Correlation Between Programmed Death Receptor-1 Expression in Tumor-Infiltrating Lymphocytes and Programmed Death Ligand-1 Expression in Non–Small Cell Lung Carcinoma

Paloma del C. Monroig-Bosque, MD, PhD; Brandon Driver, MD; Joel A. Morales-Rosado, MD; Michael Deavers, MD; David Tacha, PhD; Eric Bernicker, MD; Philip T. Cagle, MD; Ross A. Miller, MD

Context.—The interaction between programmed death ligand-1 (PD-L1) and programmed death receptor-1 (PD-1) on activated T cells sends an inhibitory signal that dampens the immune response. Tumors can express PD-L1 and evade the immune system. In advanced non–small cell lung carcinoma, expression of PD-1 in tumor-infiltrating lymphocytes (TILs) correlates with PD-L1 expression in tumor cells (TCs). However, this relationship has not been thoroughly explored in early disease.

Objective.—To investigate the correlation of PD-1 and PD-L1 in non–small cell lung carcinoma tumor samples, with emphasis on stage I disease.

Design.—Whole tissue sections from non–small cell lung carcinoma tumors were retrospectively evaluated by immunohistochemistry for PD-1 and PD-L1 expression. The scoring was based on the percentage of cells positive for PD-1 in TILs and PD-L1 in TCs and tumor-infiltrating immune cells (ICs).

Results.—Expression of PD-1 in TILs was observed in 147 of 161 non–small cell lung carcinoma cases (91%). The majority of cases negative for PD-1 also lacked PD-L1 in TCs. The 68 cases with highest PD-1 expression in TILs included 33 (49%) with expression of PD-L1 in TCs and ICs. Strong correlations were observed in patients with elevated PD-1 expression in TILs and PD-L1 in TCs and ICs (P = .01) and ICs (P = .003). Expression of PD-1 also correlated with increased PD-L1 in TCs and ICs when the 2 were grouped together (P < .001). Finally, stage I patients with negative PD-1 and PD-L1 expression showed trends toward increased disease-specific survival.

Conclusions.—Expression of PD-1 in TILs correlates with PD-L1 expression in both TCs and ICs. Furthermore, negative expression of PD-1 and PD-L1 suggest trends toward disease-specific survival, even in early disease stages.

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Lung cancer is the leading cause of cancer-related mortality in the United States and worldwide. The majority of cases (80%–85%) are non–small cell lung cancer (NSCLC).1 Adenocarcinoma is the most frequent cell type, accounting for up to 50% of all lung cancers. Tumor histology and biomarker expression patterns predict and correlate with responses to various agents.2–4 An array of treatments for NSCLC have been developed, yet these therapies are beneficial in subsets of patients harboring specific genomic alterations, and no significant difference in overall survival has been seen.5 Additionally, chemotherapy resistance in NSCLC is not uncommon; thus, there continues to be an unmet urgency to develop novel therapeutic strategies for patients with NSCLC.6 To this end, immunotherapy has emerged as a novel and promising approach in NSCLC patients.7 Under normal physiologic conditions, immune checkpoints such as programmed death receptor-1 (PD-1)/programmed death ligand-1 (PD-L1) dampen the immune response to maintain a balanced equilibrium. However, the exploitation and overexpression of checkpoints such as this enable tumor cell (TC) survival and proliferation, resulting in tumoral immune evasion.8–10 Immunotherapies targeting the PD-1/PD-L1 checkpoint are available, and have shown favorable safety profiles as well as marked and durable responses in advanced melanoma, renal cell cancer, and NSCLC.11,12 Nonetheless, details pertaining to the prognostic significance of PD-1/PD-L1 and overall dynamics in PD1/PDL-1 in NSCLC in Early Disease (Stage I)—Monroig-Bosque et al.
response rates of the underlying physiology within the tumor microenvironment remain to be elucidated.

