Image Analysis-Based Assessment of PD-L1 and Tumor-Associated Immune Cells Density Supports Distinct Intratumoral Microenvironment Groups in Non-Small Cell Lung Carcinoma Patients


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Translational Relevance

Better understanding of PD-L1 expression profile and its interplay with tumor-associated inflammatory cells (TAICs) will provide important insight into the pathogenesis and progression of non-small cell lung carcinoma (NSCLC), and assist in the development of biomarkers for anti-PD-1/PD-L1 therapy or other immunotherapy strategies. We investigated the immunohistochemical PD-L1 expression and TAICs density in a large series of well-characterized NSCLS specimens using image analysis approach. We demonstrated different PD-L1 expression and TAICs density patterns in NSCLC according to tumor histology and differentiation, and patients smoking and airflow limitation history. Importantly, we identified four subtypes of tumor microenvironment in this disease, and that a subset of tumors exhibiting characteristics of intrinsic induction of PD-L1 had worse outcome.
ABSTRACT

Purpose: We investigated the correlation between immunohistochemical PD-L1 expression and tumor associated immune cells (TAICs) density in non-small cell lung carcinoma (NSCLC) and correlated them with clinicopathological variables.

Patients and Methods: Tumor tissue specimens from 254 stage I-III NSCLCs (146 adenocarcinomas [ADC]; 108 squamous cell carcinomas [SqCC]) were examined. PD-L1 expression in malignant cells (MCs) and macrophages, and the density of TAICs expressing CD3, CD4, CD8, CD57, granzyme B, CD45RO, PD-1, FOXP3 and CD68 were evaluated using immunohistochemistry and image analysis.

Results: MCs PD-L1 H-score >5 was detected in 23% of ADCs and 31% of SqCCs and no significant differences were detected comparing both histologies; the median H-score in macrophages was significantly higher in SqCC than in ADC ($P < 0.001$). In ADC, high MCs PD-L1 expression and high TAIC density correlated with solid histology, smoking history, and airflow limitation. Multivariate analysis demonstrated that high CD57-positive cell density correlated with better recurrence-free survival (RFS; $P = 0.0236$; HR 0.457) and overall survival (OS; $P = 0.0261$; HR, 0.481) rates for SqCC. High CD68-positive cell density in intratumoral compartment correlated with better RFS ($P = 0.0436$; HR, 0.553) for ADC. The combination of low CD4/CD8/C68-positive cell density and PD-L1 H-score >5 in MCs identified small subset of ADCs with worse outcomes (RFS: $P = 0.036$; HR, 4.299; OS: $P = 0.00034$; HR, 5.632).

Conclusions: We detected different PD-L1 expression and TAIC density patterns in NSCLC. Distinct groups of tumor microenvironment correlated with NSCLC clinicopathological features, including outcome.
INTRODUCTION

Multiple genetic and epigenetic changes in patients with several cancer types cause resistance to immune attack of tumors by inducing specific T-cell tolerance against tumor and expression of ligands that engage inhibitory receptors and block T-cell activation, resulting in T-cell anergy or exhaustion in the tumor microenvironment (1). In this process, programmed cell death protein 1 (PD-1), a T-cell co-inhibitory receptor, and one of the protein’s ligands, programmed cell death ligand 1 (PD-L1; B7-H1 or CD274), play pivotal roles in the ability of tumor cells to evade the host’s immune system. Although virtually absent from normal cells except macrophages, PD-L1 expression can be induced in a variety of cell types, including antigen-presenting, B, T, epithelial, muscle, and vascular endothelial cells and trophoblasts (2). The physiological role of PD-L1 is to bind to PD-1 receptors expressed on the surface of activated cytotoxic T cells (2). This binding causes inhibition of interleukin-2 production and T-cell activation via reduced phosphorylation of ζ-chain-associated protein kinase 70 and protein kinase Cθ (3). PD-1/PD-L1 interaction serves as an important regulatory checkpoint for excessive adaptive immune responses to antigens and autoimmunity. Researchers have observed and evaluated PD-L1 expression in malignant cells (MCs) of a number of tumor types, including melanoma and breast, colorectal and lung cancers (4-11).

Therapies blocking the PD1/PD-L1 axis have resulted in different rates of tumor response for a variety of cancer types (12). For example, antibody-mediated blockade of PD-L1 induced durable tumor regression and prolonged disease stabilization in patients with a variety of solid tumors, including non-small cell lung carcinoma (NSCLC) (12, 13). Although these studies demonstrated correlation between immunohistochemical PD-L1 expression in NSCLC cells and clinical responses to anti-PD-1 and anti-PD-L1 antibodies (12, 13), some patients with tumors
negative for PD-L1 expression had responses to similar to those in patients with tumors positive for PD-L1 expression\textsuperscript{(14)}. Recently, investigators showed that across several cancer types, including NSCLC, patients with tumors expressing PD-L1 at high levels in both MCs and tumor-associated immune cells (TAICs) had responses to anti-PD-L1 therapy\textsuperscript{(11)}. Taken together, these findings suggested that factors other than PD-L1 in the tumor microenvironment, including tumor-infiltrating lymphocytes (TILs)\textsuperscript{(15, 16)} and tumor-associated macrophages (TAMs)\textsuperscript{(17)}, may drive responses to anti-PD-1 and anti–PD-L1 therapies and be involved in lung cancer pathogenesis and progression\textsuperscript{(10, 11, 18)}.

