1042 MODULATION OF IMMUNE LANDSCAPE IN GLIOBLASTOMA BY CXCL12 INHIBITION

[1]Chen Cam-El Makranz*, [2]Meili Zhang, [2]Dionne Davis, [2]Hua Song, [2]Nicole Briceno, [2]Wei Zhang, [2]Mark Gilbert, [2]Masashi Watanabe. [1]*National Institutes of Health, Rockville, MD, USA*; [2]*National Cancer Institute, Bethesda, MD, USA*

Background Glioblastoma is the most common aggressive primary brain tumor, with a median overall survival of 14–16 months despite treatment. Immunotherapy has not been highly effective in glioblastoma, likely related to an immunosuppressive tumor microenvironment (TME) and impaired immune cell trafficking. The CXCL12-CXCR4 chemokine axis polarizes lymphocytes and myeloid cells to their immunosuppressive phenotype and control trafficking of immunosuppressive and effector immune cells to and from TME.[1–7] Glioblastoma TME is rich in CXCL12 and its receptor CXCR4; both correlate with poor prognosis.[8] In this study we aimed to address the role of CXCL12 and its inhibition by NOX-A12 (with and without immune checkpoint inhibitors, ICI) in modulating the immune landscape in mice bearing GBM.

Methods C57BL/6 mice bearing intracranial GL261 tumors (n=40) were randomized to either vehicle, NOX-A12, anti-PD1 and anti-CTLA4 (dual ICI) or combined NOX-A12 and dual ICI, and followed for survival and tumor growth. For immune cell characterization, blood was collected on day 16 (early) and 28 (late); brain extracted at terminal endpoint; and analyzed by 23 color FACS assay.

Results Inhibition of CXCL12 by NOX-A12 resulted in late increase in frequency and CXCR4 expression of peripheral neutrophils, associated with decrease of effector memory CD4 and NK cells in peripheral blood. Dual ICI treatment showed an early increase in peripheral blood monocytes and CD169 expression, with later decreased CD169 and CD206, associated with early increase of both regulatory and effector memory CD4 cells and PD-1 expression, and later increase in CD8 lymphocytes in blood. Combined NOX-A12 and dual ICI caused an early increase in PDL-1 on peripheral monocytes with early MHC-I increase and late CD206 decrease in $Ly6C^{low}$ monocytes and CD169 decrease in $Ly6C^{high}$ monocytes. Brains from terminal endpoint after NOX-A12 showed increased $Ly6C^{low}$ monocytes and B-cells and increased TIM3 on activated CD4 lymphocytes. Compared to ICI, NOX-A12 elevated CXCR4 on CD4 T cells, and combination NOX-A12 and ICI decreased exhausted CD4 cells. However, CXCL12 inhibition did not result in an obvious tumor growth inhibition or survival benefit over ICI treatment in this preclinical model.

Conclusions Inhibition of CXCL12 resulted in a time-dependent array of changes in peripheral blood and tumor-bearing brains but no clear survival benefit. Whether immune-related brain edema, or insufficient activation of immune cells in TME contributed to this result will be clarified by comparing response between intracranial and flank tumor, to better understand the mechanism of immune cell trafficking and polarization mediated by CXCL12 in GBM.

Acknowledgements We acknowledge NOXXON Pharma AG for providing of Nox-A12

REFERENCES

1. De Filippo K, SM Rankin. CXCR4, the master regulator of neutrophil trafficking in homeostasis and disease. *Eur J Clin Invest*, 2018;**48 Suppl 2**(Suppl Suppl 2): e12949.
2. Mercurio L, *et al.* Targeting CXCR4 by a selective peptide antagonist modulates tumor microenvironment and microglia reactivity in a human glioblastoma model. *J Exp Clin Cancer Res*, 2016;**35**:55.
3. Wang Z, *et al.* Carcinomas assemble a filamentous CXCL12-keratin-19 coating that suppresses T cell-mediated immune attack. *Proc Natl Acad Sci U S A*, 2022;**119**(4).
4. Seo YD, *et al.* Mobilization of CD8(+) T Cells via CXCR4 Blockade Facilitates PD-1 Checkpoint Therapy in Human Pancreatic Cancer. *Clin Cancer Res*, 2019;**25** (13):3934–3945.
5. Fearon DT. The carcinoma-associated fibroblast expressing fibroblast activation protein and escape from immune surveillance. *Cancer Immunol Res*, 2014;**2** (3):187–93.
6. Feig C, *et al.* Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci U S A*, 2013;**110**(50):20212–7.
7. Yang J, *et al.* Loss of CXCR4 in Myeloid Cells Enhances Antitumor Immunity and Reduces Melanoma Growth through NK Cell and FASL Mechanisms. *Cancer Immunol Res*, 2018;**6**(10):1186–1198.
8. Yeo ECF, *et al.* The Role of Cytokines and Chemokines in Shaping the Immune Microenvironment of Glioblastoma: Implications for Immunotherapy. *Cells*, 2021;**10**(3).

Ethics Approval All animal experiments and protocols were reviewed and approved by National Cancer Institute-Bethesda Animal Care and Use Committee (IACUC Number: NOB-005–3)

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1042