ADI-270: an armored allogeneic “off-the-shelf” CAR γδ T cell therapy targeting CD70+ cancers

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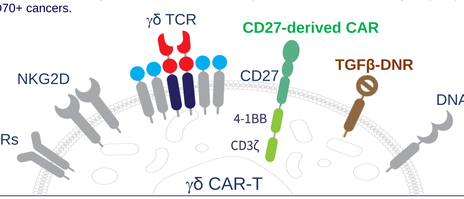
INTRODUCTION

CD70 represents a compelling target for the development of CAR T cell therapies due to its high expression in multiple solid and hematological malignancies; while in normal tissues it is transiently expressed by a subset of activated lymphocytes. CAR T efficacy in solid tumors has been a key challenge in the field; one emerging strategy to improve clinical responses is to employ alternative cytotoxic effector cells with multifunctional tumoricidal activity. γδ T cells combine innate and adaptive immunity to kill malignant cells, and their infiltration into various cancers, including those expressing CD70, significantly correlates with survival. Strategies targeting CD70 by engineering its natural receptor (CD27) as the antigen-recognition moiety of a CAR have demonstrated superior preclinical antitumor activity compared to scFv-based approaches. Additional armoring of CAR T cells to mitigate the immunosuppressive tumor microenvironment can further enhance activity. Here we report the manufacturability and functionality of ADI-270, an allogeneic γδ T cell product expressing a CD27 natural receptor third-generation CAR armored with a dominant negative TGFβRII that has the potential to reduce host vs graft (HvG) susceptibility, for targeting CD70+ cancers.



METHODS

Healthy donor PBMCs were used to activate, expand, and engineer Vδ1 T cells to express CD70 CAR. *In vitro* phenotype, cytokine profile, and antitumor functionality of ADI-270 were determined using flow cytometry, multiplex cytokine assays, and cell-based cytotoxicity assays against cell lines with varying expression levels of CD70. Human tumor xenograft models in immunodeficient mice were used to evaluate *in vivo* efficacy after a single dose of ADI-270. In addition, *ex vivo* analysis was performed to evaluate the proliferation and phenotype of ADI-270 in the tumor and non-tumor tissues.

Generation of ADI-270

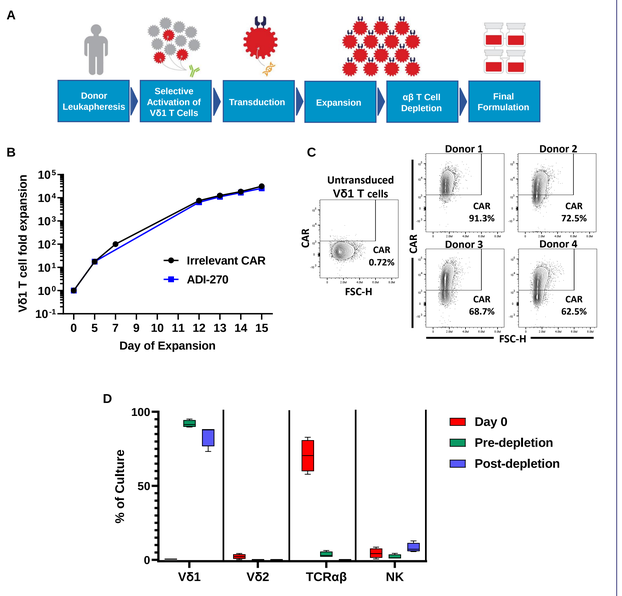


Figure 1. Selective activation and expansion of Vδ1 T cells from healthy donor-derived PBMCs using an agonistic mAb. **(A)** Flow chart highlighting the key steps in the generation of ADI-270. **(B)** The ADI-270 generation process results in a substantial fold-expansion of Vδ1 T cells with no effect of fratricide when compared to irrelevant CAR control during the expansion. **(C)** Contour plots displaying the transduction efficiency of ADI-270 derived from 4 different donors as measured by flow cytometry. **(D)** % cell composition throughout the expansion of ADI-270 derived from 4 different donors analyzed using flow cytometry.