Researchers in a number of studies have characterized PD-L1 protein expression in surgically resected stage I-IV NSCLC tumors using immunohistochemistry (IHC) or immunofluorescence\textsuperscript{(7, 8, 11, 15, 16)} with formalin-fixed, paraffin-embedded tumor specimens and correlated the findings with outcome. Although most of the studies demonstrated that high PD-L1 protein expression correlated with better outcome than did low expression\textsuperscript{(8, 11, 18, 19)}, some authors reported no association of PD-L1 expression with outcome\textsuperscript{(9, 15)} or that the expression correlated with poor prognosis\textsuperscript{(10)}. Most of these studies differed in the type of specimens used (whole histological sections versus tissue microarrays [TMAs]), type of protein expression analysis (IHC versus immunofluorescence), and quantification assessment (image analysis versus microscopic observation)\textsuperscript{(7-11, 15, 16, 18, 19)}. Researchers in only a few studies have attempted to correlate the expression of PD-L1 with TAICs density and characteristics, particularly TILs, using a limited number of IHC markers (e.g., CD8, CD45RO)\textsuperscript{(15, 19)} or simple histological evaluation\textsuperscript{(7, 11)}. To date, no published studies have included assessment of a comprehensive panel of immune markers in NSCLC cases using image analysis to characterize the association among PD-L1 expression, immune cell response and clinicopathological features of tumors to
determine distinct subgroups of NSCLC and patients’ outcome. Therefore, in the present study, we characterized the IHC expression of a large panel of immune markers PD-L1, PD-1, and eight others and assessed the TAIC density in both the peritumoral and intratumoral compartments in surgically resected NSCLC specimens. To assess this objectively, we performed quantitative image analysis and correlated the findings with the patients’ clinical features and their tumors’ pathological and molecular features.

MATERIAL AND METHODS

Cases and Specimens

Formalin-fixed, paraffin-embedded histological sections of surgically resected primary NSCLC tumors obtained from 254 patients who underwent surgery with curative intent from 1997 to 2012 at The University of Texas MD Anderson Cancer Center were used. The study patients had either adenocarcinoma (ADC; N = 146) or squamous cells carcinoma (SqCC; N = 108) and did not receive neoadjuvant therapy. Tumor staging was performed using the staging system from the 7th American Joint Committee on Cancer (AJCC)(20). For ADC, predominant histologic pattern (solid, lepidic, acinar, papillary, and micropapillary) was determined according to the 2015 World Health Organization classification(21). For SqCC grading, we used well, moderate and poorly differentiated categories. In a subset of cases (N=130; 97 ADCs and 33 SqCCs) PD-L1 IHC expression was assessed in paired whole tissue sections and TMA samples. Of these, all three TMA cores were available in 73 ADC and 24 SqCCs, while the rest of the cases had two cores available for analysis. The TMA sections were prepared using three 1.0-mm tissue cores obtained from the center, middle and periphery of the tumor, as described previously(22). Data on nonreversible airflow limitation, defined as a ratio of the forced
expiratory volume in 1 second to the forced vital capacity of less than 0.7\(^{(23)}\), were available for a large subset of our patients (N = 179). EGFR and KRAS mutation data obtained using Sanger sequencing were available in 91% (N = 137) of ADCs. This study was approved by the MD Anderson Institutional Review Board.

**IHC staining**

Four-micron-thick sequential histological tumor sections were obtained from a representative formalin-fixed, paraffin-embedded tumor block and used for IHC analysis. IHC was performed using an automated staining system (BOND-MAX; Leica Microsystems, Vista, CA, USA) with antibodies against PD-L1 (clone E1L3N, dilution 1:100; Cell Signaling Technology, Beverly, MA, USA), CD3 (T cell lymphocytes; dilution 1:100; Dako, Carpinteria, CA, USA), CD4 (helper T cell; Novocastra; clone 4B12, dilution 1:80; Leica Biosystems, Buffalo Grove, IL, USA), CD8 (cytotoxic T cell; clone CD8/144B, dilution 1:20; Thermo Fisher Scientific, Waltham, MA, USA), CD57 (natural killer T cell; clone HNK-1, dilution 1:40; BD Biosciences, San Jose, CA, USA), granzyme B (cytotoxic lymphocytes; clone F1, ready to use; Leica Biosystems), CD45RO (memory T cell; clone UCHL1, ready to use; Leica Biosystems), PD-1 (clone EPR4877-2, dilution 1:250; Abcam, Cambridge, MA, USA), FOXP3 (regulatory T cell; clone 206D, dilution 1:50; BioLegend, San Diego, CA, USA), and CD68 (macrophages; clone PG-M1, dilution 1:450; Dako). Expression of all of the markers in cells was detected using a Novocastra Bond Polymer Refine Detection kit (Leica Microsystems) with a diaminobenzidine reaction to detect antibody labeling and hematoxylin counterstaining. Positive and negative controls were used for PD-L1 IHC expression (human embryonic kidney 293 cell line transfected and non-transfected with PD-L1 gene, and human placenta and tonsil FFPE tissues).
during each run IHC staining using autostainers. For the TAICs IHC expression, human tonsil FFPE tissues with and without primary antibody were used as positive and negative controls, respectively with each run IHC staining.

**Image analysis**

To measure the IHC expression of the different markers and quantify the inflammatory cells expressing the slides, containing whole tumor sections or immunohistochemically stained TMA sections, were digitally scanned at ×200 magnification using a ScanScope Aperio AT Turbo slide scanner (Leica Microsystems). The images were visualized using the ImageScope software program (Leica Microsystems) and analyzed using the Aperio Image Toolbox and GENIE image analysis tool (Leica Microsystems). The pathologist who performed the image analysis was blinded to patients’ outcome. After training the software by a pathologist (Supplementary Figure 1), membranous PD-L1 expression in malignant epithelial cells (MCs) and macrophages was analyzed using a cell membrane staining algorithm, and the staining intensity scored as 0 (no staining), 1+ (weak staining), 2+ (moderate staining), or 3+ (strong staining) and extension (percentage) of expression were determined (Figure 1). The PD-L1 H-scores for tumor tissues were determined by multiplying the staining intensity and reactivity extension values (range, 0-300). The densities of cells expressing CD3, CD4, CD8, CD57, granzyme B, CD45RO, PD-1, and FOXP3 were evaluated using the Aperio nuclear algorithm and CD68 using Aperio cytoplasmic algorithm (Figure 2, and Supplementary Figure 2) and counting the cells positive for them in five random square areas (1 mm² each) in both intratumoral and peritumoral compartments. While five intratumoral regions were available in all cases, and 24 tumors did not have peritumoral regions for analysis. Histology assessment of each 1 mm² was performed to
ensure that tumor tissue (at least 80% MCs, and tumor stroma) was included in the selected intratumoral region, and only non-malignant cells were included in the peritumoral compartment. For this analysis, each area examined was overlapped with the sequential IHC slides to quantify each marker at the same location of the tumor specimens. The average total number of cells positive for each marker in the five square areas was expressed in density per mm². Similar to PD-L1 H-score from 5 intratumoral areas, the TMA was scored as the median PD-L1 H-score average of all cores from each case. In addition, as has been proposed by Teng and colleges(24) four different types tumor microenvironment we were able to identify based on the density of TILs and the expression of PD-L1, as follow: type I (adaptive immune resistance), type II (immunologic ignorance), type III (intrinsic induction), and type IV (tolerance). Combining PD-L1 expression in MCs (>5% was considered positive) with the density of cells expressing CD3 using three levels (tertile) divided based on regular values of distribution by the statistical software (moderate and severe density were considered positive), we were able to identify the frequency of those four subtypes of tumor’ microenvironment in our NSCLC cases.

