CHARACTERIZATION AND CLINICAL SIGNIFICANCE OF THE INTRA AND INTER-TUMOR SPATIAL IMMUNE HETEROGENEITY USING 3-DIMENSIONAL MULTIPLEXED PHENOTYPING IN NON-SMALL CELL LUNG CANCER

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Background Tumor infiltrating lymphocytes (TILs) can be identified in the tumor microenvironment (TME) of most solid malignancies including non-small cell lung cancer (NSCLC). TILs mediate cancer-cell recognition, elimination and affect patient prognosis. To date, the composition and spatial distribution of immune cells in the TME of primary tumors and secondary metastatic locations remain largely unexplored. In addition, the study of the TME in tissue sections is limited by its 2-dimensional nature. Here, we used a 3-dimensional tissue reconstruction approach to study major TIL subpopulations and their spatial interactions in primary NSCLCs and paired metastases.

Methods We established a retrospective cohort of 97 synchronous primary and metastatic lesions from 19 individuals with advanced NSCLC obtained during autopsy procedures. Each lesion was sampled three times in different areas and represented in tissue microarray (TMA) format, resulting in a total of 291 individual tumor cores. We obtained 50 consecutive 5µm-thick TMA sections that were stained using a multiplex quantitative immunofluorescence panel to detect tumor epithelial cells and major TIL subpopulations (DAPI/CK/CD4/CD8/CD20). After slide digitalization, the images were pre-processed into grey-scale and spatially aligned by autograd registration to minimize data loss, which consists of the mean square error based on pixel-level intensity and dissimilarity of structures detected automatically by convolution and pooling operations at different resolutions. Images underwent single-cell segmentation and phenotyping using novel in-house developed computational models and were integrated into 3-dimensional projections for visualization and analysis.

Results In primary NSCLCs, the total TIL density using 3-dimensional volumetric analysis was 8,623 cells/mm³ for CD4+ helper T-cells, 8,436 for CD8+ effector T-cells, and 5,654 for CD20+ B-lymphocytes. In metastatic lesions, the TIL density was significantly lower with 7,890, 6,747 and 2,944 cells/mm³, respectively. The inter-tumor spatial immune heterogeneity between primary and metastases assessed by the coefficient of variation inter-quartile range was significantly higher than the intra-tumor heterogeneity of primary lesions. Associations between spatial TIL heterogeneity and clinicopathologic variables such as anatomic tumor location and patient gender were identified. Spatial analyses of results using graph neural networks and orthogonal validation of quantitative TIL analysis are ongoing.

Conclusions We established a strategy for systematic and quantitative 3-dimensional analysis of major TIL populations in paired synchronous primary and metastatic NSCLC with single-cell resolution. Our results reveal prominent inter-tumor spatial immune heterogeneity, suggesting the progressive acquisition of immune evasion properties during NSCLC progression. The utilization of 3-dimensional microscopy coupled with advanced computational analysis can unveil novel aspects of the immune TME.

Ethics Approval This study was carried out in accordance with the principles of the Declaration of Helsinki and all tissue and clinical information were used in a de-identified fashion after approval from the Yale Internal Review Board (Yale Human Investigation Committee) protocols #950508219 and #1608018220, which approved the patient consent forms or waiver of consent.