Original Study

Programmed Cell Death Ligand 1 Immunohistochemistry: A Concordance Study Between Surgical Specimen, Biopsy, and Tissue Microarray

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Abstract

Programmed cell death ligand 1 (PD-L1) expression within the same lung cancer tissue is variable. In this study we evaluated if the PD-L1 expression on small biopsy specimens represent the PD-L1 status of the corresponding resection specimen. Our results indicate a relative good agreement between biopsy and surgical specimens, with a discordance in approximately 10% of the cases.

Background: The immunohistochemical analysis of programmed cell death ligand 1 (PD-L1) expression in tumor tissue of non–small-cell lung cancer patients has now been integrated in the diagnostic workup. Analysis is commonly done on small tissue biopsy samples representing a minimal fraction of the whole tumor. The aim of the study was to evaluate the correlation of PD-L1 expression on biopsy specimens with corresponding resection specimens. **Materials and Methods:** In total, 58 consecutive cases with preoperative biopsy and resected tumor specimens were selected. From each resection specimen 2 tumor cores were compiled into a tissue microarray (TMA). Immunohistochemical staining with the antibody SP263 was performed on biopsy specimens, resection specimens (whole sections), as well as on the TMA. **Results:** The proportion of PD–L1-positive stainings were comparable between the resection specimens (48% and 19%), the biopsies (43% and 17%), and the TMAs (47% and 14%), using cutoffs of 1% and 50%, respectively ($P > .39$ all comparisons). When the resection specimens were considered as reference, PD-L1 status differed in 16%/5% for biopsies and in 9%/9% for TMAs (1%/50% cutoff). The sensitivity of the biopsy analysis was 79%/82% and the specificity was 90%/98% at the 1%/50% cutoff. The Cohens κ value for the agreement between biopsy and tumor. was 0.70 at the 1% cutoff and 0.83 at the 50% cutoff. **Conclusion:** The results indicate a moderate concordance between the analysis of biopsy and whole tumor tissue, resulting in misclassification of samples in particular when the lower 1% cutoff was used. Clinicians should be aware of this uncertainty when interpreting PD-L1 reports for treatment decisions.

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Introduction

The treatment of lung cancer patients has dramatically changed with the introduction of immunotherapy. Checkpoint inhibitors against the immune regulatory element programmed cell death 1 (PD-1) and its ligand (PD-L1) have been approved for the treatment of advanced non–small-cell lung cancer (NSCLC).[1-5] However, only approximately 20% of patients develop a long-term response. Higher PD-L1 expression in tumor cells is associated with benefit of anti–PD-1/PD-L1 therapy in most randomized clinical trials.[1-4,6-8] Consequently, corresponding PD-L1 immunohistochemical assays has been recommended as companion or complementary diagnostic tests before starting treatment and is now implemented in the clinical routine.

Many studies have compared available PD-L1 assays and shown only slight differences in their performance when approved methods

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are applied.[6,9-11] Also, efforts have been done to minimize inter-observer variability between pathologists. Another compelling cause of variability might be related to tumor heterogeneity and is therefore intrinsic to tumor biology.[12-14] The PD-L1 analysis is usually done on a tiny biopsy specimen, representing only a minimal fraction of the whole tumor tissue. Thus, biopsy specimens from different tumor regions might show different PD-L1 status, ultimately leading to different treatment recommendations.

With this background, the aim of this study was to evaluate how well a biopsy specimen represents the whole tumor tissue, when PD-L1 expression is evaluated using the approved SP263 PD-L1 assay (Ventana Medical Systems, Tucson, AZ). Because many studies have used tissue microarrays (TMAs) to determine PD-L1 expression for specific scientific questions, we also wanted to evaluate how well the results on the basis of a TMA correlate with the resection specimen and the biopsy-derived PD-L1 status.

