ADI-002: an IL-15 armored allogeneic "off-the-shelf" Vδ1 gamma delta CAR T cell therapy for solid tumors targeting glypican-3 (GPC-3)

Amani Makkouk, Xue (Cher) Yang, Taylor Barca, Anthony Lucas, Mustafa Turkoz, Jonathan T.S. Wong, Kevin P. Nishimoto, Mary M. Brodey, Maryam Tabrizizad, Smitha R.Y. Gundurao, Lu Bai, Arun Bhat, Zili An, Stewart Abbot, Daulet Satpayev, Marissa Herrman

Adicet Bio, Menlo Park, CA 94025 US

Background

Glypican-3 (GPC-3) is an oncofetal protein that is highly expressed in various solid tumors, including hepatocellular carcinoma (HCC), but is rarely expressed in healthy adult tissues and serves as a therapeutic target of interest. Chimeric antigen receptor (CAR) T cell therapy has established clinical benefit in hematologic malignancies but, to date, limited efficacy in solid tumors.[1]

Gamma delta (γδ) T cells are highly cytolytic lymphocytes that can recognize and kill tumor cells in an MHC-unrestricted manner without causing graft vs host disease (GvHD).[2] The Vδ1 γδ T cell subset preferentially localizes in peripheral tissues and is critical for tumor immunosurveillance.[3]

Engineering Vδ1 T cells with CARs can further enhance antitumor activity and represents an attractive and potentially safe approach to treating solid tumors. Here, we describe the first preclinical evaluation of ADI-002, a next-generation allogeneic CAR Vδ1 T cell therapy targeting GPC-3 and armored with soluble IL-15 (sIL-15), for the treatment of solid tumors. Our results show that expanded Vδ1 T cells engineered with GPC-3.CAR and sIL-15 represent a promising platform for safe and effective off-the-shelf treatment of HCC and support further investigation in the clinical setting.

GPC-3.CAR Vδ1 T cells co-expressing sIL-15 show robust expansion from healthy donor PBMCs

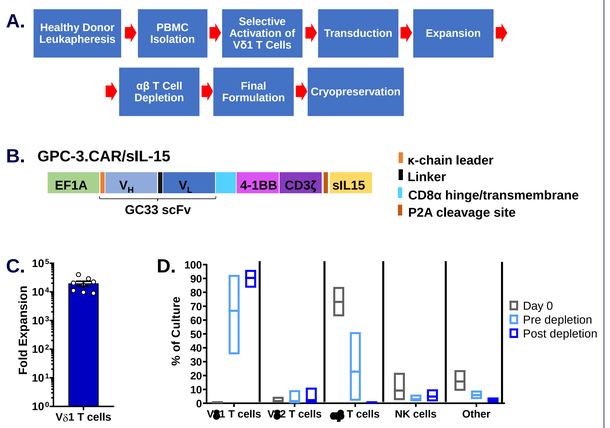

Figure 1 (A) Schematic representing the process for manufacturing 'off-the shelf' allogeneic GPC-3.CAR/sIL-15 Vδ1 T cells. **(B)** Schematic diagram of the GPC-3.CAR/sIL-15 construct. **(C)** Ex vivo culture of GPC-3.CAR/sIL-15 Vδ1 T cell results in a substantial fold-expansion of Vδ1 T cells. The mean ± SEM of 5 expansions using PBMCs from 8 different donors are shown. **(D)** Cellular composition over time of 9 lots of GPC-3.CAR/sIL-15 Vδ1 T cell products using PBMCs from 9 donors was analyzed by flow cytometry. The vast majority (~90% on average) of cells in the final product are Vδ1 T cells.

GPC-3.CAR/sIL-15 Vδ1 T cells display a less differentiated naïve-like phenotype

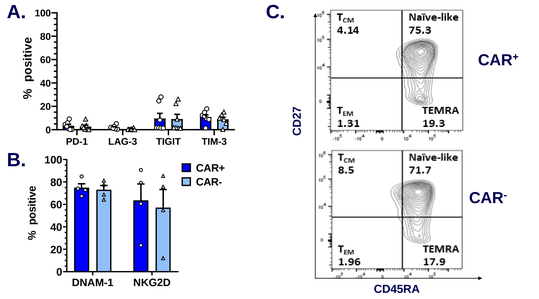

Figure 2 (A,B) Flow cytometric expression levels of exhaustion markers (A) and innate receptors (B). Data shown as mean ± SEM of 4-7 banks using PBMCs from 3 donors. **(C)** Representative flow plots showing memory cell subsets in CAR+ and CAR- GPC-3.CAR/sIL-15 Vδ1 T cells, which primarily exhibit a naïve-like or less differentiated T cell memory phenotype.

