Optimize *In Vivo* research with the right humanized mouse model

Symposium TACONIC BIOSCIENCES

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Use of Humanized Immune System Mice in Immuno-oncology research and beyond

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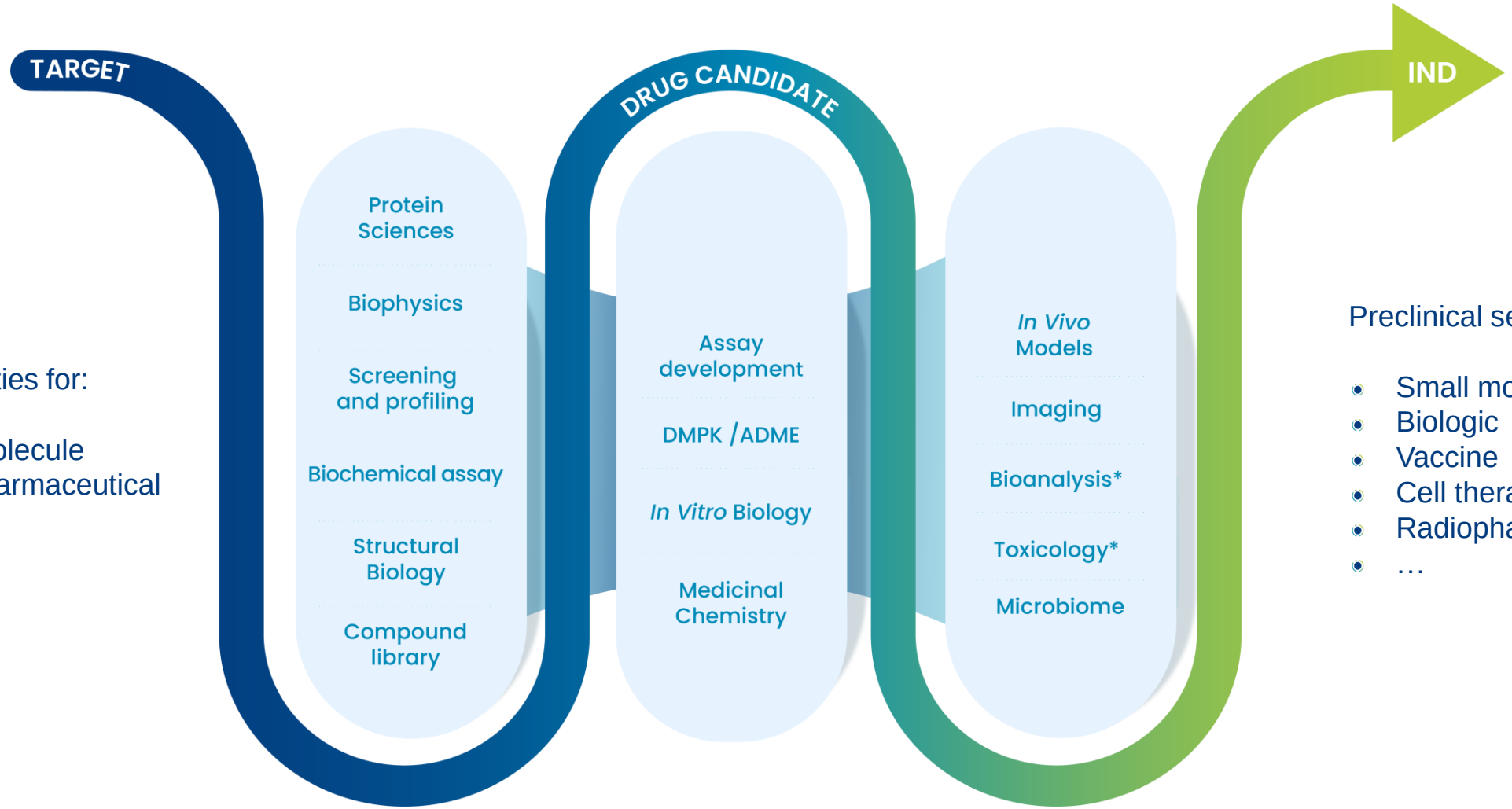
* Acquisition of ZoBio in 2024

A CONTINUUM OF EXPERTISE FROM TARGET TO IND

Full capabilities for:

- Small molecule
- Radiopharmaceutical



Preclinical services for:

- Small molecule
- Biologic
- Vaccine
- Cell therapy
- Radiopharmaceutical
- …

* GLP & non-GLP

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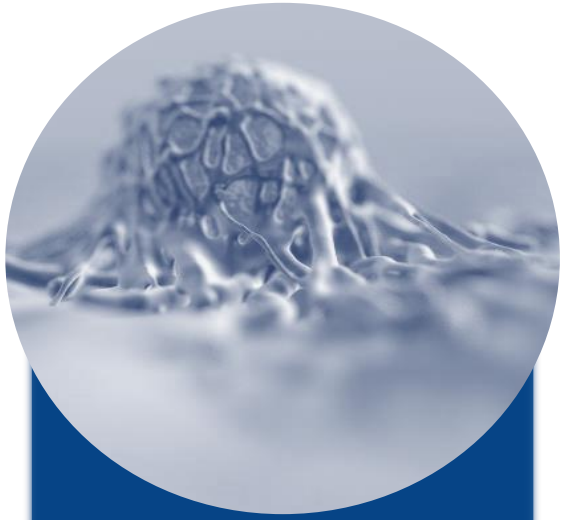
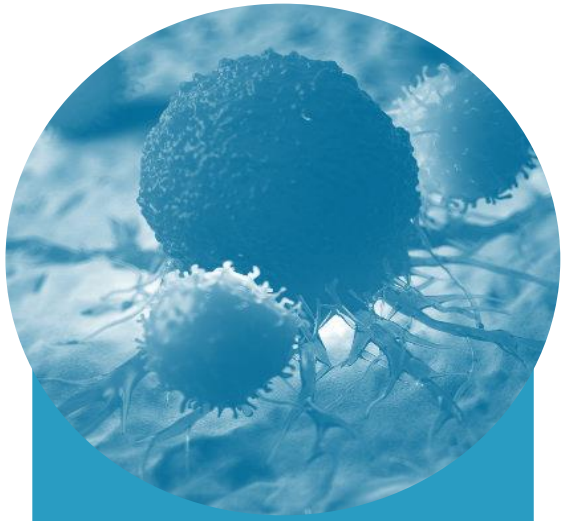
WITH A STRONG EXPERTISE IN PHARMACOLOGY

A large collection of models positioned on therapeutic areas with high medical needs



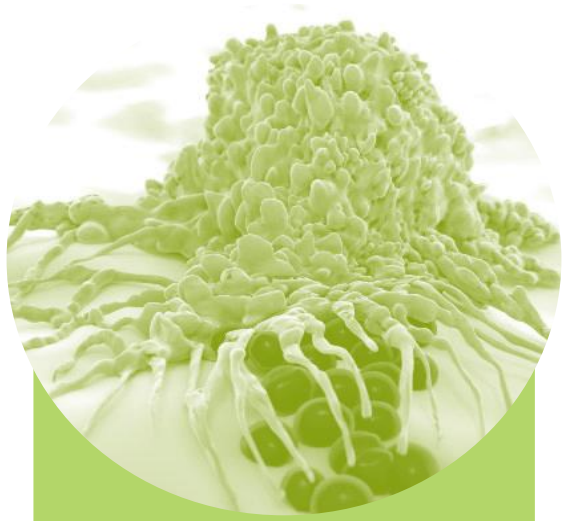
ONCOLOGY

All cancer types
PDX, CDX,
Syngeneic models
Orthotopic models
….



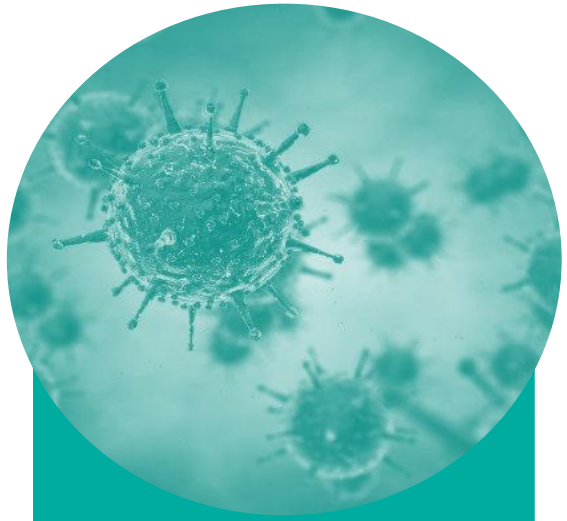
IMMUNO-ONCOLOGY

CAR-T cells
Oncovirus
Bispecific antibodies
Checkpoint inhibitors
…



INFLAMMATION

Fibrosis
Psoriasis
Asthma
Colitis
GvHD
…



INFECTIOUS DISEASES

Covid-19
HIV-1 and -2
Respiratory syncytial virus
Arboviruses…

Humanized mice modeling services

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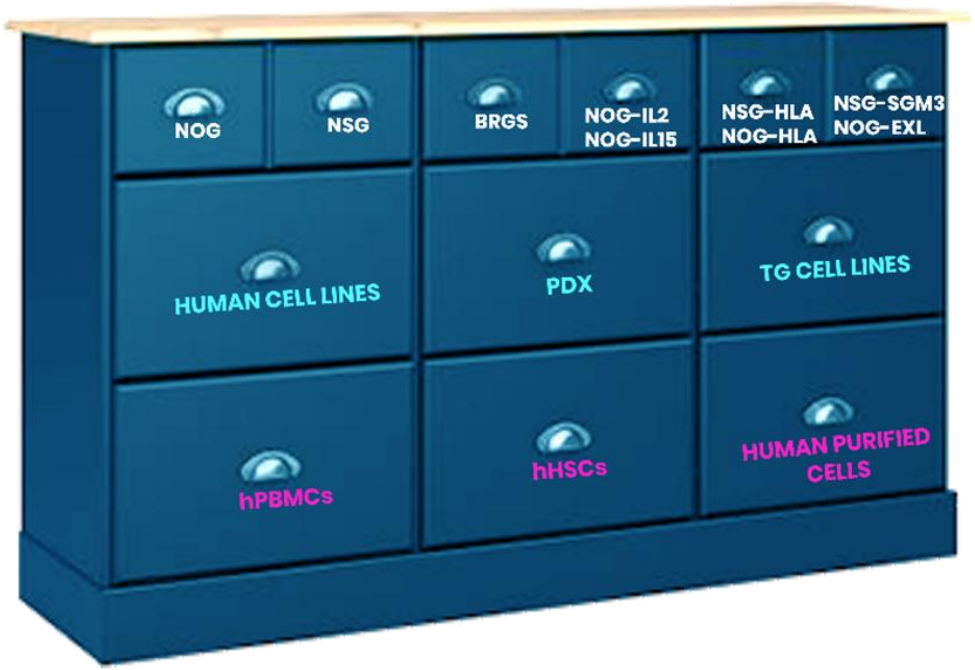
Animal models for preclinical evaluation of immunotherapies

Why use humanized mouse models?

