BALB/c-hPD1

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

Strain Type：Knock-in

Strain ID：T002726

Background：BALB/cJGpt

Description

Programmed cell death protein 1, also known as PD-1 and CD279, is a cell surface receptor that belongs to the immunoglobulin superfamily. PD1 is an immune checkpoint that negatively regulates T cell responses.

Immunomodulation targeting PD1 has important implications for anti-tumor, anti-infection, anti-autoimmune diseases and organ transplant rejection.

A large number of studies have confirmed that the expression of PDL1 on the surface of tumor cells is increased in the tumor microenvironment, and it binds to PD1 on activated T cells, transmitting negative regulatory signals, leading to apoptosis or immune disability of tumor antigen-specific T cells, thereby suppressing immune response, and promoting the immune scape of tumor cells. Blocking the PD1/PDL1 signaling pathway with antibodies has become a classic method for tumor immunotherapy [1-3].

BALB/c mouse 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 deficit models 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.

GemPharmatech using gene editing technology developed BALB/c-hPD1 humanized model independently. The coding sequence of extracellular domain of PD1 was replaced with human counterpart on BALB/c background. Intracellular region of murine PD1 was completely retained and normal intracellular signal transduction was guaranteed. hPD1 expression in homozygous BALB/c-hPD1 mice were similar to mPD1 expression in wildtype. These mice are ideal models for anti-PD1 drug evaluation and immunotherapy drug development.

Application

1. Anti-hPD1 antibody test (screening small molecule drug screening of human PD1 neutralizing antibodies, human PD1 activity inhibition)

2. Anti-tumor drug screening and efficacy test

3. Immune system related research

Data support

1. PD1 expression level detection

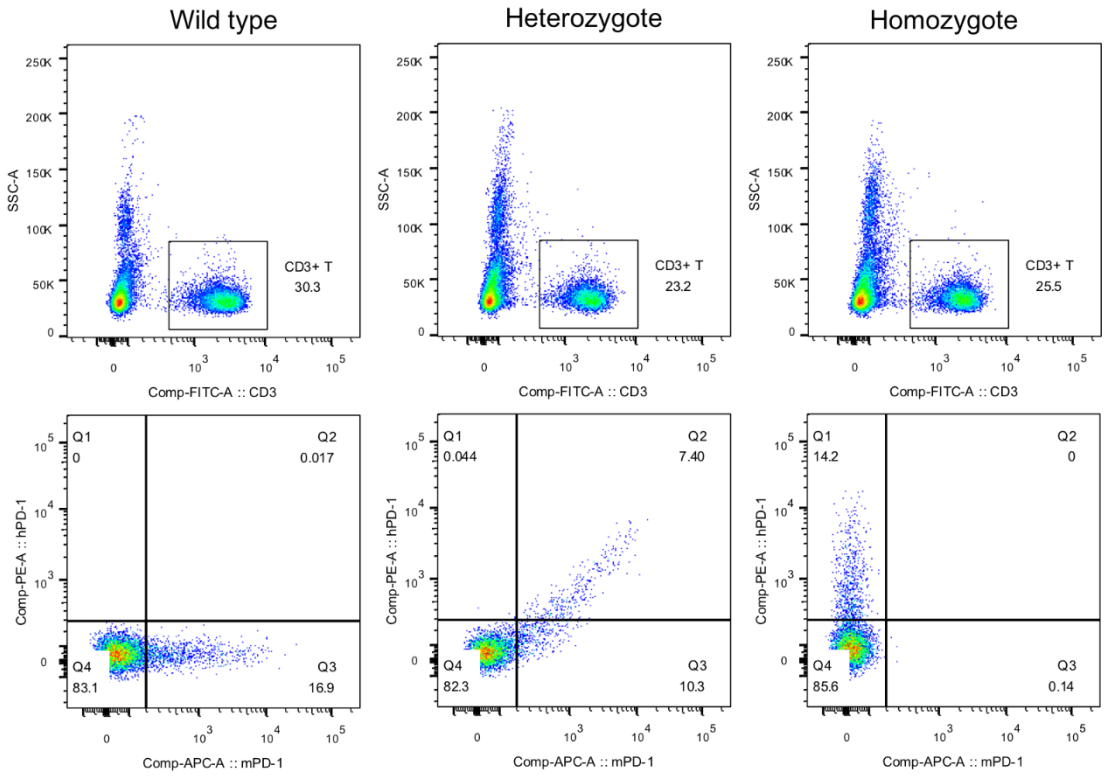

Fig 1. Detection of hPD1 expression on BALB/c-hPD1 mice.

hPD1 is expressed at comparable level in homo as mPD-1 expressing the wildtype mice.

