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Impact of cytotoxic chemotherapy on PD-L1 expression in patients with non–small cell lung cancer negative for *EGFR* mutation and *ALK* fusion

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ABSTRACT

Objectives: Immune-checkpoint inhibitors (ICIs) are now an established therapeutic option for advanced non–-small cell lung cancer (NSCLC). It has remained unclear, however, whether cytotoxic chemotherapy affects the immune microenvironment in NSCLC wild type for *EGFR* and *ALK*.

Materials and methods: We retrospectively evaluated changes in programmed cell death 1–ligand 1 (PD-L1) expression, tumor mutation burden (TMB), and $CD8^+$ tumor-infiltrating lymphocyte (TIL) density in NSCLC patients who underwent rebiopsy at the site of recurrence after postoperative platinum-based adjuvant chemotherapy, or in those who underwent rebiopsy after one or more chemotherapeutic regimens at the advanced stage. The PD-L1 tumor proportion score (TPS) and $CD8^+$ TIL density were determined by immunohistochemistry. TMB was estimated by next-generation sequencing with a cancer gene panel (409 genes).

Results: Seventeen patients with NSCLC wild type for *EGFR* and *ALK* were enrolled. Although PD-L1 TPS tended to be increased in rebiopsy samples compared with initial biopsy tissue, this difference was not significant ($P = 0.113$). Seven patients showed an increase in PD-L1 TPS, with this change being pronounced in four. Two cases in which PD-L1 TPS increased from 0 to 90% or from 0 to 95% after cytotoxic chemotherapy also showed a durable response to subsequent treatment with an ICI. No substantial correlation between PD-L1 TPS and TMB was apparent either before ($R = 0.112$) or after ($R = 0.101$) chemotherapy. A moderate correlation was detected between PD-L1 TPS and $CD8^+$ TIL density before chemotherapy ($R = 0.517$) and a negligible correlation after ($R = 0.0219$).

Conclusion: Cytotoxic chemotherapy may change the biological characteristics of tumors including PD-L1 expression level and TMB.

1. Introduction

Lung cancer is the most common cause of cancer-related death worldwide, with non–small cell lung cancer (NSCLC) accounting for ~75% of all lung cancer cases [1]. Recent insight into the molecular basis of lung cancer has led to changes in the treatment of this disease. The identification of driver genetic changes, such as those affecting the epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) genes, has already been successfully translated into clinical practice with the approval of targeted agents, including erlotinib, gefitinib, and afatinib for patients with activating mutations of *EGFR* [2–4] and crizotinib and alectinib for those with rearrangements of *ALK* [5,6]. Cytotoxic chemotherapy has remained the standard of care for advanced NSCLC without identified driver oncogenes, however, with the efficacy of such treatment having reached a plateau.

Cancer immunotherapy has also markedly changed the landscape for treatment of NSCLC in recent years. Monotherapy with nivolumab, an antibody to the immune-checkpoint protein PD-1 (programmed cell death–1), was first approved for patients with advanced NSCLC who experience disease progression during or after platinum-based chemotherapy. The approval of this regimen was based on the results of phase III clinical trials [7,8], and its use is not dependent on the expression level of PD-1–ligand 1 (PD-L1) on tumor cells. Although chemotherapy and immunotherapy have been considered antagonistic because chemotherapy induces an immunosuppressive state—as reflected by neutropenia or lymphocytopenia, for example—emerging

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evidence suggests that chemotherapy actually might also activate antitumor immunity [9,10]. Recent phase III trials for NSCLC (Keynote-189 and IMpower 150) have thus been designed to assess whether the addition of antibodies specific for PD-1 or for PD-L1 to platinum-based chemotherapy increases clinical efficacy in terms of progression-free (PFS) and overall (OS) survival (Clinicaltrials.gov identifiers: NCT02578680 and NCT02366143) [11].

Preclinical studies have shown that radiation or chemoradiation upregulates PD-L1 expression in tumor cells [12,13], with a similar effect having been observed in clinical samples [14,15]. Whether cytotoxic chemotherapeutic agents affect the immune microenvironment in terms of PD-1–PD-L1 signaling in NSCLC has remained unclear. We therefore examined changes in PD-L1 expression, tumor mutation burden (TMB), and the density of $CD8^+$ tumor-infiltrating lymphocytes (TILs) in the tumors of NSCLC patients who underwent a repeat biopsy either at the site of recurrence after postoperative platinum-based adjuvant chemotherapy or after treatment with one or more chemotherapeutic agents at the advanced stage.

