Multiplex immunohistochemistry reveals a lymphocytic immune signature associated with longer overall survival in small cell lung cancer patients

Portia L. Thomas[1,2], Sam Sivagnanam[2], Courtney Betts[2], Giovanney Gonzalez[2], Wade Iams[3], Lisa M. Coussens[2], and Christine M. Lovly[3,4]

[1]School of Graduate Studies and Research, Meharry Medical College, Nashville, TN [2]Department of Cell, Developmental and Cancer Biology, Knight Cancer Institute, Oregon Health & Science University, Portland, OR
[3]Department of Medicine, Division of Hematology-Oncology, Vanderbilt University Medical Center, Nashville, TN [4]Vanderbilt-Ingram Cancer Center, Nashville, TN

Abstract

Introduction: Small cell lung cancer (SCLC) is an aggressive neuroendocrine malignancy with limited treatment options and median survival of approximately 1 year after diagnosis, even with treatment. SCLC patients typically respond to platinum-based cytotoxic chemotherapy, but drug resistance and resultant disease progression rapidly develop, driving the 5-year patient survival rate to < 5%. The emergence of immunotherapy (IO) offers promising therapeutics for this disease. However, recently FDA approved immunotherapies only improve overall survival (OS)
of SCLC patients by 2 months (chemotherapy OS: 10 mos.; chemotherapy + IO OS: 12 mos.). Little is known about the immune landscape in SCLC. We assert that a better understanding of this immune contexture, defined as abundance, type, and location of immune cells in SCLC may provide important criteria for patient stratification.

Objectives: The primary objective of this study is to comprehensively and quantitatively identify immune cell subsets and their associated functional states in small cell lung cancer to address critical knowledge gaps in the tumor biology of the disease.

Methods: To broadly audit immune contexture in SCLC, we generated a tissue microarray containing 48 unique patient specimens with fully annotated clinical data. Next, we used an innovative multiplex immunohistochemistry (mIHC) platform consisting of a panel of 18 validated antibodies and a computational pipeline. Importantly, mIHC enables cell classification via image cytometry, for identification of immune cell subsets, including: T cells (CD3$^+$, CD4$^+$, CD8$^+$), B cells (CD20$^+$), granulocytes (CD66b$^+$) Th2-skewed monocytes and macrophages (CD11b$^+$ CD68$^+$ CD163$^+$), and dendritic cells (CD68$^-$ CD11c$^+$ HLA-DR$^+$, DCLAMP$^{+/-}$). Further, functional markers provide insight into immune checkpoints (PD-1, PD-L1), proliferation (Ki67), and T cell functionality (EOMES, granzyme B).

Results: Patients (n= 36) exhibited high variability in total immune cell infiltrates (8 – 8673 cells/mm^2) with heterogeneity in immune cell composition. Anatomic location of the biopsy was the predominate driver of tumor immune signature. Longer overall survival was associated with increased lymphocyte (CD4$^+$ T, CD8$^+$ T, and B cell) and dendritic cell infiltration and increased granzyme B-positive CD8$^+$ T cells. Future analyses aim to confirm findings in additional SCLC patient cohorts as well as identify predominating immune cell populations that correspond with clinical response to first-line therapy in stage-matched samples.

Conclusions: Understanding the SCLC immune landscape could further our understanding of
SCLC immune suppression and evasion, ultimately leading to optimal single-agent
immunotherapy deployment and rational, effective combination therapy for patients with SCLC.

Project goal: Gain a better understanding of SCLC immune evasion through an in-depth characterization of the tumor immune microenvironment.

Multiplex immunohistochemistry (mIHC) image processing workflow

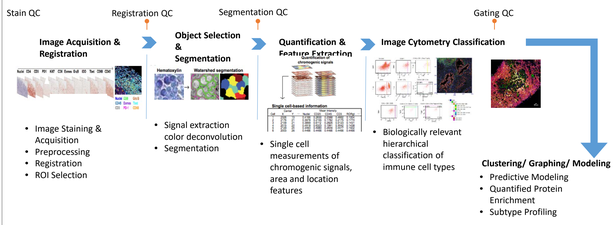

Figure 1: Comprehensive in-situ monitoring of the tumor immune microenvironment using mIHC.

Quantitative multiplex immunohistochemistry (mIHC) 'Discovery 18' panel immune cell phenotyping

1. Cell type identification by marker expression

Lineage	Identification (All populations are CD45+)
CD8$^+$ T lymphocytes	CD3$^+$ CD8$^+$
T-helper (CD4$^+$ T) cells	CD3$^+$ CD8$^-$
CD20$^+$ B cells	CD3$^-$ CD20$^+$
CD163$^-$ Macrophages (M1-like)	CD3/CD20$^-$ CD11b$^+$ CD66b$^-$ CD68$^+$ CD163$^-$
CD163$^+$ Macrophages (M2-like)	CD3/CD20$^-$ CD11b$^+$ CD66b$^-$ CD68$^+$ CD163$^+$
Immature DCs	CD3/CD20$^-$ CD11b$^{+/-}$ CD66b$^-$ CD68$^-$ CD11c$^+$ HLA class II$^+$ DC-LAMP$^-$
Mature DCs	CD3/CD20$^-$ CD11b$^{+/-}$ CD66b$^-$ CD68$^-$ CD11c$^+$ HLA class II$^+$ DC-LAMP$^+$
Neutrophils and eosinophils	CD3/CD20$^-$ CD11b$^+$ CD66b$^+$

