

Title: Nanobody-Based PSMA CAR T Cells Enable Safe Dual-Antigen Targeting of Metastatic Castration-Resistant Prostate Cancer

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Background: Metastatic castration-resistant prostate cancer (mCRPC) remains largely refractory to immunotherapy. We previously developed a second-generation STEAP1-BBζ CAR T cell that demonstrated potent antitumor activity with an acceptable safety profile in preclinical models and has advanced to a Phase I clinical trial. However, antigen escape through STEAP1 loss represents a major mechanism of resistance. Because STEAP1 and PSMA are co-expressed in approximately 70% of mCRPC tumors, dual-antigen targeting represents a promising strategy to improve therapeutic durability. Clinical translation of PSMA-directed CAR T cells, however, has been limited by toxicity, including a reported treatment-related death in a clinical trial, highlighting the need for safer PSMA-targeting CAR designs.

Methods: We engineered and optimized a second-generation nanobody-based (NB) PSMA-BBζ CAR and directly compared its function with an optimized conventional J591-based PSMA CAR. Antigen-specific activity was evaluated in vitro using parental and PSMA-knockout 22Rv1 and C4-2B models. Antitumor efficacy and toxicity were assessed in a human B-PSMA knock-in (hPSMA-KI) mouse model using tumor growth, body weight, serum cytokines, and histopathological analyses, including PSMA expression and CD3 infiltration. The optimized PSMA binder was subsequently incorporated into bicistronic STEAP1/PSMA CAR constructs to enable dual-antigen targeting.

Results: The NB PSMA-BBζ CAR mediated potent, antigen-specific antitumor activity comparable to the J591 CAR while demonstrating a substantially improved safety profile in hPSMA-KI mice. In contrast, J591 CAR T cells induced significant toxicity, including approximately 20% body weight loss within seven days of treatment, whereas the NB CAR was well tolerated. Histopathological analysis further demonstrated increased CD3+ T-cell infiltration in the brains of J591 CAR-treated mice compared with NB CAR-treated animals, suggesting reduced off-tumor toxicity with the nanobody-based construct. Dose-escalation studies identified lower J591 CAR doses that retained antitumor activity while partially mitigating toxicity, supporting ongoing binder optimization. Incorporation of the optimized nanobody-based PSMA CAR into bicistronic STEAP1/PSMA CAR constructs enabled efficient recognition of both target antigens in vitro, establishing a platform to overcome STEAP1 antigen escape.

Conclusions: We developed a nanobody-based PSMA CAR that maintains potent antitumor activity while exhibiting an improved preclinical safety profile compared with a conventional J591-based CAR. Incorporation of this optimized binder into dual STEAP1/PSMA CAR constructs provides a strategy to overcome antigen escape in mCRPC. Collectively, these findings underscore the importance of rigorous preclinical safety evaluation and rational CAR engineering to improve the therapeutic index and facilitate clinical translation of next-generation CAR T-cell therapies for solid tumors.

Funding Acknowledgements: Prostate Cancer Foundation Young Investigator Award (V.B.), and a Department of Defense Prostate Cancer Research Program Early Investigator Research Award (HT9425-23-1-0089 to V.B.).

Conflicts of Interest Disclosure Statement: J.K.L. and V.B. is an inventor on U.S. Provisional Patent Application No. 64/106,457 entitled NANOBODY-BASED CHIMERIC ANTIGEN RECEPTORS TARGETING PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA) IN PROSTATE CANCER.