BALB/c-hCD3EDG

Strain Name： BALB/cJGpt-*Cd3e, d, g*$^{tm1(hCD3E, D, G)}$/Gpt

Strain Type: Knock-in

Strain Number: T053483

Background: BALB/cJGpt

Description

Bispecific antibodies (BsAbs) combine specificities of two antibodies and simultaneously address different antigens or epitopes. BsAbs with ‘two-target’ functionality can interfere with surface receptors or associated ligands belonging to multiple pathways simultaneously, for example, cancer, proliferation or inflammatory processes. BsAbs can also place targets into close proximity, either to support protein complex formation on one cell, or to trigger contacts between cells. CD3-target-based bispecific antibodies(CD3-TCB)are designed to simultaneously bind to T cells and target cell antigens, leading to T-cell activation, proliferation, and target cell death.

To create a desired animal model for studies of CD3-TCBs, GemPharmatech developed the BALB/c-hCD3EDG model by gene editing technology. Briefly, a large human genomic fragment containing CD3E, CD3D, CD3G as well as regulatory elements is introduced into the endogenous Cd3e, Cd3d, Cd3g locus located on Chr9. Animals carrying human CD3E, CD3D, CD3G integration and mouse Cd3e, Cd3d, Cd3g removed are selected to establish the strain. The BALB/c-hCD3EDG mice successfully express human CD3E/CD3D/CD3G on the T cell surface and exhibit a normal immune system. The CD3EDG humanized mice are suitable models for preclinical studies of bispecific antibodies and related immunotherapies.

Strain strategy

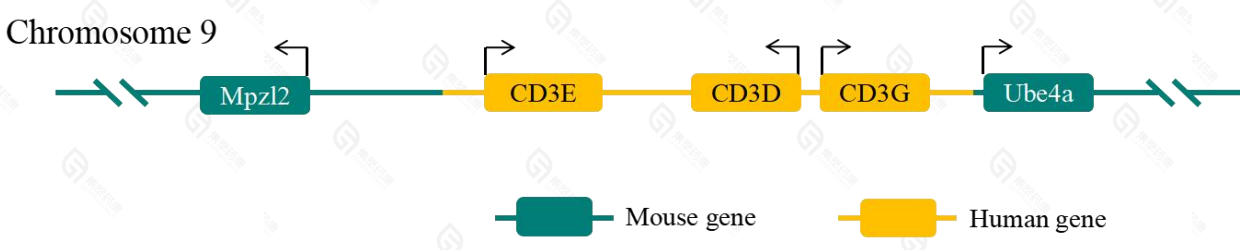

Figure 1. Schematic diagrams of CD3E/D/G humanization strategy.