2. Material and methods

2.1. Patients

We recruited consecutive patients with recurrent or advanced NSCLC who were treated with platinum-based adjuvant chemotherapy or received one or more chemotherapeutic agents, respectively. Patients met all of the following criteria: an age of at least 20 years, a histological diagnosis of NSCLC, and the availability both of clinical information and of formalin-fixed, paraffin-embedded (FFPE) tissue blocks obtained both before and after chemotherapy that were suitable for genetic analysis and immunohistochemistry (IHC). For assessment of the impact of cytotoxic chemotherapeutic agents, mainly platinum-based drugs, on PD-L1 expression level, patients harboring *EGFR* mutations or *ALK* translocations were excluded. The following clinical information was collected retrospectively: patient demographics, including sex and date of birth; date of diagnosis; and tumor characteristics, including stage (TNM classification, 7th edition), pathological diagnosis, *EGFR* mutation status, and *ALK* translocation status. *EGFR* mutations were detected with commercial assays, whereas *ALK* translocation status was determined by IHC or fluorescence in situ hybridization. Treatment history and dates of biopsy were also collected. The choice of chemotherapy regimen was made by the treating physician. Written informed consent was obtained from the patients as far as possible; patients who could not provide such consent were included if they had not previously selected an opt-out option at an informational website. This study was approved by the ethics committee of Kindai University Faculty of Medicine.

2.2. IHC of PD-L1 and CD8 in tumor specimens

Individual biopsy blocks were cut into 4-μm slices. The sections were depleted of paraffin with xylene and rehydrated with a graded series of ethanol solutions. IHC for PD-L1 was performed with the use of a PD-L1 IHC 22C3 pharmDx kit (Agilent Technologies, Santa Clara, CA) and a Dako Autostainer Link 48 platform (Dako, Carpinteria, CA) [16]. Staining intensity for PD-L1 and the percentage of PD-L1–positive tumor cells were evaluated for each sample by two pathologists (Y.N. and A.I) who were blinded to clinical outcome. Staining intensity was scored as 0 for negative, 1 for weak, 2 for moderate, and 3 for strong. The tumor proportion score (TPS) was calculated as the percentage of viable tumor cells showing partial or complete membrane staining (≥ 1) relative to all viable tumor cells present in the sample [17].

IHC and counting of $CD8^+$ TILs were performed as previously described [18]. At least one and a maximum of five scanned fields of tumor regions at an absolute magnification of 200× were randomly chosen for each TIL count. TILs were counted by two pathologists (Y.N. and A.I), and the density of TILs in the tumor was calculated by dividing the number of TILs by the sum of the area (mm^2) of the viewed fields. For TIL analysis, all fields of small biopsy specimens were evaluated as intratumoral regions. In the case of surgical specimens, both intratumoral and peritumoral regions were evaluated, but TILs of intratumoral regions were used for final analysis.

2.3. Determination of TMB with a next-generation sequencing–based target panel

The collected FFPE specimens underwent histological review, and only those containing sufficient tumor cells as revealed by hematoxylin-eosin staining were subjected to nucleic acid extraction. DNA was purified with the use of an Allprep DNA/RNA FFPE Kit (Qiagen, Valencia, CA), and the amount of isolated DNA was verified with the use of a NanoDrop 2000 device (Thermo Scientific, Wilmington, DE) and PicoGreen dsDNA Assay Kit (Life Technologies, Foster City, CA).

TMB was determined with an Ion AmpliSeq Comprehensive Cancer Panel (CCP409, Thermo Fisher) [19], which covers all coding exons of 409 cancer-related genes with four multiplexed primer pools. Bar-coded libraries were generated from 40 ng of DNA with CCP409 and an Ion AmpliSeq Library Kit version 2.0. The purified libraries were sequenced with an Ion Torrent Proton instrument, Ion Proton Hi-Q Sequencing Kit, and Ion PI v3 Chip (all from Life Technologies). DNA sequencing data were accessed through the Torrent Suite v.5.8 program (Life Technologies). Reads were aligned with the hg19 human reference genome, and potential mutations were called with the use of Variant Call Format ver. 5.8. Raw variant calls were filtered with a quality score of < 100 and depth of coverage of < 19 and were manually checked with the Integrative Genomics Viewer (IGV; Broad Institute, Cambridge, MA). Germline mutations were excluded with the use of the Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB) and the Exome Aggregation Consortium database. To calculate TMB, we counted synonymous and nonsynonymous single-base substitutions and small insertions-deletions (indels) in coding regions. TMB was expressed as the rate of true variants per million bases of CCP409 regions of interest.

