Optimize *In Vivo* Research with the Right Humanized Mouse Models

Agenda

Talk #1: Introduction to Humanized Immune System (HIS) Mouse Models

Speaker:
Ditte Olsen, PhD
Scientific Solutions Consultant
Taconic Biosciences

Talk #2: Humanized Mice Modeling Services

Speaker:
Caroline Mignard, PhD
Senior Study Director
Oncodesign Services

Talk #3: Methods to Generate a Humanized Mouse

Speaker:
Ditte Olsen, PhD
Scientific Solutions Consultant
Taconic Biosciences

Talk #4: Breeding and Handling of Humanized Mice

Speaker:
Julie Torvund-Jensen, PhD
Associate Director
Taconic Biosciences

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Symposium: Optimize *In Vivo* Research with the Right Humanized Mouse Models

Talk 1: Introduction to Humanized Immune System (HIS) Mouse Models

Ditte Olsen, PhD
Scientific Solution Consultant
Taconic Biosciences

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Agenda

- Types of humanization
- The use of super immunodeficient mice
  - The “Nod Scid Gamma” Landscape
- How to generate HIS mice and the pros and cons of various methods
- Different models – different strengths
- Limitations of HIS mice
- Considerations when choosing model

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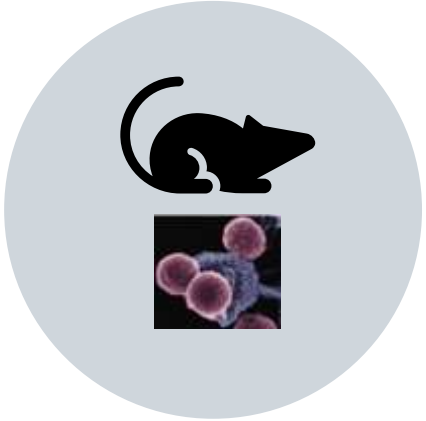
Humanized Mouse Models

Humanized rodents bridge some of the translational gaps across the species barrier

- Human tissue grafts
- Purified human immune cell populations
- Mixed human immune cells
- Human stem cells



Human cells/tissue **engrafted** into the mouse

Human genes **inserted** into the mouse genome

- Human transgenes
- Human minigene knock-ins
- Partial human gene knock-ins
- Full genomic replacements

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Humanized Mouse Models

Humanized rodents bridge some of the translational gaps across the species barrier

- **Human tissue grafts**
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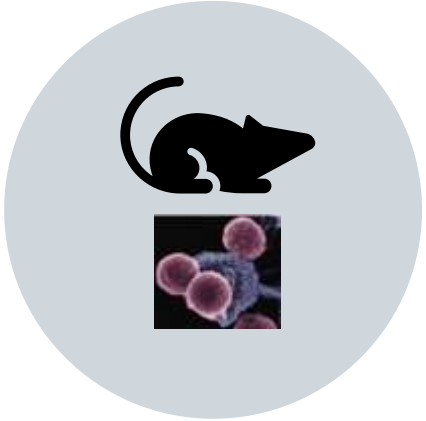
- **Human stem cells**



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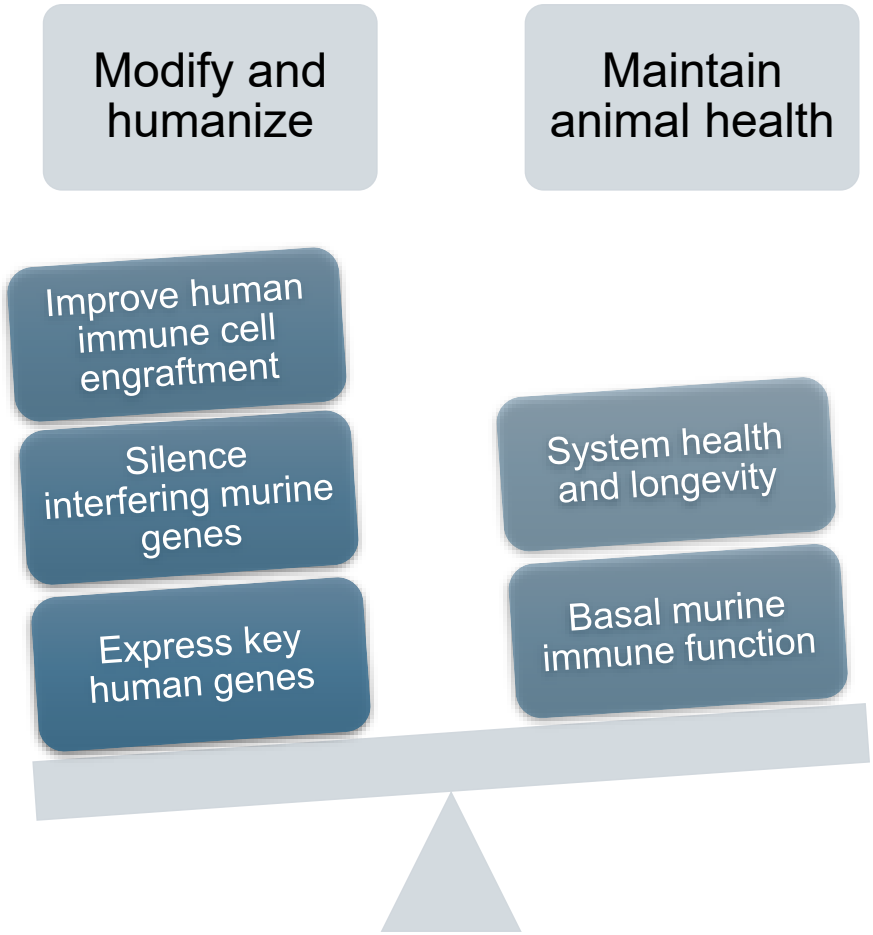
Improving Experimental Outcomes with Novel Models

Balancing desired modifications with model health outcomes

Desirable changes to humanize for improved project outcomes…



…must be balanced with ongoing functionality of murine systems

It is not possible to fully humanize a mouse

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Choosing the Right Mouse Strain to Humanize

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CIEA NOG Mouse®

The first and most versatile SUPER IMMUNODEFICIENT MODEL on the market

1983

NOD mouse

- **Polymorphic SIRPα**
- **Complement C5 deficient**
- **Impaired dendritic cell function**
- **Impaired macrophage function**

1985

Scid mouse

- **B and T cell deficient**

1990

NOD scid mouse

- **Polymorphic SIRP-α**
- **Complement C5 deficient**
- **Impaired dendritic cell function**
- **Impaired macrophage function**
- **B and T cell deficient**

1997

IL2rγ^{null} mouse

- **NK cell deficient**
- **Impaired cytokine signaling**

1998

CIEA NOG mouse®

- **Polymorphic SIRPα**
- **Complement C5 deficient**
- **Impaired dendritic cell function**
- **Impaired macrophage function**
- **B and T cell deficient**
- **NK cell deficient**
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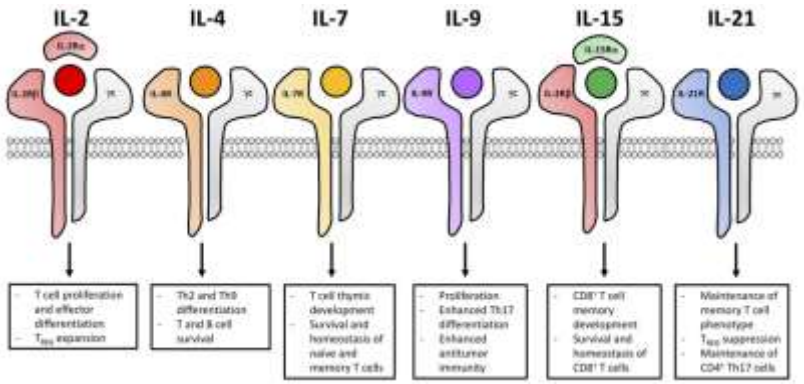

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Figure from: Dwyer C.J, et. al. Fueling Cancer Immunotherapy With Common Gamma Chain Cytokines. Front Immunol 2019 10:263. doi: 10.3389/fimmu.2019.00263.

The “Nod Scid Gamma” Landscape

NOG mouse® (**NO**D/Scid, IL2R**Gamma**), CIEA/Taconic

NOD/SCID/γ_c^{null} mouse: an excellent recipient mouse model for engraftment of human cells

Mamoru Ito, Hidefumi Hiramatsu, Kimio Kobayashi, Kazutomo Suzue, Mariko Kawahata, Kyoji Hioki, Yoshito Ueyama, Yoshio Koyanagi, Kazuo Sugamura, Kohichiro Tsuji, Toshio Heike, and Tatsutoshi Nakahata

BLOOD, 1 NOVEMBER 2002 • VOLUME 100, NUMBER 9 3175

NSG™ mouse, (**N**OD/**S**cid, IL2R**Gamma**), Jackson

Human Lymphoid and Myeloid Cell Development in NOD/LtSz-*scid IL2Rγ*^{null} Mice Engrafted with Mobilized Human Hemopoietic Stem Cells[1,2]

The Journal of Immunology, 2005, 174: 6477–6489.

