Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies

M. Ilie¹,², E. Long-Mira¹,², C. Bence¹, C. Butori¹, S. Lassalle¹,², L. Bouhlel²,³, L. Fazzalari², K. Zahaf¹, S. Lalvée¹, K. Washetine⁴, J. Mouroux²,⁵, N. Vénissac⁵, M. Poudenx³, J. Otto⁶, J. C. Sabourin⁷, C. H. Marquette²,³, V. Hofman¹,²,⁴, P. Hofman¹,²,⁴

¹Laboratory of Clinical and Experimental Pathology, Pasteur Hospital, Nice; ²IRCAN Team 3, INSERM U1081/UMR CNRS 7284, Faculty of Medicine of Nice, University of Nice Sophia Antipolis, Nice; ³Department of Pneumology; ⁴Hospital-Related Biobank BB-0033-00025; ⁵Department of Thoracic Surgery, Pasteur Hospital, Nice; ⁶Department of Pneumology, Centre Antoine Lacassagne, Nice; ⁷Laboratory of Cancer Genetics, Department of Pathology, Rouen University Hospital, Rouen, France

Background: High expression of programmed death ligand-1 (PD-L1) on tumor cells (TC) and/or on tumor-infiltrating immune cells (IC) is associated with a high response rate in patients with advanced nonsmall-cell lung cancer (NSCLC) treated with PD-L1 inhibitors. The use of a PD-L1 immunohistochemical (IHC) test in determining the responsiveness to immunotherapy has raised the question of the reliability and reproducibility of its evaluation in lung biopsies compared with corresponding resected surgical specimens.

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Patients and methods: PD-L1 expression in TC and IC was assessed in 160 patients with operable NSCLC on both whole surgical tissue sections and matched lung biopsies, by using a highly sensitive SP142 IHC assay. The specimens were scored as TC 0–3 and IC 0–3 based on increasing PD-L1 expression.

Results: PD-L1 expression was frequently discordant between surgical resected and matched biopsy specimens (the overall discordance rate = 48%; 95% confidence interval 4.64–13.24) and x value was equal to 0.218 (poor agreement). In all cases, the biopsy specimens underestimated the PD-L1 status observed on the whole tissue sample. PD-L1-positive IC tumors were more common than PD-L1-positive TC tumors on resected specimens. The discrepancies were mainly related to the lack of a PD-L1-positive IC component in matched biopsies.

Conclusions: Our results indicate relatively poor association of the PD-L1 expression in TC and IC between lung biopsies and corresponding resected tumors. Although these results need to be further validated in larger cohorts, they indicate that the daily routine evaluation of the PD-L1 expression in diagnostic biopsies can be misleading in defining the sensitivity to treatment with PD-L1 targeted therapy.

Key words: PD-L1, immunotherapy, immunohistochemistry, biopsy, surgical resected specimen, NSCLC

Introduction

Early phase I/II trials with immunotherapeutic agents have showed clinical efficacy in both chemo-naive and previously treated advanced nonsmall-cell lung cancer (NSCLC), including tumors with adenocarcinoma (ADC) and squamous cell carcinoma (SCC) histology [1, 2]. In particular, MPDL3280A is a human monoclonal antibody that is directed against the immune checkpoint receptor programmed death-1 (PD-1) ligand 1 (PD-L1), disrupting PD-L1 binding to its receptors PD-1 and B7.1 [1, 3].

In a phase I study, MPDL3280A (atezolizumab) (anti-PD-L1) has recently demonstrated promising response rates in NSCLC that correlated with PD-L1 expression on tumor-infiltrating immune cells (IC) and/or tumor cells (TC) [4]. In addition, treatment with MPDL3280A doubled overall survival (OS) compared with docetaxel in previously treated patients with PD-L1-positive squamous and non-squamous NSCLC, according to results from the phase II POPLAR study presented at the 2015 ASCO Annual Meeting [5, 6]. In patients with the highest level of PD-L1 expression (TC3/IC3), the median OS with atezolizumab was not reached compared with 11.1 months for docetaxel [hazard ratio (HR) = 0.46; 95% confidence interval (CI) 0.19–1.09]. In this same group, the median progression-free survival was 7.8 versus 3.9 months, for the anti-PD-L1 antibody and docetaxel, respectively (HR = 0.57). The objective response rate was 38% with immunotherapy and 13% with chemotherapy. Based on these early stage studies, atezolizumab received a breakthrough therapy designation from the Food and Drug Administration as a potential treatment of patients with PD-L1-positive NSCLC following progression on prior therapy, including chemotherapy and targeted therapies. The latest data suggest that PD-L1 protein expression measured by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded tissue samples (FFPE) may be used as a companion diagnostic test to predict clinical response to PD-L1/PD-L1 directed therapy [5, 6].

