NCG-MHC-dKO

Strain Name： NOD/ShiLtJGpt*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}*H2K1*^{em2Cd10}*H2D1*^{em2Cd6in16de11in5} *H2Ab1* ^{em2Cd1}/Gpt
Strain type： Knock-out
Strain number： T050886
Background： NOD/ShiLtJGpt

Description

NOD/ShiLtJGpt genetic background mice have natural immune defects such as complement system, macrophage defects. Mutations in the Prkdc gene result in impaired VDJ recombination (Scid mice), resulting in failure of T and B cell development to mature, manifesting as a severe combined immunodeficiency of cellular and humoral immunity. IL2 receptor gamma (IL2rγ) chain is a common receptor subunit for the immunologically important cytokines IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Knockout mice with the IL2rg gene encoding IL2rγ have severely reduced immune function in the organism, particularly with almost no activity of NK cells. NCG, a highly immunodeficient model produced by knocking out the Prkdc and Il2rg genes in NOD/ShiLtJGpt mice by gene editing techniques, has a more homogeneous background, is long-lived, lacks mature T, B and NK cells and lacks complement activity.

MHC class I molecules are distributed on the surface of almost all nucleated cells, but the level of expression varies considerably between cells of different tissues. An important physiological function of class I molecules is to exert a limiting effect on the antigen recognition function of $CD8^+$ T cells, i.e. They are involved in the delivery of antigens to $CD8^+$ T cells. $CD8^+$ T cells can only recognise antigens bound to the same class I molecules (mostly endogenous cellular antigens, such as virus-infected cells, tumour cells, etc.), a phenomenon known as MHC restriction. For example, when a virus infects a cell, the viral antigen can be broken down into short peptide fragments which are expressed on the cell surface after binding to a class I molecule synthesised in the endoplasmic reticulum in order to be recognised by $CD8^+$ T cells. MHC class I molecules mainly mediate the cytotoxic effects of Tc cells and are also critical transplant antigens.

MHC class II molecules have a more restricted distribution and are mainly expressed on antigen-presenting cells such as B cells, monocytes-macrophages and dendritic cells, but also on spermatocytes and some activated T cells. Some cells that do not normally express class II molecules can be induced to express class II molecules by cytokines during the immune response, and therefore the expression of class II molecules is considered to be a marker of antigen-presenting ability. The function of class II molecules is mainly to deliver processed antigen fragments to CD4 T cells during the initiation phase of the immune response. Just as CD8 T cells can only recognise antigen fragments bound to MHC class I molecules, CD4 T cells can only recognise antigen fragments bound to class II molecules. Class II molecules are mainly involved in the presentation of exogenous antigens, but under some conditions they can

also present endogenous antigens. In tissue or organ transplantation, class II molecules are important target antigens for causing graft rejection, including causing host-versus-graft reactions (HVGR) and graft-versus-host reactions (GNHR). In the immune response, class II antigens mainly coordinate the interactions between immune cells and regulate humoral and cellular immune responses.

NCG-MHC-dKO strain of mice was obtained by knocking out H2-related genes and disrupting MHC class I and class II molecules by gene editing techniques using the NCG as a background. The mice combine severe immunodeficiency mutations (Scid), Il2rγ null and lack of MHC class I/II molecules to minimise the graft-versus-host disease (GvHD) response and extend the window period of the experiment and to ensure normal immune cell ratios and accuracy of antibody molecular evaluation. It can be used for the preparation of humanised mouse models such as PBMC or HSC, for human cell tissue transplantation, for the inoculation of human derived tumours for drug screening, for the study of the human haematopoietic and immune systems, and for studies related to graft-versus-host disease (GvHD).

Application

1. For the preparation of humanized mouse models of the immune system
2. For human-derived cellular tissue transplantation
3. Inoculation of human-derived tumors for screening of relevant drugs
4. For research on the human haematopoietic and immune systems
5. For graft-versus-host disease (GvHD) related research

Model strategy

This model uses CRISPR/Cas9 technology for gene editing of the H2-K1 gene and the principle is schematically shown below：

Figure 1. Schematic diagram of H2-K1 gene knockout in NCG-MHC-dKO mice.

