2016

# Onco-Hu™ Models: Humanized NSG™ and NSG™-SGM3 Mice for Immuno-Oncology

JAX Mice, Clinical and Research Services
*The Jackson Laboratory*

# *Onco-Hu™ Models: Humanized NSG™ and NSG™-SGM3 Mice for Immuno-Oncology*


## ABSTRACT

Understanding the interactions between human immune cells and tumors is paramount when devising treatment strategies that prevent tumor evasion of immune cells and improve cytotoxic responses. NSG™* and NSG™-SGM3* mice are a proven host for engraftment of human tumors or establishment of human immunity following hematopoietic stem cell transplantation. Here, we provide evidence that NSG and NSG-SGM3 mice engrafted with hematopoietic stem cells (collectively referred to here as "humanized NSG mice") can support the growth of allogeneic human tumors. The human tumors respond to standard-of-care chemotherapeutics, targeted therapies, and to immune check-point inhibitors clinically proven to initiate cytotoxic activity towards the tumor. Tumor-bearing, humanized NSG mice (the Onco-Hu™* mouse offered exclusively from The Jackson Laboratory) are a new and valuable preclinical testing platform for immuno-oncology.


The Jackson Laboratory

*Leading the search for tomorrow's cures*

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## *Immuno-Oncology: A New Paradigm for Patient Care*

The traditional approach to cancer treatment utilizes broad-acting chemical agents that are toxic to rapidly dividing cells. This chemotherapeutic approach can be successful but is complicated by an array of off-target toxicities and risks inducing drug resistance. Targeted therapies are emerging as new standards-of-care (SOCs) but often result in resistances that leave room for improvement. Mammalian immune systems have efficient, highly specific mechanisms for eliminating pathogen-infected cells and cancerous cells. In response, tumors have developed their own suite of mechanisms to evade immune detection.

These results support the use of humanized NSG and NSG-SGM3 mice as a new preclinical bridge for immuno-oncology therapies.

Fortunately, researchers are gaining a better understanding of the interaction between immune effector cells and tumors. New, promising treatment strategies that stimulate durable, immune-mediated tumor regression are now being used clinically. While these new immuno-oncology strategies are highly encouraging, they are not broadly efficacious across all cancers. Research to improve immuno-oncology-based therapies would benefit from a humanized, small animal model testing platform. The platform would allow scientists to better understand human immune and tumor cell interactions and enable preclinical testing of new therapies that have a higher likelihood for clinical success. Here, we review methods tumors use to evade immune-mediated elimination, current immunotherapies being tested in the clinic, and limitations to developing novel therapies given the mouse models currently available. Importantly, we demonstrate the utility of a new mouse platform – Onco-hu – that allows the study of patient-derived xenograft (PDX) tumors in the context of a humanized immune system for immuno-oncology preclinical research (Figure 1). Humanized NSG mice (both NSG and NSG-SGM3 hosts) support the growth of PDX tumors, respond to SOC treatments, and exhibit immune-mediated tumor regression following treatment with a check-point inhibitor. These results support the use of humanized NSG mice as a preclinical bridge for new immuno-oncology therapies.

## *Tumor Evasion of Human Immunity*

The immune system is a major regulator of anticancer therapy response and resistance. Many early tumors are thought to be eradicated by host immune cells following recognition of unique antigens expressed on the tumor cell's surface. Immune cell involvement is

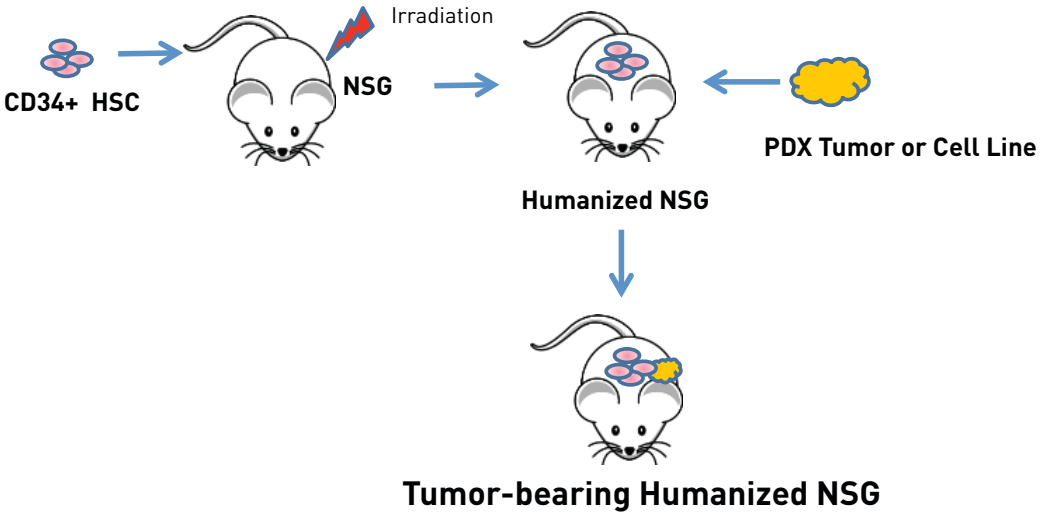



**Figure 1. Onco-Hu: A revolutionary immuno-oncology mouse platform.** Tumor-bearing humanized NSG mice. CD34+ hematopoietic stem cells are intravenously injected into sublethally irradiated 3-week old immunodeficient NSG or NSG-SGM3 mice. After confirmation of human leukocyte engraftment (including T cells) in peripheral blood at 15 weeks of age, mice are then engrafted with patient-derived xenograft tumors or tumor cell lines. Study enrollment for immuno-oncology testing may begin shortly after tumors and reach a desired size.

largely dictated by tumor type and molecular signature (Coffelt and de Visser, 2015). Indeed, advanced patient-derived tumor samples often carry human immune effector cells as passengers. One such population is tumor infiltrating lymphocytes (TILs), which are typically CD8+ cytotoxic T lymphocytes (CTLs). TILs have been isolated from tumors, expanded *ex vivo*, and adoptively transferred back into the patient to take advantage of their tumor-specific killing ability (Restifo *et al.*, 2012). The adoptive immunotherapy approach has been successful in treating some patients with metastatic melanoma (Rosenberg *et al.*, 2011) and has expanded to the treatment of common epithelial cancers (Rosenberg and Restifo, 2015). However, it is not universally effective in eradicating tumors. The absence or loss of tumor killing activity, both pre- and post-adoptive transfer, can be explained by multiple modes of immune suppression.

