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The frequency of high PD-L1 expression is low in lung adenocarcinoma patients from Northeast Brazil

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Abstract

Background: As Immune checkpoint inhibitors (ICPIs) are changing the standard-care in lung cancer with good clinical activities and durable responses, its indication must be based on the appropriate patient selection once only a fraction of patients has a response to these costly drugs. In larger cohorts the expression of programmed cell death–ligand 1 (PD-L1) has been associated with good clinical response of ICPIs in lung adenocarcinoma where the rate of high PD-L1 expression (defined as PD-L1tumor proportion score ≥ 50%) is ~ 30%, but once rare studies are available addressing the frequency of PD-L1 in populations outside those cohorts, we aimed to report the prevalence of PD-L1 and the frequency of patients with high PD-L1 expression utilizing data from a major pathology laboratory in Northeastern Brazil.

Methods: We retrospectively evaluated the PD-L1 expression in 158 surgically resected primary lung adenocarcinoma including 158 with anaplastic lymphoma kinase (ALK) expression. PD-L1 and ALK expression were evaluated by immunohistochemical analysis with the SP263 and D5F3 assays, respectively.

Results: Of the 158 samples analyzed, 94 (59.5%) had a PD-L1 tumor proportion score (TPS) < 1%, 38 (24.0%) had a PD-L1 TPS of 1–49% and 26 (16.5%) had a PD-L1 TPS of ≥50%. ALK expression was detected in 21 (13.3%) of the 158 tumor samples and 5 (3.2%) of them had a PD-L1 TPS of ≥50%.

Conclusion: The frequency of strong PD-L1 expression was lower than that previously reported in the trials where PD-L1 expression was used as a biomarker for patient selection. Also, considering that a subset of patients with ALK positivity had a strong PD-L1 expression, further studies will be required to examine the efficacy of PD-1/PD-L1 inhibitors in such patients.

Keywords: ALK, PD-L1, Immunotherapy, Lung adenocarcinoma, Biomarker, Brazil

Background

Accounting for 1.64 million deaths in 2013 (Leidl et al., 2016), lung cancer is still the leading cause of cancer-related death worldwide (Siegel et al., 2018). Non-small cell lung cancer (NSCLC) is the most prevalent subtype accounting for approximately 85% of all lung cancers with the majority of NSCLC patients presenting with an inoperable advanced disease at the time of diagnosis. In Brazil, 31.270 new cases of lung cancer are expected in 2018 (Brasil M da S, 2018), highlighting the substantial burden and need for novel treatment approaches and prevention.

Traditional platinum-based chemotherapy used to be the standard first-line treatment for lung cancer, but with low response rate (20–35%) and significant toxicities. More recently, targeted agents have been developed against specific oncogenic driver alteration, such as epidermal growth factor receptor (EGFR) and rearrangement of those encoding anaplastic lymphoma kinase (ALK) and leading the development of small molecule tyrosine kinase inhibitors (TKIs). The use of TKIs in NSCLC harboring these genetic mutations have obtained improvement in response rate and time

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to progression, but these benefits are temporary due to the onset of drug resistance to TKIs from various molecular mechanisms (Rihawi et al., 2017; Bruckl et al., 2017). Moreover, many NSCLC patients lack a genetic mutation with alterations that could be druggable by TKIs or other targeted therapies, such as mutations in the KRAS gene.

In the last decades, increasing knowledge of cancer biology has taking advantage of immunosuppressive mechanisms of tumors to evade immune surveillance in lung cancer, including 'immune check points' which are receptors on T cells that regulate the immune response. (Dong et al., 2002) Immune checkpoint inhibitors (ICPIs) that have been approved for treatment of NSLCL include monoclonal antibodies against CTLA-4 or PD-1/PD-L1 that restore T-cell activation enabling an antitumor immune response (Pardoll, 2012). One of the most important breakthroughs in cancer treatment of the past decade, ICPIs are revolutionizing lung cancer treatment as the rising number of studies shows improvements in overall survival and impressive clinical responses in NSCLC patients. (Rihawi et al., 2017)

Despite significant antitumor activity and survival benefits documented in patients with advanced NSCLC treated with ICPIs, only 15–20% of unselected patients have tumor response to these antibodies and a significant number of patients display primary resistance to these drugs (Shukuya & Carbone, 2016).