- **Animal models: useful to study the complex biological processes that underlie normal human physiology and disease**
- **Mouse: most commonly used model organisms for biological research**
- **Immunocompetent syngeneic models have been useful in preclinical evaluation of immunotherapies**
- **But biological differences between mice and humans: limiting factors**
- **Need for animal models recapitulating more accurately features of human biology, modeling the interaction between the human immune system and disease such as cancer:**
  - Various methods developed to « humanize » mice
  - Mouse genetically modified to carry human transgenes
  - and/or engrafted with human cells or tissues
- **HERE: Humanized mice = immunodeficient mice engrafted with human immune components combined or not with other target cell populations (e.g. tumor cells)**

A personalized toolbox with expanding options

- **No longer an exotic tool, tailor-made humanized mouse solutions are available in different formats for different purposes**
- **Strong added value for the evaluation of human-specific immuno-therapies**



- **State-of-the-art immuno-deficient hosts**
  - NSG, NCG, NOG, BRGSF
  - NSG-SGM3, NOG-EXL, BRGSF / BRGSF-A2, NOG-FcγR KO
- **Applicable to a variety of oncology models**
  - Established cell lines from our catalog
  - Hematologic malignancies
  - Patient Derived Xenograft
- **Multiple humanization strategies**
  - PBMC
  - Isolated subsets : NK cells, T cells…
  - Hematopoietic Stem Cells (hCD34+)
- **Experience that goes beyond oncology**
  - GvHD studies
  - Antigen recall response
  - Cytokine secretion assays
  - Inflammation diseases
  - Safety assessment (i.e. CRS)
  - **Pros and cons to take into account**

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Adoptive transfer models: PBMC-engrafted mice

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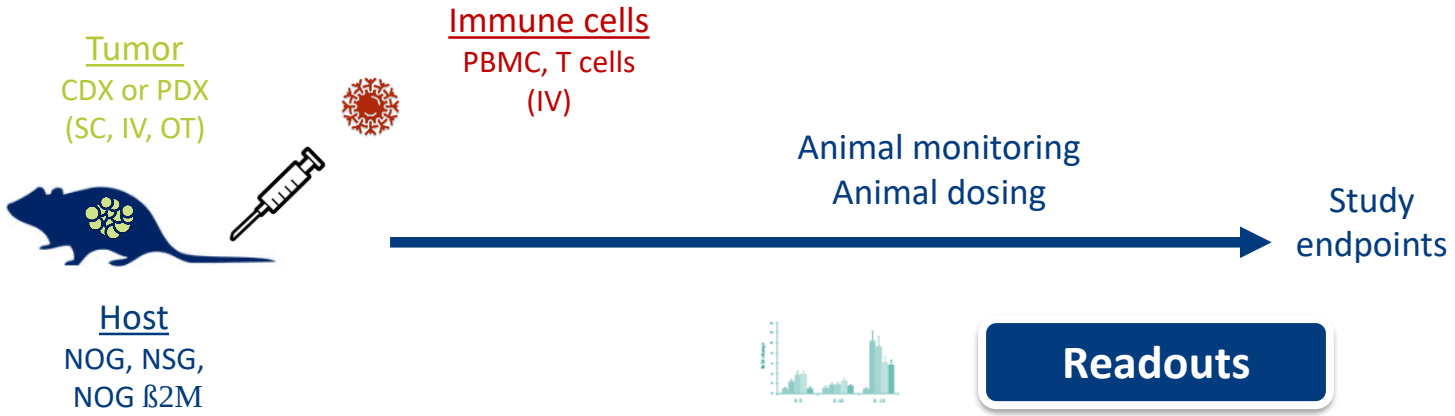
PBMC-engrafted mice for IO research

Representative study design



Readouts

- **Body weight and tumor volume**
- **Survival time and clinical scoring**
- **Flow cytometry**
- **Biomarker/target monitoring**
- **Histology**
- **Imaging (BLI)**

- Synchronization of humanization/tumor growth
- Donor-to donor variation in tumor response and GvHD onset
- Frozen PBMC are available to re-use same donors across multiple experiments

Primary T cell engraftment

Appropriate model for
BiTEs and T cell engagers

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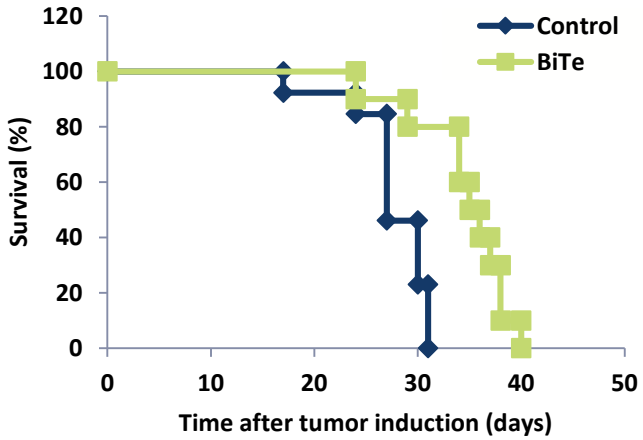
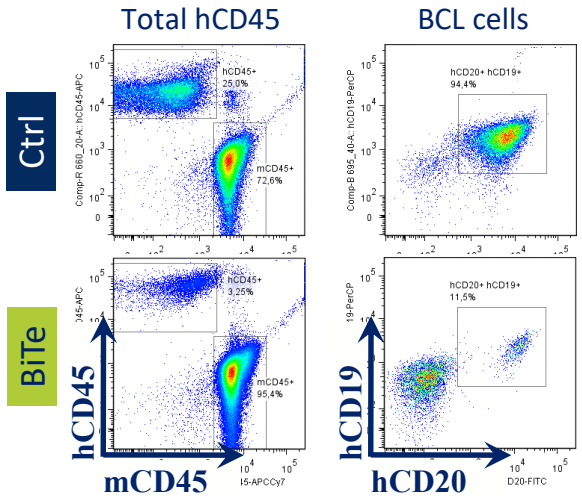
PBMC-engrafted mice for evaluation of Bi-specific T-cell engager

- Case study example (IV tumor model)
- BiTE in humanized mice implanted with B Cell lymphoma

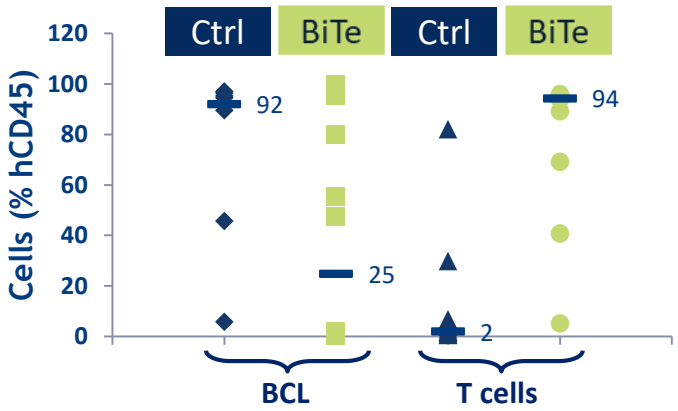
Survival increase



Tumor cell depletion



Immune cell recruitment



flow cytometry analysis (bone marrow samples)

- Death of mice is related to tumor development
- Efficacy if BiTE is demonstrated by a significant reduction of tumor burden in bone marrow
- T cell recruitment is observed in bone marrow from mice treated with the BiTE

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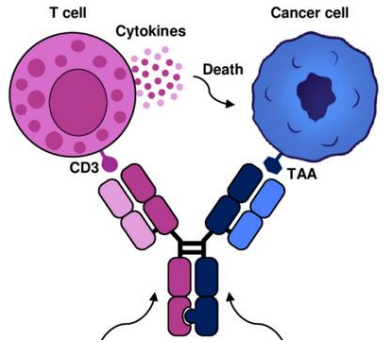
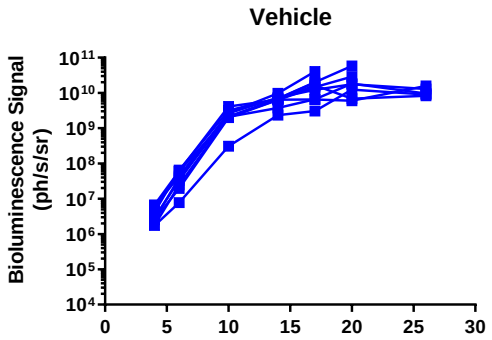
PBMC-engrafted mice for evaluation of Bi-specific T-cell engager

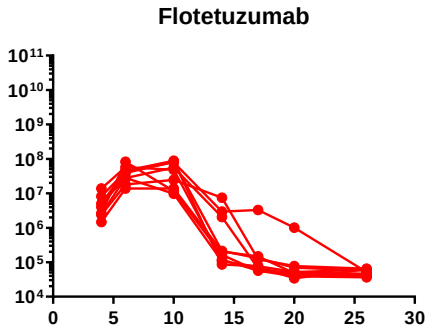
- Case study example (IV tumor model/T cells)
- BiTE in humanized mice implanted with AML

