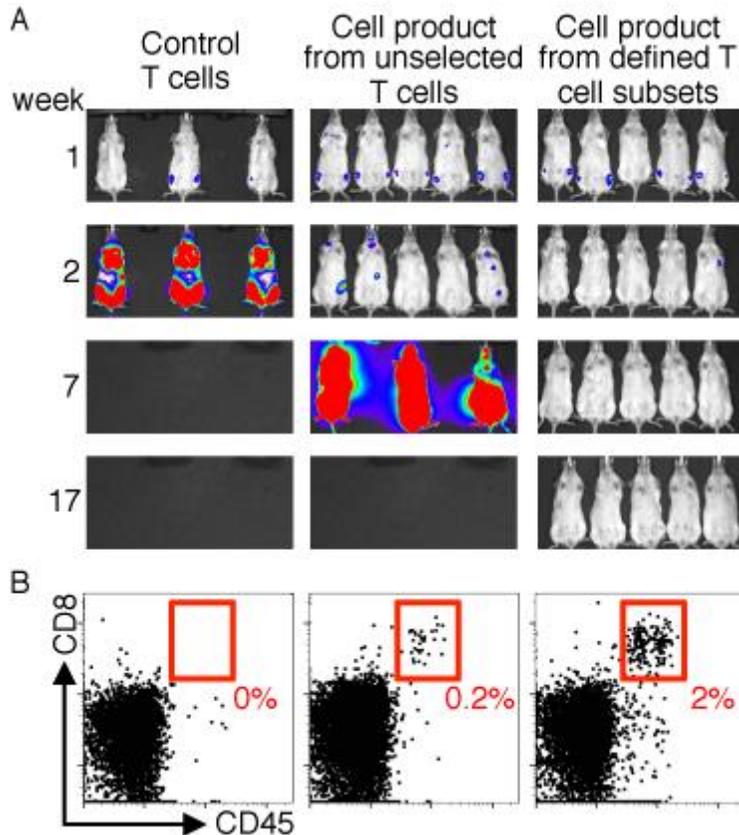# 4+8=0: Synergy from defined cell populations in CAR-T-cell therapy

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T cell products derived from defined patients' T cell subsets have enhanced antitumor potency. (A) Bioluminescence imaging of tumor growth in mice that had received 1 million control T cells or CD19 CAR-T cells prepared from either unselected cells or from defined T cell subsets (CD4 naïve/CD8 central memory). (B) Frequency of transferred CD8+ T cells (human CD45+/CD8+) in peripheral blood of mice one week after T cell transfer. Flow cytometry data from mice with median T cell frequency are shown for each group.

Image provided by Dr. Daniel Sommermeyer

One of the difficulties in treating cancer is that tumors arise from a patient's own cells, which allows them to evade the human immune system more easily than infections by bacteria or viruses. However, there are few therapies that perform better than the natural human immune system, because of its long-term protection, which lasts years to decades. To this end, researches are hotly pursuing cancer therapies that enable patient immune cells to recognize cancer cells and elicit antitumor activities. One such approach is through T cells modified with a chimeric antigen receptor (CAR) that can recognize and target cancer cells with high specificity. One half of this fusion protein is the cytosolic domain of CD3ζ, which is the activating part of the T-cell receptor complex. The appropriate antigen-binding domain must be chosen to ensure the immune response against healthy tissue is minimal. In currently successful CAR-T-cell therapy CD3ζ is fused to the binding domain of

an antibody that targets CD19. CD19 is expressed solely in healthy and malignant B cells and thus can target cancers while minimally damaging healthy hematopoietic stem cells and other healthy tissue. The DNA coding for this CAR is introduced into the genome of T cells isolated from patients or donors, and these CAR-T cells are then transferred into leukemic patients, with great success. Research efforts thus far have focused on optimizing CARs for increased potency; however, in a recent publication in *Leukemia*, Dr. Sommermeyer and his colleagues in the Riddell Laboratory (Clinical Research Division) instead focused on the properties of T cells that were being genetically modified.

Historically T cells have been isolated from patients and donors by their unique expression of CD3, resulting in an extremely diverse population of T cells. Early in the differentiation process, T cells express both CD4 and CD8, however their expression becomes mutually exclusive during differentiation. Within both CD4 and CD8 lineages, there also exist naïve, effector, and memory T cells, which contribute uniquely to antitumor activity. Thus under current CAR-T-cell therapy, every patient has been treated with a different population of T cells. Researchers verified this by measuring the ratios of CD4 and CD8 T cells in patients and donors and found patients who had previously received cytotoxic chemotherapy were depleted of CD4 T cells. Moreover, there was a general decrease in the number of naïve T cells of either CD4 or CD8 lineages. In general, the populations varied dramatically among individuals, emphasizing the heterogeneity of CAR-T-cell therapy.

To understand the contribution of each cell type towards antitumor activity, researches measured lysis of cancer cells and production of inflammatory cytokines. They observed that CD8 CAR-T cells performed robustly at lysing tumor cells, while CD4 CAR-T cells dramatically outperformed at cytokine production. Activity of CD4 CAR-T cells was further characterized by isolating three subtypes: naïve (4N) and two classes of memory cells, central (4CM) and effector memory (4EM). Using the same tests as above, as well as measuring survival in mouse leukemia models, the researchers found that 4N and 4CM cells had the most potent antitumor activity. The same panel of tests was performed on CD8 naïve (8N), central memory (8CM), and effector memory (8EM) T cells. CD8 CAR-T cells generally outperformed CD4 CAR-T cells, and among the subtypes, 8CM were more potent in vivo. Finally, combination of 8CM with each CD4 subtype was tested. 8CM in combination with either 4N or 4CM (1:1) demonstrated synergistic antitumor activity, whereby the 3 times fewer mixed cells achieved the same antitumor activity of either population alone. When testing the persistence of CAR-T cells in the blood stream, significantly more CAR-T cells were detected when the 8CM+4N mix was used for treatment. These findings were then directly compared to the current clinical protocols using mouse models, and demonstrated that an undefined

CAR-T-cell population extended lifespan 2-3 times, yet a mix of 8CM+4N resulted in full remission in all mice.

The success of this work isn't simply the increased antitumor activity, "This work demonstrates that certain T cell subsets are superior for adoptive therapy and that formulating optimal compositions can ensure uniform efficacy and dramatically reduce the cell doses needed to achieve tumor eradication, which improves the feasibility and lowers the cost of therapy. We are already using this approach in a clinical trial at Fred Hutch, which demonstrates that lower cell doses can also reduce toxicity", said Dr. Riddell. This exciting research helps remind us cancer therapeutics are in a new frontier beyond simply remissions, but onto reducing toxicity and cost of care.

Sommermeyer D, Hudecek M, Kosasih PL, Gogishvili T, Maloney DG, Turtle CJ, Riddell SR. 2015. Chimeric antigen receptor-modifed T cells derived from defined CD8$^+$ and CD4$^+$. *Leukemia.* doi: 10.1038/leu.2015.247. [Epub ahead of print.]