HIGHLY MULTIPLEXED, SINGLE-CELL SPATIAL PHENOTYPING OF EPSTEIN-BARR VIRUS ASSOCIATED LYMPHOMAS

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The Epstein-Barr virus (EBV), a group 1 carcinogen, is a causative factor in nine different cancers causing ~165,000 deaths globally each year. Almost all adults carry EBV asymptomatically with the virus persisting primarily in rare memory B cells for the lifetime of the host. However, in some infected individuals, for reasons that are unknown, EBV may contribute to the development of several malignancies including classical Hodgkin's Lymphoma (cHL), Diffuse-Large B Cell Lymphoma (DLBCL) and Burkitt Lymphoma (BL) among others. There is already evidence from cell-line studies to suggest that the tumour microenvironments of these diseases regulate viral gene expression and vice versa. However, a high-plex unbiased quantification of primary EBV-associated tumours has yet to be explored which could potentially unlock specific therapeutic targets for virus-associated cancers. Here, we have deployed a spatial biology approach for comprehensive study of the immune microenvironment of EBV-infected lymphomas. Using AKOYA's CODEX system, we designed and implemented a 40-plex antibody panel detecting biomarkers *in situ* at single cell resolution (Figure 1). The panel contains EBV specific antibodies targeting latent viral infection, as well as different immune cell lineages, cell activation states, immune checkpoints and tissue structures. This design allows simultaneous identification of infected tumour cells within their microenvironment, while, crucially, maintaining spatial relationships between them. Our data will provide an initial understanding of how the immune microenvironment of virus-infected cells influences tissue pathology and, ultimately, the development of cancer and its resistance to therapy.

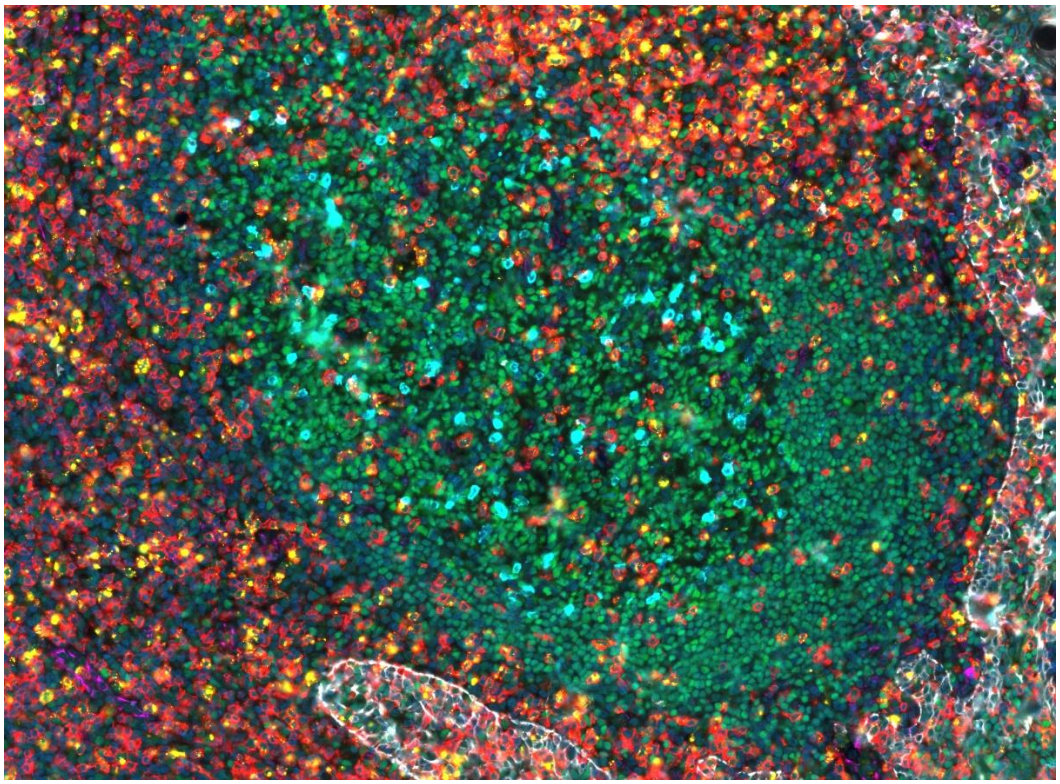

Figure 1: 7-colour image (40-plex antibody panel) of human infectious mononucleosis tonsil.