Evaluation of non-gene edited allogeneic "off-the-shelf" Vδ1 γδ CAR T cells targeting CD20 for B cell malignancies

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BACKGROUND

High clinical response rates have been observed with αβ CAR T therapies, but opportunities for improvement remain[1]. Off-the-shelf, allogeneic CAR T cell immunotherapies offer potential for immediately accessible cell therapies for patients. Strategies for investigating alternative cytotoxic effector cells with intrinsic tumoricidal activity, like γδ T cells, may improve depth and breadth of CAR T responses. Tumor targeting by allogeneic γδ CAR T therapy is complemented by innate and adaptive mechanisms[2]. ADI-001 is an allogeneic CD20-targeted γδ CAR T cell therapy currently being evaluated in patients with B cell lymphomas (NCT04735471)[3].

OBJECTIVE

To characterize the function and phenotype of ADI-001, a non-gene edited allogeneic Vδ1 γδ CAR T cell therapy targeting CD20, designed for the treatment of patients with B cell lymphomas.

Manufacturing of allogeneic CD20 CAR Vδ1 γδ T cells

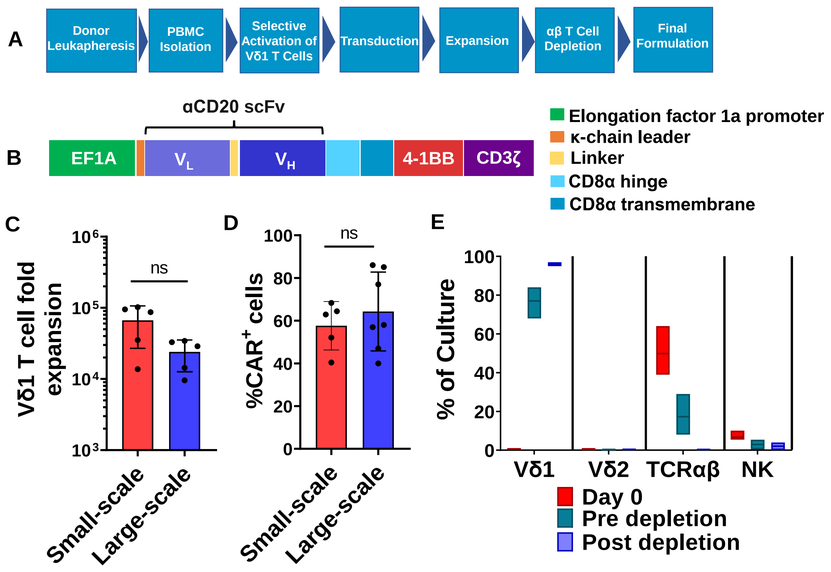

Figure 1. Selective activation and expansion of Vδ1 γδ T cells using agonistic mAb from healthy donor-derived PBMCs. **(A)** Flow chart highlighting the key steps in the manufacturing of allogeneic CD20 CAR+ Vδ1 γδ T cells. **(B)** Schematic diagram of the second generation CD20 CAR. **(C)** CD20 CAR+ Vδ1 γδ T cell manufacturing process resulted in a substantial fold-expansion of Vδ1 γδ T cells. **(D)** Average percentage of Vδ1 γδ T cells expressing the CD20 CAR from manufacturing runs as measured by flow cytometry. **(E)** % Cell composition throughout expansion of CD20 CAR+ Vδ1 T cell products derived from 4 different donors analyzed by flow cytometry. Paired t-test was used to assess statistical significance.

Predominant naïve-like T cell memory phenotype, NKR and chemokine receptor expression in CD20 CAR Vδ1 γδ T cells

