Development of Humanized Mouse Models for Biomedical Research

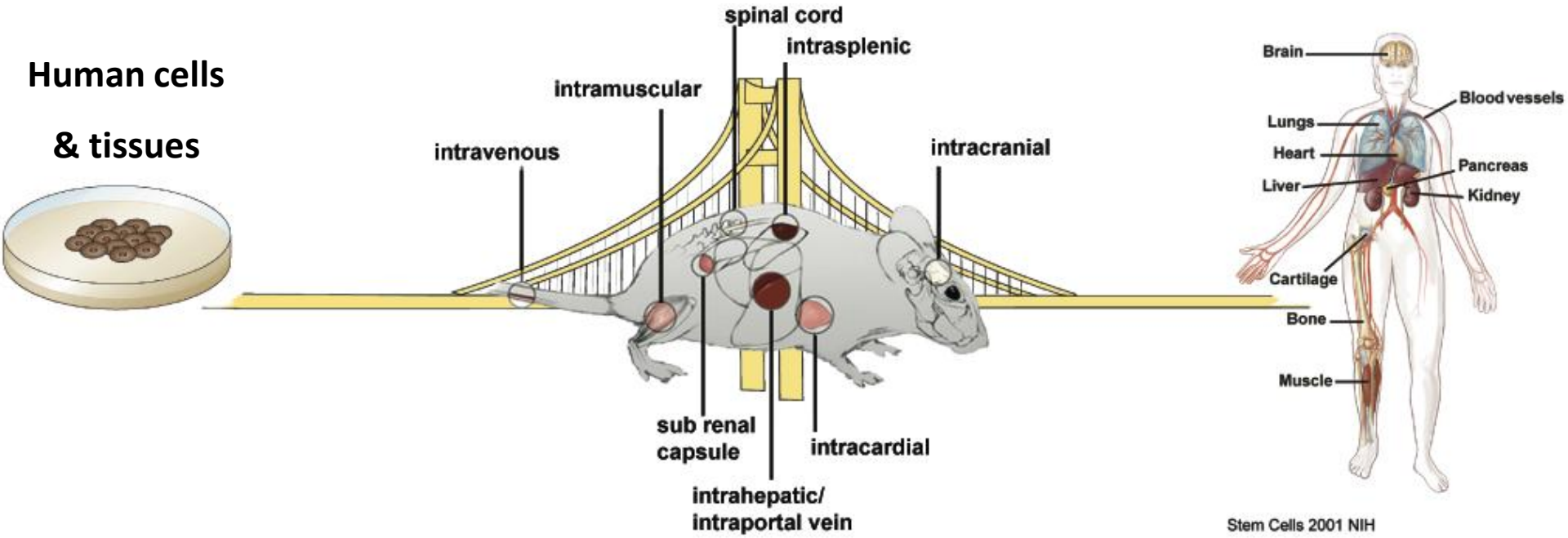
Michael A. Brehm

Diabetes Center of Excellence

Dale Greiner
David Harlan
Rita Bortell
Philip Dilorio

The Jackson Laboratory

Leonard Shultz



Application of Humanized Mice for Biomedical Research

-Cancer biology

-Regenerative medicine

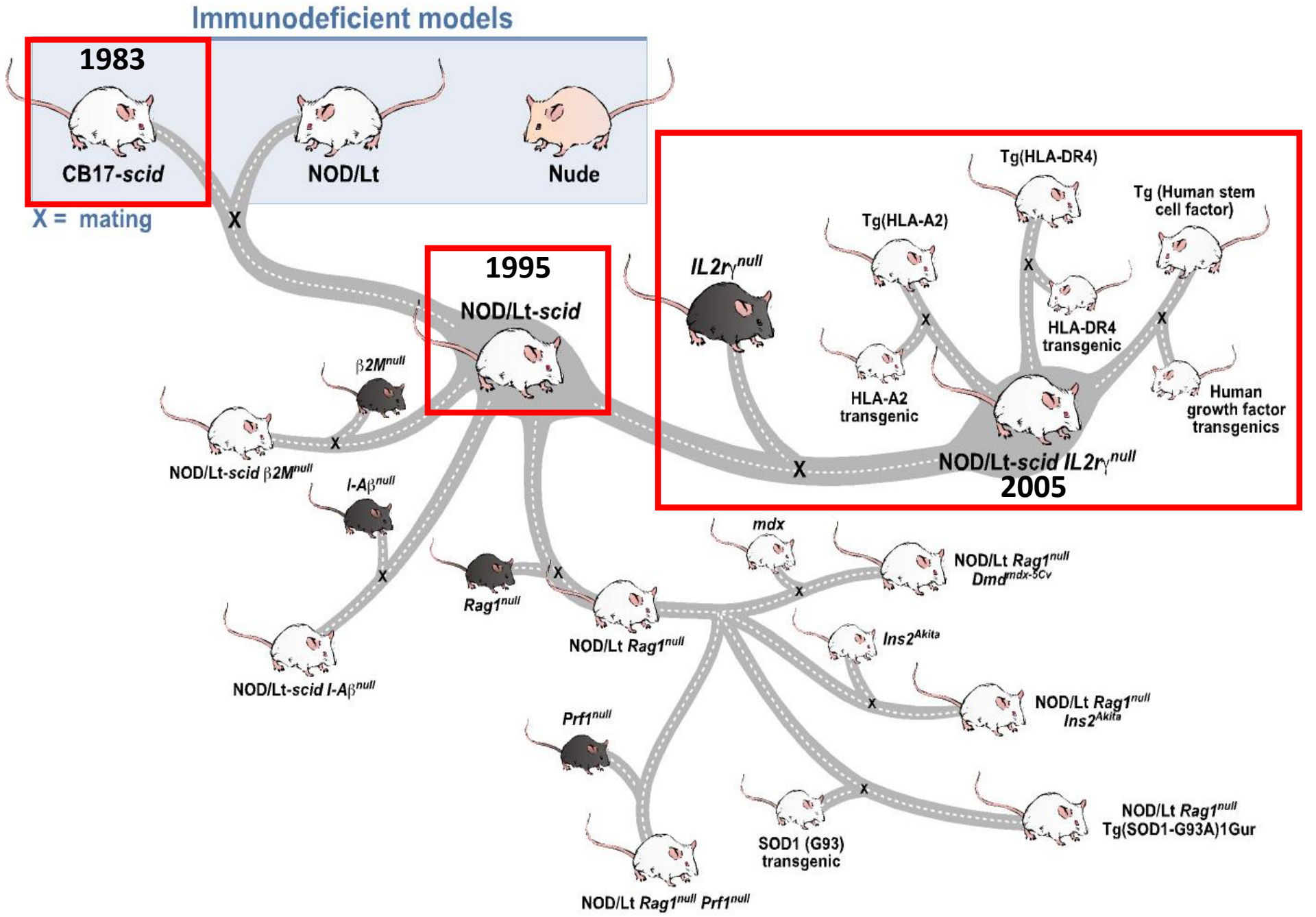
-Human hematopoiesis

-Infectious diseases

-Immunity and Autoimmunity

-Transplantation

The Road to Humanized SCID Mice

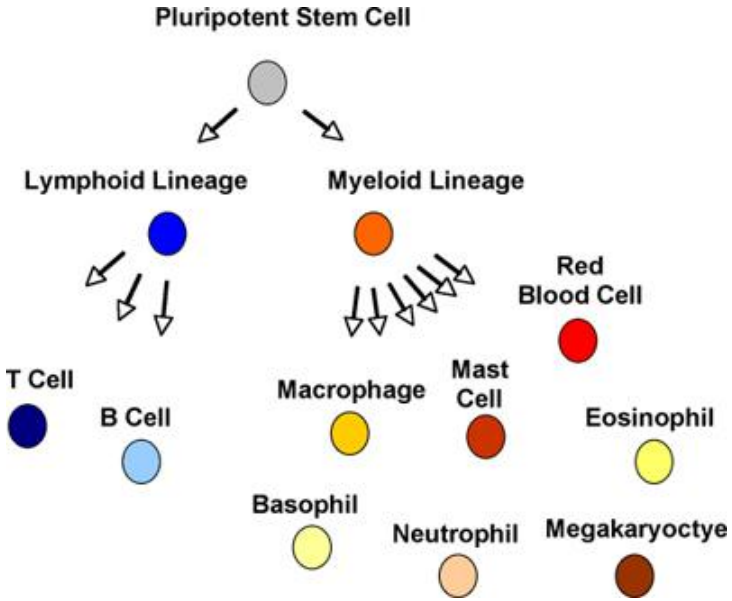


NOD-*scid IL2rγ*^{null} (NSG) Mouse

Shultz, 2005. *J. Immunol*.; Ishikawa, 2005. *Blood.*

- **Complete absence of *IL2rg* gene**
  - **-long life span**
  - **-further impairment of innate immunity**
  - **-complete absence of NK cells**

- **NOD-*scid IL2rγ*^{null} mice engraft at high levels with human cells: 10-90% human cells in periphery**
  - **-Develop all hematopoietic lineages of cells:**
    - **T cells**
    - **B cells**
    - **NK cells**
    - **Dendritic cells**
    - **Macrophages**
