JAX™ WEBINARS

Welcome Colleagues!

TODAY'S PRESENTATION:

Comparing Immunodeficient Mice for Cancer, Immunity and Transplant Research

START TIME:

10:00 AM SGT February 26 (Singapore)

Janine Low-Marchelli, Ph.D.

Manager, Technical Information Services

Senior Technical Information Scientist

Organized by:

InVivos
BRINGING LIFE TO YOUR RESEARCH

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JAX MISSION

To discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health.

EMPOWERING SCIENTIFIC EXCELLENCE

Our scientific expertise is derived directly from JAX faculty and scientific researchers, who are embarking on ground-breaking research in addition to providing cutting-edge models and powerful preclinical services to researchers worldwide.

Explore the Latest Innovations

JAX MICE AND SERVICES

Precise models, powerful services,
and scientific expertise

MODEL ACCESS

- Mice Models
- Immunodeficient Models
- Humanized Models
- Preconditioned Models
- PDX Tumors & Tissue Bank
- Aged Models
- Model Generation
- Cohort Generation

TARGET VALIDATION/ TAILORED CHARACTERIZATION

- Genotyping
- Phenotyping
- Surgical Services
- Expression Analysis
- Necropsy Studies
- Preconditioning

PRECLINICAL SOLUTIONS

- In Vivo Pharmacology
- Safety & Toxicity Studies
- FcRn Studies
- Natural History Studies
- Neurobehavioral Studies
- Breeding and Importation

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Learning Goals

- Match genetic modification, strain characteristic to immunological phenotype
  - Nude, *scid* (and Rag1/Rag2), Il2rg
  - Effect of genetic background on immunodeficiency
- Identify appropriate immunodeficient strain to use based on research application
- Implement appropriate housing conditions for your immunodeficient strains

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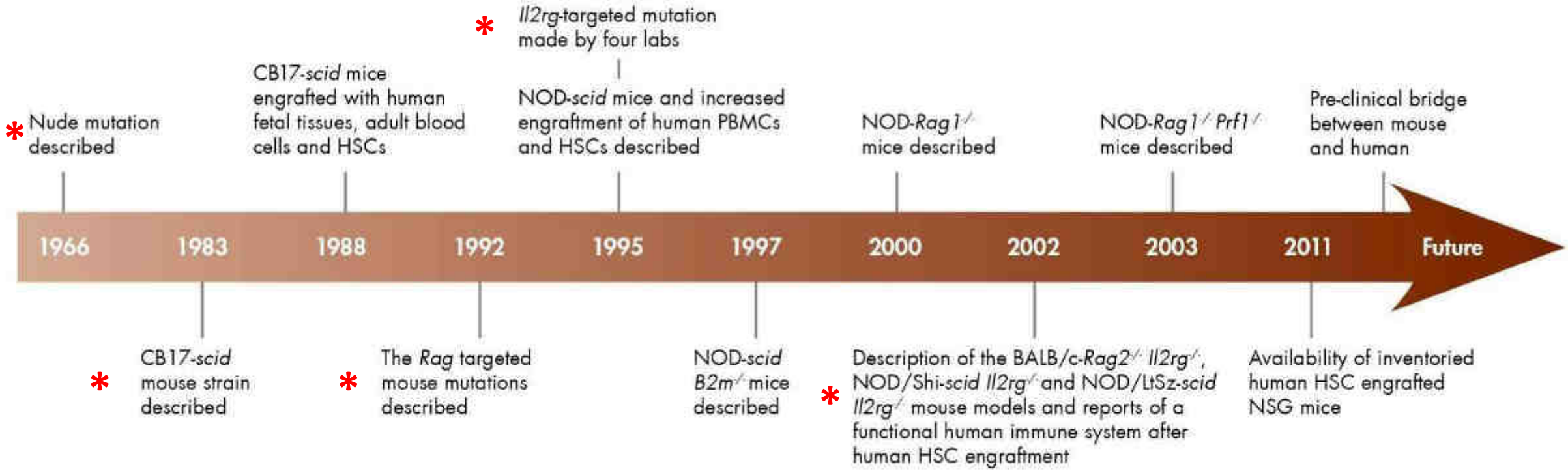
Timeline | **Immunodeficient and humanized mouse development**



B2m, ß$_2$-microglobulin; HSC, haematopoietic stem cell; *Il2rg*, interleukin-2 receptor γ-chain; NOD, non-obese diabetic; PBMC, peripheral-blood mononuclear cell; *Prf1*, perforin 1; *Rag*, recombination-activating gene; *scid*, severe combined immunodeficiency.

Shultz LD et al. 2007. *Nat Rev Immunol* 7(2):118-30. PMID:17259968

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COMPREHENSIVE IMMUNODEFICIENT SUITE

	NOD *scid* gamma (NSG™) *	NOD Rag gamma (NRG)	NOD *scid* gamma (IL3, GM-CSF, SCF (NSG-SGM3)	NOD *scid* *	BALB *scid* *	B6 Rag1	Outbred and Inbred Nude *
Name & Stock Number	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (005557)	NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ (007799)	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ (013062)	NOD.CB17-*Prkdc*^{scid}/J (001303)	CByJmn.CB17-*Prkdc*^{scid}/J (001803)	B6.129S7-*Rag1*^{tm1Mom}/J (002216)	J:NU (007850) NU/J (002019)
Mature B cells	Absent	Absent	Absent	Absent	Absent	Absent	Present
Mature T cells	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Dendritic Cells	Defective	Defective	Defective	Defective	Present	Present	Present
Macrophages	Defective	Defective	Defective	Defective	Present	Present	Present
Natural killer cells	Absent	Absent	Absent	Defective	Present	Present	Present
Complement	Absent	Absent	Absent	Absent	Present	Present	Present
Leakiness	Very low	Absent	Absent	Very low	Very low	Absent	N/A
Irradiation tolerance	Low	High	Low	Low	Low	High	High
Lymphoma Incidence	Low	Low	Low	High (thymic lymphoma)	High (thymic lymphoma)	Low	Low
Benefits	• 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	• Long-term multilineage hematopoietic stem cell repopulation similar to NSG™ mice • Engrafts human PBMC without irradiation similar to NSG™ • Engrafts a wide range of solid and hematological cancers	• Increased CD4+ FoxP3+ regulatory T cell population • Enhanced human myelopoiesis and terminal differentiation, • Increased efficiency of engrafting human acute myeloid leukemia (AML)	• Higher take-rates for slow-growing cancer cell lines than BALB *scid* or nude xenograft models • Xenotransplantation of some solid human tumors • Adoptive transfer from strains on NOD background enables study of cell function & track cell movement • About 36 weeks median survival	• Allows allogeneic and xenogeneic cancer cell lines & tissues • Engrafts hematopoietic cancer cell lines, some primary cells • Improvements in engraftment efficiency over nude models for some cancer cell lines	• Radiation resistant, providing an alternative to *scid* mutants • Adoptive transfer from strains on B6 background permits to study cell function and track cell movement	• Engraftment of human & mouse tumor cell lines • Easy assessment of subcutaneous tumor growth due to lack of fur
Considerations	• No thymic lymphomas—can be used for long and short-term experiments • Sensitive to irradiation	• No thymic lymphomas—can be used for long-term experiments • Requires higher dose of irradiation to obtain human HSC engraftment	• Compromised human stem cell regeneration • Suppression of human erythropoiesis • Reduction of human B-lymphopoiesis	• Develops thymic lymphomas by 8-9 months—best used in short-term experiments • Sensitive to irradiation	• Innate immunity intact • NK cell activity limits engraftment • Sensitive to irradiation	• Innate immunity intact • Poor host for primary cell transplantation	• Innate immunity intact • Little engraftment of hematopoietic cancer cells • Not suitable for primary cell transplantation
References	Ishikawa et al. 2005 (PMID: 15920010) Shultz et al. 2005 (PMID: 15879151)	Pearson et al. 2008 (PMID: 18785974) Brehm et al. 2010 (PMID: 20096637) Maykot et al. 2014 (PMID: 24798995)	Nicolini et al. 2004 (PMID: 14628073) Wunderlich et al. 2010 (PMID: 20686503) Billerbeck et al. 2015 (PMID: 21252091)	Shultz et al.1995 (PMID: 7995938)	Nonoyama et al. 1993 (PMID: 8473734)	Mombaerts et al.1992 (PMID: 1547488)	

