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Comparison of Clinic-Pathological, Molecular Features and PD-L1 Status in a Series of Non-small Cell Lung Cancers: Are Real Life Data Similar to Clinical Trials results?

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Abstract

Objective: Immunotherapy is a promising treatment option for a subset of lung cancers as it utilizes the host's own immune system to attack tumors cells. Selection of patients who are likely to respond to immunotherapy is based on PD-L1 expression, a specific biomarker. Clinic-pathological correlation of PD-L1 status and NSCLC has been explored in several studies and large clinical trials. However, there is discrepancy of data as several antibodies are available. We looked at a series of lung tumors to study the association between PD-L1 expression and patient characteristics in our daily setting to improve the selection of patients more likely to express this marker. Results were compared to those available in literature using the same antibody.

Methods: We analysed PD-L1 status (using Dako clone 22C3) in 170 non-small cell lung cancers and correlated their PD-L1 status with clinical, pathological and molecular characteristics focusing in particular on EGFR and ALK status.

Results: We found a statistically significant association between PD-L1 status, histological pattern in the adenocarcinomas subtype and stage of the disease.

Conclusion: Our results support the current findings that PD-L1 expression more frequently occurs in advanced stage disease and certain histological pattern. Our data also confirmed longer survival in PD-L1 positive patients.

Highlights

- Immunotherapy is a promising option for the treatment of NSCLC
- PD-L1 status detected by immunohistochemistry is linked to immunotherapy response.
- There are many clones available but only 22C3 is approved as companion diagnostic
- Patient selection can be affected by the antibody used.

Keywords: PD-L1 clone 22C3; Non-small cell lung carcinoma; Immunotherapy; Pembrolizumab

Introduction

In the era of targeted therapy, selection of lung cancer patients based on the characteristics of their tumour is vital. However, despite the significant improvement in progression free survival associated with targeted therapies for a subset of tumours which harbour Epidermal Growth Factor Receptor (EGFR) mutations or anaplastic lymphoma kinase (ALK) rearrangements, it has been demonstrated that resistance eventually develops in these tumors [1] hence the need for new alternative routes. More recently, there has been an increased interest in immune checkpoint blockade to boost antitumor immunity [2] and there have been significant advances in the treatment of lung cancer with the introduction of cancer immunotherapy.

The focus is currently on Programmed cell death-1 (PD-1) an immune-checkpoint protein that is expressed primarily on the surface of activated T cells. PD-1 regulates the activation of T lymphocytes by binding to its ligands PD-L1 and PD-L2, which may be expressed by tumour cells. PD-L1 expression protects tumour cells from the immune system; therefore inhibition of PD/PD-L1 binding allows the immune system to mount an attack against tumors cells [3].

The Food and Drug Administration (FDA) approved Pembrolizumab, a humanized monoclonal IgG4-κ isotype antibody that binds to PD-1 receptor preventing the interaction with both its ligands (PD-L1 and PD-L2), for the treatment of patients with

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metastatic non-small cell lung carcinoma (NSCLC) with disease progression [4-7].

Pembrolizumab was shown to improve progression-free survival in patients with previously treated metastatic squamous and non-squamous non-small cell lung cancer (NSCLC) [8]. Only patients whose tumour expresses PD-L1 can receive this therapy and PD-L1 status can be easily determined by immunohistochemistry. Currently, several antibodies and platforms are available to detect its expression and several studies have been done to compare them [9-13]. Amongst that PD-L1 IHC 22C3 pharmaDX assay has obtained regulatory status as a companion diagnostic for Pembrolizumab [14-16]. However, the majority of the data regarding patient characteristics and their correlation with PD-L1 status come from clinical trial setting rather than real life cases and results are often discordant leaving the our question unanswered. We report our data from a series of consecutive lung tumours analysing PD-L1 status using the 22C3 pharmaDx protocol and correlate it with clinic-pathological parameters. The results were compared to those of other studies and clinical trials available in literature where the same antibody was used.

Methods

We retrieved all cases of non-small cell lung cancer from six hospitals and discussed at our Lung Multidisciplinary Team Meeting that were tested for PD-L1expression between November 2016 and December 2017. One hundred and seventy-nine (179) cases were selected, predominantly lung biopsies. When material was insufficient or inadequate, PD-L1 testing was performed on corresponding resected tumours (28 cases) or metastatic lymph nodes (17 cases).

PD-L1 Immunohistochemistry and scoring

The test was performed with PD-L1 IHC 22C3 pharmDx TM assay (Dako), a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC), using EnVision FLEX visualization system on Autostainer Link 48. Both negative and positive controls (tonsillar tissue) were included for each case.