GPC-3.CAR/sIL-15 Vδ1 T cells exert robust antitumor activity even in the presence of soluble GPC-3

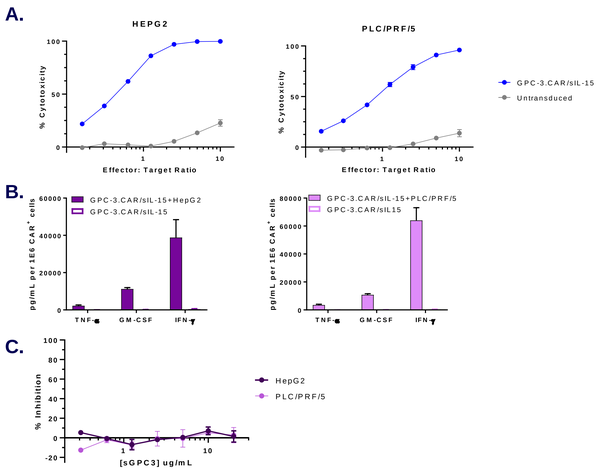

Figure 3 (A) Untransduced or GPC-3.CAR/sIL-15 Vδ1 T cells were co-cultured with luciferase-expressing GPC-3^{hi} (HepG2) (*left*) or GPC-3^{lo} (PLC/PRF/5) (*right*) cells across varying E:T ratios (1:6–10:1). Data shown as mean ± SEM of triplicates and represent two banks. **(B)** Cytokine production by GPC-3.CAR/sIL-15 Vδ1 T cells after co-culture with HepG2 cells *(left)* or PLC/PRF/5 cells *(right)* (2:1 E:T) . Data shown as mean ± SEM of triplicates and represent 2 banks. **(C)** Cytotoxic potential of GPC-3.CAR/sIL-15 Vδ1 T cells against HepG2 and PLC/PRF/5 cells (1:1 E:T) was assessed in the presence of soluble GPC-3 (0.3 ug/mL-20 ug/mL) in an 18-hr cytotoxicity assay. Data shown as mean ± SEM of triplicates and represent 2 banks.

Co-expression of sIL-15 sustains GPC-3.CAR Vδ1 T cell proliferation

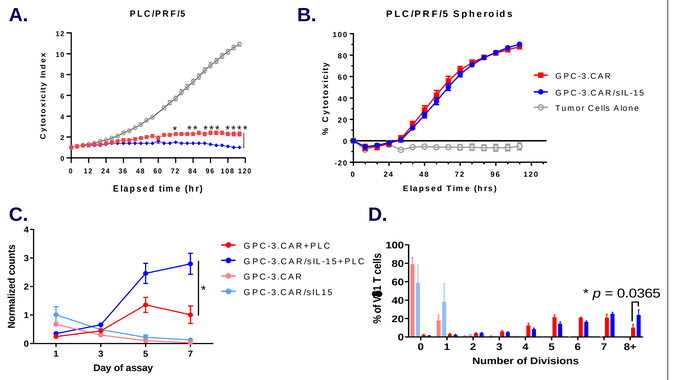

Figure 4 (**A**) Cytotoxic potential of GPC-3.CAR and GPC-3.CAR/sIL-15 Vδ1 T cells against PLC/PRF/5 cells. 2.5:1 E:T in a 120-hr Incucyte cytotoxicity assay. Cytotoxicity Index was calculated by dividing the total red object area (mm^2/well) of all time points by the value at time = 0. Data shown as mean ± standard deviation of triplicates and represent 2 banks each for GPC-3.CAR and GPC-3.CAR/sIL-15 Vδ1 T cells. (**B**) Cytotoxic potential of GPC-3.CAR and GPC-3.CAR/sIL-15 Vδ1 T cells against PLC/PRF/5 spheroids (2:1 E:T) in a 120-hr Incucyte cytotoxicity assay. (**C,D**) Proliferative potential assessed on days 1, 3, 5, and 7 from (**B**). For **B-D**, data shown as mean ± SEM of at least 2 replicates and represent 3 GPC-3.CAR and 2 GPC-3.CAR/sIL-15 Vδ1 T cell banks, respectively. *P* values were calculated using one-way ANOVA with Tukey post hoc (**A** and **C**) or two-tailed unpaired Student's t-test (**D**). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

GPC-3.CAR/sIL-15 Vδ1 T cells maintain a primarily naïve-like phenotype and proliferate exclusively in tumor *in vivo*

