Abstract #46 | Poster

Single-nucleus RNA-sequencing of central nervous system lymphoma (CNSL)

Y. Su[1, 2], H. Dong[2], F. Ullrich[1], K. Keyvani[3], S. Hartmann[4], C. Reinhardt[1], B. V. Tresckow[1], F. Rambow[2], C. Mlynarczyk[5], E. Kocakavuk[1, 2]

[1] *University Hospital Essen, Department of Hematology and Stem Cell Transplantation, Essen, North Rhine-Westphalia, Germany*
[2] *University Hospital Essen, Institute for AI in Medicine, Essen, North Rhine-Westphalia, Germany*
[3] *University Hospital Essen, Department of Neuropathology, Essen, North Rhine-Westphalia, Germany*
[4] *University Hospital Essen, Department of Pathology, Essen, North Rhine-Westphalia, Germany*
[5] *Yale University, Yale School of Medicine, New Haven, Connecticut, United States of America*

Introduction
Central nervous system (CNS) lymphoma is a rare, aggressive malignancy with a poor prognosis. Limited patient availability restricted our comprehensive understanding of the disease. We thus developed a sophisticated multi-omics platform to characterize malignant B-cell states and tumor-microenvironment interactions, with emphasis on cancer-immune and cancer-neuronal interaction axes.

Methods
We integrated three publicly available single-cell RNA sequencing datasets of CNS lymphoma with our *in-house* cohort (n = 8). After Harmony integration, we performed quality control and annotated cell types using cell markers and unsupervised clustering. Then, we inferred cell-to-cell communication with inferred copy number variations using inferCNV and CopyKat to identify malignant B cells. We also used non-negative matrix factorization (NMF) to discover B cell subclusters. Next, we plan to incorporate whole-genome and whole-exome sequencing datasets (n = 288) in the future.

Results
Our preliminary analysis successfully integrated public and *in-house* cohorts without significant batch effects. After integrating and filtering out potential doublets, we retained over 100,000 high-quality cells. Then, we corrected batch effects using Harmony and performed unsupervised clustering, and identified major cell types including T cells (n = 39,145), B cells (n = 90,809), neurons (n = 5,012), myeloid cells (n = 13,840), and smaller cell populations. Cell type proportions varied greatly across samples, indicating extensive inter- and intratumoral heterogeneity. One outlier sample with low tumor content stood out due to its large neuronal (>70%) cell population, indicating a low tumor purity sample. Unsing inferCNV, we observed loss of the HLA-region on chromosome 6 and widespread gain on chromosome 12, which were consistently altered among many samples. We further deconvoluted the malignant tumor cells into different subclusters with different characteristics. Using NMF, we identified seven meta programs with significant differences among proliferating/cycling B cells, germinal center-like B cells, and plasmablast-like cells.

Outlook
We have successfully generated single-nucleus RNA-sequencing data from challenging tissue samples of CNSL patients and integrated these data with publicly available datasets. Our goal is to gain a comprehensive understanding of cancer-neuron-immune interactions and link these data with genetic and clinical variables.