Materials and Methods

Patient Population

The study was based on the Uppsala Lung Cancer Cohort, consisting of lung cancer patients surgically treated at Uppsala University Hospital between the years 2006 and 2010.[15] Cases were selected when a preoperative biopsy and subsequent surgical specimen were available as formalin-fixed paraffin embedded samples. Cases with too little material left in the biopsy blocks, or too few tumor cells, were excluded. Finally, 58 patients were included in this study (Table 1).

Tissue Microarray Construction and Immunohistochemistry

A TMA of all 58 cases was constructed with two 1-mm cores from one tumor block of each of the resection specimens as previously described.[16] New 4-μm sections of the resection specimens, the biopsies, and the TMA were stained with hematoxylin and eosin. Immunohistochemical staining using the commercially available Ventana PD-L1 assay (clone SP263; Ventana Medical Systems) was performed using a Ventana Benchmark Ultra with OptiView Universal DAB Detection kit (Ventana Medical Systems), in accordance with the manufacturers' instructions. Placental tissue was included as positive control. All slides were evaluated independently by one senior pathologist (P.M.) and one senior resident pathologist (H.E.). Both were blinded to the others' results and the material was evaluated in mixed order. Only a fraction of positive tumor cells were evaluated and the specimens were scored as 0, 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% positive cells, respectively. After the blinded individual scoring, both pathologists evaluated inconsistent cases together and determined a consensus score. The study was conducted in adherence to the Declaration of Helsinki and approved by the regional ethical review board in Uppsala (D-nr 2012/532).

Table 1 Patient Characteristics

Characteristic	Value
Age, Years	
Median	61
Range	40-82
Sex	
Female	32 (53)
Male	26 (47)
Histology	
ADC	37 (64)
SCC	17 (29)
Other	4 (7)

Data are presented as n (%) except where otherwise noted. Cases were selected from patients with resected NSCLC, for whom also a preoperative biopsy was available.
Abbreviations: ADC = adenocarcinoma; NSCLC = non–small-cell lung cancer; SCC = squamous-cell carcinoma.

Statistical Analysis

To test if there was a statistically significant difference between the proportions of positive scores of whole sections (reference) and scores of biopsies and TMA cores, respectively, 2-proportions tests were conducted, using the function "prop.test" in R (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria). Test results with P values $< .05$ were considered to be statistically significant. For the 2-proportions test, the sample number ("number of trials") must be large enough to allow for normal approximation. A sample number of 58 was considered to be sufficient. The analysis was done separately for the 2 thresholds ("$\geq$1" and "$\geq$50", respectively).

The sensitivity of PD-L1 detection was calculated as the ratio between the number of correctly detected as positive (positive on the whole slide) and the number of actual positive results, for "biopsy" and "TMA." Likewise, the specificity of PD-L1 detection was calculated as the ratio between the number of correctly annotated as negative and the number of actual negative results. Both measures were calculated for the 2 thresholds ("$\geq$1" and "$\geq$50").

To measure the correctness of PD-L1 detection (whole slide annotation as reference) for biopsies as well as for TMA cores, we calculated Cohen κ using the library "irr" in R (R Foundation for Statistical Computing). K values of 1 indicate perfectly correct agreement, whereas a κ of 0 connotes that the agreement might have been achieved by chance only. The R library also provides a significance test, with low P values indicating that the rater agreement is significantly different from what would have been achieved by pure chance.

Results

The study population consisted of 58 patients, 32 female and 26 male, between the age of 40 and 82 years, and comprised 37 adenocarcinomas, 17 squamous cell carcinomas, and 4 cases of other subhistology (Table 1). The distribution of PD-L1 positivity is illustrated in Table 2. The PD-L1 scores for each case and each tissue material are shown in Supplemental Table 1 in the online version.

Positive staining with cutoff $\geq$1% was observed in 28 (48%), 25 (43%), and 27 (47%) cases for resection specimens, biopsies, and TMAs, respectively. For the cutoff $\geq$50% were 11 (19%), 10 (17%), and 8 (14%) cases PD-L1 positive in the resection specimens, biopsies, and TMAs, respectively (Figure 1). The differences between the proportion of positive samples were not statistically significant (resection specimen vs. biopsy vs. TMA: $\geq$1% cutoff: $P = .39$; $\geq$50% cutoff: $P = .71$).