ADI-270 expresses a less differentiated T cell memory phenotype with minimal activation/exhaustion-associated markers

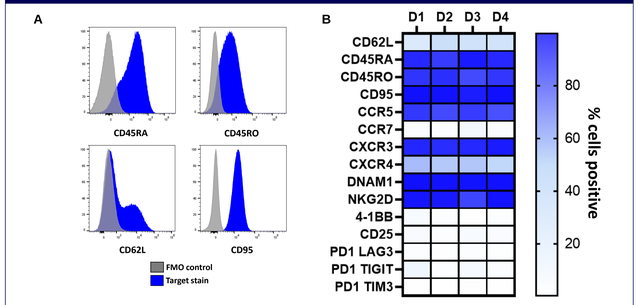


Figure 2. (A) ADI-270 exhibits a less differentiated T cell memory phenotype assessed by flow cytometry. **(B)** Heatmap showing percentages of various markers from ADI-270 (4 different donors) including T cell memory markers, multiple chemokine receptors, NKRs, activation markers, and the co-expression of PD1 and another co-inhibitory receptor.

ADI-270 is armored against immunosuppressive effects of TGF-β with a dominant-negative receptor (dnTGFβRII) “bolt-on”

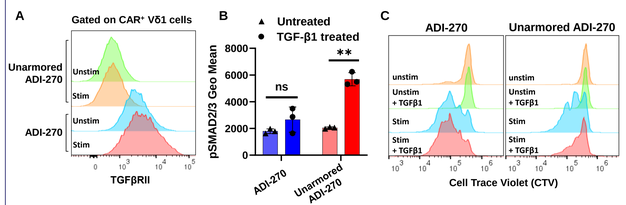


Figure 3. **(A)** Detection of TGFβRII surface expression in ADI-270 with or without “bolt-on” using flow cytometry. TGFβRII expression was evaluated pre- and post-stimulation with immobilized rhuCD70 (5μg/mL) after an 18-hr incubation. **(B)** ADI-270 with the “bolt-on” showed lower intracellular staining levels of pSMAD2/3 (Geo Mean) in the presence of TGF-β1 (20ng/mL) compared to unarmored ADI-270 without “bolt-on”, confirming the functionality of the dnTGFβRII. Statistical analysis was performed using paired t-test ** p<0.005. **(C)** CTV labeled ADI-270 and unarmored ADI-270 were stimulated with CD70+ A498 tumor cells for 7 days in the presence and absence of TGF-β1 (20ng/mL). Increased cell proliferation is indicated by the dilution of the CTV dye (histogram plots) compared to unstimulated controls. ADI-270 becomes resistant to the effects of TGF-β1 compared to unarmored ADI-270. The data is a representative of 3 different donors.

ADI-270 is armored against TGF-β1 mediated alterations to express an activation profile

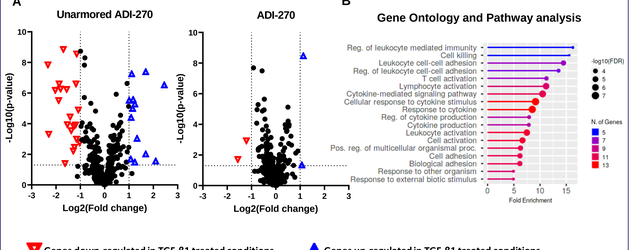


Figure 4. (A) Volcano plots of differentially expressed genes (DEGs) from ADI-270 vs unarmored ADI-270 activated with rhuCD70 (5μg/mL) in the presence or absence of TGF-β1 (20ng/mL) for 24 hrs from 2 different donors. Blue triangles represent DEGs upregulated in TGF-β1 treated vs untreated conditions. Red triangles represent DEGs downregulated in TGF-β1 treated vs untreated conditions. Gene expression were quantitated using the Nanostring nCounter® CAR T cell Characterization panel. **(B)** Gene Ontology analysis was performed using ShinyGO 0.76.1 (http://bioinformatics.sdstate.edu/go/) to identify the biological pathways associated with DEGs that were downregulated in the presence of TGF-β1 from unarmored ADI-270 cells without the dnTGFβRII “bolt-on”.

