Adjuvant Chemotherapy Increases Programmed Death-Ligand 1 (PD-L1) Expression in Non−small Cell Lung Cancer Recurrence

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Abstract

We sought to determine the effect of chemotherapy on programmed death-ligand 1 (PD-L1) expression in non−small-cell lung cancer and variability upon PD-L1 expression on initial tumor and recurrence. Our results suggest that chemotherapy might increase PD-L1 expression and demonstrate variability from primary tumor to recurrence.

Background: Despite recent studies, the effect of chemotherapy on programmed death-ligand 1 (PD-L1) expression remains controversial. In this study, we investigated whether PD-L1 expression is affected by platinum-based chemotherapy. Furthermore, we evaluated correlation of PD-L1 expression with oncogenic driver alterations. **Materials and Methods:** We retrospectively evaluated changes in PD-L1 expression by immunohistochemical (IHC) analysis in resected specimens and in biopsies at non−small cell lung cancer recurrence in patients receiving or not adjuvant chemotherapy after surgical resection. Four IHC score groups were defined: TC0 $< 1\%$, T $\geq 1\%$ and $< 5\%$, TC2 $\geq 5\%$ and $< 50\%$, and TC3 $\geq 50\%$. **Results:** Thirty-six patients with adenocarcinoma were included. Twenty (56%) patients underwent adjuvant chemotherapy, and 16 (44%) patients did not receive adjuvant chemotherapy. PD-L1 expression was present in 10 (28%) of 36 initial tumor specimens. From patients receiving adjuvant chemotherapy, 7 (35%) of 20 tumor biopsies showed significant upregulation in PD-L1 expression at recurrence. In contrast, from patients with no adjuvant therapy, only 2 (12.5%) of 16 showed a change in PD-L1 expression. Six (17%) of 36 patients were PD-L1-negative in the primary tumor and turned positive at recurrence. KRAS mutation was present in 70% of patients expressing PD-L1. **Conclusion:** PD-L1 expression in non−small cell lung cancer can change from primary to recurrence, implicating the need for re-biopsy at recurrence. Moreover, chemotherapy might increase expression of PD-L1, supporting a combinatorial therapy with chemotherapy and anti-PD(L)1 treatment.

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Introduction

The emerging field in cancer immunotherapy has revolutionized therapy strategy for non−small cell lung cancer (NSCLC); nevertheless, NSCLC remains one of the cancer types with the highest mortality rate worldwide and accounts for almost 80% of all lung cancers.[1,2] Surgical resection remains the key component in operable early stage or locally advanced NSCLC followed or not by adjuvant chemotherapy.[3] As the identification of common driver mutations in NSCLC, such as epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), has already been integrated in clinical guidelines as targeted drug therapy, recent advances in oncologic therapy have led to new therapeutic approaches including immunotherapy targeting checkpoint inhibitors such as programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) inhibitors. PD-1 is a key immune-checkpoint molecule expressed by activated T-cells in order not to overshoot during an

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infection.[4] Interaction of PD-L1 on the tumor cells with PD-1 on T-cells abrogates T-cell function and prevents the immune system from attacking the tumor cells, resulting in tumor cell proliferation and immune escape.[5] However, blocking the interaction between PD-1 and PD-L1 enhances T-cell response and therefore antitumor activity.[6] The PD-1 inhibitor pembrolizumab has been approved as first-line therapy for metastatic NSCLC with PD-L1 expression $\geq$ 50 % of the tumor cells and as second-line therapy in cases expressing $>$ 1 % PD-L1.[7,8] Yet, PD-L1 expression on tumor cells and on immune cells is a dynamic process explaining the variability in PD-L1 expression and difficulty to predict response to checkpoint inhibitors.[9] Patients with NSCLC with PD-L1 expression $<$ 1 % are treated with platinum-based standard chemotherapy as first-line therapy. They covalently bind DNA and crosslink DNA stand, thus inhibiting DNA replication and transcription resulting in apoptosis.[10] In the past, chemotherapies consisting mainly of cytotoxic drugs were thought to induce cell death without a major impact on the immune system. However, recent data describe a more comprehensive mode of action with immunomodulatory effects being part of the response. Platinum-based chemotherapies enhance the immune-stimulatory capacity of dendritic cells, inducing the infiltration of cytotoxic T-cell lymphocytes and the ablation of regulator T-cells and possibly upregulating major histocompatibility complex class I.[11,12] These findings demonstrate that combination therapies might represent a novel therapeutic approach in NSCLC. The design of such combination therapies mandates a deeper understanding of the underlying biological processes. First results of combined standard platinum-based chemotherapy with the PD-1 inhibitor nivolumab have shown encouraging overall survival rates and acceptable tolerability.[13]

Nonetheless, the impact of standard platinum-based chemotherapy on PD-L1 expression in patients with NSCLC remains controversial.[14-17]

The aim of this study was to investigate whether platinum-based chemotherapy might induce PD-L1 expression in the tumor tissue. In addition, we assessed whether genetic driver alterations correlate with PD-L1 expression.

Materials and Methods

Patients

We retrospectively collected and reviewed the medical records of patients with histologically confirmed NSCLC, who underwent surgical resection followed or not by adjuvant chemotherapy and presented NSCLC recurrence. Adjuvant chemotherapy was administrated according to tumor classification, including TNM status (TNM staging system). Specifically, clinical data were collected from 2007 until 2017 at University Hospital Zurich.