    - **Red blood cells**



Major Strain Platforms

NSG	**NOD-*scid IL2r*γ^{null}**	**Jackson Lab**
NOG	**NOD-*scid IL2r*γ^{Trunc}**	**Central Institute for Experimental Animals**
BRG	**BALB/c-*Rag2*^{null} *IL2r*γ^{null}**	**Yale/Univ. Hosp. Zurich**
H2^{d}RG	**Stock-H2^{d}-*Rag2*^{null} *IL2r*γ^{null}**	**Pasteur Institute**

LD Shultz, et.al., 2007. *Nat. Rev. Immunol.* 7:118

Brehm *et al Clin. Immunol*, 135:84-98, 2010

Human Immune System Models

- **Hu-PBL-SCID mice: *scid* mice injected with human peripheral blood mononuclear cells (PBMC)**

- **Hu-SRC-SCID mice: *scid* mice that have been sublethally irradiated and injected with hematopoietic stem cells (HSC)**
  - ***scid* repopulating cells (SRC) = $CD34^+$ cells**

- **SCID-Hu mice: *scid* mice that have been engrafted with human fetal liver and thymus under the renal capsule**
  - **BLT with autologous CD34+ cells from liver**

LD Shultz, et.al., 2007. *Nat. Rev. Immunol.* 7:118

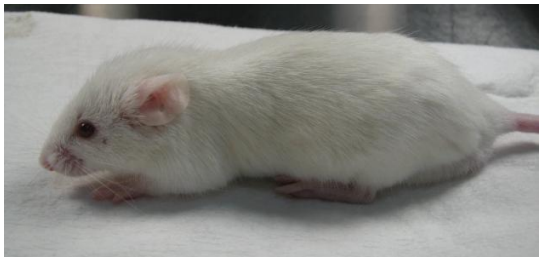
Model of Xenogeneic-GVHD Mediated by Human PBMC