NCG mouse (**N**OD **C**RISPR Prkdc IL2R**Gamma**), Charles River & GemPharmatech

B-NDG mouse (**B**iocytogen **N**OD/Sci**D** IL2R**Gamma**), Envigo

NXG mouse (**N**OD **X**enograft **G**amma), Janvier

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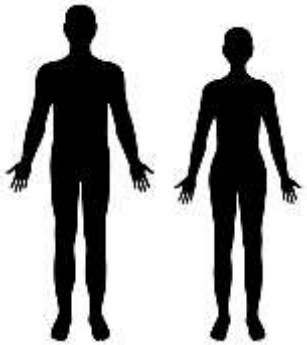
Methods to Generate HIS Mice

PBMC

Adult peripheral blood



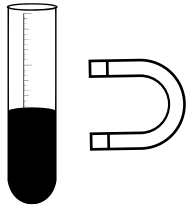
Peripheral Blood Mononuclear Cells

Human peripheral blood mononuclear cell (PBMC)

- Mature human immune cells
- Human immune cells developed in a human and recognize their murine host as foreign

CB CD34+ HSC

Umbilical Cord Blood



CD34+ Cord Blood Cells isolated via MACS

Human hematopoietic stem cells (HSC)

- Multipotent primitive cells that can develop into all types of blood cells
- Commonly isolated from umbilical cord blood, but can also be obtained from fetal liver, adult bone marrow or peripheral blood
- Human immune cells develop within the mouse and recognize the host as self

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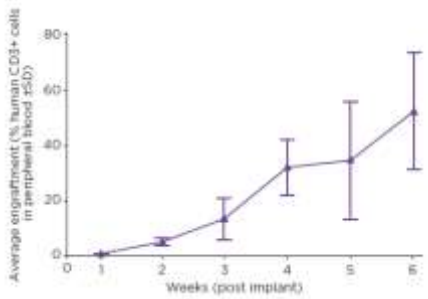
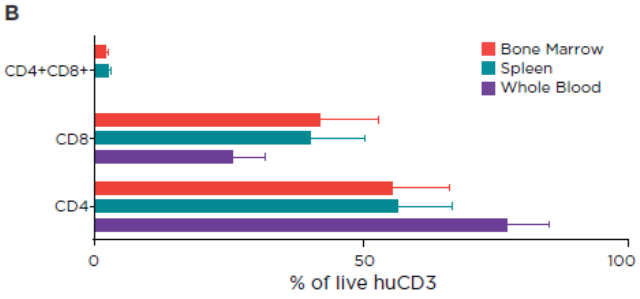
HIS Mice via PBMC Engraftment

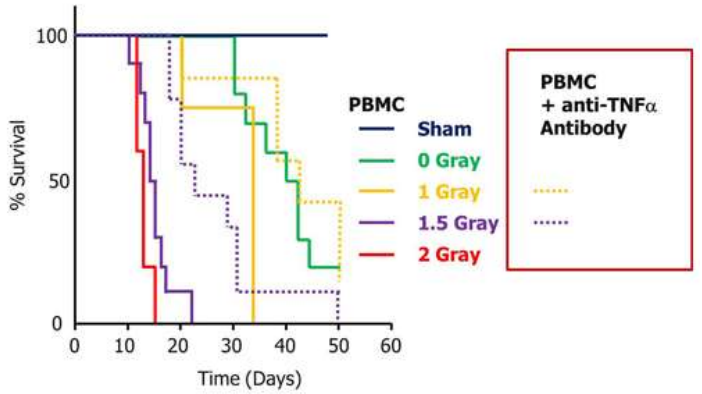
NOG Humanization with PBMC

Myeloablation (optional) | **PBMC i.v. 1x10^7** | **Ship**





Graft vs host disease



Day 0
6-10 weeks old

Day 1

Day 2

Week 3-6

- Study window: 3-4 weeks
- Myeloablation preconditioning improves engraftment but accelerates GvHD
- PBMC cell dose: 5M or 10M
- May perform PBMC engraftment at point-of-receipt, including after tumor installation

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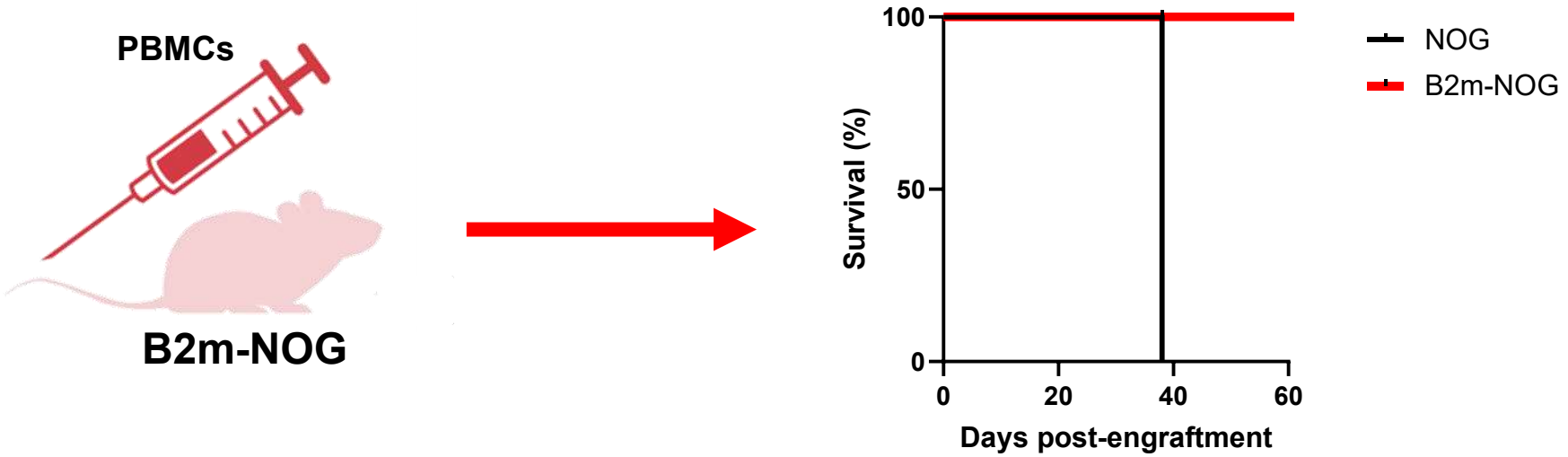
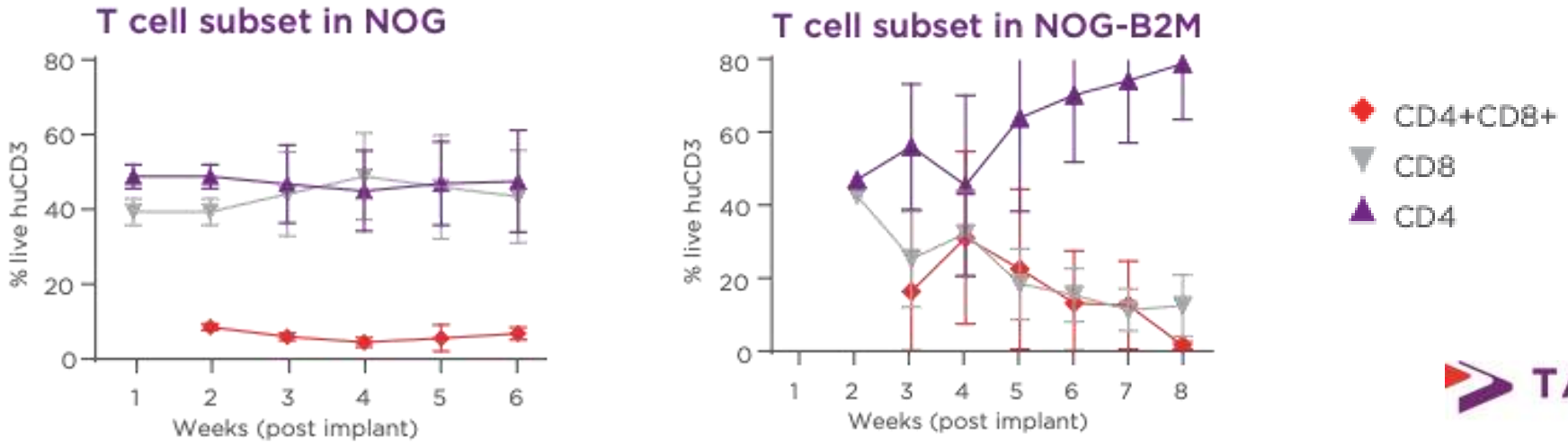
Increased Survival Window in B2m-NOG + PBMCs



- B2m-NOG mice survive significantly longer than other severely-immunodeficient strains following human PBMC engraftment (1 x 10^7 human PBMC)



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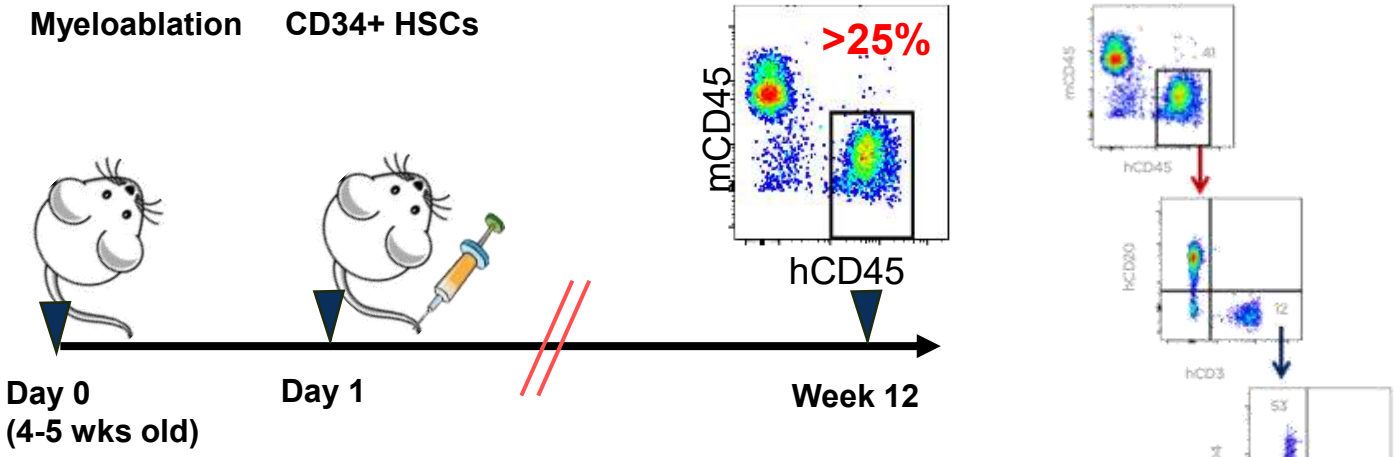
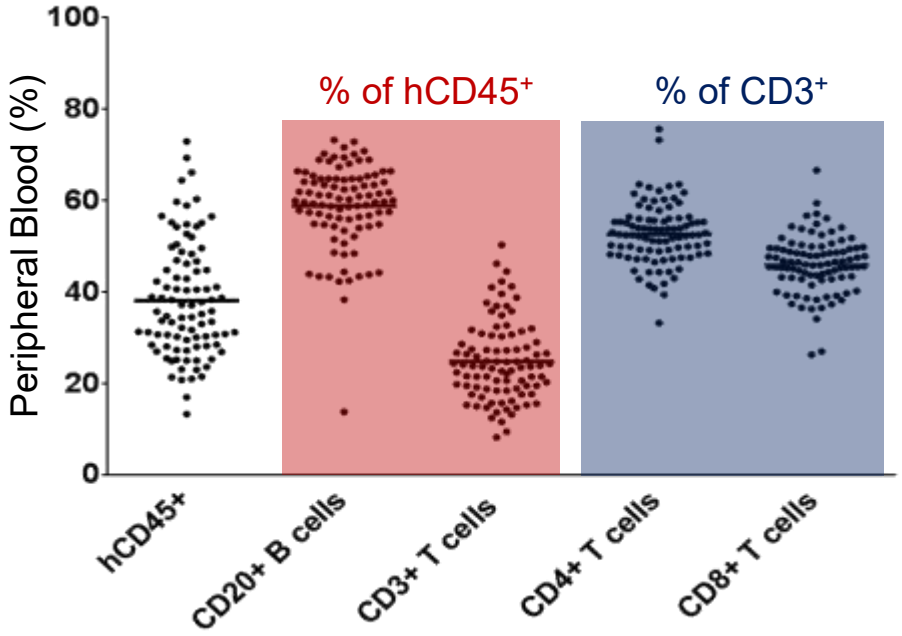
Models For Life.

