NCG-hIL15

Strain Name: NOD/ShiLtJGpt-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}*Il15*$^{em1Cin(hIL15)}$/Gpt
Strain type: Knock-in
Strain number: T004886
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, 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]. Loss of Prkdc gene leads to the inability of V(D)J recombination to occur, resulting in the inability of T cells and B cells to mature. IL2rg is a common subunit of various interleukin cytokine receptors, and the inactivation of IL2rg leads to the loss of six different cytokine signaling pathways[3], resulting in NK cell defects [4].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 reconstitution. The NCG has a long life cycle of >89 weeks, which is beneficial for long-term transplantation and pharmacodynamic evaluation.

IL15 (interleukin-15) is a pleiotropic cytokine produced by activated monocytes-macrophages, epidermal cells, fibroblasts and many other cells, exhibiting biological activity similar to IL2. IL15 can activate T cells, B cells and NK cells, and mediate the proliferation and survival of these cells[5, 6]. NCG-hIL15 strain, knocked in the humanized IL15 gene on an NCG strain, can support the colonization and activity of human NK cells. The NCG-hIL15 mice verified by phenotypic analysis can be matched with other cytokines humanized mouse strains and also will become an important model for the reconstitution of the human immune system.

Model strategy

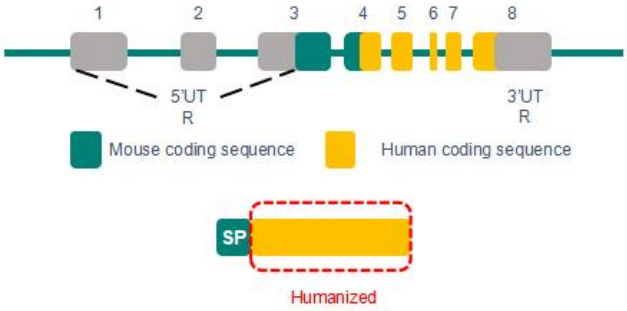

Figure 1. NCG-hIL15 model strain strategy.

Application

1. Study on the mechanism of NK cell development
2. Studies on tumor immunotherapy in which NK cells or NK and T cells act in combination
3. Antibody-dependent NK cell-mediated toxicity study (ADCC)
4. Efficacy validation of CAR-NK
5. Human hematopoietic and immune system research

Data support

1. IL15 mRNA expression in different tissue of NCG-hIL15 mice

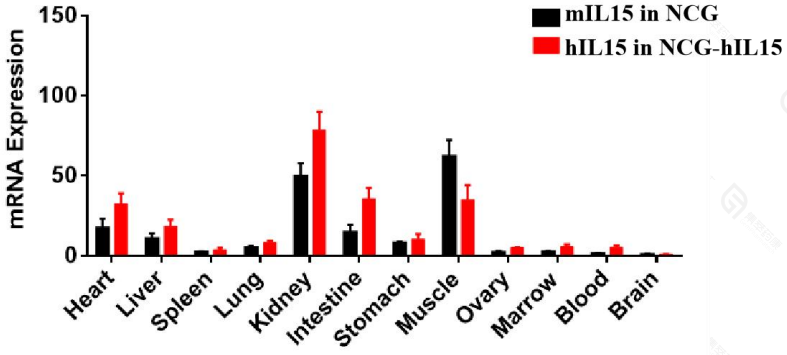

Fig 2. mRNA expression of hIL-15 in NCG-IL15 and mIL-15 in NCG by Q-PCR

The mRNA expression of hIL15 in NCG-IL15 humanized mice was consistent with mIL15 in NCG background mice by Q-PCR.

2. hIL15 expression in peripheral blood of NCG-hIL15 mice.

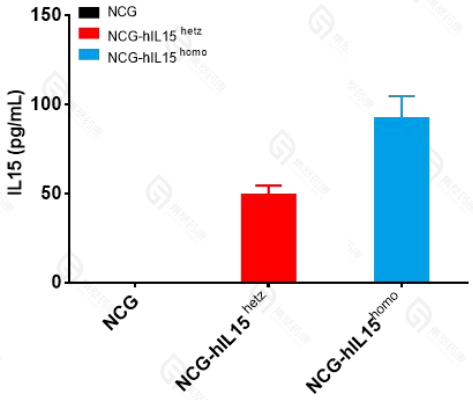

Fig 3. human IL-15 protein level in plasma

Higher level of hIL15 was detected in the peripheral blood plasma of NCG-IL15 mice (physiological level) by ELISA, while not detected in the peripheral blood plasma of NCG mice.

