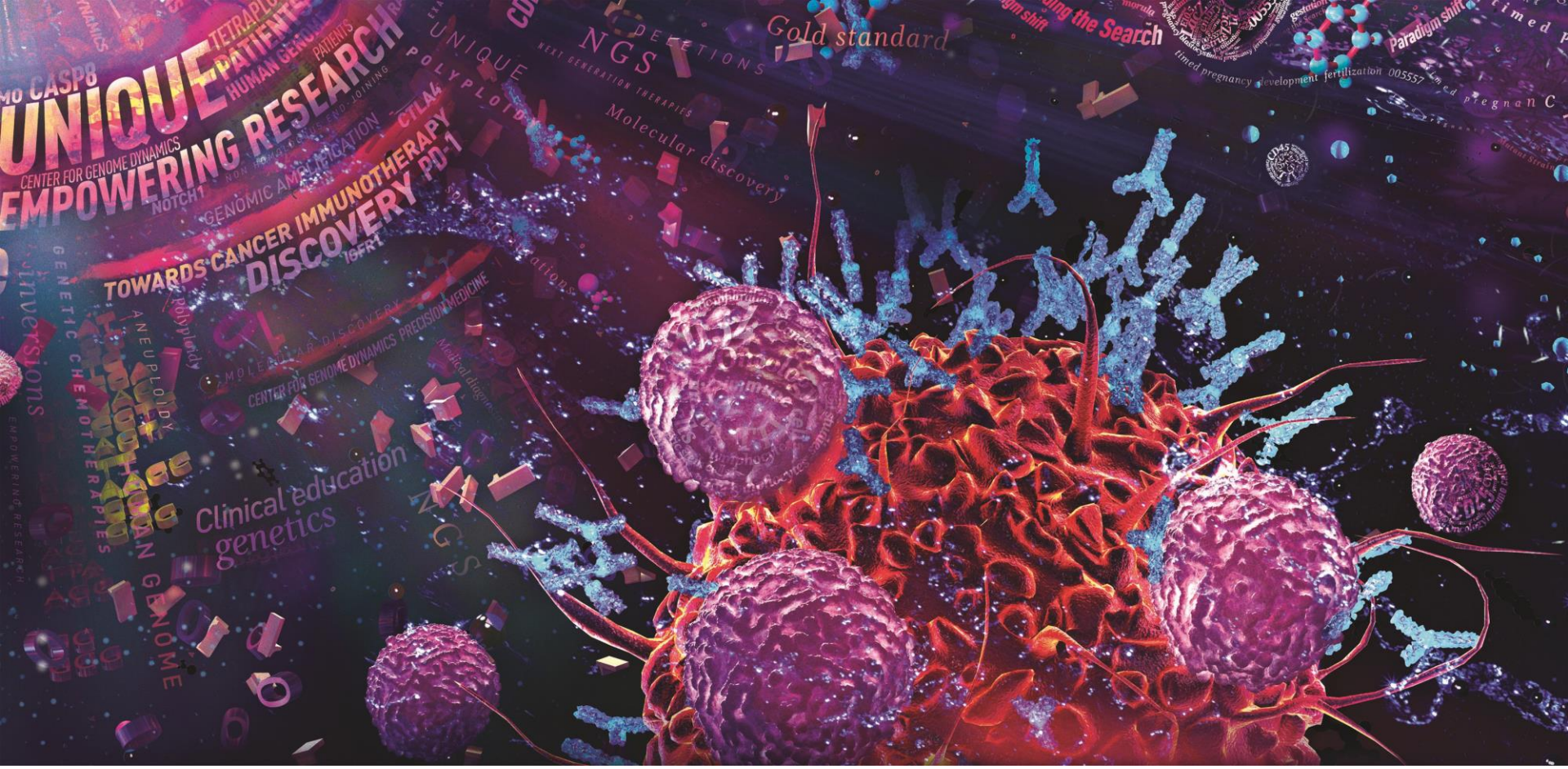


# ONCO-HU™ MICE FOR IMMUNOTHERAPEUTIC DRUG DISCOVERY

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*Senior Technical Information Scientist*

The Jackson Laboratory

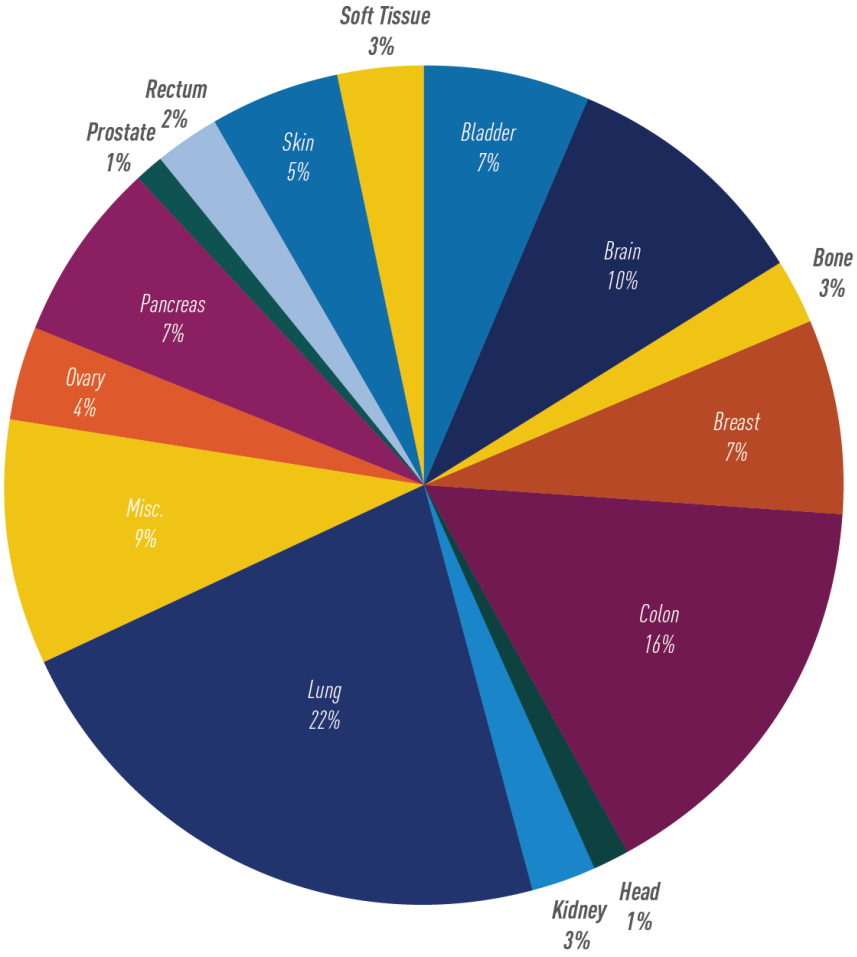
# Presentation Outline

- Capabilities
- Humanization
  - Hu-NSG™ versus Hu-NSG™-SGM3
- Immuno-oncology responses in Onco-Hu™
  - Anti-PD1; Pembrolizumab (Keytruda)
  - Anti-DLL4; Demcizumab

# Onco-Hu™:
## Humanized Mouse & PDX Capabilities

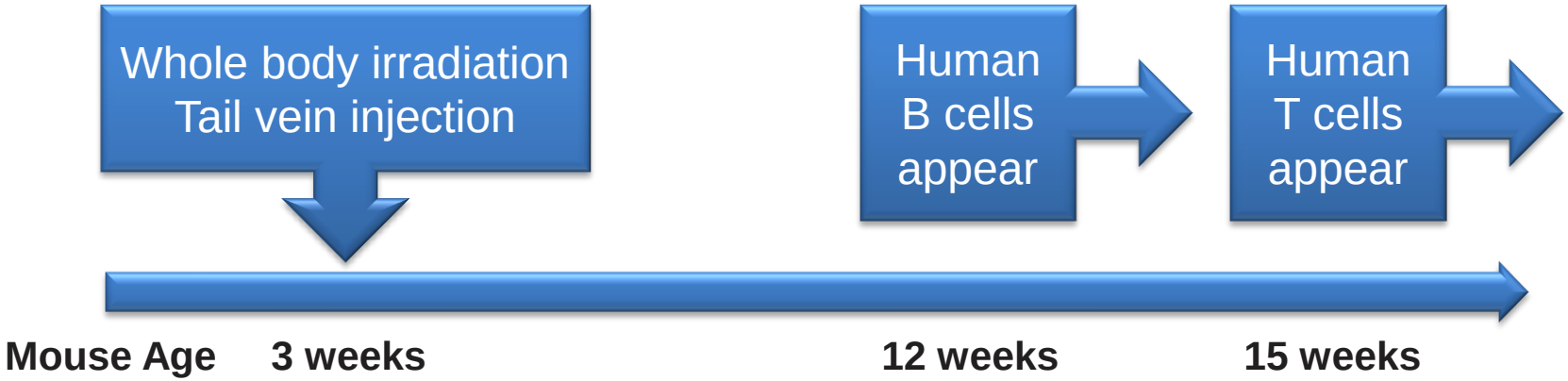
- **Humanized Mouse Portfolio**
  - **CD34+ and custom stem cells**
  - **NSG, NSG-SGM3 and other NSG derivatives expressing human cytokines or HLA molecules**
- **PDX Experience**
  - **Over 400 PDX tumors all P5 or earlier**
  - **PDX Live**
- **Access options**
  - **Delivery of models – humanized mice with or without tumors**
    - **CD34+ engrafted mice are "off-the-shelf" ready**
  - **Execution of studies**