HIS Mice via CD34+ Hematopoietic Stem Cell Engraftment

NOG Humanization with HSCs





- HSCs are cord blood-derived (CD34+ HSCs)
- QC for every mouse (% CD45 chimerism)
- B cells (non-functional) and T cells
- Myeloid and NK are marginal

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First-Generation HIS Mouse Models

HIS mice bridge translational gaps across the species barrier

PBMC-engrafted

- Short-term studies (~4 weeks)
- T cell predominant
- B cells nonfunctional
- Interference from GvHD
- Suitable for some T cell-engaging therapeutics

CD34+ HSC-engrafted

- Long-term studies (50+ weeks)
- T cell predominant
- B cells nonfunctional
- Incomplete immune T cell development in mouse thymus
- Suitable for some T cell-engaging therapeutics

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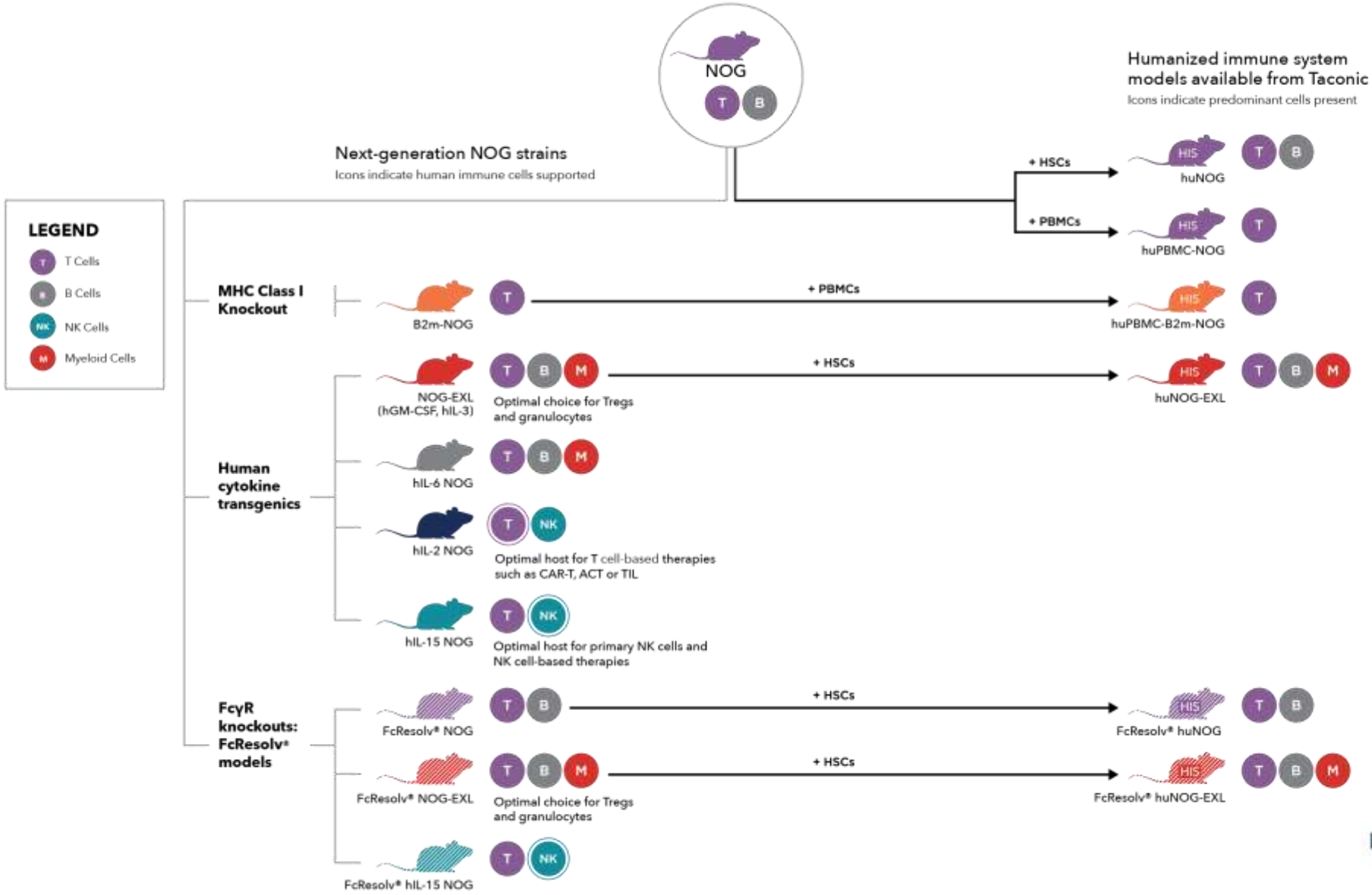
The NOG Portfolio



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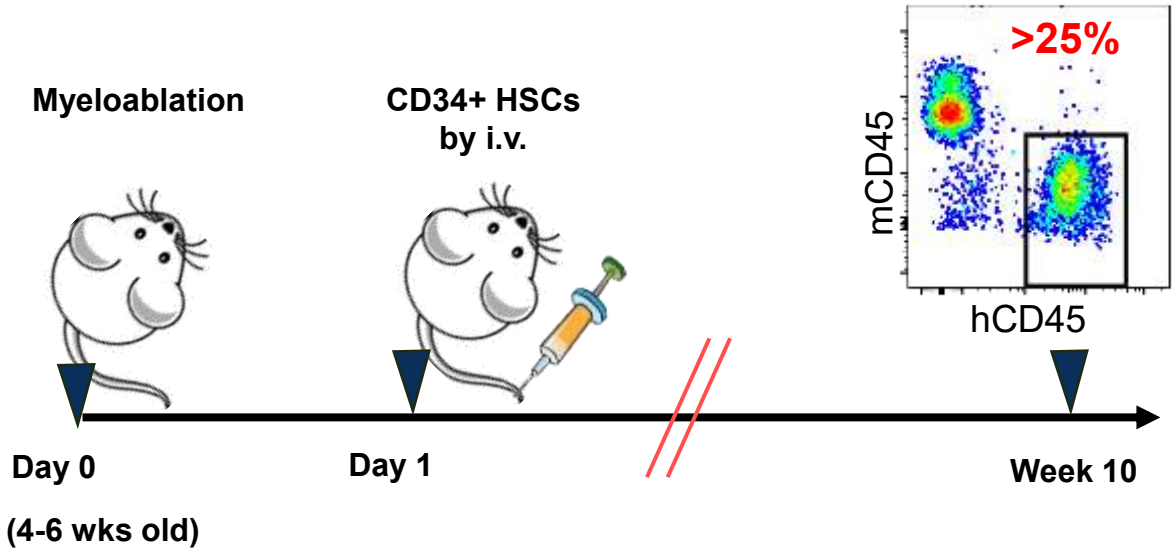
NOG-EXL

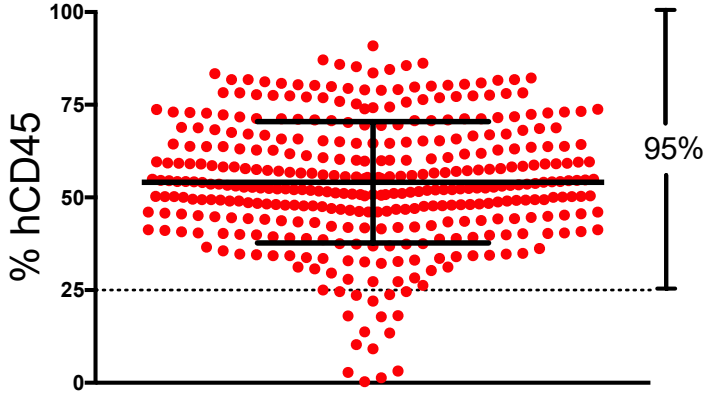
huNOG-EXL

Human CD34+ HSC-engrafted NOG-EXL

Advantages

- Pre-validated donors available for predictable engraftment
- Standard Access animals receive QC by 10-color Flow Cytometry at 10 WPE
- Early Access (EA) animals using pre-validated donors can be shipped as early as 2 WPE, QC optional
- HLA data by request
- Access to animals made with different HSC donors
- Customizations available by request





