BALB/c-hHER2

Strain Name: BALB/cJGpt-*Her2*$^{em1Cin(hHER2)}$/*Gpt*
Strain type: Knock-in
Strain ID: T009819
Background: BALB/cJGpt

Description

HER2, also known as ERBB2, is a transmembrane receptor with tyrosine kinase activity but without a known ligand[1-2]. It belongs to the human epidermal growth factor receptor family that are involved in regulating cell growth, survival and differentiation. Overexpression of HER2 was found to occur in human breast cancer (BC), and HER2 signalling and transforming functions leading to the formation of aggressive tumor cells[3].

The discovery that amplification or overexpression of HER2 was associated with extremely poor survival in BC ultimately led to the development of drugs targeting HER2. The dependence of the tumour on HER2, coupled with effective HER2-targeted drugs such as trastuzumab, pertuzumab and most recently, tucatinib and trastuzumab deruxtecan (T-DXd), have contributed to these survival improvements in patients with HER2-positive (HER2+) BC[4]. Besides, HER2-directed therapies have been used to treat other HER2-expressing tumor types such as gastric and lung cancers[5-6]. Recently, a specifically engineered HER2-directed antibody drug conjugate (ADC), named Enhertu has been jointly developed and commercialised by AstraZeneca and Daiichi Sankyo. The clinical results showed that Enhertu met the prespecified target for objective response rate (ORR) and demonstrated durable response across multiple HER2-expressing advanced solid tumours.

The BALB/c-hHER2 humanized model was created at GemPharmatech using gene editing technology whereby the coding sequence of the extracellular domain of the HER2 gene was replaced with the human counterpart on BALB/cJGpt background. The intracellular region of murine HER2 was completely retained for normal intracellular signaling transduction. This mouse will be useful for evaluation of drugs that targeting HER2.

Strategy

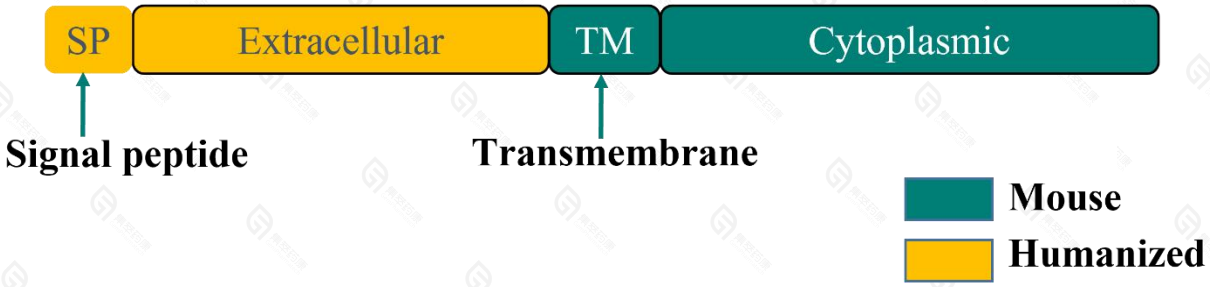


Fig.1 Schematic diagram of HER2 humanization strategy in BALB/c-hHER2 mice.

Application

1. Evaluation of efficacy and safety of human HER2 drugs
2. Anticancer drug research and development
3. Development of cancer vaccines

Supporting data

1. HER2 mRNA expression analysis

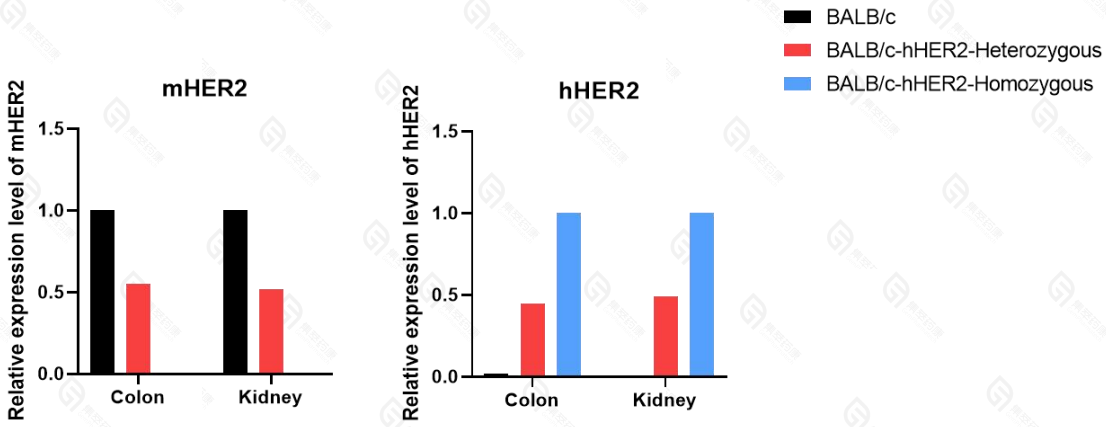


Fig.2 Quantitative PCR analysis of HER2 mRNA expression in BALB/c-hHER2 mice.

