

**Promab Biotechnologies' CAR-T new product development programs are being designed for pre-clinical and future clinical applications.**

**CAR-T cells can be used for:**

1. Compound screening
2. Antibody screening
3. Co-stimulatory and activation domain comparison
4. Personalized medicine and donor variations for CAR-T screening
5. Checkpoint inhibitors
6. Safety switches and regulators of CAR-T functions
7. Pre-clinical in vivo models
8. Treg and T memory cells in CAR-T setting
9. CAR-T signaling, tumor microenvironment
10. Proof of concept studies for clinical trials

**The structure of CAR from Promab's available CAR-T cells targeting CD19 with inducible caspase-9:**

The inducible caspase-9-CD19 CAR construct consists of FKBP12 gene with mutation F36 to V coding two 12 kDa FK506 binding proteins with HA tag at the end fused to truncated Caspase9 (135-416; lacking pro-domain), followed by T2A peptide and then CD19scFv-CD28- CD3ζ without signaling peptide and with GGGS-Flag. This construct can be used as a negative control for PM-CAR1006-1M (it expressed same length as icasp-CD19 without tags but is non- functional protein). In the presence of dimerization agent, AP20187 or AP1903, caspase-9 is activated inducing cell killing. Introduction of inducible caspase-9 can be used for increasing safety of CAR-T cells in the presence of dimerization agent.



**Figure 1.** CAR-T cells expressing the above construct are available from Promab targeting CD19 with inducible caspase-9. Both genes are separated with T2A peptide sequence allowing independent expression of both gene products. ScFv, single chain variable fragment. HA and Flag tags are indicated at the end of each gene.

To date Promab generated 2nd or 3rd generation CAR and CAR controls (2nd generation of CAR is shown in Figure 1, CAR-T cells and CAR-Natural Killer (NK) effector cells against cancer target cells that show excellent functionality, including dose-dependent and target cell- specific cytotoxic activity (Figures 2, 3).

These cells can be tested with CAR-T in cytotoxic assays and used for testing modulators of immune checkpoint inhibitors (PD-1, CTLA-4 pathways) or activators of immune response, small molecules affecting T cell or Treg activity.

## Data

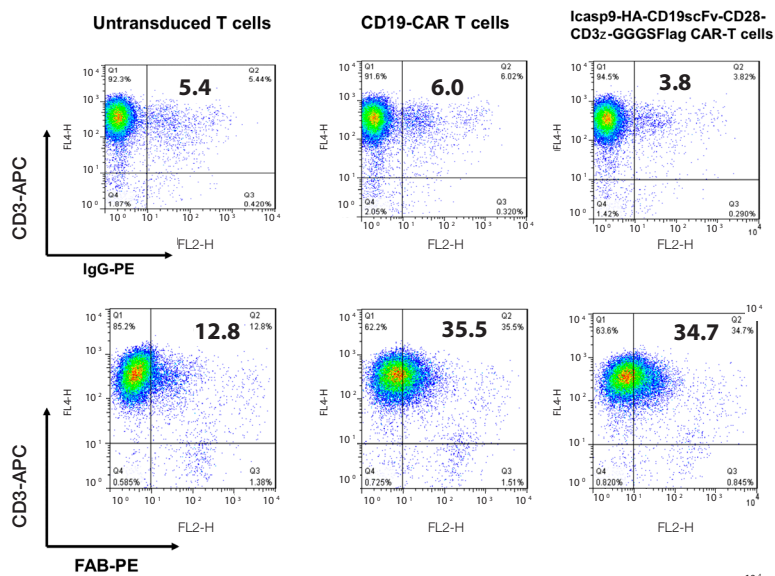
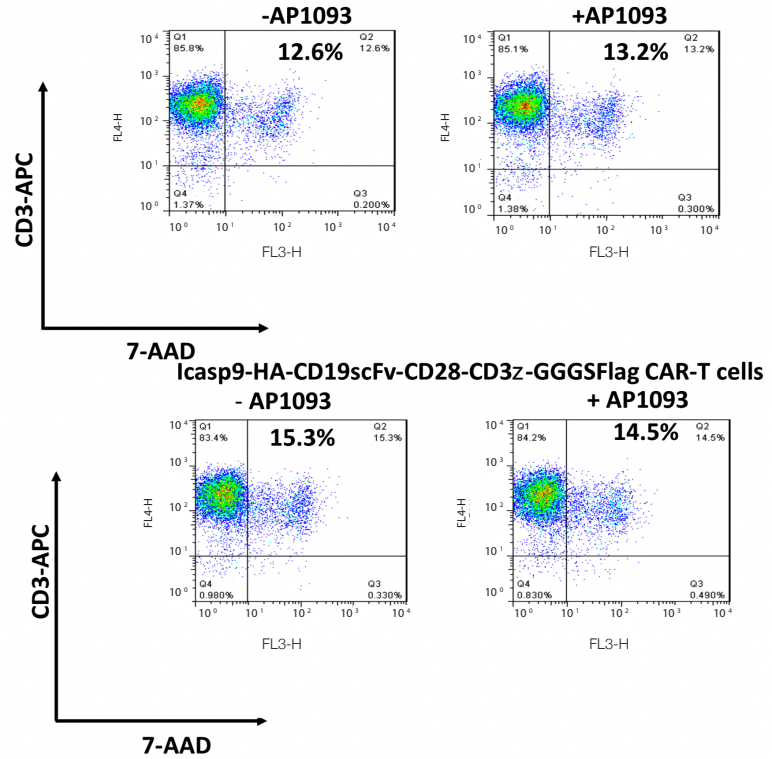


Figure 2. Transduction efficiency of icasp9-HA-CD19scFv-CD28-CD3zeta-GGGSFlag CAR-T cells and CD19-CAR-T cells. Ab showing expression of scFv.



## Data

### Non-transduced T cells



**Figure 3.** Addition of chemical inducer of dimerization (CID) AP1903 100 nM for 24 hours did induce CAR-T cell killing.