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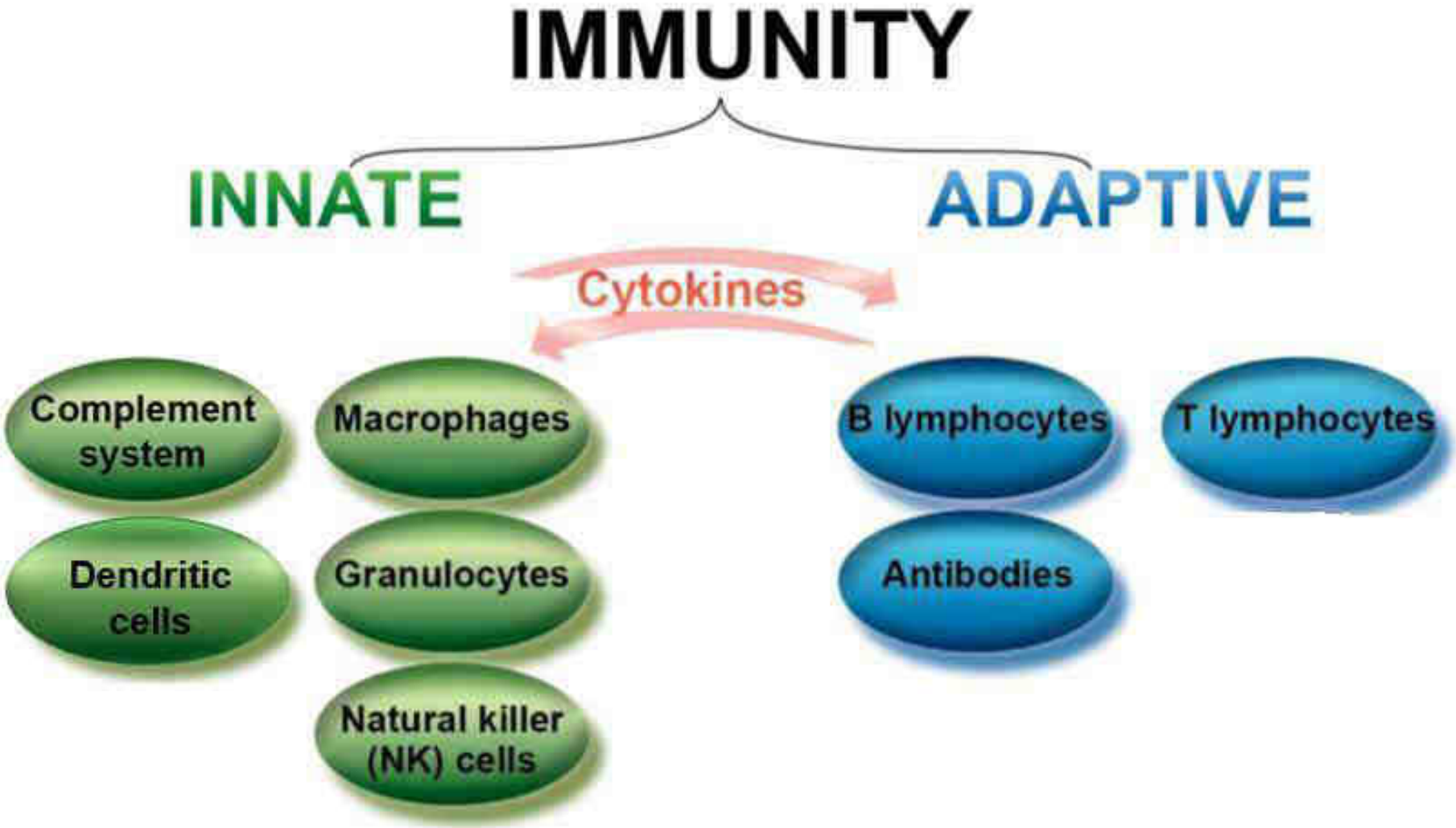
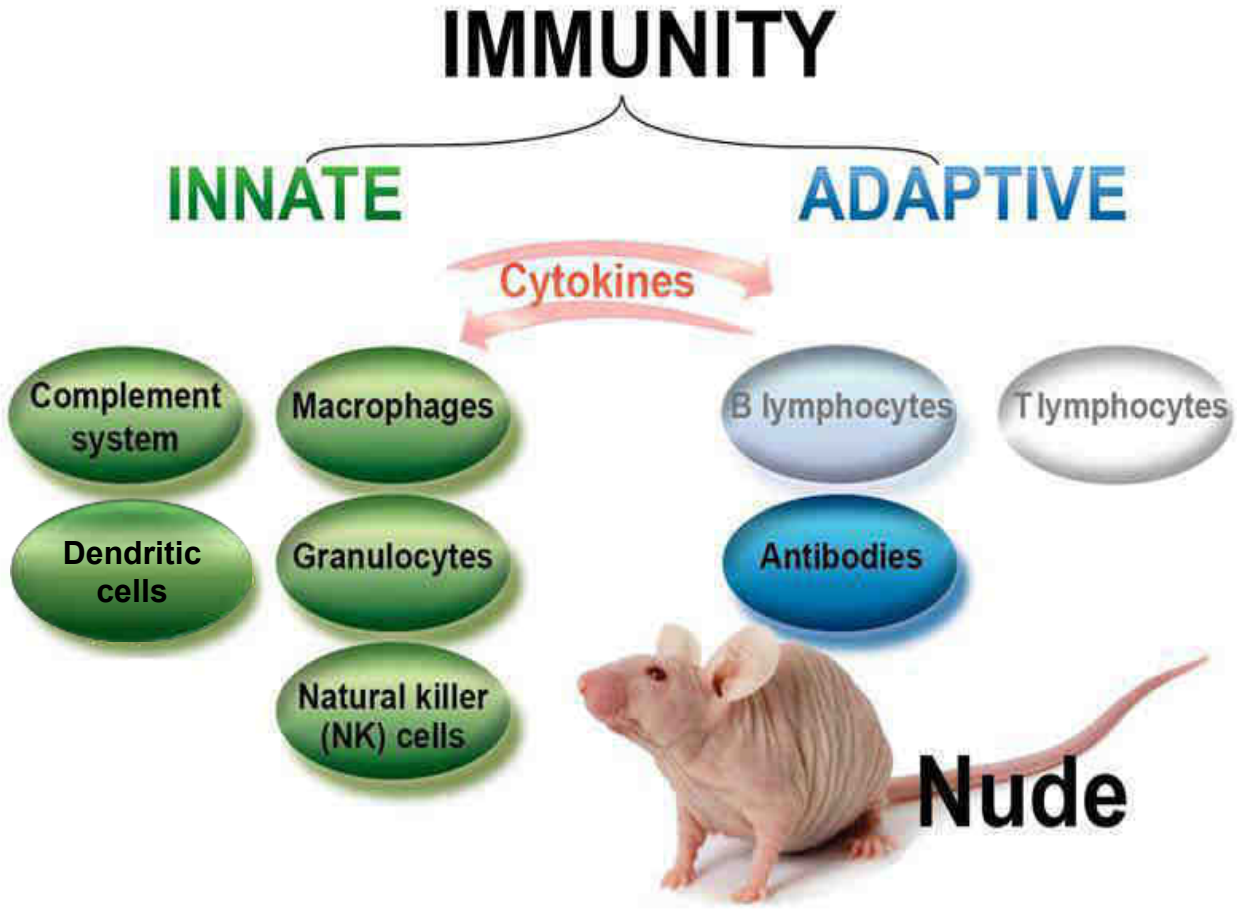
Considerations to Select an Immunodeficient Strain

- What is your experimental goal?
  - How much immunocompetency is required?
  - How much immunodeficiency is required?
    - What kind of cells are being engrafted (what species, immortalized cell line/PDX, hematopoietic)?


IMMUNITY
INNATE
ADAPTIVE
Cytokines
Complement system
Macrophages
B lymphocytes
T lymphocytes
Dendritic cells
Granulocytes
Antibodies
Natural killer (NK) cells

Nude Mice

- Nomenclature
  - Inbred Nude NU/J (002019)
  - Outbred Nude J:NU (007850)
- Immunological Deficiencies
  - Mutation: *Foxn1*^{nu}
  - Athymic and T cell Deficient



Nude Mice

- Applications and Benefits
  - Engraftment of human & mouse tumor cell lines
  - Well published/characterized
  - Hairless phenotype facilitates tumor growth measurements

- Considerations
  - Innate immunity intact
  - May not be suitable for primary cell transplantation or hematological malignancies

Flanagan SP. 1966. *Genet Res* 8(3):295-309. PMID:5980117

Pantelouris EM. 1973. *Differentiation* 1(6):437-50. PMID:4547146

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Easy Evaluation of Therapeutic Response in Nude Mice

