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Positive Conversion of PD-L1 Expression After Treatments with Chemotherapy and Nivolumab

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Abstract. Background/Aim: Few studies have so far described the conversion of programmed death-ligand 1 (PD-L1) after treatment. In particular, the effect of nivolumab on the PD-L1 expression has never been reported. We investigated the changes in PD-L1 expression after chemotherapy and nivolumab treatment and herein describe the detailed clinical course. Patients and Methods: We retrospectively examined the PD-L1 expression in resected specimens and in the re-biopsy specimens of patients with non-small cell lung cancer by immunohistochemical analysis. Results: Four patients underwent a re-biopsy after treatment. Of those, three showed positive conversion of the PD-L1 expression. One patient underwent a re-biopsy after chemotherapy and nivolumab treatment, and the other two cases underwent a re-biopsy after chemotherapy, radiotherapy and nivolumab treatment. Conclusion: These cases suggest the positive conversion of PD-L1 expression after treatments including nivolumab, suggesting that PD-L1 expression must be assessed in not only the resected specimens, but also in the re-biopsied ones.

Lung cancer is the leading cause of cancer-related death worldwide (1). Recently, chemotherapy and molecular targeted therapy have led to the improved clinical outcome of lung cancer patients. However, despite those advances in therapies, the prognosis remains poor, and a novel treatment strategy is needed. Inhibitors against the immune checkpoint system, such as programmed cell death 1 (PD-1) and programmed death-ligand 1 (PD-L1), have been shown to improve the outcomes of patients with non-small cell lung cancer (NSCLC) (2-7).

The PD-1 receptor expressed on activated T cells is engaged by the tumor-expressed ligand PD-L1 to limit the T cell effector functions within tissues and promote the immune escape of the tumor (8). To identify the patients likely to respond to PD-1 or PD-L1 inhibitors, the relationship between the PD-L1 expression on tumor cells and the effect of immune checkpoint inhibitors has been reported (2-7, 9, 10). In the CheckMate 017 study of nivolumab *versus* docetaxel in advanced squamous NSCLC, the PD-L1 expression had no relationship with the prognosis or predicted clinical benefit (3). However, many other studies have reported the expression of PD-L1 to be associated with a good response against immune checkpoint inhibitors (2, 4-6, 9, 10).

Thus, the expression of PD-L1 has attracted increasing attention, since it helps determine the optimal treatment strategy, including specific immunotherapy; however, few studies have examined the conversion of PD-L1 after treatment. In addition, its detailed clinical course is almost unknown. In this translational study, we investigated changes in the PD-L1 expression in NSCLC before and after chemotherapy and nivolumab, and the detailed clinical course was reported.

Materials and Methods

Study patients. Eligible patients underwent surgical resection for primary NSCLC between January 2008 and December 2016 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Of those, four patients who had shown disease progression during anticancer treatment including nivolumab after resection underwent a re-biopsy to assess the PD-L1 expression of the postoperative recurrence lesion. We retrospectively examined the PD-L1 expression in the surgically resected specimens and in the re-biopsy specimens of these patients using an immunohistochemical analysis.

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Table I. *Patient characteristics*.

Patient number	Age	Gender	PS	PY	Surgical procedure	Pathological stage	Histology	Reccurence stage
1	75	Male	0	54	Lobectomy	pT1bN2M0	Adenocarcinoma	rT0N3M1b (OSS,LYM)
2	60	Male	0	66	wedge resection	pT2aN1M1a	Carcinosarcoma	-
3	70	Male	1	35	Lobectomy	pT2aN0M0	Adenocarcinoma	rT0N3M1a (PUL,PLE, OSS)
4	60	Male	0	39	Pneumonectmy	ypT1aN0M0	Squamous cell	rT0N1M1b (OSS)

PS: Performance status; PY: pack year index.

Table II. *Changes in the programmed cell death 1 expression of each patient*.

Patient number	Treatment before the re-biopsy	Re-biopsy lesion	TPS of primary resected lesion	TPS of recurrence re-biopsy lesion
1	Chemotherapy, nivolumab	Axillary lymph node	0	25
2	Chemotherapy, RTx, nivolumab	Lung	0	60
3	Chemotherapy, RTx, nivolumab	Bone	5	30
4	Chemotherapy, nivolumab	Bone	0	0

TPS: Tumor proportion score of programmed cell death 1 expression.