This model uses CRISPR/Cas9 technology for gene editing of the H2-Ab1 gene and the principle is schematically shown below：

Figure 2. Schematic diagram of H2-Ab1 gene knockout in NCG-MHC-dKO mice.

This model uses CRISPR/Cas9 technology for gene editing of the H2-D1 gene and the principle is schematically shown below:

Figure 3. Schematic diagram of H2-D1 gene knockout in NCG-MHC-dKO mice.

Data support

1. MHC I/II detection

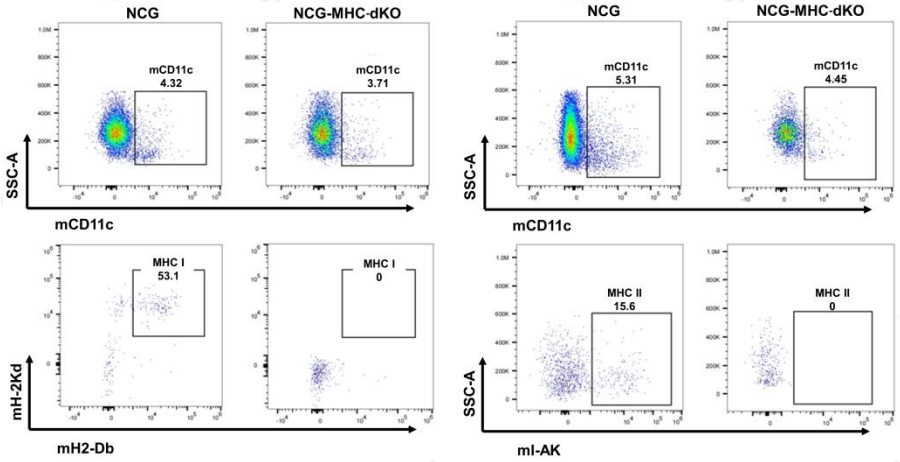

Figure 4. Detection of mouse MHCI and MHCII protein expression in NCG-MHC-dKO Mice.
Peripheral blood was collected from 6-8 weeks old NCG and NCG-MHC-dKO mice and analyzed by flow cytometry for the expression of MHC I (mH-2Kb/mH2-Db; left) and MHC II (mI-Ak; right) in dendritic cells (mCD11c), neither of which was detected in NCG-MHC-dKO mice.

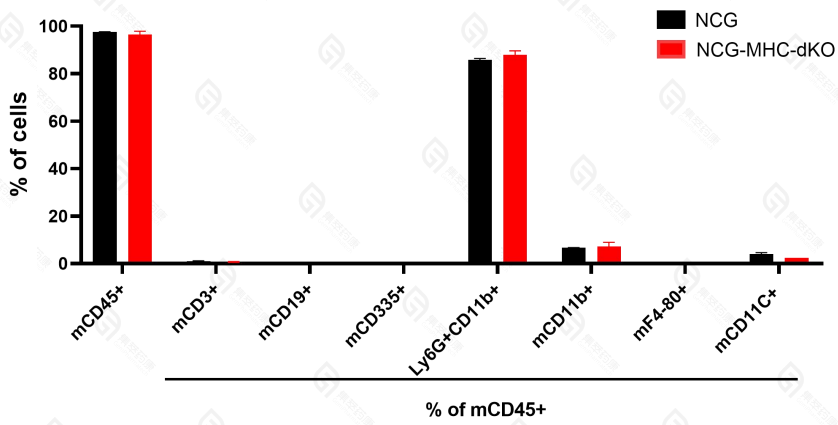

Figure 5. Detection of immune cell fractions in NCG/NCG-MHC-dKO mice.

After knocking out MHC I/II in NCG-MHC-dKO mice, the immune cell subpopulations was detected by FACS. The results showed that T, B, NK, monocytes, neutrophils and dendritic cells were almost identical to those of NCG mice.

