Title: Potentiating the efficacy of immune check-point inhibitors in glioblastoma by inhibition of CXCL12

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BACKGROUND: The tumor immune microenvironment (TIME) in glioblastoma (GBM) is rich in CXCL12, a chemokine known for stimulating angiogenesis. CXCL12 also controls immune cell trafficking and promotes polarization to an immunosuppressive phenotype. We postulated that inhibiting CXCL12 will modulate the immunosuppressive TIME in GBM, thereby augmenting the efficacy of immunotherapy agents like immune checkpoint inhibitors (ICIs). We examined the immunomodulatory and therapeutic efficacy of CXCL12 inhibition, using a small molecule NOX-A12, with and without ICIs in pre-clinical murine GBM models. METHODS: B6 immunocompetent mice bearing intracranial (i.c.) or subcutaneous (s.c.) SB28 tumors received either vehicle, NOX-A12, anti-PD1 and anti-CTLA4 (dual ICI) or NOX-A12 + dual ICI (combination). Tumor growth was measured by IVIS imaging and immune cells in brain were analyzed using high dimensional flow cytometry. Survival was analyzed using Kaplan-Meier curves and log rank test. RESULTS: Combination treatment resulted in tumor regression and long-term survival in 40% of s.c. tumor bearing mice compared to 10% treated with dual ICI only. Three of 4 mice rechallenged with contralateral s.c. tumor remained tumor free while all naive mice reached endpoint. TIME from treated s.c. tumors revealed increase in CD4 and effector memory CD8 T cells by combination treatment compared to dual ICI. In i.c. GBM tumors, while no survival benefit or growth inhibition was seen, combination treatment induced an early increase in effector memory CD8 T cells and PD-L1^{+} B cells, and a later increase in MHC-II^{+} microglia compared to dual ICI. CONCLUSION: Inhibition of CXCL12 enabled the increase of effector cytotoxic T cells by ICI, improving survival in the s.c. GBM model, but not in i.c. GBM model. This might be due to differences in CXCL12 effect between extra and intra-CNS tumor or due to robust immune response causing excessive brain edema and death. These will be further explored in ongoing and future studies.