Total RNA was isolated from colon and kidney of wild-type mice, heterozygous and homozygous BALB/c-hHER2 mice, qPCR method was used to detect the mRNA expression of HER2. The results showed that the expression of mHER2 mRNA was detectable in wild-type and hetrozygous BALB/c-hHER2 mice. The expression of hHER2 mRNA was only detectable in heterozygous and homozygous BALB/c-hHER2 mice, and the relative expression of hHER2 of homozygous BALB/c-hHER2 mice was nearly twice that of heterozygous mice.

2. HER2 protein expression analysis

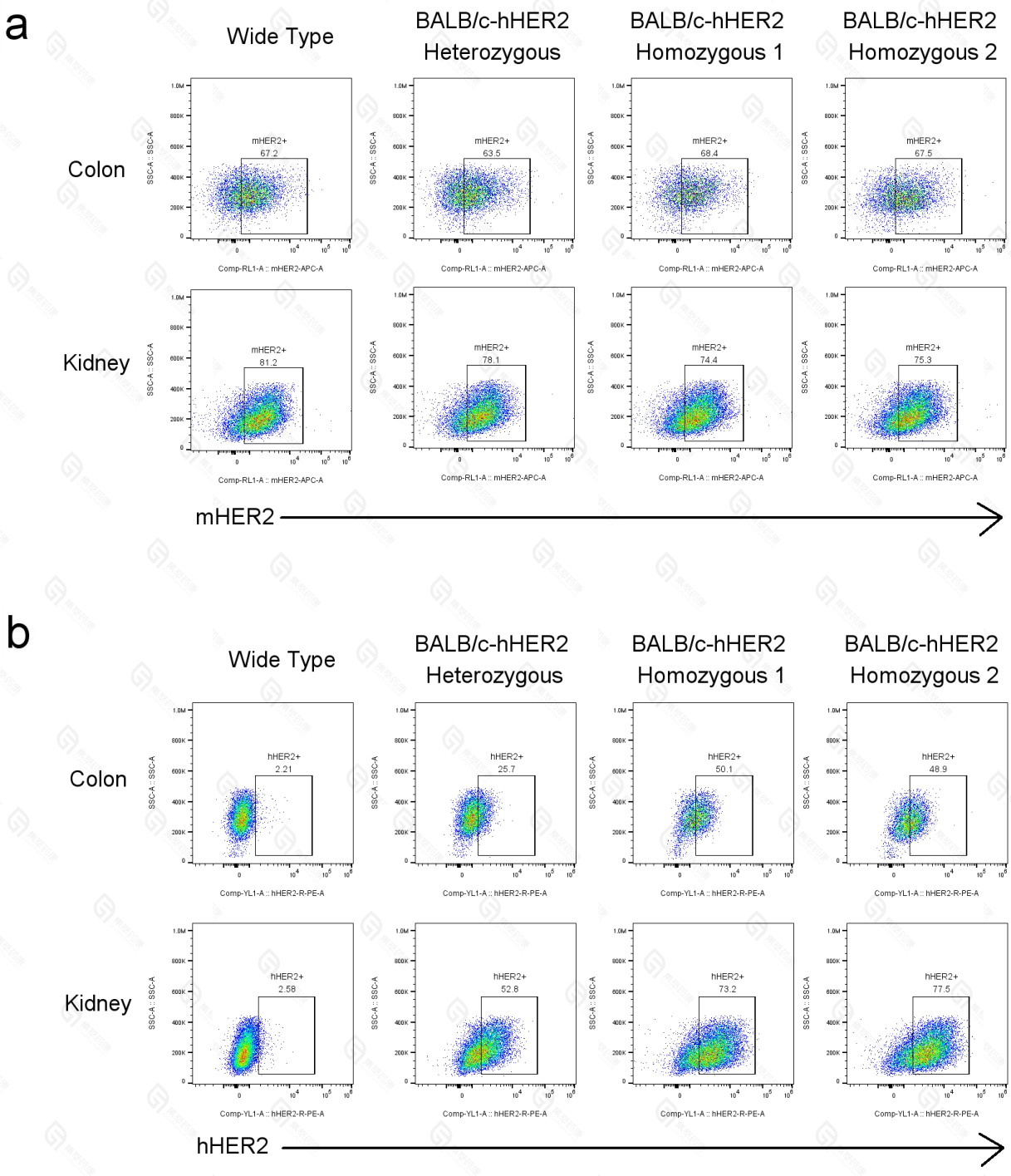


Fig.3 Detection of HER2 expression in BALB/c-hHER2 mice.