2. Human PBMC reconstitution in NCG-MHC-dKO mice

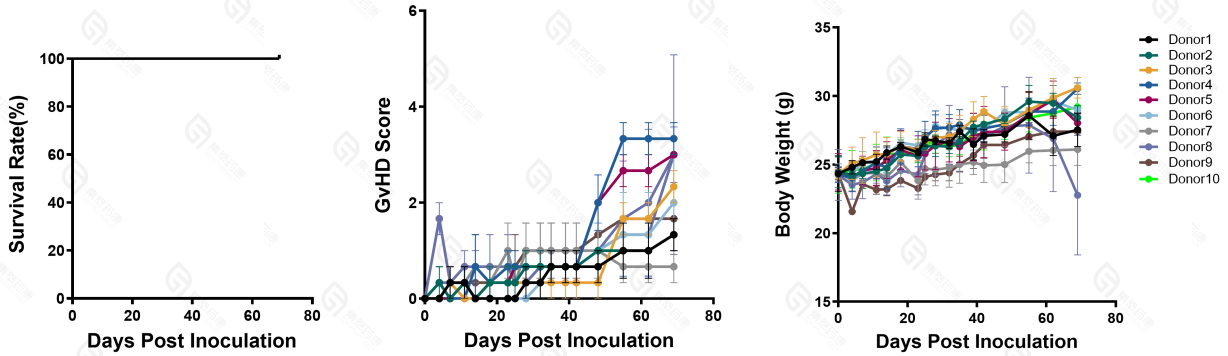

Figure 6. Survival Rate, GvHD score and Body Weight changes of NCG-MHC-dKO mice after transplanted with huPBMC.

NCG-MHC-dKO mice (n=3/group) were inoculated with 10 different huPBMC donors separately ($1x10^7$, 200 μL, i.p.). During the observation period, NCG-MHC-dKO mice showed little GvHD symptoms with no severe loss of body weight and long survival rate. However, after 50 days of huPBMC reconstitution, several groups of NCG-MHC-dKO mice had slightly GvHD symptoms, which mainly included the status of activity, posture, hair and skin of the mice.

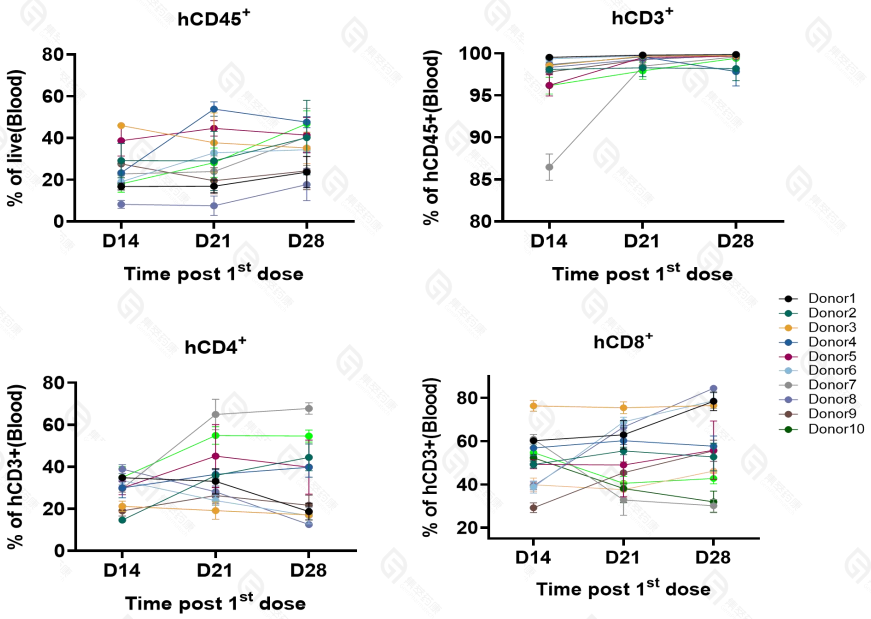

Figure 7. huPBMC Reconstitution efficiency in NCG-MHC-dKO mice.

NCG-MHC-dKO mice (n=3/group) were inoculated with 10 different huPBMC donors separately ($1x10^7$, 200 μL, i.p.). Peripheral blood of NCG-MHC-dKO mice was collected on Day 14, 21 and 28 after huPBMC engraftment for flow cytometry analysis, respectively. The results showed that all 10 different huPBMC donors were able to reconstitute the human immune system in NCG-MHC-dKO mice well, and the reconstituted cells were mainly human T cells, which means PBMC reconstitution efficiency is relatively high and stable in the NCG-MHC-dKO model.

