evotec

#RESEARCHNEVERSTOPS

Evotec

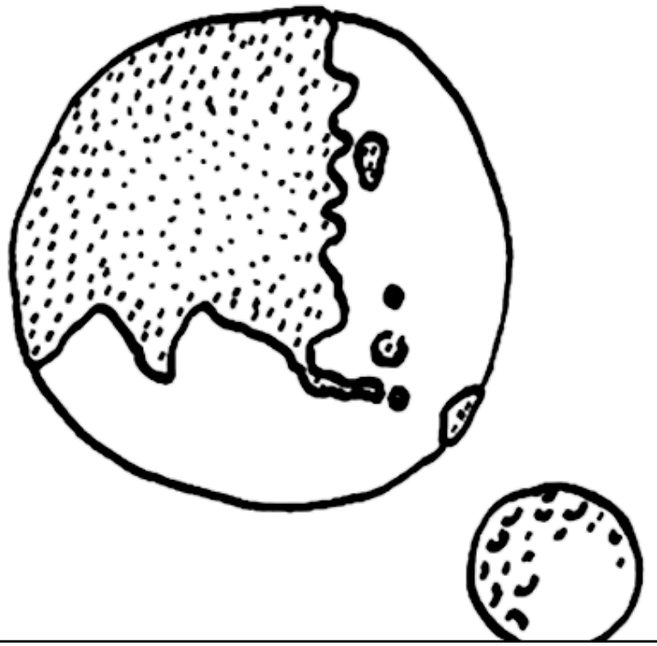
Translational Biology Department
In vivo expertise and capabilities

Oncology and Immuno-Oncology

Agenda

***In vivo* capabilities, PK and early tox platforms to support the projects**

In vivo Oncology and Immuno-oncology expertise



An experienced team of *in vivo* scientists

State-of-the-art animal facilities in Toulouse

- Group of over ≈100 scientists, 2 in-house veterinarians
- AAALAC-accredited animal facility
- Disease area expertise
  - ***Oncology and immuno-oncology***
  - ***Immunology and inflammation***
  - ***BSL3 infectious disease*** *(Tuberculosis, SARS-Cov-2 …)*
- Drug discovery and research services (non-GLP) include:
  - **PK** studies (supported by **formulation** assay/screening)
  - **PK/PD** and **efficacy evaluation**
  - **Early discovery toxicology**
    (MTD, TK, necropsy, histopathology & safety biomarkers)
  - **Biomarker** discovery and hypothesis testing/validation

➢ **Scientifically tailored approaches to discovery programs, continuous and interactive exchanges for decision making**

Area: ≈4,000 m^2 state-of-the-art animal facility
Mouse capacity: 46,440
Rat capacity: 5,400
Gerbils & hamsters: 1090
Rabbits: & Guinea pigs: 540

- Dedicated procedure & surgery rooms, drug preparation room
- X-ray irradiation and imaging

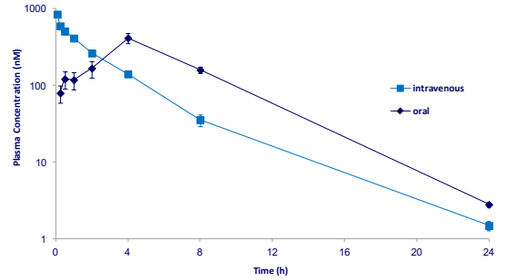
Fast & high quality pharmacokinetic data

Evotec PK platform

Obtaining PK data is a key requirement for the evaluation of compounds *in vivo*

- Data analysis using Phoenix® WinNonlin® 6.3 software
- Evotec can offer cassette PK screening of up to 5 compounds simultaneously
- Extensive Dx formulation experience to guide choice of vehicle
- Administration routes; intravenous (bolus and infusion), per oral, intraperitoneal, subcutaneous, intra cerebrospinal, intramuscular, pulmonary, intratracheal (nebulized, aerosolized, dry powder)
- Sampling types; jugular vein cannulation, cardiac puncture, tail vein micro-sampling, microdialysis
- Matrices; blood, plasma, CSF, BALF, tissues, bile, urine and faeces

Cycle time: 5 business days from compound receipt to data



PK Parameter	Intravenous	Oral
Dose (mg/Kg)	1.83 ± 0.04	4.07 ± 0.02
C_0 / C_{max} (ng/mL)	492 ± 25	206 ± 80
T_{max} (h)	-	4
V_{dss} (L/Kg)	6.9 ± 0.3	-
CL (mL/min/Kg)	34.6 ± 0.3	-
Liver Blood Flow (%)	48.1 ± 0.5	-
AUC_{inf} (ng.hr/mL)	880 ± 28	1248 ± 380
Bioavailability (%)	-	64 ± 0.2

Exploratory *in vivo* toxicity studies

Evotec early tox platform (non GLP) to derisk projects and explore safety ratio

Study design

- Rats or mice
- Control and 3 dose groups
- Male and female animals
- Daily/twice daily dosing (oral)
- Duration: 4-14 days

1	**General evaluation**	• Clinical signs • Body weight • Food consumption
2	**Clinical pathology**	• Hematology (RBC and WBC counts) • Blood clinical chemistry • Safety biomarker evaluation (or discovery)
3	**Toxicokinetics**	• Microsampling • Blood and tissue concentrations; • PK parameters (C_{max}, AUC, …)
4	**Pathology**	• Organ weight & macroscopic examination • Histopathology

Determination of

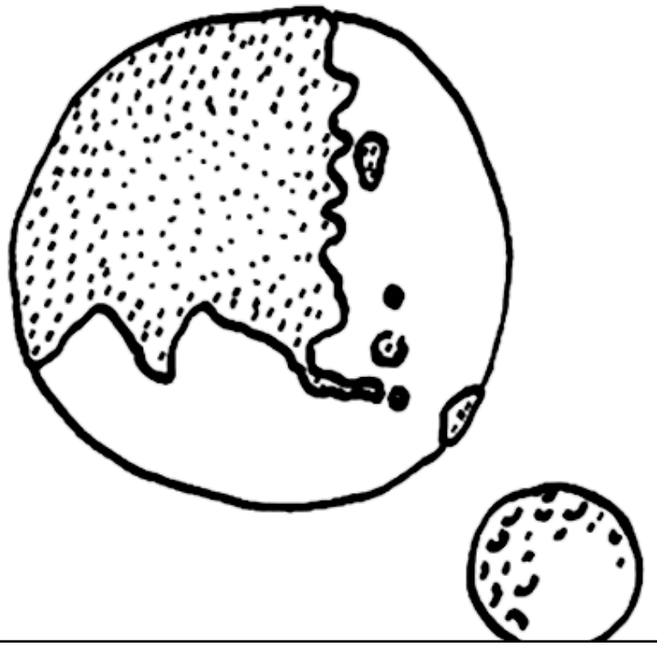
- Type and severity of injury
- MTD
- NOAEL
- Dose-exposure relationship
- Therapeutic index
- Reversibility

