BALB/c-hCD3E

Stain Name：BALB/cJGpt-Tg(hCD3E BAC)/Gpt

Strain Type：Transgenic

Strain Number：T001550

Background：BALB/cJGpt

Description

CD3E encoded the CD3-epsilon polypeptide, which together with CD3-gamma, -delta and -zeta, and the T-cell receptor alpha/beta and gamma/delta heterodimers, forms the T-cell receptor-CD3 complex. This complex plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways [1,2]. CD3ε plays a critical role in formation and function of the TCR-CD3 complex. T cell bispecific antibody (TCB), an important class of drug against variety of tumors, binds to a tumor associated antigen (TAA) and human CD3ε (hCD3ε), and directs specific killing of tumor cells carrying the TAA[5]. However, therapeutic TCBs usually don't cross-reactive to mouse CD3E as hCD3ε shares only 47% homology with mouse CD3ε (mCD3ε) in the extracellular domain, thus there is an unmet need for suitable animal models to evaluate the therapeutic efficacy of TCB candidates.

BALB/c-hCD3E model was established by injection of BAC clone harboring whole human CD3E gene and its regulatory elements into BALB/c zygotes. These transgenic mice co-express human and mouse CD3ε in over 90% of its T-cells and had normal immune system compared to wild-type BALB/c mice. Moreover, we knocked out mCd3e in BALB/c-hCD3E mice to generate BALB/c-hCD3E/mCd3e-KO mice that displayed a marked reduction in the number of splenic T cells, as well as percentages and numbers of $CD4^+$ and $CD8^+$ T cells. Importantly, strong tumor inhibition of anti-mCTLA4 was observed in BALB/c-hCD3E but not BALB/c-hCD3E/mCd3e-KO mice. These data indicates that mCD3ε is indispensable for T cell normal function and the BALB/c-hCD3E mice carrying intact mCD3ε are ideal models for efficacy study of T cell bispecific antibodies.

Application

1.Study on the development and activation of T cells

2.Screening the T cell bispecific antibody

Data support

1. The hCD3E expression and immune system analysis in the BALB/c-hCD3E mice

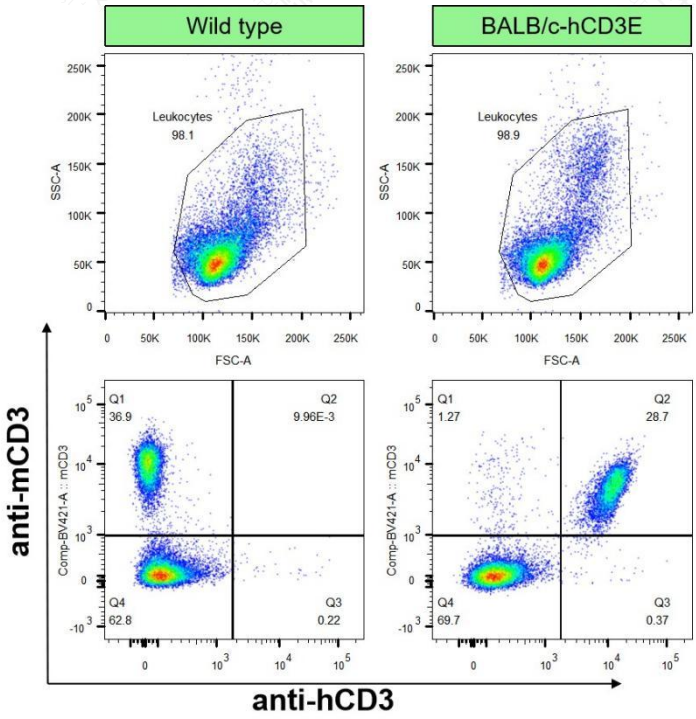

Fig.1 Detection of hCD3E expression in BALB/c-hCD3E.

hCD3E was expressed on the surface of T cells, and 96% CD3+ T cells are hCD3E and mCD3E co-expressing cells in BALB/c-hCD3E mice.