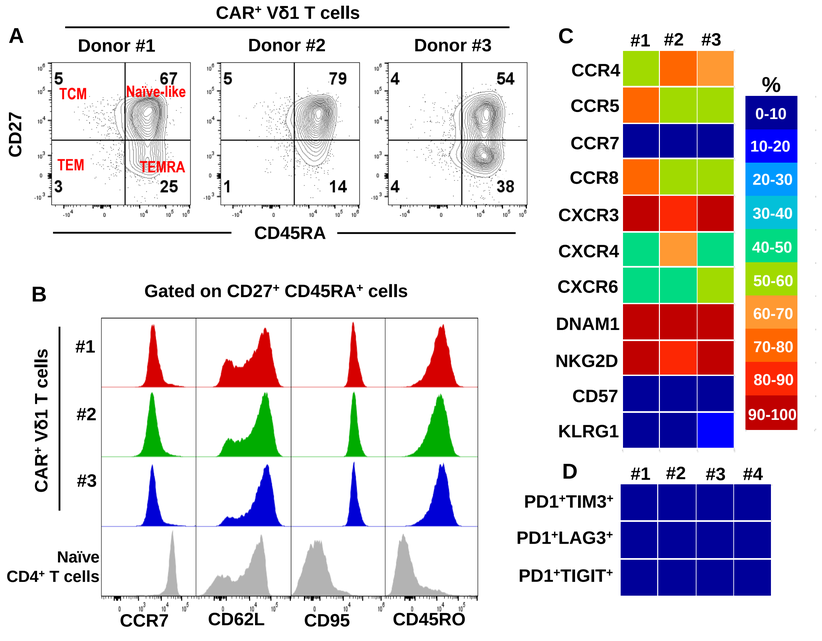

Figure 2. (A and B) Majority of CAR+ Vδ1 γδ T cells exhibited a naïve-like T cell memory phenotype assessed by flow cytometry. **(C)** Heatmap showing percentages of CAR+ Vδ1 γδ T cells in CD20 CAR+ Vδ1 γδ T cell products that express multiple chemokine receptors, natural killer (NK) cell receptors, and terminal differentiation markers. **(D)** Heatmap showing percentages of CAR+ Vδ1 γδ T cells in CD20 CAR+ Vδ1 γδ T cell products that co-express PD-1 and another co-inhibitory receptor.

CD20 CAR Vδ1 γδ T cells exhibit potent cytotoxicity and proliferation against B cell lymphoma cell lines

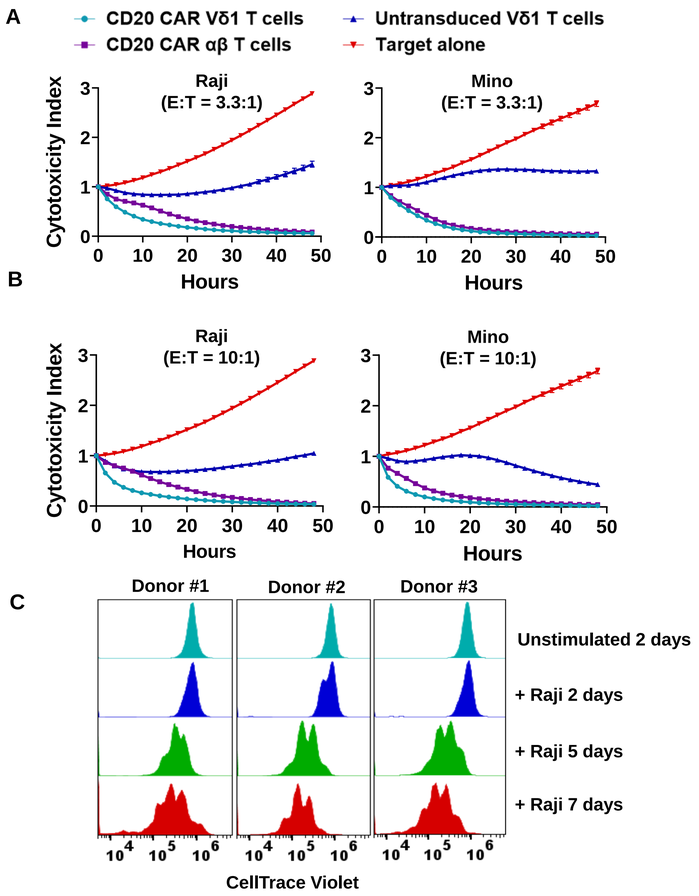

Figure 3. (A and B) Cytotoxic potentials of untransduced Vδ1 γδ T cells, CD20 CAR-transduced Vδ1 γδ T cells (71.5% CAR+), and CD20 CAR-transduced αβ T cells (68% CAR+), all derived from the same healthy donor, were evaluated against CD20+ target cells in a 48-hour Incucyte Immune Cell Killing Assay, in which T cells were co-cultured with NucR-expressing Raji or Mino target cells at E:T ratios of 3.3:1 and 10:1. **(C)** Proliferative potential of CD20 CAR+ Vδ1 γδ T cells following three rounds of target antigen exposure in a 7-day culture period.