Applications

1. Efficacy study of human CD3-target-based bispecific antibodies
2. Cancer immunotherapy research

Supporting data

Detection of CD3 expression

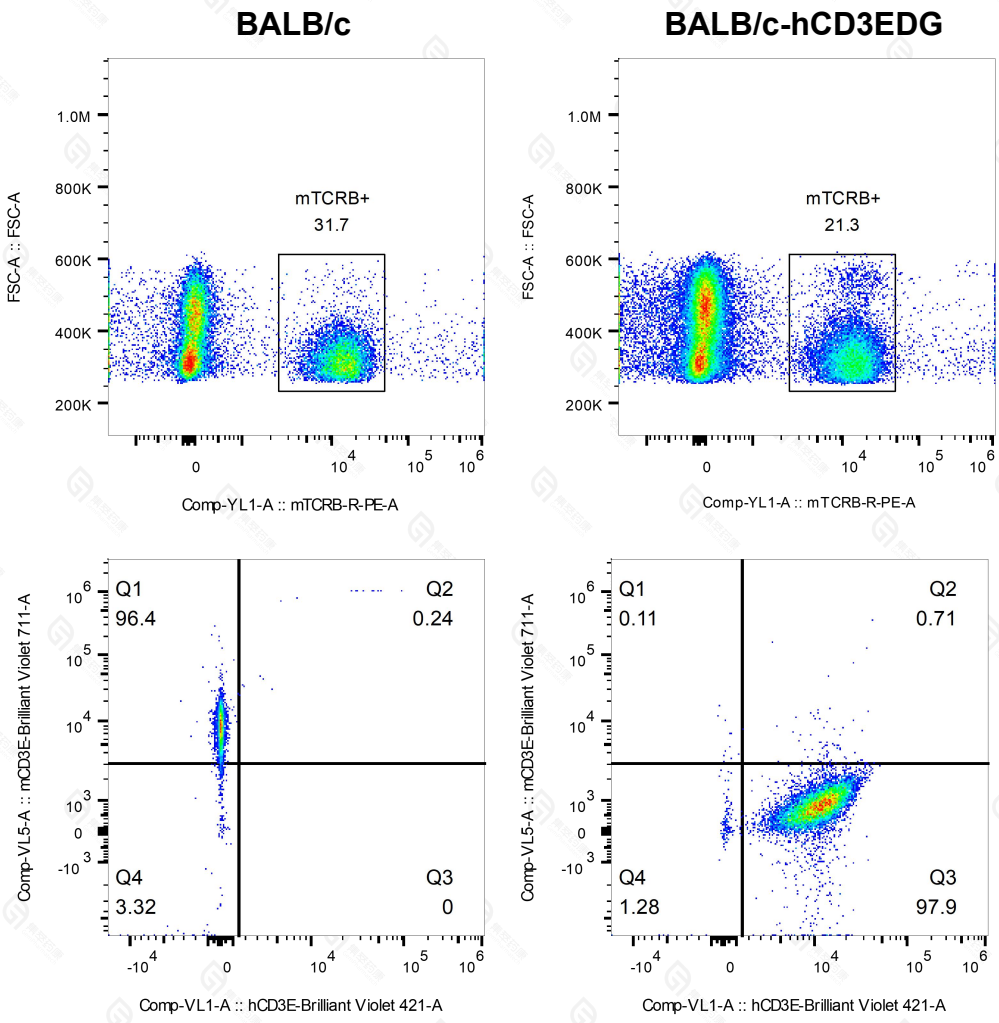

Figure 2. Human CD3 expression detection.

The hCD3E expressing cell ratio in total T cells in homozygous BALB/c-hCD3EDG mice blood is comparable to that of mCD3E of expressing cell ratio in wild-type BALB/c mice blood. Top panel: mTCRb$^+$ expressing ratio in live cells. Bottom panel: mCD3E$^+$/hCD3E$^+$ expressing ratio in total T cells.