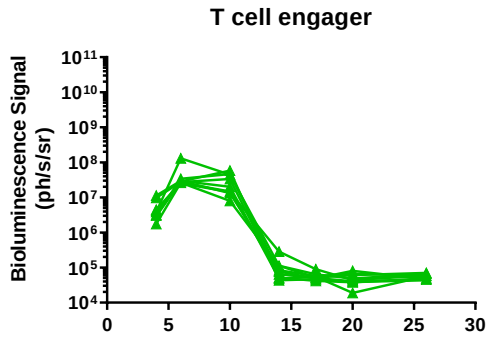


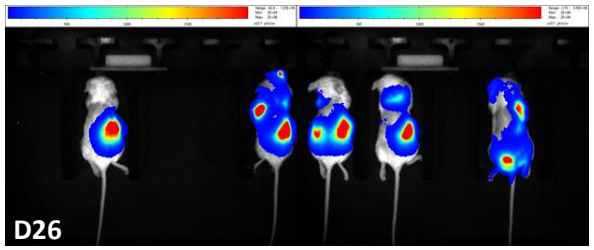
Tumor burden reduction (BLI)

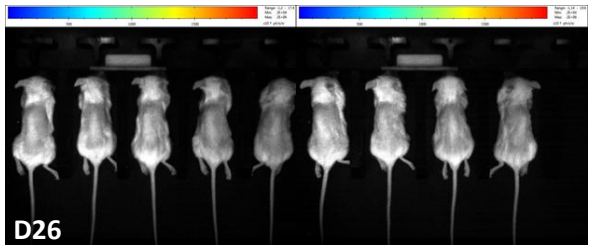
AML cells-Luc-GFP

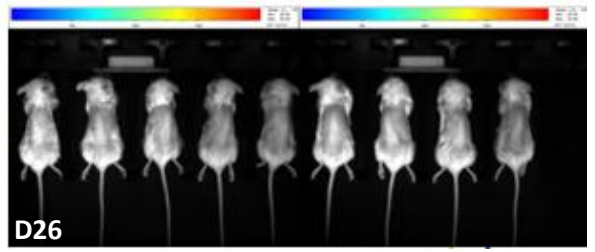












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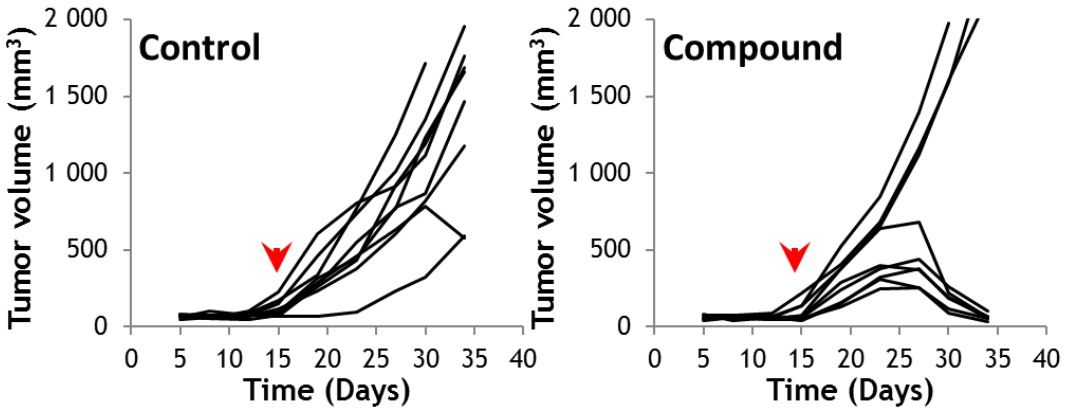
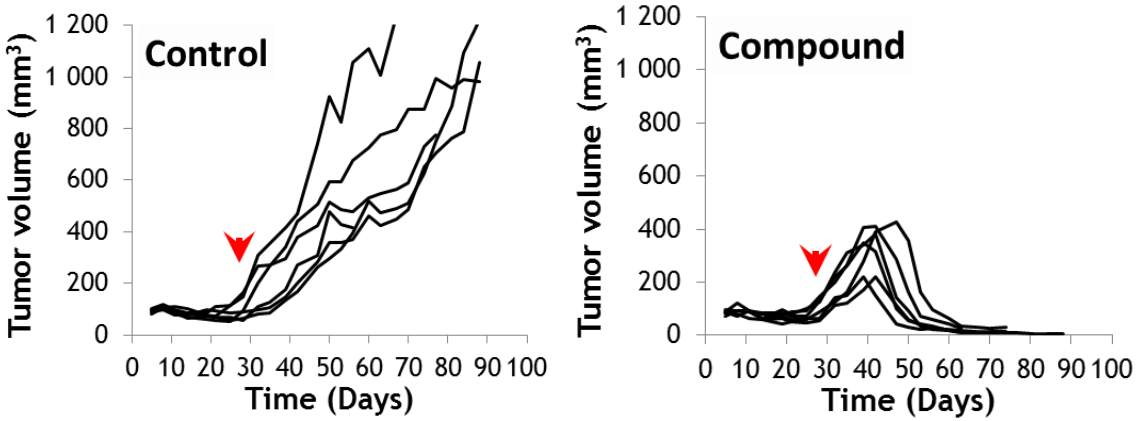
PBMC-engrafted mice for evaluation of Bi-specific T-cell engager

- Case study example (SC tumor model)
- BiTE in humanized mice implanted with human blood or breast tumor cells

Hematological tumor



Breast tumor



- In both cases SC tumor growth is significantly reduced in animals treated with BiTE

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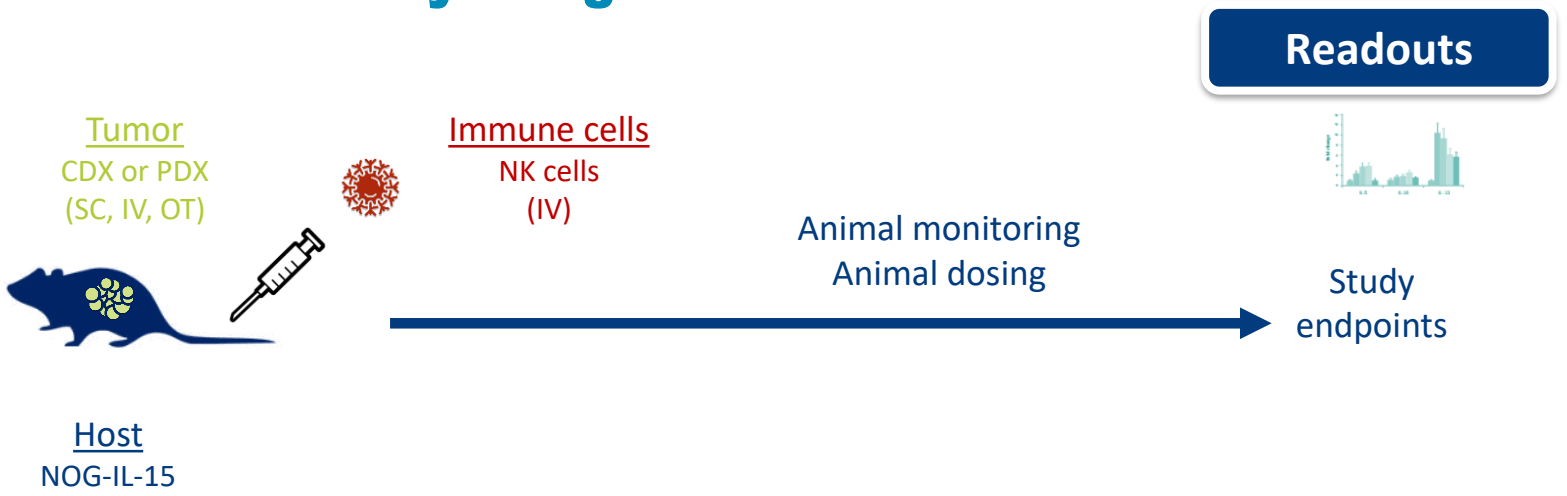
Adoptive transfer models: NK-engrafted mice

Human NK cell-engrafted mice

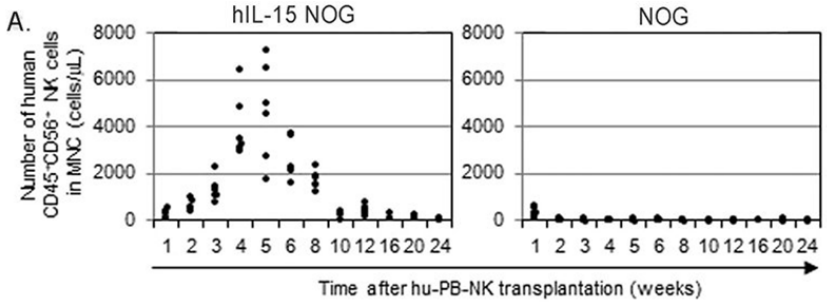
Representative study design



Readouts

- **Body weight and tumor volume**
- **Survival time and clinical scoring**
- **Flow cytometry (NK marker expression)**
- **Biomarker/target monitoring**
- **Histology**
- **Imaging (BLI)**

- Synchronization of humanization/tumor growth (considering kinetics of engraftment and persistence of NK cells)
- Donor-to donor variation in tumor response
- Possibility to use frozen expanded NK cells to re-use same donors across multiple experiments



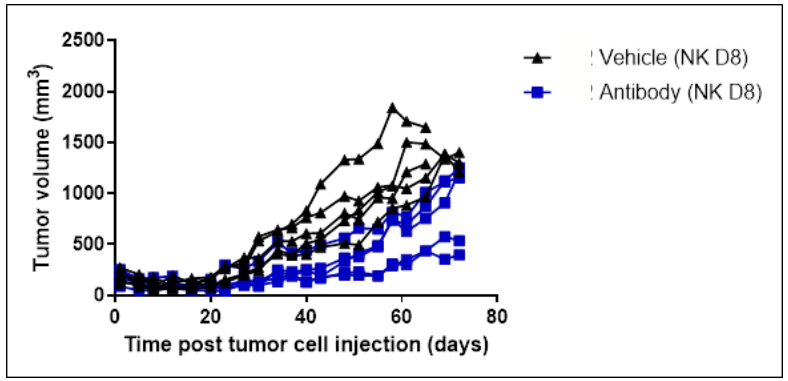
NK cell engraftment

Appropriate model for NK cell targeting therapies

NK cell-engrafted mice for evaluation of NK-cell engager

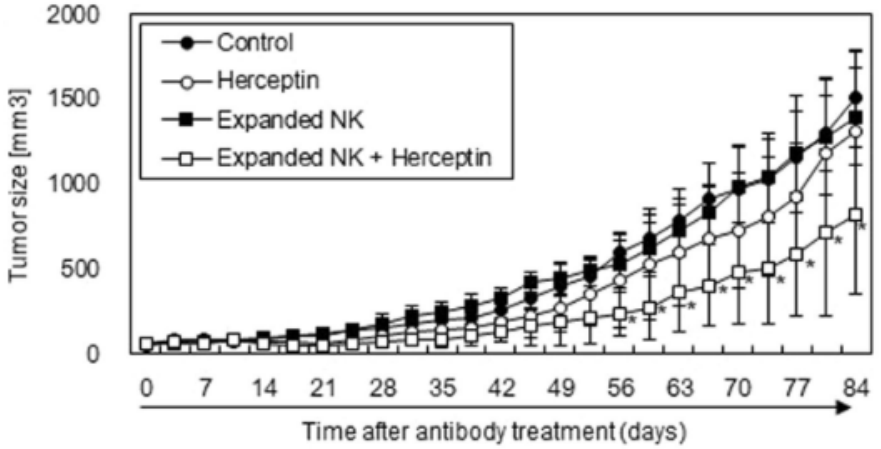
- Case study example (SC tumor model)
- Test Antibody in NK-humanized mice implanted with solid human tumor