ADI-270 exhibits a favorable cytokine profile with proinflammatory and chemoattractive cytokines

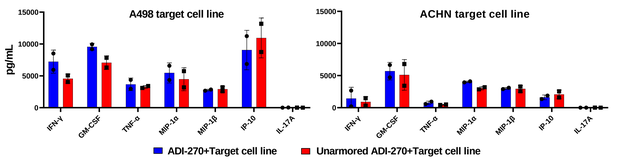


Figure 5. Cytokine and chemokine production by ADI-270 after a 24-hour co-culture with CD70+ tumor cell lines, A498 and ACHN. IFN-γ, GM-CSF, TNF-α, MIP-1α, MIP-1β, IL-17A, and IP-10 were measured in culture medium supernatants using the Luminex platform. Minimal cytokine and chemokine secretion was seen with ADI-270 and target alone (data not shown). Data shown is the mean (pg/mL) using ADI-270 derived from two different donors.

ADI-270 has enhanced functional persistence against renal cell carcinoma cell lines

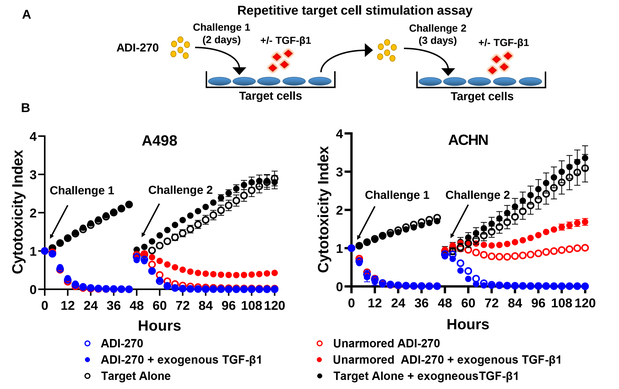


Figure 6. **(A)** Diagram of the *in vitro* repetitive stimulation assay to measure the cytolytic activity of ADI-270 co-cultured with target cell lines in the presence and absence of TGF-β1 (20ng/mL). ADI-270 was stimulated with target cell line for 48 hours, then transferred into a new vessel with freshly plated target cells for an additional 72 hours. **(B)** Cytotoxic potentials of unarmored ADI-270 (red circles) and ADI-270 (blue circles) were evaluated against CD70+ tumor cell lines A498 and ACHN in the repetitive stimulation Incucyte Immune Cell Killing Assay. The Cytotoxicity Index was calculated by dividing the total NIR object area (mm²/well) of all time points by the value at time of tumor challenge (challenge 1 or challenge 2 time points).

ADI-270 significantly inhibits tumor growth in a renal cell carcinoma xenograft model in NSG mice

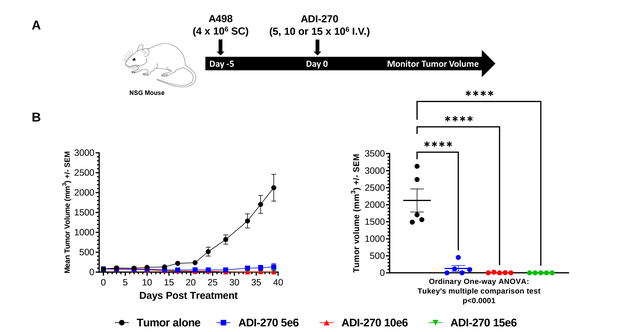


Figure 7. **(A)** Study schematic **(B)** *In vivo* efficacy of a single IV dose of ADI-270 in a subcutaneous A498 xenograft model in NSG mice (n = 5 per group). Average tumor volumes for the duration of the study (left) and statistical comparison between treatment groups and the vehicle control group at the end of the study (Day 39) (right) using Tukey's multiple comparison test.