PD-L1 protein expression in NSCLC was determined by using Tumour Proportion Score (TPS), which is the percentage of viable tumour cells showing partial or complete membrane staining at any intensity. The specimen was considered to have PD-L1 expression if TPS ≥ 1% and high PD-L1 expression if TPS ≥ 50%. PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab).

Cancer panel

Next generation sequencing using the Ion Torrent Cancer Hotspot Panel performed mutation screening. The assay comprises 207 amplicons in 50 oncogenes frequently mutated in solid tumours. DNA was extracted from formalin fixed paraffin embedded (FFPE) tissue using the QiagenQIAmp DNA FFPE tissue kit. The sensitivity is 5% at a minimum read depth of 200. A base quality of >10 is required for variant calling. Reference Sequence NM_002524.4, NM_004985.3, NM_005228.3 and NM_006218.2 were used to screen the NRAS, KRAS, BRAF, EGFR and PIK3CA genes respectively where A of the ATG start site is designated 1.

Cytogenetics analysis

Interphase FISH analysis was undertaken on all FFPE tumours in order to investigate the presence of an ALK rearrangement using a dual colour break apart probe for ALK. Targeted analysis was performed in conjunction with an informative immunostaining and incorporated at least 100 cells from three separate regions of the samples.

Statistical methods

The median and range was used to summarise continuous variables such as survival (months). Tables were produced for categorical variables. Chi-square was used to compare categorical variables such as PD-L1 status and gender. Kaplan-Meier graphs with the log-rank test were used to compare survival between PD-L1 status. SPSS v22 was used for the analysis.

Results

179 cases were tested for PD-L1, of which 170 (95%) had sufficient material to perform the test, 95 (55.88%) patients were male and 75 (44.12%) were female, mean age was 60 years old (range 24 to 90 years). 27 (15.9%) patients were never-smokers whilst 143 (84.1%) were current or ex-smokers. 143 (84.1%) patients have stage III and IV disease whilst 27 (15.9%) have stage I-II disease.

107 (63%) patients expressed PD-L1, of which 57 (33.5%) were strong positive (≥50% of tumour cells positive) and 50 (29.4%) weak positive (1% to <50% cells positive). 63 (37.1%) cases did not show PD-L1 expression. There was no evidence of an association between PD-L1 status and gender or between PD-L1 and smoking history as shown in Table 1.

All cases were classified according to 2015 WHO criteria based on histological features and immunohistochemistry. Overall 135 (79.41%) cases were classified as adenocarcinoma (ADCs), 23 (13.53%) as squamous carcinomas (SQCC), and 12 (7.06%) showed other histology.

90/112 (80.4%) of subjects that have <50% of tumour cells express PD-L1 or have negative PD-L1 status, have adenocarcinoma as histological subtype, while 45/58 (77.6%) of subjects that have >50% of tumour cells express PD-L1 have adenocarcinoma as histological

	PD-L1 TPS<50%	PD-L1 TPS>50%	*P* Value
Smoking Status			
Current/Ex-smoker	93	50	0.592
Never Smoker	19	8	
Gender			
Male	65	30	0.432
Female	47	28	
Stage			
Early (I+II)	24	3	**0.006**
Advanced (III+IV)	88	55	
Tumour type			
Adenocarcinoma	90	45	
Squamous cell carcinoma	15	8	0.841
Others	7	5	
Histological pattern (ADCs only)			
Solid	36	33	
Acinar	40	10	**0.001**
Lepidic/papillary	14	2	
EGFR status			
Wild-type	76	44	0.283
Mutated	14	4	

Table 1: Correlation between PDL1 status and clinic-pathological data.

subtype. There was no evidence of an association between PD-L1 and tumors type as shown in Table 1.

Amongst ADCs the histological patter in 69 (51.1%) cases was predominantly solid pattern of growth whilst 50 (37%) cases showed predominant acinar pattern and 16 (11.9%) cases lepidic or lepidic/papillary pattern. In the subgroup of ADCs, 14/90(15.6%) of subjects that have <50% of tumour cells expressing PD-L1 have lepidic or lepidic/papillary pattern of growth, while 2/45 (4.44%) of subjects that have >50% of tumour cells express PD-L1 have lepidic or lepidic/papillary pattern of growth. There is strong evidence of an association between PD-L1 and pattern of growth (p<0.0001) as shown in Figure 1.

