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

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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 TGFβ induced suppression extensively demonstrated to exist in prostate cancer. Upon binding PSMA, our CAR supplies 4-1BB and CD3ζ signaling. We therefore created anti-PSMA CAR T Cells that coexpress the dominant negative TGFβ receptor II (dnTGFRβII).

Having focused on safety and efficacy of these anti-PSMABBz CAR T cells that express dnTGFRβII (dnTGFRβII-T2A-PBBZ), we demonstrate evidence of dnTGFRβII functionality. The dnTGFRβII functions to prevent SMAD signaling induced by TGFβ, 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 dnTGFRβII-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 TGFβ 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 TGFβ 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 dnTGFRβII-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.

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