CD20 CAR Vδ1 γδ T cells significantly inhibit *in vivo* tumor growth in B cell lymphoma xenografts

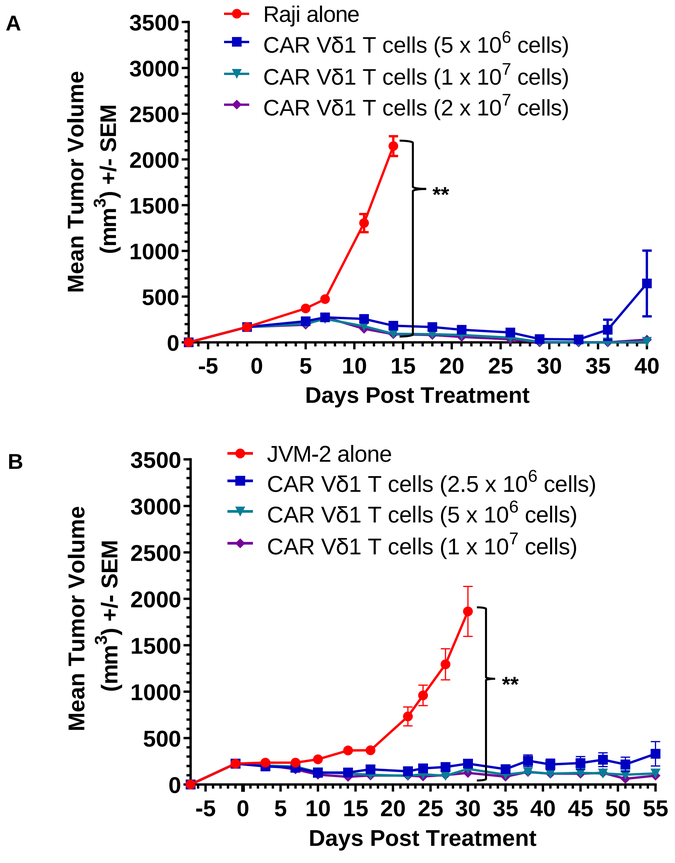

Figure 4. *In vivo* efficacy of three different doses of viable CD20 CAR+ Vδ1 γδ T cells in combination with 13,000 IU IL-2 in **(A)** SC Raji Burkitt Lymphoma and **(B)** SC JVM-2 Mantle Cell Lymphoma model in NSG mice (n = 5 per group). Kruskal-Wallis test was used to assess statistical significance among the groups (**P*-value < 0.01).

Innate and adaptive immune contributions for Vδ1 γδ CAR T cell targeting of B cell lymphomas

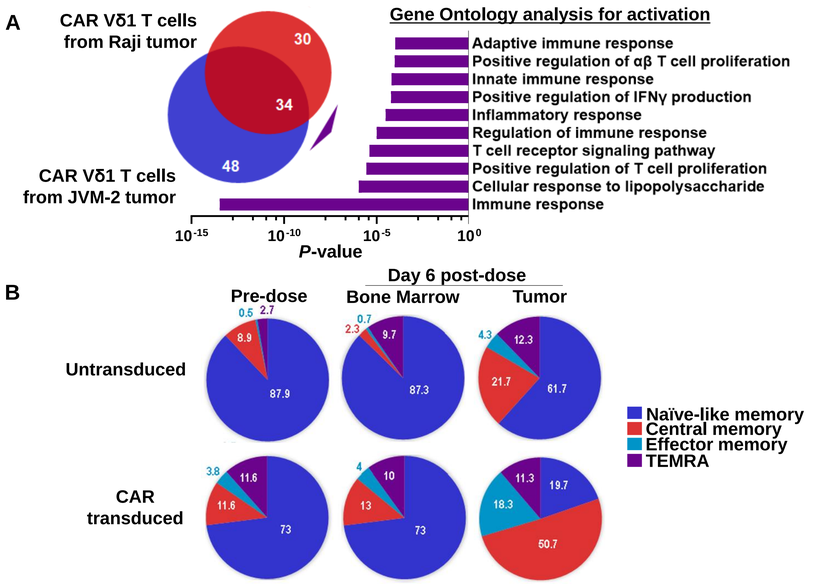

Figure 5. (A) Venn diagrams of differentially expressed genes (DEGs) from Raji (red circle) and JVM-2 (blue circle) tumors treated with CD20 CAR+ Vδ1 γδ T cells (day 6 post-dose). The overlapping area represents DEGs that are shared between the two tumor models. Gene expression were quantitated using the Nanostring nCounter® CAR T Characterization and Human Immunology Panels. Gene Ontology analysis was performed using Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/) to identify the biological pathways associated with DEGs that are unique to each tumor model and that are shared by the two tumor models **(B)** T cell memory phenotype analysis of untransduced and CD20 CAR+ Vδ1 γδ T cells in bone marrow and tumor harvested from Raji tumor-bearing mice on day 6 post-treatment.

