C57BL/6-hCTLA4

Strain Name: C57BL/6JGpt -*Ctla4*$^{em1Cin(hCTLA4)}$/Gpt
Strain Type: Knock-in
Strain ID: T003362
Background: C57BL/6JGpt

Description

CTLA4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152, is a member of the immunoglobulin superfamily. CTLA4 constitutively expressed on regulatory T cells （Treg）, are immunosuppressive, and generally accompany with effector T cells induction and proliferation decrease. The function of CTLA4 is currently considered to control Treg localization. CTLA4 competes with immunoactivator receptor CD28 for the same set of ligands: CD80(B7-1) and CD86(B7-2). CTLA4 was found to bind CD80 and CD86 with a higher affinity compared to CD28 thus enabling it to outcompete CD28. CTLA4 transmits an inhibitory signal to T cells, inhibiting immune responses against cancer. Blocking CTLA-4, and thus freeing B7 for interaction with the co-stimulatory molecule CD28, resulted in the rejection of tumors and induced immunity to a secondary tumor challenge [1-2].

The CTLA4 protein contains an extracellular domain for receptor/ligand binding, and the transmembrane region is responsible for signal transduction. The coding sequence of extracellular region of CTLA4 was replaced with human counterpart by CRTSPR/Cas9 technology on C57BL/6 background. Abundance of hCTLA4 expression in homozygous C57BL/6-hCTLA4 mouse is similar to wild-type. The CTLA4 humanization mice are ideal models for anti-CTLA4 drug evaluation and immunotherapy drug development.

Strategy

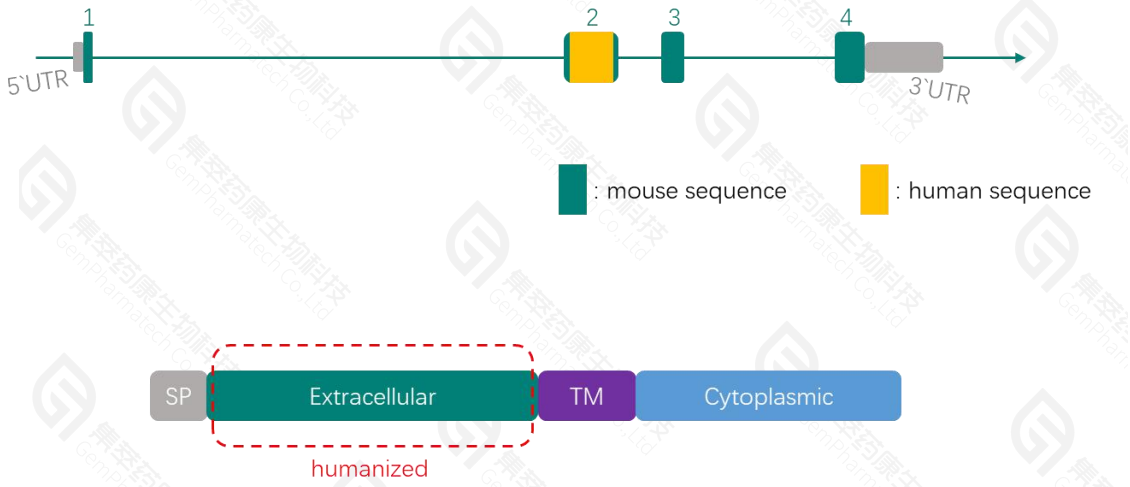

Fig.1 Schematic diagram of CTLA4 humanization strategy in C57BL/6-hCTLA4 mice.

