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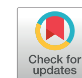
Research article

Correlation between PD-L1 expression (clones 28-8 and SP263) and histopathology in lung adenocarcinoma



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A R T I C L E I N F O

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A B S T R A C T

Lung cancer is the leading cause of cancer-related death worldwide. Recent advances in the management of non-small cell carcinoma are focused on the discovery of targeted therapies and novel immunotherapy strategies for patients with advanced disease. Treatment with anti PD-(L)1 immune checkpoint inhibitors requires the development of predictive biomarkers to select those patients that can most benefit from these therapies. Several immunohistochemical biomarkers have been developed in different technological platforms. However, the most useful and accessible for the daily clinical practice need to be selected. The objective of this study was to compare PD-L1 expression by automated immunohistochemistry in lung adenocarcinoma (ADC) FFPE samples with clones 28-8 and SP263 performed with the BenchMark GX automated staining instrument. To further determine inter-observer agreement between two pathologists, and to correlate the results with histologic and pathology variables. FFPE tissue from 40 samples obtained from patients with lung ADC were reviewed retrospectively. Among all studied specimens, 53% of samples presented <1% of positive tumor cells with the 28-8 clone and 50% had <1% of PD-L1 expression in tumor cells with the SP263 clone; PD-L1 expression between ≥1 and <5% was observed in 18% and 24%; ≥5 and <50% PD-L1 expression in 18% and 21%; and ≥50% PD-L1 expression in 11% and 5% of samples, respectively. Similar results between antibodies were observed in 84% of cases for each of the four PD-L1 cutoff groups (Pearson's score 0.90, $p < 0.00001$). The interobserver degree of agreement calculated with Kappa was 0.75 (95%CI: 0.57–0.93), $z = 7.08$; $p < 0.001$. Lepidic, acinar and mucinous patterns had predominantly <1% PD-L1 expression, and the solid pattern subtype had high levels of PD-L1 staining using both clones. PD-L1 expression in less than 1% of tumor cells was similar in stages I/II compared to III/IV. No significant differences were observed in PD-L1 staining and quantification pattern between IHC antibodies 28-8 and SP263.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. In Argentina, 10,296 new cases are diagnosed and 9,254 people die every year [2]. Non-small cell lung carcinomas (NSCLC) are divided in 3 main categories: adenocarcinoma (ADC), squamous cell carcinoma (SCC) and large cell carcinoma [3]. NSCLC is characterized by the presence of genetically distinct and dynamic subpopulations within the same tumor, which can have an impact on treatment outcomes. To select patients for targeted therapies like kinase inhibitors, we need test for driver alterations involving *EGFR*, *ALK*, *ROS1*, and *BRAF* as standard practice for patients with advanced tumors [4, 5, 6, 7]. The discovery of immune-checkpoints inhibitor blockade of CTLA4 and the PD-(L)1 axis has enabled novel treatments in a wide range of tumor types. Immune surveillance is essential to prevent the development of cancer and is associated with the expression of neo-antigens by tumor cells as result of somatic mutations in genes, viral antigen presentation [7, 8, 9].

The use of immunohistochemical analysis for the determination of PD-L1 has been proposed as a prognostic and predictive biomarker for anti-PD-1 and anti-PD-L1 monoclonal antibodies in the clinical scenario of advanced NSCLC. The Food and Drug Administration (FDA) requires the development of diagnostic tests, either as “companion” or compulsory for such a drug, or “complementary”, which means recommended (eg. PD-L1 28-8 antibody [Abcam] using the DAKO detection system).

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There are several anti PD-L1 antibodies in practice, which are being developed as biomarker tests including: 22C3 (Dako' Platform), 28-8 (pharm Dx, Dako's Platform), SP142 (Spring Bioscience, Ventana's Platform), E1L3N and E1J2J (Cell Signaling Technologies, Ventana's Platform), SP263 (Ventana's Platform), 7G11 (Boston University), EPR1161-2 (Epitomics-Abcam); etc [10]. Available companion diagnostic tests use specific assays with different clones, staining protocols, automated platforms, scoring interpretation and target cells (tumor and/or immune cells). In addition, different PD-L1 cutoffs are being selected for anti PD-(L)1 treatment in the first or second line therapy, and PD-L1 expression is a dynamic marker subject to temporospatial heterogeneity.

