



We help you discover life

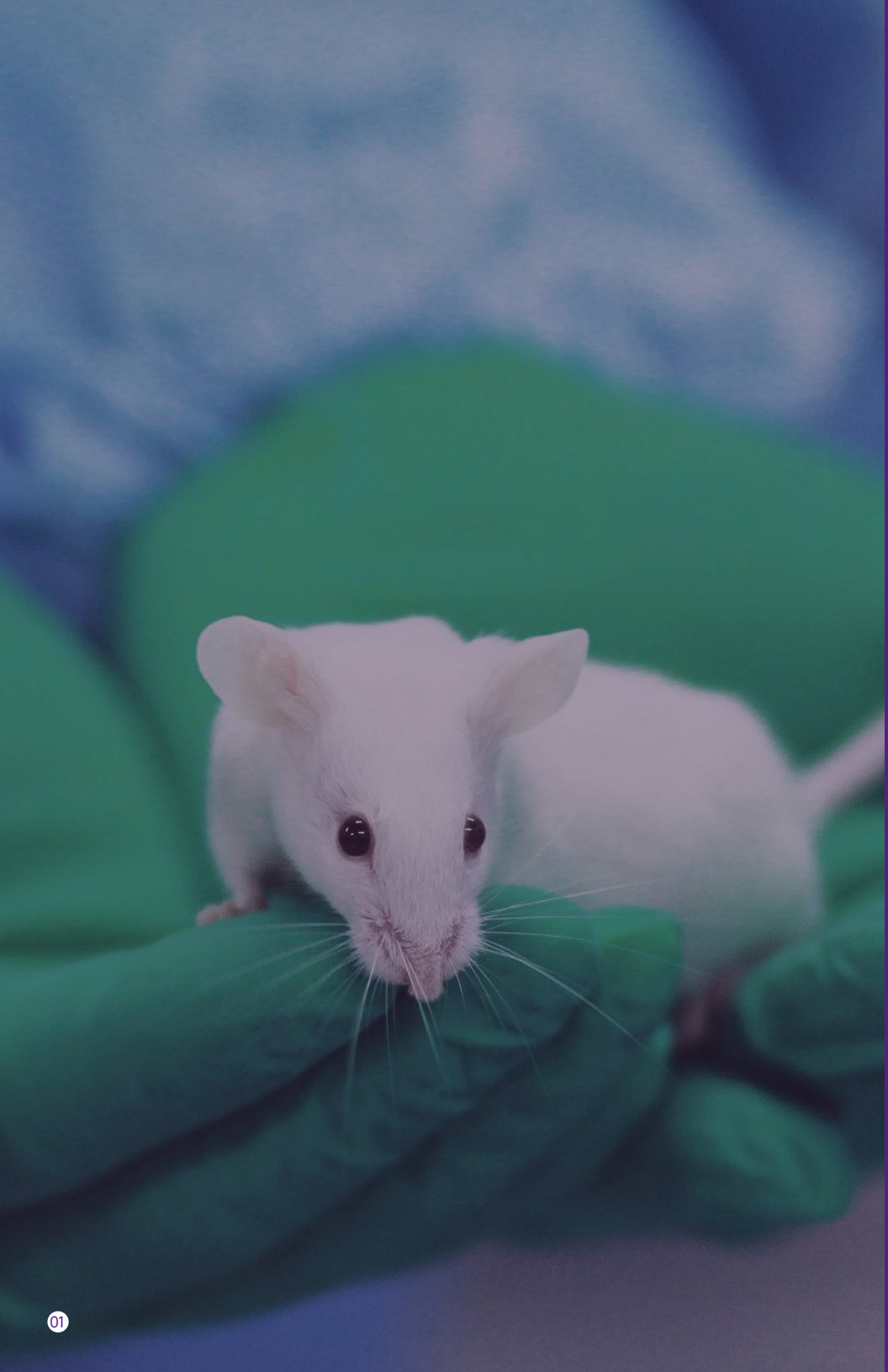


Cyagen: Your Partners in Innovation

NKG Immunodeficient Mice

Explore the mechanism of tumor occurrence and development, drug screening and efficacy evaluation

- Immunodeficient NKG mice:
highly effective in supporting PDX and HIS model implantation
- Continue to expand the NKG series product line to meet diverse research needs



CONTENTS

Introduction to NKG Mice	03
NKG Mice Validation Data	06
Application of NKG Mice	06
Next Generation NKG Mice	10
Case Studies	14
FAQs	27
Publications	33

Introduction to NKG Mice



NKG is a currently recognized mouse model with an extremely high degree of immunodeficiency, independently developed by Cyagen by knocking out the Il2rg gene on the NOD-Scid background strain. NKG mice exhibit a severely compromised immune system, characterized by the absence of mature T, B, and NK cells, diminished complement activity, and impaired macrophage phagocytic capacity against human cells. The incidence of T and B cell leakage remains exceptionally low in aged NKG mice, and they also demonstrate minimal lymphoma development, rendering them highly suitable for both short-term and long-term experimental applications.

►► NKG and Its Variants

Product Type	Product Number	Product Name	Features and Research Applications
Absence of T, B, NK Cells	C001316	NKG	■ Advanced immunodeficient mouse model with significantly higher survival rate for human cell and tissue transplants, capable of engrafting a higher proportion of normal or cancerous human cells and tissues ■ Suitable for research in oncology, immunology, autoimmune diseases, immunotherapy vaccines, GvHD/transplantation, and drug safety assessment
	C001416	NKG B2m KO	■ Postpones GvHD (Graft-versus-host disease) development, significantly prolonging the experimental window for longitudinal studies ■ Used in the establishment of humanized immune system mouse models, GvHD research
	C001498	NKG-H2-Ab1 KO	■ Effective reconstruction of CD8+ T cells, improved survival post-PBMC transplantation ■ Used for PBMC immune system reconstitution and GvHD research
	C001559	NKG-MHC I/II dKO	■ Efficiently delays the onset of Graft-versus-host disease (GvHD) ■ Used for PBMC immune system reconstitution and GvHD research
	C001513	NKG-hIL15	■ Enables effective reconstruction of NK cells ■ Used for immunotherapy, drug screening research
	I001175	NKG-Kit*V831M	■ Hematopoietic stem cell (HSC) reconstitution can be free from irradiation ■ Used for HSC immune system reconstitution, oncology, immunology, and drug safety evaluation, etc

Product Type	Product Number	Product Name	Features and Research Applications
Absence of T, B, NK Cells	I001176	NKG-hIL6	- Enhances monocytes and macrophages - Used for immune system reconstitution, oncology, immunology, and drug safety evaluation, etc
	I001177	NKG-SGM3	- Enhances the differentiation of myeloid cells - Used for immune system reconstitution, oncology, immunology, and drug safety evaluation, etc
	C001695	NKG-SGM3/hIL6/Kit*V831M	- Enhance myeloid cell differentiation and monocytes/macrophages. Enable irradiation - free HSC reconstitution - Used for HSC immune system reconstitution, oncology, immunology, and drug safety evaluation, etc

►► Other Immunodeficient Mice and Rats

Product Type	Product Number	Product Name	Features and Research Applications
Absence of T, B, NK Cells	C001367	B6RG	- NK cell activity is almost lost - Used for immune system deficiency, cancer, toxicology, xenotransplantation research
	C001436	BRG	- NK cell activity is almost lost - Used for oncology, immunology, infectious diseases, stem cell biology research
	IR1023	SDRG	- Absence of mature T cells, B cells, and circulating NK cells - Used for oncology and immunology
Absence of T, B Cells	C001197	Rag1 KO	Used for transplantation of primary cells, hematoma cells, and other tumor types
	C001324	Rag2 KO	Used for transplantation of primary cells, hematoma cells, and other tumor types
	CR005	SD-Rag2 KO	Used for transplantation of primary cells, hematoma cells, and other tumor types
Absence of $\alpha\beta$ T Cell Receptor	C001332	Tcra KO	Used for immunology, inflammation, autoimmune research
Absence of NK Cells	C001374	B6-Il2rg KO	Used for oncology, immunology, infectious diseases, stem cell biology research

Product Type	Product Number	Product Name	Features and Research Applications
Absence of B Cells	C001344	B6-Ighj KO	■ Used for immunology, inflammation, autoimmune, B cell deficiency research
	C001345	BALB/c-Ighj KO	■ Used for immunology, inflammation, autoimmune, B cell deficiency research
	C001340	B6-Ighm KO	■ Used for immunology, inflammation, autoimmune, B cell deficiency research
	C001341	BALB/c-Ighm KO	■ Used for immunology, inflammation, autoimmune, B cell deficiency research
	C001342	B6J-Ighm Ighd-DKO	■ Used for immunology, inflammation, autoimmune, B cell deficiency research
	C001343	BALB/c-Ighm Ighd-DKO	■ Used for immunology, inflammation, autoimmune, B cell deficiency research
Il12a Deficiency	C001213	Il12a KO	■ Used for cardiovascular, autoimmune, infectious disease research, and studies of immune cells/systems
Il12rb1 Deficiency	C001214	Il12rb1 KO	■ Used for autoimmune, infectious disease research, and studies of immune cells/systems
Alox5 Deficiency	C001215	Alox5 KO	■ Used for hematopoietic regulation, myeloid leukemia pathology, atherosclerosis mechanisms, immune and inflammation response regulation, hematologic tumor research
Igha Deficiency	C001394	B6-IgA KO	■ Used for research on selective IgA deficiency, IgA deficiency related autoimmune diseases, allergic diseases, IgA mechanisms, and functions
IgG1 Deficiency	C001390	B6-IgG1 KO	■ Used for IgG1 deficiency diseases, hypogammaglobulinemia related to IgG deficiency, IgG mechanisms, and functions research

