395 ENGINEERING NON-ACTIVATED CAR T CELLS WITH ENHANCED POTENCY AGAINST ADVANCED CANCERS

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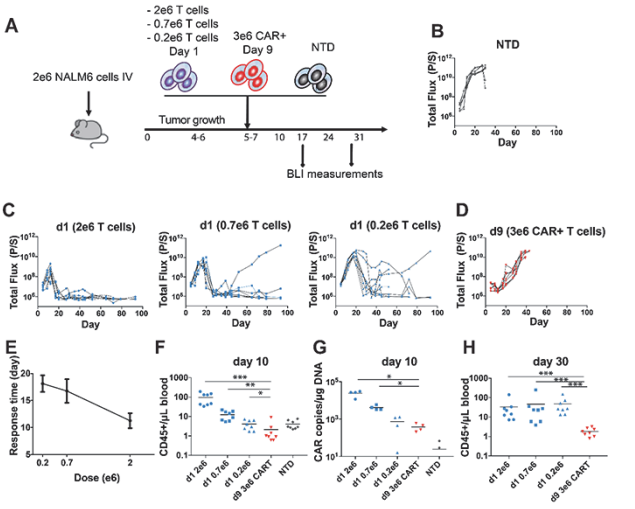
Background Chimeric antigen receptor (CAR) T cells can generate durable clinical responses in B-cell hematologic malignancies. The effectiveness of this approach led to several FDA-approved CAR T cell therapies against cancer. Despite their therapeutic promise in blood-based malignancies, CAR-T cells do not effectively kill solid tumors. Several factors contribute to success including tumor entry, CAR engagement, T cell expansion, and persistence. In several solid tumor models, CAR T cells can enter solid tumors and undergo antigen-specific stimulation. "Failure" coincides with a diminished ability to proliferative and persist.

Methods CAR T cell manufacturing is an iterative process with several important steps. Initially, T cells are activated through their T cell receptor, followed by lentiviral transduction and expansion ex vivo for several weeks. Unfortunately, activating and expanding CAR T cells in this manner drives differentiation with a corresponding loss of anti-tumor activity. Here we provide evidence that functional CAR T cells can be generated within 24 hours without any need for T cell activation or ex vivo expansion. We show that the efficiency of viral transduction in this process is substantially influenced by the formulation of the medium and the surface area-to-volume ratio of the culture vessel.

Results Remarkably, these cells exhibited higher anti-leukemic in vivo activity per cell than standard CAR T cells (activated and expanded for 9 days prior to adoptive transfer) in preclinical models of human leukemias (figure 1). Our preliminary findings position the non-activated system as a superior alternative against solid tumors where terminally differentiated T cells are so often ineffective. Our findings imply that quiescent T cells retain key attributes that influence engraftment and survival following adoptive transfer.

Conclusions In summary, the ability to generate genetically-modified T cells with superior therapeutic potential in a shorter time period has important implications for CAR-T manufacturing and therapy. Our results indicate that extended ex vivo culture is unnecessarily costly and redundant. Leveraging the unique ability of lentiviral vectors to enter and integrate into the genome of non-dividing cells, effective CAR-T cell products with durable engraftment in vivo can be generated in as little as one day. Minimizing ex vivo manipulation also conserves limited resources such as human serum and manufacturing space as expansion occurs entirely in vivo. Finally, more rapid product generation will lead to a shorter period of time between apheresis collection and re-infusion of CAR T cells. This will be of particular benefit to those patients with rapidly progressive disease.



Abstract 395 Figure 1 Non-activated CAR T cells induce durable remission
(A) Schematic of the xenograft model and CART19 cell treatment in NSG mice. (B-D) serial quantification of disease burden by bioluminescence imaging. (B) Total bioluminescence flux in mice treated with non-transduced (NTD) control non-activated T cells. (C) Total bioluminescence flux in mice treated with a single high (2x106), medium (0.7x106) or low (0.2x106) dose of non-activated T cells. (D) Total bioluminescence flux in mice treated with 3x106 CAR+ T cells stimulated with anti-CD3/CD28 microbeads and expanded over 9 days. There are 8 mice in each group. (E) Time to initial anti-leukemic response (i.e. first reduction in bioluminescence) after infusion of non-activated CART19 in relationship to T cell dose. (F) Absolute peripheral blood CD45+ T cell counts in blood collected from mice at day 10 following T cell transfer. (G) Vector copy number in peripheral blood collected at day 10 measured by qPCR and normalized to DNA concentration. H) Absolute peripheral blood CD45+ T cell counts in blood collected from mice on day 30 following T cell transfer. *P< 0.05, **P< 0.01 and ***P< 0.001. The mean of each group is indicated by the solid black line. Groups were compared using the two-tailed, unpaired Mann–Whitney test.

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