ADI-212: A next-generation gene-edited and armored allogeneic CAR γδ T cell therapy targeting PSMA for prostate cancer

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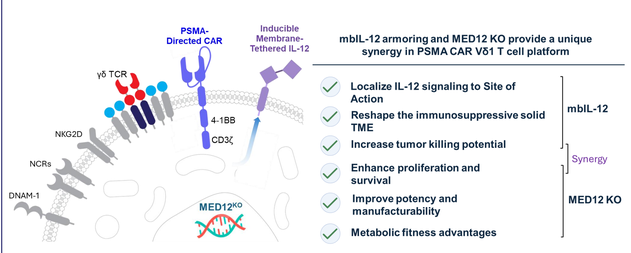
Background

Metastatic castration-resistant prostate cancer (mCRPC) remains a challenging disease with poor prognosis and reduced survival. Prostate-specific membrane antigen (PSMA), a transmembrane homodimeric glycoprotein highly expressed in prostate cancer cells, particularly in advanced CRPC, is an attractive therapeutic tumor target. Strategies targeting PSMA, such as radioligand therapy, represent promising advances in the evolving landscape of mCRPC treatment options. However, a significant unmet need remains for PSMA-directed therapies. To address these limitations, we developed ADI-212, an allogeneic CAR-T cell product comprised of gamma delta (γδ) T cells to leverage their natural antitumor immunity and tissue tropism. ADI-212 is an optimized, next-generation gene-edited and armored clinical candidate designed to enhance potency in solid tumors and deliver multiple anti-tumor mechanisms of action to the tumor microenvironment (TME). ADI-212 is engineered to express a novel CAR binder that targets a membrane-distal conformational PSMA epitope to support enhanced tolerability and tumor-specific recognition. Additionally, we have integrated a synergistic combination of membrane-tethered IL-12 (mbIL-12) armoring and CRISPR/Cas9 gene-editing to disrupt subunit 12 of the mediator complex (MED12), resulting in robust ADI-212 antigen-specific proliferation, tumor cell killing upon rechallenge, and survival, while also conveying the potential to reshape the immunosuppressive microenvironment.

ADI-212



ADI-212 is an allogeneic γδ CAR T cell product engineered with a second-generation CAR targeting PSMA, armored with a membrane-tethered form of the immunomodulatory cytokine IL-12, and gene-edited to knock out MED12, a negative regulator of T cell effector function.

ADI-212 scFv is designed to recognize functional PSMA complexes

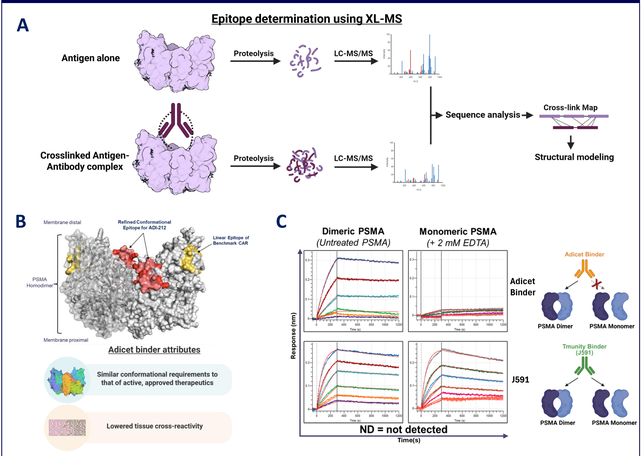


Figure 1. (A) Schematic describing the process of conformational epitope mapping using cross-linking mass spectrometry (XL-MS). **(B)** Binding interface of the scFv used in ADI-212 (red) displayed on a surface representation of the homodimeric X-ray crystal structure of the PSMA extracellular domain (PDB ID: 1ZBL), in comparison to that of the benchmark J591 CAR (yellow). **(C)** Bio-layer interferometry sensograms showing binding of the benchmark antibody J591 and the ADI-212 binder to various concentrations of recombinant human PSMA protein either untreated (dimeric conformation) or treated with 2mM EDTA (monomeric conformation), demonstrating that ADI-212 scFv binds only functional PSMA dimer.