Breast tumor growth



- *In vivo* antitumor activity of antibody candidate is demonstrated by a reduced tumor growth in comparison with vehicle group

Gastric tumor growth



From Katano et al., **2017**

- Tumor growth is significantly suppressed in the mouse group receiving NK cells and anti-Her2 therapy

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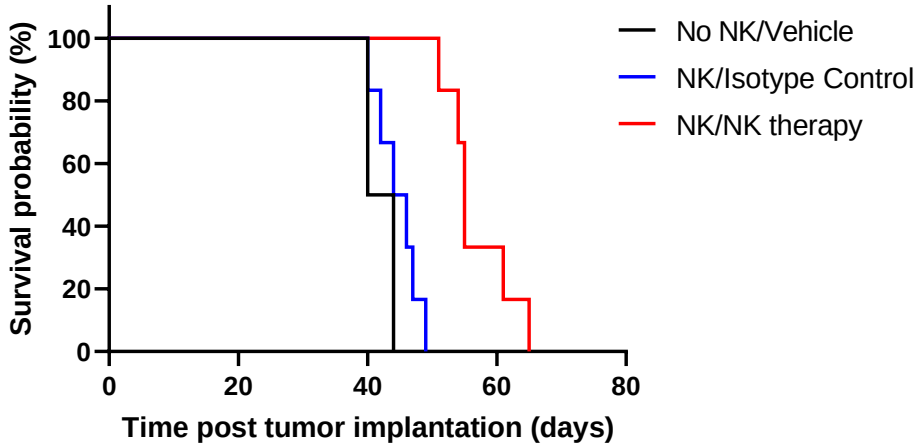
NK cell-engrafted mice for evaluation of NK-cell engager

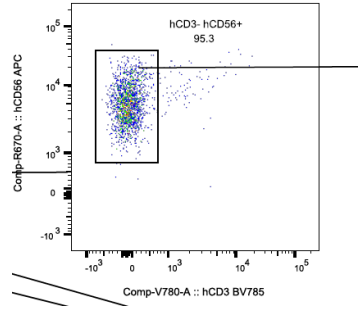
- Case study example (IV tumor model)
- NK-cell engager in NK-humanized mice implanted with human multiple myeloma

Survival increase



- *In vivo* antitumor activity of NK therapy is demonstrated by a highly increased survival time of tumor bearing mice receiving expanded NKs and therapy

NK cell engraftment



- Flow cytometry analysis evidenced the reconstitution of animals with human NK cells (hCD45+ hCD3- hCD56+ cell population in spleen from humanized NOG-hIL15 mouse at D35)

Adoptive transfer models: CAR- injected mice

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CARs evaluation

Representative study design

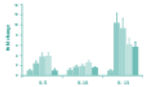
Tumor
CDX or PDX
(SC, IV, OT)

Host
NOG, NSG, BRGSF
NOG-IL-15

Immune cells
CAR T/CAR NK cells
(IV)

Animal monitoring
Animal dosing

Study
endpoints



Readouts

- **Body weight and tumor volume**
- **Survival time and clinical scoring**
- **Flow cytometry**
- **Biomarker/target monitoring**
- **Histology**
- **Imaging (BLI)**

- Capabilities for preparation of CAR T cells
- Use of HSC-HIS models, which additionally allow for CAR T therapy-induced CRS toxicity to be analyzed

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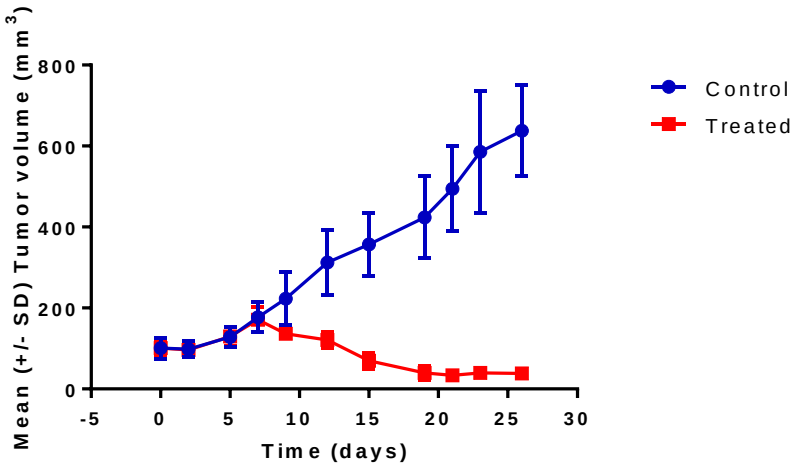
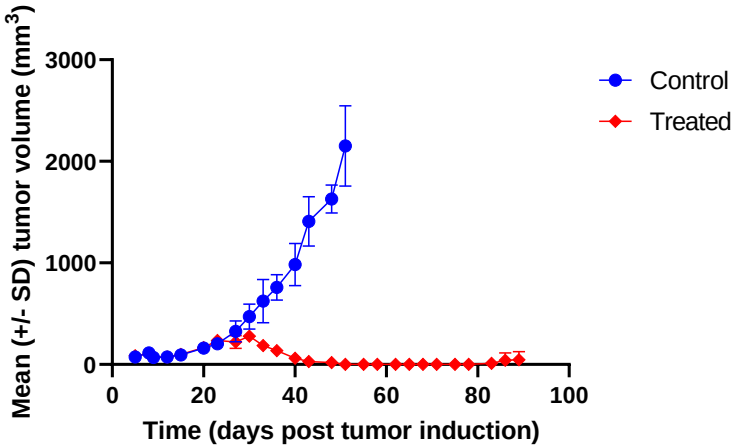
Case study example

- Evaluation of CAR T cell therapy in a solid tumor model

Tumor growth (CDX)



Tumor growth (PDX)



Engineered T cell therapy induced a large tumor regression in solid tumor models

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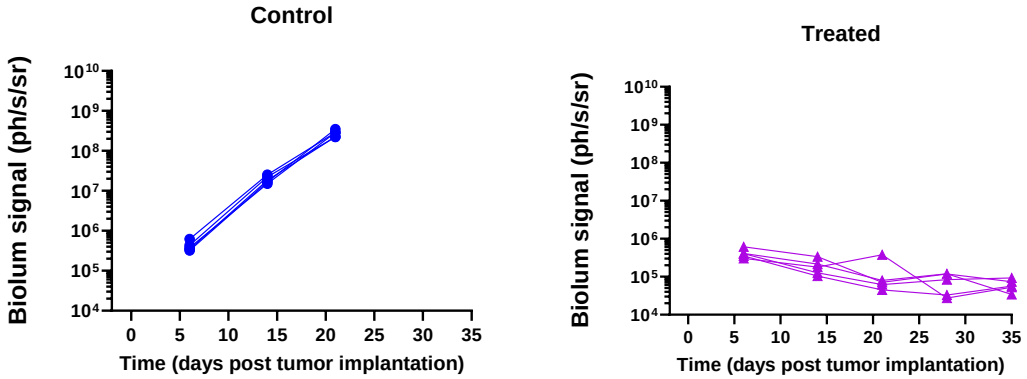
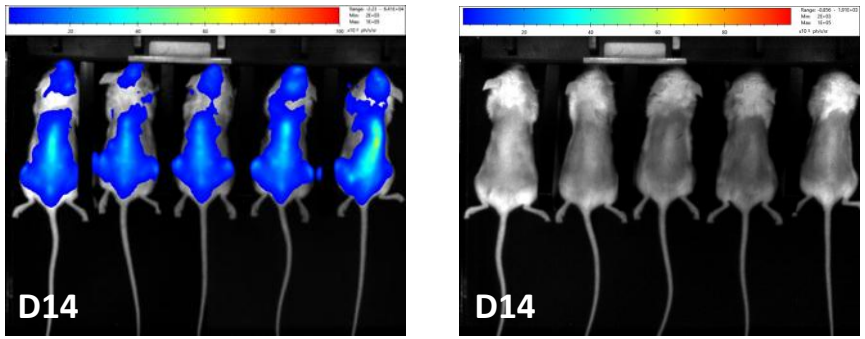
Case study example

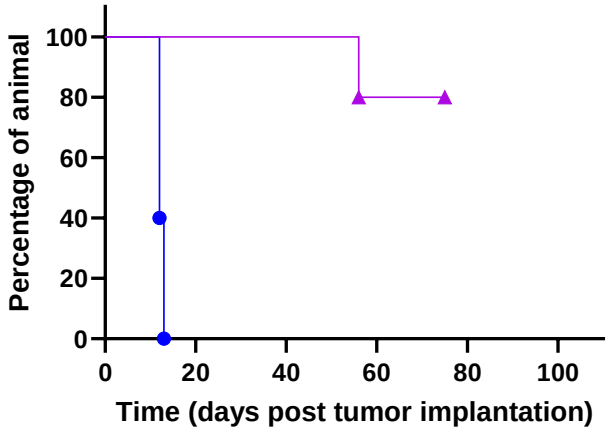
- Evaluation of CAR T cell therapy in a hematological tumor model