2.4. Statistical analysis

Descriptive statistics were applied to patient and tumor characteristics. The Wilcoxon signed-rank test was applied to compare PD-L1 TPS, TMB, or $CD8^+$ TIL density between the first biopsy and rebiopsy specimens of the study patients, and Fisher's exact test was adopted to assess the difference in the percentage of samples with a TPS of $> 1\%$ between the two sets of samples. The relation between PD-L1 TPS and either TMB or $CD8^+$ TIL density for tumor tissue obtained before or after chemotherapy was examined with Spearman's correlation coefficient. All tests were two-tailed, and a P value of < 0.05 was considered statistically significant. PFS was calculated from the date of initiation of chemotherapy either to the date of disease progression or to the date of last contact. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [20].

3. Results

3.1. Patient characteristics

Electronic medical records from April 2014 to October 2016 at the Department of Medical Oncology of Kindai University Hospital were reviewed. The review identified 17 NSCLC patients who underwent rebiopsy at the site of recurrence after postoperative platinum-based adjuvant chemotherapy or who underwent rebiopsy after treatment with one or more chemotherapeutic agents at the advanced stage. The

Table 1
Characteristics of the Enrolled Non–Small Cell Lung Cancer Patients.

No.	Sex	Age (Years)	Smoking Status	Stage at Diagnosis	Surgery	Histology	*EGFR* status	*ALK* status	Initial biopsy site	Rebiopsy site	Interval between initial and rebiopsy (months)	No. of chemotherapy regimens between initial and rebiopsy
1	M	76	Smoker	IIIA	+	Ad	WT	WT	rt S10	rt axillary node	33	1
2	M	76	Smoker	IIA	+	Ad	WT	WT	lt upper lobe	rt S10	12	2
3	M	67	Smoker	IIA	+	Sq	WT	WT	rt upper lobe	rt S6	29	1
4	F	58	Never	IIB	+	Ad	WT	WT	lt lower lobe	rt S6	45	4
5	M	66	Smoker	IIA	+	Sq	WT	WT	rt upper lobe	rt S10	50	1
6	M	61	Smoker	IIB	+	Ad	WT	WT	rt upper lobe	brain	26	1
7	M	73	Smoker	IB	+	Ad	WT	WT	lt upper lobe	rt S3	25	1
8	M	77	Smoker	IV	–	Ad	WT	WT	rt S1	rt S1	6	1
9	M	73	Smoker	IV	–	Ad	WT	WT	Lung (unknown biopsy site)	lt upper lobe	11	4
10	M	54	Never	IA	+	Ad	WT	WT	lt lower lobe	lt S1 + 2	156	4
11	F	79	Never	IIIA	+	Sq	WT	WT	rt lower lobe	liver	43	1
12	F	40	Never	IIA	+	Ad	WT	WT	rt lower lobe	lt S5	52	3
13	M	55	Never	IIA	+	Ad	WT	WT	lt upper lobe	lt S8	116	5
14	M	67	Smoker	IIIB	–	Sq	WT	WT	lt S1 + 2	lt S1 + 2	3	1
15	F	49	Smoker	IV	+	Ad	WT	WT	rt upper lobe	rt cervical lymph node	69	6
16	M	58	Smoker	IIB	+	Ad	WT	WT	lt lower lobe	rt S10	60	3
17	M	64	Smoker	IV	–	Ad	WT	WT	rt S1	rt S1	43	1

Abbreviations: No, number; M, male; F, female; Ad, adenocarcinoma; Sq, squamous cell carcinoma; *EGFR*, epidermal growth factor receptor gene; WT, wild type; *ALK*, anaplastic lymphoma kinase gene; rt, right; lt, left.

characteristics of the study patients are shown in Table 1. The median age of the patients was 66 years (range, 40–79 years), 13 (76.5%) were men, and 5 (29.4%) were never smokers and 12 (70.6%) current or former smokers. Clinical stage at diagnosis was IA (n = 1, 5.9%), IB (n = 1, 5.9%), IIA (n = 5, 29.4%), IIB (n = 3, 17.6%), IIIA (n = 2, 11.8%), IIIB (n = 1, 5.9%), and IV (n = 4, 23.5%). Adenocarcinoma was the most common histology (n = 13, 76.5%), followed by squamous cell carcinoma (n = 4, 23.5%). The tumors of all 17 patients were negative for both *EGFR* mutation and *ALK* fusion. The location of the first biopsy was the lung in all patients, whereas that of the rebiopsy was the lung in 13 patients (76.5%), lymph nodes in 2 patients (11.8%), liver in 1 patient (5.9%), and brain in 1 patient (5.9%). The median number of chemotherapy regimens between first biopsy and rebiopsy was 1 (range, 1–6), with most (n = 16, 94.1%) patients being treated with platinum-based therapy during this period and the remaining one patient (case 13) receiving only nonplatinum chemotherapy (Supplementary Table S1). With curative intent, 12 (70.6%) patients received surgery between the first biopsy and rebiopsy.

3.2. Change in PD-L1 TPS in tumor samples obtained before and after chemotherapy

Among the 34 tumor specimens (17 pairs of samples), PD-L1 expression was evaluable in 33, with the remaining sample containing insufficient tumor tissue. The median PD-L1 TPS was 0 at initial biopsy and 5 at rebiopsy, with this difference not being statistically significant ($P = 0.113$, paired Wilcoxon signed-rank test) (Fig. 1A). A trend toward increased positivity for PD-L1 expression (TPS of > 1%) was apparent in the rebiopsy samples (n = 11, 64.7%) compared with the initial biopsy samples (n = 7, 43.8%) (P = 0.30, Fisher's exact test).

Seven (41.2%) of the 17 patients (cases 1, 5, 7, 8, 10, 12, and 14) showed an increase in TPS, with four (23.5%) of these individuals (cases 1, 7, 8, and 10) showing a marked increase in the median value from 10% to 90%. PD-L1 staining for these latter four cases is shown in Fig. 2. To minimize the effects of heterogeneity, we evaluated the change of the PD-L1, TMB, and CD8 + TILs expression levels among 13 patients whose initial and rebiopsy site was restricted to lung fields. Of these patients, no specific trend was shown compared with total cohort (Supplementary Fig. 1A). A trend toward increased positivity for PD-L1 expression (TPS of > 1%) was apparent in the rebiopsy samples (n = 9, 69.2%) compared with the initial biopsy samples (n = 4, 33.3%) ($P = 0.115$, Fisher's exact test).

3.3. Change in TMB in tumor tissue obtained before and after chemotherapy

Four patients were excluded from TMB analysis because their tumor samples were not evaluable as a result of degradation. For the remaining 13 patients, paired pre- and postchemotherapy tumor tissue was available for analysis (Fig. 1B). The median TMB in the initial biopsy and rebiopsy samples was 6.4 and 5.6 mutations per megabase, respectively ($P = 0.722$, paired Wilcoxon signed-rank test). TMB was evaluable in three of the four cases in which PD-L1 TPS underwent a marked increase, with one case showing a small decrease in TMB, one case a large decrease, and one case a small increase. In 13 study patients whose biopsy and rebiopsy were performed in lung lesions, no specific trend of change of TMB was shown compared with total patients (Supplementary Fig. 1B). The median TMB in the initial biopsy and rebiopsy samples was 8.0 and 5.6 mutations per megabase, respectively ($P = 0.400$, paired Wilcoxon signed-rank test).

3.4. Change in intratumoral $CD8^+$ TIL density in tumor tissue obtained before and after chemotherapy

Given that two patients did not have adequate tumor tissue for evaluation of $CD8^+$ TIL density, the analysis was performed with 15 pairs of pre- and postchemotherapy tumor samples (Fig. 3). The median $CD8^+$ TIL density was 8/mm^2 in the initial biopsy samples and 10/mm^2 in the rebiopsy samples ($P = 0.889$, paired Wilcoxon signed-rank test). Three of the 15 evaluable patients (cases 6, 7, and 14) showed large decreases in $CD8^+$ TIL density after chemotherapy. $CD8^+$ TIL density was evaluable in three of the four cases in which PD-L1 TPS increased markedly, with one case showing a decrease in $CD8^+$ TIL density, one an increase, and one no change. In 13 study patients, whose biopsy and rebiopsy were performed in lung lesions, no specific trend of $CD8^+$ TIL density was shown compared with total patients (Supplementary Fig. 1C).

