BALB/c-hPD1/hCTLA4

Strain Name：BALB/cJGpt-*Pdcd1*$^{em1Cin(hPDCD1)}$*Ctla4*$^{em1Cin(hCTLA4)}$/Gpt

Strain Type：Knock-in

Strain ID：T003720

Background：BALB/cJGpt

Description

PDCD1（Programmed cell death protein 1，PD1）, a member of the extended CD28/CTLA-4 family of T cell regulators, is involved in the regulation of T-cell function during immunity and tolerance. Immunomodulation targeting PD1 has been showed to have important implications in tumor defection, infection, autoimmune diseases and organ transplant rejection.

A large number of studies have confirmed that PDL1 is highly expressed on the surface of tumor cells in tumor microenvironment. PD1 binding of PDL1 transmits negative regulatory signals and leads tumor antigen-specific T cells apoptosis, thereby T cells were suppressed to immune response and promotes the escape of tumor cells. Blocking the PD1/PDL1 signaling pathway with antibodies has become a classic method for tumor immunotherapy [1].

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 [2-3].

BALB/c background can serve as a host and transplant almost all popular murine tumor cell lines that

currently available (e.g., CT26,4T1,H22,Renca). Additionally, different from immune-deficient strain such as NCG and NSG, this strain has sound functional immune system which could mimicking some human immune reactions. Therefore, this BALB/c-hPD1 strain will be a good model for anti-tumor drug evaluation and efficacy test.

The coding sequence of extracellular region of PD1 and CTLA4 is replaced with human counterparts by CRISPR/Cas9 technology on C57BL/6 background. Abundance of hPD1 and hCTLA4 expression in homozygous C57BL/6-hPD1/hCTLA4 mice is similar to the wild-type. This strain is used for anti-tumor drug evaluation and immunotherapy drug development.

Strategy

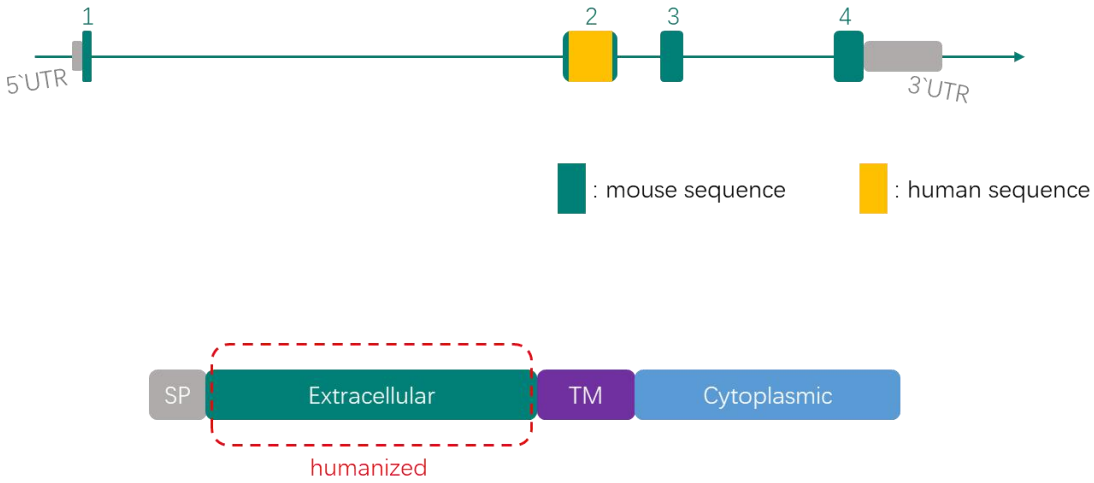
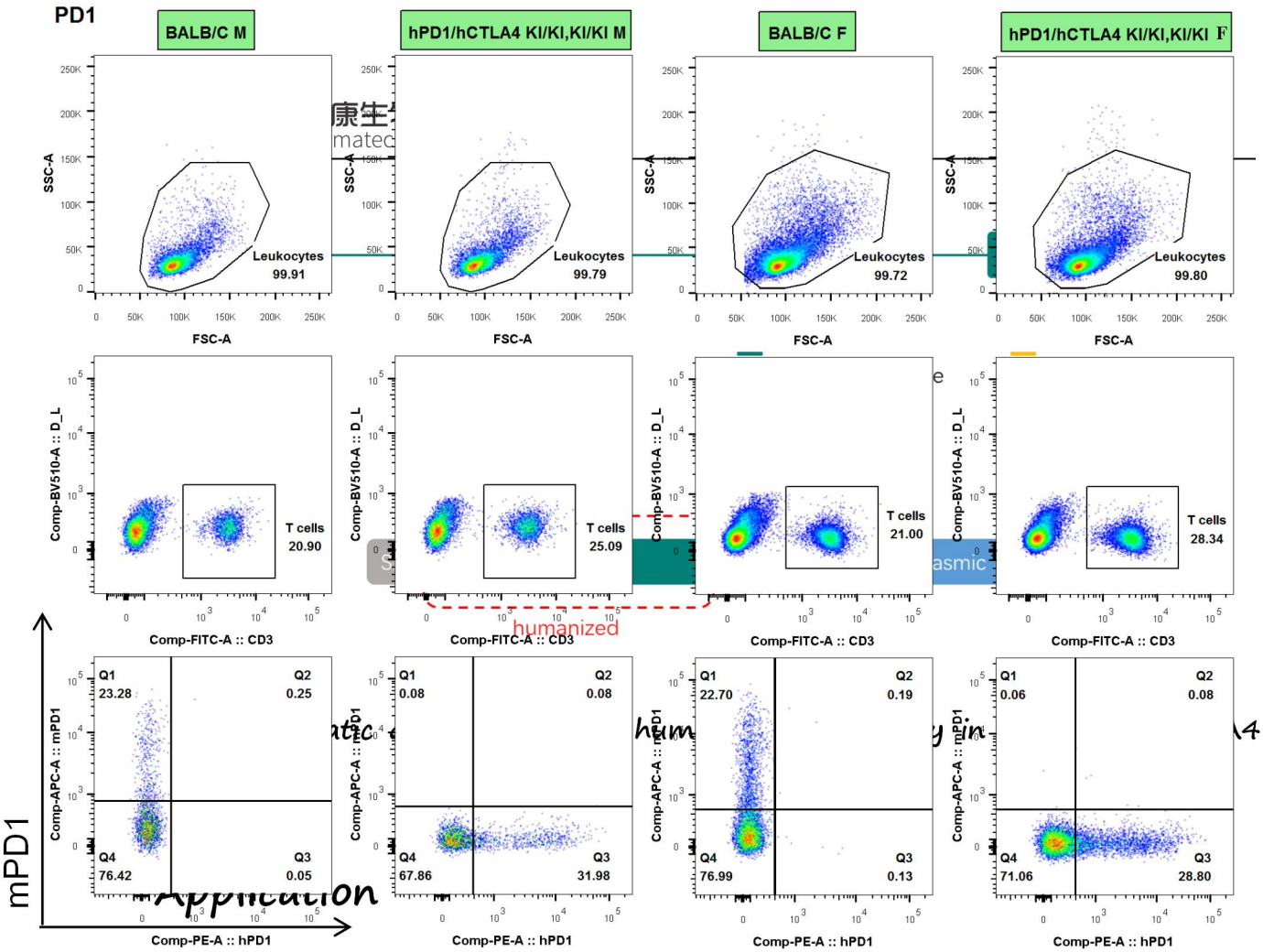


Fig.1 Schematic diagram of CTLA4 humanization strategy in B6-hPD1/hCTLA4 mice.



