

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]. AUCell [4] 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 [Wilcox tests, p < 0.001]. Negative logFC was observed for macrophages, TNF-alpha, hypoxia, and proliferation, but positive logFC for T-cells and microglia.

Conclusion

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.

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Insights from Single Cell RNAseq

A public dataset of 1.1M GBM associated single cell transcriptomes [6] was analyzed to explore correlations between GBM phenotype, immune, and tumor hallmark signatures. Five major cell types were considered (Fig. 1). Gene expression signature scores were computed across malignant cells, and their correlation structure was explored (Fig. 2). Broad trends included:

Figure 1

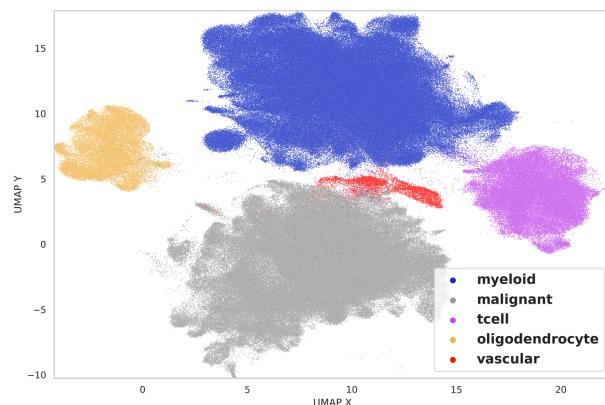
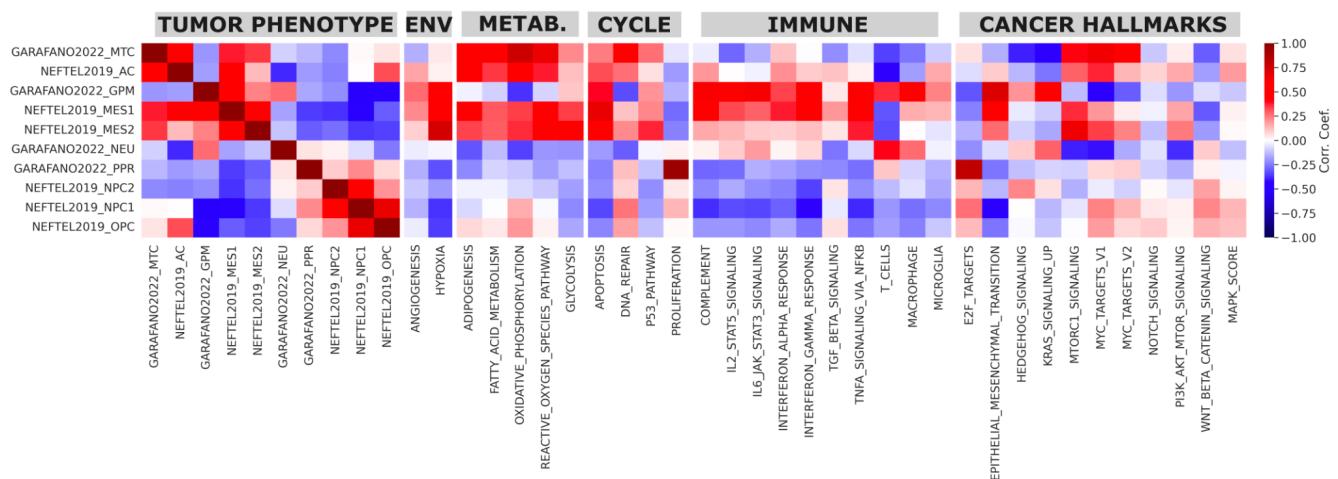


Figure 2



Mesenchymal: MES [3] and GPM [7] signatures correlated with hypoxia, apoptosis, and interferon response. Glycolytic MES types had upregulated macrophage genes, and protein synthesis through MTOR. GPM anti-correlated with OXPHOS, but had elevated KRAS activity.

Proneural/OPC: scores for NPC1, NPC2 and OPC [3] correlated with each other, but not other signature scores.

Neural/Proliferative: the NEU type [7] correlated with T cell expression markers.

Gene Signature Analysis of Visium Spots

Figure 3 and 4

Visium data for 10 subjects was processed using STLearn. Each spot was represented by 41 gene signature scores, representing GBM phenotype, immune cells, and environment. Spots were aggregated across subjects, integrated using Harmony, and clustered at low resolution.