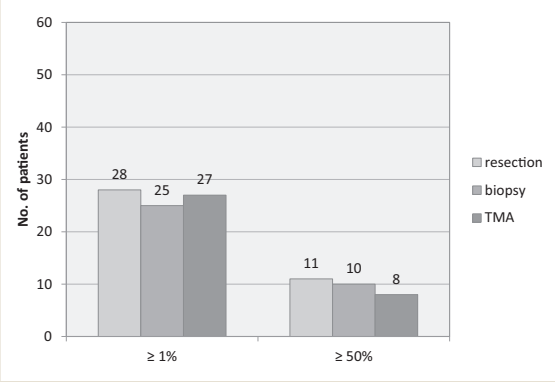
Table 2 Distribution of PD-L1 Positivity

	PD-L1 ≥1%		PD-L1 ≥50%	
	Positive	Negative	Positive	Negative
Resection				
Patient n	28	30	11	47
Histology				
ADC	15	20	5	30
SCC	10	8	4	14
Other	3	2	2	3
Biopsy				
Patient n	25	33	10	48
Histology				
ADC	13	24	5	32
SCC	9	8	4	13
Other	3	1	1	3
TMA				
Patient n	27	31	8	50
Histology				
ADC	14	23	3	34
SCC	10	7	4	13
Other	3	1	1	3

Programmed death ligand 1 positivity is given at cutoff ≥1% and ≥50%, respectively, for each analyzed tissue sample (TMA, biopsy, and resection specimen) for 58 cases.
Abbreviations: ADC = adenocarcinoma; PD-L1 = programmed death ligand 1; SCC = squamous-cell carcinoma; TMA = tissue microarray.

The correlation between the scores was calculated using the Cohen κ (inter-rater reliability). We evaluated the concordance with the annotation of the resection specimens as the reference at the clinically relevant cutoffs of ≥1% and ≥50%. For the biopsy the agreement was good with a κ value of 0.69 at the ≥1% cutoff and very good with a value of 0.83 at the ≥50 % cutoff. The agreement between the resection specimens and TMA was higher at the ≥1% cutoff with a κ value of 0.83, but lower at the ≥50% cutoff with a κ value of 0.69.

Figure 1 Frequency of PD-L1 Positivity at a Cutoff of ≥1% and ≥50%, Respectively, for Each Analyzed Tissue Sample (TMA, Biopsy, and Resection Specimen [Tumor]) for 58 Cases



Abbreviations: PD-L1 = programmed death ligand 1; TMA = tissue microarray.

When the PD-L1 score of the resection specimen was set as the reference, 9 cases (16%) were wrongly annotated in the biopsy for the ≥1% cutoff, and 3 cases (5%) with the ≥50% cutoff. The corresponding values for the TMA were better with only 5 cases (9%) in total annotated incorrectly for the ≥1% cutoff and the ≥50% cutoff. Examples for inconsistent cases between resection specimen, biopsy, and TMA are illustrated in Figure 2.

The sensitivity and specificity of the biopsy-based PD-L1 analysis compared with the analysis on the resection specimen as reference were 79% and 90%, respectively, for the ≥1% cutoff, and 82% and 98%, respectively, for the ≥50% cutoff.

The results for the TMA-based PD-L1 analysis were again slightly better, especially for the lower cutoff, with a sensitivity and specificity of 89% and 93%, respectively, for the ≥1% cutoff, and 64% and 98%, respectively, for the ≥50% cutoff.

