Lung Cancer 132 (2019) 36–38

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

The metastatic site does not influence PD-L1 expression in advanced non-small cell lung carcinoma

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ARTICLE INFO

Keywords:
PD-L1 expression
Metastatic sites
NSCLC

ABSTRACT

Introduction: PD-L1 expression by immunohistochemistry (IHC) testing with Tumor Proportion Score (TPS) ≥50% and ≥1% is required to be eligible for first- and second-line Pembrolizumab treatment for metastatic non-small cell lung cancer (NSCLC) respectively. Stage IV NSCLC often presents with metastasis to multiple distant sites which are easily accessible for biopsy. Knowing whether PD-L1 IHC TPS can be indifferently measured from different metastatic site is therefore an important clinical question. In this study, we evaluated PD-L1 expression in NSCLC from varied distant metastatic sites.

Methods: A total of 580 NSCLC specimens of distant metastases were retrieved for study, including 35 paired samples from two different metastatic sites. The metastatic sites included brain, bone, remote lymph nodes, serous membranes (pleura, pericardium and peritoneum), extra-thoracic solid organs and skin/soft tissues. The samples were cytology cell blocks, small biopsies or surgical resections. IHC was performed using Dako PD-L1 IHC 22C3 pharmDx. A total of 100 viable tumor cells was required for adequacy. TPS ≥ 50% and 1–49% were defined as high and low PD-L1 expression respectively.

Results: PD-L1 TPS scores were not significantly different across a range of distant metastatic sites nor between metastases in paired samples.

Conclusion: Our results suggest that the PD-L1 TPS scoring is similar across different metastatic sites and any site biopsied will yield necessary information for guiding clinical management.

1. Introduction

The treatment of non-small cell lung cancer (NSCLC) has significantly changed over the past decade with the identification of actionable molecular alterations such as *EGFR* mutation, *BRAF* mutation, *ALK* or *ROS1* gene translocation. Recently, immune therapy has emerged as a key treatment strategy. Four PD-1/PD-L1 check point inhibitors Nivolumab, Pembrolizumab, Atezolizumab and Durvalumab have been approved by FDA for advanced NSCLC as either first line, second line or combined treatment for advanced NSCLC. Currently, PD-L1 immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) tissue blocks is used to select NSCLC patients for Pembrolizumab treatment. Only patients with PD-L1 tumor proportion score (TPS) ≥50% are eligible for first-line, and TPS 1–49% for 2nd line Pembrolizumab treatment [1,2].

In the real world, the choice of samples used for PD-L1 testing is mainly driven by tissue accessibility. Stage IV and locally advanced NSCLC often present with multiple metastases. Biopsy or cytology samples from remote metastatic sites such as extra-thoracic organs are often collected for diagnosis, symptom relief or biomarker testing. However, whether PD-L1 expression from one remote metastatic site differs from that of another site was still unknown. It has been reported that despite a strong heterogeneity of PD-L1 TPS in the primary tumor, the concordance of PD-L1 expression between the primary and metastatic tumors in negative (TPS 0%) and high-expression groups (TPS ≥50%) is high at 71–88% [3,4]. However, knowing whether PD-L1 TPS by IHC can be indifferently measured from any metastatic site is clinically important. In this study, we evaluated PD-L1 expression in NSCLC sampled from different metastatic sites.

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https://doi.org/10.1016/j.lungcan.2019.04.009
Received 25 March 2019; Received in revised form 3 April 2019; Accepted 7 April 2019

2. Methods

2.1. Specimens and immunohistochemistry

A total of 580 consecutive cases of stage IV NSCLC with available metastatic tumor samples and PD-L1 testing at Jewish General Hospital (JGH) between June 2016 and April 2018 were included in this study. The metastatic sites included brain, bone, non-regional lymph nodes, serous membranes (pleura, pericardium and peritoneum) and organs outside the chest (liver, adrenal gland, skin, soft tissue). Of these cases, thirty-five were found to have additional specimens from different metastatic sites and designated as paired specimens. In addition, we also included 101 specimens of regional supraclavicular lymph nodes from stage IV or stage IIIB/C NSCLC. The samples included cytology cell blocks, small biopsies or surgical resections. All surgical resections, small biopsies and most of the cytology specimens were fixed with 10% buffered neutral formalin (BNF). Rare cytology cases were fixed by short period of CytoLyt first then 10% BNF. All tissue blocks were cut into 4 μm thick sections and then stained with Dako PD-L1 22C3 PhamDx according to manufacturer's instruction.