**PD-L1 mRNA analysis**

Total RNA extracted from the NSCLC specimens with fresh frozen tissue available was used to measure PD-L1 gene expression using an available probe with a human WG-6 v.3 Expression BeadChip (Illumina, San Diego, CA, USA). Resulting data set for 104 primary ADCs and 39 primary SqCCs (SPORE data set; GSE41271) were deposited in the Gene Expression Omnibus repository(25).

**Statistical methods**
The chi-square test or Fisher exact test was used to examine differences in categorical variables, and the Wilcoxon rank-sum test and Kruskal-Wallis test were used to detect differences in continuous variables between groups of patients. The recurrence-free survival (RFS) and overall survival (OS) distributions for the patients were estimated using the Kaplan-Meier method. RFS was defined as the interval from surgery to recurrence or last contact, and OS was defined as the interval from surgery to death or last contact. Both RFS and OS data were censored at 5 years if a patient was alive or died beyond 5 years. A log-rank test was performed to determine the difference in survival between the groups. Regression analysis of the RFS and OS data was performed using Cox proportional hazards model. The statistical software programs SAS (version 9.2; SAS Institute, Inc., Cary, NC, USA) and S-Plus (version 8.04; TIBCO, Palo Alto, CA, USA) were used to perform the computations for all analyses.

RESULTS

PD-L1 protein expression

Clinicopathological and molecular data on the patients are shown in Table 1. Our image analysis-based examination of whole tumor sections obtained from the 146 ADC and 108 SqCC patients demonstrated that the median PD-L1 H-score in MCs was low for both tumor histologies (median, 1.29 for ADC versus 0.95 for SqCC). We found no significant differences in PD-L1 H-score between the two histologies \( (P = 0.326) \) (Figure 1).

Additionally, we determined the distribution of lung tumors using five cutoff percentages of MCs with PD-L1 expression according to histology: >1%, 87 ADCs (60%) and 52 SqCCs (48%); >5%, 34 ADCs (23%) and 34 SqCCs (31%); >10%, 31 ADCs (21%) and 29 SqCCs (27%); >20%, 21 ADCs (14%) and 25 SqCCs (23%); and >50%, 9 ADCs (6%) and 12 SqCCs.
(11%). Whereas SqCC specimens exhibited higher PD-L1 expression than did ADC specimens at all cutoff percentages examined, the difference was statistically significant at only greater than 5% ($P = 0.003$), greater than 10% ($P < 0.0001$), and greater than 20% ($P = 0.020$).

PD-L1 expression in cells with characteristics of TAMs was higher than that in MCs for both tumor histologies, whereas SqCC specimens exhibited significantly higher PD-L1 H-scores in macrophages than did ADC specimens (median, 39.89 and 18.08, respectively; $P < 0.001$).

**Comparison of IHC PD-L1 expression in whole histological tumor sections and TMA sections**

To compare IHC PD-L1 expression levels in whole histological tumor sections and TMA sections, we looked at the H-scores for MCs only and for MCs and TAMs together in a subset of ADC (N = 97) and SqCC (N = 33) specimens. In our analysis of MCs only, we found that PD-L1 expression in whole histological sections correlated positively and significantly with that in TMA sections for both histologies (ADC: $r = 0.417$, $P < 0.0001$; SqCC: $r = 0.438$, $P = 0.0108$).

In addition, in our analysis of MCs and TAMs in five randomly selected 1-mm$^2$ areas of tumor specimens, we again found that PD-L1 expression in whole histological sections correlated positively and significantly with that in TMA sections (ADC: $r = 0.617$, $P < 0.0001$; SqCC: $r = 0.502$, $P = 0.003$). Taken together, these data suggested that the three TMA cores were good surrogates for whole tumor sections in PD-L1 expression analysis of NSCLC. Additionally, we observed a positive and significant correlation between the expression of PD-L1 in MCs and TAMs in whole histological tumor sections and that in the five 1-mm$^2$ areas of the specimens (ADC: $r = 0.575$, $P < 0.0001$; SqCC: $r = 0.733$, $P < 0.0001$).
Correlation between PD-L1 protein and PD-L1 gene expression

To assess the correlation between PD-L1 protein and PD-L1 gene expression in NSCLC cases, we studied 104 ADC and 39 SqCC specimens with available data on PD-L1 mRNA expression. We found that PD-L1 mRNA expression in whole tumor specimens correlated positively and significantly with PD-L1 protein expression for both NSCLC histologies when we assessed the PD-L1 protein expression in the entire population of tumor cells, including both MCs and TAMs, in five random areas of the whole tumor specimens (ADC: \( r = 0.448, P < 0.0001; \) SqCC: \( r = 0.634, P < 0.0001 \)) and TMA sections (ADC: \( r = 0.443, P < 0.0001; \) SqCC: \( r = 0.428, P = 0.02 \)). Similarly, we found positive and significant correlations of PD-L1 gene and protein expression when we examined protein expression in MCs only in whole histological tumor sections (ADC: \( r = 0.323, P = 0.0008; \) SqCC: \( r = 0.398, P = 0.012 \)). These data indicated that PD-L1 mRNA expression is a good potential surrogate for PD-L1 protein expression in whole NSCLC tumor tissue.

Correlation between PD-L1 expression and clinicopathological features of NSCLC

In ADC specimens, the PD-L1 H-score greater than the median in MCs correlated with smoking history, airflow limitation, and tumor differentiation. The PD-L1 H-score was significantly higher in ever-smokers (mean, 11.41; median, 1.45, \( P < 0.0001 \)) than in never-smokers (mean, 1.31; median, 0.56, \( P < 0.0001 \)). In comparison, the PD-L1 H-score in, adults who have smoked at least 100 cigarettes in their lifetime and quit smoking at least a year before surgery, former smokers (mean, 10.24; median, 1.43) did not differ significantly from that in current smokers (mean, 12.82; median, 1.64; \( P = 0.918 \)). Also, ADC patients with irreversible airflow limitation (forced expiratory volume in 1 second/forced vital capacity ratio \(<0.7\) ) had a
significantly higher PD-L1 H-score in MCs in whole tumor specimens (median, 1.44 versus 1.11; \( P = 0.048 \)) and five random areas of the tumor specimens (median, 30.78 versus 15.98; \( P = 0.014 \)) than did patients without airflow limitation (Supplementary Table 1). ADC specimens with a solid tumor histological pattern had significantly higher PD-L1 H-scores in MCs (\( P = 0.021 \); average, 15.46; median, 1.69) than did specimens with a non-solid tumor histological pattern (mean, 3.57; median, 1.13). In addition, as we detected at the protein level, we found that PD-L1 gene expression was significantly higher in ADC specimens with a solid tumor histology than in those with a non-solid tumor histology (\( P < 0.0001 \)). PD-L1 expression in MCs was lower in \( EGFR \)-mutant ADC specimens (mean H-score, 5.97; median H-score, 1.39) than in ADC specimens wild-type for \( EGFR \) (mean H-score, 9.65; median H-score, 1.07), but the difference was not significant (\( P = 0.071 \)). We did not detect an association between PD-L1 expression and \( KRAS \) mutation in ADC specimens. We observed no other correlations between PD-L1 expression and other clinical and pathological features for either histological type of NSCLC.

**TAIC density**

Our image analysis-based IHC examination of TAICs included that of immune markers identifying TILs (CD3 positive), helper T-cells (CD4 positive), cytotoxic T cells (CD8 or granzyme B positive), natural killer T cells (CD57 positive), memory T cells (CD45RO positive), regulatory T cells (PD-1 or FOXP3 positive) and TAMs (CD68 positive) in five randomly selected 1-mm\(^2\) areas of tumor specimens. Representative images of immunohistochemical stains of TAICs for these markers are shown in Figure 2. We found that both ADC and SqCC specimens had various TIL densities, particularly when we examined the
intratumoral and peritumoral compartments separately (Table 2; Supplementary Table 2). Unexpectedly, we found in both tumor types and compartments examined that the number of CD4+CD8 positive cells was slightly larger than the number of CD3 positive cells, and that the number of cells expressing granzyme B was significantly lower than cells expressing CD8. In the peritumoral compartment, SqCCs exhibited higher density of cells expressing all markers except PD-1 in TILs, with statistically significantly higher levels of cells expressing CD3 ($P = 0.0010$), CD8 ($P = 0.0020$), granzyme B ($P = 0.0490$), CD45 ($P < 0.0001$), and FOXP3 ($P = 0.0010$), than that in ADCs. In the intratumoral compartment, the differences in TAICs density between the two tumor histologies were less noticeable. We did observe that ADC specimens had significantly higher densities of CD3 ($P = 0.0030$), CD4 ($P < 0.0001$), and CD57 ($P < 0.0001$) positive cells than SqCC specimens’ did. Similar to TILs, the density of cells expressing CD68 was significantly higher in the peritumoral compartment than in intratumoral areas for both tumor histologies ($P < 0.0001$) and significantly higher in the intratumoral region in ADC than in SqCC specimens ($P < 0.0001$). These findings indicated that the densities of TILs and TAMs vary according to the tumor compartment and NSCLC histology.

**PD-L1 expression and TAIC density**

When we examined the correlation of PD-L1 H-score in MCs with TIL and TAM densities in whole tumor histological sections, we found different patterns of correlation according to NSCLC histology and tumor compartment. In ADC specimens, density of cells expressing the majority of TAIC markers examined exhibited a positive significant correlation with expression of PD-L1 in MCs in the intratumoral (seven of nine comparisons) and peritumoral (eight of nine comparisons) compartments (Supplementary Table 3). The cells
expressing markers whose level was not significantly correlated with PD-L1 expression in ADCs were positive for CD68 in both compartments and CD45RO in the intratumoral region. In SqCC specimens, we found lower correlation of PD-L1 expression with TAIC markers’ expression, with four of nine markers (CD3, CD8, granzyme B, and CD45RO) significantly and positively correlated with PD-L1 expression in the intratumoral area and one marker (CD3) significantly and positively correlated with PD-L1 expression in the peritumoral region (Supplementary Table 3). The PD-L1 H-score in TAMs exhibited a lower rate of correlation with marker expression in TAICs for both NSCLC histologies and in both tumor compartments than did the PD-L1 expression in MCs (Supplementary Table 4).

Combining PD-L1 expression in MCs with the density of cells expressing CD3 as proposed by Teng and colleges\(^{(24)}\) we were able to identify the four subtypes of tumor’s microenvironment in our NSCLC cases as showed in the Table 3. This analysis showed that 29% in ADC and 26% in SqCC had an immune ignorance phenotype, and that pattern most frequently observed (ADC 48% and SqCC 43%) was tumors with immune tolerance pattern defined as positive TILs without PD-L1 expression in MCs. Cases with positive PD-L1 expression and positive TILs defined as tumors with adaptive immune resistance pattern were found in 19% of ADC and 31% of SqCC. Tumors with the intrinsic induction patterns were less frequently detected (ADC 3% and SqCC 7%). Similar percentages of cases in each group were found when the analysis included CD4 and CD8 positive TILs, as well as CD3 and CD68 positive TAICs (data not shown).

**Correlation between TAIC density and clinicopathological features of NSCLC**
TAIC density correlated with clinical and pathological tumor features only in ADC specimens. Specifically, these specimens with any solid tumor histology had significantly higher levels of TILs in the intratumoral (cells expressing CD3, CD8, granzyme B, and PD-1) and peritumoral (cells expressing CD3, CD4, CD8, granzyme B, CD45RO, PD-1, and FOXP3) compartments than did ADC specimens with non-solid tumor histologies (Supplementary Table 5). Tumor specimens obtained from ever-smokers had significantly higher granzyme B expression in both compartments and higher peritumoral CD8 expression than did those obtained from never-smokers, whereas tumor specimens obtained from never-smokers had higher intratumoral CD4 expression. Overall, tumor specimens obtained from current smokers had a significantly higher TAIC density (cells positive for CD3, CD8, CD57, granzyme B, PD-1, and FOXP3), mostly in the peritumoral compartment, than did those obtained from former smokers and never-smokers (Supplementary Table 5). Patients with airflow limitation had overall higher numbers of TAICs in both compartments than did patients without airflow limitation (Supplementary Table 1). Furthermore, we observed significantly higher TILs expressing granzyme B and FOXP3 in the intratumoral compartment and cells expressing CD3, CD4, CD8, granzyme B, and CD45RO in the peritumoral compartment in tumors from patients with airflow limitation (Supplementary Table 2). Tumors larger in diameter than the median (3.1 cm) exhibited greater overall TAIC densities in the peritumoral region (cells expressing CD3, CD8, granzyme B, PD-1, or FOXP3) than in the intratumoral region (CD4), whereas smaller tumors had higher densities of CD4-positive cells in the intratumoral compartment than in the peritumoral one. Of note, ADC specimens with \textit{EGFR} mutations had more intratumoral cells expressing CD57 and CD45RO and peritumoral cells expressing CD3 and CD4 than did
specimens wild-type for *EGFR*. We did not detect a correlation between TAIC density and *KRAS* mutation in ADC specimens.

**Correlation between immune markers expression and NSCLC prognosis**

PD-L1 expression H-score and percentage of expression at various cut-off levels in MCs was not significantly correlated with the outcome of NSCLC in our multivariate analysis. However, in ADC specimens, our univariate analysis demonstrated that PD-L1 H-scores higher than the median (*P* = 0.070; HR, 1.746 [95% confidence interval (CI), 0.955-3.190]) and greater than 5 (*P* = 0.051; HR, 1.128 [95% CI, 1.000-1.274]) were non-significantly correlated with a poor OS duration (Figure 3, A). In SqCC specimens, PD-L1 H-scores higher than the median in macrophages correlated significantly with poor OS durations (*P* = 0.036; HR, 0.548 [95% CI, 0.312-0.961]) and indicated a nonsignificant trend of poor RFS durations (*P* = 0.063; HR, 0.565 [95% CI, 0.309-1.033]) in our univariate analysis (Figure 3, B).

Additionally, the density of TAICs expressing several immune markers in tumor specimens correlated with NSCLC outcome in univariate and multivariate analyses. For the ADC patients, intratumoral densities of cells expressing CD4 and FOXP3 greater than the median density were significantly correlated with good RFS and OS durations, respectively, in univariate analysis (data not shown). For the SqCC patients, peritumoral densities of cells expressing CD57 greater than the median density were significantly associated with good RFS and OS durations, respectively, in both univariate (data not shown) and multivariate (RFS: *P* = 0.0236; HR, 0.457 [95% CI, 0.232-0.900]; OS: *P* = 0.0261; HR, 0.481 [95% CI, 0.258-0.918]) analyses after adjustment for tumor stage and adjuvant therapy (Figure 3, C and D). Additionally, our multivariate analysis demonstrated that intratumoral CD68-positive cell
densities greater than the median correlated with good RFS durations for ADC patients ($P = 0.0436$; HR, 0.553 [95% CI, 0.311-0.983]) (Figure 3, E) and a nonsignificant trend of correlation with poor RFS durations for SqCC patients ($P = 0.0811$; HR, 1.751 [95% CI, 0.033-3.287]). Furthermore, the combination of CD4/CD8/C68-positive cell densities lower than the median and PD-L1 H-scores greater than 5 in MCs identified a small subset of ADC patients with poor outcomes (RFS: $P = 0.036$; HR, 4.299 [95% CI, 1.415-13.059]; OS: $P = 0.00034$; HR, 5.632 [95% CI, 2.015-15.737]) (Figure 3, F).

**DISCUSSION**

In this study, we examined archived tumor specimens obtained from a large cohort of patients with stage I-III NSCLC, both ADC (N = 146) and SqCC (N = 108), for IHC expression of PD-L1 in MCs and macrophages and density of TAICs (TILs and TAMs) expressing CD3, CD4, CD8, CD57, granzyme B, CD45RO, PD-1, FOXP3, and CD68 in intratumoral and peritumoral compartments using image analysis. We detected PD-L1 H-scores >5 in MCs in 23% of the ADC specimens and 31% of the SqCC specimens and found that the median H-score in macrophages was markedly higher in SqCC specimens than in ADC specimens. In ADC specimens, high PD-L1 expression in MCs and high TAIC densities correlated with solid tumor histology, airflow limitation, and smoking history. For TAICs expressing most of the immune markers tested, the cell density was significantly higher in peritumoral than in intratumoral compartments for both tumor histologies, and SqCCs exhibited higher peritumoral densities than did ADCs. Multivariate analysis of outcome demonstrated that the combination of low CD4/CD8/C68-positive cell density and high PD-L1 expression in malignant cells identified a small subset of ADC patients with poor RFS and OS durations. We concluded that the PD-L1
protein expression pattern and TAIC density differ in surgically resected ADC and SqCC specimens and that TAIC density varies according to tumor compartment. Importantly, in the present study, we identified distinct tumor environment patterns in NSCLC, and several immune markers whose expression correlated with outcome of surgical resection tumors.

As described previously (7, 8, 15, 16, 19), we observed relatively low levels of expression of PD-L1 in MCs in NSCLC specimens. In particular, SqCCs had slightly higher levels of PD-L1 expression in MCs than did ADCs, particularly for cutoff percentages greater than 5%, 10%, and 20%. The clinically relevant threshold for IHC PD-L1 expression in NSCLC cells has yet to be defined, and researchers have examined various cutoffs of PD-L1 positivity, including at least 5% (7, 9, 26), 10% (15), and 50% (8, 16) cells and greater than the median H-score (10, 27). The cutoff most frequently used to assess IHC PD-L1 expression in MCs in NSCLC patients given PD-1/PD-L1 inhibitors has been at least 5% cells, and this cutoff value has been associated with durable tumor regression and prolonged disease stabilization in response to anti-PD-L1 treatment in advanced metastatic NSCLC cases (14). In the present study, 23% of the ADC specimens and 31% of the SqCC specimens had more than 5% of MCs with PD-L1 expression, which was not markedly different from the percentages reported by others (7, 27). Additionally, we found high levels of PD-L1 expression in cells with characteristics of macrophages for both NSCLC histologies, with markedly higher expression in SqCC than in ADC specimens. To the best of our knowledge, we are the first to specifically analyze and quantify TAMs expressing PD-L1 in NSCLC specimens using IHC. PD-L1 expression in macrophages is biologically relevant and may be associated with tumor progression. As shown in a study using an experimental inflammatory macrophages mouse model (28), PD-L1 expression was induced in macrophages using the same cytokines, such as interferon-γ and vascular endothelial growth factor, involved
in the PD-L1 upregulation of human cancer cell lines\(^{(29)}\), leading to effective suppression of T-cell immunity.

Comparison of different reports of PD-L1 expression in NSCLC cases in the literature is hindered by the use of several analytical methodologies. In most studies, investigators undertook PD-L1 analysis using whole tissue sections\(^{(7, 9, 10, 16, 19, 26, 27)}\), and some studied TMAs\(^{(8, 11, 15)}\). Examination of whole tissue sections may lead to more frequent identification of PD-L1 positive cases than that using more limited sampling with a TMA. However, the present study surprisingly demonstrated that PD-L1 expression in MCs in whole tumor sections correlated markedly and positively with the expression in TMA sections for both NSCLC types. In addition, the data on whole tumor sections correlated with that obtained via examination of five randomly selected 1-mm\(^2\) areas of tumor specimens. Taken together, these data demonstrated for the first time that TMA sections and randomly selected areas of tumor specimens are reliable surrogates for whole tumor sections in sampling approaches to assessing PD-L1 expression in NSCLC patients.

We examined PD-L1 in MCs and macrophages and TAICs density using a quantitative computer-based image analysis system. In most of the previously reported studies of NSCLC, researchers used semiquantitative PD-L1 and TAIC density scoring systems based on microscopic observation of slides by a trained pathologist\(^{(7-10, 15, 16, 19, 26, 27)}\). Visual evaluation of immunohistochemically stained tissue sections remains a rather subjective process characterized by significant intraobserver and interobserver variability and reduced reproducibility, which digital image analysis can overcome\(^{(30, 31)}\).

The PD-L1 H-score in MCs correlated with smoking history and airflow limitation in ADC patients. The H-score was markedly higher in ever-smokers than in never-smokers, but we
detected no difference between former and current smokers. Additionally, we found markedly higher PD-L1 expression in MCs in patients with airflow limitation, a condition closely associated with chronic obstructive pulmonary disease\(^{(23)}\), than in patients without it, suggesting a link between inflammatory processes affecting the lungs of smokers and immune response statuses of tumors.

In our study, specimens of both NSCLC histologies had higher numbers of TAICs in the peritumoral compartment than in the intratumoral region. Also, we found distinct TIL and TAM infiltration patterns in specimens of both histologies when we examined both compartments, with SqCC specimens exhibiting higher numbers of TILs expressing immune markers (except PD-1) than those in ADC specimens in the peritumoral compartment. We hypothesized that nonhomogeneous or clustered secretion of cytokines and chemokines at the invasive tumor margin may enhance the recruitment of different TAIC subtypes to the tumor microenvironment\(^{(32)}\). Interestingly, PD-L1 expression in MCs was markedly correlated with the TAIC density in both tumor compartments for both NSCLC histologies. These observations are supported by previous findings that different immune cells, such as CD8- and CD4-positive cells and natural killer cells, are capable of inducing PD-L1 expression in MCs via interferon-\(\gamma\) production\(^{(33)}\). Curiously, although we found a significant correlation between PD-L1 IHC expression in MCs and infiltration of PD-1 positive cells in ADC, this was not observed in SqCC suggesting different microenvironment mechanism(s) of immune resistance between these two NSCLC histology types. In our study, we characterized the four types of tumor microenvironment described by Teng et al\(^{(24)}\) using as criteria for TIL density levels of CD3-positive cells combined with PD-L1 expression in MCs. We found that most tumors demonstrate an immune tolerance phenotype defined as positive TILs without PD-L1 expression and tumors
with intrinsic induction pattern defined as positive PD-L1 expression and lack of TILs are rarely observed in surgically resected NSCLCs.

Our TAICs quantitative image analysis of NSCLC encountered a couple of unexpected findings. The number of CD4+CD8 positive cells was slightly larger than the number CD3 positive cells in both tumor types and compartments examined. Potential explanations to this finding include that CD4 is expressed in various immune cells such as monocytes/macrophages, eosinophils, CD34+ progenitor cells and natural killer cells\(^{34}\), and that double CD4/CD8 positive T cells have been described in several pathological conditions as well as in normal individuals\(^{35}\). Also, we found that the number of cells expressing granzyme B was significantly lower than the cells expressing CD8. Interestingly, it has been reported a decreased proportion of granzyme B positive cells in lung tumors as a result of soluble mediators, not identified yet, secreted probably by cancer cells\(^{36}\).

Additionally, we observed that TAIC density correlated with clinical and pathological tumor features only in ADC specimens. In these specimens, the presence of any solid tumor histology, smoking history, and airflow limitation was correlated with higher overall numbers of TILs in intratumoral and peritumoral compartments than was a non-solid tumor histology. Smoking status and pulmonary disorders with airflow limitation such as chronic obstructive pulmonary disease are associated with greater risk of lung cancer than patients without those characteristics and are characterized by abundant and deregulated inflammation\(^{37}\). The selective recruitment of TILs (CD3 positive), helper T cells (CD4 positive), cytotoxic T cells (CD8, granzyme B) and, memory T cells (CD45RO positive), to the peritumoral compartment of these lung tumors suggests that these TAICs, which are located around the tumors, are less affected by
tumor-derived inhibitory factors than are cells located in direct contact with MCs in the intratumoral compartment.

Although the present study did not demonstrate that PD-L1 expression in MCs is an independent prognostic factor for NSCLC, our univariate analysis demonstrated a nonsignificant trend of correlation of a PD-L1 H-score greater than 5 in ADC specimens with poor OS duration as reported previously\(^{15, 26}\). Notably, we report herein for the first time that a PD-L1 H-score higher than the median in macrophages correlated significantly with poor OS duration and demonstrated a nonsignificant trend of poor RFS rate in SqCC patients in univariate analysis. Accumulating studies have demonstrated that increased TAM density is associated with poor prognosis for NSCLC\(^{38, 39}\), suggesting that macrophages are targets for therapy for this cancer. Recently, a study showed that a fraction of macrophages in peritumoral stroma from patients with hepatocellular carcinoma expressed PD-L1 and was attenuated by blocking PD-L1 activity in these cells; the accumulation of these macrophages in the peritumoral stroma also predicted poor prognostic survival in these patients\(^{40}\).

Increased TIL numbers have been associated with increased survival durations for both early-stage and advanced NSCLC\(^{11, 41, 42}\). In the present study, the TAIC density correlated with NSCLC outcome in univariate and multivariate analyses. The presence of increased numbers of cells expressing intratumoral CD4 or FOXP3 and macrophages in ADC specimens and of increased numbers of cells expressing intratumoral and peritumoral CD57 in SqCC specimens were associated with OS or RFS. Only the numbers of cells expressing CD4, CD57 or FOXP3 independently indicated better prognosis in ADC patients in multivariate analysis, though. However, studies using IHC analysis of TILs in surgically resected NSCLC specimens have had conflicting results. One study demonstrated an association between increased numbers of
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cytotoxic (CD8 positive) but not helper T (CD4 positive) cells with increased survival duration (43), whereas others had opposite results or did not demonstrate a survival benefit (44, 45). Similar to our findings, increased natural killer cell (CD57-positive) infiltration in several types of tumors has been associated with good clinical outcome (46, 47), including for lung SqCC (48).

A key understudied issue in lung cancer is the potential for TAIC density to predict response to immunostimulatory therapies and patient outcomes. Studying three important immune cell populations helper T-cells (CD4 positive), cytotoxic T-cells (CD8 positive), and macrophages (CD68 positive) (49), we found that the combination of low CD4/CD8/CD68-positive intratumoral density and high PD-L1 expression in MCs identified a small subset of ADCs (6%) with worse outcome than other tumors. This subset of tumors exhibited characteristics of intrinsic induction of PD-L1. Importantly, the majority of ADCs (58%) exhibited prominent CD4/CD8/CD68-positive cells with high or low PD-L1 expression in MCs. Investigators recently showed that in melanoma and NSCLC cases, tumors with high PD-L1 expression in MCs and high densities of TAICs exhibited durable tumor regression and prolonged disease stabilization when treated with anti-PD-1/PD-L1 drugs (50), representing a group of patients who would benefit from this type of therapy.

In summary, in this study, we showed that multiple factors may be correlated with PD-L1 expression in NSCLC MCs and macrophages. We identified different patterns of PD-L1 protein expression and detected the TAIC density in surgically resected ADC and SqCC specimens. We found that TAIC density varied according to the compartment of the tumor examined and that ADC specimens with a solid tumor histology, obtained from smokers, or obtained from patients with airflow limitation had higher expression of immune markers than patients without these characteristics. Also, we identified several immune markers whose expression correlated with
outcome of NSCLC; however, future validation of our finding using similar cohort of patients is needed.
REFERENCES


39. Chen JJ, Yao PL, Yuan A, Hong TM, Shun CT, Kuo ML, et al. Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and


FIGURE LEGENDS

**Fig 1.** Microphotographs of representative examples of IHC PD-L1 expression in lung ADC and SqCC specimens. Four levels of staining (brown) are shown: (A) strong (staining score, 3+), (B) moderate (staining score, 2+), (C) weak (staining score, 1+), and (D) negative (staining score, 0).

**Fig 2.** Microphotographs of representative examples the 10 immune markers in lung ADC and SqCC specimens. *Red lines* divide lung tissue into two compartments: intratumoral (*right*) and peritumoral (*left*); *arrows* indicate cells expressing each immune marker (brown).

**Fig 3.** Kaplan-Meier curves illustrating the prognostic effect on OS and RFS of expression of immune markers in lung ADC and SqCC specimens.
Figure 1

A

ADC

SqCC

B

C

D

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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.
Figure 2
Figure 3

A Adenocarcinoma: Malignant Cells PD-L1 (H-score)

B Squamous Cell Carcinoma: Macrophages PD-L1 (H-score)

C Squamous Cell Carcinoma: Intratumoral CD57

D Squamous Cell Carcinoma: Peritumoral CD57

E Adenocarcinoma: Intratumoral CD68

F Adenocarcinoma: Intratumoral Immuno-score

[PD-L1 H-score & (CD4+CD8+CD68)]

P-value < 0.001

High (>5) PD-L1 + High Inflammatory cells (E / N = 6 / 26)
High (>5) PD-L1 + Low Inflammatory cells (E / N = 8 / 8)
Low (≤5) PD-L1 + High Inflammatory cells (E / N = 12 / 51)
Low (≤5) PD-L1 + Low Inflammatory cells (E / N = 19 / 41)