The present study was approved by our institutional review board.

Immunohistochemical analyses. Immunohistochemistry (IHC) was performed for four surgical resected specimens and four re-biopsy specimens of NSCLC patients using the Dako Autostainer Link 48 platform (Santa Clara, CA, USA) and an automated staining protocol validated for the PD-L1 IHC 22C3 pharmDx assay, as described previously (11). The primary antibody was monoclonal mouse Anti-PD-L1, Clone 22C3. Scoring was recorded as the percentage of PD-L1-positive tumor cells among the total tumor cells (tumor proportion score; TPS).

Results

Four patients underwent a re-biopsy after the treatment including nivolumab. Of those, 3 (75.0%) showed the positive conversion of the PD-L1 expression (Tables I and II).

Patient 1 was a 76-year-old male smoker with postoperative recurrence of lung adenocarcinoma. The primary lesion existed in the right lower lobe, and right lower lobectomy was performed (pathological stage: T1bN2M0 (TNM 7th edition), epidermal growth factor receptor (*EGFR*): wild type, rearrangement of the anaplastic lymphoma kinase gene (*ALK*): negative; Figure 1A and B). Approximately 4.8 months after the operation, multiple bone, axillary and mediastinal lymph node metastases were seen. First-line chemotherapy (platinum-based pemetrexed) had to be discontinued after three months because of disease progression. Although the PD-L1 expression was negative in the resected specimen of the primary lesion (Figure 1C), second-line treatment with nivolumab led to stable disease. The patient then experienced disease failure after seven cycles of nivolumab. Third-line treatment was docetaxel; however, a relapse of multiple axillary and mediastinal lymph nodes was seen approximately seven months later (Figure 1D), and a re-biopsy of the axillary lymph node showed positive PD-L1 expression (TPS 25%; Figure 1E and F).

Patient 2 was a 60-year-old male smoker who had been preoperatively diagnosed with non-small cell lung cancer (NSCLC, *EGFR*: wild type, rearrangement of the *ALK*: negative; Figure 2A). Because intraoperative cytology of the pleural effusion was positive for malignant cells, only wedge resection of the primary lesion in the right upper lobe was performed to confirm the histological findings. The histological examination of the specimen showed malignant osteoid stroma and adenocarcinoma, and the diagnosis was carcinosarcoma (Figure 2B). Approximately 30 months after the first-line (Carboplatin+Paclitaxel+Bevacizumab), second-line (cisplatin+S-1+RTx [60 Gy/30 Fr.]) and third-line (docetaxel) treatments, nivolumab (3 mg/kg, every 2 weeks) was administered. Although the patient achieved a stable disease status for 6.2 month, a relapse of the pulmonary lesion was observed after 13 cycles of nivolumab treatment (Figure 2D). The PD-L1 expression was negative in the resected archival specimen (Figure 2C); however, a re-biopsy of the pulmonary lesion showed high PD-L1 expression (TPS 60%; Figure 2E and F); the patient is now undergoing treatment with pemblorizumab.

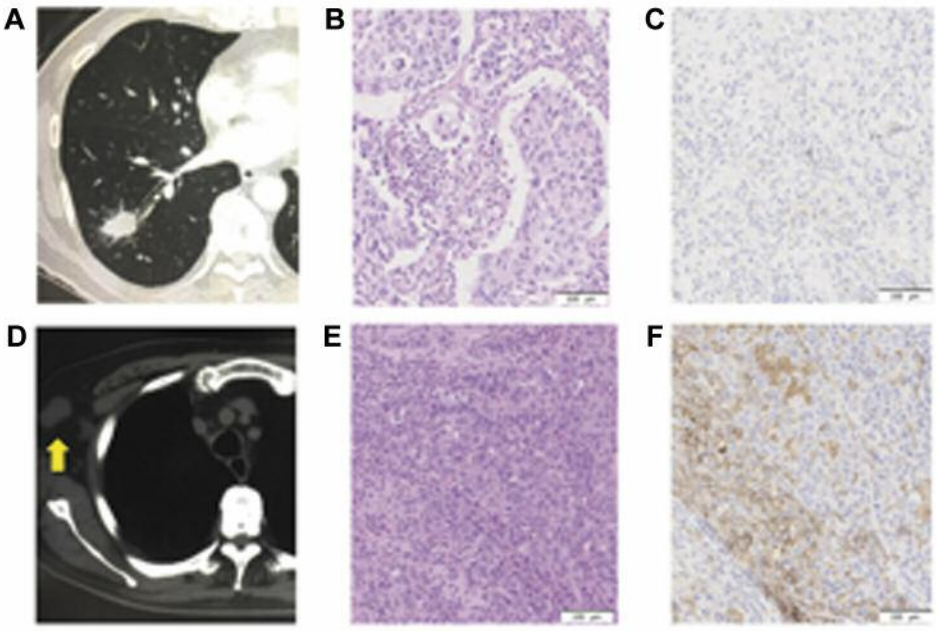

Figure 1. *Chest computed tomography (CT) images and the histological (hematoxylin eosin staining) and immunohistochemical findings of the first case. CT imaging shows the primary lung lesion in the right lower lobe prior to operation (A). The resected specimen shows the histology of adenocarcinoma (B). Immunohistochemistry using 22C3 revealed that the tumor did not express programmed death-ligand 1 (PD-L1; C). CT imaging after third-line treatment shows a swollen axillary lymph node (D), and a re-biopsy specimen shows the histology of metastasis of adenocarcinoma (E) and positive PD-L1 expression (F). Scale bar: 100 μm.*

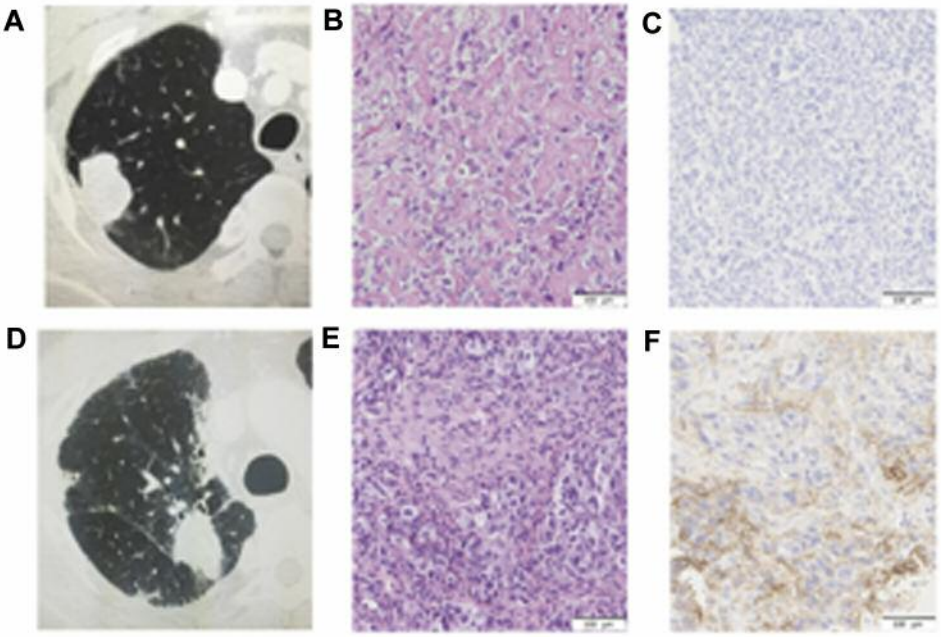

Figure 2. *Chest computed tomography (CT) images and the histological (hematoxylin eosin staining) and immunohistochemical findings of the second case. Chest CT imaging shows the primary lung lesion in the right upper lobe prior to the operation (A). Polygonal and spindle-shaped carcinoma cells accompanied by malignant osteoid stroma were observed in the sarcomatous component (B). Immunohistochemistry using 22C3 showed no programmed death-ligand 1 (PD-L1) expression (C). CT imaging shows the pulmonary lesion after 13 cycles of nivolumab (D). A re-biopsy of the lesion shows the finding of metastasis of carcinosacroma and high PD-L1 expression (E, F). Scale bar: 100 μm.*

Patient 3 was a 70-year-old male smoker with postoperative recurrence of lung adenocarcinoma (*EGFR*: wild type, rearrangement of the *ALK*: negative). Although the resected specimen showed weak positivity of PD-L1 expression (TPS: 5%), after treatment (first line: platinum-based pemetrexed; second line: docetaxel; third line: gemcitabine+navelbine; fourth line: nab-paclitaxel; fifth line: nivolumab) and palliative radiotherapy (30 Gy) for rib metastasis, a re-biopsy of the rib metastasis showed a stronger positive PD-L1 expression (TPS: 30%).

