

huHSC-NCG-M/hIL15(IN)

Strain Name: huCD34+ HSC-NOD/ShiLtJGpt-

Prkdc^{em26Cd52}Il2rg^{em26Cd22}Il15^{em1Cin(hIL15)}Rosa26^{em1Cin(hCSF2&IL3&KITLG)}/Gpt

Strain type: Knock-in

Strain number: T072273

Background: NOD/ShiLtJGpt

Description

The NOD/ShiLtJGpt mouse has natural immunodeficiency due to defects in the complement system and macrophages^[1]. Mutations in the *Prkdc* gene lead to impaired VDJ recombination (scid mouse), resulting in severe combined immunodeficiency of cellular and humoral immunity due to the inability of T and B cells to mature^[2]. The IL2 receptor gamma (IL2ry) chain is a common receptor subunit for the cytokines IL2, IL4, IL7, IL9, IL15, and IL21, which play important roles in the immune system. Knockout of the *Il2rg* gene encoding IL2ry severely impairs immune function, especially the activity of NK cells^[3]. The NCG mouse model with high immunodeficiency was created by knocking out *Prkdc* and *Il2rg* genes using gene editing technology, which has a relatively homogeneous background, long lifespan, lack of mature T, B, and NK cells, and lack of complement activity^[4].

To date, NCG is the most complete mouse immunodeficiency model and is very suitable for cell-derived xenotransplantation (CDX), patient-derived xenotransplantation (PDX), human peripheral blood mononuclear cells (PBMCs), and human hematopoietic stem cells (CD34+ HSC) transplantation for human immune reconstitution^[4]. However, when the human immune system was engrafted, due to interspecies differences, mouse cytokines often do not act well on human hematopoietic cells, resulting in limited development of myeloid cells. To improve the engraftment of human immune cells, genes encoding human stem cell factors (SCF, also known as KITLG), granulocyte/macrophage colony-stimulating factor 2 (GM-CSF, also known as CSF2), and interleukin-3 (IL-3) were introduced into NCG mice to obtain the NCG-M humanized mouse model. This mouse model can better promote the reconstitution of myeloid cells^[5-6] and improve the engraftment efficiency of acute myeloid leukemia cells from patients^[7-8].

IL15 is a multifunctional cytokine produced by activated monocytes/macrophages, keratinocytes, fibroblasts, and other cells. It has biological activity similar to IL2 and plays a role in activating T cells, B cells, and NK cells, mediating proliferation and survival functions^[9].

^{10]}. Therefore, we performed humanization of the IL15 gene in the NCG mouse model to obtain the NCG-hIL15 strain with independent intellectual property rights. This mouse model can support the settlement and activity of human NK cells.

In summary, to obtain a mouse model capable of reconstituting a more diverse population of human immune system, GemPharmatech Co., Ltd. successfully developed the NCG-M/hIL15 mouse model by crossbreeding NCG-M and NCG-hIL15 mice, which stably express human KITLG, CSF2, IL-3, and IL15 at the same time. The NCG-M/hIL15 model can simultaneously reconstitute functional human T cells, NK cells, macrophages, and other myeloid cells, which will aid in the development or combination therapy evaluation of CD3 agonists or inhibitors, ADCC-type drugs, and macrophage-related drugs.

Model strategy

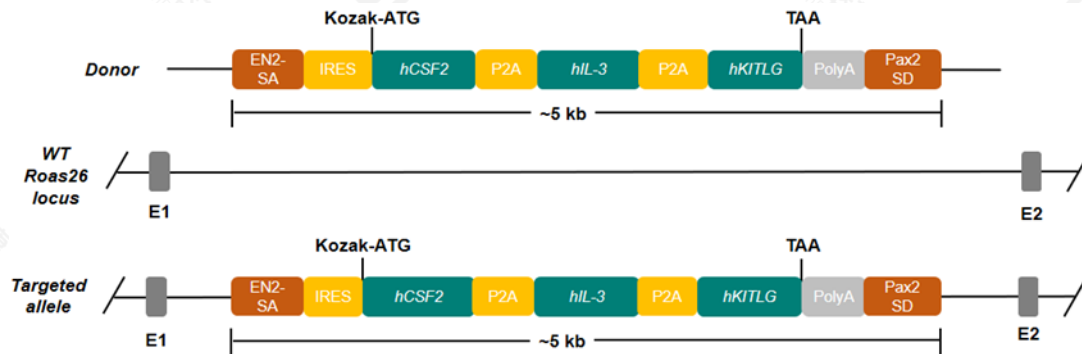


Figure 1. NCG-M mouse model strategy.