1. Immune cell ratio assay

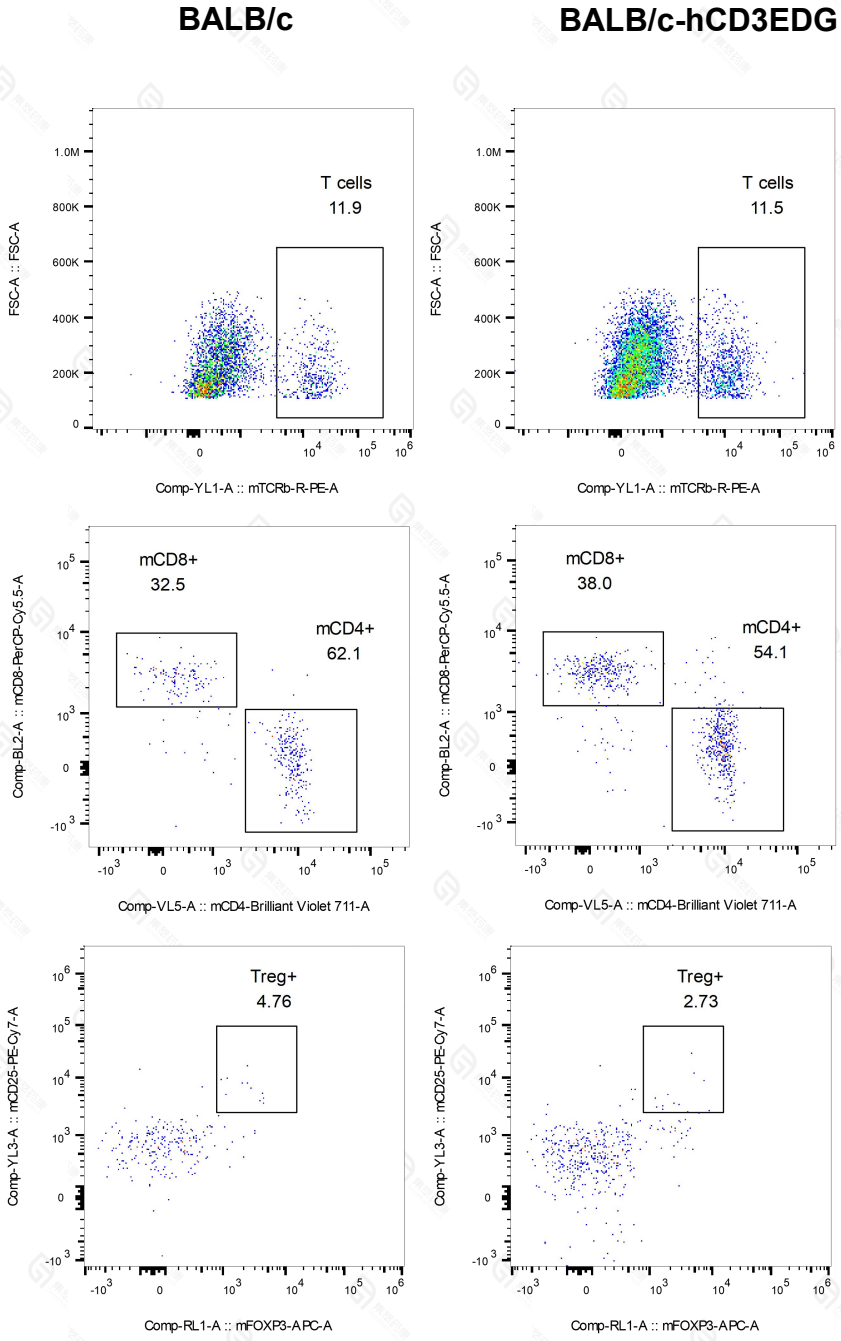

Figure 3. T lymphocytes and subgroup ratio detection.

The ratio of T lymphocytes and their subgroups in the blood of BALB/c-hCD3EDG mice is similar to that in wild-type BALB/c mice. Top panel: mTCRb+ ratio in live cells. Middle panel: mCD8+/mCD4+ ratio in total T cells. Bottom panel: Treg ratio in mCD4+ T cells.

