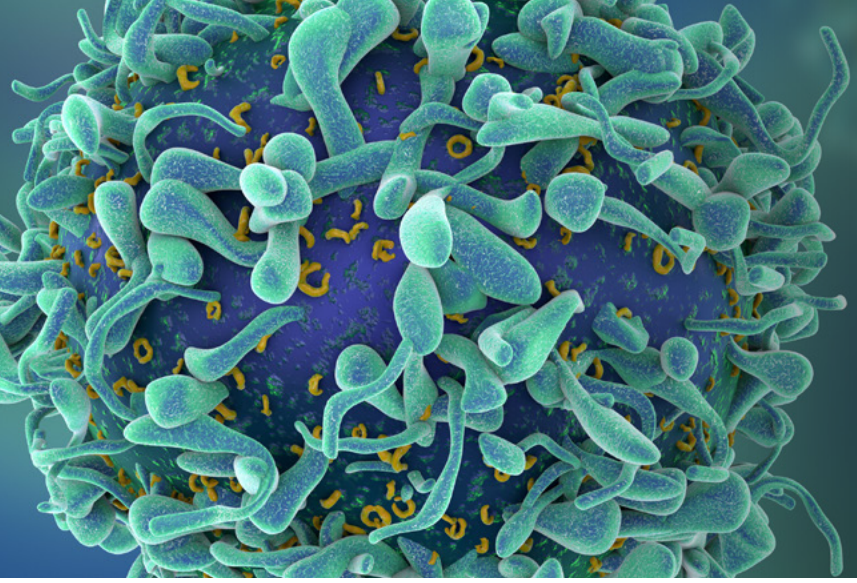


ENVIGO

Case study

Validation of the Immunodeficient B-NDG Mouse for Generating PDX and CDX Tumor Models

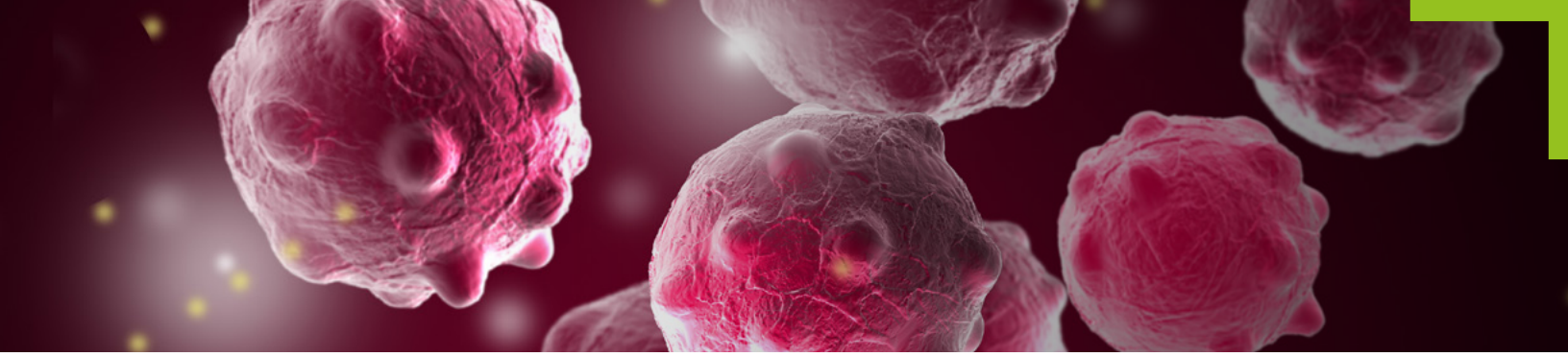
Introduction

Immunodeficient mice play an important role in oncology research, serving as a platform to generate powerful *in vivo* models that can be employed to test investigational agents and understand tumor biology.

Not all strains of immunodeficient mice are created equal. Different genetic backgrounds or targeted genes can lead to varying levels of immune dysfunction and ability to accept human cells. For example, athymic nude mice lack T-cells, while SCID (C.B-17/IcrHsd-*Prkdc*^{scid}) mice show severe defects in T- and B-cell development. However, residual immune function or "leakiness" as the mice age can limit their utility as hosts for human cells.

Advances in technology have led to the development of mice with highly compromised immune systems that support a broader range of human cells. These next-generation models have been widely adopted in oncology research to generate patient-derived xenograft (PDX) and cell line xenograft (CDX) models in which cells derived from cancer patients are directly grown and passaged *in vivo* or cultured *in vitro* prior to implantation. PDX models have been particularly valuable, as they better recapitulate many of the features of human tumors, including pathohistological, genomic and gene expression profiles, and drug response (Xu 2019).