PD-L1 expression by immunohistochemistry (IHC) has emerged as an important factor, playing a major role in patient selection. As a predictive biomarker, PD-L1 helps to optimize therapy decisions by providing information on the probability of obtaining response to the PD-1/PD-L1 inhibitors such as pembrolizumab, nivolumab and atezolizumab (Passiglia et al., 2016; Abdel-Rahman, 2016). Concurrently, studies have suggested that PD-L1 expression can also work as a prognostic biomarker as it can provide information on the likely course of lung cancer, allowing estimation about the outcome of the patient (Kobayashi et al., 2018; Schmidt et al., 2015; Wang et al., 2015). Even being useful, PD-L1 IHC has limitations as a predictive biomarker such as the efficacy of anti-PD-1/PD-L1 agents in subsets of PD-L1 negative patients (Aguiar et al., 2016). To overcome such issue, Tumor Mutational Burden (TMB) has been studying as an independent predictor of response to immunotherapy complementary to PD-L1 (Goodman et al., 2017). Besides TMB, new savvy approaches are being studied and in a near future more sophisticated biomarkers should be available.

To date, four PD-L1 IHC assays are available: 22C3 and 28–8 pharmDx assays on Dako platforms, and SP142 and SP263 on Ventana platforms (Hirsch et al., 2017; Adam et al., 2018). When faced with calls for one PD-L1 test for all ICPIs it is crucial to know how comparable these assays are in order to safeguard the integrity of therapy decision making. Considering this, multiple initiatives aiming harmonization among these tests have been made involving multiple stakeholders. The results from these studies have suggested the use of 22C3, 28–8 and SP263 as interchangeable tests for the assessment of tumor cell PD-L1 expression. (Hirsch et al., 2017; Adam et al., 2018; Abdul Karim et al., 2017; Scheel et al., 2016; Rimm et al., 2017)

In Brazil, the PD-1 inhibitors pembrolizumab (Keytruda, Merck & Co) and nivolumab (Opdivo, Bristol-Myers Squibb) and the PD-L1 inhibitor atezolizumab (Tecentriq, Genentech/Roche) are approved by the Brazilian Health Regulatory Agency (ANVISA) for patients with advanced NSCLC after the failure of first-line therapy. Pembrolizumab is also approved in the first-line setting for patients with advanced NSCLC whose tumors have a strong PD-L1 expression, tumor proportion score (TPS) ⩾ 50% with no EGFR or ALK genomic aberrations. More recently, on June 2018, pembrolizumab in combination with conventional platinum-based chemotherapy was approved by the ANVISA for first-line therapy for patients with metastatic NSCLC. (BRASIL, 2018)

Due to the costly price of these drugs, the implementation of novel and more effective therapies must be accompanied by the appropriate selection of patients most likely to benefit from it. In addition, patient selection is important to avoid exposure to toxic effects and ineffective drugs and prevent the inappropriate allocation of health resource.

Here we discuss the importance of biomarker selection PD-L1 in lung adenocarcinoma, showing the prevalence of PD-L1 and the rate of patients expressing high PD-L1 expression (TPS ≥ 50%) utilizing data from a major pathology laboratory in Northeastern Brazil.

Methods

Clinical samples

We retrospectively evaluated tumor samples of biopsies and surgical resections from patients with primary lung adenocarcinoma at Argos Laboratory (Fortaleza-CE, Brazil). Of these, only those cases with available information regarding PD-L1 and ALK expression were selected.

PD-L1 expression

PD-L1 IHC was carried out on 5 μm sections, using the Ventana SP263 clone (Roche Diagnostics, Cat#740–4907), OptiView Amplification Kit and OptiView DAB IHC Detection Kit on Ventana Benchmark GX equipment (Roche Diagnostics, Switzerland). Hematoxylin was used as counterstaining. The PD-L1 TPS was calculated as the percentage of at least one hundred viable tumor cells with partial or complete staining. PD-L1 positivity was defined by the

Table 1 Characteristics of patients with lung adenocarcinoma (n=158)

Factor	value or number of patients (%)
Age (years)	
Median (range)	66
Range	36 – 94
Gender	
Male	65 (41.1)
Female	93 (58.9)
Sample origin	
Lung	96 (60.8)
Pleura	26 (16.5)
Lymph node	16 (10.1)
Bone	9 (5.7)
Other	11 (7.0)

positive percentage of any staining intensity using percentage increments of 10%.