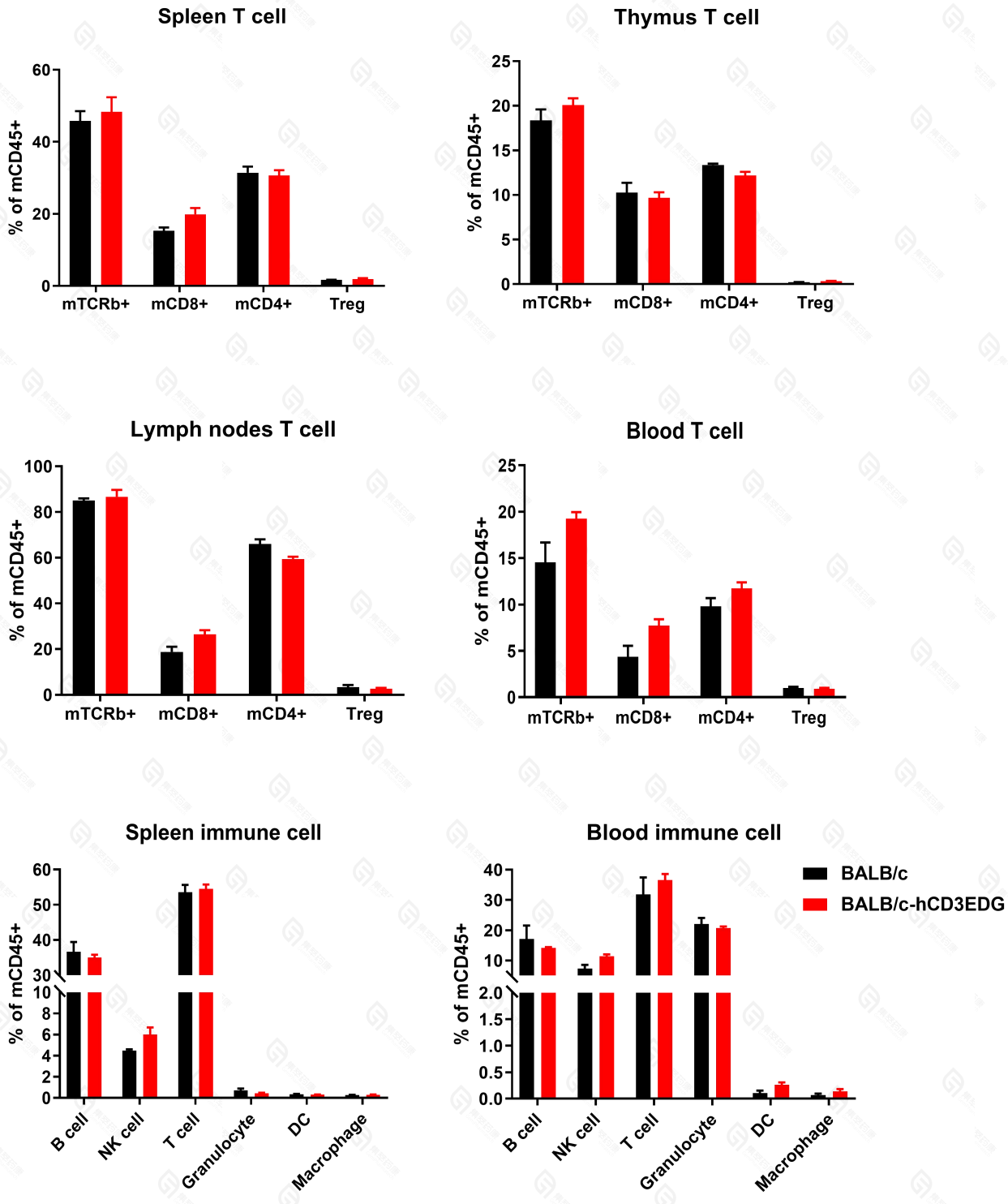

Figure 4. Detection of immune cell ratio in BALB/c-hCD3EDG mice.

The ratio of T cells, B cells, NK cells, granulocytes, DC and macrophages in different tissues of BALB/c-hCD3EDG mice is similar to that of wild-type BALB/c mice (n=3). There is no significant difference between BALB/c and BALB/c-hCD3EDG in any immune cell types. B cell: mB220^{+}; NK cell: mCD335^{+}; T cell: TCRβ^{+}; Granulocyte: mGr-1^{+} mCD11b^{+}; DC: mGr-1^{+}mCD11c^{+}mCD11b^{-}; Macrophage: mGr-1^{+} mF4/80^{+}mCD11b^{-}