Bioluminescence imaging





Survival increase



Engineered T cell therapy reduced bioluminescence signal in disseminated blood tumor bearing mice, resulting in an increased survival time

hCD34+ engrafted mouse models

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hCD34+ engrafted mice for IO research

Representative study design

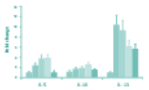
Tumor
CDX or PDX
(SC, IV, OT)

CD34+
humanized mice

Host
NOG, NSG, NOG-EXL, BRGSF
(support the engraftment of different immune population of interest)

Animal monitoring
Animal dosing

Study endpoints



Readouts

- **Body weight and tumor volume**
- **Survival time and clinical scoring**
- **Flow cytometry**
- **Biomarker/target monitoring**
- **Histology**

Appropriate model for a broad collection of I/O drugs

Engraftment of the major immune population depending on the mouse strain

- Choice of the right strategy depending on the population of interest
- Donor-to donor variation in humanization level and tumor response

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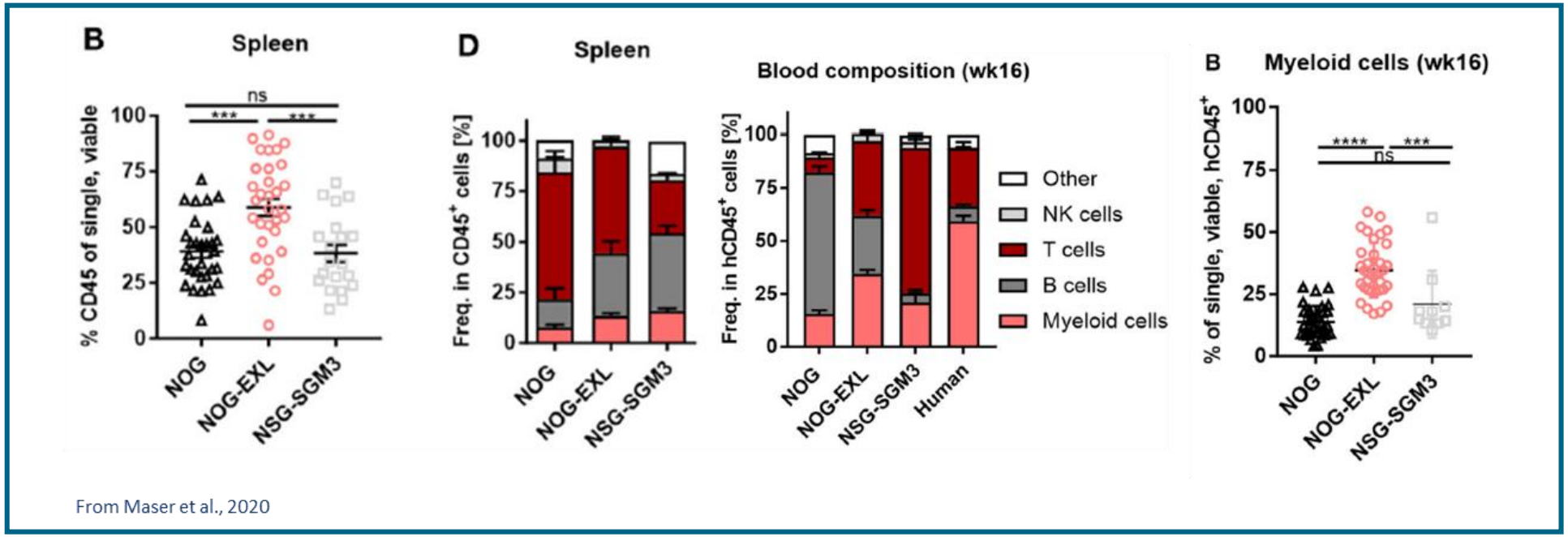
Immune cell engraftment

- Immune cell engraftment and immune cell profile varies among mouse strains
- Cytokine expression drives immune cell engraftment then use of transgenic mice expressing human cytokines is helpful to increase differentiation of myeloid lineages and NK cells (functional immune cells)



From Maser et al., 2020

- Depending on immune subsets of interest and mechanism of action of compound to be tested the choice of mouse strain is important

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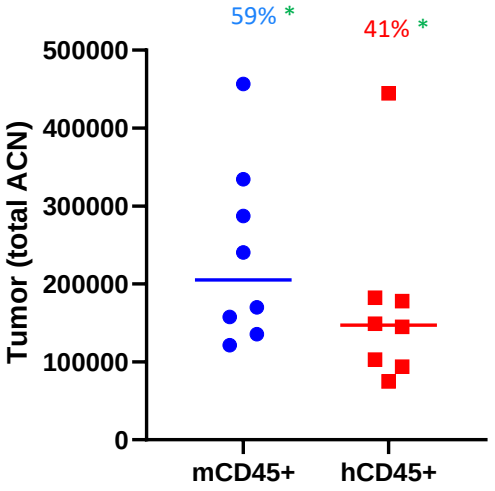
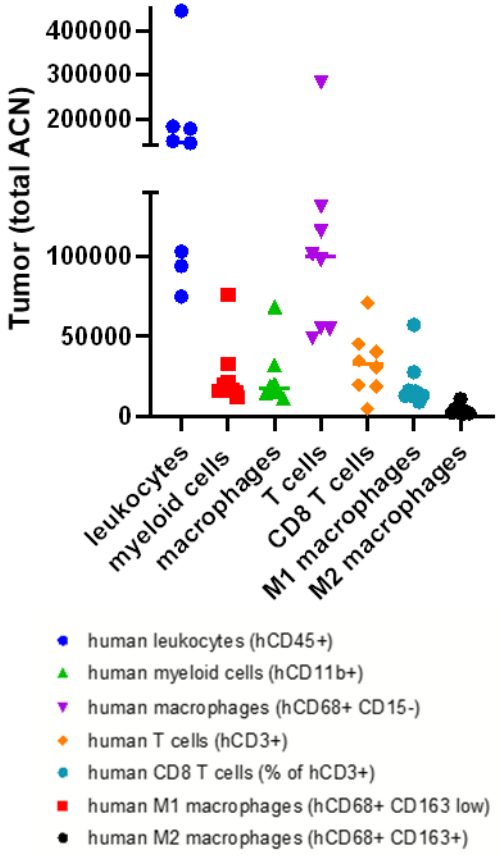
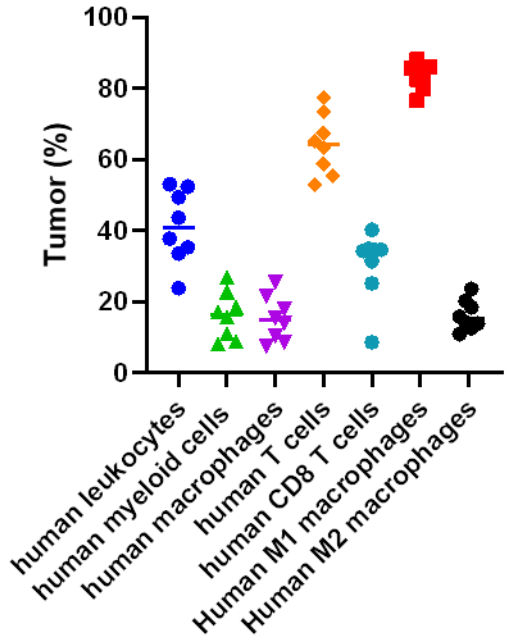
Tumor infiltration with immune cells

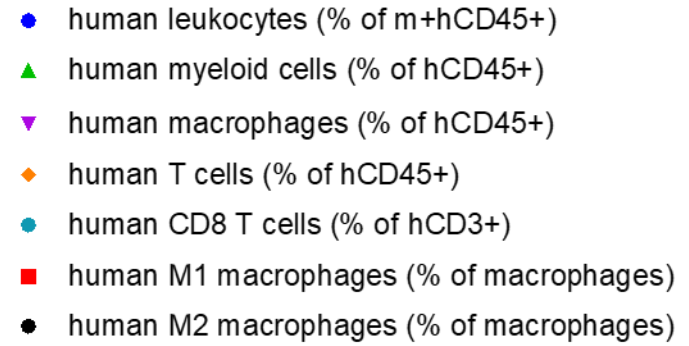
- **Human tumors implanted into hCD34+ humanized NOG-EXL mice are infiltrated with human immune cells**
  - Example : Human breast xenograft 15-16 weeks post humanization and FACS analysis 5 weeks after tumor implantation (Tumor volume ranged from 740 to 1100 mm^3)
  - Human immune subpopulations in tumor samples



* Expressed as % of h+m hCD45+ cells

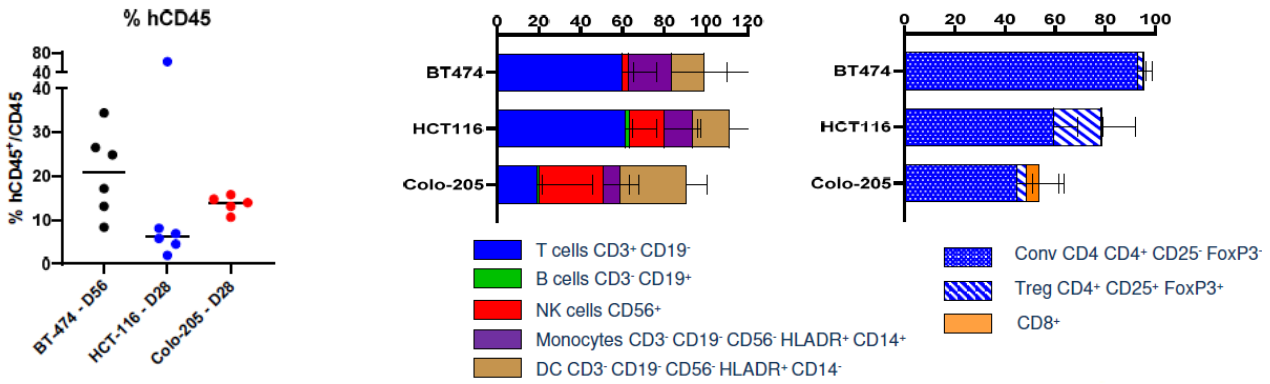






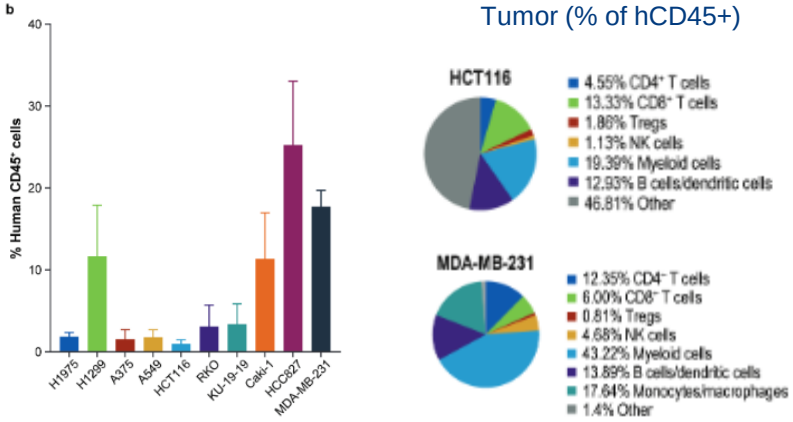
Tumor infiltration with immune cells

- **Immune cell composition varies among tumors (and with tumor burden…)**
  - Examples of CDX (BRGSF-HIS mice here)