Given the diversity of testing platforms, worldwide efforts are made to "harmonize" PD-L1 testing to facilitate clinical decision-making. Thus, the National Cancer Institute in France developed a national validation study with different antibodies and platforms searching for technical equivalences [11]; the International Pulmonary Pathology Society [12]; the Colonia Score in Germany [13]; the Blueprint PD-L1 Assay Comparison Project [14, 15] and the Harmonization study in Israel [16].

The objective of this study was to compare PD-L1 expression by automated immunohistochemistry in lung adenocarcinoma (ADC) FFPE samples in our country with anti PD-L1 clones 28-8 and SP263 performed with the BenchMark GX automated staining platform. Interobserver agreement between two observers was analyzed and results were correlated with pathological data.

2. Materials and methods

We retrospectively studied forty non-matched biopsies from patients with lung ADC, fixed in 10% buffered formalin, paraffin embedded, and then cut into sections of 4 μm. These samples underwent immunohistochemistry testing using PD-L1 rabbit monoclonal antibody, clones 28-8 (Abcam, Cambridge, UK) and SP263 (Ventana Medical Systems Inc, Tucson, USA). Immunohistochemical staining was performed with BenchMark GX immunoautomate (Ventana Medical Systems Inc, Tucson, USA), OptiView DAB IHC Detection Kit and OptiView Amplification Kit (Ventana Medical Systems Inc, Tucson, USA). Staining was evaluated by two pathologists with expertise in thoracic pathology, IHC and PD-L1 assessment. Both pathologists blinded to clinical data scored the proportion of PD-L1 in tumor cells for each biopsy independently. For tumor cells, the proportion of PD-L1 positive cells was estimated as the percentage of PD-L1 positive tumor cells over the total tumor cells. Although ADC is a heterogeneous tumor type and several histological patterns may coexist in the same sample, PD-L1 staining was evaluated in the whole slide, irrespective of cell type. Interobserver agreement between two observers was evaluated using the Kappa test in each of the four groups where the results were divided based on cutoffs from recently published studies (<1%, ≥1 to <5%, ≥5 to <50% and ≥50%) [17, 18]. Alpha significance level was p = 0.05. No binary limit was applied.

The study protocol was approved by the Institutional Ethics Committee. This study was performed in compliance with the good clinical practice (GCP), as defined by the International Conference on Harmonization (ICH). This protocol fully complies with the International Declaration on human genetic data, approved unanimously and by acclamation, by the UNESCO General Conference 32nd session, October 16, 2003. All data obtained were handled with absolute confidentiality according to national legislation (Ley de protección de Datos Personales, Habeas Data), and could only be accessed by the researchers involved in the study or the members of the institutional Ethics Committee.

3. Results

A total of 40 patients were included, 18 males and 22 females, with a mean age of 65 years (range: 31–84). Twenty-three were surgically resected specimens and 17 were biopsy specimens (endoscopic, core and fine needle aspiration). Cancer stage at diagnosis was: Ia 17%, Ib 25%, IIa 8%, IIIb 3%, and IV 47%.

Regarding PD-L1 staining, 53% were classified as having <1% of PD-L1 positive tumor cells using the 28-8 clone, and 50% of the samples had <1% of PD-L1 positive tumor cells with the SP263 clone. Using the 28-8 clone, 18% of samples were classified as having ≥1% to <5% PD-L1 positive tumor cells and 24% of samples were classified in this cutoff using SP263 antibody. Similarly, 18% of samples were scored as having ≥5% to <50% PD-L1 positive tumor cells using the 28-8 clone, and 21% using the SP263 clone. In addition, 11% and 5% of samples had a ≥50% PD-L1 expression in tumor cells using the 28-8 and SP263 clones, respectively. Matching results were observed in 84% of cases in all four categories, showing a high level of correlation between assays (Pearson's score 0.90, $p < 0.00001$).

Overall, 47% of lung ADC samples were PD-L1 positive (≥1%) with 28–8 antibody, and 50% with SP263 antibody. Using a cutoff of >5% PD-L1 positive tumor cells, 29% were positive using the 28-8 antibody and 26% of samples using SP263 antibody. The association between PDL1 and histological pattern is shown in Tables 1 and 2. Lepidic, acinar and mucinous patterns predominantly showed low PD-L1 expression (PD-L1 TPS <1%); however, the solid pattern had high levels of PD-L1 staining with both clones. Table 3 showed the relationship between PD-L1 results and clinical stage. Interobserver degree of agreement calculated with Kappa was 0.75 (95%CI: 0.57–0.93), $z = 7.08$; $p < 0.001$.