3. T/B/NK cell ratio assay

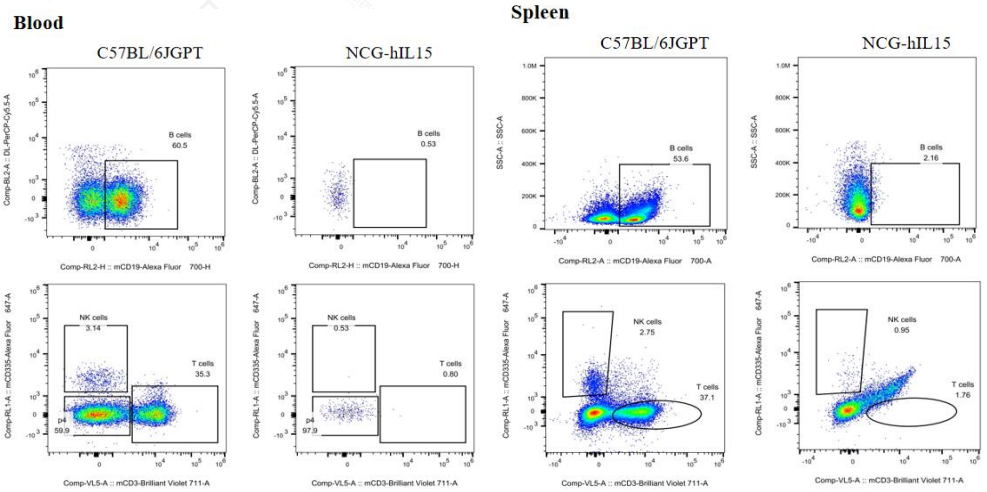

Fig 4. Detection of T/B/NK cell ratio in NCG-hIL15 mice.

The peripheral blood and spleens were collected from 5-week-old C57BL/6JGpt and NCG-hIL15 female mice were detected by flow cytometry. The proportions of T cells ($CD3^+$), B cell ($CD19^+$) and NK cells ($CD335^+$) were determined. The results indicated that, compared with C57BL/6JGpt, NCG-hIL15 mice had almost no T, B and NK cells, and the immune deficiency was more complete.

4. huHSC-NCG-hIL15 moulding process

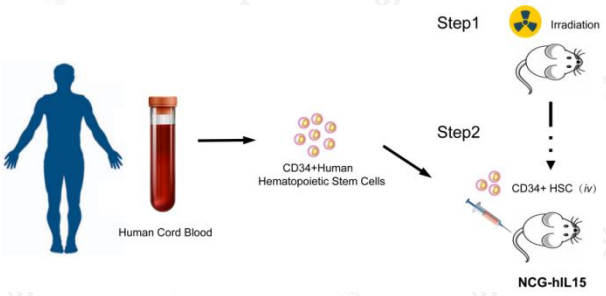

Figure 5. The huHSC-NCG-hIL15 reconstitution process.

5. huHSC reconstitution in NCG-hIL15 mice

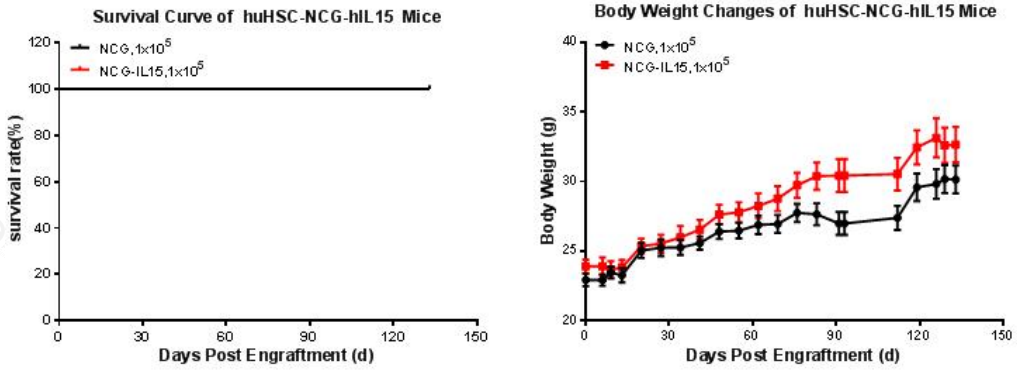

Fig 6. Survival and weight changes in NCG-hIL15 mice after huHSC reconstitution.

The body weights of huHSC-NCG and huHSC-NCG-hIL15 mice were both steadily increased during immune reconstitution.