Vehicle

Merodantoin

MCF-7 breast cancer cell line

Gulliya 1994 *Cancer* PMID:8082074

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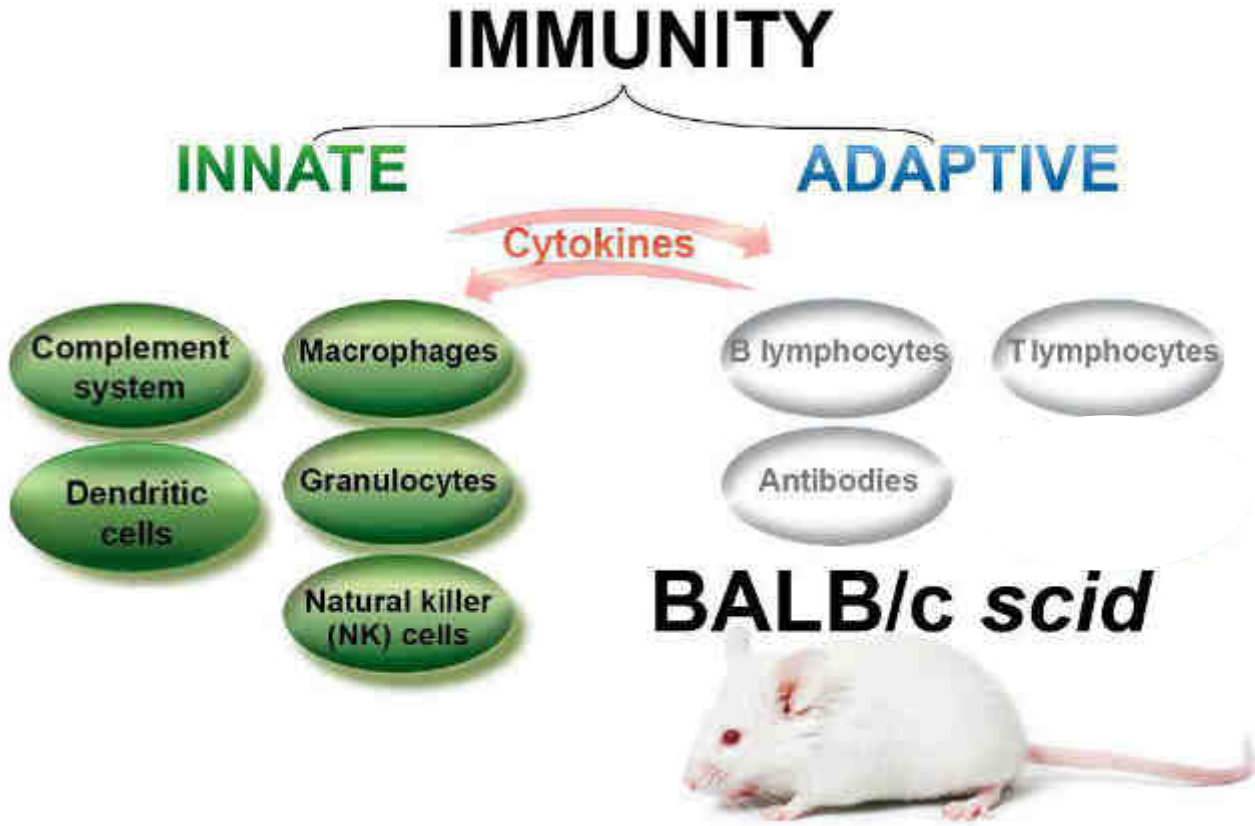
BALB/c *scid* Mice

- Nomenclature
  - CBySmn.CB17-*Prkdc*^{scid}/J (001803)
  - Similar to original C.B-17 *scid*
- Immunological Deficiencies
  - Mutation: *scid* “severe combined immune deficiency”
  - Impairs V(D)J recombination
  - No mature B and T cells

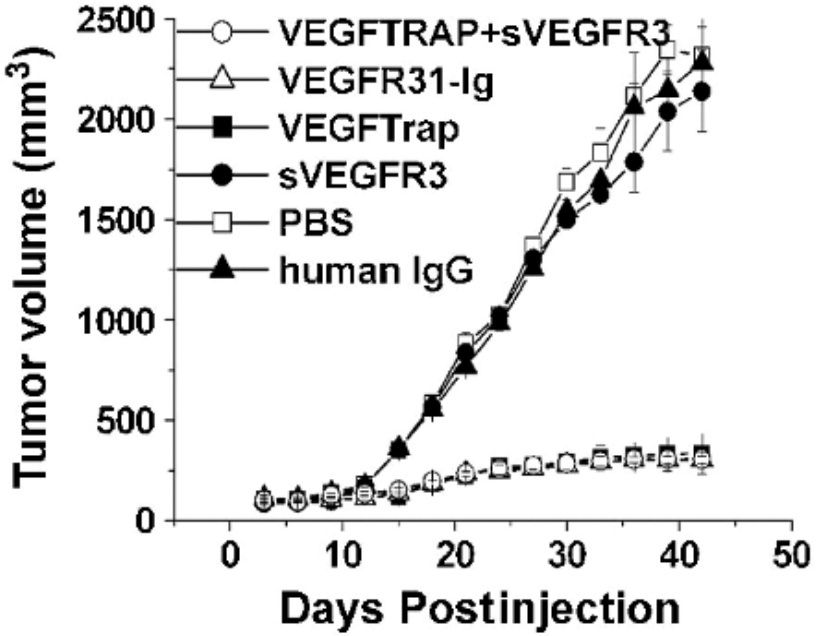


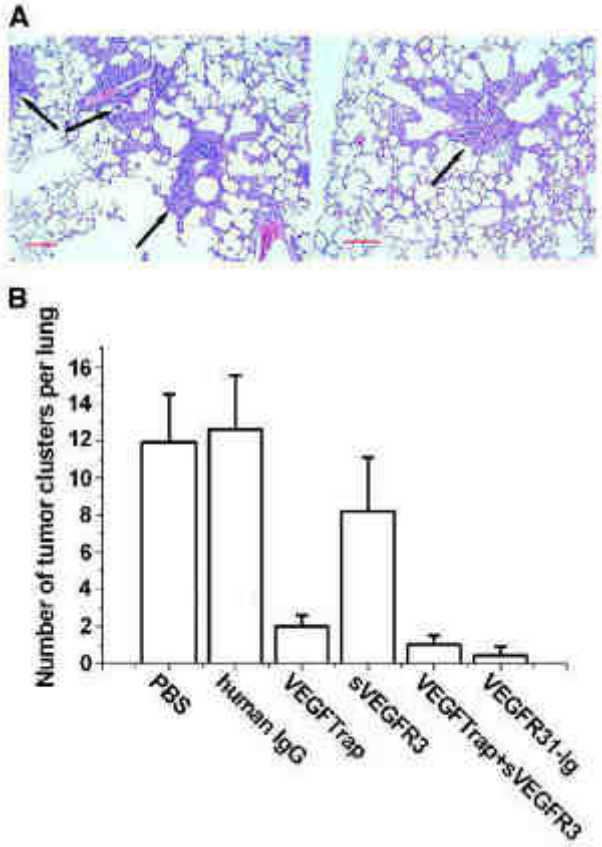
BALB/c *scid* Mice

- Applications and Benefits
  - Xenograft host for cancer cell lines
  - Efficacy testing of therapeutic antibodies
  - Adoptive transfer from BALB/c donors
  - Common inbred background simplifies creation of compound immunodeficient mutants

- Considerations
  - Innate immunity intact
  - scid side effects: radiation sensitivity; genotoxic drugs may have higher toxicity
  - High scid leakiness; mice may develop T and B cells as they age