This article highlights the B-NDG mouse, a new addition to the portfolio of commercially available immunodeficient research models. Representing the next generation of ultra-immunodeficient mice, the B-NDG model provides researchers with a new option for evaluating novel and standard therapeutics and investigating tumor–stroma interactions. B-NDG mice have been referenced in over 20 peer-reviewed publications, and Envigo is working closely with others and conducting internal studies to fully characterize their utility in producing CDX and PDX models for oncology and immuno-oncology research.



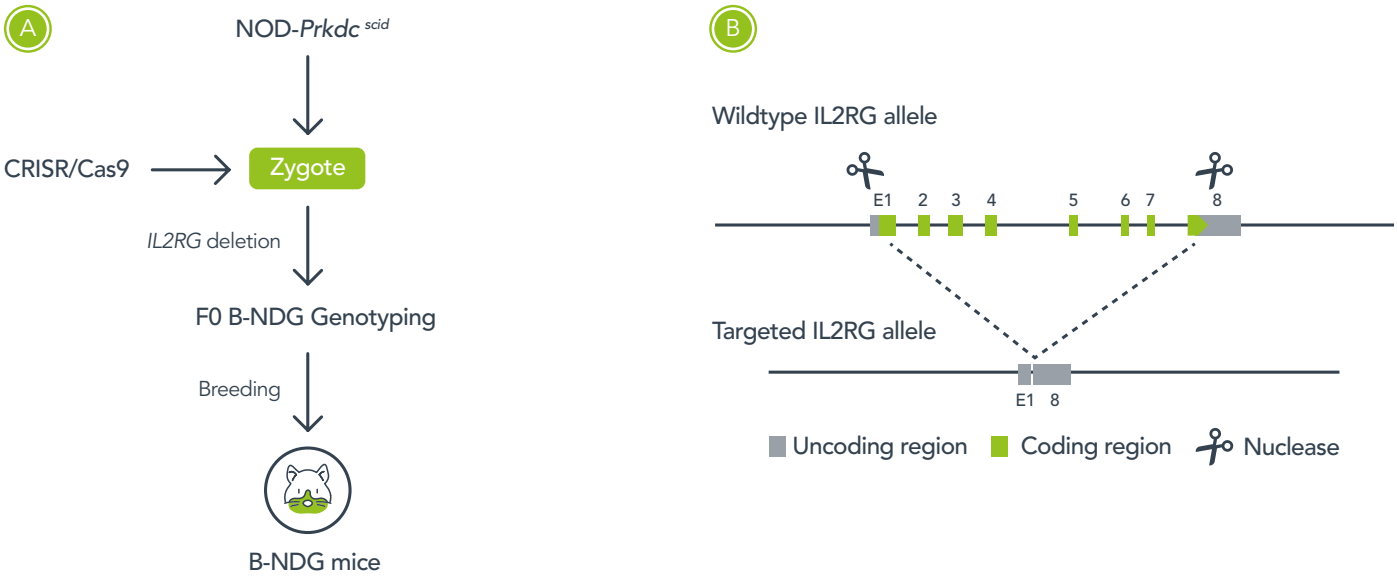
The B-NDG model

B-NDG mice are an albino strain in which the *IL2RG* (common gamma chain) gene is deleted from NOD.SCID (NOD.CB17 SCID) mice (Figure 1A). The model was generated by Biocytogen and is now commercially available from Envigo through a license established in 2019.

Deleting the *IL2RG* gene leads to the absence of functional receptors for a variety of cytokines that are critical for Natural Killer (NK) cell activity, including IL-2, IL-4, IL -7, IL-9 and IL -15 (Sugamura 1996). The *Prkdc* (protein kinase DNA-activated catalytic) mutation results in B- and T-cell deficiencies, and the NOD (non-obese diabetic) background is associated with numerous immune defects. Together, these mutations render B-NDG mice an ultra-immunodeficient model that is ideally suited for human cell engraftment.

Another highly immunodeficient strain is JAX® NSG™ mice (exclusively distributed by The Jackson Laboratory), which carry an alteration of the *IL2RG* gene on a NOD.SCID background. These mice were generated using older recombinant DNA technology and feature the replacement of part of exon 3 and all of exons 4–8 with a neomycin resistance cassette. This approach results in a null mutation of the *IL2RG* gene with partial and complete loss of the extracellular domain and transmembrane/cytoplasmic domains of the protein, respectively. In contrast, B-NDG mice were independently designed and generated using newer CRISPR-Cas9 technology to delete the entire coding region of the gene, reducing the risk of leakiness in cytokine signaling (Figure 1B).

Figure 1. Generation of the B-NDG mouse model. (A) The steps for deleting the *IL2RG* gene from NOD.SCID mice. (B) Schematic of how the entire coding region of the mouse *IL2RG* gene was deleted using CRISPR-Cas9.



