ADI-925: an allogeneic off-the-shelf Chimeric Adapter (CAd) γδ T cell therapy targeting NKG2D ligand-expressing cancers

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INTRODUCTION

γδ T cells are a clinically active cytotoxic effector subtype with intrinsic tumoricidal activity and are correlated to improved survival in solid and hematologic malignancies. γδ T cells target tumors through innate and adaptive mechanisms and can be further enhanced by chimeric antigen receptor (CAR) engineering.

The innate receptor NKG2D is highly expressed on γδ T cells and recognizes a family of target proteins commonly upregulated on a wide range of tumors, including MICA, MICB, and ULPBs 1-6. NKG2D specifically associates with intracellular DAP10, a binding partner necessary for signal transduction and activation. We recently described a novel form of cell engineering incorporating an enhanced intracellular DAP10 chimeric adaptor (CAd) protein designed to amplify potency of γδ T cells for tumor targeting via endogenous receptor activation.

Here we report the development and functional characterization of ADI-925, an allogeneic Vδ1 γδ T cell product expressing a DAP10 CAd with broad tumor targeting across varied patterns of NKG2D ligand expression. ADI-925 showed enhanced NKG2D expression after target engagement, diverse tumor cell targeting, robust proliferation, and long-term tumor control across multiple donors and cell expansion processes.

Enhancing γδ Innate Effector Activity with Chimeric Adaptors (CAds)

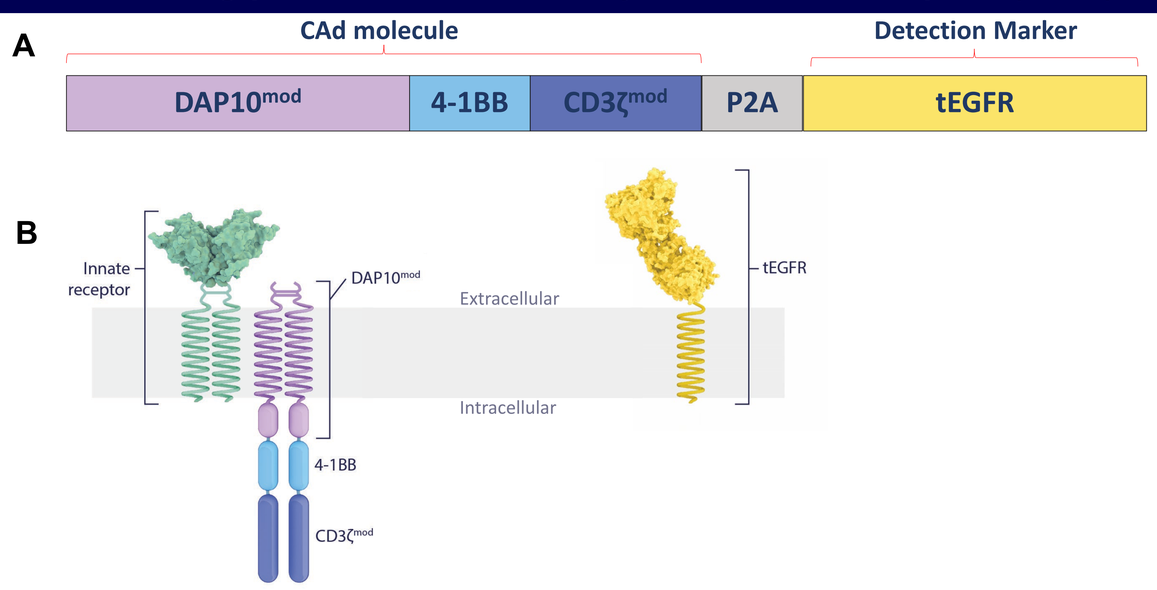
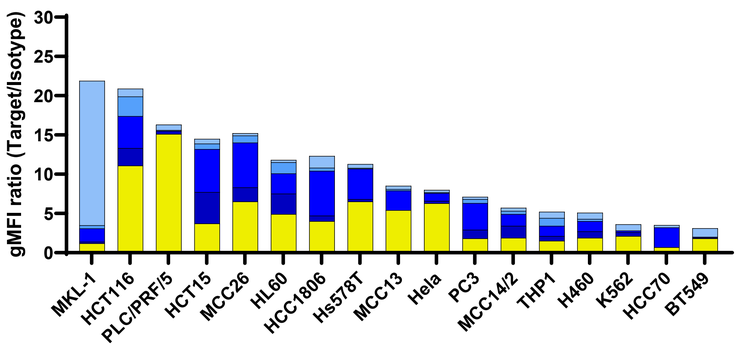


