Fraunhofer IZI

Fraunhofer Institute for Cell Therapy and Immunology IZI

SAXOCELL®

CLUSTERS 4 FUTURE
Innovationsnetzwerke für unsere Zukunft

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SaxoCell Sparkmeeting 11.05.2023

Induction of Graft-versus-Host disease in mouse models

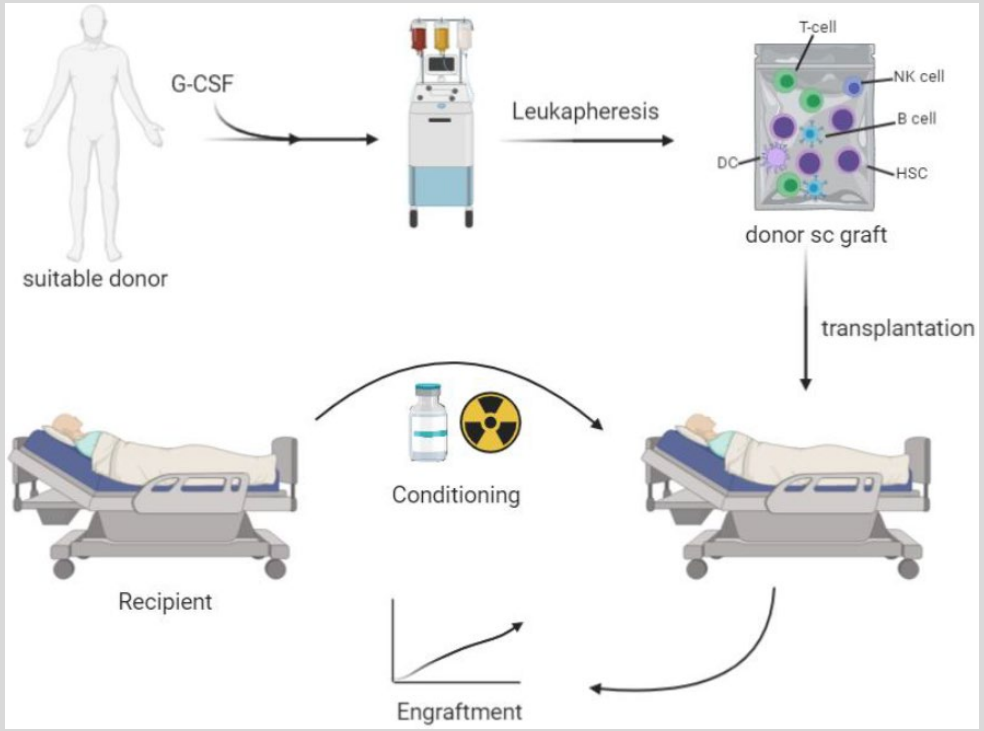
Background on Graft-versus-Host disease I

Hematopoietic cell transplantation is a treatment option for mostly hematological diseases

Allogeneic setting (allo-HCT)

Examples of indications for HCT:

- *Leukemias*
  - acute myeloid leukemia (AML)
  - acute lymphoblastic leukemia (ALL)
  - chronic myelogenous (CML)
  - myelodysplastic syndromes (MDS)
  - chronic lymphocytic leukemia (CLL)
- *Lymphoid malignancies*
  - diffuse large B cell lymphoma (DLBCL)
  - follicular lymphoma (FL)
  - Hodgkin lymphoma (HL)
  - multiple myeloma (MM)
- *Other diseases*
  - aplastic anaemia (AA)



Main purposes of HCT are…

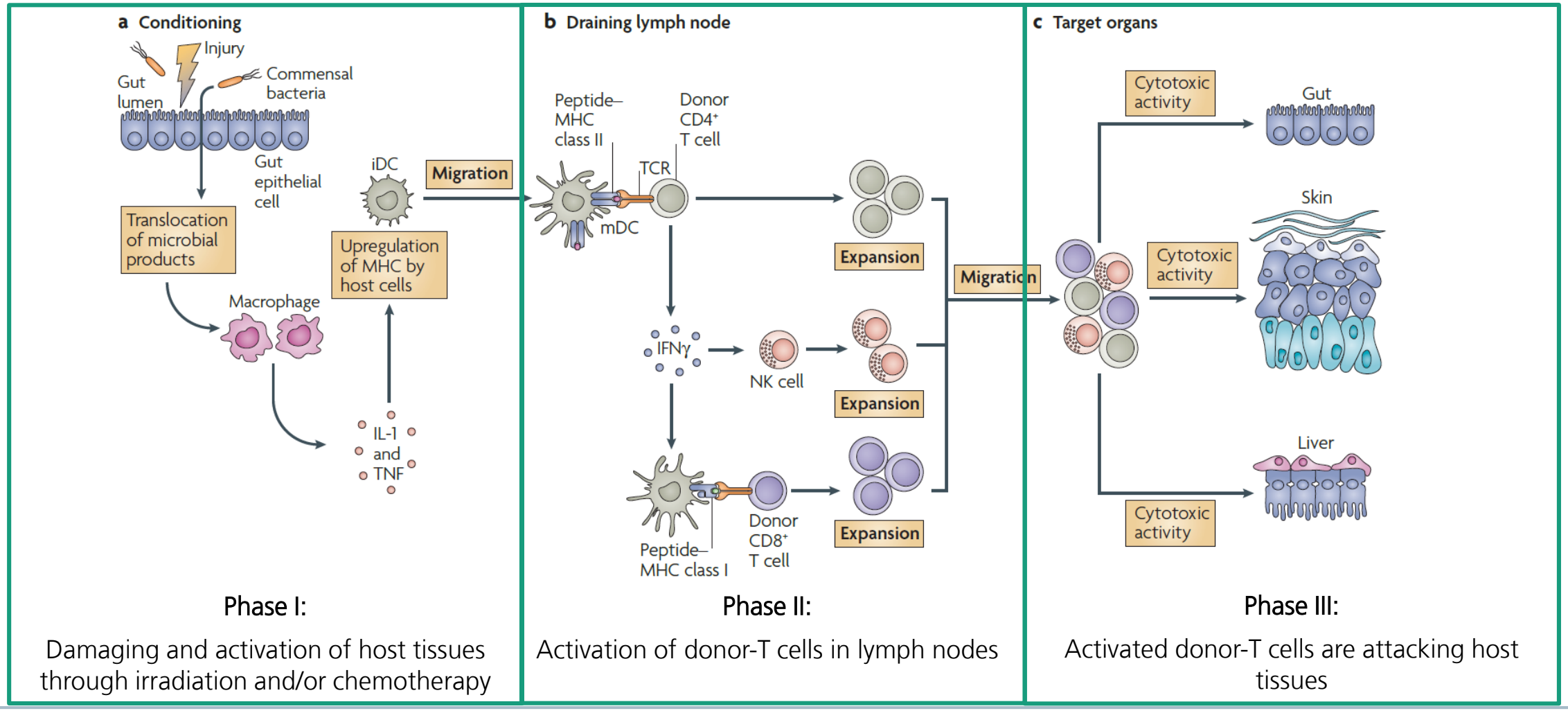
1) the **reconstitution** of the hematopoietic system after high dose chemotherapy and radiation therapy
2) the **eradication** of remaining cancer cells by NK and T cells

(Graft-versus-Leukemia effect)

1) Duarte et al., Bone Marrow Transplantation, 2019
2) Gaballa, Thesis, 2020

Background on Graft-versus-Host disease II

Problem: Graft attacks host tissues



Phase I:

Damaging and activation of host tissues through irradiation and/or chemotherapy

Phase II:

Activation of donor-T cells in lymph nodes

Phase III:

Activated donor-T cells are attacking host tissues

Adapted from Roncarolo et al., Nature Reviews Cancer, 2018

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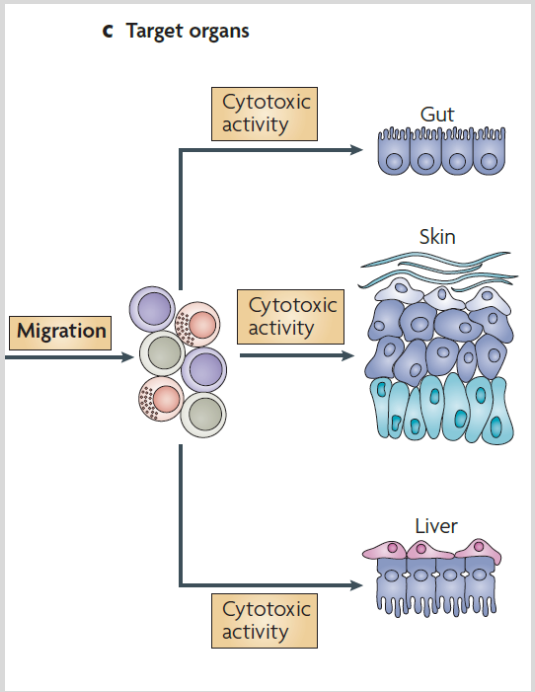
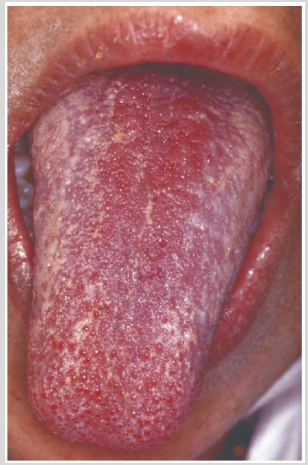
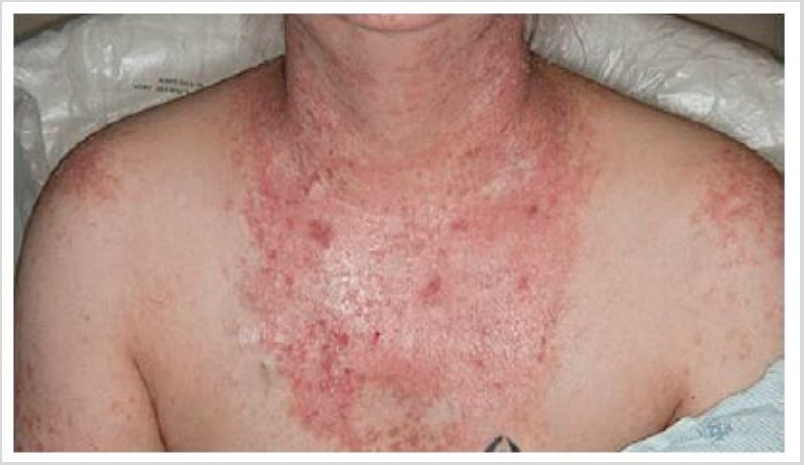
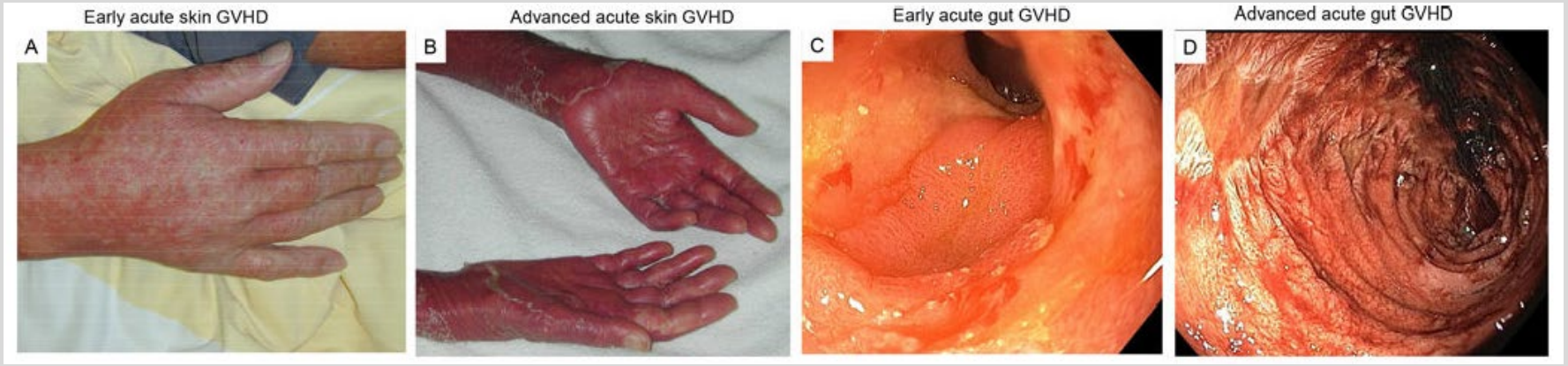
Background on Graft-versus-Host disease III