Example data from multiple combined lots

n= 371
mean = 54%
SD = 16%

Average n/lot size=43

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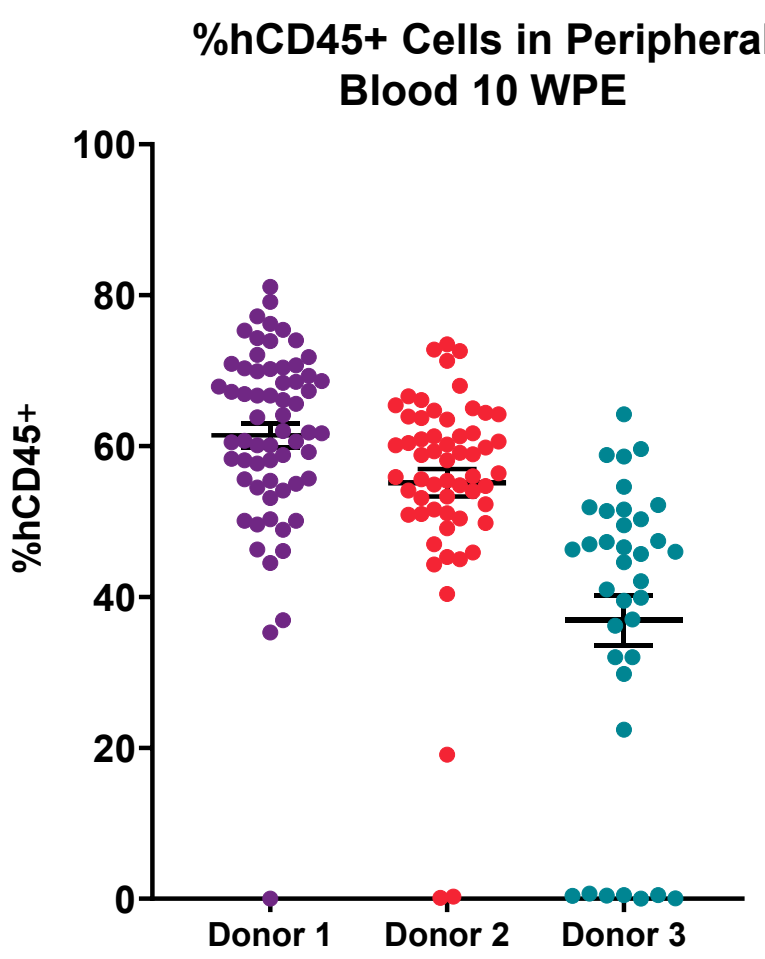
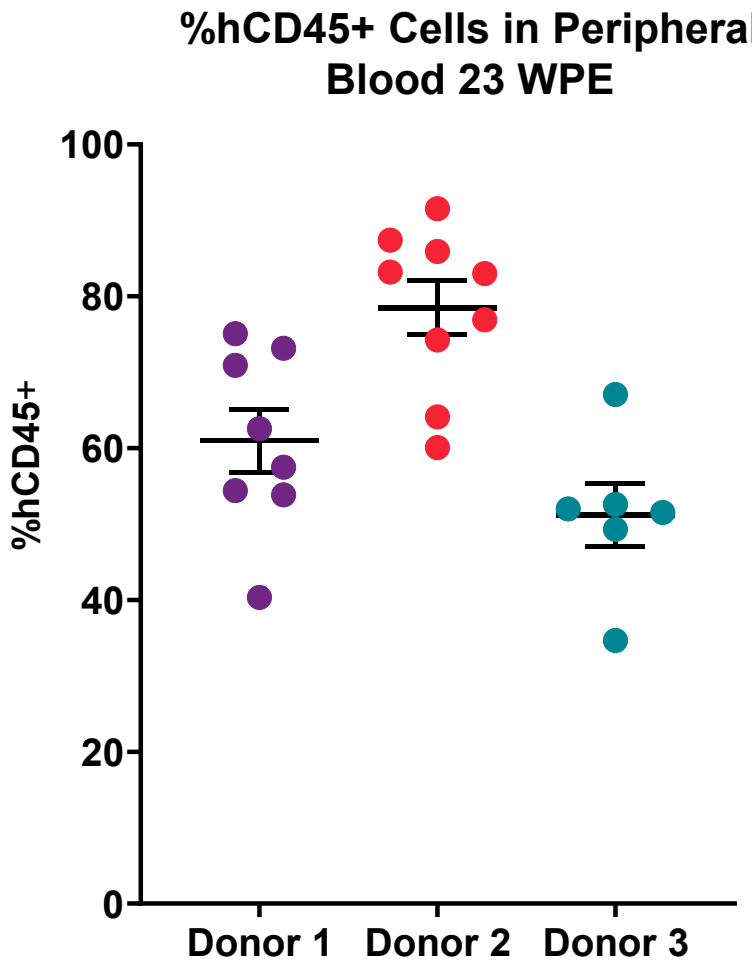
Immune Cell Engraftment in huNOG-EXL Over Time

Human chimerism in peripheral blood is stable





Taconic internal study

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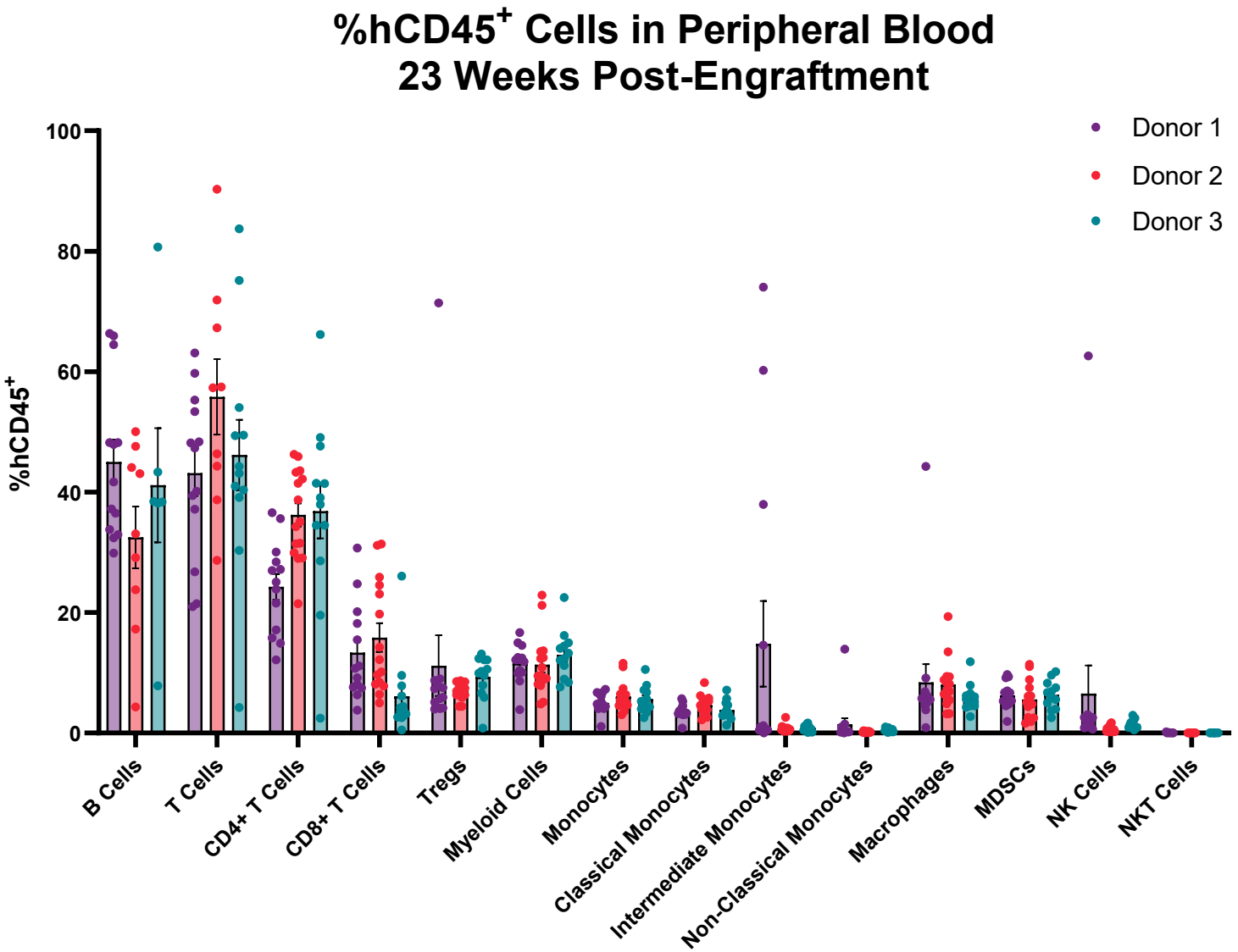
Immune Cell Engraftment in huNOG-EXL at 23 WPE



