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LONGITUDINAL SPATIAL PROFILING OF PEDIATRIC HIGH-GRADE GLIOMA REVEALS A HETEROGENEOUS AND DYNAMIC IMMUNE MICROENVIRONMENT WITH THERAPEUTIC IMPLICATIONS

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Background Pediatric high-grade glioma (pHGG) is a deadly childhood cancer, with five-year survival under 20%. Conventional therapy consisting of radiation and, where possible, surgical resection remains the standard of care. Successful immunotherapy has been limited in pHGG, hindered by an incomplete understanding of the molecular mechanisms driving an immunosuppressive microenvironment. We investigated tumor-immune interactions and immune cell infiltration with single-cell and spatial resolution, with the goal of informing improved immunotherapies in pHGG.

Methods We studied pHGG tumors from seven patients, with four of seven patients sampled at two timepoints, before and after conventional therapy. Highly multiplexed immunofluorescence imaging was performed measuring >50 protein markers at single-cell resolution using the PhenoCycler-Fusion platform (Akoya Biosciences).

Results Gliomas are highly heterogeneous, with four tumor-intrinsic cellular states previously identified in adult HGG that recapitulate the cellular ontogeny of the developing brain: astrocyte-like (AC), mesenchymal-like (MES), neuronal precursor cell-like (NPC), and oligodendrocyte precursor cell-like (OPC). Most tumors contained all four states at varying proportions, confirming the applicability of this model to pHGG. These cell states underscored substantial within tumor and between patient heterogeneity. Furthermore, multiplexed imaging analysis revealed spatial segregation of tumor states, suggesting that anatomic context may influence tumor cell heterogeneity. Although tumor cell state composition changed considerably in some patients following conventional therapy, no single state was consistently favored across patients, suggesting that personalized therapies might be required to account for tumor heterogeneity and evolution.

We next asked if immune cell infiltration varied between patients and how this may be associated with tumor cell states. Resident microglia were enriched in normal brain, whereas bone marrow-derived macrophages (BMDM) were enriched in tumor areas. Tumor/normal boundaries represented a distinct immune microenvironment with altered myeloid cell infiltration. In contrast, CD3+ T cell infiltration was variable across patients, but generally low, with a substantial fraction of T cells associated with vasculature and with minimal penetration into the tumor. Distinct tumor cell states were associated with differential expression of immune checkpoint markers. For example, CD44, a marker of the MES cell state, was associated with higher co-expression of CD47.

Conclusions Although traditionally described as immunologically 'cold' tumors, our studies reveal a complex and dynamic immune microenvironment in pHGG. The heterogeneity of pHGG tumors, including tumor cell state, checkpoint marker expression, and immune cell infiltration, represents a challenge for effective and durable immunotherapy. This study provides a foundation for rational design of personalized immunotherapies that target the unique microenvironment of pHGG.