SYNTHETIC PD-1 CO-STIMULATORY MOLECULE PRESERVES A CYTOLYTIC CAR T CELL IMMUNE SYNAPSE WHILE REDUCING TONIC SIGNALING

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Background It is well established that solid tumors upregulate checkpoint inhibitor ligands to suppress anti-tumoral immunity. This in turn can have a negative impact on the functionality of cell therapeutics such as chimeric antigen receptor (CAR) T cells by promoting exhaustion and cell death. In tumors such as glioblastoma (GBM), pro-inflammatory cytokines produced by T cells further contribute to this feedback loop, resulting in increased expression of PD-L1 and PD-L2. We have developed CAR T cells to modulate PD-1/PD-L1 (or PD-L2) signaling through engagement with a synthetic PD-1 molecule. Here, we hypothesize that the first generation CAR T cells with synthetic PD-1 co-stimulatory molecule form a functional immune synapse with tumor cells while maintaining a more physiological signaling state.

Methods HER2-specific CAR T cells expressing a synthetic PD-1 co-stimulatory molecule were generated from primary human T cells using a bicistronic vector and retroviral gene delivery. Appropriate control T cells were manufactured in parallel from the same donors under similar conditions. CAR T cell and HER2+ LN229-GBM cell conjugates (doublets) were studied using imaging flow cytometry for CAR immune synapse (CARIS) formation and composition. Phosphorylation of CD3ζ was assessed at rest and over time after exposure to tumor cells by western blotting. Supernatant from T cell and HER2+ GBM co-cultures were analyzed using a multiplex assay to evaluate patterns of cytokine release.

Results Imaging of the CARIS revealed that PD-1 is recruited within 60 minutes of CAR T cell and tumor cell engagement. Synthetic PD-1 receptor was recruited to the CARIS within 60 minutes with tumor cells exhibiting constitutive/inducible PD-L1 or PD-L2 expression. Further, first generation CAR T cells with a PD-1 co-stimulatory molecule demonstrated enrichment of actin, indicative of “mature” immune synapse formation, comparable to a second-generation CAR. At baseline, PD-1 co-stimulatory molecule expressing CAR T cells with split signaling exhibit lower phosphoCD3ζ compared to second generation control CAR T cells. Consistent with our imaging findings, CAR T cells with split signaling demonstrated robust CD3ζ phosphorylation similar to that of second generation CAR T cells by 60 minutes. Upon additional assessment, we found that CAR T cells with split signaling showed a pattern of pro-inflammatory cytokine secretion distinct from second generation CAR T cells.

Conclusions Co-stimulation through synthetic PD-1 molecule confers a favorable functional profile to CAR T cells and may enhance their safety, longevity and antitumor function after adoptive transfer.

Ethics Approval This study was approved by Baylor College of Medicine’s Ethics Board; approval number H-15280.