MED12 KO improves ADI-212 manufacturing yield and promotes a stem cell-like memory T cell phenotype

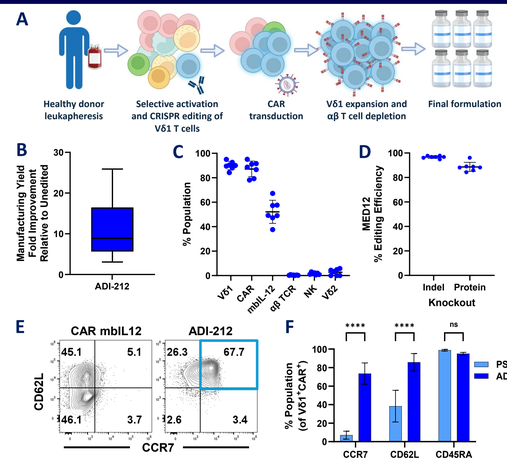


Figure 2. **(A)** Overview of ADI-212 manufacturing. **(B)** Improvement in ADI-212 manufacturing yield relative to donor-matched unedited control. **(C)** Immune composition of ADI-212 final product, with high Vδ1 purity and transgene (CAR, mbIL-12) expression. **(D)** High ADI-212 MED12 editing efficiency at the gene and protein level, as assessed by TIDE and flow cytometry, respectively. **(E-F)** High expression of memory receptors CCR7, CD62L, and CD45RA on Vδ1 CAR+ cells in ADI-212 cells relative to donor-matched unedited cells. Wilcoxon signed-rank test, p < 0.0001

mbIL-12 is induced upon antigen-specific ADI-212 activation

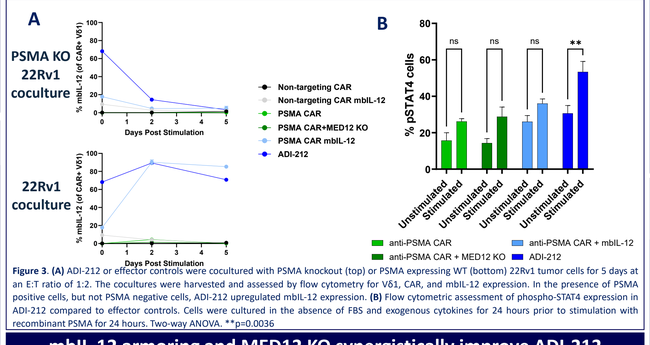


Figure 3. **(A)** ADI-212 or effector controls were cocultured with PSMA knockout (top) or PSMA expressing WT (bottom) 22Rv1 tumor cells for 5 days at an E:T ratio of 1:2. The cocultures were harvested and assessed by flow cytometry for Vδ1, CAR, and mbIL-12 expression. In the presence of PSMA positive cells, but not PSMA negative cells, ADI-212 upregulated mbIL-12 expression. **(B)** Flow cytometric assessment of phosphe-STAT4 expression in ADI-212 compared to effector controls. Cells were cultured in the absence of FBS and exogenous cytokines for 24 hours prior to stimulation with recombinant PSMA for 24 hours. Two-way ANOVA. **p=0.0036

mbIL-12 armoring and MED12 KO synergistically improve ADI-212 proliferation and *in vitro* tumor cytotoxicity

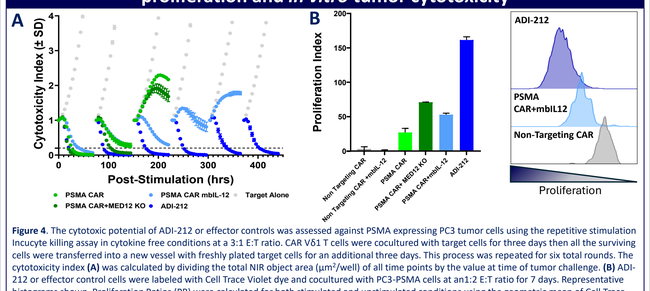


Figure 4. The cytotoxic potential of ADI-212 or effector controls was assessed against PSMA expressing PC3 tumor cells using the repetitive stimulation Incucyte killing assay in cytokine free conditions at a 3:1 E:T ratio. CAR Vδ1 T cells were cocultured with target cells for three days then all the surviving cells were transferred into a new vessel with freshly plated target cells for an additional three days. This process was repeated for six total rounds. The cytotoxicity index **(A)** was calculated by dividing the total NIR object area (μm²/well) of all time points by the value at time of tumor challenge. **(B)** ADI-212 or effector control cells were labeled with Cell Trace Violet dye and cocultured with PC3-PSMA cells at an1:2 E:T ratio for 7 days. Representative histograms shown. Proliferation Ratios (PR) were calculated for both stimulated and unstimulated conditions using the geometric mean of Cell Trace fluorescence at day 7 divided by the geometric mean of Cell Trace fluorescence on day 0. Proliferation Index = $PR_{stimulated}$ - $PR_{unstimulated}$.

