Onco-Hu™ Models: Humanized Mice for Evaluation of Immuno-Oncology Therapeutics

Technical Information Services

June 14, 2018
The Jackson Laboratory’s Mission

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

Biomedical Research
Genetics, genomics and biology of human disease

Resources & Services
PRECLINICAL: Mouse models & research services (mouse breeding, model generation, cryopreservation, *in vivo* pharmacology, humanized mice, and more)

CLINICAL: Clinical genomics services and JAX Clinical Knowledgebase (JAX-CKB)

Education & Training
Courses, conferences, internships, pre-doctoral and post-doctoral training, live webinars, on-demand videos, on-site seminars and other programs
Online Resources to Expedite Research

- **JAX® Mice Database**
  www.jax.org/mouse-search

- **Mouse Genome Informatics**
  www.informatics.jax.org

- **Mouse Phenome Database**
  www.jax.org/phenome

- **Others, including:**
  JAX-Clinical Knowledgebase
  Mouse Tumor Biology Database
JAX® Mice
The Gold Standard for Biomedical Research

- NIH-funded resource
- >9,500 strains and growing
  - >3 million mice shipped annually
- Unsurpassed genetic quality & animal health
- Best characterized & referenced ~100 new pubs/week
- Common inbred strains (C57BL/6J, BALB/cJ, B6.SJL-Ptpca
  Pepcb/BoyJ) support development/collection of specialty strains and other valuable community research resources
Onco-Hu™: Humanized Mice for Evaluation of Immuno-Oncology Therapeutics

- Characteristics of humanized NSG™ and NSG™-SGM3 mice
- Onco-Hu™: Overview
- Onco-Hu™: Efficacy data
Onco-Hu™: Humanized Mice for Evaluation of Immuno-Oncology Therapeutics

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What Makes NSG™ Such a Good Host?

NSG™, NOD scid gamma

NOD background contributes:
- Absence of hemolytic complement
- Reduced dendritic cell function
- Defective macrophages
- Optimal human hematopoietic stem cell engraftment (Sirpa allele)

Scid mutation prevents development of mature T and B cells

Il2rg deficiency
- Eliminates signaling from 6 distinct interleukins (IL-2, IL-4, IL-7, IL-9, IL-15, & IL-21)
- Blocks NK cell development

Considerations:
scid side effects: radiation sensitivity; genotoxic drugs may have higher toxicity
NSG™ vs. NSG™-SGM3 Mice

**NOD scid gamma (NSG™)**

NOD.Cg-Prkdc\textsuperscript{scid} Il2rg\textsuperscript{tm1Wjl/SzJ} (005557)
- The current gold standard for reconstitution of the human immune system
- Excellent functional T cell responses
- Limited human myeloid lineage development

**NSG™-SGM3**

NOD.Cg-Prkdc\textsuperscript{scid} Il2rg\textsuperscript{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ (013062)
- Promotes improved AML engraftment efficiency
- Improves normal human myeloid cell development after HSC transplantation

Experimental Timeline for Humanization

Human cord blood derived CD34+ cells engrafted in female mice

- Whole body irradiation
- Tail vein injection
- NSG: 3 weeks
- NSG-SGM3: 4 weeks
- B cells mature ahead of T cells
- Myeloid cells peak at 15 weeks in NSG-SGM3
- Validation of Engraftment by FACS
- 16 weeks
- 12 weeks
- Mature T cell development
- ~18 weeks
- ~18 weeks

In Vivo Pharmacology Services
Humanization of NSG™ vs. NSG™-SGM3

Direct Comparison Experimental Design

- **Mice**
  - Female NSG™, 3 weeks of age, 140 cGy (N=20)
  - Female NSG™-SGM3, 4 weeks of age, 100 cGy (N=9)

- **Donor Cells**
  - Hu CD34+ HSC, ~130,000 cells/mouse
  - All mice transplanted with cells from the same donor

- **Blood Collection**
  - PBL collected 4, 6, 9, 12, 15 & 18 weeks post Tx
  - Flow for huCD45, huCD33, huCD19, huCD3, huCD4, huCD8, huT<sub>reg</sub>
Human Immune Cells in Peripheral Blood of Hu-NSG™ vs. Hu-NSG™-SGM3: Absolute Counts

- Greater total cell numbers of huCD45 in NSG™-SGM3
- Greater numbers of myeloid cells in NSG™-SGM3
- Earlier B cell development and higher numbers of T cells in NSG™-SGM3

