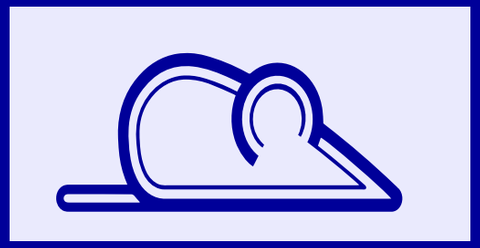
evotec

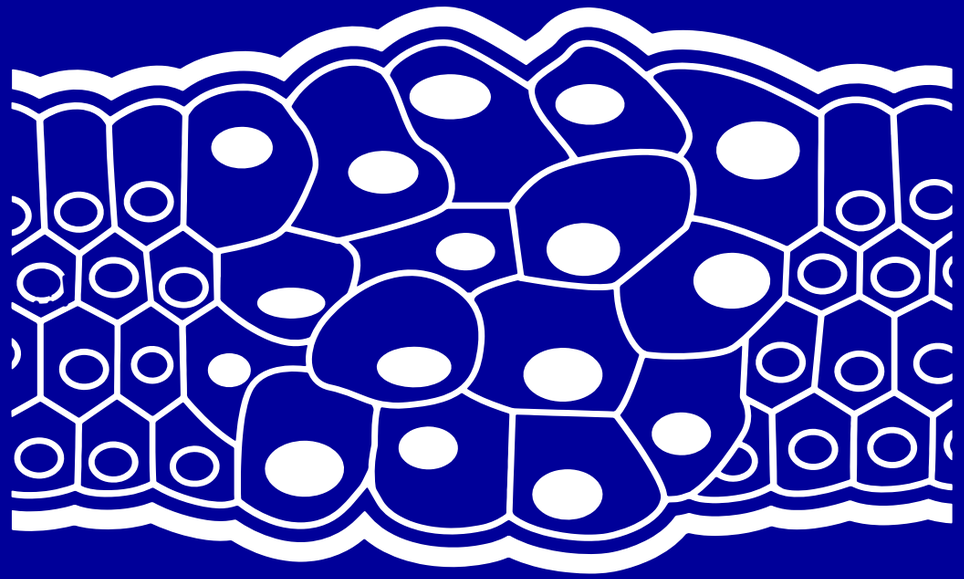
#RESEARCHNEVERSTOPS

AACR 2024

Oncology and Immuno-oncology

In vivo / ex vivo Expertise & Capabilities





Agenda

1. Oncology and Immuno-oncology *in vivo*
2. Oncology models
   - *In vivo target validation*
   - *PD / Efficacy*
   - *Tailored model*
3. Immuno-oncology models
   - *Syngeneic mouse tumour models*
   - *humanised mouse tumour models*
   - *Mouse models for cancer vaccines*

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Evotec offers integrated solutions up to IND and manufacturing

Integration benefits applied all through the value chain

Sourcing of novel ideas	Target ID/ validation	Hit identify-cation	Hit-to-Lead & Lead Opti-mization	Pre-clinical	Phase I	Phase II	Phase III	Approval	Market
• Exploratory biology • Hit-finding technologies • Chemistry • DMPK • Comp Mgt.	• Biology • Design/Chemistry • DMPK • PK:PD	• Translational Biology • Design/Chemistry • DMPK/physical chem • PK:PD • Safety	• Translational Biology • Process Chemistry • Analytical Chemistry • Formulation • Safety	• Process Chemistry • Analytical Chemistry • Formulation • Packaging					

- Interdisciplinary integration and seamless team working and evolution
- Innovation, high science, technology and problem-solving
- Knowledge and experience of successful practitioners
- Under “ONE Evotec” roof offering, unique breadth, capacity, knowhow, track record
- Operational excellence to drive rapid progress and successful outcomes

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

In vivo team of ~90 staff

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

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

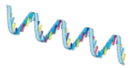
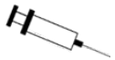
Over 30 scientists dedicated to Oncology including 13 scientists specialized in Immuno-Oncology

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

Build a tailored *in vivo* approach for the project

Models adapted to the cancer treatment modality

Cancer Therapy Modalities

- Small molecules 
- Oligonucleotides (ASO, siRNA ...) 
- Vaccines 
- Antibodies[1], Ab-conjugated, Bi-specific T-cell Engagers (BiTEs) 
- Adoptive cell therapies: TIL, chimeric antigen receptor (CAR) or engineered TCR 
- Cytokines therapies 
- Oncolytic viruses 

Our *in vivo* models

Syngeneic models

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

Xenografts in humanised models

- Immunocompromised mice with a human immune system
- immunotherapy efficacy and pharmacodynamics in a **human immune-tumour context**

Xenografts cell line derived models

- s.c. or orthotopic in immunodeficient mice
- **human cancers** with the relevance of an *in vivo* host

Specific models

- Chemically-induced tumour models
- Chemo-induced alopecia
- Vaccine models

- **General evaluation and clinical pathology:** clinical signs, body weight, food consumption, hematology (RBC and WBC counts)
- **Tumour growth:** digital caliper system, *in vivo* imaging (bioluminescence)

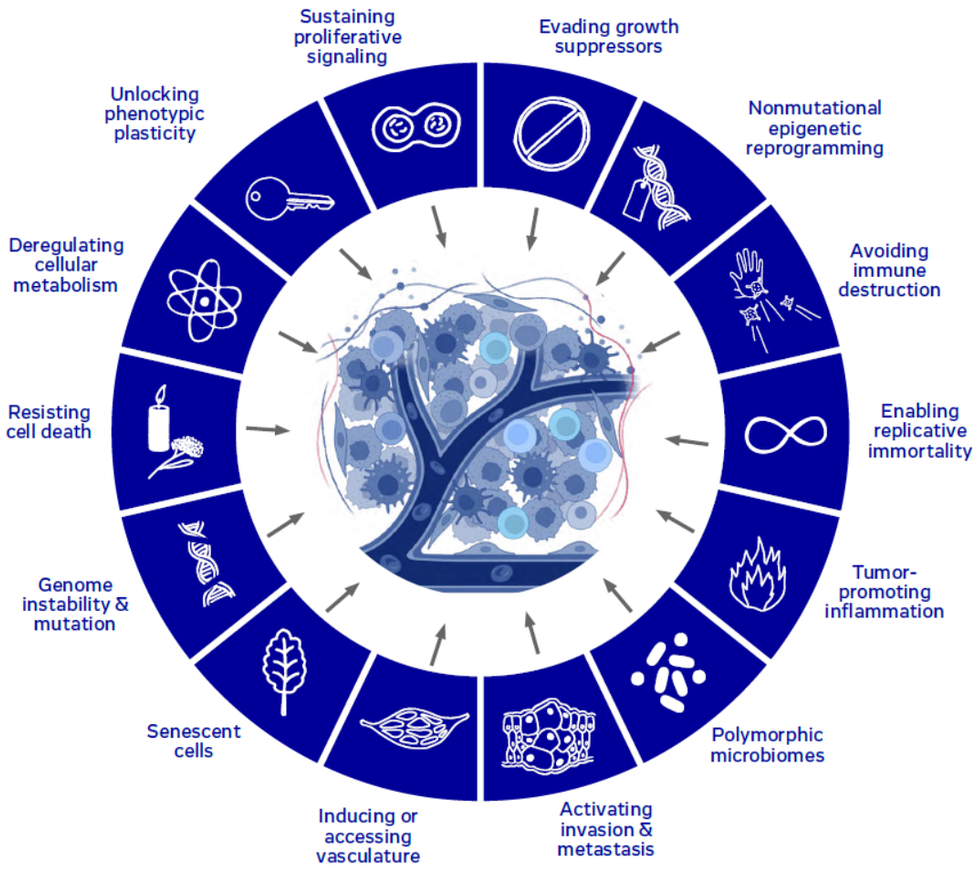
- **Target engagement – PD/Biomarkers** modulation in relation to **compound exposure or biodistribution**
- **Survival / Relapse efficacy models:** Therapeutic index-Driver of efficacy