Applications for CDX and PDX model development

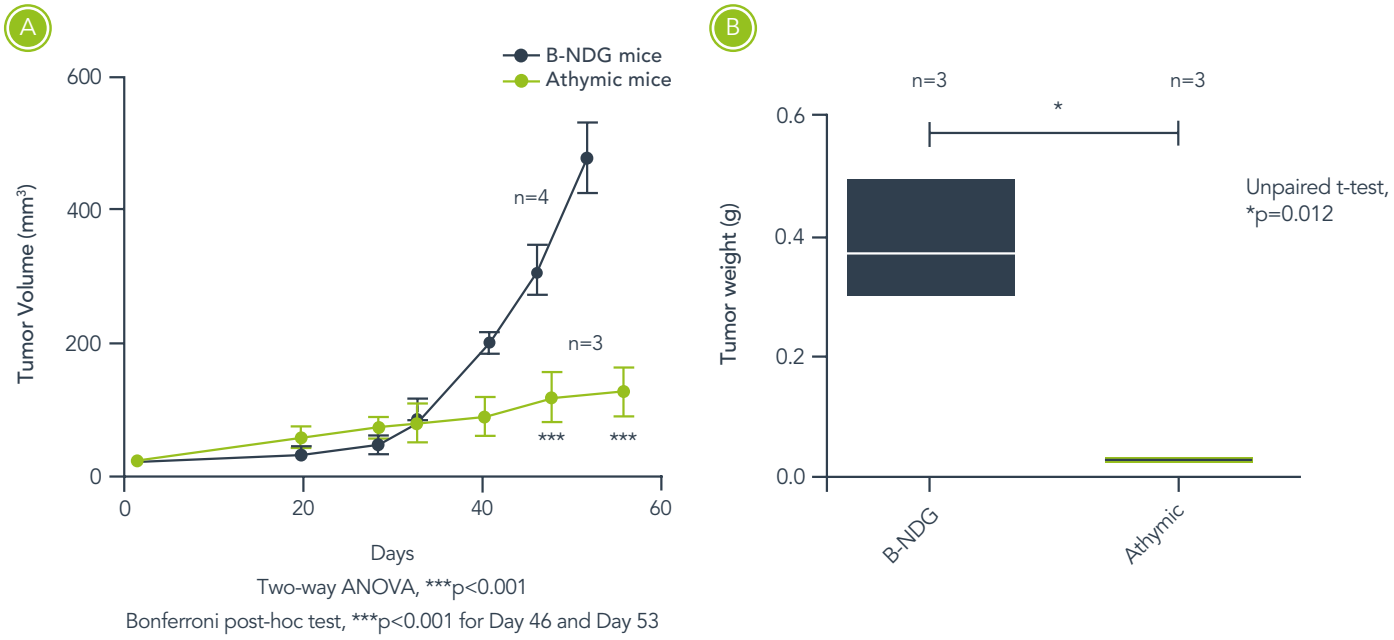
CDX: Lung Cancer

Dr. Rosa Bosch and Dr. Alberto Villanueva at Xenopat SL (Spain) and Dr. Álvaro López-Medrano from Plebiotic SL (Spain) collaborated to compare the growth kinetics of a human non-small-cell lung cancer (NSCLC) cell line using B-NDG and athymic nude mice. The researchers implanted tumor tissue subcutaneously in the flank and closely monitored tumor size for 60 days.

After an initial period of comparable growth over the first four weeks, tumors in the B-NDG animals consistently reached a significantly larger average volume by ~40 days post-injection (Figure 2A). This trend continued until the end of the study, with growth stabilizing in athymic mice below 200 mm^3, whereas the average final tumor volume in B-NDG mice was greater than 400 mm^3. The tumor weights for the two groups of animals are shown in Figure 2B.

The performance of B-NDG in this study indicates that the model is superior to athymic mice in supporting NSCLC human tumor cell growth and ideal for testing anticancer agents for their ability to delay tumor growth.

Figure 2: (A) Tumor growth of human NSCLC cells in B-NDG and athymic mice. (B) Orthotopic growth of NSCLC cells in B-NDG and athymic mice.