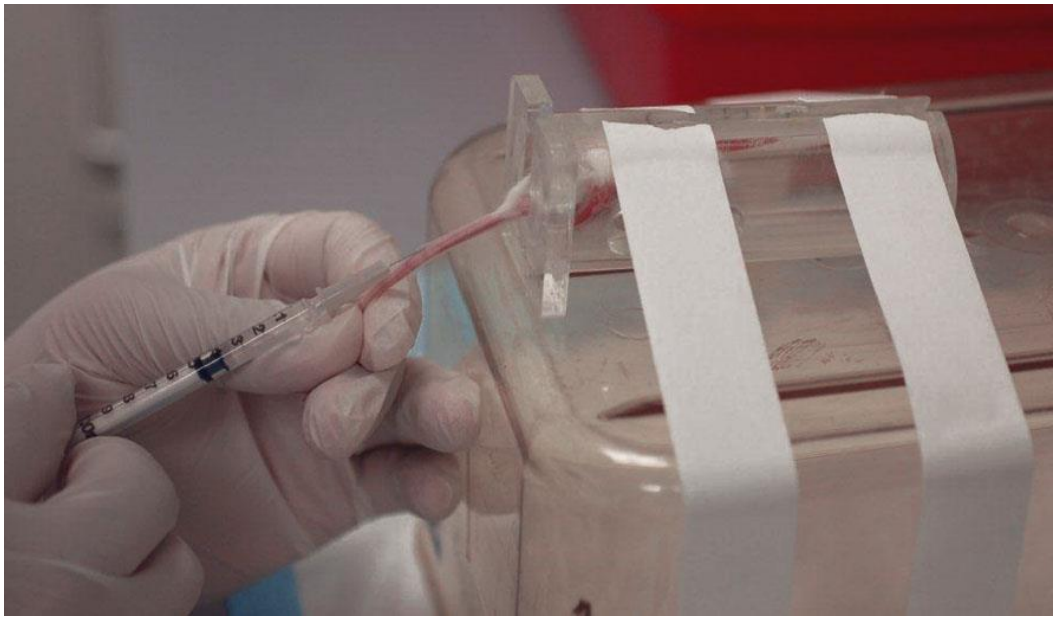
# PDX Models Established




**>400 clinically relevant PDX tumors, with orthotopic engraftment capabilities**

# Creating Humanized Mice: Timeline



# NSG™ vs NSG™-SGM3

## NOD scid gamma (NSG™)

NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (005557)

- **Highly immunodeficient**
- **The current gold standard for reconstitution of the human immune system**
- **Limited human myeloid lineage development etc.**

## NSG™-SGM3

NSG-Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ (013062)

- **Improves normal human myeloid cell development after HSC transplantation**
- **Promotes improved AML engraftment efficiency**

# Reconstituting the Human Immune System in NSG™ vs NSG™-SGM3

## Experimental Design

**Mice:**

- Female
- NSG: 3-week old, n=20
- NSG-SGM3: 4-week old, n=9

**Irradiation:**

- NSG: 140 cGy
- NSG-SGM3: 100 cGy

**HuCD34+ HSC:**

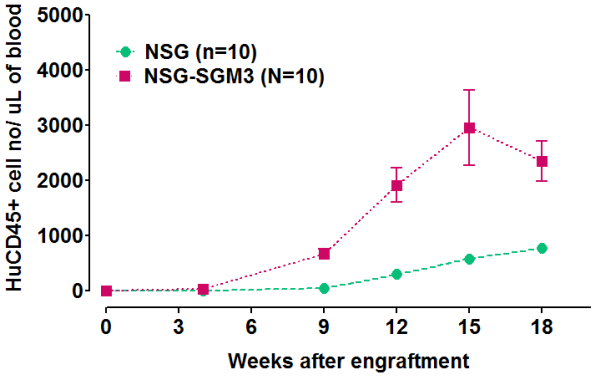
- ~130,000 cells/mouse from the same donor

**Blood collection:**

- 4, 6, 9, 12, 15 & 18 weeks post engraftment to check major human leukocyte lineages
- flow panels: hCD45, hCD33, hCD19, hCD3, hCD4, hCD8, hTreg

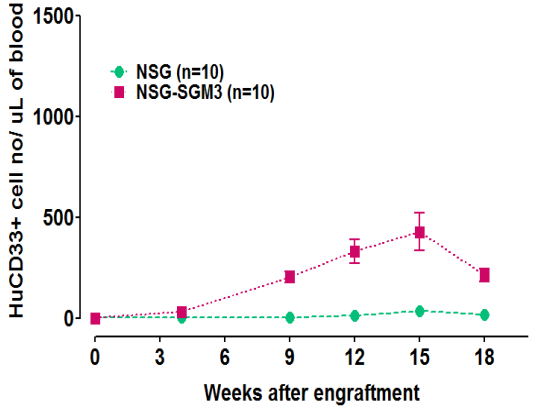
# Human Immune Cells in Peripheral Blood of NSG™ vs. NSG™-SGM3: Absolute Counts

Total Human Donor (cells/µl)

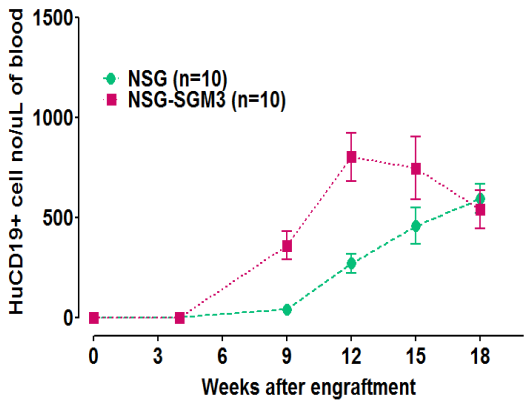



- Greater total cell numbers of huCD45 in NSG-SGM3
- Greater numbers of myeloid cells in NSG-SGM3
- Equivalent B cells, but higher numbers of T cells in NSG-SGM3

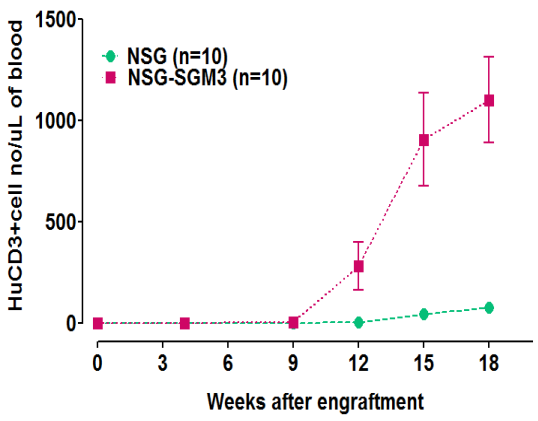
HuCD33 Myeloid Cells (cells/µl)




HuCD19 B Cells (cells/µl)

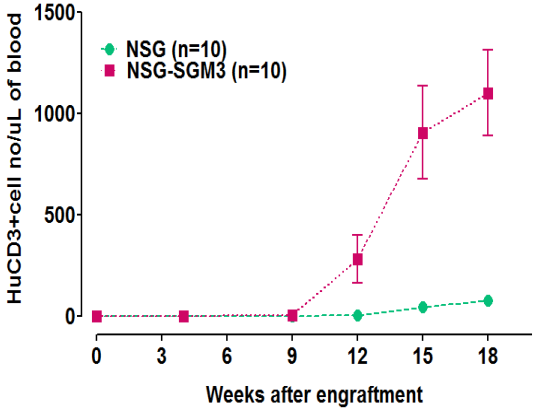



HuCD3 T Cells (cells/µl)



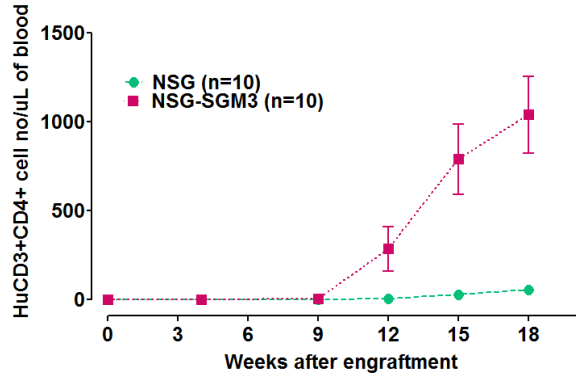
# Human Immune Cells in Peripheral Blood of NSG™ vs. NSG™-SGM3: Absolute Counts

HuCD3 T Cells (cells/µl)

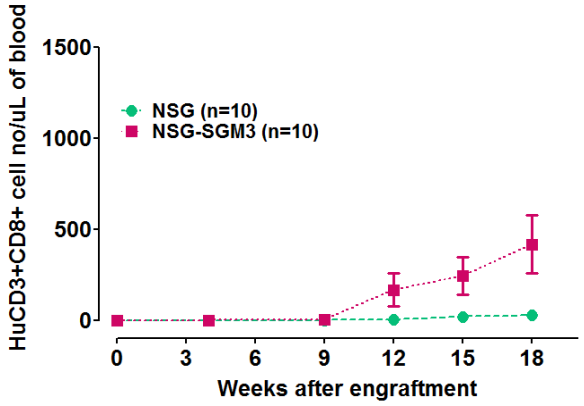



- Greater numbers of huCD3 T cells in NSG-SGM3
- Greater expansion of huCD4 T cells in NSG-SGM3 (including regulatory T cells)
- Greater expansion of huCD8 T cells in NSG-SGM3

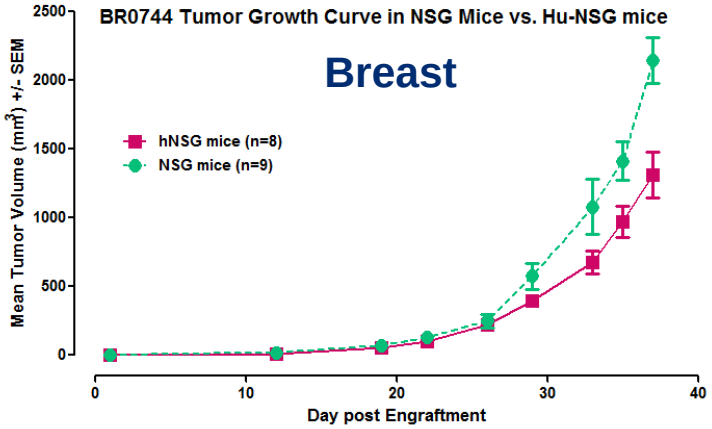
HuCD4 Helper T Cells (cells/µl)

