NCG-M

Strain Name： NOD/ShiLtJGpt-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}*Rosa26*$^{em1Cin(hCSF2\&IL3\&KITLG)}$/Gpt
Strain Type： Knock-in
Strain Number： T036669
Background： NOD/ShiLtJGpt

Description

Severe immune-deficient strain NCG is established by CRISPR/Cas9 technology. Prkdc (Protein kinase, DNA activated, catalytic polypeptide) and Il2rg (Common gamma chain receptor) genes are knocked out on NOD/ShiltJGpt background. The genetic background of NOD/ShiltJGpt makes this line have natural immunodeficiency, such as complement system and macrophage defects [1]. At the same time, the Sirpa on NOD/ShiltJGpt has high affinity with human CD47, making it more suitable for colonization of human grafts (e.g. tumors and human cells) than other strains [2]. Therefore, NCG is the most thorough mouse model of the immune-deficient to date, and is very suitable for Cell derived xenograft (CDX), Patient derived xenograft (PDX), human peripheral blood mononuclear cells (PBMC) and human hematopoietic stem cell ($CD34^+$HSC) transplantation for immune reconstruction [3]. however，in the reconstruction of human immune system, mouse cytokines often have poor effects on human hematopoietic cells due to species differences. Although reconstructed T cells respond well, the development of myeloid immune cells is limited.

In order to improve the implantation of human immune cells, three human cytokine genes, including human stem cell factor (SCF, also named as KITLG), granulocyte/macrophage colony stimulating factor 2 (GM-CSF, also named as CSF2) and interleukin-3 (IL-3) were introduced into NCG mice, and the obtained NCG-M humanized mouse model could better promote the expansion of myeloid cells as well as the T cells, B cells and NK cells [4, 5], and improve the migration efficiency of AML[6, 7]. The NCG-M mice verified by phenotypic analysis can be matched with other cytokines humanized mouse strains and also will become an important model for the reconstruction of the human immune system.

Applications

1. Supporting the reconstruction of multiple lineages of human immune cells, including myeloid cells and regulatory T cell populations, after $CD34^+$ hematopoietic stem cell transplantation in mice.

2.To establish a mouse model of acute myeloid leukemia (AML) PDX (patient-derived xenotransplantation).

3. Immune-oncology therapy.

4. Cytokine storm and immunotoxicity studies.

Data support

1. hCSF2, hIL3 and KITLG expression in NCG-M mice

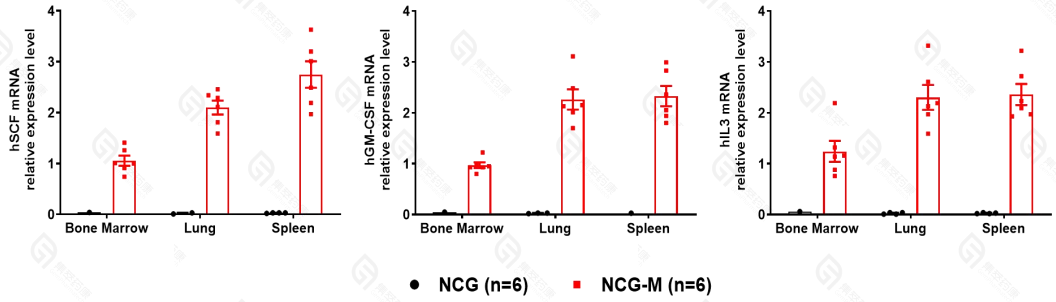


Fig.1 Detection of human hCSF2, hIL3 and KITLG mRNA expression in NCG and NCG-M mice.