Recently, He et al. investigated NSCLC tumor microenvironment by analyzing PD-1 and PD-L1 protein expression and their correlation with tumor-infiltrating lymphocytes (TILs). Their data set included surgically resected specimens from NSCLC patients, the majority with advanced-stage and nodal disease, including patients with diagnosed metastasis. Their cohort had all histotypes (squamous cell carcinoma, adenocarcinoma, large cell carcinoma, NSCLC not otherwise specified, and others), the majority of which were squamous cell carcinoma. Their results showed that high expression of PD-1 in TCs significantly correlated with high expression of PD-L1 in a high percentage of TILs. Furthermore, they found that patients whose TCs were PD-L1 negative had a tendency toward longer relapse-free survival compared to patients whose TCs were PD-L1 positive. To date, there are no current studies focusing on the correlations of PD-1 and PD-L1 proteins in stage I disease, which can be treated and theoretically cured by surgery. Moreover, advancements in imaging technologies and patient screening are allowing early detection of lung cancer, and the prognostic relevance of PD-1/PD-L1 is not known for this patient group. Thus, in this study we evaluated the protein expression of PD-1 and PD-L1 in NSCLC patients with emphasis on the most common type, adenocarcinoma, but also including some squamous cell carcinomas. Most of the patients we selected had a diagnosis of an early/limited disease stage, few had evidence of nodal metastasis, and only one had distant metastasis. All of our patients were treated by surgical resection with no adjuvant therapy. We then investigated the expression of PD-1/PD-L1 using previously established categories for immunohistochemistry scoring, and assessed the correlation between the expression of PD-1 in TILs and PD-L1 in TCs and tumor-infiltrating immune cells (ICs). We also evaluated the prognostic significance of the PD-1/PD-L1 immune checkpoint in NSCLC patients of all disease stages.

**MATERIALS AND METHODS**

**Study Samples**

Institutional review board approval of research protocols for this project was obtained through Houston Methodist Hospital Research Institute (Houston, Texas). Surgical pathology blocks were obtained from a series of NSCLCs from attempted curative surgical resections of lung cancers between 1975 and 1991 at Houston Methodist Hospital for which 5-year or greater survival data were available. Because these were attempted curative surgical resection specimens, the great majority were expected to represent early-stage NSCLC. Retrospective chart review was performed to obtain pathology reports, including original cell type diagnosis, and clinical data, including patient age, sex, smoking history, and survival status. Routine hematoxylin-eosin sections were obtained from these blocks and reviewed for histologic diagnosis.

Tumors lacking both adenocarcinoma and squamous differentiation were reclassified according to 2015 World Health Organization criteria. Tumors with sarcomatoid or neuroendocrine morphology were also excluded. Subtypes were recorded for adenocarcinoma cases according to 2015 World Health Organization criteria. Tumor staging was modified if necessary to be consistent with the 7th American Joint Committee on Cancer Staging Manual.

**Immunohistochemistry**

Tissue sections on glass slides were cut to 4 to 5 μm, deparaffinized, and hydrated in a series of gradient alcohols. A peroxidase block was performed, and tissue sections were then retrieved in a modified citrate buffer using a pressure cooker (Decloaking Chamber, Biocare Medical, Pacheco, California) at 110°C for 15 minutes. Tissue sections were then cooled for 20 minutes and placed in buffer.

Immunohistochemistry for PD-L1 was carried out with an automated stainer (Leica Bond III, Leica Biosystems, Buffalo Grove, Illinois) using anti–PD-L1 (clone SP142) obtained from Spring Bioscience (Pleasanton, California). Incubation time was 30 minutes and dilution was 1:1000. Expression of PD-L1 was scored semiquantiatively according to percentage of PD-L1–positive TCs (TC0 for <1%, TC1 for 1%–4%, TC2 for 5%–49%, and TC3 for ≥50%) and percentage of tumor area with PD-L1–positive ICs (IC0 for <1%, IC1 for 1%–4%, IC2 for 5%–9%, IC3 for ≥10%) as previously described. Immunohistochemistry for PD-1 was performed using a PD-1 antibody (NAT105, Biocare), which was diluted at 1:50 and incubated for 1 hour at room temperature, followed by a 2-step...
Table 2. Programmed Death Receptor-1 (PD-1) Is Significantly Expressed in Non–Small Cell Lung Cancer (NSCLC)

| PD-1 Expression | NSCLC (N = 161) | Adenocarcinoma (n = 124) | Squamous Cell Carcinoma (n = 37) |
|-----------------|-----------------|--------------------------|-------------------------------|
| IHC0, No. (%)   | 14 (9)          | 10 (8)                   | 4 (11)                        |
| IHC1, No. (%)   | 39 (24)         | 30 (24)                  | 9 (24)                        |
| IHC2, No. (%)   | 40 (25)         | 29 (23)                  | 11 (30)                       |
| IHC3, No. (%)   | 68 (42)         | 55 (44)                  | 13 (35)                       |

Abbreviation: IHC, immunohistochemistry score.