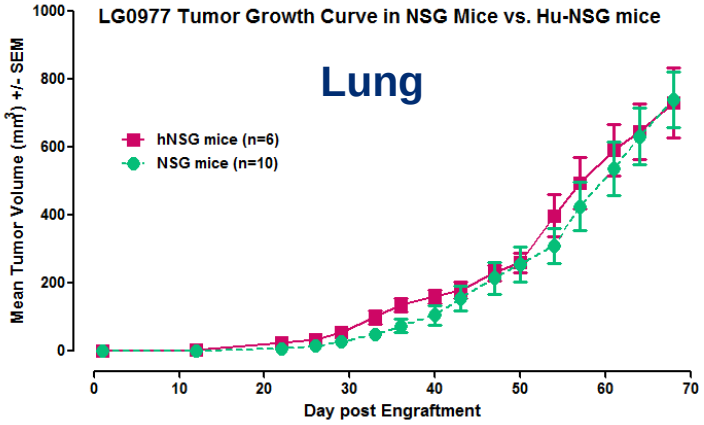


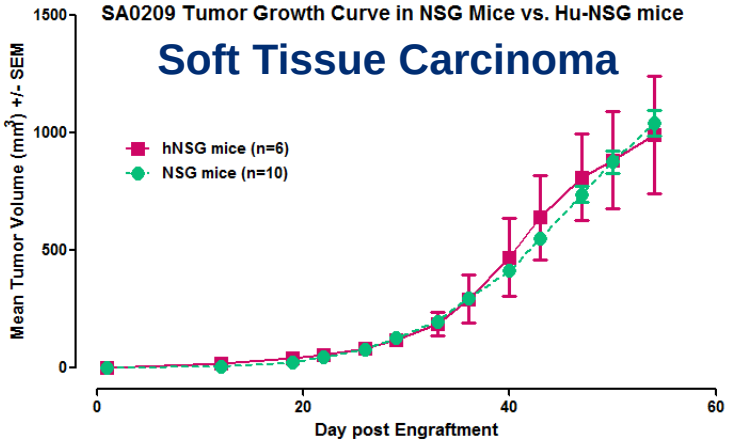

HuCD8 Cytotoxic T Cells (cells/µl)



# Hu-NSG™ Mice: Minimal Impact on PDX Growth Kinetics



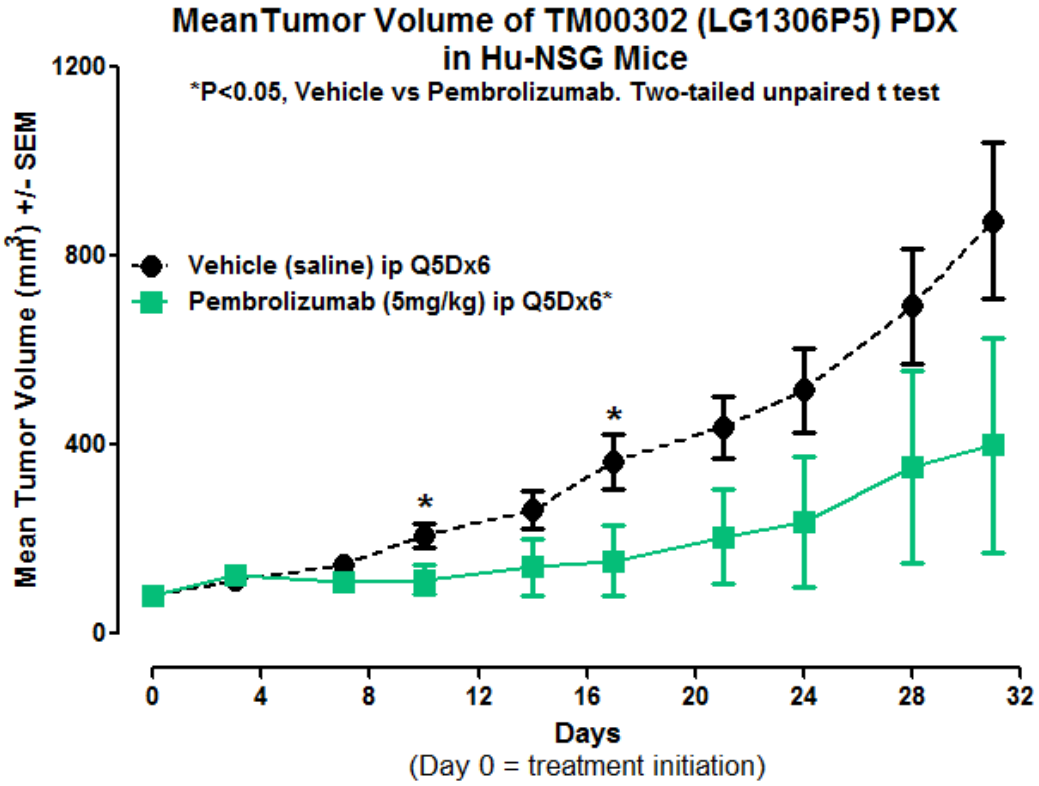




- No HLA match testing performed
- 100% take rate in NSG™ or Hu-NSG™ mice
  - ~15% PDX tumor rejection
  - ~85% PDX/ cell line tumor growth
  - ~70% of these we have statistically significant tumor growth reduction with anti-PD1 (Keytruda) treatment

# Employing Humanized Mice for Testing of Immune Modulators that Target Human Tumors

- Three week old NSG or NSG-SGM3 mice are engrafted through the tail vein with purified human CD34+ HSCs
- Twelve weeks later the circulating human CD45+ cell population is quantitated and confirmed to be at least 25% of the cell population
- Human PDX tumors or human cell lines expressing PD-L1 are then engrafted subcutaneously into the mice
- When tumors reach 70-90 $mm^3$ mice are grouped and treated with therapeutics including Pembrolizumab for 21 to 28 days

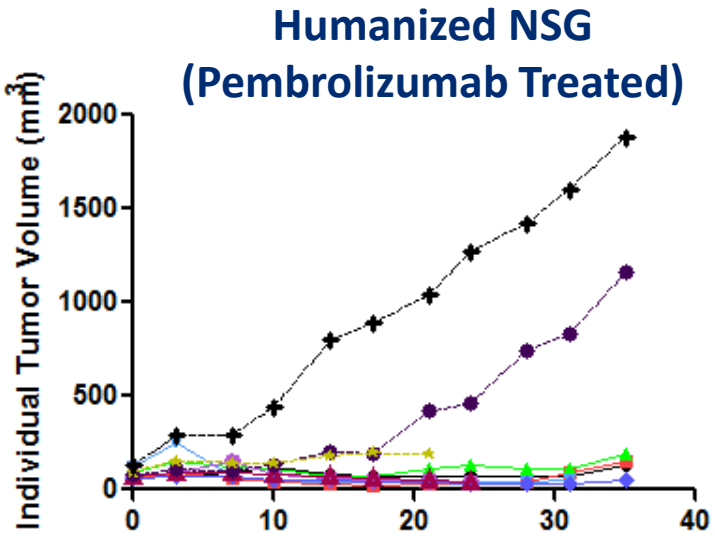
# Hu-NSG™ Mice: Efficacy Results of Pembrolizumab (Keytruda) on Lung (LG1306) PDX Tumors

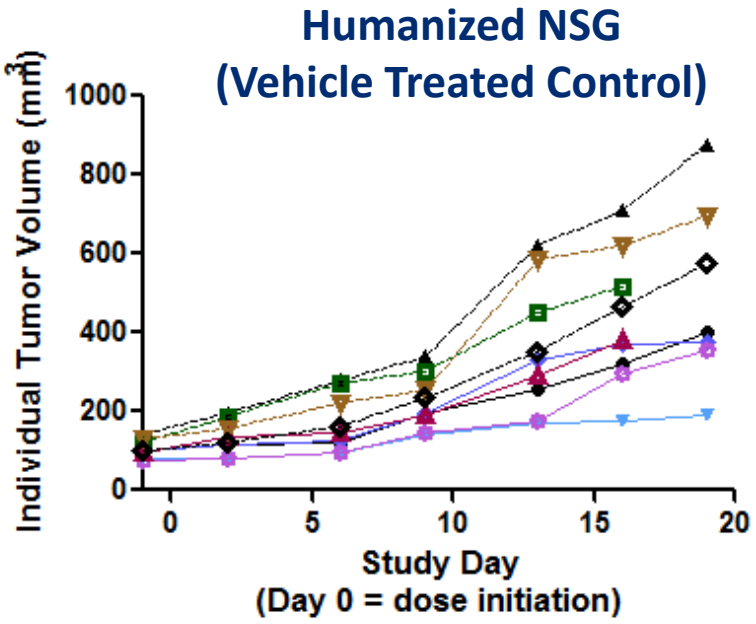



- Fresh tumor tissue engraftment
- HuCD45+ more than 20%
- LG1306 PD-L1 surface expression: 89.1%

| HLA match | CD34+HPC donor | |
|---|---|---|
| Tumor | 1 | 2 |
| LG1306 | HLA-DRB4, DQA1, DQB1 | No match |

