Background  Glioblastomas (GBM) account for ~60% of adult primary brain tumors. With few advances in therapeutics, median overall survival remains 15-months post-diagnosis. Immunotherapies may provide therapeutic benefit; however, no predictive immune features have informed therapeutic stratification. Radiographic tumor contact with the lateral ventricle (C-GBM) correlates with 7-months worse prognosis compared to patients with ventricle non-contacting GBM (NC-GBM), yet the influence of ventricle contact on anti-tumor immunity is unknown. This study characterized the GBM immune microenvironment and identified targetable mechanisms of immunosuppression correlating with worse outcomes in C-GBM patients.

Methods  Primary glioblastoma tissue was provided with written informed consent in accordance with the Declaration of Helsinki and with approval of the Vanderbilt Institutional Review Board (IRB #131870). Seventeen patients presented with primary, IDH-wildtype C-GBM and 15 with NC-GBM. Machine learning integrated 1) mass cytometry immunophenotyping, 2) metabolic phenotypes, 3) immune cytokine response patterns and induced intracellular signaling networks, and 4) matched multiplex immunohistochemistry on FFPE embedded tissue to identify phenotypic, functional, and spatial biomarkers correlating with patient outcome.

Results  C-GBM tumors were enriched in STAT3-driven CD32 +CD44+HLA-DR+ monocyte-derived macrophages (MDM) compared to NC-GBM (19 ± 8% vs. 6 ± 2%; p<0.001) and depleted in lymphocytes including subsets of T, B and NK cells (2.9 ± 1% vs. 7.6 ± 2%; p<0.001) and tissue-resident microglia (1.8 ± 0.3% vs. 7 ± 3%; p<0.001). Moreover, 45% of exhausted T cells in C-GBM co-expressed the checkpoint receptors PD-1 and TIGIT despite exhibiting metabolic activity consistent with retained functional capacity. As an orthogonal approach, we used multiplex IHC to identify the spatial distribution of immune cells throughout the GBM tumor tissue. K-means clustering identified 10 immunological niches in GBM tumors. Macrophage-tumor niches were most common in C-GBM (17.93% of niches), followed by T cell-microglia-tumor niches (17.72%). Within NC-GBM niches, T cell-T cell interactions were more prevalent in NC-GBM tumors (log odds ratio = 0.90) and correlated with improved survival outcome.

Conclusions  These findings suggest that factors within the periventricular space negatively influence the immune microenvironment within GBM tumors. Clinically targetable immune biomarkers (e.g. PD-1) were identified in C-GBM. Notably, this work highlights the potential impact of radiologic assessment of lateral ventricle contact as a guide for clinical trial design for immunotherapies in neuro-oncology based on tumor proximity to the lateral ventricle wall.

Ethics Approval  Primary glioblastoma tissue was provided with written informed consent in accordance with the Declaration of Helsinki and with approval of the Vanderbilt Institutional Review Board (IRB #131870).

Consent  No sensitive or identifiable information is included in this study.