



PM-CAR2009

Engineered Target Cell Lines

T-cells are functionally divided into helper T-cells, which are CD4⁺CD8⁻ ("T4"), and cytotoxic T-cells, which are CD8⁺CD4⁻ ("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_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_{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

T8 cells **PBMC** Q2 0.252 10 Q1 46.0% 10 PBMC.0001 T8.003 10 10 FL2-H::CD4 PE 10 10 10 10 100 10 Tcm cells Tcm cells T4 cells Q1 65.3% 10 Q1 14.0% Q2 77.7% 10 Q2 21.0% 104 Q1 2.15% Q2 90 6 T4.002 Tcm.004 Tem.005 103 10 10 FL2-H::CD4 PE FL2-H::CD4 PE 102 10 10 -CD4 10 CD4 Q3 10 0.095% CD3

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

ProMab Biotechnologies, Inc. | 2600 Hilltop Drive, Building B, Suite C320, Richmond, CA 94806 USA Toll free 1-866-339-0871 | 1-510-860-4615 (Sales) | 1-510-860-4614 (Technical Support) | Fax 1-510-740-3625 | info@ProMab.com | www.ProMab.com



NON-TRANSDUCED CD4⁺ T_{EM} CELLS Engineered Target Cell Lines

Data



T4 cells **Tcm cells** Tcm cells 10⁴ Q1 6.52% T4.002 Q2 52.9% 104 Q1 63.7% Q2 20.3% 10 Q2 91.4% Tcm.007 Q1 8.17% Tem.008 FL1-H::CD45RO FITC FL1-H::CD45RO FITC 10³ 10³ 10 memory T cells 91.0% memory T cells 51% memory T cells 19.9% CD45RO 102 10 10 10 10 10 Q3 0.128% Q3 0.3959 100 10º 10º 10 10 104 104 10 T4.006 Tcm.007 Tem.008 10³ FL2-H::CCR7 PE 103 FL2-H::CCR7 PE 10 CCR7+ 24.0% CCR7 40.7% CCR7+ 80.5% 10² 102 10² CCR7 101 10 10¹ 100 100 10° 10 10 10 10 10 100 101 10² 103 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⁺, non-T cells are CD3⁻, and monocytes are CD3⁻CD4low. Then, the T4, $\rm T_{cm}$ and $\rm T_{em}$ subsets were stained with antibodies against CD3, CD45RO and CCR7 (lower panel).