Colon and kidney tissues were collected from wild-type mice, heterozygous and homozygous BALB/c-

hHER2 mice, and analyzed HER2 expression by flow cytometry. Expression of mHER2 was detectable in wild-type mice, heterozygous and homozygous BALB/c-hHER2 mice（Fig 3a). Expression of hHER2 was only detectable in heterozygous and homozygous BALB/c-hHER2 mice, and the proportion of hHER2 in $CD45^-$cells of homozygous BALB/c-hHER2 mice was nearly twice that of heterozygous mice（Fig 3b). Annotation: mHER2 antibody is cross-reactive with both human and mouse.

3. Immunization of BALB/c-hHER2 mice with recombinant hHER2 proteins

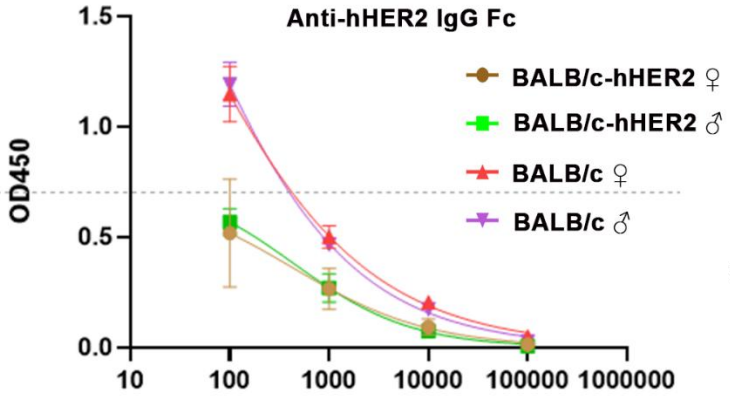
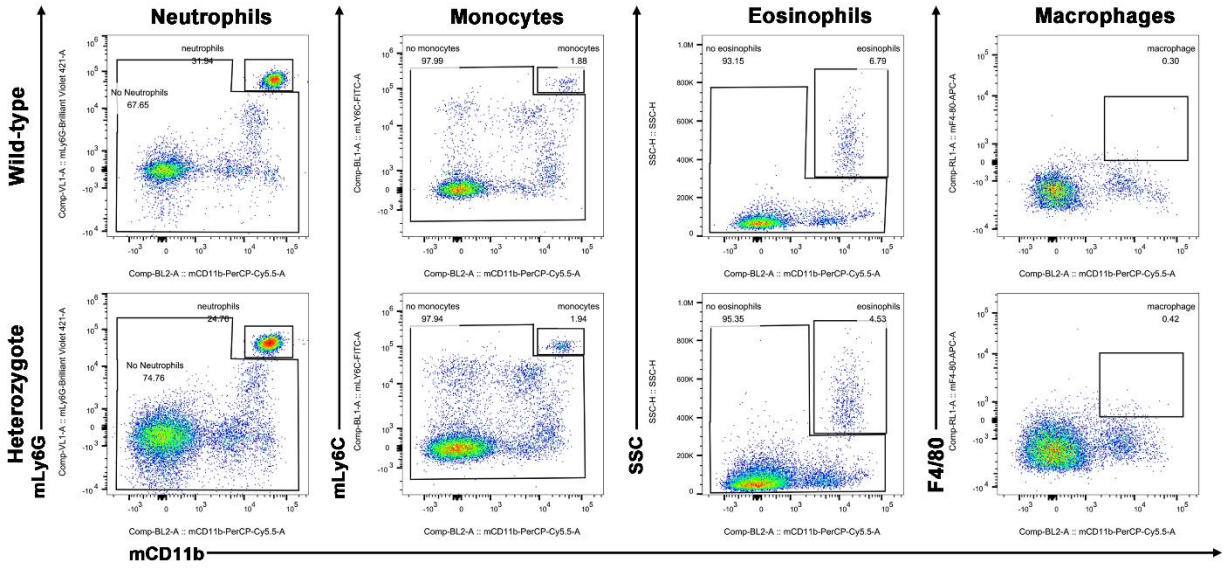


Fig.4 The levels of anti-hHER2 antibodies in BALB/c-hHER2 mice(collaboration data). BALB/c and heterozygous BALB/c-hHER2 mice were immunized with human recombinant HER2 protein. The results showed that the levels of anti-hHER2 antibodies in heterozygous BALB/c-hHER2 mice were much lower than those in BALB/c mice, suggesting that our heterozygous BALB/c-hHER2 mice endogenously express human HER2 protein.