Figure 1. (A) Linear schematic of the CAd construct used to transduce ADI-925. Multiple modifications in the DAP10 protein domain and the CD3ζ costimulation domain were tested throughout the following experiments. Truncated EGFR (tEGFR) was included as a detection marker to track transduction efficiency since the vast majority of the CAd molecule is intracellular. **(B)** Illustration of the CAd molecule expressed in ADI-925. Partnering of the CAd with innate receptor molecules, such as NKG2D, results in tumor cell recognition through the extracellular binding domain of the innate receptors and augmented signal transduction through the CAd.

NKG2D Ligands are Ubiquitously Expressed Across a Broad Range of Cancer Types



Cancer Type	Target Cells
Merkel Cell	MKL-1, MCC26, MCC14/2
AML	HL60, THP1
Liver	PLC/PRF/5
Prostate	PC3
Colon	HCT116, HCT15
Breast	Hs578T, HCC70, BT549
Cervical	Hela
NSCLC	H460
CML	K562

Figure 2. A panel of cancer cell lines derived from a variety of hematologic and solid tumors were assessed for NKG2D ligands by flow cytometry. The 5 antibodies used for staining detected: MICA/MICB, ULBP1, ULBP2/5/6, ULBP3, and ULBP4. Mean fluorescence intensity was compared against the relevant isotype controls. Cancer cell lines were stained in triplicate.

ADI-925 Cytotoxicity is Mediated by Endogenous NKG2D

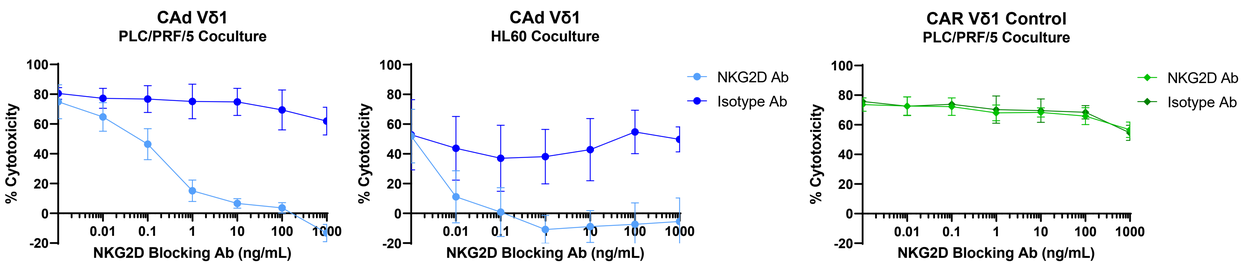


Figure 3. ADI-925 CAd+ (blue) or control CAR+ (green) Vδ1 T cells were preincubated with various dilutions of either anti-NKG2D antibody (clone 1D11) or isotype control (1ug/ml-0.01ng/mL) prior to coculture with luciferase labeled target cells (PLC/PRF/5 or HL60). After ~18 hours, target cell killing was assessed by measuring luciferase signal. NKG2D-mediated cytotoxicity can be assessed by comparing % cytotoxicity with isotype preincubation (darker curves) to % cytotoxicity with NKG2D antibody preincubation (lighter curves).