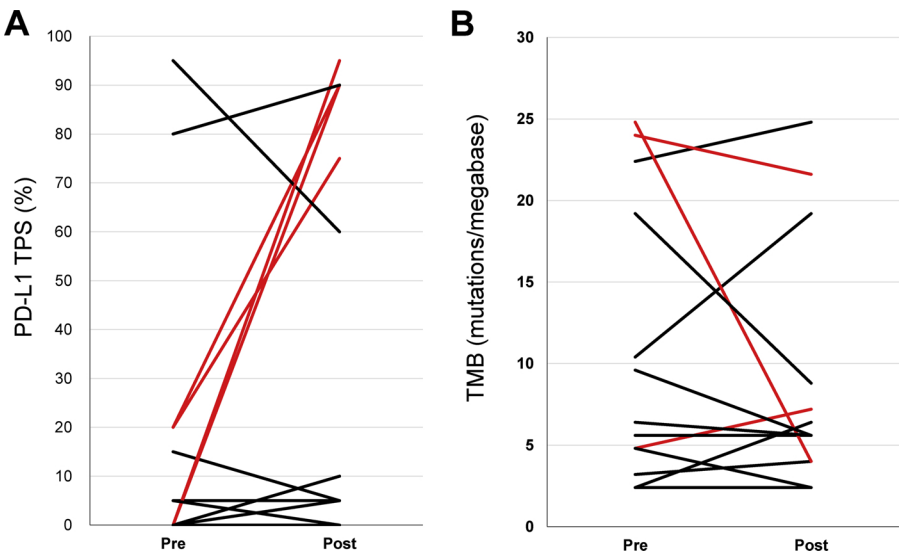


Fig. 1. Changes in PD-L1 tumor proportion score (TPS) and tumor mutation burden (TMB) between before (pre) and after (post) cytotoxic chemotherapy. Initial biopsy and repeat biopsy samples were analyzed for programmed cell death 1–ligand 1 (PD-L1) TPS (A) and TMB (B). Red lines in (A) indicate four cases (cases 1, 7, 8, and 10) in which the PD-L1 TPS increased markedly; those in (B) correspond to three of these four cases (cases 1, 7, and 8). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Relation between PD-L1 TPS and either TMB or CD8+ TIL density for tumor tissue collected before or after chemotherapy

We analyzed the relation between TMB and PD-L1 TPS for tumor tissue obtained before or after chemotherapy with Spearman's correlation coefficient. No substantial correlation between the two parameters was apparent either before ($R = 0.112$) (Fig. 4A) or after ($R = 0.101$) (Fig. 4B) chemotherapy. Tumors with a low PD-L1 TPS and high TMB and those with a high PD-L1 TPS and low TMB were not rare in this cohort.

We also analyzed the relation between $CD8^+$ TIL density and PD-L1 TPS for tumor tissue collected before or after chemotherapy. A moderate correlation was detected between these two parameters before chemotherapy ($R = 0.517$) (Fig. 4C) and a negligible correlation after ($R = 0.0219$) (Fig. 4D). The most common pattern was a low $CD8^+$ TIL density and low PD-L1 TPS both before and after chemotherapy.

3.6. Treatment efficacy of PD-1 inhibitors

Six patients (cases 2, 10, 11, 12, 13, and 17) received nivolumab and one patient (case 8) received pembrolizumab after rebiopsy (Supplementary Table S1). The best overall response for treatment with these antibodies to PD-1 was a partial response in cases 8, 10, and 17; stable disease in cases 11 to 13; and progressive disease in case 2 (Fig. 5A). Of note, three (cases 8, 10, and 17) of these seven patients showed a durable clinical benefit (> 20 months) of such treatment. In case 8, the PD-L1 TPS increased from 0% before to 90% after chemotherapy whereas the TMB decreased slightly from 24.0 to 21.6 mutations per megabase (Fig. 5B). Similarly, in case 10, the PD-L1 TPS increased from 0% before to 95% after chemotherapy, although TMB was not evaluable because of sample degradation (Fig. 5B). In the remaining case (case 17) to show a prolonged benefit of PD-1 inhibitor treatment, the PD-L1 TPS was only 5% in the rebiopsy sample (Fig. 5B).

4. Discussion

In this study, we sought to evaluate whether cytotoxic chemotherapy modulates the immune response to tumor cells in patients with NSCLC negative for *EGFR* mutation and *ALK* fusion who underwent rebiopsy at the site of recurrence after postoperative platinum-based adjuvant chemotherapy or in those who underwent rebiopsy after treatment with one or more chemotherapeutic agents at the advanced stage. Positivity for PD-L1 expression (TPS of > 1%) was considerably higher in the rebiopsy tissue samples compared with the initial biopsy samples, although the difference was not statistically significant. Of interest, the PD-L1 TPS increased markedly in four patients, two of