# Hu-NSG™ Mice: Efficacy Results of Pembrolizumab (Keytruda) on Lung (LG1306) PDX Tumors



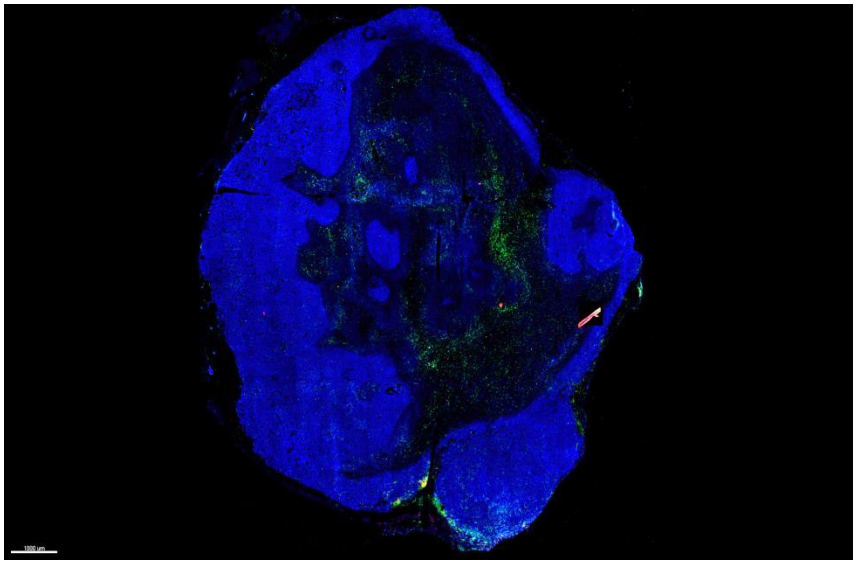




- PDX have highly variable growth
- Two non-responders, each from a different donor
  - Fresh tumor tissue engraftment
  - HuCD45+ more than 20%
  - LG1306 PD-L1 surface expression: 89.1%

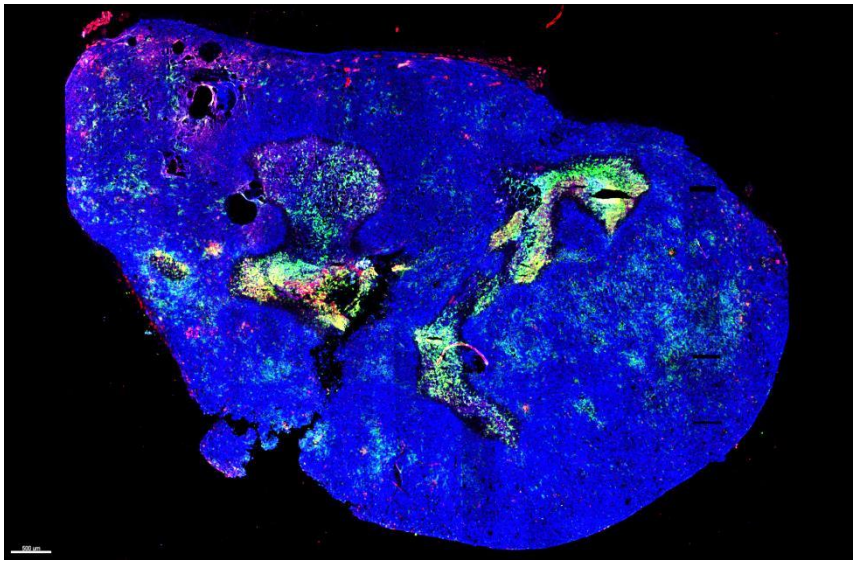
| HLA match | CD34+HPC donor | |
|---|---|---|
| Tumor | 1 | 2 |
| LG1306 | HLA-DRB4, DQA1, DQB1 | No match |

# Immune Cell Infiltration of Onco-Hu™: Hu-NSG™ Bearing Lung (LG1306) PDX

**Vehicle**



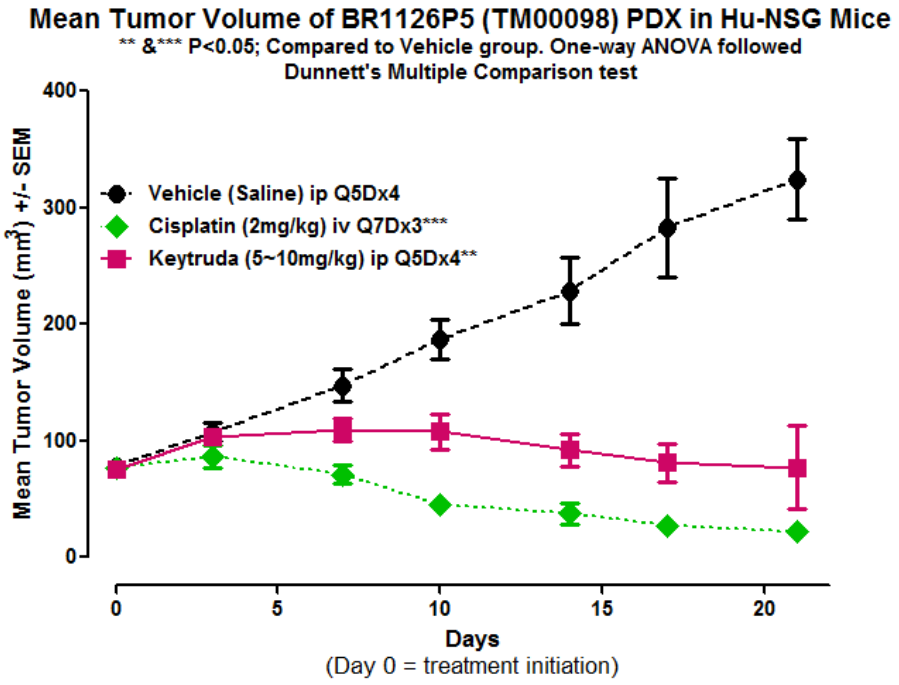
**Pembrolizumab**



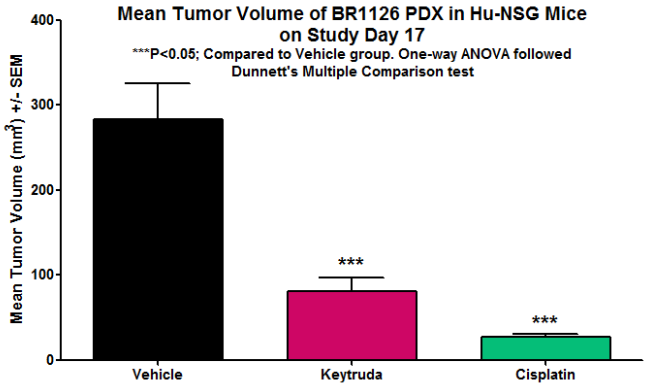
**CD45 CD8 Cytokeratin**

**Pembrolizumab-treated mice displayed increased total immune cell and CD8⁺ T lymphocyte tumor infiltration compared to vehicle-treated mice**

# Hu-NSG™ Mice: Pembrolizumab and Cisplatin Inhibit Growth of Breast (BR1126) PDX

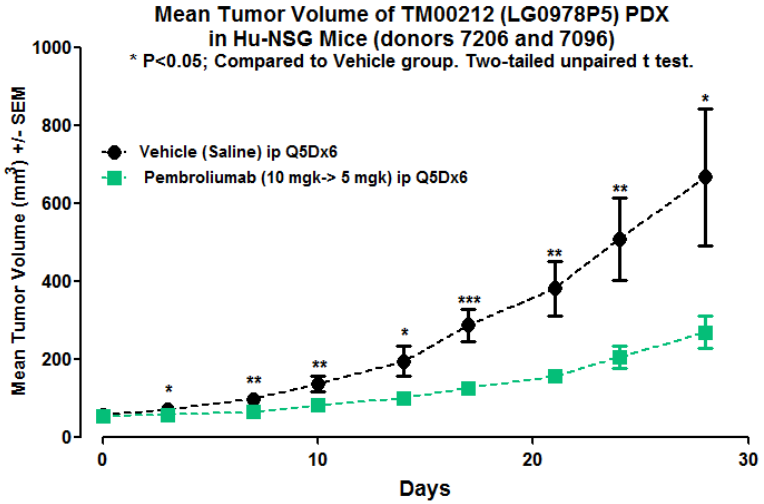


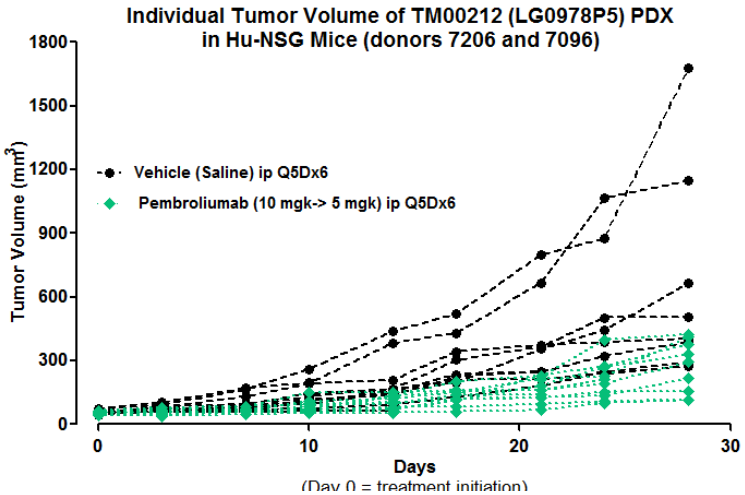

- Fresh tumor tissue engraftment
- HuCD45+ in Hu-NSG mice: >25%
- BR1126 PD-L1 surface expression: 56.9%