Intravenous Injection of $10x10^6$ Human PBMC

NOD-*scid IL2r*γ^{null} (NSG) Mice

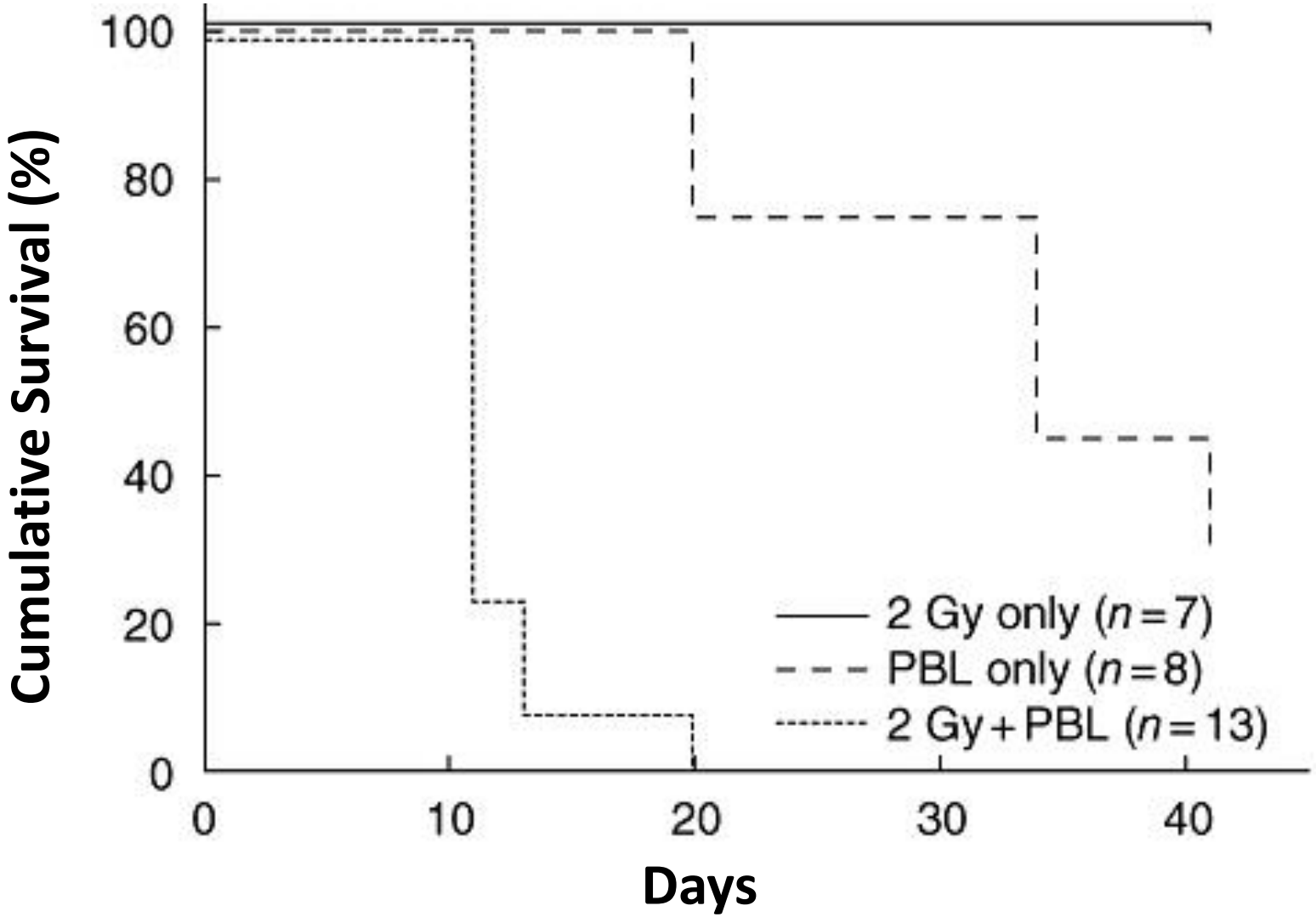
+ or - radiation

Follow for engraftment and disease



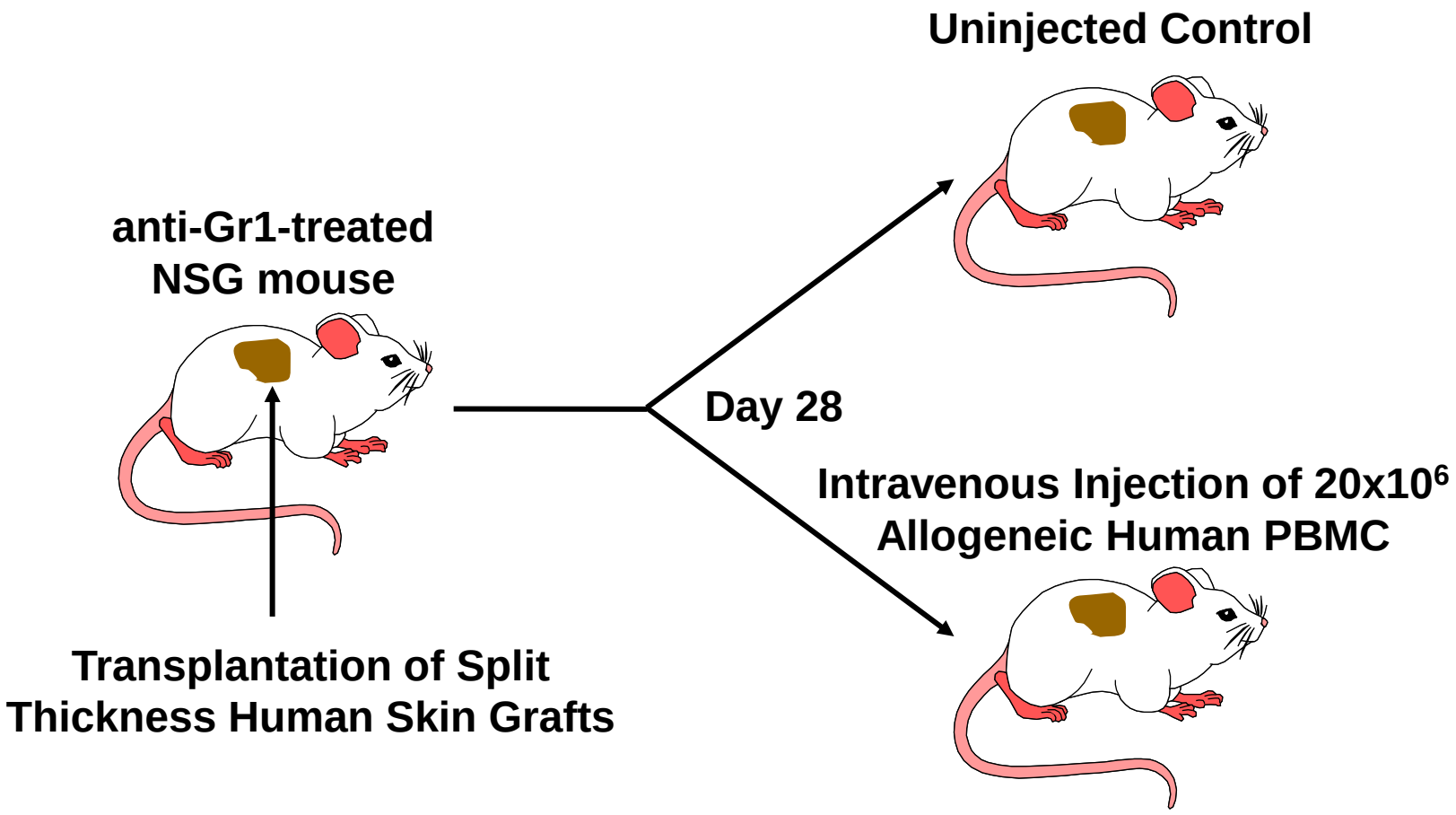
-hair loss/erythema
-hunched posture
-weight loss
-death