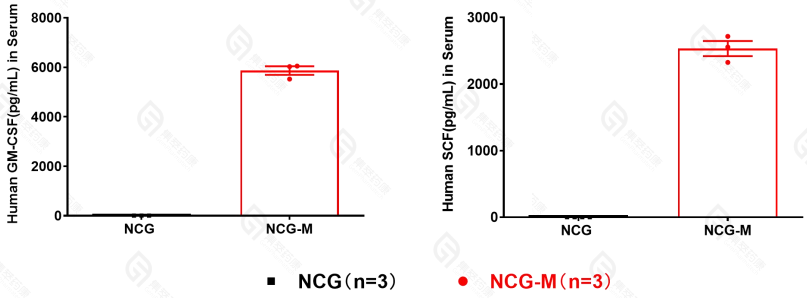


Fig.2 Detection of human hCSF2, hIL3 and KITLG ptrtein expression in NCG and NCG-M mice.

Bone marrow, lung, spleen and serum samples from NCG and NCG-M mice were collected for mRNA expression and protein expression of human SCF, GM-CSF, and IL-3. The mRNA expression of SCF, GM-CSF, and IL-3 were detected in NCG-M mice but not in NCG mice (Fig.1). The protein expression of human SCF, GM-CSF were detected in NCG-M mice(Fig.2). Since murine IL3 is hard to be detected in serum at the physiological level in NCG mice, human IL3 protein level expression in the NCG-M model couldn't be detected which is understandable.

2. Immunophenotypes of huHSC-NCG and huHSC-NCG-M mice

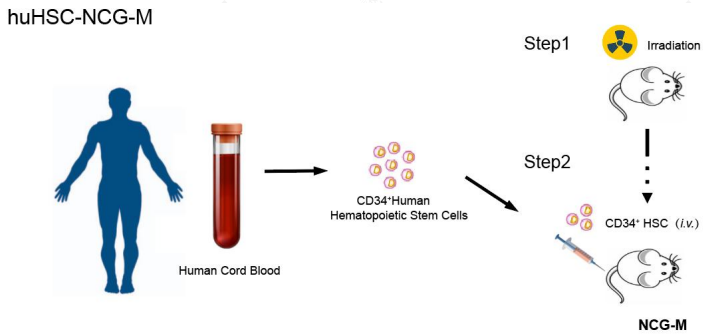


Fig.3 The reconstitution process of human immune systems in NCG-M mice.

