

VASCULAR NORMALIZATION IMPROVES THE DELIVERY AND EFFICACY OF EGFRvIII-CAR-T CELLS IN MOUSE GLIOBLASTOMA

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Background Chimeric antigen receptor (CAR)-T cells has revolutionized the treatment of hematological malignancies.¹ However, they have shown limited or no efficacy in patients with glioblastoma (GBM) or other solid tumors due to poor infiltration into tumors and immunosuppressive tumor microenvironment (TME).^{2,3} We previously showed that blocking vascular endothelial growth factor (VEGF) signaling normalizes tumor vessels, reprograms the immunosuppressive TME into an immunostimulatory milieu, and improves the efficacy of immunotherapy.^{4,5} Here, we tested the hypothesis that anti-VEGF therapy (B20) can improve the delivery and efficacy of CAR-T cells in immunocompetent orthotopic GBM mouse models.

Methods Two syngeneic mouse GBM cell lines (CT2A and GSC005) were used in the study. They were engineered to express EGFRvIII, one of the most common neoantigens in human GBM.^{6,7} CAR-T cells were designed to recognize EGFRvIII. Orthotopically injected, GBM-bearing C57BL/6 mice were treated with B20 (2.5 mg/kg, every 3 days for 4 doses), followed by EGFRvIII-CAR-T injection. We used intravital imaging with two-photo microscopy to track the infiltration of CAR-T cells into the tumor and flow cytometry to measure the number and function of CAR-T and other immune cells.

Results Combination of B20 with CAR-T cell treatment prolonged survival of GBM-bearing mice compared to CAR-T cell therapy alone. Intravital imaging revealed that B20 normalized tumor vasculature (figure 1A) and a combination of B20 increased the number of infiltrated CAR-T cells up to 4-fold compared to the CAR-T therapy without B20 (figure 1B). Flow cytometry analysis resulted in an increased population of IL-2+ or IFN- γ + CAR-T cells, indicating that B20 increased the anti-tumor function of injected CAR-T cells (figure 1C). Moreover, the population of endogenous lymphocytes was increased after B20 therapy. Increased Granzyme B+ TNF- α + CD8 T cells (Cytotoxic T lymphocytes; CTLs) and decreased FoxP3+ CD4 T cells (Regulatory T cells; Tregs) were observed after B20 treatment indicating that the TME was remodeled to increase the effect of CAR-T therapy (figure 1D).

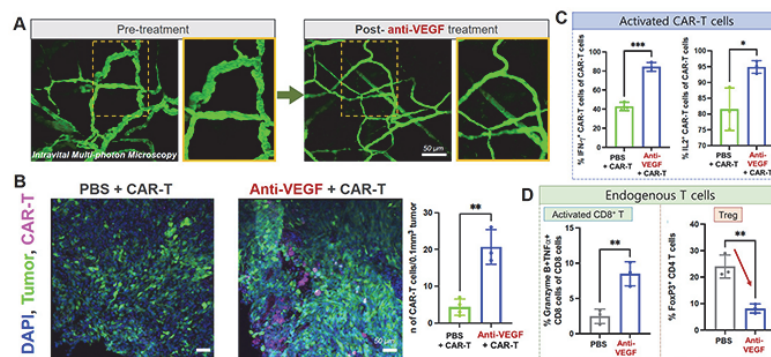
Conclusions Our strategy improved the efficacy of CAR-T therapy in GBM mouse models by increasing the CAR-T cell infiltration and reprogramming TME by increasing the activation of CAR-T cells and endogenous effector T cells.⁸ Our findings provide compelling data and a rationale for the clinical evaluation of combining anti-VEGF agents with CAR-T cells for GBM patients. Furthermore, we are expanding this approach to improve CAR-T therapy for breast cancer brain metastasis, which shares similar immunosuppressive brain TME features.

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Abstract 249 Figure 1 (A) Intravital image by multiphoton microscopy of brain tumor vasculature two days after B20 (2.5 mg/kg) treatment. (B) Intratumoral distribution of EGFRvIII-CAR-T cells inside of GFP-GSC005 GBM tumors imaged by multiphoton microscopy. Images were taken 24 hours after the injection of CAR-T cells. Measurement of CAR-T cell (pink) number in GFP-GSC005 tumors (green) treated with PBS or B20 (2.5 mg/kg). (C) Percentage of IL2+ and IFN- γ + CAR T cells inside the GSC005 GBM tumors. (D) Percentage of the cytotoxic Granzyme B+TNF- α + CD8 T cells and regulatory FoxP3+ CD4 T cells in the GSC005 tumors. Error bars show \pm SEM. Statistical analysis was performed using Student's t-test. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$.