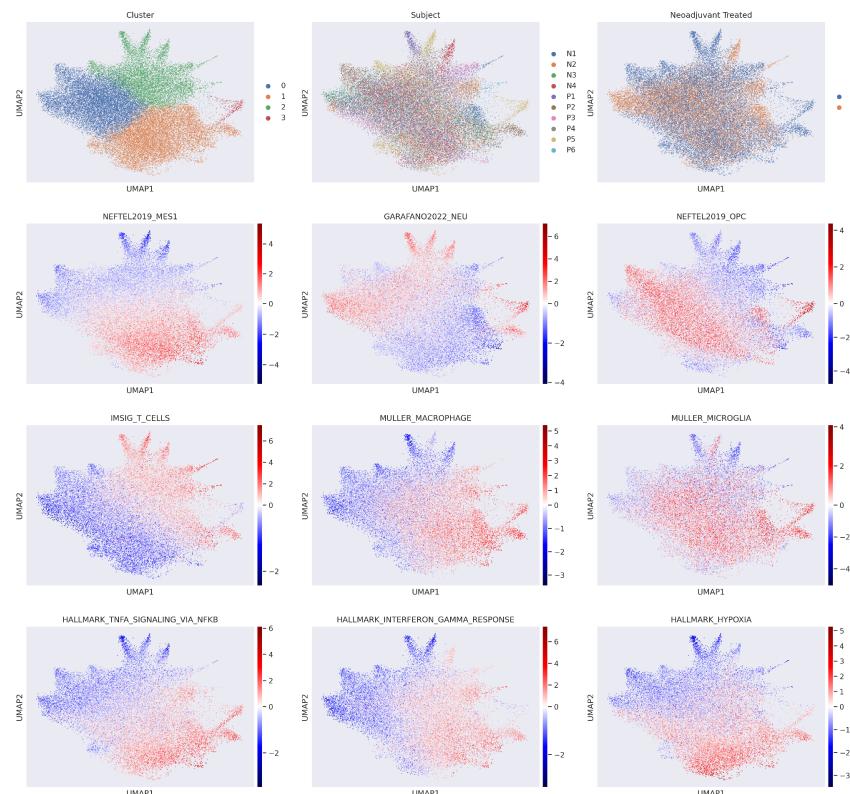
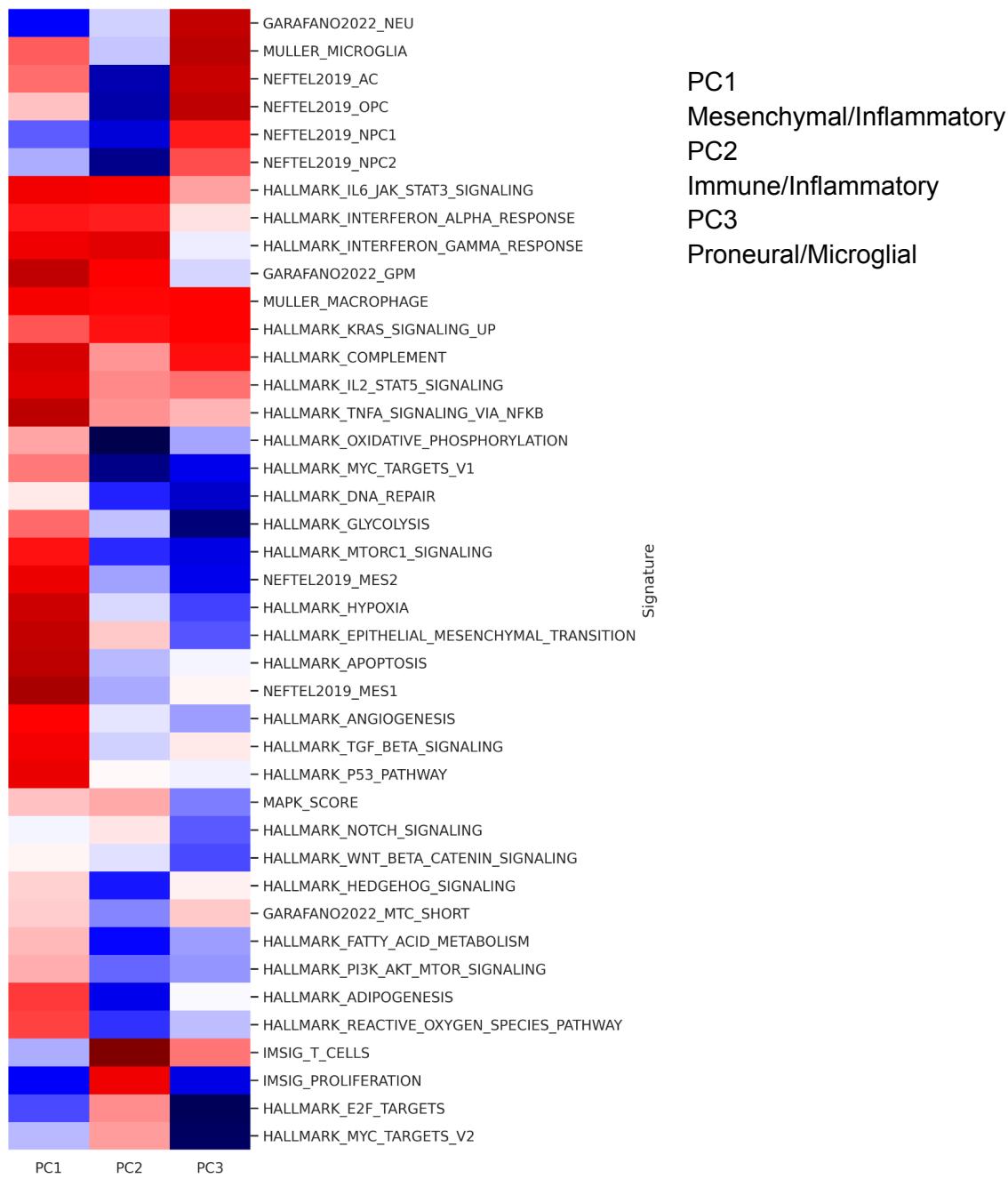