Clinical symptoms









Hymeset al., J Am Acd Dermatol. 2012
Findler, The NEJM 2003
Zeiser et al., The NEJM 2017

Background on Graft-versus-Host disease IV

Some Facts

In 2021, 27,155 allogeneic HCT were reported by European centers[1].

30–50% of the patients develop acute GvHD[2].

50% of all patients develop chronic GvHD and 25% of them will die[2].

Treatment costs between 75.000 and 225.000 € per patient[3].

1) Passweg et al., Bone Marrow Transplantation, 2023
2) Carreras et al., The EBMT Handbook, 2019
3) Mischak-Weissinger et al., Stem Cell Transplantation (Oral presentation), 2005

Background on Graft-versus-Host disease V

Prevention of GvHD through immunosuppression with drugs and graft manipulation

Table 3: Medications Used in Prophylaxis and Treatment of GVHD

Medication	Action	Adverse Effects
Corticosteroids	Direct lymphocyte toxicity; suppress pro-inflammatory cytokines (TNF-alpha)	Hyperglycemia, acute psychosis, severe myopathy, cataract development, avascular necrosis
Methotrexate (MTX)	Antimetabolite; aids in inducing tolerance after BMT; may downregulate T lymphocytes by inhibiting proliferation	Significant renal, hepatic, and gastrointestinal toxicities
Cyclosporine A (CSA)	IL-2 suppressor; blocks calcium-dependent signal transduction distal to engagement of T cell receptor	Renal and hepatic insufficiency, hypertension, hyperglycemia, headache, nausea and vomiting, hirsutism, gum hypertrophy, seizure with severe toxicity
Tacrolimus (FK506, Prograf)	Similar to CSA	Similar to CSA
Mycophenolate mofetil (MMF)	Inhibits de novo purine synthesis; lymphocytes are highly dependent on de novo synthesis	Body aches, abdominal pain, nausea and vomiting, diarrhea, neutropenia
Antithymocyte globulin	Polyclonal immunoglobulin capable of destroying human T cells	Anaphylaxis, serum sickness
Sirolimus (Rapamune)	Inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine stimulation	Muscle aches, hypertension, cytopenias especially thrombocytopenia, renal insufficiency, peripheral edema
Pentostatin (Nipent)	Potent transition state inhibitor of the enzyme adenosine deaminase (ADA) found in lymphoid cells, especially T cells	Nausea/vomiting, fever, leucopenia, myalgias, hepatic dysfunction, adjust for renal insufficiency
Hydroxychloroquine (Plaquenil)	Antimalarial; beneficial in autoimmune disorders; exact mechanism of action not known	Irreversible retinal damage, headache, mild GI symptoms
Soriatane (Acitretin)	Retinoid used to treat psoriasis	Must not be used in women who plan on getting pregnant, erythema and breakdown of skin, elevation in LFTs and lipids, dry eyes, dry skin
Daclizumab (Zenapax)	IL2 receptor antagonist; in circulation impairs response of immune system to antigenic challenges	Increased mortality when used with steroids

Significant renal, hepatic, and gastrointestinal toxicities

Renal and hepatic insufficiency, hypertension, hyperglycemia, headache, nausea and vomiting, hirsutism, gum hypertrophy, seizure with severe toxicity

In addition: T cell depletion of grafts

Increased risk for…

- infections
- defective cytokine production
- reduced engraftment
- relapse of underlying disease (reduced GvL effect)

1) Barton-Burke et al., Oncology (Williston Park), 2008
2) Li Pira et al., Blood Reviews, 2016

Unmet medical need

- Current treatments for GvHD can be effective but have **significant side effects**.
- While survival rates for cell transplants have improved over the years, GvHD remains a major cause of **morbidity** and **mortality**.
- **Research** aimed at improving patient outcomes, reducing the incidence and severity of GvHD is still needed.
- **Animal models** for GvHD research are available at the IZI.

Immunodeficient Mice Strains

Levels of Immunodeficiency

The Jackson Laboratory

charles river
Exclusive distributor of JAX™ Mice in Europe.

Mice

NSG®

- NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ^{rr}

NOD SCID

- NOD.CB17-*Prkdc*^{scid}/J^{rr}
- NOD.CB17-*Prkdc*^{scid}/NcrCrl

Fox Chase SCID® Beige

- CB17.Cg-*Prkdc*^{scid}*Lyst*$^{bg-J}$/Crl

SCID

- Fox Chase: CB17/Icr-*Prkdc*^{scid}/IcrIcoCrl

Inbred Nude

- BALB/c Nude Crl: CAnN.Cg-*Foxn1*^{nu}/Crl
- BALB/c Nude J: CByJ.Cg-*Foxn1*^{nu}/J^{rr}

Outbred Nude

- Athymic Nude: Crl:NU(NCr)-*Foxn1*^{nu}
- CD-1® Nude: Crl:CD1-*Foxn1*^{nu}
- NMRI Nude: Crl:NMRI-*Foxn1*^{nu}
- Nude Mouse: Crl:NU-*Foxn1*^{nu}
- Swiss Nude: Crl:NU(Ico)-*Foxn1*^{nu}

	NSG®	NOD SCID	Fox Chase SCID® Beige	SCID	Inbred Nude	Outbred Nude
Mature B cells	✗	✗	✗	✗	✓	✓
Mature T cells	✗	✗	✗	✗	✗	✗
Dendritic cells	!	!	✓	✓	✓	✓
Macrophages	!	!	✓	✓	✓	✓
Natural killer cells	✗	!	!	✓	✓	✓
Hemolytic complement	✗	✗	✓	✓	✓	✓
Leakiness	Very Low	Low	Low	Low	N/A	N/A
Radiation tolerance	Low	Low	Low	Low	High	High
Spontaneous tumour incidence (type)	Low	High (thymic lymphoma)	High (thymic lymphoma)	High (thymic lymphoma)	Low	Low
Features and research applications	• Engrafts the widest range of solid and hematological cancers, including ALL and AML • Most sensitive host for cancer stem cells when compared to NOD SCID or nude mice • Longer lifespan than NOD SCID; supports long-term engraftment studies and capabilities; >89 weeks median survival • Amenable to humanization	• Higher take-rates for slowgrowing cancer cell lines than SCID or Nude models • Xenotransplantation of some solid human tumours • Adoptive transfer from strains on NOD background enables study of cell function and track cell movement	• Engrafts hematopoietic cancer cell lines • Suitable for therapeutic antibody testing due to functional complement	• Engrafts hematopoietic cancer cell lines, some primary cells • Allows allogeneic and xenogeneic cancer cell lines and tissues • Improvements in engraftment efficiency over nude models for some cancer lines	• Engraftment of human and mouse tumour cell lines • Easy assessment of subcutaneous tumour growth due to lack of fur • Less genetic and phenotypic variability compared to outbred mice. Allows for more consistent and reproducible growing of many allogeneic cell lines • Not as hardy or robust as outbred mice	• Engraftment of human and mouse tumour cell lines • Easy assessment of subcutaneous tumour growth due to lack of fur • More genetic and phenotypic variability as compared to inbred mice • Hardier and more robust as compared to inbred mice
Considerations	• No thymic lymphomas – can be used for long and short-term experiments • Sensitive to irradiation	• Develops thymic lymphomas by 8–9 months - best used in short term experiments • Poor radiation tolerance • 36 weeks median survival	• BEIGE mutation leads to defective NK cells • Provides alternative to NOD SCID	• NK activity limits engraftment • Poor radiation tolerance • Innate immunity intact	• Innate immunity intact • Little engraftment of hematopoietic cancer cells • Not suitable for primary cells	• Innate immunity intact • Little engraftment of hematopoietic cancer cells • Not suitable for primary cells
Degree of Immunodeficiency	Highest					Lowest