ADI-925 Maintains High NKG2D Surface Expression After Target Engagement

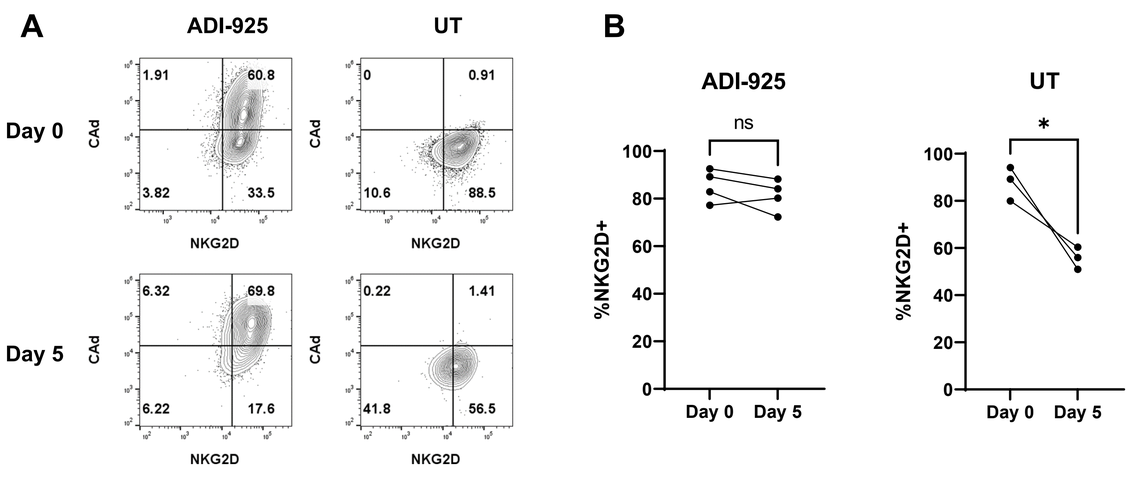


Figure 4. (A) NKG2D and CAd expression in ADI-925 or untransduced Vδ1 T cells before (Day 0) and after coculture with PLC/PRF/5 target cells for 5 days (Day 5). **(B)** Dynamics of NKG2D expression across multiple batches of ADI-925 or untransduced Vδ1 T cells before (Day 0) and after 5-day coculture with PLC/PRF/5 target cells. Each line represents a unique batch of effector cells. *p=0.043; ns=not significant.

ADI-925 Demonstrates Robust Anti-Tumor Activity with Kinetics Similar to CAR Vδ1 T Cells