6. Immunophenotypes of huHSC-NCG-hIL15 mice

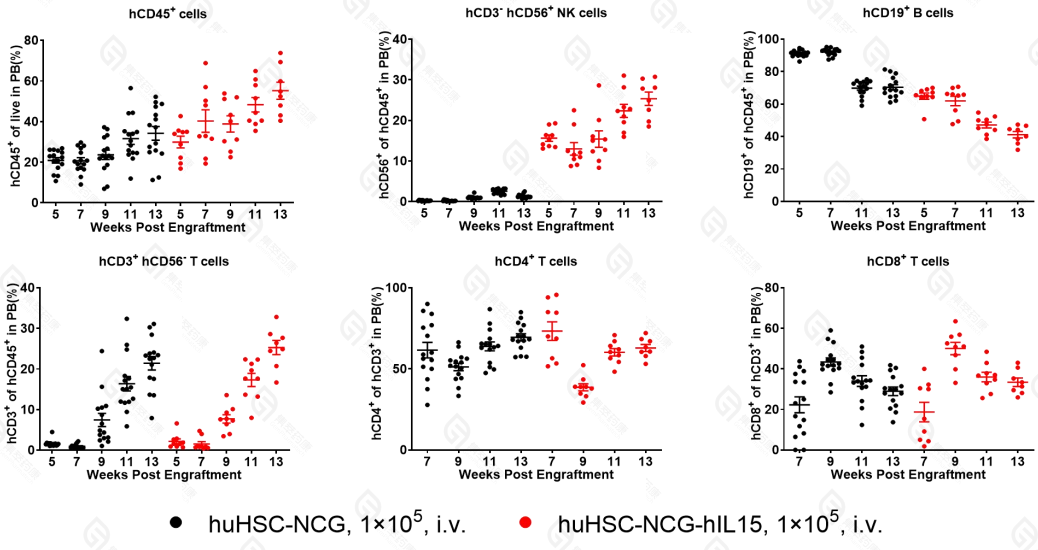

Fig 7. Reconstitution of different immune cells in peripheral blood of huHSC-NCG-hIL15 mice.

Peripheral blood of huHSC-NCG and huHSC-NCG-hIL15 mice was collected and detected by flow

cytometry at weeks 7, 9, 11 and 13 after HSC engraftment. The levels of hCD45, hCD3, hCD19, hCD4, hCD8 and hCD56 were measured. $hCD45^+$ leukocytes in the peripheral blood of huHSC-NCG-hIL15 mice reached more than 20% at around 5 weeks as the immune reconstitution process progressed, with $hCD3^+$ T cell levels gradually increasing and $hCD4^+$ and $hCD8^+$ T cell subsets differentiated. The reconstituted levels of $CD56^+$ NK cells were significantly higher in huHSC-NCG-hIL15 mice compared to huHSC-NCG mice.

7. Function of NK cells in peripheral blood of huHSC-NCG-hIL15 mice

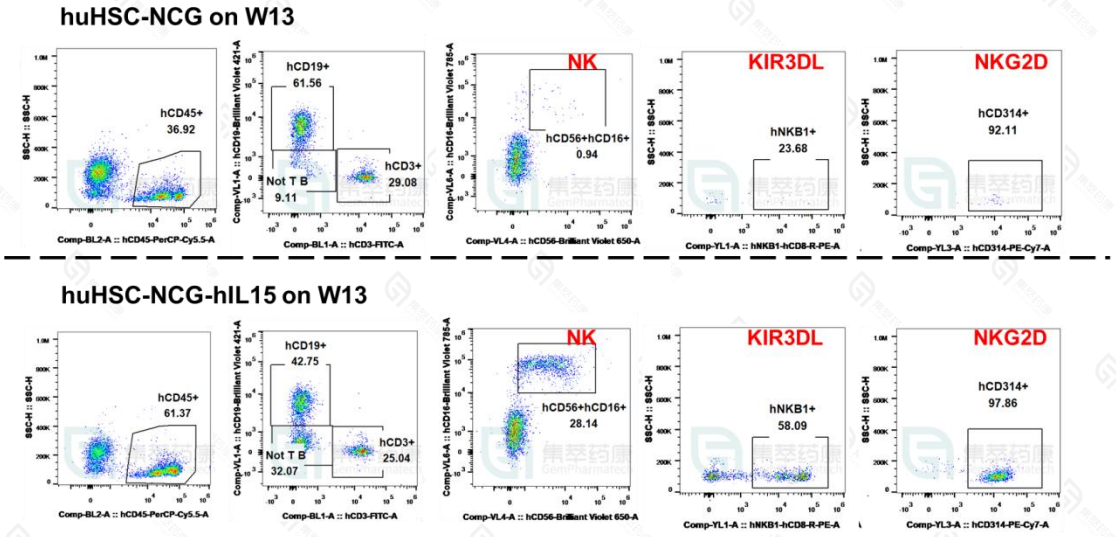

Fig 8. Expression of typical NK cell functional genes of huHSC-NCG-hIL15 mice.