4. Discussion

The prescription of different anti PD-(L)1 drugs for the same disease depends on IHC PD-L1 testing with a specific antibody and platform. Pathologists have to face the challenge of working with different antibody clones, staining protocols, platforms, scoring systems and cutoffs [19]. Other issues with an impact on PD-L1 assessment include: tumor heterogeneity, dynamic nature of PD-L1 expression, which varies between anatomical sites, time of biopsy, type of treatments, epitopes with high sensitivity to fixation and composition of tumor microenvironment [20, 21].

In the initial findings from the Blueprint Programmed Death Ligand 1 Immunohistochemistry Assay Comparison Project, three experts, independently, evaluated the percentages of tumor and immune cells staining positive in 39 NSCLCs using 22C3, 28-8, SP142 and SP263. This comparison revealed a similar percentage of stained tumor cells between 22C3, 28-8 and SP263 assays, whereas the SP142 assay exhibited overall

Table 1. PD-L1 results and histological patterns with clones 28.8 and SP263.

% of positive tumor cells	clone 28.8		clone SP263	
	cases	Histological pattern	cases	Histological pattern
<1	53%	acinar/lepidic 66%mucinous 17%solid 17%	50%	acinar/lepidic 64%mucinous 22%solid 14%
≥1 to <5	18%	acinar/lepidic 100%	24%	acinar/lepidic 88%solid 12%
≥5 to <50	18%	beside other patterns,solid pattern 66%	21%	beside other patterns,solid pattern 63%
≥50	11%	acinar 50%solid pattern 50%	5%	acinar 50%solid pattern 50%
Cut-off ≥5 %, PD-L1 +:	29%		26%	
Cut-off ≥1 %, PD-L1 +:	47%		50%	

Table 2. PD-L1 results and histological patterns in surgically resected and biopsy specimens.

% of positive tumor cells	Histological pattern (surgically resected specimens)	Histological pattern (biopsy specimens)
<1	acinar/lepidic:70% mucinous: 10% solid: 20%	acinar/lepidic:67% mucinous: 33% solid: 0%
≥1 to <5	acinar/lepidic: 100%	0%
≥5 to <50	beside other patterns, solid pattern:80%	papillary: 50% solid:50%
≥50	acinar: 100%	solid: 100%

Table 3. PD-L1 percentage and its relationship with clinical stage.

% of positive tumor cells	Stage I/II	Stage III/IV
<1	41%	59%
≥1 to <5	86%	14%
≥5 to <50	83%	17%
≥50	25%	75%

lower PD-L1 staining [14]. Subsequently, the Blueprint 2 project corroborated these findings with a larger number of cases and observers. However, most specimens were obtained surgically, rather than by percutaneous biopsies, which are the most frequent type of diagnostic specimens in advanced NSCLC [15]. A collaborative study performed in Israel by Neuman *et al.* assessed the harmonization for the use of 22C3 clone on Ventana's platform. Neither in Argentina, nor in Israel is the Dako platform and/or the In Vitro Diagnostic (IVD) kit easily available. Therefore, Neuman *et al.* performed a comprehensive staining calibration on the BenchMark XT platform using Dako's prediluted 22C3 anti-PD-L1 primary antibody with two Ventana detection systems: Forty one 41 NSCLC cases independently evaluated by two pathologists proving that same PD-L1 IHC algorithm can be reliably applied to Ventana's BenchMark XT platform, and that all of the strongly positive cases had high interobserver and intraobserver agreement [16].

Adam *et al.* further showed that laboratory-developed tests (LDTs) have various levels of agreement when compared with three commercial assays. Those using SP263 clone had the greatest agreement across all platforms, whereas some LTDs with 28–8, 22C3, and E1L3N showed good correlation with the three commercial assays for tumoral cells only [22]. Assessment of tumor cells (TCs) score in NSCLC was highly reproducible using the SP263 assay, showing the accuracy of this assay in patient selection for anti-PD-1/PD-L1 therapy. The overall diagnostic sensitivity and specificity analyses indicated that the relative analytical sensitivities of the Food and Drug Administration-approved kits for tumor cell scoring, most specifically in non-small cell lung cancer, were as follows: Ventana PD-L1 (SP142) had the lowest sensitivity/specificity, followed by PD-L1 IHC 22C3 pharmDx, PD-L1 IHC 28-8 pharmDx and PD-L1 SP263 Ventana assay with the highest score [23].