1 Monoclonal antibody binding to cancer cell surface target and leading to cancer cell death (e.g. trastuzumab) and Checkpoint inhibitors (e.g. pembrolizumab)
TIL: Tumour-Infiltrating Lymphocyte, TCR: T-Cell Receptor, s.c.: subcutaneous

A broad range of *ex vivo* assays/readouts

To further characterize the response *in vivo*

Therapeutic targeting of cancer hallmarks



Our key readouts

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

IHC: Immunochemistry, ISH: *In Situ* Hybridization, CK19: Cytokeratin 19, MSD & HTRF: Meso Scale Discovery & Homogeneous Time Resolved Fluorescence, ELISA: Enzyme-Linked Immunoassay, ELISpot: Enzyme-linked Immune absorbent Spot
Figure adapted from Hanahan D. Hallmarks of cancer: New Dimensions. Cancer Discovery (2022)

Rely on cutting-edge technologies to fully exploit *in vivo* studies

Innovative technical platforms

In vivo and live-cells imaging systems

Bioluminescence
Fluorescence
3D tomography
(IVIS Spectrum)

X-ray imager
(Faxitron MX20)

Laser Doppler
(MoorLDI2)

Live-Cell Analysis
Instrument (Incucyte S3)

Flow cytometry and haematology

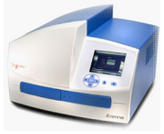
Flow cytometry analyzer up 30 parameters / 5 lasers
(BD Canto II, BD Fortessa X20, Biorad ZE5 and BD Symphony)

Cell separator
(autoMACS Pro)

Cell sorter
(FACSAria Fusion)

Gene/mRNA/protein analysis

Viia7, QuantStudio



Singulex

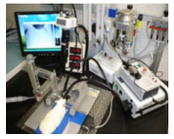
MSD&HTRF
technology ELISA

Automated Western
Device (Jess)

Mass spectrometry

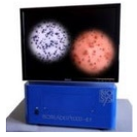
Bio-analysis

Proteomics



Metabolomics/
Microdialysis

Analyzer system



ELISpot
reader

Haematology analyzer
(Procyte Dx)

Histology: tissue sample preparation, classical staining, IHC/ISH/TMA, scan and digital analysis

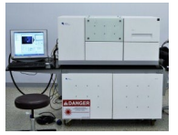
Dehydration

Paraffin
embedding

SLEE
autostainer

Leica
BondRX

Ventana auto-
stainers Ultra/XT



Opera

Operetta

Zeiss Axioscan

OperaPhenix

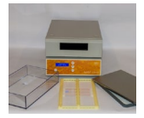
Tissue slicing

Vibratome

Microtome

Cryo-Microtome

Cryostat



ACD-Bio ISH system

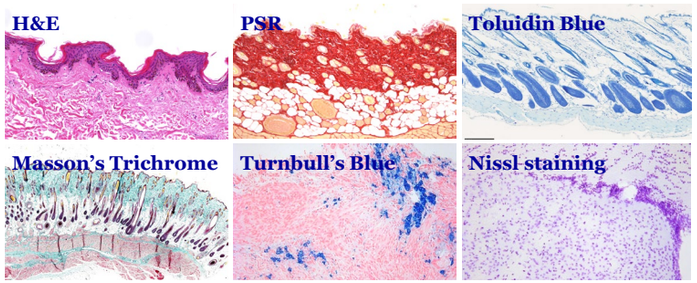
Manual
microscopes

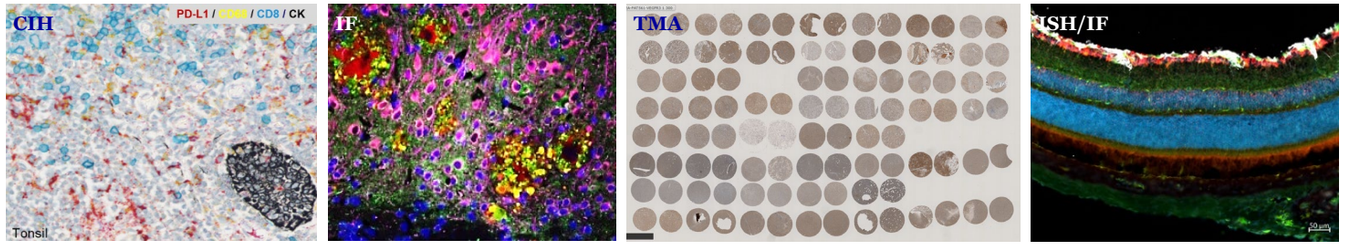
MSD & HTRF: Meso Scale Discovery & Homogeneous Time Resolved Fluorescence, IHC: Immunochemistry, ISH: *In Situ* Hybridization, TMA: Tissue Micro-Array

Highly efficient platform for histological processing and analysis

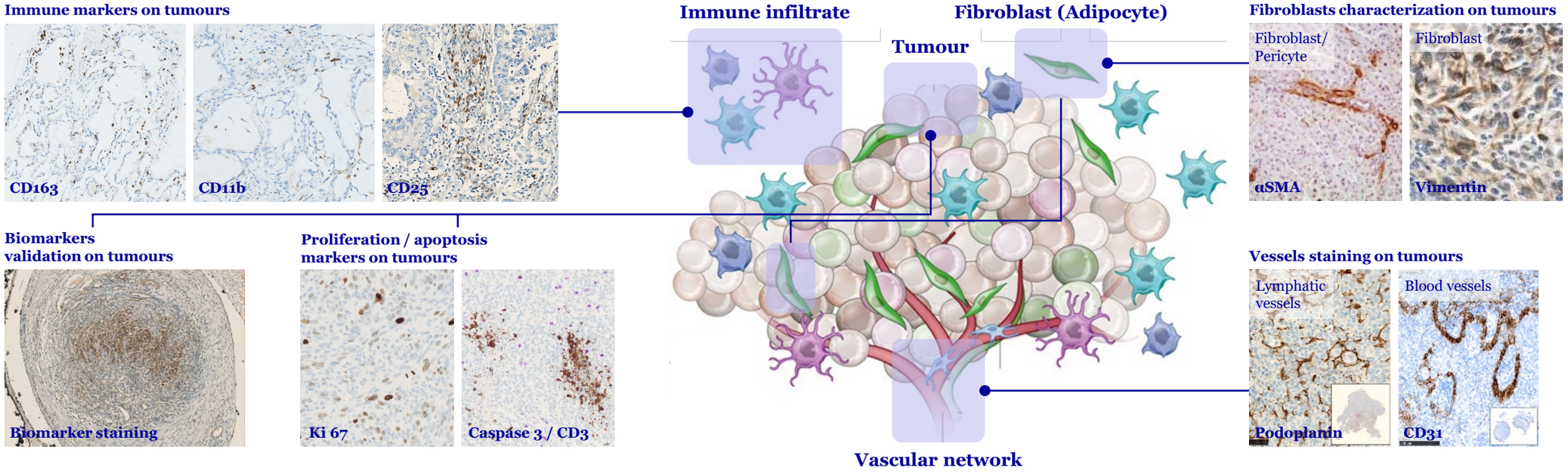
Different workflows tailored to the partner needs

Evotec's Histological capabilities





Analysis of the Tumour Micro-Environment (IHC): from biomarkers validation to MoA exploration



H&E: Haematoxylin & Eosin, PSR: Picrosirius Red, CIH: Chromogenic Immunohistochemistry, IF: Immunofluorescence, TMA: Tissue Micro-Array, ISH: *In Situ* Hybridization, IHC: Immunochemistry, MoA: Mechanism of Action
Adapted from Junttila & de Sauvage, Influence of tumour micro-environment heterogeneity on therapeutic response, *Nature*, **2013**, 501, 346-354
IHC performed on Ventana platform (Discovery & Ultra), image analysis on NanoZoomer