2. T/B/NK cell ratio assay

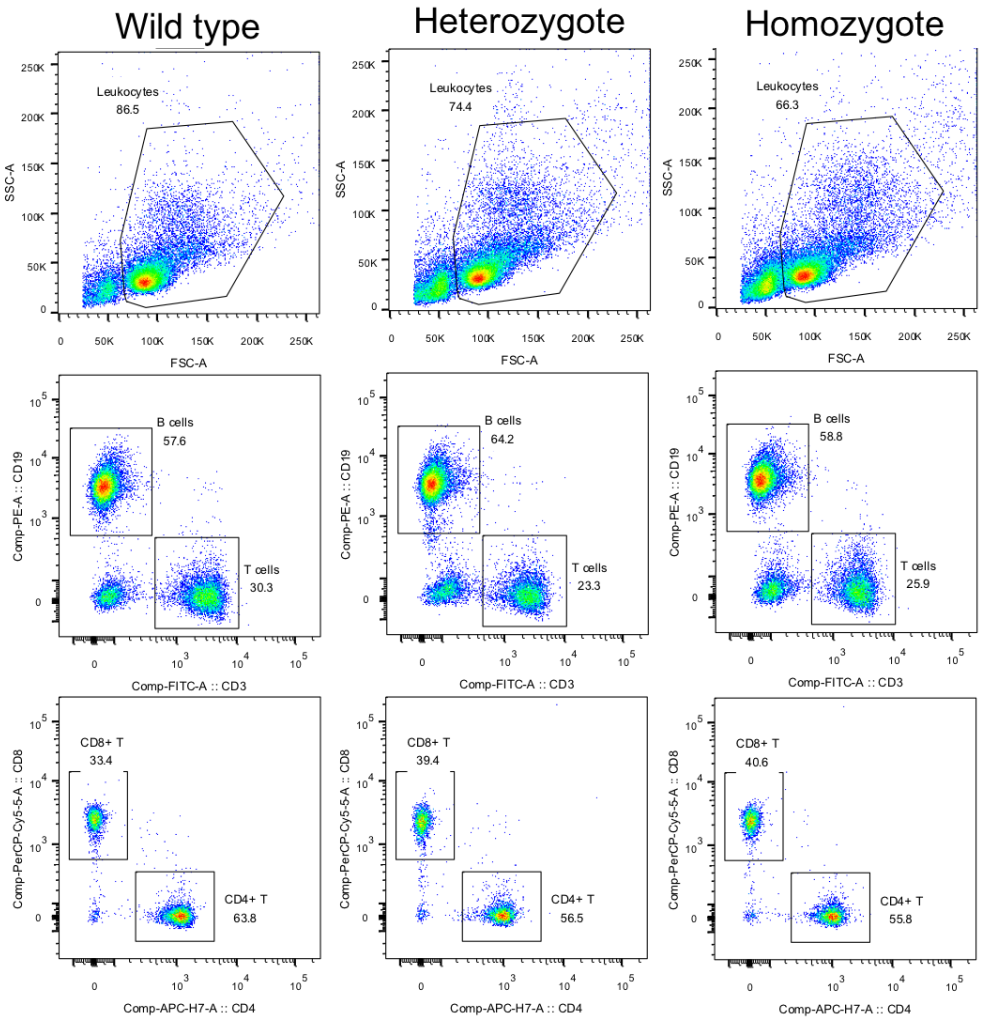

Fig 2. Detection of T/B/NK cells proportion on BALB/c-hPD1 mice.