ALK expression

All cases were carried out with well-established standard operating procedures incorporated at pathology laboratory. In summary, 4 μm-thick sections were cut from Formalin-fixed paraffin-embedded (FFPE) and stained in the Ventana Benchmark GX equipment (Roche Diagnostics, Switzerland), using approved anti-ALK Rabbit Monoclonal Primary Antibody, clone D5F3 (Roche, Cat#790–4796), and utilizing OptiView Amplification Kit (Roche Diagnostics, Cat#760–099) and OptiView DAB IHC Detection Kit (Roche Diagnostics, Cat#760–700). Counterstaining was performed with hematoxylin and negative controls were also assessed. ALK staining was graded as follows: weak, for absent or barely perceptible expression in rare cells; moderate, for strong cytoplasmic staining tumor cells (multifocal expression); strong, homogeneously strong cytoplasmic staining in most cells. Only strong staining was considered as ALK positive.

Statistics analysis

Statistical analysis was performed using SAS software (Cary, NC). The relationships between PD-L1 expression and other variable were examined with Fisher's exact test. For all analyses, a P-value < 0.05 was considered to be statistically significant.

Results

A total of lung 158 lung adenocarcinoma patients were included in the present study, comprising 65 (41.1%) men and 93 (58.9%) women. The median age of all patients was 66.5 years (range 36–94 years) as showed in Table 1.

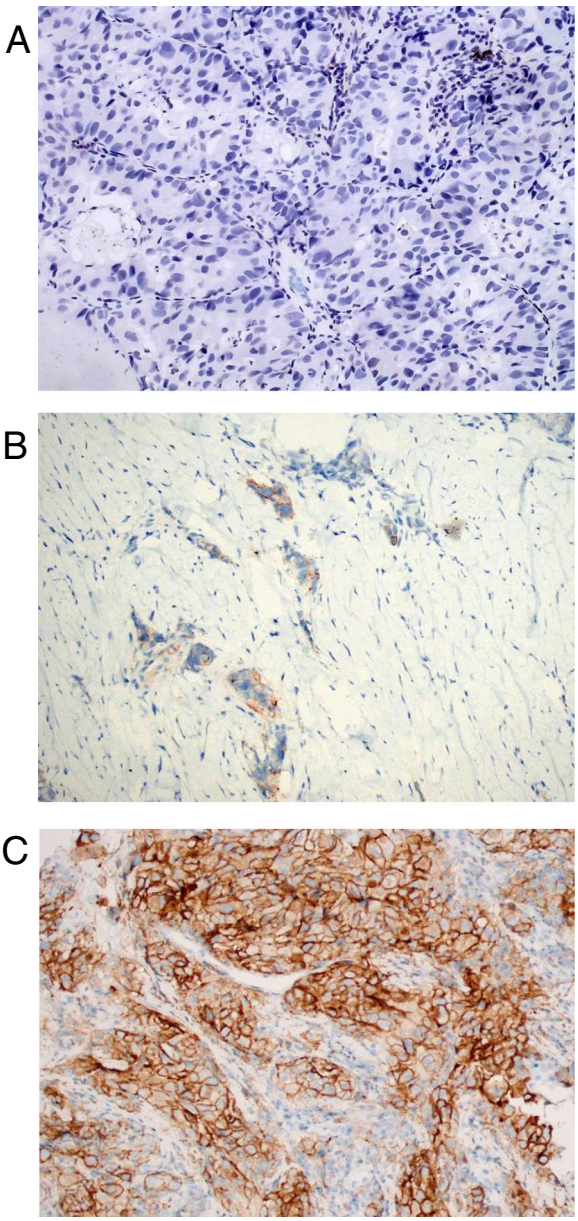

Fig. 1 Representative figures of immunohistochemical staining for PD-L1 in resected tumors from patients with primary lung adenocarcinoma (400x magnification). (**a**) PD-L1 TPS < 1%. (**b**) PD-L1 TPS 2%. (C) PD-L1 TPS ≥50%. PD-L1: programmed cell death-ligand 1, TPS: tumor proportion score

Among the 158 patients included in this study, 94 (59.5%) had PD-L1 TPS < 1% and 64 (40.5%) had a PD-L1 TPS of ≥1%. PD-L1 positivity was not associated with patient age, gender, biopsy sample origin or ALK status. Patients expressing any degree of PD-L1 higher than TPS of 1% were divided into groups with a PD-L1 TPS of 1–49% (n = 38, 24.0%) or ≥ 50% (n = 26, 16.5%) referred as high PD-L1 expression, thus comprising 3 groups whose a representative patterns of

