antibodies to identify cytotoxic or regulatory T cells, respectively. Tumor cells were labeled with a pan-Keratin antibody. Images were acquired using a Leica SP8 confocal microscope. FIJI3 and IMARIS were used for image processing.

**Results** We identified both cytotoxic and regulatory T cell populations in the TME using thin sections and whole-mount. However, using whole-mount after tissue clearing allowed us to better evaluate the spatial distribution of the T cell populations in relation to the tumor structure. Furthermore, tissue clearance facilitates the imaging of larger volumes using multiplex immunofluorescence.

**Conclusions** Analysis of large lung tissue volumes provides a better understanding of the location of immune cell populations in relation to the TME and allows to study heterogeneous immune infiltration on a per-lesion base. This valuable information will improve the characterization of the TME and the definition of cancer-immune phenotypes in NSCLC.

**REFERENCES**

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**50 SPATIALLY RESOLVED MOLECULAR INVESTIGATION OF TRIPLE NEGATIVE BREAST CANCER AND ITS IMMUNE MICROENVIRONMENT**

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**Background** Triple negative breast cancer (TNBC) accounts for 10–20% of all diagnosed breast cancer cases in the US and is characterized by loss of HER2, estrogen receptors, and progesterone receptors. TNBC is an aggressive, complex disease with a poor prognosis due to resistance to traditional therapies. Understanding the underlying biology and tumor microenvironment is critical to the development of diagnostic biomarkers and to guide the search for effective therapies. Here, we demonstrated the ability of the 10x Genomics Visium Spatial Gene Expression Solution to elucidate the immunological profile and microenvironment of TNBC samples in conjunction with standard pathological techniques.

**Methods** Spatial transcriptomics technology complement pathological examination by combining the benefits of histological stains with the throughput and deep biological insight of RNA-seq. We investigated serial sections of TNBC by using the 10x Genomics Visium Spatial Gene Expression Solution to spatially resolve the samples’ cellular composition and expressed microenvironment. Visium incorporates ~5000 molecularly barcoded, spatially encoded capture probes in spots over which a tissue section is placed and imaged. The samples are permeabilized and native mRNA is captured. Imaging and RNA sequencing data are processed together, resulting in whole transcriptome gene expression mapped to the tissue image.

**Results** We captured spatial patterns of gene expression and mapped the information back to H&E-stained images with regional annotations. Serial sections were then subject to fluorescence immunohistochemical staining for immune infiltrate paired with spatial gene expression capture. Subsequently, we combined these data with 3’ single-nuclei RNA-seq from the same tumor, generating expression profiles that were used to automatically annotate cell-types across the sections. This allowed for an understanding of the tumor microenvironment that could not be captured by image-based techniques alone. We resolved subgroups of spatially and biologically distinct immune, stem, and cancer progenitor cells. Finally, we digitally annotated tumor and normal tissue regions using expressed genetic mutations alone. Annotated tumor regions expressed more deleterious mutations than normal regions and we were able to automatically cluster regions of tumor vs. normal cells without any prior histopathological information. We also found intratumor gradients of mutational burden in oncogenes as well as non-cancer associated loci.

**Conclusions** Taken together, we demonstrated that Visium can provide a powerful complement to traditional histopathology, enabling both targeted panels and whole-transcriptome discovery of gene expression. This spatially resolved molecular information provides an unprecedented view into the tumor microenvironment and a powerful new tool for discovery of new biomarkers and therapeutic targets.

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**51 SMALL CELL/LYMPHOHISTIOCYTIC MORPHOLOGY IS ASSOCIATED WITH CD8 POSITIVITY, RETAINED T CELL MARKERS, A TREND OF DECREASED PD-L1 EXPRESSION, BUT NOT OUTCOME IN ADULTS WITH ALK + ALCL**

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**Background** Several morphologic patterns of ALK+ anaplastic large cell lymphoma (ALCL) are recognized: common, small cell, lymphohistiocytic, Hodgkin-like, and composite patterns. Small cell (SC) and lymphohistiocytic (LH) patterns are thought to be closely associated with poorer outcome in children with ALK+ ALCL.2 However, the clinicopathologic and prognostic features of SC/LH patterns of ALK+ ALCL are not yet reported in adults. Recently, we found PD-L1 expression in a large subset of ALK+ ALCL cases, however, PD-L1 expression in SC/LH versus non-SC/LH ALCL has not been reported.