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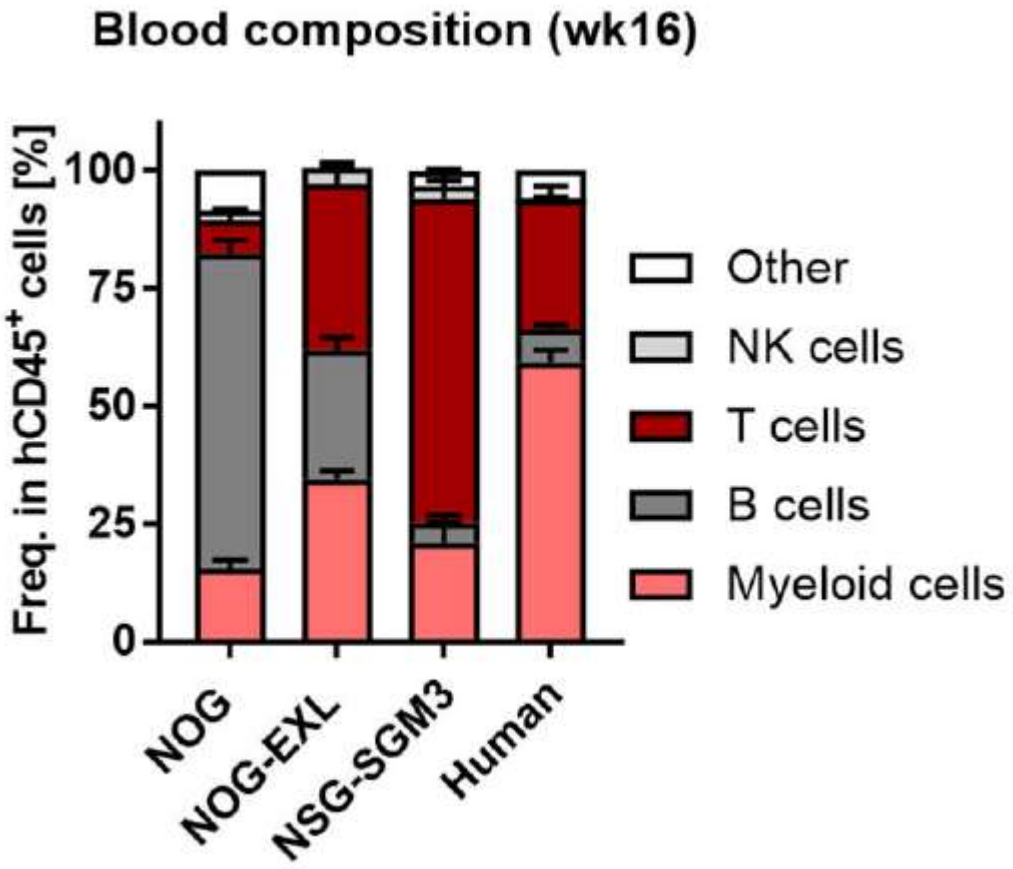
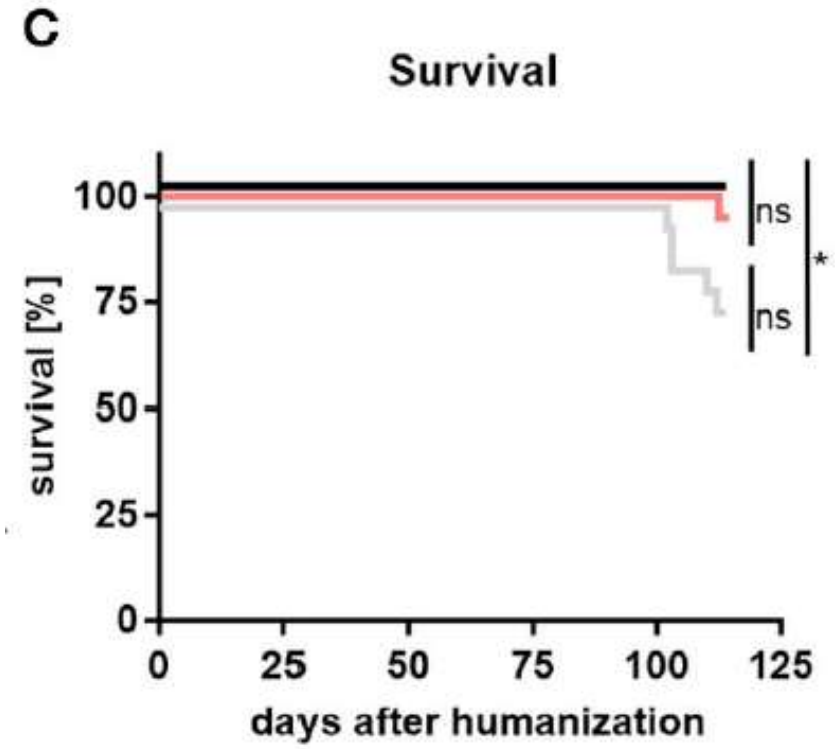
CD34+ HSC Engrafted NOG vs. NOG-EXL vs. NSG-SGM3

NOG-EXL better mimic human composition



NOG-EXL survive longer



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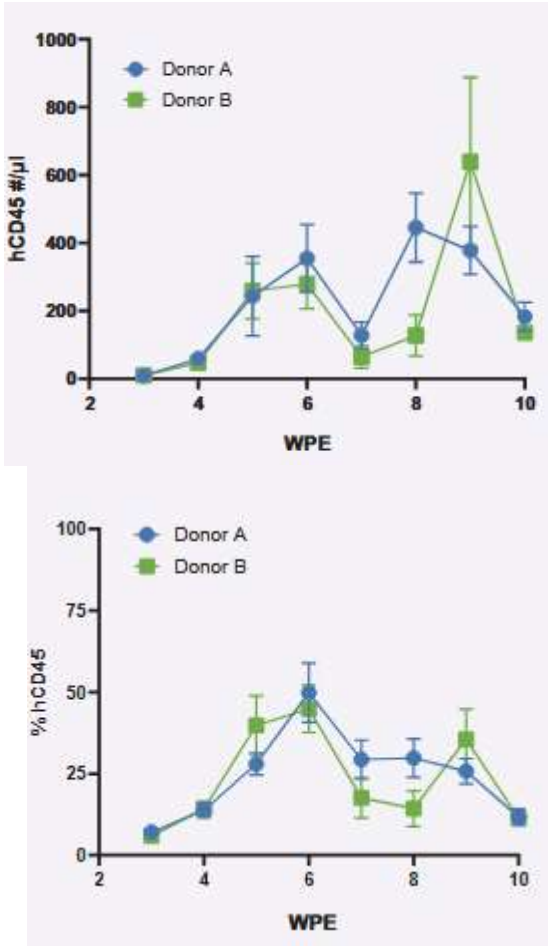
Maser I-P, et al (2020) The Tumor Milieu Promotes Functional Human Tumor-Resident Plasmacytoid Dendritic Cells in Humanized Mouse Models. Front. Immunol. 11:2082. doi: 10.3389/fimmu.2020.02082.

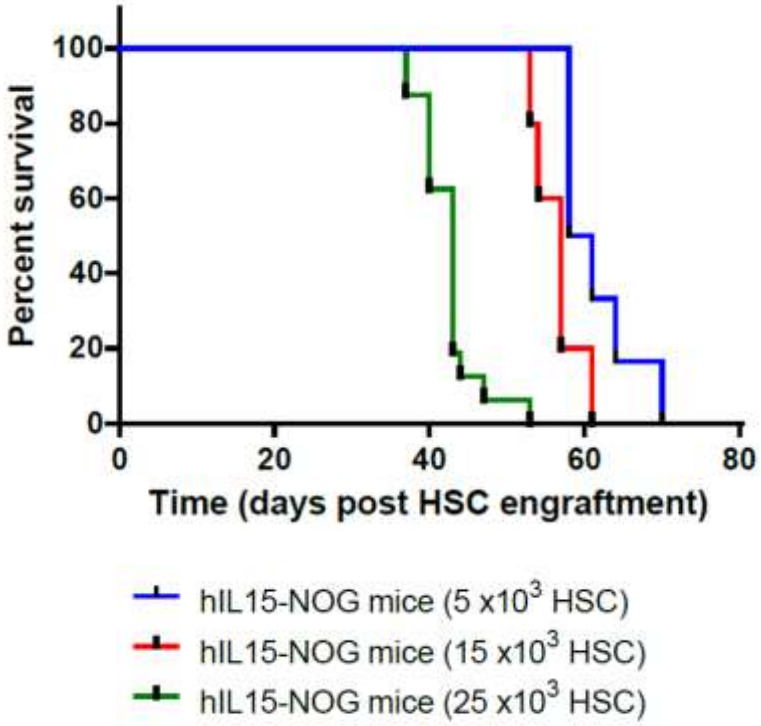
Importance of Choosing the Right Model and Cell Type

hIL-15 NOG – Excellent for NK Cell Engraftment

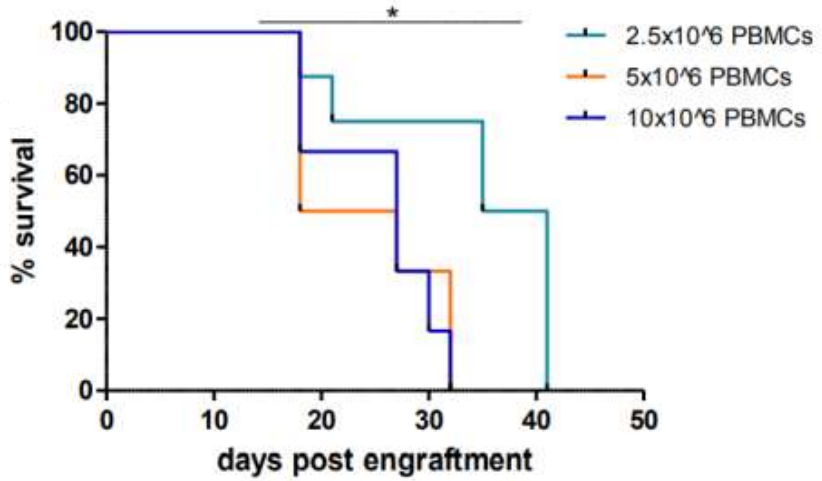
NK cell engraftment



HSC engraftment



PBMC engraftment



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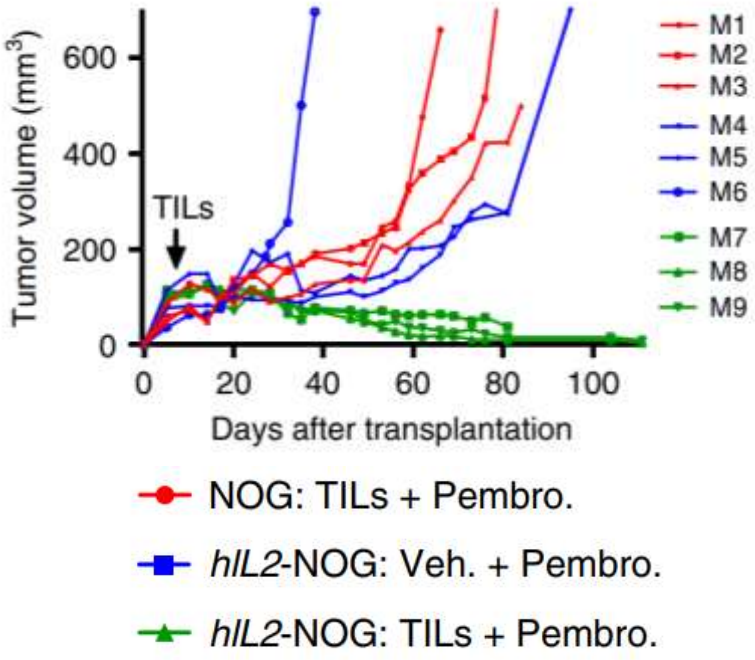
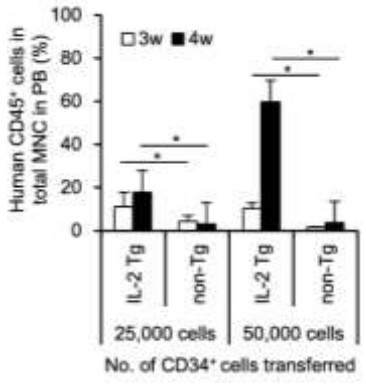
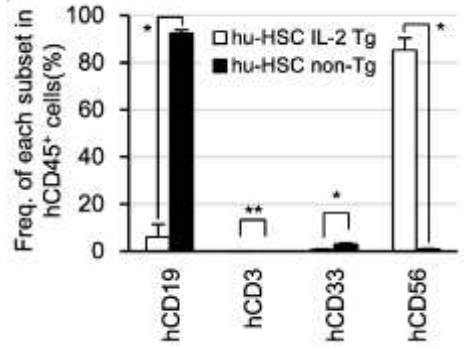
hIL-2 NOG – a Useful Tool for Studying T-cell Therapy

