282 DUAL ANTIGEN TARGETING CAR T CELLS ARMORED WITH A DOMINANT-NEGATIVE TGF-β RECEPTOR II ENHANCE ANTITUMOR POTENCY BY OVERCOMING TGF-β IMMUNOSUPPRESSION IN GBM

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Background We have completed two CAR T cell clinical trials for glioblastoma (GBM) and have identified several key challenges to therapeutic efficacy, including the inherently heterogenous genomic landscape and the immunosuppressive tumor microenvironment (TME) found in GBM. Our previous studies showed that EGFR variant III (EGFRvIII)-targeting monovalent CAR T cells reduced target-positive tumor cell populations, but tumor recurrence resulted from target-negative tumor cells, highlighting the limitation of single-target approaches in heterogenous tumors. With regards to the highly immunosuppressive TME in GBM, we found that transforming growth factor-β (TGF-β) is consistently highly expressed in both GBM tumor cell lines and patient tumor tissues. Tumor-derived TGF-β can induce tumorigenesis and present as a major driver of suppression of the anti-GBM response.

Methods We used two parallel scFv constructs, independently targeting both IL13Rα2 and EGFRvIII, in combination with a dominant negative (dn) TGFb receptor II. The CART-EGFR-IL13Rα2-dnTGFbRII construct was designed to explore possible additive effects in both *in vitro* and *in vivo* GBM model systems to limit tumor escape, rescue the immune cells' functions, and overcome the immunosuppressive GBM TME. The CART-EGFR-IL13Rα2-dnTGFbRII construct broadened the targeted tumor cell repertoire, blocked TGFβ signaling, and served as a sink for free TGFβ in the GBM TME to overcome the suppressive function of TGFb.

Results CART-EGFR-IL13Rα2-dnTGFbRII T cells blocked the suppressive pSmad2/3 signaling pathway, leading to increased proliferative activity with repeated stimulation co-culture experiments in the tumor-mimicking conditions when compared to CART-EGFR-IL13Rα2 constructs *in vitro*. Despite initially lower activation levels, CART-EGFR-IL13Rα2-dnTGFbRII enhanced CAR expression and converted CART cells into an effector and effector memory cell phenotype during the intermediate and final cytotoxic stages, respectively, thereby aiding in the eradication of tumor cells. In an immunodeficient mouse model, tri-modular CAR T cells eradicated tumor cells more efficiently and mice exhibited a longer median survival when compared those treated with the bicistronic CART-EGFR-IL13Rα2 cells, lacking the dnTGFb receptor II module.

Conclusions Overcoming the adaptive changes in the local GBM TME and addressing antigen heterogeneity will be required to improve clinical efficacy of CAR T-directed strategies. Our combination work showed that bicistronic CART constructs cooperate with a truncated TGFb receptor II module efficiently. In summary, the dominant-negative TGFβRII CART-EGFR-IL13Rα2 tri-modular structure is a promising strategy to address the clinicopathologic challenges of antigenic heterogeneity and the immunosuppressive TME in GBM we have observed in our two GBM CART cell trials at UPenn.

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