BALB/c-hTIGIT/hPVRIG

Strain Name: BALB/cJGpt-Tigit$^{em1Cin(hTIGIT)}$Pvrig$^{em1Cin(hPVRIG)}$/Gpt
Strain Type: Knock-in
Strain ID: T058266
Background: BALB/cJGpt-hTIGIT

Description

TIGIT (T cell immunoglobulin and ITIM domain), a type I transmembrane protein with immunoglobulin structural domain and ITIM motif, is mainly expressed on the surface of T cells and NK cells. In vitro blockade of TIGIT enhances the activation and degranulation of NK and T cells[1]. In a variety of syngeneic mouse models, in vivo administration of TIGIT-blocking monoclonal antibodies significantly slowed the growth and metastasis of colon cancer, breast cancer and melanoma. In addition, dual PD1/TIGIT blockade effectively enhanced T cell proliferation and activation and inhibited tumor growth[2].

PVRIG (poliovirus receptor-related immunoglobulin domain-containing protein) is a poliovirus receptor-related immunoglobulin structural domain-containing protein. This protein, as a single transmembrane protein of the PVR family, is mainly expressed on $CD8^+$ T cells and $CD16^+$ and $CD16^-$NK cells[3].PVRIG binds to the ligand PVRL2 (CD112/NECTIN-2) on antigen-presenting cells and tumor cells, exerting an inhibitory effect on T cell activation and attenuating NK cell killing, which in turn contributes to tumor cell immune escape.

In vitro experimental studies have shown that blocking PVRIG promotes T cell proliferation and activation, and in addition, the combination with TIGIT and PD1 blockers can achieve more significant anti-tumor effects[4]. In the $PVRIG^{-/-}$ mouse model, PVRIG deficiency enhanced $CD8^+$ T cell proliferation and effector functions and slowed tumor growth in mice in vivo, and the combination of PD-1 blockers produced a synergistic antitumor response[5]. Blocking PVRIG also promotes NK cell killing ability of breast cancer cell lines and enhances Trastuzumab-mediated ADCC function[6]. Therefore, PVRIG is a promising immune checkpoint target for cancer therapy, and the combination study with TIGIT is also of great importance.

Gempharmatech uses gene editing technology on BALB/c-hTIGIT background mice, the portion of the extracellular region encoding the murine Pvrig gene was replaced with the corresponding human-derived PVRIG gene fragment, while retaining the signal peptide

and intracellular signal transduction region of the corresponding murine-derived gene, ensuring that the correct cell signaling was not affected. The completed BALB/c-hTIGIT/hPVRIG will be an ideal animal model for the evaluation of human TIGIT and PVRIG targeting drugs.

Strategy

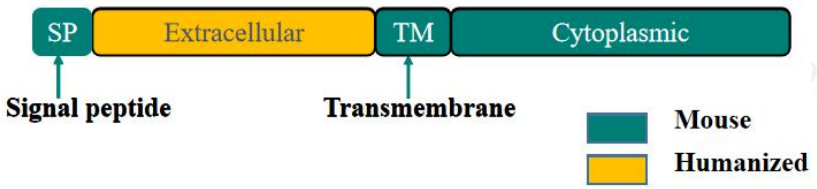


Figure 1. Schematic diagram of TIGIT humanization strategy in BALB/c-hTIGIT/hPVRIG mice.

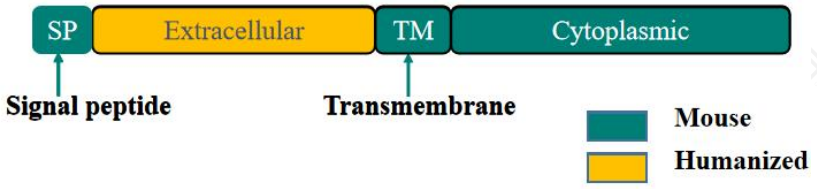


Figure 2. Schematic diagram of PVRIG humanization strategy in BALB/c-hTIGIT/hPVRIG mice.

Application

1. Efficacy and safety evaluation of TIGIT, PVRIG inhibitors.
2. Evaluation of the efficacy of human PVRIG-targeted drugs and in combination with human TIGIT-targeted drugs.

