huHSC-NCG-hIL15

Strain: huCD34+HSC-NOD/ShiLtJGpt-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}*Il15*$^{em1Cin(hIL15)}$/Gpt

Product name: huHSC-NCG-hIL15

Strain type: Immune reconstitution

Strain code: T038070

Background: NOD/ShiLtJGpt

Strain Features

HSC humanized mice are the powerful models for assessment of new drugs based on immune modulation. These models establish human immune system by engraftment of human hematopoietic stem cells (HSC) into severe immunodeficient mice(e.g. NCG). NCG mice that have been sublethally irradiated are injection with human HSCs derived from umbilical cord blood, bone marrow, fetal liver through tail vein or intraperitoneal, which allow them to develop mature human immune cells with multi-lineage, including T cells, B cells, NK cells and myeloid cells[1]. The engrafted human immune cells with the co-transplanted cancer cells mimic the tumor microenvironment and display immune response manifesting in patients[2,3]. With long survival cycle and stable reconstituted human immune system, the model could be used for long-term in vivo studies for drug effectiveness, which make it the ideal platform for preclinical drug evaluation.

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. 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 [4, 5]. NCG-hIL15 strain, knocked in the humanized IL15 gene on an NCG strain, can support the colonization and activity of human NK cells. Our HSC humanized NCG-hIL15 mice (huHSC-NCG-hIL15), combined with PDX and CDX models, could facilitate the investigation of tumor progression under human immune modulation, and evaluate drug effectiveness for anti-tumor, especially the immune-based therapeutic strategies for cancer treatment.

HSC humanization Strategy

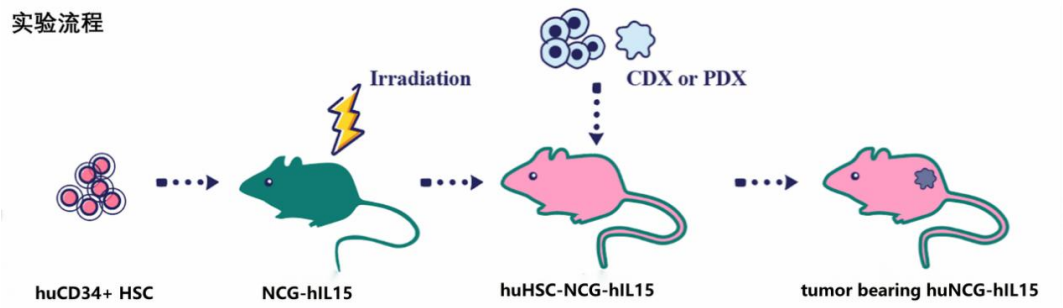

Fig.1 The establishment of huHSC-NCG-hIL15 mice

Application

1. Cell derived xenograft (CDX), Patient derived xenograft (PDX).
2. Immune-oncology therapy.
3. Human hematopoietic and immune system research

Data support

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

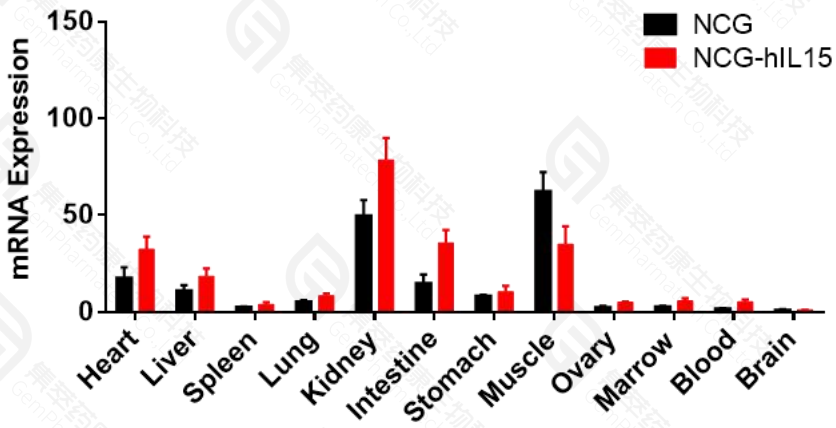

Fig.2 Comparison of mRNA expression of hIL-15 in NCG-IL15 and mIL-15 in NCG by Q-PCR

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

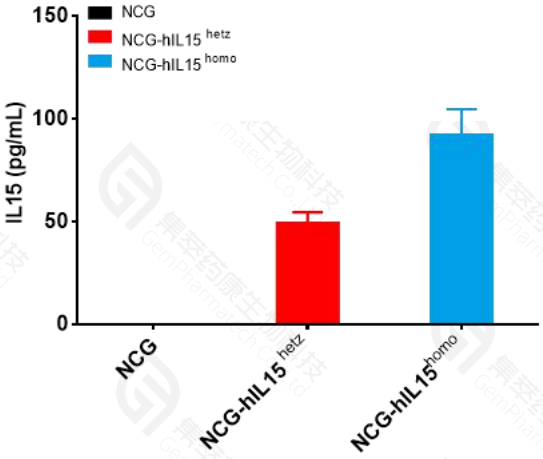

Fig.3 Comparison of human IL-15 concentrations in plasma from NCG and NCG-IL15 mice