Discussion

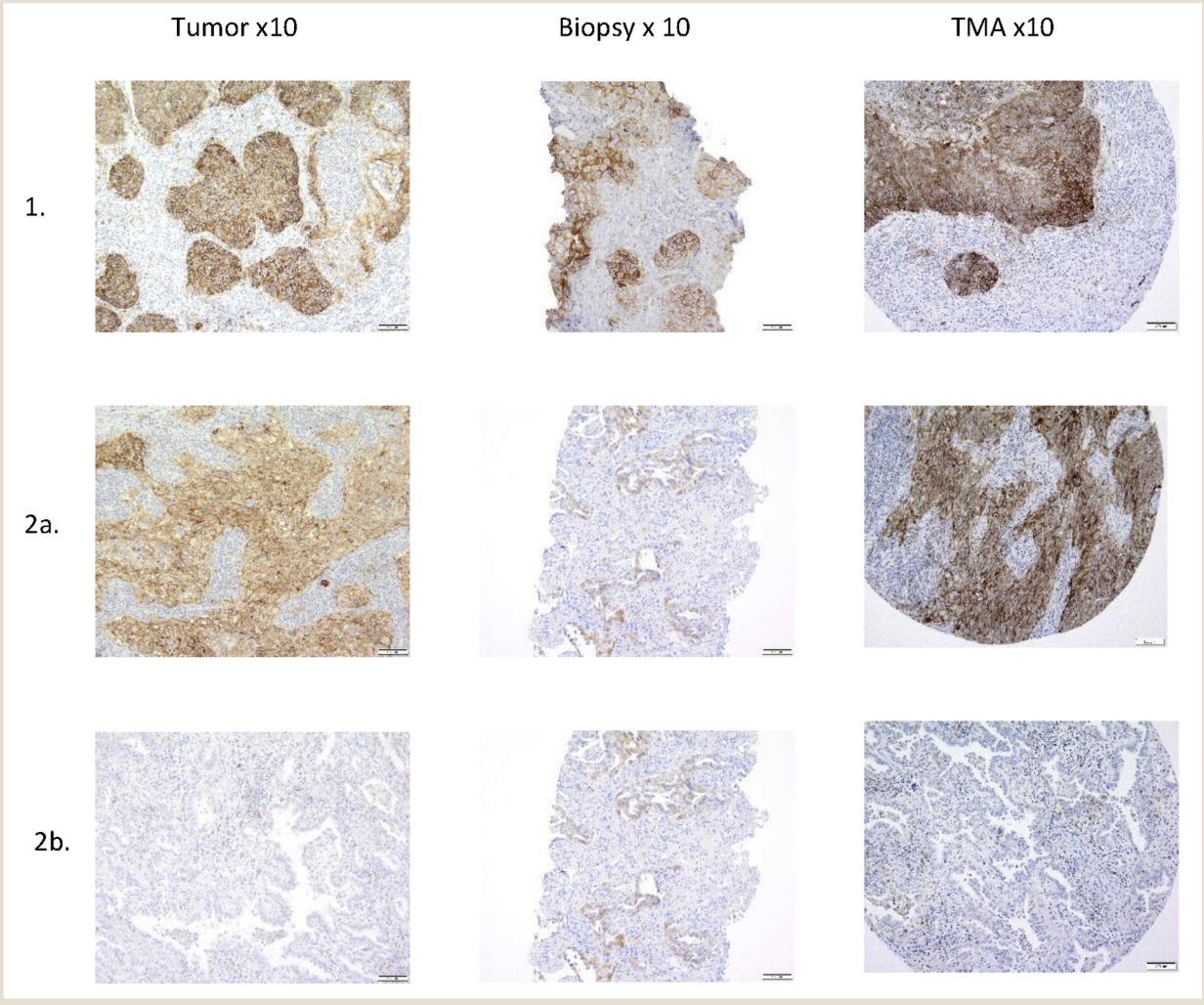
Our findings indicate that the PD-L1 status evaluated on a small fragment of the whole tissue is usually representative for the results on the basis of the resection specimen. However, a certain proportion, in our study approximately 5% to 16% of cases, will deviate resulting ultimately in a different treatment recommendation. The deviating cases showed PD-L1 levels around the cutoff levels, which means that the absolute deviation of PD-L1 levels were in general small. In practice, this means that it is very unlikely that a biopsy with a high PD-L1 score is negative in the resection specimen or vice versa. However, a biopsy with 2% PD-L1 positivity could be negative, or a biopsy that is negative could be weakly positive, in the resection specimen. This uncertainty of the PD-L1 assay should be considered when interpreting PD-L1 pathology reports, in particular at lower cut points. Information about the mutational status, smoking habits, or comorbidity should be included in decision-making for the best treatment option for each individual patient.