Data Support

1. Analysis of spleen Immune cell subpopulations in BALB/c-hTIGIT/hPVRIG mice

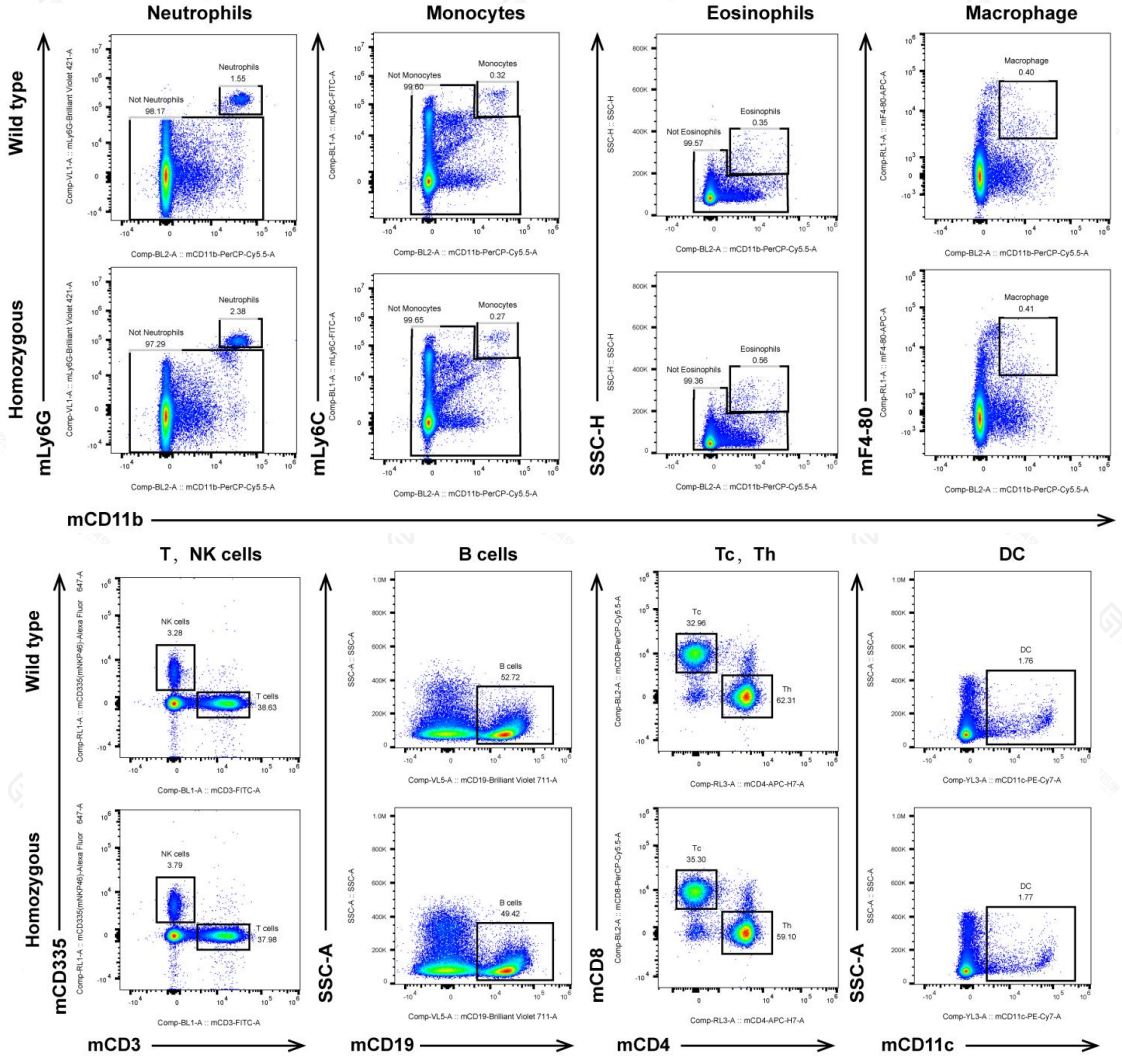


Figure 3. BALB/c and BALB/c-hTIGIT/hPVRIG spleen leukocyte subpopulation ratio assay. Spleen was taken from female BALB/c and BALB/c-hTIGIT/hPVRIG mice for flow cytometric analysis to assess Immune subpopulations. As shown in Figure 3, the percentages of T cells, NK cells, B cells, neutrophils, monocytes, and dendritic cells in BALB/c-hTIGIT/hPVRIG mice were similar to those in BALB/c, indicating that the replacement of mTigit and mPvrig by hTIGIT and hPVRIG did not alter the development, differentiation, and distribution of these cells in spleen.