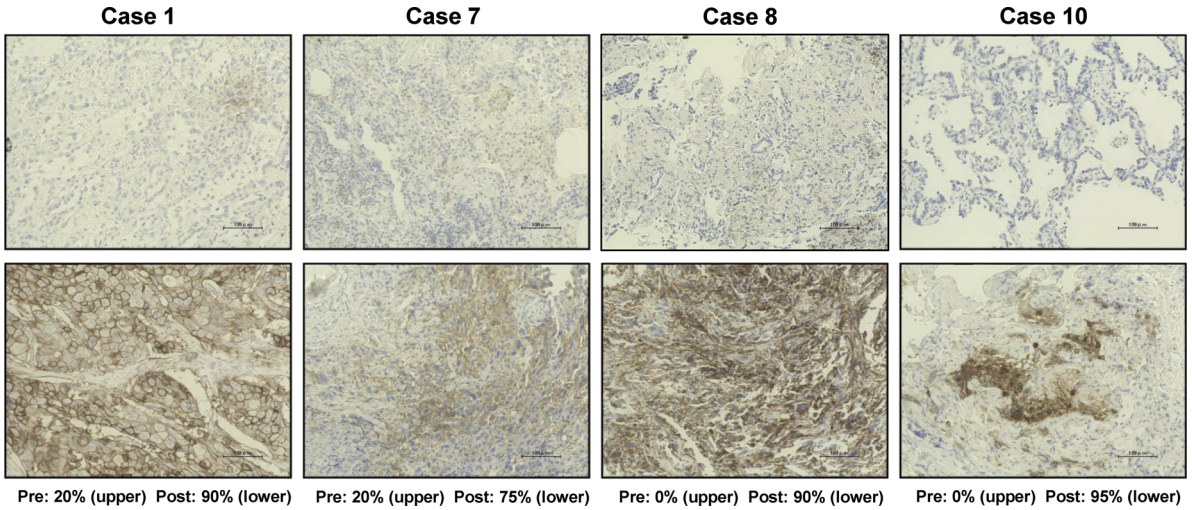


Fig. 2. Marked increase in PD-L1 staining between before (pre) and after (post) cytotoxic chemotherapy for four patients. Immunohistochemical staining of programmed cell death 1–ligand 1 (PD-L1) is shown together with the PD-L1 tumor proportion score (TPS) for four patients. Scale bars, 100 μm.

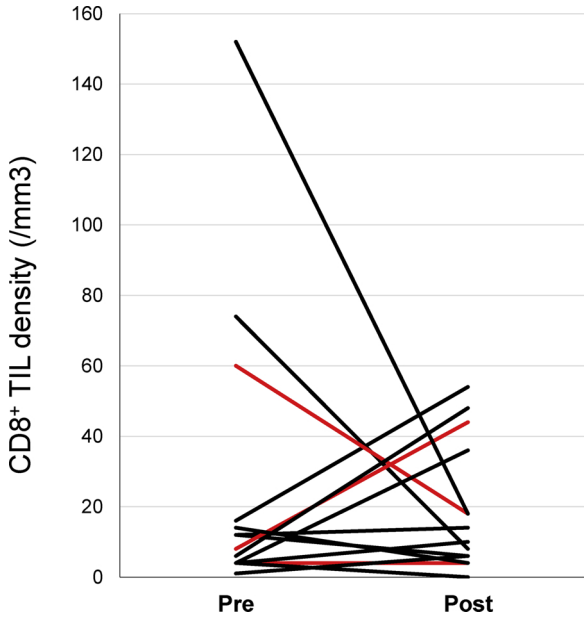


Fig. 3. Changes in intratumoral CD8$^+$ tumor-infiltrating lymphocyte (TIL) density between before (pre) and after (post) cytotoxic chemotherapy. The red lines correspond to three (cases 1, 7, and 8) of the four patients highlighted in Fig. 1A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

whom showed a durable response to PD-1 inhibitor treatment after the PD-L1 TPS had increased from 0 to 90% (case 8) or from 0 to 95% (case 10). Most patients underwent initial and repeat biopsy at the same site, suggesting that phenotypic and functional heterogeneity may arise among cancer cells within the same tumor as a potential consequence of genetic change or environmental differences either induced by chemotherapy or associated with tumor progression.

Nivolumab monotherapy failed to improve PFS compared with standard platinum-based chemotherapy as first-line treatment for NSCLC with a PD-L1 expression level of at least 5% in the CheckMate 026 trial [21]. In contrast, recent phase III trials for NSCLC in the front-line setting (Keynote-189 and IMpower 150) were designed to assess whether the addition of antibodies specific for PD-1 or for PD-L1 to platinum-based chemotherapy improves clinical efficacy in terms of PFS and OS. Given that sensitivity to immunotherapy might change during treatment course, we have focused on the effect of cytotoxic chemotherapy on such sensitivity. Indeed, recent studies showed that chemotherapy, radiation, or chemoradiation increases PD-L1 expression in tumor cells in vitro [12,13] or in clinical samples [14,15]. Consistent with these previous results, we observed a trend toward increased PD-L1 expression in rebiopsy tissue compared with initial tissue samples, although this difference did not achieve statistical significance.

