Humanized PD-1 Mouse

Strain Name: C57BL/6-Pdcd1<sup>tm1(hPDCD1)/Smoc</sup>  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

![Flow Cytometry Diagram](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](image-url)

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 changes 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)Smoc (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 mm³. 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 OX40 Mouse

Strain Name: C57BL/6-Tnfrsf4
Strain Background: C57BL/6
Cat. No.: IT-HU-00041

OX40 is a co-stimulatory molecule expressed on the surface of activated cytotoxic T cells and regulatory T cells. Administration of agonistic, anti-OX40 antibody increases proliferation of peripheral blood CD4⁺ and CD8⁺ T cells, thereby creating a tumor microenvironment that is more favorable to anti-tumor immune responses. Accumulating preclinical evidence supports the application value of anti-OX40 antibodies in cancer therapy, and several such agonistic antibodies are now tested in early stage of clinical trials. The humanized OX40 mice developed by SMOC provide a translational model that enables the in vivo efficacy evaluation of human-specific therapeutic OX40 antibodies.

Construction strategy

The humanized OX40 mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human OX40 as well as the transmembrane and intracellular domains of mouse OX40 was placed immediately downstream of the start codon of the mouse endogenous OX40 gene, followed by a poly(A) signal. Thereby, the extracellular domain of the mouse OX40 was replaced by its human counterpart while the rest of the mouse gene remained untouched.

Validation data

- Flow cytometry (FACS) analysis data of lymph node T-cells collected from humanized OX40 mice
Figure 1. Expression of OX40 in the spleen lymphocytes of humanized OX40 mice is detected by FACS.

The spleen lymphocytes of heterozygous humanized OX40 mice were activated by anti-CD3 and anti-CD28 for 48 hours, and then collected for staining. Along with a group undergoing no stimulation, the expression of murine and human OX40 was detected by FACS. The results showed that the active expression of human OX40 can be detected in both activated CD4+ and CD8+ T lymphocytes collected from heterozygous humanized OX40 mice, and the expression trend of human OX40 and murine Ox40 was similar.

- In vivo validation in a MC38 tumor-bearing model of humanized OX40 mouse.

- Case study 1
Figure 2. OX40 antibody showed dose-dependent anti-tumor activity in human OX40 knock-in mice bearing MC38 tumors. The OX40 antibody was obtained from Innoventbio.
Case study 2
Figure 3. IBI101 showed dose-dependent anti-tumor activity and enhanced tumor-specific CD8+ T cell response in human OX40 knock-in mice bearing MC38 tumors. a Tumor growth curve of mice treated with different doses of IBI101 alone or in combination with anti-mouse PD-1 antibody. Different doses of IBI101 and anti-mouse PD-1 antibody were administrated as indicated by the arrow heads after MC38 cells implantation. b Animal body weights were measured during the time course of the experiment. c Mice were injected with h-IgG (10 mg/kg), IBI101 (10 mg/kg), anti-PD-1 (0.5 mg/kg) alone or IBI101 (10 mg/kg) + anti-PD-1 (0.5 mg/kg) at day 10 and 14 post tumor cell implantation. At day 17, tumor and spleen were collected and analyzed by flow cytometry for the absolute counts of the indicated cell subsets in tumor and d proportions of indicated cell subsets in CD45+ splenocytes. Flow cytometry results showing the proportions of cytokine-secreting cancer-specific CD8+ and CD4+ T cells from tumor (e) and spleen (f) (n≥5) (In collaboration with Innoventbio)
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  PD-1/PD-L1
CD40  PD-1/TIGIT
CD47  PD-1/TIM3
CD73 (NT5E)  PD-L1
CTLA4 (C57BL/6)  PD-L1/CTLA4
CTLA4 (BALB/c)  PD-L1/LAG3
KDR  PD-L1/OX40
LAG3  PD-L1/TIGIT
OX40  SIRPA
OX40/CTLA4  SIRPA/CD47
PD-1 (C57BL/6)  TIGIT
PD-1 (BALB/c)  TIM3 (C57BL/6)
PD-1/4-1BB  TIM3 (BALB/c)
PD-1/CD40  TNFRSF1B
PD-1/CTLA4
PD-1/LAG3
PD-1/OX40

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).