Therapeutic Ab Efficacy in Tumor Growth and Metastasis using BALB/c-scid Mice





Zhang D et al. 2010. *Cancer Res* 70:2495-2503. PMID:20197464

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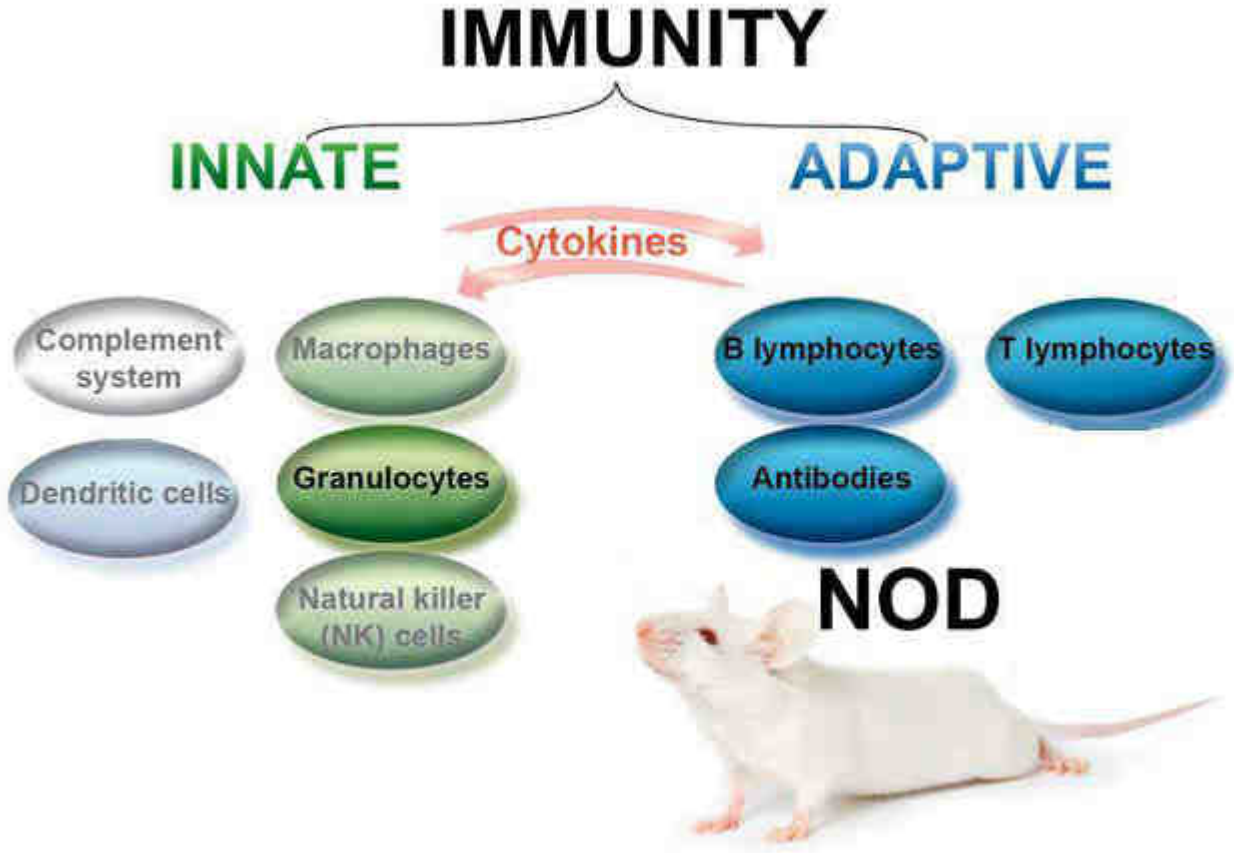
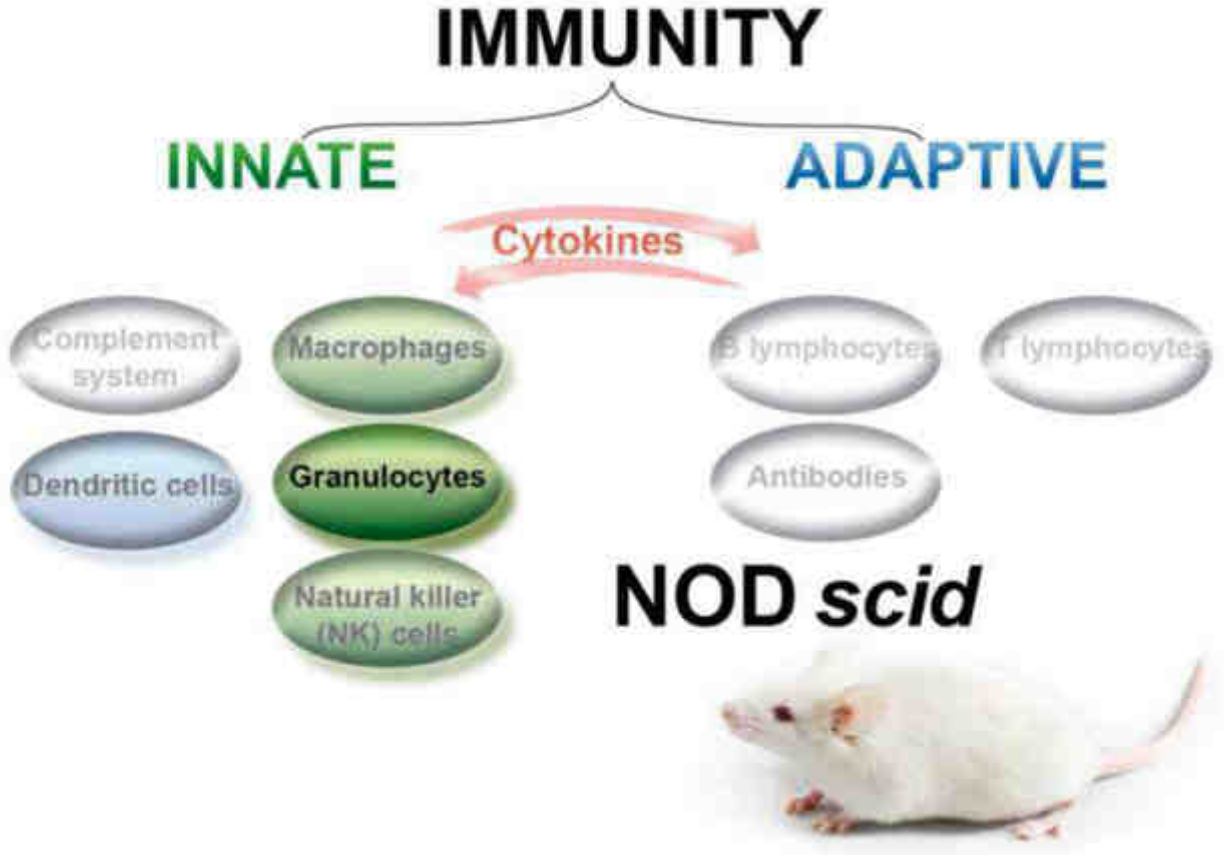
NOD *scid* Mice

- Nomenclature
  - NOD.CB17-*Prkdc*^{scid}/J (001303)
- Immunological Deficiencies
  - NOD genetic background
    - Absence of hemolytic complement
    - Reduced dendritic and NK cell function
    - Less responsive macrophages
    - Optimal human hematopoietic stem cell engraftment (Sirpa allele)



NOD *scid* Mice

- Nomenclature
  - NOD.CB17-*Prkdc*^{scid}/J (001303)
- Immunological Deficiencies
  - Mutation: *scid* “severe combined immune deficiency”
  - Impairs V(D)J recombination
  - No mature B and T cells



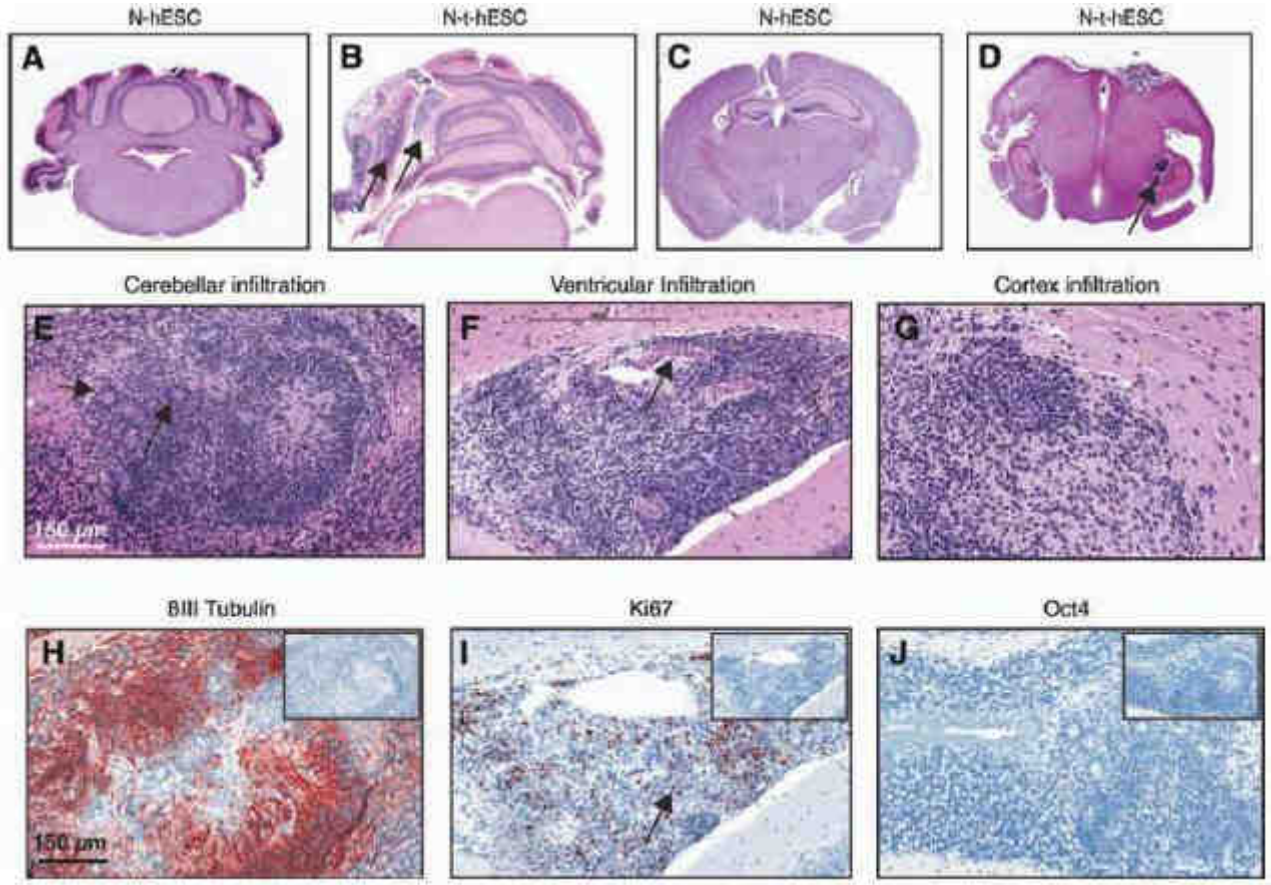
NOD *scid* Mice

- Applications and Benefits
  - Xenotransplantation of human tumors
  - Engrafts some hematopoietic cancer cell lines
  - Adoptive transfer recipient for study of autoimmune type 1 diabetes
  - Significantly less scid leakiness compared to other backgrounds
- Considerations
  - Short life span (thymic lymphoma by ~9 months)
  - Residual innate immunity (NK cell function)
  - scid side effects: radiation sensitivity; genotoxic drugs may have
  - higher toxicity