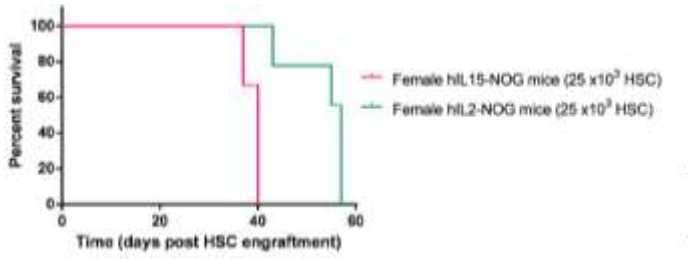
CAR-T cell engraftment



HSC engraftment

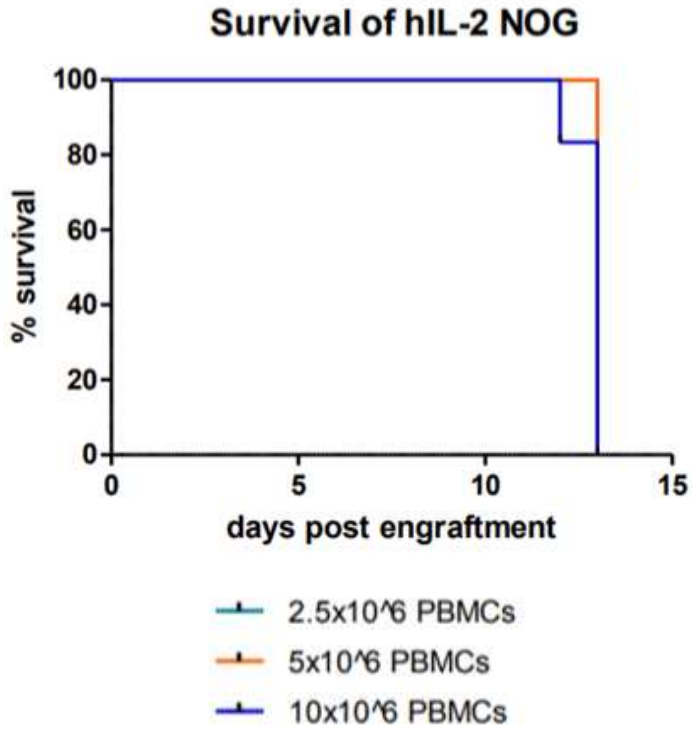






- Heavily skewed towards NK cells
- 40-60 day survival post-engraftment

PBMC engraftment



< 2 Week survival post-engraftment

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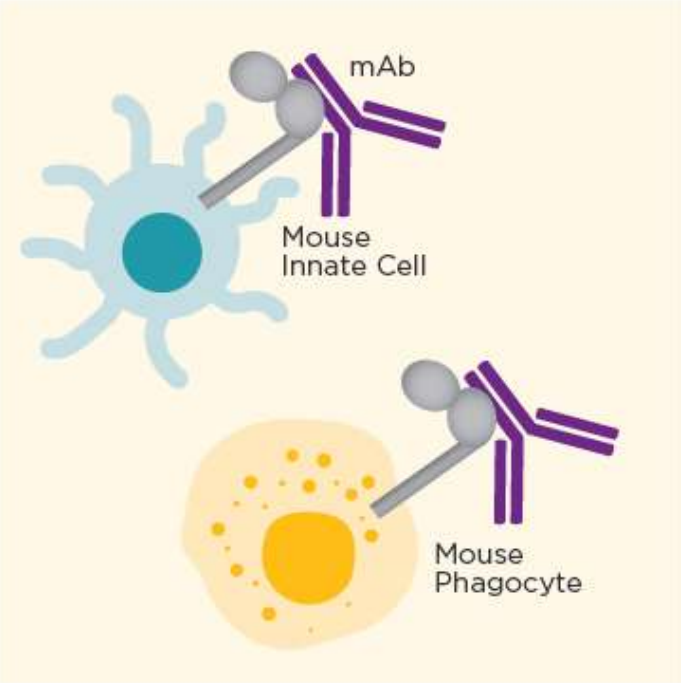
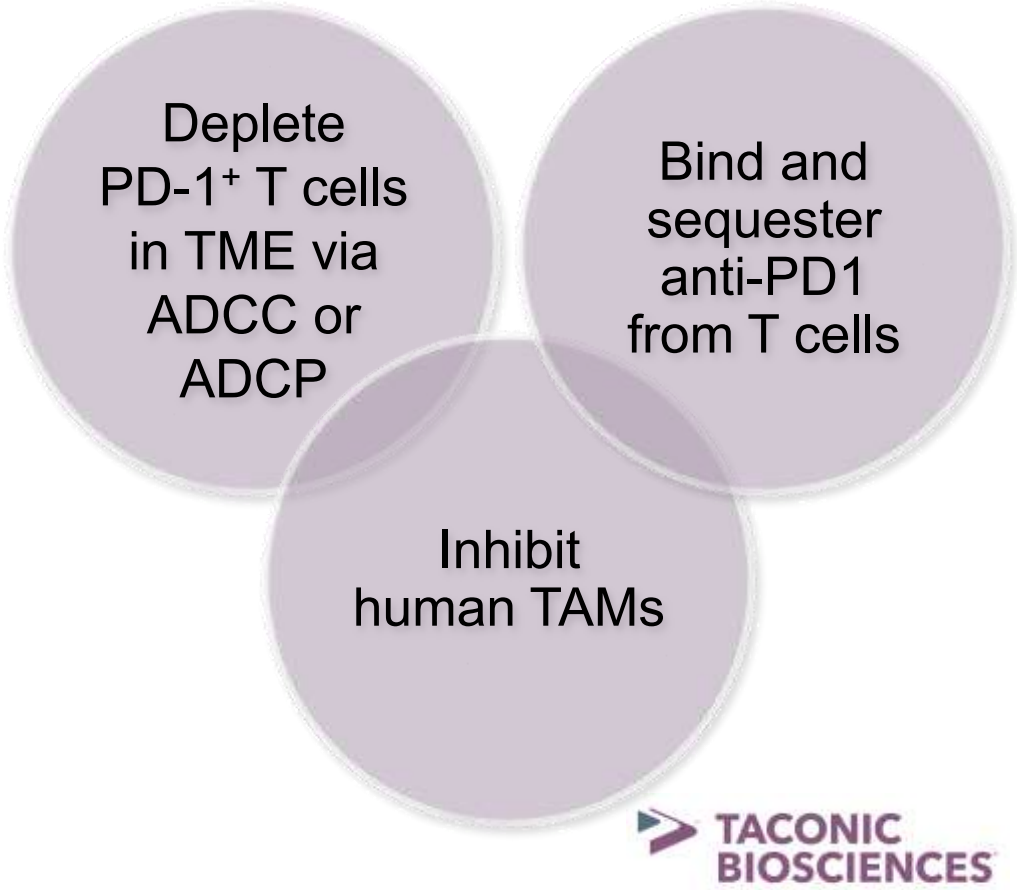
Jespersen et al, Clinical responses to adoptive T-cell transfer can be modeled in an autologous immune-humanized mouse model, Nat Commun. 2017 Sep 27;8(1):707 doi: 10.1038/s41467-017-00786-z
Mignard et al. (Oncodesign; AACR 2014) Volden et al. (EPO Berlin; AACR 2018)

Residual Murine Immune Cells can Functionally Interact with Human Biologics

All super-immunodeficient mice have residual murine immune cells

Human mAbs (e.g. anti-PD-1) can cross-react with murine FcγRs to confound results in preclinical models





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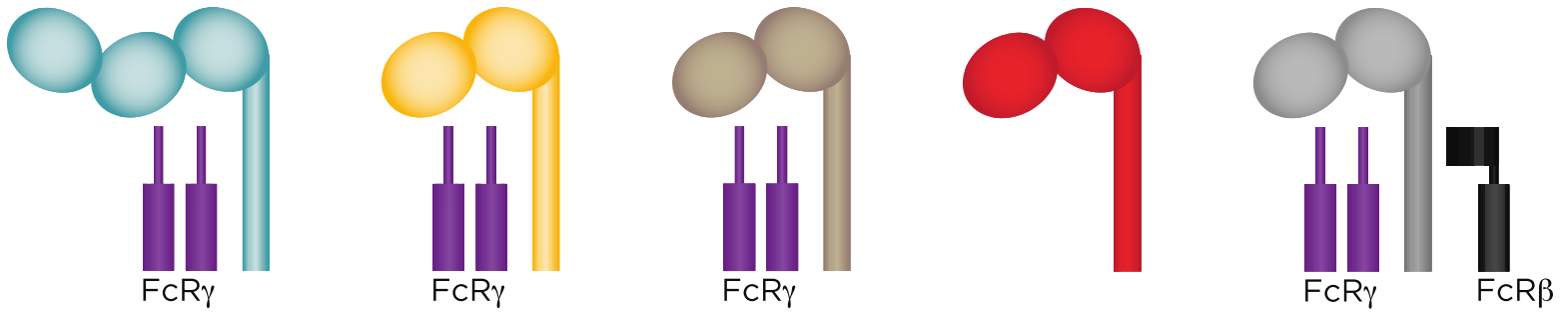
Human IgGs Differentially Activate Murine FcγRs