MED12 KO in ADI-212 promotes increased metabolic transcriptional pathways

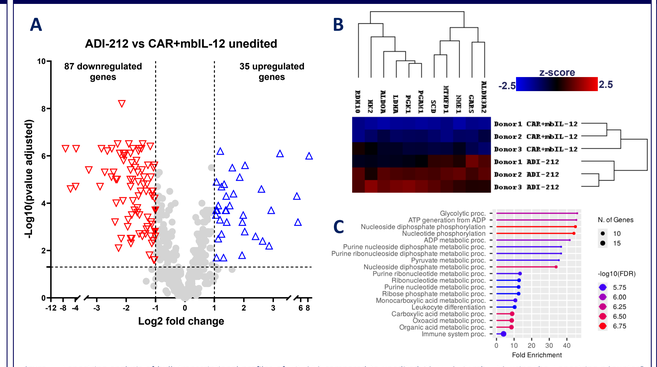


Figure 5. Nanostring analysis of bulk transcriptional profiles of ADI-212 compared to unedited PSMA CAR+mbIL-12 using the Nanostring nCounter® CAR T cell Characterization Panel. **(A)** Volcano plots of differentially expressed genes (DEGs) upregulated or down regulated in ADI-212. DEGs (-$\log_{10}$(adjusted p-value) >1.3 and $\log_2$(fold change) >1 or < -1 **(B)** Heat map of DEGs associated with metabolism. **(C)** Gene ontology analysis was performed using ShinyGO 0.85 (https://bioinformatics.sdstate.edu/go/) to identify pathways associated with DEGs that were upregulated in ADI-212.

ADI-212 expansion and cytotoxicity is resilient to suppressive Tregs

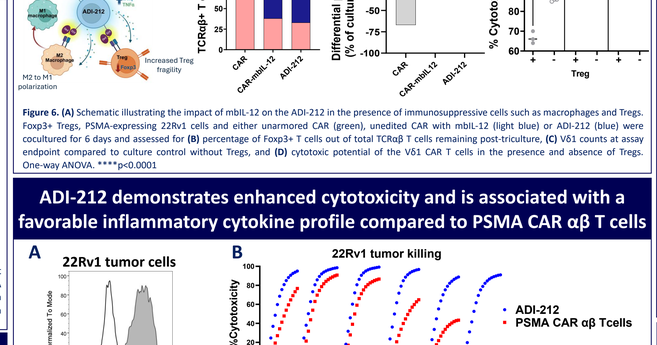


Figure 6. (A) Schematic illustrating the impact of mbIL-12 on the ADI-212 in the presence of immunosuppressive cells such as macrophages and Tregs. Foxp3+ Tregs, PSMA-expressing 22Rv1 cells and either unarmored CAR (green), unedited CAR with mbIL-12 (light blue) or ADI-212 (blue) were cocultured for 6 days and assessed for **(B)** percentage of Foxp3+ T cells out of total TCRαβ T cells remaining post-triculture, **(C)** Vδ1 counts at assay endpoint compared to culture control without Tregs, and **(D)** cytotoxic potential of the Vδ1 CAR T cells in the presence and absence of Tregs. One-way ANOVA. ****p<0.0001

ADI-212 demonstrates enhanced cytotoxicity and is associated with a favorable inflammatory cytokine profile compared to PSMA CAR αβ T cells

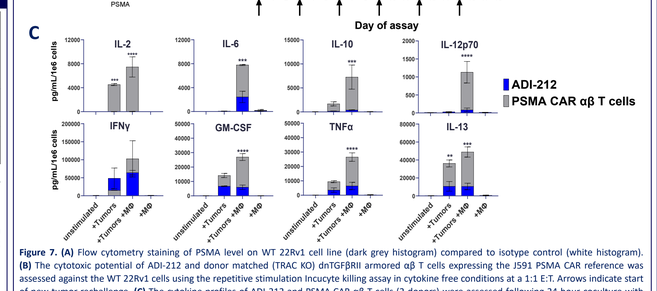


Figure 7. (A) Flow cytometry staining of PSMA level on WT 22Rv1 cell line (dark grey histogram) compared to isotype control (white histogram). **(B)** The cytotoxic potential of ADI-212 and donor matched (TRAC KO) dnTGFβRII armored αβ T cells expressing the J591 PSMA CAR reference was assessed against the WT 22Rv1 cells using the repetitive stimulation Incucyte killing assay in cytokine free conditions at a 1:1 E:T. Arrows indicate start of new tumor rechallenge. **(C)** The cytokine profiles of ADI-212 and PSMA CAR αβ T cells (2 donors) were assessed following 24-hour coculture with 22Rv1 tumor cells in the presence or absence of macrophages using the Luminex platform. Two-way ANOVA, **p<0.01, ***p<0.001, ****p<0.0001.

