



Animal Model Workshop

Development of Humanized Mice to Study Human NK cells In Vivo

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Program in Molecular Medicine
Diabetes Center of Excellence

September 11-13, 2025



Development of Novel Humanized Mouse Models



Diabetes Center of Excellence

Dale Greiner

**Hematological
Disorders**

**Pancreatic
Beta-Cell
Biology**

David Harlan

**Infectious
Diseases**

The Jackson Laboratory

Immunology

James Keck

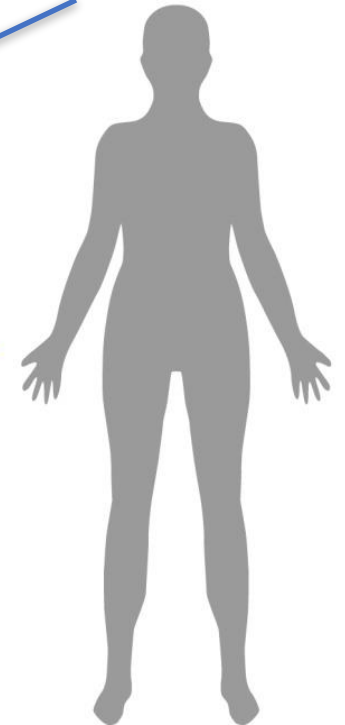
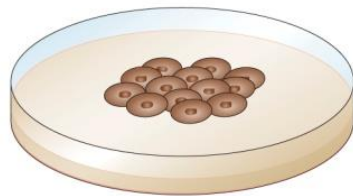
Brian Soper

Li-Chin Yao

Cancer

Autoimmunity

**Regenerative
Medicine**



Major Immunodeficient Strain Platforms For Humanization		
<u>MODEL</u>	<u>STRAIN</u>	<u>SOURCE</u>
NSG	NOD- <i>scid IL2rg^{null}</i>	Jackson Laboratory
NRG	NOD- <i>Rag1^{null} IL2rg^{null}</i>	
NOG	NOD- <i>scid IL2rg^{Trunc}</i>	Central Institute for Experimental Animals /Taconic
NCG	NOD-CRISPR <i>Prkdc Il2rg</i>	Charles River Lab
NSI	NOD-TALEN <i>Prkdc Il2rg</i> Ye, 2015 <i>J Hema Onco</i>	South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou
BRG	BALB/c- <i>Rag2^{null} IL2rg^{null}</i> “MISTRG” Rongvaux, 2014 <i>Nat Biotech</i> 32;364	Yale/Univ. Hosp. Zurich
BRG-S	BALB/c- <i>Rag2^{null} IL2rg^{null} NOD^{SIRPa}</i>	Pasteur Institute/Univ. of Amsterdam
B6-RG	C57BL/6- <i>Rag2^{null} IL2rg^{null} CD47^{null}</i>	Rocky Mountain Laboratories, NIAID, NIH

Human Immune System Engrafted Mice

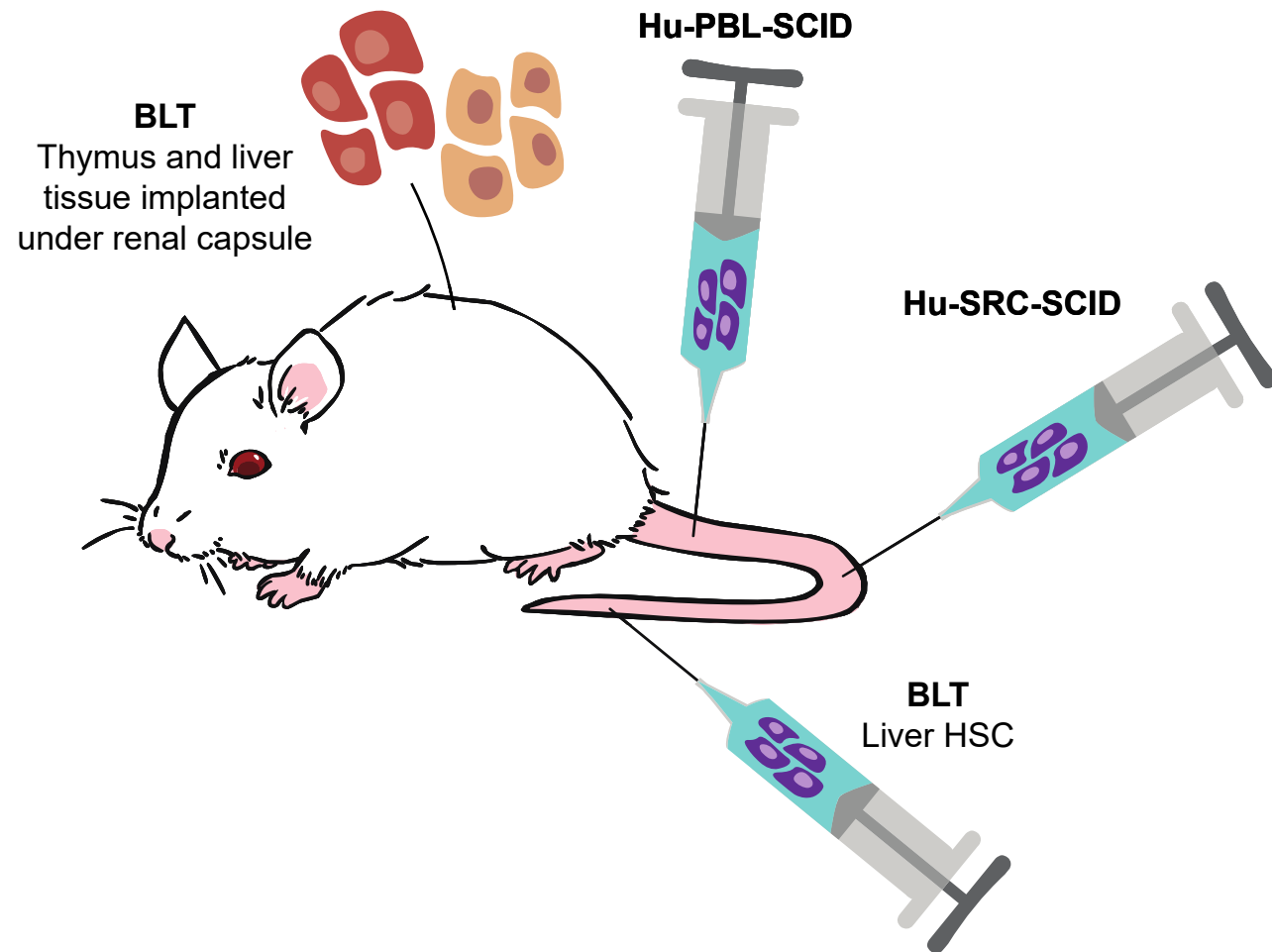
- 1) Hu-PBL-SCID mice: *immunodeficient* mice injected with human peripheral blood mononuclear cells (PBMC)^{Mosier, 1988. *Nature*, 335:256}
-NSG MHC-DKO (PMC6404556)

- 2) Hu-SRC-SCID mice: *immunodeficient* mice that have been sublethally irradiated and injected with hematopoietic stem cells (HSC)

scid repopulating cells (SRC) = CD34⁺ cells
Lapidot, 1992. *Science*, 255:1137

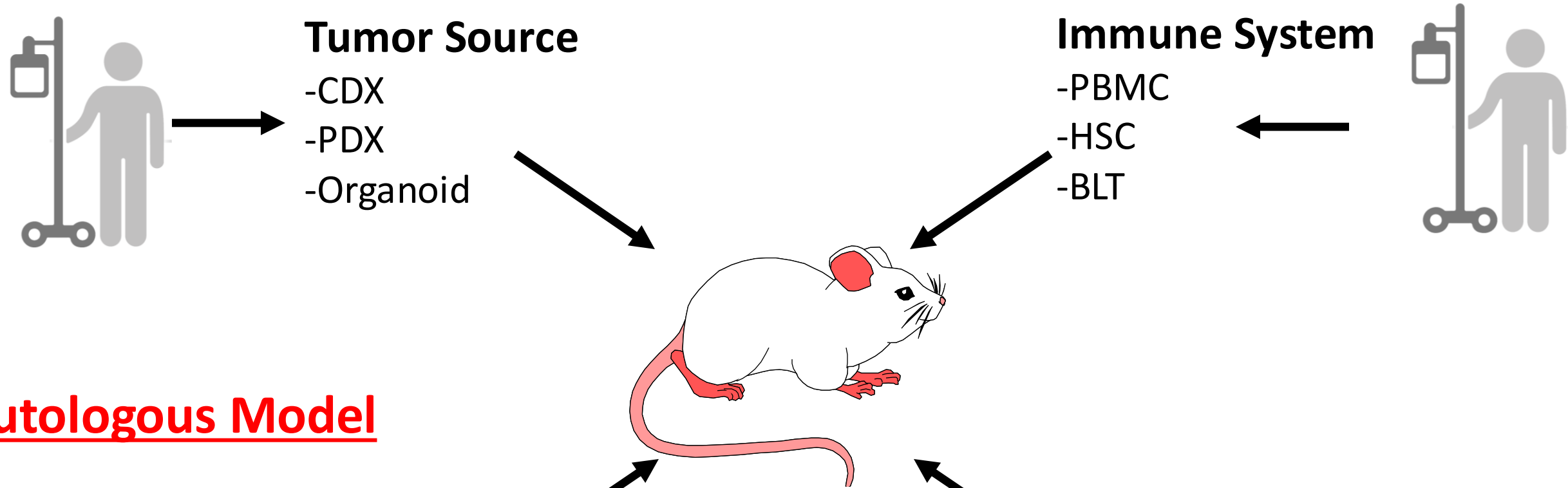
- 3) Human Thymus Models: *immunodeficient* mice that have been engrafted with human thymus under the renal capsule and injected with HSC (BLT, NeoThy, SC-TEC)

McCune, 1988. *Science*, 241:1632

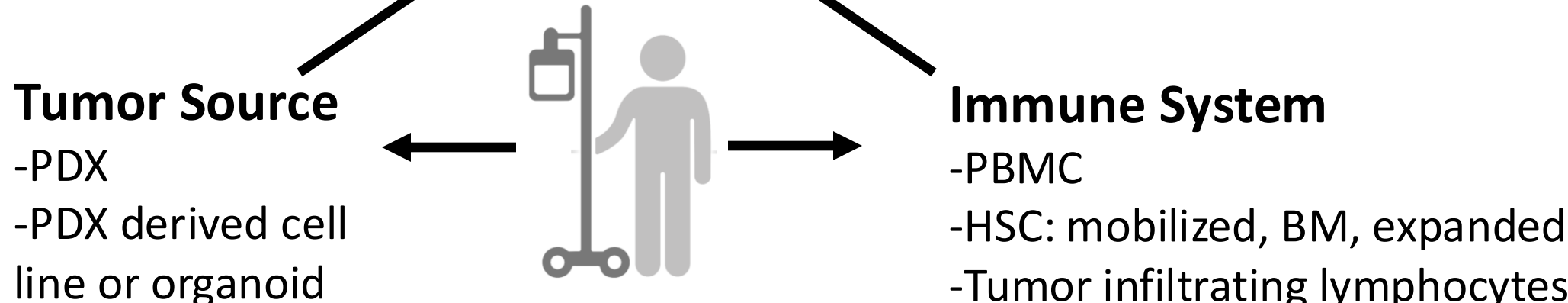


Humanized Models to Study Immuno-Oncology

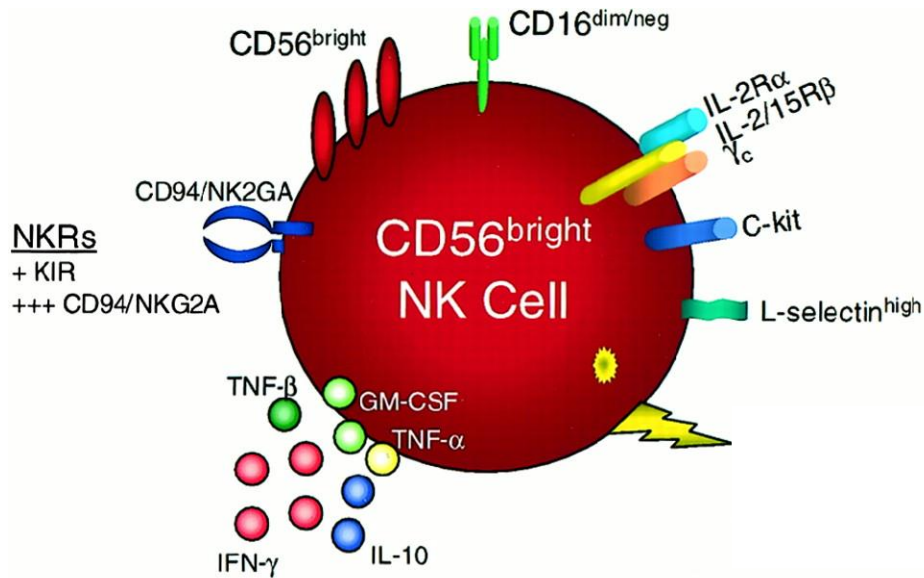
Allogeneic Model



Autologous Model



Humanized Mouse Models with Enhanced NK cell Development



-Cytotoxic

- ADCC
- Direct receptor mediated killing

-Cytokine producers

- Inflammatory
- Regulatory

Exogenous IL15

- Injection of IL-15:IL-15Ra complexes enhances NK cell development in HSC-engrafted BRG mice; Huntington, *JEM* 206,2009
- Hydrodynamic injection of plasmids expressing human IL15 and FLT3L enhances NK cell development in HSC engrafted NSG mice; Chen, *PNAS* 106:51, 2009