AUC = Are under the curve; MTD = maximal tolearted dose; NOAEL = no observed adverse effect level; PK = pharmcokinetics; RBC = red blood cells; WBC = white blood cells

Agenda

In vivo capabilities, PK and early tox platforms to support the projects

***In vivo* Oncology and Immuno-Oncology expertise**



Fully integrated Oncology drug discovery

Evotec Oncology platform

- Target validation / deconvolution
- RNAi/CRISPR screen
- Phenotypic screening

Target ID / validation

- Omics data mining
- Tumour microenvironment
- Cancer metabolism and targeted therapy resistance

- HTS / VS
- Rapid Fire™/MST
- *In silico* modelling
- Bioinformatics platform
- Medicinal chemistry
- ADME
- *In vitro* biology
- High content imaging

Drug discovery platform

- Global metabolomics
- Antibody discovery, humanisation and optimisation

- Biomarker discovery and validation
- Targeted proteomics and metabolomics
- IHC, FACS, MSD
- *In vivo* pharmacology (orthotopic, syngeneic)
- Formulation & PK
- Microdialysis
- Discovery toxicology

Clinical translation

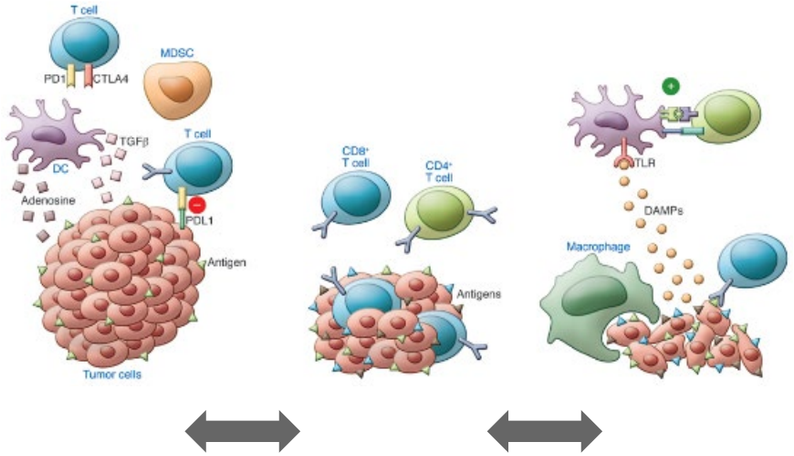
- Fresh tumour and blood access
- Biobank access
- *In vivo* imaging
- Histopathology
- Early clinical trial design
- Phase I sample access

Focusing on innovative targets with first-in-class potential

Evotec oncology themes

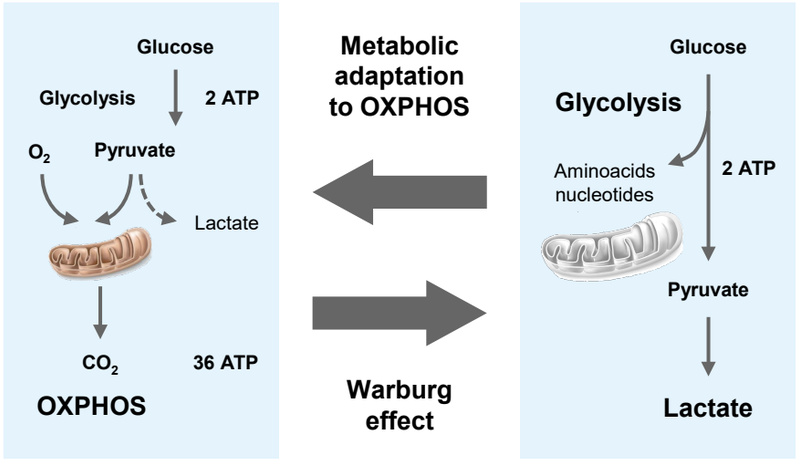
Tumour Microenvironment

Targeting for ex mechanisms of immune exclusion from the tumours



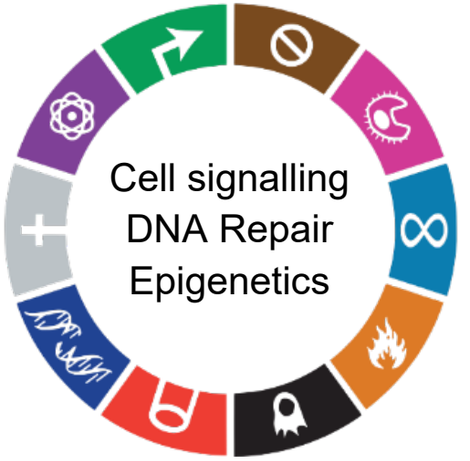
Cancer Metabolism

Targeting mechanisms of metabolic adaptation



Tumour survival and proliferation

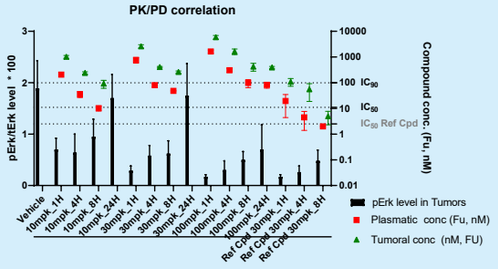
Targeting mechanisms underpinning tumour survival and proliferation

