

TGF β Signaling Blockade Enhances the Functionality of PSMA Targeted CAR Human T Cells for the Eradication of Metastatic Prostate Cancer

Christopher C. Kloss¹, Aaron Zhang¹, Jihyun Lee¹, and Carl H. June¹

¹ Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania.

The recent efficacies demonstrated using Chimeric Antigen Receptor (CAR) mediated immunotherapy to treat hematological malignancies have been met with great enthusiasm. Unfortunately, the ability to target solid tumors like prostate cancer with CAR T cells has been less successful. The major parameter to achieve in using CAR T cells to treat prostate cancer is overcoming the immunosuppression that is created by the tumors to inhibit CAR T cells. Our efforts have aimed to create PSMA specific CAR T cells that are resistant to the TGF β induced suppression extensively demonstrated to exist in prostate cancer. Upon binding PSMA, our CAR supplies 4-1BB and CD3 ζ signaling. We therefore created anti-PSMA CAR T Cells that coexpress the dominant negative TGF β receptor II (dnTGFR β II).

Having focused on safety and efficacy of these anti-PSMABBz CAR T cells that express dnTGFR β II (dnTGFR β II-T2A-PBBZ), we demonstrate evidence of dnTGFR β II functionality. The dnTGFR β II functions to prevent SMAD signaling induced by TGF β , therefore resisting upregulation of CD25 and CTLA-4 by T cells. When co-cultured with tumor cells *in vitro*, efficient antigen specific lysis is induced by the PBBZ CAR and the dnTGFR β II-T2A-PBBZ CAR T cells exhibit up to 15 fold overall proliferation than PBBZ alone CAR T cells over 42 days. This allows for superior levels of T cell persistence in the peripheral blood of NSG mice when compared to T cells expressing the anti-PSMA CAR alone. Most importantly, these CAR T cells are very effective at eradicating systemic PSMA⁺ PC3 prostate cancer cells *in vivo*. These studies suggest proper resistance to TGF β by CAR modified T cells that show great promise to eradicate metastatic prostate cancer in the clinic.

Our current studies focus on the following aims: 1) investigating the immunological mechanism for the enhanced functionality of TGF β resistant T cells and 2) investigating the role of nectin-like protein mediated immunosuppression of T cells in prostate cancer. In order to achieve aim 1, we are investigating the differences in cytokine secretion, global gene expression, and phenotype of T cells both *in vitro* and *in vivo* of dnTGFR β II-T2A-PBBZ T cells compared to the PBBZ T cells. In order to achieve aim 2, we are using the CRISPR system to disrupt expression of nectin-like proteins alone or in combination with PD-1 to determine the level of immunosuppression conferred by these molecules in the context of prostate cancer both *in vitro* and *in vivo*. These studies will develop methods to supply antigen specific T cells to solid tumors that can be engineered to be exclusively resistant to immunosuppression while stimulated using a CAR. These methods will allow for targeting prostate cancer systemically by efficiently inducing anti-tumor immune responses that we believe will provide great clinical benefit to patients with advanced prostate cancer.

Disclosure of Conflict of Interest:

C.C. Kloss reports having ownership interest in patents owned by Memorial Sloan-Kettering Cancer Center and licensed to Juno Therapeutics and Fate Therapeutics. A. Zhang reports no conflicts. J. Lee reports no conflicts. C.H. June reports receiving commercial research grants from Novartis and has ownership interest in patents owned by University of Pennsylvania and licensed to Novartis.

Funding Acknowledgments:

This study was supported by the Prostate Cancer Foundation All-Star Killer T-Cell Special Challenge Award 2014 – 2016 and the 2016 PCF Young Investigator Award.