Peripheral blood of huHSC-NCG and huHSC-NCG-hIL15 mice was collected at 13 weeks (W13) post-reconstitution, and the expression of functional proteins on the surface of reconstituted human NK cells was detected by flow cytometry. Compared with huHSC-NCG, huHSC-NCG-hIL15 mice showed significantly higher levels of reconstituted human NK cells and could express KIR3DL, NKG2D and other key functional NK proteins.

8. Functional validation of reconstituted NK cells in vitro and in vivo

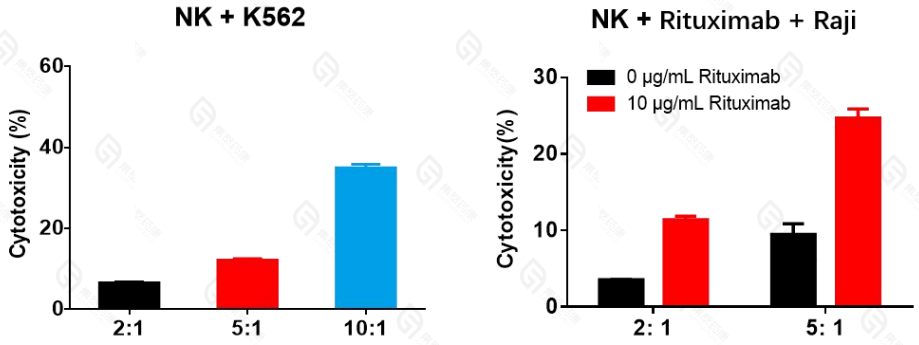

Fig 9. Detection in vitro killing test and ADCC effects by the reconstitution NK cells in huHSC-NCG-IL15 mice.

As shown in the figure on the left, the killing ability of reconstitution NK cells to K562 cells enhanced with the increase of effect-target ratio. The right figure showed that, the killing ability of reconstitution NK cells

against Raji cells increased with the increase of effect-target ratio. These data suggested that huHSC-NCG-IL15 mice could reconstitute functional NK cells.

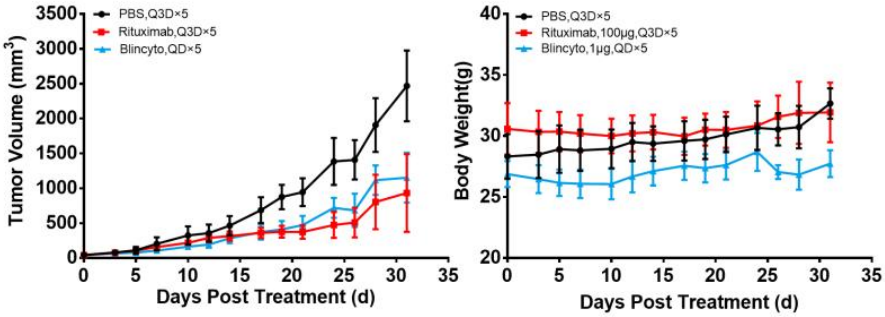

Fig 10. In vivo efficacy study in huHSC-NCG-IL15.

The huHSC-NCG-IL15 mice were inoculated subcutaneously with Raji cells. When tumors reached an average volume of 40-50 mm^3, mice were treated with control(black), Rituximab antibody and Blincyto antibody. Rituximab antibody and Blincyto antibody had obvious inhibitory effect on tumor growth (TGI=59.67%, TGI=48.95%). Indicating that huHSC-NCG-IL15 mice are the ideal animal models to evaluate the efficacy of human anti-tumor antibody that based on T and NK cell.

9. huPBNK-NCG-hIL15 moulding process

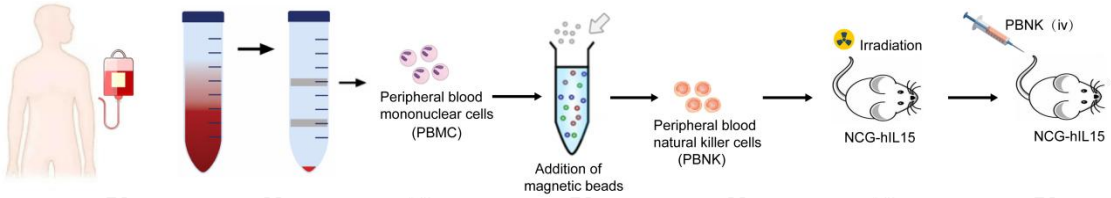

Fig 11. huPBNK-NCG-hIL15 reconstitution process.