Out of 135 ADCs, 18 (13.3%) cases showed an EGFR mutation. 14/90 (15.6%) of subjects that have <50% of tumour cells express PD-L1 or have negative PD-L1 status showed EGFR mutation, while 4/45 (8.9%) of subjects that have >50% of tumour cells express PD-L1 showed EGFR mutation. There was no evidence of an association between PD-L1 and EGFR statistically (Table 1).

Four patients tested for ALK translocation showed rearrangement and of these 2 showed also strong expression of PD-L1. In addition, 35 cases showed KRAS mutation (missense mutation in exon 2 and less frequently in exon 4) and of these 13 (37.1%) were strongly positive for PDL1. Seven cases showed BRAF mutation (missense mutation in exon 11 and less frequently in exon 15) and of these 3 (43%) were strongly positive for PD-L1. Statistical tests were not conducted due to the paucity of the sample for these data.

88/112 (78.6%) of subjects that have <50% of tumour cells express PD-L1 are at advanced stage, while 55/58 (94.83%) of subjects that have >50% of tumour cells express PD-L1 are at advanced stage. There is evidence of an association between PD-L1 and stage (p=0.006).

48 patients amongst those with any PD-L1 expression were treated with Pembrolizumab (mainly as second line treatment) and 13 (27.1%) were dead at the end of follow-up. Overall 72 (42.4%) patients were dead at the end of the follow-up.

The median survival for patients with <50% of tumour cells express PD-L1 is 24.07 months. The median survival for patients with >50% of tumour cells express PD-L1 (eligible for treatment) is 34.63 months. Kaplan-Meier survival estimates shows no evidence of a difference in survival between patients with <50% and >50% of PD-L1 positive tumour cells (p=0.266) as shown in Figure 2.

The median survival for patients with <50% of tumour cells express PD-L1 treated with Pembrolizumab is 35.50 months. Kaplan-Meier survival estimates shows no evidence of difference in survival between patients with <50% of PD-L1 positive tumour cells treated with Pembrolizumab and those not treated (p=0.245).

The median survival for patients with <50% of tumour cells express PD-L1 treated with Pembrolizumab was not reached. Kaplan-Meier survival estimates shows evidence of difference in survival between patients with >50% of PD-L1 positive tumour cells treated with Pembrolizumab and those not treated (p=0.032) as shown in Figure 3.

Discussion

Cancer immunotherapy utilizes a person's immune system to specifically attack cancer cells; therefore, causing less collateral damage to normal tissue compared to standard treatments.

However patient selection is crucial due to possible side effects and different cut off for different antibodies have been proposed generating confusion amongst pathologists and difficulties in data analysis [17]. We compared results from research in clinical trial context to our routine samples using the same antibody. Data show tumour proportion score (TPS) of ≥50 positive tumour cells is associated with better response rate to Pembrolizumab and therefore these patients are candidates for treatment. Our data support this cut off as patients with TPS >50% show a better prognosis. Nevertheless, responses have been reported in patients with lower TPS (1% to 49%) but larger studies are still investigating this.

Compared to three clinical trials (KEYNOTE-001, KEYNOTE-010

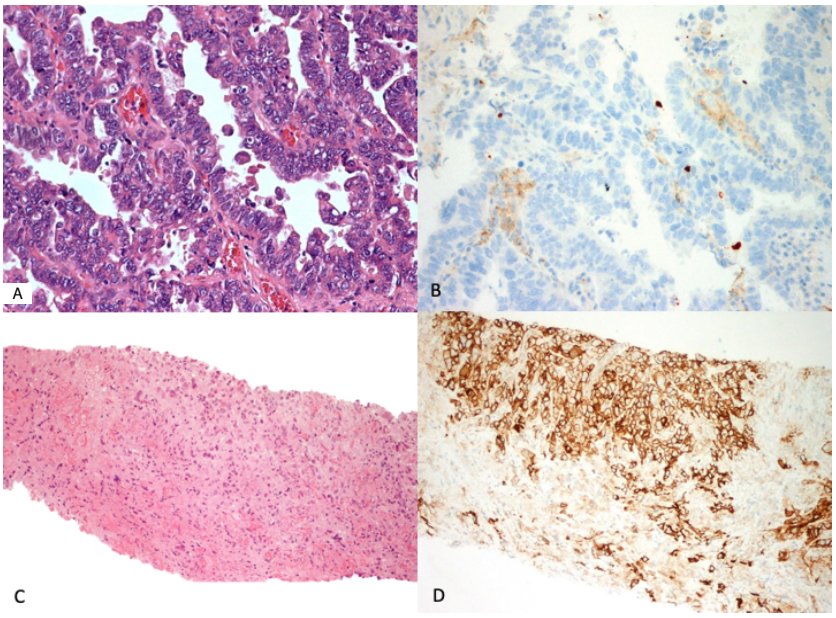

Figure 1: (A and B) Adenocarcinoma with predominantly lepidic growth pattern showing negative staining for PD-L1 (H&E and IHC 40X). C and D: Adenocarcinoma with predominantly solid growth pattern showing positive staining for PD-L1 (H&E and IHC 10X).

