PD-L1 and molecular biomarker expression in non-small cell lung cancer in Tunisian patients

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Abstract

In cancer treatment, PD-1 and PD-L1 inhibitors are thriving. Activated T lymphocytes express PD-1; it works with its ligand PD-L1 to limit T lymphocyte activation and prevent autoimmune disease. The expression of molecular biomarkers and PD-L1 in lung cancer determines the appropriate treatment strategy for patients with lung cancer. The purpose of this study was to look at the prevalence of molecular biomarkers and PD-L1 expression in a large group of Tunisian patients with advanced non-small cell lung cancer. We conducted an observational retrospective study in which medical/treatment history data were extracted retrospectively from medical records and archived tissue samples between January 1, 2019, and December 31, 2021. We gathered 157 patients who had recently been diagnosed with non-small cell lung carcinoma. In 36.9% of the cases, there was no molecular genotyping. *EGFR* (28.6%), *KRAS* (5.73%), and *ALK* gene rearrangement were the most common genotyping mutations (3.8%). *ROS1* rearrangement was not present. There was a link between *EGFR* and gender, *HER* and age, and *KRAS* and biopsy tissue origin. Six of the tested cases with PD-L1 met the cut-off (^{3}50%). PD-L1 positivity was more common in solid-type adenocarcinoma (1.9%) than in acinar or papillary adenocarcinoma. There were no significant differences in PD-L1 expression across clinical and demographic parameters. High PD-L1 expression and molecular abnormalities were found in one case of *EGFR,* one case of *BRAF*, and one case of *KRAS* (three cases). All of the other specimens with abnormalities had a PD-L1<50%. *ALK*, *ROS1*, *BRAF*, *KRAS*, and *MET* were found to be significantly associated with PD-L1 expression. Our study is one of the country's largest, describing a large panel of biomarkers and their clinicopathologic/histopathologic associations in Tunisian lung cancer patients. We have the same molecular profile as European patients with an *EGFR* mutation, which is not the most common genotype abnormality in Tunisian patients. There is only one mutation at any given time. The expression of PD-L1 is determined by the histologic type and the origin of the biopsy tissue.

Introduction

Cancer is a major public health issue and the second leading cause of death worldwide, after cardiovascular disease [1]. Lung cancer is the second leading cause of cancer in both men and women, as well as the leading cause of cancer-related deaths in both men and women worldwide [2]. Tunisia is a country in North Africa with a land area of 163,610 km^2 and a population of approximately 11 million people. Lung cancer is more common in men than in

women in Tunisia. The male lung cancer death rate is 27.8/100,000, while the female lung cancer death rate is 1.65/100,000 [3].

Lung cancer is a heterogeneous disease. Almost all lung cancers are carcinomas. The predominant histological types are adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and large cell carcinoma. Since the 2021 publication of the 5th edition volume of the WHO classification of thoracic tumors, the histopathological classification remains intact, except for newly described entities such as the SMARCA4-deficient undifferentiated tumor characterized by a strong association with tobacco consumption and by a very poor prognosis [4]. Adenocarcinoma is the most frequent non-small cell lung carcinoma (NSCLC) accounting for more than 50% [5,6]. It has precise molecular characterization, which is the key to improving understanding of the tumor pathogenesis, determining the prognosis, and defining an individualized treatment plan based on predictive biomarkers.

EGFR mutations and *ALK* fusions were the only driver mutations that required routine clinical testing for non-squamous non-small cell carcinoma. More driver genes with available drugs have been identified as target therapies for patients harboring driver mutations in these genes [7,8].

PD-L1 down-regulates immune responses primarily in peripheral tissues and acts to suppress anti-tumor immunity. The determination of molecular profile and PD-L1 expression has led to growing interest in identifying additional targetable oncogenes in NSCLC.