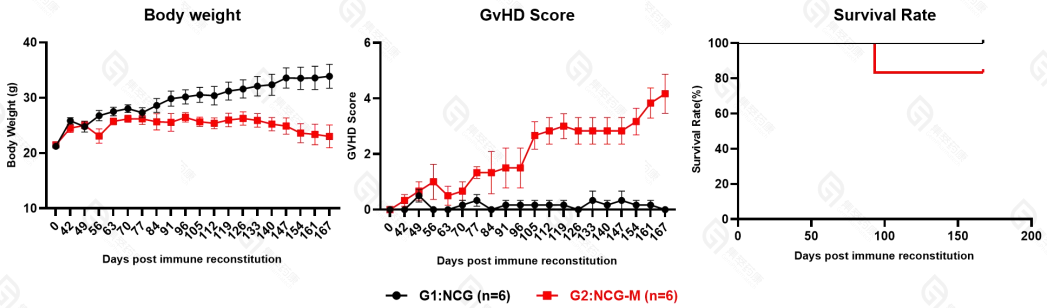
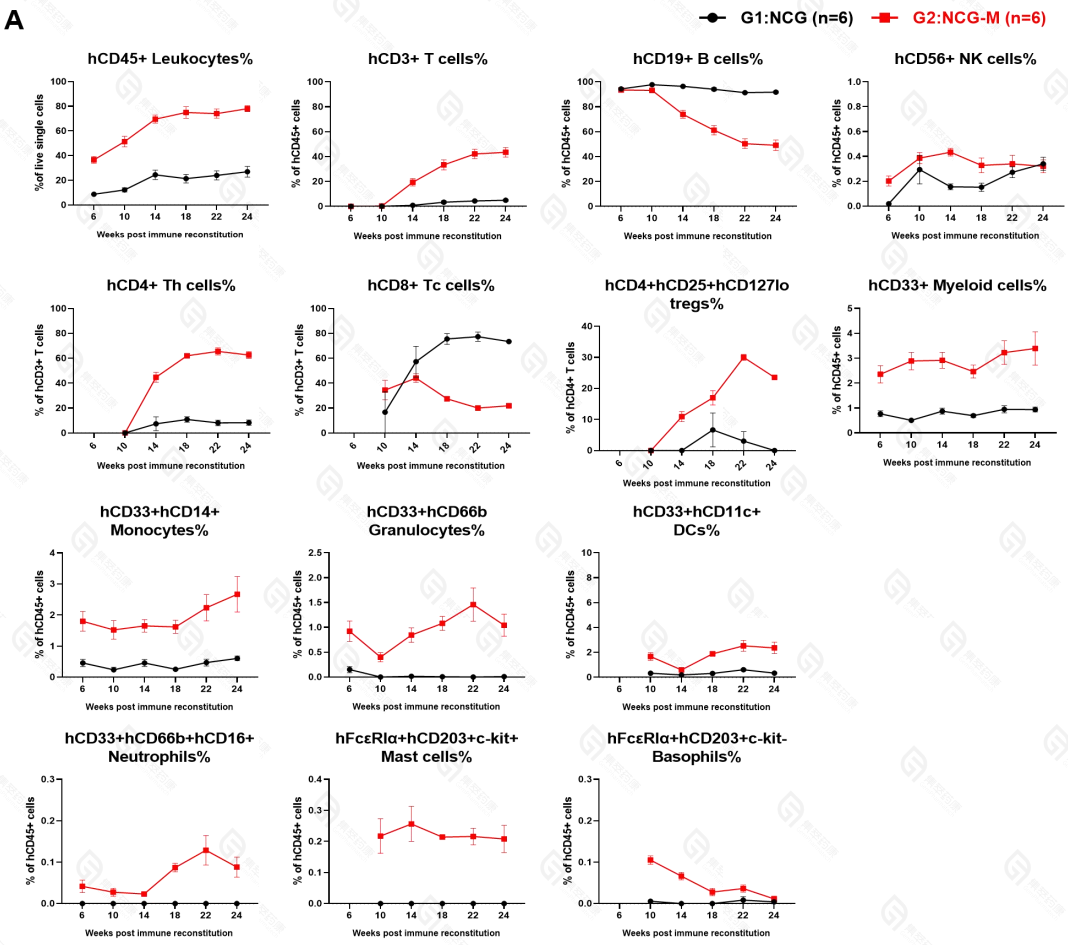


Fig.4 Body weight, GvHD score and survival curve of huHSC-NCG and huHSC-NCG-M mice.