ADI-212 *in vivo* tumor control is enhanced by synergy of mbIL-12 armoring and MED12 KO

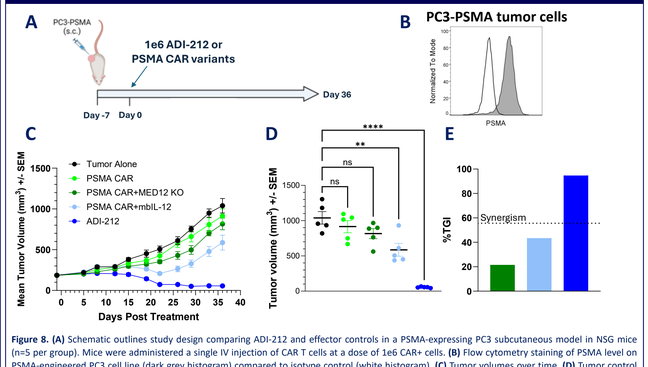


Figure 8. (A) Schematic outlines study design comparing ADI-212 and effector controls in a PSMA-expressing PC3 subcutaneous model in NSG mice (n=5 per group). Mice were administered a single IV injection of CAR T cells at a dose of 1e6 CAR+ cells. **(B)** Flow cytometry staining of PSMA level on PSMA-engineered PC3 cell line (dark grey histogram) compared to isotype control (white histogram). **(C)** Tumor volumes over time. **(D)** Tumor control at study endpoint. Difference from tumor alone calculated with one-way ANOVA with Dunnett's multiple comparisons. **p=0.0025, ****p<0.0001 **(E)** At study endpoint, % tumor growth inhibition (TGI) was calculated. Synergy of mbIL-12 and MED12 KO was demonstrated using the Bliss Independence model (Mao et al. *Cancer Res Commun.* 2023).

ADI-212 demonstrates potent and durable tumor growth control *in vivo*

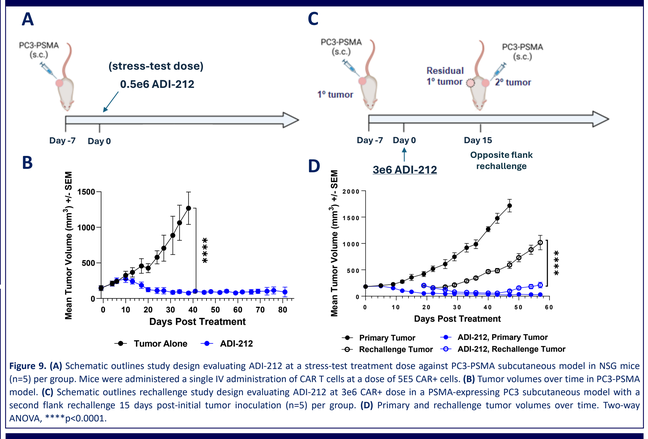


Figure 9. (A) Schematic outlines study design evaluating ADI-212 at a stress-test treatment dose against PC3-PSMA subcutaneous model in NSG mice (n=5) per group. Mice were administered a single IV administration of CAR T cells at a dose of 5E5 CAR+ cells. **(B)** Tumor volumes over time in PC3-PSMA model. **(C)** Schematic outlines rechallenge study design evaluating ADI-212 at 3e6 CAR+ dose in a PSMA-expressing PC3 subcutaneous model with a second flank rechallenge 15 days post-initial tumor inoculation (n=5) per group. **(D)** Primary and rechallenge tumor volumes over time. Two-way ANOVA, ****p<0.0001.

Summary & Conclusions

- ADI-212 is an allogeneic Vδ1 PSMA CAR-T cell therapy armored with the unique, synergistic combination of membrane tethered IL-12 and MED12 KO intended for patients with treatment refractory mCRPC.
- ADI-212 utilizes a novel scFv based CAR that binds a membrane-distal conformational PSMA epitope that is distinct from the linear epitope for the J591 scFv previously tested in αβ CAR-T clinical trials.
- ADI-212 shows PSMA antigen-specific mbIL-12 expression and STAT4 phosphorylation, that provide a highly regulated, localized delivery of IL-12 that minimizes the potential for systemic toxicities.
- ADI-212's mbIL-12 armoring and MED12 KO improve PSMA antigen-specific proliferation and cytotoxicity, resulting in robust *in vitro* cytotoxicity and *in vivo* tumor control with chronic stimulation.
- ADI-212 retains potent effector function in the presence of suppressive cells prevalent in the TME, including Tregs, due to IL-12 signaling and upregulation of metabolic pathways with MED12 KO.
- Compared to αβ CAR-T cells expressing the J591 PSMA CAR previously tested in the clinic, ADI-212 demonstrates enhanced cytotoxicity and a favorable cytokine profile, including in the presence of macrophages, supporting its expected enhanced efficacy and tolerability profile.
- In conclusion, ADI-212 is a next-generation CAR-T product, combining multiple anti-tumor mechanisms that drives robust cytotoxicity, persistence, and ability to reshape the suppressive tumor microenvironment.