Two immunosuppressive pathways that have been implicated in TIL inhibition are mediated by cytotoxic T lymphocyte antigen 4 (CTLA-4) and by programmed death 1 (PD-1) (Ahmadzadeh *et al.*, 2009; Baitsch *et al.*, 2011). Both are known as "check-point" pathways because they prevent the loss of immune tolerance under normal conditions. Human monoclonal antibodies Yervoy (Ipilimumab) and Tremelimumab have been developed to bind CTLA-4 and prevent its interaction with CD80 or CD86 on antigen presenting cells, causing a block in T-cell inhibitory signaling. The current consensus is that CTLA-4 blockade allows CD80 or CD86 to bind with CD28, thereby activating a key T-cell co-stimulatory pathway. Ipilimumab and Tremelimumab are the first "checkpoint inhibitors" to advance into clinical trials for treating melanoma (Pardoll, 2012). Both antibodies promote longer-term patient survival in a fraction of patients, a fact that has stimulated widespread interest to find methods to activate other native immune modalities to target tumors.

PD-1 and its ligand PD-L1 are part of another key check-point pathway that has been manipulated to

enhance CTL-mediated tumor destruction (Ostrand-Rosenberg *et al*., 2014; Pauken and Wherry, 2015). The development of anti-PD-1 blocking antibodies was spurred by three key reports: mice with a null mutation in PD-1 develop an autoimmune syndrome (Nishimura *et al*., 1999), TILs upregulate PD-1 and subsequently become anergic (Ahmadzadeh *et al*., 2009), and many tumor types express PD-L1 (Zou *et al*., 2008). In early clinical trials, 28% of advanced melanoma patients who were refractory to anti-CTLA-4 antibody treatment responded to an anti-PD-1 monoclonal antibody (Hamid *et al*., 2013). Further, when both anti-PD-1 and anti-CTLA-4 check-point inhibitors were combined, 53% of patients responded favorably (Wolchok *et al*., 2013). Immunotherapeutics that inhibit immune regulatory pathways such as anti-CTLA-4 and anti-PD-1, have initiated a new era in the treatment of cancer (Coffer and de Visser, 2015). As of December 2015, there are over 20 anti-PD-1/PD-L1 antibodies in clinical trials (Gandini *et al*., 2016). In fact, as of 2016, two anti-PD-1 check-point inhibitors have been approved for the U.S. market: Prembrolizumab (Keytruda) and Nivolumab (Optivo). Both are currently entering clinical trials to test their efficacy against abroad range of tumor types. Since CTLA-4 and PD-1 regulate distinct inhibitory pathways, concurrent combination therapy is often more efficacious than either treatment alone (Sharma and Allison, 2015).

Tumors use other mechanisms to evade immune responses. For example, the highly inflammatory microenvironment of the tumor recruits tissue-resident macrophages and peripheral blood monocytes. These myeloid cells receive tumor-derived signals that alter gene expression and phenotype (Gabrilovich *et al*., 2012 and Ostuni *et al*., 2015). One myeloid cell subpopulation that develops in the tumor is the tumor-associated macrophages (TAM). TAMs are an abundant population of leukocytes in solid tumors. In many settings, TAMs fuel, rather than limit, tumor progression (Ostuni *et al*., 2015). TAMs suppress TIL activity and increase tumor angiogenesis by the mechanisms presented in Table 1, creating a hospitable microenvironment that favors cancer outgrowth. TAMs also recruit and stimulate regulatory T cells ($T_{reg}$) and T-helper 2 cells ($T_H2$) lymphocytes contributing to strong immunosuppressive actions in the tumor. These cells are normally associated with maintenance of immune tolerance.

**Table 1. Tumor Associated Macrophage (TAM) Immune Suppression**

| TAM Phenotype | Effect |
|---|---|
| Increased PD-L1 | Suppressed CTL activation |
| Increased IL-10 | Stimulation of $T_H2$ cells |
| Decreased IL-12 | Suppression of T cells |
| Increased CCL22 | Attraction of $T_{Reg}$ cells |
| Increased Tek (TIE2) | Increased angiogenesis |

**Table 2. Myeloid Derived Suppressor Cell (MDSC) Immune Suppression**

| MDSC Phenotype | Effect |
|---|---|
| Increased ARG1 & NOS2 (iNOS) | Suppressed CTL activation; TCR down-regulation; decreased proliferation |
| Increased Reactive Oxygen Species (ROS) | Suppressed CTL activation; TCR down-regulation; decreased IL-2R signaling |
| Increased ADAM17 | Decreased CD62L on CD4 & CD8 T cells; impaired lymph node trafficking |
| Co-stimulation & Soluble factors | Activation and expansion of $T_{Reg}$ cells |

Other myeloid cells found in tumors include myeloid-derived suppressor cells (MDSCs). MDSCs represent a heterogeneous group of immature cells that include precursors of macrophages, granulocytes, and dendritic cells, defined by their ability to suppress T cell proliferation and to promote angiogenesis (Coffelt and de Visser, 2015). As shown in Table 2, MDSCs use a spectrum of immunosuppressive mechanisms to help tumors evade immunity. Most of their effects are directed at suppressing T cells.

Other immune cell populations important in tumor immunity include dendritic cells (DCs) and natural killer (NK) cells. DCs are considered "professional antigen presenting cells" and are capable of processing unique tumor-specific antigens to activate T and B cells. DCs, therefore, are at the center of research devoted to developing tumor vaccines and to expanding tumor-specific CTLs *ex vivo* for subsequent adoptive immunotherapy (Palucka *et al.*, 2012). NK cells have unique cell-surface receptors that are important for immune surveillance of self-tissues and whose activities are mediated by binding of HLA class I antigen-presenting molecules that are found on most normal cells and tumors. Tumors that retain HLA class I expression evade NK cell-mediated cytotoxicity, but those that lose expression are no longer recognized by NK cells as "self" and are killed. Compounds that promote NK cell activation and adoptive immunotherapies that use allogeneic NK cells are active areas of preclinical and clinical investigation (Vivier *et al.*, 2012).

## *New Therapeutics in the Pipeline*

As the information above suggests, our knowledge of immune cell function and interactions with tumor cells is improving. Delineation of the basic mechanisms that underlie these interactions has uncovered several potential strategies through which immune cells might be manipulated to enhance their antitumor activity. Some of the most promising examples are outlined in Table 3.

**Table 3. Current Approaches to New Immuno-Oncology Therapeutics**

| | |
|---|---|
| **Monoclonal Antibodies** | Block ligands & receptors between cells |
| | Bind receptors to activate pathways |
| | Target cells for destruction by ADCC |
| | Antibody-drug conjugates |
| **Bispecific T-cell Engager (BiTE) Antibodies** | Promotion of T cell killing of target |
| **Chimeric Antigen Receptors (CARs)** | Promotion of T or NK cell killing of target |
| **Adoptive Cell Transfer** | Tumor infiltrating lymphocytes (TIL) |
| | TCR gene therapy |
| | Allogeneic donor lymphocyte infusion (DLI) |
| | Allogeneic NK cell infusion |
| | Antigen loaded dendritic cells |
| **Vaccination** | Tumor-specific antigen stimulation |

For example, monoclonal antibodies can block or enhance ligand-receptor interactions between cells, act as agonists or antagonists, target cellular destruction by antibody-dependent cellular cytotoxicity (ADCC), and deliver conjugated drug payloads to specific target cells. Bispecific T-cell Engager (BiTE) therapy uses binding specificity regions from two antibodies fused into a single molecule. These molecules directly bind CTLs to antigens on tumor cells to enhance tumor killing (Stone, *et al*., 2012 and Laszlo *et al*., 2014).