NSG Mice Succumb to Xeno-GVHD Mediated by Human PBMC

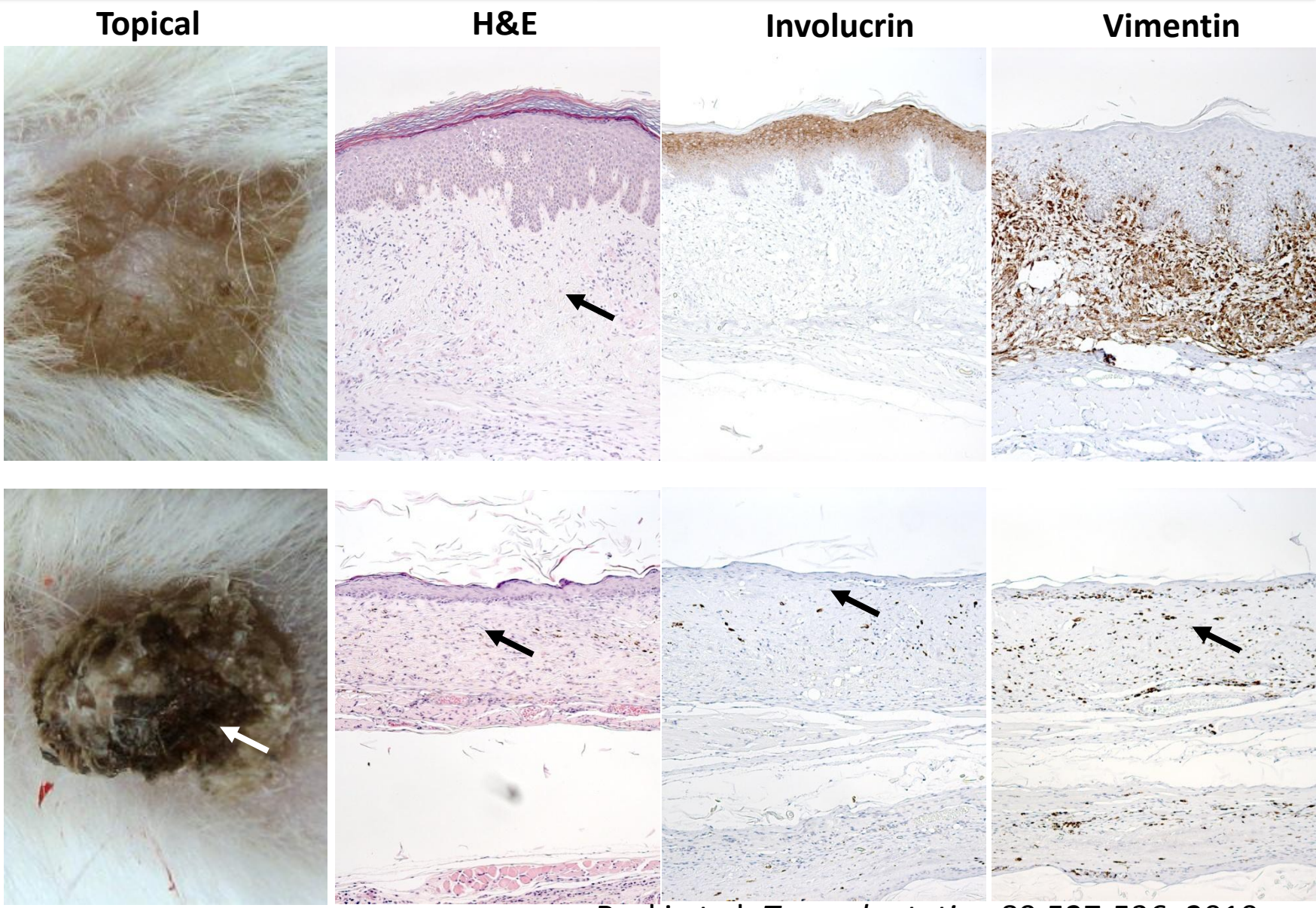


King *et al., Clin Exp Immunol* 157:104, 2009

Hu-PBL-SCID Mouse Model to Study the Rejection of Human Skin Allografts



Injected Human PBMC Reject Human Skin Allografts in NOD-*scid IL2r*γ^{null} Mice (day 28)



Racki et al, *Transplantation* 89:527-536, 2010

Reduction of Xeno-GVHD in the Hu-PBL-SCID Model

- **Study human T cell function in the absence xeno-GVHD**

- **Majority of engrafted cells recovered from blood and spleen of NOD-*scid IL2r*γ^{null} injected with human PBMC are T cells**
  - Our hypothesis is that mice lacking murine MHC will have reduced susceptibility to xeno-GVHD

- **NOD-*scid IL2r*γ^{null} *Ab*^{0}**
- **NOD-*scid IL2r*γ^{null} β*2M*^{null}**
- **NOD-*scid IL2r*γ^{null} *KbDb*^{null}**

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Variables For Creating Humanized Mice to Study Human Immune Responses

1. Age of the recipient

2. Strain background

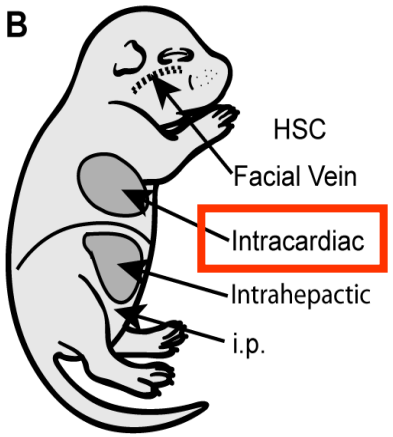
3. Source of human tissues and cell dose

4. Injection route

Brehm *et al Clin. Immunol*, 135:84-98, 2010

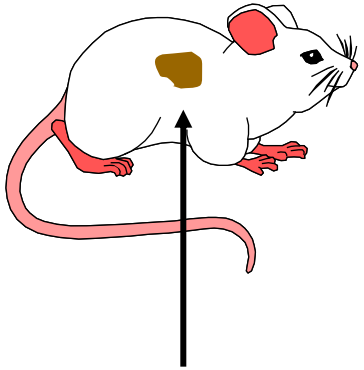
Hu-SRC-SCID Mouse Model to Study the Rejection of Human Skin Allografts