**Total Human Donor (cells/µl)**

**HuCD33 Myeloid Cells (cells/µl)**

**HuCD19 B Cells (cells/µl)**

**HuCD3 T Cells (cells/µl)**
Human Immune Cells in Peripheral Blood of Hu-NSG™ vs. Hu-NSG™-SGM3: Absolute Counts

- Greater numbers of huCD3 T cells in NSG™-SGM3
- Greater expansion of huCD4 T cells in NSG™-SGM3
- Greater expansion of huCD8 T cells in NSG™-SGM3
Onco-Hu™: Humanized Mice for Evaluation of Immuno-Oncology Therapeutics

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Hu-NSG™ and PDX Capabilities

- Hu-NSG™ Portfolio
  - CD34+
  - NSG™, NSG™-SGM3 and other cytokine expressing NSG™ derivatives

- PDX Experience
  - Over 400 PDX tumors all P5 or earlier
  - PDX Live

- Access options
  - Delivery of models – humanized mice with or without tumors
    - CD34+ engrafted mice are “off-the-shelf” ready
  - Execution of studies
JAX® PDX Library
Wide Selection of Genetically Unique Tumors

- Soft Tissue - 3%
- Bladder - 7%
- Brain - 10%
- Bone - 3%
- Breast - 7%
- Colon - 16%
- Head - 1%
- Kidney - 3%
- Lung - 22%
- Misc. - 9%
- Ovary - 4%
- Pancreas - 7%
- Prostate - 1%
- Rectum - 2%
- Skin - 5%

>400 clinically relevant PDX tumors
Lung Adenocarcinoma EGFR L858R: Acquired TKI resistance
Lung Adenocarcinoma EGFR L858R: PDX tumor drug response in NSG™
Onco-Hu™ Are Humanized Tumor-Bearing NSG™ and NSG™-SGM3 Mice:
The next step in cancer modeling

NSG™ (005557)
NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ

NSG™-SGM3 (013062)
NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>
Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ

Onco-Hu™ (Humanized with Tumor)
(When tumors reach 70-120 mm<sup>3</sup> mice are treated with therapeutics for 21 to 28 days)
Humanized Mice Evaluate Immune Cell Modulation, Recognition is Likely Allogeneic

Yervoy (Ipilimumab); anti-CTLA-4

Keytruda (Pembrolizumab); anti-PD-1

- Pembrolizumab (Keytruda)
  - Anti-PD1 mAb
- Ipilimumab (Yervoy)
  - Anti-CTLA-4 mAb
- ImaginAb, Inc.
  - Anti-OX40 mAb

Many other immuno-oncology targets may also prove efficacious (and in combination), but we need a humanized platform for pre-clinical analysis.
Immuno-Oncology Drug Development: PD-1 Signaling Blockade

1992 Identification of PD-1
1999 Discovery of PD-L1
2000 PD-L1 interacts with PD-1
2001 PD-L1 Ab enhances T cell proliferation
2002 PD-1 and ligand regulate tumor immunity
2005 PD-1 and PD-L1 mAbs treat animal tumors
2010 Phase I results for PD-1 blockade
2014 Pembrolizumab and Nivolumab approved for melanoma
2015 Nivolumab approved for non-small cell lung cancer

Efficacy shown in 9 Organ systems:
- Head and Neck
- Skin
- Lymph node
- Breast
- Lung
- Liver
- Kidney
- Bladder
- Ovary

Ongoing, more than 150 Clinical trials

Trial breakdown:
- Combination therapies: ~13%
- Ipilimumab: An anti-CTLA-4 inhibitor
- FDA approved in 2011

Humanization of NSG™ Mice Has No Significant Impact on PDX Growth Kinetics

No difference between NSG™ and Hu-NSG™ mice on tumor growth curve

- No HLA match testing performed
- Fresh tumor tissue engraftment
- 100% take rate in NSG™ or Hu-NSG™ mice
- HuCD45+ more than 20%

Wang et al., 2017 FASEB PMID: 29146734
Onco-Hu™: Humanized Mice for Evaluation of Immuno-Oncology Therapeutics

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Hu-NSG™ MDA-MB-231 Mice: Suppression of Breast Tumor Growth by Pembrolizumab

- MDA-MB-231 (TNBC) cell surface expression of PD-L1: 49.2%
- Increased infiltration of CD8+ T cells post treatment