Key: ✓ Present; ! Defective; ✗ Absent; ^ High; v Low; vv Very Low

Fraunhofer IZI

Center for Experimental Medicine at Fraunhofer IZI

Central animal facility headed by Dr. Franziska Lange

State-of-the-art animal house

Standardized hygiene levels and individually ventilated cage (IVC) systems

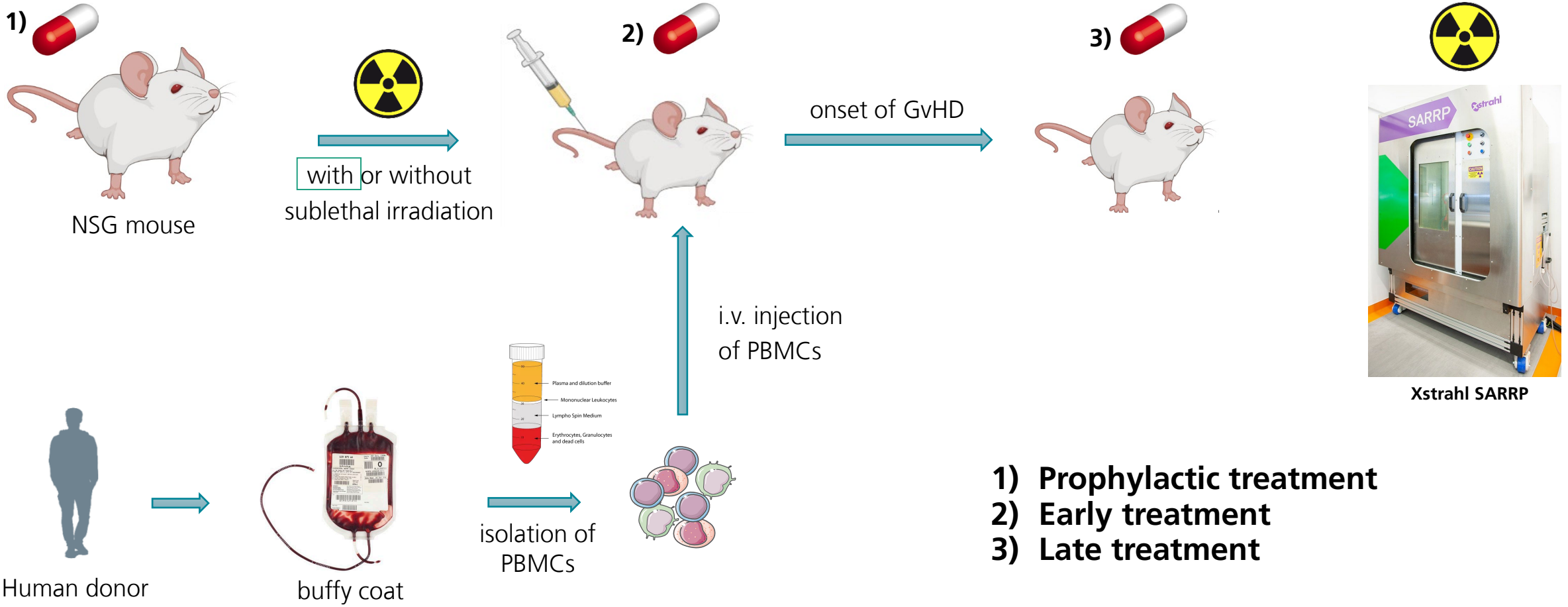
All experimental work can be carried out under sterile conditions

Fraunhofer IZI

Xenogenic murine model for GvHD

Current GvHD Model at IZI

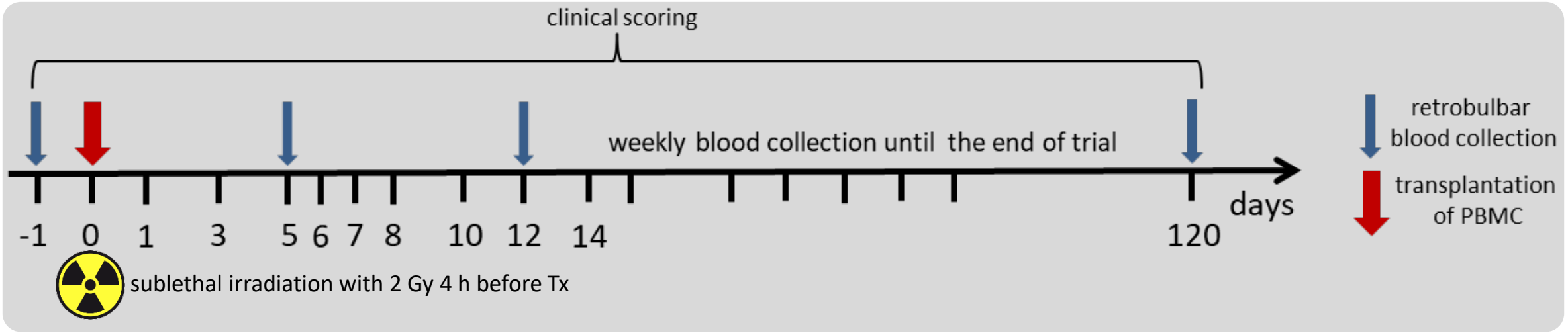
NSG mice transplanted with PBMC obtained from healthy donors



1) **Prophylactic treatment**
2) **Early treatment**
3) **Late treatment**

Experimetal design

GvHD Model with irradiation

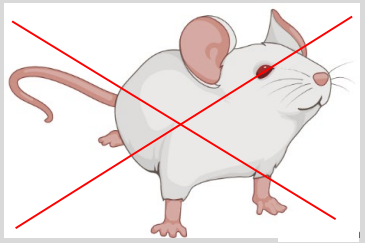


Clinical scoring is based on the evaluation of

- Body weight
- Activity
- Posture
- Fur texture
- Symptoms (texture of skin and feces)