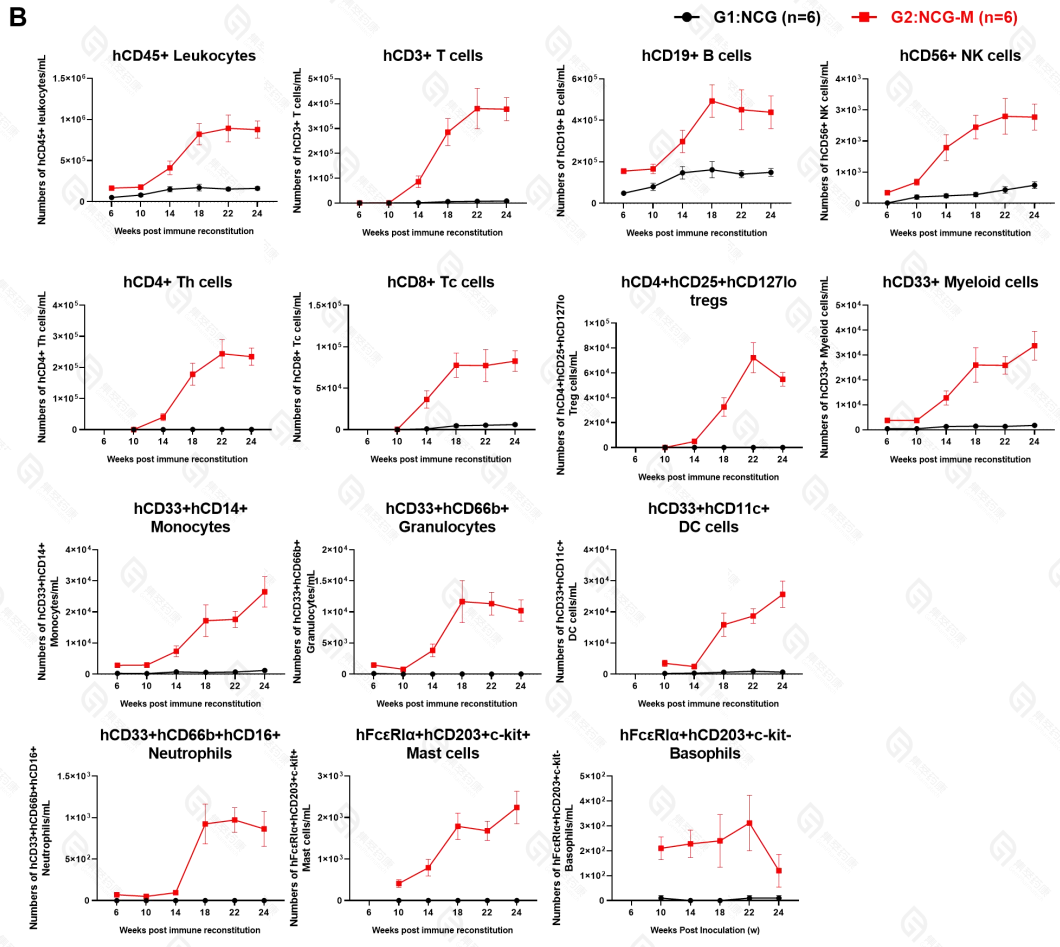
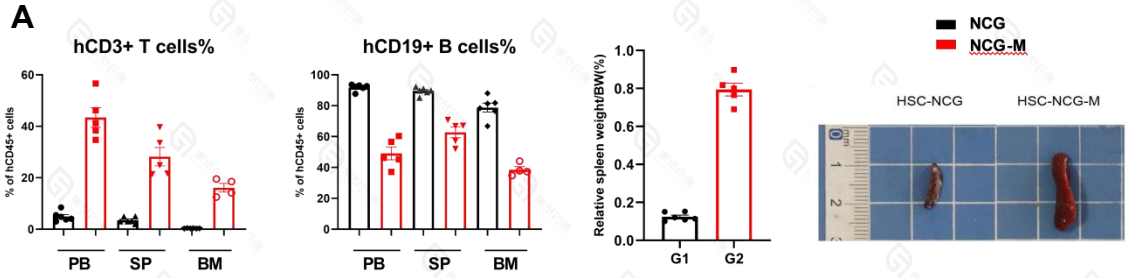


Fig.5 Reconstruction of different types of immune cells in peripheral blood of huHSC-NCG and huHSC-NCG-M mice.

NCG and NCG-M mice were engrafted with 5×10^4 $CD34^+$ hematopoietic stem cells via intravenous injection after irradiation. Peripheral blood (PB) was collected at weeks 6, 10, 14, 18, 22 and 24 post engraftment to characterize immunophenotypes in huHSC-NCG and huHSC-NCG-M mice by flow cytometry. Compared with huHSC-NCG mice, more diversed humanized immune system were reconstituted, especially producing the $hCD33^+$ myeloid lineages and $hCD14^+hCD33^+$ monocytes populations in huHSC-NCG-M mice as well as with the higher human leukocytes level.