A previous study that examined the impact of neoadjuvant chemotherapy (NAC) on PD-L1 expression in NSCLC patients undergoing tumor resection found that PD-L1 positivity of tumor cells decreased after NAC [22]. The discrepancy in the direction of the change in PD-L1 expression level between this previous and our present study is probably due to differences in patient characteristics, treatment, or timing of PD-L1 evaluation. All patients in our study were wild type for both *EGFR* and *ALK*, whereas 34% of patients in the prior study harbored activating mutations of *EGFR*, with most of these individuals having been treated with EGFR tyrosine kinase inhibitors. A meta-analysis of the association between PD-L1 expression and driver gene mutations in NSCLC patients found that PD-L1 expression in *EGFR* mutation–positive tumors is lower than that in those wild type for *EGFR* [23]. Moreover, the EGFR tyrosine kinase inhibitor erlotinib down-regulated PD-L1 expression in NSCLC cell lines harboring *EGFR* mutations [24]. In the previous NAC study [22], rebiopsy was performed after NAC followed by surgery, with most of the rebiopsy tissue likely being sensitive to chemotherapy, whereas in the present study rebiopsy was performed in patients who had relapsed after surgery followed by adjuvant chemotherapy, with such rebiopsy tissue likely being resistant to chemotherapy. Knockdown of PD-L1 was shown to result in an increased sensitivity to cisplatin in lung cancer cells, with the expression level of PD-L1 being higher in cisplatin-resistant cells [25], consistent with our present results.

Whole-genome sequencing of primary and relapsed tumor specimens from patients with acute myeloid leukemia revealed an increase in the number of transversions, probably due to DNA damage induced by cytotoxic chemotherapy, after relapse [26]. Whole-exome sequencing of gliomas at initial diagnosis and recurrence also revealed that exposure to the alkylating drug temozolomide directly induced a hypermutational state and genomic instability in the tumor cells [27]. In

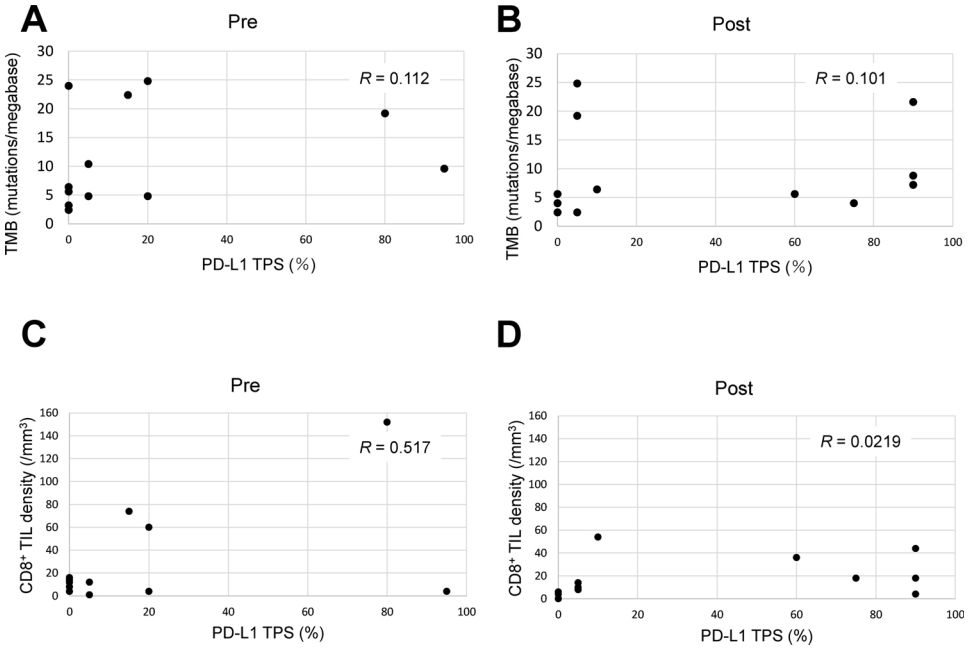
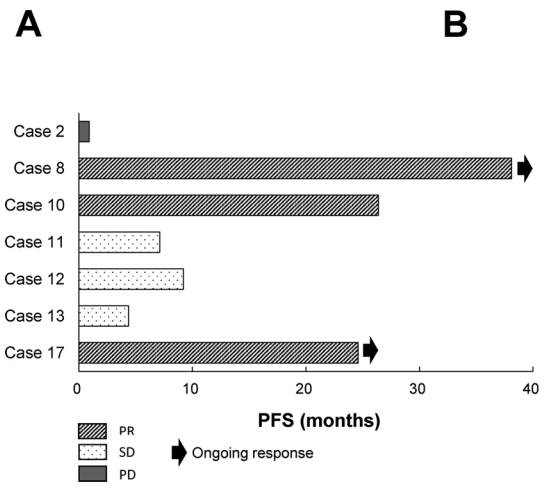