2. Interrogation of functional state of immune cells

Classification	Marker
Proliferation	Ki67
Cytotoxicity	Granzyme-B$^+$
T cell memory phenotype	EOMES
Immune regulation	PD-1, PD-L1

Table 1: Discovery 18 panel | Multiplexed immunohistochemical platform enabling simultaneous evaluation of 18 biomarkers in one formalin-fixed paraffin-embedded tissue section (PMID: 32122539)

Baseline demographics of SCLC patients matched for age, gender, and race

	Poor survivors <7mos n = 21	Average Survivors 7mos – 24mos n = 18	Exceptional Survivors >24mos n = 9
Overall survival (median)	2.3 mos	12.4 mos	38.1 mos
Stage at diagnosis			
Limited-stage SCLC	5 (24%)	9 (50%)	9 (100%)
Extensive-stage SCLC	16 (76%)	9 (50%)	0 (0%)
Site of biopsy			
Lung	7 (33%)	7 (39%)	7 (78%)
Lymph node	4 (19%)	5 (28%)	0 (%)
Other†	10 (48%)	6 (33%)	2 (22%)
First-line therapy			
Platinum+etoposide	11 (52%)	16 (89%)	8 (89%)
Other	2 (10%)	2 (11%)	1 (11%)
No systemic therapy	8 (38%)	0 (0%)	0 (5%)

† = liver, bone, brain, neck, bowel, and adrenal

Values shown are n (%) unless otherwise stated.

Table 2: Exceptional responders TMA | SCLC tissue microarray containing patients (n = 48) with exceptional survival status (overall survival ≤7mos or overall survival ≥24mos); median overall survival = 15.3 mos.

Immune composition density of SCLC patients show wide heterogeneity in total CD45+ infiltrate

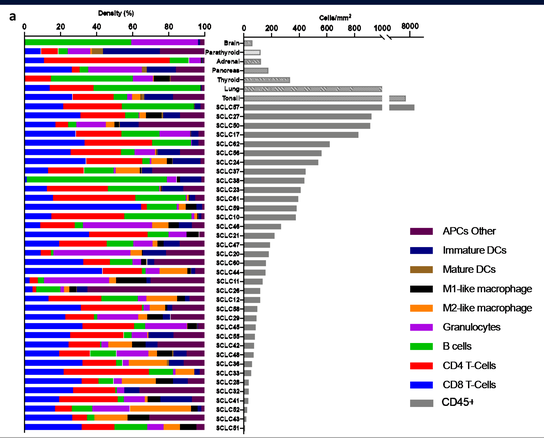

Figure 2: Immune composition density in SCLC tissue | a) Right: Density of identified immune cells (positive cells/mm^2 tissue) in SCLC patients (n = 36) and control tissues (n = 7) Left: Scaled density (immune cell type/total immune cells) of immune cells in SCLC patients (n = 36) and control tissues (n = 7).

Increased T cells (CD8$^+$ T-cells and CD4$^+$ T-cells) and antigen-presenting cells (dendritic cells) in SCLC tumor tissue is associated with better overall survival in patients

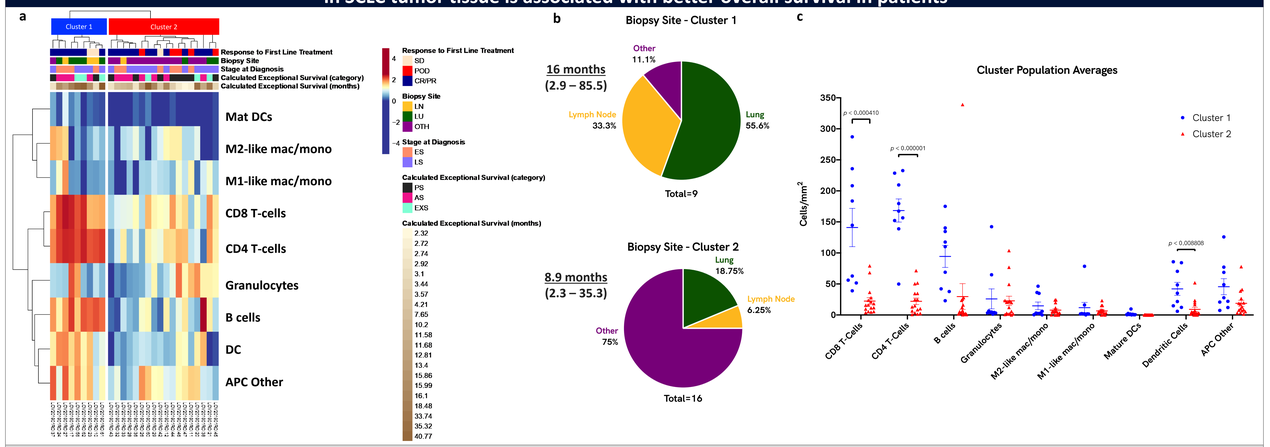

Figure 3: Analysis of immune infiltrates in unsupervised patient clusters | a) Unsupervised clustering of SCLC patients using log10 density of immune infiltrates b) Percentage of biopsies from each site in each cluster c) Immune density population averages (positive cells/mm^2 tissue) in patient clusters. SD, stable disease; POD, progression of disease; CR, complete response; PR, partial response; LN, lymph node; LU, lung; OTH, other; ES, extensive-stage; LS, limited-stage; PS, poor survivor; AS, average survivor; EXS, exceptional survivor; DC, dendritic cell