Table 4. Expression of Programmed Death Receptor-1 (PD-1) in Tumor-Infiltrating Lymphocytes Correlates With Programmed Death Receptor Ligand-1 (PD-L1) Expression in Tumor-Infiltrating Immune Cells (ICs) in Non–Small Cell Lung Cancer

| PD-1 | PD-L1, No. (%) |
|------|----------------|
|      | IC0            | IC1/2/3               |
| IHC0 (n = 14) | 13 (93) | 1 (7)                |
| IHC1 (n = 39) | 32 (82) | 7 (18)               |
| IHC2 (n = 40) | 27 (68) | 13 (33)              |
| IHC3 (n = 68) | 36 (53) | 32 (47)              |

Abbreviation: IHC, immunohistochemistry score.

Table 3. Expression of Programmed Death Receptor-1 (PD-1) and Programmed Death Receptor Ligand-1 (PD-L1) Expression in Tumor Cells (TCs) in Non–Small Cell Lung Cancer

| PD-L1, No. (%) |
|----------------|
| PD-1 TC0 TC1 TC2/3 |
| IHC0 (n = 14) | 14 (100) 0 (0) 0 (0) |
| IHC1 (n = 39) | 29 (74) 7 (18) 3 (7) |
| IHC2 (n = 40) | 25 (63) 9 (23) 6 (15) |
| IHC3 (n = 68) | 35 (51) 16 (24) 17 (25) |

Abbreviations: IHC, immunohistochemistry score; TC, tumor cell.

RESULTS

Following reclassification by 2015 World Health Organization criteria, a total of 124 adenocarcinomas and 37 squamous cell carcinomas were included in the study (161 cases total). The clinicopathologic features of the NSCLC study population were as follows: median age 64 years (range, 35–90 years), male to female ratio of 1.48, and median smoking pack-years of 52 (range, 1–200) (Table 1). As expected, the majority (13 of 14; 93%) of cases lacking PD-1 expression in TILs concurrently lacked PD-L1 expression in TCs (Table 3). In addition, increased PD-1 expression scores correlated with increased PD-L1 expression in TCs. Approximately half of the samples having a PD-1 IHC3 score had a PD-L1 TC score of TC1, 2, or 3 (Table 3). The correlation between PD-1 expression in TILs and PD-L1 expression in TCs was statistically significant ($\chi^2$; P = .01).

The majority (13 of 14; 93%) of cases lacking PD-1 expression in TILs also lacked PD-L1 expression in ICs (Table 4). Similar to what was observed with PD-1 expression in TCs, increased PD-1 expression scores correlated with increased PD-L1 expression in ICs, with approximately 47% of ICs having PD-L1 scores of IC1, 2, or 3. Correlation between PD-1 expression in TILs and PD-L1 expression in ICs was statistically significant ($\chi^2$; P = .003). Additionally, high PD-1 expression strongly correlated with high PD-L1 expression in both TCs and ICs ($\chi^2$; P < .001) (Table 5). Finally, the monotonic relationship of the variables (whether linear or not) was assessed and found to be statistically significant for the percentage of PD-1 in TILs with PD-L1 in TCs (Spearman rank correlation factor = 0.29, P < .001), and PD-1 in TILs with PD-L1 in ICs (Spearman rank correlation factor = 0.31, P < .001). For lung adenocarcinomas, PD-1 expression in TILs at a 5% cutoff was not associated with age, sex, smoking pack-years, TNM stage, or histologic subtype (data not shown).
However, lung adenocarcinomas with PD-1 expression in TILs greater than 5% were weakly associated with higher histologic grades (data not shown). For prognostic implications, we evaluated disease-specific survival according to PD-1/PD-L1 scores in the NSCLC tumor samples. A score lower than 1% was considered negative and any percentage of 1% or higher was considered positive for both PD-1 and PD-L1. Our results showed a trend toward increased disease-specific survival in patients with negative PD-1 (P = .21, hazard ratio [HR] = 0.76; 95% CI, 0.3758–1.557) and PD-L1 (P = .45, HR = 0.42; 95% CI, 0.161–1.099) scores. We further stratified the patients by disease stage and evaluated disease-specific survival in stage I patients (Figure, A and B). Our results suggest a trend toward increased disease-specific survival in stage I patients with PD-1 negative scores (P = .21, hazard ratio = 0.76; 95% CI, 0.3758–1.557). A limited trend was also observable in stage I patients with negative PD-L1 expression compared with positive (P = .45, hazard ratio = 0.42; 95% CI, 0.161–1.099). No correlation was found between PD-1 in TILs and PD-L1 in ICs.

**DISCUSSION**

Tumor cell exploitation of immune checkpoint pathways is thought to facilitate cancer tolerance and immune system evasion. The PD-1/PD-L1 receptor–ligand pair comprises a dominant immune checkpoint pathway that is known to contribute to tumor immune evasion in several cancer types, particularly NSCLC. Recent studies have shown that immune checkpoint inhibitors against proteins such as PD-1 and PD-L1 have been proven successful in clinical trials...
treatment of NSCLC patients. Nonetheless, their roles as prognostic biomarkers to predict treatment response and survival advantages have not been fully elucidated.

In this vein, He et al. investigated the relationship between PD-1 expression in TILs and PD-L1 expression in surgically resected NSCLC tumors. As part of their data set, they had more patients who had a diagnosis of squamous cell carcinoma (52.3%) and fewer with a diagnosis of adenocarcinoma (28.8%). In addition, less than half of their patients were stage I (41.7%) and more patients were higher stage (stage II, 25.2%; stage III, 28.1%; stage IV, 5.0%). With their cohort, their results showed that high expression of PD-1 in TILs significantly correlated with increased expression of PD-L1 in TCS. Furthermore, they determined there was a trend associating negative expression of PD-1 and of PD-L1 (independently) increased relapse-free survival. 

In this study, we investigated the correlation of PD-1 in TILs and PD-L1 in TCs. We further evaluated the correlation of PD-1 expression with PD-L1 in ICs. This is an important observation, as PD-L1 expression in TILs is thought to diminish antitumor immunity similarly to PD-L1 expression in TCs. In addition, we evaluated the prognostic significance of PD-1 and PD-L1 in NSCLC patients, focusing on stage I disease.

Compared with the study from He et al., our series includes a larger number of adenocarcinomas (77%), the most commonly diagnosed NSCLC type, and a larger percentage of early-stage cancers, particularly stage I. In our cohort, the patients had surgical resection, with no previous neoadjuvant treatment. Upon staging, most of them were disease stage I (75%), with T1 (52%); very few (18%) had nodal extension, and only one had distant metastasis. We hypothesized that PD-1 and PD-L1 could potentially be relevant in early disease such as stage I.

Our results are in good agreement with the results of the prior study by He and colleagues, despite differences in study population and study design. We found a significant correlation between PD-1 in TILs and PD-L1 in TCs (P = .01). Furthermore, our results extend the observation in NSCLC, as we evaluated and confirmed that PD-1 expression in TILs correlates with PD-L1 expression in ICs (P = .003). When grouped together, PD-L1 expression in TCs and ICs correlates with expression of PD-1 in TILs (P < .001). Additionally, we evaluated the prognostic significance of PD-1 and PD-L1 in NSCLC. In patients of all stages, we found that there was a trend toward disease-specific survival in those who were negative, compared with positive, for PD-1 expression in TILs (data not shown). Similar trends were seen in patients who were negative for PD-L1 in TILs (data not shown). Given that no previous studies had evaluated the prognostic value of this immune checkpoint in early-stage NSCLC, we further explored if these trends were seen in patients with a diagnosis of stage I disease. Our results showed that trends toward disease-specific survival were seen in patients with negative PD-1 scores compared with positive ones (P = .21). Similar trends were also seen toward disease-specific survival in patients negative for PD-L1 expression in TCS (P = .45). No trends were seen regarding PD-L1 expression in ICs. There was no difference between these results and results when our data set was stratified by lung cancer type (adenocarcinoma versus squamous cell carcinoma). We also did not see any correlation between the clinicopathologic factors of the patients evaluated and expression of PD-1 or PD-L1.

Our study design includes 2 additional strengths. First, we used the same PD-L1 immunohistochemical scoring used in the POPLAR clinical trial, and PD-1 scoring that stratifies into 4 groups rather than a single cutoff. Regarding the latter, this stratification helped to show the correlation between PD-1 expression in TILs and PD-L1 expression in the tumor. Second, we used a different antibody (compared with the study from He et al.) to perform immunohistochemistry, and our findings confirmed their results (refer to Materials and Methods).

In summary, our study showed a correlation between PD-1 in TILs and PD-L1 in TCs and in ICs. Further, in patients with stage I NSCLC, and negative PD-1 as well as PD-L1 expression in TILs, there was a trend toward disease-specific survival. The results of our study support contemplating the use of immune checkpoint blockers in patients with limited/localized disease, not only advanced disease stages. Our results are novel, because the majority of the PD-L1 clinical studies have focused on advanced-stage NSCLC. Further studies are needed to address these findings.