2. Response of splenocytes to anti-CD3 stimulation

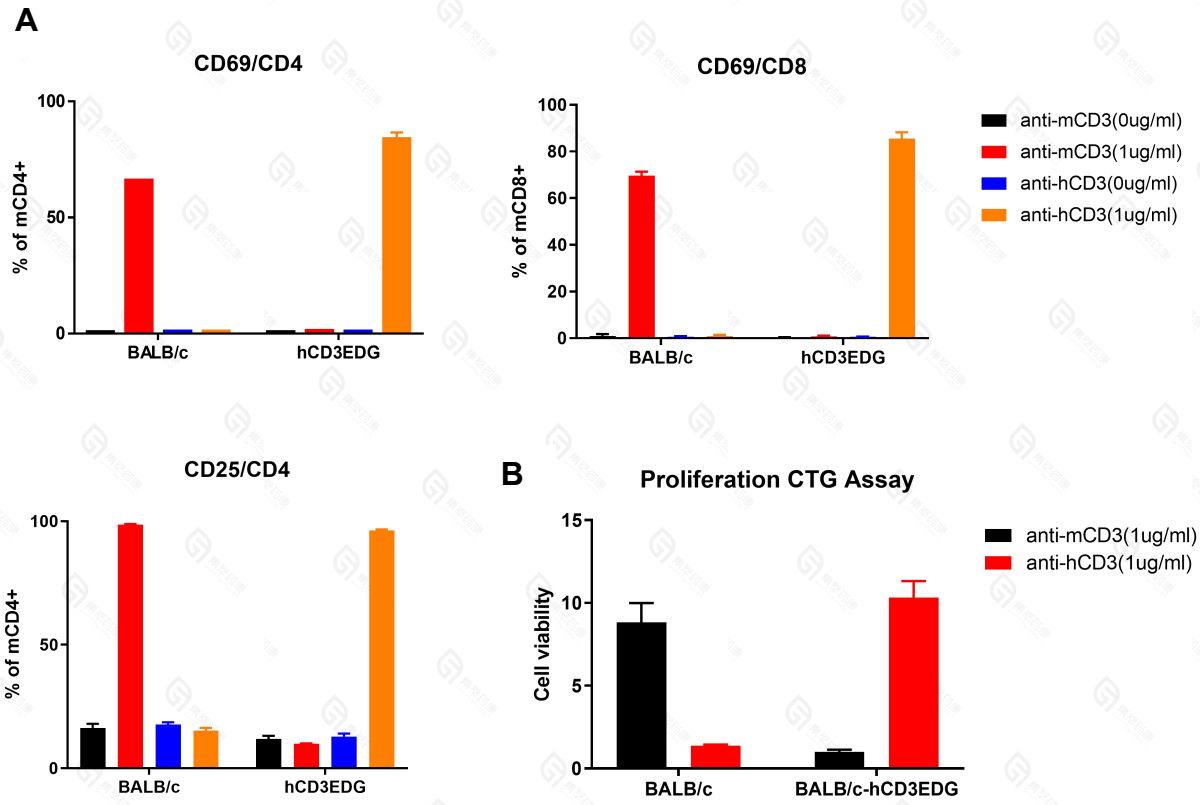

Figure 5. Proliferative response to anti-CD3 stimulation.

Splenocytes were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and treated with or without 1μg/mL anti-CD3 for 48h. A.T cell stimulation was tested by flow cytometry. T cell activation in BALB/c-hCD3EDG mice was significantly up-regulated by anti-hCD3E antibody, similar to the activation level shown in BALB/c mice treated with anti-mCD3E antibody.

B.Splenocyte proliferation was analyzed by CTG assay, and reported as the mean cell viability(n=3), Cell viability=(RLU values for treated - RLU values for blank)/(RLU values for non-treated - RLU values for blank). The splenocytes from BALB/c-hCD3EDG could only be stimulated by anti-hCD3. And the BALB/c-hCD3EDG splenocytes proliferation treated with anti-hCD3 is comparable to BALB/c treated with anti-mCD3.

3. Preclinical in vivo efficacy evaluation of anti-CD3/CD20 BsAb (Mosunetuzumab analogue)

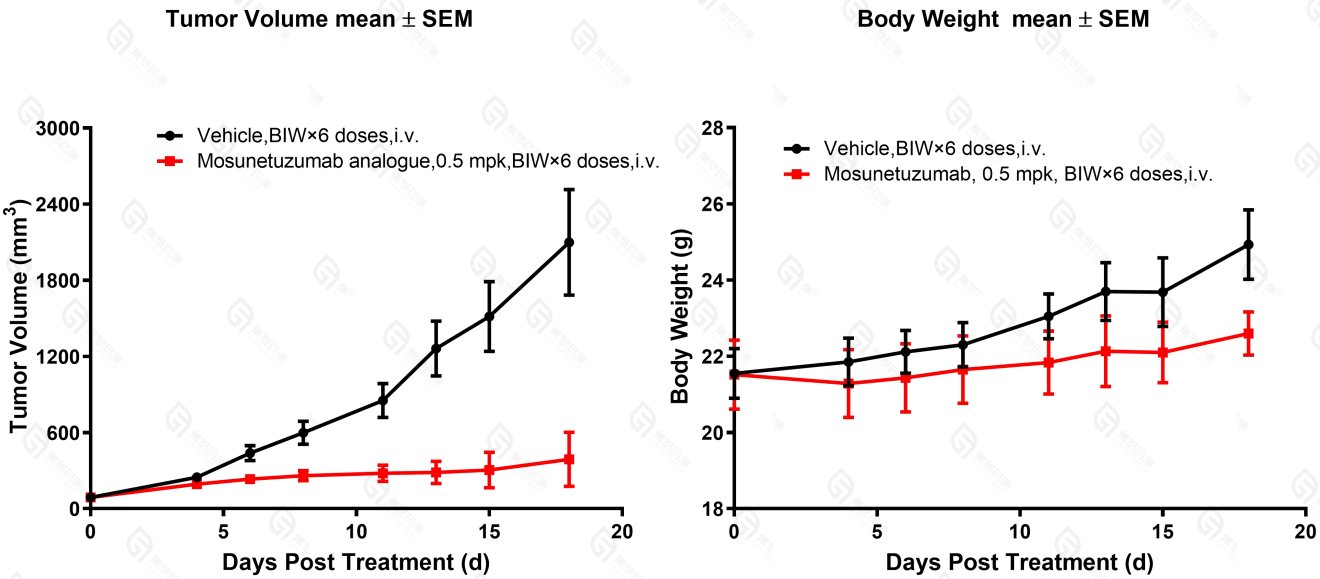

Figure 6. Preclinical in vivo efficacy evaluation of Mosunetuzumab analogue in mouse B cell lymphoma line, A20-hCD20(humanized CD20).