2. .T/B/NK cell ratio assay in BALB/c-hCD3E mice

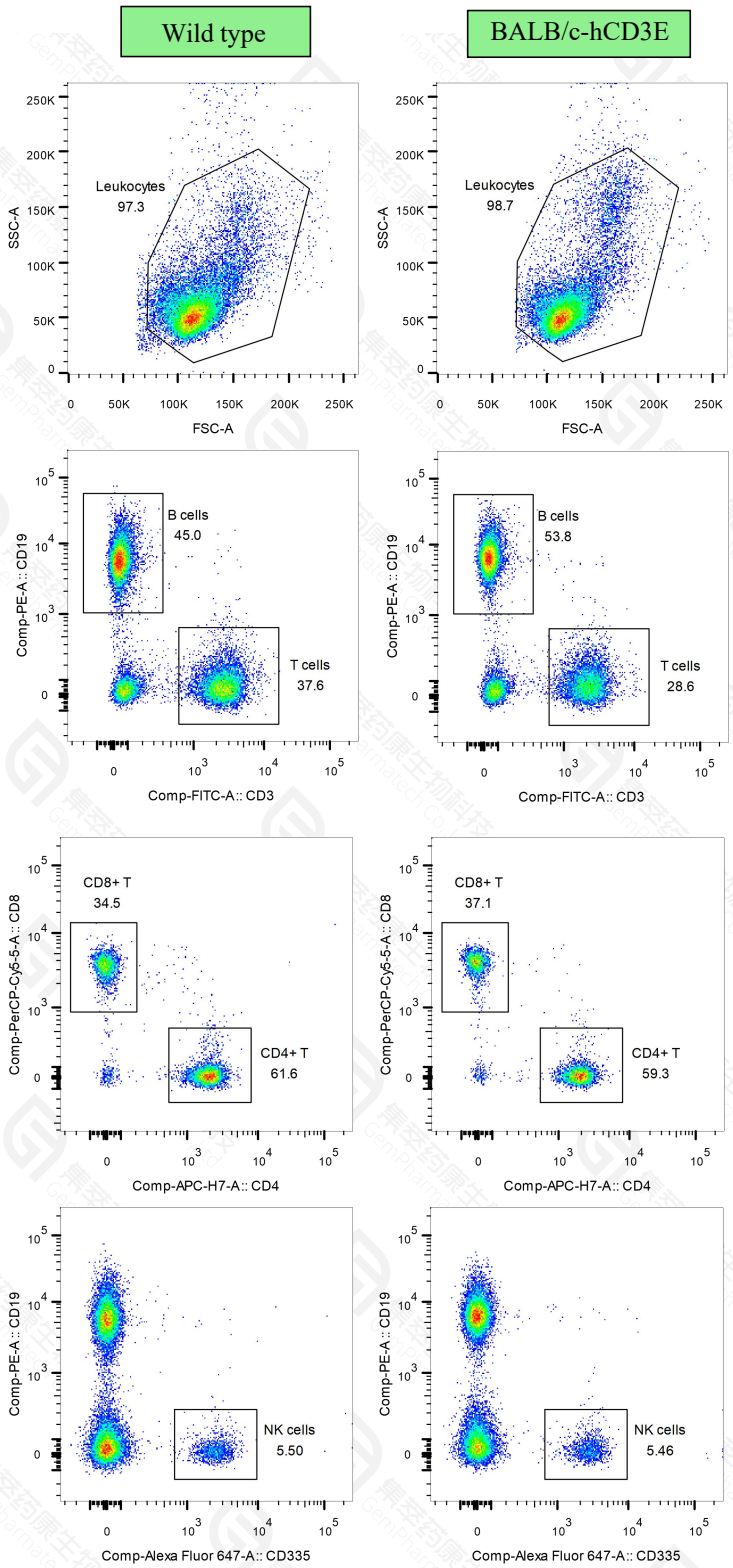

Fig.2 Detection of T/B/NK cell ratio in BALB/c-hCD3E mice.

No significant differences of T/B/NK cell ratio were observed between BALB/c-hCD3E and wild type mice.