Only a few other studies addressed these aspects of PD-L1 testing in clinical diagnostic tests.[12,17-19] Heymann et al[19] compared the PD-L1 status of cytological material, biopsies, and surgical specimens with the 22C3 assay and reported a concordance in 21 of 23 (91%) samples. Kitazono et al[18] compared a larger series of 79 biopsies with resected specimens. When in the "hybrid-score" any staining (polyclonal antibody) was defined as positive, a concordance of 92% was achieved. Ilie et al[17] used the PD-1 clone SP142 and reported only a poor concordance between surgical tissue sections and matching lung biopsy specimens. However, not only the tumor cell positivity, but also the immune cell staining was annotated and included in the PD-L1 score, which often led to the discrepancy between biopsy and resection specimen.

The Blueprint group, a cooperation to compare diagnostic PD-L1 assays, recently presented the results of the phase B2.[20] They compared PD-L1 staining on 31 resection specimens and corresponding small biopsies and fine needle aspirates. K Fleiss scores were mostly of >0.7 and regarded as good. Again, the interobserver agreement was in the same range (>0.7), showing that several variables might contribute to an uncertain PD-L1 scoring.

Our study showed a concordance that is in the same range as in the previously mentioned studies, using the approved PD-L1 assay SP263 and comparing biopsies and tumor cores in a TMA in parallel, in a cohort of consecutive patients.

Figure 2 Programmed Death Ligand 1 Immunohistochemistry. Representative Images Illustrate Different Staining Results on 2 Different Cases. Most Cases Showed Consistent PD-L1 Status in the Resected Specimen, Biopsy, and TMA (1). Some Cases Showed Tumor Heterogeneity. Image 2a and 2b are From the Same Tumor, With 2 Different Tumor Components (Solid and Glandular). Although the Biopsy Specimens Only Represent the Glandular Component (PD-L1: 70%), the TMA Included Areas With Higher (PD-L1: 100%), and Lower PD-L1 Expression (PD-L1: 1%; Average PD-L1 Score Was 60% for Resected Specimens and TMA)



Abbreviations: PD-L1 = programmed death ligand 1; TMA = tissue microarray.

Some limitations should be considered, when interpreting these results. Although the cohort comprised 58 patients, a higher number would increase the statistical power. Furthermore, we defined the "ground truth" as the PD-L1 score of just 1 whole tumor section from the resection specimen. Therefore, the evaluated tumor area was still only a small proportion of the whole tumor and a combination of several whole section scores would be optimal. However, we believe that our findings are representative and provide a realistic estimate of the "real-world" concordance.

Many studies on the diagnostic accuracy of PD-L1 testing have mainly focused on the comparability of different PD-L1 assays.[6,9-11,21-24] Most of the findings indicated that the antibody clone (with exception of SP142) used for assessment plays a minor role, as long as the manufacturers' protocols on the corresponding platforms were used. In addition, although the interobserver variability was relatively small, some cases were scored differently because of the pathologist. Our findings also showed that tumor heterogeneity adds a small proportion of uncertainty to the final PD-L1 score. In the end, the product of 3 factors (assay, observer, tumor heterogeneity) will bias the score. With the careful assumption that all of these 3 factors imply a 10% failure rate, approximately 27% of cases were annotated incorrectly as false positive or negative. With this background, it is not surprising that the immunohistochemical PD-L1 assay is not the best to select patients for therapy, although the biomarker per se might be optimal.