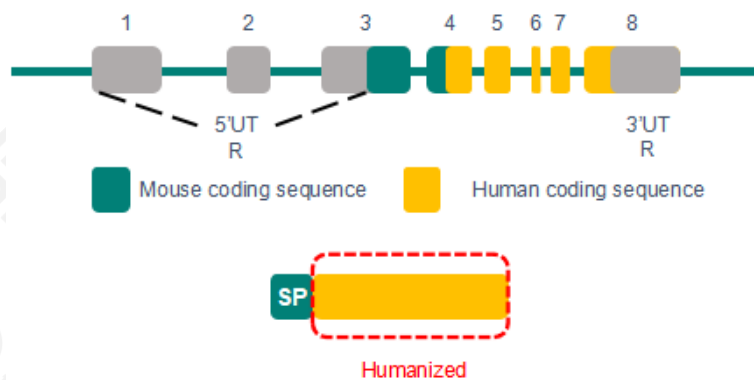


Figure 2. NCG-hIL15 mouse model strategy.

Applications

1. Support reconstitution of human multi-subset immune cells (myeloid, T/NK cells) in mice post CD34⁺ hematopoietic stem cell transplantation

2. Establish acute myeloid leukaemia (AML) PDX mouse models
3. Human tumor inoculation for relevant drug screening
4. Study tumor immunotherapy of NK cell combined with T cell/macrophage
5. Research on cytokine storm and immunotoxicity.

Data support

1. mRNA expression analysis of human GM-CSF, SCF, IL3 and IL15 in NCG-M/hIL15 mice