3. Cytokine release assay in BALB/c-hCD3E mice

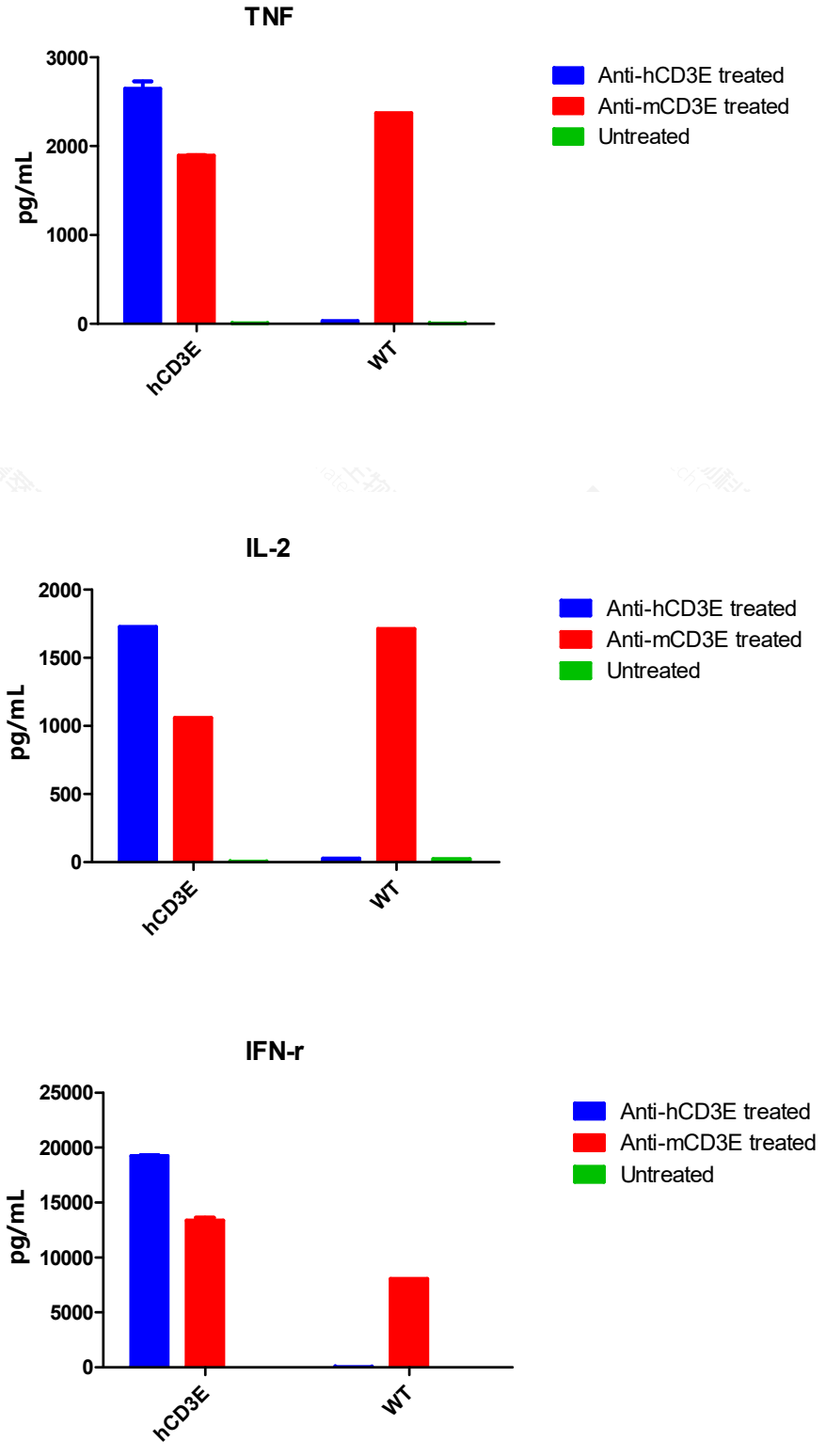

Fig.3 Detection of cytokine release assay in BALB/c-hCD3E mice.

BALB/c-hCD3E mice respond to anti-hCD3E antibody and anti-mCD3E antibody, and BALB/c widy-type mice just respond to the stimulation of anti-mCD3E antibody. IL2, IFN-γ and TNF level was elevated strikingly after the stimulation with anti-hCD3 or anti-mCD3 in BALB/c-hCD3E mice, and cytokine levels were elevated similarly with BALB/c wide type mice after the stimulation with anti-mCD3.