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. HuHSC reconstruction in NCG-hIL15 mice

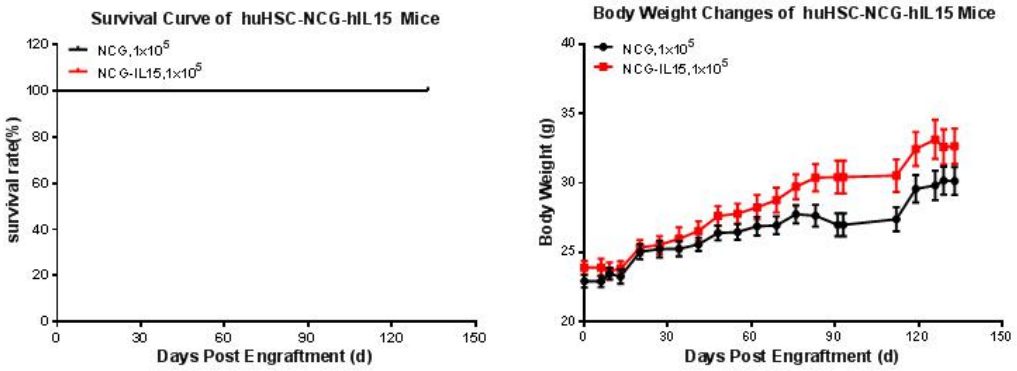

Fig.4 Survival and weight changes in mice after HuHSC reconstruction

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

2. Immunophenotypes of huHSC-NCG and huHSC-NCG-hIL15 mice

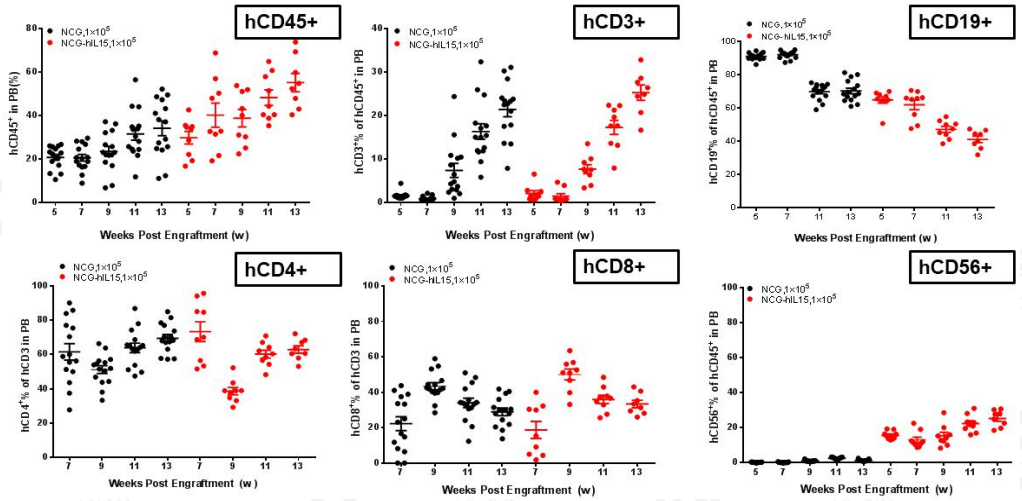

Fig.5 Reconstruction of different types of immune cells in peripheral blood of huHSC-NCG and huHSC-NCG-hIL15 mice

Peripheral blood was collected at weeks 7, 9, 11 and 13 post engraftment to characterize immunophenotypes in huHSC-NCG and huHSC-NCG-hIL15 mice by flow cytometry.

During the humanization process, hCD45+ cells have been over 20% since week 5 in huHSC-NCG-hIL15 mice. Meanwhile, hCD3+ T cells were gradually increased and developed hCD4+ T and hCD8+ T subpopulation. Compared with huHSC-NCG mice, CD56+ NK cells displayed higher reconstitution level.