3. Half-life of hIgG

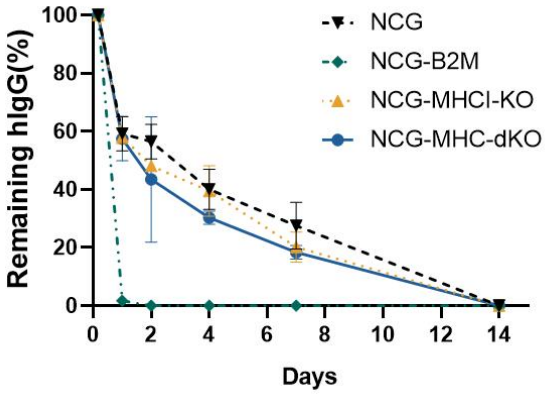

Figure 8. Changes in the half-life of human IgG in the sera of NCG, NCG-B2M, NCG-MHCI-KO and NCG-MHC-dKO mice.

Six-week-old female mice were injected with 200 ug of human IgG (hIgG) via intraperitoneal (n=3/group). Plasma was collected from the mice at various time points and ELISA assays were performed to detect changes in the levels of circulating human IgG. Results revealed that hIgG clearance was faster in NCG-

B2M mice compared to NCG mice, while NCG-MHC-dKO mice had a similar hIgG half-life comparing to NCG mice.

4. Efficacy study in PBMC reconstitution mouse model

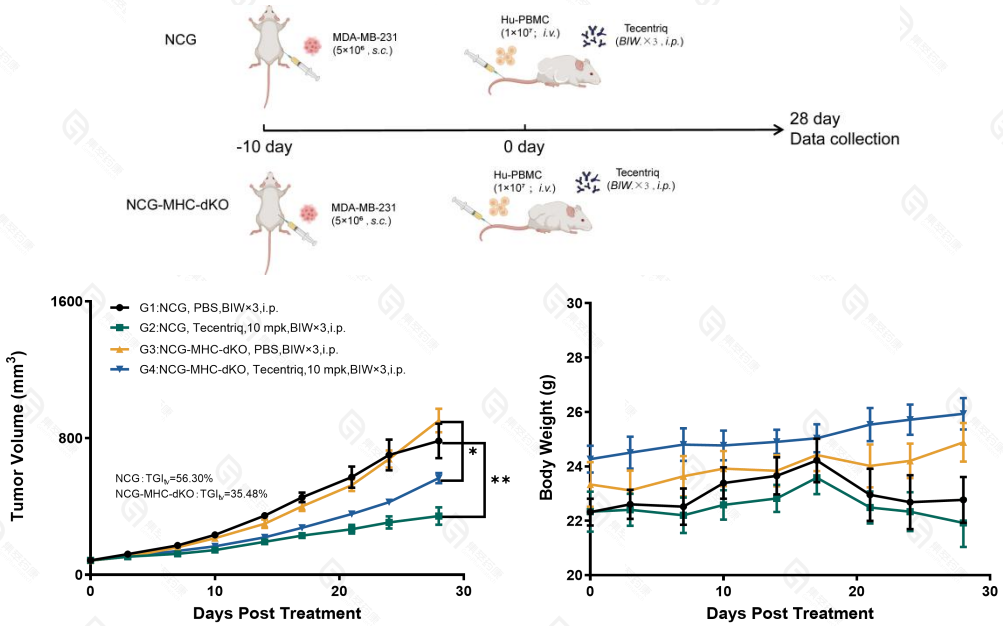

Figure 9. Evaluation of the efficacy of Tecentriq in the NCG-MHC-dKO mice after huPBMC reconstitution.

Six-week-old mice were inoculated subcutaneously with the MDA-MB-231 breast cancer cell line and randomly grouped (n=6/group) when tumours had grown to approximately 80-100 mm^3. Mice were inoculated with huPBMC on the day of grouping and then administered PBS or Tecentriq (BIW x 3, i.p) and monitored for body weight and tumour size from the time of dosing until 28 days. Tecentriq was shown significantly inhibit MDA-MB-231 tumour growth in NCG-MHC-dKO mice, and the body weight of the mice were stable.