NKG Mice Validation Data



► Absence of B, T and NK cells

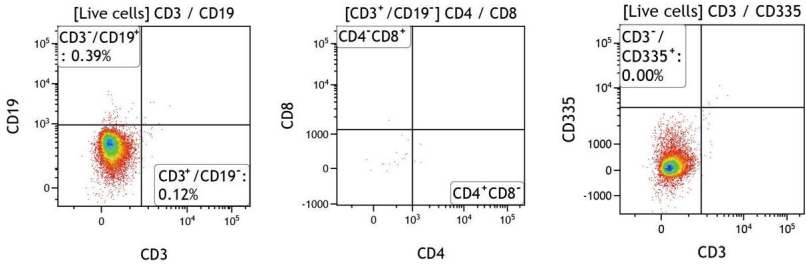


Figure 1. Severe loss of B, T, and NK cells in peripheral blood, manifesting as severe immunodeficiency

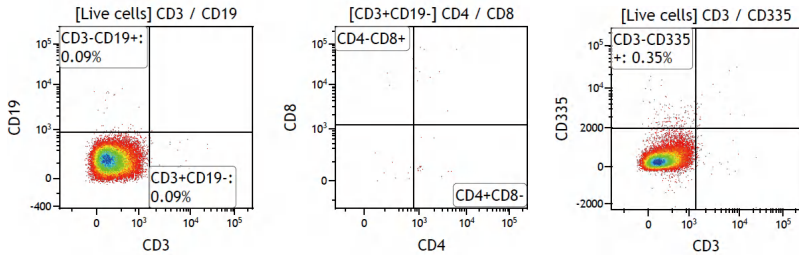


Figure 2. Severe loss of B, T, and NK cells in spleen tissue, manifesting as severe immunodeficiency

Application of NKG Mice



- 1 Compared with NOD-Scid mice, NKG mice have significantly improved survival rates of human cell and tissue transplants and are able to engraft a higher proportion of normal or cancerous human cells and tissues.
- 2 They can efficiently transplant human hematopoietic stem cells (HSCs), peripheral blood mononuclear cells (PBMCs), patient-derived xenografts (PDXs), adult stem cells, and tissues.
- 3 They can be used for research on tumors, immunity, autoimmune diseases, immunotherapy vaccines, GvHD/transplantation, and safety assessment.

► Constructing a Human Immune System Reconstruction Model

Cyagen separately transplanted human peripheral blood mononuclear cells and hematopoietic stem cells into NKG mice to construct immune system reconstituted mice; and huHSC-NKG mice underwent a series of iterative upgrades. Utilizing advanced neonatal mouse technology, these mice can develop various human immune cells, especially the fully humanized immune system mouse model huHSC-NKG-ProF, which can reconstitute lymphoid lineage cells such as T, B, and NK cells, as well as myeloid lineage cells such as dendritic cells (DCs), monocytes, macrophages, and granulocytes.

Mouse Name	Reconstruction Time	Immune Reconstitution Status	Research Applications
huHSC-NKG-ProF	8 weeks, humanization rate 40-60%	Reconstitution of lymphoid T, B, and NK cells, as well as myeloid dendritic cells (DC), monocytes, macrophages, and granulocytes.	Tumor immunology research; autoimmune disease research; drug metabolism and toxicity research
huHSC-NKG-ProM	8 weeks, humanization rate 40-60%	Reconstitution of lymphoid T and B cells, as well as myeloid monocytes.	Tumor immunology research; autoimmune disease research; drug metabolism and toxicity research
huHSC-NKG-ProN	8 weeks, humanization rate 40-60%	Reconstitution of lymphoid T, B, and NK cells.	Tumor immunology research; drug metabolism and toxicity research
huHSC-NKG-hIL15	Rapid reconstitution by week 3 post-transplantation	Reconstitution of multiple immune cells, especially effective reconstitution of human NK cells.	Research on NK cell development mechanisms and related tumor immunotherapy development; antibody-dependent NK cell-mediated cytotoxicity (ADCC) research; human immune system and hematopoietic system research
huHSC-NKG	8 weeks, humanization rate >50%	Reconstitution of lymphoid T and B cells.	Tumor immunology research; autoimmune disease research; drug metabolism and toxicity research
huPBMC-NKG	3 weeks, humanization rate >40%	Predominantly T cells, accounting for over 90%.	Tumor immunology research; anti-GvHD drug research; infectious disease research; gene therapy; research on non-cross-reactive drug targets; immunogenicity testing

HSC Immune System Reconstitution Model – Taking Humanized Immune System Mouse huHSC-NKG-ProF as an Example

Model Validation

a Peripheral Blood

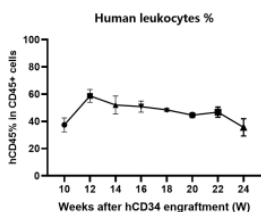


Figure 3. Humanization rate after human HSC transplantation into NKG mice

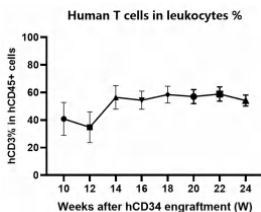


Figure 4. Proportion of human T cells and B cells after human HSC transplantation into NKG mice

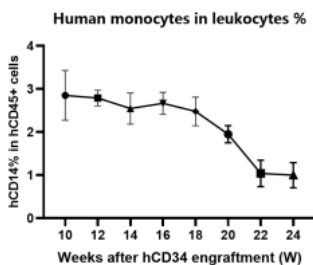
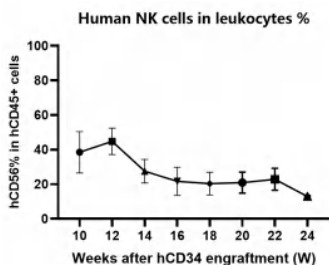
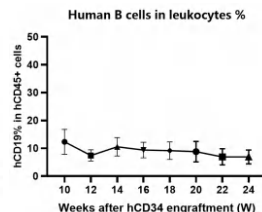


Figure 5. Reconstitution of human NK cells and monocytes in adult NKG mice after human HSC

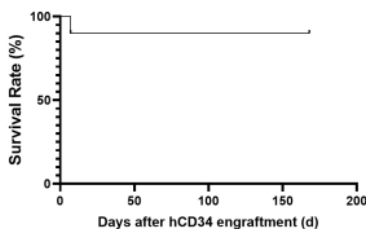
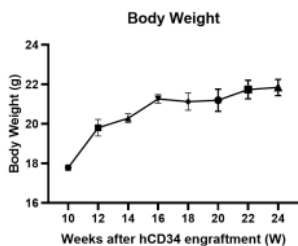


Figure 6. Body weight and survival curve of NKG mice after human HSC transplantation

b Spleen

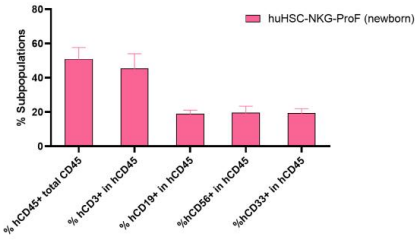


Figure 7. Humanization rate and subpopulations of immune cells in the spleen

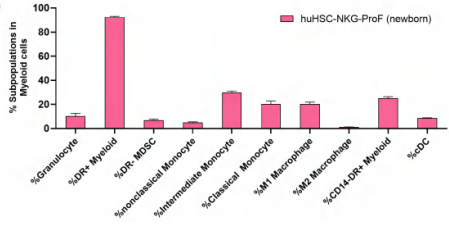


Figure 8. Analysis of myeloid cell subtypes in the spleen

Tumor-Infiltrating Immune Cell Analysis

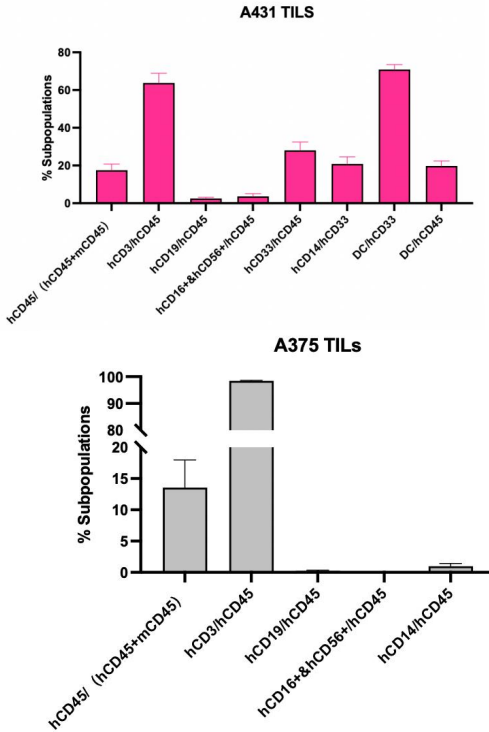


Figure 9. Different tumor cell lines have different types of infiltrating immune cells

Next Generation NKG Mice



NKG-MHC I/II dKO Mice (B2m and H2-Ab1 genes knocked out on the NKG mouse background): Can effectively delay the onset of graft-versus-host disease (GvHD) after PBMC transplantation, and can be used for long-term studies of PBMC immune system reconstitution.