Two-way ANOVA, ***p<0.001

Bonferroni post-hoc test, ***p<0.001 for Day 46 and Day 53

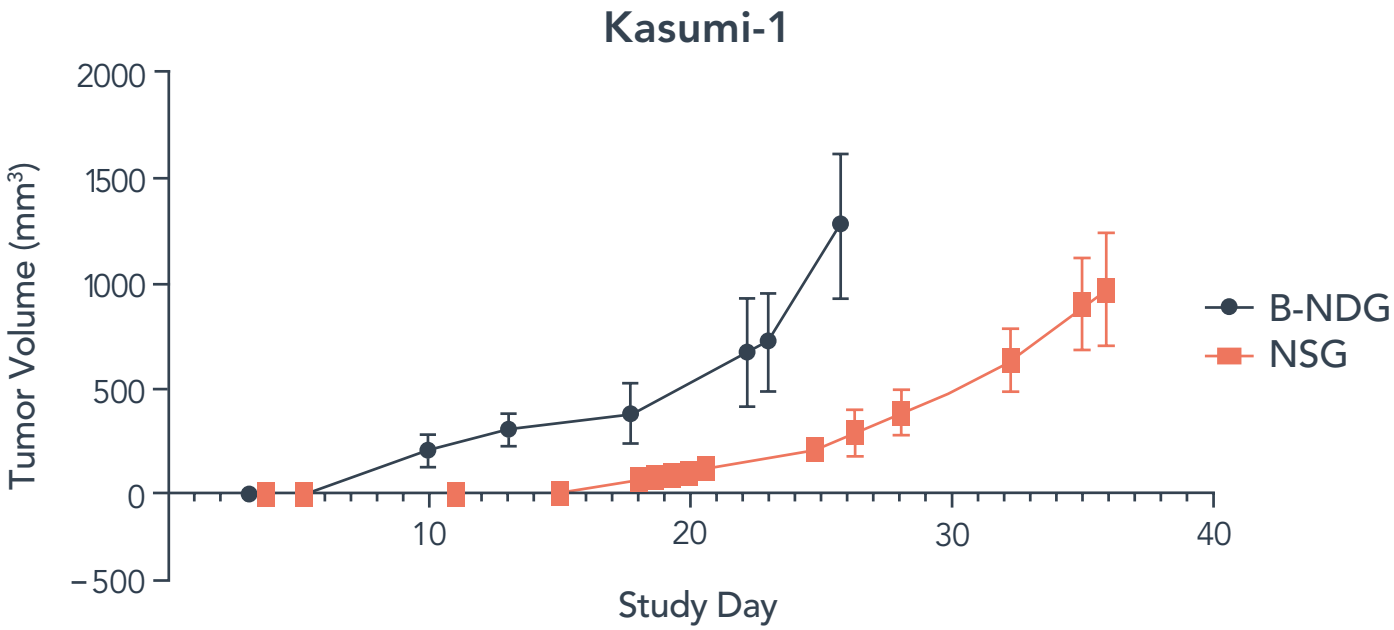
CDX: Acute Myeloid Leukemia (AML)

In a different comparator study, scientists at Crown Bioscience, Inc. (a JSR Life Sciences Company) assessed the growth of human leukemia cells in B-NDG and NSG™ mice. Animals were maintained in a barrier under controlled environmental conditions and consumed Teklad Global Rodent Diet 2919 (19% protein). Approximately 1.0 x 10^7 Kasumi-1 AML cells (sourced from ATCC) were mixed with Corning Matrigel® GFR (1:1 dilution) and inoculated subcutaneously in the right flank of six-week old female B-NDG and NSG™ mice (*n* = 10 in each group). Tumors were measured multiple times per week.

Tumor growth in the B-NDG cohort was substantially faster and reached the volume goal much earlier in the study (Figure 3). At the study endpoint, tumors in the B-NDG mice had an average volume of approximately 1,000 mm^3, compared to the slower-growing tumors in NSG™ mice, with an average volume of 500 mm^3.

The study results demonstrate that B-NDG mice were superior for CDX model development using the Kasumi-1 human tumor cell line. The better performance of the B-NDG model ultimately led to faster project turnaround (~10 days earlier) and cost savings due to shorter study duration and less time spent in the barrier.

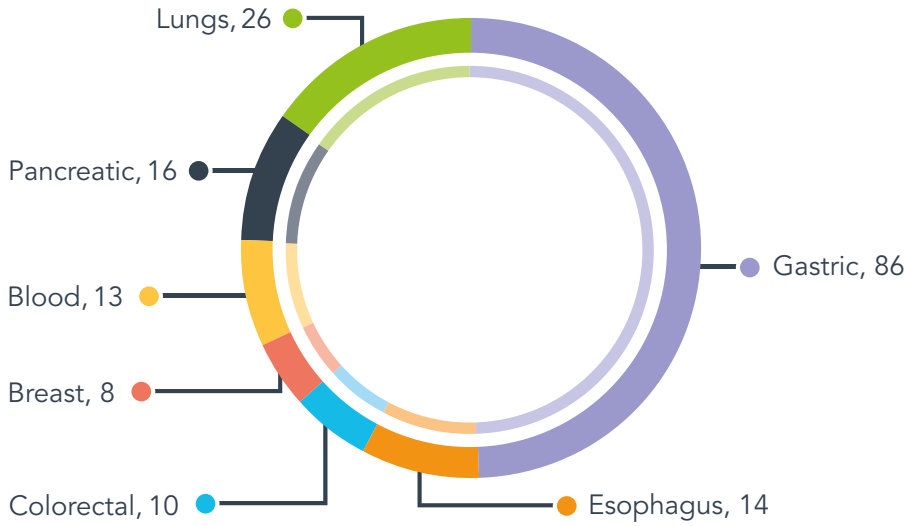
Figure 3: Tumor growth rate of human Kasumi-1 AML cells in B-NDG and NSG™



Patient-Derived Xenografts (PDXs)

The B-NDG mouse model has been successfully used to establish more than 170 PDX models across a variety of tumor types, including both solid and hematological malignancies (Figure 4). The results of studies conducted in some of these models are presented in the sections below.

