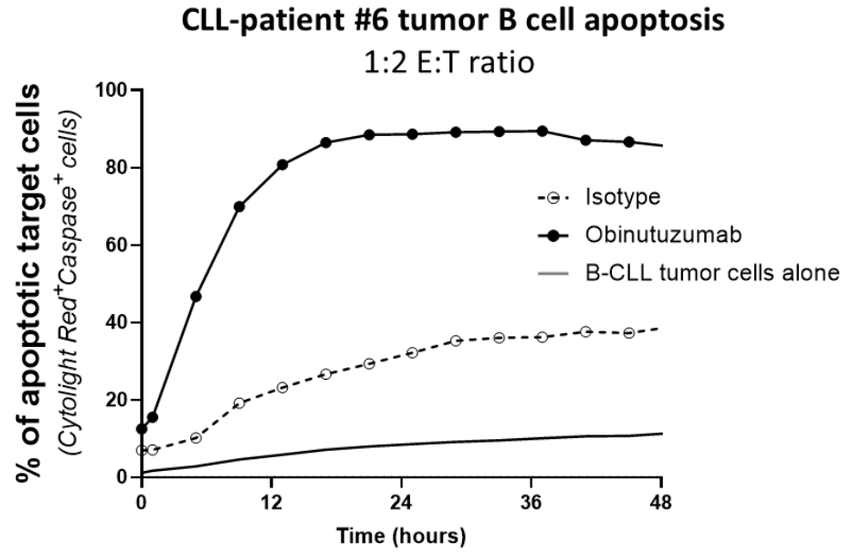


iNK are too potent to have a window for measuring ADCC with tumour cell line





Methods: *Evaluation of patient PBMC death by FACS (viability dye) after 4 hours of co-culture at various E:T ratios*



Methods: *Evaluation of apoptosis of CLL-patient-derived tumor B cells co-cultured with iNK cells ± biologics at 10µg/mL (Caspase 3/7 activity measured by Incucyte)*

B leukemic tumor cells are defined as $CD19^+$ $CD5^+$ cells (60% - 80%) of total PBMCs in patients

Overcoming limitations of tumour cell lines using patient' samples for cell therapy projects and IO discovery

From whole blood assays to Clinical Trial Support

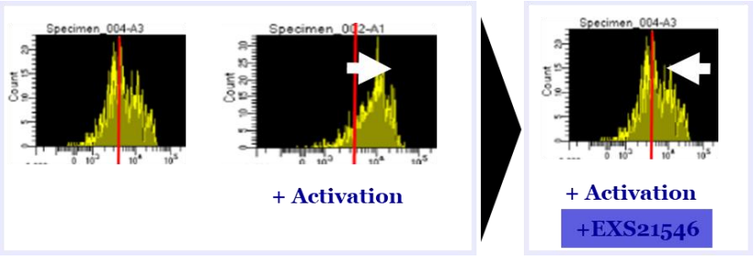
Ensuring Pharmacodynamic biomarkers in the clinic

Background

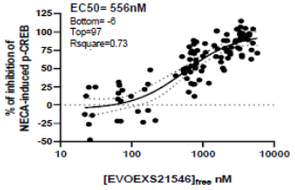
Develop a target engagement assay to demonstrate that EXS21546 is mechanistically active at the right dose

Whole Blood Assay Set-up

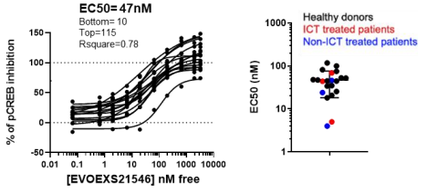
pCREB staining on CD8⁺ T-cells for one healthy donor



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

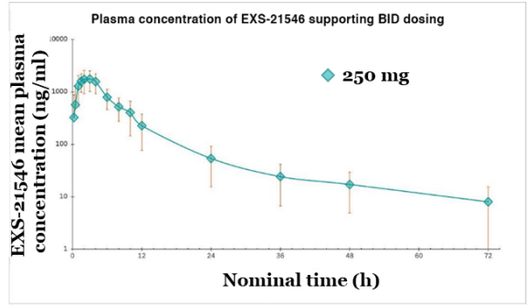
SAD dose level 1
SAD dose level 2
SAD dose level 3
SAD dose level 4

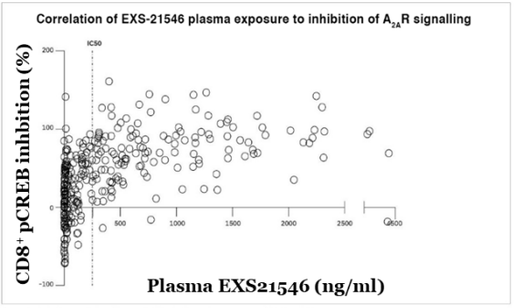
Part 2:
Multiple ascending dose (MAD)

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





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)

TICIMEL (NTC03293784): Monitoring Treatment Effects

Efficacy biomarkers: cytokines quantification

Background

Cytokines are easily quantified and characterised and concentration can be modified by drug treatment

Main experimental settings

in pre-clinical assays drug, leads to reduction of circulating cytokines

Outcome

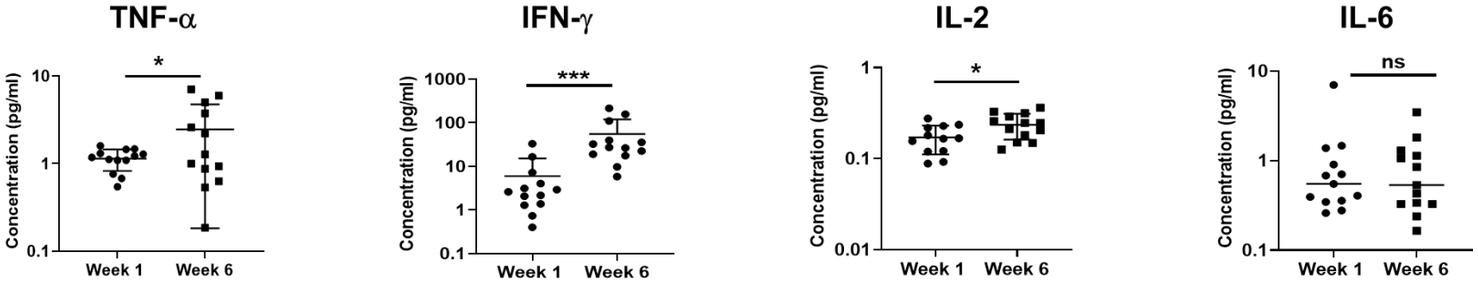
Panels of inflammatory cytokines has been evaluated in patients as an efficacy endpoint biomarkers

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

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