Innate-Enhanced Chimeric Adaptors (CAd): A Newly-Described Approach for Augmenting Potency of γδ T Cell Immunotherapy

Marissa Herrman, PhD, Taylor Barca, BS, Xue (Cher) Yang, MS, Maryam Tabrizizad, BS, Morgan Smith-Boeck, BS, Louise Kiru, PhD, Jonathan Wong, BS, Ramandeep Kaur, BS, Smitha Rao Yelaguli Gundurao, MSc, Gauri Lamture, PhD, Aruna Azameera, MSc, Kevin P. Nishimoto, PhD, Arun Bhat, PhD, Blake T. Aftab, PhD
Adicet Bio, Inc. Redwood City, CA 94065

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.

Here we describe 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. Vδ1 T cells transduced with CAds comprising various DAP10 domain modifications, a 4-1BB signaling domain and modified CD3ζ co-stimulation, showed enhanced tumor cell targeting, long term tumor control and T cell proliferation across a diverse panel of cancer cell types.

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

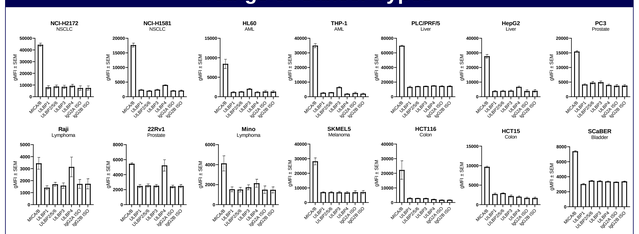

Figure 1. A panel of cancer cell lines derived from a variety of hematologic and solid tumors were assessed for NKG2D ligands by flow cytometry. The 5 antibodies used for staining detected: MICA/MICB, ULBP1, ULBP2/5/6, ULBP3, and ULBP4. Mean fluorescence intensity was compared against the relevant isotype controls. Cancer cell lines were stained in triplicate.

Enhancing γδ Innate Effector Activity with Chimeric Adaptors (CAds)

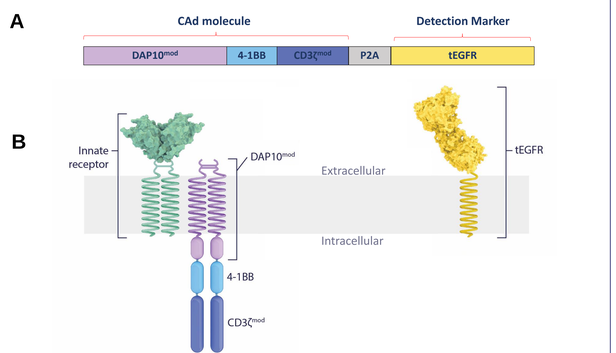

Figure 2. (A) Linear schematic of the CAd construct used to transduce Vδ1 T cells. Multiple modifications in the DAP10 protein domain and the CD3ζ costimulation domain were tested throughout the following experiments. Truncated EGFR (tEGFR) was included as a detection marker to track transduction efficiency since the vast majority of the CAd molecule is intracellular. **(B)** Illustration of the CAd molecule expressed in Vδ1 T cells. 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.

CAd Vδ1 T Cells Can Be Efficiently Expanded and Transduced

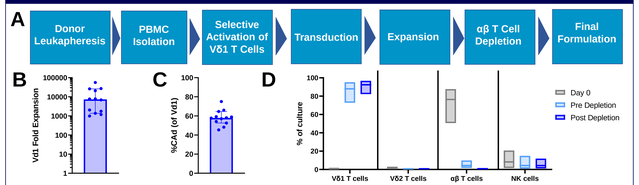

Figure 3. (A) Schematic representing the process for generating 'off-the shelf' allogeneic CAd Vδ1 T cells. Ex vivo culture of Vδ1 T cell results in a substantial fold-expansion **(B)** and robust CAd transduction **(C)** of Vδ1 T cells. 12 independent cultures using PBMCs from 7 different donors are shown. **(D)** Cellular composition over time of CAd+ Vδ1 T cells.