Additionally, the ability to genetically engineer lymphocytes to express conventional T-cell receptors or chimeric antigen receptors (CARs) has extended the application of adoptive cell transfer immunotherapies (Rosenberg and Restifo, 2015). CAR T-cell therapy is an exciting and powerful technology that was designated as a Breakthrough Therapy in 2014 by the U.S. Food and Drug Administration (FDA). A CAR is composed of the antigen-recognition portion of an antibody conjugated to the transmembrane and signaling domains of a T-cell receptor. When expressed on T-cells isolated from a patient, CARs recognizing cancer-associated antigens can be infused back into the bloodstream. Such CAR T-cells have the ability to decrease tumor burden and increase survival. CAR T-cell therapy has made great strides in treating CD19 positive leukemias and lymphomas which have previously failed all other chemotherapeutic interventions (Dai, *et al*., 2016). Future developments in CAR therapy will focus on the unique barriers to treating solid tumors, expressing CARs in NK cells, and combination therapies with check-point inhibitors (Beavis *et al*., 2015, Vanneman and Dranoff, 2012, Rezvani *et al*., 2015, and John *et al*. 2013).

Allogeneic (i.e., mismatched) donor lymphocyte infusion (DLI) creates a graft-versus-tumor effect and has been successful in treating many patients with leukemia. A similar approach is being explored using allogeneic NK cells (Vivier *et al*., 2012). Greater knowledge of tumor-specific antigens has been accumulating such that tumor

antigen peptides might be used to prime DCs and thereby stimulate their adaptive immune responses prior to adoptive transfer. Alternatively, these antigens might be injected directly as potential vaccines to amplify endogenous tumor-specific T-cell responses (Miller and Sadelain, 2015). All of these new approaches require appropriate *in vivo* preclinical evaluation prior to testing in humans in order to validate their mechanism of actions, efficacy, and safety.

## *The Challenge of Developing New Therapeutics*

Laboratory mice represent one of the most valuable research platforms for characterizing *in vivo* immune-tumor interactions and for testing new therapeutics. Immune-competent mice can be genetically engineered to develop tumors spontaneously, or can be transplanted with tumors from a syngeneic donor or tumor cell line. Mouse genetics, immunity, and tumor development share many similarities with humans, but distinct differences exist. For example, humans and mice differ in peripheral blood lymphocyte distribution and their neutrophils respond differently in innate and adaptive immune stimulation (Mestas and Highes, 2004). MDSCs in mice are primarily of the less-immunosuppressive polymorphonuclear variety, while those in humans are commonly the more suppressive monocytic type (Gabrilovich *et al*., 2012). The etiology of tumor development in the two species is also not necessarily identical (Rangarajan and Weinberg, 2003).

Additionally, mice and humans exhibit differences in receptor-ligand homologies and affinities such that reagents showing efficacy in the mouse do not necessarily show the same efficacy in the clinic. Inbred mice, also, are genetically uniform, which greatly enhances their scientific utility with respect to drug specificity and therapeutic reproducibility, but humans are genetically diverse. Human genetic diversity can dilute efficacy or uncover previously unobserved off-target toxicities. In some cases, a therapeutic developed in the mouse may need to be re-engineered and re-tested in a more human-specific system. Such efforts can be expensive and time-consuming.

Given the challenges of testing novel immuno-oncology therapies in mice, researchers at The Jackson Laboratory have employed a highly immunodeficient mouse, called NSG (NOD.Cg-Prkdc$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ, 005557), that is capable of engrafting human hematopoietic stem cells and supports the development and function of multiple aspects of human immunity (jax.org/humanized-mice).

In addition, JAX *In Vivo* Pharmacology Services has recently expanded its humanized mouse models by employing a new NSG variant, NSG-SGM3 (NOD.Cg-Prkdc$^{scid}$ *Il2rg*$^{tm1Wjl}$ Tg(CMV-IL3, CSF2, KITLG)1Eav/MloySzJ,

013062). NSG-SGM3 mice are NSG mice that express human hematopoietic cytokines: stem cell factor (SCF), also known as Steel Factor gene and Kit Ligand, granulocyte/macrophage-stimulating factor (GM-CSF), and interleukin-3 (IL-3), all driven by a human cytomegalovirus promoter/enhancer sequence. Triple transgenic NSG-SGM3 mice constitutively produce 2-4 ng/ml serum levels of human SCF, GM-CSF, and IL-3, providing cell proliferation and survival signals that become more measurable over time. These mice support the stable engraftment of CD33+ myeloid lineages, and several types of lymphoid cells. As a result, NSG-SGM3 mice allow superior engraftment of diverse hematopoietic lineages compared to other existing models.

Both NSG and NSG-SGM3 mice support co-engraftment of both human immune cells and a wide range of human PDX tumors. We demonstrate that PDX tumors grown in the context of humanized immune systems respond to standard-of-care chemotherapeutics and to the checkpoint inhibitor Keytruda (Prembrolizumab), establishing humanized NSG mice as a new preclinical platform for testing human-specific immuno-oncology therapeutics.

## *Meeting the Need with NSG and NSG-SGM3 Mice*

At The Jackson Laboratory, humanized mice are created by first sub-lethally irradiating NSG (hu-CD34-NSG™) or NSG-SGM3 (hu-CD34-NSG™-SGM3) mice at 3-4 weeks of age, followed by subsequent tail vein injection of human CD34+ HSCs isolated from cord blood. Peripheral blood (PBL) engraftment is validated 12 weeks later. NSG mice support multi-lineage engraftment and human immune cell homing into nearly all of the appropriate organs and tissues, including host bone marrow, thymus, spleen, and PBL (as summarized in Figure 2 and Table 4, Ishikawa *et al.*, 2005; Tanaka *et al.*, 2012).