1. *Efficacy and toxicological evaluation of human CTLA4 inhibitor and human PD1 inhibitor;*
2. *Combo pharmaceutical efficacy evaluation of CTLA4 and PD1 inhibitor;*
3. *Research and Development of anti-cancer mechanism;*
4. *Study of autoimmune diseases.*

Data support

1. **PD1 and CTLA4 Protein expression analysis**

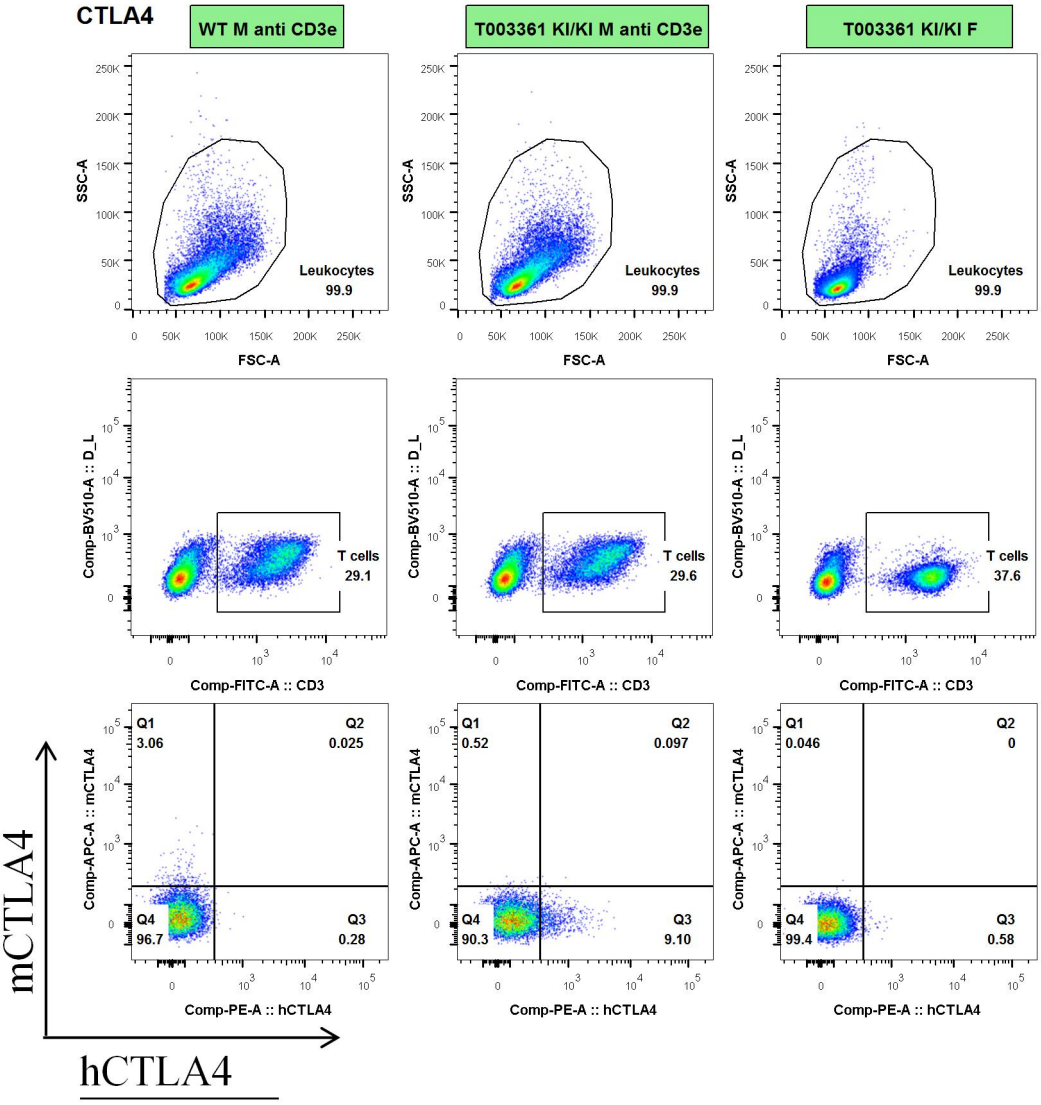


Fig 3. Detection of hCTLA4 and hPD1 expression in BALB/c-hPD1/hCTLA4 mice. In the absence of stimulation, BALB/c-hPD1/hCTLA4 mice can express hPD1 at comparable levels as mPD1 repressing in wild-type mice. After anti-CD3e stimulation, BALB/c-hPD1/hCTLA4 mice can successfully express hCTLA4 on the surface of T cells.