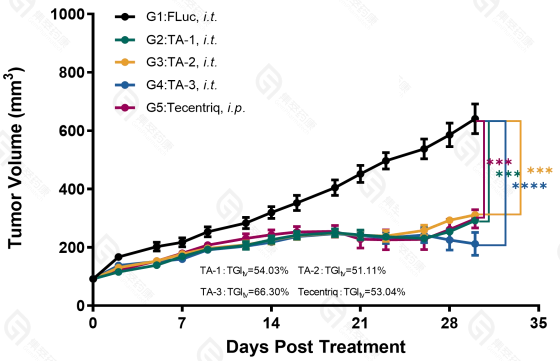

Figure 10. Evaluation of the efficacy of human mRNA drug and Tecentriq in the NCG-MHC-dKO mice after huPBMC reconstitution.

Mice were inoculated subcutaneously with the MDA-MB-231 breast cancer cell line at 6 weeks old. When tumours were approximately 60 mm^3, each mouse was injected intraperitoneally with 1 x 10^7 huPBMC cells. When tumours were approximately 80-100 mm^3, mice were randomly grouped (n=6/group) and

given different doses of the testing drug (TA) and Tecentriq as indicated. Body weight and tumour were measured over 30 days from the initiation of dosing. Results revealed that both human cytoking mRNA drug and Tecentriq significantly inhibited MDA-MB-231 tumour growth in NCG-MHC-dKO mice.

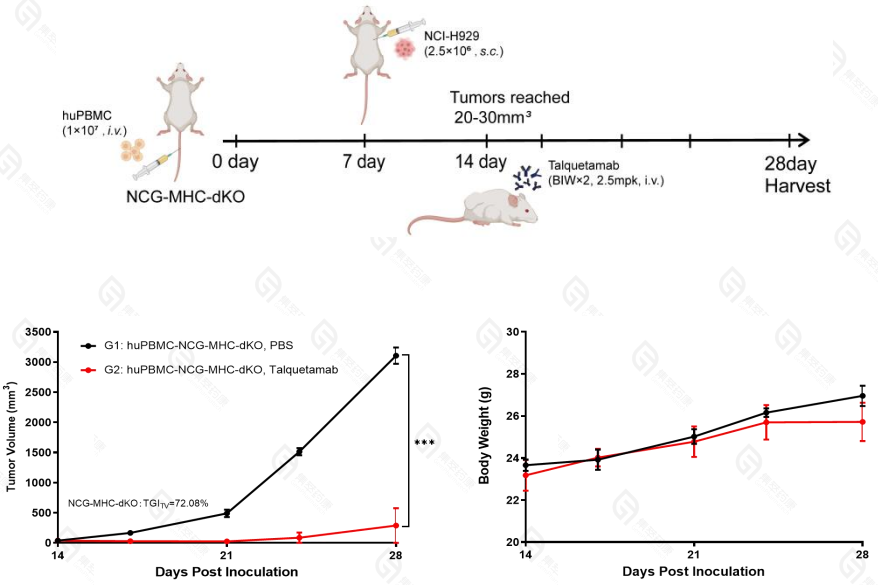

Figure 11. Evaluation of Talquetamab efficacy in the NCG-MHC-dKO mice after reconstitution of huPBMC.

Mice were injected intraperitoneally with 1 x 10^7 huPBMC cells at 6-8 weeks old. Seven days after reconstitution, mice were subcutaneously seeded with the NCI-H929 multiple myeloma cell line. When tumours were approximately 20-30 mm^3, mice were randomly grouped (n=5/group) and Talquetamab (GPRC5D x CD3 bispecific antibody) was dosed as shown. Changes in GvHD scores and tumours were observed from the initiation of dosing until 28 days. Talquetamab was found to significantly inhibit tumour growth in NCG-MHC-dKO mice, and the weight of the mice were stable during treatment, indicating that the NCG-MHC-dKO model is an ideal model for evaluating CD3 bispecific antibodies.

References

1. Sprent J, Webb SR: Function and specificity of T-cell subsets in themouse. Adv Immunol 41:39—133, 1987
2. Madsen L, et al., Mice lacking all conventional MHC class II genes. Proc Natl Acad Sci U S A. 1999 Aug 31;96(18):10338-43
3. Brehm, Michael A., et al. "Lack of acute xenogeneic graft-versus-host disease, but retention of T-cell function following engraftment of human peripheral blood mononuclear cells in NSG mice deficient in MHC class I and II expression." The FASEB Journal (2018): fj-201800636R.