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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Engineering Off-the-Shelf Gamma Delta CAR T Cells for the Treatment of Acute Myeloid Leukemia

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Chimeric antigen receptor T cell therapy (CART) therapy has shown remarkable success in the treatment of B cell acute lymphoblastic leukemias (B-ALL) and lymphomas. However, CART therapies for acute myeloid leukemia (AML), where 5-year survival rates are significantly lower than for B-ALL, are only in their infancy. CD33-CART have potent activity against AML in preclinical models and a first-in-child/first-in-human phase 1/2 CD33-CART clinical trial for AML is ongoing in the Pediatric Oncology Branch of the National Cancer Institute (NCT03971799). Nonetheless, published outcomes suggest a modest efficacy of approximately 50% (Shahzad et al., *Front Immunol* 2023), highlighting the critical need to develop new strategies to improve CART accessibility and a more robust anti-AML response. We hypothesized that off-the-shelf gamma delta ($\gamma\delta$) CD33 CART cells could potentially overcome current challenges for the treatment of AML. $\gamma\delta$ lineage T cells are unconventional lymphocytes whose functions are not restricted to MHC-mediated antigen presentation; they are primed for immediate responses, including tumor killing. Furthermore, allogeneic$\gamma\delta$ T cells have the potential to induce robust anti-tumor cytotoxicity without causing graft versus host disease (GVHD).
Here, we generated $\gamma\delta$ CAR T cells from healthy donor elutriated lymphocytes by activation with zoledronic acid and IL-2 for 7-14 days. Within 9 days post stimulation, the vast majority of lymphocytes were Vδ2+ and 30-40% were successfully transduced with a lentiviral CD33 CAR construct harboring the 4-1BB costimulatory domain. Importantly, and unlike conventional alpha beta (ab) T lymphocytes, >98% of these $\gamma\delta$ CD33CAR T cells expressed IFNγ under basal conditions. This characteristic likely accounted for the efficient *in vitro* killing of AML cell lines by untransduced $\gamma\delta$ T lymphocytes under conditions of high effector/target (E/T) ratios. While untransduced $\gamma\delta$ T cells did not exhibit cytotoxicity following repeat AML stimulations, $\gamma\delta$ CD33CAR T lymphocytes exhibited proficient *in vitro* cytotoxicity, with killing rates that were more rapid than those initiated by ab CD33 CART (Figure 1). These characteristics were associated with a prolonged metabolic activity of $\gamma\delta$T cells; $\gamma\delta$ CD33 CART expressed high levels of the GLUT1 glucose transporter for >14 days post activation whereas GLUT1 levels on ab CD33 CART returned to resting within 10 days. High GLUT1 levels were linked to efficient killing under conditions of basal glucose levels. Most notably, $\gamma\delta$ CD33CAR T lymphocytes achieved high *in vivo* cytotoxicity, assessed using bioluminescent AML cell line xenografts in humanized NSG mice. Together, these data highlight the feasibility of generating allogeneic $\gamma\delta$ CD33CART with a strong anti-AML cytotoxic response.