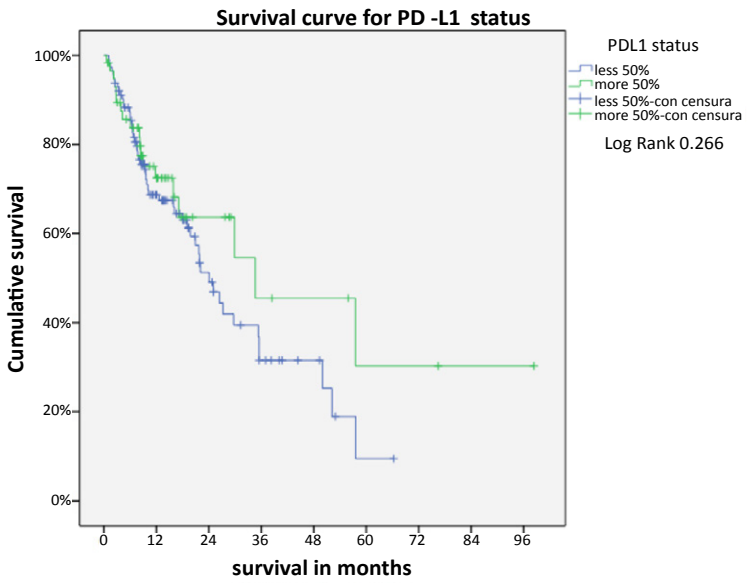

Figure 2: Kaplan-Meier survival estimate. Comparison between PD-L1 expression, survival and outcome.

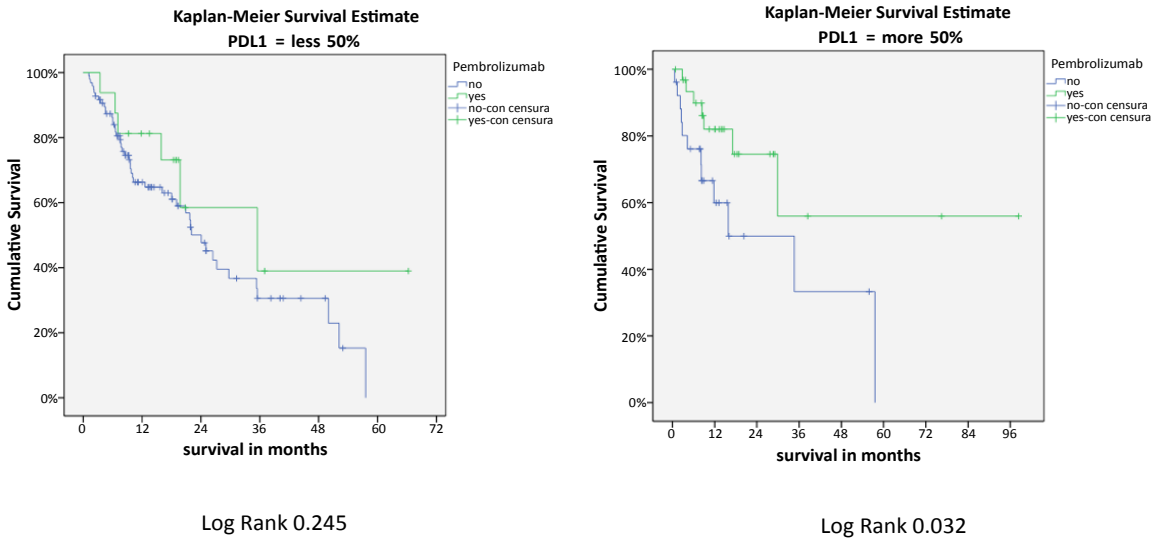

Figure 3: Kaplan-Meier survival estimate. Left: comparison between PD-L1 expression (TPS <50%), treatment option (Pembrolizumab vs. chemotherapy) and outcome. Right: comparison between PD-L1 expression (TPS >50%), treatment option (Pembrolizumab vs. chemotherapy) and outcome.