The ratio of T and B cells in spleen of BALB/c-hPD1 mice did not change significantly, and there was no significant different in mature T cells ($CD4^+/CD8^+$).

3. Anti-tumor pharmacological efficacy

Tumor inhibition effect of anti-human PD1 antibody KEYTRUDA® (Pembrolizumab) on subcutaneous CT26 tumor bearing BALB/c-hPD1 mouse model

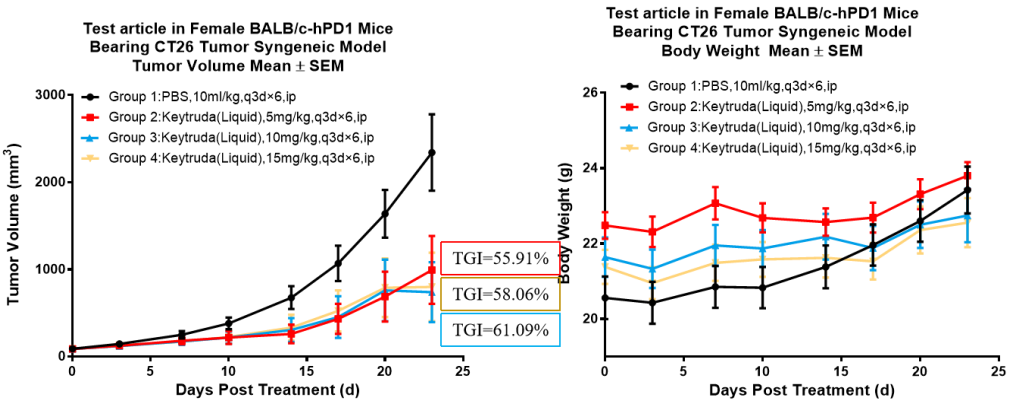

Fig 3. In vivo efficacy trial based on BALB/c-hPD1.

BALB/c-hPD1 mice at the age of 6-8 weeks were inoculated subcutaneously with murine colon cancer CT26 cells. When the tumors were grown to an average volume of about 100 mm^3, mice were randomly divided into a Vehicle (control) group and KEYTRUDA® drug groups (n=8), and treated with the corresponding drugs. The drug was administered every 3 days for a total of 6 times. The results showed that KEYTRUDA® with different dosages inhibited tumor growth similarly (KEYTRUDA® medication group TGI=55.91% 61.09% and 58.06%) (Fig. 3 Left). The body weight trends of the four groups of mice were close (Fig. 3 Right). The results demonstrated that BALB/c-hPD1 mice is an ideal model for assessing the in vivo efficacy of human PD1 antibodies.

Tumor inhibition effect of Anti-hPD1 antibody KEYTRUDA® (Pembrolizumab) alone and KEYTRUDA® combining with Ent (HDAC small molecule inhibitor) on subcutaneous CT26 tumor bearing BALB/c-hPD1 mouse model

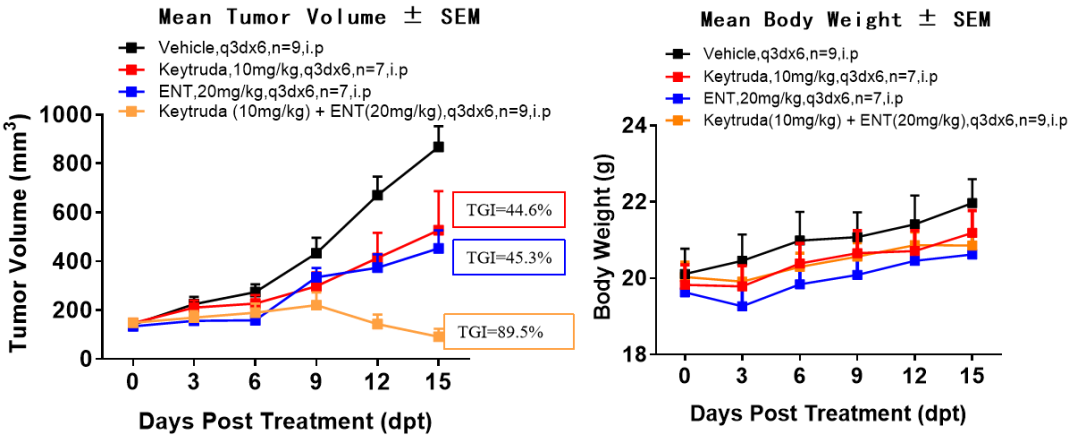

Fig 4. In vivo efficacy trial of drug combination on BALB/c-hPD1 mice.