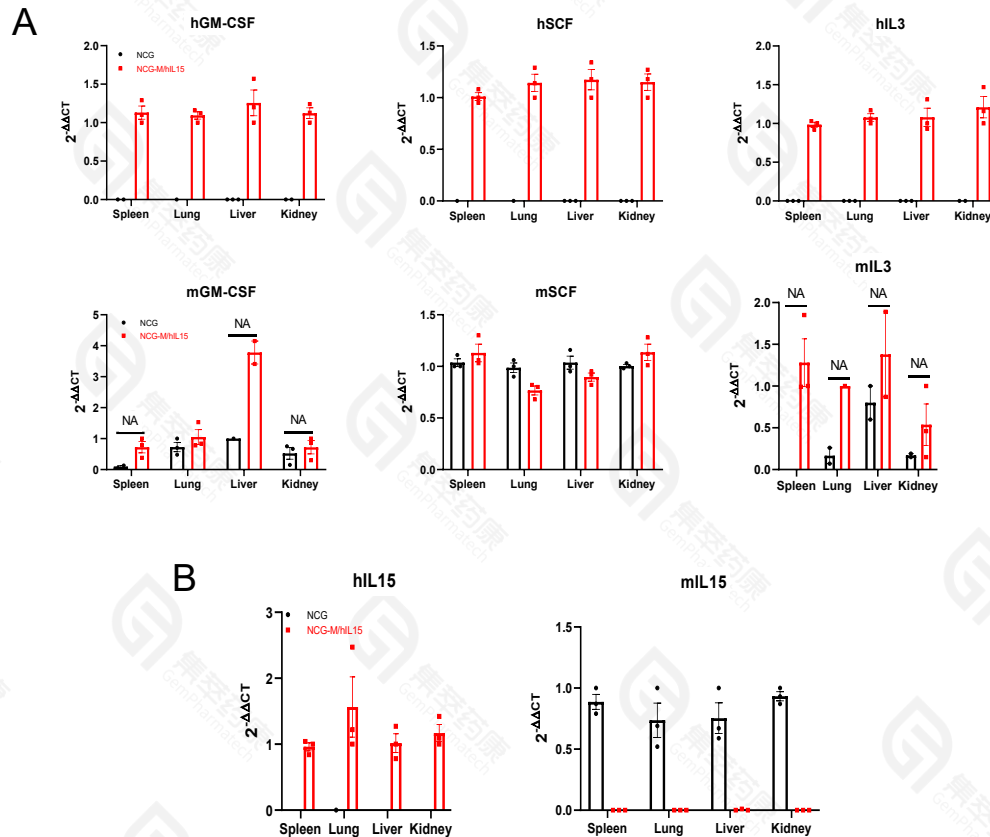


Fig 3. Detection of mRNA expression of human GM-CSF, SCF, IL3 and IL15 in NCG-M/hIL15 mice.

The mRNA expression of human GM-CSF, SCF, IL3 (Figure3A) and IL15 (Figure3B) in NCG-M/hIL15 mice was consistent with mouse GM-CSF, SCF, IL3 and IL15 in NCG mice by Q-PCR.

2. Analysis of human GM-CSF, SCF, IL3 and IL15 protein expression in the serum of NCG-M/hIL15 mice

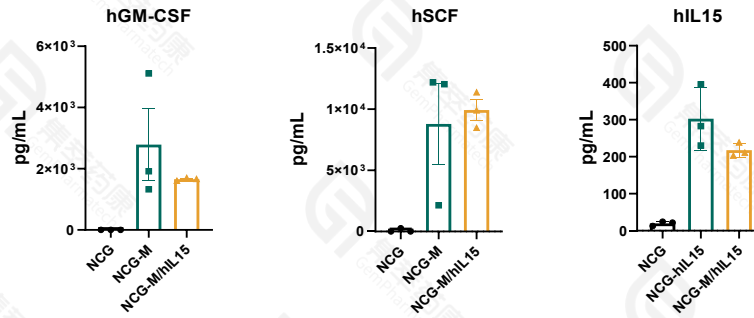


Fig 4. Detection of human GM-CSF, SCF, IL3 and IL15 protein expression in the serum of NCG-M/hIL15 mice.

The serum of peripheral blood of 6 weeks old mice was collected for ELISA, and the protein expression of human GM-CSF, SCF and IL15 was detected in NCG-M/hIL15 mice. The protein expression of human IL3 was detected at a lower value than the lower limit of the Elisa assay. The detection of mIL3 protein expression in peripheral blood of immunologically competent mice at unstimulated physiological levels has proven to be challenging. Consequently, in conjunction with the observed mRNA transcript levels of hIL3 in various tissues, it is hypothesized that hIL3 may function in vivo at relatively low expression levels.

3. Analysis of immune cell subpopulations in NCG-M/hIL15 mice

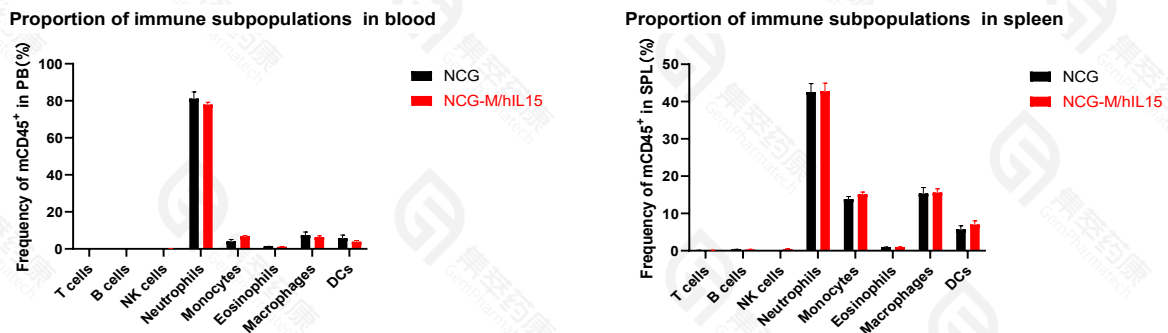


Fig 5. Detection of immune cell subpopulations in the peripheral blood (left) and spleens (right) of NCG-M/hIL15 mice.