Mouse FcR	FcγRI	FcγRIII	FcγRIV	FcγRIIb	FcεRI*
Expression Pattern	Dendritic Cells, Monocytes/ Macrophages (±)	Dendritic Cells, Natural Killer Cells, Monocytes/ Macrophages, Neutrophils	Monocytes/ Macrophages, Neutrophils	Dendritic Cells, Monocytes/ Macrophages, Neutrophils	Mast Cells, Basophils
Human Ig Relative Binding Affinity to Mouse FcR	hIgG1: ↑↑↑↑ hIgG2: - hIgG3: ↑↑↑↑ **hIgG4:** ↑↑↑	hIgG1: ↑ hIgG2: ↑ hIgG3: ↑↑ **hIgG4:** ↑	hIgG1: ↑↑↑ hIgG2: - hIgG3: ↑↑↑ **hIgG4:** ↑	hIgG1: ↑↑ hIgG2: ↑ hIgG3: ↑↑ **hIgG4:** ↑	hIgE: -
Human FcR Ortholog	hFcγRI	hFcγRIIa	hFcγRIIIa	hFcγRIIb	

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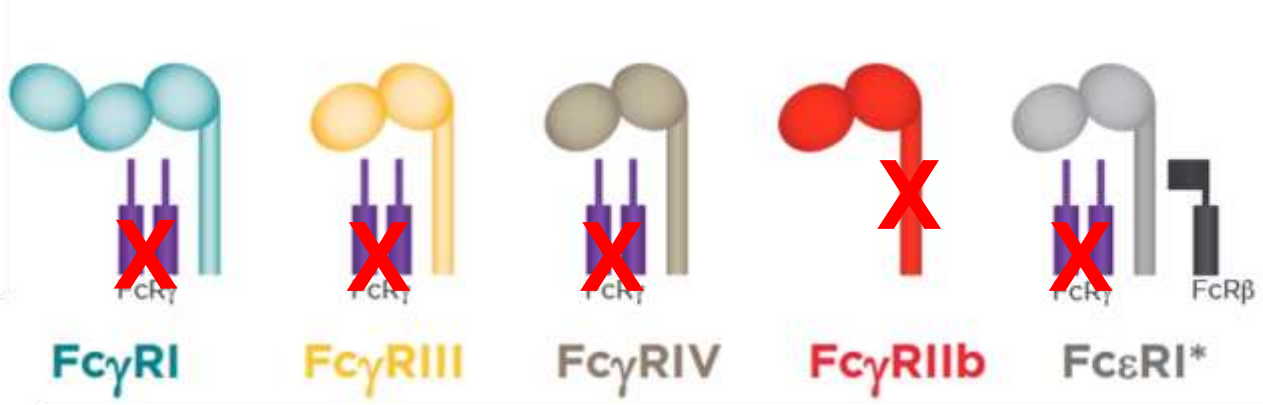
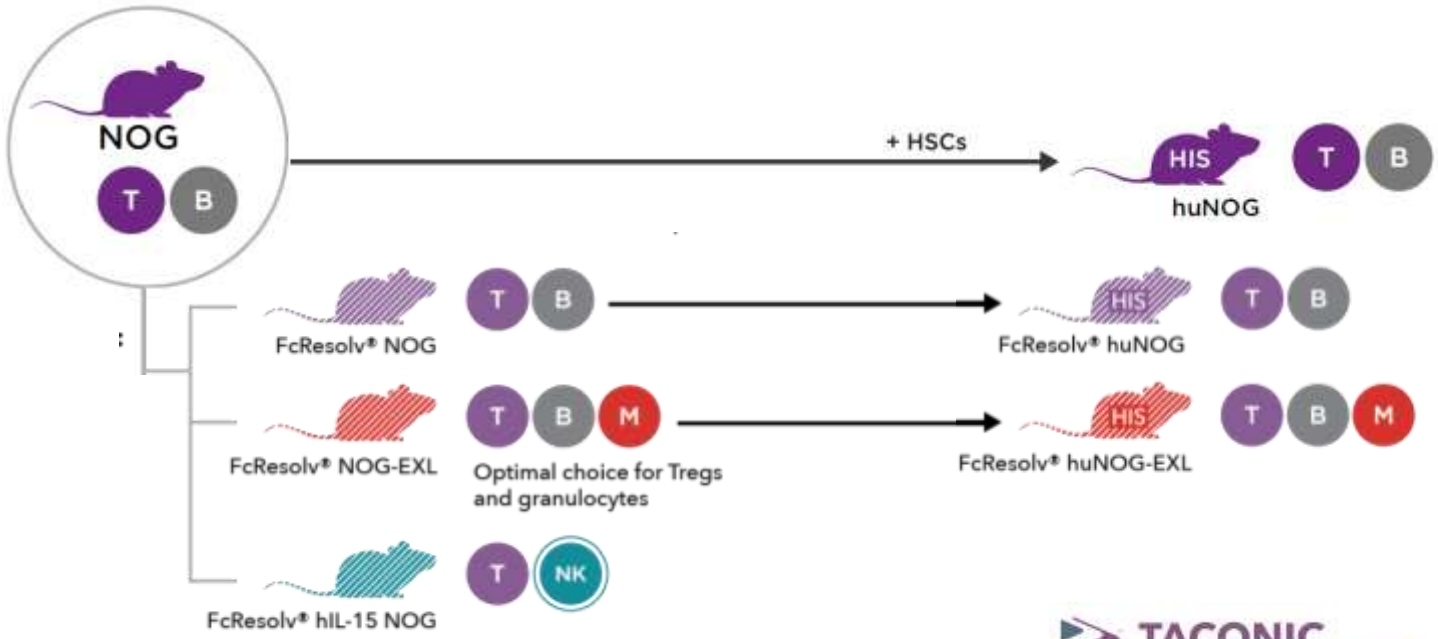
Solution: FcResolv® NOG

- Severely immunodeficient NOG mouse
- KO of common FcRγ subunit and FcγRIIb to prevent all Fc-mediated effector functions
- Can be used in a similar fashion to the NOG mouse, including for xenograft and immune system humanization experiments.
- Removes confounding effects to improve accuracy





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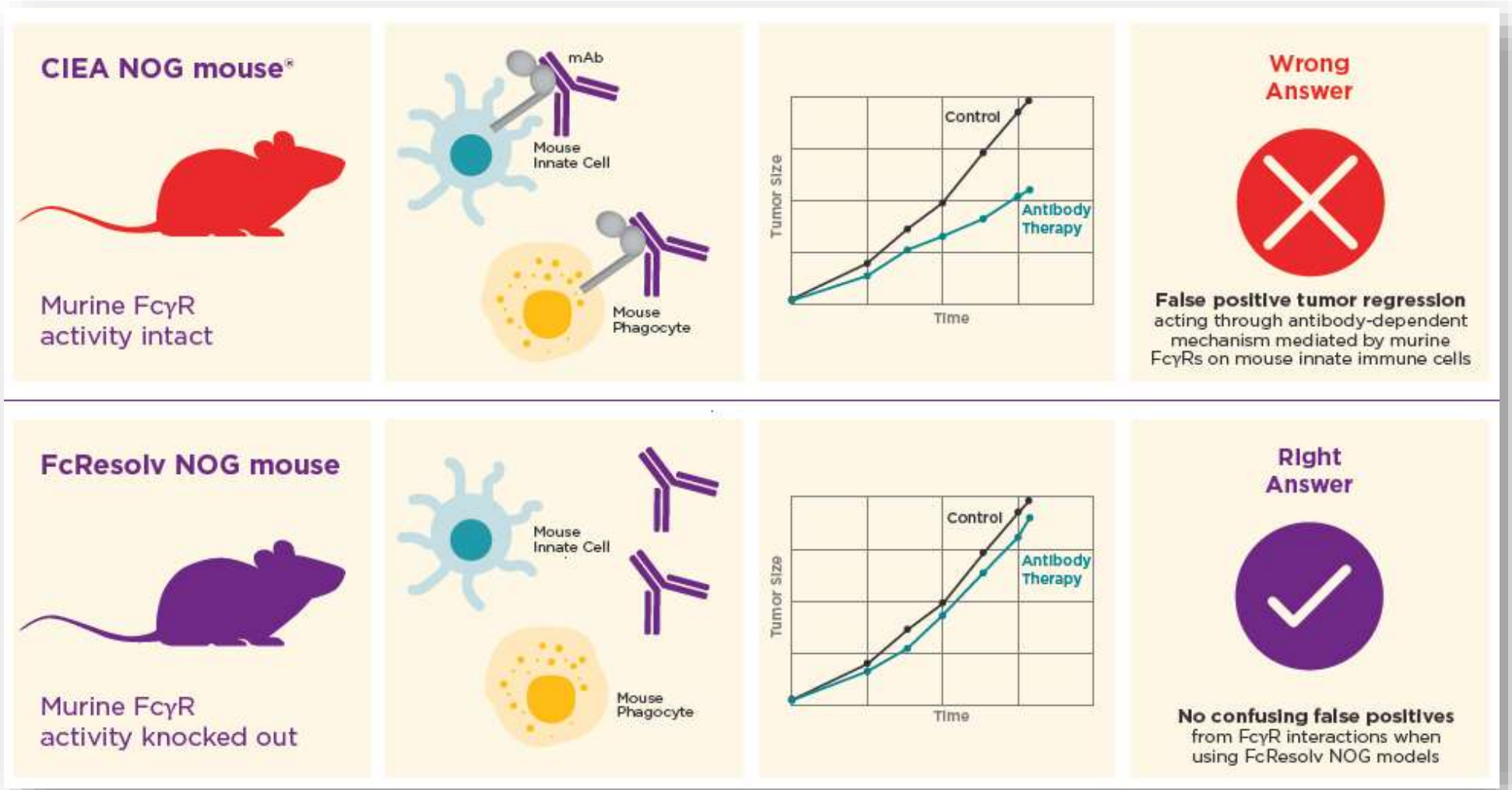
False Positives Generated by Murine Innate Cell-Mediated ADCC or ADCP