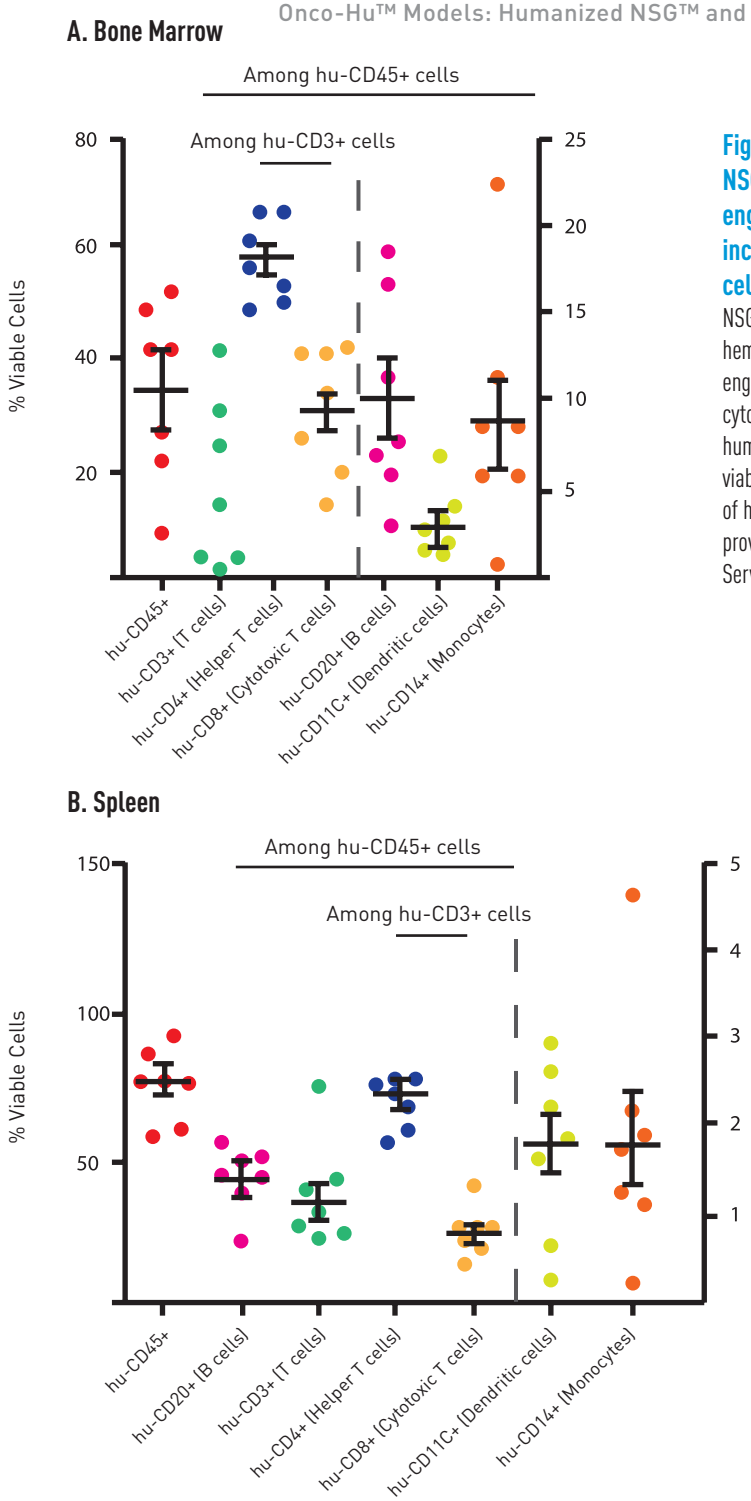



**Figure 2. Humanized CD34+ NSG mice support multilineage engraftment of human cells, including B cells, T cells, dendritic cells and monocytes.**
NSG mice injected with human hematopoietic stem cells show very robust engraftment efficiencies measured by flow cytometry using cell specific markers of human leukocytes [A] Percent of human viable cells in bone marrow and [B] Percent of human viable cells in spleen. Data provided by JAX® *In Vivo* Pharmacology Services.

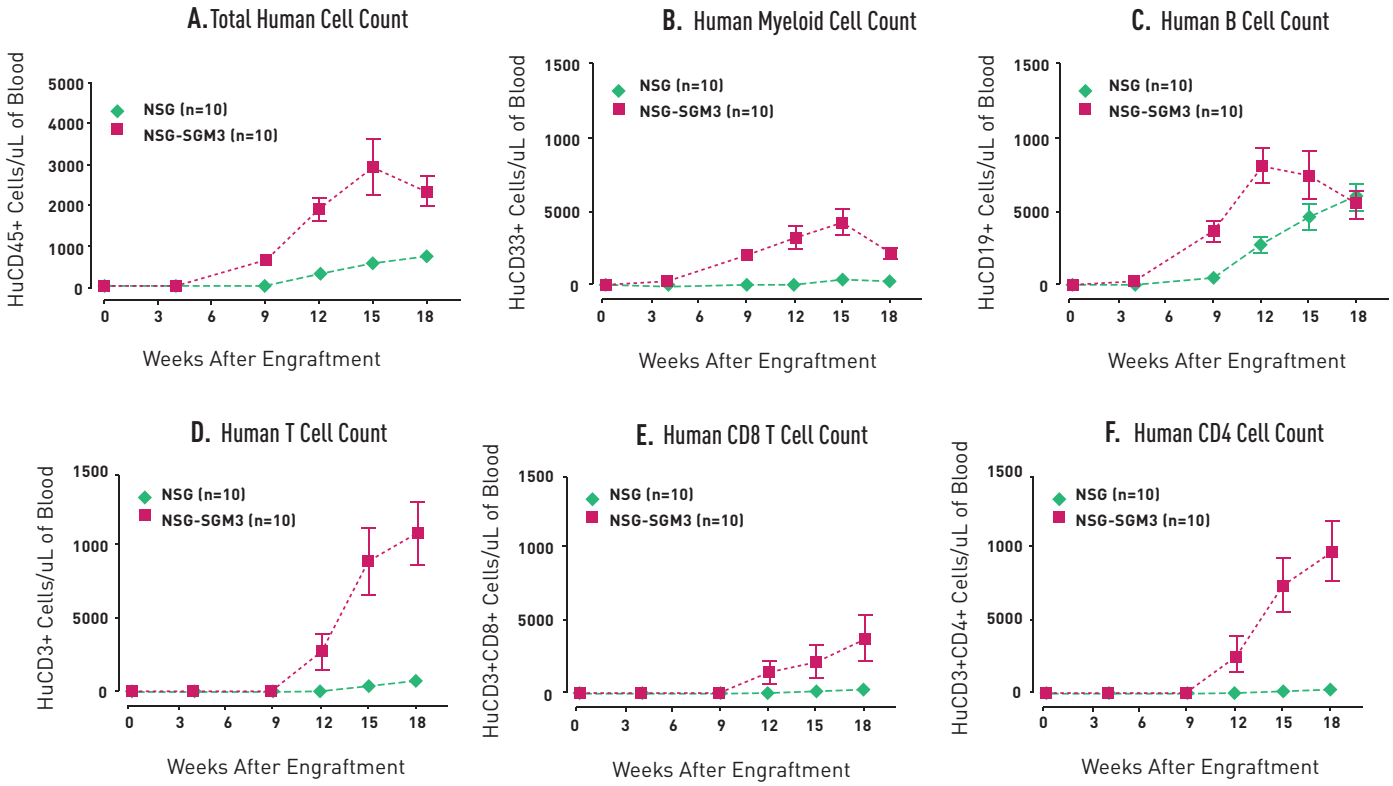
| Myeloid Cells | Lymphoid Cells |
|---|---|
| Macrophages | CD4+ & CD8+ T cells |
| Monocytes | $T_{Reg}$ |
| Neutrophils | B cells |
| Basophils | NK cells |
| Mast cells | |
| Erythroid progenitors | |

**Table 4. Human Multi-Lineage Engraftment in hu-CD34-NSG**
Data provided by JAX® *In Vivo* Pharmacology Services, The Jackson Laboratory.