Currently, PD-L1 testing is mainly carried out on biopsy specimens, which may not be representative of the tumor as a whole. However, NSCLC tumors have been noted to show significant tumor heterogeneity for PD-L1 expression [7, 8]. Therefore, expression heterogeneity may result in inaccurate results, particularly if testing is carried out on a small tissue specimen such as bronchial or transthoracic biopsies alone [2]. The resultant false negatives could lead to under-treatment of patients. In addition, evaluation of PD-L1 positivity by IHC is not well defined and subject to antibody and assay variability and interpretative subjectivity.

Better understanding of the frequency of discrepancies and characteristics, which may lead to discrepancies, can impact on clinical decision making. Given the potential heterogeneity of NSCLC, and potential significance of false-negative results as well as the small amount of tissue analyzed in biopsies, we evaluated the correlation of immunohistochemical (IHC) expression of PD-L1 between biopsy and corresponding surgical specimen by using a highly sensitive PD-L1 IHC assay developed for use in clinical trials.

Materials and methods

Study population

The study population consisted of patients with operable and resectable NSCLC, who underwent both diagnostic lung biopsies and definitive surgical resection between January 2006 and July 2015 at the Departments of Pneumology and Thoracic Surgery of the Pasteur Hospital Nice, France. To be eligible for the study, patients should not have received chemotherapy or radiotherapy before surgery. The study was approved by the local ethics committee (Centre Hospitalier Universitaire de Nice, France). The patients received the necessary information concerning the study and consent was obtained.

The diagnostic procedures included bronchial biopsies during fiberoptic bronchoscopy for 110 (69%) patients, percutaneous computed tomography (CT)-guided bronchopulmonary biopsy for 38 (24%) patients, and transthoracic mediastinal lymph node biopsy for 12 (7%) patients (supplementary Material, available at Annals of Oncology online). An institutional database of NSCLC patients was queried and identified retrospectively 160 eligible patients (Table 1).

Immunohistochemical procedures

FFPE tissue sections of 4-µm thickness were stained for PD-L1 with an anti-human PD-L1 rabbit monoclonal antibody (clone SP142; Ventana, Roche Group, Tucson, AZ) on an automated staining platform (Benchmark ULTRA; Ventana) using a concentration of 1:60, as recently described [1]. An OptiView DAB IHC Detection Kit (Ventana) and an OptiView Amplification Kit (Ventana) were used according to the manufacturer’s recommendations for the visualization of the bound anti-PD-L1 primary antibody; sections were counter-stained with hematoxylin. Each IHC run
Table 1. Clinicopathological characteristics of NSCLC patients included in the study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient demographics (N = 160)</strong></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>64 (41–85)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>111 (69%)</td>
</tr>
<tr>
<td>Female</td>
<td>49 (31%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>106 (66%)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>32 (20%)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>22 (14%)</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>4.7 (1.8–13)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>33 (21%)</td>
</tr>
<tr>
<td>Lung adenocarcinoma subtypes</td>
<td></td>
</tr>
<tr>
<td>Acinar-predominant</td>
<td>53 (33%)</td>
</tr>
<tr>
<td>Solid with mucin-predominant</td>
<td>29 (18%)</td>
</tr>
<tr>
<td>Papillary-predominant</td>
<td>23 (14%)</td>
</tr>
<tr>
<td>Micropapillary-predominant</td>
<td>13 (8%)</td>
</tr>
<tr>
<td>Lepidic-predominant</td>
<td>9 (6%)</td>
</tr>
<tr>
<td>pTNM stage</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>27 (17%)</td>
</tr>
<tr>
<td>IIA</td>
<td>16 (10%)</td>
</tr>
<tr>
<td>IIB</td>
<td>24 (15%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>53 (33%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>40 (25%)</td>
</tr>
</tbody>
</table>

TNM, tumor node metastasis.

PD-L1 immunohistochemical evaluation

PD-L1 expression was evaluated on TC and tumor-infiltrating IC, as recently described (supplementary Material, available at Annals of Oncology online) [1, 5, 9]. IHC staining of both biopsies and surgical specimens was independently assessed by four senior lung pathologists (MI, EL, VH and PH) blinded to clinical data. When discrepancy between the four pathologists was noted, the slides were reviewed in order to obtain a consensus, and the highest score was validated for analysis.

Statistical analysis

Statistical analysis was carried out by using the SPSS software system (version 16.0, SPSS, Inc., Chicago, IL), including $\chi^2$ test. For the comparison of the dichotomized expression values, we calculated the proportion of discordance between the two procedures together with 95% confidence intervals, as well as the Cohen’s $\kappa$ coefficient of agreement. $P$ values < 0.05 were considered statistically significant.

Results

Given the complexity of the PD-L1 scoring algorithm, we determined concordance between individual analyses by different pathologists. In our cohort, the level of discordance between the four independent pathologists was 6%.