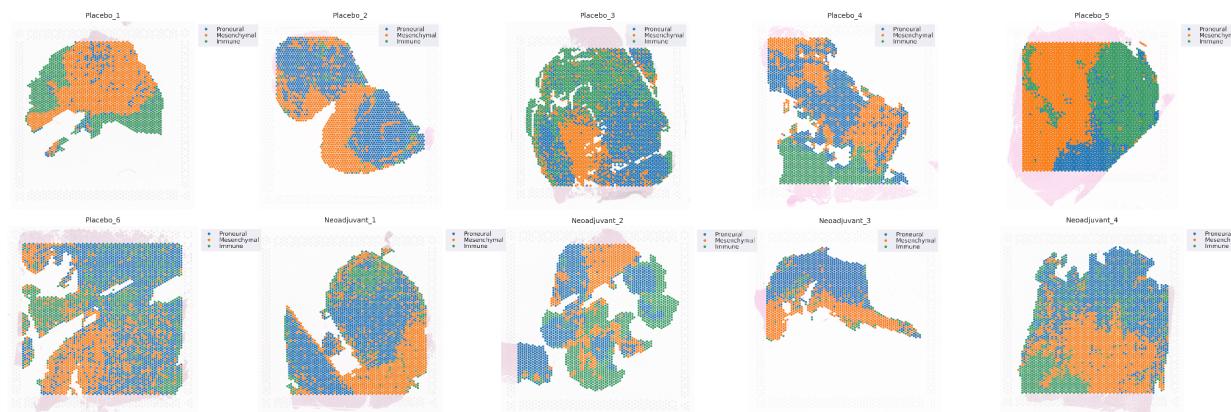
Figure 3 shows UMAP projections of spots with scores. Data roughly clustered along a mesenchymal / proneural axis. Scores highly correlated, and PCA was applied to compactly describe expression profiles (Figure 4):