Figure 4: PDX models established in B-NDG mice.

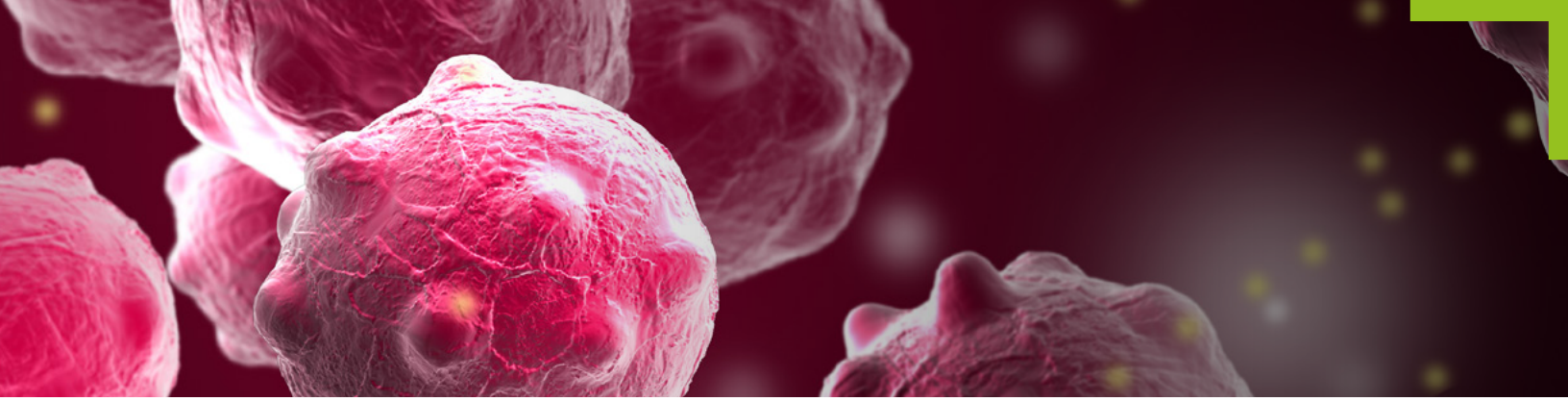


Key ● Gastric ● Esophagus ● Colorectral ● Breast ● Blood ● Pancreatic ● Lung

Want to find out more?

To learn more about Envigo's Tumor models and services, please visit:

envigo.com/tumor-models-and-services



PDX: Breast Cancer and Melanoma

Envigo carried out an in-house comparative study to evaluate the growth of breast cancer and melanoma PDX models in three strains of mice:

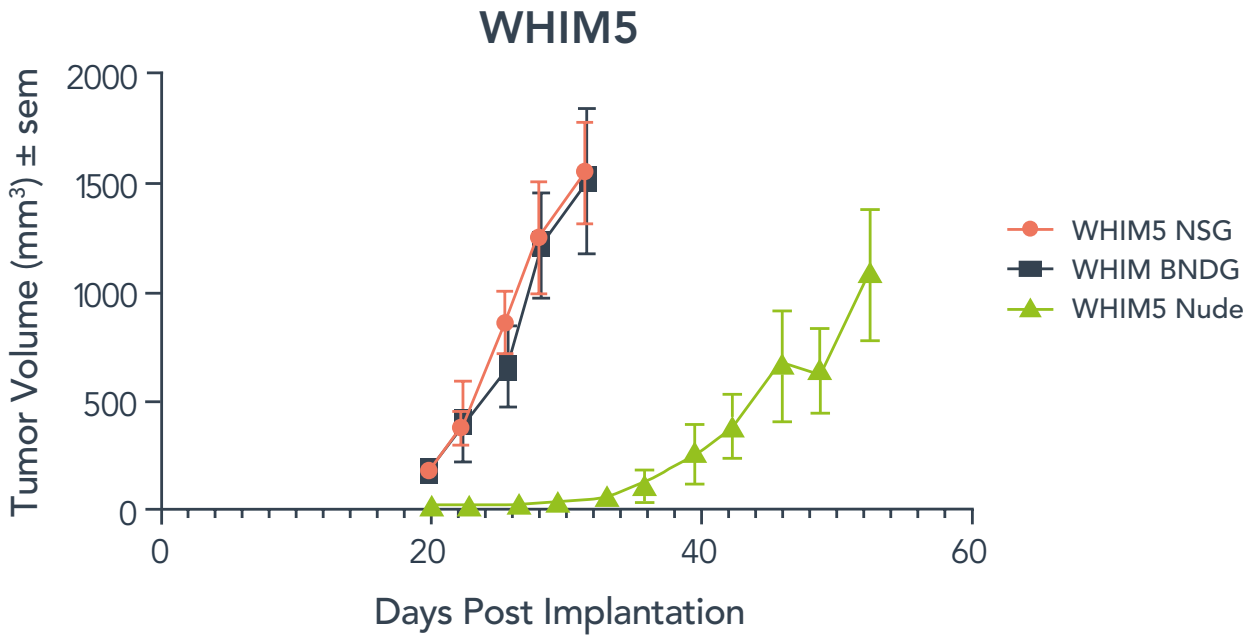
+ B-NDG
+ NSG™
+ Athymic nude

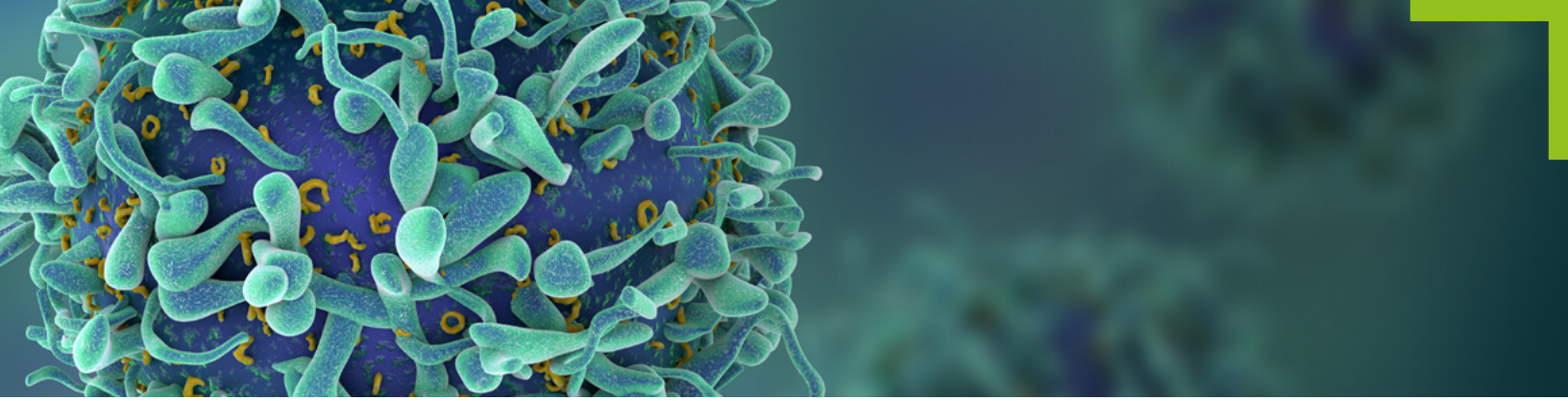
For these experiments, $1.5x10^6$ WHIM5 (breast cancer) or WM-4071-2 (melanoma) PDX cells were mixed with Corning Matrigel® GFR (1:1 dilution) and implanted into the subcutaneous space of the right flank of eight-week old female mice.

Tumors were measured multiple times per week. Mice were housed in individually vented cages and consumed Teklad Global Rodent Diet 2919 throughout the study.

The growth rate of WHIM5 tumors was similar between the B-NDG and NSG™ models. Both groups achieved nearly the same final average volume, approximately 1,500 mm^3 (Figure 5). In contrast, the tumor size and rate of tumor growth were less in the athymic nude mouse group.

Figure 5: Tumor growth rate of WHIM5 PDX cells inoculated into B-NDG, NSG™, and athymic mice.

