

## INNATE-ENHANCED CHIMERIC ADAPTORS (CAD): A NEWLY-DESCRIBED APPROACH FOR AUGMENTING POTENCY OF $\gamma\delta$ T CELL IMMUNOTHERAPY

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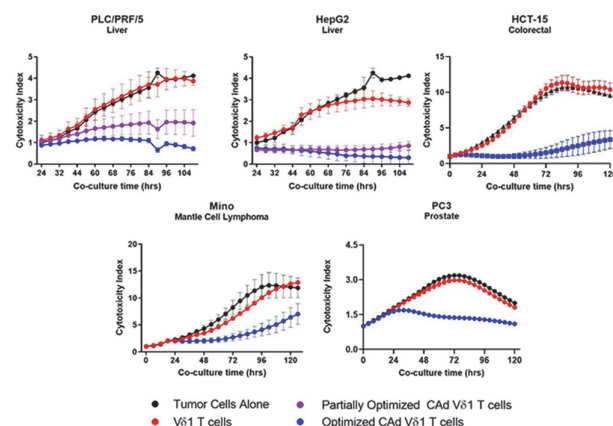
**Background**  $\gamma\delta$  T cells are a clinically active cytotoxic effector subtype with intrinsic tumoricidal activity and are correlated to improved survival in solid and hematologic malignancies.  $\gamma\delta$  T cells target tumors through innate and adaptive mechanisms. Notably, the innate receptor NKG2D is highly expressed on  $\gamma\delta$  T cells and recognizes a family of target proteins commonly upregulated on tumors. 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 (CAAd) protein, comprising DAP10 domain modifications and inclusion of 4-1BB and modified CD3 $\zeta$  co-stimulation, designed to amplify potency for tumor targeting via endogenous NKG2D receptors.

**Methods** V $\delta$ 1 T cells were expanded from donor PBMCs and transduced with enhanced DAP10 CAAds. CAAd expression and association with NKG2D were confirmed by western blot and blocking studies. In vitro characterization included co-culture assays, flow cytometric phenotyping, and cytokine production by multiplexed immunoassay. Anti-tumor potency of CAAd V $\delta$ 1 T cells was evaluated in a panel of human tumor xenograft models. Xenograft and murine tissues were analyzed for V $\delta$ 1 homing and proliferation by flow cytometry.

**Results** CAAd-enhanced V $\delta$ 1 T cells showed robust expansion and transduction, routinely reaching >80% V $\delta$ 1 purity prior to depletion steps, while maintaining a primarily naïve-like phenotype. CAAd-enhanced V $\delta$ 1 T cells displayed robust in vitro proliferation, cytokine production, and cytotoxic activity across a broad array of solid and heme tumor lines with significantly increased potency compared to unmodified V $\delta$ 1 T cells (figure 1). *In vivo*, CAAd-enhanced V $\delta$ 1 T cells primarily accumulated and specifically proliferated in tumors, with a single dose demonstrating control of tumor burden (figure 2).

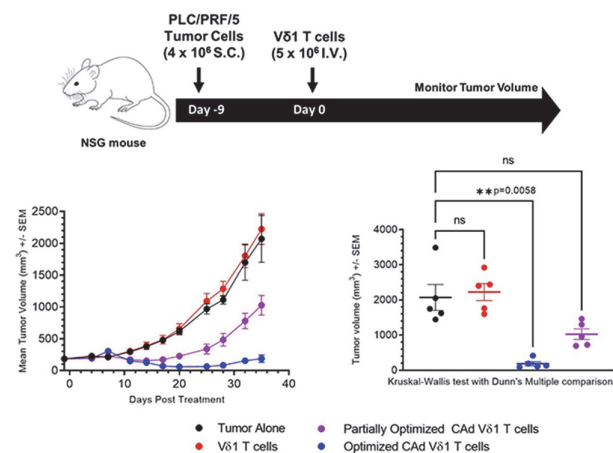
**Conclusions** Innate-enhanced V $\delta$ 1 T cells engineered with a newly-described DAP10-based chimeric adaptor technology represents a potent and potentially broadly-applicable ‘off-the-shelf’ cell therapy with marked increase in cytolytic activity via endogenously expressed innate cytotoxicity receptors. These data support continued development and further investigation in the clinic across a range of hematologic and solid tumor indications.

**Ethics Approval** All mouse experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and followed all institutional and national guidelines with appropriate protocol review and approval.



**Abstract 198 Figure 1** in vitro cytotoxicity with innate-enhanced V $\delta$ 1 T cells

An array of tumor cell lines across multiple indications were cocultured with unmodified V $\delta$ 1 T cells or innate-enhanced DAP10 CAAd V $\delta$ 1 T cells and tumor cell growth was tracked using Incucyte. The cell line and tumor origin are indicated in the title of each graph. The difference between partially optimized CAAd V $\delta$ 1 and optimized CAAd V $\delta$ 1 groups is incorporation of additional DAP10 modifications into the CAAd construct design



**Abstract 198 Figure 2** in vivo efficacy in hepatocellular carcinoma model

Top panel schematic provides details of study design and bottom panel reports tumor volumes throughout study (left) and statistical comparisons of treatment groups relative to tumor alone control at study termination (right). The difference between partially optimized CAAd V $\delta$ 1 and optimized CAAd V $\delta$ 1 groups is incorporation of additional DAP10 modifications into the CAAd construct design

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