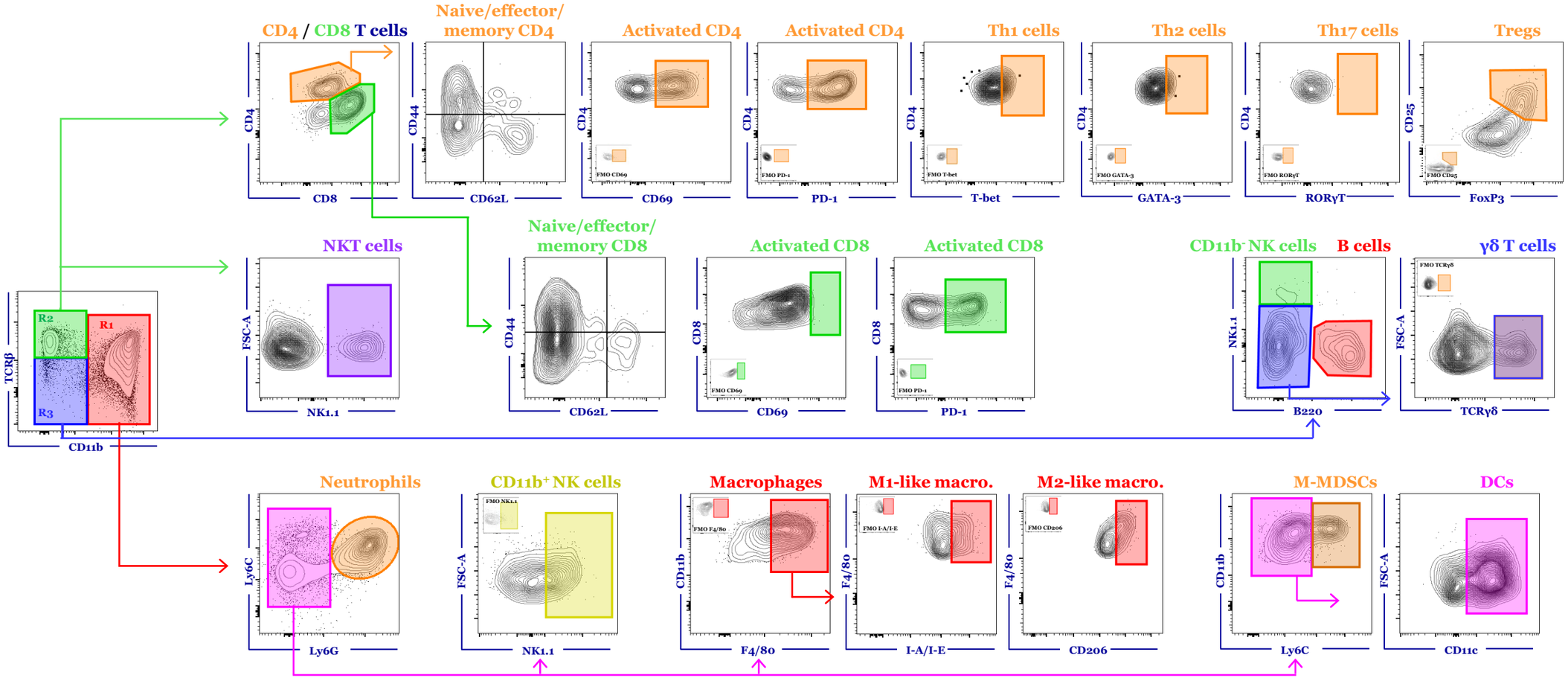
Deep expertise in multiparametric flow cytometry

Off-the-shelf or custom flow cytometry panels

Immune response profiling:

from simple to complex phenotypic and functional analysis

26-parameters panel allowing identification of multiple immune cell types in tumour, lymph node, spleen or blood[1]



1 Plots from a tumour sample
NK: Natural Killer cells; DC: Dendritic Cells; M-MDSCs: Monocytic Myeloid-Derived Suppressor Cells

In vivo imaging to follow therapeutic effect

Animal models supported by *in vivo* imaging

Evotec's *in vivo* imaging capabilities

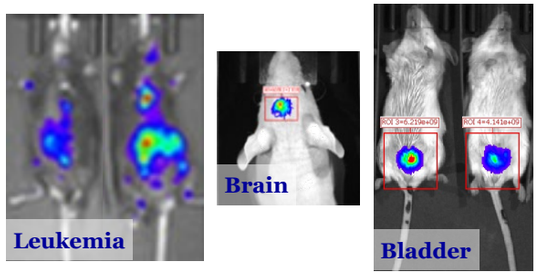
- Non invasive imaging of internal tumour growth (orthotopic tumour models), metastasis spreading and microbial invasion
- Tracking of fluorescence imaging probes
- Monitoring of tissue-vascularisation: blood flow measurement and vascular network visualisation

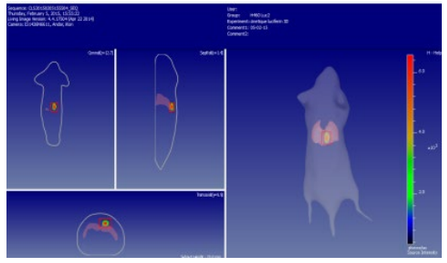
Optical *in vivo* imaging:

- Bioluminescence
- Fluorescence

IVIS Spectrum

Orthotopic tumours imaging



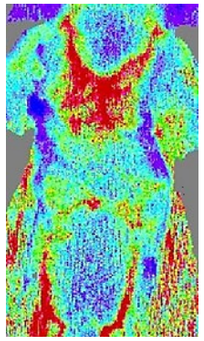


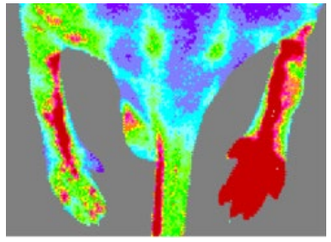
Laser Doppler monitoring:

High spatial resolution & depth penetration in tissues (IR 830 nm)

MoorLDI2

Blood flow imaging



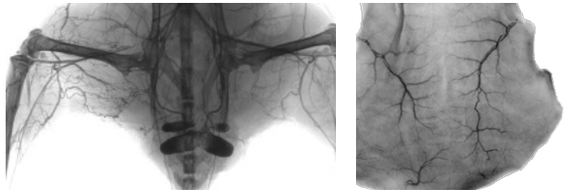


X-Ray imaging:

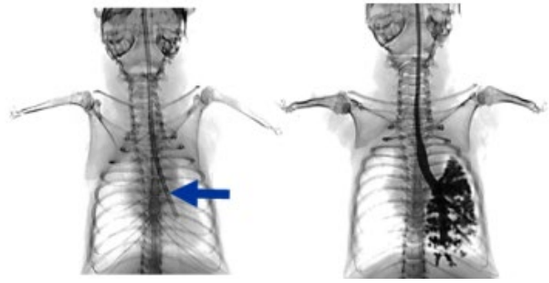
Radiography of bone tissue, some soft tissues & vascular network

Faxitron MX20

Vascular network imaging



Intrabroncheal instillation imaging



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   - *humanised mouse tumour models*
   - *Mouse models for cancer vaccines*

Extensive expertise in preclinical tumour models

Subcutaneous & orthotopic models in immuno-deficient /competent mice

- **Model set up based on project need**
  - Choice of the cancer cell type: based on therapeutic indication, molecular profile, *in vitro* work, …
  - In immunocompetent, immunocompromised or humanised mice
- **Orthotopic implantation can be considered to foster the original location**
  - Skills in breast, lung, liver, bladder, ovary, leukemic cells implantation, …
  - Luciferase-engineered cells for time-course follow up of tumour growth by bioluminescence
- **PDX models outsourced on demand (Master agreements with a number of providers)**
  - Model identification based on target indication
  - Study design: dose and schedule, sampling time points
  - Study follow up with the CRO
  - *Ex vivo*, PK analysis of the samples

Examples of tumour indications for which models have been set up

Tissue	Site of inoculation	Species
Bladder	s.c. / Intravesical instillation	Mouse / Human
Brain	s.c.	Human
Breast	Mammay fat pad	Mouse / Human
Colon	s.c.	Mouse / Human
Fibrosarcoma	s.c.	Mouse
Kidney	s.c.	Human
Leukemia	Intravenous	Mouse
Lung	s.c. / Transpleural / Intratracheal / Intracranial	Mouse / Human
Lymphoma	s.c.	Mouse / Human
Ovary	s.c. / i.p. for peritoneal carcinomatosis	Mouse / Human
Pancreas	s.c.	Human
Skin	s.c. / Intradermal	Mouse / Human

For some cell lines a license may be required

In vivo target validation study in tumour xenograft model

Case study: inducible shRNA KD to validate a protein as a possible therapeutic target

Study design

NSG mice
(immunodeficient)

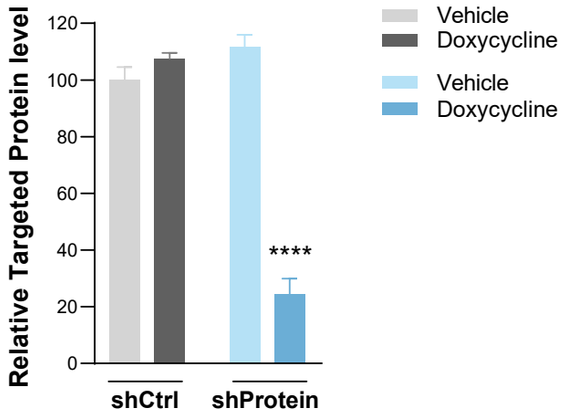
body weight and tumour volume (3x/week)

0 — 53 days ▶ Tumour collection for Jess analysis

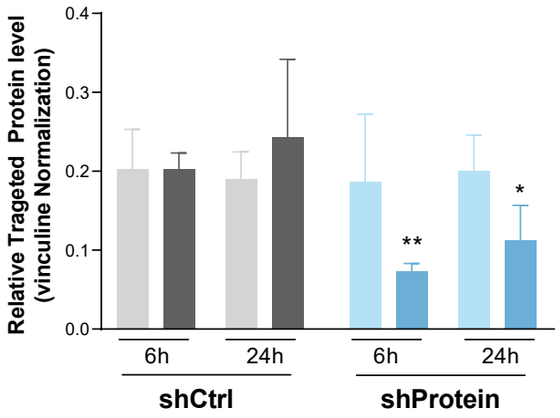
- ▶ **shCtrl or shProtein cells inoculation (s.c.)**
- ▶ **Mice randomization on tumour volume** (~150 mm³)
- ▶ **Doxycycline ttmt (BID) to induce shRNA expression**

shRNA induction decreases Targeted Protein level in the tumour *in vitro* and *in vivo*

In vitro data

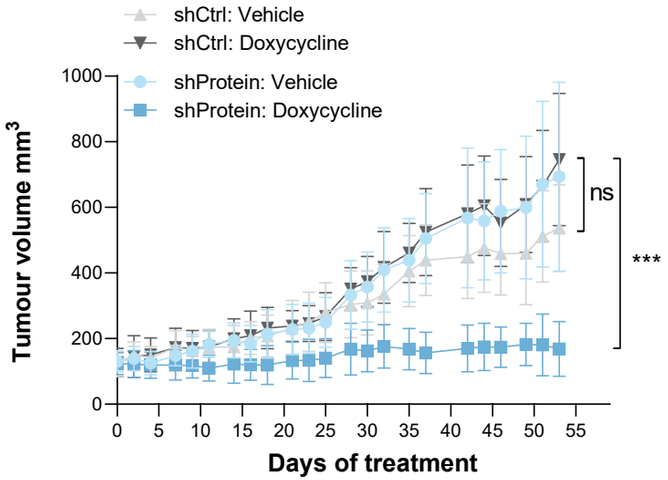


In vivo data (tumour)



Targeted Protein KD translates to tumour growth inhibition and validates the protein as a possible therapeutic target

Tumour growth



s.c.: subcutaneous, shRNA: short hairpin RNA, Ctrl: control
Results are expressed as mean ± SD. Statistical analysis: One-way ANOVA with Tukey's multiple comparisons test (ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.0001)

PD data to design dosing schedule in efficacy study

Case study: PD response in tumours predicts the dosing regimen in efficacy study

Pharmacodynamic study

Study design

o days ▶ Time-course sampling

- ▶ **Cells inoculation (s.c.)**
  - ▶ **Mice randomization on tumour volume** (~400 mm³)
  - ▶ **Single dosing (1, 3, 10 or 30 mg/kg, p.o.)**

Efficacy study

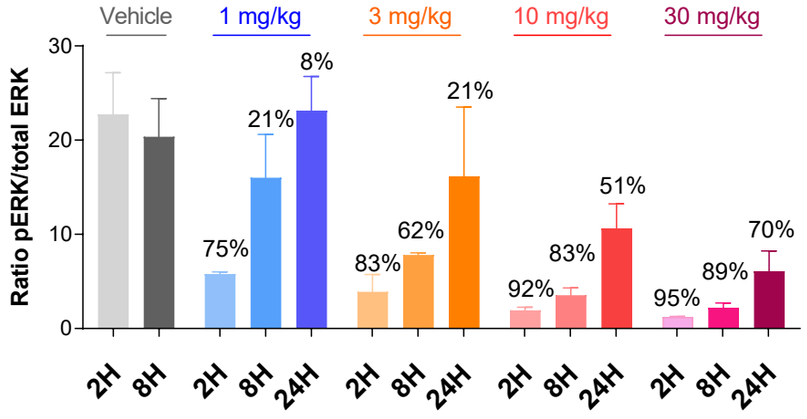
Study design

o days ▶ Endpoint

- ▶ **Cells inoculation (s.c.)**
  - ▶ **Mice randomization on tumour volume** (~150 mm³)
  - ▶ **Repeated Treatment**

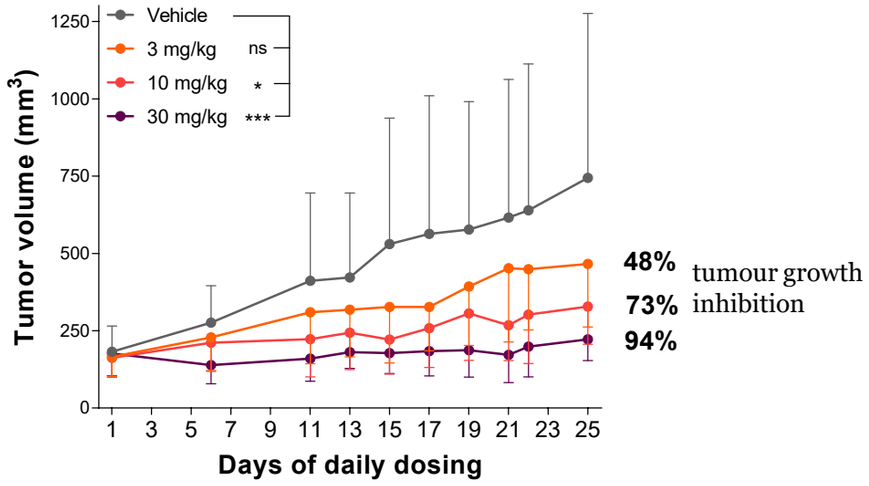
Sustained >50% pErk inhibition until 24h at 30 mg/kg

pERK modulation after single p.o. dosing



PD data were used to define the dose and schedule in the efficacy study

Tumour stasis was observed after daily dosing at 30 mg/kg



s.c.: subcutaneous, p.o.: *per* os

Results are expressed as mean ± SD. Statistical analysis: Mixed-effects model with Dunnett's multiple comparisons test on log transformed data vs Vehicle (ns $p>0.05$, * $p<0.05$ and *** $p<0.001$)

Efficacy and PK/PD in tumour xenograft model

Case study: dose response of an inhibitor (targeted therapy small molecule)

Study design

NSG mice

mice body weight and tumour volume

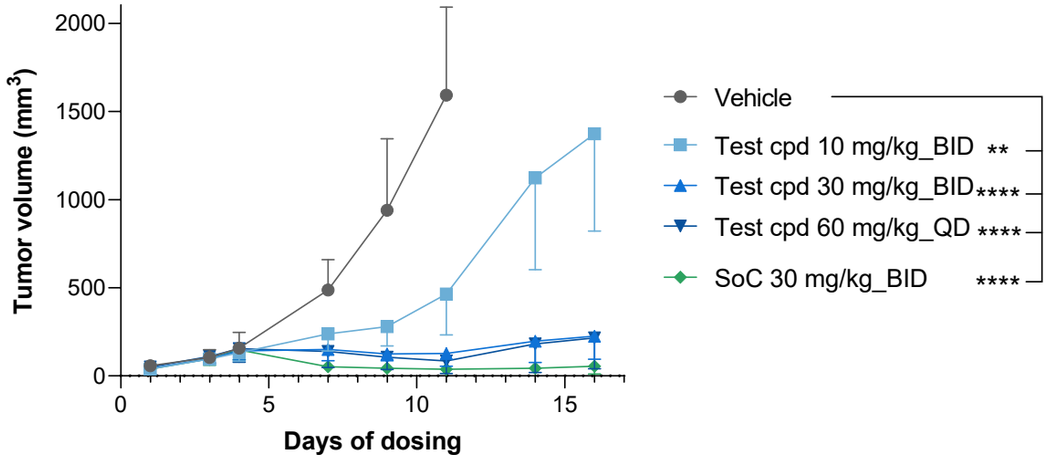
0 — 16 days

Samples collection: Plasma, tumour and tissues for bio-analysis

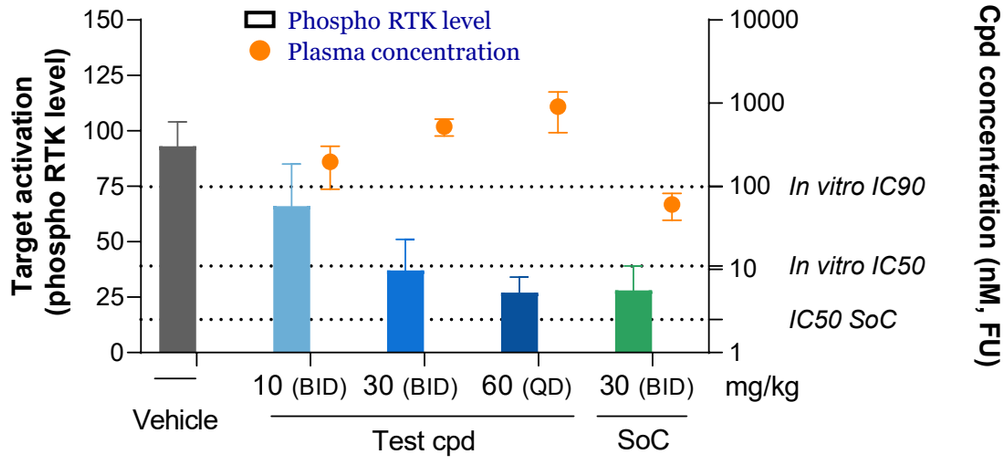
- **Cells inoculation (s.c.)**
- **Mice randomization on tumour volume** (~150 mm³)
- **Start treatment with Test compound (10, 30 or 60 mg/kg, QD or BID) or SoC (30 mg/kg, BID)**

Correlation between tumour growth inhibition, target engagement in the tumour and plasma cpd concentrations

Tumour growth



Target engagement in tumours (2h post last-dosing)



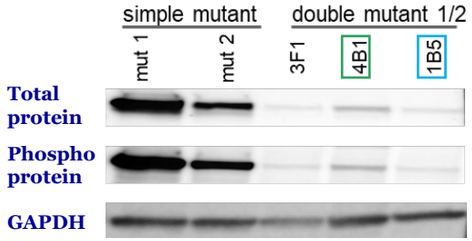
s.c.: subcutaneous, SoC: Standard of Care
Results are expressed as mean ± SD. Statistical analysis: Mixed-effects model with Dunnett's multiple comparisons test vs Vehicle (** p<0.01 and **** p<0.0001)

Tailored models when needed for the project

Case study: cells expressing a double mutant oncogenic protein fusion of interest

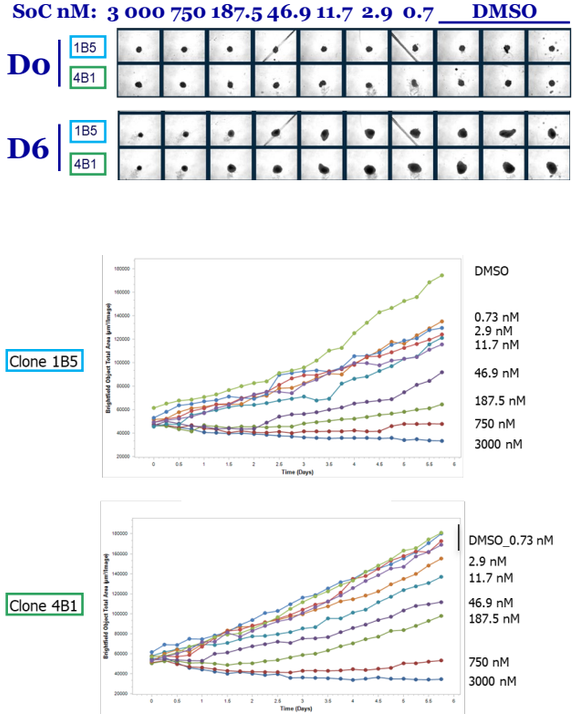
Cell transduction, cloning and characterization | ***In vitro* 2D and 3D functional assays for validation with SoC** | ***In vivo* mouse model set up** | ***In vivo* PK/PD and Efficacy studies**

Protein fusion expression levels:

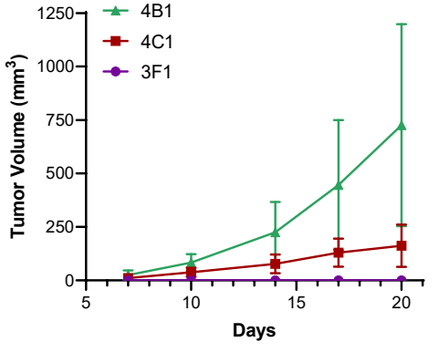


Preselection of clones

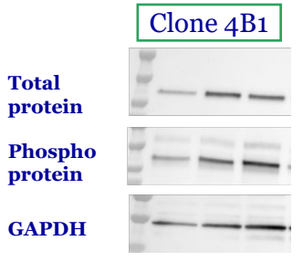
Tumour spheroid growth:



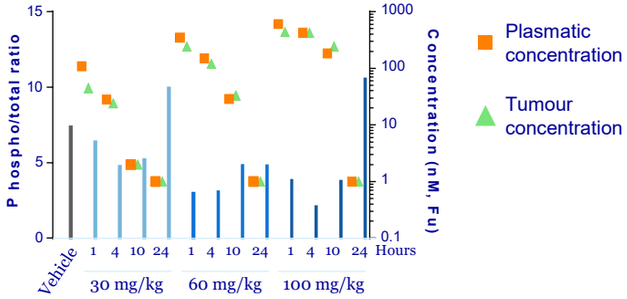
Tumour growth of preselected clones:



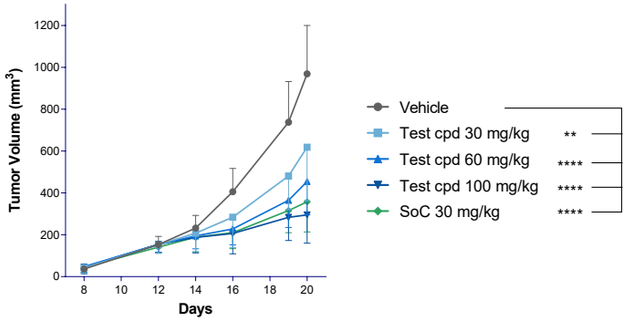
Tumour expression level:



PK/PD study (on 4B1 selected clone):



Efficacy study (on 4B1 selected clone):



sSoC: Standard of Care

Results of tumour growth are expressed as mean ± SD. Statistical analysis: Mixed-effects model with Dunnett's multiple comparisons test vs Vehicle (** p<0.01 and **** p<0.0001)

Agenda

1. Oncology and Immuno-oncology *in vivo*
2. Oncology models
   - *In vivo target validation*
   - *PD / Efficacy*
   - *Tailored model*
3. Immuno-oncology models
   - *Syngeneic mouse tumour models*
   - *humanised mouse tumour models*
   - *Mouse models for cancer vaccines*

Evotec expertise in syngeneic mouse models for IO

Custom-tailored cell line tumour models can be developed on request

Indication	Cell line	Mouse strain	Inoculation site	Immune CheckPoint Therapy (ICT) response based on			
Colorectal	CT26	BALB/c	Ectopic (s.c.)	αPD-1	–	αCTLA-4	αPD-1+ αCTLA-4
	MC38	C57Bl6	Ectopic (s.c.)	αPD-1	αPD-L1	αCTLA-4	αPD-1+ αCTLA-4
Bladder	MB49	C57Bl6	Ectopic (s.c.) Orthotopic (intravesical)	αPD-1	–	αCTLA-4	–
Breast	4T1	BALB/c	Orthotopic (mammary f.p.)	αPD-1	–	αCTLA-4	–
	EMT6	BALB/c	Orthotopic (mammary f.p.)	–			
Fibrosarcoma	MCA205	C57Bl6	Ectopic (s.c.)	αPD-1	αPD-L1	–	
Lymphoma	EG7-OVA	C57Bl6	Ectopic (s.c.)	–	αPD-L1	–	
Pulmonary	LLC1/LL2	C57Bl6	Ectopic (s.c.)	–			
Melanoma	B16	C57Bl6	Ectopic (s.c.)	–			
Renal cell carcinoma	Renca	BALB/c	Ectopic (s.c.)	–			
Ovarian peritoneal carcinomatosis	ID8	C57Bl6	Orthotopic (i.p.)	–			

T/C ratio high intermediate non responder

Cell Quality Control (performed before each inoculation)

- Test for mycoplasma contamination (PCR)
- Cell count and size distribution
- Control doubling time
- Essential surface markers expression: MHC-I and PD-L1 (flow cytometry)

s.c.: subcutaneous, f.p.: fat pad and i.p.: intraperitoneal
T/C ratio (%) = (mean Treated-tumour weight or volume / mean Control-tumour weight or volume) x 100
For some cell lines a license may be required.

Combining ICTs enhances anti-tumour response

Case study: efficacy and Immune response in MC38 colon carcinoma mouse model

Study design

MC38 cancer cells
in C57Bl6 mice (n=11-12/group)

mice body weight and tumour volume (3x/week)

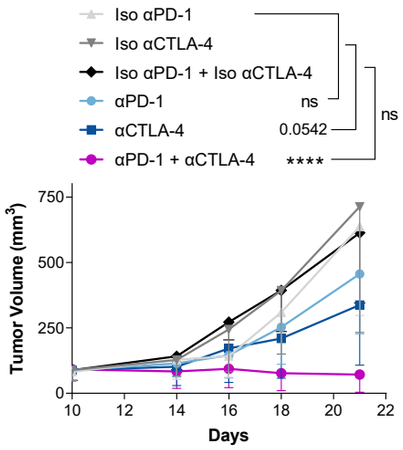
0 — 10 — 14 — 18 — 21 days

- ▶ **Cells inoculation (s.c.)**
- ▶ **Mice randomization on tumour volume**
- ▶ **Start treatment ICTs (i.p. 10 mg/kg), Q4dx8d**

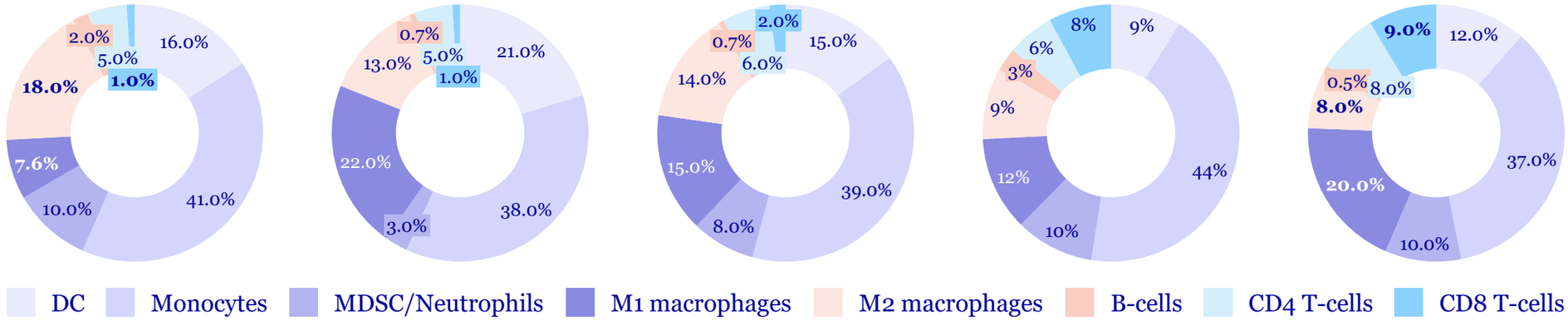
Endpoint tumour:
- Weight
- Immune cells infiltration (flow cytometry)

MC38 is sensitive to ICT treatment. Response is associated with CD8+ T-cells infiltration and M1 polarization

Tumour growth (mean ± SD)



Evaluation of immune cell infiltrates within the tumour (flow cytometry)



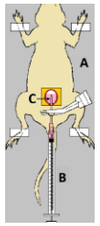
s.c.: subcutaneous, ICT: Immune Checkpoint Therapy, DC: Dendritic Cells, MDSC: Myeloid-Derived Suppressor Cells
Results are expressed as mean ± SD. Statistical analysis: Mixed-effects model with Sidak's multiple comparisons test (ns $p>0.05$ and **** $p<0.0001$)

Evaluating immunotherapy in orthotopic model

Case study: ICTs efficacy in the orthotopic MB49 bladder cancer model

Study design

Intravesical instillation (i.ves.) of tumour cells: a non-invasive procedure



MB49-Luc cancer cells in C57Bl6/J mice (n=10/group)

body weight and bioluminescence

0 — 3 — 75 days

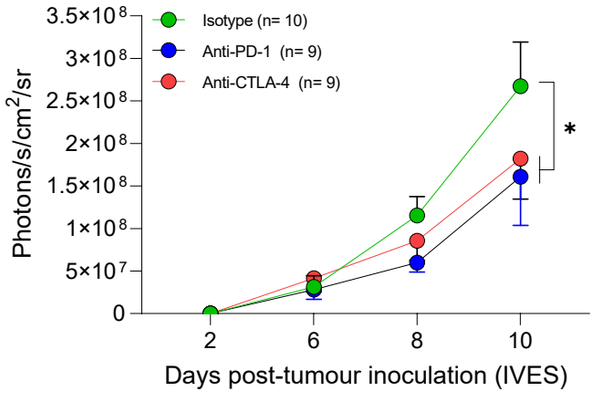
► **Cells inoculation (i.ves.)**

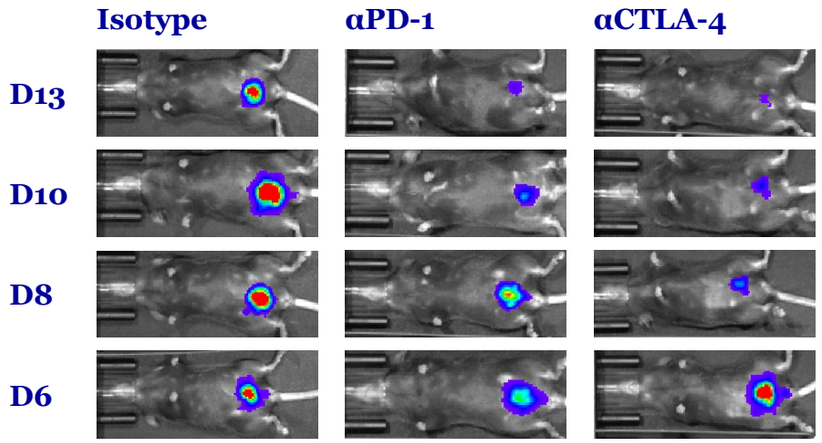
► **start treatment ICTs (i.p.), Q3dx4**

Endpoint tumour:
- Weight
- Immune cells infiltration (flow cytometry)

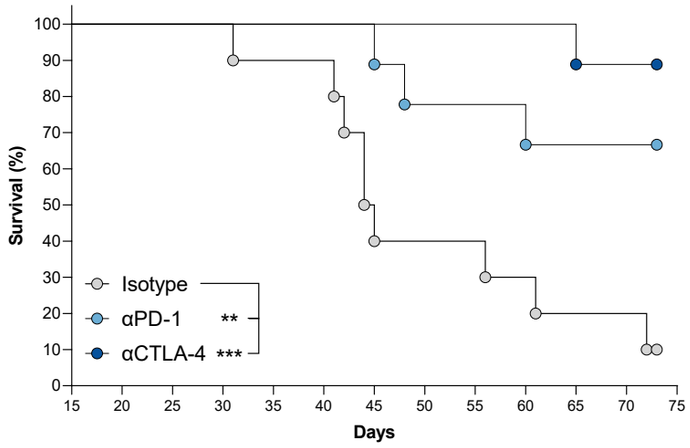
Orthotopic tumour models provide a clinically relevant, organ-specific TME. BLI allows non-invasive monitoring of tumour growth and real time tracking of tumour response

Tumour growth monitoring by bioluminescence(BLI) (mean ± SD and representative images[1])





Survival



1 for each group, follow up images of the same mouse are presented
i.ves.: intravesical; ICT: immune checkpoint therapy; TME: tumour microenvironment; BLI: bioluminescence
Statistical analysis: Mixed-effects model with Dunnett's multiple comparisons test on log transformed data vs Isotype group (** p<0.01) and Log Rank Test Mantel Cox vs Isotype group (** p<0.01 and *** p<0.001)

Mice humanised for the immune system (Humice)

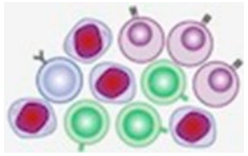
Mouse generation and providers

hCD34⁺ humanised mice model

BRGSF-His

- GenOway

BALB/c background: resistant to radiation



Functional human lymphoid and myeloid cell compartments

RAG2$^{-/-}$

No murine B- and T-cells

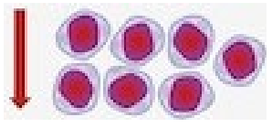
IL2Rγc$^{-/-}$

No murine NK cells

SIRPα^{NOD}

Inhibited murine macrophages

Flk2$^{-/-}$

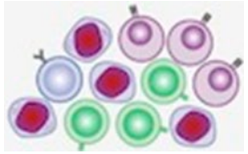


Strongly reduced myeloid cell compartment

NOD

- Taconic
- Janvier Labs
- Jackson Lab.
- Transcure (busulfan)

NOD background



Functional human lymphoid and myeloid cell compartments

NOD/Shi

Defects in:
Ag presentation, T cell repertoire, NK cell function, macrophage cytokine production

SCID

No murine B- and T-cells

IL2Rγc$^{-/-}$

No murine NK cells

hCD34 humanised mice	Providers	Genetic Backgrounds
BRGSF-his	GenOway	BALB/c
huNOG	Taconic	NOD
Hu-NCG	Transcure	NOD
huNXG	Janvier Labs	NOD
Hu-NSG	The Jackson Laboratory	NOD

Information given by the provider

- Donor ID
- Mouse ID
- Reconstitution in human leukocytes
- Reconstitution in human B-cells
- Reconstitution in human T-cells & subpopulations
- Week of engraftment

Parameters to be taken into consideration when designing the study

Note: illustrations obtained from Genoway website

Evaluate PD and efficacy in a human immune-tumour context

BRGSF-His mice model

Human immune cells can be activated *in vivo*

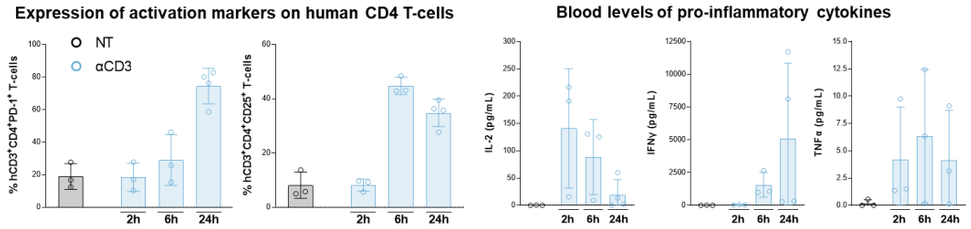
αCD3 PD model of *in vivo* T-cells activation in humanised mice (n=3/time point)

BRGSF-his mice (F, 24-week-old)

αCD3 (1 µg, i.p.)

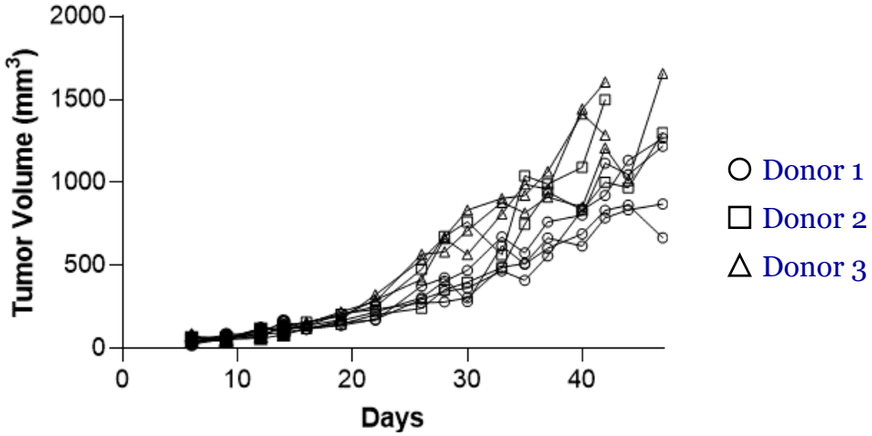
0 +2 +6 +24 hours

- Spleen T-cells activation (FACS)
- Cytokines blood level (MSD)



Full tumour take and homogeneous tumour growth

MDA-MB-231 TNBC model

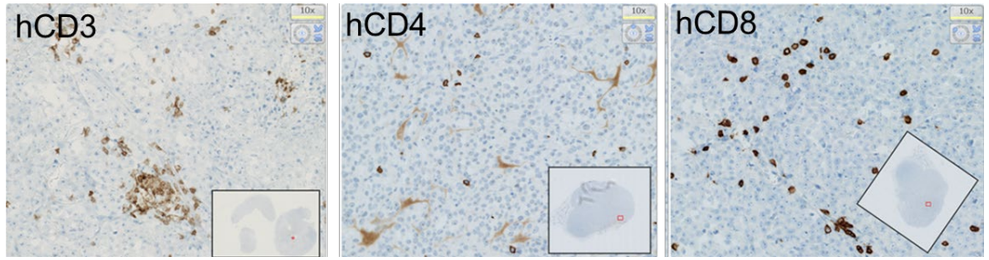


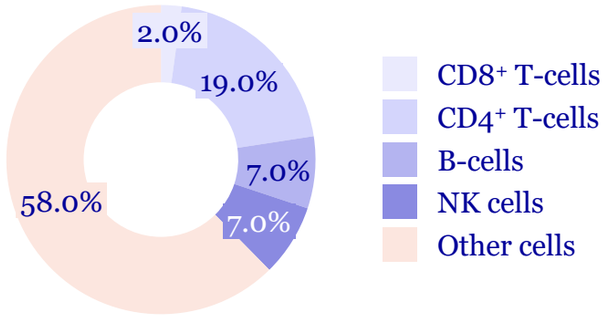
○ Donor 1
□ Donor 2
△ Donor 3

Similar results were obtained with A375 melanoma cells

Human immune cells infiltrate human tumours (IHC and FACS)

A375 melanoma model





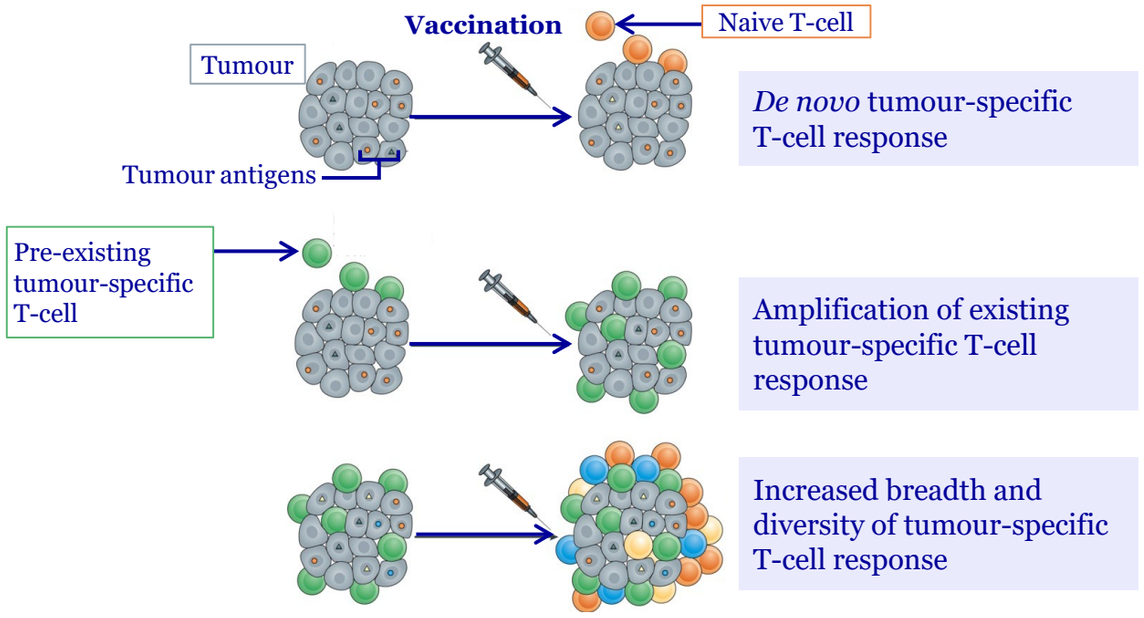
- $CD8^+$ T-cells
- $CD4^+$ T-cells
- B-cells
- NK cells
- Other cells

i.p.: intraperitoneal, PD: Pharmacodynamic, MSD: Meso Scale Discovery, FACS: Fluorescence-Activated Single Cell Sorting (flow cytometry), IHC: Immunochemistry and TNBC: Triple Negative Breast Cancer
Results are expressed as mean ± SD or individual data.

Therapeutic cancer vaccines

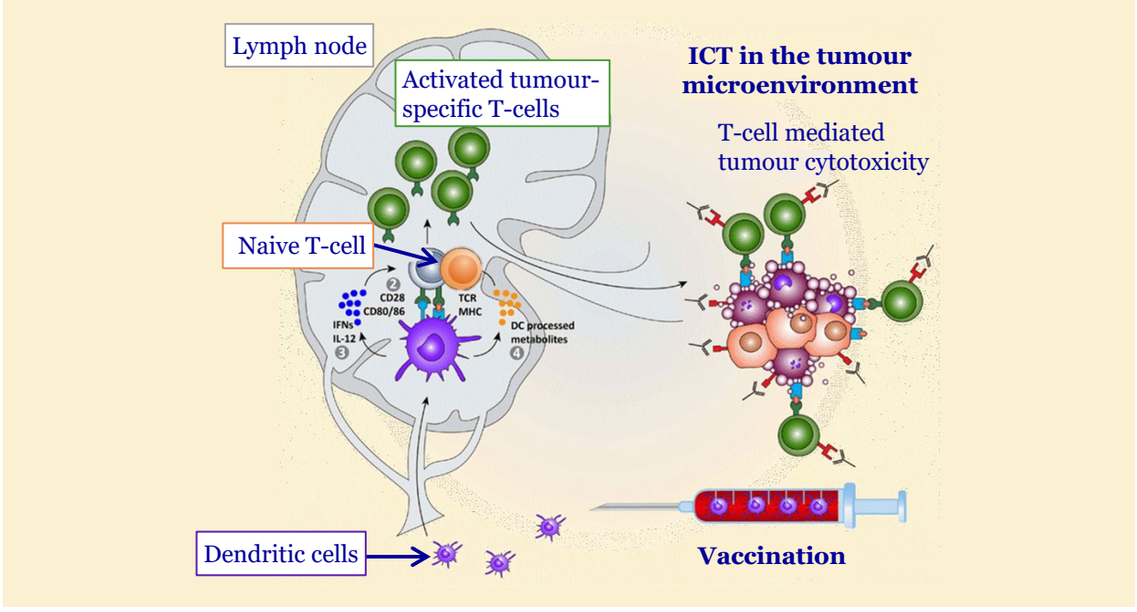
Vaccine immunogenicity: a critical step for antitumour response

A wide range of vaccination approaches to generate and/or amplify antitumour immunity



Cancer vaccines as effective partners in combination with ICT[1]

(potential to generate new tumour antigen-specific T-cell responses and amplify existing responses)



Regardless of the approach, efficacy of cancer vaccines relies on induction of an immune response to vaccination

1 given their potential to both generate new antigen-specific T-cell responses against tumour cells and amplify existing responses
Adapted from Zhuting Hu *et al., Nature Review Immunology,* 2018, **18**: 168, and Vasaturo Angela *et al., Frontiers in Immunology,* 2013, **4**:417

Immunogenicity assessment of vaccine candidates

Model to evaluate/optimize specific T-cell response to candidate vaccines

Study design

CD1 outbred mice
(n=8/group)

Immunization with candidates

0 → 14 days

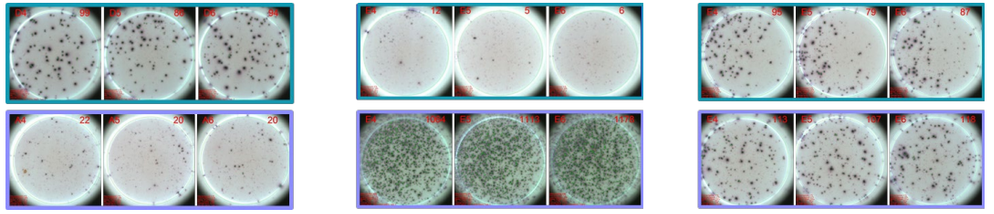
IFN-γ ELISpot
(on splenocytes)

Treatment	Admin. route	Restimulation
Vaccine_1 (Ag.A)	i.m	Pool Ag.A or Pool Ag.B
Vaccine_2 (Ag.AB)	i.m	
Vaccine_3 (Ag.B)	i.m	

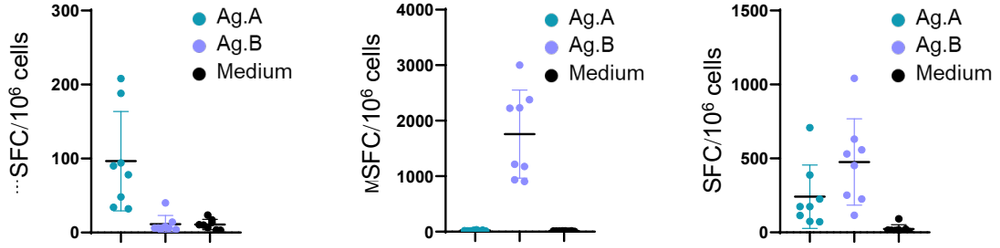
Vaccination induces development of Ag-specific CD8⁺ T-cells

IFN-γ producing CD8⁺ T-cells in response to a specific Ag:

Representative ELISpot images:



Quantification:
Results are expressed as median of triplicat IFN-γ secreting cells, measured as spot forming cells (SFC) per million splenocytes



i.m.: intramuscular, Ag: Antigen, SFC: Spot Forming Cell, ELISpot: Enzyme-linked Immune absorbent Spot
Results are expressed as mean ± SD.

#RESEARCHNEVERSTOPS

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