Cytokine Transgenic Mice

- NOG-IL2 show enhanced NK cell development after HSC engraftment; Katano, *J Immunol* 194:3513, 2015
- SRG-15 (BRG mouse expressing human *SIRPA* and *Il15*) show enhanced NK cell development after HSC engraftment; Herndler-Brandstetter, *PNAS* 114:45, 2017
- NOG-IL15 show enhanced NK cell survival after PBMC or NK cell injection; Katano, *Sci Rep* 7:1, 2017
- NSG-IL15 mice, Aryee. 2022. *FASEB J.* 36(9):e22476

“NK cells in renal cell carcinoma and its implications for CAR-NK therapy”-Li et al, *Front Cell Dev Biol.* 2025. PMC11882582

HSC-Engrafted NSG-Tg(Hu-IL15) mice show increased human NK cells

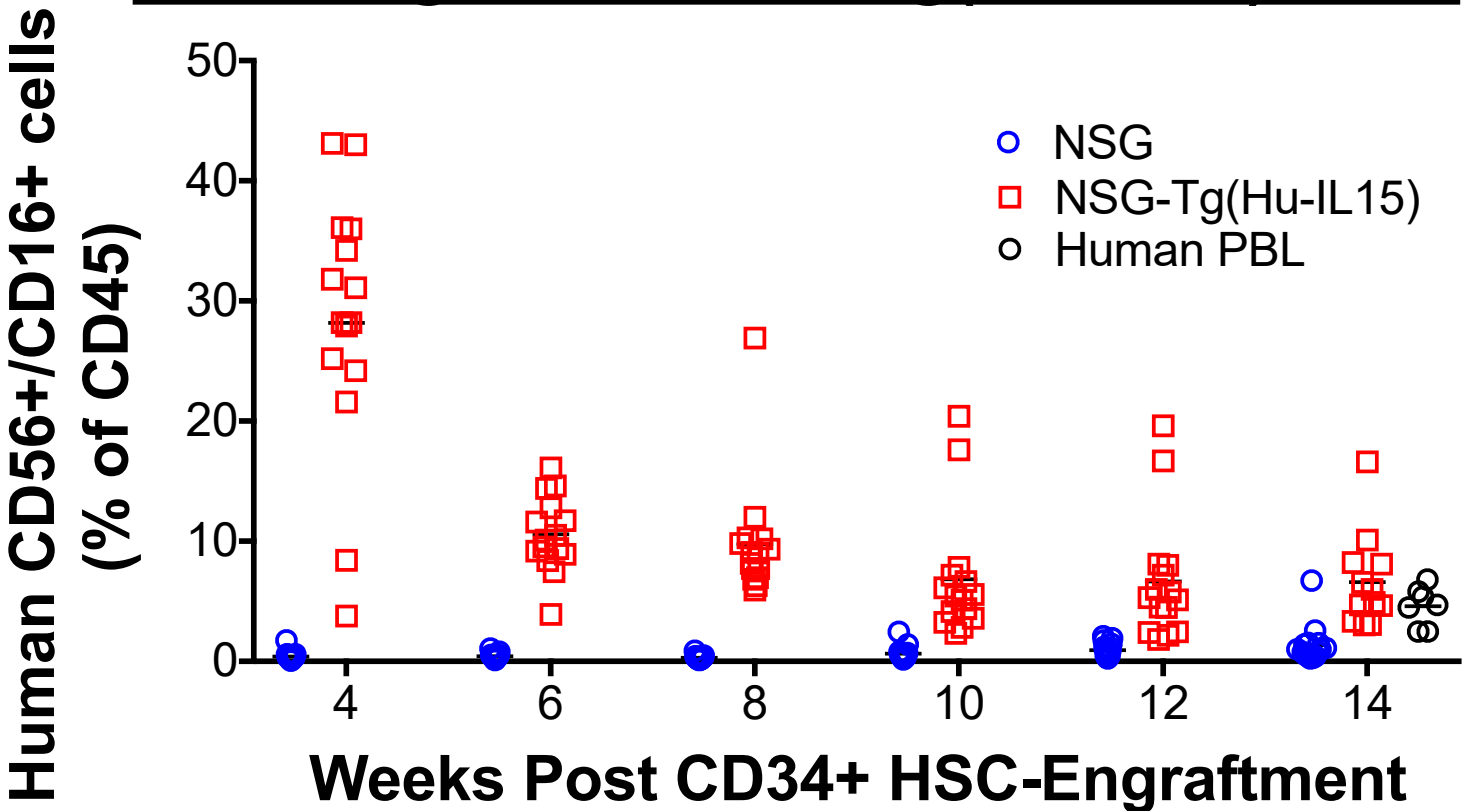
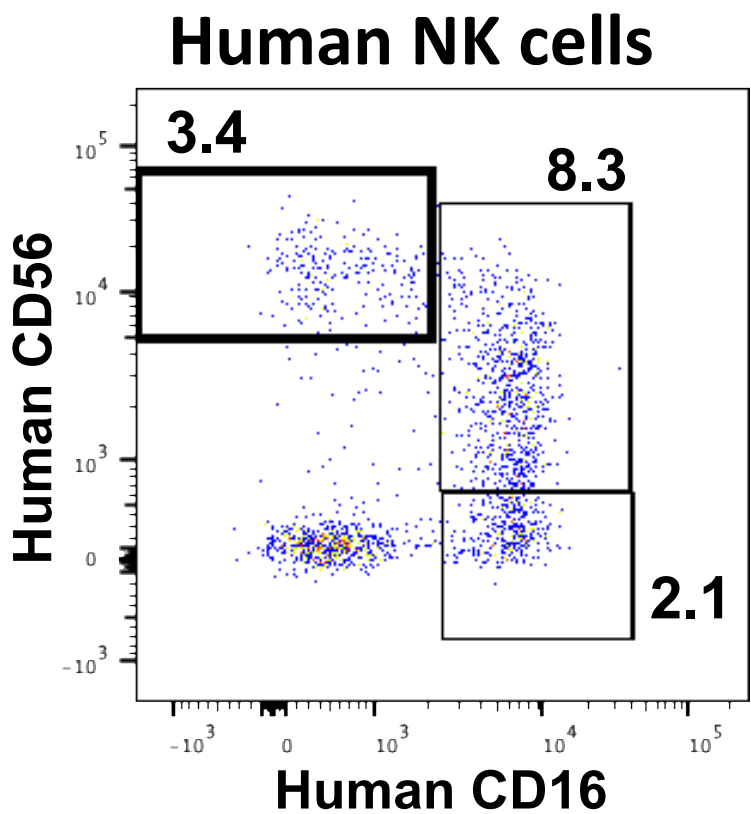
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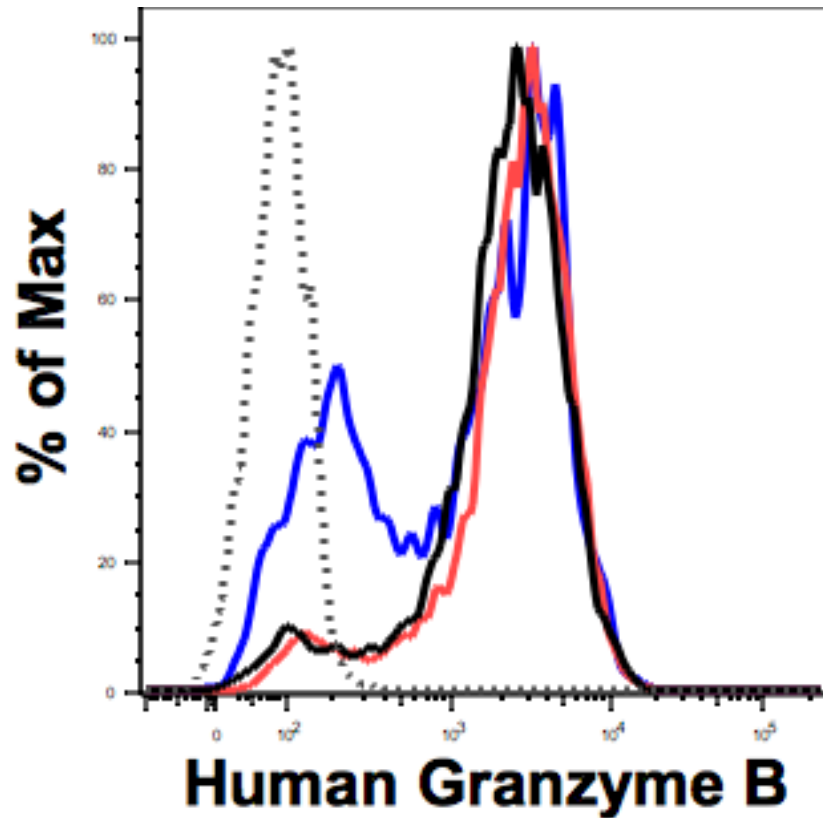


HSC-Engrafted NSG-Tg(Hu-IL15) mice



Human NK cells from HSC-Engrafted NSG-Tg(Hu-IL15) mice are cytotoxic

Granzyme B+ NK Cells
(Blood 16 Weeks)

