CXCL12 Inhibition Modulates The Tumor Microenvironment in Glioblastoma To Potentiate Immunotherapy Response

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Introduction

- Glioblastoma is the most common aggressive primary brain tumor, with a median overall survival of 14-16 months despite heavy treatment.
- Immunotherapy has not been effective in glioblastoma, likely related to an immunosuppressive tumor microenvironment (TME) and impaired immune cell trafficking, manifest as a paucity of effector T cells in the tumor.
- The CXCL12-CXCR4 chemokine axis plays a role in attracting immunosuppressive myeloid cells to TME and excluding effector T lymphocytes, inducing an immunosuppressive TME (Fig 1).
- Glioblastoma TME is rich in CXCL12 and its receptor CXCR4; both correlate with poor prognosis.

Aim

Examine the effect of CXCL12 blockade on tumor immunity in conjunction with immune checkpoint inhibition in the immuno-resistant SB28 mouse glioma model.

Methods

C57BL/6 mice bearing intracranial or subcutaneous SB28 tumors were randomized to 4 treatment groups (n=10 each) (Fig 2,6):

Group 1 : Vehicle

Group 2 : NOX-A12 (CXCL12 inhibitor)

Group 3 : anti-PD1 and anti-CTLA4 (dual ICI),

Group 4 : Combined NOX-A12 and dual ICI (combination)

Mice were followed for survival and tumor growth.

For immune cell characterization, tumors were analyzed by high-dimensional flow cytometry.

Fig 1: CXCL12 Role in Chemotaxis and Polarization

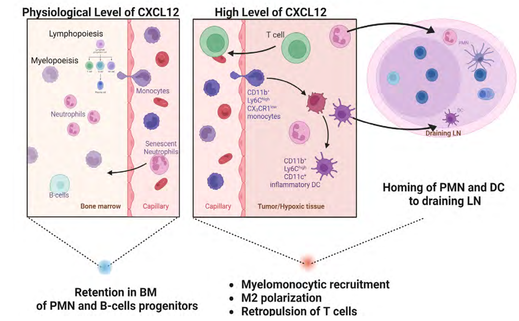
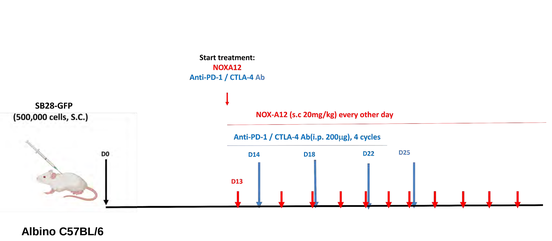
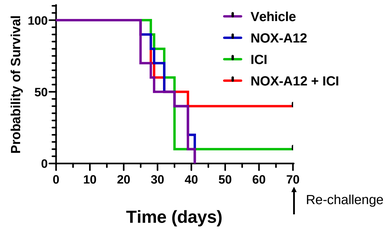


Fig 2: Experimental Design in s.c. Tumor Model



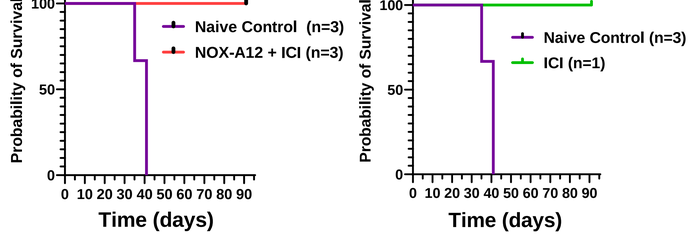
Schematic presentation of experimental design. Mice were injected s.c with SB28 tumor cells. When tumor size reached 6-7mm (day 13) mice were randomized, and treatment started according to the mentioned schedule.

Fig 3: More long-term Survivors with Combination Treatment in s.c. Tumors



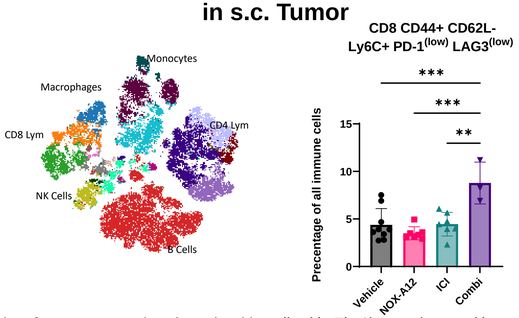
Survival analysis comparison between treatment groups of s.c. tumor bearing mice.