Newborn NSG injected With 5x10^4 CD34$^+$ cells



12-16 weeks

HSC-engrafted NSG

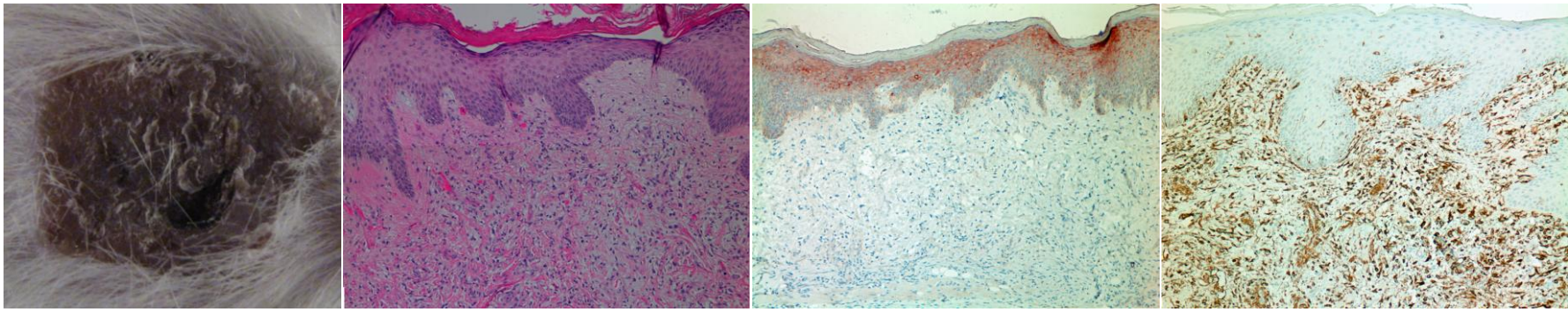


Transplantation of Split Thickness Human Skin Grafts

Rejection of Allogeneic Human Skin After Transplantation onto HSC-Engrafted NSG Mice (Day 28)

NSG

Topical | H&E | Involucrin | Vimentin



HSC-Engrafted NSG

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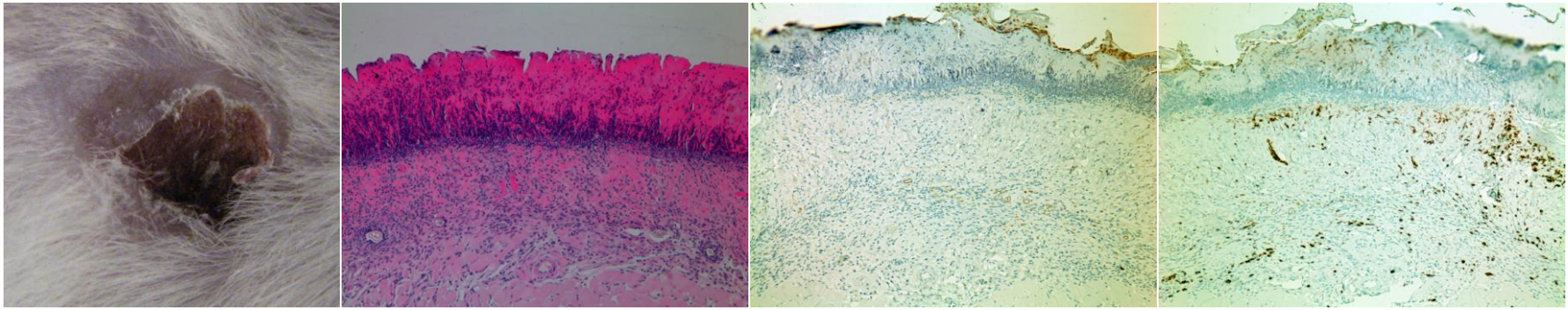

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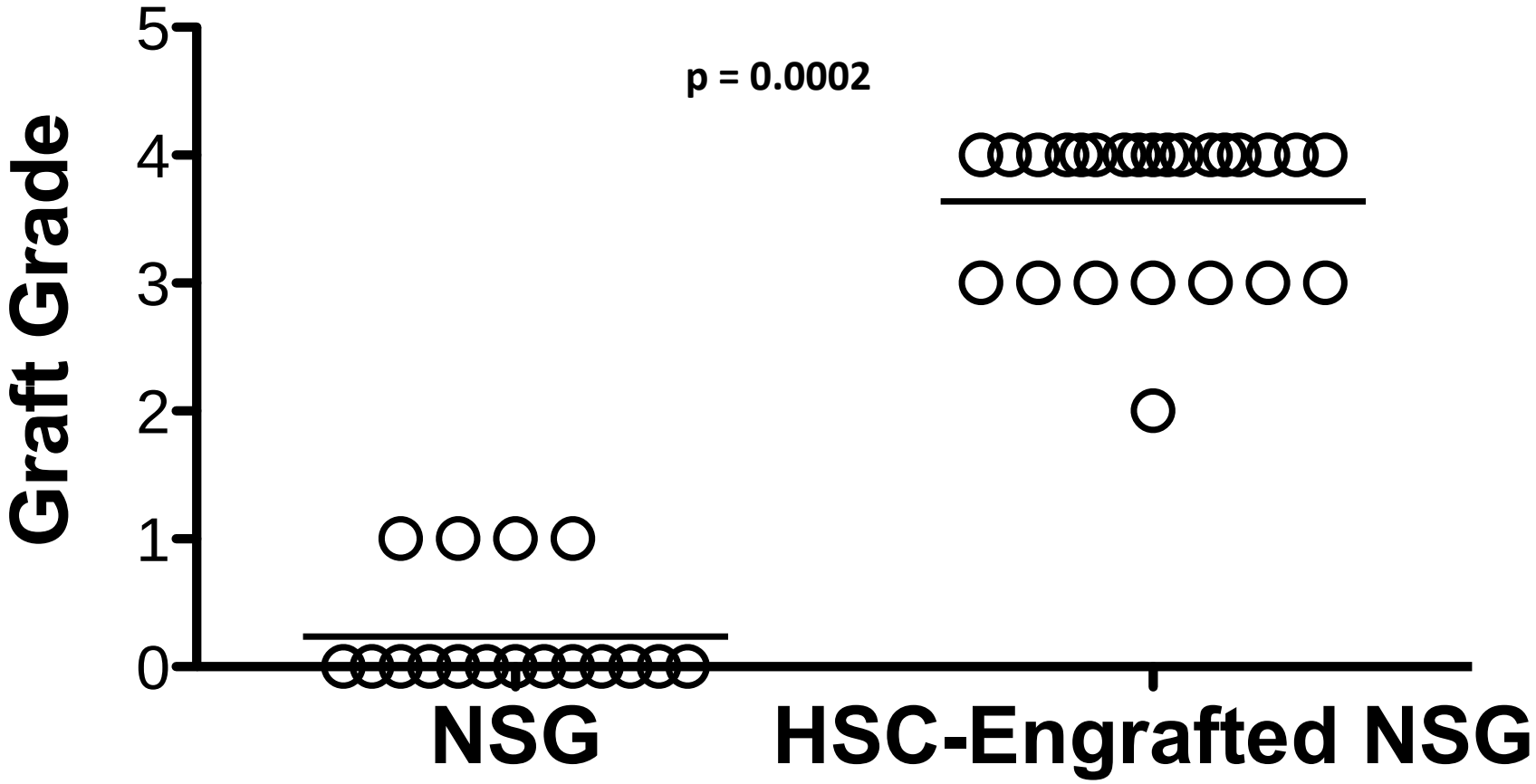