**Mean Tumor Volume of MDA-MB-231 Model in Hu-NSG Mice**

**P**=0.003; **P**=0.0005 & ****P<0.0001; compared to vehicle group, Two-tailed unpaired t test

- Vehicle (Saline) ip Q5Dx6
- Pembrolizumab (10–5mg/kg) ip Q5Dx6

Days (Day 0 = treatment initiation)

**Vehicle**

**Pembrolizumab**
Hu-NSG™ MDA-MB-231 Mice: Characterization of Human Tumor Infiltrating Cells & PD-1 Levels

- Pembrolizumab treatment:
  - Does not increase human cell percentages in peripheral blood
  - Does not typically lead to greater tumor infiltration

- Pembrolizumab is targeting human immune cells in the tumor and blocks binding of anti-PD-1 FACS reagent

- % HuCD45 Cells in Blood

- % HuCD45 Cells in Tumor

- % PD-1 on HuCD45 Cells in Tumor
Hu-NSG™ MDA-MB-231 Mice: Efficacy Of Pembrolizumab Is CD8⁺ T Cell Dependent

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

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

Wang et al., 2017  FASEB PMID: 29146734
Hu-NSG™-SGM3 MDA-MB-231 Mice: Suppression of Breast Tumor Growth by Pembrolizumab

- Engrafted with 5x10^6 cells/mouse s.c. with matrigel
- MDA-MB-231 cell surface expression of PD-L1: 95.1%

In Vivo Pharmacology Services | THE JACKSON LABORATORY
**Hu-NSG™ BR1126 TNBC PDX Mice:** Pembrolizumab Inhibits Tumor Growth

- Pembrolizumab (Keytruda); anti-PD-1 mAb
- Tumor PD-L1 surface expression: 56.9%
- Hu-CD45 engraftment in Hu-NSG™ >25%

### Mean Tumor Volume of TM00098 (BR1126P5) PDX in Hu-NSG Mice

* P=0.0127; Compared to Vehicle group. Two-tailed unpaired t test.

- Vehicle (Saline) ip Q5Dx4
- Pembrolizumab (10-5mg/kg) ip Q5Dx4

<table>
<thead>
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<th>HLA match</th>
<th>CD34+HPC donor</th>
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<tbody>
<tr>
<td>Tumor</td>
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</tr>
<tr>
<td>BR1126</td>
<td>HLA-C, DPA1</td>
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</tbody>
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**In Vivo Pharmacology Services** | **THE JACKSON LABORATORY**
Hu-NSG™ LG1208 NSCLC Lung PDX Mice:
No Inhibition of Tumor Growth By Pembrolizumab

<table>
<thead>
<tr>
<th>HLA match</th>
<th>CD34+ HPC Donor</th>
<th>LG1208 HLA-DRB4, DPA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>CD34+ HPC Donor</td>
<td>LG1208 HLA-DRB4, DPA1</td>
</tr>
</tbody>
</table>

Tumor volume of LG1208 PDX in Hu-NSG Mice

- Vehicle
- Pembrolizumab

(days 0 = treatment initiation)
Hu-NSG™ LG1208 NSCLC Lung PDX Mice: PD-1 and PD-L1 Expression in Tumor Tissue

Pembrolizumab entered tumors and bound to CD45+ PD-1+ leukocytes

- PD-1 Expression: Activated T cells, Tregs, B cells, NK cells and monocytes; occasionally on tumor cells
- PD-L1 Expression: Mainly on tumor cells and normal tissues; also on T cell, B cells, macrophages, DCs
**Hu-NSG™-SGM3 BR1126 TNBC PDX Mice:** Pembrolizumab & Doxorubicin Inhibit Tumor Growth

- **HuCD45+ in whole blood:** 50-88%
- **HuCD3+/HuCD45:** average 34%
- **BR1126 PD-L1 surface expression:** 56.9%

![Graph showing mean tumor volume of BR1126P4 PDX in HuCD34-SGM3 Mice](image)

**Mean Tumor Volume of BR1126P4 PDX in HuCD34-SGM3 Mice**

* & ** P<0.05; Compared to Vehicle group. One-way ANOVA followed Dunnett’s Multiple Comparison test

- **Vehicle (Saline) ip Q5Dx5**
- **Doxorubicin (2mg/kg) iv Q7Dx2**
- **Pembrolizumab (5mg/kg) ip Q5Dx5**