2. T/B/NK cell ratio assay

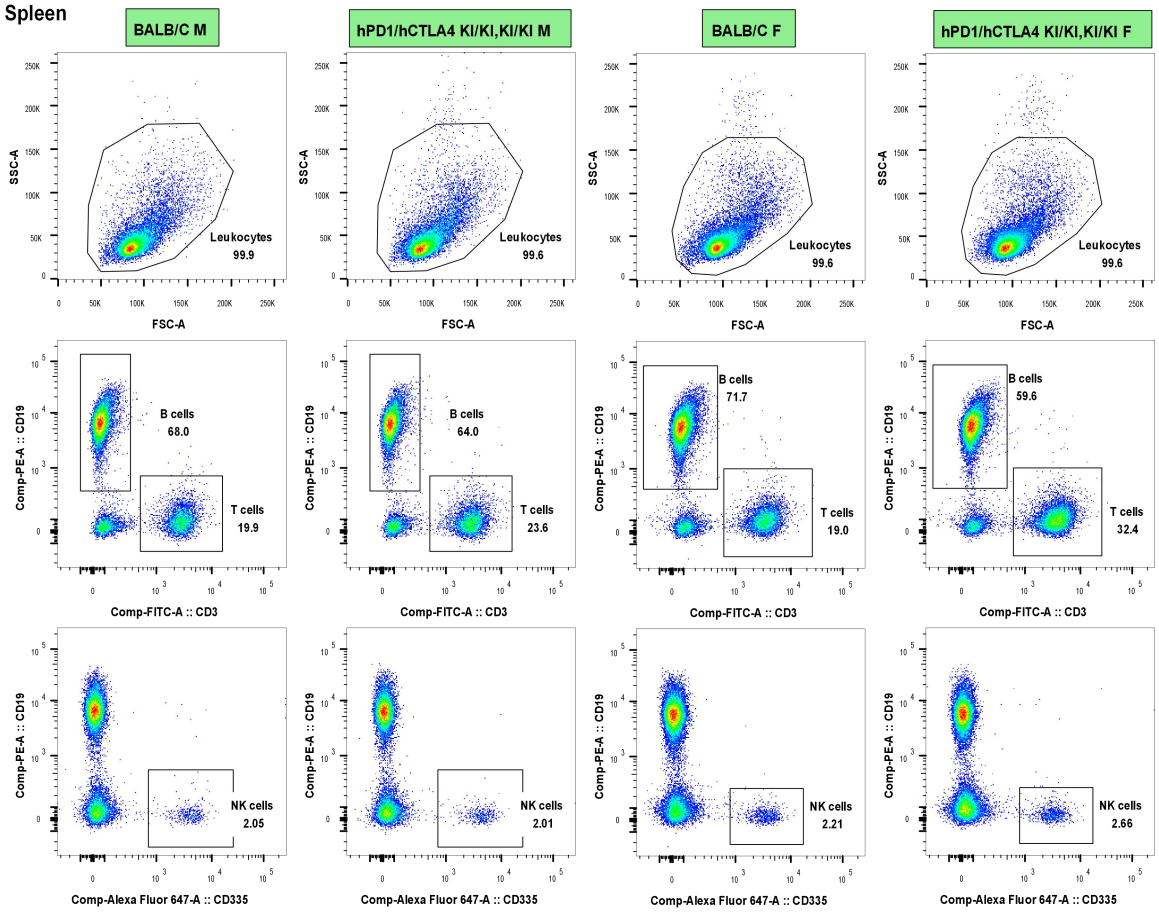


Fig4. Detect the proportion of T/B/NK cells in BALB/c-hPD1/hCTLA4 mice. In peripheral blood and spleen there was no obvious difference of T/B/NK cells proportion between wild-type and homozygote mice.