Testing PD-L1 is now recommended for advanced-stage non-neuroendocrine carcinomas, as new studies concluded that patients with a PD-L1 tumor proportional score of ³50% are eligible for first-line treatment with the anti-PD1therapy pembrolizumab [9]. Also, atezolizumab is approved for first-line treatment in patients with PD-L1>50%, demonstrating a survival advantage over platinum therapy.

Advances in descriptions of genomic aberrations in non-small cell carcinomas have profoundly changed therapeutic strategies. Lung cancer is no longer a single tumor type diagnosis but is defined by a combination of factors, including histology and biomarker status. Currently, in lung adenocarcinoma, predictive testing for *EGFR*, *ALK*, *ROS1*, and *BRAF* gene abnormalities, regardless of sex, race, smoking history, or other risk factors, is prioritized over other molecular predictive tests [10].

This approach for targeted therapies in lung cancer allows the right patients to receive the most active therapy, while those who are unlikely to benefit can be spared the cost and potential morbidity associated with ineffective therapeutic interventions. Thus, the evaluation of genomic aberrations is important to manage advanced NSCLC. Hence, the role of pathologists in making the histologic subtype of NSCLC is major for determining eligibility to establish genomic aberrations and therapeutic strategies.

Besides, although the therapeutic impact of the discovery of these alterations has now been widely demonstrated, the epidemiological data associated with each of these biomarkers remain insufficiently studied. In Tunisia, due to the difficult economic situation, daily practice of molecular testing is rare, which led to a lack of data concerning Tunisian patients.

We performed this study to evaluate retrospectively the association between PD-L1 and driver mutations among a large series of Tunisian patients with advanced-stage NSCLC. We aimed also to describe the baseline demographics and clinicopathologic characteristics among patients with lung cancer and to examine the association between PD-L1 expression, molecular profile, and other clinicopathological parameters.

We postulate that classic driver oncogene aberrations and high PD-L1 expression do not often coexist, which made us wonder about the generation of distinct subgroups of patients, which may allow for optimal pairing of systemic therapies with disease characteristics.

Materials and Methods

We retrospectively collected data about 157 patients with histologically confirmed advanced stage IIIA/B NSCLC or oligometastatic and genotyped for at least one molecular biomarker over 3 years between 2019 and 2021 (Figure 1) Staging was established according to the 8th edition of tumor-nodes-Metastases classification. Patients and NSCLC pairs were excluded if genotyping was not performed. We included only the 157 patients who had undergone a genotyping of their disease. Histological diagnosis was obtained by either endoscopic, computed tomography-guided percutaneous biopsy or surgically. Only adenocarcinoma, squamous cell carcinoma with no history of smoking [11], carcinosarcoma, and large cell histological subtypes were considered. Pathologic data, molecular profile, PD-L1 expression, and clinical characteristics were amassed from retrospective chart extraction.

Patients with no available archival tumor tissue for PD-L1 (insufficient material) or molecular profile testing or with tissue samples of poor quality based on total and viable tumor content and/or bad fixation were excluded.

Data required for exploratory endpoints will be extracted from the medical records, when available, and the designated exploratory endpoints will be assessed according to data availability.

Surgical and core needle biopsies were processed using standard techniques: 10% neutral buffered formalin fixation and paraf-