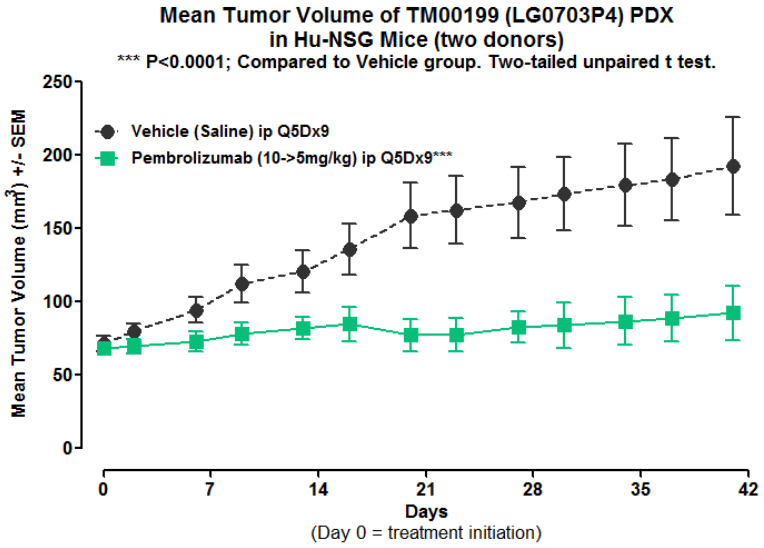


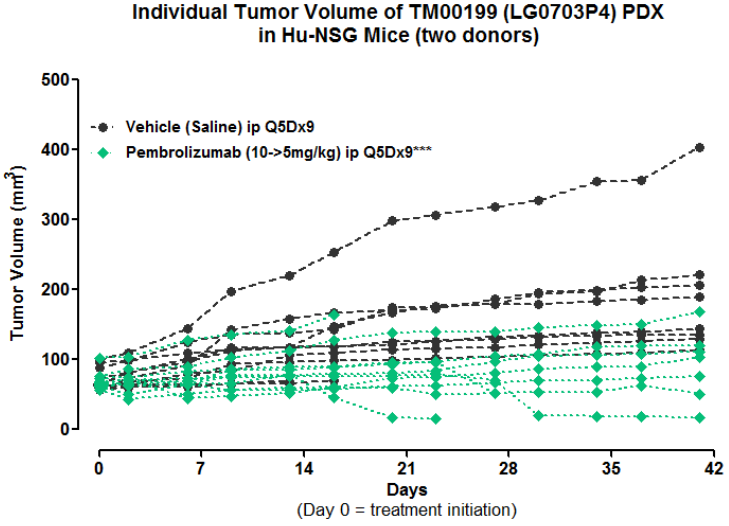

| HLA match | CD34+HPC donor | | |
|---|---|---|---|
| Tumor | 1 | 2 | 3 |
| BR1126 | HLA-C, DPA1 | HLA-A,DQA1, DPB1, DPA1 | HLA-C, DPA1 |

# Additional Lung PDX Models for Immuno-Oncology



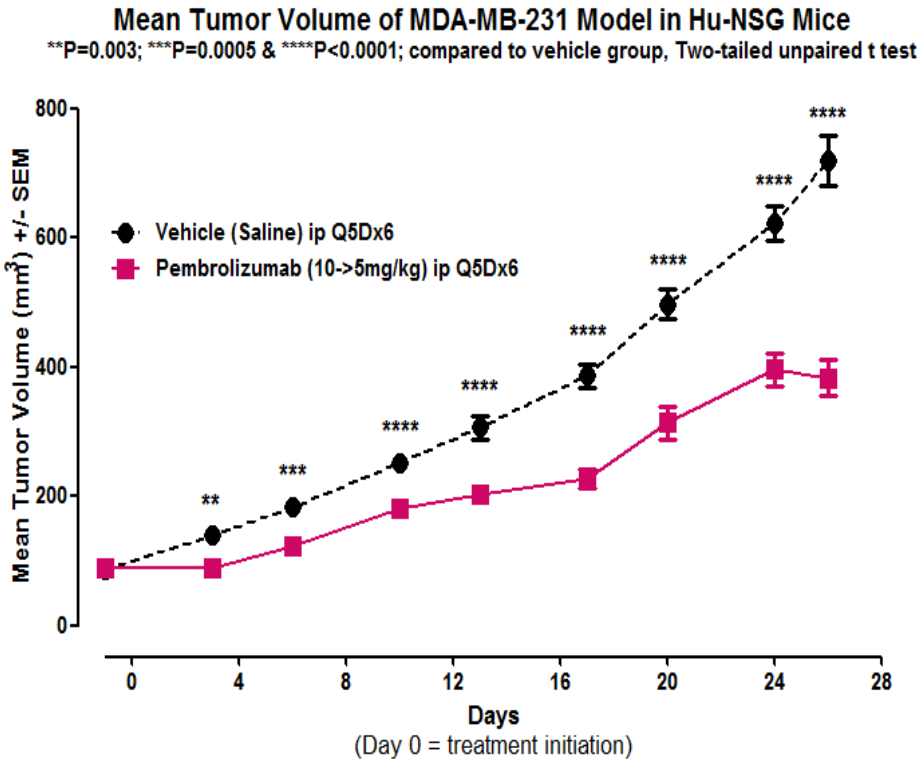



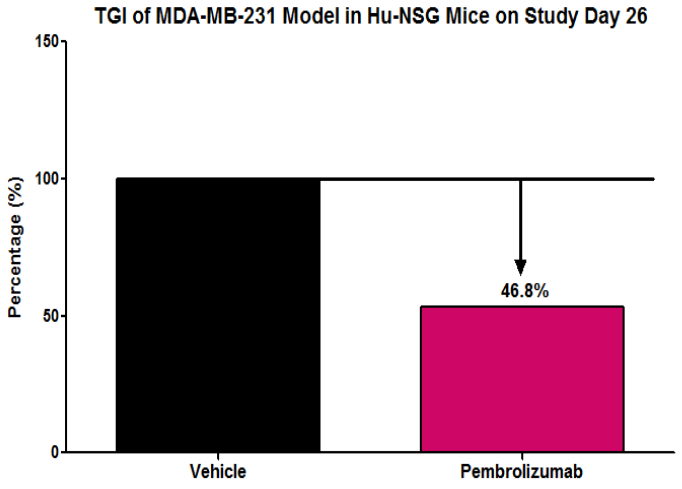



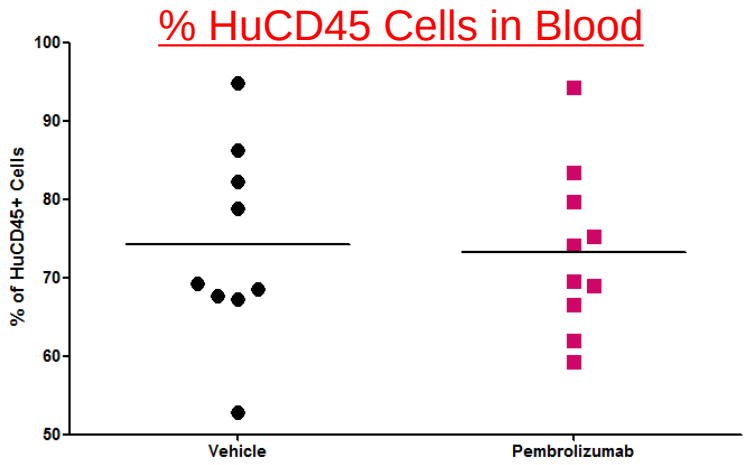
# Hu-NSG™ Mice: Suppression of MDA-MB-231 Breast Tumor Growth by Pembrolizumab

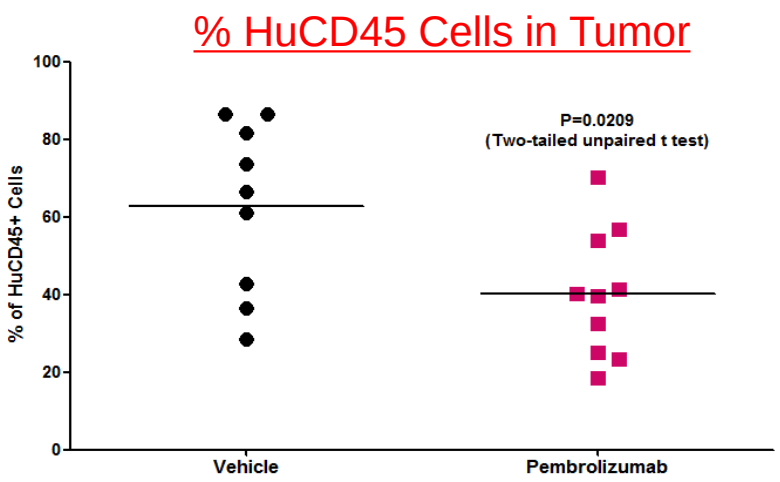
- Engrafted with $5x10^6$ cells/mouse s.c. with matrigel
- MDA-MB-231 cell surface expression of PD-L1: 49.2%

