DISSECTING THE SPATIAL HETEROGENEITY OF SARS-CoV-2-INFECTED TUMOUR MICROENVIRONMENT REVEALS A LYMPHOCYTE-DOMINANT IMMUNE RESPONSE IN A HBV-ASSOCIATED HCC PATIENT WITH COVID-19 HISTORY

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Background We previously reported the presence of SARS-CoV-2 RNA in the hepatic tissues of recovered patients¹ but the spatial immune profile of SARS-CoV-2 infection remains poorly understood. To address this, here we performed deep spatial profiling in tumour-adjacent normal hepatic tissue from a HBV-associated hepatocellular carcinoma (HCC) patient with history of COVID-19.

Methods We obtained tissue from curative resection of a HCC patient 85 days post-recovery from COVID-19. Spatial immune profiling was performed by multiplex immunohistochemistry (mIHC)² and more deeply using the Visium spatial transcriptomics platform complemented with signatures derived from single-cell RNA sequencing (scRNA-seq) and published signatures.

Results SARS-CoV-2 nucleocapsid and spike proteins were detected in a tumour-adjacent normal hepatic section in a spatially-restricted pattern (figure 1A and B) and higher abundance of lymphocytes but not macrophages were observed in regions with virus detection (figure 1C). We employed spatial transcriptomics and scRNA-seq to further characterize the immune microenvironment of SARS-CoV-2 post-infection. Unsupervised clustering and automatic annotation³ of Visium spots revealed that the distribution of SARS-CoV-2 viral proteins partially coincided with a memory T-cell signature (figure 1D). Quantification of Visium transcriptomic spots using an independent transcriptomic signature based on genes differentially upregulated in immune cells in SARS-CoV-2 infection⁴ (figure 1E) resulted in an enrichment pattern similar to the SARS-CoV-2 protein distribution. Additionally, a signature derived from scRNA-seq of hepatic tumour-infiltrating lymphocytes after *ex vivo* peptide stimulation using a pool of SARS-CoV-2 peptides showed a strongly associated distribution, in line with a SARS-CoV2-specific immune response⁵ whereas that from using a pool of HBV peptides resulted in an anti-correlated distribution (figure 1F). These illustrate the ability of spatial transcriptomics to quantify with microenvironment-level resolution the SARS-CoV-2-specific immune response. Recapitulating the mIHC protein data, deconvolution of immune populations⁶ revealed marked spatial associations between SARS-CoV-2 viral presence and the distributions of lymphocytes but not of macrophages (figure 1G).

Conclusions We believe this is the first deep profiling report of non-post-mortem samples which adopts a multi-modal approach combining mIHC, spatial transcriptomics, and transcriptomic signatures derived from scRNA-seq to interrogate the *in situ* immune response to viral infection. Applying this to SARS-CoV-2 infection, we detected tissue spatial heterogeneity in viral presence and an associated lymphocyte-dominant immune response in the COVID-19-recovered patient, in contrast to post-mortem observations of scarce lymphocytes in cases of severe COVID-19.⁷ Ongoing work including further

validation of the findings in local and overseas cohorts and their correlation with patient clinical outcomes.

Abstract 626 Figure 1 Spatial heterogeneity of SARS-CoV-2 infection uncovers an association with a dominant lymphocytic response

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