## 203 PRECLINICAL DISCOVERY AND CHARACTERIZATION OF ALLOGENEIC ANTI-PSMA γδ CAR T THERAPY FOR PROSTATE CANCER

Nitya Ramadoss*, Aruna Azameera, Alexander Teague, Jonathan Wong, Atrish Bagchi, Tonya Capillo, Morgan Smith-Boeck, Michael Salum, Erika Meaddough, Elizabeth Speltz, Matthew Hoopes, Katherine Wang, Ramandeep Kaur, Smitha Gundurao, Marissa Herrman, Yvan Chanthery, Blake Aftab, Kevin Nishimoto, Arun Bhat. *Adicet Bio, Inc., Redwood City, CA, USA*

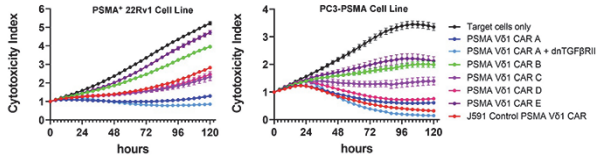
**Background** Prostate-specific membrane antigen (PSMA) is a transmembrane glycosylated homodimer overexpressed in >80% of prostate cancers. PSMA expression is increased in advanced stages of the disease, making it an attractive therapeutic target. Clinically, autologous anti-PSMA αβ CAR T cells have shown initial efficacy with significant CRS-like dose-limiting toxicities. Compared to αβ T cells and other innate cells, γδ T cells are associated with multifunctional innate and adaptive targeting and differentiated biodistribution into tumor-associated tissues. Additionally, γδ CAR T cells have demonstrated enhanced tumoricidal activity and tailored activation-induced cytokine profiles that may decrease toxicities associated with CRS. We characterized γδ 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 Vδ1 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 Vδ1 T cells using flow cytometry. Potency was also assessed in NSG mice bearing subcutaneous PSMA-expressing xenografts.

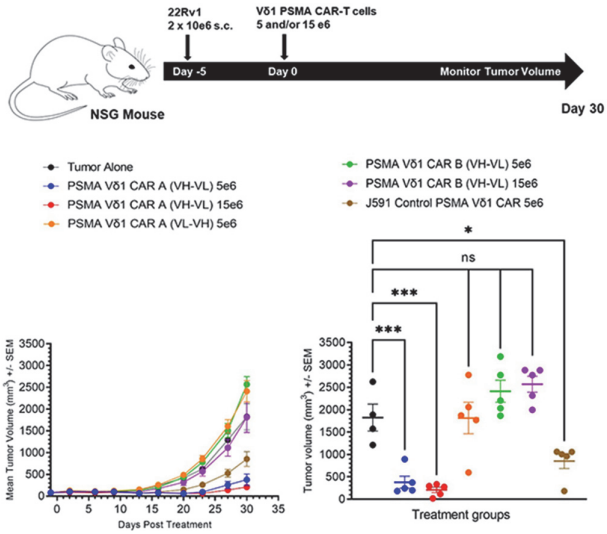
**Results** Anti-PSMA scFvs ranged in apparent affinities from the low nanomolar to the sub-micromolar range. CAR-expressing Jurkat cells showed target-specific NFAT activation and low tonicity. CAR-engineered Vδ1 T cells demonstrated robust expansion, *in vitro* cytotoxicity and antigen-specific proliferation against PSMA$^+$ cell lines in a manner comparable to, or greater than, J591 scFv-based Vδ1 CAR. *In vitro* potency correlated with the release of multiple effector cytokines. Notably, IL-6 and IL-10 production by anti-PSMA Vδ1 CAR T cells was negligible, while TNFα production was low, further supporting the potential of the γδ CAR T platform to demonstrate a favorable cytokine-associated safety profile. Anti-PSMA Vδ1 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 Vδ1 CAR T cells was investigated upon co-expression of dominant negative TGFβRII (dnTGFβRII). In xenograft models, anti-PSMA Vδ1 CAR T cells demonstrated potent anti-tumor efficacy.

**Conclusions** We have engineered, screened and demonstrated preclinical efficacy for "off-the-shelf" PSMA-targeting γδ CAR T cells. Resulting γδ 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 Vδ1 CAR-T cells
Demonstration of in vitro potency at 1:1 E:T ratio of PSMA-targeting Vδ1 CAR-T cells (including CAR A co-expressing dnTGFβRII) when co-cultured with PSMA-expressing target cell lines, 22Rv1 (left) and TGFβ-secreting PC3 cells engineered to express PSMA (right)




**Abstract 203 Figure 2** in vivo potency of Vδ1 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 Vδ1 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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