Other biomarkers are emerging, among them tumor mutational burden is one of the most mature used in several recent trials as companion diagnostic tests.[25,26] However, the analysis is technically challenging and few labs have the required molecular and bioinformatic expertise to perform it. Also, cutoffs used in the clinical trials differ, meaning that the results have to be adapted to the planned treatment. Finally, the overall performance is not better than PD-L1 and rather would supplement the PD-L1 status. Many other biomarkers have been suggested, including gene expression signatures using quantitative real-time polymerase chain reaction[27] or immune cell counts using multiplex assays.[28] The results have been promising but not validated in independent cohorts.

Conclusion

Our study revealed that tumor heterogeneity of PD-L1 protein expression did affect PD-L1 scoring on biopsies and tissue cores of TMAs in a certain proportion of cases. For research studies with larger number of cases this uncertainty might not influence scientific conclusions, but for the individual patient the incorrect results will influence a vital therapy choice.

Clinical Practice Points

- Tumor heterogeneity can lead to variable PD-L1 protein expression when different tumor regions are analyzed.
- An immunohistochemical analysis of the diagnostic biopsy results in most of the cases to the same PD-L1 status as in the corresponding resection specimen.
- Despite the overall agreement, in the biopsies in approximately 10% of the cases might be wrongly annotated, as positive or negative, because of tumor heterogeneity.
- Clinicians should be aware of this inconsistency of PD-L1 results, in particular when the proportion of PD-L1 expression is around the cutoffs of 1% and 50%. This should be considered when immunotherapy is planned.

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Disclosure

The authors declare that they have no conflicts of intrests.

Supplemental Data

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Supplemental Data

Supplemental Table 1 Distribution of PD-L1 Positivity

ID	Tumor Type	Resection, %	Biopsy, %	TMA, %	Sex
2	SCC	0	0	0	F
18	ADC	0	0	0	F
19	ADC	0	0	0	F
30	ADC	0	0	0	F
66	ADC	0	0	0	M
71	ADC	0	0	0	F
98	SCC	100	50	80	M
103	ADC	0	0	0	F
108	ADC	2	1	1	F
114	ADC	0	0	0	F
120	ADC	3	20	1	M
179	SCC	0	0	0	M
186	ADC	70	70	90	F
215	ADC	4	30	6	F
278	SCC	4	0	5	M
285	ADC	0	0	0	F
287	ASCC	2	10	1	F
296	SCC	0	0	0	F
297	ASCC	0	0	0	M
350	SCC	5	0	10	F
357	SCC	90	100	90	M
381	ADC	0	0	0	M
400	ADC	1	2	10	M
403	ADC	0	0	0	M
404	ADC	0	5	1	F
410	ADC	0	0	0	F
478	SCC	2	20	1	F
482	ADC	20	0	1	F
485	SCC	0	1	0	M
488	SCC	100	100	100	F
491	ADC	10	10	0	M
494	ADC	40	80	50	M
532	ADC	50	90	30	F
538	SCC	0	0	0	M
565	ADC	0	0	0	F
600	ADC	30	0	30	M
617	ADC	0	5	0	M
621	ADC	60	70	60	F
635	ASCC	0	0	0	F
638	ADC	0	0	0	M
641	LCC	70	80	40	F
649	ADC	4	40	1	F
657	SCC	100	100	60	F
661	SCC	0	0	0	M
679	ADC	1	0	0	F
688	ADC	0	0	0	M
701	ADC	50	0	30	F
719	SCC	0	0	0	M
731	ADC	0	0	0	F
732	ADC	0	0	0	M
741	ADC	60	5	30	M
755	SCC	5	10	10	M
778	ADC	0	0	0	F
787	SCC	20	30	1	F
795	ADC	0	0	0	F
807	LCC	100	90	100	M
857	SCC	2	5	0	M
860	SCC	0	0	1	M

The PD-L1 positivity for each case.
Abbreviations: ADC = adenocarcinoma; ASCC = adenosquamous carcinoma; F = female; LCC = large cell carcinoma; M = male; SCC = squamous-cell carcinoma; TMA = tissue microarray.