Humanized PD-1 Mouse

Strain Name: C57BL/6-Pdcd1^tm1(hPDCD1)Smoc  Strain Background: C57BL/6  Cat. No.: IT-HU-00015

Programmed cell death protein 1, also known as PD-1 or CD279, is a cell surface receptor on activated T cells. PD-1 is an important immune checkpoint molecule that negatively modulates T cell responses upon the binding of its ligand, PD-L1. Increasing evidence indicates that the PD-L1 expression on the surface of tumor cells is up-regulated in tumor micro-environment. The binding of PD-L1 to PD-1 on activated T cells results in an apoptosis or immune disability of tumor antigen-specific T cells, thereby suppressing anti-tumor immune responses. The blockade of PD-L1 binding to PD-1 reverses T cell exhaustion and thus strengthens anti-tumor activity, which has become a classic method for enabling tumor immunotherapy.

Construction strategy

On the C57BL/6 background, the full-length coding sequence of human PDCD-1 gene was placed immediately downstream of the start codon of the mouse endogenous Pdcd1, followed by a poly(A) element. This guarantees an exclusive expression of human PD-1 in the humanized PD-1 mice.

Validation data

- Data from flow cytometry (FACS) analysis

![Image of flow cytometry data](image.png)

Figure 1. Expression of PD-1 in the activated spleen lymphocytes of humanized PD-1 Homozygous mice is detected by FACS.
• In vivo validation in a MC38 tumor-bearing model of humanized PD-1 mice

Figure 2. In vivo anti-tumor effect of an anti-human PD-1 antibody in a humanized mouse model of PD-1. Anti-human-PD-1 drugs significantly inhibited the growth of MC38 tumors in PD-1 mice, demonstrating that the humanized PD-1 mice can be used to assess the anti-human PD-1 antibody.

A. Mean volume ± SEM of tumor tissues (completed in cooperation with Genscript).

In vivo validation of anti-tumor efficacy in a MC38 tumor-bearing model of humanized PD-1 mice. Homozygous humanized PD-1 mice were inoculated with MC38 colon cancer cells. After the tumors grew to 100 mm$^3$, the animals were randomly assigned into a control group and a treatment group (n=8). The drug was given twice a week for a total of 4 administrations. The results showed that Keytruda, a drug targeting human PD-1, exerted a very significant anti-tumor effect (p<0.001), demonstrating that the humanized PD-1 mice are a good in vivo model for validating the efficacy of antibodies targeting human PD-1.
B. Mean volume ± SEM of tumor tissues. C. Mean body weight ± SEM of mice (data were obtained in cooperation with PharmaLegacy).

In vivo dose validation of anti-tumor efficacy in a MC38 tumor-bearing model of humanized PD-1 mice. Homozygous humanized PD-1 mice were inoculated with MC38 colon cancer cells. After the tumors grew to about 90 mm$^3$, the animals were randomly assigned into a control group and a treatment group (n=9). The results showed that the antibodies targeting human PD-1 showed a very significant antitumor effect (p<0.001), and such antitumor effect is dose-dependent.

Figure 3. In vivo dose validation in a MC38 tumor-bearing model of humanized PD-1 mice. Homozygous humanized PD-1 mice were inoculated with MC38 colon cancer cells. After the tumors grew to 100 mm$^3$, the animals were randomly assigned into a control group and a treatment group (n=8). The drug was given twice a week for a total of 4 administrations.

**Case Study**

As one of the first five anti-PD-1 drugs with BLAs accepted by CFDA, Sintilimab has shown potent T cell stimulating activity and significant anti-tumor efficacy. (read more)
Figure 4. In vivo hPD-1 knock-in mouse model to test anti-tumor efficacy of Sintilimab. (A) Tumor growth inhibition (TGI) of MC38 tumors in hPD-1 knock-in mice of individual animals treated with different doses of Sintilimab. (B) Effect of Sintilimab on percentage change in mouse body weight (mean). (C) Changes in ratios of tumor infiltrating CD4+, CD8+ and Treg cells. For d8: IgG (n = 2); Sintilimab (n = 2). For d14, n = 3 for all groups. P values were calculated using a two-tailed t-test method.
Construction strategy

The BALB/c-Pdcd1 [em1(hPDCD1)Smo] (abbreviated as BALB/c-hPD1) mouse genetic stock was developed by first crossing BALB/c females with B6-hPD1 males.

Validation data

Figure 1. Expression of PD-1 in the activated spleen lymphocytes of homozygous humanized PD-1 BALB/c KI mice is detected by FACS.
Figure 2. In vivo validation of homozygous BALB/c-hPD1 mice. The homozygous BALB/c-hPD1 mice were inoculated with CT26 cells, and randomly assigned to different groups (n=7) when the tumor grew to a volume of 100 mm$^3$. A combinatorial treatment of anti-hPD1 antibody Keytruda and entinostat (ENT; a class I HDAC inhibitor) demonstrated a noticeable efficacy improvement compared to the same dose of single agent (A) without affecting the animal body weight (B).
Figure 3. In vivo validation of homozygous BALB/c-hPD1 mice. The homozygous BALB/c-hPD1 mice were inoculated with CT26 cells, and randomly assigned to different groups (n=6) when the tumor grew to a volume of 65 mm$^3$. A combinatorial treatment of anti-hPD1 antibody Opdivo and anti-mCTLA antibody demonstrated a noticeable efficacy improvement compared to the same dose of single agent (A) without affecting the animal body weight (B). (In collaboration with Crownbio)
Humanized LAG3 Mouse

**Strain Name:** B6.129-Lag3<sup>tm1(hLAG3)/Smoc</sup>  **Strain Background:** B6.129  **Cat. No.:** IT-HU-00049

LAG3 (lymphocyte activating 3, also known as CD223) has been shown to act as a co-inhibitory molecule expressed on activated T cells, NK cells, B cells, and plasmacytoid dendritic cells. LAG3 is an immune checkpoint receptor that binds to the antigen-MHC complex to present antigens to T cells. Experiments have shown that LAG3 negatively regulates T cell proliferation as well as the development of lasting memory T cells.