2. Detection of TIGIT expression in spleen T cells

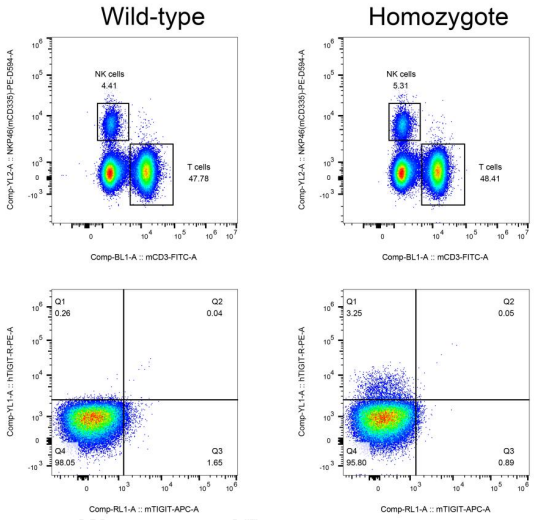


Figure 4. TIGIT expression in spleen T cells of BALB/c-hTIGIT/hPVRIG mice.

The expression of TIGIT in the spleen of BALB/c mice and BALB/c-hTIGIT/hPVRIG homozygous mice was detected by flow cytometry. The results are shown in Figure 4, the expression of hTIGIT was detectable in T cells from the spleen of BALB/c-hTIGIT/hPVRIG mice, and only mTigit expression was detectable in BALB/c mice.

3. Detection of TIGIT expression in spleen Treg cells

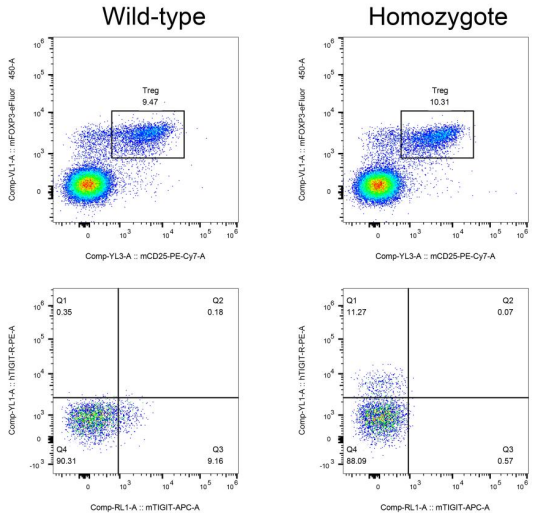


Figure 5. TIGIT expression in spleen Treg cells of BALB/c-hTIGIT/hPVRIG mice.

The expression of TIGIT in the spleen of BALB/c mice and BALB/c-hTIGIT/hPVRIG homozygous mice was detected by flow cytometry. The results are shown in Figure 5, the expression of hTIGIT was detectable in Treg cells from the spleen of BALB/c-hTIGIT/hPVRIG mice, and only mTigit expression was detectable in BALB/c mice.

4. Detection of PVRIG expression in spleen NK cells

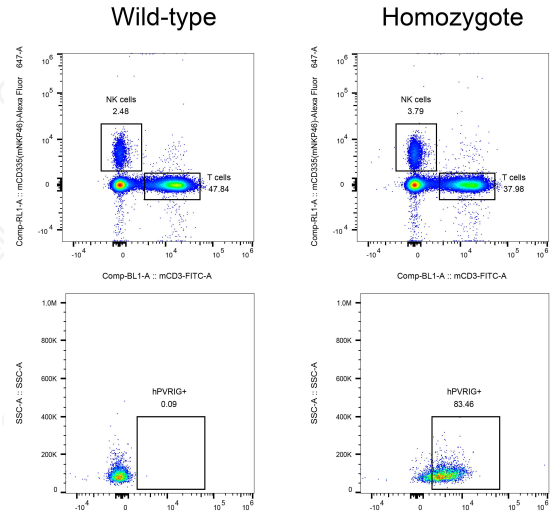


Figure 6. PVRIG expression in spleen NK cells of BALB/c-hTIGIT/hPVRIG mice.

The expression of hPVRIG in the spleen of BALB/c mice and BALB/c-hTIGIT/hPVRIG homozygous mice was detected by flow cytometry. The results are shown in Figure 7, the expression of hPVRIG could be detected in NK cells from spleen of BALB/c-hTIGIT/hPVRIG mice.