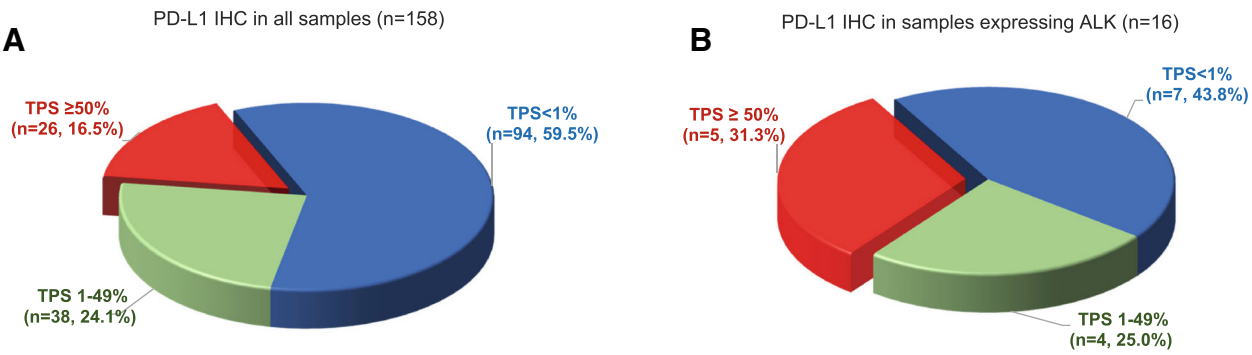

Fig. 2 PD-L1 TPS determined by IHC for lung adenocarcinoma patients (**a**) PD-L1 TPS for all 158 samples (**b**) PD-L1 TPS for the 16 samples expressing ALK. ALK: anaplastic lymphoma kinase, PD-L1: programmed cell death-ligand 1, TPS: tumor proportion score

immunohistochemical staining for PD-L1 are shown in Fig. 1. Figure 2a shows a visualization distribution of the 3 groups of PD-L1 IHC expression comprising all samples analyzed.

Of the 158 specimens analyzed, 16 (10.1%) showed ALK positivity. As depicted in Fig. 2b, among patients expressing ALK, 5 had PD-L1 TPS ≥50% and 4 PD-L1 TPS of 1–49% Association between patient pathologic features and PD-L1 TPS stratified as < 1, 1–49%, or ≥ 50% are shown in Table 2.

Discussion

Considering the rising importance of immunotherapies to treat cancer patients and the relevance of specific biomarkers in selecting patients more likely to respond to these therapies, it is critical to understand the frequency of PD-L1 expression in lung adenocarcinoma from the diverse geographical region. In the present study, we found that PD-L1 TPS > 1 was present in 40.5% of patients, a number very close to another Brazilian study describing a rate of 37.9% of PD-L1 positivity. (Dix Junqueira Pinto et al., 2016) When PD-L1 TPS > 1 was divided into two groups (TPS 1–49% and ≥ 50%) the frequency of patients expressing PD-L1 TPS ≥50% was 16.5%, a rate inferior to that described by other studies whose frequency of high level of PD-L1 expression (TPS ≥50%) was estimated to be approximately 30% (Rangachari et al., 2017; Mok et al., 2015; Brahmer et al., 2015).

PD-L1 TPS ≥50% is a crucial parameter to be considered when choosing the therapeutic options available to the patient with lung cancer or even other specific types of cancer (Jørgensen, 2018). For instance, most pembrolizumab trials for NSCLC treatment were based on patients whose tumors had PD-L1 TPS ≥50%, among others. Currently PD-L1 expression is used to stratify patients between first and second line PD-1/PD-L1 checkpoint inhibition immunotherapy (Brahmer et al., 2015). New promising clinical trials assessing PD-L1 inhibitors

Table 2 Association analysis for PD-L1 expression (TPS< 1%, 1-49%, or TPS ≥50%) with gender, age and sample site origin

	PD-L1 TPS < 1% (n=94)	PD-L1 TPS 1-49% (n=38)	PD-L1 TPS 50% (n=26)	p-value
Age				
>70	62	21	19	0.7353
≤70	32	16	8	
Gender				
Female	57	22	14	0.7409
Male	37	15	12	
Site sample origin				
Lung	59	18	19	0.1073[a]
Pleura	16	9	1	
Lymph node	8	5	3	
Other	13	6	3	

PD-L1 programmed cell death–ligand 1, *TPS* tumor proportion score
[a]Lung *versus* other

durvalumab and avelumab are underway, both are drugs whose indication will be also based on the level of PD-L1 expression (Hirsch et al., 2017).

To date, there are no studies in Brazil specifically describing the PD-L1 TPS ≥50% patients, the only exception is made for an abstract where the authors, by using 22C3 pharmDx, found the rate of PD-L1 TPS ≥50% to be 15.5% (Gelatti et al., 2018), a number that almost matched the result found in the present series (15.5% vs 16.5%).