**Construction strategy**

The coding sequence for the extracellular domain of mouse endogenous LAG3 was completely replaced by the human LAG3 counterpart, leading to the expression of a chimeric LAG3 protein.

**Validation data**

![Expression of LAG3 in activated spleen lymphocytes of humanized LAG3 mice](image)

Figure 1. Expression of LAG3 in the activated spleen lymphocytes of humanized LAG3 mice is detected by FACS. Stimulated spleen lymphocytes of homozygous humanized LAG3 mice. The results showed that the active expression of humanized LAG3 can be detected in both activated CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes collected from homozygous humanized LAG3 mice. (Completed in collaboration with CrownBio).
Figure 2. Flow cytometry analysis of tumor infiltrating lymphocytes (TILs) after humanized LAG3 mice were inoculated with MC38 tumor cells (data were obtained in cooperation with GenScript).

FACS detection of LAG3 expression in tumor infiltrating lymphocytes collected from humanized LAG3 mice. Homozygous humanized LAG3 mice were inoculated with MC38 colon cancer cells. After the tumors grew to 1000 mm$^3$, tumor infiltrating lymphocytes were isolated and detected by FACS to measure the expression of humanized LAG3 in CD4+ and CD8+ T cells. The results showed that the active expression of humanized LAG3 was detected in the tumor infiltrating lymphocytes collected from humanized LAG3 mice. In particular, 41.1% of CD4+ positive cells were also positive for humanized LAG3.
Figure 3. In vivo validation of anti-tumor efficacy in a MC38 tumor-bearing model of humanized LAG3 mouse. Homozygous humanized LAG3 mice were inoculated with MC38 colon cancer cell lines. After the tumors grew to 50 mm³, the animals were randomly assigned into different groups (n=5). The results showed that the anti-tumor effect was not observed when the antibody targeting human LAG3 was administered alone. However, a significant anti-tumor effect was observed when the antibody targeting human LAG3 was administered together with the anti-PD1 antibody.
Figure 4. In vivo validation of anti-tumor efficacy in a MC38 tumor-bearing model of humanized LAG3 mouse. Homozygous humanized LAG3 mice were inoculated with MC38 colon cancer cell lines. After the tumors grew to 70-80 mm³, the animals were randomly assigned into different groups (n=8). The results showed a significant anti-tumor effect was observed when the antibody targeting human LAG3 was administered together with Tencentriq.
Immune Checkpoint Humanized Mouse Models

Being recognized as a top scientific breakthrough in 2013, cancer immunotherapy is predicted to be one of the most promising research areas for improving patient outcomes. Although many immunotherapy breakthroughs may still lie ahead, important clinical advances have been made in the past few years for some of the deadliest cancers, reaffirming the potential of immunotherapy for many types of patients.

However, it is worth noting that drug candidates developed to interfere with human proteins may not comparably interact with their murine counterparts. It is therefore critical to develop humanized mouse models to enable in vivo efficacy evaluation of cancer immunotherapies.

Immune Checkpoint Humanized Mouse Models available at ingenious targeting laboratory

- 4-1BB
- CD40
- CD47
- CD73 (NT5E)
- CTLA4 (C57BL/6)
- CTLA4 (BALB/c)
- KDR
- LAG3
- OX40
- OX40/CTLA4
- PD-1 (C57BL/6)
- PD-1 (BALB/c)
- PD-1/4-1BB
- PD-1/CD40
- PD-1/CTLA4
- PD-1/LAG3
- PD-1/OX40
- PD-1/PD-L1
- PD-1/TIGIT
- PD-1/TIM3
- PD-L1
- PD-L1/CTLA4
- PD-L1/LAG3
- PD-L1/OX40
- PD-L1/TIGIT
- SIRPA
- SIRPA/CD47
- TIGIT
- TIM3 (C57BL/6)
- TIM3 (BALB/c)
- TNFRSF1B

And more to come!

To get to know more about these models, visit our website www.genetargeting.com or contact our scientific experts at inquiry@genetargeting.com
About ingenious targeting laboratory

ingenious targeting laboratory (ingenious) has been a leading global provider of custom genetically modified mouse, rat, and rabbit models for over 20 years. As one of the very first mouse gene targeting companies, our trusted service is built on two decades’ worth of successful animal model creation for investigators, organizations, and companies worldwide. Our models have been published in hundreds of journals including Science, Nature, and Cell, making us one of the most validated and respected production companies in the industry. We are excited to add catalog mouse models to our service repertoire by means of our collaboration with Shanghai Model Organisms Center (SMOC).