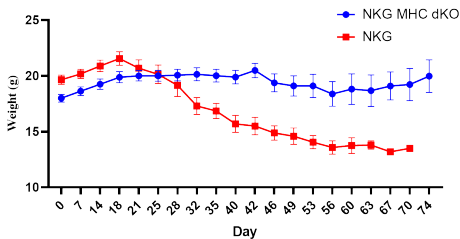


Figure 10. Body weight changes in NKG-MHC I/II dKO mice after PBMC transplantation

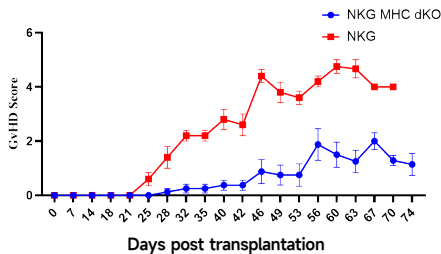


Figure 11. GvHD scores in NKG-MHC I/II dKO mice after PBMC transplantation

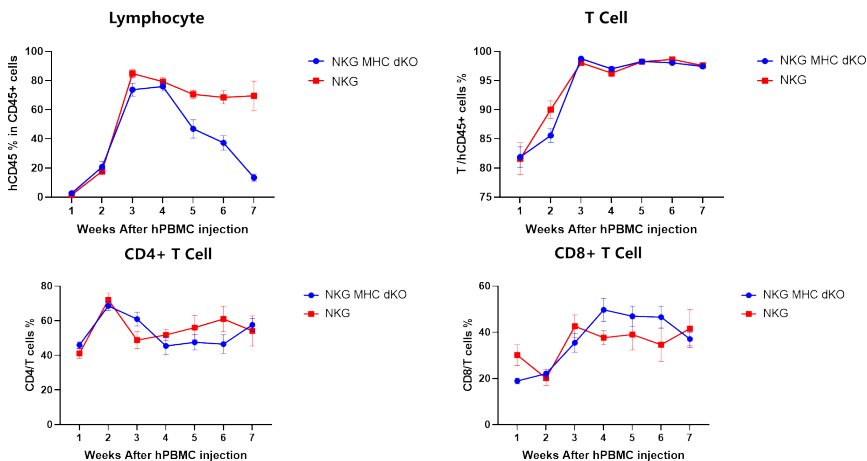


Figure 12. Reconstitution of human lymphocytes and T cells in NKG-MHC I/II dKO mice after PBMC transplantation

Note: NKG-MHC I/II dKO mice were obtained by crossing NKG B2m KO mice (Product No.: C001416) with NKG-H2-Ab1 KO mice (Product No.: C001498), both of which can be provided separately.

■ NKG-hIL15 Mice (human IL15 gene introduced on the NKG mouse background): Can be used for studying NK cell development mechanisms, developing NK cell-related tumor immunotherapies, and antibody-dependent cell-mediated cytotoxicity (ADCC) research.

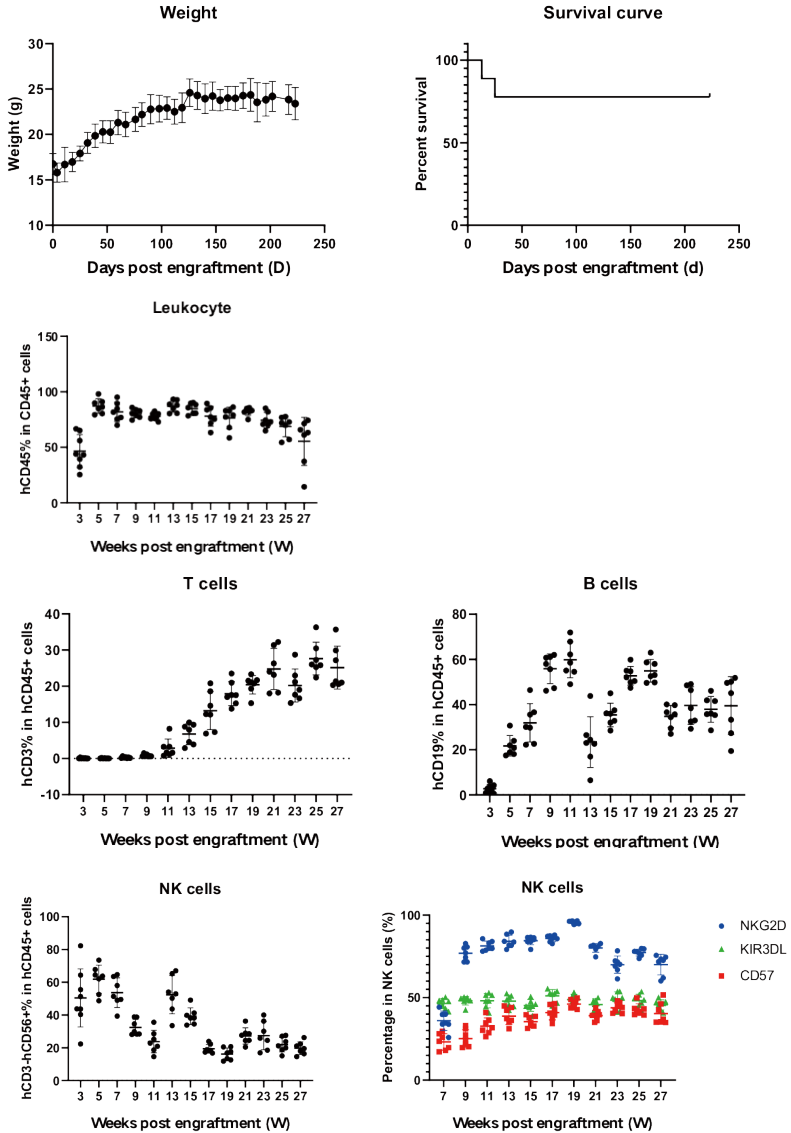


Figure 13. Survival and reconstitution of NKG-hIL15 mice after huCD34+ HSC transplantation

NKG-Kit*V831M Mice (Kit gene mutated on the NKG mouse background): Can avoid irradiation during HSC reconstitution, preventing the side effects of irradiation on the hematopoietic, gastrointestinal, and nervous systems in mice, and can be used for the development and drug evaluation of various tumor immunotherapies.

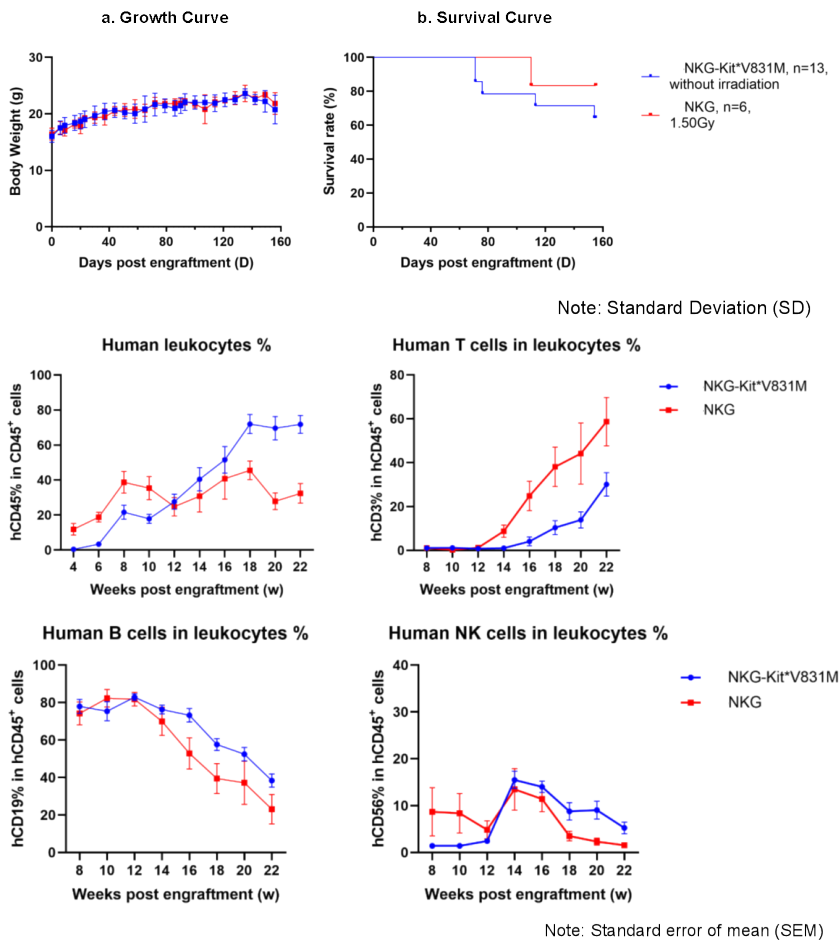


Figure 14. Survival and reconstitution of NKG-Kit*V831M mice after huCD34⁺ HSC transplantation

Note: The reconstitution level of different batches is donor-dependent, and differences between donors may lead to variations in the reconstitution level.

NKG-hIL6 Mice (human IL6 gene introduced on the NKG mouse background): Can efficiently and rapidly reconstitute human CD45+ leukocytes during HSC reconstitution, and can be used for the development and drug evaluation of various tumor immunotherapies.

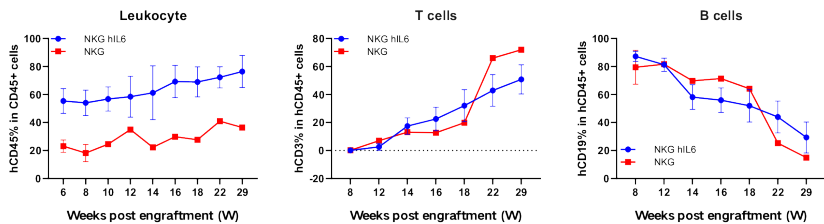


Figure 15. Reconstitution of NKG-hIL6 mice after huCD34+ HSC transplantation

NKG-SGM3 Mice (human SCF (KITLG), GM-CSF (CSF2), and IL3 genes introduced on the NKG mouse background): Can effectively enhance the differentiation of myeloid cells, improve the transplantation efficiency of hematopoietic and acute myeloid leukemia cells, and can be used for immuno-oncology, immunology, and infectious disease research.

