# Increased proliferation and specificity of tumor associated antigen specific T-cells using checkpoint inhibitor during stimulation

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## Background:

Malignant primary brain tumors are a leading cause of cancer mortality in children with few therapeutic options. Tumor associated antigen specific T cells (TAA-T cells) have the advantage of targeting any tumor peptide of interest, including intracellular proteins. However, expansion and cytolytic function of in vitro expanded TAA-T cells are suboptimal. In vitro expansion of TAA-T cells requires multiple stimulations with the target antigen rendering the T-cells susceptible to exhaustion.

## Objective:

We aim to determine if repeated and prolonged stimulation of TAA-T cells ex vivo leads to upregulation of exhaustion markers. Subsequently, we aim to determine whether checkpoint blockade during TAA-T cell expansion leads to increased proliferation and specificity of target specific T cell population.

## Methods:

T cells from healthy donors were harvested from peripheral blood and expanded in vitro. Mature dendritic cells (DCs) were pulsed with PRAME peptides and were used to stimulate the T cells. The specificity of these expanded cells were tested using IFN-g ELISPOT.

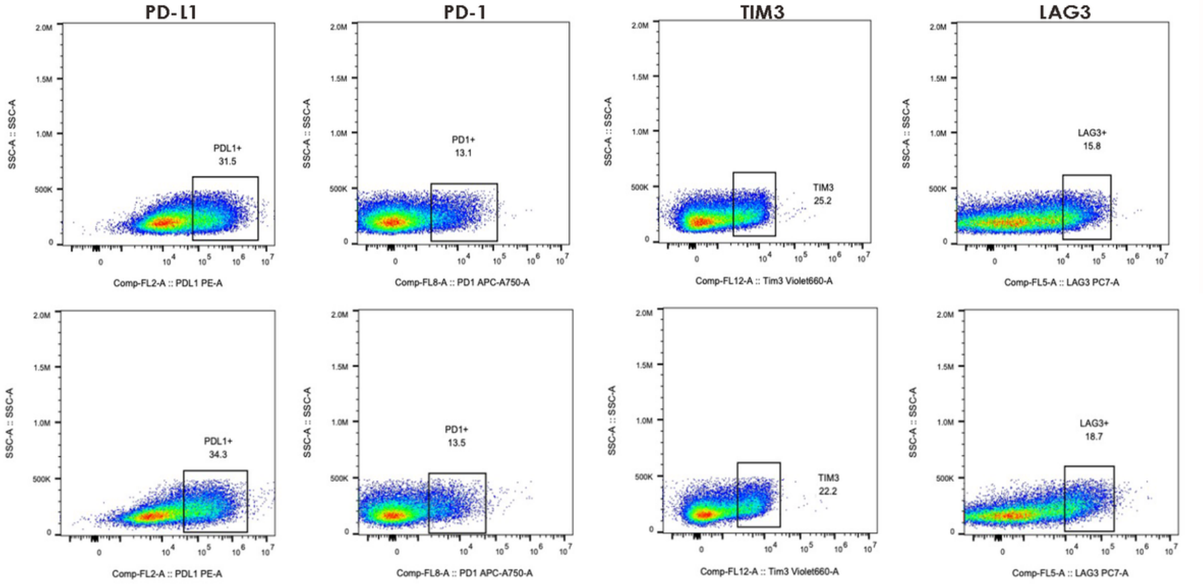


Figure 1. Exhaustion markers on T cells post stimulation 2 (above) and post stimulation 3 (below)

## Results:

Flow cytometry on TAA-T cells post-stimulation 2 and 3 showed multiple inhibitory receptors including PD-1, PD-L1, TIM3 and LAG3. T cells expanded more with PD-L1 inhibitor during stimulations ($27.5 \times 10^6$ with PD-L1 inhibitor at 10 ug/mL and $19.5 \times 10^6$ without PD-L1 inhibitor). The specificity for PRAME was significantly higher using PD-L1 inhibitor during stimulations ($638.7 \pm 33.9$ SFC/1x$10^5$ cells with PD-L1 inhibitor at 10 ug/mL and $6.3 \pm 2.3$ SFC/1x$10^5$ cells without PD-L1 inhibitor).

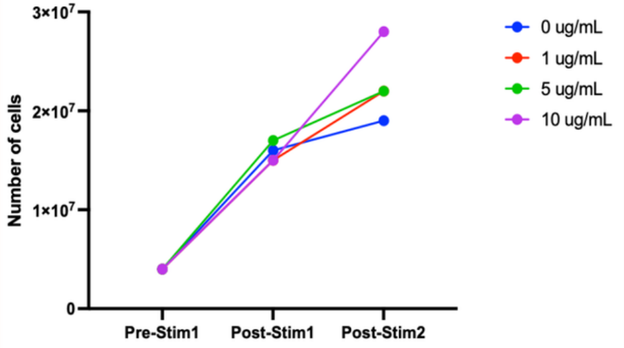


Figure 2. T cell expansion with different PD-L1 concentrations during stimulations

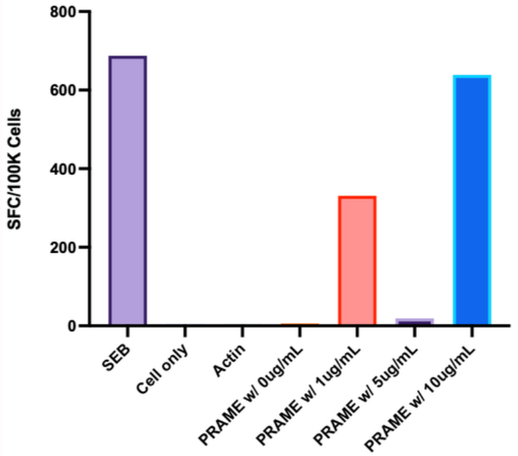


Figure 3. ELISPOT of post-Stim2 TAA-T cells with PD-L1 inhibitor at different concentrations

## Conclusions:

These results indicate checkpoint blockade during T cell stimulations could lead to increased proliferation and specificity of TAA-T cells and possibly shorten the time of TAA-T cell generation. Future work will focus on testing the cytotoxicity of the TAA-T cells generated with checkpoint blockade and trialing different concentrations and combinations (i.e PD-1 and CTLA-4) of the checkpoint inhibitors. We also seek to determine the mechanism of decreased specificity at a certain concentrations of anti-PD-L1.