221 DIGITAL ASSESSMENT OF STROMAL B CELL AGGREGATES AS A BIOMARKER OF RESPONSE TO IMMUNE CHECKPOINT INHIBITION IN UNRESECTABLE MELANOMA

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Background Tertiary lymphoid structures (TLSs) have been associated with response to immune checkpoint inhibitors (ICIs) in multiple solid tumors.[1–3] TLS definitions have varied across reports and TLS identification has typically required expert pathologist assessment, introducing subjectivity and variability. Multiplex immunofluorescence (mIF) analysis can provide quantitative measures of the frequency and spatial orientation of immune cell populations, with the potential to inform standardized biomarkers and more objective reporting of TLS-like features. Using a 12-plex mIF panel, we evaluated the frequency and spatial proximity of stromal $CD20^+$ B cells as predictive biomarkers of ICI response in melanoma, and compared their performance to that of pathologist-assessed lymphoid aggregates (PALA).

Methods Pre-treatment whole-tissue sections from 53 ICI-treated patients with unresectable melanoma were stained with a mIF panel including CD8, CD3, CD20, PD1, PD-L1, CD68, FoxP3, TCF1/7, TOX, Ki67, LAG-3, and Sox10/pancytokeratin. Cell segmentation and tissue classification were performed using HALO (Indica Labs), categorizing all cells into stromal and intratumoral compartments. Frequencies of 27 pre-specified immune phenotypes were calculated as the fraction of all cells within each compartment. Spatial proximity of phenotype pairs was calculated using a hypothesized interaction distribution (HID)[4] with a pairing distance of 30 µm. A board-certified pathologist blinded to the mIF results independently scored cases for the presence of lymphoid aggregates based on hematoxylin and eosin stains. Clinical endpoints included best overall response (BOR) and progression-free survival (PFS) by RECIST v1.1.[5]

Results Higher stromal $CD20^+$ frequency was associated with BOR ($p = 0.00028$) and longer PFS ($p = 0.001$) among all cases, as well as among non-lymph node metastases ($n = 41$; $p = 0.006$, $p = 0.006$ respectively). We identified 12 cases with both high $CD20^+/CD20^+$ HID scores and high stromal $CD20^+$ frequency as containing digital B-cell aggregates (dBCA). dBCA presence provided greater discrimination for PFS ($p = 0.0008$) compared to stromal $CD20^+$ frequency alone. There was moderate agreement between dBCA and PALA (Kappa = 0.50), and PALA positivity alone was not significantly associated with BOR or PFS. dBCA+ cases were enriched for multiple stromal T cell populations, including $CD3^+CD8^-TCF1/7^+$ ($p = 5.7 \times 10^{-6}$), and $CD3^+CD8^-$ ($p = 3.92 \times 10^{-6}$).

Conclusions Digital assessment of stromal B cell frequency and spatial distribution warrant further investigation as potential biomarkers of response to ICI therapy in advanced melanoma. Further characterization of T-cell populations associated with B-cell aggregates may provide additional insights into how these structures facilitate ICI-mediated anti-tumor responses.

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Ethics Approval This study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center; approval number 19–114.

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