4. Anti-tumor efficacy test in BALB/c-hCD3E mice

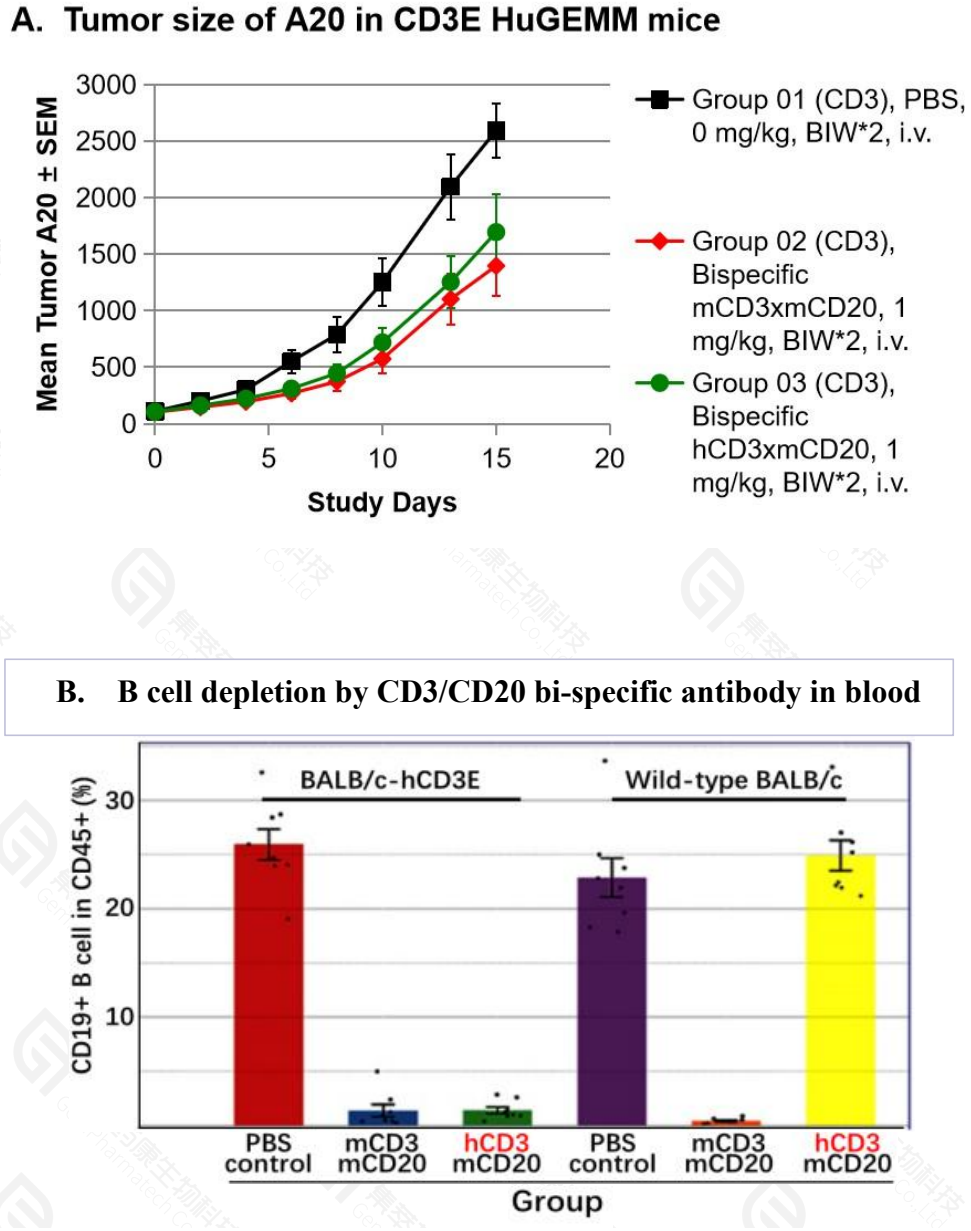

Fig.4 In vivo efficacy test based on BALB/c-hCD3E mice (Data collected cooperating

with CrownBio). (A)Tumor size of A20 in hCD3E mice (Mean volume ± SEM), (B) B cell depletion by CD3/CD20 bi-specific antibody in blood.

A20-CD20 cells in logarithmic growth phase were inoculated into BALB/c-hCD3E mice (6-8 weeks old) by subcutaneous injection . When the average volume of tumors reached to 100 mm3, all mice were randomly allocated to three different study groups(n=8), mice were treated with PBS(black), 1mg/kg Bispecific mCD3xmCD20(red), 1mg/kg Bispecific hCD3xmCD20(green) every 3 days for a total of 4 times. The results showed that Bispecific hCD3xmCD20(TGI=42%) and Bispecific mCD3xmCD20(TGI=50%) inhibited tumor growth when used alone. The B cells in the blood of the mice were destroyed.