Reaching a certain threshold score

Removal of animals from experiment

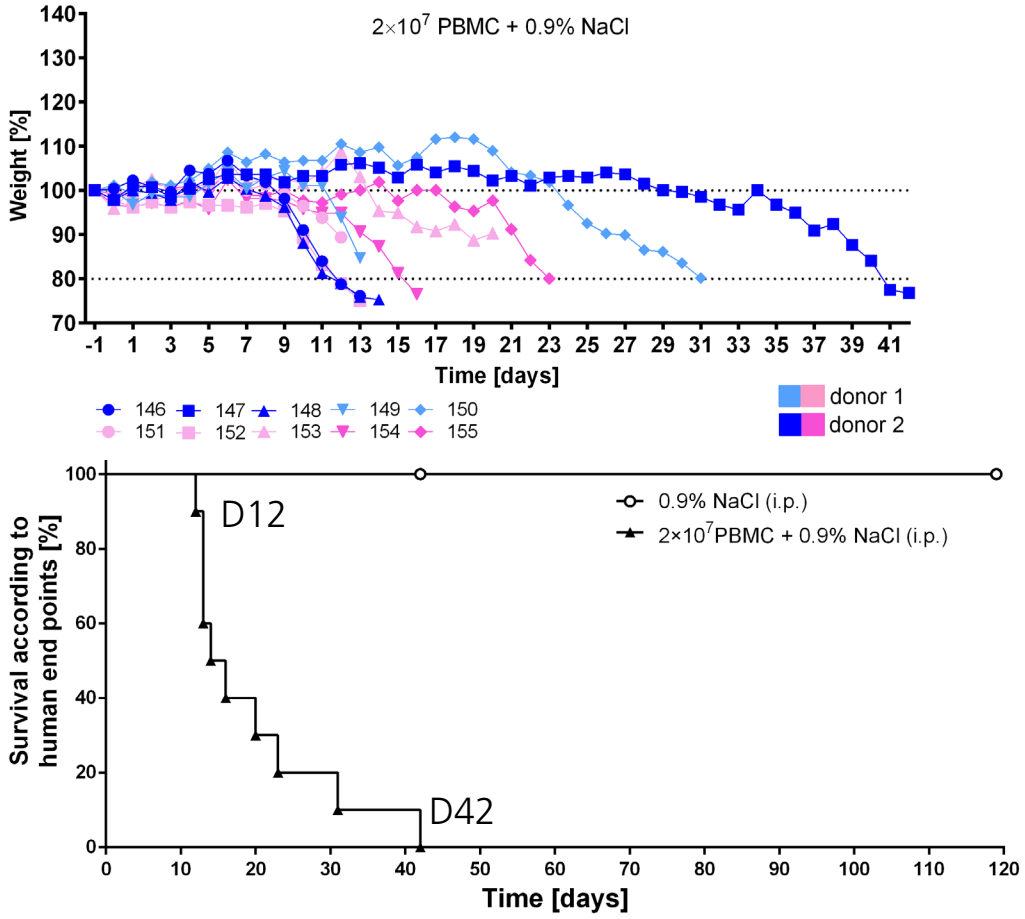


Gy = Gray
Tx = Transplantation

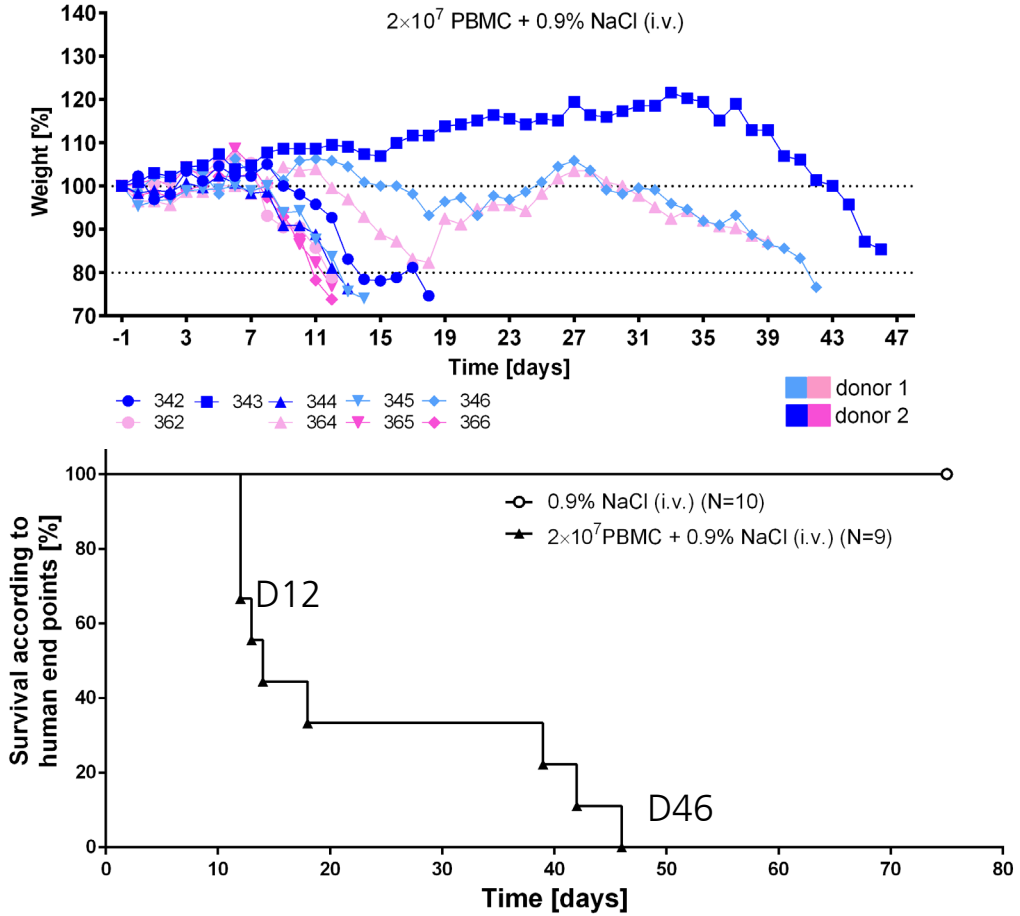
Results I

Body weight and survival

Exp. 1: 2 different human donors



Exp. 2: 2 different human donors



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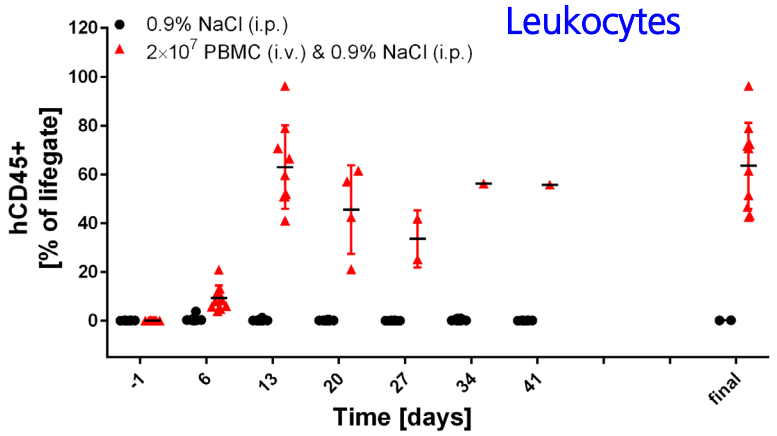
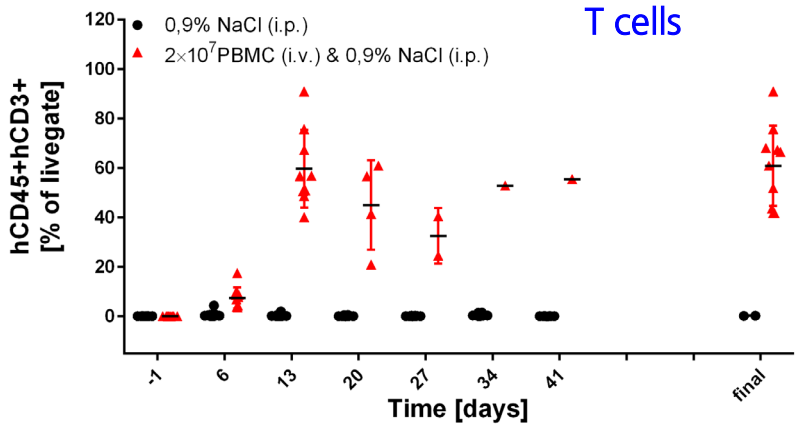
Results II

Flow cytometric analysis - Engraftment of PBMC

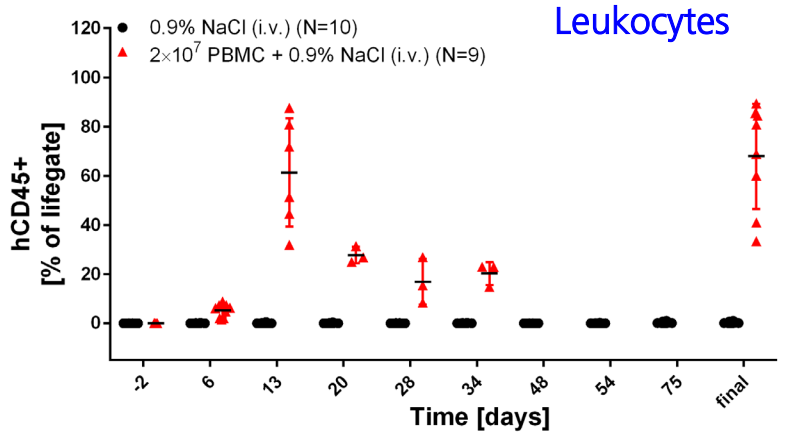
Exp. 1



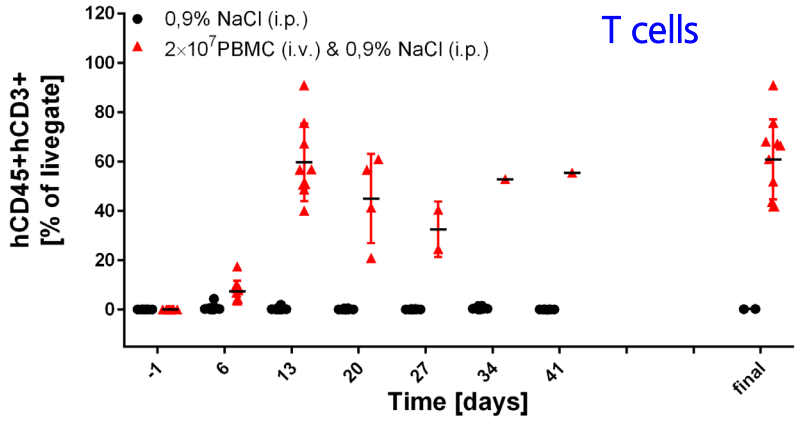
D12
D42



Exp. 2



D12
D46



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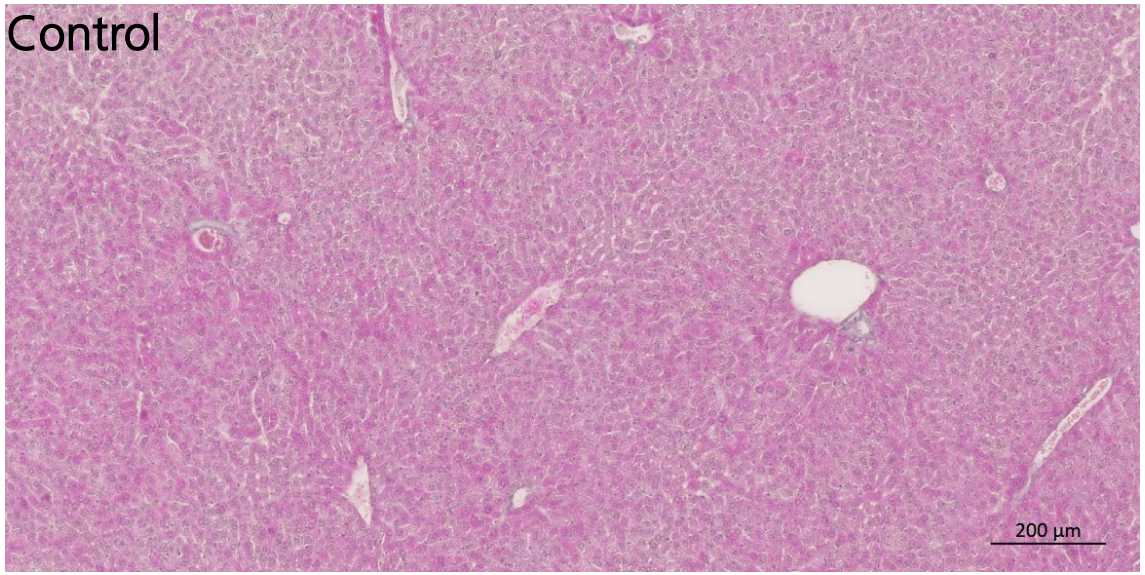
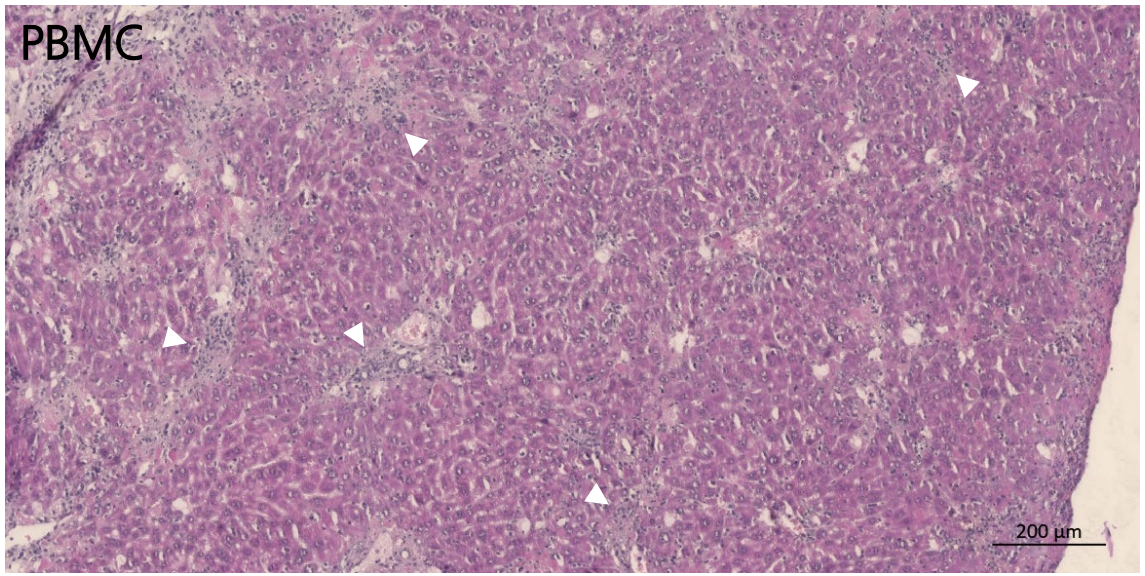
Results III