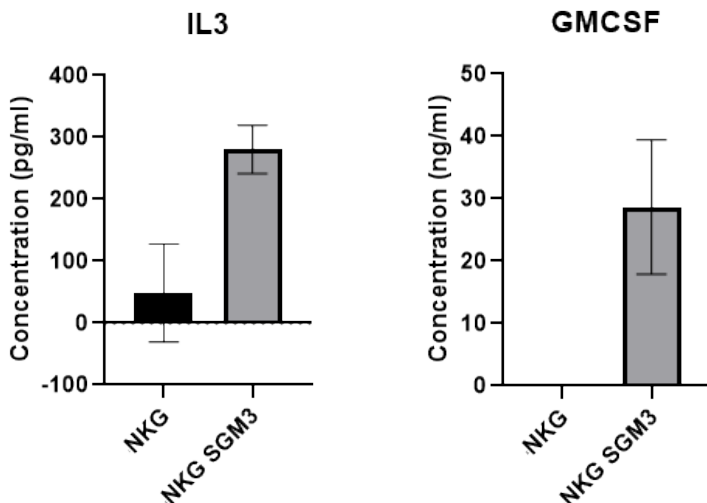


Figure 16. Detection of human IL3 protein and human GM-CSF protein expression



a mRNA Tumor Vaccine Treatment of Melanoma A375

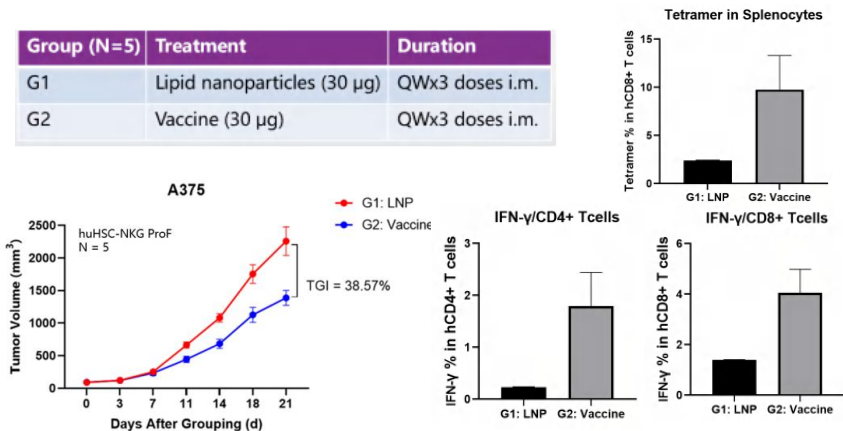


Figure 17. After mRNA tumor treatment, the tumor inhibition rate of melanoma A375 reached 38.75%. Antigen-specific T cell detection showed a significant increase in the proportion of Tetramer+ and IFN-γ+ cells in the vaccine treatment group, indicating that huHSC-NKG-ProF mice possess functional human T cells and dendritic cells. This mouse model can be an effective tool for preclinical efficacy studies of tumor vaccines.

b Rituximab Treatment of Burkitt's Lymphoma Raji

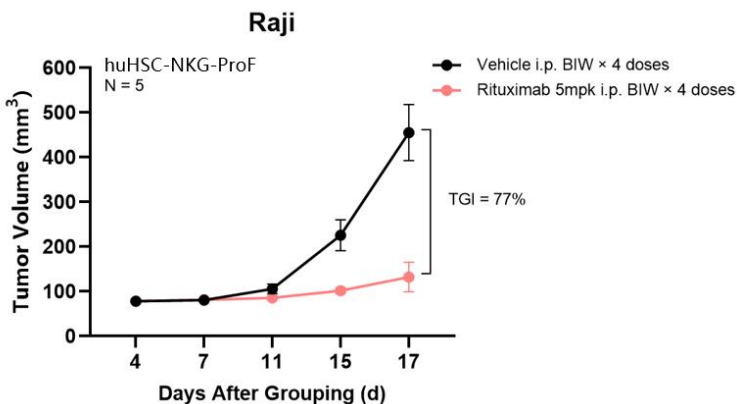


Figure 18. After 4 doses of Rituximab, the tumor inhibition rate reached 77%, showing a significant therapeutic effect, indicating that huHSC-NKG-ProF mice possess functional human NK cells.

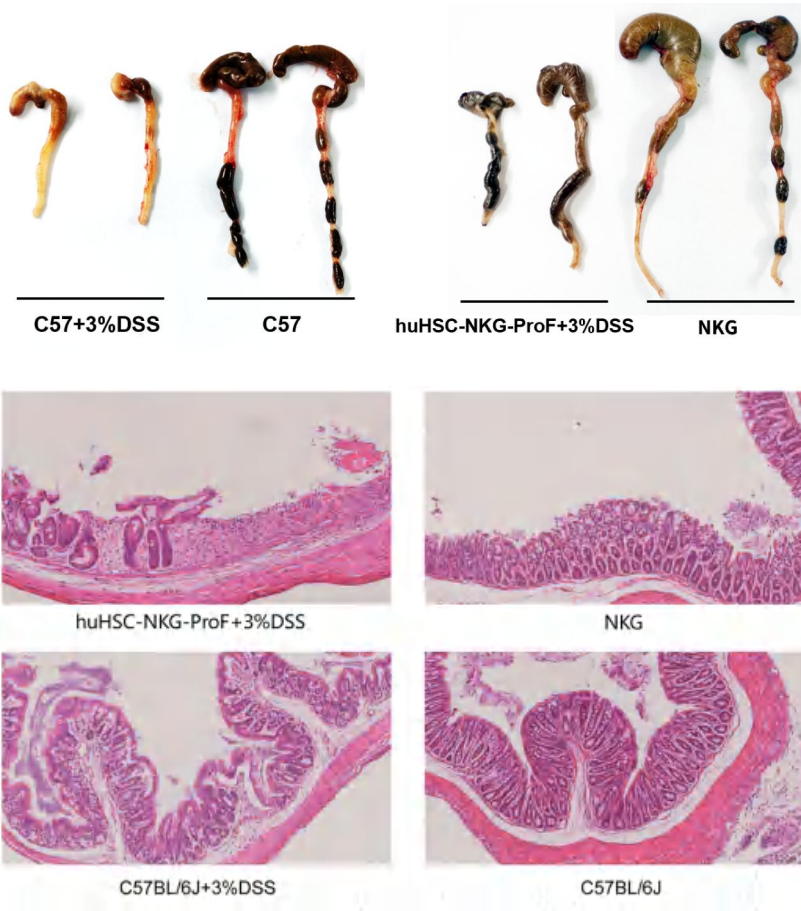


Figure 19. In this experiment, C57BL/6 and huHSC-NKG-ProF mice were fed with 3% DSS. After 7 days, huHSC-NKG-ProF mice showed similar colitis phenotypes to C57BL/6 mice, including ulcers, destruction of the intestinal mucosa, tissue edema and congestion, and destruction of glandular structures, indicating that huHSC-NKG-ProF can be used to construct a humanized colitis model.

HSC Immune System Reconstitution Model – Taking the Humanized Immune System Mouse huHSC-NKG as an Example

Bispecific Antibody Efficacy Study Case

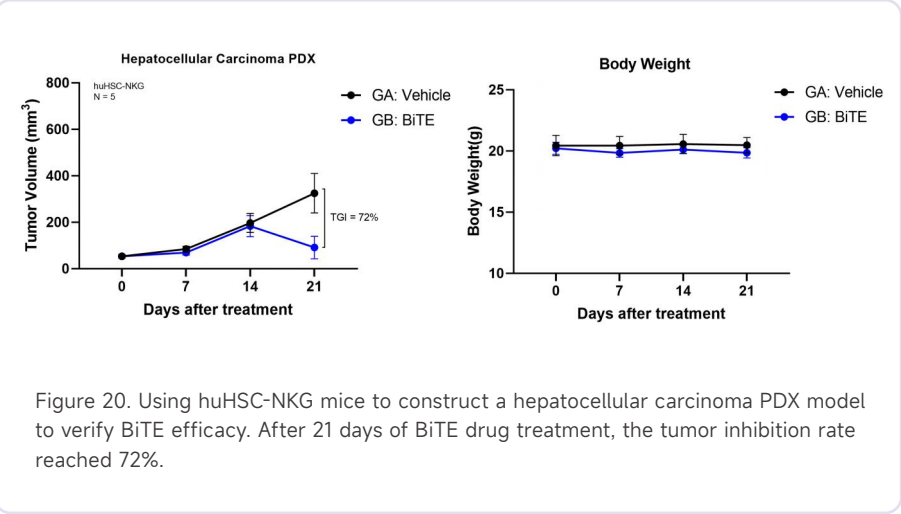


Figure 20. Using huHSC-NKG mice to construct a hepatocellular carcinoma PDX model to verify BiTE efficacy. After 21 days of BiTE drug treatment, the tumor inhibition rate reached 72%.