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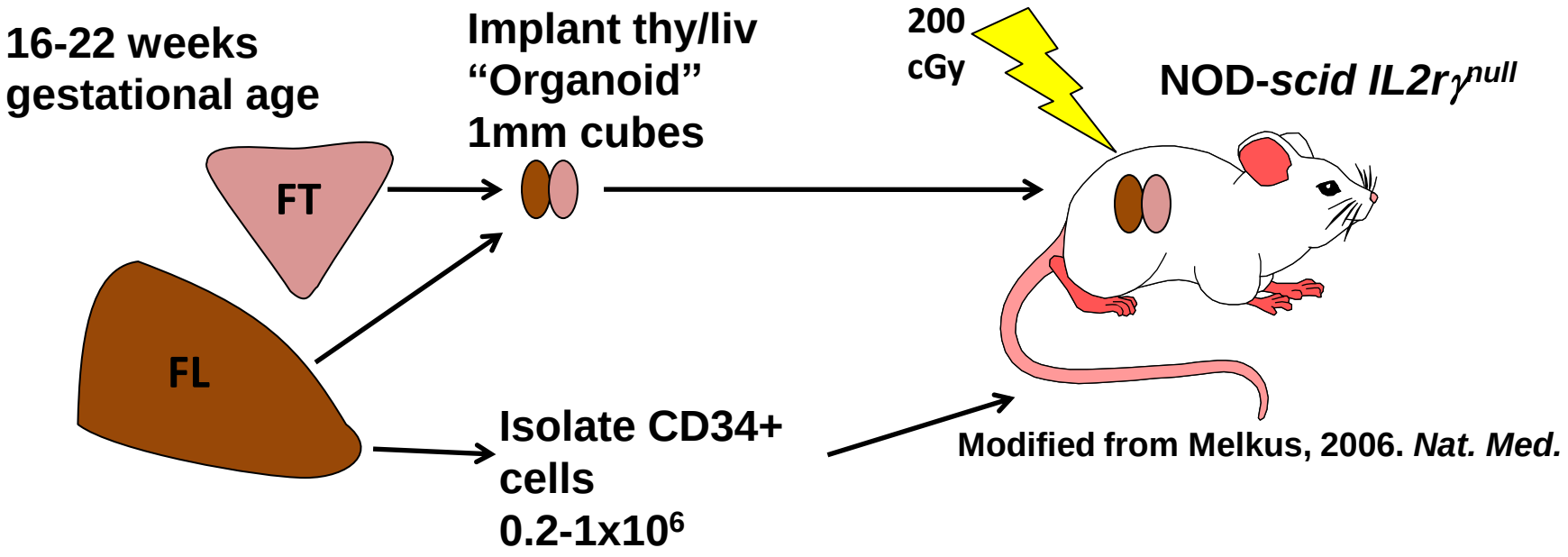
LD Shultz, et.al., 2007. *Nat. Rev. Immunol.* 7:118

BLT Mouse Model
Bone Marrow/Liver/Thymus



-Develops robust immune system comprised of multiple lineages

-Sustained, high level T cell development

-T cells educated on autologous thymic tissues

-Detectable T and B cell responses to viral infection (EBV and HIV)

Melkus, 2006. *Nat. Med.*; Sun, 2007, *J. Exp. Med.*; Brainard, 2009, *J. Virol.*

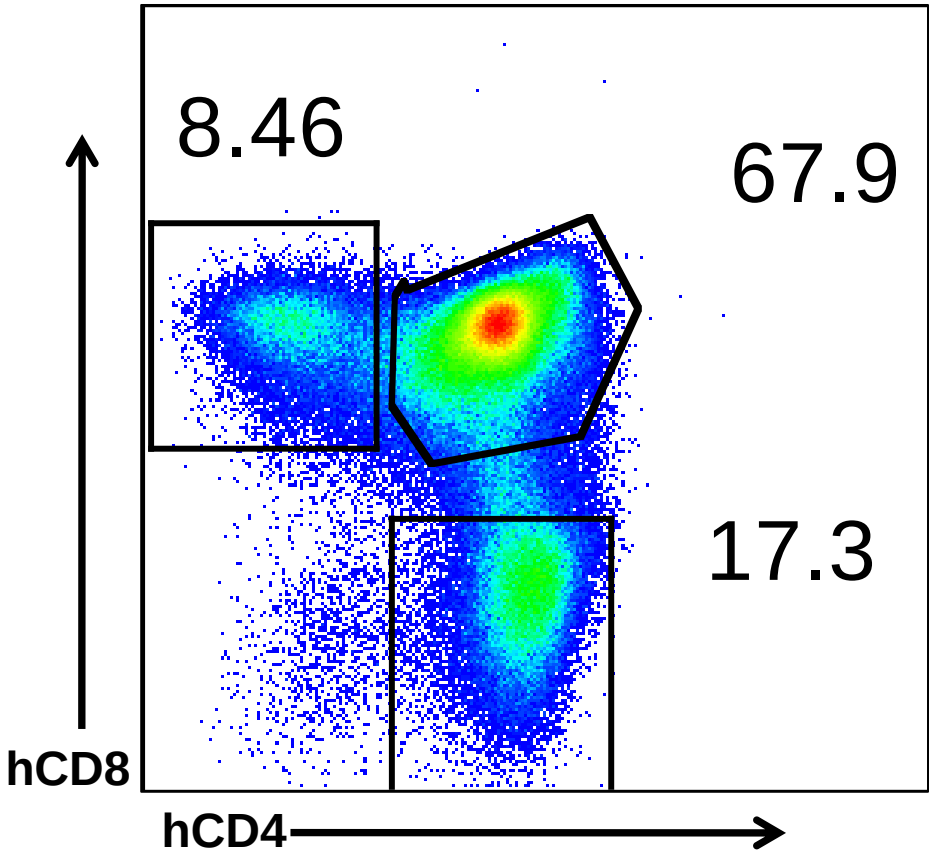
-Rejection of pig islets: Tonomura, 2008. *XenoTranspl.*