Application

1. Efficacy evaluation of human CTLA4 inhibitor
2. Toxicological evaluation of human CTLA4 inhibitor
3. Screening of human CTLA4 agonist (treatment of autoimmune diseases and transplant rejection)

Data supports

CTLA4 Protein expression analysis

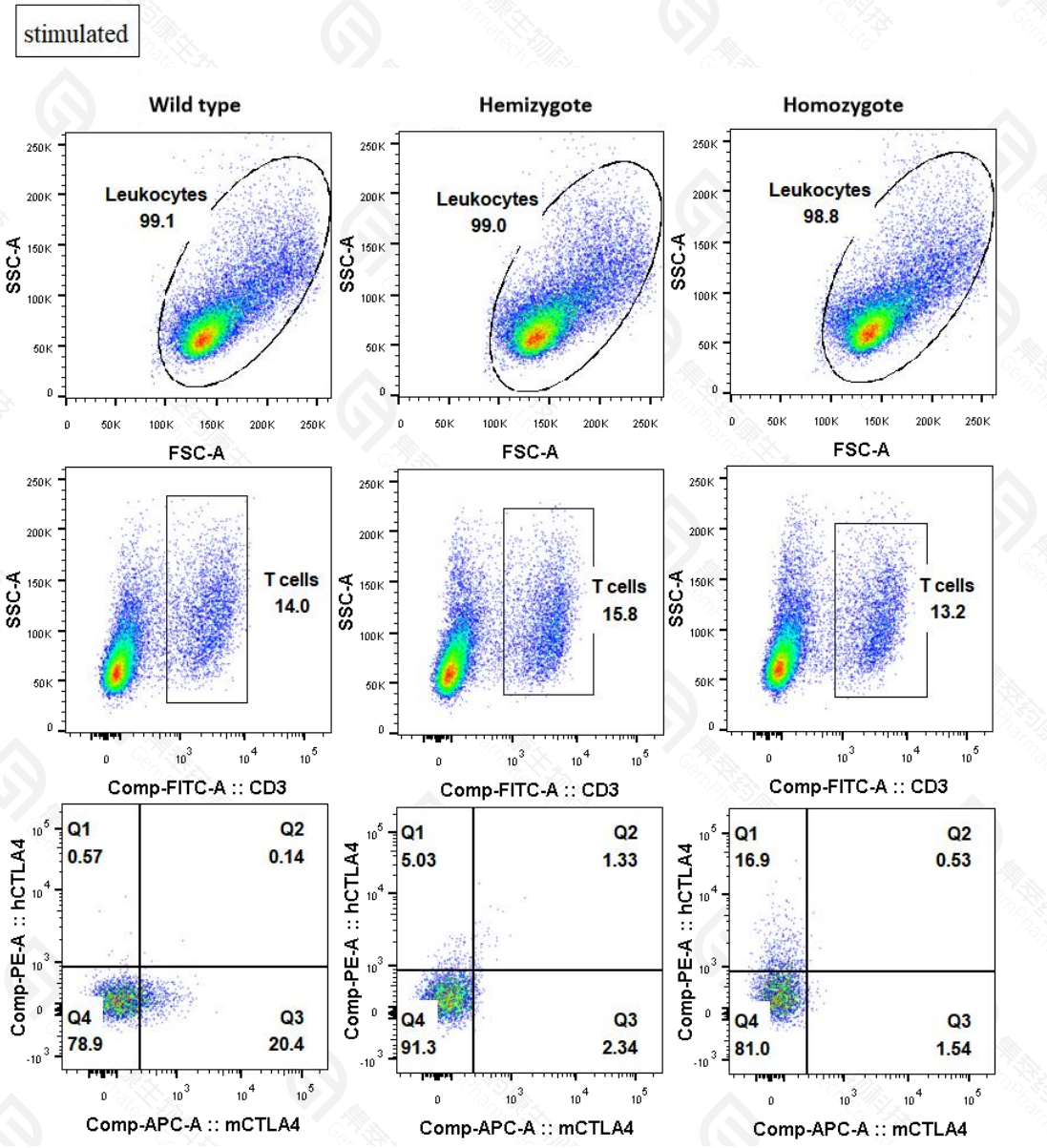

Fig 2. Detection of CTLA4 expression in C57BL/6-hCTLA4 mice.

hCTLA4 is expressed in homo C57BL/6-hCTLA4 mice similar expression level with mCTLA4 in wildtype mice after treated with anti-Cd3e antibody.