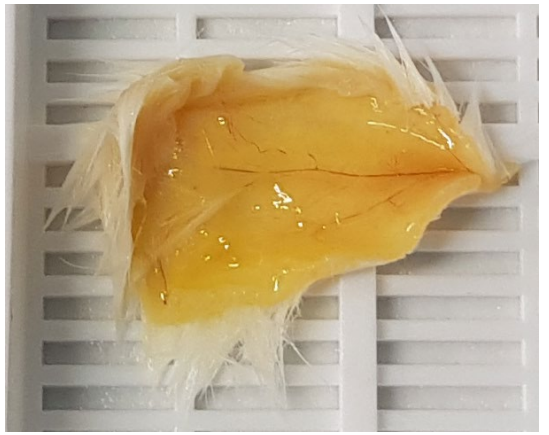
Hepatic GvHD

H&E staining





Icterus



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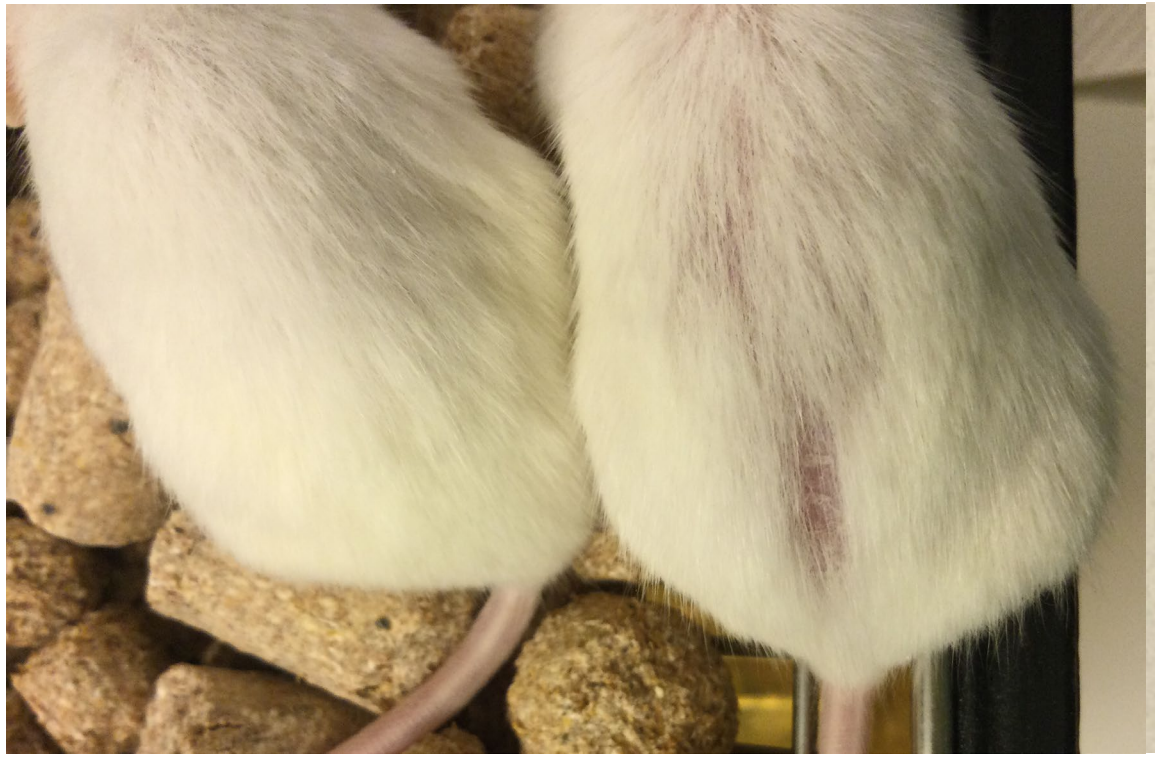
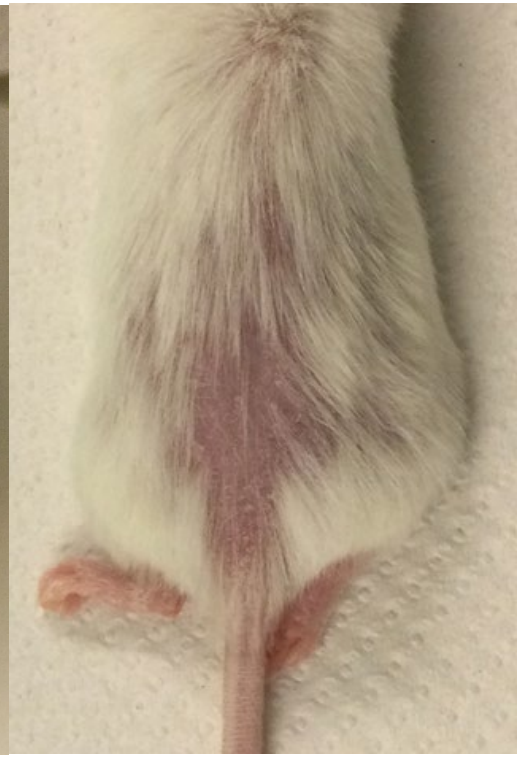
Results IV

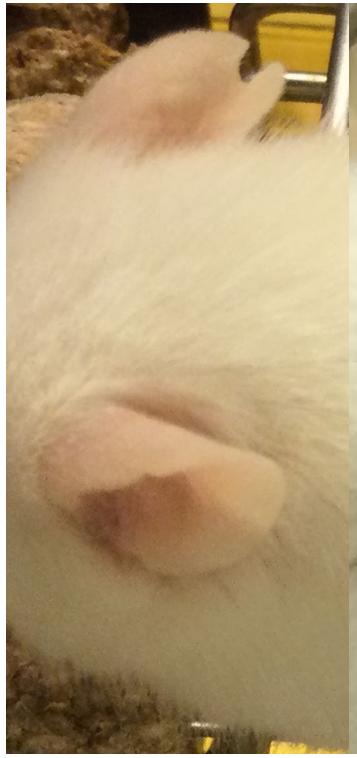
Visual signs of GvHD

Examples for GvHD of the skin

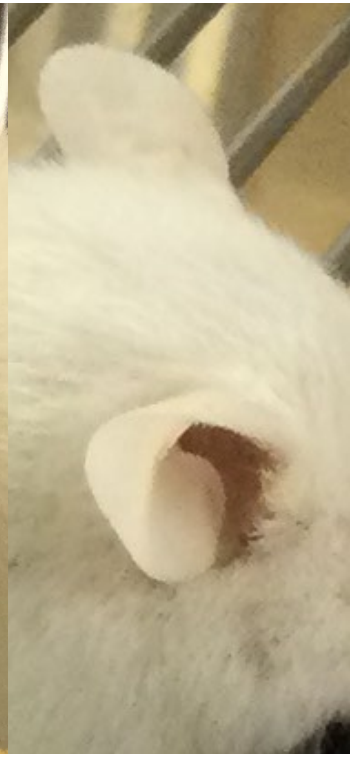




Non-anemic



Anemic



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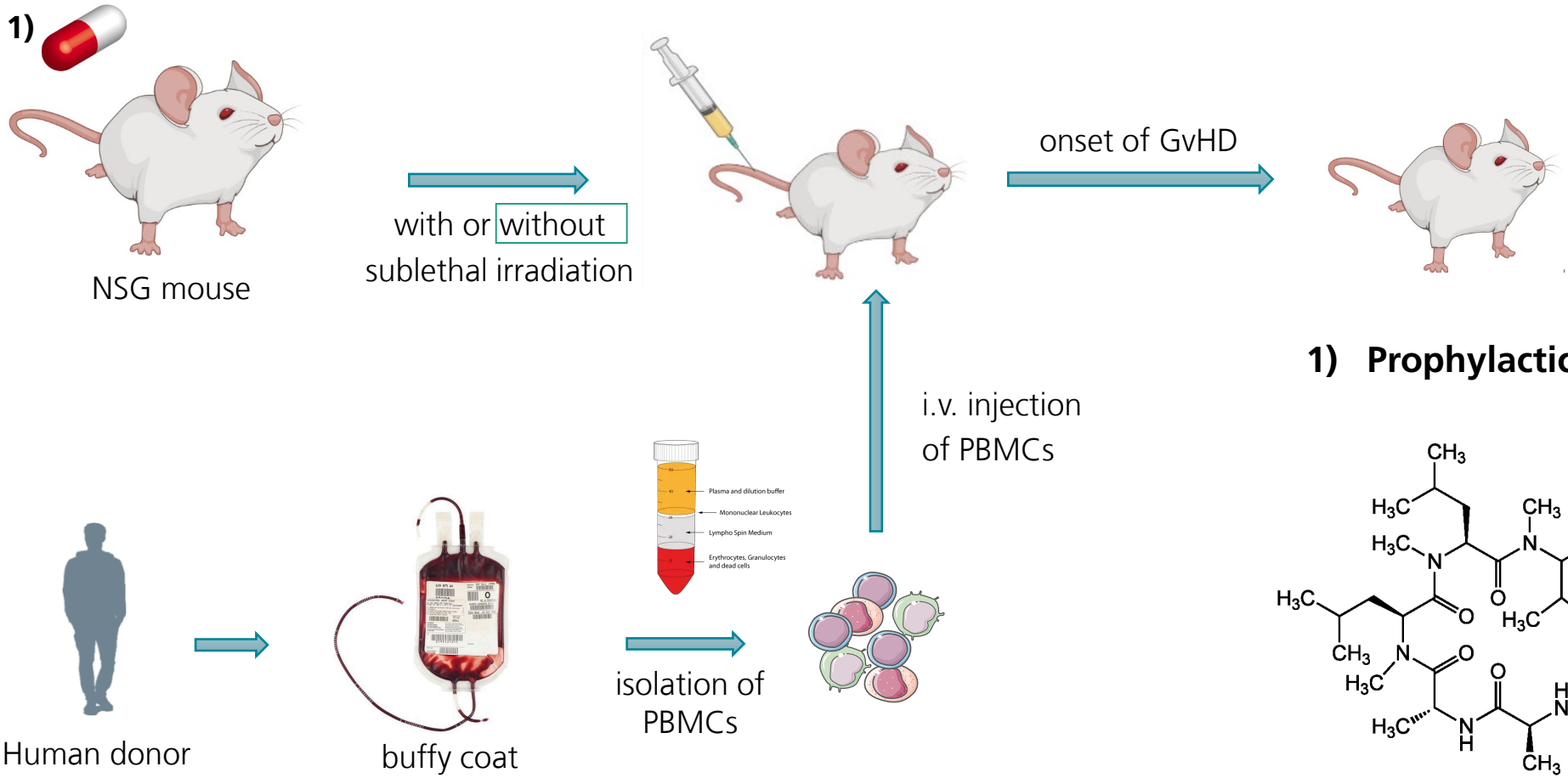
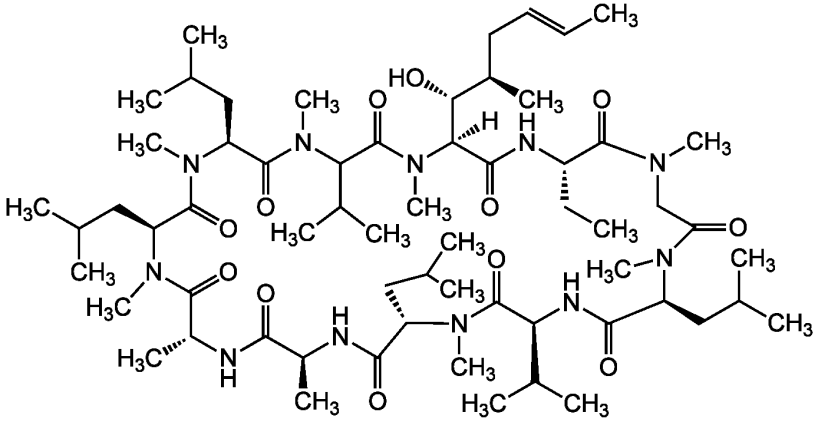
Xenogenic murine model for GvHD

