

TRI-CAR: Aim, Arm, and Armor-CAR-T Against Prostate Cancer

Authors and Affiliations:

Nour Shobaki^{1,2}, Menelik Duey¹, Yutong Deng¹, Kendall Cavalier¹, Jidapa Janpathompong¹, Julia Nguyen¹, Martina Spiga¹, Khatuna Gabunia¹, Decheng Song¹, Ting-Jia Fan¹, Mei Qing Ji¹, Shane Mackey¹, Charles-Antoine Assenmacher⁷, David Schultz⁸, Elizabeth Kropf⁹, Yael Day⁹, Fang Chen⁹, Mohamad Abedi¹⁰, Vivek Narayan⁶, Andrew Rech^{1,2,4,5}, Julie Jadowsky¹, Julie Barber-Rotenberg¹, Wei Liu¹, Neil Sheppard¹, Joseph Fraietta^{1-5,9}, John Scholler¹, Anne Chew^{1,2}, Carl June^{1,2,4,5}

¹ Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA.

² Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA.

³ Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA.

⁴ Abramson Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104, USA.

⁵ Parker Institute for Cancer Immunotherapy, University of Pennsylvania, Philadelphia, PA, 19104, USA.

⁶ Division of Hematology/Medical Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

⁷ Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.

⁸ High-Throughput Screening Core, University of Pennsylvania, Philadelphia, PA, USA.

⁹ Translational and Correlative Studies Laboratory, Center for Cellular Immunotherapies, University of Pennsylvania, Philadelphia, PA, USA.

¹⁰ Department of Bioengineering, School of Engineering, University of California, Los Angeles, CA, USA.

Background

Metastatic castration-resistant prostate cancer (mCRPC) remains uniformly lethal. CAR T-cell therapy has transformed hematologic malignancies, but its translation to mCRPC has been limited by three unresolved barriers: PSMA-targeted CAR T cells and bispecifics have caused grade ≥ 3 cytokine release syndrome and neurotoxicity in clinical trials (NCT03089203, NCT04227275, NCT03972657); tumor antigen heterogeneity allows antigen-negative escape; and TGF β within the tumor microenvironment (TME) suppresses T-cell function. No single-mechanism strategy addresses all three at once. This abstract updates our 2024 and 2025 PCF Retreat presentations with new *in vivo* efficacy, immune-memory, and safety data from a now-completed preclinical package.

Methods

We engineered TRI-CAR, a single lentiviral construct integrating three functions: a STEAP2-directed CAR that restricts targeting to prostate tissue (Aim); a secreted α PSMA $\times$ CD28 bispecific antibody (BiAb) that recruits and co-stimulates bystander T cells against STEAP2-negative/PSMA-positive escape variants (Arm); and a dominant-negative TGF β receptor II (dnTGF β RII) that confers resistance to TME-mediated immunosuppression (Armor). The lead construct was selected through three sequential rounds of screening across antigen-defined human prostate cancer lines. We then characterized it *in vitro* (cytotoxicity, cytokine secretion, BiAb secretion and function, SPR binding kinetics, SMAD2/3 signaling) and *in vivo*, using subcutaneous, intraosseous bone-metastasis, and tumor-rechallenge NSG xenograft models, with histopathology, clinical chemistry, and plasma cytokine profiling.

Results

Across eight or more human T-cell donors, TRI-CAR transduced efficiently ($\sim 38\%$ CAR+) despite its large size and mediated potent, antigen-dependent cytotoxicity, with median kill times of 21–31 hours depending on the model ($p < 0.0001$), driven by STEAP2-CAR-dependent killing of STEAP2⁺ tumors and BiAb-dependent killing of PSMA⁺ tumors. Cytotoxicity was accompanied by strictly antigen-restricted

cytokine secretion (IFN- γ , IL-2, TNF- α , IL-6). dnTGF β RII blocked TGF β signaling, evidenced by loss of SMAD2/3 phosphorylation. The secreted BiAb bound PSMA and CD28 with high affinity (KD $\sim$ 11.4 nM and $\sim$ 38 nM, respectively), and endogenous secretion outperformed exogenous delivery. *In vivo*, TRI-CAR produced significant, durable tumor control in subcutaneous LNCaP models across two independent donors ($p < 0.0001$ vs. control), and mice achieving complete remission resisted tumor rechallenge, indicating durable immunologic memory. In an intraosseous bone-metastasis model of clinically established and advanced disease, a stringent test of efficacy, TRI-CAR drove marked tumor regression. Against STEAP2-negative/PSMA-positive tumors, the secreted BiAb alone controlled tumor growth, a result reproducible across independent donors and CAR T-cell doses. Comparative histopathology showed fewer systemic immune infiltrates than PSMA-CAR controls, with preserved hepatic and renal function and no cytokine-release-syndrome-like signature in any model.

Conclusions

TRI-CAR is, to our knowledge, the first platform to combine prostate-restricted targeting, a CAR-secreted costimulatory bispecific, and TGF β resistance in a single construct. It achieves potent antitumor activity, including against bone metastasis, with a favorable safety margin relative to PSMA-CAR. A patent application has been filed, and TRI-CAR is now advancing through IND-enabling studies toward a planned clinical trial.

Funding Acknowledgements

This work was supported by the Prostate Cancer Foundation (PCF) Tactical Award (2023) and the PCF Young Investigator Award (2024).

Conflicts of Interest Disclosure Statement

N.S., C.J., J.S., and W.L. are inventors on a patent application related to this work (Combination immunotherapy of STEAP2 chimeric antigen receptor T cells delivering anti-CD28 bispecifics targeting prostate-specific membrane antigen; University of Pennsylvania; filed February 2025). The other authors declare no conflicts of interest.