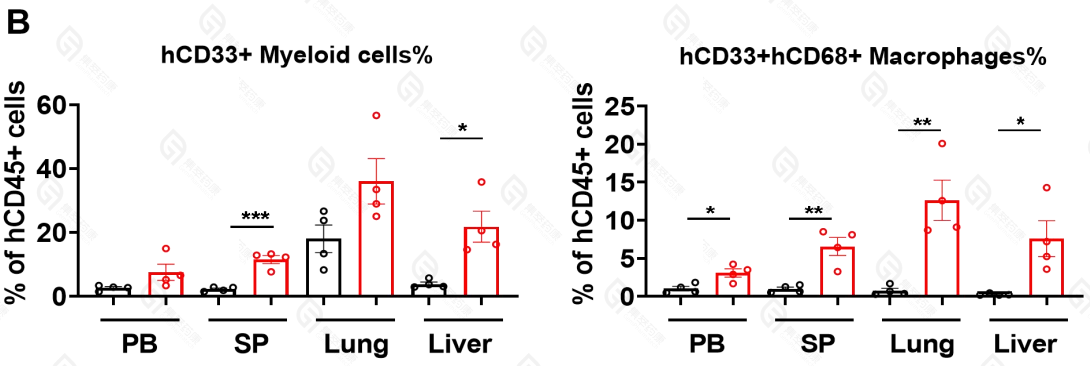


Fig.6 Reconstruction of different types of immune cells in peripheral blood of huHSC-NCG and huHSC-NCG-M mice.

PB and various tissues were collected from huHSC-NCG and huHSC-NCG-M mice at endpoint (24w post immune reconstitution) and analyzed by FACS. Improved engraftment of human T cells reconstitution were evident in the huHSC-NCG-M mice versus huHSC-NCG mice in different tissues and spleens were markedly enlarged in huHSC-NCG-M mice (Fig.A).

PB and various tissues were collected from huHSC-NCG and huHSC-NCG-M mice at 14w post immune reconstitution and analyzed by FACS. Improved engraftment of human myeloid cells and macrophages reconstitution were evident in the huHSC-NCG-M mice versus huHSC-NCG mice in different tissues (Fig.B).

3. Survival curve of huHSC-NCG-M mice

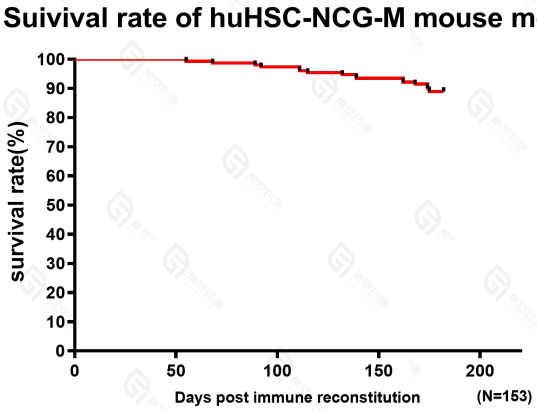


Fig.7 Survival curve of huHSC-NCG-M mice.

Female NCG-M (4-6 weeks) mice were engrafted with $4x10^4$ $CD34^+$ HSC (from nine different donors) via intravenous injection after irradiation. HuHSC-NCG-M mice ($hCD45^+$%>25% at 6w post immune reconsitution analyzed by facs in peripheral blood) mostly can survive 26 weeks after engraftment.

References

1.Shultz LD, Schweitzer PA, Christianson SW, et al. (1995). "Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice". J. Immunol. 154 (1): 180.
2.Takenaka K, Prasolava TK, Wang JC, et al. (2007). "Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells". Nat. Immunol. 8 (12): 1313.
3.Cao X, Shores EW, hu-Li J, et al. (1995). "Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain". Immunity. 2 (3): 223.
4.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.
5.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.
6.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.
7.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.