5. Detection of PVRIG expression in spleen T cells

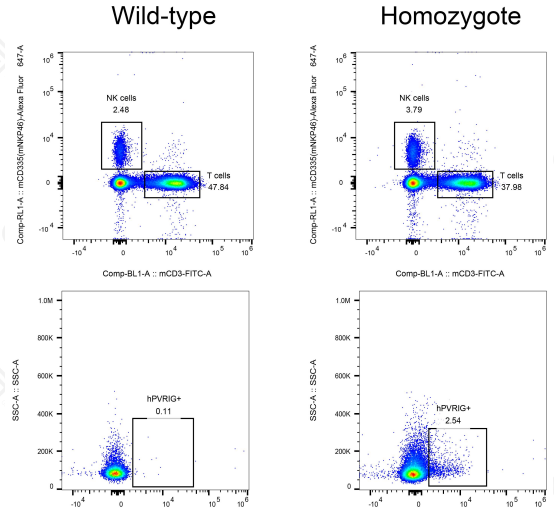


Figure 7. PVRIG expression in spleen T cells of BALB/c-hTIGIT/hPVRIG mice.

The expression of hPVRIG in the spleen of BALB/c mice and BALB/c-hTIGIT/hPVRIG homozygous mice was detected by flow cytometry. The results are shown in Figure 8, the expression of hPVRIG could be detected in T cells from spleen of BALB/c-hTIGIT/hPVRIG mice.

6. Anti-tumour efficacy data

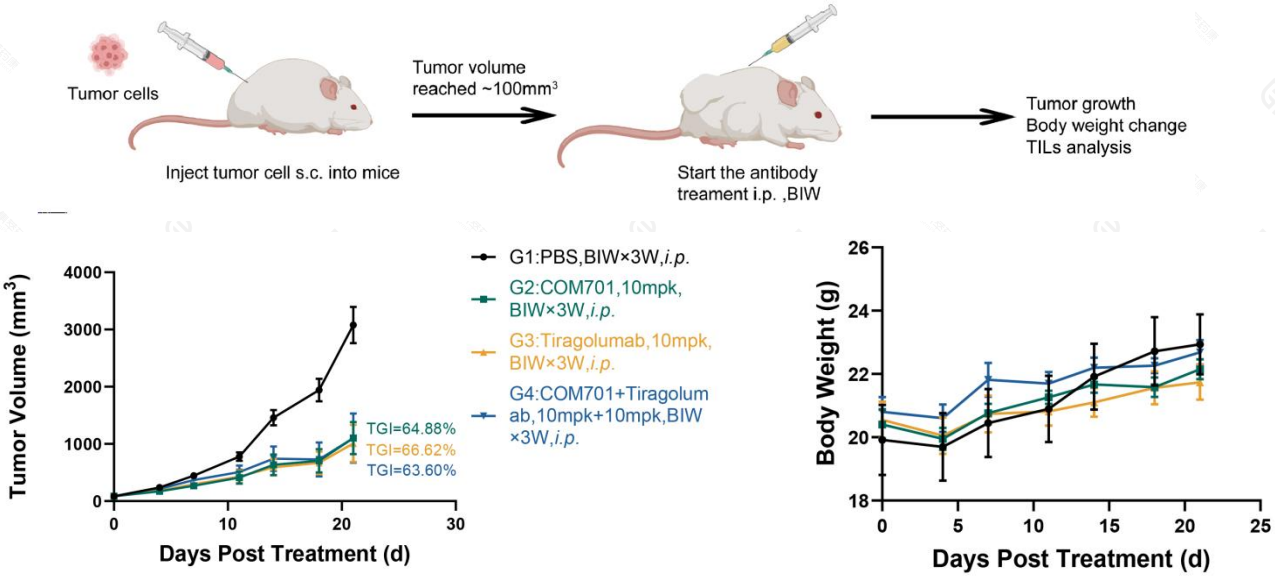
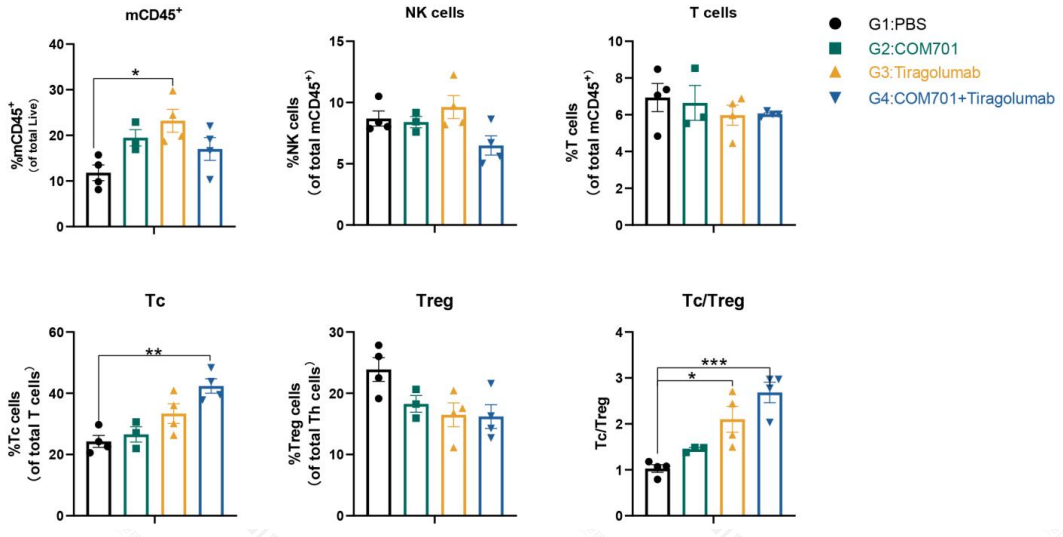