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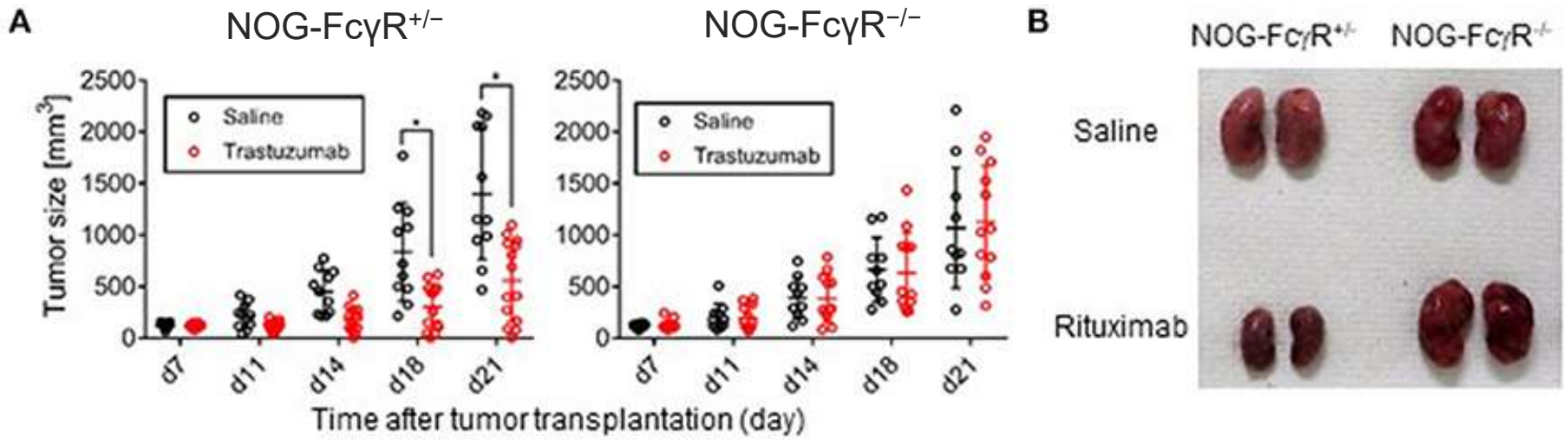
FcγR Knockout Resolves False Positives

Abrogation of mouse innate cell mediated antibody dependent cytotoxicity in NOG-FcγR$^{-/-}$ mice



4–1ST tumor

Daudi tumor

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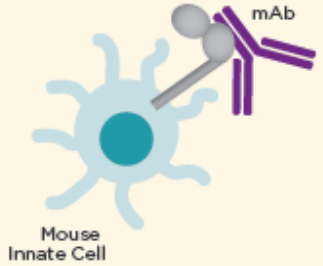
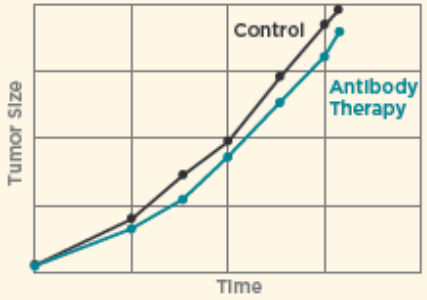
Katano I, Ito R, Kawai K and Takahashi T (2020) Improved Detection of *in vivo* Human NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity Using a Novel NOG-FcγR-Deficient Human IL-15 Transgenic Mouse. *Front. Immunol.* 11:532684. doi: 10.3389/fimmu.2020.532684. Licensed under CC BY 4.0

False Negatives Generated through Interaction Between Mouse FcγR and a Therapeutic's Fc Domain

CIEA NOG mouse®

Murine FcγR activity intact



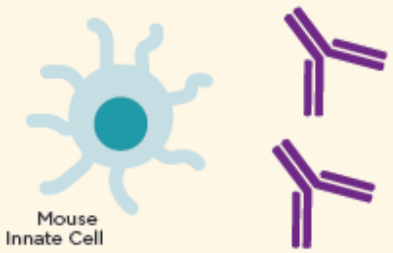


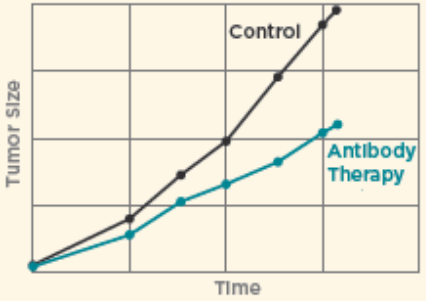
Wrong Answer

False negative efficacy result due to interference by murine FcγRs

FcResolv NOG mouse

Murine FcγR activity knocked out





Right Answer

No confusing false negatives when using FcResolv NOG models

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Limitations of HIS Mice

- HIS mice are sensitive and requires special handling and care
- Human T cells are educated in a mouse thymus → mouse MHC restricted
- B cell responses are suboptimal
- Residual murine immunity – some issues can be solved with the FcResolv™ models
- Potential development of GvHD, MAS or CRS

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HIS Model Considerations

Humanized Immune System Required?
- HSCs
- PBMCs
- Isolated cell types (e.g. NK cells)

Cell Type of Interest?
- T Cells
- Macrophages
- NK Cells

Tumor Properties?
- CDX
- PDX

Study Timeline?
- Short vs. Fast-Growing Tumor
- Short vs. Long Treatment

Model Availability & Lead Time
- Standard Access Models
- Early Access Models
- Specialized Engraftments

Inter- and Intra-Donor Variability
- # Mice/Donor
- # Donors

Animal Facility/Husbandry
- Housing
- Acclimation Time
- Microbiome

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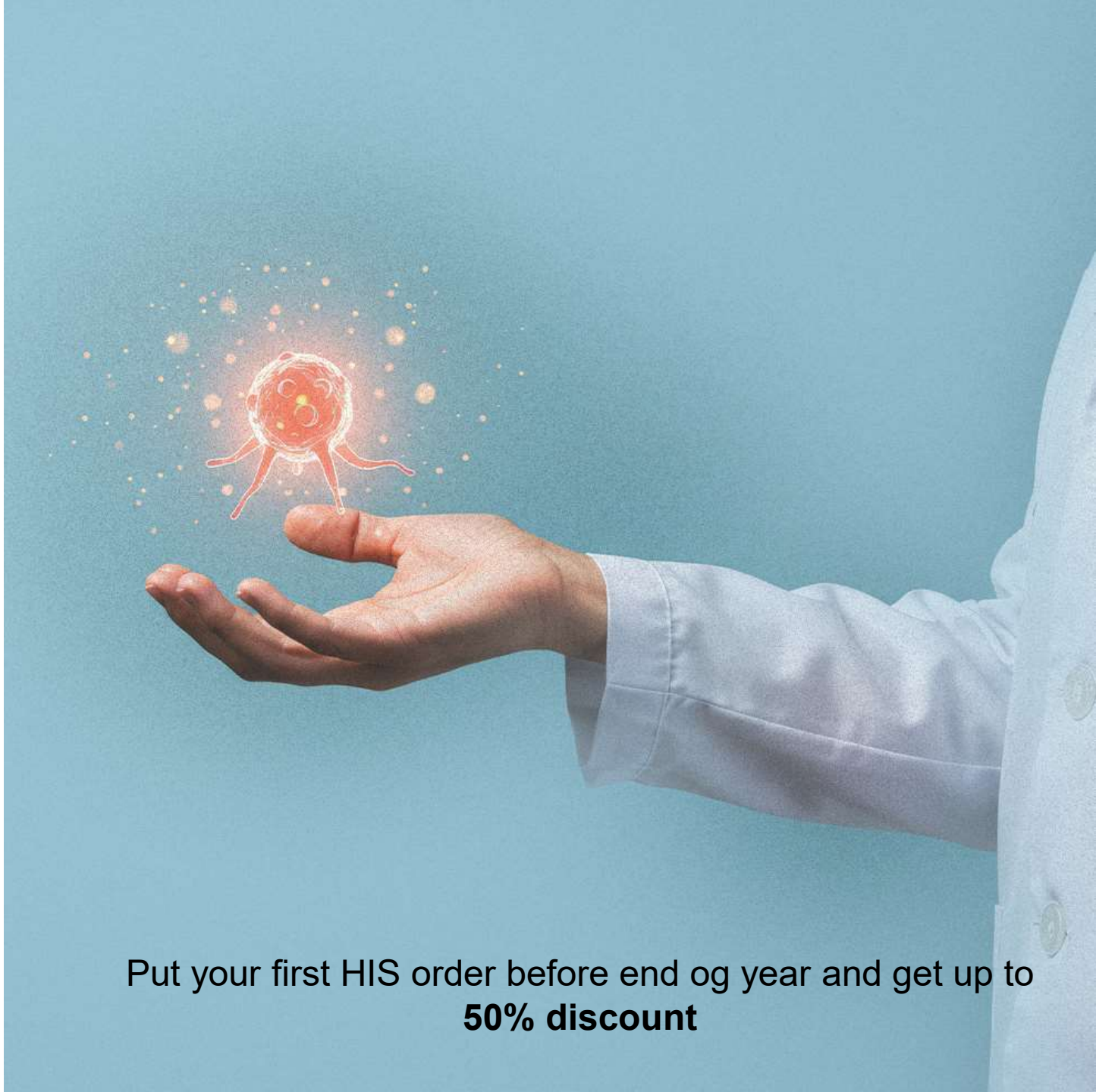
Taconic Expands Humanised Immune System Model (HIS) Production to Europe

September 2025

Strengthening our global Humanisation Centres of Excellence with new production in Leverkusen, Germany



Put your first HIS order before end og year and get up to **50% discount**

Thank You

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