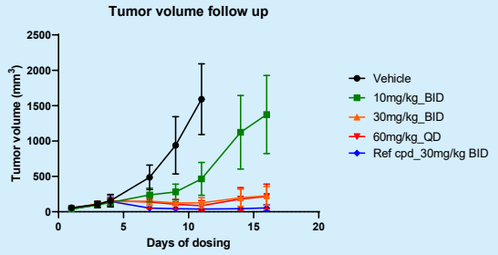


➢**For single agent therapies or combination with SoC therapies**

Oncology and immuno-oncology *in vivo*

A mix of proprietary assets and validated assays



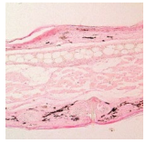


Mouse or rat tumor models	• **Human xenograft models:** s.c. & orthotopic models in **immunodeficient and humanized** mice • **Syngeneic models:** s.c. & orthotopic implantation of tumour cells in immunocompetent mice. suited for **immuno therapy & combination studies**
	• Tailored models with **PK and PD readouts** with biostatistical support – **Target engagement, biomarker or mechanism of action** studies – **Efficacy**
Specialised models	• ***in vivo* T-cell proliferation assay** • **Adoptive cell transfer** (in development) • **GvHD model** • **Chemo-induced alopecia** (hair loss)
Readouts	• **Sampling:** tumour, blood, urine, organs • **End points:** tumour size, haematology, clinical chemistry, phosphoprotein analysis (MSD Technology, Western blot, ELISA), mRNA analysis, flow cytometry, histology, immunohistochemistry • **Tumour imaging:** *in vivo* imaging (bioluminescence, fluorescence), X-ray imaging, Laser doppler, quantitative image analysis • **Compound blood exposure:** Bioanalysis (Mass spectrometry; ELISA for Biologics) • **Early toxicology:** blood biochemistry, haematology, organ specific & target specific safety biomarkers

Clinically relevant readouts for *in vivo* studies

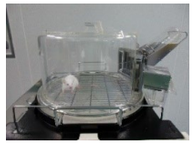
Technological resources for biomarker discovery and validation

Organ and fluid collection


Mouse ear (von Kossa)

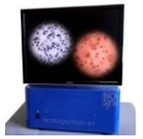

Rat caecum

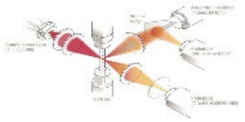

Blood, plasma


Metabolic cages

Flow cytometry and haematology

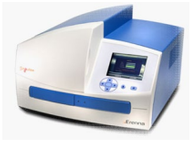

Flow cytometry (FACS Canto II, Aria…)


ELISpot



Procyte Dx Haematology analyzer

Gene/mRNA/protein analysis


Viia7, QuantStudio


Singulex


Automated Western Device (Jess)


MSD technology ELISA

Histology, IHC, ISH (RNAscope), Duolink PLA, Visiopharm


Ventana Automated histoembedder


Rotary microtome


Roche Ventana Benchmark Discovery Ultra


Digital slide scanner (Nanozoomer)

In vivo imaging systems


Bioluminescence
Fluorescence
3D tomography
(IVIS Spectrum)

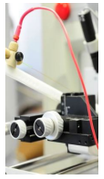

X-ray imager
(Faxitron MX20)

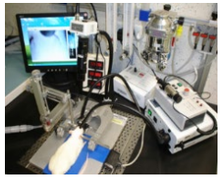

Laser Doppler
(MoorLDI2)

CREFRE
Imaging

Mass spectrometry


Bioanalysis

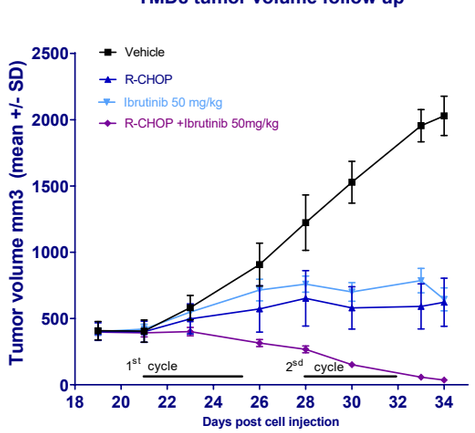

Proteomics

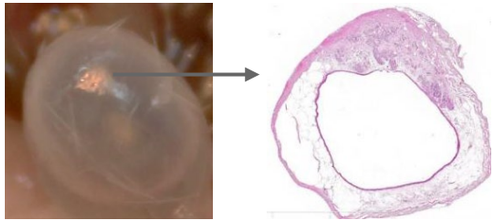

Metabolomics /
Microdialysis

Human and murine tumor models

Development of cell line-derived models on demand

- **XENOGRAFT tumour models**
  - Choice of the cancer cell type: based on therapeutic indication, molecular profile, *in vitro* work...
  - In immunocompromised or humanized mice or rat
- **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
- **Examples of cancer cell lines already worked on** U87 (glioblastoma), H520, H1299, H460, NIH-H1417, NIH-H69 and DMS114 (Lung), HT-1080 (fibrosarcoma), HCT116 (colon), KYSE-520 (esophageal SCC), lymphoma (TMD-8, OCI-LY3), RT112 (Bladder), HEP3B & HEPG2 (hepatocarcinoma), MV4;11 (acute leukemia), TMD8 (lymphoma)…
- **PDX models outsourced on demand**



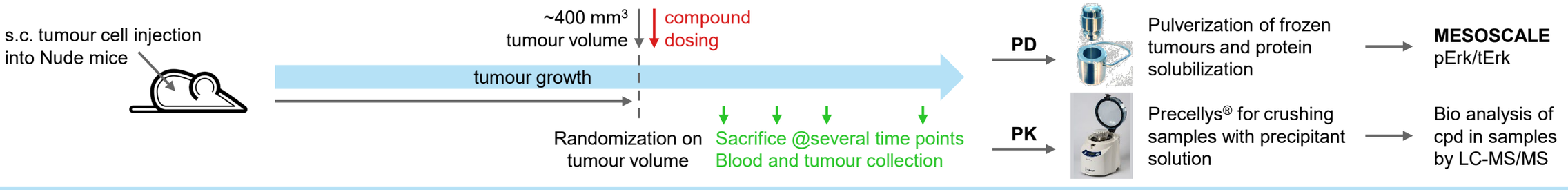


TCC97-7 tumour in urothelium

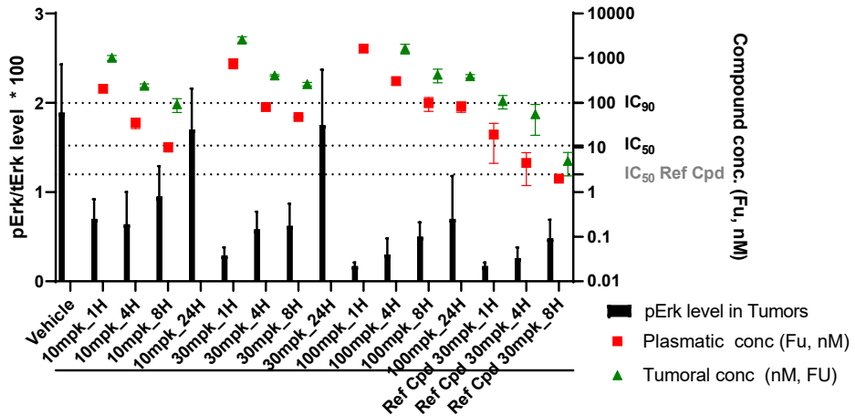
- **SYNGENEIC tumour models**
  - Integrity of the tumour microenvironment (immune populations, stroma/fibroblasts)
  - Responses to standard of care therapy and immune checkpoint therapies (PD-1, CTLA4 and PDL-1) available for combination studies
- **Examples of cancer cell lines worked on (s.c. or orthotopic)**
  - Breast 4T1; EMT6 (mammary fat pad)
  - Colon CT26 & MC38
  - Lung LL2 (transpleural implantation)
  - Skin B16-BL6 (epidermis)
  - Kidney Renca-Luc (renal capsule)
  - Ovary ID8 (i.p.)
  - Leukemia C1498, lymphoma E.G7-OVA
  - Fibrosarcoma MCA205…