Figure 8. In vivo efficacy studies in BALB/c-hTIGIT/hPVRIG mice.

BALB/c-hTIGIT/hPVRIG mice at 6-8 weeks of age were subcutaneously inoculated with mouse colon cancer CT26 cells. When the average volume of the tumour reached about 100 mm^3, all mice were randomly divided into four groups, Vehicle (black), anti-PVRIG (green), anti-TIGIT (yellow) and combined anti-PVRIG antibody/anti-TIGIT antibody (blue), twice a week for 6 times. The results showed significant inhibition of tumour growth with both single and combination drugs (anti-PVRIG TGI=64.88%, anti-TIGIT TGI=66.62% and combination therapy TGI=63.60%).

7. Analysis of TILs



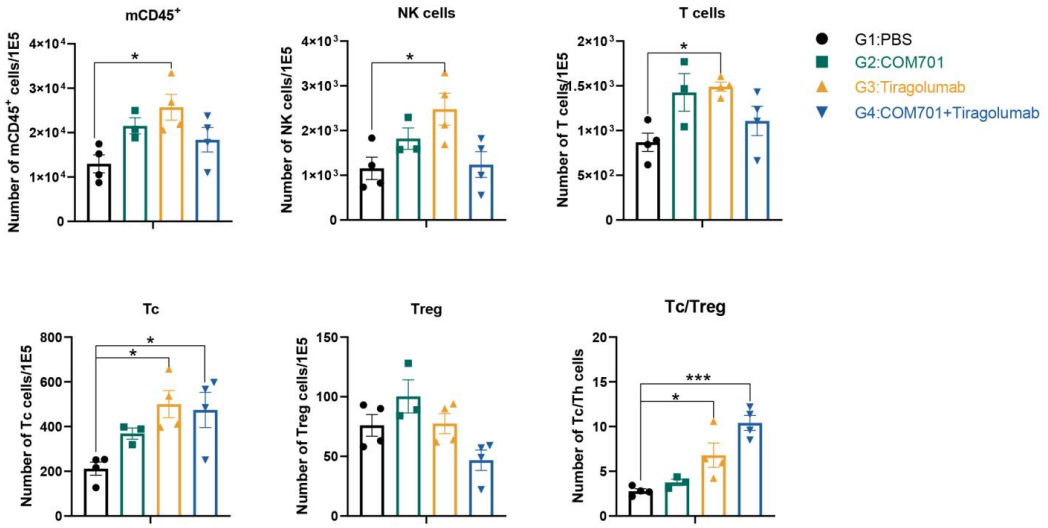


Figure 9. Analysis of tumour TILs in BALB/c-hTIGIT/hPVRIG mice.

TILs analysis showed an increase in mCD45$^+$, CD8$^+$ T and NK cells and a decrease in Treg cells in the administered group compared to the PBS group. The data showed that Tiragolumab and COM701 significantly inhibited tumour growth by enhancing the expression of CD8$^+$ T and NK cells and suppressing Treg cells. Taken together, the results suggest that BALB/c-hTIGIT/hPVRIG is a good model for evaluating human PVRIG antibody or the combination of human TIGIT and PVRIG antibody.

References

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