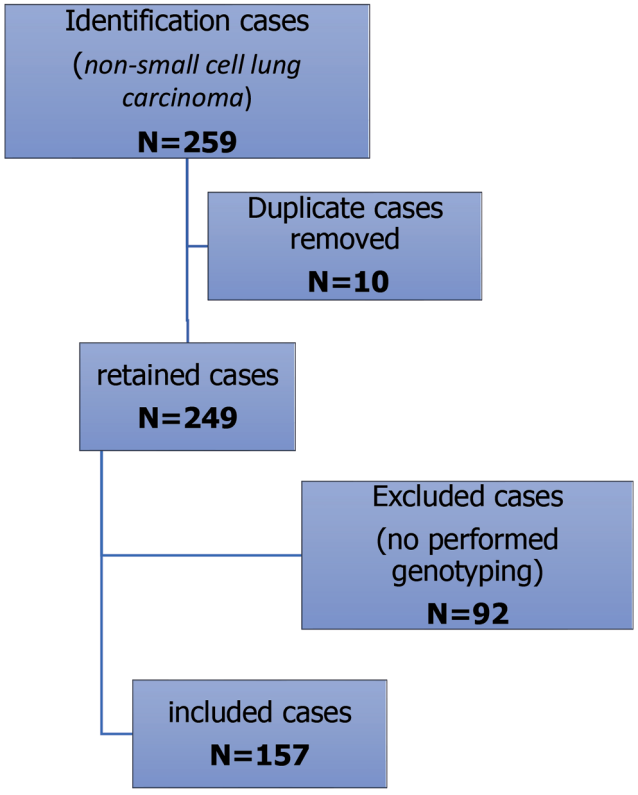

Figure 1. Flowchart of the population.

fin embedding. Bone specimens were embedded in an acid decalcification following formalin fixation. Once a diagnosis was established on histologic and/or immune-histologic staining profiles as recommended in [12], the residual material in the formalin-fixed paraffin-embedded tissue blocks was submitted for molecular analysis. When multiple tissue blocks were available, the one with the highest tumor cellularity was chosen, without additional tumor microdissection or enrichment.

PD-L1 immunohistochemistry testing was performed using Test GeneAb PD-L1 (clone IHC411). Placental villi were considered for external positive staining control. PD-L1 percentage was calculated as the percentage of at least 100 viable tumor cells with complete or partial membrane staining of any intensity. Expression was categorized into <1% (1-49%) and ≥50% of tumor cells; as immunotherapy is indicated in the first line for tumor highly expressed PD-L1, in the second line for tumor slightly expressed PD-L1 and not indicated for tumor with less than 1% [5,6].

Tumor genotype was performed, at an accredited Eurofins/Biomnis lab in France, by analyzing *EGFR* (Sanger sequencing of exons 12, 18-21), *ALK* [fluorescence *in situ* hybridization (FISH) break-apart probe), *ROS1* (FISH break-apart probe), *KRAS* (sequencing of codons 2-3 and 4), *BRAF* (sequencing of exons 11, 15), *MET* (sequencing of exons 2, 14, 16, 19) and *HER* (PathVysion HER2 DNA Probe kit HER2) in tumor samples. The considered cut-off of positivity was 5% [13].

Failure of the assays was defined as insufficient/unusable material to isolate DNA or inability to perform/complete sequencing for *EGFR* and *KRAS* mutations, and lack of hybridization signals after two attempts for *ALK* and *ROS1* FISH.

Statistical analysis was performed using SPSS® Statistics (IBM, Armonk, NY, USA). The data was analyzed to determine if there were correlations between the molecular data and the investigated parameters of the patients, using the chi-square test (or Fisher's exact test) for the categorical characteristics; and the Students' *t*-test (to compare two quantitative variables) and analysis of variance (three or more quantitative variables) for the quantitative characteristics; tests were conducted at the 0.05 significance level. Demographic data concerning the patients are listed in Table 1.

Results

Among the 157 cases, 95 were males and 62 were females with the ages ranging from 24 to 88 years (mean age = 60.3±12.29). The data about survival or smoking habits (except for squamous cell carcinoma) were not available. Specimens were obtained from the lung tissue in 125 cases (79.6%), pleural tissue in 8 cases (5.1%), lymph node tissue in 9 cases (5.7%), bone tissue in 5 cases (3.2%), liver tissue in 4 cases (2.5%) and from the brain tissue in 2 cases (1.3%). The diagnosis was established in bronchial biopsies in 79 cases (50.3%), in transthoracic biopsies in 64 cases (40.8%), and in surgical resection specimens in 10 cases (6.4%). There were 128 samples of adenocarcinoma (81.5%), 3 samples of squamous cell carcinoma (1.9%), 2 samples of large cell carcinoma (1.3%), and 3 samples of mucinous carcinoma (1.9%). Adenocarcinoma was acinar in 39 cases, solid in 88, and papillary in 1 case.