Increased CD4$^+$ T-cells in lung biopsies are associated with better overall survival in patients

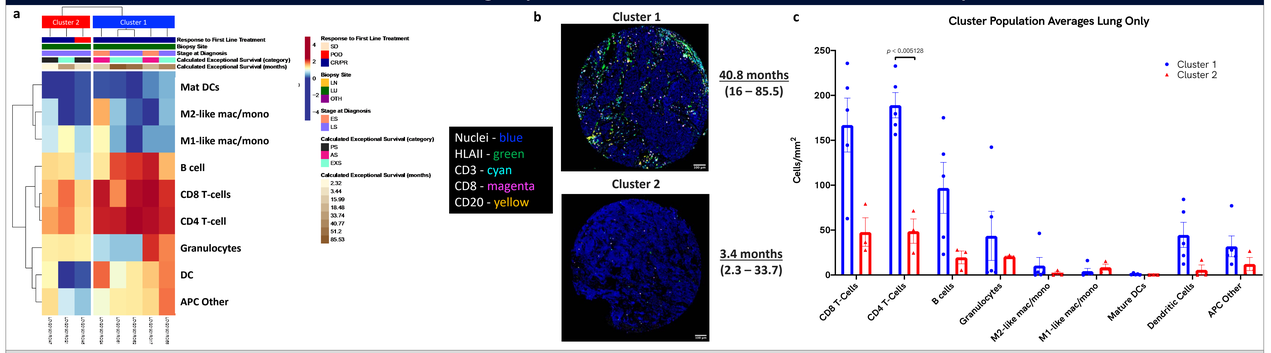

Figure 4: Unsupervised analysis of patient clusters from lung biopsies only | a) Unsupervised clustering of SCLC patients with lung biopsies using log10 density of immune infiltrates b) Representative patient lung biopsies from cluster 1 (ROI56) and cluster 2 (ROI45) showing infiltrates c) Quantified averages of immune infiltrates (positive cells/mm^2 tissue) in patient clusters. SD, stable disease; POD, progression of disease; CR, complete response; PR, partial response; LN, lymph node; LU, lung; OTH, other; ES, extensive-stage; LS, limited-stage; PS, poor survivor; AS, average survivor; EXS, exceptional survivor; DC, dendritic cell

Increased CD8$^+$ T cell functionality is associated with better overall survival

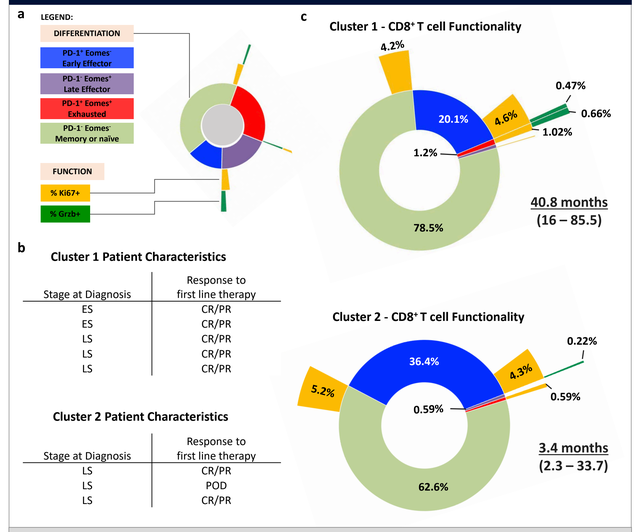

Figure 5: Sunburst graphs of CD8$^+$ T cell functionality| a) Representative diagram of CD8$^+$ T cell functionality graph b) Patient characteristics (stage and response to first line therapy) of cluster 1 and 2 c) Depicted averages of CD8$^+$ T cell functionality in cluster 1 (n = 5) and cluster 2 (n = 3) SCLC patients with lung biopsies only. ES, extensive-stage; LS, limited-stage; CR, complete response; PR, partial response; POD, progression of disease

Future Directions

Future Directions

1. Confirm findings of increased T cells and dendritic cells in long-term survivors in additional cohorts of SCLC patients.
2. Distinguish with more power the difference between primary (lung) and metastatic sites.
3. Identify predominating immune cell populations that correspond with clinical response to first-line therapy in stage-matched samples.

Conclusion: Patients exhibit a high variability of immune cell infiltrates, although overall total infiltrates seem to be low in comparison to control tissues. Patients have high variability in composition and myeloid/lymphoid proportions. Biopsy site tends to be the predominate driver of immune signature.

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