BALB/c-hPD1 mice at the age of 6-8weeks were inoculated subcutaneously with murine colon cancer CT26 cells. When tumors reached an average volume of 100 mm^3, mice were randomly divided into four groups. Mice were treated with control placebo(black), Keytruda®(red), Entinostat(blue), and Combination of Keytruda® and Entinostat (orange) every 3 days for a total of 6 times. The results proved that KEYTRUDA® or Entinostat inhibited tumor growth when used alone (TGI indicated 44.6% and 45.3%, respectively). Particularly, tumor size of the Keytruda/ENT group was significantly smaller comparing with single drug treatment（TGI=89.5%）(Fig4 Left). The trends of body weight changes were similar in all groups (Fig4 Right). The results demonstrated that BALB/c-hPD1 mice is an ideal model for assessing the in vivo efficacy of human PD1 antibody in combination with other drugs.

KEYTRUDA®（Pembrolizumab）：

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

Entinostat (Ent)：

Entinostat is a type I HDAC selective inhibitor of Syndax Pharmaceuticals, which acts on HDAC1, HDAC2 and HDAC3.

4. Physiological and biochemical characteristics

（1）Blood routine analysis

Parameter	Units	Males	Females
Hematology			
Age	weeks	16	16
WBC	K/uL	3.78±1.53	4.44±1.46
RBC	M/uL	8.81±1.54	9.40±1.23
Hb	g/L	135.29±24.57	150.29±20.66
HCT	%	43.88±9.04	46.94±6.65
MCV	fL	49.58±2.27	49.85±1.75
MCH	Pg	15.37±0.95	15.97±0.52
MCHC	g/L	310.75±24.13	320.42±8.88
RDW	%	19.82±2.80	18.58±0.69
PLT	K/uL	882.33±149.09	786.21±144.75
MPV	fL	4.68±0.46	4.85±0.32
NE#	K/uL	1.04±1.03	0.74±0.42
NE%	%	25.11±11.67	16.09±5.42
LY#	K/uL	2.35±0.73	3.37±0.98
LY%	%	64.41±10.09	76.81±7.45
EO#	K/uL	0.02±0.02	0.01±0.02
EO%	%	0.40±0.48	0.40±0.80
MO#	K/uL	0.37±0.25	0.32±0.31
MO%	%	9.93±5.57	6.58±4.01
BA#	K/uL	0.01±0.02	0.00±0.01
BA%	%	0.14±0.35	0.11±0.33

（2）Blood biochemistry

Parameter	Units	Males	Females
Biochemistry			
Age	weeks	16	16
ALT	IU/L	48.29±29.32	36.75±16.84
AST	IU/L	77.63±18.81	90.38±19.86
TP	g/L	55.69±3.12	56.65±2.93
ALB	g/L	31.69±3.18	39.10±6.48
AKP	IU/L	103.42±30.22	149.13±17.19
TBIL	umol/L	1.33±0.37	1.39±0.29
BUN	mmol/L	5.31±1.05	4.45±1.00
CREA	umol/L	9.92±2.47	6.66±1.03
CHOl	mmol/L	20.74±2.21	19.84±1.26
TG	mmol/L	2.44±0.09	2.52±0.09
HDL-C	mmol/L	3.46±0.62	3.37±0.67
LDL-C	mmol/L	3.36±0.48	2.57±0.27
Ca	mmol/L	1.02±0.42	0.62±0.30
P	mmol/L	2.72±0.36	2.06±0.21
Fe	umol/L	0.29±0.07	0.30±0.08
GLU	mmol/L	41.03±10.77	34.02±6.21

References

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.