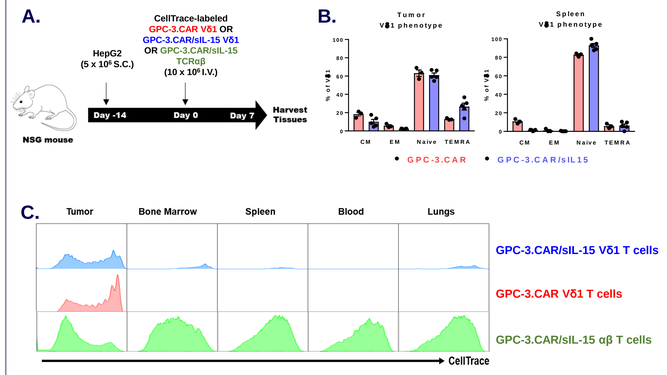

Figure 5 (A) Schematic for *ex vivo* analysis of Vδ1 and TCRαβ T cells in HepG2 tumor-bearing NSG mice. (**B**) Differentiation potential of GPC-3.CAR and GPC-3.CAR/sIL-15 Vδ1 T cells in the spleen and tumor. Data shown as mean ± SEM for 3-5 mice/group. **(C)** Proliferative potential of GPC-3.CAR and GPC-3.CAR/sIL-15 Vδ1 and TCRαβ T cells in tumor, bone marrow, spleen, blood, and lungs.

GPC-3.CAR/sIL-15 Vδ1 T cells mediate superior *in vivo* antitumor activity compared to GPC-3.CAR Vδ1 T cells

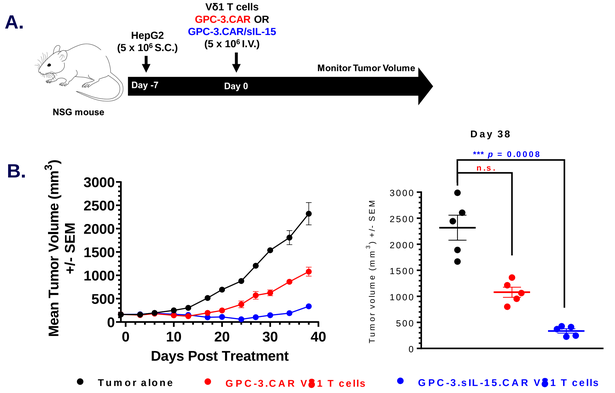

Figure 6 *In vivo* efficacy of a single dose of CAR Vδ1 T cells in HepG2 tumor-bearing NSG mice. **(A)** Study schematic. **(B)** Tumor growth kinetics (*left*) and tumor volumes on Day 38 (*right*). Data shown as mean ± SEM for 5 mice/group. Kruskal-Wallis with Dunn's post hoc was used to assess statistical significance among the groups at the last time point when all control mice were alive. ***p<0.001, n.s. not significant. GPC-3.CAR/sIL-15 Vδ1 T cells were superior to GPC-3.CAR Vδ1 T cells at controlling tumor growth. No GvHD related pathological changes were observed by immunohistochemistry (data not shown).

Summary and Conclusions

- **ADI-002 is an off-the-shelf allogeneic γδ T cell therapy targeting GPC-3 via a chimeric antigen receptor and is armored with a secreted form of IL-15 (GPC-3.CAR/sIL-15) designed to enhance proliferation and potency against solid tumors.**
- **ADI-002 is manufactured using a γ-retrovirus, encoding the CAR and sIL-15, coupled to a core process largely similar the currently established clinical manufacture of ADI-001, a first-in-class CD20-targeted γδ CAR-T (Fig 1A, B).[4]**
- **ADI-002 demonstrates high purity of T cells expressing the Vδ1 γδ TCR and co-expressing NKG2D innate receptors, and demonstrates a predominantly naïve-like phenotype (Fig 1D, Fig 2B, C)**
- **ADI-002 demonstrates potent activation and cytotoxicity against GPC-3-expressing cancer cell lines that is sustained in the presence of soluble GPC-3 (Fig 3A-C).**
- **Armoring of ADI-002 with sIL-15 enhances target stimulated expansion and proliferation potential (Fig 4A,B)**
- **Overall, ADI-002 demonstrates enhanced potency both in vitro and in vivo while maintaining low alloreactive potential and represents a promising approach for the treatment of GPC-3-positive cancers (Fig 5 and 6)**

References

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4. www.clinicaltrials.gov NCT04735471