Genotyping of the molecular profile of the tumor tissue showed that about 36.9% of the cases had no mutations in the genes tested. In the remaining cases, the most frequent genotyping mutation was observed with the *EGFR* (28.6%), followed by the *KRAS* (5.73%), followed by *ALK* gene rearrangement (3.8%), followed by *BRAF* (1.2%), *MET* (0.6%) and *HER* (0.6%), while *ROS1* rearrangement was not present at all in this series. Among 45 cases with *EGFR* mutations, 19 had an exon 19 deletion (12% of total), 17 had an exon 20 insertion (10.8%), 5 had an exon 21 L858R mutation (3.2%), 1 patient had a mutation of exon 18 (0.6%) and 2 patients had a double alteration with exon 20 insertion and exon 18 mutation (1.3%). In our series, one patient had firstly a targetable *EGFR* mutation, received initial therapy with a first *EGFR* tyrosine kinase inhibitor (TKI), and was becoming later resistant by developing a new T790M point mutation.

For *KRAS* gene abnormality, one patient had a mutation of EXON 3 (p.GLn61 protein alteration) (0.6%) and 8 patients had an Exon 2 mutation (5.1%). *KRAS* mutation was researched in 78 cases and the mutation was found in 9 cases (11%). Only one case showed Met mutation in the 14th Exon. However, this mutation is not actionable with target therapy. Two cases of *BRAF* mutations were shown in our series and are subject to target therapy. These mutations are

Table 1. Demographic and clinical characteristics of patients with non-small cell lung carcinoma.

Variables	Category	n	%
Age	24-88	157	Mean age = 60.3±12.29
Gender	Male	95	60.5
	Female	62	39.4
Biopsy tissue origin	Lung	125	79.6
	Pleura	8	5.1
	Lymph node	9	5.7
	Brain	2	1.3
	Liver	4	2.5
	Bone	5	3.2
	Not precised	4	2.5
Biopsy type/preparation	Bronchial biopsy	79	50.3
	Intrathoracic scan biopsy	64	40.8
	Surgical resection	10	6.4
	Not precised	4	2,5
Histologic type	Adenocarcinoma	128	81.5
	Squamous cell carcinoma	3	1.9
	Large cell carcinoma	2	1.3
	Mucinous carcinoma	3	1.9
	Not precised	22	14

non-V600 E mutations. No case exhibited more than one mutation at the same time.

Statistical analyses showed the presence of significant differences between *EGFR* and gender, *HER* and age, and *KRAS* and Biopsy tissue origin. There was the absence of a significant relationship between the genomic abnormalities and the other clinicopathological parameters (Table 2).

PD-L1 was not tested in 69 cases (43.9%). Among the remaining tested cases, it achieved the cut-off (³50%) in 6 cases (3.8%), was less than 1% in 7 cases (4,4%), and between 1 and 49% in 68 cases (43.3%). High PD-L1 expression was more likely observed in solid type (3.18%) (Figure 2) than acinar or papillary (0%) adenocarcinoma. PD-L1 expression showed significant differences across only the histologic type (p=0.037) (Table 2). High PD-L1 expression and molecular abnormalities overlapped *EGFR* (1 case), *BRAF* (1 case), *ALK* (4 cases), and *KRAS* (3 cases). All the other specimens harboring abnormalities had a PD-L1<50%.

For molecular biomarkers, *ALK*, *ROS1*, *BRAF*, *KRAS*, and *MET* showed significant association with the expression of PD-L1 (p=0.001, p=0.023, p=0.006, p=0.005, and p=0.018, respectively). This was not the same for *EGFR* and *HER* (Table 3).