3. Anti-tumor Efficacy Test

Evaluation of in vivo efficacy of Anti-hCTLA4 and Anti-hPD1 by subcutaneous inoculation of CT26 model in BALB/c-hPD1/hCTLA4 mice

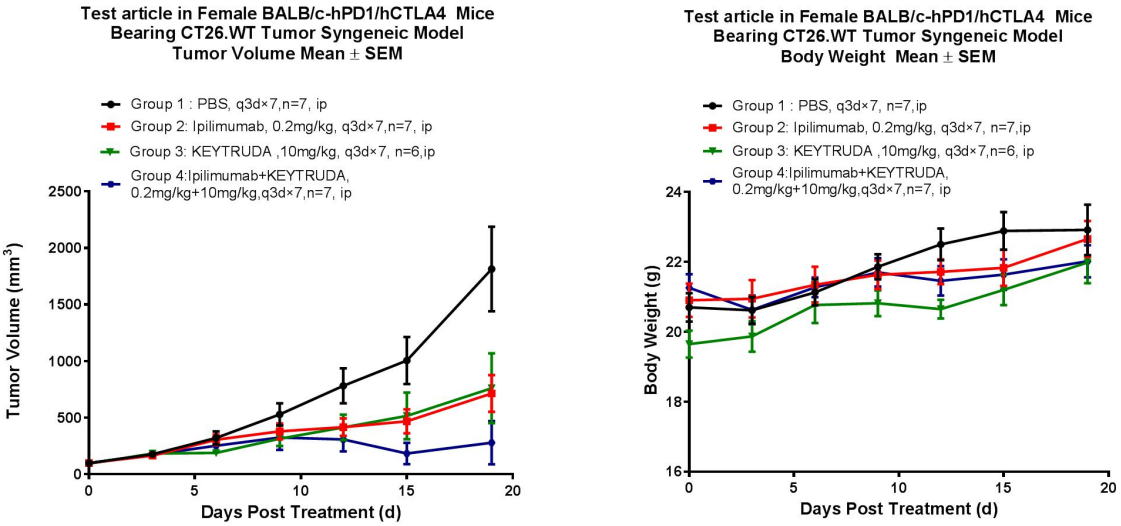


Fig.4 *In vivo* efficacy test based on BALB/c-hPD1/hCTLA4.Significant tumor inhibition observed on homologous BALB/c-hPD1/hCTLA4 mice bearing murine colon carcinoma ct26 tumor after the Ipilimumab and Keytruda combo treatment.

BALB/c-hPD1/hCTLA4 mice were inoculated subcutaneously with Murine colon cancer CT26 cells. When tumors reached an average volume of 100 mm^3, mice were treated with Vehicle (black, n =5), Yervoy (red, 0.2mg/kg, n =7), Keytruda (green, 10mg/kg, n=7) and combo treatment (blue, 0.2mg/kg + 10mg/kg, n=7) every 3 days for a total of 7 times. The results showed that both Yervoy (TGI=64%) and Keytruda (TGI=58%) has a partial inhibitory effect on tumor growth, while combo treatment has a significant inhibitory effect on tumor growth (TGI=96%), indicating BALB/c-hPD1/hCTLA4 mice an ideal animal model to evaluate the efficacy of human CTLA4 antibody and PD1 antibody.

YERVOY®（Ipilimumab）:

A marketed CTLA4-blocking antibody manufactured by Bristol-Myers Squibb for antitumor.

KEYTRUDA®（Pembrolizumab）:

A marketed PD1-blocking antibody manufactured by Merck & Co.

Reference

1. Flemming, A. "Cancer: Pd1 Makes Waves in Anticancer Immunotherapy." Nat Rev Drug Discov 11 8 (2012): 601.
2. Migden, M. R., et al. "Pd-1 Blockade with Cemiplimab in Advanced Cutaneous Squamous-Cell Carcinoma." N Engl J Med 379 4 (2018): 341-51.

3. Zhou, Q., et al. "Coexpression of Tim-3 and Pd-1 Identifies a Cd8+ T-Cell Exhaustion Phenotype in Mice with Disseminated Acute Myelogenous Leukemia." Blood 117 17 (2011): 4501-10.
4. 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.
5. Blank, Christian U., and Alexander Enk. "Therapeutic use of anti-CTLA-4 antibodies." *International immunology* 27.1 (2014): 3-10.