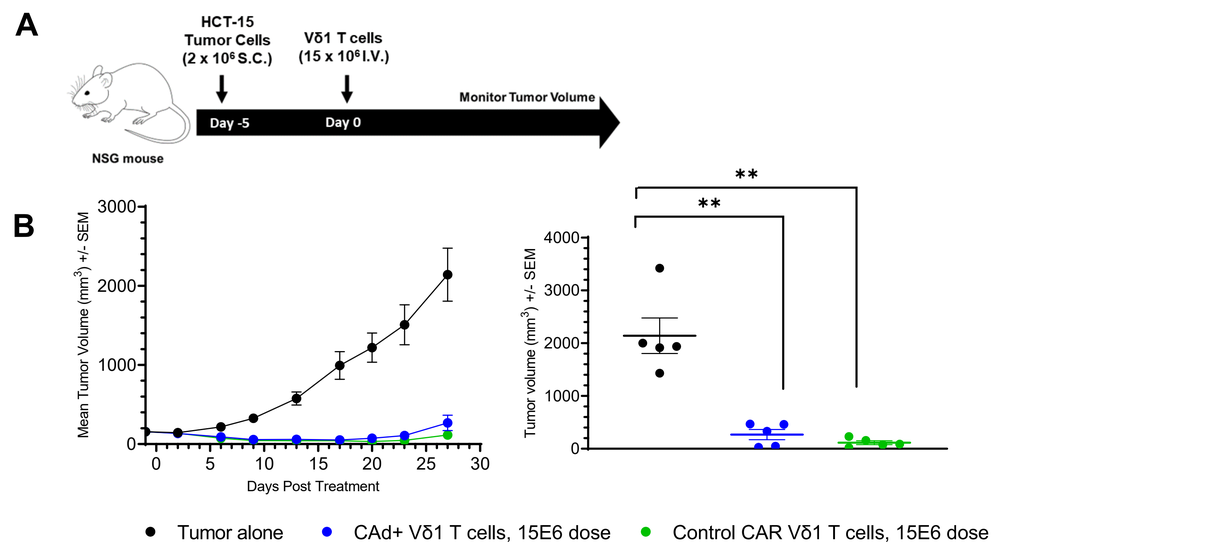


Figure 5. (A) Study schematic for in vivo efficacy of ADI-925 in an HCT-15 xenograft model. **(B)** Tumor growth kinetics (left) and tumor volumes on Day 27 (right). Data shown as mean ± SEM for 5 mice/group. A Kruskal-Wallis test with Dunn's multiple comparisons was used to assess final statistical significance amongst complete cohorts for each treatment. **p<0.01

ADI-925 Can Be Efficiently Expanded and Transduced Using a Scaled Process

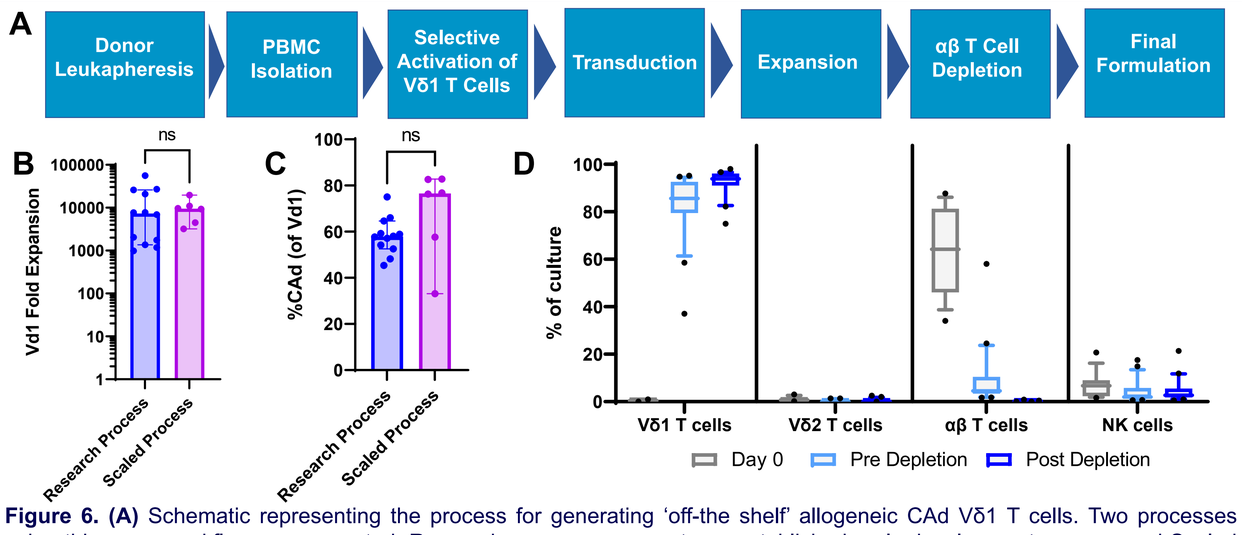


Figure 6. (A) Schematic representing the process for generating 'off-the shelf' allogeneic CAd Vδ1 T cells. Two processes using this same workflow are presented. Research process represents an established early development process and Scaled Process represents a process that is suitable for clinical manufacturing. Ex vivo culture of Vδ1 T cells results in a substantial fold-expansion **(B)** and robust CAd transduction **(C)** of ADI-925 using both processes. Twelve independent cultures using PBMCs from 7 different donors are shown are shown for the Research Process and 6 independent cultures using PBMCs from 6 different donors are shown for the Scaled Process. **(D)** Cellular composition over time for both processes. ns=not significant

ADI-925 Generated From a Scaled Process is Potent Against an Array of Tumor Targets and Secretes Proinflammatory Cytokines