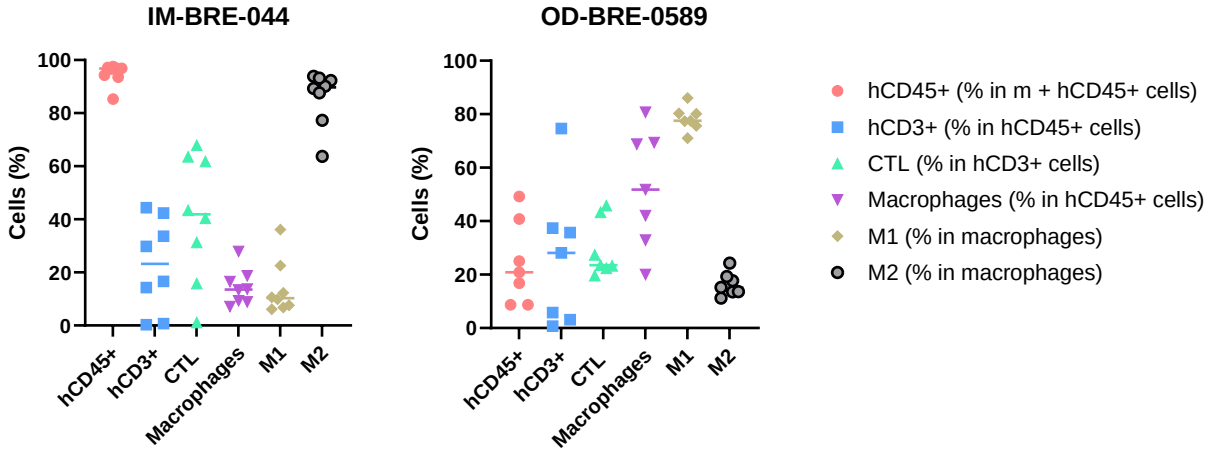


Characterization of human cancer xenografts in humanized mice

Jonathan Rios-Doria, Christina Stevens, Christopher Maddage, Kerri Lasky, Holly K Koblish



  - Examples of PDX (NOG-EXL-HIS mice here)



Patient Tumor	Sub-type	CD45	CD3	CD8	FOXP3
OD-BRE-589	TNBC	sparse	+	spares	0
IM-BRE-044	TNBC	++	+++	+++	++

Tumor infiltration in humanized mice is correlated with immune cell infiltrate in originating patient tumor

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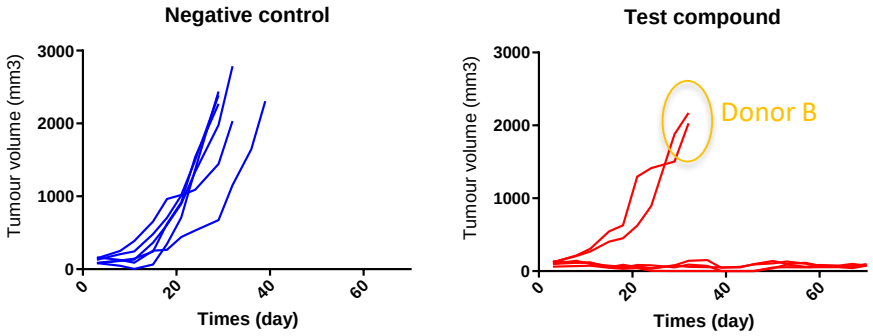
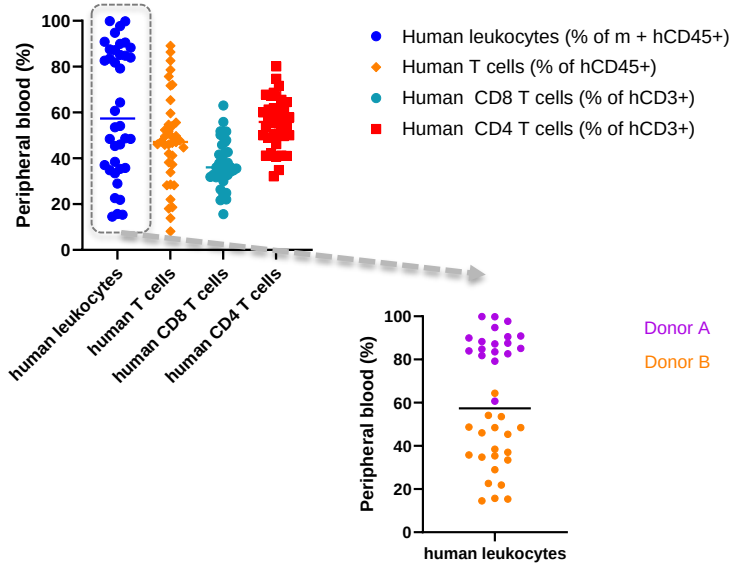
hCD34+ engrafted mice for IO research

- Case study (SC tumor model)
- BiTE Ab in humanized NSG mice implanted with SC human myeloma

Antitumor efficacy



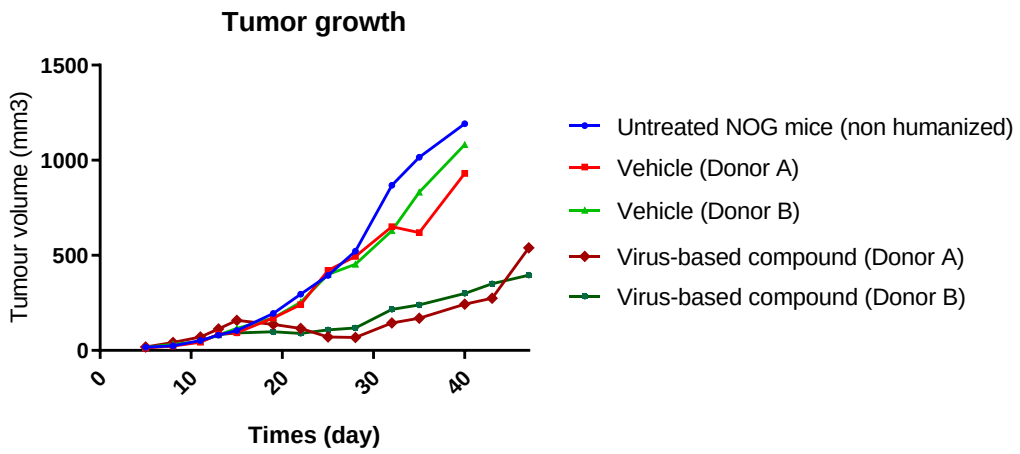
T-cell engraftment



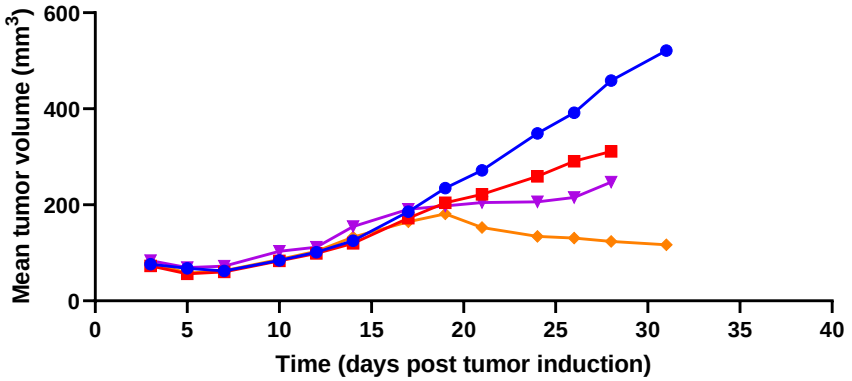
hCD34+ engrafted mice for IO research

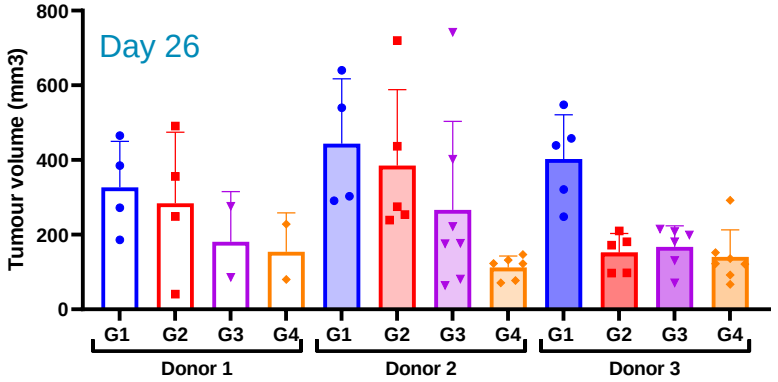
- Case study (SC tumor model)
- Virus-based compound in humanized NOG-EXL mice implanted with SC colon tumor

Antitumor efficacy (monotherapy)



Antitumor efficacy (combined therapy)