Fig 4: Long-term Survivors are Resistant to Tumor Rechallenge



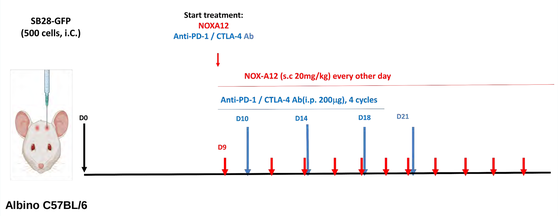
Survival analysis of combination and ICI -treated cured mice (from Fig 3) after s.c. tumor re-challenge compared to age-matched naive mice.

Fig 5: Combination Treatment Increases CD8 Subset in s.c. Tumor



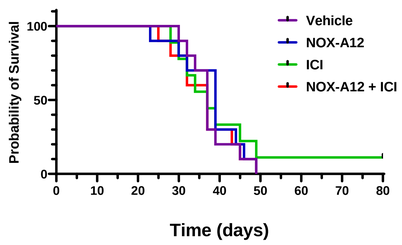
Flow data from s.c. tumor bearing mice (described in Fig 3) at end-stage (time point of euthanization) reveals 20 immune clusters in TME by tSNE analysis (left) performed with OMIQ software (Dotmatics). Comparison of clusters abundance between treatment groups reveals increase in CD8 subset by combination treated compared to ICI treated mice (preliminary results). Statistical analysis used ANOVA with post-hoc Tukey`s multiple comparison test (*P<0.05 ** P<0.005 ***P<0.0005).

Fig 6: Experimental Design in i.c. Tumor Model



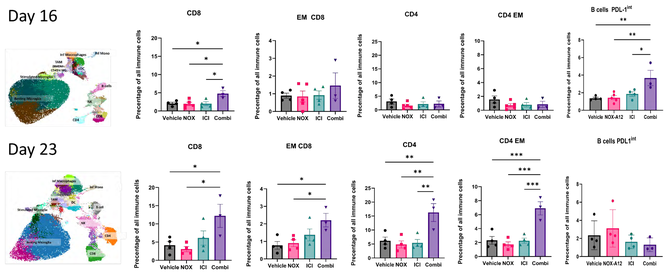
Schematic presentation of experimental design. Mice were i.c. injected with SB28 tumor cells. Treatment (NOX-A12, ICI or combination) started at day 9 after mice were randomized based on IVIS BLI measurement.

Fig 7: No Survival Benefit in i.c. Tumors by Combination Treatment Over ICI



Survival analysis comparison between treatment groups of i.c. tumor bearing mice shows no survival benefit by combination treatment.

Fig 8: Combination Treatment Increases CD4, CD8 and B cells in i.c. Tumor



UMAP clustering done by OMIQ software (Dotmatics) of flow data from i.c. tumor bearing mice at day 16 (top) and day 23 (bottom) presenting 30 immune clusters in TME (left). Comparison of cluster abundance between treatment groups shows significant effects on T and B cell lymphocytes. Statistical analysis used ANOVA with post-hoc Tuckey`s multiple comparison test (** P<0.005 ***P<0.0005).

Conclusions

- The combination of CXCL12 blockade and dual ICI showed a synergistic effect in improving long-term survival and inducing resistance to rechallenge with SB28 s.c tumors.
- While monotherapy with CXCL12 blockade or dual ICI did not affect T cell populations in TME, combining CXLC12 inhibition with ICI increased activated cytotoxic T cells in tumor tissues in both s.c. and i.c. models, demonstrating a synergy between two treatments to induce a favorable TME for anti-tumor immune responses.
- Although the combination treatment enhanced T cell trafficking, it did not improve survival in the i.c. GBM model, suggesting an additional inhibitory mechanism that needs to be targeted to improve survival. .

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