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References
1. Gridelli C, Rossi A, Carbone DP, et al. Non-small cell lung cancer. Nat Rev Dis Primers. 2013;1:15009. doi:10.1038/nrdp.2013.9.2009.Review.
2. Travis WD, Brambilla E, Riely GJ. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol. 2013; 31(8):992–1001.
3. Jamal Hanjani M, Wilson GA, McGowanahana N, et al. Tracking the evolution of non-small-cell lung cancer. N Engl J Med. 2017;376(2):2109–2121.
4. Kim J, Jung SJ, Choi CM, Ro JY. Correlation of histologic subtypes and molecular alterations in pulmonary adenocarcinoma: therapeutic and prognostic implications. Adv Anat Pathol. 2016;23(5):330–338.
5. Sagliotti GV, Bironzo P, Vansteenkiste JF. Addressing the unmet need in lung cancer: the potential of immuno-oncology. Cancer Treat Rev. 2015;41(6):465–475.
6. Di Maio M, Chiodini P, Georgoulis V, et al. Meta-analysis of single agent chemotherapy compared with combination chemotherapy as second-line treatment of advanced non-small-cell lung cancer. J Clin Oncol. 2009;27(11): 1336–1414.
7. Festino L, Botti G, Lorigan P, et al. Cancer treatment with anti-PD1/PD-L1 agents: is PD-L1 expression a biomarker for patient selection? Drugs. 2016;76(9): 925–945.
8. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer. 2016;16(5):275–287.
9. Gordon SR, Maute RL, Dulken BW, et al. PD-1 expression by tumor-associated macrophages inhibits phagocytosis and tumor immunity. Nature. 2017;543(7653):495–499.
10. Lafuente-Sanchis A, Zúñiga A, Estors M, et al. Association of PD-1, PD-L1, and CTLA-4 gene expression and clinicopathologic characteristics in patients with non-small-cell lung cancer. Clin Lung Cancer. 2016;18(2):109–116.
11. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369(2):122–133.
12. Reck M, Quan D, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013;369(2):134–144.
13. He Y, Rozeboom L, Rivard CJ, et al. PD-1, PD-L1 protein expression in non-small cell lung cancer and their relationship with tumor-infiltrating lymphocytes. Med Sci Monit. 2017;23:1208–1216.
14. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomized controlled trial. Lancet. 2016;387(10030):1837–1846.
15. Brown AE, Sinoh D, Fukuoka J, et al. Tissue-preserving antibody cocktails to differentiate primary squamous cell carcinoma, adenocarcinoma, and small cell carcinoma of lung. Arch Pathol Lab Med. 2013;137:1274–1281.
16. Travis WD, Brambilla E, Nicholson AG, et al. The 2015 World Health Organization classification of lung tumors: impact of genetic, clinical and radiologic advances since the 2004 classification. J Thorac Oncol. 2015;10(9):1243–1260.
17. Compton CC, Byrd, DR, Garcia-Agülar J, et al. AJCC Cancer Atlas. 7th ed. New York, NY: Springer-Verlag; 2012:311–328.
18. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70.
19. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674.
20. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373(2):123–135.
21. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced nonsquamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373(17):1627–1639.
22. Gubens MA. Immunotherapies for lung cancer. J Natl Compr Canc Netw. 2017;15:692–695.
23. American Association for Cancer Research. First Anti-PD-L1 drug approved for NSCLC. Cancer Discov. 2016;6(12):OF1.
24. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375(19):1823–1833.
25. Sorscher S. Pembrolizumab in non-small-cell lung cancer. N Engl J Med. 2017;376(10):996–997.
26. Zhao T, Li C, Wu Y, Li B, Zhang B. Prognostic value of PD-L1 expression in tumor infiltrating immune cells in cancers: a meta-analysis. PloS One. 2017;12(4):e0176822. doi:10.1371/journal.pone.0176822.
27. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515:563–567.
28. Sun JM, Zhou W, Choi YL, et al. Prognostic significance of PD-L1 in patients with non-small cell lung cancer: a large cohort study of surgically resected cases [published online ahead of print April 18, 2016]. J Thorac Oncol. 2016;11(7):1003–1011. doi:10.1016/j.jtho.2016.04.007.