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PD-L1 testing on the EBUS-FNA cytology specimens of non-small cell lung cancer*

Gang Wang[a], Diana N. Ionescu[a], Cheng-Han Lee[a], Tadaaki Hiruki[a], Renelle Myers[b], Tawimas Shaipanich[b], Stephen Lam[b], Barbara Melosky[c], Chen Zhou[a,*]

[a] Department of Pathology, BC Cancer Vancouver Centre, University of British Columbia, Vancouver, BC, Canada
[b] Department of Integrative Oncology, BC Cancer Research Center, University of British Columbia, Vancouver, BC, Canada
[c] Department of Medical Oncology, BC Cancer Vancouver Centre, University of British Columbia, Vancouver, BC, Canada

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ABSTRACT

Objectives: The FDA approved PD-L1 tests for anti-PD-L1 immunotherapy are for surgical or histology specimens. It is not clear if cytology specimens could be used for PD-L1 testing to guide immunotherapy. In this study, we assess the suitability of EBUS-FNA cytology specimens for the testing of PD-L1.

Materials and methods: Consecutive patients with Non-small cell lung cancer (NSCLC) underwent EBUS procedure between January 1, 2017 and March 31, 2018 for PD-L1 testing were included. The cell blocks of EBUS-FNA cytology specimens were used for PD-L1 testing using Dako 22C3 phamDx antibody according to the Dako protocol. PD-L1 protein expression in tumor cells is determined by using Tumor Proportion Score (TPS).

Results and conclusion: Of the 265 EBUS-FNA specimens from 262 patients sent for testing, 230 (86.8%) were adequate for PD-L1 testing. Of the 34 NSCLC patients with both histology and EBUS-FNA cytology specimens tested for PD-L1, the results from different specimen types had a concordance of 91.3%. The PD-L1 results from 16 paired specimens from the same anatomic site had 100% agreement. The rates of PD-L1 TPS ≥ 50% were significantly higher in the metastatic tumors in the lymph nodes than in the lung primary lesions. Therefore, EBUS-FNA cytology specimen is suitable for PD-L1 testing in patients with advanced NSCLC. The metastatic tumors in mediastinal lymph nodes appear to have higher PD-L1 expression than primary lesions.

1. Introduction

According to Canadian Cancer Statistics 2017, lung cancer is one of the most common malignancies, accounting for 14% of all newly diagnosed cancers and it is also the leading cause of cancer-related mortality, accounting for 25% of all cancer deaths [1]. The recently introduced immune checkpoint inhibitors have dramatically changed the treatment and management of NSCLC. The FDA approved anti–PD-1 immune checkpoint inhibitor pembrolizumab for the treatment of NSCLC requires testing PD-L1 as a companion test done by the immunohistochemistry (IHC) using Dako clone 22C3 pharmDx kit [2–4].

Currently, the evaluation of PD-L1 expression in tumor cells is typically performed on surgical or histological specimens because clinical trials of checkpoint inhibitors approval by FDA to date have required biopsies or resection specimen for PD-L1 IHC. However, many patients with advanced NSCLC are diagnosed by small-volume cytology or biopsy specimens obtained by endobronchial ultrasound guided transbronchial fine needle aspiration (EBUS-FNA) and, in fact, these samples may be the only material available for testing without putting a patient through further invasive sampling. The use of EBUS-FNA has become the procedure of first choice to diagnose and stage locally metastatic lung cancer and is considered the preferred initial diagnostic procedure for the diagnosis of suspected lung cancer [5–7]. Herein, the feasibility and efficacy of PD-L1 testing using EBUS-FNA cytological specimens of NSCLC were explored in this study.

2. Materials and methods

2.1. Patient sample selection

The current study was approved by the institutional review board of the University of British Columbia. Between January 1, 2017, and

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* Corresponding author at: Department of Pathology, British Columbia Cancer Vancouver Centre, 600 West 10th Avenue, Vancouver, British Columbia, V5Z4E6, Canada.
E-mail address: czhou@bccancer.bc.c (C. Zhou).

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Table 1
Patients with both surgical and EBUS-FNA specimens from the same anatomic site.