Shultz LD et al. 1995. *J Immunol* 154(1):180-91. PMID:7995938

Banuelos SJ et al. 2004. *Clin Immunol* 112(3):273-83. PMID:15308121

NOD *scid* Mice Propagate Medulloblastoma-like Tumors



Werbowetski-Ogilvie TE et al. 2012. *Stem Cells* 30(3):392-404 PMID:22213600

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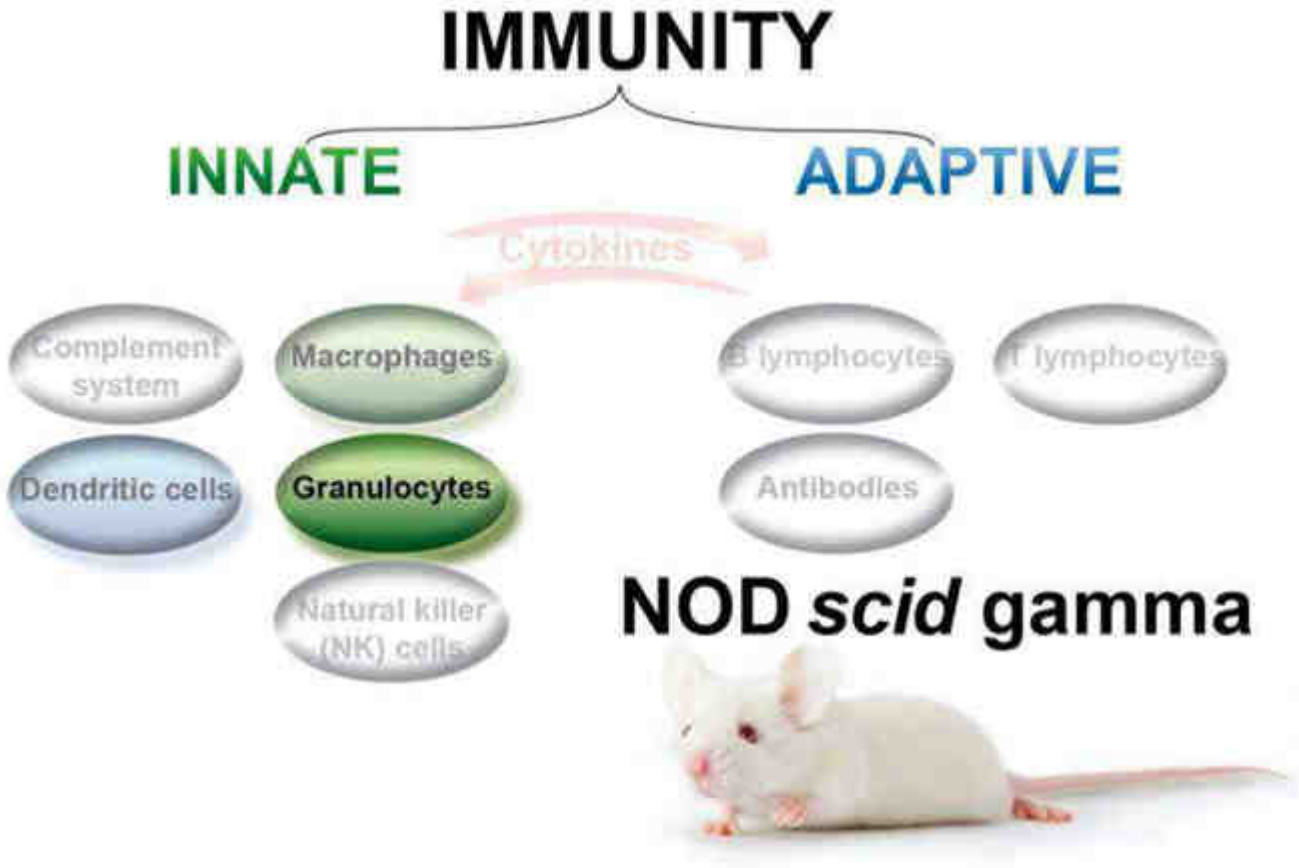
NSG™, NOD *scid gamma* Mice

- Nomenclature
  - NOD.CB17-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (005557)
- Immunological Deficiencies
  - Same as NOD *scid* mice
  - Additional Mutation: *Il2rg*^{tm1Wjl} deficiency eliminates signaling from 6 interleukins and blocks NK cell development



NSG™, NOD *scid gamma* Mice

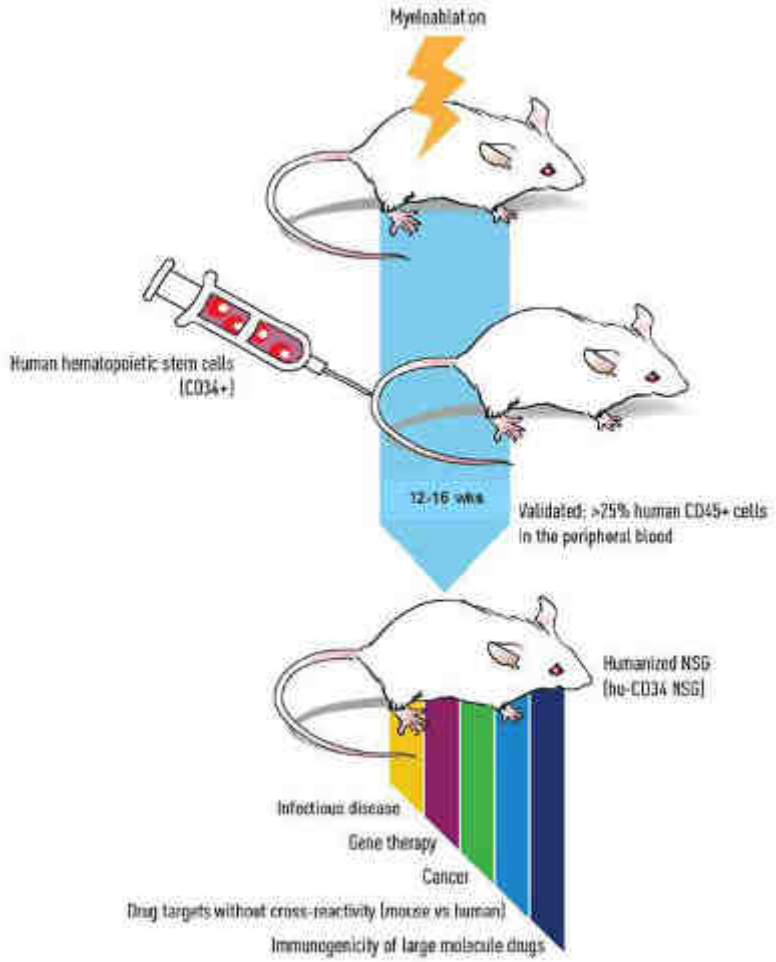
- Applications and Benefits
  - Xenotransplantation of human tumors
  - Optimal human hematopoietic stem cell engraftment (Sirpa allele)
  - No scid-associated leakiness
  - Longer life span than NOD-scid (> 16 months)
- Considerations
  - scid side effects: radiation sensitivity; genotoxic drugs may have higher toxicity

Shultz LD et al. 1995. *J Immunol* 154(1):180-91. PMID:7995938

Banuelos SJ et al. 2004. *Clin Immunol* 112(3):273-83. PMID:15308121

NSG™ Research Applications

- Primary tumor engraftment
- Hematological cancers
- Human hematopoiesis
- Infectious disease
- Cell replacement therapy for type 1 diabetes
- Immuno-oncology



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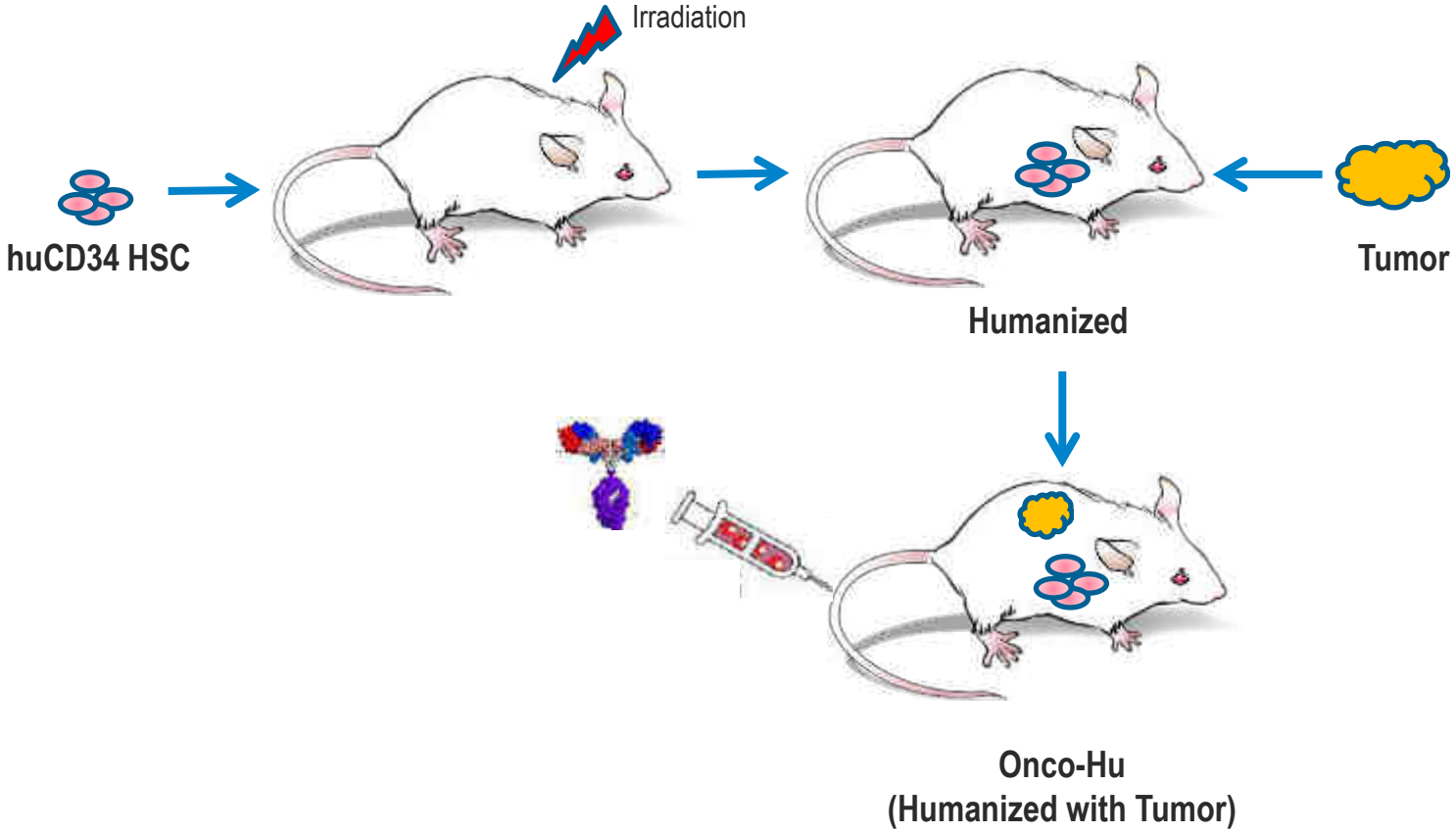
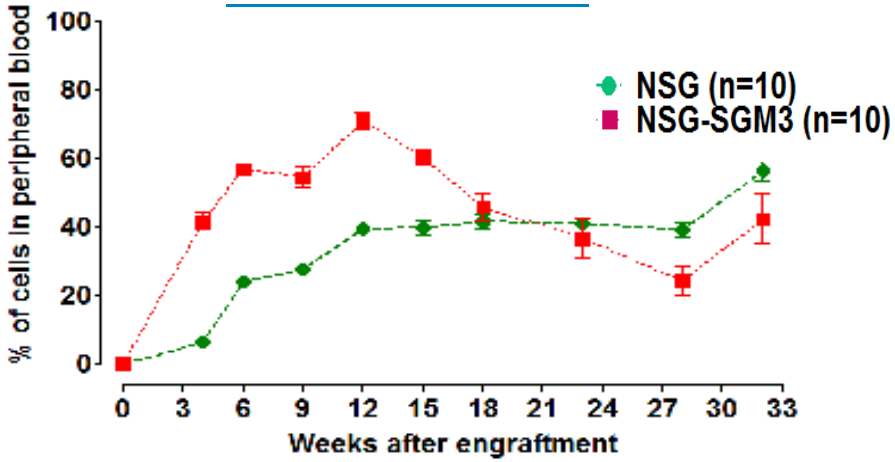
Immuno-oncology applications in NSG™ Mice



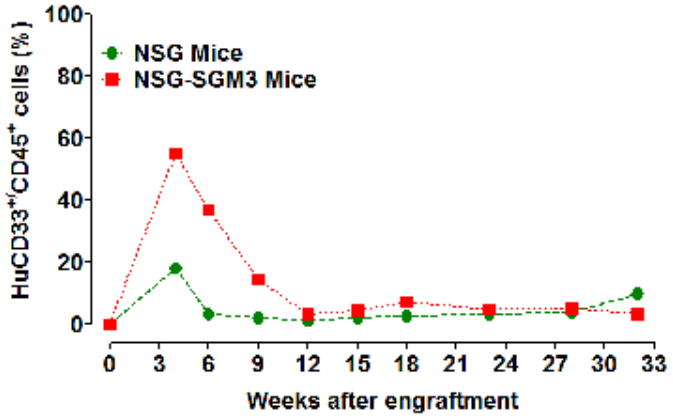
Human Immune Cells in Peripheral Blood of Hu-NSG™ vs Hu-NSG-SGM3™: Percent of HuCD45

- Greater early expansion of huCD45 in NSG-SGM3
- Greater early expansion of myeloid cells in NSG-SGM3
  - Higher CD33+ cell counts (cells/µl)
- Greater percent B cells, but higher percent T cells in NSG-SGM3