In our study there was a concordance between the 28-8 and SP246 PD-L1 clones in 84% of the cases (Pearson's score 0.90, $p < 0.00001$). However, as Williams *et al.*, we adopted VENTANA PD-L1 (SP263) Assay in our clinical practice as a reliable and reproducible assay [24]. In our experience, especially in solid pattern tumors with abundant immune cells, 28-8 stains both populations too intensely, thus making quantification difficult. On the other hand, the SP263 assay stains sections delicately and allows more reliable identification of tumor cells. Nevertheless, our interobserver degree of agreement calculated with Kappa was 0.75 (95%CI: 0.57–0.93), $z = 7.08$; $p < 0.001$).

Both clones performed adequately PD-L1 expression to the histological pattern. Regarding the relationship between PDL1 and histological pattern, it can be hypothesized that poorly differentiated ADC (solid pattern) could present a higher tumor mutational burden (TMB) which could result in enhanced immunogenic tumors. On the other hand, well differentiated tumors (lepidic, acinar, etc.) usually have less genetic alterations and consequently lower levels of neoantigens presentation. However, TMB and PD-L1 tumor expression correlate poorly and are considered independent biomarkers of treatment response [26]. In this study TMB assessment was not performed on tumor samples.

Comparison of surgical (complete tumors) and biopsy specimens reveals that the focal solid pattern seen in surgical specimens fails to be seen in biopsy specimens. Since half of the cases with <1% stained cells were small samples (biopsies), these results should be interpreted as a consequence of the heterogeneous staining phenomenon. Ilie *et al.* reported on the possible difference in PD-L1 expression when comparing whole surgical tissue sections and matched lung biopsies using SP142. They found that PD-L1 expression was frequently discordant between both types of specimens (overall discordance rate = 48%, 95% confidence interval 4.64–13.24 and Kappa = 0.218) [25]. In all cases, biopsy specimens underestimated the PD-L1 status observed in the whole tissue sample. Their findings would indicate a poor association of PD-L1 expression in tumor and immune cells between lung biopsies and corresponding resected tumors. Moreover, the daily routine evaluation of PD-L1 expression in diagnostic biopsies can be misleading in defining the treatment with PD-(L)1 inhibitors26.

PD-L1 expression <1% had a similar distribution between stages I/II, compared to stages III/IV. However, the expression ≥1 to <50 was 85% for I/II stage, ≥50% in 75% of stage III/IV cases. These results would reveal a trend that needs further confirmation with a larger number of samples.

This is a real-life study in a developing country [22]. Therefore, PD-L1 assay selection is mainly based on both the platform and trained pathologists availability. Approaches to harmonizing testing methods are therefore crucial in ensuring appropriate treatment selection for our patients.

Our study has several limitations. Firstly, the number of studied samples is rather low. However, this is the largest study presented in our region. Secondly, only two PD-L1 testing platforms were evaluated, and therefore the results of this study cannot be extrapolated to other PD-L1 antibodies (22C3, SP142). Thirdly, since this study was not performed in other subtypes of non-small cell lung cancers, these results should not be extrapolated to squamous-cell carcinomas.

In conclusion, immunostaining with anti PD-L1 clones 28-8 and SP263 has high levels of correlation, in concordance with other studies. This correlation is maintained across different histological subtypes and clinical stage, however PD-L1 staining could be underestimated in small samples. PD-L1 testing needs to be cost-effective, developed with a holistic approach to be applied in multiple indications to meet patients' needs. However, it must be interpreted in the context of other tumor and patient immunologic factors with an impact on the response and prognosis with immunotherapy, such as tumor mutation burden, microsatellite instability, neoantigens, gene signatures and intratumoral inflammation [27].

Declarations

Author contribution statement

A. García and V. Denninghoff: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

G. Recondo, G. Recondo, M. Greco, M. de la Vega, F. Perazzo and A. Avagnina: Performed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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