G1 : Vehicle
G2 : Virus-based compound
G3: BiTE
G4 : Virus-based compound + BiTE

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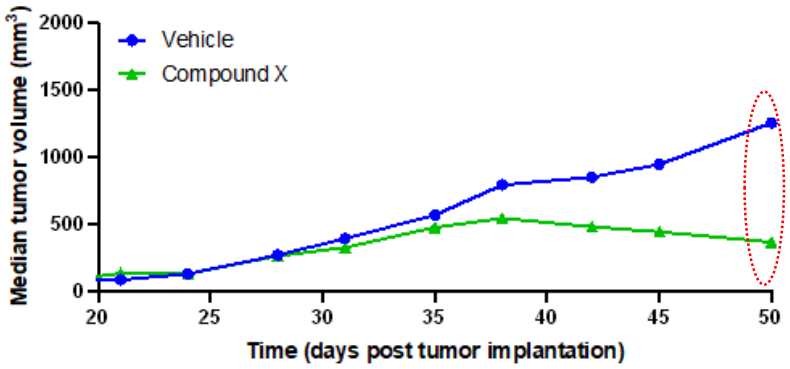
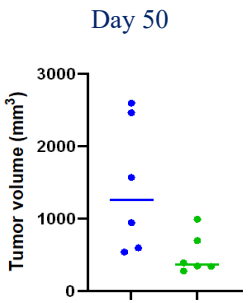
hCD34+ engrafted mice for IO research

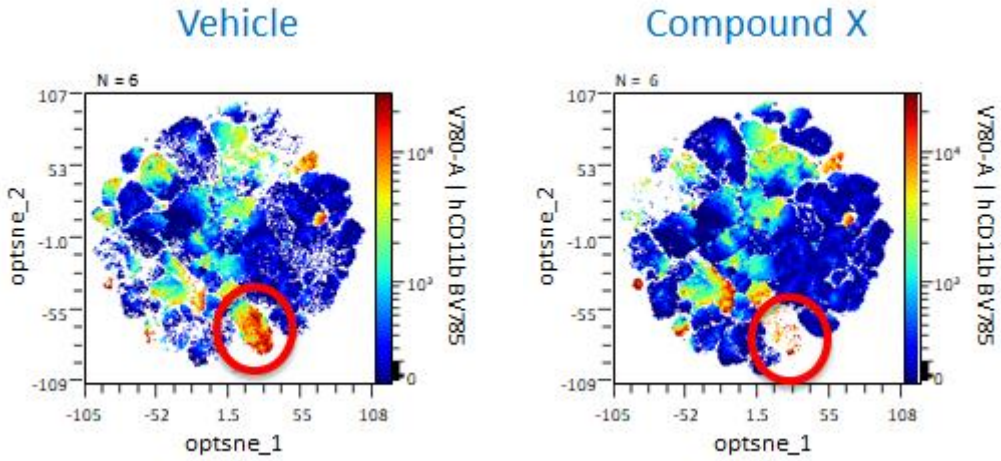
- Case study (SC tumor model)
- Antibody-based therapy in humanized NOG-EXL mice implanted with human breast carcinoma

Antitumor efficacy (PDX)





Tumor infiltration (Immune cells)



End of treatment, flow cytometry analysis (14 colors)

Clear visualization of the impact of treatment on myeloid cells (CD11b+)

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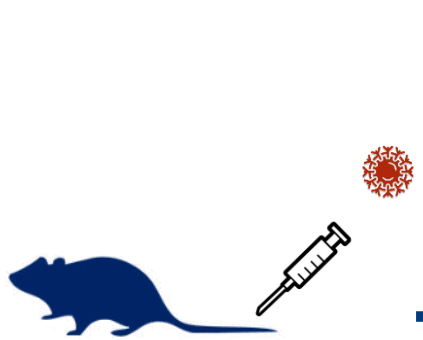
Other applications

Systemic inflammation

- Cytokine Release Syndrome (CRS) and xenogeneic Graft versus Host Disease (GvHD)
- PBMC or hCD34+ engrafted mice
- Representative study design

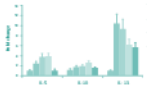


Host
NOG, NSG for GvHD
NOG-EXL, BRGSF for CRS

Immune cells (PBMC or hCD34+ cells)

Animal monitoring
Animal dosing

Study endpoints



Readouts

- **Body weight**
- **Survival time and clinical scoring**
- **Flow cytometry (T cell activation markers)**
- **Biomarker (cytokine release)**
- **Histology**

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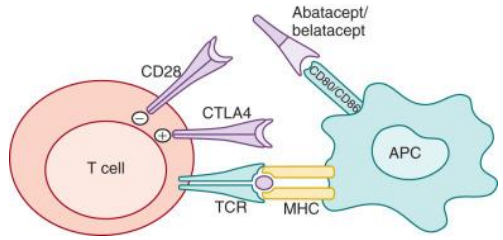
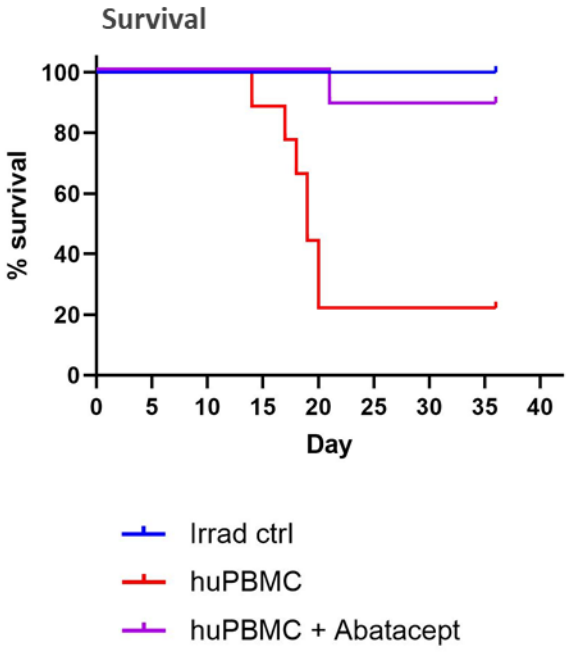
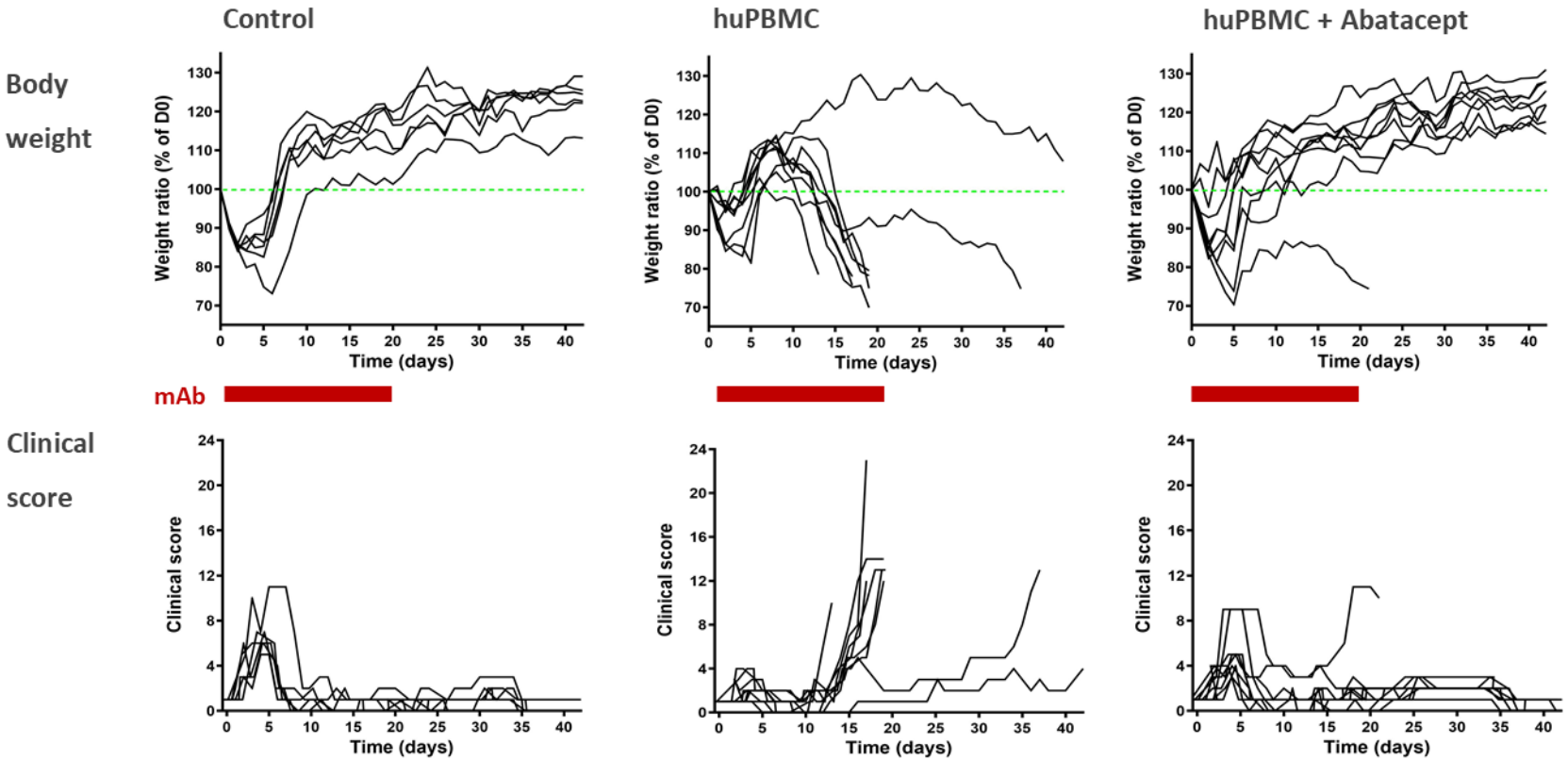
Xenogeneic Graft versus Host Disease (GvHD)

- Human mature blood cells into immunodeficient mice (NOG)
- Acute disease linked to hyper-activation of human T cells against mouse tissues
- Validation of Abatacept (CTLA4-IgG1Fc, T cell immuno-modulator) reference compound