Compared to the humanized NSG™ model, humanized NSG™-SGM3 mice have increased cell counts of the following human immune cell populations (as summarized in Figure 3, and Wunderlich *et al.*, 2010; Billerbeck *et al.*, 2011)

- CD33+ myeloid cells
- Hematopoietic stem cells
- Myeloid progenitor cells
- Mast cells
- Myeloid dendritic cells
- T cells (CD3+)
- T helper cells (CD4+)
- T cytotoxic cells (CD8+)
- T regulatory cells (CD4+, CD25+, FoxP3+)

**Figure 3. Comparison in engraftment between NSG and NSG-SGM3 (cells/µL) as measured in peripheral blood.**
(A) Total huCD45+ donor cell count, (B) Total huCD33+ myeloid cell count, (C) Total huCD19+ B cell count, (D) Total huCD3+ T cell count, (E) Total huCD3+CD8+ T cell count, and (F) Total huCD3+CD4+ T cell count. Data provided by JAX® *In Vivo* Pharmacology Services, The Jackson Laboratory.



## T Cells

In addition to human immune cell development, hu-CD34-NSG have been functionally validated. In particular, immature human T-cell progenitors (CD4+ CD8+ double positive cells) produced in the bone marrow of hu-CD34-NSG mice are found in the thymus along with mature CD4+ and CD8+ single positive T cells (Ishikawa *et al.*, 2005). The mature CD4+ T-helper cells and CD8+ CTLs then leave the thymus and populate the PBL and spleen. Together, these observations demonstrate that the cells are undergoing thymic TCR selection (self-versus-non-self) and homing to appropriate tissues.

To confirm the cytotoxic activity of the human T cells, these cells were harvested from the spleens of engrafted mice, expanded clonally *ex vivo* by growth with allogeneic target cells, and then challenged with allogeneic target cells in a chromium release cytotoxicity assay (Ishikawa *et al.*, 2005). CD4+ T cells recognized targets in the context of class II antigens and CD8+ T cells recognized targets in the context of class I antigens, as expected of normal, mature human T cells.

Hu-CD34-NSG mice also demonstrate delayed-type hypersensitivity (DTH), another measure of T cell function. In this assay, hu-CD34-NSG mice were sensitized with two abdominal applications of dinitrofluorobenzene (DNBF) and one week later were challenged by topical application of DNBF to the ear pinna. An *in vivo* T cell mediated pro-inflammatory response was observed by measuring the swelling of the pinna. Moreover, this response was blocked by hydrocortisone (JAX *In Vivo* Pharmacology Services; data not shown).

## B Cells

In addition to T cell function, B cell function in hu-CD34-NSG mice was examined by immunization with ovalbumin to stimulate immunoglobulin production. The human B cells responded by producing ovalbumin-specific IgM but only minimal IgG (Ishikawa *et al.*, 2005). A current limitation of this platform is a paucity of human cell populations within peripheral lymph nodes and poor development of germinal centers. These both are necessary for the development of the memory and plasma B cells that are responsible for robust immunoglobulin class switching to IgG. Additionally, NSG mice are C5 complement deficient. Although the absence of hemolytic complement activity supports more robust human cell engraftment in NSG, it prevents complement dependent cytotoxicity (CDC) responses stimulated by antibody binding to target cells.

## Myeloid Cells

The hu-CD34-NSG mice also develop the full spectrum of myeloid cell lineages with their appropriate functional activities (Tanaka *et al.*, 2012). Human macrophages isolated from the bone marrow and lung of engrafted mice are capable of phagocytosis of fluorescent beads

and are cytotoxic to *S. typhimurium* when stimulated with interferon gamma (INF-γ). Macrophages develop pro-inflammatory responses to bacterial lipopolysaccharides (LPS) through Toll-like receptors. The human monocytes/macrophages in hu-CD34-NSG express TLR2 and TLR4, and when the mice are challenged with LPS, multiple human pro-inflammatory molecules are detected in plasma, demonstrating an *in vivo* functional response (Tanaka *et al.*, 2012).

Neutrophils play an important role in innate immunity and in tumor biology, by developing into MDSC. Human neutrophils, defined by the markers CD15+, CD33 low, and HLA-DR-, represent 35% of the human cells in bone marrow and 1-5% in the spleen of hu-CD34-NSG mice (Tanaka *et al.*, 2012). Human CD66b+ neutrophils account for less than 1% in PBL but increase to 2.6% after *in vivo* treatment with granulocyte colony stimulating factor (Coughlin *et al.*, 2012). The marker CD63 is expressed in azurophilic granules of neutrophils, and human neutrophils in mice treated with LPS increase CD63 expression, indicating degranulation activity. LPS also stimulates an increased respiratory response and causes human neutrophils to accumulate in the lung, mimicking bacterial-induced sepsis. These results demonstrate the human neutrophils in hu-CD34-NSG mice are functional.

## Natural Killer Cells

Lastly, hu-CD34-NSG mice have CD3-NKp46+ NK cells that represent 1-3% and 7% of human cells in the spleen and lung, respectively (Strowig *et al.*, 2010). The majority of these NK cells are immature NKp46+ CD56- cells, but they can be induced to mature into NKp46+CD56+ cells when treated with interleukin 15 (IL-15). Human NK cells isolated from hu-CD34-NSG spleens degranulate and produce IFN-γ *in vitro* when treated with IL-15 and provided K562 cells as targets. K562 is a human erythroleukemia cell line that expresses NK recognition molecules NCR, NKG2D, and DNAM. These data provide evidence that humanized NSG mice produce NK cells that can be activated and respond to cells recognized as non-self.

Given the abundance of functional human immunity reported in the hu-CD34-NSG mice, it was predicted that these mice would rapidly reject any non-HLA matched human PDX tumor that was co-engrafted subcutaneously. As described above, however, there is ample published evidence for multiple mechanisms by which tumors evade immunity. The following data provide clear evidence that hu-CD34-NSG mice can efficiently engraft both human cancer cell lines and diverse human PDX tumors that are not HLA matched to the hematopoietic donor cells used to humanize the NSG host.