4. Analysis of blood immune cell subpopulations in BALB/c-hHER2 mice



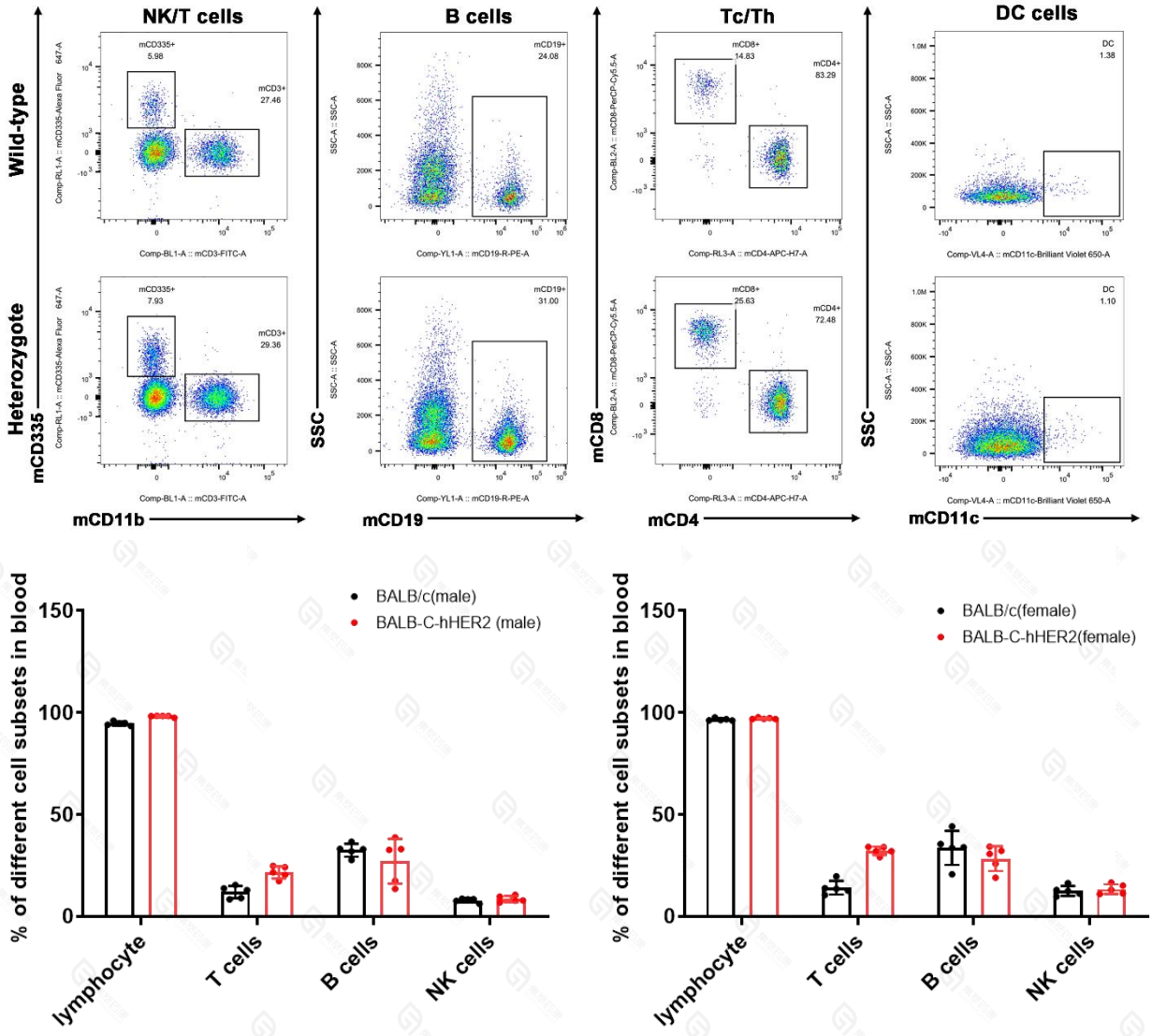