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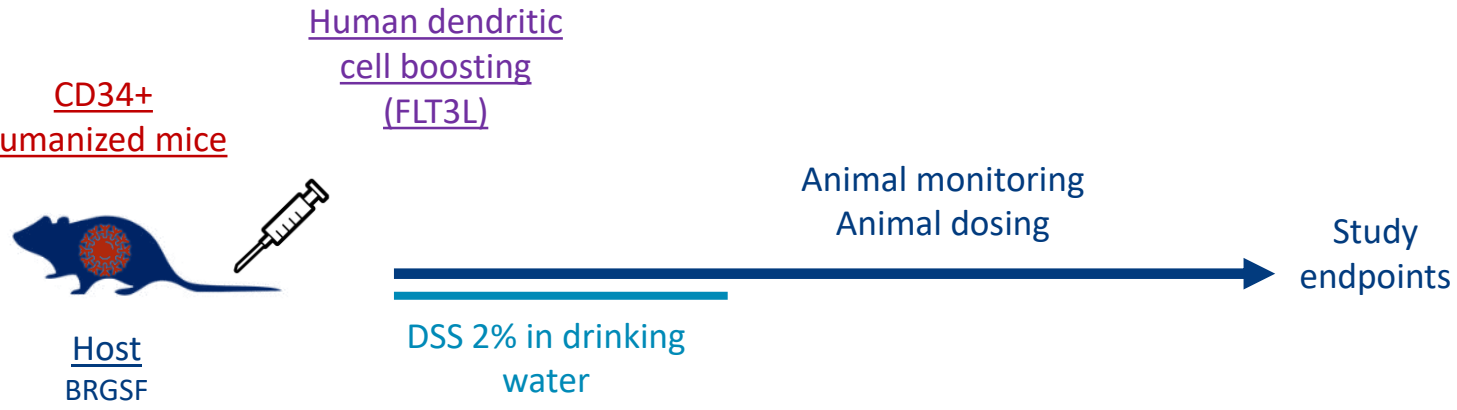
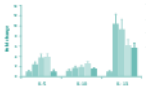
Inflammation of Gastro Intestinal tract : colitis

- **Colitis refers to inflammation of the inner lining of the colon**
- **There are numerous causes of colitis including infections, inflammatory bowel disease (Crohn's disease, ulcerative colitis), ischemic colitis, allergic reactions, and microscopic colitis**
- **Symptoms of colitis in patients depend upon the cause and may include:**
  - Abdominal pain
  - Cramping
  - Diarrhea with or without blood in stool
- **Representative study design:**



DSS = Dextran Sodium Sulfate (used in mice to reproduce colitis symptoms)



Readouts

- **Disease Activity Index (BW, Diarrhea, Blood in feces)**
- **Colon weight/Length ratio**
- **Human/Mouse gene expression analysis (proximal and distal colon)**
- **Human/mouse serum cytokine measurements**
- **Colon histology and scoring (leucocyte clusters, epithelial erosion, goblet cells depletion & edema)**

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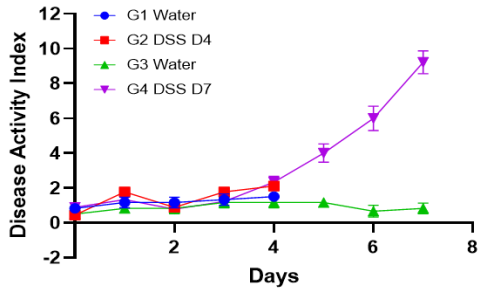
DSS-induced colitis in the mouse

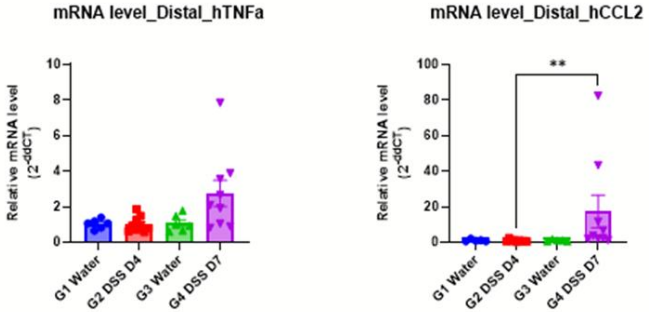
- Model validation

Disease Activity Index

Score	Body weight loss (%)	Stool consistency	Blood in Feces
0	0	Normal	Absence
1	1-5	Soft	Hemoccult +
2	6-10	Very soft	Hemoccult ++
3	11-15	Diarrhea	Bloody
4	>15	Severe diarrhea	

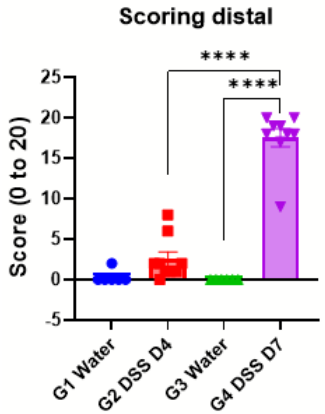


Cytokine & chemokine gene induction

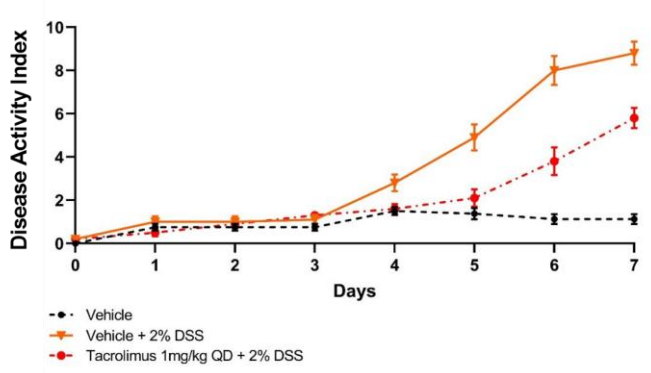


Colon histomorphometric analysis

Score	Leucocytic cell clusters (in lamina propria)	Epithelial cell damage = Erosion	Depletion of Goblet cells	Edema
0	None	None	None	None
1	Minimal < 10%	Minimal	Minimal < 20%	Minimal
2	Mild 10-25%	Mild	Mild 21-35%	Mild
3	Moderate 26-50%	Moderate	Moderate 36-50%	Moderate
4	Marked > 50%	Marked	Marked > 50%	Marked



Response to drug



Partial efficacy of Tacrolimus (FK-506 immuno-suppressor) is observed in the acute DSS-induced mouse model

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Auto-immune diseases : B cell depletion

- B cell are recognized as playing a role in a variety of auto-immune diseases such as rheumatoid arthritis, SLE (lupus)
- B cell depletion therapy (BCDT) has been employed to treat auto-immune diseases for about 20 years
- BCDT approaches include monoclonal antibodies that target B cell surface antigens (CD19 or CD20), B-cell specific CAR T treatment and more recently B-cell targeting bispecific T-cell engagers
- Based on their efficacy and their safety record in cancer, various BCDT approaches are now being repurposed for use in auto-immune diseases in which B cells play a central role

B cell depletion

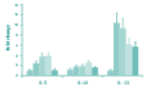
- **Representative study design**

CD34+
humanized mice

Host
NOG, NSG, NOG-EXL, BRGSF

Animal monitoring
Animal dosing

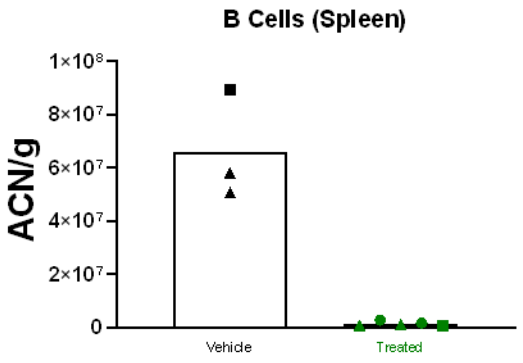
Study
endpoints

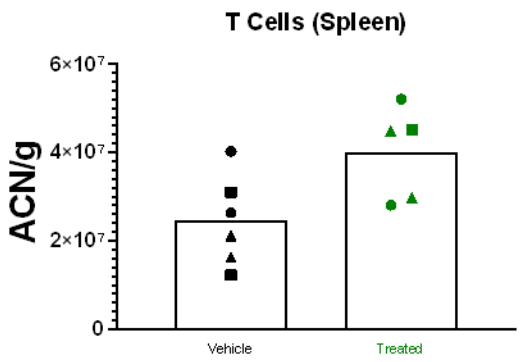


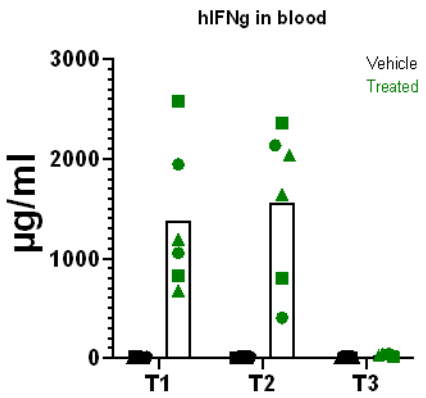
Readouts

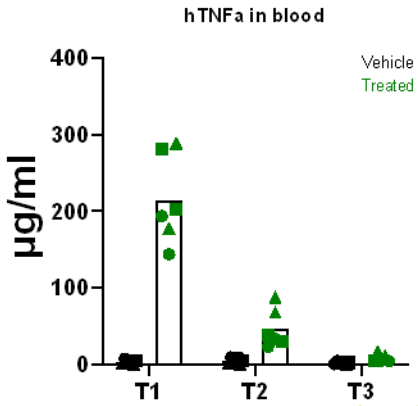
- **Body weight**
- **Flow cytometry (B cell & activation markers)**
- **Biomarker (cytokine release)**
- **Histology**

- **Case study (B cell targeting bispecific TCE)**









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To summarize

To keep in mind…

- **HIS models: useful tools but…**
- **Main limitations of humanized mouse models:**
  - lack of lymph node structures and germinal centers
  - restricted development of mature innate immune cells
  - lack of HLA molecules, limited ability to generate antigen-specific antibody responses
- **Selection of the most appropriate mouse model: essential consideration to optimize the translational potential of studies with humanized mice**
- **Limitations of each specific model need to be considered to ensure an effective study as much as possible**
- **Several parameters should be considered when designing studies with humanized mice:**
  - Engraftment strategy and immune cells of interest
  - Mouse strain
  - Tumor model (target expression, tumor growth, TME)
- **Recommendations:**
  - Use of multiple donors (donor-to-donor variation)
  - Pilot validation experiment with the selected mouse model to confirm that it is appropriate

An animal model remains an experimental model which cannot always answer all questions at once we must be critical when searching for the right animal model

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