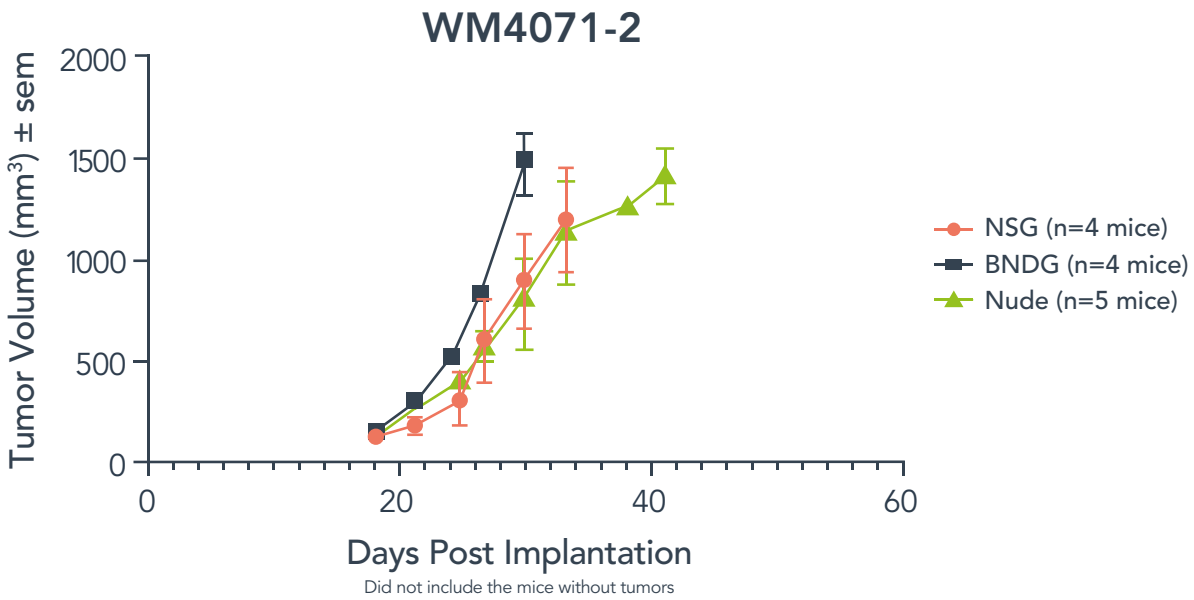




For the melanoma PDX study, the B-NDG group showed a slightly more rapid tumor growth compared to both the NSG™ and athymic mice, reaching a larger average volume earlier in the assessment window (Figure 6).

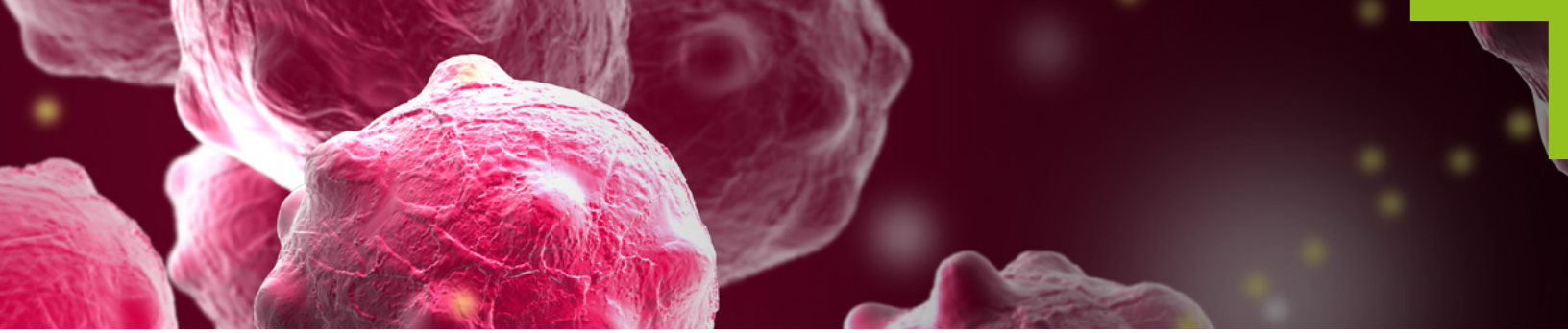
In comparison to its performance in the breast cancer PDX study, growth of the melanoma PDX model was robust in the athymic nude animals, showing similar growth kinetics and tumor volume as the NSG™ model.

Figure 6: Tumor growth rate of WM4071-2 PDX cells inoculated into B-NDG, NSG™, and athymic mice.



These findings suggest that B-NDG and NSG™ both perform well in establishing PDX models and, depending on tumor type, the B-NDG model has the advantage of more efficient tumor growth rates.

Although athymic mice consistently showed less robust PDX growth, they remain an invaluable tool, particularly for fast-growing cell lines or fluorescently labeled/bioluminescent cells or whole-body imaging.