P-value = 0.002

P-value = 0.001

P-value = 0.022

P-value = 0.02

P-value = 0.016

P-value = 0.02

P-value = 0.066

P-value = 0.085
Table 1. Characteristics of the 254 NSCLC patients

<table>
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<th>Characteristic</th>
<th>Category</th>
<th>ADC (N = 146)</th>
<th>SqCC (N = 108)</th>
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<td>Sex</td>
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<td></td>
<td>Female</td>
<td>70 (48)</td>
<td>44 (41)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>76 (52)</td>
<td>64 (59)</td>
</tr>
<tr>
<td>Tobacco history</td>
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</tr>
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<td>18 (12)</td>
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<tr>
<td>Yes</td>
<td>128 (88)</td>
<td>108 (100)</td>
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<tr>
<td>Smoking status</td>
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<td></td>
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<tr>
<td>Never</td>
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<td>--</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>70 (48)</td>
<td>52 (48)</td>
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</tr>
<tr>
<td>Current</td>
<td>58 (40)</td>
<td>56 (52)</td>
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<tr>
<td>T1</td>
<td>42 (29)</td>
<td>31 (29)</td>
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</tr>
<tr>
<td>T2</td>
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<td>N0</td>
<td>104 (71)</td>
<td>61 (56)</td>
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<td>N1</td>
<td>24 (16)</td>
<td>32 (30)</td>
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<tr>
<td>N2</td>
<td>18 (12)</td>
<td>15 (14)</td>
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<td>AJCC stage*</td>
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<tr>
<td>I</td>
<td>84 (58)</td>
<td>43 (40)</td>
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<tr>
<td>II</td>
<td>36 (25)</td>
<td>41 (38)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>26 (18)</td>
<td>24 (22)</td>
<td></td>
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<tr>
<td>Histologic features</td>
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<tr>
<td>ADC**</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Solid</td>
<td>81 (55)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Lepidic</td>
<td>20 (14)</td>
<td>--</td>
<td></td>
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<tr>
<td>Acinar</td>
<td>16 (11)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>20 (14)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Micropapillary</td>
<td>9 (6)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>SqCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>--</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>--</td>
<td>57 (53)</td>
<td></td>
</tr>
<tr>
<td>Poorly</td>
<td>--</td>
<td>49 (45)</td>
<td></td>
</tr>
<tr>
<td>KRAS status†</td>
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<td></td>
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<tr>
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<td>47 (32)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>97 (66)</td>
<td>--</td>
<td></td>
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<tr>
<td>EGFR status‡</td>
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<tr>
<td>Mutant</td>
<td>21 (14)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
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<td>Adjuvant therapy</td>
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<tr>
<td>No</td>
<td>81 (55)</td>
<td>65 (60)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>65 (45)</td>
<td>43 (40)</td>
<td></td>
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<tr>
<td>Vital status at 5 years</td>
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<tr>
<td>Alive</td>
<td>91 (62)</td>
<td>45 (42)</td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>55 (38)</td>
<td>63 (58)</td>
<td></td>
</tr>
<tr>
<td>Recurrence status at 5 years</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>89 (61)</td>
<td>62 (57)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>57 (39)</td>
<td>46 (43)</td>
<td></td>
</tr>
</tbody>
</table>

*American Joint Committee on Cancer.
**World Health Organization of Lung Cancer.
†Mutation status unknown in two cases.
‡Mutation status unknown in eight cases.
Table 2. Median TAIC density by mm² in the intratumoral and peritumoral compartments in lung ADC and SqCC specimens

<table>
<thead>
<tr>
<th>Marker</th>
<th>Intratumoral ADC</th>
<th>Intratumoral SqCC</th>
<th>Intratumoral P*</th>
<th>Peritumoral ADC</th>
<th>Peritumoral SqCC</th>
<th>Peritumoral P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>1505.80</td>
<td>1090.00</td>
<td>0.0030</td>
<td>1636.00</td>
<td>2213.70</td>
<td>0.0010</td>
</tr>
<tr>
<td>CD4</td>
<td>1217.50</td>
<td>832.00</td>
<td>&lt;0.0001</td>
<td>1425.40</td>
<td>1648.90</td>
<td>0.0700</td>
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<tr>
<td>CD8</td>
<td>866.40</td>
<td>693.20</td>
<td>0.1430</td>
<td>1040.80</td>
<td>1365.60</td>
<td>0.0020</td>
</tr>
<tr>
<td>CD57</td>
<td>377.47</td>
<td>208.00</td>
<td>&lt;0.0001</td>
<td>475.40</td>
<td>508.00</td>
<td>0.7600</td>
</tr>
<tr>
<td>Granzyme B</td>
<td>236.50</td>
<td>254.10</td>
<td>0.8070</td>
<td>410.60</td>
<td>486.60</td>
<td>0.0490</td>
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<tr>
<td>CD45RO</td>
<td>846.37</td>
<td>913.33</td>
<td>0.1370</td>
<td>1191.80</td>
<td>1725.60</td>
<td>&lt;0.0001</td>
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<tr>
<td>PD-1</td>
<td>521.40</td>
<td>499.63</td>
<td>0.1860</td>
<td>951.80</td>
<td>870.90</td>
<td>0.5810</td>
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<td>FOXP3</td>
<td>357.10</td>
<td>335.70</td>
<td>0.6470</td>
<td>435.00</td>
<td>591.80</td>
<td>0.0010</td>
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<tr>
<td>CD68</td>
<td>330.81</td>
<td>201.56</td>
<td>&lt;0.0001</td>
<td>445.54</td>
<td>428.69</td>
<td>0.7740</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test analysis.*
Table 3. Distribution of types of microenvironment in NSCLC based on intratumoral MCs PD-L1 expression and density of CD3-positive TILs.

<table>
<thead>
<tr>
<th>PD-L1 Expression</th>
<th>ADC</th>
<th>Intratumoral TILs</th>
<th>SqCC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Negative (%)</td>
<td>Positive (%)</td>
<td>Total</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>43 (29)</td>
<td>70 (48)</td>
<td>113 (77)</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>5 (3)</td>
<td>28 (19)</td>
<td>33 (23)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>48 (33)</td>
<td>98 (67)</td>
<td>146 (100)</td>
</tr>
</tbody>
</table>

Note: PD-L1 expression (negative, defined as < 5% positive MCs membranous expression; positive, > 5%) and intratumoral TILs determined by CD3 positive cells density (negative, defined as mild [lowest tertile] TIL density; positive, defined as moderate and severe density [middle and highest tertiles]).
Clinical Cancer Research

Image Analysis-Based Assessment of PD-L1 and Tumor-Associated Immune Cells Density Supports Distinct Intratumoral Microenvironment Groups in Non-Small Cell Lung Carcinoma Patients

Edwin R Parra, Carmen Behrens, Jaime Rodriguez-Canales, et al.

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