and KEYNOTE-024) we found a slightly higher strong positive expression of PD-L1 (range 23.2% to 30.2%) and slightly lower weak positive expression (range 37.6% to 37.9%) in our series. This is different from what Lin et al. [18] has reported in their work but could be explained with their higher number of early stage cases (93 cases stage I-II whilst we have only 27 cases). Conversely in other studies where PD-L1 IHC 22C3 was used, positive PD-L1 staining in more than 50% tumour cells was reported more frequently in population with advanced stage (III and above) similar to our findings [19]. However, the authors found no statistically significant differences. We showed statistically significant differences between advanced stage (III-IV) and PD-L1 expression in agreement with other studies [20,21]. We did not find any statistical difference between PD-L1 expression, gender and smoking history, which could be due to the sample size. Our findings are however in line with a large metanalysis done by Pan et al. [22] whilst differ from Takada et al. who demonstrated correlation with PD-L1 and smoking-related adenocarcinomas [23]. Regarding histological subtypes, Lin et al. showed significant differences with squamous histology and also Cooper et al. [24] found more frequently expression of PD-L1 in squamous carcinomas but results were not significant. In our series we observed staining for PD-L1 in more than 50% of tumour cells in 77.59% of ADCs compared to 13.79% of squamous carcinomas but we did not find statistically significant differences between PD-L1 and histological subtypes. This could be the result of the imbalance of our population, which however is reflective of the higher frequency of ADCs amongst NSCLC.

Amongst the ADCs we found a statistically significant difference

between PD-L1 expression and pattern of growth. The different expression of PD-L1 between different pattern of growth in adenocarcinomas has also been reported by Ng kee Kwongand et al. and Yeo et al. [25,26]. The authors also reported absence of PD-L1 staining in ADCs with predominant lepidic pattern. In our series we found similar results although similarly to Ng kee Kwongand a significant number of cases examined were biopsies. It can be pointed out that the histological pattern seen on biopsies may not be representative of the whole tumour but again this is mostly the material available in day-to-day practice and also what has been used in clinical trials.

We did not find any statistical differences between cases with actionable mutations and PD-L1 unlike Cho and Garon; however Cho analysed a cohort of EGFR-mutant tumours only. Our findings are in line with other studies and the limited number of mutated cases can explain the absence of significance. However, our sample shows similar proportion of mutated adenocarcinomas as expected in a mainly Caucasian population. Our data support the finding that PD-L1 expression and EGFR/KRAS/BRAF mutation or ALK translocation are not mutually exclusive and patients should be tested regardless of their molecular status especially if progressing because they could benefit from immunotherapy if the criteria are met [27].

There is great discrepancy regarding the prognostic impact of PD-L1 expression in NSCLC in literature. This may be explained with the use of different antibodies at different cut offs, as well as differences in the study population (Caucasian vs. Asian etc…) [28] or in tumour stage (resected cases versus advanced cases). Evidences suggest that a TPS equal or greater than 50% is associated with better OS, progression free survival (PFS) and response rate (RR) then lower TPS [29]. We found that a TPS equal or greater than 50% is associated with a higher median survival when compared with a lower TPS although this is not statistically significant. In particular our survival curve showed that within the first 20 months the two groups of patients have similar survival but after those patients with TPS more than 50% have better survival. When we add also data regarding the treatment, those patients with TPS >50% receiving Pembrolizumab have better prognosis than those not treated. This is in line with Garon et al. However other studies using the same 22C3 PD-L1 antibody showed inconsistent results [30].

We also found that amongst patients with advance stage disease, those with a TPS more than 50% have slightly better survival than those with TPS <50% although we did not find statistically significant difference.

Conclusion

In conclusion we compared findings from large clinical trials and other large series to our experience with the same antibody. Our study showed that patients with advanced stage disease are more likely to express PD-L1 in more than 50% tumour cells whilst ADCs with lepidic pattern are unlikely to do so. Also, we confirmed that patients with TPS more than 50% have better survival compared to those with TPS less than 50%. This is also seen when only advance stage patients are analysed although no statistically significant correlation was reached. We also demonstrate that a better OS is definitely achieved when treatment in given to patients with TPS >50%. The main limitation of this study is the limited number of cases together with the heterogeneity within the study population and the use of a single antibody. Further studies and results from ongoing trials are needed to validate/support the current findings and interpret those obtained with other antibodies.

Authors' contribution

MA and VP contribute to the conception and design of the study, analysis and interpretation of data, performed the statistics and wrote the manuscript. EM, KR, JE, CA, and RR contribute to the acquisition of data. DS, GN and VP contribute to interpretation of data and reviewed the histological cases. BM, BS, RC, AJ, NJ, RM, CS, DA, HO, PD, LC, NDT were involved in clinical attendance, reviewed for important intellectual content the manuscript. All gave final approval of the version to be submitted.

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