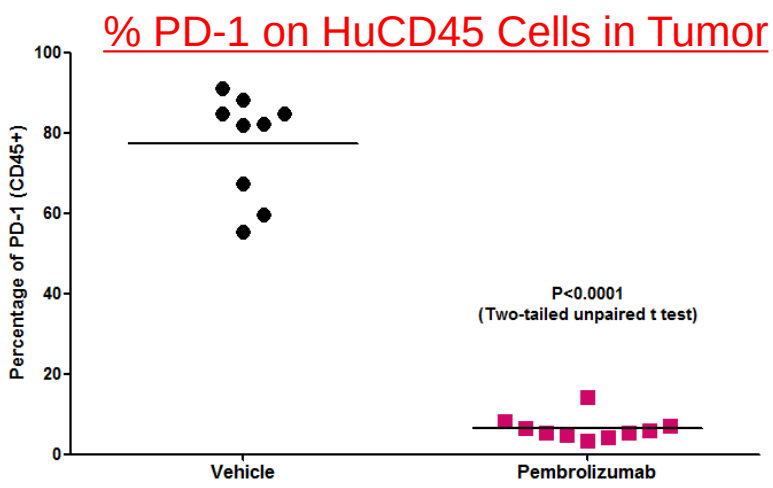


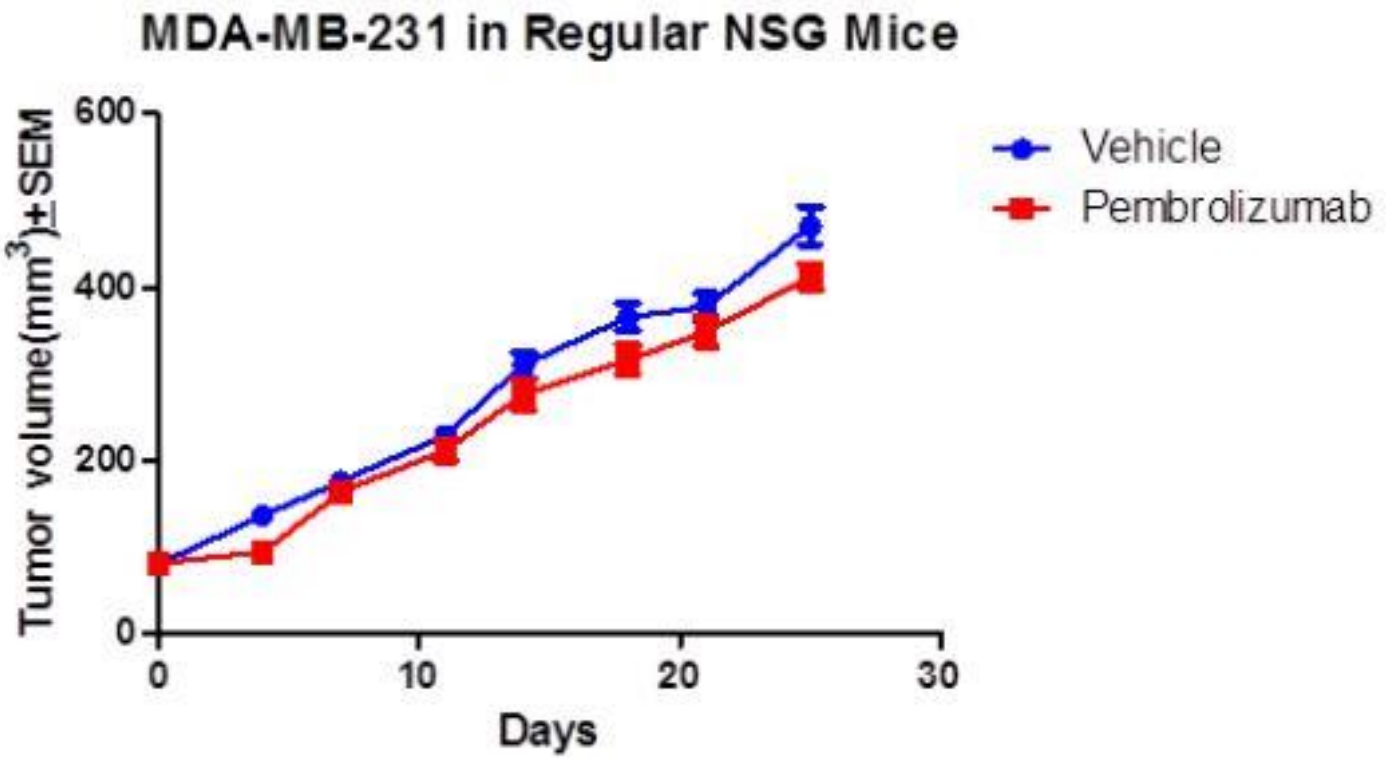
# Hu-NSG™ Mice: Characterization of Human CD45 Cells and PD-1 Levels in MDA-MB-231 Tumors



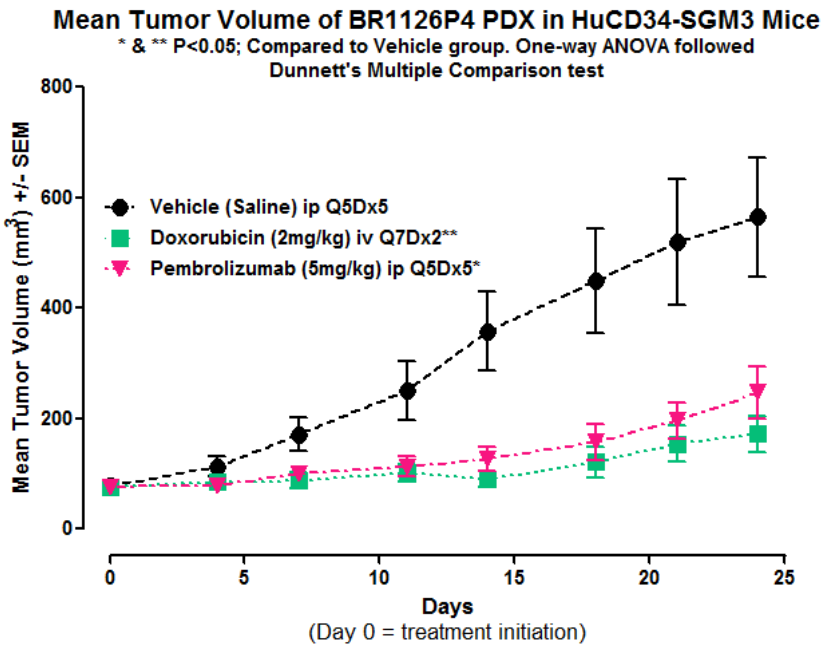



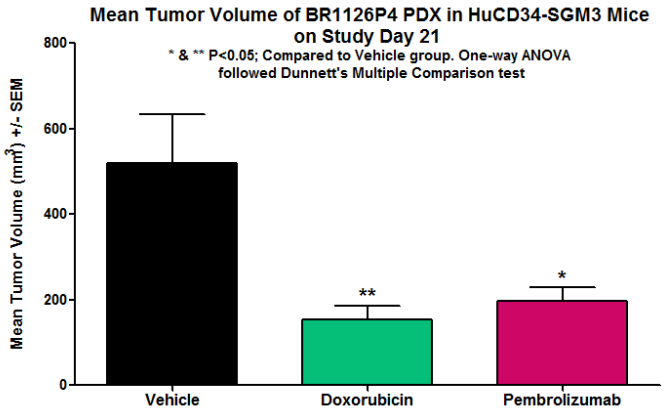
# MDA-MB-231 Does Not Respond to Pembrolizumab in Non-humanized NSG



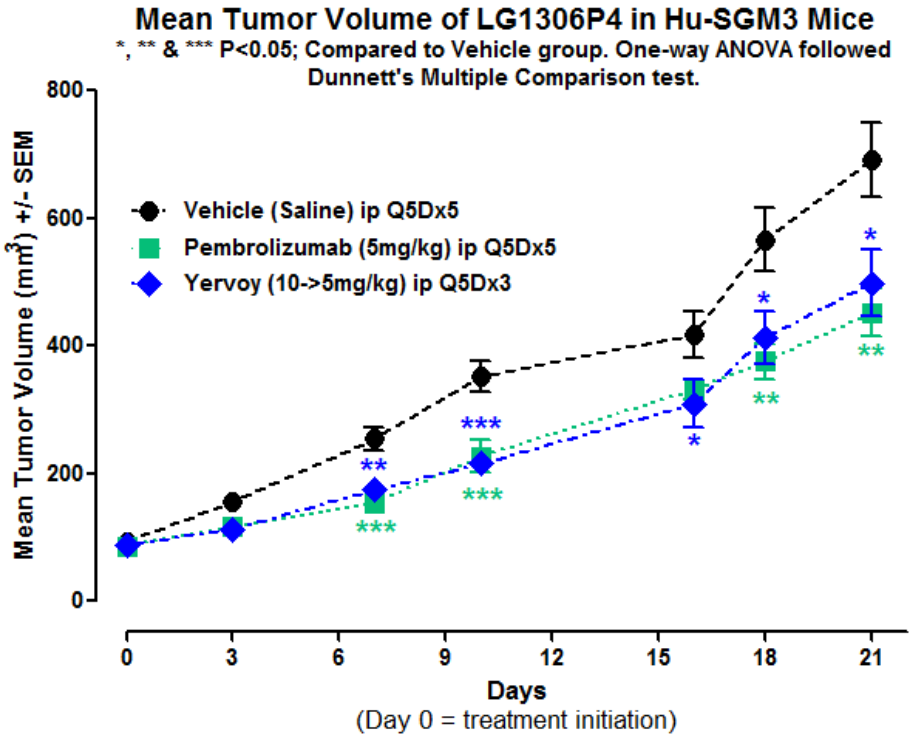
# Hu-NSG™-SGM3: Pembrolizumab and Doxorubicin Inhibit Growth of Breast (BR1126) PDX