The proportions of immune subpopulations in the peripheral blood and spleens of NCG-M/hIL15 mice were very similar to those in NCG mice. Both NCG-M/hIL15 and NCG mice were severely immunodeficient, with no T cells, B cells and NK cells.

4. The process of huHSC-NCG-M/hIL15 reconstitution

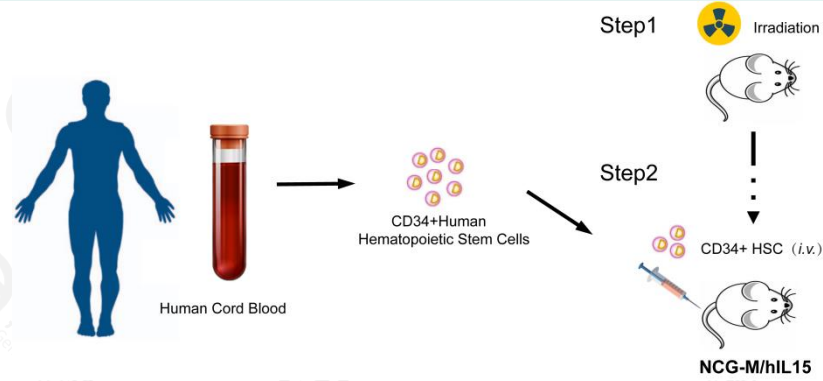


Figure 6. The huHSC-NCG-hIL15 reconstitution process.

5. The lifespan of huHSC-NCG-M/hIL15 mice

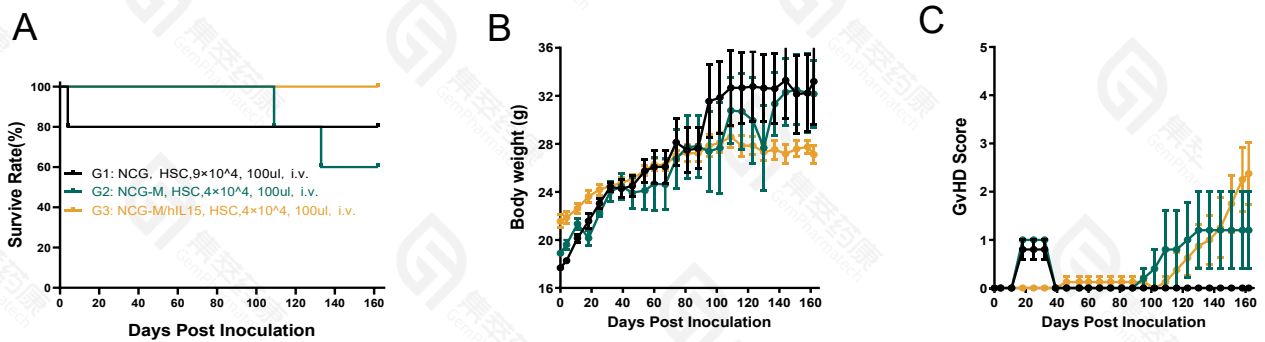
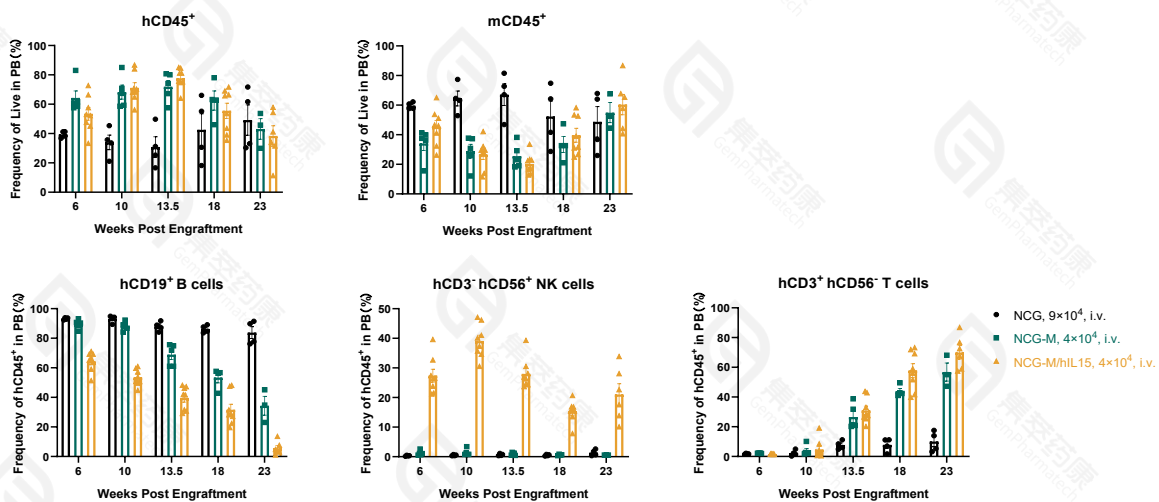