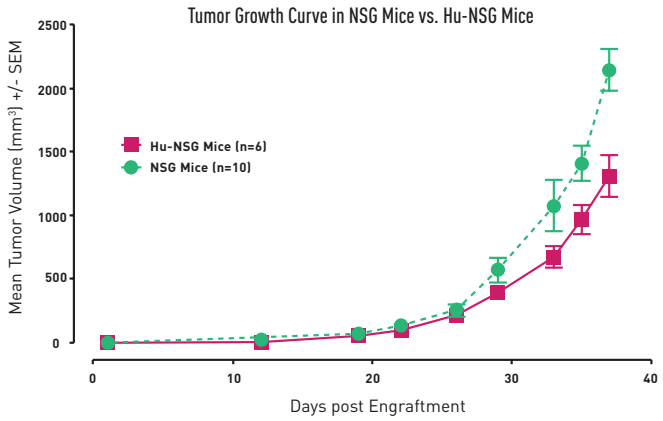
# *Onco-Hu: Humanized NSG and NSG-SGM3 Mice Engraft Non-HLA Matched Human Tumors*

Initial experiments evaluated whether human tumors would grow in humanized NSG mice that had been engrafted with allogenic human HSC. Breast, lung, and sarcoma PDX tumors were implanted subcutaneously into NSG or hu-CD34-NSG mice with established and functionally mature human immune cells derived from an HLA mismatched human HSC donor (Figure 4). All three tumors showed robust growth and no obvious indication of rejection. At study termination, tumors were analyzed by flow cytometry for the presence of TILs. Interestingly, all three tumors contained human CD4+ and CD8+ T cells, but few CD19+ B cells were detected (Figure 5). The failure of the TILs to slow tumor growth in the hu-CD34-NSG recipients suggests that these T-cells may have become anergic, similar to strategies tumors use to evade human immunity, as described above.

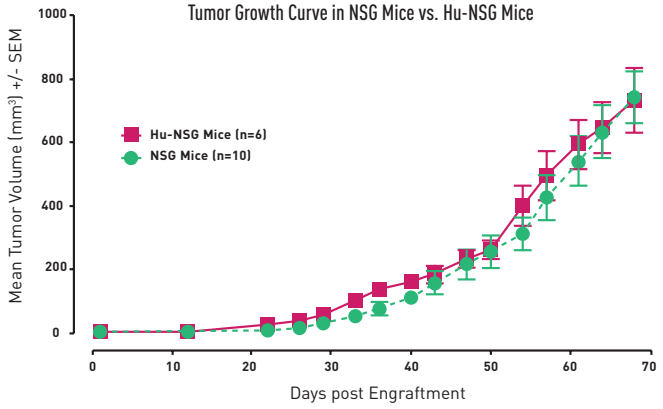
These results demonstrate that hu-CD34-NSG mice support non-HLA matched tumor growth and that the presence of human immune cells does not significantly impact tumor take or growth rates.

**Figure 4. Presence of human immunity does not impact human tumor growth.** [A] Human breast (TM00090), [B] lung (TM00213), and [C] sarcoma (TM00381) PDX grew at similar rates in NSG and humanized NSG recipients. PDX tumors were not HLA matched to the donor hematopoietic cells. Data provided by JAX® *In Vivo* Pharmacology Services, The Jackson Laboratory.

**A. Breast**




**B. Lung**




**C. Sarcoma**

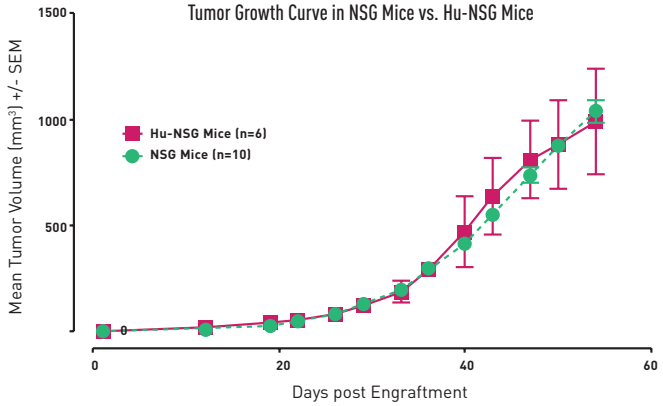



**Figure 5. PDX tumors grown in hu-CD34-NSG mice contain tumor infiltrating lymphocytes.** [A] Human breast (TM00090), [B] lung (TM00213), and [C] sarcoma (TM00381) PDX contain CD4+ and CD8+ T-cells, but minimal CD19+ B cells. The presence of TILs did not appear to influence tumor growth. Data provided by JAX® *In Vivo* Pharmacology Services, The Jackson Laboratory.

# *Human Tumors in Onco-Hu Mice Respond to Therapy*

The ability of the humanized NSG mice to support the growth of non-HLA matched human tumors was an important finding in the development of this preclinical testing platform. Next, we asked whether engrafted tumors would respond to clinically relevant standard-of-care (SOC) treatments and whether immuno-oncology checkpoint inhibitors could reactivate anti-tumor responses in the resident human immune cells.

In a first set of experiments, allogenic human breast cancer PDX tumor fragments, which exhibited PD-L1 cell surface expression on 56.9% of cells by flow cytometry, were engrafted into hu-CD34-NSG or hu-CD34-NSG-SGM3 mice and were subsequently treated with vehicle, SOC treatments (Cisplatin or Doxorubicin), or PD-1 inhibitor, Keytruda (Figure 6). Cisplatin, Doxorubicin, and Keytruda significantly reduced tumor growth compared to the vehicle control, indicating that both SOC and novel immunotherapeutics show efficacy in tumor-bearing hu-CD34-NSG and hu-CD34-NSG-SGM3 mice.