The T/B/NK cell ratio detection

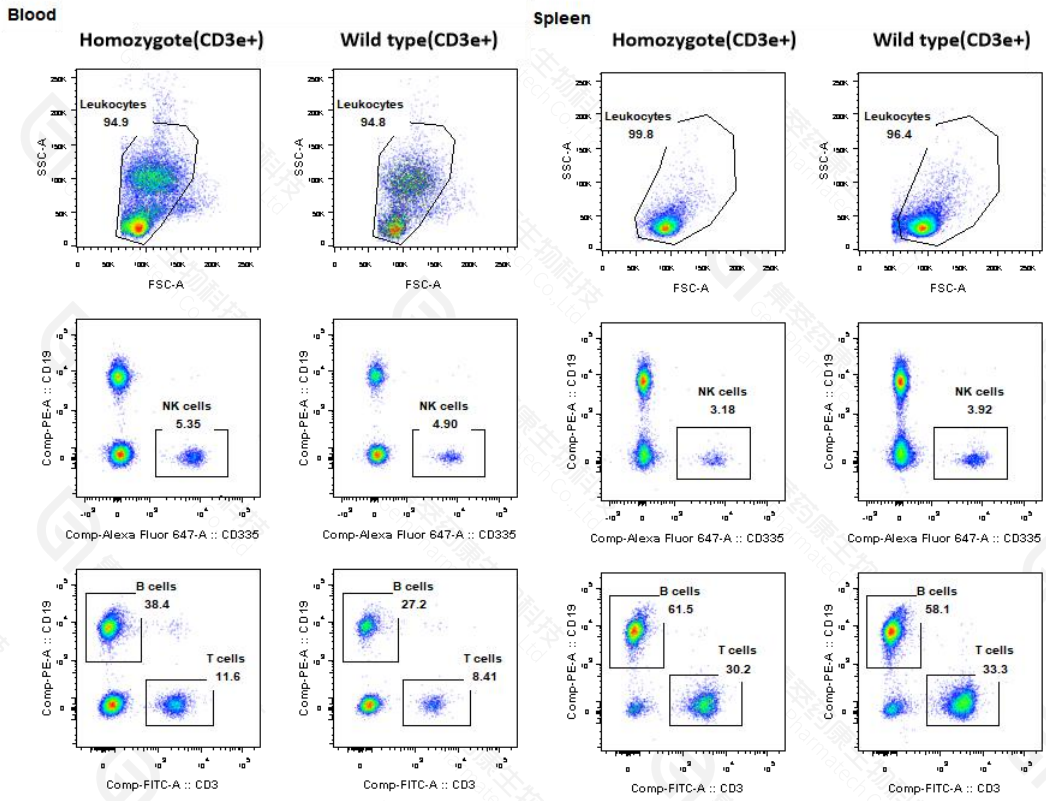

Fig3. Detect the proportion of T/B/NK cells in C57BL/6-hCTLA4 mice.

In peripheral blood and spleen, there was no obvious difference of T/B/NK cells proportion between wild-type and homozygote C57BL/6-hCTLA4 mice.