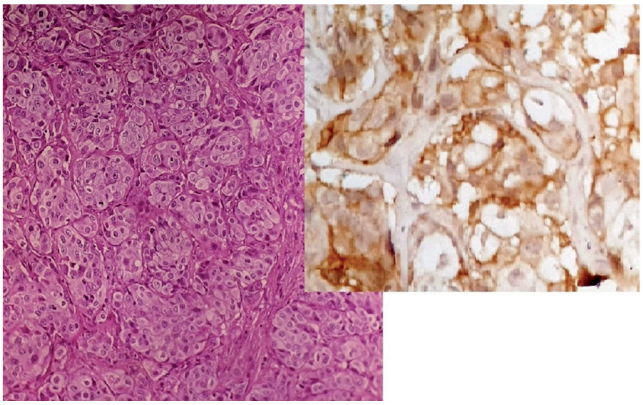

Figure 2. Positive PD-L1 expression in lung adenocarcinoma. Left: solid adenocarcinoma (hemotoxylin and eosin ×20). Right: positive PD-L1 expression in lung adenocarcinoma (immunohistochemistry ×40).

Discussion

Despite a large number of clinical studies on anti-PD-L1 immune checkpoint inhibitors and the fact that molecular profiles and PD-L1 have become established predictive biomarkers to identify patients most likely to benefit from targeted therapy and immune checkpoint inhibitor-based treatment combinations in multiple indications, molecular mutational profiles and PD-L1 frequency data in patients with NSCLC in Tunisia are limited. To the best of our knowledge, our study is the first report to evaluate the molecular profile and the frequency of PD-L1 expression in NSCLC in a developing country.

The major results of our study are as follows: i) the most frequent genotype abnormality in Tunisian patients is the mutation of *EGFR*; ii) there is no more than one mutation at the same time; iii) statistical analyses showed the presence of significant differences between *EGFR* and gender; iv) there is no significant relationship between molecular profile and biopsy type preparation; v) PD-L1 expression showed no significant differences across clinical pathological parameters; vi) PD-L1 expression was significantly associated with *ALK* and *ROS1* rearrangement and *BRAF*, *KRAS* and *MET* mutations and not for *EGFR* and *HER* abnormalities.

Despite the number of enrolled patients in this study, our results merit further validation to be considered representative of this geo-

Table 2. Statistical relationship between molecular abnormality and clinicopathological parameters.

Variables	Category	Molecular profile 'number' (p*)						
		EGFR	*ALK*	*ROS1*	*BRAF*	*KRAS*	*HER2*	*MET*
Age	[24-88]							
	p	0.234	0.187	0.379	0.314	0.168	0.028	0.275
Gender	Male	21	5	0	2	7	0	0
	Female	25	4	0	0	2	1	1
	p	0.027	0.087	0.340	0.623	0.603	0.276	0.446
Biopsy tissue origin	Lung	35	8	0	2	6	0	1
	Pleura	4	0	0	0	0	1	0
	Lymph node	3	1	0	0	0	0	0
	Brain	0	0	0	0	1	0	0
	Liver	1	0	0	0	0	0	0
	Bone	0	0	0	0	0	0	0
	p	0.334	0.467	0.518	0.216	0.042	0.279	0.199
Biopsy tissue origin	Bronchial biopsy	25	8	0	2	5	1	1
	Intrathoracic scan biopsy	16	1	0	0	3	0	0
	Surgical resection	2	0	0	0	1	0	0
	p	0.712	0.154	0.505	0.06	0.095	0.722	0.07
Histologic type	Adenocarcinoma	35	8	0	2	8	0	1
	Squamous cell carcinoma	1	0	0	0	0	0	0
	Large cell carcinoma	0	0	0	0	0	0	0
	Mucinous carcinoma	1	0	0	0	0	0	0
	Not precised	7	1	0	0	1	1	0
	p	0.242	0.921	0.335	0.137	0.137	0.169	0.130

*Cut off of p>0.05.

graphical region. More population-based data is required before we can draw definite conclusions.