Using the described scoring algorithm, 118 resected specimens (74%) were considered positive (TC1/2/3 and/or IC1/2/3), with TC3- or IC3-expressing tumors representing 38% of cases, whereas 41 biopsy samples (26%) were considered positive (TC1/2/3 and/or IC1/2/3), with TC3- or IC3-expressing tumors representing 18% of cases (Figures 1 and 2). The overall discordance rate was 48% (95% CI 4.64–13.24) and $\kappa$ value was equal to 0.218 (poor agreement) (Figures 3 and 4). The discordance rate for high PD-L1 scoring groups (TC3/IC3) was 20% (95% CI 9.90–16.52), and the $\kappa$ value was equal to 0.528 (moderate agreement). The concordance analysis based on PD-L1 expression of TC only (TC1, TC2, TC3 versus TC0) demonstrated a discordance rate of 19% (95% CI 14.42–39.37; 23% positive resection samples versus 7% positive biopsy specimens), and the $\kappa$ value was equal to 0.396 (poor agreement).

In all cases, the biopsy specimens underestimated the PD-L1 status observed for the whole tumor section (Figure 3). Interestingly, in 75% of cases the discordance was related to the positivity of PD-L1 on tumor-infiltrating IC in resection specimens and negative or under evaluated in matched biopsies, whereas 25% of discordant cases had positive PD-L1 TC and IC in resection specimens only (Figure 4 and supplementary Figure S1, available at Annals of Oncology online). There was no significant difference between discordance rates based on TC1–3/IC1–3 or TC only scoring groups according to histological subtypes: (i) the discordance rate for ADC cases was 46% based on TC1–3/IC1–3 scores (69% positive resection samples versus 23% positive biopsy specimens); the discordance rate for ADC cases was 20% based on TC scores (23% positive resection samples versus 3% positive biopsy specimens); (ii) the discordance rate for SCC cases was 58% based on TC1–3/IC1–3 scores (94% positive resection samples versus 36% positive biopsy specimens); and (iii) the discordance rate for ADC cases was 12% based on TC scores (21% positive resection samples versus 9% positive biopsy specimens) (Figure 4 and supplementary Figure S1, available at Annals of Oncology online).

The number of biopsy cores varied between 1 and 12 (mean number, 4). There was a trend toward a significant difference between the average number of biopsy fragments in discordant versus concordant cases (3.4 versus 6.8; $P = 0.07$).

Based on the analysis of resection specimens, PD-L1-positive IC tumors (79%) were more common than PD-L1-positive TC tumors (31%). In addition, IC3-positive tumors demonstrated a high frequency of IC infiltrates, whereas TC3-positive tumors had a lower frequency of immune infiltrates.

TC typically showed membranous staining with a variably strong component of cytoplasmic staining (Figure 2). The distribution of PD-L1-positive TC within a given tumor sample was typically diffuse and focal. The intensity of staining was however homogenous.

Tumor-infiltrating IC with a clearly distinguishable cytoplasm, such as macrophages and dendritic cells, showed a...
membranous staining pattern for PD-L1 (supplementary Figure S2, available at Annals of Oncology online). This was more difficult to determine for cells of small lymphoid morphology with scant amounts of cytoplasm, seen as strong dot-like signals, as recently described [1]. PD-L1-positive tumor-infiltrating IC present in TC3-positive tumors were mainly located in the stromal bands dissecting the tumor mass or within tumor-infiltrating IC small aggregates or as single cells scattered in stroma.

PD-L1-positive tumor-infiltrating ICs present in IC3-positive tumors were mainly disposed as aggregates within the tumor, stroma and tumor/stroma interface, as well as aggregates toward the periphery of the tumor.

We evaluated the correlation of PD-L1 expression with the clinicopathological features of the 160 patients, and found no significant association with age, gender, smoking status, tumor size, histological subtype or tumor stage (P > 0.05).

discussion

The aim of our study was to compare the IHC expression of PD-L1 on TC and tumor-infiltrating ICs between preoperative biopsies and matched surgical specimens in NSCLC. Diagnostic biopsy, bronchial, percutaneous CT-guided or transbronchial mediastinal lymph node biopsies, is a very useful means of obtaining information about tumor biology that could define and orientate the therapeutic strategy in the neoadjuvant or metastatic setting of NSCLC. This is important, taken the large proportion of NSCLC patients with inoperable disease at the time of diagnosis. However, because of the focal nature of PD-L1 expression within many tumors and the emerging information about intratumoral genetic heterogeneity [10], as well as the modulation of PD-L1 expression by cytotoxic agents, radiotherapy, and targeted therapies, if small needle biopsies or dispersed single-cell cytology specimens are obtained, a false-negative evaluation could potentially result [7, 11].