Current GvHD Model at IZI

NSG mice transplanted with PBMC obtained from healthy donors

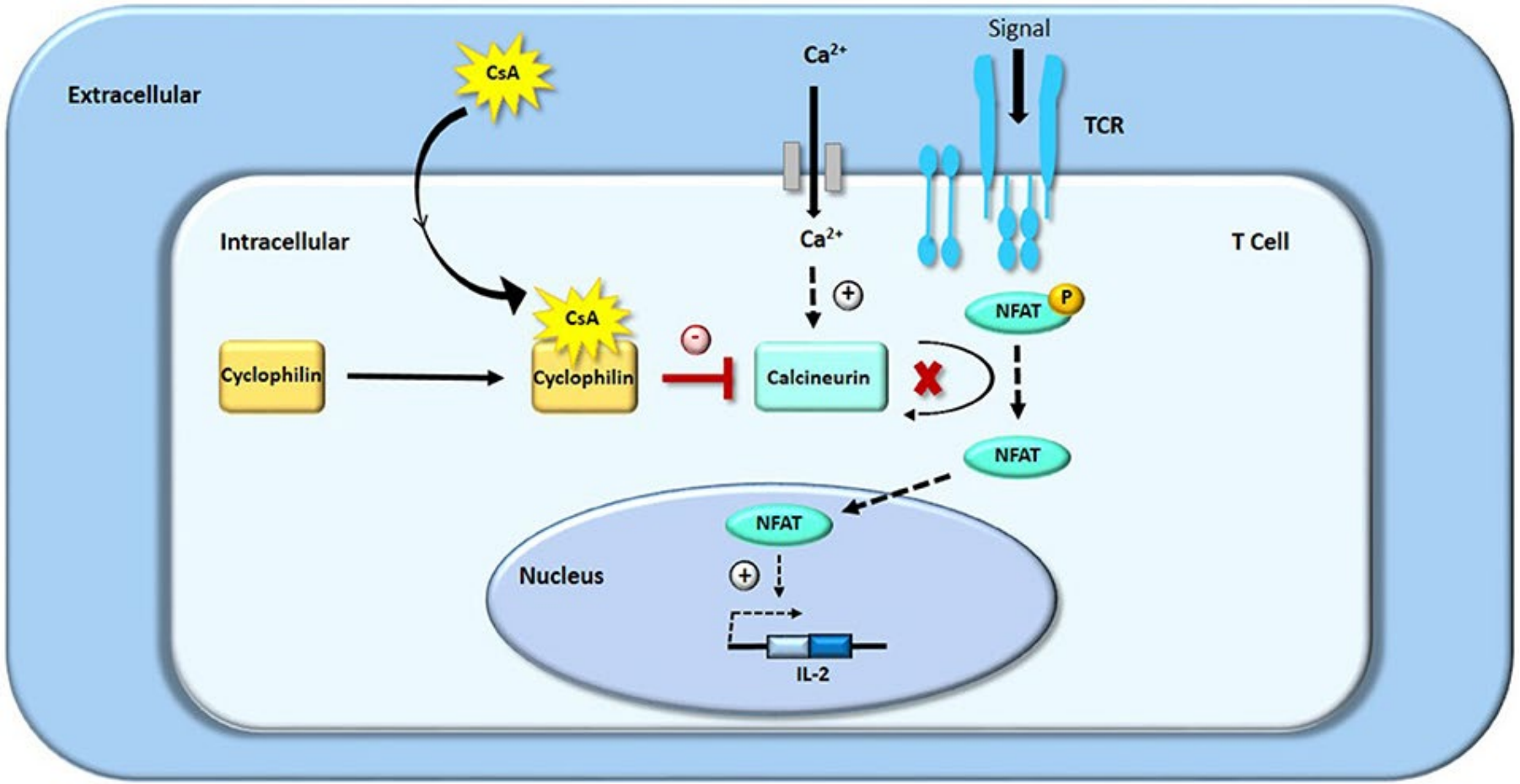


1) Prophylactic treatment with CsA



Cyclosporin A

Mode of action



Inhibition of T-cells proliferation by blocking IL-2 transcription.

Flores et al, Front. Immunol, 2019

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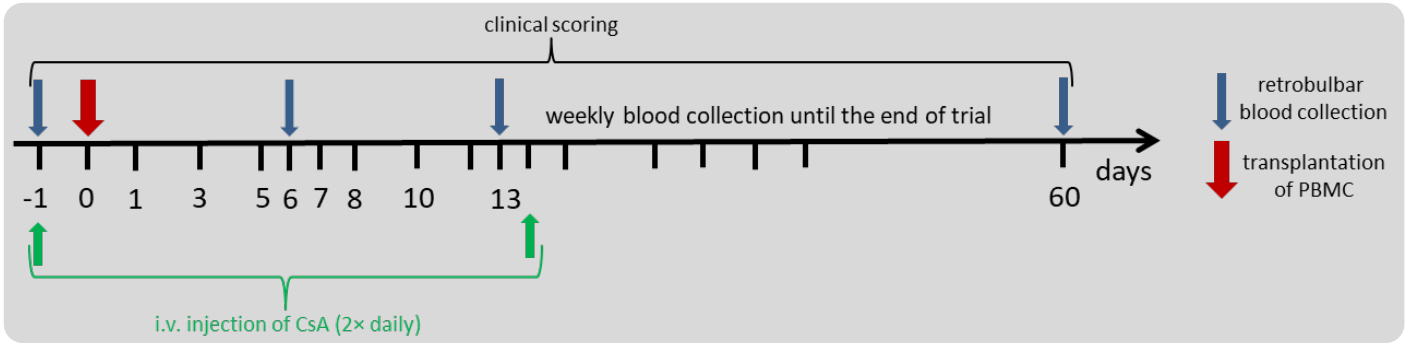
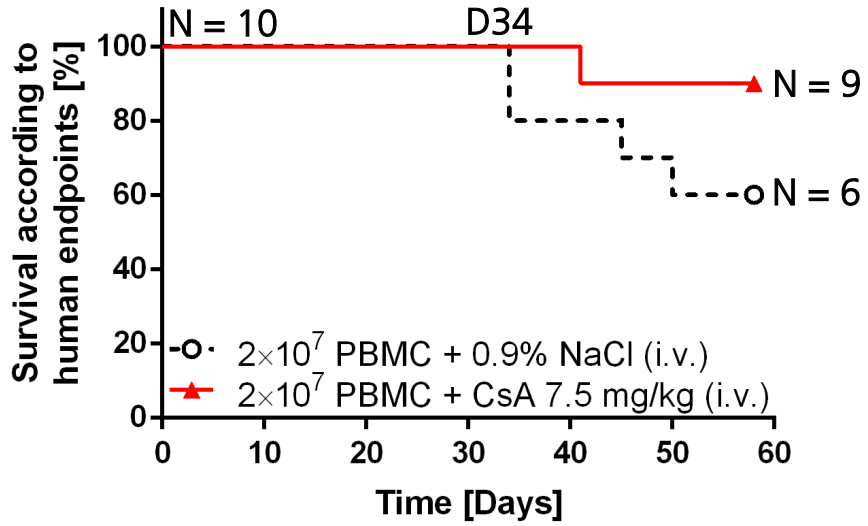
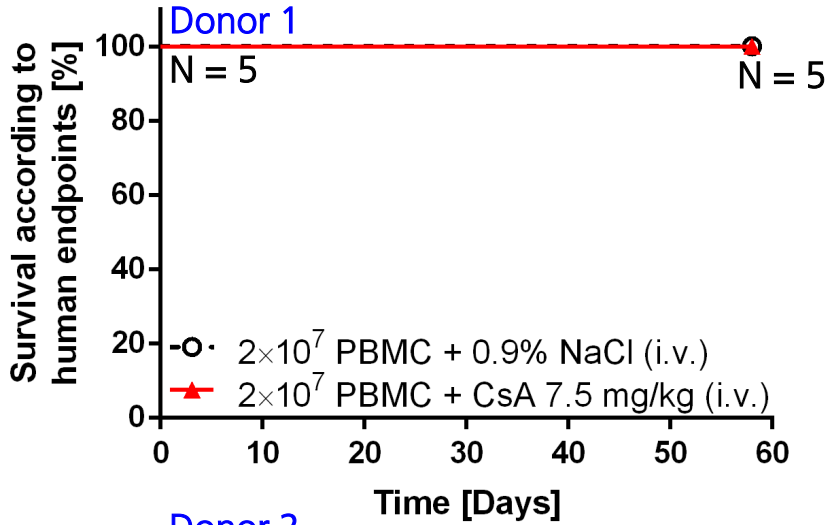
Results I

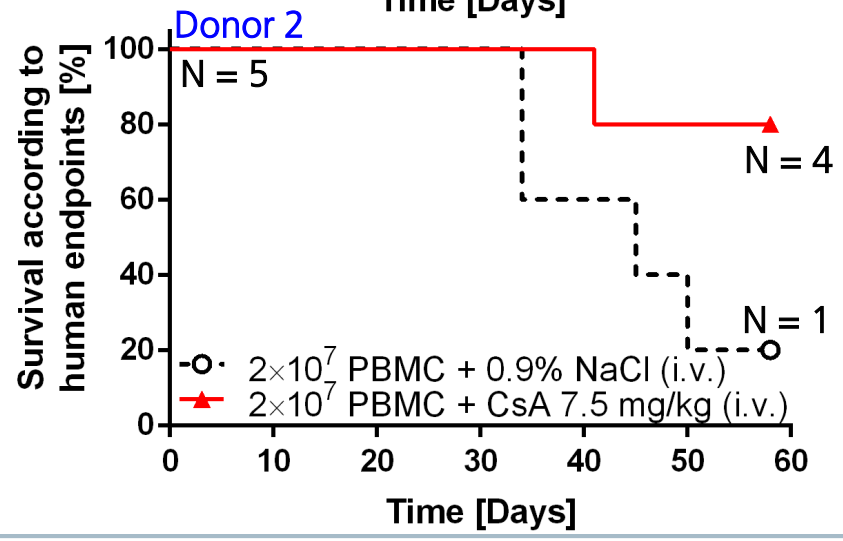
Experimental design and survival



2 different human donors







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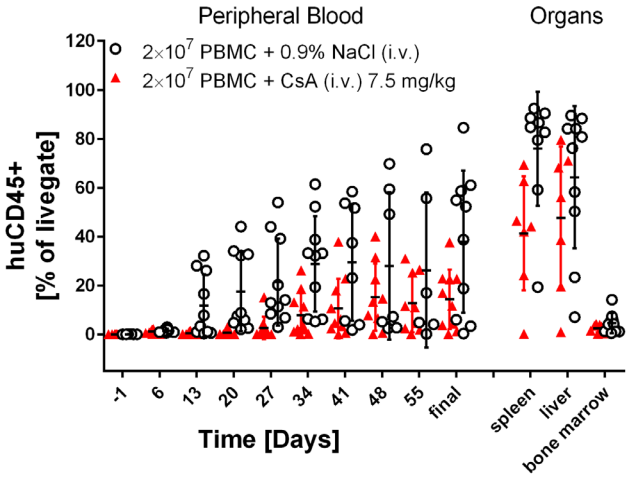
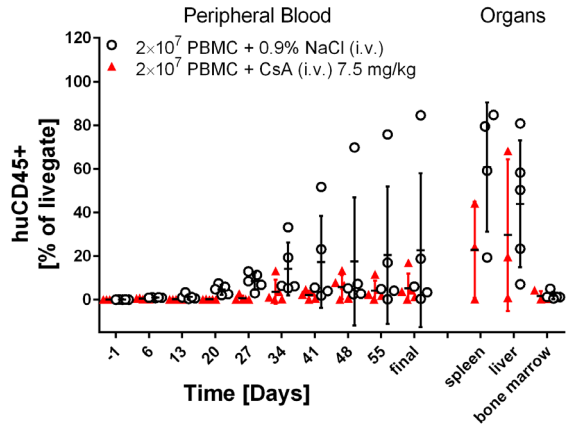
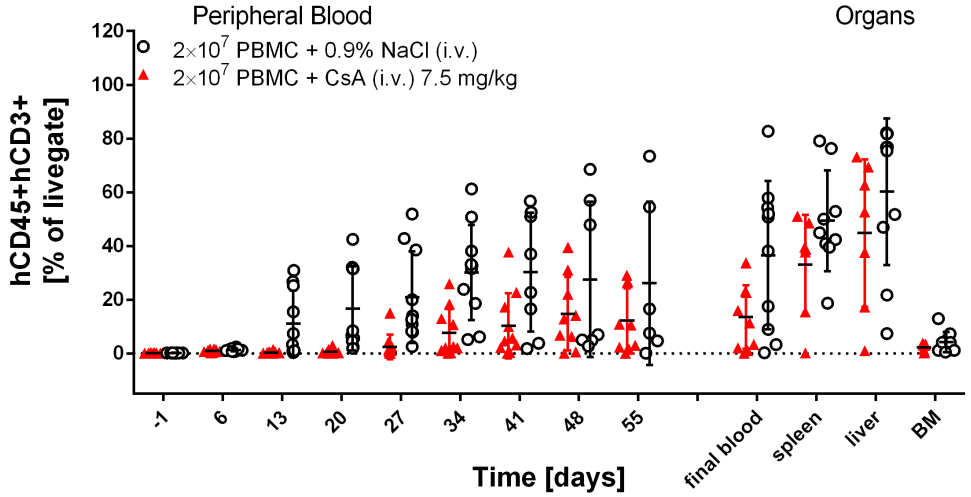
Results II

