

Engineered Target Cell Lines

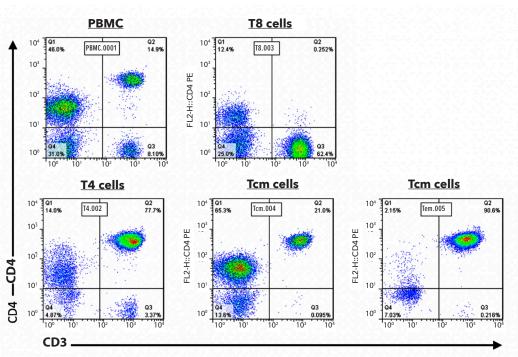
T-cells are functionally divided into helper T-cells, which are CD4<sup>+</sup>CD8<sup>-</sup> ("T4"), and cytotoxic T-cells, which are CD8<sup>+</sup>CD4<sup>-</sup> ("T8"). However, recent studies have indicated that, at least in vitro, cultured helper T-cells can exhibit similar levels of cytolytic activity as cultured cytotoxic T cells.

## T cells can also be divided based on differentiation status into 4 subsets:

- 1. Naïve ("T<sub>n</sub>", containing T<sub>scm</sub>), which are CD27<sup>+</sup> CCR7<sup>+</sup> CD45RO<sup>-</sup>
- 2. Central-memory ("T<sub>cm</sub>"), which are CD27<sup>+</sup> CCR7<sup>+</sup> CD45RO<sup>+</sup>
- 3. Effector-memory ("T<sub>em</sub>"), which are CD27<sup>-</sup> CCR7<sup>-</sup> CD45RO<sup>+</sup>
- 4. Effector ("T<sub>eff</sub>"), which are CD27<sup>-</sup> CCR7<sup>-</sup> CD45RO<sup>-</sup>.

Human PBMC are separated into 4 subsets using antibodies and magnetic beads. The subsets were:  $CD4^+CD8^-T$  cells (T4),  $CD8^+CD4^-T$  cells (T8),  $CD4^+$  central-memory T cells ( $T_{cm}$ ) and  $CD4^+$  effector-memory T cells ( $T_{em}$ ). Immediately after isolation, the subsets and the PBMC were analyzed by flow cytometry for the composition of the subsets. Each of the above subsets can be used for CAR transduction for functional analysis of proliferation, CAR expression, memory and cytotoxic function.

## Data



Example of separation of four fractions by FACS (Figure 1)

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Data



Tcm cells T4 cells **Tcm cells** 10<sup>4</sup> Q1 6.52% T4.002 Q2 52.9% 104 Q1 63.7% Q2 20.3% 104 Q2 91.4% Tcm.007 Q1 8.17% Tem.008 FL1-H::CD45RO FITC FL1-H::CD45RO FITC 10<sup>3</sup> 10<sup>3</sup> 10 memory T cells 51% memory T cells 19.9% memory T cells 91.0% CD45RO 102 10 10 10 10 10 Q3 0.3959 Q3 0.128% 100 10º 10º 10 10 104 104 10 T4.006 Tcm.007 Tem.008 10<sup>3</sup> FL2-H::CCR7 PE 103 FL2-H::CCR7 PE 10 CCR7+ 24.0% CCR7 40.7% CCR7+ 80.5% 10<sup>2</sup> 102 10<sup>2</sup> CCR7 101 10 10<sup>1</sup> 100 100 10° 10 10 10 10 10 100 101 10<sup>2</sup> 103 10 10 101 10 10 CD3

(Figure 1) Flow cytometric analysis of the PBMC and T cell subsets. First, the cells are stained with antibodies against CD4 and CD3 (top panel). T cells are CD3<sup>+</sup>, non-T cells are CD3<sup>-</sup>, and monocytes are CD3<sup>-</sup>CD4low. Then, the T4,  $\rm T_{cm}$  and  $\rm T_{em}$ subsets were stained with antibodies against CD3, CD45RO and CCR7 (lower panel).