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

Christopher C. Kloss[1] Ph.D., Aaron Zhang[1], and Carl H. June[1] M.D.

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

Harnessing the immune system to eradicate cancer has demonstrated dramatic responses in many facets of the immunotherapy field. However, many cancers elude our efforts to unleash any type of significant immune response. Nonetheless these studies have supported that given the proper understanding of the disease, immunotherapies hold tremendous potential. We have learned that a likely reason for these anergic immune responses is due to multiple mechanisms of immunosuppression and immune evasion. Therefore, we hypothesized that by engineering PSMA targeted CAR T cells to be resistant to immunosuppressive signaling pathways (i.e. TGFβ), potent T cell immune responses will be generated resulting in long-term eradication of prostate cancer.

Our previous efforts established that by expressing dnTGFβRII in PSMA specific CAR T cells, it enhances 1) proliferation, 2) cytokine secretion, 3) tumor eradication, 4) numbers and memory phenotype of CAR T cells in vivo. These data were used to initiate a Phase I clinical trial currently opened and waiting to infuse these same CAR T cells into metastatic prostate cancer patients. We then wanted to investigate the mechanism of how blocking TGFβ allows for these enhanced parameters.

To investigate these mechanisms, we produced triplicate samples at 4 weekly timepoints post weekly antigen stimulation for A) anti-PSMA CAR T cells and B) anti-PSMA CAR and dnTGFBRII T cells and isolated RNA for whole mRNA transcript microarrays. From these microarray data, we identified significantly differentially expressed genes. Due to consistency of fold change between timepoints and highest fold change, we focused our efforts on TH1, TH2, and immunosuppression associated genes.

Being guided by these data, we then used these samples for luminex and multi-parameter flow cytometry analysis. The cytokine profiling revealed that the dnTGFBRII group had generally significantly higher secretion of various cytokines. Flow cytometric analysis unraveled that there is a dramatic drop in CD8+ T cells, differential memory phenotype T cell subsets, increase in "naïve" like CCR7+ T cells, and a dramatic decrease in immunosuppressive cells.

From these data, we support that by expressing the dnTGFβRII receptor in CAR T cells we have enhanced in vitro T cell proliferation that from microarray analysis has T cells with globally higher expression of genes associated with immunosuppression, TH1, and TH2 function. We found that dnTGFβRII T cells had enhanced cytokine secretion, a reduction in total CD8+ T cells, more memory T cells, , and a dramatic reduction in immunosuppressive cells. These data correlate with the previous enhanced proliferation, cytokine secretion, tumor eradication, and memory T cells in vivo previously demonstrated.

Disclosure of Conflict of Interest:

Funding Acknowledgments: