

PRECLINICAL DISCOVERY AND CHARACTERIZATION OF ALLOGENEIC ANTI-PSMA $\gamma\delta$ CAR T THERAPY FOR PROSTATE CANCER

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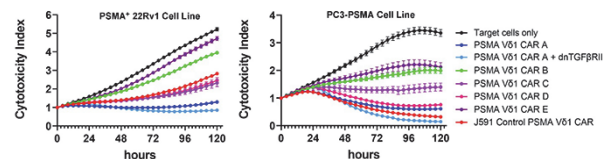
Background Prostate-specific membrane antigen (PSMA) is a transmembrane glycosylated homodimer overexpressed in >80% of prostate cancers. PSMA expression is increased in advanced stages of the disease, making it an attractive therapeutic target. Clinically, autologous anti-PSMA $\alpha\beta$ CAR T cells have shown initial efficacy with significant CRS-like dose-limiting toxicities. Compared to $\alpha\beta$ T cells and other innate cells, $\gamma\delta$ T cells are associated with multifunctional innate and adaptive targeting and differentiated biodistribution into tumor-associated tissues. Additionally, $\gamma\delta$ CAR T cells have demonstrated enhanced tumoricidal activity and tailored activation-induced cytokine profiles that may decrease toxicities associated with CRS. We characterized $\gamma\delta$ T cells modified from a set of novel scFv-based CARs targeting PSMA for prostate cancer.

Methods We used phage panning to identify a library of anti-PSMA scFv sequences, which were reformatted into CARs in VH-VL and VL-VH orientations and screened in Jurkat-Lucia™ NFAT cells to assess CAR expression and activation in the context of target cell-based stimulation. We transduced a set of functional CARs into V δ 1 T cells, a primarily tissue-resident subset, activated and expanded from healthy donor PBMCs. We performed *in vitro* cell-based cytotoxicity assays and phenotypic assessments of CAR V δ 1 T cells using flow cytometry. Potency was also assessed in NSG mice bearing subcutaneous PSMA-expressing xenografts.

Results Anti-PSMA scFvs ranged in apparent affinities from the low nanomolar to the sub-micromolar range. CAR-expressing Jurkat cells showed target-specific NFAT activation and low tonicity. CAR-engineered V δ 1 T cells demonstrated robust expansion, *in vitro* cytotoxicity and antigen-specific proliferation against PSMA⁺ cell lines in a manner comparable to, or greater than, J591 scFv-based V δ 1 CAR. *In vitro* potency correlated with the release of multiple effector cytokines. Notably, IL-6 and IL-10 production by anti-PSMA V δ 1 CAR T cells was negligible, while TNF α production was low, further supporting the potential of the $\gamma\delta$ CAR T platform to demonstrate a favorable cytokine-associated safety profile. Anti-PSMA V δ 1 CAR T cells were also found to be predominantly naïve, with low levels of exhaustion marker expression. Additionally, *in vitro* (figure 1) and *in vivo* (figure 2) potency of anti-PSMA V δ 1 CAR T cells was investigated upon co-expression of dominant negative TGF β R2 (dnTGF β R2). In xenograft models, anti-PSMA V δ 1 CAR T cells demonstrated potent anti-tumor efficacy.

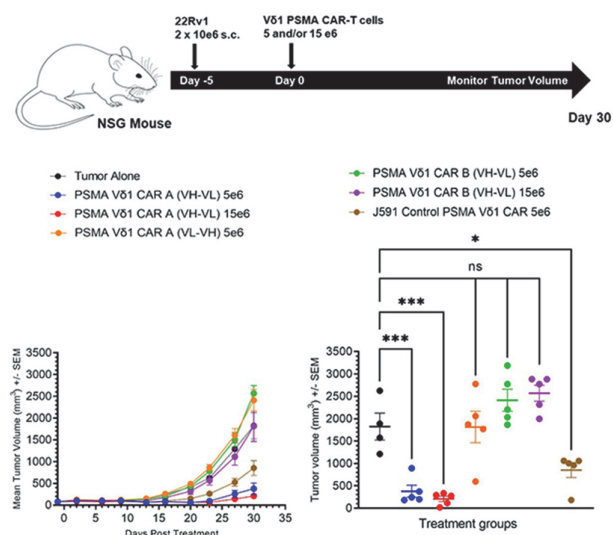
Conclusions We have engineered, screened and demonstrated preclinical efficacy for “off-the-shelf” PSMA-targeting $\gamma\delta$ CAR T cells. Resulting $\gamma\delta$ CAR T constructs identified here are candidates for further preclinical and clinical development in the context of armoring technologies.

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 203 Figure 1 In vitro cytotoxicity of PSMA-targeting V δ 1 CAR-T cells

Demonstration of *in vitro* potency at 1:1 E:T ratio of PSMA-targeting V δ 1 CAR-T cells (including CAR A co-expressing dnTGF β R2) when co-cultured with PSMA-expressing target cell lines, 22Rv1 (left) and TGF β -secreting PC3 cells engineered to express PSMA (right)



Abstract 203 Figure 2 *in vivo* potency of V δ 1 CAR-T cells in 22Rv1 PCa model

Demonstration of *in vivo* potency in a 22Rv1 PCa xenograft model (low, heterogenous PSMA expression) with PSMA-targeting V δ 1 CAR-T cells. Schematic outlines the study design (top panel). Graphs detail tumor volumes determined for the entire study duration (bottom, left panel) as well as statistical comparison of treatment groups relative to the untreated tumor alone control at study termination (bottom, right panel).

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