-Rejection of non-self human pancreas: unpublished data, Lafferty et.al.

Thymus Development in BLT mice at 16 Weeks Post-Implant

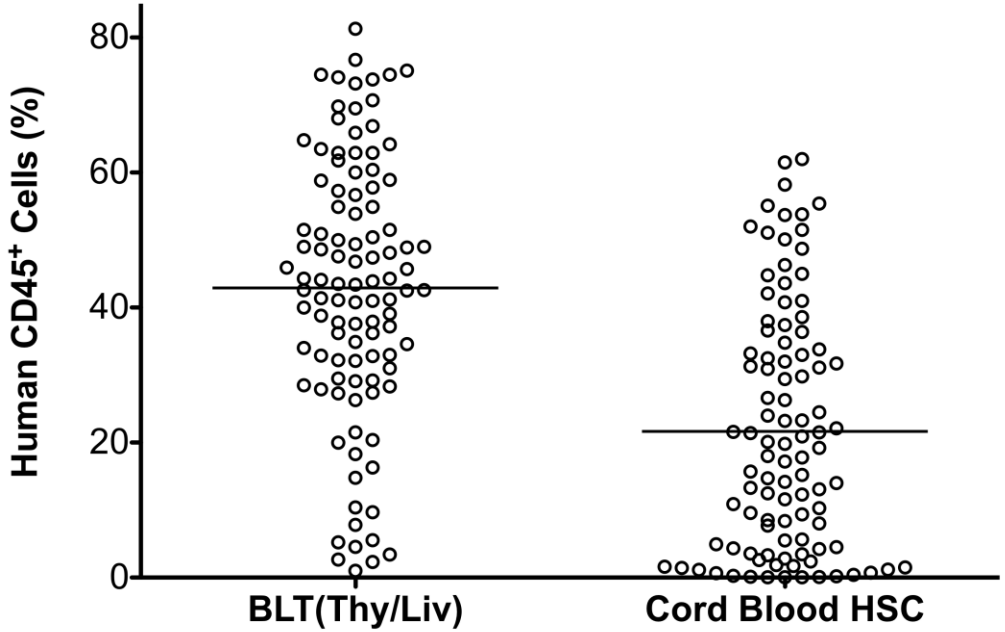
Thymic Organoid

Thymocyte subsets

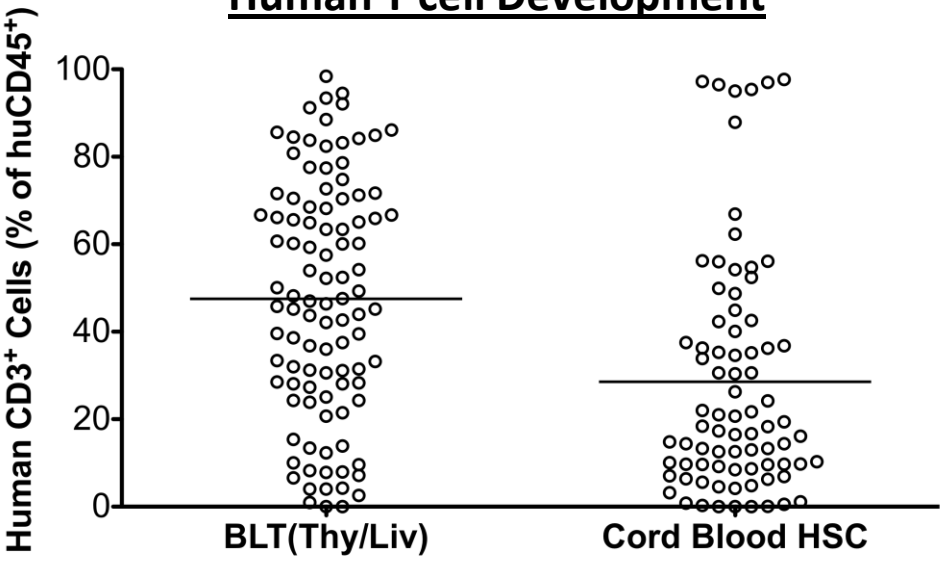


Human Cell Engraftment is Superior in the BLT Mouse Model (PBL)