2.2. PD-L1 immunohistochemistry scoring

Specimens were considered adequate when a total of 100 or more viable tumor cells were present. Tumor cell PD-L1 expression was scored by 3 pathologists (HW, AS, MR) as previously reported method [5]. As per recommendations, tumor cells with weak (1+), moderate (2+) or strong (3+) partial or complete membranous staining were counted as stained cells. The percentage of stained viable tumor cells over total tumor cells (tumor cell proportion score -TPS) was used to categorize the specimens into 3 groups: TPS < 1% (negative), 1–49% (low expression) and > 50% (high expression).

2.3. Statistical analysis

Analyses were completed using IBM SPSS Statistics for Windows version 24.0 (SPSS, Chicago, IL). Adequacy of the samples and correlations of PD-L1 TPS expression between different metastatic sites were analyzed using the Chi Square test. Comparison of paired PD-L1 TPS in NSCLC between different remote metastatic sites used a paired-sample t-test. Comparison of paired PD-L1 TPS scores expressed as negative, low, and high between different remote metastatic sites used a non-parametric related-samples marginal homogeneity test. The significance level for all analyses was set at $P \leq 0.05$.

3. Results

3.1. PD-L1 expression in NSCLC from remote metastatic sites and cervical lymph nodes

Most specimens, 547/580 (94.3%), were adequate for PD-L1 testing. There was no significant difference in specimen adequacy between different metastatic sites ($P = 0.45$). The distribution of PD-L1 expression in NSCLC from remote metastatic sites was 37.6% for high expression, 28.7% for low expression and 33.7% for negative expression. These results did not differ between metastatic sites ($P = 0.25$, Table 1). Supraclavicular lymph nodes were included in study since they are often involved in stage IV and stage IIIB/IIIC disease, and are often sampled for diagnosis and biomarker testing due to their superficial location. Among 101 samples of supraclavicular lymph nodes, high, low and negative expression of PD-L1 was found in 43.6%, 25.7% and 30.7% of the cases respectively, comparable to NSCLC sampled from other sites ($P = 0.53$).

Table 1
PD-L1 expression in NSCLC from different metastatic sites.

Metastatic sites	≥50% *n* (%)	PD-L1 TPS 1–49% *n* (%)	0% *n* (%)	Total *n* (%)
Serous membranes	83 (36.7)	63 (27.9)	65 (28.8)	211 (100)
Bone	32 (37.2)	20 (23.3)	27 (31.4)	79 (100)
Remote lymph nodes	10 (38.5)	7 (26.9)	7 (26.9)	24 (100)
Brain	19 (29.7)	15 (23.4)	30 (46.9)	64 (100)
Skin/Soft tissue	18(32.7)	13 (23.6)	23 (41.8)	54 (100)
Organs outside chest	44 (35.8)	39 (31.7)	32 (26.0)	115 (100)
Total	206 (37.6)	157 (28.7)	184 (33.7)	547 (100)

TPS: Tumor proportion score.
$P = 0.25$.

3.2. PD-L1 expression in paired specimens from different metastatic sites

Thirty-five patients with 2 samples from different metastatic sites were identified. Most of the specimens were from remote metastatic sites, while metastatic NSCLC from mediastinal lymph nodes were also included and represented 17.4% of the total paired cases. Thirty-two pairs (91.5%) were sampled within 1-year interval, and 3 pairs (8.5%) were sampled between 1–2 years. Twenty-nine (82.9%) paired specimens showed a concordant PD-L1 expression between two metastatic sites. There was no significant difference in the paired TPS scores between the metastatic sites whether PD-L1 TPS was kept continuous or distributed in 3-tiers expression groups ($P = 0.99$ and $P = 0.79$ respectively). Overall, 6 cases showed discordant results between paired specimens. Of these 6 cases, 3 pairs differed between high expression and low expression; and 3 pairs differed between low expression and negative (Table 2).