Non-edited Vδ1 γδ CAR T cells show decreased HvG potential compared to gene-edited platform variants

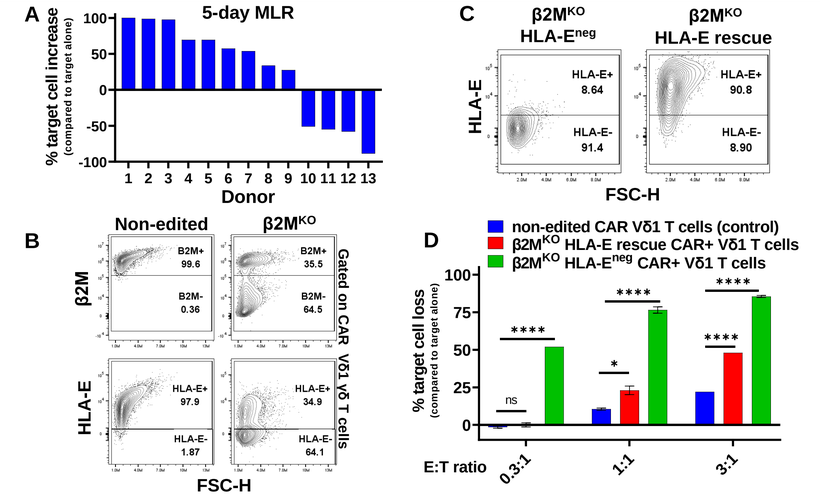

Figure 6. (A) Assessment of allo-susceptibility using enriched primary lymphocytes from allogeneic PBMC donors (responders) against non-edited CD20 CAR Vδ1 γδ T cells (targets) in a 5-day MLR. **(B)** Generation of β2M KO CAR+ Vδ1 γδ T cells was assessed using flow cytometry. **(C)** β2M KO with single-chain HLA-E rescue expression was assessed using flow cytometry after cell sorting. **(D)** NK cell allo-susceptibility against non-gene edited CD20 CAR+ Vδ1 γδ T cells was compared to β2M KO with and without single chain HLA-E rescued CD20 CAR+ Vδ1 γδ T cells. Enriched, activated, and expanded allogeneic NK cells (responders) were co-cultured with CFSE-labeled CD20 CAR+ Vδ1 γδ T cells (targets) for 18-hrs and cell killing was assessed using flow cytometry.
(****P*-value < 0.001, **P*-value < 0.05, NS = not significant).

CONCLUSIONS

- PBMC-derived Vδ1 γδ T cells were successfully activated, expanded, and genetically engineered using established manufacturing processes.
- ADI-001 demonstrated a predominantly naïve-like T cell memory phenotype and expressed multiple chemokine and natural killer cell receptors.
- ADI-001 exhibited robust *in vitro* and *in vivo* tumor growth inhibition in multiple human lymphoma cell lines.
- Adaptive and innate mechanisms contribute to the anti-tumor activity of ADI-001
- Vδ1 γδ CAR T cells may be relatively resilient to host vs graft rejection when compared to gene-edited approaches (β2MKO with or without HLA-E overexpression), based on mixed lymphocyte reactions with mismatched allogeneic PBMC and NK donor lymphocytes.
- These findings demonstrate preclinical proof-of-concept for ADI-001 as an allogeneic CAR-T therapy. A phase 1 trial using ADI-001 to treat R/R B cell NHL patients is currently under investigation (NCT04735471)[3].

References

1. McCreedy BJ, Senyukov VV, Nguyen KT. Off the shelf T cell therapies for hematologic malignancies. *Best Pract Res Clin Haematol* 2018; **31**: 166-175.
2. Bonneville M, O'Brien RL, Born WK. γδ T cell effector functions: a blend of innate programming and acquired plasticity. *Nat Rev Immunol* 2010; **10**: 467-478.
3. A Study of ADI-001 in B Cell Malignancies (GLEAN-1). *https://www.clinicaltrials.gov/ct2/show/NCT04735471*