Abstract #38 | Poster

Zonation mapping of the neuro-immune-tumor crosstalk in primary central nervous system lymphoma via spatial transcriptomics

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Introduction
Primary central nervous system lymphoma (PCNSL) is an aggressive and rare subtype of diffuse large B-cell lymphoma (DLBCL). Despite advances in the genetic subtyping of systemic DLBCL, the spatial architecture, ecotypes, and cellular origins of PCNSL remain incompletely understood.

Methods
We used in situ spatial transcriptomics (10X Genomics, Xenium), employing a a panel of 370 pre-designed and 50 custom genes, to profile five PCNSL specimens. This enabled us to perform high-resolution spatial mapping of single cells. We identified major major cell clusters using published markers and through integration with our in-house single-nucleus RNA sequencing (snRNA-seq) data. We then performed level-2 annotation of malignant B cells and T cells.
To characterize the cellular organization within the tumor microenvironment systematically, we developed a generalized zonation analysis framework that captures continuous and discrete niche architectures. This approach integrates spatial coordinates and cell type annotations to project all samples into a unified reduction map, enabling cross-sample comparability. Using this framework, we performed zonation-aware clustering, trajectory inference, and quantitative analyses of cellular abundance and morphology.

Results
We profiled approximately 2.8 million cells, including the following: neurons (n = 13,411), glial cells (n = 761,443), tumor-associated macrophages (n = 496,646), fibroblasts (n = 204,711), T cells (n = 147,166), dendritic cells (n = 66,515), malignant B cells (n = 1,628,793), and other cell types. Subclustering of malignant B cells and T cells revealed distinct transcriptional subtypes based on genetic signatures. These subtypes were further validated through marker staining and cellular morphology. This approach led to the identification of novel clusters, including a previously unrecognized necrotic tumor subtype characterized by CENPV expression. Our zonation analysis further demonstrated that tumor cells, macrophages, and T cells are not randomly distributed. For example, neurons were found to predominantly interact with active tumor regions but were excluded from necrotic zones.

Keywords
PCNSL, spatial transcriptomics, tumor microenvironment, neuro-immune-tumor interactions, zonation analysis