4. Discussion

Over 50% of NSCLC cases are locally advanced or stage IV disease. The patients often present with multiple metastasis and are mainly managed by systemic treatment. Immunotherapy has become a key player in the management of advanced NSCLC. Immunohistochemistry testing for PD-L1 is currently used to select patients who are more likely to respond to checkpoint inhibitor Pembrolizumab and therefore eligible for first or second line treatment with Pembrolizumab. However, little was known on whether sampling of NSCLC from different metastatic sites may affect the determination of PD-L1 expression and treatment. In this study, we found that no significant difference was observed in the distribution of PD-L1 expression categories across different metastatic sites.

More specifically, in more than 80% of paired tumor specimens from two different metastatic sites, PD-L1 expression was concordant using a 3-tiers category. The cause of discordant PD-L1 expression in the other 20% is unknown. The heterogeneous immunostaining pattern of PD-L1 expression in tumors may be a major reason. Previous studies have found PD-L1 expression is heterogeneous and dynamic [3,4,6]. Nakamura et al found that the majority of specimens with PD-L1 positive segments exhibited intratumoral heterogeneous expression [3]. Gagne et al found that if using TPS > 50% as a threshold, 26.5% of patients may have false-negative PD-L1 result as compared with another core biopsy [6]. The discrepancy of PD-L1 expression may also be seen between primary tumors and metastasis or relapsed NSCLC or renal cell carcinoma [4,7]. The current study is the first to compare the PD-L1 expression between metastatic sites. The discrepancy in PD-L1 expression in our results is within the range of reported discordant rates between two different samples of primary tumors and most likely due to heterogeneity. Despite discrepancy of PD-L1 expression was seen between some paired samples from different metastatic sites, the difference was only between two adjacent TPS categories. There was no change in category from negative to high expression and vice versa.

Table 2
Discordant PD-L1 expression in paired cases.

Case #	1st specimen			2nd specimen			Treatment between Two specimens
	Site	Type	TPS (%)	Site	Type	TPS (%)	
1	Med LN	FNA	40%	Cervical LN	FNA	90%	No
2	Med LN	FNA	0%	Pleura	Cytology CB	10%	No
3	Adrenal gland	FNA	30%	Cervical LN	Core bx	70%	No
4	Cervical LN	Core bx	0%	Kidney	Core bx	10%	No
5	Med LN	FNA	5%	Skin	Core bx	0%	No
6	Med LN	FNA	90%	pleura	Cytology CB	10%	No

FNA: fine needle aspiration; Med: mediastinal; LN: lymph node; bx: biopsy; CB: cell block.
$P = 0.99$.

Repercussions on treatment selection were therefore mild, as patients who are not selected for 1st line Pembrolizumab treatment would still have the opportunity to be treated with Pembrolizumab in the 2nd line setting under current guidelines.

We observed a non-significant trend of lower PD-L1 expression in brain metastasis as compared with other samples. Similar to our observation, Mansfield et al found lower expression of PD-L1 by tumor cells or immune cells in brain metastases compared with their paired specimens from primary lung cancers [8]. It suggests that when PD-L1 expression tested on a metastasis from the brain has TPS < 50%, repeating test on another available non-brain specimen maybe a reasonable approach.

Previous studies suggested that chemotherapy and radiation may release tumor antigens and increase PD-L1 expression on tumor cells [9,10]. The effect of immunotherapy on PD-L1 expression is not well known. However the changes in PD-L1 expression after chemoradiation varied in the reports. In our study, 35 paired specimens were sampled sequentially during a period of 3 months to 2 years. Of these patients, 7 were treated with either chemotherapy or chemoradiation; 3 were treated with short period of (2–6 months) either Nivolumab or Pembrolizumab; 3 were treated with tyrosine kinase inhibitors. There was no change in PD-L1 expression after treatment. All 6 paired cases of discordant results were from patients had no treatment between two testing.

In conclusion, this study is the first to evaluate PD-L1 testing on metastatic tumor specimens and showed a comparable distribution of 3 TPS categories of PD-L1 expression in a variety of metastatic sites. Our results suggest that PD-L1 IHC may be tested on samples from any accessible distant metastatic sites or cervical lymph nodes.

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