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

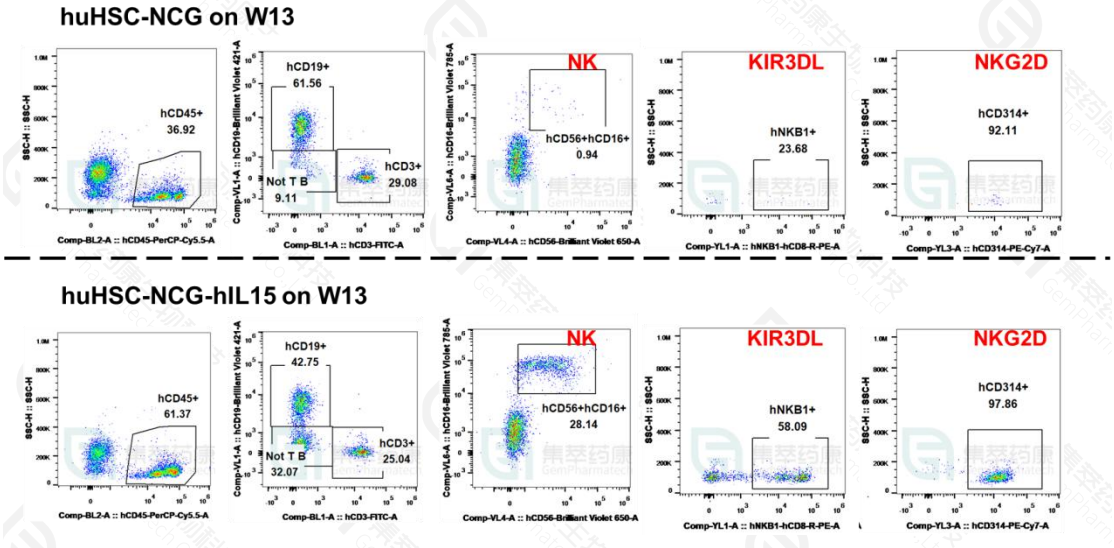

Fig.6 Expression of typical NK cell functional genes of huHSC-NCG and huHSC-NCG-hIL15 mice

Peripheral blood was collected at week 13 post engraftment to characterize NK cell functional genes in huHSC-NCG and huHSC-NCG-hIL15 mice by flow cytometry, including CD56, CD16, KIR2DL and NKG2D. Compared with huHSC-NCG, the humanization of NK cell reconstruction level was significantly increased in huHSC-NCG-hIL15 mice, and the functional proteins KIR2DL and NKG2D were also detected.

4. Data validation

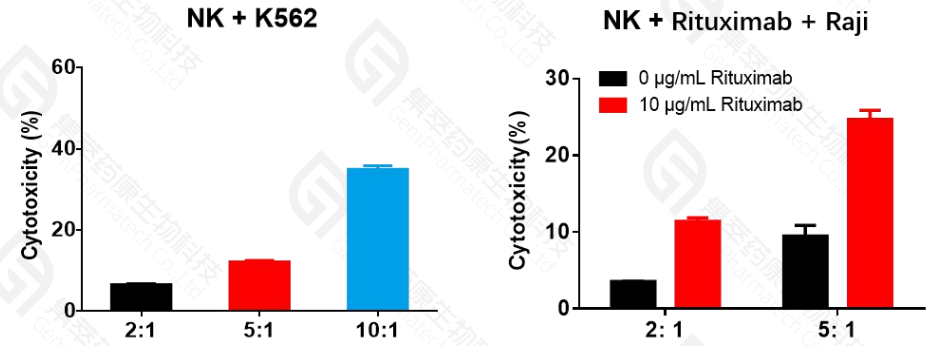

Fig.7 Detection the in vitro killing test and ADCC effects by the reconstruction NK cells in HuHSC-NCG-IL15 mice

As shown in the figure on the left, the killing rate of reconstructed NK cells against K562 cells in NCG-IL15 humanized mice increased with the increase of effect-target ratio.

The right figure showed that, the killing rate of reconstructed NK cells in NCG-IL15 humanized mice against Raji cells increased with the increase of effect-target ratio.

These data suggested that HuHSC-NCG-IL15 mice could reconstruct functional NK cells.

In vivo efficacy test of Rituximab and Blincyto against the Human lymphoma in huHSC-NCG-IL15. Mean Tumor Volume ± SEM

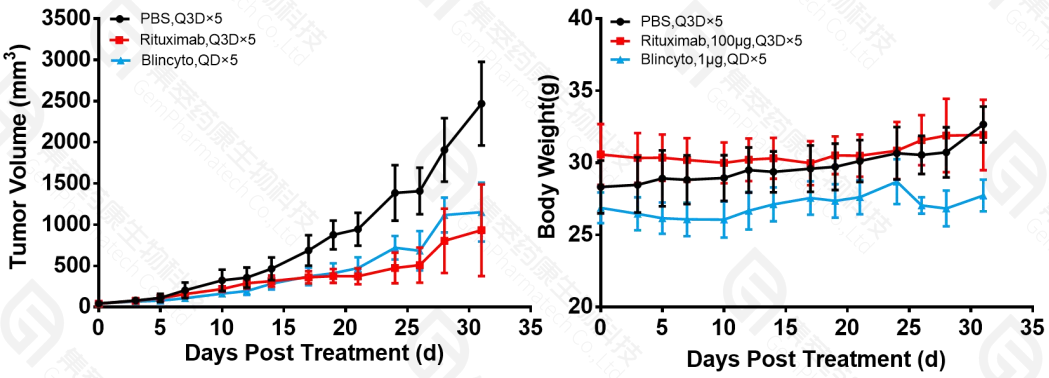

Fig.8 In vivo efficacy test in huHSC-NCG-IL15.

The huHSC-NCG-IL15 mice were inoculated subcutaneously with Raji cells. When tumors reached an average volume of 40-50 mm3, 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 model to evaluate the efficacy of human anti-tumor antibody that based on T cell and NK cell.

References

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4. 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.
5. 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.