Patients No.	Age	Sex	smoking	Dx	stage	Bx site	PD-L1 in Bx	FNA site	PD-L1 in FNA
1	43	F	never smoker	AC	IV	4R	≥50%	4R	≥50%
2	46	F	never smoker	AC	III	L lung	≥50%	L lung	≥50%
3	60	F	ex-smoker	AC	III	11L	≥50%	11L	≥50%
4	61	M	smoker	AC	IV	7R/12R	< 1%	7R/12R	< 1%
5	62	M	never smoker	AC	III	L lung	≥50%	L lung	≥50%
6	63	F	never smoker	AC	III	11R	1-49%	11R	1-49%
7	64	M	ex-smoker	AC	IV	7R	1-49%	7R	1-49%
8	67	M	ex-smoker	AC	III	R lung	1-49%	R lung	1-49%
9	70	F	ex-smoker	AC	IV	L lung	1-49%	L lung	1-49%
10	70	M	ex-smoker	AC	IV	hilar LN	≥50%	hilar LN	≥50%
11	72	M	ex-smoker	SCC	III	R lung	< 1%	R lung	< 1%
12	74	F	never smoker	AC	IV	R lung	1-49%	R lung	1-49%
13	78	F	never smoker	AC	III	4R	≥50%	4R	≥50%
14	78	M	ex-smoker	AC	IV	R lung	≥50%	R lung	≥50%
15	81	M	ex-smoker	SCC	IV	7R	1-49%	7R	1-49%
16	83	M	ex-smoker	NSCLC	IV	11R	≥50%	11R	≥50%

EBUS, endobronchial ultrasound; Dx, diagnosis; Bx, biopsy; FNA, fine needle aspiration; AC, adenocarcinoma; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; LN, lymph node.

March 31, 2018, a total of 262 patients with NSCLC underwent transbronchoscopic FNA for which PD-L1 testing was clinically requested.

2.1.1. EBUS-FNA procedure

As per routine standard of practice, after obtaining informed consent, linear EBUS bronchoscopy and biopsy of mediastinal or hilar lymph nodes were performed under conscious sedation and local anesthesia to the upper airways using a 21 G needle (ViZiShot 2, Olympus America Inc, Center Valley, Pennsylvania). For peripheral tumors, FNA was done using a 21 G needle (PeriView FLEX, Olympus America Inc, Center Valley, Pennsylvania) via a guide sheath after confirmation of the lesion using radial ultrasound.

2.2. Cytology sample processing

After aspiration of a sample, the needle was removed from the bronchoscope and the sample was discharged into CytoLyt solution (Hologic Inc, USA), visible cores of tissue mixed with blood clot were immediately removed from the Cytolyt container by gently sucking them with a plastic pipette and were placed into a container with 5% buffered formalin. The Cytolyt container was used for preparation of a ThinPrep cytology slide in ThinPrep 5000 Processor (Hologic Inc, USA), the remaining Cytolyt fluid was used for preparation of a cell block (Cytolyt cell block). The cores in formalin container were used to prepare another cell block (formalin cell block). The formalin cell block was used for the testing of PD-L1, which contains tumor cells initially fixed in Cytolyt and then immediately post fixed in formalin.

2.3. Cytopathology evaluation and diagnosis of NSCLC

ThinPrep cytology slides and the two H&E slides from each Cytolyt and formalin cell blocks were examined by cytopathologists. The EBUS cytology specimen was considered adequate if the ThinPrep slide has significant number of lymphocytes or anthracosis, or tumor; and /or the cell block contains lymphoid tissue or tumor. If malignant cells were present, the tumor was subtyped if possible based on cytomorphology, tumor morphology and/or additional IHC on the cell block.

2.4. PD-L1 IHC evaluation

PD-L1 IHC testing was performed using Dako clone 22C3 pharmDx kit and Dako Automated Link 48 platform (Dako Canada Inc., Canada). PD-L1 TPS was calculated as the percentage of at least 100 viable tumor cells with complete or partial membrane staining.

2.5. Statistical analysis

Continuous variables were reported as the mean (± standard deviation) and were compared using the Student *t*-test (GraphPad Software Inc, USA). Categorical variables were expressed as a frequency (percentage) and were compared using a Fisher exact test (GraphPad Software Inc, USA). All tests were 2-sided. A p value of < 0.05 was set as the standard for determining statistical significance.

3. Results

3.1. Patient clinical and pathologic characteristics

A total of 265 specimens from 262 patients had PD-L1 testing requested on EBUS-FNA samples. There were 143 female and 119 male patients, with an average age of 68.7 years (± 10.2 years), 193 Caucasian and 69 non-Caucasian, 102 never smokers and 160 smokers (ex-smoker or current smoker). The diagnoses include 203 adenocarcinomas, 17 squamous cell carcinomas, 4 adenosquamous carcinomas, 2 adenoid cystic carcinomas and 36 NSCLC, not otherwise specified. 199 specimens were from EBUS-FNA of the mediastinal lymph nodes and 66 specimens were from lung lesions. There is no significant difference in gender, ethics, smoking status or diagnosis between these two sources of specimens.