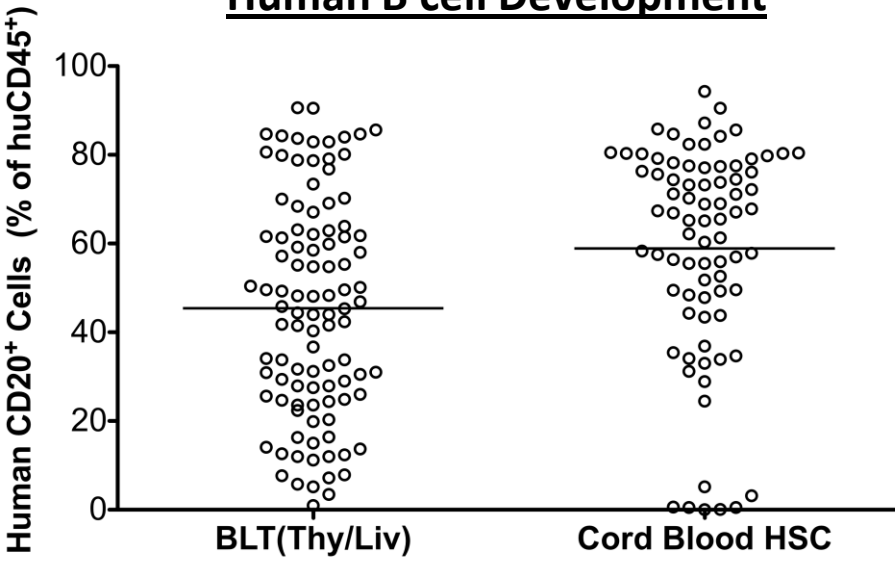
Total Human Cell Engraftment



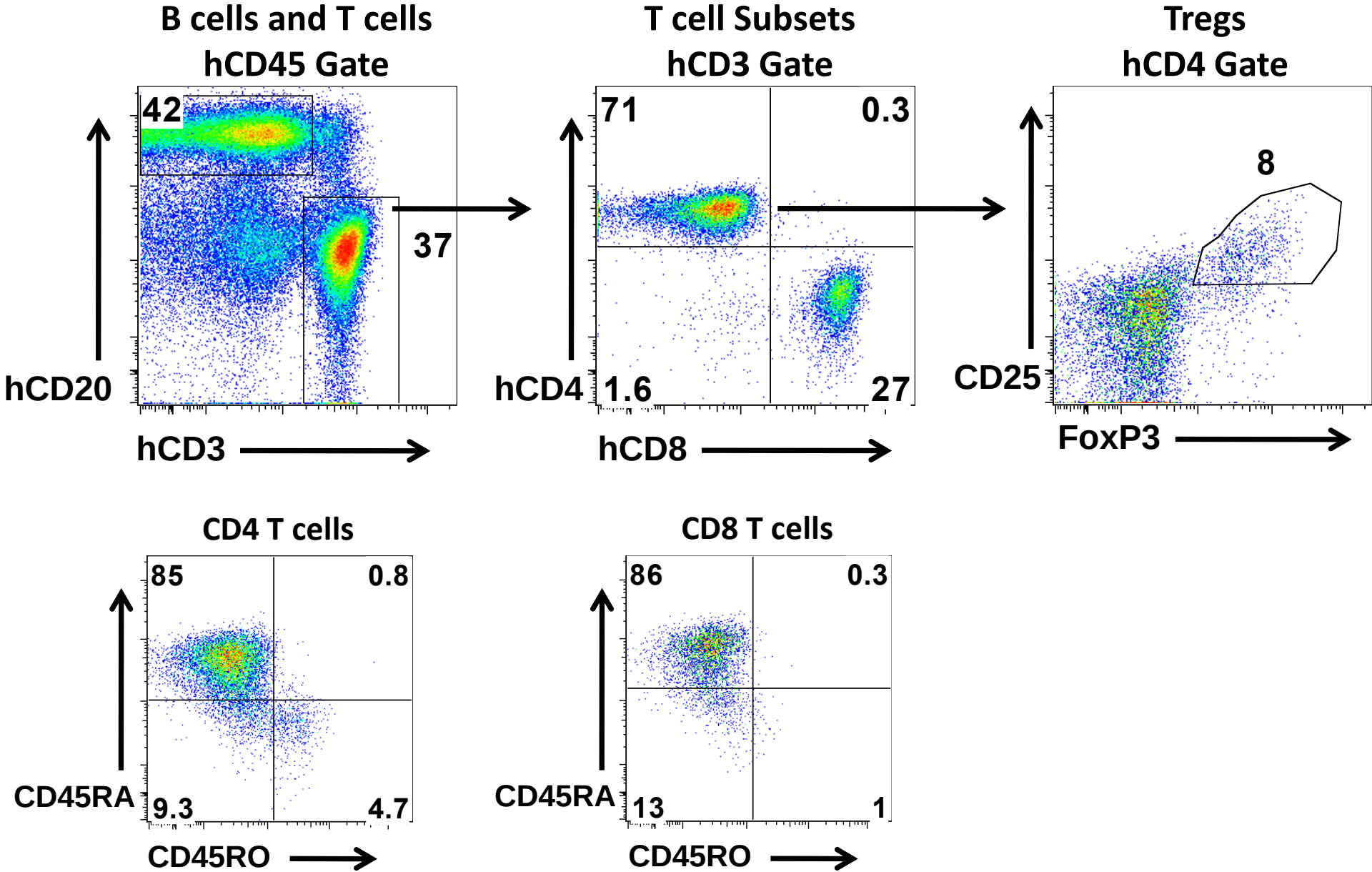
Human T cell Development



Human B cell Development



Peripheral T cell Development (spleen at 16 weeks)



Summary

1. **Documented that human PBMC will induce a xeno-GVHD in NSG mice**

1. **Immunodeficient mice bearing targeted mutations in the IL2r common γ-chain are the optimal recipients of human HSC**

1. **Humanized NSG mice (Hu-PBL-SCID and Hu-SRC-SCID) can be used to study the rejection of allogeneic tissues by human immune cells**

1. **BLT model allows for robust and consistent engraftment of human cells, including multiple hematopoietic lineages**

   -T cells are educated on human thymic epithelium

Limitations of HSC Engraftment in NSG Mice

1. **Lack of HLA molecules for T cell education**
2. **Species specificity of cytokines and growth factors**

   -Improve engraftment of human HSC: NSG-Tg(hu-mSCF)

   -Improve T cell function: NSG-Tg(huIL7)

   -Inability of B cells to class switch: NSG-Tg(huBLyS), NSG-Tg(huIL7)

   -Functionality of innate immune cells: NSG-Tg(huCSF1), NSG-TripTg (SCF, IL3, GM-CSF)

3. **Limited lymph node development: NSG-Tg(huIL7)**
4. **Residual murine innate immunity: NSG-TLR4^{null}, NSG-NCF1^{null}, NSG-MyD88^{null}, NSG-Tg(huSIRPa)**
5. **Immune cell homing: intergrins and chemokines**

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Beta Cell Biology Consortium

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dedicated to finding a cure

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