The tissue *EGFR* positivity rate among our patients was 28.6% which is similar to that reported in Indian studies but higher than most Western reports [14]. The frequency of *EGFR* mutations is much higher in individuals of Asian origin (45-50%) than in individuals from Western Europe (10-15%) or North America (15-20%) [15]. This is explained by the fact that chromosome 17 which contains *EGFR* oncogene driver is longer in Asian than in other people [4]. If we considered activated *EGFR* oncogene driver (*i.e.*, alteration of EXON19 and 21) (15.2%), which are predictive of good response to TKI, our results are comparable to the French series. The same result was obtained for *ALK* rearrangement which is similar to other reported series (5% in Western and 1.45-7.6% in Indian individuals) [16]. Activated driver *EGFR* and *ALK* rearrangement are predictive of good response of the first line of TKI treatment.

However, more than half of patients who first have targetable *EGFR* mutation and who receive a first- or second-generation *EGFR* TKI as first therapy, will develop a new T790M point mutation which confers resistance to this treatment. These patients can switch to *EGFR* TKI Osimertinib which is a good option as the standard second-line therapy for a patient with an acquired T790M mutation. In our series, this was noticed in two patients. We found that EGFR expression was more observed in female patients than in males. This expression was significantly associated with gender (p=0.027). We supposed that can be due to a smoking lifestyle, even if we do not have all the information about smoking for all patients. However, in Tunisia, smokers are often men than women. However, EGFR was not associated with histologic type (p=0.242), biopsy type/preparation (p=0.712), biopsy tissue origin (p=0.334), and age (0.234). This could be explained by the number of the low number of patients with *EGFR* mutations *vs*. patients without *EGFR* mutations.

For *KRAS* gene abnormalities (mutation of Exon 2 or Exon 3), observed mutations in our series are not eligible for target therapy. However, Exon 3 mutation with alteration of p.GLn61 protein is associated with a poor prognostic. Lung adenocarcinomas are usually associated with tobacco smoking, and *KRAS* mutations have been found to occur at a higher frequency in tumors in smokers compared to those in non-smokers. However, the "smoking" lifestyle of our patients couldn't be studied as the data was lacking. We note also the recent emergence of specific inhibitors for KRASG12C mutations [17]. In our series, *KRAS* mutation was not systematically researched in the first recorded patients, as *EGFR* mutation could be proposed in isolated analysis on tumor tissue. However, since the new international recommendations, a large panel including the *KRAS* was indicated by next-generation sequencing. Mutation of *KRAS* was observed in 9 cases in our series. It was significantly associated only with biopsy tissue origin (p=0.042).

METex14 skipping mutations are poor prognostic factors of overall survival. In our series, one patient had this mutation. *BRAF* V600 mutation is associated with worse outcomes. The frequency of *BRAF* mutation is similar to other series. BRAF mutations are rare in NSCLC, occurring in 1-5% of cases [17]. *ROS1* rearrangement was not observed in our series. *ROS1* alterations are rare molecular drivers of NSCLC that can be effectively treated with a variety of *ROS1*-targeted drugs [18].

One case of *HER* amplification was observed in our series, but this genetic alteration is not eligible for target therapy. Overexpression of the HER2 receptor has been known for a long time in breast cancer where it appears in approximately 20% of cases. In this pathology, targeted treatments with anti-HER2 antibodies or TKI have demonstrated their effectiveness. In NSCLC, the presence of a HER2 mutation is found in 2% of cases.

