

SPATIAL-TRANSCRIPTOMIC ANALYSIS OF NEOADJUVANT CHECKPOINT IMMUNOTHERAPY IN RECURRENT GLIOBLASTOMA

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Background Glioblastomas (GBMs) are a prevalent form of brain tumor with median overall survival of 1-1.5 years, and inevitable recurrence after initial surgical resection and chemotherapy. Less than half of patients are eligible for surgery at recurrence.¹ In many other cancers, checkpoint immunotherapies are efficacious, but GBMs resist treatment. Multiple resistance mechanisms may be at play and overcoming them is essential to improve immunotherapy response. The high heterogeneity of GBM, both in cellular phenotype and immune landscape, likely contributes to resistance. Although the GBM tumor-immune transcriptome is well-studied, spatial organization is an open frontier.

Methods Spatial transcriptomic samples were analyzed from a two-arm randomized trial for recurrent GBM patients (NCT04201873). One arm received neoadjuvant and adjuvant PD-1 mAb (“neoadjuvant” group), while the other received placebo (“placebo” group). Both groups received adjuvant autologous tumor lysate-pulsed DC vaccination. Tissue samples (N=10) were taken from on-study surgical resection, with 4 patients in the neoadjuvant group, 6 in placebo. The Visium spatial assay was applied to produce 3,140 +/- 969 transcriptomic “spots” per sample, 31,395 in total. Gene signature analysis quantified each spot’s tumor-immune composition. Signatures were obtained from MSigDB Hallmarks, ImSig,² and Neftel et. al.³ AUCell⁴ was used to score signatures at each spot.

Results Analysis of glioma subtype signatures showed mesenchymal scores (MES1 and MES2) highly correlated [Pearson coefs. > 0.77], while astrocyte (AC), oligodendrocyte precursor (OPC), and neural precursor (NPC1 and NPC2) scores formed a second correlated group [Pearson coefs. > 0.5]. There was no discernable difference in correlation structure between neoadjuvant and placebo patients.

MES1-high spots had a characteristic signature of high macrophage, hypoxia, angiogenesis, glycolysis, interferon-gamma response, TGF-beta, and TNF-alpha scores [Pearson coefs. > 0.5]. AC, OPC and NPC-high spots correlated weakly to immune and vascular signatures [Pearson coefs. < 0.35]. Microglia did not correlate highly to other scores [Pearson coefs < 0.35].

Differential expression showed negative log fold-change (logFC) in glioma subtype scores for neoadjuvant patients, for all types except NPC2 [Wilcoxon tests, p < 0.001]. Negative logFC was observed for macrophages, TNF-alpha, hypoxia, and proliferation, but positive logFC for T-cells and microglia.

Conclusions Spatial-transcriptomic analysis suggests that patients treated with neoadjuvant immunotherapy have lower mesenchymal subtype scores, with lower proliferation, but higher T-cell scores. Subsequent analysis will relate neoadjuvant immunotherapy with spatial heterogeneity of T-cells, myeloid subtypes, glioma subtypes, and vascularization, to understand resistance mechanisms.

Trial Registration NCT04201873

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