57 patients also had surgical specimens tested for PD-L1 expression (Supplemental Table 1). Of the 57 paired specimens, 31 were obtained concurrently, 16 cytology specimens were obtained before the surgical specimens, and 10 cytology specimens were obtained subsequent to the surgical specimens. In patients whose cytology samples preceded surgical sampling, the median time between specimens was 41 days (range, 6–143 days), during which 1 patient received radiation therapy, 1 patient received chemotherapy and 2 patients received both radiation and chemotherapy. In patients whose cytology samples were obtained after surgical sampling, the median time between specimens was 75 days (range, 13–1310 days), during which 3 patients received radiation therapy, 1 patient received chemotherapy, 1 patient received both radiation and chemotherapy and another patient received targeted therapy with EGFR inhibitors. In these 57 patients, 16 of them had surgical specimen (resection or biopsy) and EBUS-FNA specimen from the same anatomic site (Table 1, Fig. 1), including 9 pairs from hilar lymph nodes and 7 pairs from lung.

3.2. EBUS-FNA specimens have high success rate for PD-L1 testing

Of the overall 265 EBUS-FNA specimens (262 patients) sent for PD-

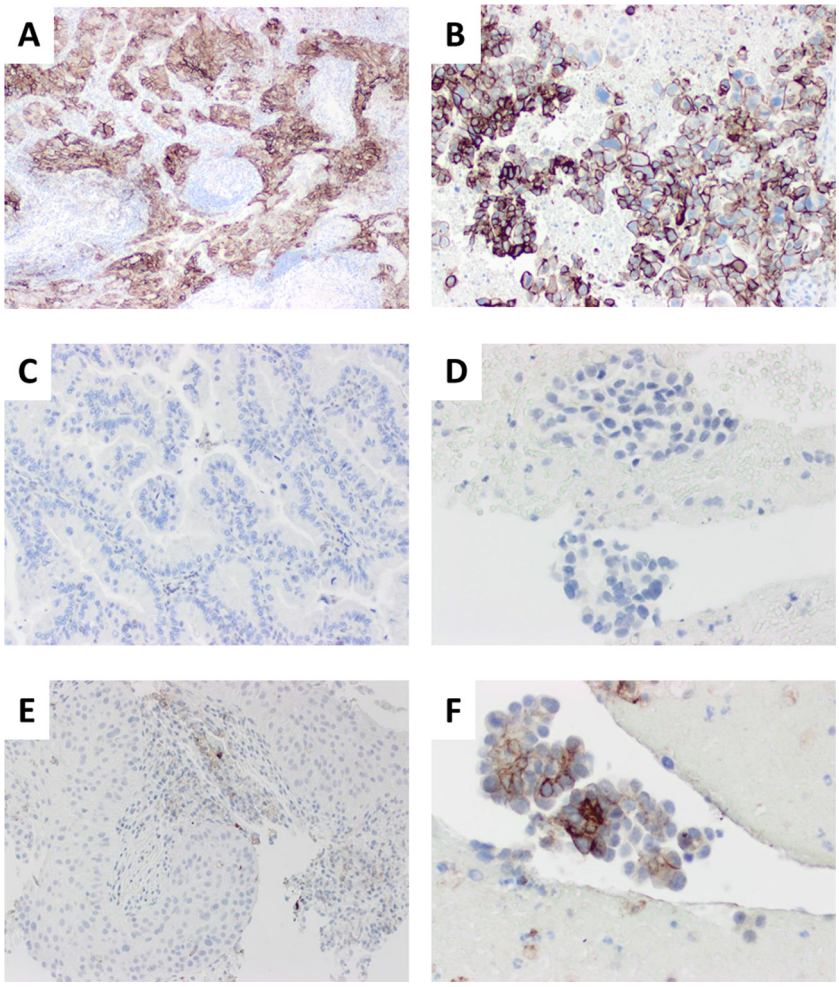

Fig. 1. PD-L1 expression in surgical specimens vs. cytology specimens, and in tumor at primary site vs. at metastatic lymph nodes tested by using Dako 22C3 antibody: PD-L1 expression is ≥50% in biopsy site (a) and is also ≥50% in FNA cell block specimen (b) at same site; PD-L1 expression is < 1% in surgical specimen (c) and in matched cytology specimen (d); PD-L1 expression is < 50% in primary tumor (e) and is ≥50% in metastatic mediastinal lymph node (f).

Table 2
PD-L1 expression in NSCLC at primary lung site vs. at metastatic hilar/mediastinal lymph nodes.