Construction of a Systemic Lupus Erythematosus (SLE) Disease Model

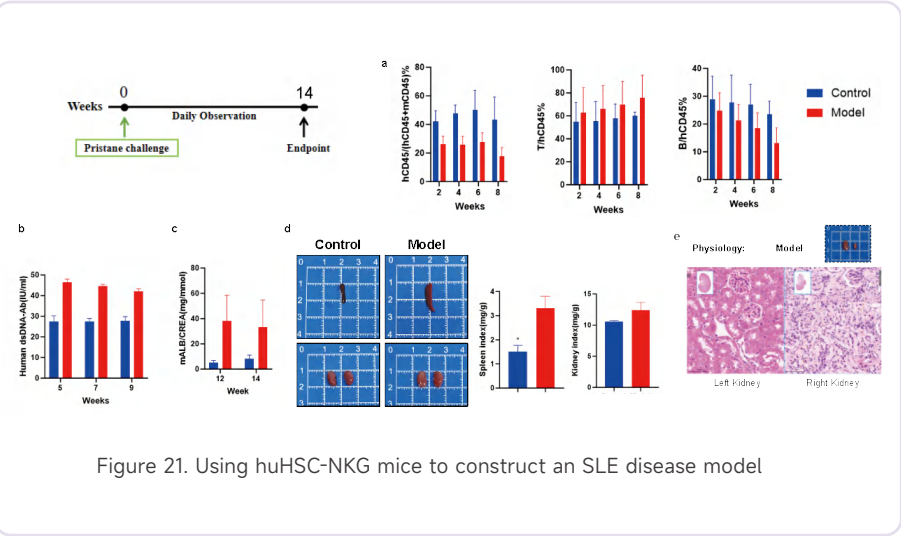


Figure 21. Using huHSC-NKG mice to construct an SLE disease model

PBMC Immune System Reconstitution Model – huPBMC-NKG Mice

Model Validation

a Survival Curve

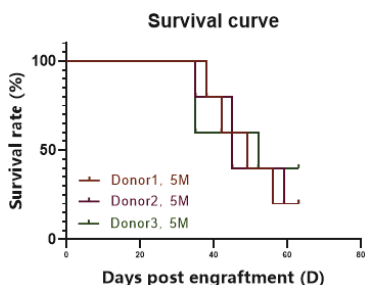


Figure 22. Survival curve of huPBMC-NKG mice

b Human Leukocyte Reconstitution Rate

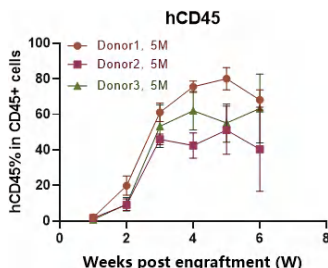


Figure 23. Changes in the content of human CD45+ cells in the peripheral blood of NKG mice after human PBMC transplantation

c Human T Cell Reconstitution Rate

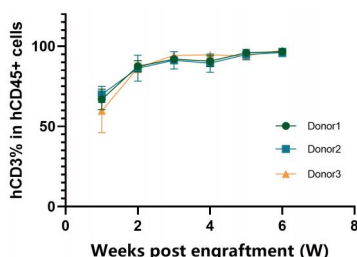


Figure 24. Changes in the content of human CD3+ T cells in the peripheral blood of NKG mice after human PBMC transplantation

d GvHD Reaction

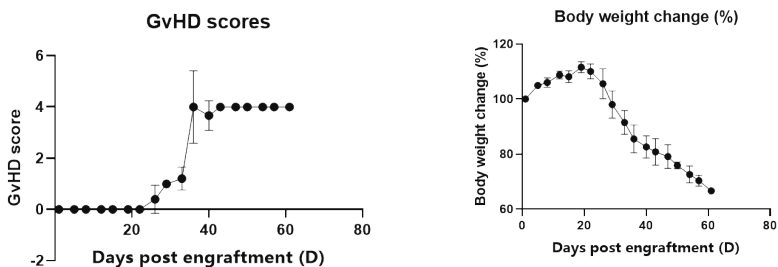


Figure 25. GvHD scores and changes in body weight of NKG mice after human PBMC transplantation

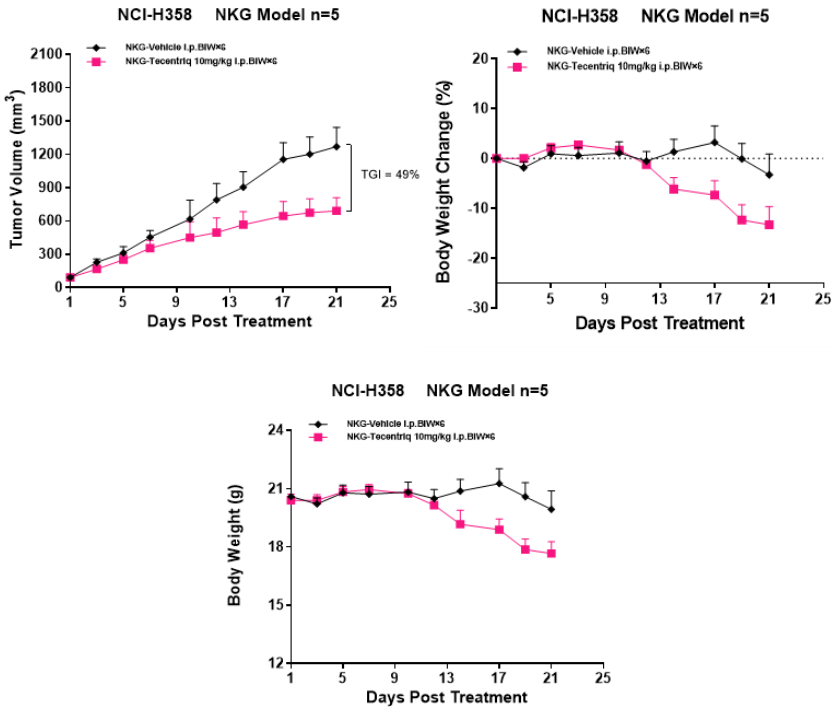


Figure 26. Using huPBMc-NKG mice to construct a lung cancer CDX model to verify Tecentriq efficacy

► Construction of Human Tumor Cell Line Xenograft (CDX) Models

Cyagen has established various effective tumor cell line transplantation efficacy models including human and mouse origins. The recipients used are immunodeficient mice such as NKG (NOD-Scid IL2rynull), NOD-Scid, BALB/c nude, etc., or wild-type mice such as BALB/c and C57BL/6. The inoculation sites are generally subcutaneous, intravenous, or orthotopic.

With over 200 tumor cell lines proven to establish robust in vivo tumor models in our mice, tumor mice from Cyagen cover over 90% of the market demand. Characterized by its comprehensive variety, large quantity, and superior quality, our NKG-based CDX model demonstrates remarkable "universality." We have successfully assisted numerous clients in completing IND applications.

Tumor Type	Cell Line
Lung Cancer	NCI-H1299, PC-9, NCI-H1975, NCI-H460, A549, A549-luc, Calu-3, Calu-3-luc, NCI-H23, NCI-H23-luc, NCI-H1703-luc, HCC827, NCI-H1703, NCI-H441, NCI-H1650, NCI-H292, NCI-H520, NCI-H441-luc, NCI-H446, NCI-H2228, NCI-H69, HCC827-luc, NCI-H226, EBC-1, CORL23
Colon Cancer	Colo205, HT29, LOVO, HCT116, HCT116-luc, SW620, SW620-luc, SW480, SW480-luc, HCT-15, DLD-1, DLD-1-luc, LS174T
Bladder Cancer	5637, 5637-luc, UMUC3, RT4, SW-780
Lymphoma	Raji
Breast Cancer	MDA-MB-231, BT474, MCF-7, MDA-MB-468
Ovarian Cancer	Hela, SK-OV-3, OVCAR-3, Caov-3
Pancreatic Cancer	AsPC-1, PANC-1, PANC-1-luc, BxPC-3, AsPC-1-luc, HPAF-II, CFPAC-1
Brain Cancer	U-87MG, U-87MG-luc
Gastric Cancer	HGC-27, MGC803-luc, MGC803, MKN45, NCI-N87, NUGC-3, NUGC-4, AGS
Hepatocellular Carcinoma	HepG2, HUH-7, Hep3B, HepG2-luc, Hep3B-luc, SK-HEP-1
Esophageal Cancer	KYSE-150, KYSE-150-luc
Melanoma	A375
Skin Cancer	A431
Renal Cell Carcinoma	786-O, caki-1, ACHN, 786-O-luc
Leukemia	MV-4-11
Myeloma	MM.1S, RPMI8226
Prostate Cancer	22RV1, PC-3
Neuroblastoma	SH-SY5Y

Subcutaneous Transplantation Cases

a Subcutaneous Transplantation of Human Renal Clear Cell Carcinoma Cells (Caki-1) and Lenvatinib (RTK Inhibitor) Pharmacodynamic Study

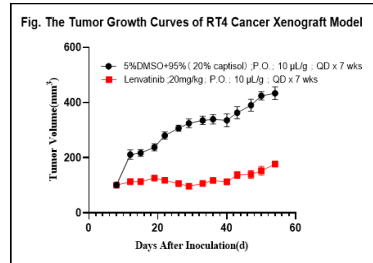
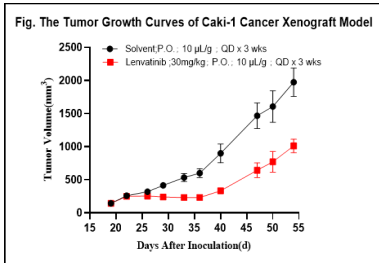