**Methods** Among 102 adult patients with ALK+ ALCL seen at our institution from January 1, 2007 through August 30, 2018, 18 (18%) cases had a SC and/or LH pattern. The clinical, pathologic, and outcome data were compared between SC/LH and non-SC/LH ALK+ ALCL cases using Fisher’s exact test. Overall survival (OS) was analyzed using the Kaplan-Meier method and compared using the log-rank test.

**Results** There were no significant differences in clinical features including age, gender, nodal versus extranodal involvement, B symptoms, stage, leukocytosis/lymphocytosis, and serum LDH levels between patients with SC/LH versus non-SC/LH ALK+ ALCL. Compared to non-SC/LH cases, SC/LH ALCL was more often positive for CD2 (92% vs. 36%, p = 0.0007), CD3 (81% vs. 15%, p = 0.0001), CD7 (80% vs. 37%, p = 0.03), and CD8 (54% vs. 7%, p = 0.0006). SC/LH ALCL showed a trend of decreased PD-L1 expression than non-SC/LH cases (24% vs. 46%, p = 0.11). There were no significant differences in the expression of CD4, CD5, CD25, CD43, CD45, CD56,
TCR A/B, TCR G/D, cytotoxic markers, EMA, Ki-67 proliferation index. The induction chemotherapy and response rate in patients with SC/LH ALK+ ALCL were similar to patients with non-SC/LH ALK+ ALCL. After a median follow-up of 30.5 months (range, 0.3–224 months), there was no significant difference in OS between patients with SC/LH versus non-SC/LH ALK+ ALCL (p = 0.88).

Conclusions In adults with ALK+ALCL, the SC/LH morphologic pattern is associated with a CD8+ T cell immunophenotype and retention of expression of T cell markers (CD2, CD3, and CD7). The trend of decreased PD-L1 expression in SC/LH ALCL suggests that these patients may not be ideal candidates for PD-L1 immunotherapy. The SC/LH patterns of ALK+ ALCL have no impact on the prognosis of adult patients which is in contrast to the reported association of the SC/LH patterns with poorer outcome in children with ALK+ ALCL.

Ethics Approval The study was approved by the Institutional Review Board at MD Anderson Cancer Center, Approval number: PA16-0897

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ADVANCED T LYMPHOCYTE ANALYSIS SYSTEM (ATLAS)
FOR IN-DEPTH IMMUNOLOGICAL INTERROGATION IN
REAL-WORLD CONDITIONS, A METHODOLOGICAL
STRATEGY

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Background Humans are genetically diverse and possess a rich immunological history. It is logical to consider that these factors may lead to differences in individual immunological responses to therapy when diagnosed with cancer. The successful implementation of immune-based therapies against cancer has brought the need to develop strategies to create meaningful profiles that faithfully depict the patient’s immunological status. We report an in-depth immunological interrogation methodology, termed ATLAS. This system was designed to generate an accurate representation of the patient’s immunological landscape that can be used during various time points during immune-checkpoint inhibitor (ICI) therapy.

Methods We selected data from our prospective registry trial at Loyola University Medical Center to design individual immunological profiles of patients diagnosed with locally advanced or metastatic solid tumors planning to receive ICI. Only metastatic melanoma patients samples pre-ICI therapy are included in this first analysis. Twenty mL of peripheral blood were collected. Giving consideration to scientific rigor and limited sample availability, the assays were designed in miniaturized forms. ATLAS includes classical peripheral blood mononuclear cells (PBMCs) composition and T cell phenotypic and transcriptional analysis. To depict T cell functionality, we examined multiple parameters such as T cell receptor (TCR) signaling threshold, cell proliferation and NF-κB activation, at steady-state and in response to cell activation. To obtain both a broad and T cell-specific view, we quantified circulating chemokines and cytokines in plasma and from activated T cells.

Results For this first methodologic demonstration, patient characteristics are depicted in table 1. Data from different ATLAS assays were used to create individual immunological profiles presented as a dashboard for each patient. Distributional plots and measures of center (mean, median) and spread (range, variance) were used to eliminate low-information parameters from the figures. Data visualizations compared individual patients to the sample median for continuous parameters and

![Abstract 52 Figure 1](link)