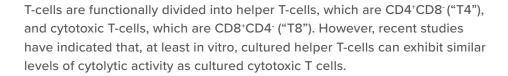


NON-TRANSDUCED CD8+ CD4-T CELLS

Engineered Target Cell Lines

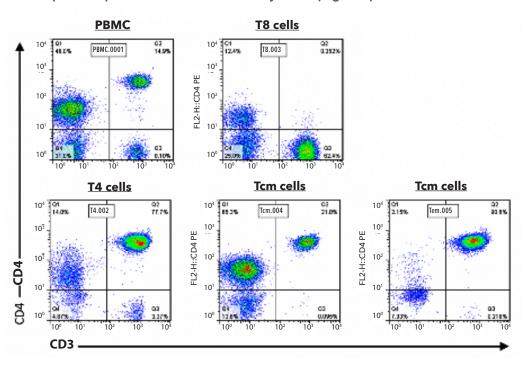


T cells can also be divided based on differentiation status into 4 subsets:

- 1. Naïve ("T_n", containing T_{scm}), which are CD27⁺ CCR7⁺ CD45RO⁻
- 2. Central-memory ("T_{cm}"), which are CD27⁺ CCR7⁺ CD45RO⁺
- 3. Effector-memory ("T_{em}"), which are CD27⁻ CCR7⁻ CD45RO⁺
- 4. Effector ("T_{eff}"), which are CD27 CCR7 CD45RO.

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 $_{\rm cm}$) and CD4 $^+$ effector-memory T cells (T $_{\rm em}$). 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)



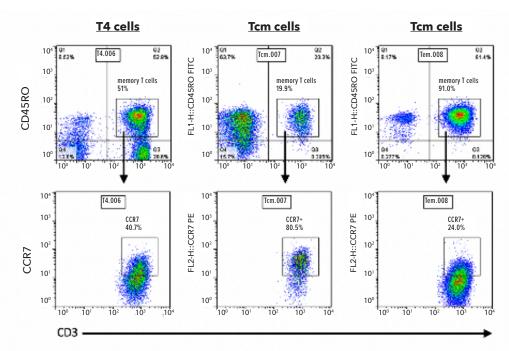




NON-TRANSDUCED CD8+ CD4-T CELLS

Engineered Target Cell Lines

Data



(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 $^{+}$, non-T cells are CD3 $^{-}$, and monocytes are CD3 $^{-}$ CD4low. Then, the T4, T $_{\rm cm}$ and T $_{\rm em}$ subsets were stained with antibodies against CD3, CD45RO and CCR7 (lower panel).