**In Vivo Pharmacology Services** | THE JACKSON LABORATORY
Hu-NSG™-SGM3 LG1306 Lung PDX Mice: Pembrolizumab & Ipilimumab Inhibit Tumor Growth

- HuCD45+ in whole blood: 36-81%
- HuCD3+/HuCD45: average 14.3%
- LG1306 PD-L1 surface expression: 89.1%
OX40 in Anti-Tumor Immunity

CD4+ T Cells
- CD4+ T cell engages tumor peptide through MHC II, OX40 upregulated
- OX40-specific mAb binds and induces an activation signal
- Cytokine production

CD8+ T Cells
- Cytokine production by CD4 cell provides “help” to CD8+ CTL
- CD8 CTL produces perforin and granzyme, killing tumor

Hu-NSG™ MDA-MB-231 Mice: Suppression of Breast Tumor Growth by Anti-OX40

Jean Gudas
VP, Research & Development
ImaginAb, Inc.

Two Donors (#5038, #5040)

Mean Tumor Volume (mm$^3$)

Study Days

Group 1 (Vehicle)

Group 3 (anti-OX40)

*p < 0.05

**p < 0.01

***p < 0.005

****p < 0.0001

2-tailed unpaired t-test
ADCC & ADCP Mediated Depletion of Treg’s by mDTA-1 (anti-GITR mAb)

Mouse Syngeneic Tumor Studies

DTA-1 (mouse anti-GITR mAb) Decreased Suppression of CTL’s Tumor Cell Killing

Adapted from: Targeting regulatory T cells in tumor immunotherapy
Hu-NSG™ SK-MEL-5 Mice: Suppression of Human Melanoma by MK-4166 (anti-GITR mAb)

Depletion of Treg’s in Tumor

Suppression of Tumor Growth

Increased Effector Cytokines in Tumor

Mahne et al., 2016  Cancer Res  PMID: 28122327
Immuno-Oncology Summary

- NSG™ and NSG™-SGM3 are a proven platform for engraftment of the human immune system

- PDX growth is not grossly effected by HLA-type matching
  - Tumor growth kinetics are similar in humanized and non-humanized hosts
  - ~15% of PDX tumors fail to grow in humanized mice

- Human immune effector cells infiltrate human tumors in both Hu-CD34-NSG™ and Hu-CD34-SGM3
  - CD4 and CD8 T cells and CD19 B cells

- Hu-CD34-NSG™ and Hu-CD34-SGM3™ PDX respond to anti-tumor agents; anti-PD-1, anti-CTLA4, anti-OX40, and anti-GITR
Acknowledgements

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  - James Keck, Minan Wang, Li-Chin Yao, Mingshan Cheng and Danying Cai

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  - Karolina Palucka

- **JAX – Mammalian Genetics**
  - Lenny Shultz, Rick Huntress, Carol Bult, Susie Airhart and Ed Liu

- **UMASS**
  - Dale Greiner and Mike Brehm
JAX® Mice & Services: Leading Experts in Mouse Modeling

- CRISPR/Cas9 Mouse Model Generation
- Common Inbred and Specialty JAX® Mice
- Aged C57BL/6J Mice (25-78 wks)
- Inbred, Outbred and B6J Nude Mice
- Syngeneic Mice Studies for Cancer Research (i.e., Allograft Mouse Tumor Studies)
- Mouse Genome Scanning
- NSG™ & NRG Mouse Model Variants
- Mouse Cryopreservation and Recovery
- Basic and Complex Mouse Breeding, Speed Congenics, and Rederivation
- Humanized Mice, Patient-Derived Xenograft Preclinical Models and Therapeutic Drug Evaluation
- Neurobiology Models and Resources
Upcoming JAX Webinars™

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

- Aged B6 Mice: An Essential Tool for Preclinical Discovery in Human Disease
  - June 21, 2018, 1:00 pm ET; 5:00 pm GMT

- Accelerating Immunotherapy Development with Onco-Hu™ Mice and Flow Cytometry *(jointly presented by The Jackson Laboratory and BD Biosciences)*
  - June 28, 2018, 1:00 pm ET; 5:00 pm GMT

- Differences among B6 Substrains and the Research Impact
  - July 12, 2018, 1:00 pm ET; 5:00 pm GMT

- Advanced Cre-lox: Generating Reporters, Inducible Mice, and Disease Models
  - July 19, 2018, 1:00 pm ET; 5:00 pm GMT

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