TUMOR MICROENVIRONMENT PROFILING IN RELAPSED/REFRACTORY ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA USING CODEX MULTIPLEX IMAGING

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Introduction

Nodal T follicular helper cell lymphoma, angioimmunoblastic type (nTFHL-AI), is a peripheral T-cell lymphoma where the tumor microenvironment (TME) contributes to disease progression and therapy resistance. The role of Epstein-Barr virus (EBV)-positive B cells, identified by EBER in situ hybridization, in modulating the TME remains unclear.

Objectives/ AIM

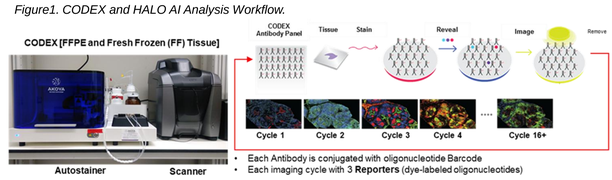
- To characterize the immune microenvironment in relapsed/refractory AITL.
- To explore the spatial and phenotypic differences based on EBER (EBV) status.
- To assess the utility of CODEX multiplex imaging in clinical TME studies.

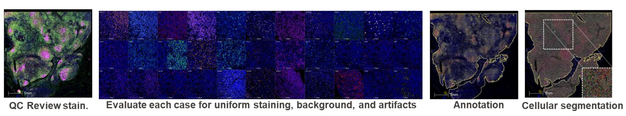
Methods

Table 1. Correlation with clinicopathological characteristics of Nodal T Follicular Helper Cell Lymphoma, Angioimmunoblastic Type.

Diagnosis	Sex	Age	Vital Status	EBER	p53 Mutated	RHOA mutated	Response
AITL1	Female	67	Died	Positive	Yes	No	Responder
AITL2	Male	65	Died	Positive	Yes	No	Non-responder
AITL3	Female	80	Alive	Positive	No	No	Responder
AITL4	Male	64	Alive	Negative	No	No	Responder
AITL5	Female	70	Alive	Negative	No	Yes	Responder
AITL6	Female	32	Alive	Positive	No	No	Responder
AITL7	Female	77	Died	Positive	No	No	Responder

Figure1. CODEX and HALO AI Analysis Workflow.





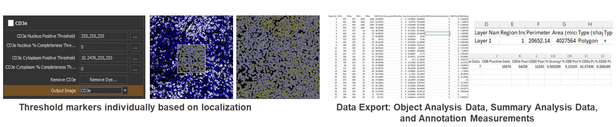
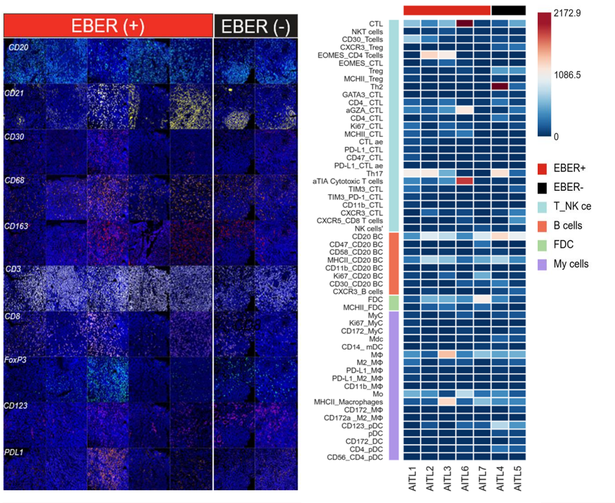
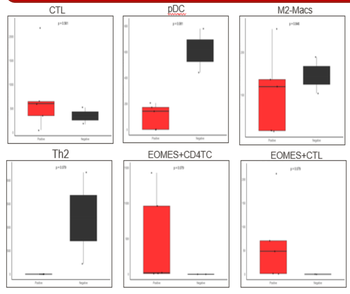


Figure2. Representative multiplex immunofluorescence (mIF) images (left) showing expression of CD20, CD21, CD30, CD68, CD163, CD3, CD8, FoxP3, CD123, and PD-L1 in AITL cases stratified by EBER status. Heatmap (right) summarizes quantitative analysis of immune and stromal phenotypes across individual cases (ATL1–ATL7), highlighting differences between EBV-positive and EBV-negative groups.

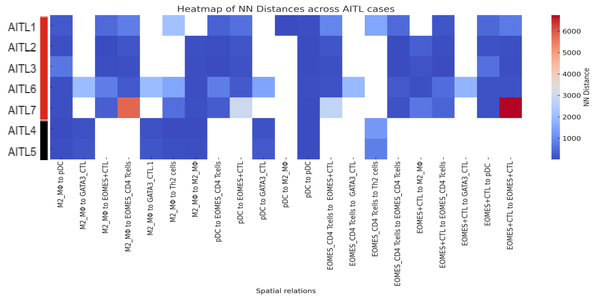


Results



EBER⁺ :
↑ CD4⁺EOMES⁺,
↑ CD8⁺EOMES⁺,
Trend toward ↑ CTL.

EBER⁻:
↑ Th2 cells,
↑ CD68⁺CD163⁺
↑ CD123⁺ pDCs



- EBER⁺ cases (AITL1–3, 6, 7): Demonstrated reduced NN distances, reflecting closer spatial associations among multiple immune cell phenotypes.
- EBER⁻ cases (AITL4–5): Exhibited longer NN distances, consistent with less compact cellular interaction

Summary

- In this study of seven angioimmunoblastic T-cell lymphoma (AITL) cases, we observed distinct immune microenvironment profiles according to EBV status. EBER⁺ cases (n=5) were enriched in EOMES⁺ CD4⁺ and CD8⁺ T cells, while EBER⁻ cases (n=2) demonstrated higher densities of GATA3⁺ Th2 cells, CD68⁺CD163⁺ macrophages, and CD123⁺ plasmacytoid dendritic cells. Overall, these findings highlight the heterogeneity of the AITL microenvironment and underscore the importance of considering EBV status in both biological investigations and clinical trial design.

Discussion

- Our findings suggest that EBV fundamentally reshapes the tumor microenvironment in AITL, driving two distinct immune landscapes.
- In EBER⁺ cases, chronic viral stimulation may promote T-cell exhaustion and immune suppression, while in EBER⁻ cases, the disease appears to evolve within a Th2/pDC/macrophage-enriched context driven by intrinsic TFH biology.
- Although the sample size is small (n = 7), limiting statistical power, these observations are biologically meaningful and hypothesis-generating.
- They support the idea of EBV-stratified therapeutic strategies, with checkpoint blockade potentially more relevant for EBV-positive cases, and Th2/myeloid-targeted therapies being of interest in EBV-negative cases. Validation in larger cohorts is warranted.

References

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