Figure 27. Subcutaneous transplantation to establish a human renal clear cell carcinoma model and perform Lenvatinib (RTK inhibitor) pharmacodynamic validation

b Subcutaneous Transplantation Model of Human Solid Tumor Cell Lines

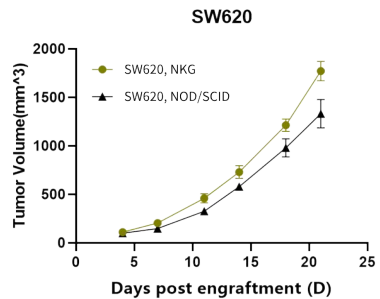
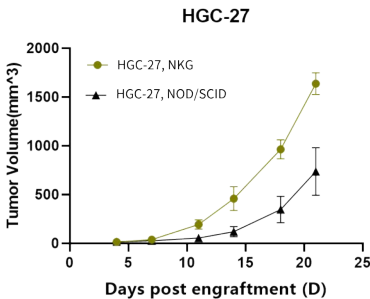
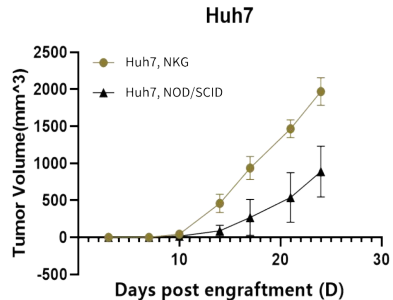
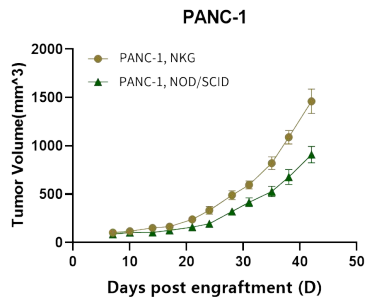


Figure 28. Tumor growth curves after subcutaneous xenograft of various tumor cells (a. Human pancreatic cancer cell line PANC-1; b. Human liver cancer cell line Huh7; c. Human gastric cancer cell line HGC-27; d. Human colorectal cancer cell line SW620)

Tail Vein Transplantation Cases

a Tail Vein Transplantation of Human B Lymphoblastic Leukemia Cells (NALM-6 with Luciferase & EGFP) and Blincyto Pharmacodynamic Study

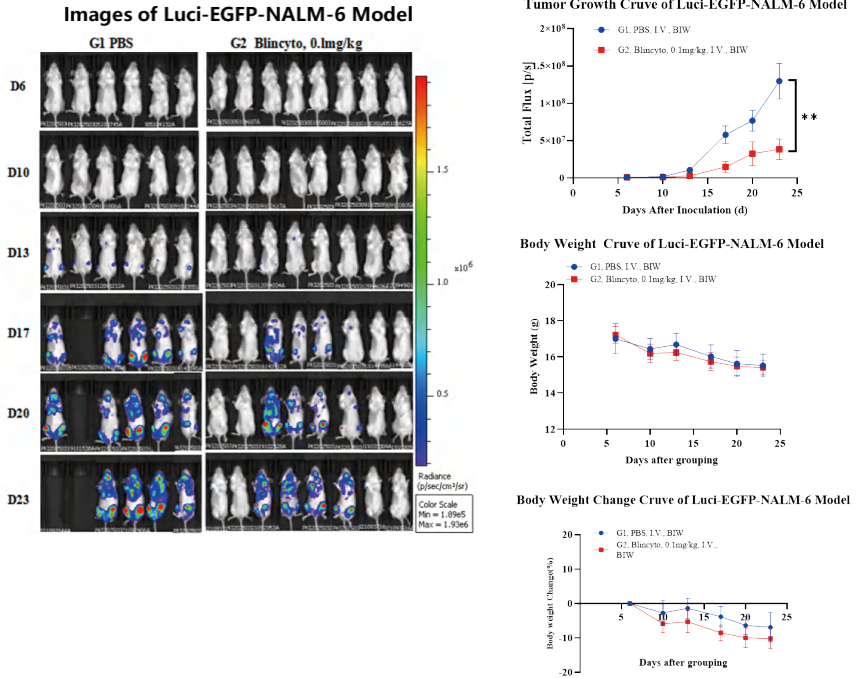


Figure 29. Tail vein transplantation to establish a human B lymphoblastic leukemia model and perform CD3&CD19 TCE (T Cell Engager, targeting CD19&CD3 bispecific antibody) pharmacodynamic validation

b Construction of Hematological Tumor Cell Transplantation Models

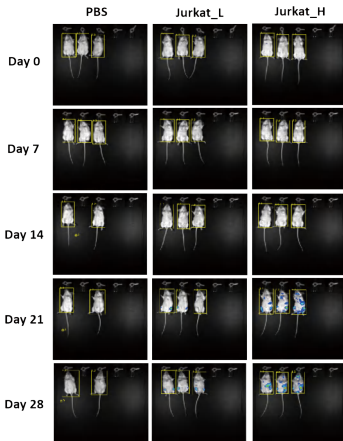


Figure 30. Tail vein transplantation to establish a human T lymphoblastic lymphoma model. Luciferase-labeled human T lymphoblastic lymphoma cell line Jurkat was transplanted into NKG mice via tail vein injection, and tumor growth was monitored by bioluminescence detection.

c Verification of Paclitaxel's Anti-tumor Efficacy Using a Human Lymphoma (Raji-Luc) CDX Model

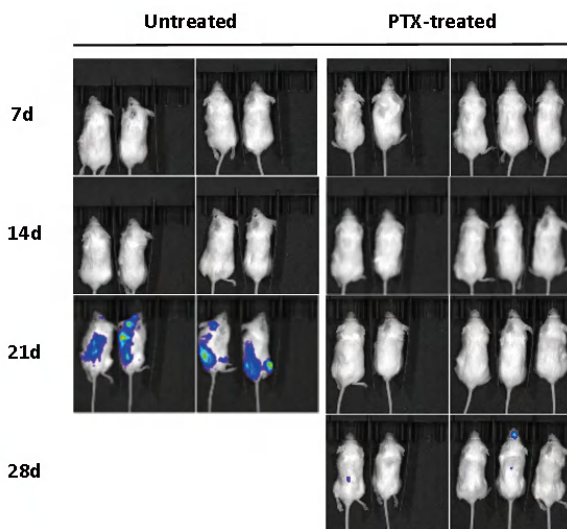


Figure 31. Verification of the anti-tumor efficacy of anti-tumor drugs using NKG mice with transplanted tumors

Orthotopic Transplantation Cases

a Orthotopic Model of Bladder Cancer 5637-luc

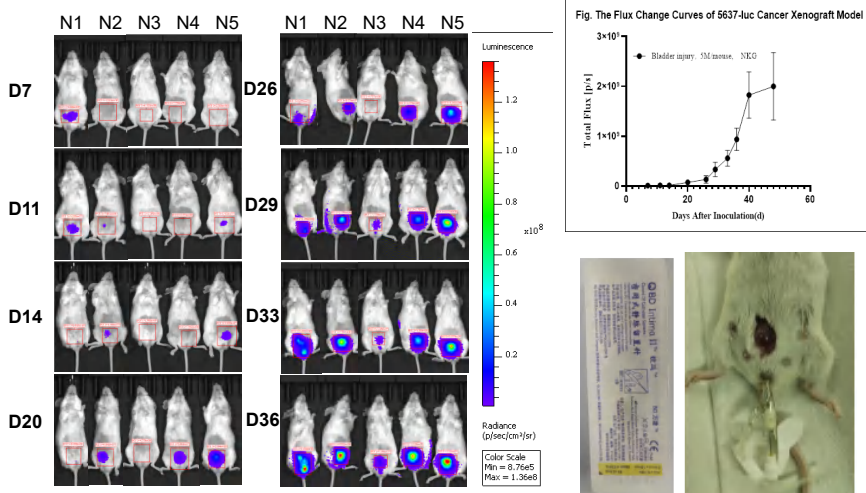


Figure 32. Orthotopic transplantation to establish a bladder cancer model

b Orthotopic Liver Model of Human Liver Cancer Hep3B-luc

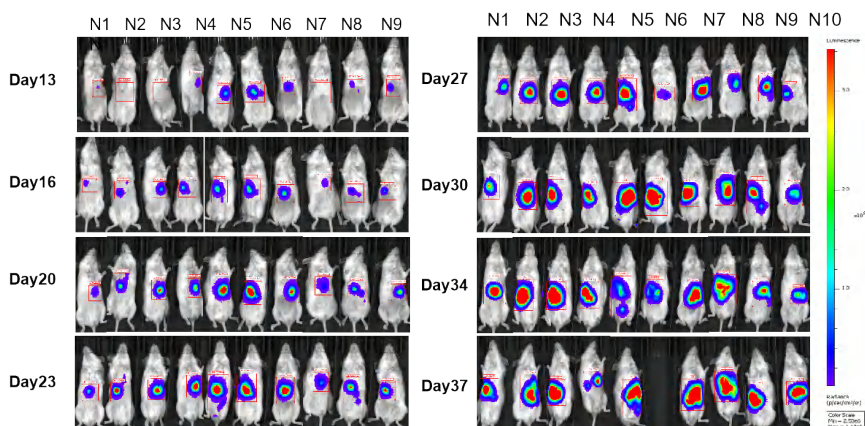


Fig. The Flux Change Curves of Hep3B-luc Cancer Model

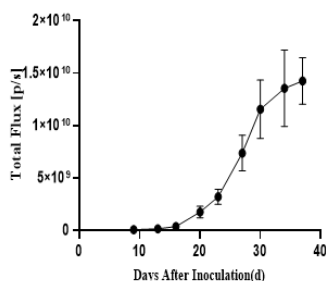


Fig. The Survival of Hep3B-luc Cancer Model

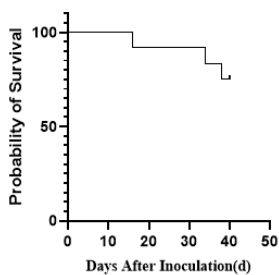


Figure 33. Orthotopic transplantation to establish a liver cancer model. This orthotopic liver model breaks through the traditional tumor block inoculation method by using cells for in vivo inoculation.