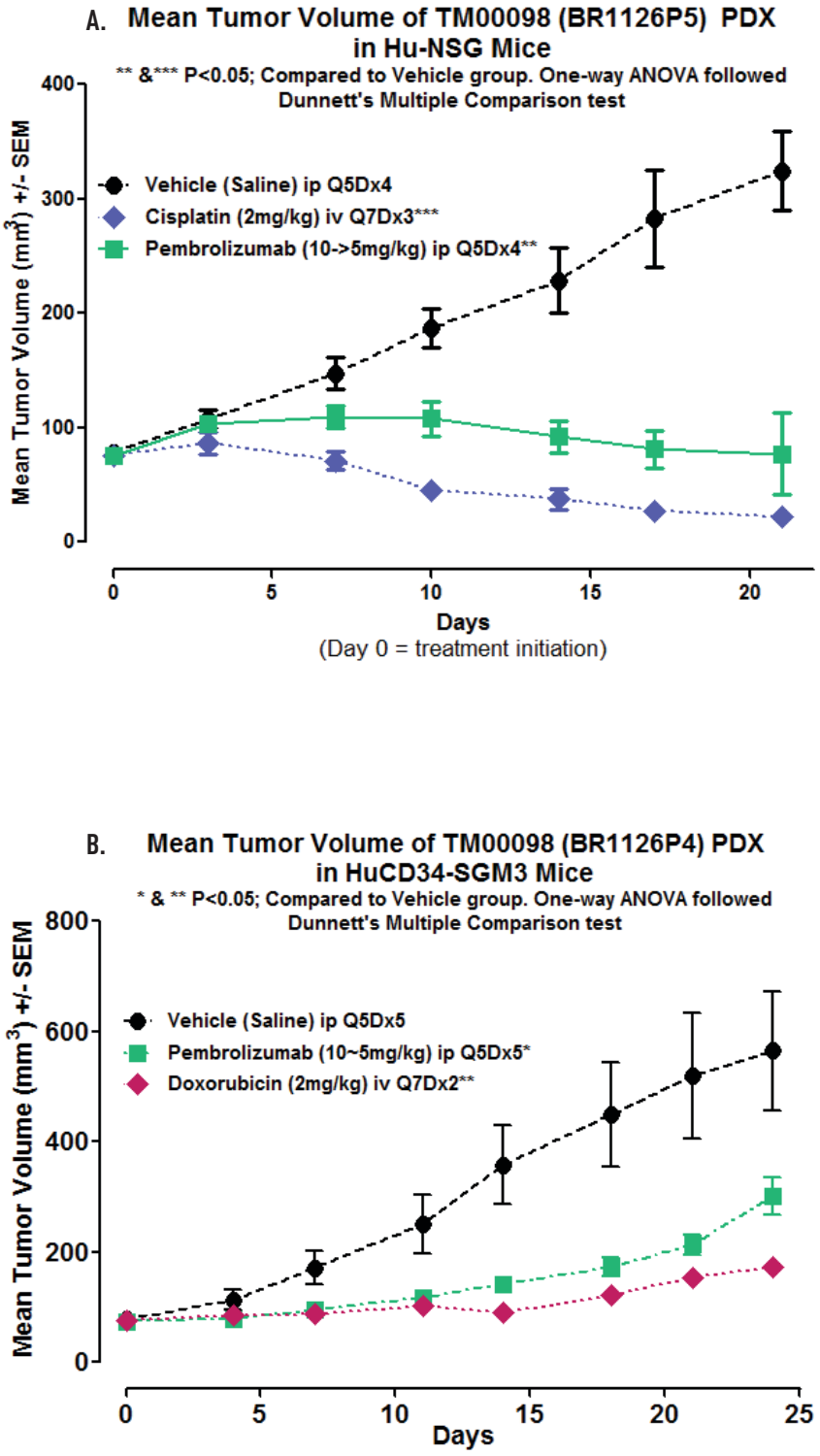



**Figure 6. PD-L1 expressing allogenic PDX triple negative breast cancer responds to standard of care therapy and Keytruda.**
Human PDX breast tumor (model TM00098) grown in either hu-C34 NSG (A) or hu-CD34-NSG-SGM3 mice (B) were treated with either standard of care therapies (Cisplatin or Doxorubicin, as indicated in figure) or Keytruda and compared to vehicle group. P<0.05 by one-way ANOVA followed by Dunnett's Multiple Comparison Test.

Similar experiments were performed using allogenic human non-small cell lung cancer (NSCLC) PDX fragments, which exhibited PD-L1 cell surface expression on 89.1% of cells by flow cytometry (Figure 7). These PDX tumors were engrafted into hu-CD34-NSG and hu-CD34-NSG-SGM3 mice and treated with vehicle, SOC, Keytruda or CTLA-4 inhibitor, Yervoy. As before, both SOC and check-point inhibitors significantly reduced tumor growth compared to the vehicle control. In the hu-CD34-NSG mice, NSCLC PDX tumors were collected at study completion and analyzed by immunofluorescence. CD8+ T-cells had infiltrated Keytruda-treated tumors indicating that tumor regression may be mediated by cytotoxic T-cells (data not shown).

Together, these experiments demonstrate that human tumors engrafted in hu-CD34-NSG and hu-CD34-NSG-SGM3 mice are able to respond to standard-of-care chemotherapeutics. An even more significant finding, however, is that the engrafted tumors appear to evade human immunity much as they do in the patients from which they were derived. Moreover, treatment with a TIL check-point inhibitor presumably releases T-cells from anergy and stimulates their cytotoxicity towards the tumor. These are all very preliminary experiments and additional work is needed to verify the increased TIL-mediated cytotoxicity in the Keytruda-treated samples. Nevertheless, these data clearly show the hu-CD34-NSG and hu-CD34-NSG-SGM3 mice as powerful platforms for gathering new insights into the interactions of human immune cells and tumors and for testing immuno-oncology and combination therapies.

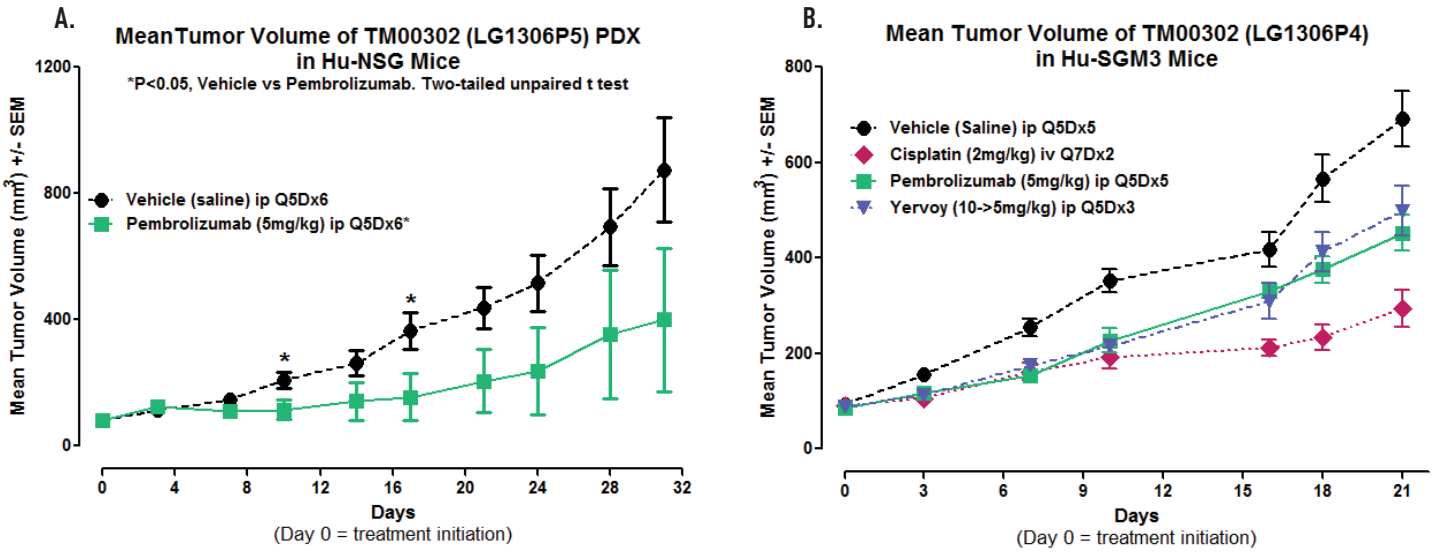



**Figure 7. Allogenic non-small cell lung cancer (NSCLC) PDX responds to checkpoint inhibitor blockade and increases tumor infiltration of cytotoxic T-cells.**
Human PDX lung tumor (model TM00302) grown in either hu-CD34-NSG (A) or hu-CD34-NSG-SGM3 mice (B) were treated with either standard of care therapy (Cisplatin) or checkpoint inhibitors (Keytruda or Yervoy) and compared to vehicle group.

