METHODS

1. Generation of humanized NSG™ and NSG™-SGM3 mice and examination of the engraftment kinetics in peripheral blood.

**Mice:**

NSG™ (005557): 3-week old, n=10
NSG™-SGM3 (013062): 4-week old, n=10

**Whole body irradiation:** 140 cGy for NSG™ vs. 100 cGy for NSG™-SGM3.

**HuCD34+ HPC injection:** 130,000 cells/mouse from the same donor.

**FACS analysis:** RO bleeds at 4, 6, 9, 12, 15, and 18 weeks post-engraftment. The flow panels include hCD45, hCD33, hCD19, hCD3, hCD4, and hCD8. 50 µl counting beads (Molecular Probes) were added to the stained samples before FACS according to the manufacturer’s instructions.

2. Efficacy studies in humanized NSG™ and NSG™-SGM3 mice.

**Cancer models (with high PDL1 levels):** We selected mouse cohorts based on some degree of HLA matching between tumors and CD34+ donors. Two PDX models BR1126 (TM00098) and LG1306 (TM00302) were transplanted subcutaneously in the mouse right flank. Re-suspended MDA-MB-231 cancer cells were mixed with Matrigel at 1:1 ratio and injected into mammary fat pad (6 X 10⁶ cells/mouse). Mice were grouped when tumors reached 60-200 mm³.

**Drug treatment:** Mice were IP dosed with Pembrolizumab (Merck) at 5 mg/kg or Ipilimumab (BMS) at 10 mg/kg on Day 0 and then 5 mg/kg every 5 days for up to 30 days. Tumors were harvested at the terminal point for flow-cytometry analysis to check immune cell infiltrates and PD-1 levels.

**Data Collection:** Body weight, clinical observations, and tumor volume were recorded twice weekly.

**Statistics analysis:** One-way ANOVA followed Dunnett’s Multiple Comparison test or Student’s t-test.

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**FIGURE 1. Human immune cell reconstitution in peripheral blood of Hu-NSG™-SGM3 and Hu-NSG™ mice.**
FIGURE 4. (cont.) Effect of Pembrolizumab on BR1126 (breast carcinoma) bearing Hu-NSG™-SGM3 mice.

FIGURE 5. Effect of Pembrolizumab and Ipilimumab on LG1306 (lung carcinoma) bearing Hu-NSG™-SGM3 mice.
FIGURE 6. Effect of anti-OX40 mAb, provided in collaboration with ImaginAb, on MDA-MB-231 (breast carcinoma) bearing Hu-NSG™ mice. Anti-OX40 mAb enhances T cell costimulation and cytotoxic response to tumors.

FIGURE 7. Effect of Demcizumab (21MR), provided in collaboration with OncoMed Pharma, on OMP-LU121 (non-small cell lung cancer) bearing Hu-NSG™-SGM3 mice. Demcizumab (anti-DLL4) disrupts Notch1 mediated monocyte chemotaxis and angiogenesis.
FIGURE 2. Effect of Pembrolizumab on MDA-MB-231 (breast adenocarcinoma) bearing Hu-NSG™ mice.

FIGURE 3. Effect of Pembrolizumab on MDA-MB-231 (breast adenocarcinoma) bearing Hu-NSG™-SGM3 mice.

FIGURE 4. Effect of Pembrolizumab on BR1126 (breast carcinoma) bearing Hu-NSG™-SGM3 mice.
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ABSTRACT

Humanized mice engrafted with tumors enable in vivo investigation of the interactions between the human immune system and human cancer. We have recently found that humanized NOD-scid IL2Rγnull (NSG™) mice bearing patient-derived xenografts (PDX) allow efficacy studies of check-point inhibitors. Next generation NSG™ strains include triple transgenic NSG™ mice expressing human cytokines KITLG, CSF2, and IL-3 (NSG™-S, NSG™-SGM3). Here we provide a direct comparison of humanization levels between NSG and NSG™-SGM3 mice engrafted with CD34+ human hematopoietic progenitor cells (HPCs) from the same donor. Three cancer models, previously shown to respond to anti-PD1 therapy in hu-NSG™ mice, were engrafted into partially HLA-matched hu-NSG™-SGM3 mice at 9 weeks post engraftment, when circulating hCD3+ T cells reach 10% of the hCD45+ cell population. Treatment with the anti-PD-1 receptor antibody Pembrolizumab (Keytruda) significantly reduced tumor growth in these models. Thus, PDX-bearing hu-NSG™-SGM3 mice—The Jackson Laboratory’s Onco-hu® mouse model—might serve as a new and improved platform for preclinical immuno-oncology efficacy.

BACKGROUND

1. Hu-NSG™ portfolio

Six plus years of experience in generating humanized mice: CD34+ and custom stem cells for humanization.

Hu-NSG™ and Hu-NSG™-SGM3 mice readily available.

NSG™, NSG™-SMG3, and other cytokine expressing NSG™ variants.

2. PDX

Over 400 PDX tumors all passage 5 or earlier.

PDX Live™: Live PDX tumor-bearing mice readily available.

3. Access options for Onco-hu™

Models: Delivery of humanized mice with or without tumors; NSG™ and NSG™-SGM3 mice engrafted with CD34+ HPCs are both readily available.

Execution of drug efficacy studies.