C Orthotopic Pancreatic Model of PANC-1

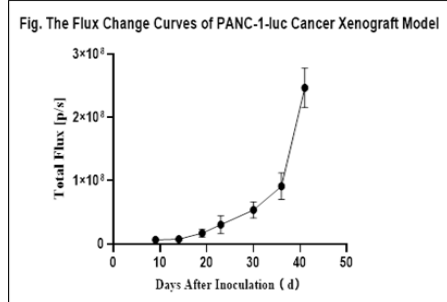
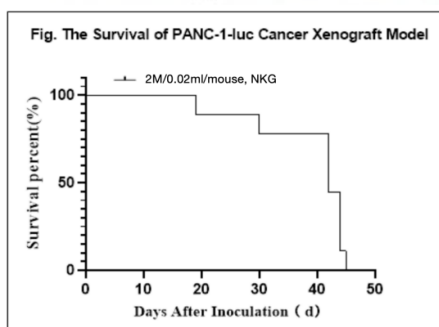
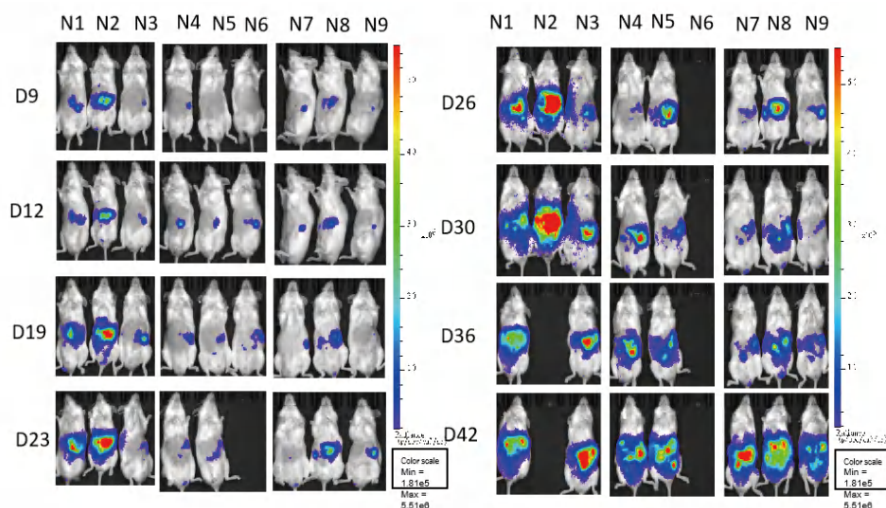


Figure 34. Detection of bioluminescence, mouse survival, and average total bioluminescence values after transplanting the human pancreatic cancer cell line PANC-1 into NKG mice.

d Orthotopic Lung Model of NCI-H441

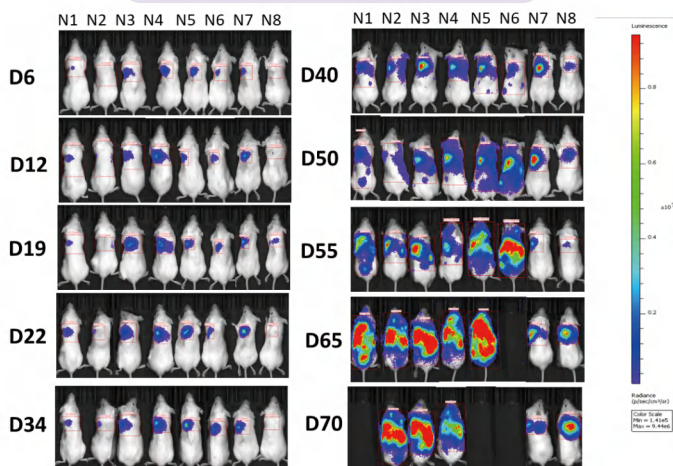


Fig. The Flux Change Curves of NCI-H441-luc Cancer Xenograft Model

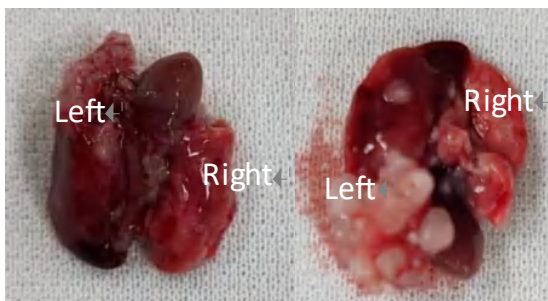
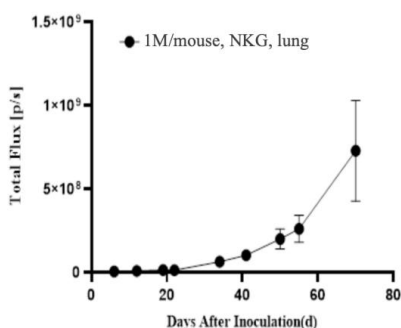
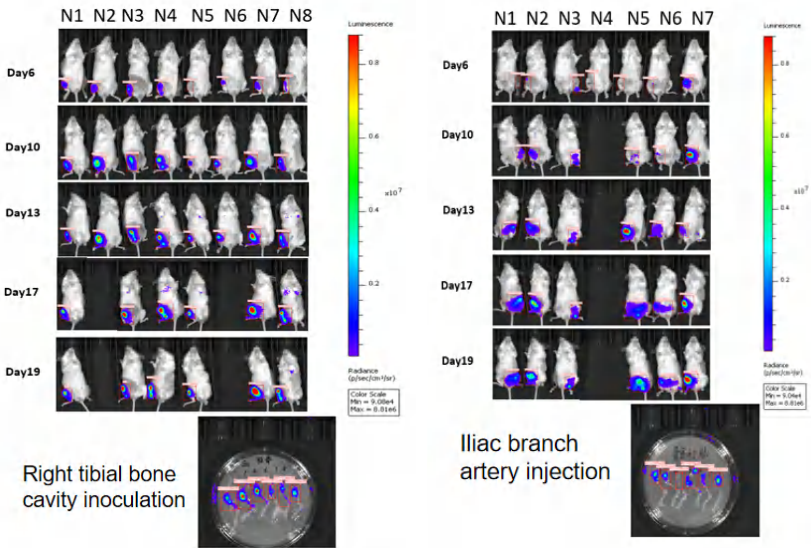


Figure 35. Detection of bioluminescence, average total bioluminescence values, and dissection observation of mouse lung tissue after transplanting the human lung cancer cell line NCI-H441 into NKG mice. The results showed that the NCI-H441 orthotopic lung model was successfully established.

Metastasis Model: MCF-7-luc Breast Cancer Bone Metastasis Mouse Model



Left: Intraosseous inoculation of MCF-7-luc into the right tibia
 Right: Intracarotid artery injection of MCF-7-luc

Figure 36. Establishment of a breast cancer bone metastasis mouse model using NKG mice

► Construction of Patient-Derived Xenograft (PDX) Models

PDX is a tumor model in which tumor tissue from tumor patients is transplanted into severely immunodeficient mice and allowed to grow in vivo. Its biological characteristics are more completely maintained, and it has high clinical similarity. Cyagen can provide PDX models for head and neck squamous cell carcinoma, gastrointestinal tumors, breast tumors, etc.

Frequently Asked Questions



► Immunodeficient Models

Q Do NKG mice exhibit T, B, and NK cell leakage? That is, the phenomenon where immunodeficient mice occasionally show functional T, B, and NK cells.

A The incidence of T and B cell leakage is extremely low; the incidence of NK cell leakage is lower than in normal mice, but it is not completely absent.

This is because NKG mice carry the *Prkdcscid* mutation. The *Prkdc* gene mainly encodes the catalytic subunit of DNA-dependent protein kinase, which is an important gene involved in double-strand DNA break repair and the recombination of the variable (V), diversity (D), and joining (J) segments of immunoglobulins and T cell receptors. *Prkdcscid* leads to impaired DNA repair, consequently affecting the normal development and maturation of T and B cells. Even if a small number of T and B cells successfully develop, their numbers are insufficient to mount an effective immune response.

At the same time, the *Il2rg* gene in NKG mice is completely knocked out. The *Il2rg* gene encodes the common gamma chain of the IL-2 receptor, which is a shared subunit of receptors for various cytokines such as IL-15. The development and maturation of NK cells are highly dependent on IL-15 signaling. Therefore, the deletion of the *Il2rg* gene leads to severely impaired NK cell development and function. However, other cytokines may partially compensate for the absence of IL-15; or there might be trace amounts of IL-15 signaling, which could allow a small number of NK cells to develop. To ensure the quality of the colony, Cyagen regularly conducts QC monitoring of the NKG mouse colony, including strain function monitoring, health monitoring, genetic monitoring, and breeding monitoring.

Q Do NKG mice develop spontaneous tumors?

A The probability of NKG mice developing spontaneous tumors is very low, with occasional individual cases of spontaneous lymphoma.

Q Do NKG mice develop diabetes?

A Diabetes is usually divided into Type 1 and Type 2 diabetes. Type 1 diabetes is caused by the autoimmune system mistakenly attacking and destroying insulin-producing beta cells in the pancreas, leading to insufficient insulin secretion. Type 2 diabetes is mainly caused by insulin resistance and the loss of function of pancreatic beta cells, resulting in elevated blood sugar, and is usually the result of a combination of genetic and environmental factors.