The statistical analysis of the relationship between molecular abnormalities and clinicopathological parameters showed that EGFR mutation was significantly associated with gender (p=0.027), *KRAS* mutation to biopsy tissue origin (p=0.042), and *HER* to age (p=0.024). *ROS1*, *MET*, and *BRAF* did not correlate with the studied variables (age, gender, biopsy tissue origin, biopsy type preparation, and histologic type). This could be explained by the low number of cases with abnormalities of these genes. How would you explain the association between gender and *EGFR* mutation

Our findings showed the absence of coexisting genomic aberrations, which is different from the results reported in other studies [19].

In one of the largest published screening cohorts for PD-L1 using the 22C3 pharmDx assay to date in the KEYNOTE-024 trial, the frequency of overlap between common driver oncogene aberra-

Table 3. PD-L1 expression in antigen-presenting cells and clinical data.

Variables	Category	PD-L1 expression				p
		<1%	1-49%	≥50%	Not tested	
Age	[24-88]	7	68	6	76	0.062
Gender	Male	4	44	5	42	0.411
	Female	3	24	1	34	
Biopsy tissue origin	Lung	7	48	4	66	0.093
	Pleura	0	6	0	2	
	Lymph node	0	5	2	2	
	Brain	0	2	0	0	
	Liver	0	3	0	1	
	Bone	0	4	0	1	
	Not precised	0	0	0	4	
Biopsy tissue origin	Bronchial biopsy	5	36	4	34	0.201
	Intrathoracic scan biopsy	2	25	2	35	
	Surgical resection	0	7	0	3	
	Not precised	0	0	0	4	
Histologic type	Adenocarcinoma	6	59	5	58	0.037
	Squamous cell carcinoma	0	2	1	0	
	Large cell carcinoma	0	2	0	0	
	Mucinous carcinoma	0	0	0	2	

tions (*i.e.*, in *EGFR* or *ALK*) and PD-L1 TPS of ≥50% was just 6% (30/500) [19]. In our study, PD-L1 positivity and molecular abnormalities overlapped *EGFR* (1 case), *BRAF* (1 case), *ALK* (4 cases), and *KRAS* (3 cases).

In the present study, PD-L1 expression was ≥50% in 3.8% and between 1-49% in 4.4%, which is lower than reported in other studies. In a recent pan-cancer analysis, the PD-L1 expression frequency was investigated in a wide variety of tumor types; in the overall lung carcinoma cohort, the PD-L1 positivity rate was 31.3% [20,21]. However, to date, little is known about the real-Tunisia frequency of PD-L1 expression in the tumor cells of unselected patients with NSCLC, and whether or not geographical differences exist.

This distribution of the PD-L1 expression with thresholds aims to determine the best use of PD-1/PD-L1 inhibitors, whether alone in the first or second line or in combination with chemotherapy. Immunotherapies are more efficient as second-line agents for patients with advanced lung cancer, as well as first-line therapy for patients with high levels (>50%) of PD-L1 expression and absence of driver mutation (*EGFR* mutations or *ALK* rearrangements). Guidelines indicate the use of nivolumab, a monoclonal antibody in patients with advanced lung squamous cell carcinoma, the use of Pembrolizumab, an anti-PD-1 antibody, in combination with chemotherapy as first-line treatment of metastatic lung adenocarcinoma and the efficacy of atezolizumab in combination with other drugs in patients with untreated lung adenocarcinoma. The adjunction of atezolizumab significantly improved progression-free survival and overall survival among patients with metastatic lung adenocarcinoma, regardless of PD-L1 expression and *EGFR* or *ALK* genetic alteration status [22].

Song *et al.* reported that PD-L1 expression is associated with advanced-stage, lymph node metastasis, solid predominant subtype, and wild-type *EGFR* gene [23]. In our series, PD-L1 expression had no significant association across clinicopathological parameters, *HER*, and *EGFR* mutations. No data exists to support the use of *BRAF* inhibition for non-*BRAF* V600E mutated lung cancer and chemotherapy or immunotherapy remains the favored option in this case. In contrast to other NSCLCs with targets (*EGFR*, *ALK*) immune checkpoint inhibitors appear to be active in those with a *BRAF* mutation irrespective of PD-L1 status or *BRAF* mutation type [17]. Data in this setting is limited and would need further investigation to clarify this point.