Case study: PK/PD study in tumour-bearing mice

pERK levels in tumours modulated by a compound targeting a RTK upstream the Erk signaling



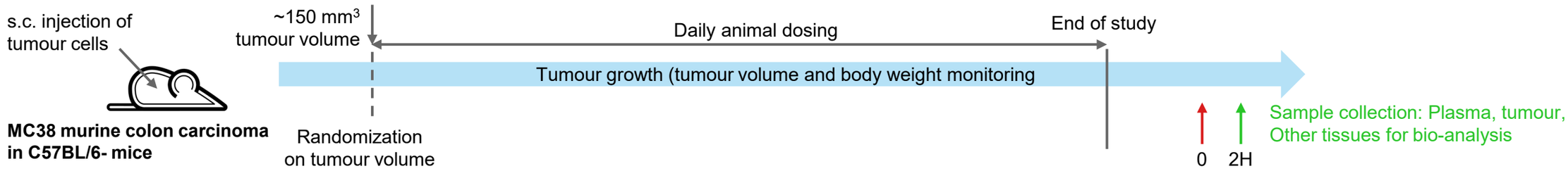
PK/PD correlation



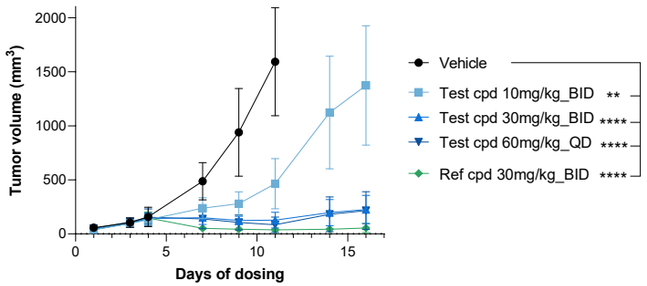
Direct PK/PD correlation established between the inhibition of the downstream signalling pathway of the target and the concentration of the compound

Pre-clinical efficacy study in oncology

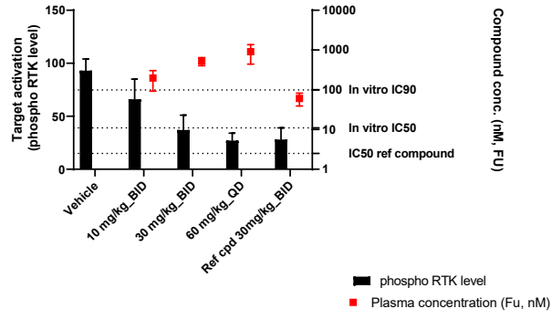
Tumour xenograft model and dose response of an inhibitor (smol targeted therapy)



Tumour volume follow up



Target engagement in tumours 2 hours post last dosing



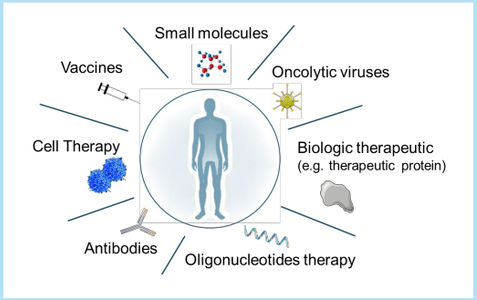
Tumour growth inhibition observed at the higher doses after chronic dosing is correlated with the target engagement of the compound in the tumours and the compound concentrations

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)

Immuno-oncology

From therapeutic efficacy to Mode of Action (MoA)

Your therapeutic strategy



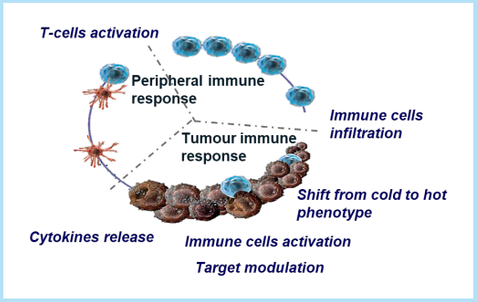
Model adapted to your need

- **Syngeneic** mouse tumour models
  - Featuring **full murine immunity** and comprehensive stroma
  - **Reduced cost / Rapid** (suitable when large group numbers is required)
- **Humanized** mouse tumour models
  - Opportunity to assess immunotherapy efficacy and pharmacodynamics in a **human immune-tumor context**

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

Broad range of sample analysis (blood, urine, organs and tumours)

MoA of your candidate



Tailored *ex vivo* assays

- ➢ **Analysis of the tumor micro-environment:** flow cytometry, IHC
- ➢ **Functional assays with immune cells:** proliferation assay, ELISpot, flow cytometry
- ➢ **Analysis of cytokines release:** MSD & HTRF technology, ELISA
- ➢ **Analysis of phosphoproteins:** MSD & HTRF technology, western blot, ELISA
- ➢ **Analysis of gene signature:** qRT-PCR, RNAseq
- ➢ **Custom assay development**

IHC = Immunochemistry
MSD & HTRF = Meso Scale Discovery & Homogeneous Time Resolved Fluorescence

Syngeneic mouse model expertise

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

Indication	Cell line	Mouse strain	Immune CheckPoint Therapy (ICT) response (based on T/C ratio)			
Colorectal	CT26	BALB/c	αPD-1	–	αCTLA-4	αPD-1+αCTLA-4
	MC38	C57Bl6/J	αPD-1	αPD-L1	αCTLA-4	αPD-1+αCTLA-4
Breast	4T1	BALB/c	αPD-1	–	αCTLA-4	–
	EMT6	BALB/c	–			
Fibrosarcoma	MCA205	C57Bl6/J	αPD-1	αPD-L1	–	
Lymphoma	EG7-OVA	C57Bl6/J	–	αPD-L1	–	
Pulmonary	LL2	C57Bl6/J	–			
Melanoma	B16	C57Bl6/J	–			
Renal cell carcinoma	Renca	BALB/c	–			
Ovarian	ID8	C57Bl6/J	–			