NOD mice are a commonly used spontaneous animal model for Type 1 diabetes, characterized by the autoimmune system attacking pancreatic beta cells, leading to insufficient insulin secretion and hyperglycemia. NOD-Scid mice, due to carrying the SCID mutation, lack functional T and B cells, so their immune system cannot attack pancreatic beta cells, thus avoiding the development of Type 1 diabetes. NKG mice are genetically engineered on the NOD-Scid background, resulting in deficiencies in T, B, and NK cells. Similarly, they will not spontaneously develop Type 1 diabetes. However, under specific experimental conditions, such as high-fat diet-induced insulin resistance, they may develop metabolic disorders similar to Type 2 diabetes.

► Human Immune System Reconstitution Models

Q What are the methods for constructing human immune system reconstituted mice?

A The main methods include the following:

- 1. Human Hematopoietic Stem Cell Transplantation (HSC):** This is the most common method, which involves transplanting HSCs into immunodeficient mice, allowing them to differentiate and reconstitute a human immune system within the mice.
- 2. Human Peripheral Blood Mononuclear Cell (PBMC) Transplantation:** This involves transplanting PBMCs (including T cells, B cells, and monocytes, etc.) into immunodeficient mice to rapidly establish a human immune system. This method can be used for short-term studies in tumor immunology, GvHD, hematological diseases, infectious diseases, and other areas requiring mature T cells.
- 3. Human Thymus Tissue Transplantation:** This involves transplanting human thymus tissue into immunodeficient mice, allowing it to provide a human T cell development environment. This method is often used in combination with HSC transplantation to enhance T cell development and function.
- 4. Human Spleen or Lymph Node Transplantation:** This involves transplanting human spleen or lymph node tissue into immunodeficient mice, providing a human immune cell development and functional environment. This method can enhance the complexity and diversity of the human immune system within the mice.

Q Why do mice die quickly after PBMC reconstitution?

A Graft-versus-host disease (GvHD) is a condition caused by T lymphocytes from the allogeneic donor graft attacking the recipient host cells. After PBMC transplantation, human T cells will attack the mouse recipient cells, leading to symptoms such as significant weight loss, hunched back, diarrhea, hair loss, and skin peeling in the mice.

Q What is the predominant type of immune cell in PBMC reconstitution?

A PBMC humanized mice have high immune reconstitution efficiency, and their human T cells proliferate rapidly. After reconstitution, the predominant immune cell type is lymphocytic T cells. Although low levels of human B cells are present in PBMC humanized mice, the proportion of human B cells gradually decreases over time due to the rapid proliferation of human T cells. Certain signaling proteins related to B cell survival have weak cross-reactivity between mice and humans, and mice lack the relevant signals to support human B cell survival. Similarly, mice also lack the relevant cytokines to support human NK cell survival, leading to a shorter survival time for human NK cells.

Q What are the precautions when using NKG mice for PBMC reconstitution?

A When performing PBMC reconstitution, it is important to select suitable donors. Different donors can affect reconstitution efficiency, so it is recommended to use the same donor within the same research project. In addition, increasing the number of enrolled mice can help reduce intra-group differences caused by individual mouse variations. It is worth noting that irradiation treatment is not required for PBMC reconstitution; in fact, irradiation can accelerate the occurrence of graft-versus-host disease (GvHD).

Q Why don't mice develop GvHD after HSC reconstitution?

A Because when human HSCs develop in mice, they produce multi-lineage human immune cell differentiation, including lymphoid T, B, and NK cells. These human immune cells develop within the mouse and develop immune tolerance to the mouse, so GvHD usually does not occur, and the mice have a longer lifespan.

Q What are the advantages of the NKG neonatal mouse HSC reconstitution process? What precautions should be taken during reconstitution?

A HSC reconstitution involves sorting out human CD34+ hematopoietic stem cells derived from umbilical cord blood and then transplanting them into NKG mice to form a human immune system. By monitoring the mouse's immune system and cellular composition, the development, maintenance, and function of the human immune system can be studied. Cyagen's optimized neonatal mouse process has the following advantages: high survival rate after irradiation, high success rate of intravenous injection, high success rate of immune reconstitution, large number of mice produced from a single donor's HSC cells, and richer reconstitution of human immune cell subtypes.

When performing HSC reconstitution, mice need to receive sub-lethal irradiation to eliminate their own immune cells. HSC reconstitution requires a longer time and higher cost, making it suitable for research that needs to focus on multi-target or multiple immune cell subtypes involved in anti-tumor mechanisms.

Q What research areas can human immune system reconstituted mice be applied to?

A Functional validation of immune checkpoint inhibitor (ICI) therapy, bispecific antibodies, therapeutic tumor vaccines, combination therapies, and other research.

► Human Tumor Cell Line Xenograft (CDX) Model

Q What is the tumor growth effect in NKG mice? What precautions should be taken?

A NKG mice exhibit certain advantages in the proliferation of mouse tumors, with longer survival times and strong tumor burden capacity in their CDX models.

	Cell Name	Inoculation Route	Inoculation Dose	Inoculation Date	Tumor Burden/ Proliferation	Animal Death Date	Survival Period
N*G Series Competitor	THP-1	i.v.	1.00E+06	July 1st	D7	July 14th	14 days
Cyagen NKG				July 22nd	D3	August 24th	33 days

The growth rate of different tumor cell lines varies. Generally, a tumor-bearing pre-experiment is conducted first to confirm the tumor engraftment rate and tumor growth. If CDX is performed on reconstituted NKG mice, attention should be paid to the interval between PBMC and tumor cell line transplantation.

Q What are the influencing factors for the uniformity of orthotopic transplantation tumor models?

A There are three main influencing factors:

1. Positive cell activity: Orthotopic models generally use luciferase-overexpressing positive cell lines. Cell culture conditions, cell viability at inoculation, and the cell's own fluorescence expression ability will all affect the uniformity of the orthotopic xenograft.
2. Host mice: Orthotopic models are highly dependent on mouse strain. Depending on the origin of the transplanted cells, human tumor cells generally use immunodeficient mice with varying degrees of immunodeficiency or human immune system reconstituted mice, while mouse tumor cells use immunocompetent mice.
3. Human error: This includes surgical factors, the uniformity of the cell suspension, and the uniformity of the injection volume. Orthotopic transplantation models in organs (except for mammary fat pad orthotopic models) generally require anesthesia and inoculation at the orthotopic site for model construction. Anesthesia and surgical factors can affect mouse survival, thereby affecting the success rate of orthotopic tumor models. The uniformity of the cell suspension and the uniformity of the injection volume affect the uniformity of the orthotopic transplantation tumor model.

Q How is an immunodeficient mouse model of the MCF-7 cell line constructed?

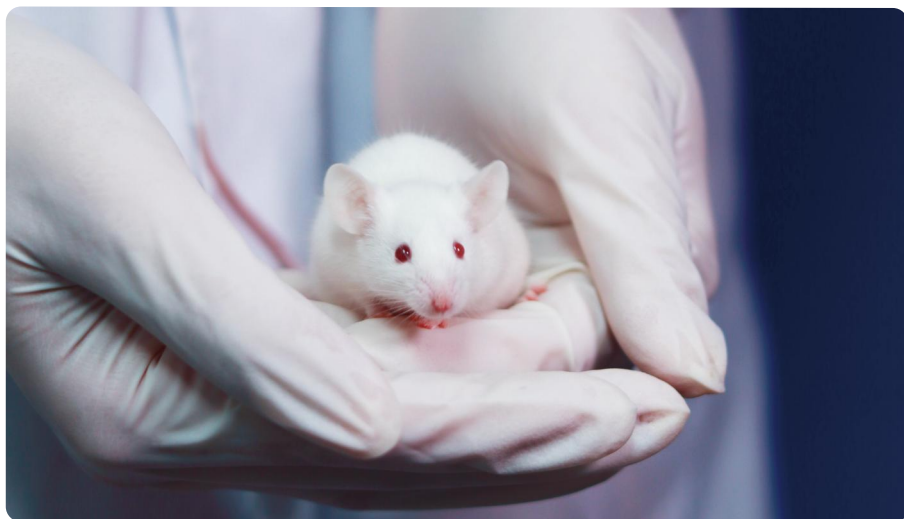
A MCF-7 is an ER⁺ breast cancer cell line. When constructing the model, estrogen pellets need to be added, and Matrigel needs to be added during cell inoculation. The in vivo growth cycle of this model is generally 7-8 weeks, reaching an experimental endpoint of 2000 mm³ tumor volume. It is recommended to choose estrogen pellets with a slow release of more than 60 days.

In vivo inoculation has high requirements for cell viability and activity status. The cell viability should be above 90%, and it is recommended to add Matrigel. This model has a high dependence on the host. The higher the degree of immunodeficiency, the lower the cell requirements for model construction: inoculation of 5M cells/mouse is sufficient on NKG mice, while 10M cells/mouse is recommended for nude mice.

Q How is internal carotid artery injection performed when constructing a hematogenous brain metastasis model?

A The injection procedure is as follows:

1. Anesthetize the mice with anesthetic.
2. Make a midline incision in the mouse's neck to expose the submandibular glands. Bluntly separate them into left and right submandibular glands to expose the sternohyoid muscle. The common carotid artery is located on the outer edge of the sternohyoid muscle. Expose the muscle and bluntly dissect the muscle layer. Gently lift the common carotid artery with forceps, being careful to separate the mandibular nerve. Separate the carotid artery by continuously lifting and sliding, and dissect along the artery towards the distal end. Prepare a surgical knot at the proximal end of the common carotid artery.
3. Locate the carotid artery bifurcation and identify and ligate the external carotid artery.
4. Lift the common carotid artery with forceps and slowly inject (note that the tumor cell suspension should not exceed 40 μ l). Finally, ligate and tie off the prepared common carotid artery.
5. Suture the skin and wait for the mice to recover.





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