Discussion

The significance of the expression of PD-L1 has been gathering increasing attention as a predictive biomarker of the efficacy of immune checkpoint inhibitors; however, two main concerns exist regarding the analysis of the PD-L1 expression: (1) the antibodies and cut-off values used to detect PD-L1 expression and (2) the type of samples, *i.e.* archival or fresh tumor samples.

A report by Herbst *et al.* in the phase three KEYNOTE-010 study showed a significantly longer overall survival (OS) in NSCLC patients previously treated with pembrolizumab than in those treated with docetaxel, regardless of whether the PD-L1 expression was assessed with archival or new tumor samples; however, the difference in the Kaplan-Meier estimates of the OS between the two drugs seemed to be clearer in the patients assessed with new tumor samples than in those assessed with archival samples (5, 12). These results suggest that fresh tumor samples might be better predictive biomarkers than archival samples.

One reason for these differences might be the change in the PD-L1 expression. Mansfield *et al.* reported on the temporal and spatial discordance of PD-L1 expression in NSCLC between the primary lesion and brain metastasis (13). Heterogeneity in the expression of PD-L1 between primary and metastatic lesions in different organs despite sharing pathological characteristics has been assessed in some malignancies (13-15). Furthermore, the PD-L1 protein antigenicity has been reported to decrease over time (16, 17). Those factors might underlie the discordance of PD-L1 expression in the current three cases.

In addition, the high discordance in PD-L1 expression between surgically-resected and biopsy samples was reported (discordance rate: 48%; 18). Although the biopsy specimens underestimated the PD-L1 status observed in the whole tissue sample because of the heterogeneity of the PD-L1 expression in the tumor (18), in our present three cases, fresh samples that showed positive conversion of PD-L1 were collected *via* a biopsy. As such, the collection method probably did not have much influence on the PD-L1 expression in the current cases.

With regard to the influence of treatment on the PD-L1 expression, some *in vitro* studies have shown that certain chemotherapeutic agents and radiation therapy lead to the up-regulated expression of PD-L1 (19-21); however, changes in the PD-L1 expression after such treatments in clinical settings have been poorly reported. In particular, the effect of nivolumab on the PD-L1 expression has never been reported. Jong Ho Cho *et al.* reported that there was no significant difference in the PD-L1 expression among several types of treatment (chemotherapy, EGFR-tyrosine kinase inhibitor, radiation therapy, or none-treatment); however, these data included resected and biopsy samples. In the current cases, the archival samples were collected during surgical resection, and the fresh samples were collected *via* a re-biopsy. Furthermore, the recurrence lesion of the second case was pulmonary metastasis, which has been reported to have high concordance in PD-L1 expression with the primary lesion (22). The treatment might therefore be the most significant factor inducing positive conversion of the PD-L1 expression, especially in the second case, and the re-biopsy of the recurrence lesion should be considered in order to assess the PD-L1 expression if anticancer treatment is performed after surgical resection.

The present retrospective study is associated with several limitations. First, our sample size was very small, and it was difficult to assess the true reasons for positive conversion of the PD-L1 expression. Future studies with larger sample sizes are warranted to understand the effect of each treatment including nivolumab and the PD-L1 expressions.

Conclusion

The current three cases are suggestive of the positive conversion of PD-L1 expression after treatment including nivolumab. These results suggest that PD-L1 expression should be assessed in not only resected specimens, but also in re-biopsied ones, even if PD-L1 protein is not observed in the archival sample, in order to determine eligible treatment strategies with immune-checkpoint inhibitors. However, whether or not the treatment-induced PD-L1 expression reflects the efficacy of immunotherapy still remains to be clarified.

Conflicts of Interest

All Authors declare no conflicts of interest in association with this study.

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