High Intermediate No responder

Cell Quality Control
(performed before each inoculation)

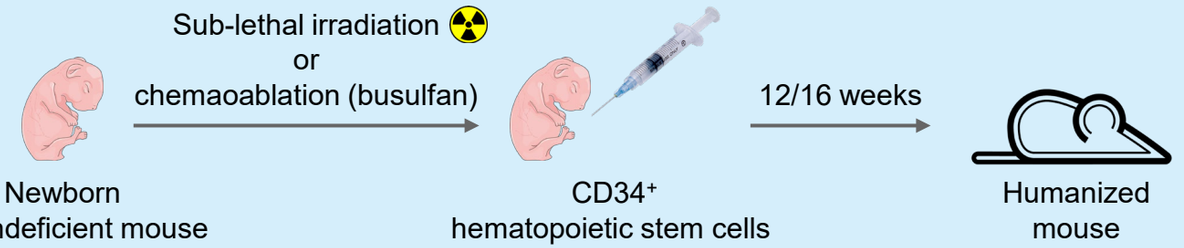
- Test for mycoplasma contamination (PCR)
- Cell count and size distribution
- Essential surface markers expression: MHC-I and PD-L1 expression (flow cytometry)
- Control doubling time

Some cell lines require license purchase

Humanized mice for the immune system (in development)

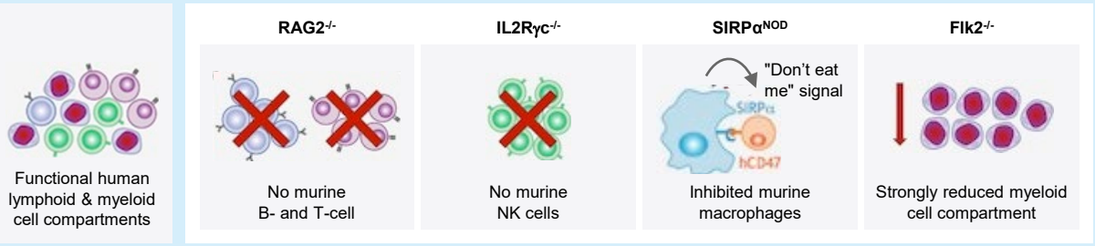
Mouse generation and providers

hCD34⁺ humanized mice model



BRGSF-His
- GenOway

BALB/c background: resistant to radiation



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

NOD background

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

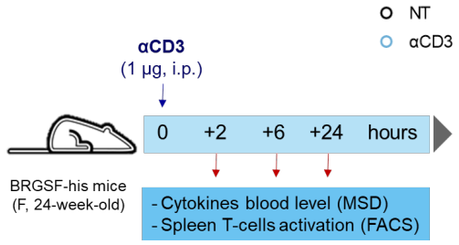
Pictures from Genoway website

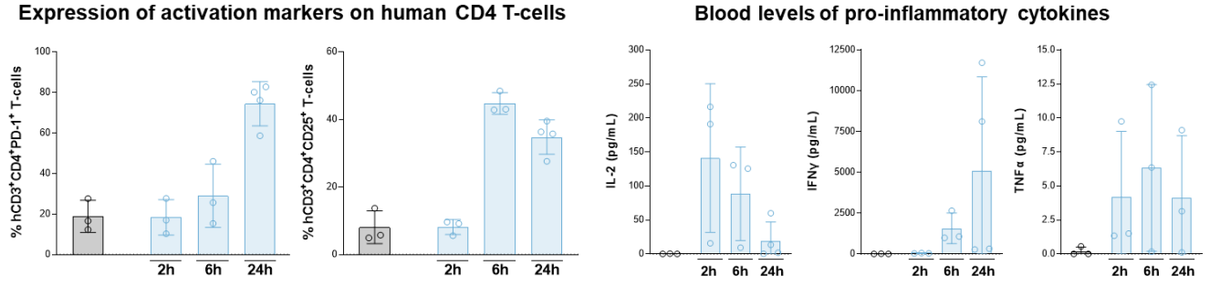
Human tumour-immune context in BRGSF-His humanized mice

Opportunity to assess PD and immunotherapy efficacy in a human immune-tumor context

Human immune cells can be activated *in vivo*

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

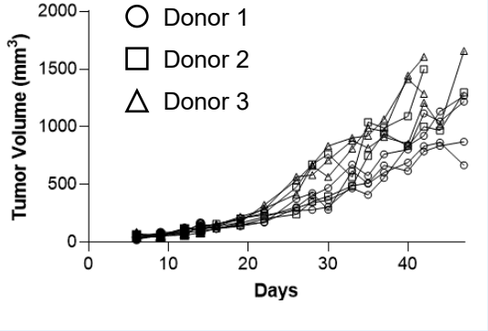




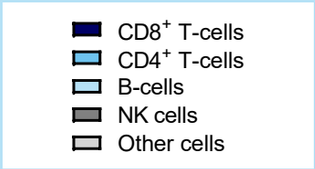
Full tumour take and homogeneous tumour growth

MDA-MB-231 TNBC model

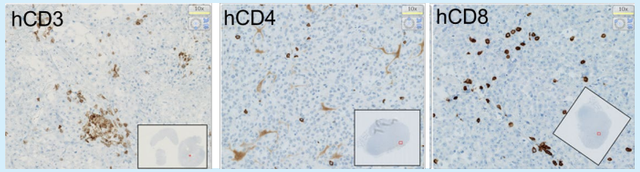
Similar results were obtained with A375 melanoma cells



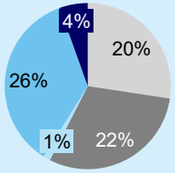
Human immune cells infiltrate human tumours (IHC and FACS)



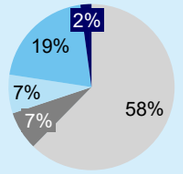
A375 melanoma model



MDA-MB-231



A375



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.