	PD-L1 TPS		
	≥50%	< 50%	Total
Lung	15 (25.8%)	43 (74.2%)	58
Lymph node	83(48.3%)	89(51.7%)	172
Total	98(42.6%)	132(57.4%)	230

TPS, tumor proportion score.

L1 testing, 35 specimens (13.2%) had inadequate material for PD-L1 testing, with a success rate of 86.8%. Within the specimens with adequate material, 98 of them (42.6%) had a PD-L1 TPS ≥ 50%, 54 specimens (23.5%) had a PD-L1 TPS between 1–49% and 78 specimens (33.9%) had a PD-L1 TPS < 1% (Table 2). Of the 57 NSCLC patients with both surgical and EBUS-FNA cytology specimens tested for PD-L1, 7 of them had inadequate tumor cells in surgical biopsy specimens but had successful testing in EBUS-FNA specimens; 15 of them had inadequate tumor cells in EBUS-FNA specimen but had successful testing in surgical specimens. Only 1 patient failed the testing in both types of specimens (Supplement Table 1).

3.3. EBUS-FNA specimens have high concordance with surgical specimens for PD-L1 testing

Of the 34 NSCLC patients with both surgical and EBUS-FNA cytology specimens tested for PD-L1, the PD-L1 results from the different specimen types agreed each other in 31 patients. Only 3 patients had different PD-L1 results between surgical specimens and EBUS-FNA specimens. In 2 of these 3 patients, the EBUS-FNA specimens from 4R mediastinal lymph node had a PD-L1 TPS ≥ 50%, while the biopsy specimens from the lung primary tumor had a PD-L1 TPS less than 1% or 1–49% (Fig. 1). For these 2 patients, both specimens are sampled concurrently. In another patient, who received targeted therapy of EGFR inhibitor after the EBUS-FNA of mediastinal lymph node and before the chest wall metastasis biopsy, the EBUS-FNA specimen from 4R mediastinal lymph node had a PD-L1 TPS of 1–49% while the biopsy specimen from the metastatic lesion of the chest wall had a PD-L1 TPS ≥ 50%. The overall agreement between the EBUS-FNA cytology specimen and surgical specimen from the same patients in PD-L1 testing was 91.2% (31/34). There were 16 NSCLC patients with surgical and EBUS-FNA cytology specimens from the same anatomic site (Table 1). The PD-L1 results from these 16 paired specimens had 100% agreement (16/16).

3.4. PD-L1 expression in tumors from lymph node versus in primary site

There were 58 EBUS-FNA specimens from the primary lung lesion

with adequate material, in which 15 specimens (25.9%) had a PD-L1 TPS ≥ 50%, 19 specimens (32.8%) had a PD-L1 TPS between 1–49% and 24 specimens (41.4%) had a PD-L1 TPS < 1%. There were 172 EBUS-FNA specimens from the hilar/mediastinal lymph nodes had adequate material, in which 83 specimens (48.3%) had a PD-L1 TPS ≥ 50%, 35 specimens (20.3%) had a PD-L1 TPS between 1–49% and 54 specimens (31.4%) had a PD-L1 TPS < 1%. The positive rates of PD-L1 (TPS ≥ 50%) were significantly higher in the tumors from the lymph nodes than in the lung primary lesions (p = 0.0034) (Table 2).

4. Discussion

We conducted this study to examine the feasibility of using EBUS-FNA samples for the evaluation of tumor PD-L1 expression in NSCLC. A total of 265 specimens from 262 patients with PD-L1 testing were included in this study. Our results showed that EBUS-FNA specimens had high success rate and high concordance with surgical specimens for PD-L1 testing. We also showed that the tumors at metastatic lymph nodes have significantly higher percentage of PD-L1 TPS ≥ 50% than in tumors at primary site.

EBUS-FNA is a safe and less invasive technique for definitive diagnosis and staging mediastinal and hilar lymph nodes in patients with lung cancers [8], and it could be used to repeat sampling of refractory tumors even after surgery. In the current environment of targeted therapy and immunotherapy, EBUS-FNA sampling is an excellent approach to obtaining tissue for the detection of EGFR mutations, ALK rearrangements, and other gene alterations by sequencing, fluorescence in situ hybridization, or polymerase chain reaction analyses [9–13]. Although a few reports suggested EBUS-FNA sampling can be applied for PD-L1 testing, the sample sizes in these studies are small [14,15]. In current study, we have overall 265 specimens from 262 patients with PD-L1 testing having a success rate of 86.8%. Additionally, of the 57 NSCLC patients with both surgical and EBUS-FNA specimens tested for PD-L1, 7 of them had inadequate material in surgical biopsy specimen but had successful testing in EBUS-FNA specimens. To the best of our knowledge, this is one of the first and so far, the largest study to examine the feasibility of using EBUS-FNA for the evaluation of PD-L1 expression. The results of the current study add to the limited body of evidence regarding the feasibility of PD-L1 testing on cell blocks from bronchoscopically acquired lung cancer specimens, especially as a complimentary sample resource for the patients who have no adequate sample from surgical or biopsy specimen.