Distinct Spatial Domains Observed

Figure 5

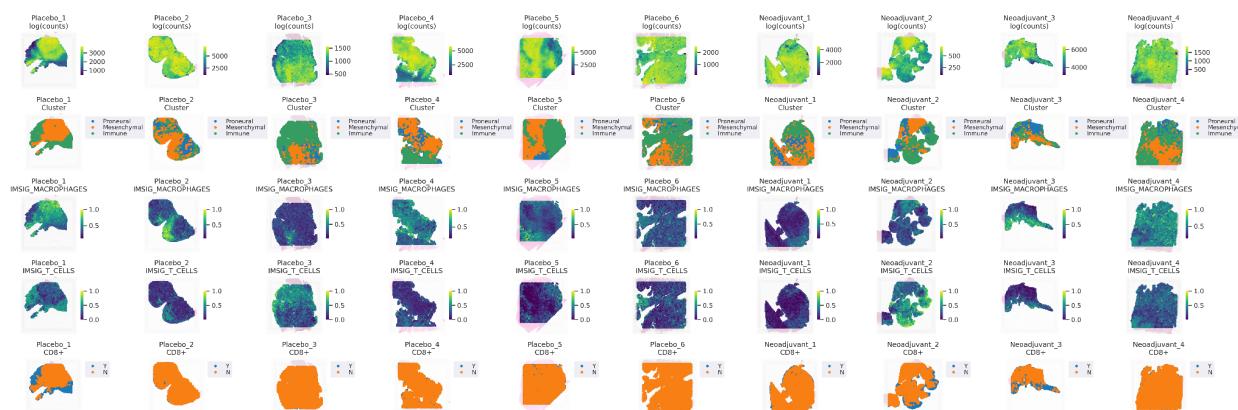
Differential expression of principal components on the spot clusters showed them to be dominantly Proneural (PC 3), Mesenchymal (PC 1), or Immune (PC 2). Spatial maps of clusters are shown in Figure 5. Immune spots were more likely to have a Proneural than Mesenchymal neighbor (average neighbor probabilities of 0.24 vs 0.16; Wilcoxon signed rank test, n=10, p=0.02).



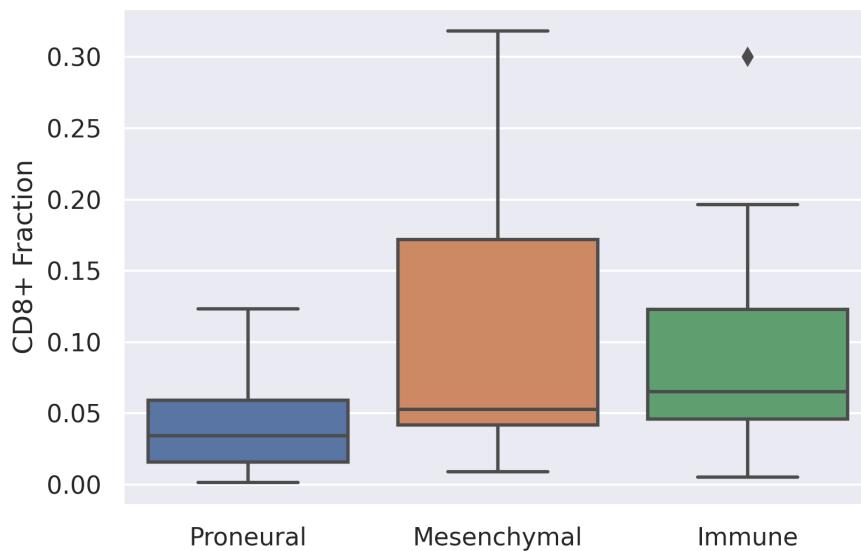
Spatial Distribution of T-cells

Figure 6 and 7

Total log counts for a visium spot negatively correlated with T cell signature score (Pearson coef.=-0.63). Potential dropout effects in low count spots challenged for identification of T cell enrichment. A tumor-infiltrating CD8 T-cell score was computed and binarized [8]. Figure 6 shows scores and the CD8+ measure ('Y'=present) for each subject.



A naive differential expression model using each spot as a sample showed higher logFC for T cell score, as noted in the abstract. However, a more careful study using CD8 fraction, accounting for a different number of spots per subject, did not demonstrate statistically significant effects on CD8+ fraction for neoadjuvant treatment (n=10, linear mixed effects, p=0.90).



When CD8+ fraction was compared across subjects, taking into account cluster, Mesenchymal cluster spots appeared to have a slightly higher CD8+ fraction than Immune or Proneural (Figure 7; n=10*3, linear mixed effects model, p=0.03 for Mesenchymal, p=0.09 for Proneural/Immune).

Conclusions

Single cell transcriptome analysis showed tumor signatures to correlate within mesenchymal and proneural phenotypes. Mesenchymal phenotypes showed clear hypoxic, glycolytic, and also macrophage signatures.

Signature analysis of Visium data from recurrent GBM patients clustered into three broad groups - proneural, mesenchymal, and immune. These cluster types formed spatially distinct domains. Immune domains were more likely to spatially associate with proneural domains.

Analysis of T-cell fraction across subjects did not show a significant effect due to neoadjuvant immunotherapy. However, regions characterized as mesenchymal showed a slightly higher T-cell fraction.

Acknowledgements and References

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Poster materials and references are available at:

https://github.com/ParkerICI/sitc2022_brain_tumor_poster