Technical expertise: immune response profiling

Off-the-shelf or custom cytometry panel to fit your needs

Flow cytometry at Evotec

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

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

Biorad ZE5
30 parameters (5 lasers)
Plate sampler (96 & 384) and tube loader

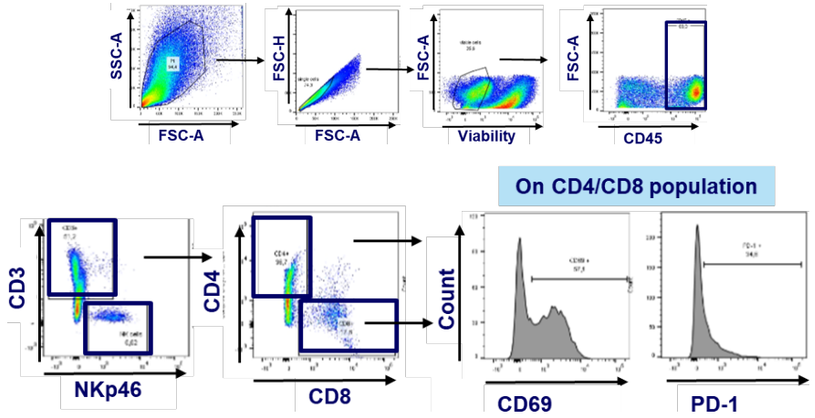
BD Symphony
30 parameters (5 lasers)
Plate sampler (96 & 384)

autoMACS Pro separator
Automated cell isolation
Magnetic cell separation

From simple to complex pheno-typing of tumour infiltrating immune cells

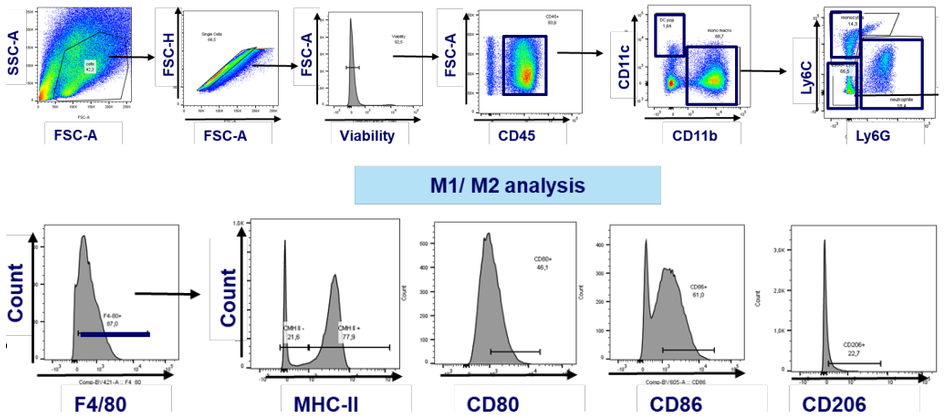
Flow panel looking at lymphoid cells

CD45, CD3, CD4, CD8, CD19, CD25, CD69, CD62L, CD44, PD-1, NKP46



Flow panel looking at myeloid cells

CD45, F4/80, Ly6G, Ly-6C, CD11b, CD11c, MHC-II, CD206, CD80, CD86



Capabilities in histological processing and analysis

Different workflows tailored to customer needs

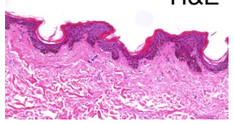
Classical Histology

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

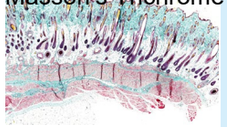
Sakura Tissue Tek

SLEE autostainer

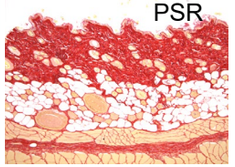
H&E



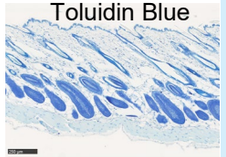
Masson's Trichrome



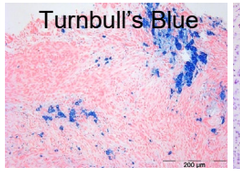
PSR



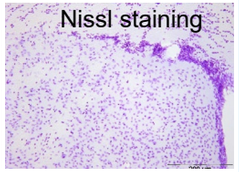
Toluidin Blue



Turnbull's Blue



Nissl staining



Tissue sample preparation: FFPE and frozen blocks

Dehydration

Paraffin embedding

Vibratome

Microtome

Cryo-Microtome

Cryostat

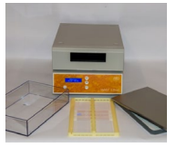
Tissue slicing

Immunohistochemistry

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

In situ hybridization

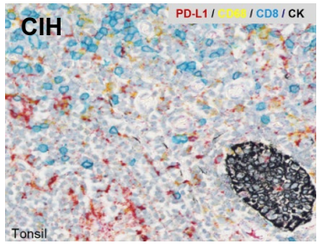
- **RNAscope:** up to 3 probes simultaneously
- Also possible in combination with IF
- **Basescope** and miRNAscope

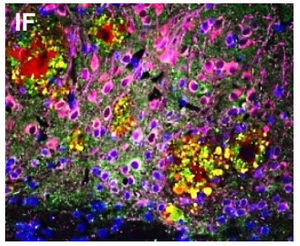


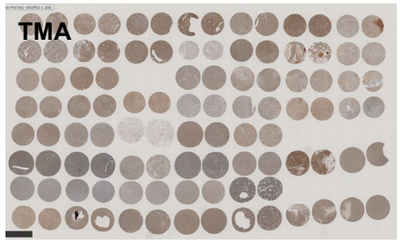
ACD-Bio ISH system

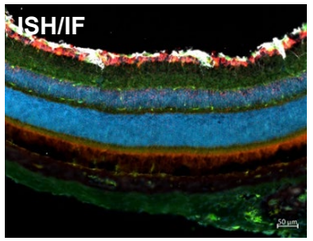
Leica BondRX

Ventana autostainers Ultra/XT





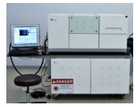




Scan & Digital Analysis

- **Scan:** bright field or fluorescence; confocal image acquisition
- **Quantification:** machine learning; tailor made image analysis using Visiopharm or Acapella

Opera



Operetta

Zeiss Axioscan

OperaPhenix

Manual microscopes

Histopathology

- Veterinary pathologist expertise
- Automated image analysis (Visiopharm®)
- Clinicial pathologist consultancy for clinical patients assessment

evotec

#RESEARCHNEVERSTOPS

Your contact:

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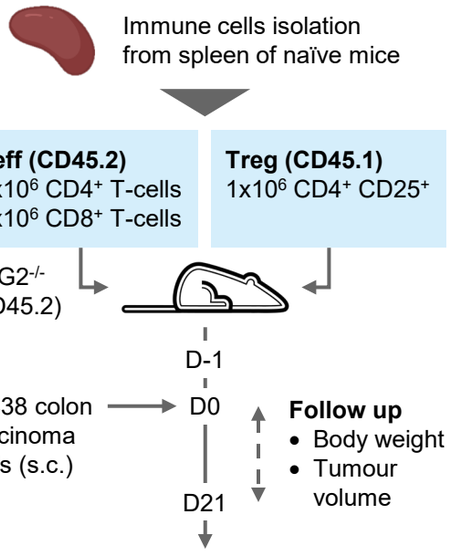
Frédérique Dol-Gleizes, PhD
Group Leader, Head *in vivo* Pharmacology group
frederique.dol-gleizes@evotec.com

Technical expertise: Adoptive Cell Transfer

Know-how in establishing models for *in vivo* target validation or cell therapy

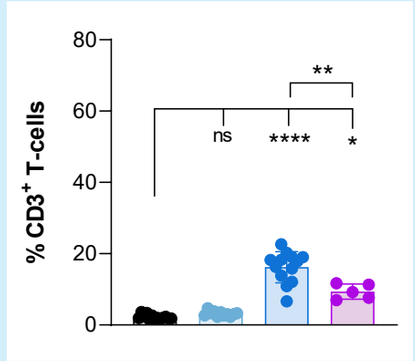
Protocol



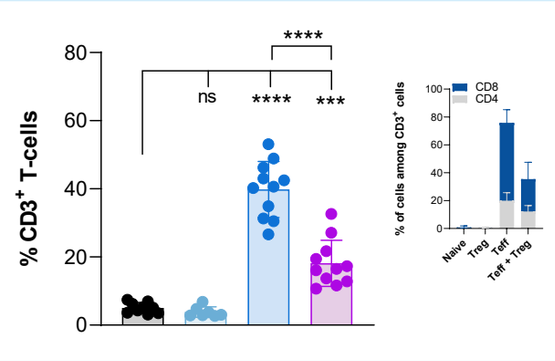
Effector T-cells induce high CD8⁺ T-cell infiltration into the tumour

Addition of Treg reduces tumour T-cell infiltration

Splenocyte reconstitution



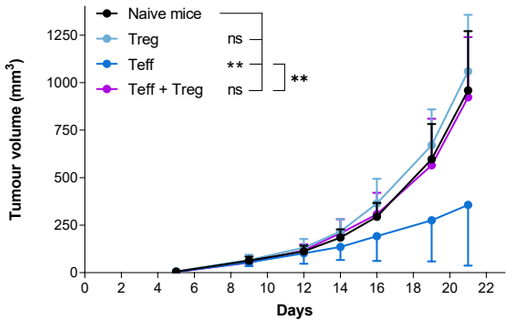
Tumour T-cells infiltration

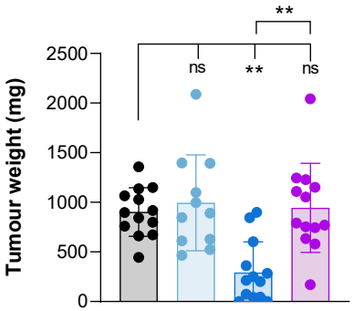


Effector T-cells control tumour growth

Addition of Treg abrogates tumour growth control by effector T-cells

Tumour growth





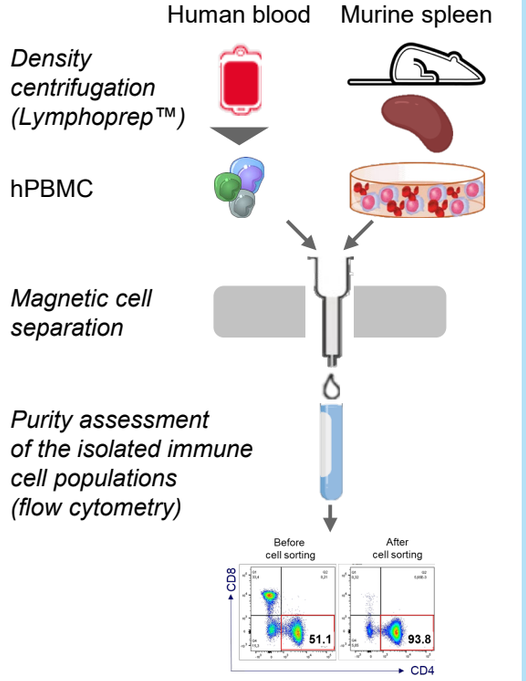
Naive Treg Teff Treg+Teff

Results are expressed as mean ± SD.
Statistical analysis: Mixed-effects model, Kruskal-Wallis test or Brown-Forsythe ANOVA test with Tukey's, Dunn's or Dunnett's T3 multiple comparisons test, respectively
(ns p>0.05, ** p<0.01, *** p<0.001 and **** p<0.0001)

Technical expertise: Isolation of immune cell populations

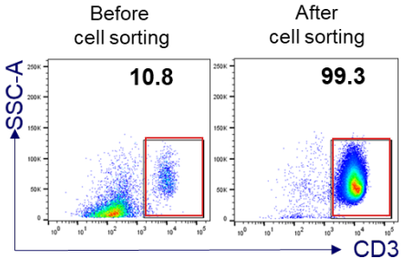
Cell sorting of immune cell populations for *ex vivo*/*in vivo* functional assays

Protocol

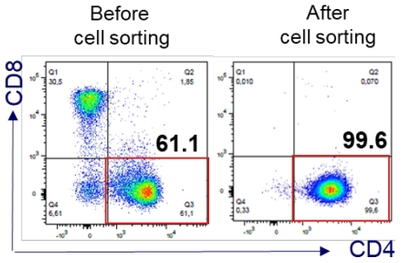


Isolation of highly pure immune cell populations allows *ex vivo* or *in vivo* functional evaluation of the sorted cells