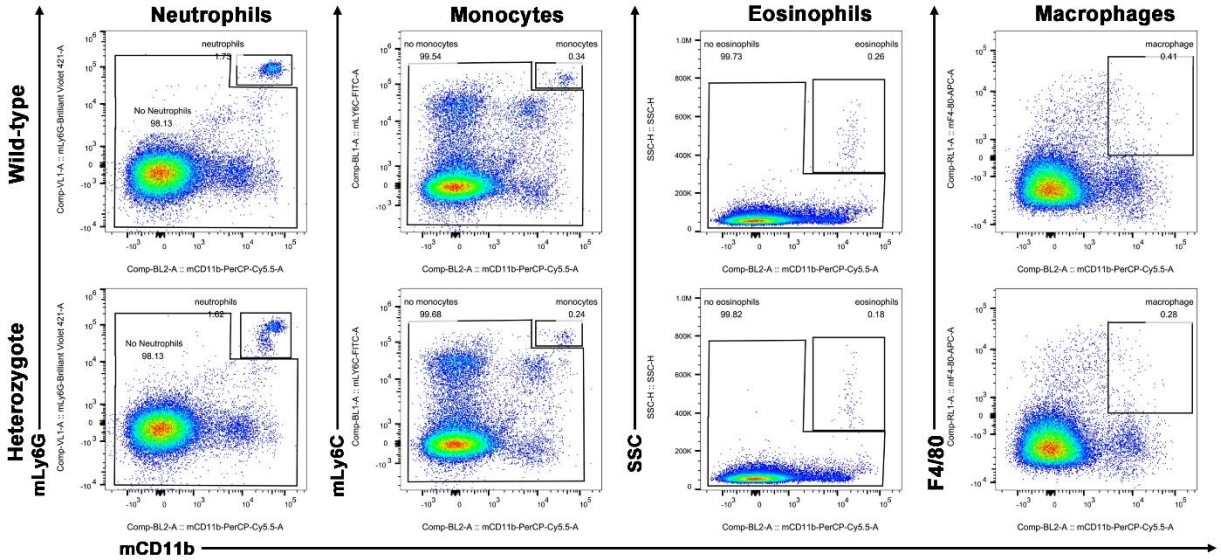
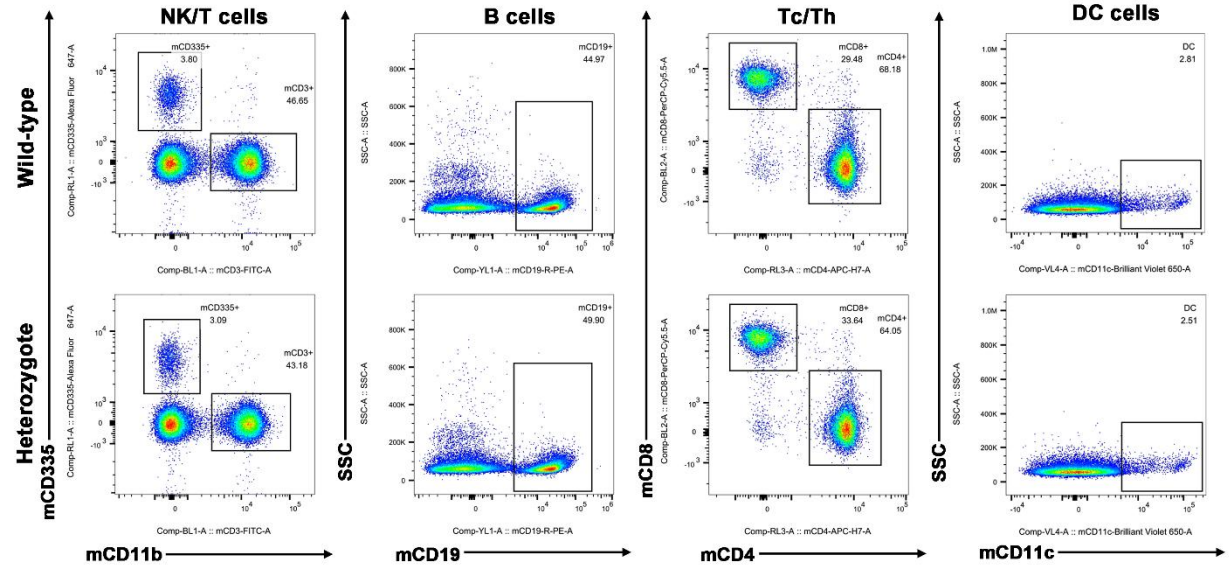