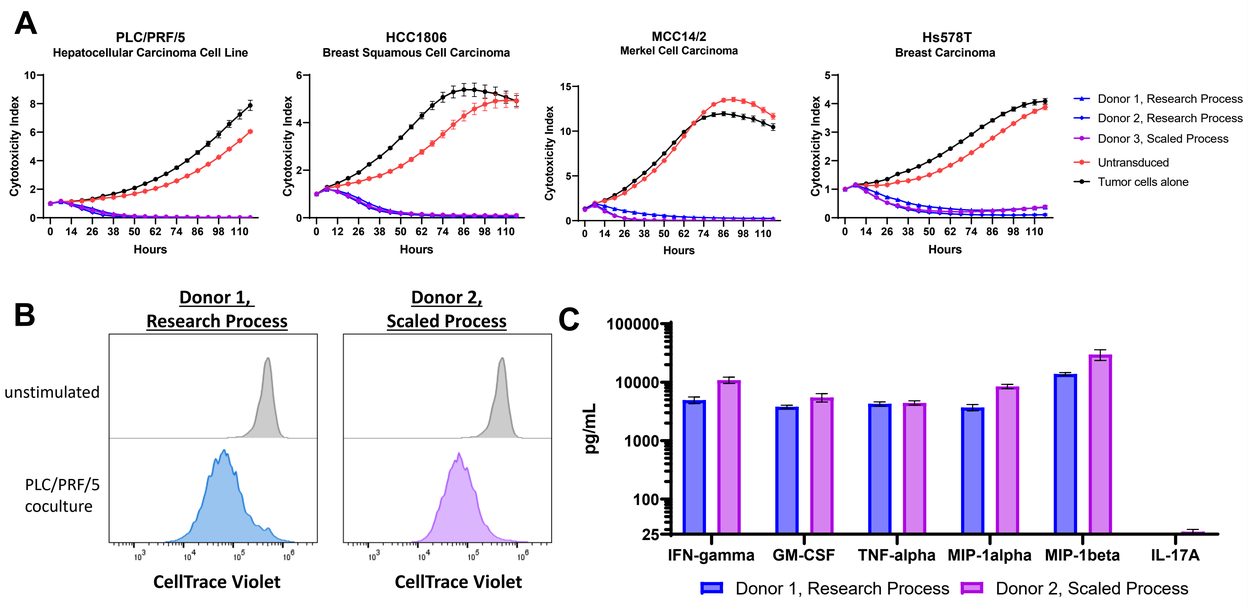


Figure 7. (A) Prolonged cytotoxic potential of multiple batches of ADI-925 (blue and purple) were evaluated against a variety of tumor cell lines in a ~120-hour Incucyte Immune Cell Killing Assay. T cells were co-cultured with NucRed-expressing target cells at submaximal E:T ratios of 5:1-1:1 depending on the cell line. The Cytotoxicity Index was calculated by dividing the total NucRed object area (mm2/well) of each time point by the value at time = 0. **(B)** Representative histograms demonstrating proliferative potential of ADI-925 across 2 donors after 5-day coculture with PLC/PRF/5 target cells as measured by dye-dilution flow cytometry. **(C)** Cytokine production of ADI-925 after 24-hour coculture with tumor targets measured using the Luminex platform.

Summary & Conclusions

- ADI-925 is an allogeneic Vδ1 T cell expressing a CAd, a novel form of cell engineering that can leverage endogenous innate immune receptors, such as NKG2D.
- ADI-925 can be robustly expanded and transduced.
- A clinically suitable manufacturing process has been developed and generates cells that maintain potent tumor targeting.
- ADI-925 maintains high levels of NKG2D expression after prolonged coculture with NKG2D ligand expressing target cells.
- ADI-925 demonstrates robust cytotoxic and proliferative potential and results in long term in vitro control across a broad array of cancer targets.
- ADI-925 exhibits a favorable cytokine profile with proinflammatory and chemoattractive cytokines.
- A single dose of ADI-925 is sufficient to control tumor in a human tumor xenograft model with kinetics similar to a CAR.
- Taken together, these data demonstrate the potentially broad application of ADI-925 as an "off-the-shelf" cell therapy in solid tumors and heme malignancies and supports investigation in the clinic.