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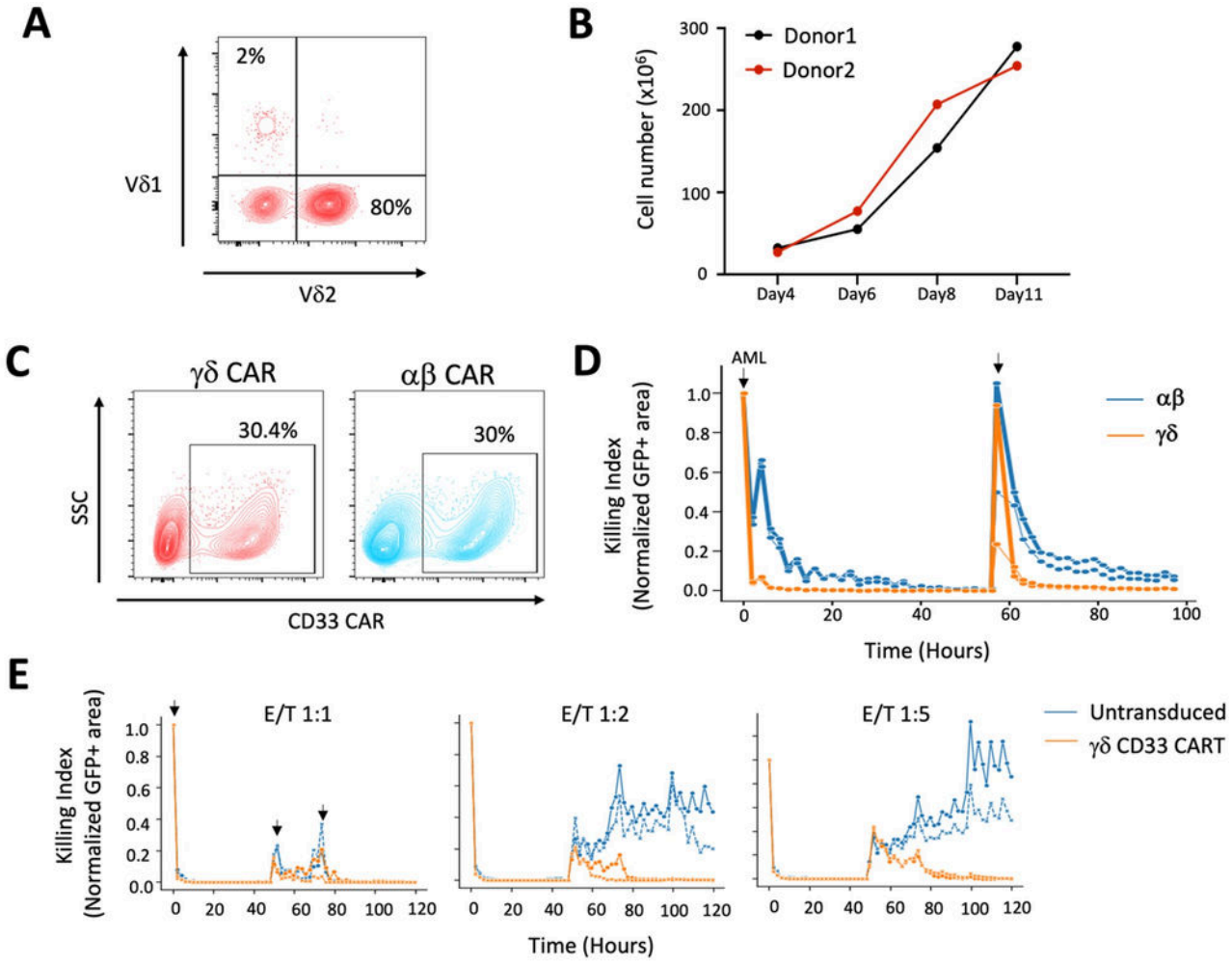

Figure 1: γδ CD33 CART exhibit more rapid killing of AML than αβ CD33 CART. (A) Representative flow cytometry profile showing the differentiation of Vδ2 γδ T cells following zoledronic acid activation of healthy donor lymphocytes. (B) Expansion profiles of γδ T cells from two representative donors following depletion of αβ T cells at day 4. (C) Representative flow cytometry profiles showing surface CD33 CAR expression on γδ and αβ T cells following lentiviral transduction. (D) Cytotoxicity assay (Incucyte) showing the killing index of γδ CD33 CART as compared to αβ CD33 CART against GFP+ MOLM-14 AML cells at an effector/target (E/T) ratio of 1:5. (E) Cytotoxicity assay comparing the reactivity of untransduced and CD33 CAR γδ T cells against GFP+ MOLM-14 AML at the indicated E/T ratios. Restimulation with MOLM-14 are indicated by arrows.

Figure 1

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