ADI-270 homes to the tumor, proliferates, and expresses activation markers

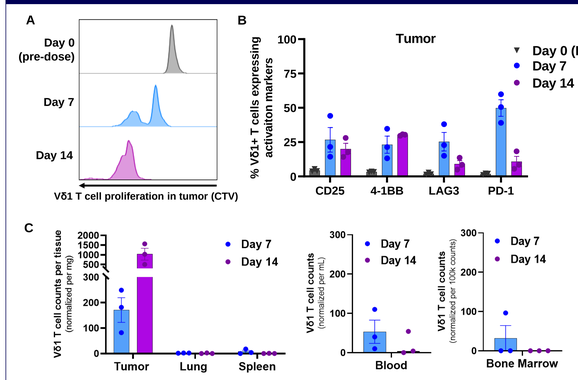


Figure 8. Based on the robust *in vivo* tumor control without incidence of xenogeneic GvHD in tumor-bearing NSG mice, the proliferation and activation of ADI-270 specifically within the tumor, with little to no accumulation in normal tissues was confirmed. Tissues from A498-bearing NSG mice receiving a single IV dose of Cell Trace Violet (CTV) labeled ADI-270 were harvested at day 7 and 14. **(A)** CTV labeled ADI-270 shows increased cell proliferation in tumor as indicated by the dilution of the CTV dye (histogram plots) compared to day 0 control. **(B)** Harvested ADI-270 were stained for activation-associated markers and **(C)** ADI-270 was seen to specifically home to the tumor when compared to lung, spleen, blood, and bone marrow at day 7 and 14.

Host vs Graft (HvG) armoring supports improved persistence of ADI-270

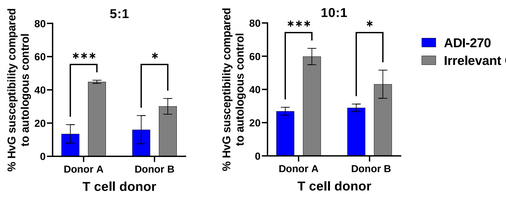


Figure 9. Stimulators (S) ADI-270 was co-cultured with Responder (R) T cells isolated from autologous and allogeneic (Donor A and B) PBMC donors at the fixed ratio of 5:1 and 10:1 (R:S) and were analyzed by flow cytometry on day 5. We observed reduced HvG susceptibility of ADI-270 compared to Vδ1 T cells expressing an irrelevant CAR suggesting ADI-270 potentially targets CD70+ activated alloreactive host T cells and can limit HvG Vδ1 T cell rejection to aid in persistence. Statistical comparison between ADI-270 and irrelevant CAR groups was determined using 2-way ANOVA.

SUMMARY & CONCLUSIONS

- ADI-270 (Vδ1 T cells modified to express an armored CD70 CAR) were successfully generated and expanded, demonstrating product expansion without indications of fratricide.
- ADI-270 expressed a less differentiated T cell memory phenotype with low expression of exhaustion markers, exhibited potent *in vitro* cytotoxicity, and was associated with a favorable cytokine and chemokine profile.
- Highly potent tumor growth inhibition was observed with ADI-270 against tumor xenografts in immunodeficient mice with evidence of selective T cell infiltration, proliferation, and activation within the tumor.
- Armoring ADI-270 with the dnTGFβRII “bolt-on” maintained activity in the presence of TGF-β.
- We observed a decrease in HvG targeting for ADI-270 compared to Vδ1 T cells expressing an irrelevant CAR suggesting that ADI-270 can dampen HvG Vδ1 rejection to better support persistence.
- In summary, ADI-270 demonstrates preclinical proof-of-concept of an armored allogeneic CD70 γδ CAR T cell therapy utilizing the CD27 natural receptor CAR format for targeting CD70+ cancers. These data support continued development and further investigation of ADI-270 in the clinic.