Our study showed that the expression of PD-L1 assessed in biopsy specimens correlated poorly with that of the corresponding resected tumor in a cohort of 160 NSCLC patients. We found discordances between negativity in the biopsy compared with the surgical specimen for a substantial percentage of patients (58/127; 46%). In all the discordant cases, the biopsy specimens underestimated the PD-L1 status assessed in the resected tissue sample. We did not find false-positive results in biopsies. When using these results in the hypothetical setting of establishing a therapeutic strategy, a significant proportion of patients might not benefit from anti-PD-L1-directed therapy in the clinical setting.
Only one study has been published concerning the comparison of the PD-L1 status between surgically resected specimens and biopsy samples for diagnosis before surgery. Kitazono et al. [12] compared 59 transbronchial biopsies, 12 transbronchial needle aspiration biopsies and 8 percutaneous CT-guided needle biopsies of NSCLC with corresponding 79 surgical specimens in terms of PD-L1 expression. The results of this study showed that PD-L1 expression in biopsy specimens correlated with that of the corresponding resected tumors, with a good concordance rate of 92%. Our study did not confirm the good correlation observed in this study [12]. The authors used the PD-L1 polyclonal antibody 4059 from ProSci (Poway, CA), whereas in our study, we used the SP142 clone from Ventana. We used the latter antibody, because (i) it is currently used as a companion diagnostic test in several clinical trials, and (ii) in our experience, this antibody showed higher sensitivity when compared with another validated antibody against the human PD-L1 protein (E1L3N, Cell Signaling Technology) (data not shown). Moreover, we used a highly sensitive and specific IHC assay measuring PD-L1 on both TC and IC that identified patients most likely to show an improved OS and response rate when treated with anti-PD-L1 agents and patients unlikely to benefit versus standard of care in phase I/II clinical trials [4, 5]. It is unclear whether the 4059 antibody is suitable in predicting the efficacy of PD-L1 blockade [12].

Several studies have demonstrated considerable intratumoral heterogeneity in terms of PD-L1 expression in surgical specimens, and this was associated with a low level of concordance with the results obtained on diagnostic biopsies across different types of cancer [7, 8, 13–15]. The identification of a PD-L1 biomarker predicting response to anti-PD-1/PD-L1 agents is highly suitable to preselect the patients most likely to benefit and spare others from unnecessary exposure to potential side-effects [16]. However, this is challenging due to the dynamic nature of the antitumor immune response and its heterogeneity across space (anatomic location), time (progression from primary to metastatic cancer) [16] and now, we may add, across the size of the specimens. In order to avoid misleading interpretations, we suggest that the IHC analysis be carried out on more than six lung biopsies, as we observed a trend toward a significant difference between the average number of biopsy fragments in discordant versus concordant cases.

The recent phase I/II studies demonstrated a pattern of improved survival after PD-1/PD-L1 pathway blockade that correlated with increasing PD-L1 expression [1, 5, 14, 15]. However, some PD-L1-negative patients also responded to treatment, raising concerns that excluding these patients from treatment might exclude potential responders [16].

It is important to note that evaluation of the PD-L1 expression in NSCLC samples using multiple antibodies that target different PD-L1 domains produced discordant patterns of expression [11]. In addition, several PD-L1 IHC assays were developed, with however poor interassay concordance [17]. These findings could possibly be due to technical reasons such as
as different antibody affinities, cross-reactivity, variable expression of distinct target epitopes, or staining techniques (manual versus automated). Moreover, the interpretation of PD-L1 IHC assay is unsatisfactory by several definitions of PD-L1 'positive' tumor (cell surface versus cytoplasmic expression, by TC only or by other cells in the tumor environment, threshold of 'positivity'), scoring increments, and definitions of PD-L1 'positive' patients (based on a single tumor biopsy, or on maximal expression in the case of multiple biopsies from an individual patient) [18]. These technical issues may have a strong impact on the wide variability in the reported prevalence of PD-L1 expression in NSCLC, from 24% to 65%, as well as on correlation analysis with clinicopathological parameters and patient outcomes [1, 5, 7, 19–22]. Our results indicate a lack of association with common clinicopathological characteristics of NSCLC, including age, gender, smoking status, histological subtype, and TNM stage, as demonstrated by a recent meta-analysis [18]. However, the selection criteria in this retrospective study are a major limitation that may introduce significant bias for correlation analysis. Whether the PD-L1 expression could be used as a prognostic predictor for NSCLC patients is still controversial, and needs further research.

In conclusion, we showed that, in a significant number of cases, evaluation of PD-L1 expression in lung biopsies can be misleading, raising thus an important issue of reliability when integrating these data into the clinical setting. In this context, the necessity of obtaining multiple biopsies from different areas of the tumor in an effort to enhance the validity of the results of IHC evaluation should be rapidly emphasized.
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disclosure
The authors have declared no conflicts of interest.

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