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. 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 intrinsically tumoricidal activity, like γδ T cells, may improve depth and breadth of CAR T responses. Targeting by alloγδ Vγδ CAR therapy is complemented by innate and adaptive mechanisms. ADI-001 is an allogeneic CD20-targeted γδ CAR T cell therapy currently being evaluated in patients with B cell lymphomas (NCT04735471).

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 CD20 CAR γδ T cells expressing the CD200 CAR from manufacturing runs as measured by flow cytometry. (E) % Cell composition throughout expansion of CD20 CAR- Vδ1 γδ T cell products derived from 4 different donors analyzed by flow cytometry. Paired t-Test was used to assess statistical significance.

Predominant naive-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 naive-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 cells 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 potential of unexpanded Vδ1 γδ T cells, CD20 CAR-transduced Vδ1 γδ T cells (71.5% CAR+), and CD20 CAR-transduced αβ T cells (68% CAR+) were confirmed to be effective against Raji and Mino target cells. PA1-γδ T cells co-cultured with NucR-expressing Raji or Mino target cells at E:T ratios of 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.

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

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 < 0.001).

CONCLUSIONS
- PBMC-derived Vδ1 γδ T cells were successfully activated, expanded, and genetically engineered using established manufacturing processes.
- ADI-001 demonstrated a predominantly naive-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 (β2M+ or without HLA-E overexpression), based on mixed lymphocyte reactions with mismatched alloγδ 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).

References