# *Accessing Humanized Mice, Onco-Hu, and PDX Tumors*

Obtaining access to human sources of hematopoietic stem cells and tissues remains a strong barrier to widespread access and construction of these valuable, humanized animal models. Similarly, HSC quality can vary considerably from donor-to-donor, which can significantly impact engraftment efficiency and, ultimately, the quality of the humanized mice created. To assist scientists in gaining ready access to these humanized models, The Jackson Laboratory has developed our humanized mice resource through which we can provide:

- Study-ready cohorts of inventoried hu-CD34-NSG and hu-CD34-NSG-SGM3 mice for immediate shipment to your institution.
- NSG mice engrafted with human peripheral blood mononuclear cells (hu-PBMC NSG and hu-PBMC NSG-SGM3 mice).
- Onco-Hu mice, humanized NSG and NSG-SGM3 mice engrafted with PDX tumors.
- Customized drug efficacy studies executed by our *In Vivo* Services scientists.

The hu-PBMC NSG and hu-PBMC NSG–SGM3 mice have not been tested for growth of human tumors, but may prove equally important in developing immuno-oncology-based therapies for aggressive hematological cancers such as AML and other leukemias (Theocharides *et al* 2016). Humanized mice from The Jackson Laboratory can be imported directly into your facility and used in your experiments following a standard 1-2 week acclimation period. The engrafted human HSCs are tested and free of HIV, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and lymphocytic choriomeningitis virus (LCMV).

Further, The Jackson Laboratory has partnered with 25 cancer clinics nation-wide to provide access to a variety of patient-derived xenograft cancer models at earlier passage stages than any other commercial PDX repository. Currently, more than 400 unique PDX oncology models from either treatment-naive or treatment-resistant patients have been established in the highly immunodeficient NSG mouse strain (Figure 8). NSG mice engraft primary human tumors that at low passage retain the genetic and phenotypic heterogeneity typically seen in human cancers, offering a distinct advantage over other PDX hosts. This preclinical platform can predict the effectiveness of novel therapeutics to treat cancer patients.

# *Perfoming Efficacy Studies Using New Immunotherapeutics*

The Jackson Laboratory can provide you with experimental cohorts of any of our PDX cancer models engrafted in NSG mice or in humanized NSG mice. Alternatively, our Ph.D.-trained study directors can conduct either standardized or fully customizable efficacy studies using these models for you at our facility. Typical studies that we perform may include pilot oncology or immuno-oncology efficacy studies for single or combination drug dosing, dose-response relationship analyses, or compound tolerability studies. To assist you in selecting a tumor model, detailed descriptions of our PDX tumors are available from the Patient Derived Xenograft Search Form on the Mouse Tumor Biology website: (tumor.informatics.jax.org).

The idea of harnessing immune cells to fight cancer is not new, but only in the past few years have scientists demonstrated what a game-changer cancer immunotherapy can be in the clinic (Mueller 2015). Currently, only a subset of patients responds to immunotherapy. It is critical to understand why. Tumor-bearing humanized NSG and NSG-SGM3 mice are a valuable preclinical testing platform to address outstanding questions in this rapidly advancing field: 1) Which tumor types will be most responsive to immunotherapy and what are the underlying mechanisms? 2) How can optimal therapy combinations be determined for individual patients? 3) What are the mechanisms behind resistance to immunotherapy and immunomodulatory agents? 4) How can resistance be anticipated and prevented? New insights will forge the way towards improving existing therapies and developing new approaches.

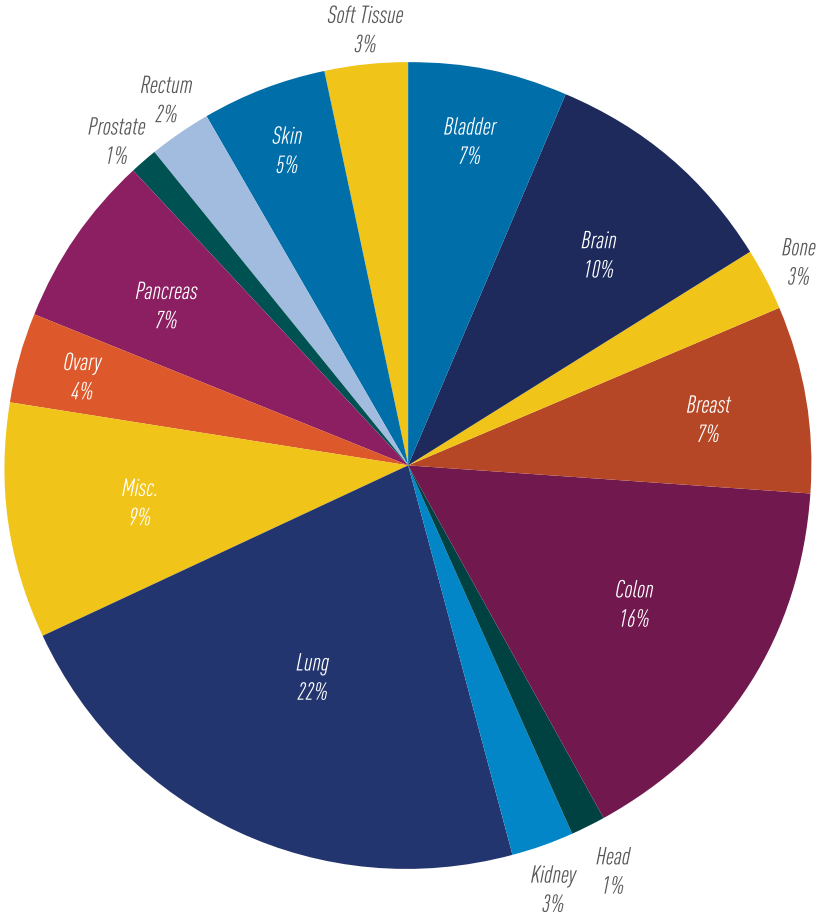



**Figure 8. PDX tumor types available from The Jackson Laboratory.** Over 400 low passage tumors have been collected and grown in NSG mice. Tumors are characterized for gene expression, gene deletion, and copy number variation. Growth characteristics and histology are available, also.

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