Anti-tumor Efficacy Test

***In vivo* Efficacy Study of Yervoy ®（Ipilimumab) in C57BL/6-hCTLA4 Mouse Model Bearing Subcutaneous Mouse MC38 Tumor**

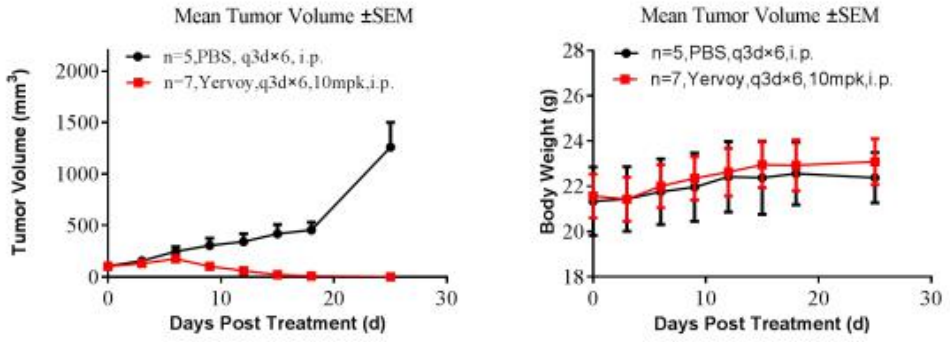

Fig.4 *In vivo* efficacy test in C57BL/6-hCTLA4.

C57BL/6-hCTLA4 mice were inoculated subcutaneously with murine colon carcinoma MC38 cells. When tumors reached an average volume of about 100 mm^3, mice were treated with vehicle or Ipilimumab (Yervoy） twice one week for 2 weeks. The results showed that CTLA4 antibody drug Yervoy has a significant inhibitory effect on tumor growth, indicating C57BL/6-hCTLA4 mice an ideal animal model to evaluate the efficacy of human CTLA4 antibody.

1. *In vivo* Efficacy of Antibody of Different Dosage

In vivo Efficacy Study of Yervoy ®（Ipilimumab) in C57BL/6-hCTLA4 Mouse Model Bearing Subcutaneous Mouse MC26 Tumor

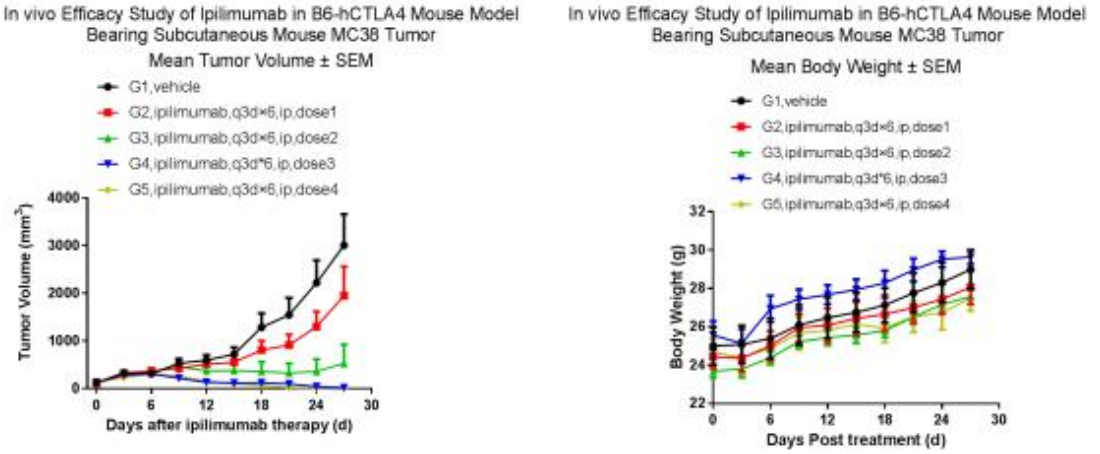

Fig 5. In vivo efficacy test of Ipilimumab dosage in C57BL/6-hCTLA4.

C57BL/6-hCTLA4 mice were inoculated subcutaneously with Murine colon carcinoma MC38 cells. When tumors reached an average volume of 100 mm^3, mice were treated with vehicle (group 1) or Ipilimumab (group 2-5） every three day for a total of 6 times. The results showed that CTLA4 antibody drug Yervoy has a significant inhibitory effect on tumor growth when mice were treated with high dose (group 3-5), while it only has partial inhibitory effect when given the low dose (group 2).

References

1. Peggs, Karl S., et al. "Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti–CTLA-4 antibodies." *Journal of Experimental Medicine* 206.8 (2009): 1717-1725.
2. Blank, Christian U., and Alexander Enk. "Therapeutic use of anti-CTLA-4 antibodies." *International immunology* 27.1 (2014): 3-10.