Fig. 4. Relation between PD-L1 tumor proportion score (TPS) and either tumor mutation burden (TMB) or intratumoral CD8$^+$ tumor-infiltrating lymphocyte (TIL) density both before (pre) and after (post) cytotoxic chemotherapy. The scatter plots show TMB and programmed cell death 1–ligand 1 (PD-L1) TPS before (A) and after (B) cytotoxic chemotherapy as well as CD8$^+$ TIL density and PD-L1 TPS before (C) and after (D) cytotoxic chemotherapy. *R* indicates Spearman correlation coefficient.



B

	PD-L1 TPS (%)		TMB (mutations/megabase)	
	Pre	Post	Pre	Post
Case 2	0	0	5.6	5.6
Case 8	0	90	24	21.6
Case 10	0	95	-	-
Case 11	5	0	4.8	2.4
Case 12	0	5	2.4	2.4
Case 13	0	0	-	-
Case 17	-	5	-	-

Fig. 5. Changes in PD-L1 tumor proportion score (TPS) and tumor mutation burden (TMB) between before and after cytotoxic chemotherapy and response to PD-1 inhibitors for patients treated with these drugs after rebiopsy. (A) Swimmer plots for patients treated with programmed cell death–1 (PD-1) inhibitors after rebiopsy. The length of each bar represents progression-free survival (PFS), with the shading pattern of the bar indicating the best response to the PD-1 antibody. PR, partial response; SD, stable disease; PD; progressive disease. (B) Changes in programmed cell death 1–ligand 1 (PD-L1) TPS and TMB for the indicated patients between before (pre) and after (post) cytotoxic chemotherapy.

contrast to these previous studies, we did not detect a significant increase in TMB between initial biopsy samples and rebiopsy tissue obtained after chemotherapy. Although the reason for this discrepancy is not clear, it may be due to differences in tumor type, patient characteristics, or chemotherapy regimens. Consistent with the lack of a significant correlation between PD-L1 expression level and TMB in the CheckMate 026 trial [21], the PD-L1 TPS and TMB showed a negligible correlation in our study. Among the seven patients treated with PD-1 inhibitors in our study, the two patients whose PD-L1 TPS increased greatly after chemotherapy achieved a partial response, whereas four of the remaining five patients whose PD-L1 TPS did not increase or was low after chemotherapy did not achieve such a response (Fig. 5). Given that the PD-L1 TPS was not substantially correlated with either TMB or $CD8^+$ TIL density, PD-L1 expression level may be an important factor in evaluation of changes to the tumor microenvironment after chemotherapy.

With regard to $CD8^+$ TIL density, we found that three patients (cases 6, 7, and 14) showed a marked decrease in this parameter after chemotherapy. A previous study of NAC for NSCLC showed that a lower $CD8^+$ TIL density in surgical specimens was related to NAC resistance [25]. Consistent with this previous observation, cases 6 and 7 in the present study relapsed early after the onset of adjuvant chemotherapy postsurgery. These observations may be indicative of a strong association between chemosensitivity and $CD8^+$ TIL density. The biological characteristics of the primary tumor can be changed after spread to distant sites, given that there are some reports demonstrating the discordance in the PD-L1 expression levels between primary and metastatic tumors [28,29]. In the data of cases in which both first and rebiopsy was performed at intra-lung, there was no specific trend regarding change of PD-L1, TMB, and $CD8^+$TIL density.

Limitations of our study include its retrospective nature and small sample size as well as heterogeneity of the participants' characteristics. Furthermore, we are not able to draw conclusions regarding the impact of changes in PD-L1 expression, TMB, or $CD8^+$ TIL density on survival. A previous study found that PD-L1 expression is not a prognostic marker for patients with advanced NSCLC treated with chemotherapy [30]. However, our results suggest that PD-L1 expression, TMB, and $CD8^+$ TIL density in tumors may change markedly in response to chemotherapy in some patients with NSCLC, with such changes likely affecting sensitivity to subsequent immunotherapy. Further study is warranted to reveal changes in the tumor microenvironment and immune system induced by various treatment regimens, and thereby to provide a basis for the development of more optimal treatment strategies for NSCLC.

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Disclosure

The authors declare no conflicts of interest.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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.2018.11.025.

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