Fig. 5 The immune cell subpopulation in blood of BALB/c and BALB/c-hHER2

Blood was taken from BALB/c and heterozygous BALB/c-hHER2 mice for flow cytometry analysis to assess immune cell subpopulations. As shown in Fig. 5, the percentages of neutrophils, monocytes, eosinophils, macrophages, B cells, T cells, NK cells and DC cells in BALB/c-hHER2 mice were similar to those in BALB/c, indicating that the replacement of mHER2 by hHER2 did not alter the development, differentiation, and distribution of these cells in blood.

5. Analysis of spleen immune cell subpopulations in BALB/c-hHER2 mice


Neutrophils
Monocytes
Eosinophils
Macrophages
Wild-type
Heterozygote
mLy6G
mLy6C
SSC
F4/80
mCD11b
neutrophils
1.71
No Neutrophils
98.13
neutrophils
1.02
no monocytes
99.54
monocytes
0.34
no monocytes
99.68
monocytes
0.24
no eosinophils
99.73
eosinophils
0.26
no eosinophils
99.82
eosinophils
0.18
macrophage
0.41
macrophage
0.28


NK/T cells
B cells
Tc/Th
DC cells
Wild-type
Heterozygote
mCD335
SSC
mCD8
mCD11b
mCD19
mCD4
mCD11c
mCD335+
3.80
mCD3+
46.65
mCD335+
3.09
mCD3+
43.18
mCD19+
44.97
mCD19+
49.90
mCD8+
29.48
mCD4+
68.18
mCD8+
33.64
mCD4+
64.05
DC
2.81
DC
2.51

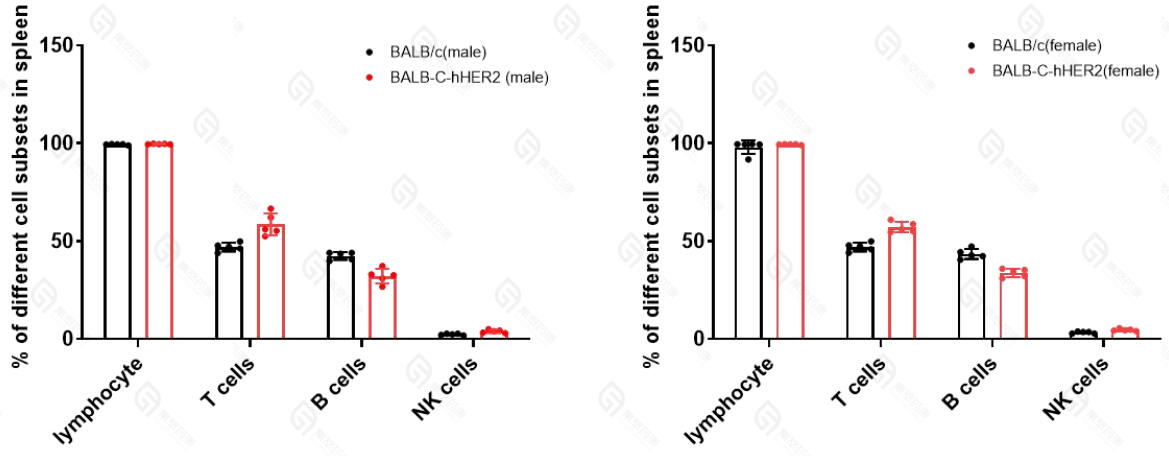

% of different cell subsets in spleen
150
100
50
0
BALB/c(male)
BALB-C-hHER2 (male)
BALB/c(female)
BALB-C-hHER2(female)
lymphocyte
T cells
B cells
NK cells

Fig. 6 The immune cell subpopulation in spleen of BALB/c and BALB/c-hHER2

Splenocytes were taken from BALB/c and heterozygous BALB/c-hHER2 mice for flow cytometry analysis to assess immune cell subpopulations. As shown in Figure 6, the percentages of T cells, NK cells, B cells, neutrophils and dendritic cells in BALB/c-hHER2 mice were similar to those in BALB/c, indicating that the replacement of mHER2 by hHER2 did not alter the development, differentiation, and distribution of these cells in spleen.