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



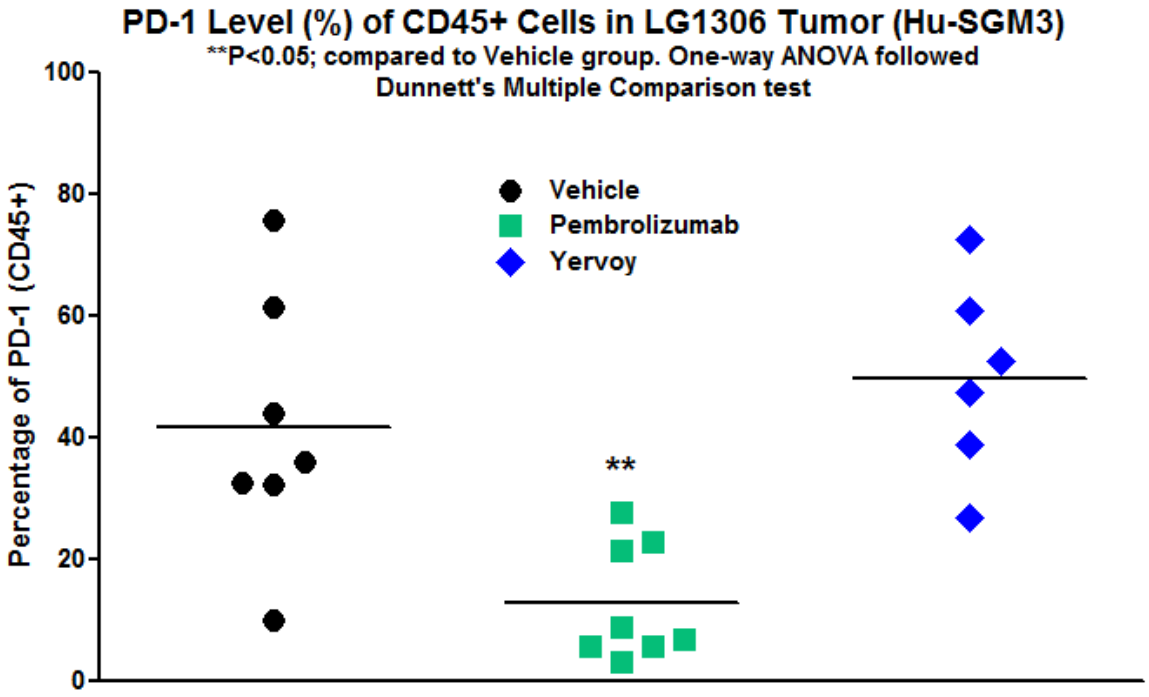
# Hu-NSG™-SGM3: Partial Growth Suppression of Lung (LG1306) PDX by Pembrolizumab & Yervoy




- Fresh tumor tissue engraftment
- HuCD45+ in whole blood: 36-81%
- HuCD3+/HuCD45: average 14.3%
- LG1306 PD-L1 surface expression: 89.1%

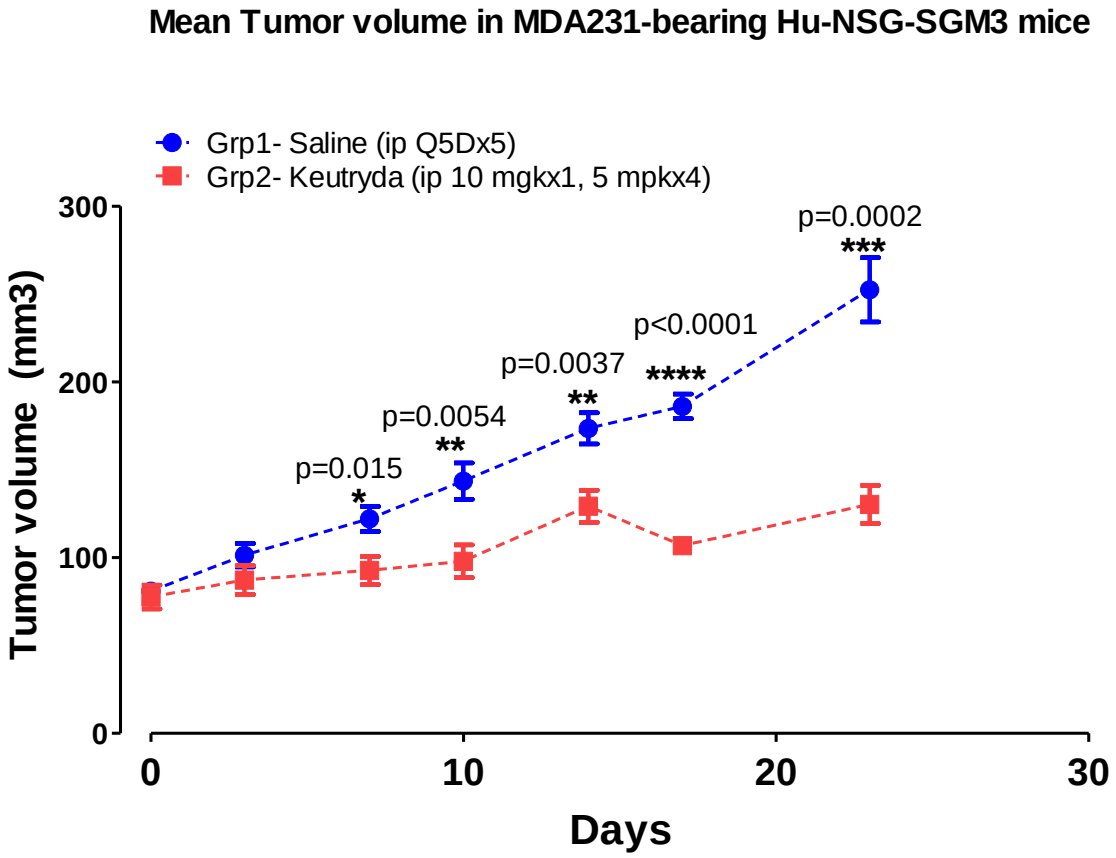
Yervoy = anti-CTLA4

# Hu-NSG™-SGM3: Expression of PD-1 in Lung (LG1306) PDX by Flow Cytometry



# Hu-NSG™-SGM3: Suppression of MDA-MB-231 Breast Tumor Growth by Pembrolizumab

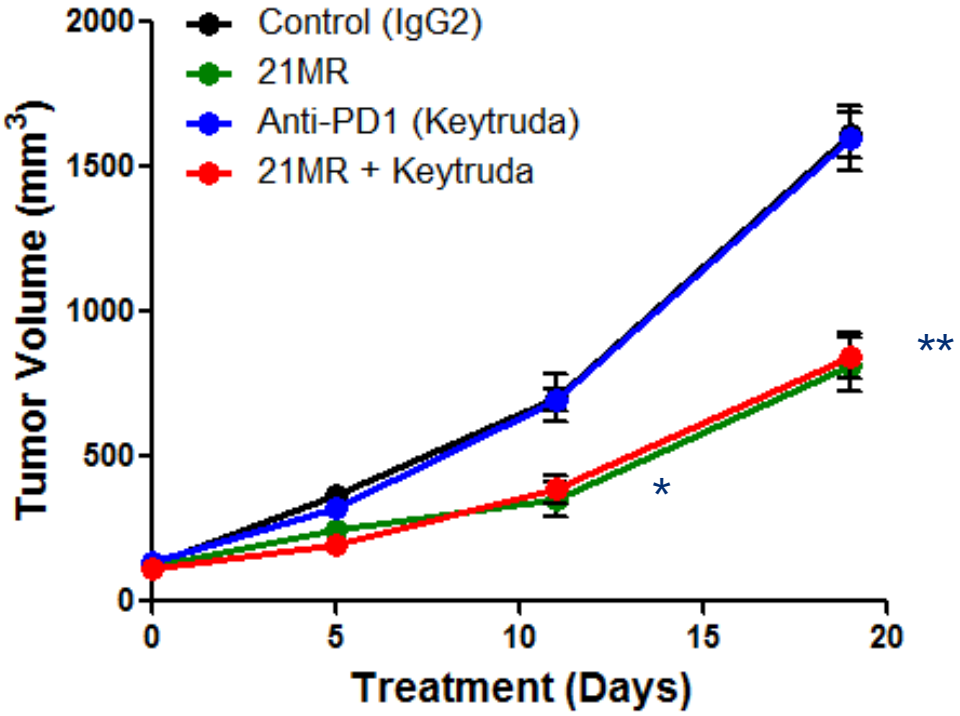
- Engrafted with $5x10^6$ cells/mouse s.c. with matrigel
- MDA-MB-231 cell surface expression of PD-L1: 95.1%



# Hu-NSG™-SGM3: Anti-DLL4 Treatment of NSCLC PDX Tumors Shows Immune Cell Engagement

- Delta-like ligand 4 (DLL4) activates the Notch pathway and is important in vessel sprouting and angiogenesis
  - DLL4 inhibition in tumor models results in hypervascularity with abnormal vessel formation
  - Disruption of angiogenesis suppresses tumor growth
  - DLL4 & Notch regulate VEGF pathway, VEGFR1 & VEGFR2 are involved in monocyte chemotaxis
- Demcizumab (anti-DLL4) is in phase 2 for NSCLC
- Data collected in collaboration with Chris Murriel and Tim Hoey, OncoMed Pharmaceuticals

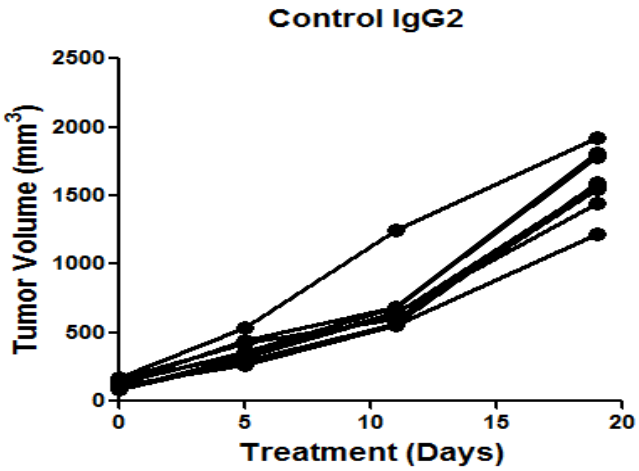
# Hu-NSG™-SGM3: Demcizumab (anti-DLL4) Significantly Inhibits NSCLC (OMP-LU121) PDX Growth

