

**TARGET EPHRIN A3 ON CANCER CELLS AND TUMOR MICROENVIRONMENT IN BRAIN CANCER**

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**Background** Glioblastoma multiforme (GBM) is the most lethal form of brain cancer with a median survival of < 2 years despite treatment advances. Evidence indicates that GBM has been shown to reprogram immune responses to promote its progression. A predominant component of the immunosuppressive tumor microenvironment (TME) is glioblastoma-associated macrophages (GAM). GAM represents up to 50% of the bulk cell population in the GBM microenvironment and is crucial in tumor progression and persistence. We and others have shown the expression of the Ephrin A3 (EphA3) receptor in both tumor and GAM. We hypothesize that dual targeting of both the cancer cell and the immunosuppressive TME with EphA3-specific chimeric antigen receptor T (EphA3-CART) cells would enhance antitumor.

**Methods** We first investigated the expression of the EphA3 receptor on GBM patient-derived xenografts using immunohistochemistry (IHC) and Western blot. We then polarize primary monocyte or THP-1 monocytic cell line into M2-like macrophages and investigated the expression of the EphA3 receptor on both flow cytometry and then studied the effect of EphA3-CART against EphA3<sup>+</sup> SNB-19 GBM cell line using the polarized macrophage. We also assessed the antitumor activity of the EphA3-CART in vivo. NSG mice were subcutaneously injected with 1 x 10<sup>6</sup> Luc+ SNB-19 GBM cell line and monitored for tumor growth using bioluminescence imaging (BLI) and/or tumor size using vernier calipers. At BLI of >1 x10<sup>8</sup> p/s and/or tumor volume of 200-300 mm<sup>3</sup>, mice were randomized and treated with 5 x 10<sup>6</sup> EphA3-CAR T cells. And were further monitored by both BLI and/or tumor size using vernier calipers.

**Results** Our immunohistochemical analysis data revealed EphA3 expression on 26.623% of the PDX representing the area of the human cancer tissue and was confirmed using the western blot analysis of the selected GBM PDX lines in our biobank (figure 1). EphA3-CART cells exhibited potent antitumor activity against EphA3<sup>+</sup> GBM cell line SNB-19 (figure 2). M2 polarized macrophage marker expressing a high level of CD206 as well as EphA3 compared to M1 polarized macrophages. EphA3-CART cells were effective in eliminating M2 macrophages (figure 3) and ameliorated M2-induced CART cell inhibition. Finally, EphA3-CART cells exhibited superior tumor control in GBM xenografts compared to the control un-transduced T cells (UTD), (figure 4).

**Conclusions** In summary, we have developed EphA3-CART cells that target both the tumor cells and GAM within GBM TME which holds promise in improving CART antitumor activity within the immunosuppressive solid tumor milieu.

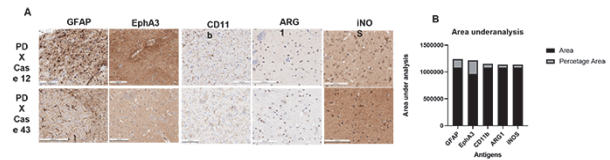


Figure 1. Results of Immunohistochemical analysis of patient derived xenograft (PDX) passed in the nude mice. Photomicrograph (A) and the result of statistical analyses (B) are shown. Immunohistochemical analyses demonstrate high expression levels of GFAP, EphA3, CD11b, ARG1 and iNOS. The EphA3 expression was shown to occupy most area. Original magnification, x20; scale bar, 100 µm.

**Abstract 414 Figure 1** Results of Immunohistochemical analysis of patient derived xenograft (PDX) passed in the nude mice. Photomicrograph (A) and the result of static analyses (B) are shown. Immunohistochemical analyses demonstrate high expression levels of GFAP, EphA3, CD11b, ARG1 and iNOS. The EphA3 expression was shown to occupy most area. Original magnification, x20; scale bar, 100 µm

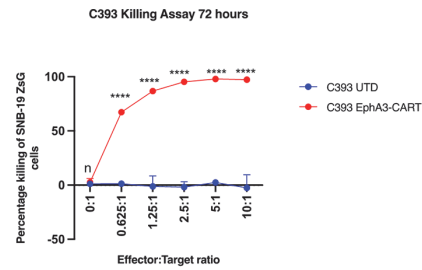


Figure 2. Cytotoxicity assay; SNB-19 ZsG was cocultured with increasing ratios of either EphA3-CART cells or the un-transduced (UTD) T cell control for 72 hours. 2-way ANOVA, \*\*\*\*P<0.0001.

**Abstract 414 Figure 2** Cytotoxicity assay; SNB-19 ZsG was cocultures with increasing ratios of either EphA3-CART cells or the un-transduced (UTD) T cell control for 72 hours. 2-way ANOVA, \*\*\*\*P<0.0001

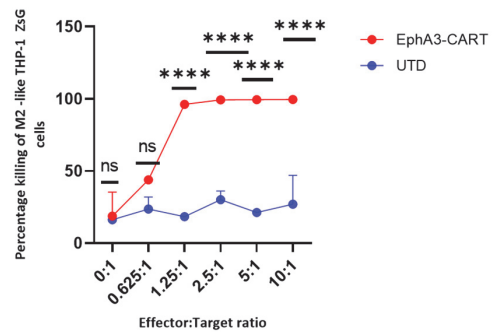
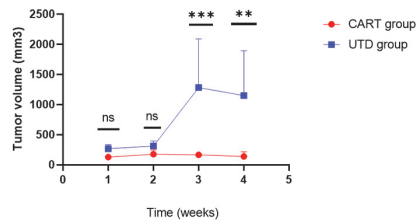


Figure 3. Cytotoxicity assay: M2-polarized THP-1 monocytic cells was cocultured with increasing ratios of either EphA3-CART cells or the un-transduced (UTD) T cell control 72 hours. 2-way ANOVA. \*\*\*\*P<0.0001.

**Abstract 414 Figure 3** Cytotoxicity assay: M2-polarised THP-1 monocytic cells was cocultured with increasing ratios of either EphA3-CART cells or the un-transduced (UTD) T cell control 72 hours. 2-way ANOVA. \*\*\*\*P<0.0001



**Figure 4.** SNB-19 GBM cell line ( $1 \times 10^6$ ) was engrafted subcutaneously to into NSG mice. and When tumor burden reached  $1 \times 10^8$  photon/second, mice were randomized to treatment of  $5 \times 10^6$  cells EphA3-CART UTD. Tumors were monitored with bioluminescence imaging and vernier callipers. 2-way ANOVA. \*\* $P < 0.05$

**Abstract 414 Figure 4** SNB-19 GBM cell line ( $1 \times 10^6$ ) was engrafted subcutaneously to into NSG mice and when tumor burden reached  $1 \times 10^8$  photon/second, mice were randomized to treatment of  $5 \times 10^6$  cells EphA3-CART UTD. Tumors were monitored with bioluminescence imaging and vernier callipers. 2-way ANOVA. \*\* $P < 0.05$

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