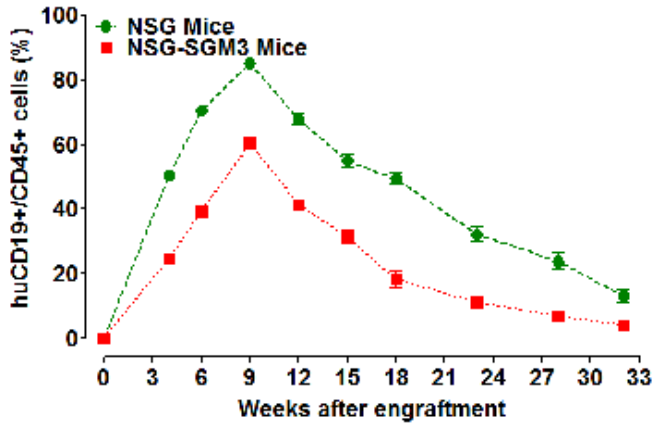
Total Human Donor



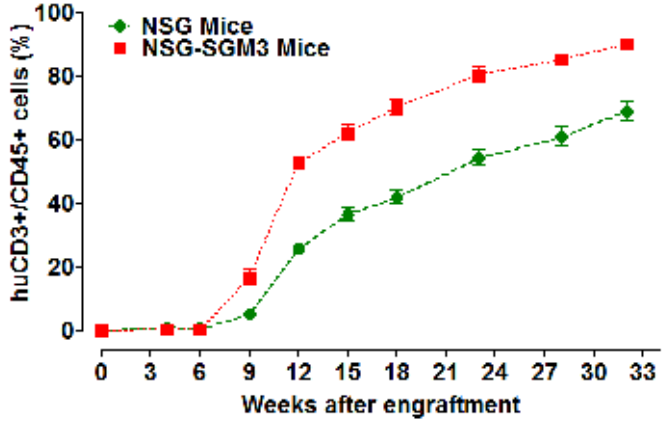
HuCD33 Myeloid Cells



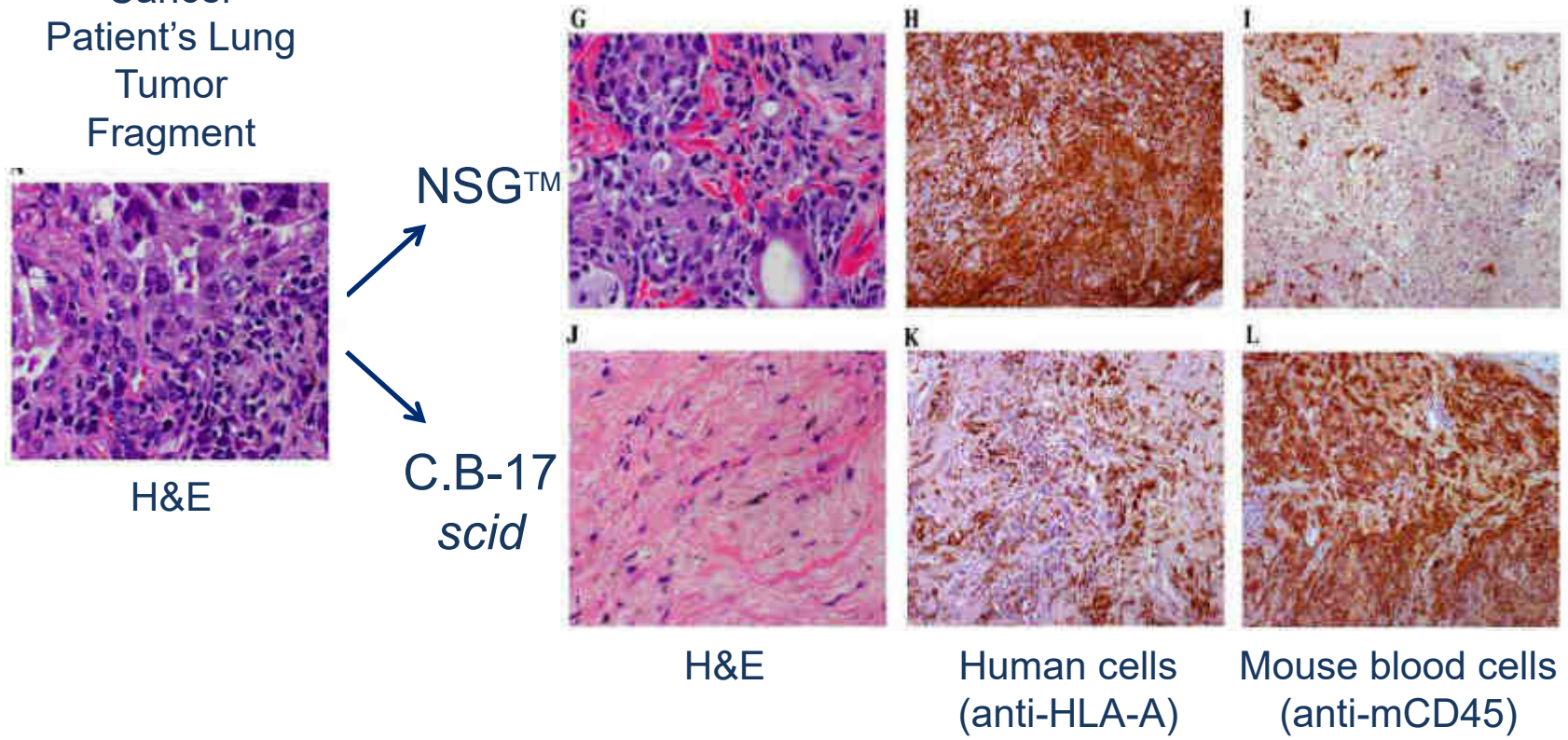
HuCD19 B Cells



HuCD3 T Cells



NSG™ Mice Preserve Patient Tumor Characteristics



Simpson-Abelson MR et al. 2008.*J Immunol* 180(10):7009-18. PMID:18453623

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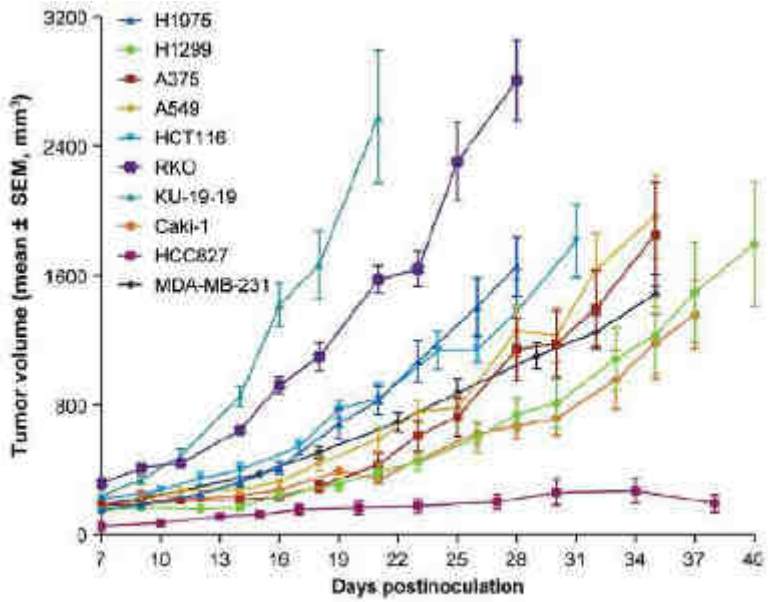
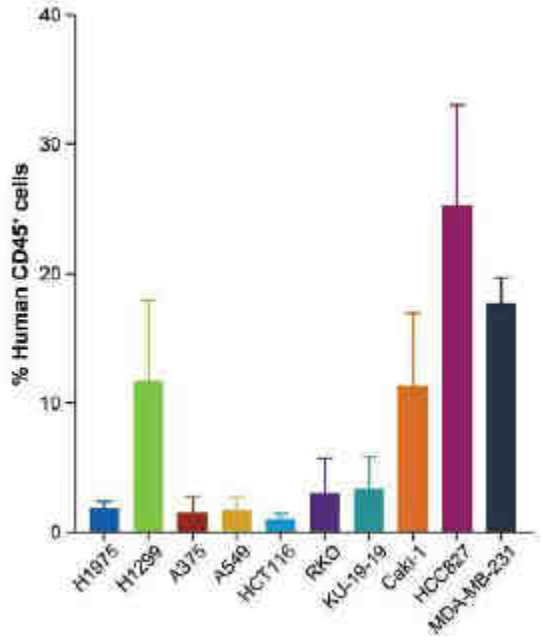
Hu-CD34 NSG™ Support The Growth of a Wide Range of Human Tumors





- Tumor growth & huCD45 infiltration not correlated with degree of HLA match between CB donor and tumor (2-4 donors per tumor)
- Similar to clinical observations, some tumors are intrinsically “cold” (low infiltration) and others are “hot” (high infiltration)

Rios-Doria et al., 2020 *J Immunother Cancer* PMID: 32217760

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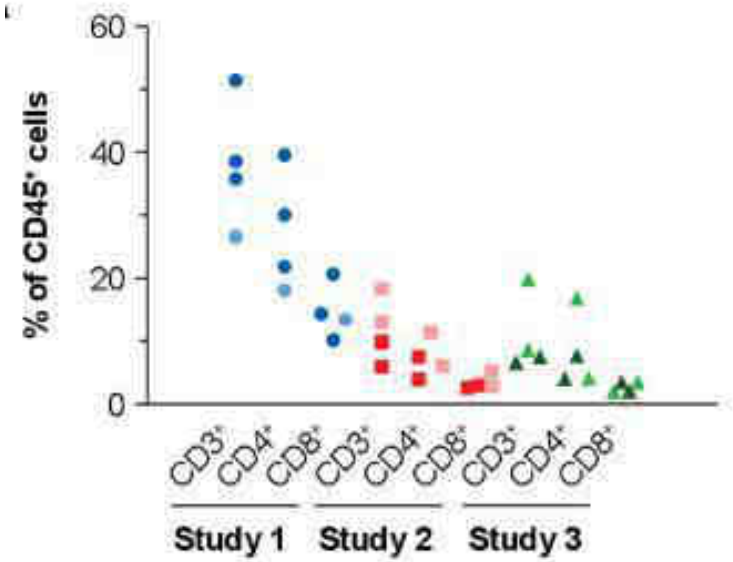
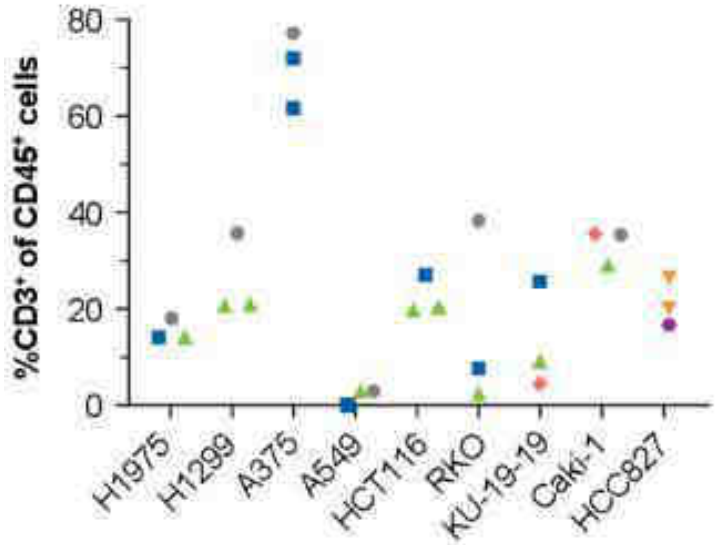
T Cell Infiltration Frequency is Driven by Tumor, Not Donor

MDA-MB-231 (2 Donors/Study)



The donor is represented by color and shape

2-3 Donors/Tumor



- Overall quantity of infiltration can vary between studies, but the frequency of TIL distribution is similar between different donors
- Each tumor type had similar TIL infiltration across different donors

Rios-Doria et al., 2020 *J Immunother Cancer* PMID: 32217760

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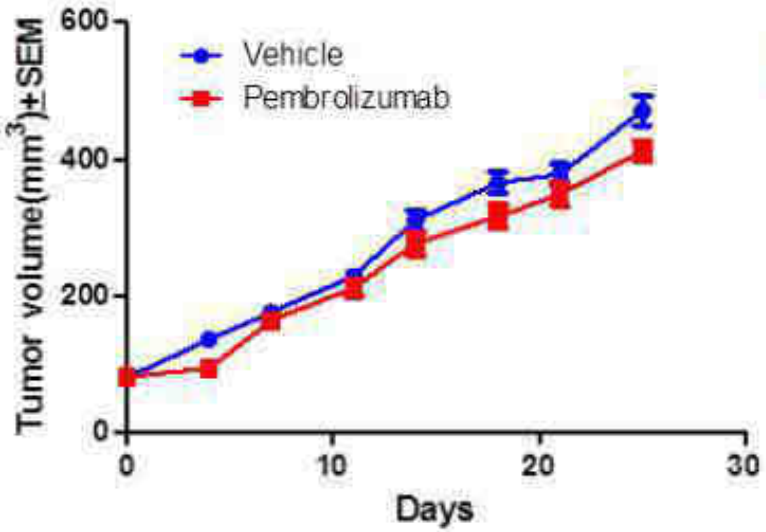
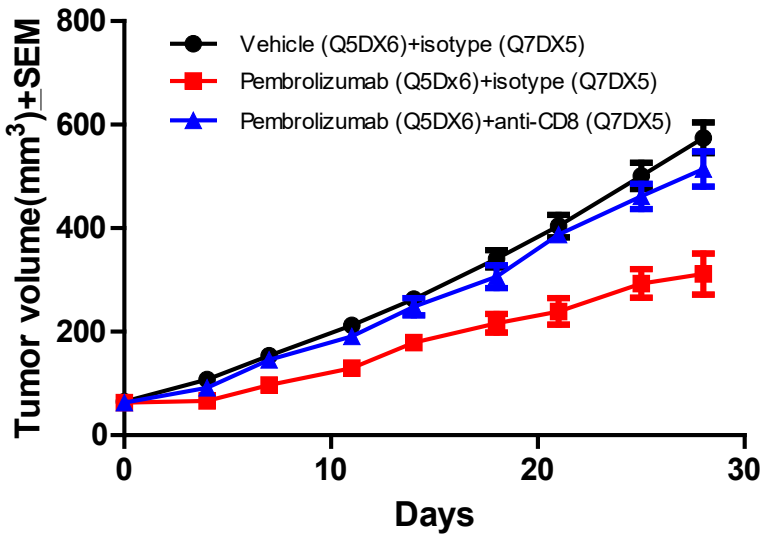
Pembrolizumab Efficacy is CD8+ T Cell Dependent in the Onco-Hu™ Model