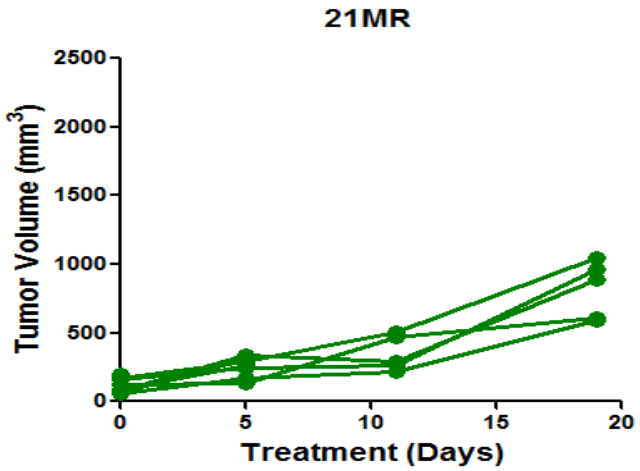


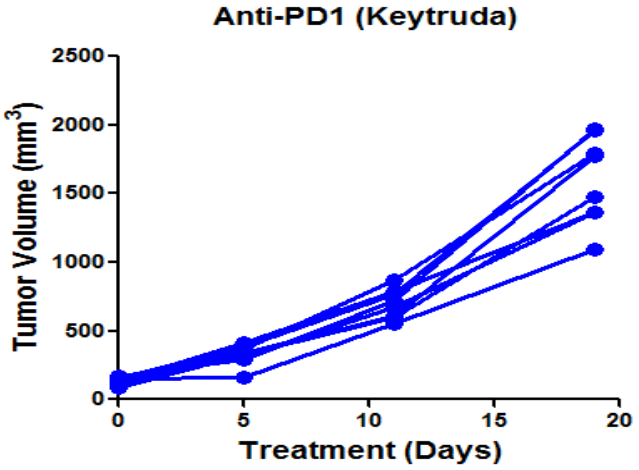

*p≤0.009 21MR ± PD1 vs Control, D11
**p≤0.0006 21MR ± PD1 vs Control, D19

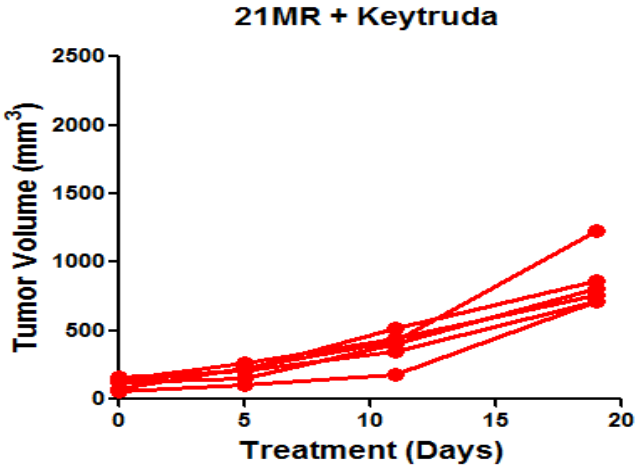
21MR = Demcizumab

# Hu-NSG™-SGM3: Demcizumab (anti-DLL4) Significantly Inhibits NSCLC (OMP-LU121) PDX Growth




21MR = Demcizumab

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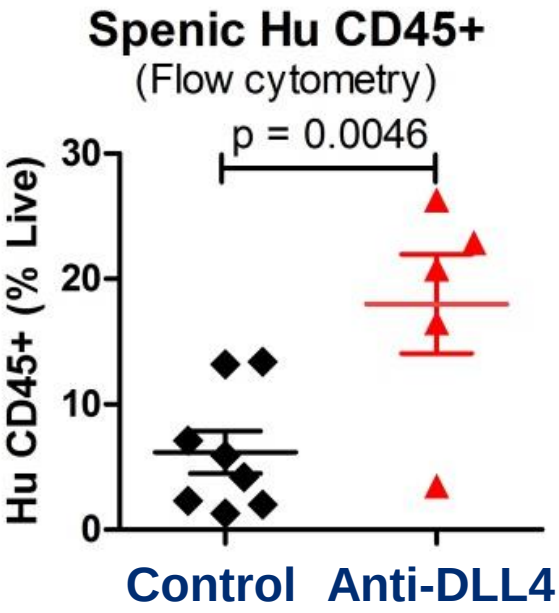
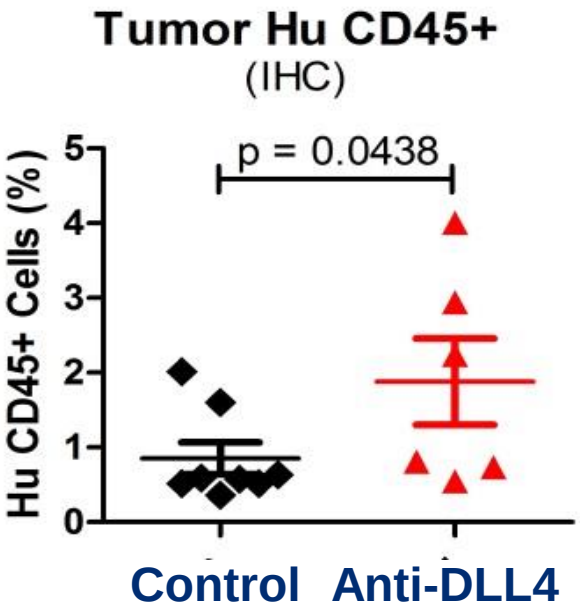
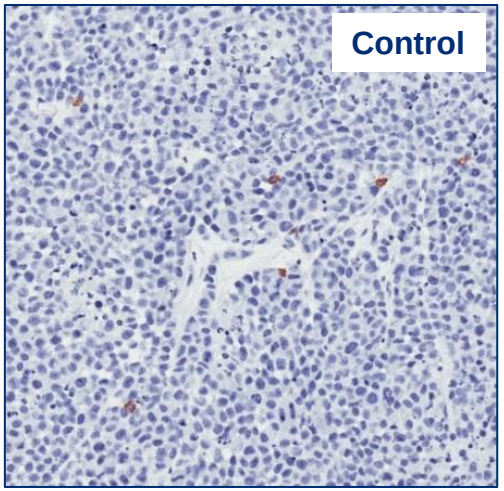
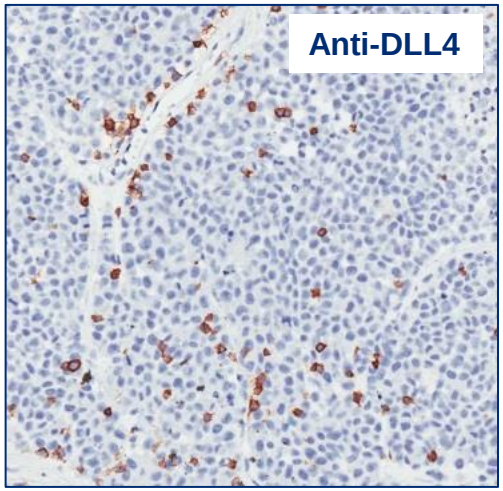
# Hu-NSG™-SGM3: Anti-DLL4 Increases Intra-Splenic and Tumor-Associated HuCD45+ Immune Cells




Tumor IHC: αHuCD45

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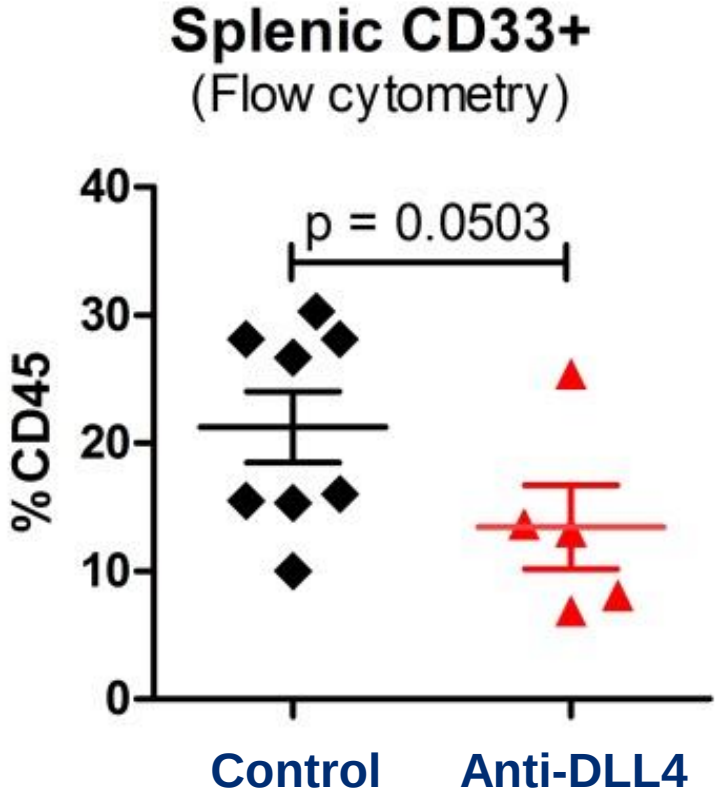
# Hu-NSG™-SGM3: Anti-DLL4 Decreases Intra-splenic Human Myeloid Cells



# Preliminary Findings and Conclusions

- PDX growth is not affected by HLA-type matching
- PDX engraftment into hu-NSG™ or hu-NSG™-SGM3 mice does not significantly impact growth kinetics
- Hu-NSG™ or hu-NSG™-SGM3 mice demonstrate immune-cell infiltration of tumors
- PDX tumors in hu-NSG™ or hu-NSG™-SGM3 mice respond to the anti-tumor agent Pembrolizumab
- Treatment of NSCLC tumors in hu-NSG™-SGM3 with anti-DLL4
  - Up-regulation of splenic and tumor huCD45$^{+}$ cells
  - Down-regulation of splenic huCD33$^{+}$ cells suggesting an increase in anti-tumor immune response
  - Hypervascularity (data not shown)

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