Fig 7. Survival rate, Body weight and GvHD score changes in huHSC-NCG-M/hIL15 mice.

Compared to huHSC-NCG and huHSC-NCG-M mice, huHSC-NCG-M/hIL15 mice showed a more stable survival rate (Fig 7A) and an upward trend in body weight (Fig 7B) during human immune system reconstitution, but showed increased GvHD symptoms (Fig 7C) due to hair loss after 100 days of reconstitution.

6. Immunophenotypes of huHSC-NCG-hIL15 mice



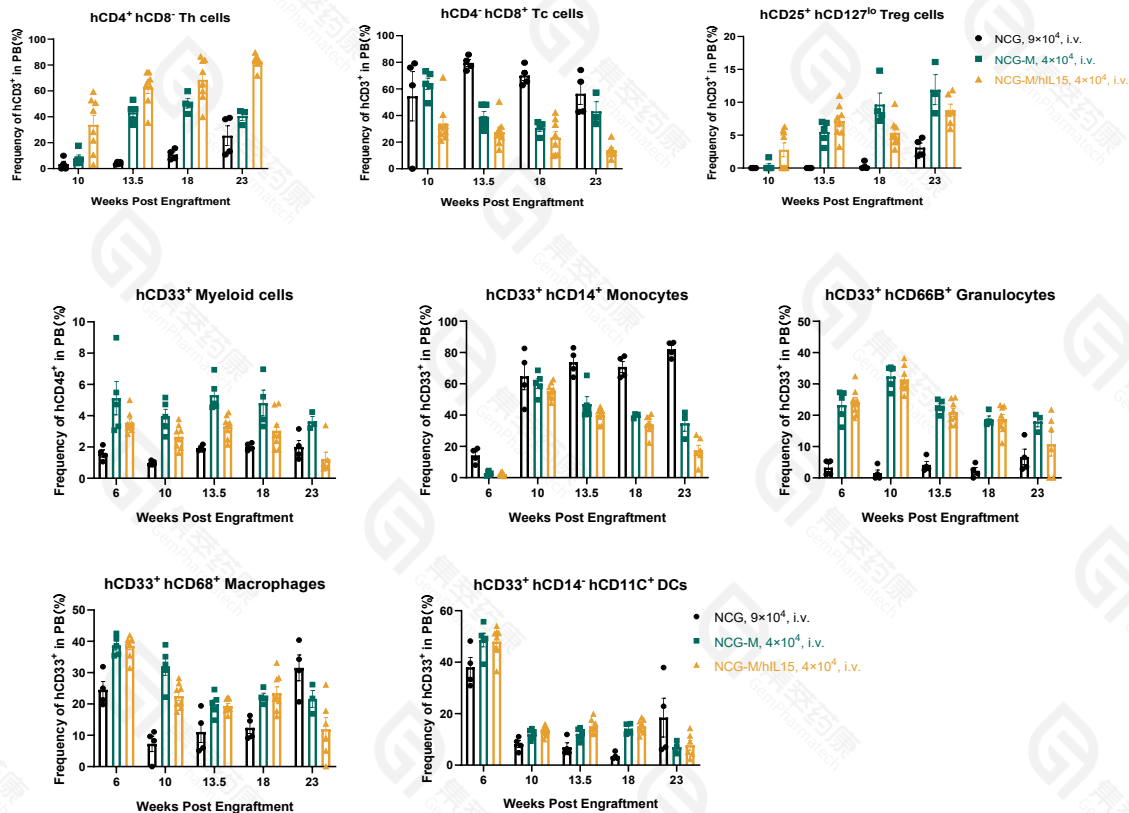


Fig 8. Reconstitution of Immune Cell Populations in the Peripheral Blood of huHSC-NGC/M/hIL15 Mice.

Peripheral blood of huHSC-NGC (n=5), huHSC-NGC-M (n=5) and huHSC-NGC-M/hIL15 (n=8) mice was collected at indicated timepoints after HSC engraftment, and the level of reconstituted human immune cells was detected by flow cytometry. The results showed that both huHSC-NGC-M and huHSC-NGC-M/hIL15 mice were able to reconstitute higher and stable levels of T cell subtypes and myeloid cells compared to huHSC-NGC mice, and huHSC-NGC-M and huHSC-NGC-M/hIL15 mice achieved these results using fewer HSC cells. Moreover, huHSC-NGC-M/hIL15 mice were able to significantly promote the reconstitution of NK cells, while almost no NK cells were reconstituted in huHSC-NGC and huHSC-NGC-M mice.

Note: Different batches or different donors of HSC can lead to variations in the level of reconstitution. The successful reconstitution standard (shipping standard) for huHSC-NGC-M/hIL15 is a proportion of hCD45+ cells $\geq 25\%$.

7. Evaluation of ADCC effect of human NK cells from huHSC-NGC-M/hIL15 mice spleen in vitro

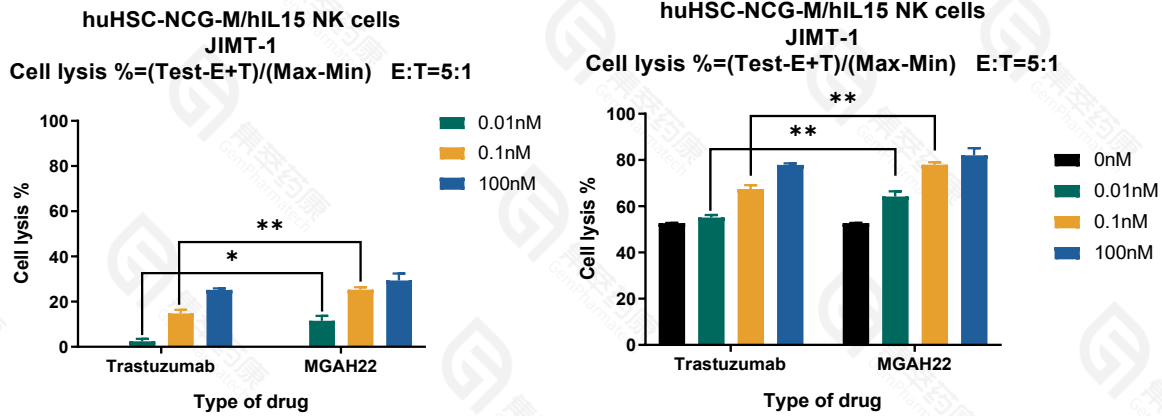


Fig 9. Evaluation of ADCC effect of NK cells from huHSC-NCG-M/hIL15 mice in vitro.

The human NK cells derived from the spleen of huHSC-NCG-M/hIL15 mice can effectively evaluate the dose-dependent effect of ADCC drugs in vitro. Additionally, it can also reflect the difference in ADCC effects between ADCC-regular (Trastuzumab) and ADCC-enhanced (Margetuximab) drugs.