CAd Vδ1 Cytotoxicity is Mediated by Endogenous NKG2D

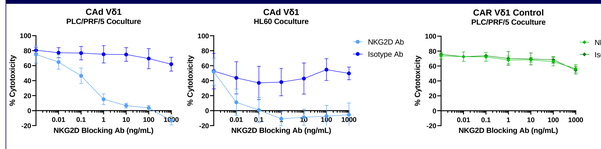

Figure 4. CAd+ (blue) or CAR+ (green) Vδ1 T cells were preincubated with various dilutions of either anti-NKG2D antibody (clone 1D11) or isotype control (1ug/ml-0.01ng/mL) prior to coculture with luciferase labeled target cells (PLC/PRF/5 or HL60). After ~18 hours, target cell killing was assessed by 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).

CAd Vδ1 T Cells Effectively Target a Broad Range of Cancer Cells

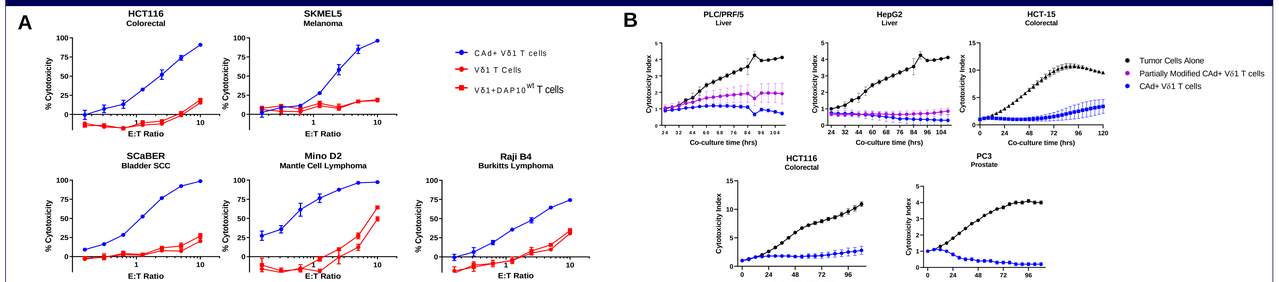

Figure 5. (A) Unengineered Vδ1 (red circles), wild type DAP10 engineered control Vδ1 (red squares), or CAd+ (blue) Vδ1 T cells were co-cultured with a variety of luciferase-expressing target cell lines across varying E:T ratios (1:6–10:1) in a short term 18-hour cytotoxicity assay. **(B)** Prolonged cytotoxic potential of CAd+ (blue and purple) Vδ1 T 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 or 2.5:1 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. The difference between partially modified CAd+ (purple) and CAd+ (blue) constructs reflect differences in mutations within the CAd sequence.

CAd Stimulation Drives Robust Cytokine Production and T Cell Proliferation

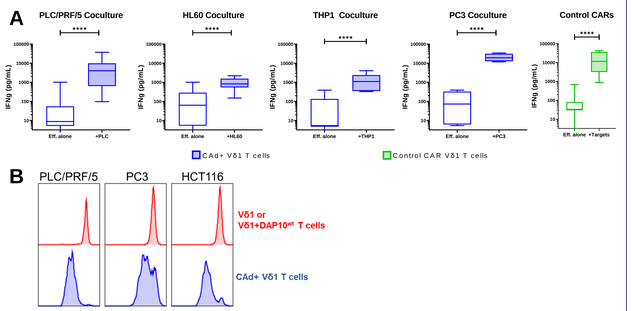

Figure 6. (A) Interferon gamma (IFNg) secretion from control Vδ1 T cells (unengineered or transduced with wild type DAP10) and CAd+ Vδ1 T cells alone and after ~18-hour coculture at submaximal E:T ratios with target cells (PLC/PRF/5, HL60, THP1, or PC3). Representative control CAR data was aggregated from three different targeting modalities after coculture with relevant target cells and are included for comparison. Statistical analysis performed using two-way ANOVA. ****p<0.0001 **(B)** Representative histograms demonstrating proliferative potential of CAd+ Vδ1 T cells after 5-day coculture with PLC/PRF/5, PC3, or HCT116 target cells as measured by dye-dilution flow cytometry .