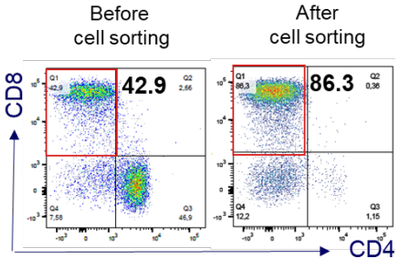
CD3⁺ T-cell sorting on hPBMC



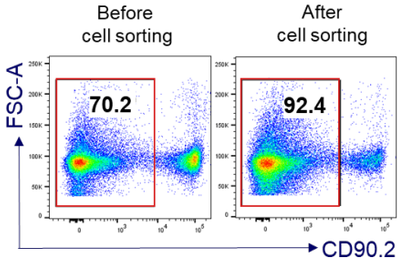
CD4⁺ T-cell sorting on hPBMC



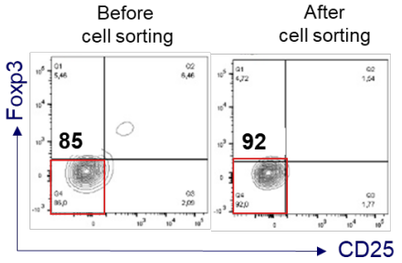
CD8⁺ T-cell sorting on hPBMC



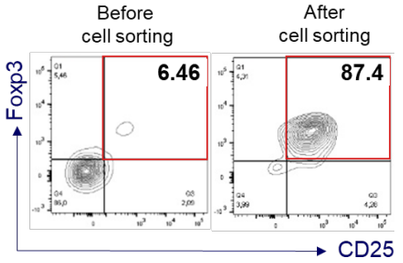
APC (CD90.2⁻) cell sorting on naive mice



T effector (CD4⁺ CD25⁻) cell sorting on naive mice



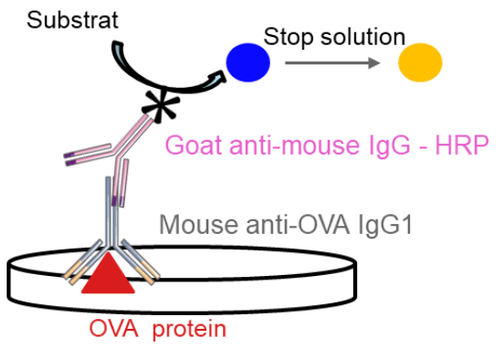
T regulator (CD4⁺ CD25⁺) cell sorting on naive mice



Technical expertise: Custom assays development

In house ELISA development to palliate lack or insensitivity of commercially available kits

Protocol

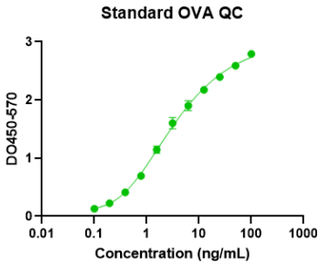


The in house ELISA is more sensitive than commercially available kits

Example: ELISA development for quantification of specific mouse IgG anti-OVA

When the project was being drawn up, commercially available kits exhibited a sensitivity of only 3 ng/mL.

Matrice: Kit buffer	STANDARD Mouse anti-OVA IgG1 Sigma SAB5300165 clone 3G2E1D9 - 5PL										
	Theoretical concentration (ng/mL)	Calculated concentration (ng/mL)			Mean concentration (ng/mL)	Replicate Accuracy (%RE)			Mean Accuracy (%RE)	Precision (%CV)	Comments
STD1	100,00	107,703916			107,70	7,70	100,00	100,00	7,70	#DIV/0!	
STD2	50,00	55,8108791	42,5081742	41,24943212	46,52	11,62	-14,98	-17,50	-6,95	17,34	
STD3	25,00	20,0377852	23,3750248	21,70352529	21,71	-19,85	-6,50	-13,19	-13,18	7,69	
STD4	12,50	11,9208955	10,6602203	12,13843957	11,57	-4,63	-14,72	-2,89	-7,41	6,90	
STD5	6,25	7,25697831	6,46535508	5,136749516	6,29	16,11	3,45	-17,81	0,58	17,04	
STD6	3,13	4,07689705	3,57528384	2,897214546	3,52	30,25	14,23	-7,44	12,35	16,84	Valid
STD7	1,56	1,75019931	1,59427169	1,419062846	1,59	12,19	2,20	-9,03	1,78	7,80	
STD8	0,78	0,77135146	0,73599712	0,647836624	0,72	-1,11	-5,64	-16,94	-7,90	8,68	
STD9	0,39	0,34148632	0,3732771	0,450708431	0,39	-12,44	-4,29	15,57	-0,39	14,46	
STD10	0,195	0,17033945	0,22490621	0,22310057	0,21	-12,65	15,34	14,41	5,70	15,04	
STD11	0,1	0,04852362	0,07894588	0,152480362	0,09	-51,48	-21,05	52,48	-6,68	57,28	



	Theoretical concentration (ng/mL)	Calculated concentration (ng/mL)		Mean concentration (ng/mL)	Mean Accuracy (%RE)	Precision (%CV)
Control +	5,00	4,40536202	5,28858295	4,85	-3,06	12,88

Upper and lower limit of detection (ULOQ and LLOQ) were determined at 50 and 0.195 ng/mL respectively.

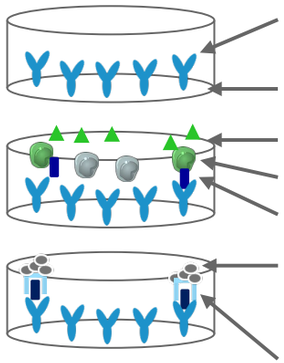
Technical expertise: Monitoring of effector T-cells functionality

IFN-γ ELISpot for detection of low-level T-cells responses

Protocol

The enzyme-linked immunospot (ELISpot) is a sensitive technique for the detection of cytokine-producing cells at the single cell level.

This assay permits the direct enumeration of low-frequency antigen-specific T-cells.

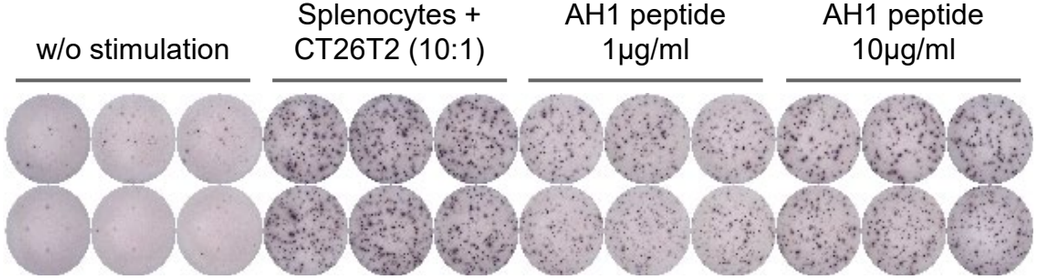


These data indicate a relationship between tumour size and the level of the tumour-specific CD8⁺ T-cell response

Example: CT26 tumour-specific CD8⁺ T-cell responses in αPD-1 mAb-treated mice (at D21)

Representative ELISpot images:

AH1 = immunodominant antigen of CT26 cells



IFN-γ ELISpot results/ tumour volume correlation:

Each dot represents an individual mouse

SFC: Spot Forming Cells