PDX: Gastric PDX

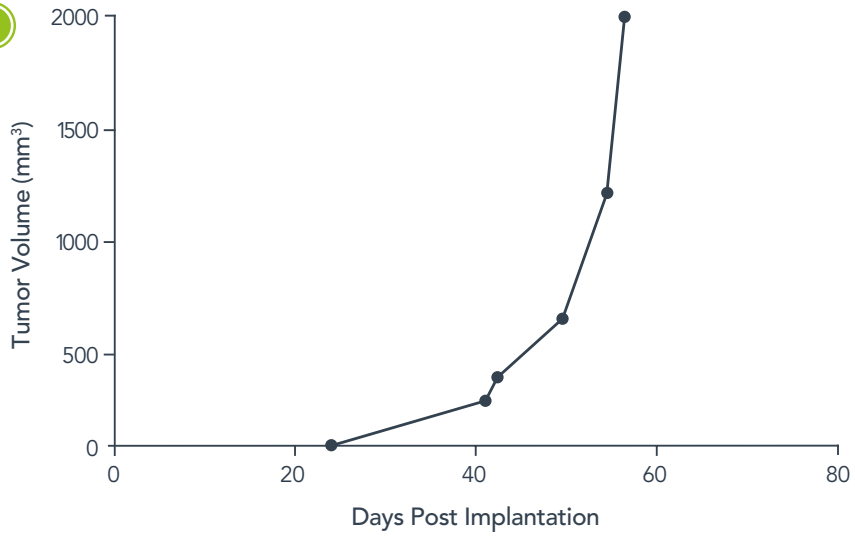
Biocytogen scientists have used B-NDG to develop a gastric cancer PDX model. In this study, B-NDG and C.B17-*scid* mice were implanted with gastric PDX cells, and tumor size was measured using calipers to 60 days post-implantation. Strikingly, none (0/6) of the C.B17-scid mice developed tumors, while PDX were successfully established in 4/6 B-NDG animals, reaching 2,000 mm^3 by 60 days (Figure 7A and 7B).

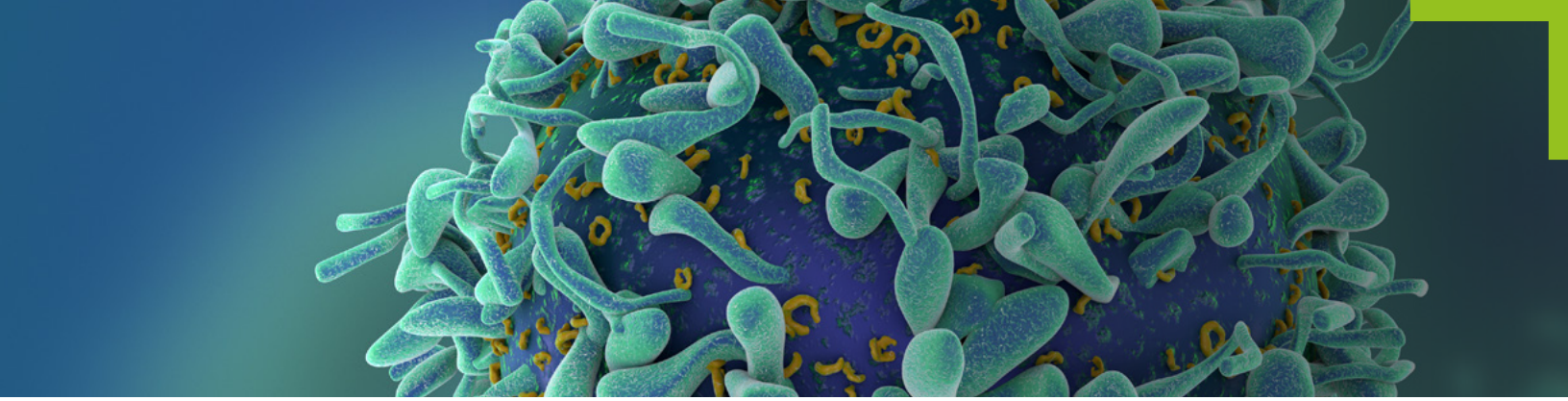
Figure 7. Comparison of gastric cancer PDX growth in C.B17 scid and B-NDG mice.
(A) Take rate and tumor growth kinetics in B-NDG mice and C.B17 scid mice.
(B) Average tumor growth in B-NDG mice 60 days after PDX implantation.

A

Gastric Cancer PDX	CB17-*scid*	B-NDG	
ID	Tumor Size to 250 mm^3	Tumor Size to 250mm^3	Tumor doubling time
1	-	40 days	7 days
2	-	56 days	5 days
3	-	66 days	10 days
4	-	-	-
5	-	-	-
6	-	40 days	5 days
	Tumor take rate 0% (0/6)	Tumor take rate 66.7% (4/6)	Average doubling time 6.75 days

B





Conclusions

In summary, the B-NDG model is unique among currently available immunodeficient strains of mice. Built using cutting-edge technology and featuring minimal rejection of human-derived cells, B-NDG mice offer an excellent platform to develop advanced *in vivo* CDX and PDX preclinical models for oncology research.

For more information on the B-NDG mouse, please read our white paper (July 2020) titled

Characterization, Development, and Applications of the Novel Ultra-Immunodeficient B-NDG Mouse.

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