CAd Vδ1 T Cells Specifically Proliferate, Accumulate, and Persist in Tumor Tissues *in vivo*

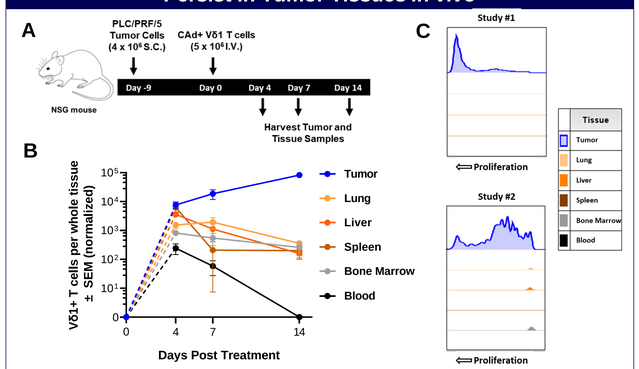

Figure 7. (A) Study schematic for kinetic *ex vivo* analysis of CAd Vδ1 T cells in PLC/PRF/5 HCC tumor-bearing NSG mice. **(B)** Quantification of Vδ1 T cells in tumor or tissues taken 4, 7, or 14 days after treatment as assessed by flow cytometry. Data represents cumulative analysis across two independent studies. **(C)** Proliferative potential of CAd Vδ1 T cells taken from tumor, lung, liver, spleen, bone marrow, and blood on day 7. HuCD45+, Vδ1+ population is shown.

CAd Vδ1 T Cells Demonstrate Anti-Tumor Activity with Kinetics Similar to CAR Vδ1 T Cells

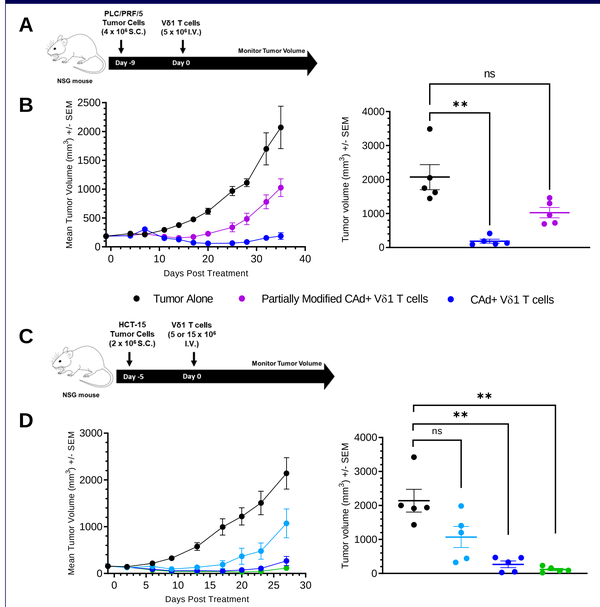

Figure 8. (A) Study schematic for *in vivo* efficacy of CAd+ Vδ1 in a PLC/PRF/5 HCC xenograft model. **(B)** Tumor growth kinetics (*left*) and tumor volumes on Day 35 (*right*). The difference between partially optimized (purple) and optimized (blue) CAd constructs reflect point mutations and select domain modifications within the CAd sequence. **(C)** Study schematic for *in vivo* efficacy of CAd+ Vδ1 and CAR+ Vδ1 in an HCT-15 xenograft model. **(D)** Tumor growth kinetics (*left*) and tumor volumes on Day 27 (*right*). For both studies, 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. n.s. = not significant.

SUMMARY AND CONCLUSIONS

- Innate-enhanced Vδ1 T cells engineered with chimeric adaptors (CAds) are potent in preclinical models
- CAds are a novel form of cell engineering that can leverage endogenous innate immune receptors, such as NKG2D.
- Innate-enhanced CAd Vδ1 T cells can be robustly expanded and transduced with established expansion protocols.
- Enhancement of CAd Vd1 cytotoxicity is primarily mediated through NKG2D.
- The cytotoxic potency and proliferative potential of unengineered Vδ1 T cells is significantly enhanced with CAd engineering and results in long term in vitro control across a broad array of cancer targets.
- CAd Vδ1 T cells exhibit a cytokine profile similar to that of CAR transduced Vδ1 T cells.
- CAd Vδ1 T cells specifically proliferate, accumulate, and persist in tumor tissues.
- A single dose of CAd Vδ1 T cells is sufficient to control multiple human tumor xenograft models.
- Taken together, these data demonstrate the potentially broad application of CAd Vδ1 T cells as an "off-the-shelf" cell therapy in solid tumors and heme malignancies and supports continued development and further investigation in the clinic.