The immune system analysis in the BALB/c-hCD3E/mCd3e KO mice

1. T/B/NK cell ratio assay in BALB/c-hCD3E/mCd3e KO mice

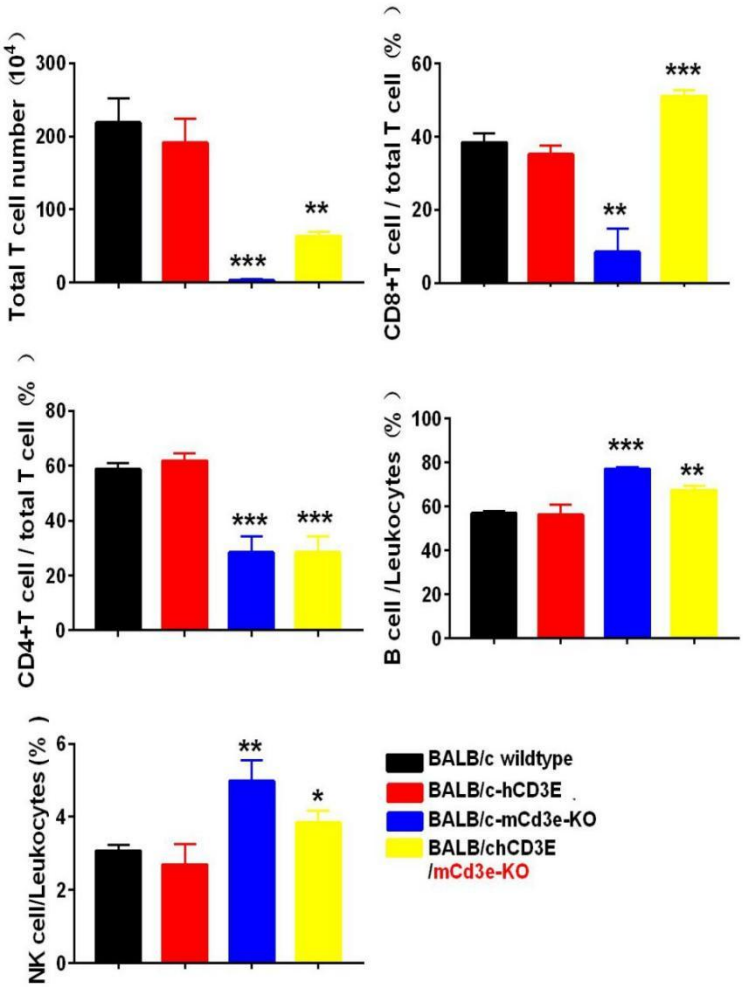

Fig.5 Detection of T/B/NK cell ratio in BALB/c-hCD3E/mCd3e mice.

Compare to wildtype and BALB/c-hCD3E mice, BALB/c-hCD3E/mCd3e-KO mice displayed a marked reduction in the number of splenic T cells and also reduced percentages and numbers of $CD4^+$ T and $CD8^+$ T cells.

2. Anti-tumor efficacy test

***In vivo* Efficacy Study of anti-mCTLA4 in BALB/c-hCD3E and BALB/c-hCD3E/mCd3e KO Mouse Model Bearing Subcutaneous Mouse CT26 Tumor**

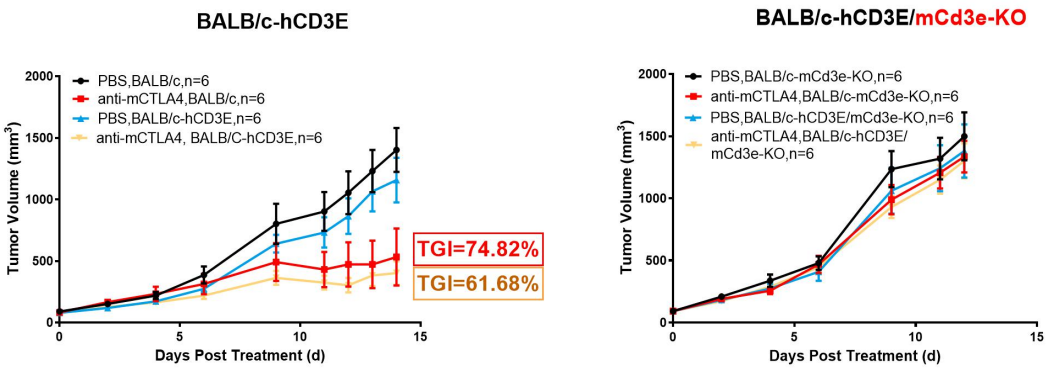

Fig.6 In vivo efficacy test in BALB/c-hCD3E and BALB/c-hCD3E/mCd3e KO mice.

BALB/c-hCD3E and BALB/c-hCD3E/mCd3e KO mice were inoculated subcutaneously with Murine colon carcinoma CT26 cells. When tumors reached an average volume of 100 mm^3, mice were treated with vehicle or anti-mCTLA4 every two day for 4 times and followed by every three day for 3 times. The results showed that anti-mCTLA4 antibodies strongly inhibited the growth of subcutaneously inoculated CT26 tumor cells in BALB/c-hCD3E(TGI=61.68) but not BALB/c-hCD3E/mCd3e-KO mice.