6. Analysis of blood immune T cell subpopulations in BALB/c-hHER2 mice

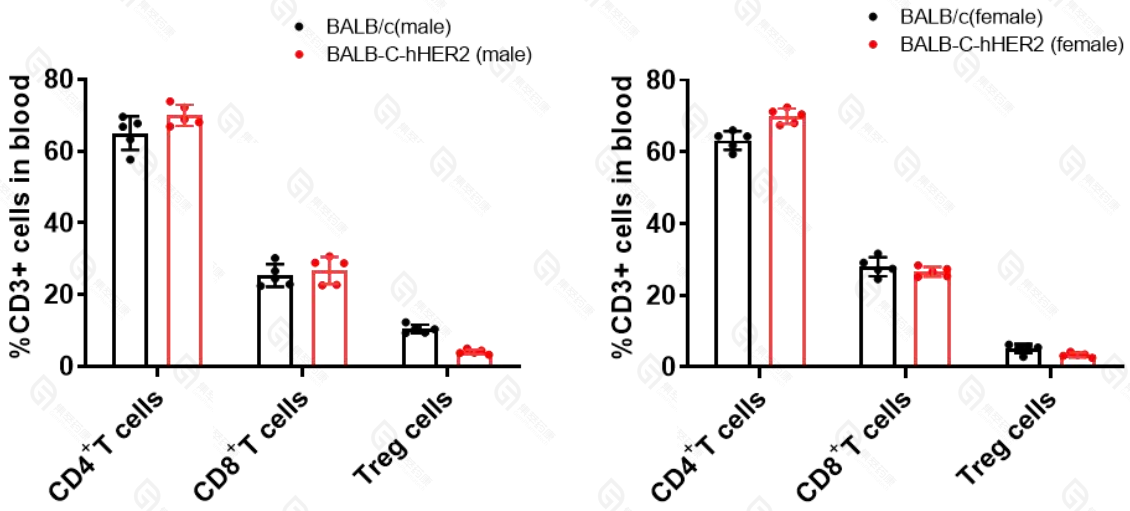


Fig. 7 The immune T cell subpopulation in blood of BALB/c and BALB/c-hHER2 mice

Blood was taken from BALB/c and heterozygous BALB/c-hHER2 mice for flow cytometry analysis to assess immune T cell subpopulations. As shown in Fig. 7, the percentages of $CD4^+$T, $CD8^+$T and Treg cells in BALB/c-hHER2 mice were similar to those in BALB/c, indicating that the replacement of mHER2 by hHER2 did not alter the development, differentiation, and distribution of these cells in blood.

7. Analysis of spleen immune T cell subpopulations in BALB/c-hHER2 mice

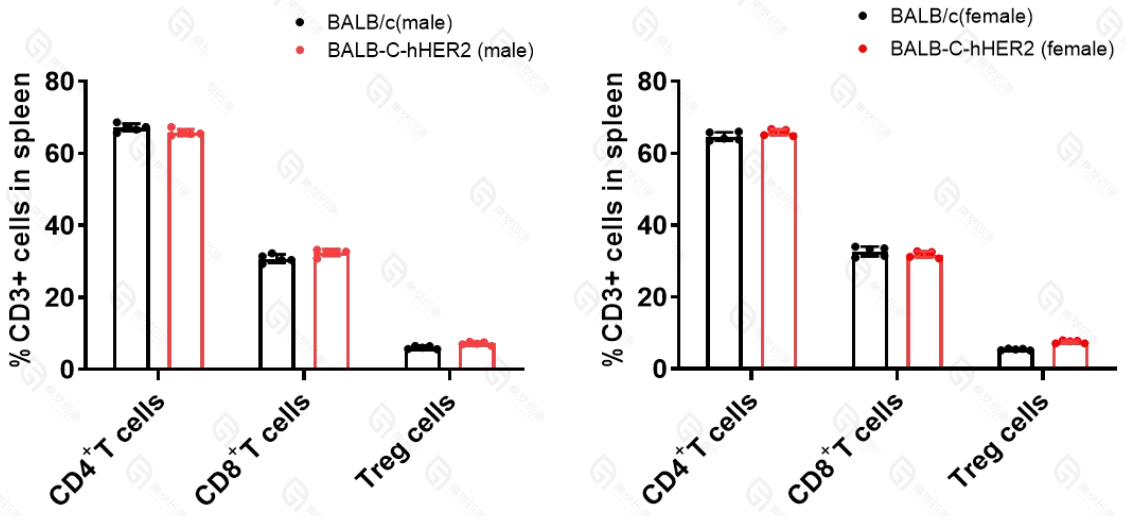


Fig. 8 The immune T cell subpopulation in spleen of BALB/c and BALB/c-hHER2 mice

Spleen was taken from BALB/c and heterozygous BALB/c-hHER2 mice for flow cytometry analysis to assess immune T cell subpopulations. As shown in Fig. 8, the percentages of $CD4^+$T, $CD8^+$T and Treg cells in BALB/c-hHER2 mice were similar to those in BALB/c, indicating that the replacement of mHER2 by hHER2 did not alter the development, differentiation, and distribution of these cells in spleen.