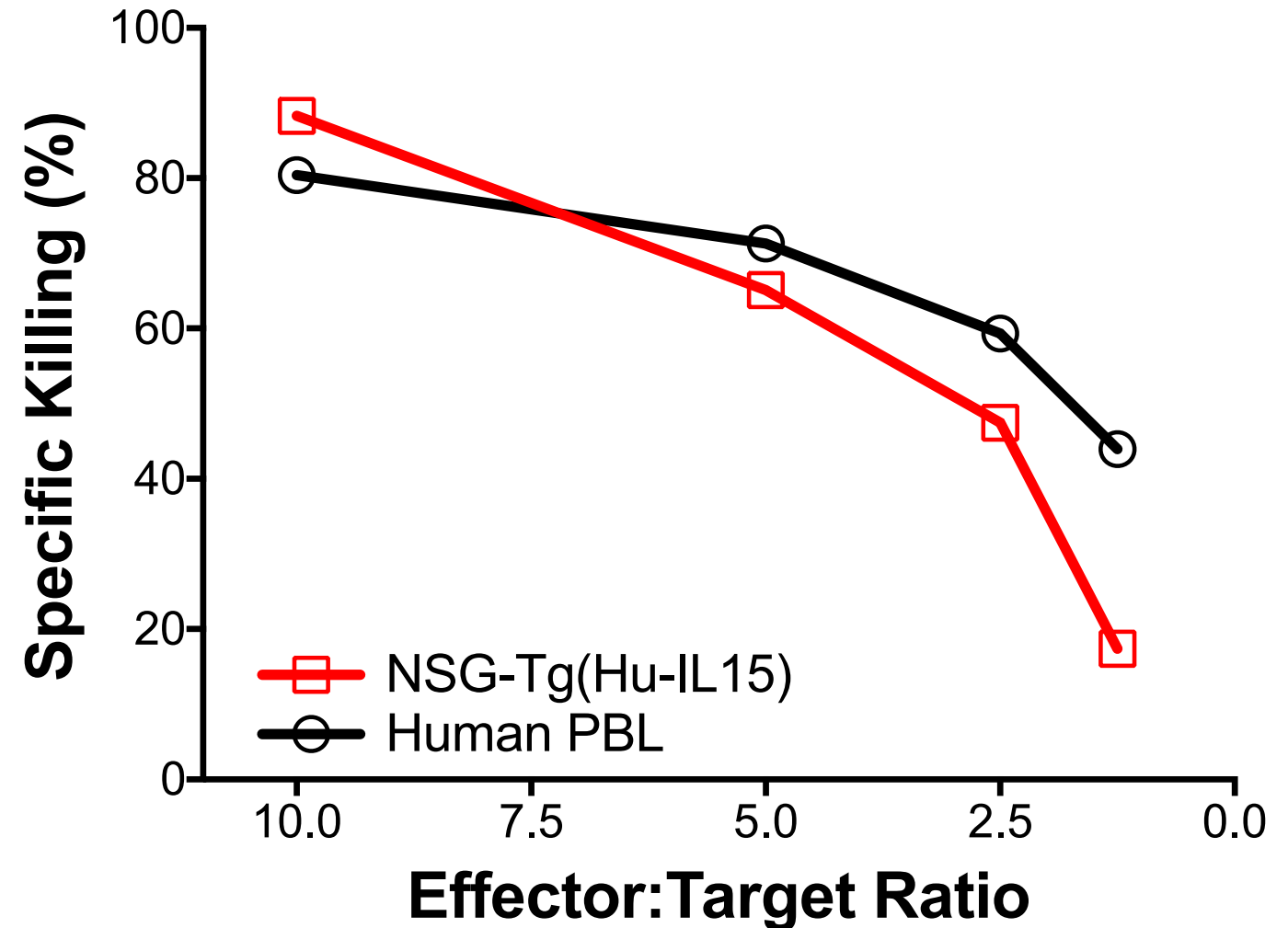


NSG

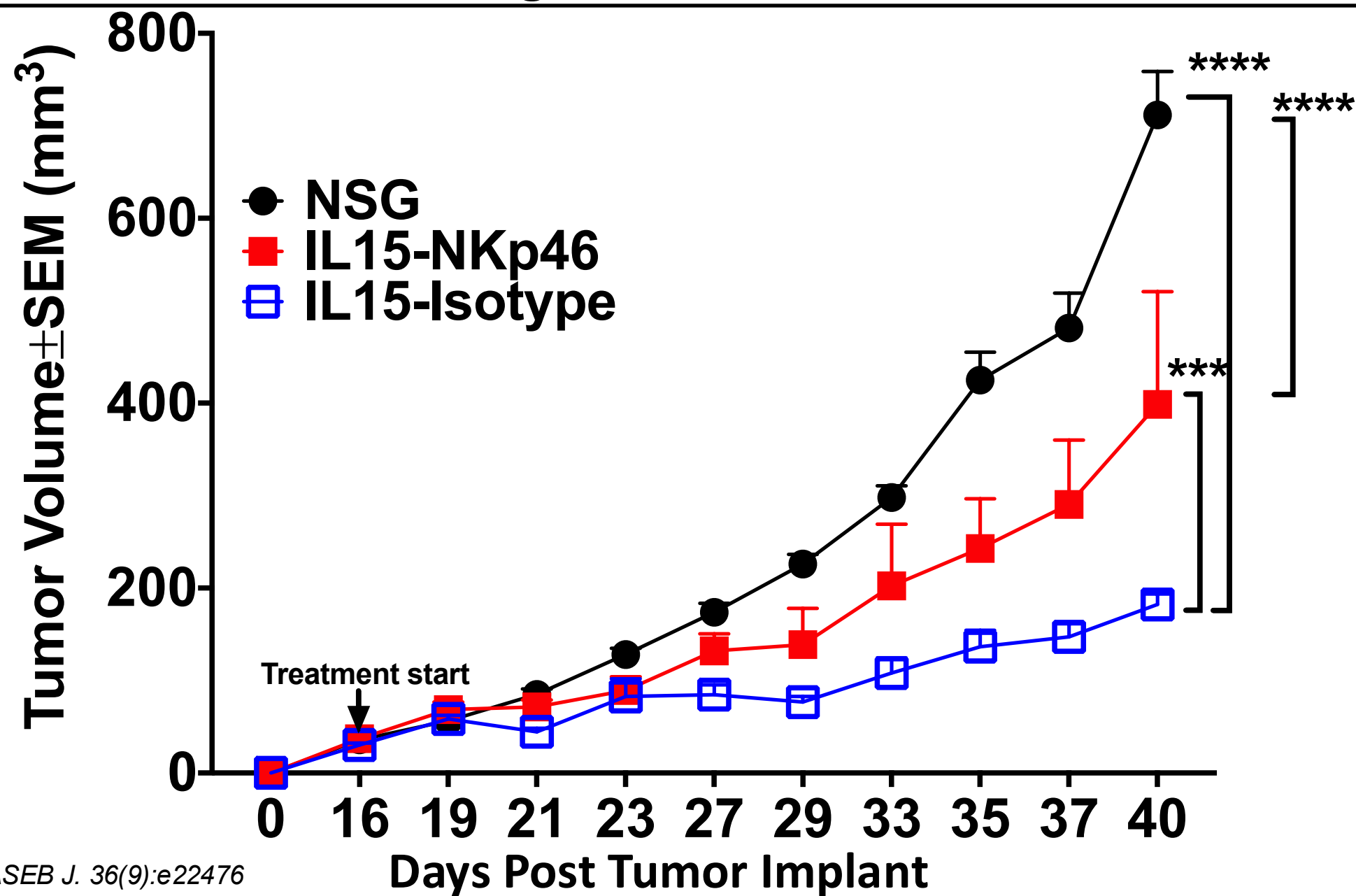
NSG-Tg(Hu-IL15)

Human PBL

In Vitro NK cell Killing (K562)



Depletion of Human NK Cells Enhances PDX Melanoma Growth in HSC-Engrafted NSG-IL15 Mice



NSG MHC-DKO Mice Expressing Human IL15 Support Engraftment of Human NK cells from PBMC