8. huHSC-NCG-M/hIL15 promotes spleen colonization of human immune cells

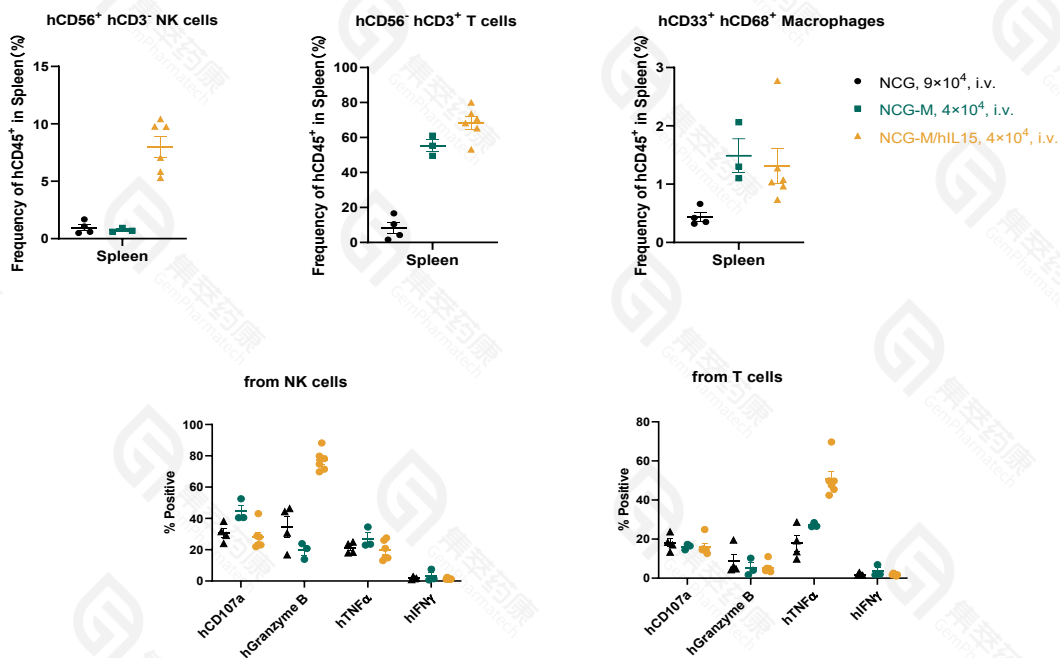


Fig 10. The spleen colonization of human immune cells in huHSC-NCG-M/hIL15 mice.

Splenocytes of huHSC-NCG, huHSC-NCG-M and huHSC-NCG-M/hIL15 mice was collected and detected by flow cytometry. Compared to huHSC-NCG, huHSC-NCG-M/hIL15 and huHSC-NCG-M mice had more T cells and Macrophages colonized in the spleen, and the spleen of huHSC-NCG-M/hIL15 had more NK cells than the other two species of mice. Furthermore, NK cells in the spleen of the huHSC-NCG-M/hIL15 mice produced more Granzyme B than those in uHSC-NCG and huHSC-NCG-M/hIL15 mice. And T cells in the spleen of the huHSC-NCG-

M/hIL15 mice produced more TNF α than the other two species of mice.

References

1. Shultz LD, et al. (1995). "Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice". *J. Immunol.* 154 (1): 180–91.
2. Takenaka K, et al. (2007). "Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells". *Nat. Immunol.* 8 (12): 1313–23.
3. Greiner DL, Hesselton RA, Shultz LD (1998). "SCID mouse models of human stem cell engraftment". *Stem Cells.* 16 (3): 166–177.
4. Cao X, et al. (1995). "Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain". *Immunity.* 2 (3): 223–38.
5. Nicolini, F. E., et al. (2004). "NOD/SCID mice engineered to express human IL-3, GM-CSF and Steel factor constitutively mobilize engrafted human progenitors and compromise human stem cell regeneration." *Leukemia* 18 (2): 341.
6. Curtis, Benson M., et al. (1991). "Enhanced hematopoietic activity of a human granulocyte/macrophage colony-stimulating factor-interleukin 3 fusion protein." *Proceedings of the National Academy of Sciences* 88 (13): 5809.
7. Feuring-Buske, M., et al. (2003). "Improved engraftment of human acute myeloid leukemia progenitor cells in beta 2-microglobulin-deficient NOD/SCID mice and in NOD/SCID mice transgenic for human growth factors." *Leukemia* 17 (4) :760.
8. Wunderlich, M., et al. (2010). "AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3." *Leukemia* 24 (10): 1785.
9. Ali, A. K., Nandagopal, N. & Lee, S. H. (2015). "IL-15-PI3K-AKT-mTOR: A Critical Pathway in the Life Journey of Natural Killer Cells". *Frontiers in immunology.* 6, 355.
10. Fehniger, T. A. et al. (2001). "Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8⁺ T cells". *The Journal of experimental medicine.* 193, 219-231.