The results demonstrate that mCD3ε is indispensable for T-cell development and function in BALB/c-hCD3E transgenic mice and these mice are a novel and valuable model to assessing the therapeutic efficacy of TCBs.

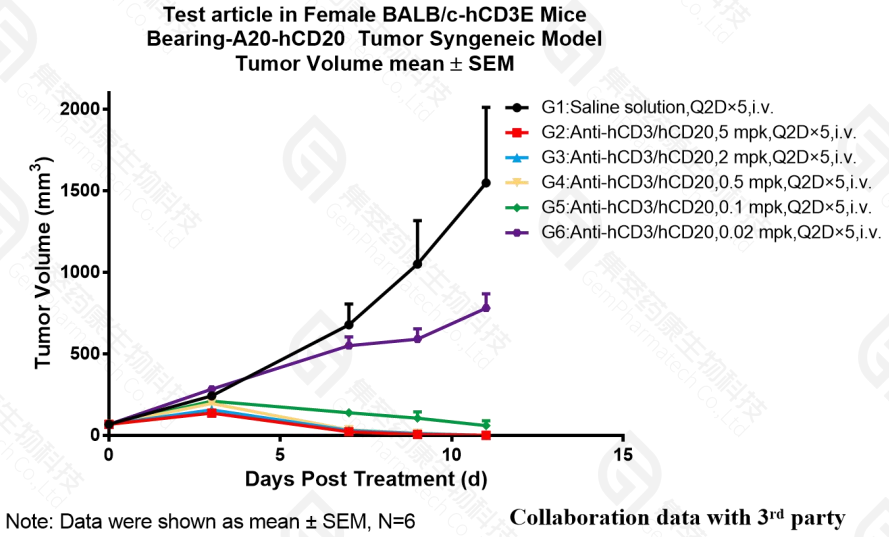

Fig.7 In vivo efficacy test based on BALB/c-hCD3E.

CD20 humanized mouse B lymphoma cells A20-HCD20 cells were cultured to logarithmic growth phase and inoculated subcutaneously into 6-8 weeks old BALB/c-hCD3E mice. Mice were divided into Vehicle groups (n=8), Anti-hCD3/hCD20（0.02mpk、0.1 mpk、0.5 mpk、2 mpk and

5 mpk）groups.When the tumor size was about 105 mm^3, mice were treated with the appropriate medication, and administrated twice a week for a total of 6 times. Data are presented in Mean ± SEM format. **Resolution:** Anti-hCD3/hCD20 groups with different doages（0.02mpk、0.1 mpk、0.5 mpk、2 mpk and 5 mpk）have different tumor inhibitory effect

Indication: BALB/c-hCD3E mice are powerful tools to evaluate the efficacy of Bispecific antibodies in vivo.

Humanized tumor cell lines available at GemPharmatech

Humanized target	Tumor cell line（BALB/c background）
CD19	A20
CD20	A20
CD33	Bcl1
IL3RA（CD123）	WEHI-3
EpCAM	4T1
GCPII（PSMA）	4T1
HER2	4T1/CT26
EGFR	4T1/CT26
BCMA	MOPC315

References

1. Yamazaki, Tetsuo, et al. "CAST, a novel CD3ε-binding protein transducing activation signal for interleukin-2 production in T cells." *Journal of Biological Chemistry* 274.26 (1999): 18173-18180.
2. Kuhn, Chantal, et al. "Human CD3 transgenic mice: preclinical testing of antibodies promoting immune tolerance." *Science Translational Medicine* 3.68 (2011): 68ra10-68ra10.
3. Soudais, Claire, et al. "Independent mutations of the human CD3–ε gene resulting in a T cell receptor/CD3 complex immunodeficiency." *Nature genetics* 3.1 (1993): 77.
4. de Saint Basile, Geneviève, et al. "Severe combined immunodeficiency caused by deficiency in either the δ or the ε subunit of CD3." *The Journal of clinical investigation* 114.10 (2004): 1512-1517.
5. Dreier, Torsten, et al. "T cell costimulus-independent and very efficacious inhibition of tumor growth in mice bearing subcutaneous or leukemic human B cell lymphoma xenografts by a CD19-/CD3-bispecific single-chain antibody construct." *The Journal of Immunology* 170.8 (2003): 4397-4402.