NSG MHC-DKO

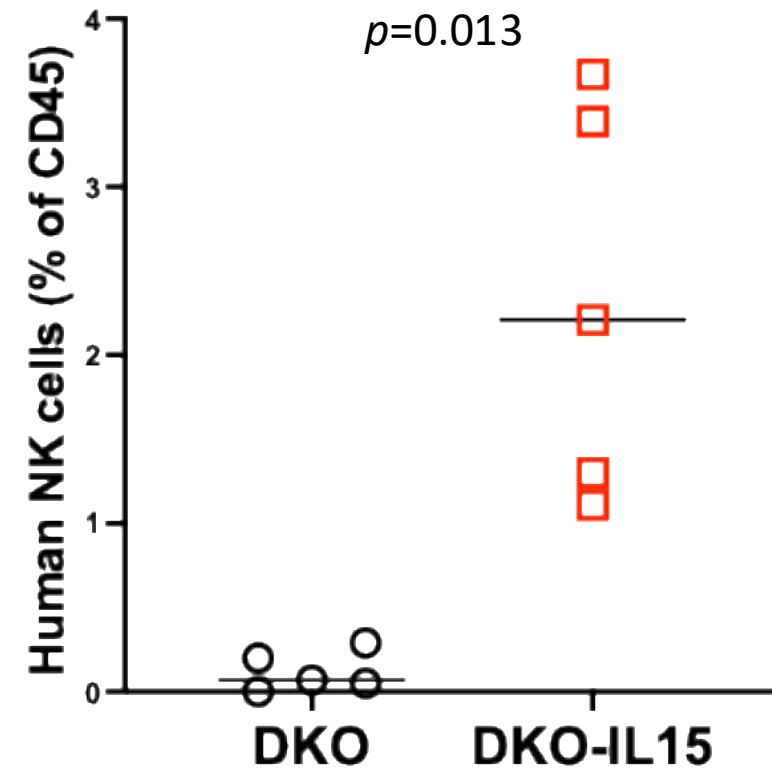
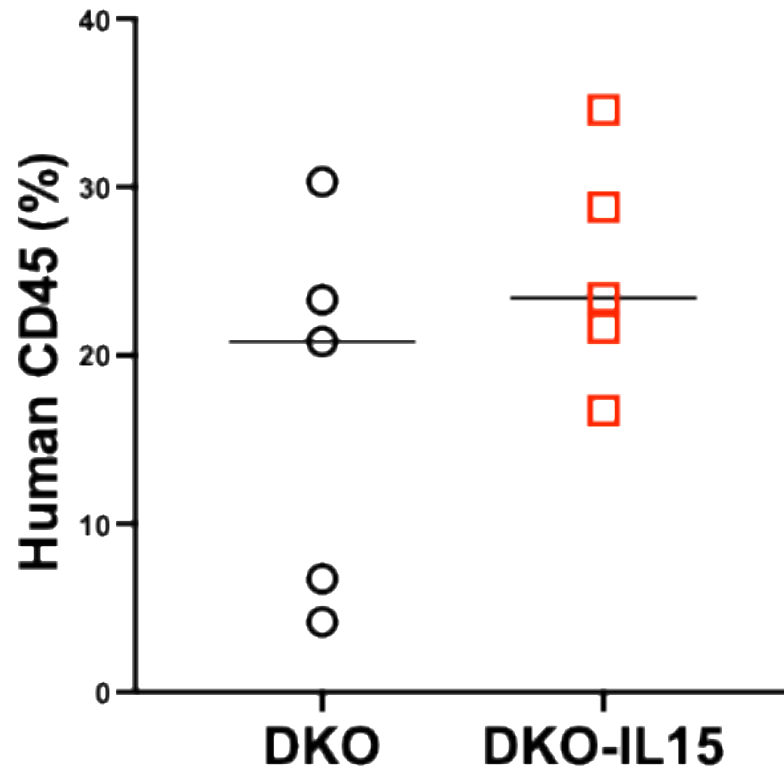
NSG MHC-DKO IL15



-IV Inject 10 million PBMC



Examine CD56dim/CD16+
NK cells in Blood at Day 14



Humanized Mouse Toolbox: Picking the right platform

Selection of human tumor model

- CDX, PDX, organoid. Understand growth kinetics!
- Genetically modified normal cells

Chuprin et al. 2023.
Nat Rev Clin Oncol.
2023
Mar;20(3):192-206

Engraftment strategy for human immune system

- PBMC derived immune cells (mature), HSC
- TIL populations
- CAR effector cells (in vitro and in vivo!)
- Pluripotent stem cells-derived HSPC

Selection of immunodeficient mouse strain

- Enhance development of specific human immune cells
- Reduce mouse immunity
- Minimize xenogeneic GVHD

Consider experimental endpoints/question for platform selection

Acknowledgements

• UMass DCOE Faculty

- David Harlan
- Dale Greiner
- Rene Maehr
- Sally Kent
- Louis Messina
- Jennifer Wang
- Tammy Nguyen
- Accalia Fu
- Michael Czech
- Roger Davis
- Silvia Corvera
- David Guertin
- Jason Kim

• UMass DCOE

- Jamie Kady
- Sean O'Brien
- Carey Zammitti
- Catherine Kooyomjian
- Anna Cerny
- Tyler Davis
- Mina Seedhom
- Suganya Veerasamy
- Makayla Brady
- Julen Goicolea Egia
- Nicolai Hathiramani
- Julia Hanssen

• Breakthrough T1D

NE COE

- David Harlan
- Jason Gaglia
- Dale Greiner
- Lenny Shultz
- Sally Kent
- Jennifer Wang
- Accalia Fu
- Jessica Spinelli
- Barbara Khan
- Rene Maehr

• The Jackson Laboratory

- Leonard Shultz
- James Keck
- Brian Soper
- Li-Chin Yao

• HMS - George Daley

• Joslin – Peng Yi

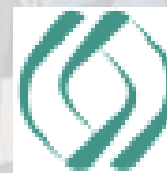
• Vanderbilt - Al Powers

• UNC - Roland Tisch

• UW - Matthew Brown



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