According to the histologic type, solid adenocarcinomas of the lung tend to be more likely to express PD-L1 than the other histologic type, suggesting that this subtype activated PD-1/PD-L1 pathways leading to the suppression of antitumor immunity. Research on the correlation between PD-L1 expression and lung carcinoma subtypes is few. PD-L1 expression was found to be significantly higher in the solid predominant subtype in studies reported by Koh *et al*. and Zhang *et al*. [24,25]. Jonas *et al*. found that there is no significant difference observed in the PD-L1 expression when comparing age, sex, diagnosis, and specimen site [26]. We found a significant correlation between PD-L1 expression across the biopsy tissue origin.

The main limitation of our study is related to its retrospective nature. Data obtained from patient medical records are sometimes incomplete. Consequently, the data obtained in this study will be less comprehensive than data obtained from a prospective, interventional clinical study. Moreover, the use of only one antibody of PD-L1 in the immunohistochemical study may be inappropriate as the tumor sample can be heterogeneous for the PD-L1 expression. Further, the inability to evaluate all the various types of mutations found among each gene type. Because of the complexity of lung cancers, the most clinically relevant mutation types were chosen for this study.

Detecting driver mutations in lung carcinomas is essential for personalized cancer treatment and prognosis. Various techniques are employed for this purpose, including traditional tissue-based methods and emerging liquid biopsy approaches like the use of circulating tumor cells (CTCs) and circulating DNA or miRNA. Next-generation sequencing on tissue biopsy also known as high-throughput sequencing, allows the identification of genetic mutations in tumor tissue samples. It provides comprehensive genomic information, including point mutations, insertions, deletions, and gene rearrangements. CTCs are cancer cells that have shed from the primary tumor and entered the bloodstream. They can be isolated from peripheral blood samples using specialized techniques. Once isolated, CTCs can be subjected to next-generation sequencing to identify driver mutations, gene amplifications, and rearrangements. This approach allows real-time monitoring of tumor evolution and can help guide treatment decisions. Circulating tumor DNA (ctDNA) consists of DNA fragments released by tumor cells into the bloodstream. Detecting driver mutations in ctDNA is a non-invasive way to assess tumor genetics. Digital polymerase chain reaction techniques can be used to quantitate specific mutations in ctDNA with high sensitivity. ctDNA can also be analyzed using targeted next-generation sequencing panels designed to detect specific mutations associated with lung cancer, such as *EGFR* mutations or *ALK* rearrangements. MiRNAs are small RNA molecules that can regulate gene expression and play a role in cancer development. These can be used to profile miRNA expression patterns in tumor tissue or blood samples. Differential miRNA expression can indicate potential driver mutations. Liquid biopsy panels are designed to detect multiple mutations simultaneously. These panels may include both DNA and miRNA targets. The choice of technique depends on various factors, including the patient's condition, tumor stage, and the availability of resources. In some cases, a combination of these techniques may be employed to obtain a more comprehensive understanding of the driver mutations in lung carcinomas. Liquid biopsies, in particular, offer the advantage of being less invasive and providing real-time information about tumor dynamics, making them valuable tools in the management of lung cancer [27,28].

Conclusions

In this report, we have studied pathological findings and the frequency of the expression of molecular biomarkers and PD-L1 expression in NSCLC in the Tunisian population. We have the same molecular profile as European patients with an *EGFR* mutation not the most frequent genotype abnormality in Tunisian. There is no more than one mutation at the same time. ThePD-L1 expression depends on the histologic type and the biopsy tissue origin.

Molecular testing and PD-L1 expression in lung cancer have meaningful implications for clinical practice. However, due to the limits of retrospective studies, prospective studies are necessary to optimize biomarker assessment and therapeutic planning in the real-world setting.

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