BALB/c-hCD3EDG mice (6-8 weeks) were subcutaneously inoculated with murine lymphoma cell line A20-hCD20 cells. Mice were randomly grouped when the average tumor volume reached 90mm3 and divided into a vehicle group and an anti-hCD3/hCD20 (Mosunetuzumab analogue) treated group (n=6). The test article was administered twice every week for a total of 6 times. Treatment of anti-hCD3/hCD20 showed significant tumor growth inhibition (TGI=81.46%, left). Mice from both groups showed overall good health as shown by their body weight (right). The results demonstrated that BALB/c-hCD3EDG mice are an excellent model for assessing the in vivo efficacy of human CD3-bispecific antibodies.

4. Humanized syngeneic tumor cell line resources

Cancer types	Parent cell line	Engineered cell line	Availability
Colorectal cancer	CT26-WT	CT26-hPDL1(Tg)-mPDL1(KO)	Available
		CT26-hHER2 (Tg)	Available
		CT26-hEGFR (Tg)-mEGFR(KO)	Available
		CT26-hPDL1(Tg)-mPDL1(KO)-mCLDN18.2(Tg)	Available
		CT26-hCD47 (Tg)-mCD47(KO)	Available
		CT26-hPDL1(Tg)-mPDL1(KO) -hCD47(Tg)-mCD47(KO)	Available
		CT26-hCD73 (Tg)-mCD73(KO)	Available
		CT26-hPDL1(Tg)-mPDL1(KO) –hCD73(Tg)-mCD73(KO)	Available
		CT26-hTNFR2 (Tg)-mTNFR2(KO)	Available
		CT26-hPDL1(Tg)-mPDL1(KO) -hCD39(Tg)-mCD39(KO)	Available

		CT26-hNectin4 (Tg)-mNectin4(KO)	Available
		CT26-hPDL1(Tg)-mPDL1(KO)-hB7H3(Tg)-mB7H3(KO)	Available
		CT26-hB7H3(Tg)-mB7H3(KO)	Available
		CT26-hPDL1(Tg)-mPDL1(KO) -hGDF15(Tg)-mGDF15(KO)	Available
		CT26-hTACSTD2(Tg)-mTACSTD2(KO)	Available
		CT26-hSSTR2(Tg)-mSSTR2(KO)	2023 Q1
		CT26-hLRRC15(Tg)-mLRRC15(KO)	2023 Q1
		CT26-hMUC17(Tg)-mMUC17(KO)	2023 Q1
Liver cancer	H22	H22-hPDL1(Tg)-mPDL1(KO) -hCD47(Tg)-mCD47(KO)	Available
		H22-hPDL1(Tg)-mPDL1(KO)	Available
		H22-hDLK1(Tg)-mDLK1(KO)	2023 Q1
Breast cancer	4T1	4T1-hPDL1(Tg)-mPDL1(KO)	Available
		4T1-hEpCAM(Tg)-mEpCAM(KO)	Available
		4T1-hGCPII (Tg)-mGCPII(KO)	Available
		4T1-hHER2 (Tg)	Available
		4T1-hEGFR (Tg)-mEGFR(KO)	Available
		4T1-hPDL1(Tg)-mPDL1(KO) -hCD47(Tg)-mCD47(KO)	Available
	EMT6	EMT6-hPDL1(Tg)-mPDL1(KO)	Available
		EMT6-hPDL1(Tg)-mPDL1(KO) -hCD47(Tg)-mCD47(KO)	Available
		EMT6-hCD39(Tg)-mCD39(KO)	Available
Lymphoma	A20	A20-hCD20(Tg)-luciferase	Available
		A20-hCD19(Tg)-mCD19(KO)	Available
		A20-hCD19(Tg)-mCD19(KO)-luciferase	Available
		A20-hGPRC5D(Tg)-mGPRC5D(KO)	2022 Q4
		A20-hCD5(Tg)-mCD5(KO)	2023 Q1
	MPC-11		Available
	MOPC315	MOPC315-hBCMA(Tg)-mBCMA(KO)-luciferase	Available
	J558	J558-mBCMA(Tg)-luciferase	Available
	BcL1 clone 5B1b		Available
Renal cancer	RENCA		Available
Leukemia	WEHI-3	WEHI-3-hIL3RA(Tg)-mIL3RA(KO)	Available
		WEHI-3-hCD33(Tg)-mCD33(KO)	Available

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. Ueda, Otoya, et al. "Entire CD3ε, δ, and γ humanized mouse to evaluate human CD3–mediated therapeutics." Scientific Reports 7 (2017): 45839.