10. huPBNK-NCG-hIL15 reconstitutes NK cells at higher levels

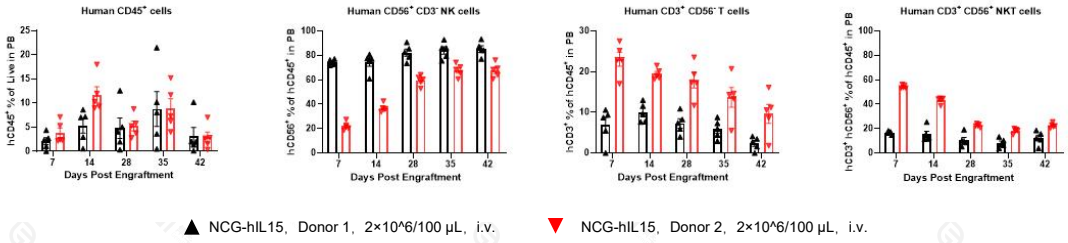

Fig 12. Reconstitution of NK cells by huPBNK-NCG-hIL15.

Two different huPBNK donors were sorted from two huPBMC donors using $CD56^+$ magnetic beads and then inoculated in NCG-hIL15 mice. Peripheral blood of huPBNK-NCG-hIL15 mice was collected at weeks 1, 2, 4, 5 and 6 after reconstitution for flow assays of hCD45, hCD3, hCD56. The results showed that the two different huPBNK donors had better reconstitution levels in NCG-hIL15 mice and the reconstitution levels of $CD56^+$ NK cells were in the range of 60%-80%.

11. huPBNK-NCG-hIL15 reconstitutes NK cells at higher level

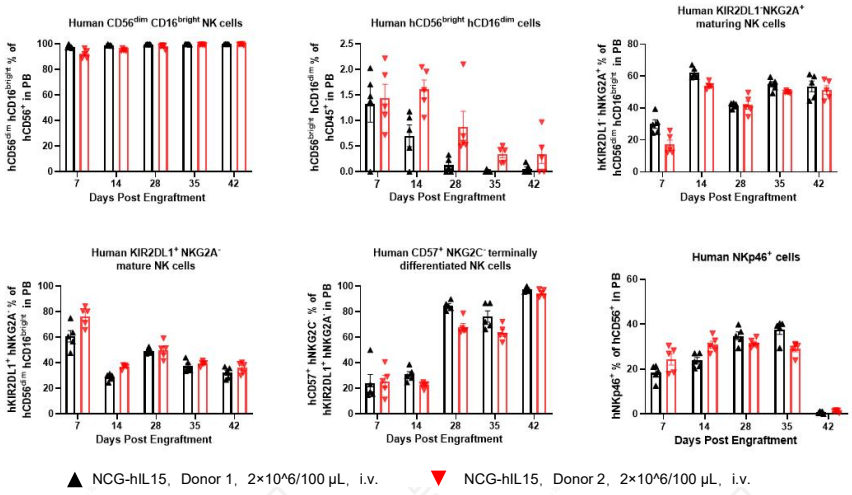

Fig 13. Subsets of NK cells in huPBNK-NCG-hIL15 mice.

Peripheral blood was collected from huPBNK-NCG-hIL15 mice at weeks 1, 2, 4, 5, and 6 after huPBNK reconstitution, and the expression of human NK cell functional protein was detected using flow cytometry. The results revealed higher levels of human functional $CD56^{dim}CD16^{bright}$ NK cells reconstituted by both donors in huPBNK-NCG-hIL15 mice. Continued study of various NK cell subsets from functional $CD56^{dim}CD16^{bright}$ NK cells revealed two different donors $KIR2DL1^{+}$ $NKG2A^{-}$ mature NK cells, $KIR2DL1^{-}$ $NKG2A^{+}$ maturing NK cells, $NKp46^{+}$ cells and $CD57^{+}$ $NKG2C^{-}$ terminally differentiated NK cells were reconstituted at relatively close levels.

References

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3. Greiner DL, Hesselton RA, Shultz LD (1998). "SCID mouse models of human stem cell engraftment". Stem Cells. 16 (3): 166–177.
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