Flow cytometric analysis - Engraftment of PBMC

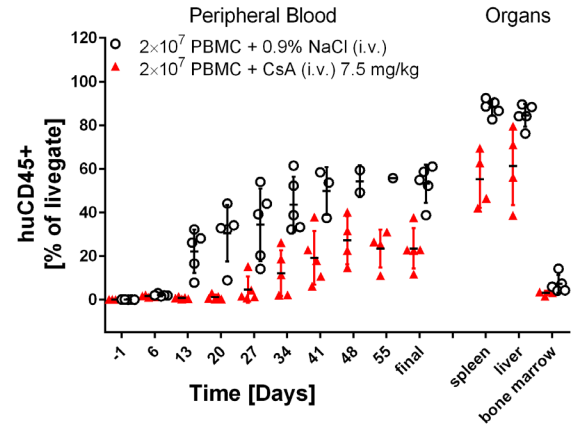


Donor 1





Donor 2



unpublished data

SaxoCell Optix

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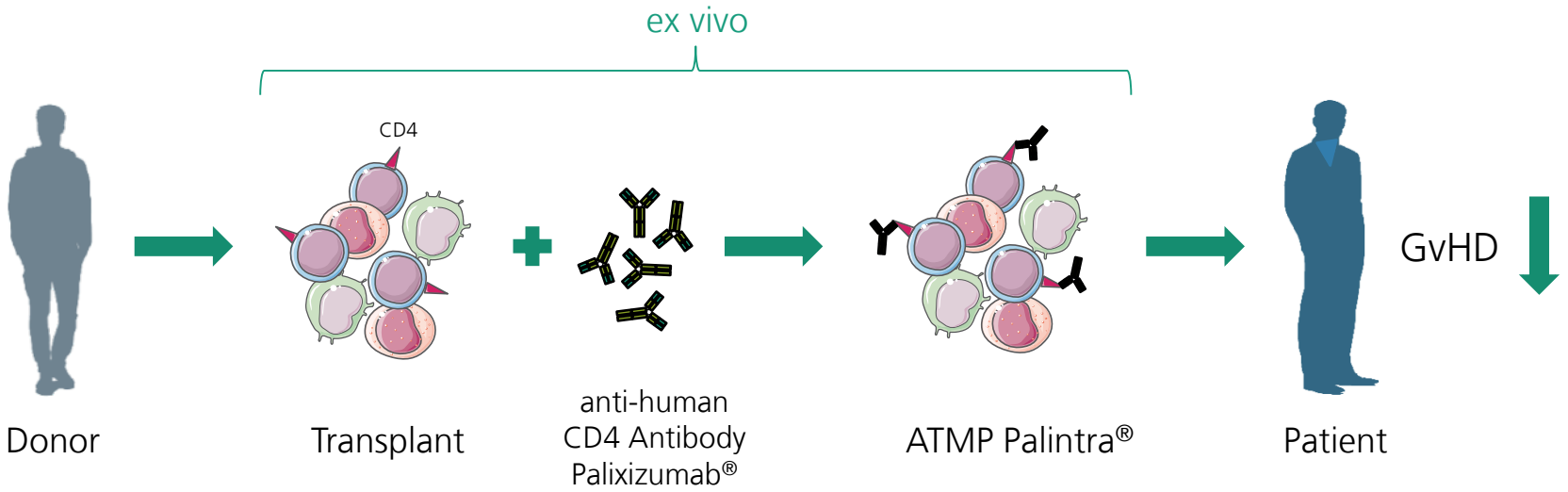
SaxoCell Optix

ATMP Palintra

Selective reduction of alloreactive T cell responses (GvHD) after hematopoietic cell transplantations



- Obtain and not decrease the anti-tumor-effect (GvL)
- Omit the toxicity caused by conventional immunosuppressive drugs
- Develop a clinically applicable, gentle therapy method

ATMP = Advanced Therapy Medicinal Product

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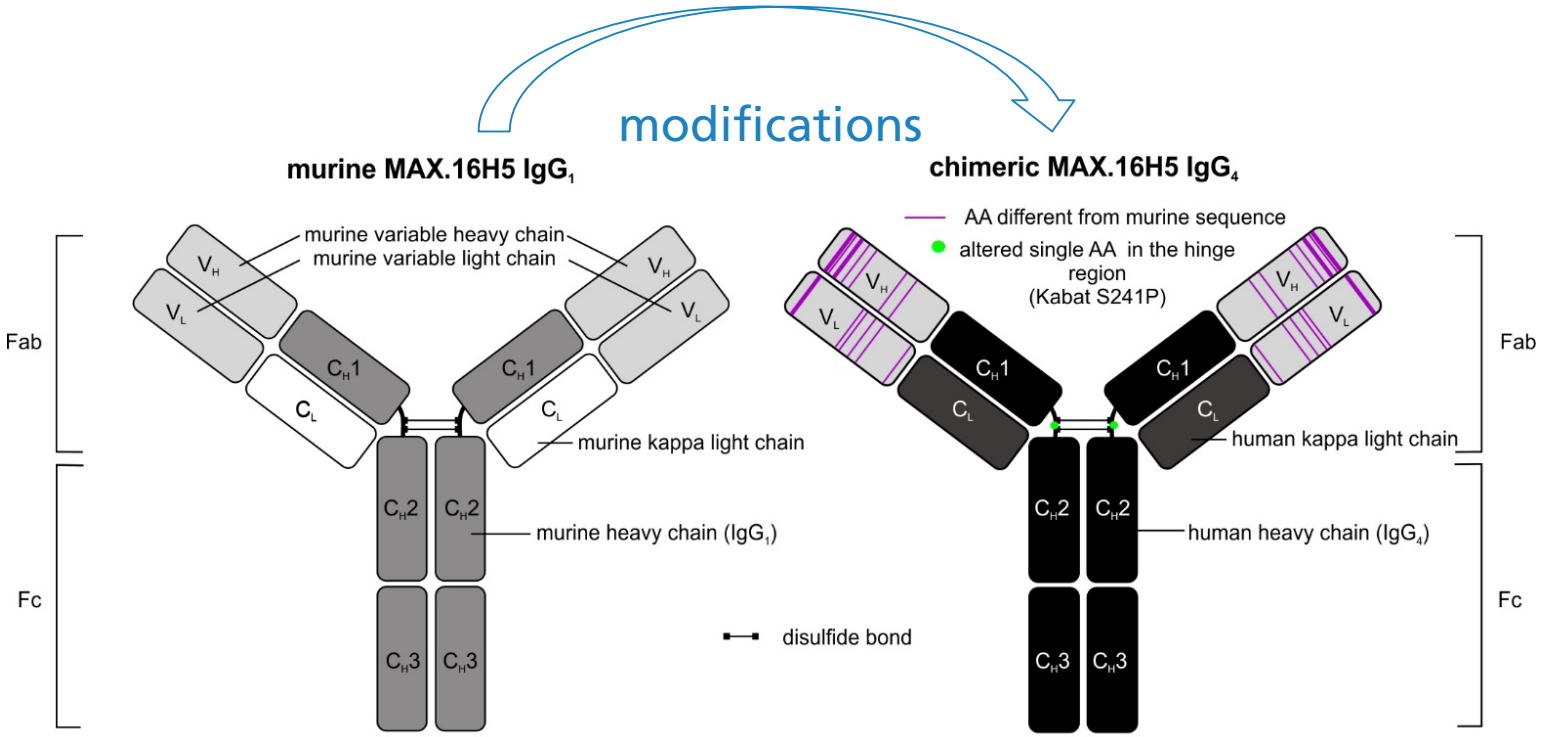
Saxocell Optix

Anti-human CD4 antibody MAX.16H5

Prevention of GvHD with preserved GvL effect using non-depleting anti-human CD4 antibodies (Ab)