MDA-MB-231 Tumor Response in Non-Humanized Mice



MDA-MB-231 Tumor Response in Hu-NSG Mice



- Hu-NSG mice engrafted with MDA-MB-231 TNBC CDX
- Depletion of human CD8+ T cells abrogates anti-PD-1 mAb response

Wang et al., 2017 *FASEB* PMID: 29146734

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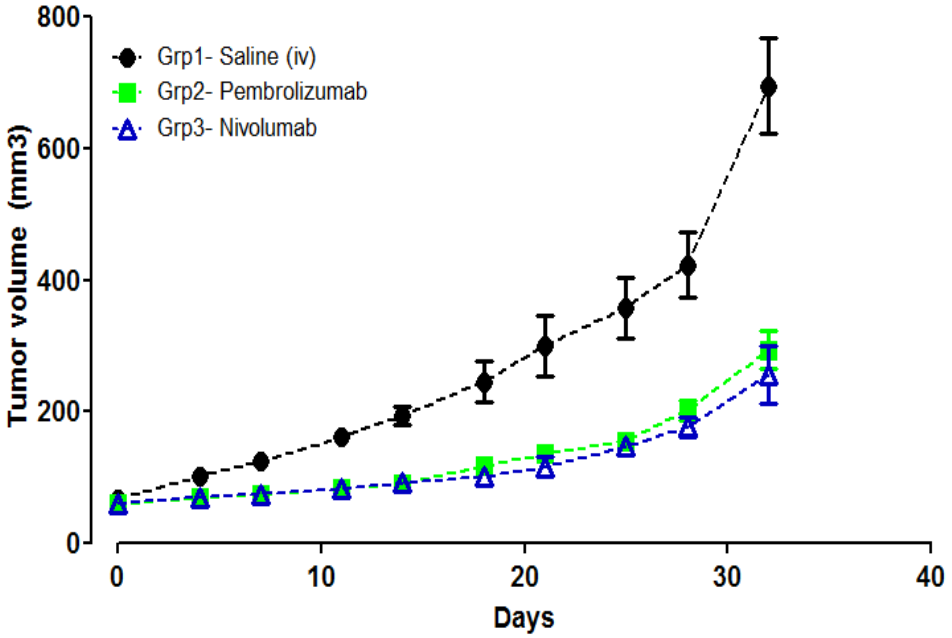
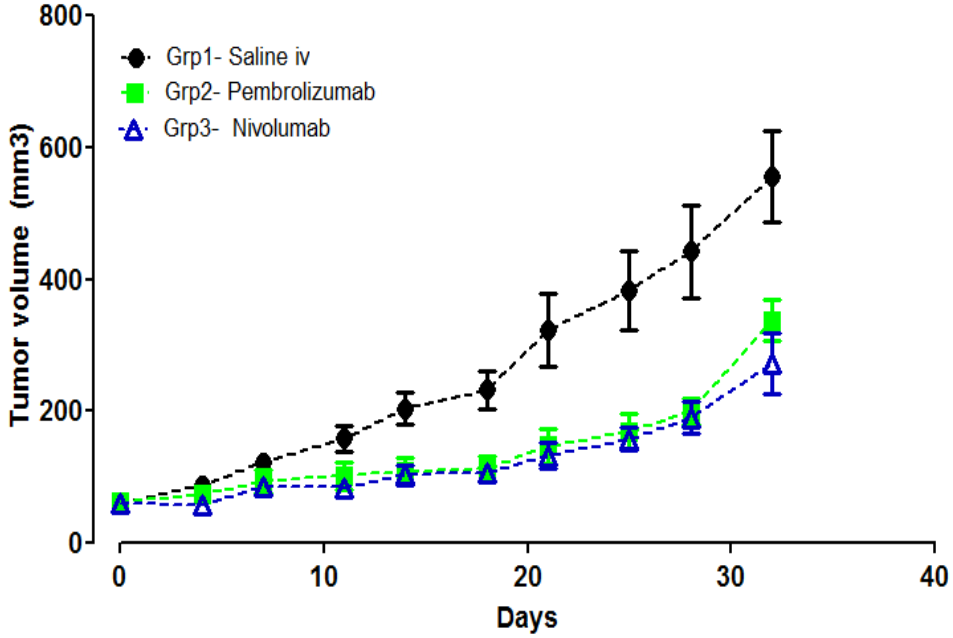
Effects of PD-1 Inhibitors on TNBC (MDA-MB-231) CDX Growth in Hu-NSG™

Mean Tumor Volume (Donor A)



Mean Tumor Volume (Donor B)



- PD-L1 level: 95.1%
- Two donors and two anti-PD-1 inhibitors
- The effect of Pembro and Nivo was similar and the drug treatment reduced tumor growth in both donors

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Considerations to Use an Immunodeficient Strain

- Where and how will you house them?
  - What does the strain need and what can the facility provide?
  - What pieces of equipment might be used and who else shares this research space?

Infectious Disease Concerns in Highly Immunodeficient NSG™ mice

- High level of immunodeficiency results in extreme susceptibility to
  - Pathogens, infectious agents that typically causes disease in immunocompetent host
  - Opportunists, potentially infectious agents that rarely cause disease in immunocompetent hosts
  - Commensals, potentially infectious agents that reside in normal host tissues without causing disease
- Common threats include
  - C bovis, Citrobacter, Enterobacter, Enterococcus spp., Klebsiella spp., Proteus, Pneumocystis murina, Pseudomonas, S. aureus, Coagulase-negative Staphylococcus spp.

Foreman O, et al. 2011. *Vet Pathol*. [PMID: 20817888]

Housing and husbandry conditions may vary depending on the level of immunodeficiency

- Barrier practices adequate to maintain nude, or even *scid* mice may not be adequate for NSG™

- Recommend:
  - PPE, dedicated shoes
  - Sanitize hands before gloving (washing)
  - Disinfect surfaces (laminar flow hoods, experimental equipment, floors, walls)
  - Sterilize tools (forceps, scissors, ear punches, etc.), bedding, food and cages
  - More frequent change cages, use of microisolators/individually ventilated cages (IVCs) with HEPA filters
  - Work under a HEPA ventilated, laminar flow hood
  - Sterilized food and water
  - Monitor for pathogens frequently (opportunistic pathogen testing by fecal, colony or dirty bedding sentinel testing)

https://www.jax.org/jax-mice-and-services/customer-support/technical-support/breeding-and-husbandry-support/special-care

Personal Protective Equipment

- Sterile scrubs, gloves, dedicated shoes and shoe cover
- Face shields, hair/beard bonnet and mask and goggles
- Sterile smock
- PAPR (Powered air purifying respirator)
- Air shower

When to work with your veterinarian

- Non-specific clinical problems, ruffled fur, hunched posture
- Unthriftiness, diarrhea, wasting, sickliness
- Weight loss, weakness, lethargy, reduced mobility
- Acute and/or premature death
- Breeding problems, including:
  - Embryonic death
  - Small litters
  - Small, weak, and/or sickly pups
  - Pup mortality

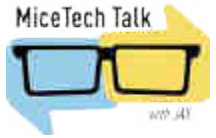
Summary

- Choose a strain that allows the right balance of immunocompetency to immunodeficiency needed to answer your research question
- Both the specific genetic mutations in a strain and the genetic background of the strain contribute to the immunological phenotype
- Housing needs may differ from strain to strain and from facility to facility; work with your facility managers and veterinarians to determine what gives the mice and your research the best chances for success

Upcoming JAX™ Webinars

Subscribe to the monthly webinar announcements email list: https://subscribe.jax.org/

- Introduction to In Vivo Platforms for Cancer Immunotherapy Research
  - Mar 4, 2021, 1:00 PM USA Eastern Time (New York)
- Improving Translational Relevance with Humanized NSG™ Mice
  - Mar 18, 2021, 1:00 PM USA Eastern Time (New York)



MiceTech Talks: 15 minute chat sessions with JAX Technical Information Scientists on mouse-based research topics. Join us on YouTube or LinkedIn. Watch past episodes.

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THANK YOU FOR THE ADVENTURE

At JAX, we enjoy the journey as much as reaching the destination, and we're so happy you joined us.

Authorized JAX™ Mice Distributor in Singapore:

InVivos

Website: www.invivos.com.sg

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JAX™ immuno-deficient strains available from INVIVOS


Outbred Nude


Inbred Nude


BALB/c SCID


NOD SCID


NSG

Common Name	Strain Nomenclature	Web Link to Data Sheet for Equivalent JAX™ Strain
Outbred Nude	Inv:NU	https://www.jax.org/strain/007850
Inbred Nude	NU/JInv	https://www.jax.org/strain/002019
Balb/c SCID	CBySmn.Cg-*Prkdc*^{scid}/J*Inv*	https://www.jax.org/strain/001803
NOD SCID	NOD.Cg-*Prkdc*^{scid}/JInv	https://www.jax.org/strain/001303
NSG	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJInv	https://www.jax.org/strain/005557

Promotional discount of 10% for JAX immuno-deficient strains till 31 Mar 2021

InVivos
BRINGING LIFE TO YOUR RESEARCH

InVivos is an authorized JAX™ Mice Propagator in Singapore