8. Blood routine test in BALB/c-hHER2 mice

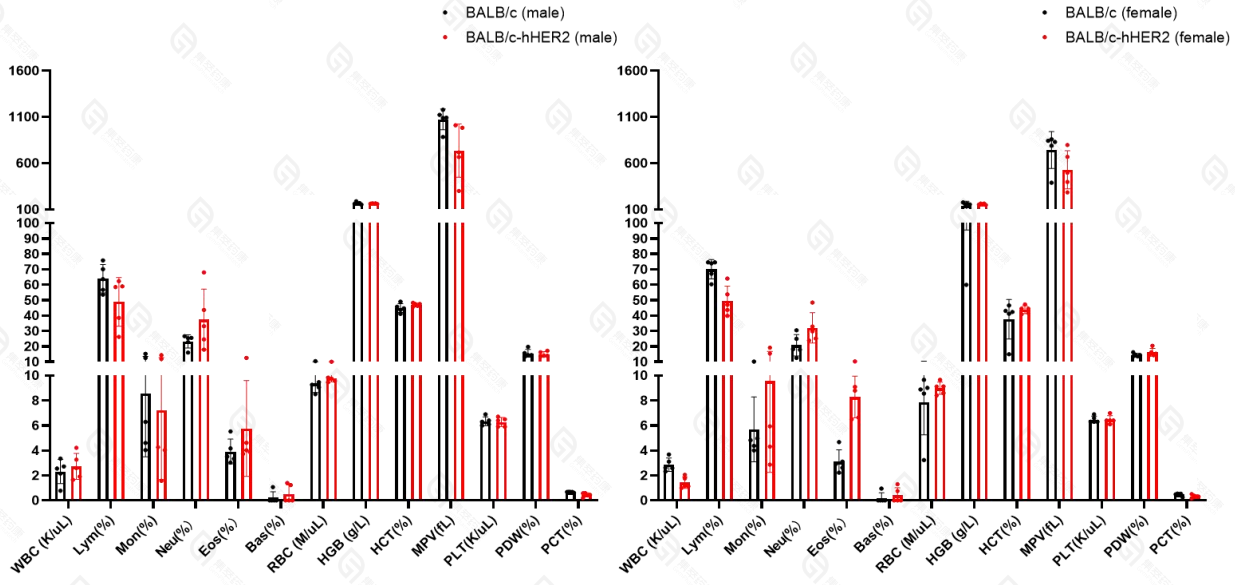


Fig. 9 The blood routine test in BALB/c and BALB/c-hHER2 mice

Blood was taken from BALB/c and heterozygous BALB/c-hHER2 mice for blood routine analysis. As shown in Fig. 9, there was no differences among the tested parameters between BALB/c and BALB/c-hHER2 mice.

9. Blood biochemistry test in BALB/c-hHER2 mice

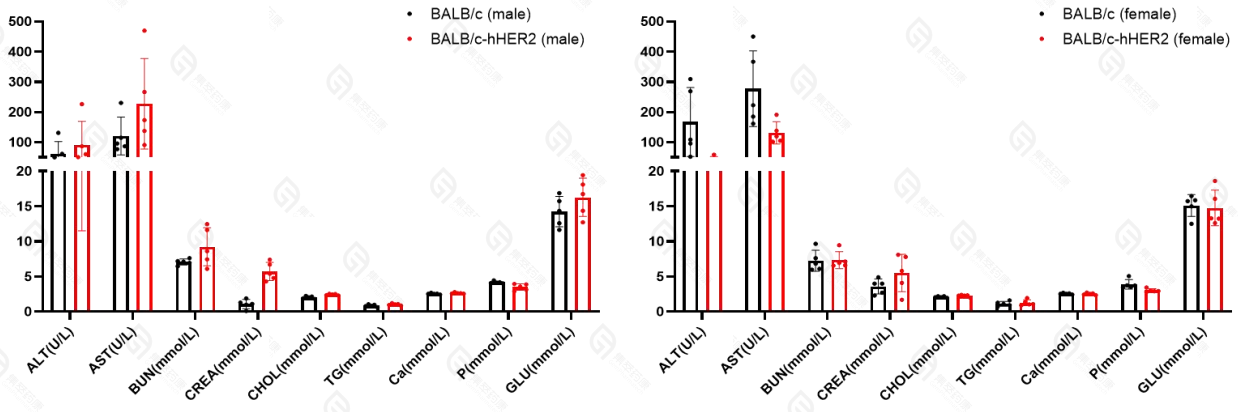


Fig. 10 The blood routine test in BALB/c and BALB/c-hHER2 mice

Serum was taken from BALB/c and heterozygous BALB/c-hHER2 mice for blood biochemistry analysis. As shown in Fig. 10, there was no differences among the tested parameters between BALB/c and BALB/c-hHER2 mice.

References

1. G. Carpenter, L. King, S.Cohen. Epidermal growth factor stimulates phosphorylation in membrane preparations in vitro. *Nature* 1978, **276**, 409-410.
2. A. Schechter, D. Stern, L. Vaidyanathan, et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* 1984, **312**, 513-516.
3. D. Slamon, G. Clark, S. Wong, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987, **235**, 177-182.
4. S. Giordano, M. Franzoi, S. Temin, et al. Systemic therapy for advanced human epidermal growth factor receptor 2-positive breast cancer: ASCO guideline update. *J. Clin. Oncol.* 2022, **40**, 2612-2635.
5. N. Iqbal. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. *Mol. Biol. Int*. 2014, **8**, 52748.
6. V. Wu, N. Kanaya, C. Lo, et al. From bench to bedside: What do we know about hormone receptor-positive and human epidermal growth factor receptor 2-positive breast cancer? *J. Steroid Biochem. Mol. Biol*. 2015, **153**, 45-53.