Few studies have been done to compare the PD-L1 testing on surgical and cytology specimens, showing the concordance rates ranging from 82% to 94% [16–19]. In Heymann's study, there was no significant difference in PD-L1 expression noted between small biopsy (25.8%) and surgical resection (25.7%) specimens, but PD-L1 expression was found to be higher in cytology specimens (39%) as compared with surgical specimens (25%) [20]. Some pre-analytical factors such as fixation may contribute to the discrepancy of the PD-L1 expression in cytology versus surgical specimens [21,22]. In current study, among 34 patients with both EBUS-FNA and surgical histology specimens, the concordance for PD-L1 expression is 91.2%. In 16 patients with surgical and EBUS-FNA specimens from the same anatomic site, including 7 primary lung lesions and 9 hilar/mediastinal lymph nodes, the PD-L1 results had 100% agreement in these paired specimens (16/16). We have to admit that there were only 16 patients with paired specimens from the same anatomic site. Large sample size of the matched cytology and surgical specimen is needed to draw more solid conclusion.

The discrepancy of the PD-L1 expression between metastatic versus primary tumor has been reported in other studies [17,23–26]. Our results demonstrated that positive (TPS ≥ 50%) for PD-L1 expression was significantly higher in the metastatic tumors from the hilar/mediastinal lymph nodes than in the lung primary lesions. 25.9% of the EBUS-FNA specimens from the primary lung lesion had PD-L1 TPS ≥ 50%, which is consistent with 23% reported in the initial phase 1 clinical trial that formally validated PD-L1 expression in NSCLC as a biomarker of clinical response to PD-1 inhibition [27]. However, 48.3% of the EBUS-FNA specimens from the hilar/mediastinal lymph nodes had a PD-L1 TPS ≥ 50%. Consistent with the above finding, in 3 patients having different PD-L1 results between surgical specimens and EBUS-FNA specimens, all of them had higher PD-L1 expression in the tumors from the higher stage sites (hilar/mediastinal lymph node versus lung primary in 2 cases, chest wall metastasis versus hilar/mediastinal lymph node in 1 case). Overall, these results suggest a moderate-to-strong correlation for PD-L1 expression between primary and metastatic tumor tissue. One of the reasons which may explain higher PD-L1 positive (TPS ≥ 50%) rate at metastatic site is that those tumors with higher PD-L1 expression could escape from immune response and attack by T lymphocytes, metastasize from primary site to mediastinal lymph nodes or distant sites. Although additional studies, with large population of the patients with paired specimens from both primary and metastatic tumors, are needed to better define this topic, if this relationship holds true, one could argue for the sampling and testing of metastatic site instead of primary cancer tissue for PD-L1 expression given the benefits of immune checkpoint inhibitors [2].

In summary, this study demonstrated a high adequacy for PD-L1 testing in EBUS-FNA samples. The higher PD-L1 positive (TPS ≥ 50%) rate in more advanced status of tumors may explain the discrepancy of the PD-L1 results between EBUS-FNA and surgical specimens. Our data suggests that EBUS-FNA is a reliable method for the evaluation of tumor PD-L1 expression in lung cancer, and the metastatic sites should be examined if possible for PD-L1 expression since it might select more patients with NSCLC who will benefit from immunotherapy.

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Precis

1. EBUS-FNA cytology specimen is suitable for PD-L1 testing in patients with advanced NSCLC. 2. Metastatic tumors in mediastinal lymph nodes have significantly higher percentage of PD-L1 TPS ≥ 50% than those in primary tumors.

Author contributions

Gang Wang: Conception and design of study, data acquisition, data analysis and interpretation, manuscript drafting and revising. Diana N. Ionescu: Conception and design of study, data acquisition, data interpretation, manuscript revising. Cheng-Han Lee: Data acquisition. Tadaaki Hiruki: Data acquisition. Renelle Myers: Data acquisition. Tawimas Shaipanich: Data acquisition. Stephen Lam: Data acquisition, data interpretation, manuscript revising. Barbara Melosky: Data interpretation, manuscript revising. Chen Zhou: Conception and design of study, data acquisition, data analysis and interpretation, manuscript revising.

Declaration of Competing Interest

All authors have no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.07.033.

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