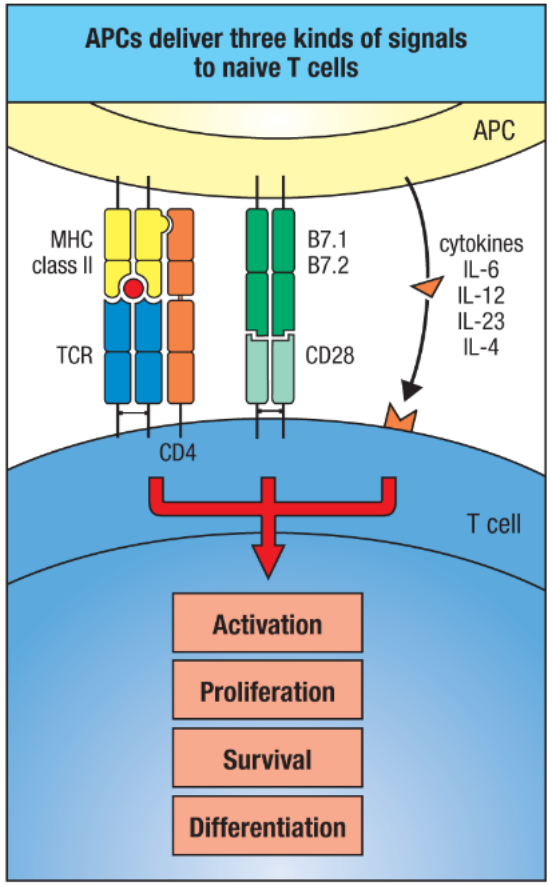


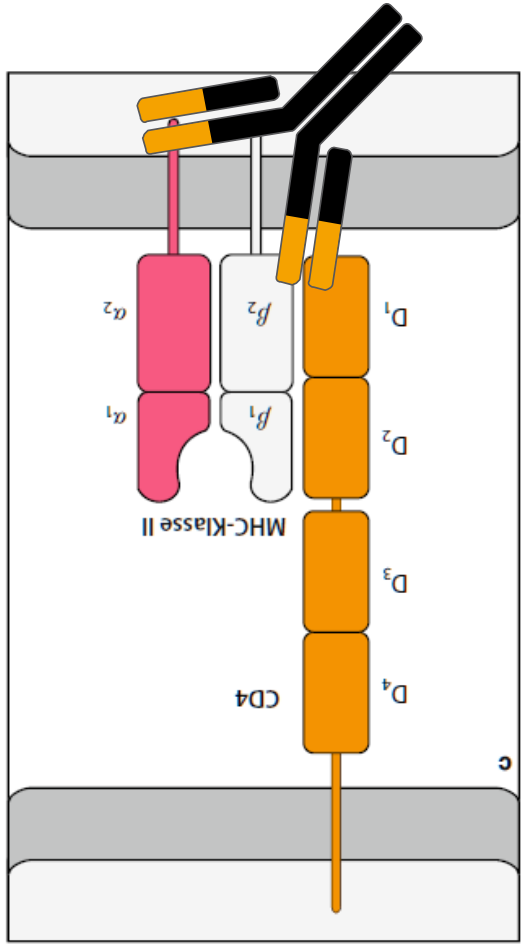
- Chimerization to reduce immunogenicity for potential clinical trials
- IgG4 is a weak activator of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC)

Stahl et al., Front. Immunol., 2019

SaxoCell Optix

MAX.16H5 – Mode of action





Primary mechanism of action of the anti-huCD4 antibody MAX.16H5:

CD4-targeted costimulatory blockade impairs TCR signalling.

Roth et al. 2023, in preparation

Janeway 10th Edition, 2022

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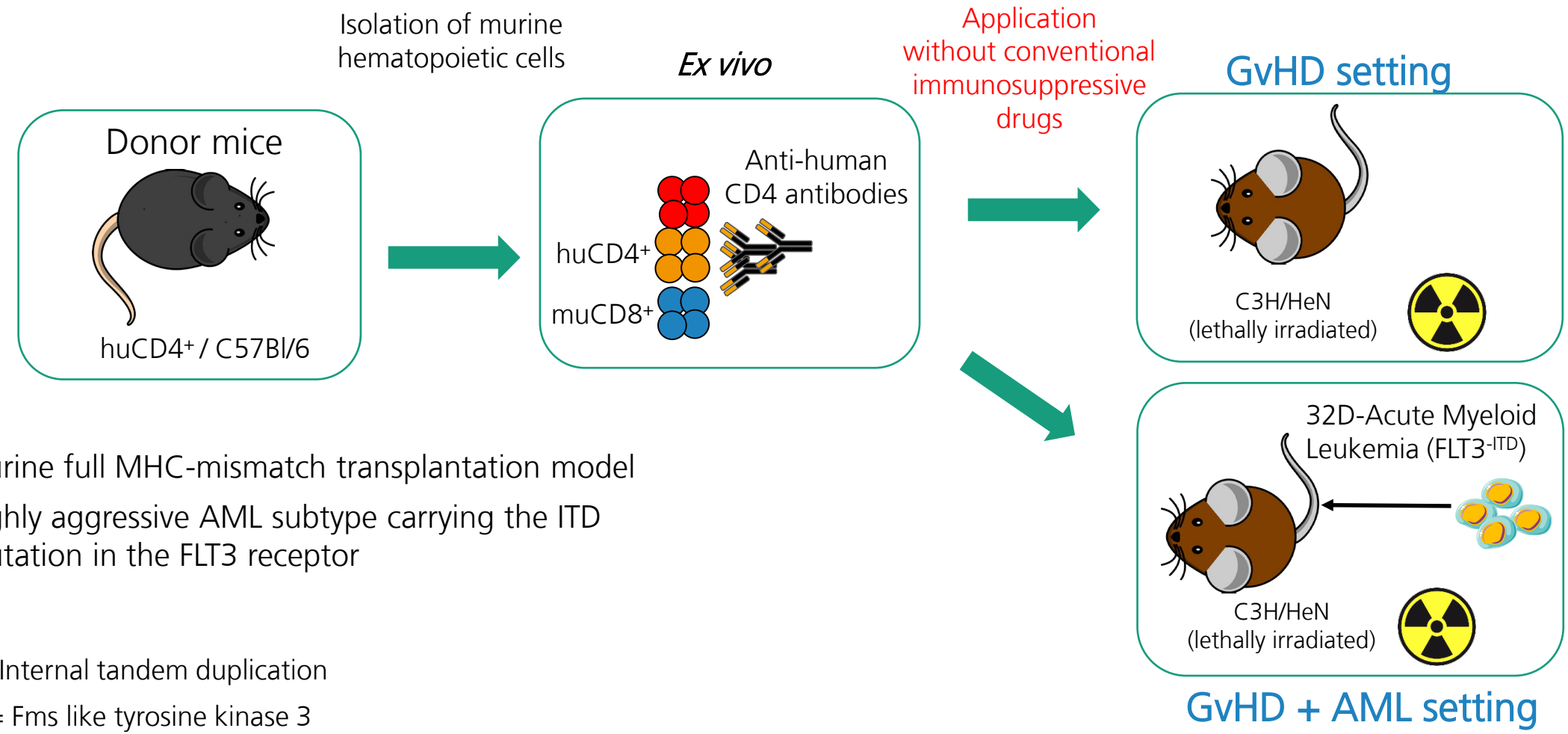
Acute Myeloid Leukemia (AML) – C3H Mouse Model

Experimental Design



- Murine full MHC-mismatch transplantation model
- Highly aggressive AML subtype carrying the ITD mutation in the FLT3 receptor

ITD = Internal tandem duplication

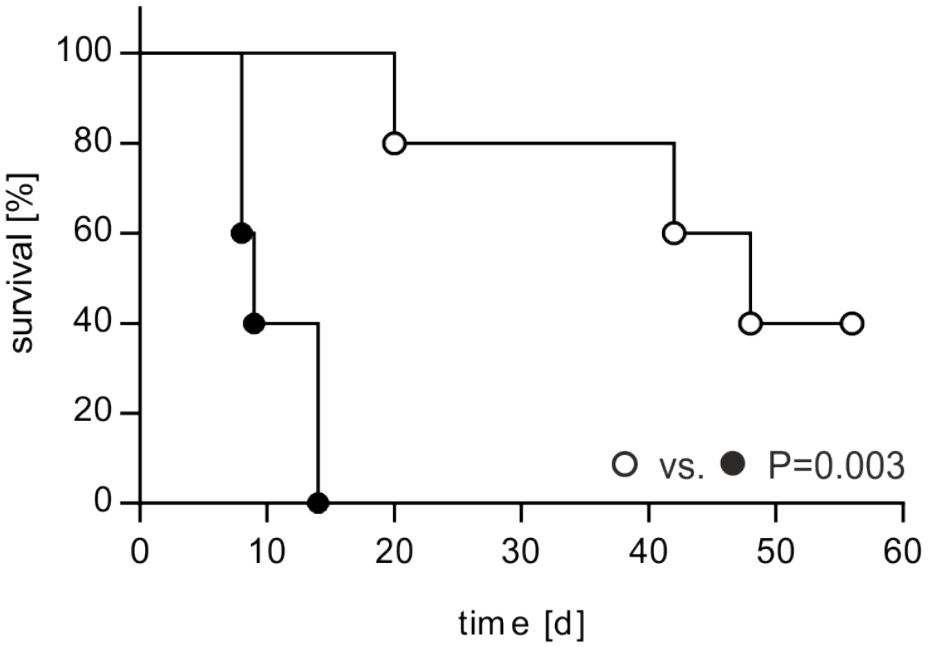
FLT3 = Fms like tyrosine kinase 3

Hilger et al., Front. Immunol., 2018

Acute Myeloid Leukemia (AML) – C3H Mouse Model II

Results - Survival

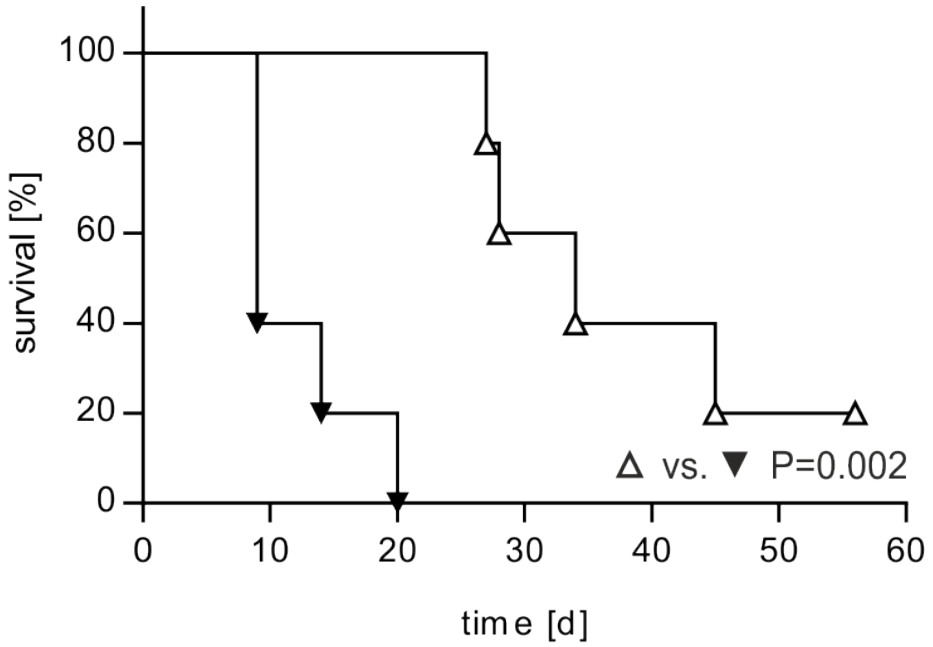
A GvHD setting



● 1×10^7 BMC + 3×10^7 SpC (n=5)

○ 1×10^7 BMC + 3×10^7 SpC + MAX.16H5 IgG_1 (n=5)

B GvHD + AML setting



▼ 1×10^7 BMC + 3×10^7 SpC + 5×10^3 32D-$FLT3^{ITD}$ (n=5)

△ 1×10^7 BMC + 3×10^7 SpC + 5×10^3 32D-$FLT3^{ITD}$ + MAX.16H5 IgG_1 (n=5)

A) Without Ab mice rapidly succumb to GvHD

B) With Ab mice survive significantly longer

Hilger et al., Front. Immunol., 2018

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Thank you for your attention!

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