The lower frequency of PD-L1 expression (TPS ≥50%) in the present study compared to published data in the literature is likely related to distinct methods and antibodies utilized. The most important concerns to the fact that the different studies used different antibodies whose distinct epitopes determine different visibilities of the same target (PD-L1 protein) (Thunnissen et al., 2017). On the other hand, studies comparing different PD-L1 IHC assays have shown that Ventana SP263, the primary antibody used in the present study, exhibits similar staining characteristics for PD-L1 staining compared to other anti-PD-L1 antibodies frequently used in the studies here mentioned such as Dako 22C3 and 28–8 pharmDx (Hirsch et al., 2017; Adam et al., 2017). Of note, most of the data estimating the frequency of PD-L1 come from larger cohorts in the phase I-III trials; however, here we presented real-world data from a single institution then it is possible that the molecular features of the studied population might contribute in a relevant way to these findings.

The KEYNOTE-024 phase III trial established the bases for pembrolizumab as first-line therapy in NSCLC patients whose tumors had a PD-L1 TPS of ≥50% with no EGFR or ALK aberrations (Brahmer et al., 2015). The subsequent approval of this drug by the US FDA following the same indications and restrictions made very difficult to know the frequency PD-L1 TPS of ≥50% in patients harboring driver oncogenes. By analyzing our data, we found that the frequency of overlap between ALK IHC expression and PD-L1 TPS of ≥50% was just 3.2% (5 cases out of 158). This number thus indicate that the rate of PD-L1 TPS ≥50% is lower in patients expressing ALK compared with those negative for the expression of this protein. Our results coincide with other series that reported that high PD-L1 expression and alterations in ROS1, ALK and EGFR rarely overlap (Rangachari et al., 2017; Herbst et al., 2016). Although the frequency of patients harboring the driver oncogene ALK and high PD-L1 expression is low in the present series, further studies will be required to examine the efficacy of PD-1/PD-L1 inhibitors in such group of patients.

Brazil became the first country in the world to approve the use of pembrolizumab in conjunction with platinum-based chemotherapy, expanding further the use of ICPIs (BRASIL, 2018), which will lead to the adoption of these drugs in the Brazilian public health system soon. By utilizing a decision-analytic model, Aguiar and colleagues showed that, without patient selection by PD-L1 expression by IHC, immunotherapy is not cost-effective (Aguiar et al., 2017). The issue that needs to be addressed is quite clear: it does not enough to provide the most modern and expensive drug, it is also required to ensure that the drug is effective in those specific group of patients.

The near absence of studies assessing the frequency of PD-L1 expression in Brazilian population reflects the difficulties that Brazil, as well other low and middle-income countries, have in accessing molecular tools to characterize tumors. However, two circumstances oblige these countries to revise its policies and priorities: the first is the very fact that immunotherapies will become the rule in cancer treatments, not only in lung cancer but soon it will play a major role in other malignancies; second, in this new drug-diagnostic co-development model where the ICPIs were developed, drug indication is mainly based on the presence of specific biomarkers and not on a conventionally defined disease indication (Jørgensen, 2018).

Conclusion

We found that the majority of the patients in our study express low levels of PD-L1 (TPS < 1%) with the frequency of PD-L1 TPS > 50% being lower than that previously reported in the clinical trials where PD-L1 expression was used as a biomarker for patient selection. Further studies are encouraged to explore the frequency of PD-L1 in Brazil and its importance in the treatment of lung adenocarcinoma.

Abbreviations

ALK: Anaplastic lymphoma kinase; EGFR: Epidermal growth factor receptor; ICPI: Immune checkpoint inhibitors; IHC: Immunohistochemistry; NSCLC: Non-small cell lung cancer; PD-L1: Programmed cell death–ligand 1; TKI: Tyrosine kinase inhibitor; TMB: Tumor Mutational Burden; TPS: Tumor proportion score

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Availability of data and materials

De-identified dataset used in the current study is available upon reasonable request to the corresponding author.

Authors' contributions

AVAS, ACSMO and FMN contributed to the collection, assembly, analysis, and interpretation of data. Drafting and critically reviewing of manuscript. MA and CDN contributed to the conception, design and planning of the study, drafting and critically reviewing of manuscript. AVAS, BAC and FT interpreted the data and contributed for drafting, critically reviewing and revising the manuscript for important intellectual content. All authors approved the final version of the manuscript to be published.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Messejana Heart and Lung Hospital under control number CAAE 65315317.0.0000.5039.

Consent for publication

Not applicable.

Competing interests
The authors declared that they have no competing interests.

Publisher's Note

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