For the defined patient cohort, we retrieved available tumor specimens to assess PD-L1 expression in both the initial tumor sample and NSCLC recurrence.

This study was approved by the local ethics committee (EK-ZH-2018-01919) and in accordance with the local laws and regulations.

Evaluation of PD-L1 Expression by IHC

We retrieved 72 pathologically confirmed primary lung cancer specimens from patients who underwent surgery and biopsy at NSCLC recurrence. For every case, all hematoxylin and eosin-stained slides were reviewed for confirmation of diagnosis; one block was then selected for PD-L1 analysis. From each block, specimens were formalin-fixed, paraffin embedded, sectioned into 4-μm sections and stained for PD-L1 with a rabbit monoclonal antibody (clone E1L3N; Cell Signaling Technology, Danvers, MA).[18,19] PD-L1 IHC was performed on all slides using Cell Signaling-E13LN monoclonal antibody. The IHC score was defined as the proportion of tumor cells with stained cell membranes. Migration of IHC group was considered as a significant change upon the PD-L1 expression. In order to evaluate change in the PD-L1 expression, 4 IHC score groups were created as defined in previous studies: TC0 $<$ 1%, TC1 $\geq$ 1% and $<$ 5%, TC2 $\geq$ 5% and $<$ 50%, and TC3 $\geq$ 50%.[20,21] Scoring was performed independently by 2 pathologists (A.S. and D.S.).

Statistical Analyses

Descriptive statistics were performed to evaluate patient and tumor characteristics. Difference in PD-L1 expression in the initial tumor sample and at NSCLC recurrence in the 2 groups (adjuvant and no adjuvant chemotherapy) was evaluated by the Pearson χ^2 test. Statistical analysis was performed with SPSS software, and a P value $< .05$ was considered as statistically significant.

Results

Patient Characteristics

A total of 36 patients were included in our study. All patients presented NSCLC recurrence after surgical resection with or without adjuvant chemotherapy (chemotherapy regimens are shown in Table 1) depending on the initial tumor classification (TNM staging system). Twenty (56%) patients underwent adjuvant chemotherapy after surgical resection. Sixteen (44%) patients had no adjuvant chemotherapy after surgical resection. All patients presented with the histologic type of adenocarcinoma. The median time to recurrence was 516 days for patients receiving adjuvant chemotherapy and 569 days for patients with no adjuvant chemotherapy.

Staging repartition was as follows: IA 7 (19%) patients, IB 5 (14%) patients, IIA 6 (17%) patients, IIB 4 (11%) patients, IIIA 13 (36%) patients, and IVb (oligometastatic) 1 (3%) patient.

The most common localization of the recurrence in both groups was mediastinal lymph nodes, followed by lung and brain. All patient characteristics are described in Table 1.

Changes in PD-L1 Expression in Tumors From Patients With or Without Adjuvant Chemotherapy After Primary Surgical Resection

In the cases undergoing adjuvant chemotherapy, 5 (25%) of 20 initial tumor specimens showed PD-L1 expression ($>$ 1%). After platinum-based chemotherapy, 7 (35%) cases showed upregulation of PD-L1 expression at NSCLC recurrence ($P = .102$) (Figure 1A-D; Table 2). After adjuvant chemotherapy, PD-L1 expression was present in 10 of 20 tumors, corresponding to 50% (Figure 2B).

In cases without adjuvant chemotherapy, initial PD-L1 expression was found in 5 of 16 tumors, corresponding to 31%. In comparison to the group with adjuvant chemotherapy, only 2 (12.5%) of 16 cases showed upregulation in PD-L1 expression at NSCLC recurrence ($P = 1.000$) (Figures 1E,F and 2A).

Table 1 Patient Characteristics

	Adjuvant Chemotherapy (n = 20), n (%)	No Adjuvant Chemotherapy (n = 16), n (%)
Median age, y (range)	67.8 (37-84)	71.3 (59-79)
Gender		
Male	14 (70)	9 (56)
Female	6 (30)	7 (44)
Smoking History		
Yes	20 (100)	13 (81.3)
No	0 (0)	3 (18.7)
Histology		
Adenocarcinoma	20 (100)	16 (100)
Stage		
IA	0 (0)	7 (44)
IB	1 (5)	4 (25)
IIA	4 (20)	2 (12.5)
IIB	3 (15)	1 (6)
IIIA	11 (55)	2 (12.5)
IVb (oligometastatic)	1 (5)	0 (0)
Chemotherapy		
Platinum-pemetrexed		
4 cycles	14 (70)	
3 cycles	2 (10)	
2 cycles	1 (5)	
Platinum-gemcitabine		
4 cycles	1 (5)	
Platinum-vinolrebin		
4 cycles	2 (10)	
Oncogenic Driver Alterations		
EGFR	0 (0)	2 (12.5)
KRAS	6 (30)	4 (25)
ALK	0 (0)	0 (0)
Localizations of Recurrence		
Lymph node	7 (35)	7 (44)
Lung	3 (15)	5 (31)
Brain	7 (35)	2 (12.5)
Other	3 (15)	2 (12.5)
Median time to recurrence, d (range)	516 (34-1215)	569 (146-1656)

Abbreviations: ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor.

Of all 36 cases, 6 patients were PD-L1-negative on initial tumor sample and were positive at NSCLC recurrence, corresponding to 17%.

PD-L1 Expression and Correlation With Oncogenic Driver Alterations

In our study cohort, we assessed the 3 most common oncogenic driver alterations (KRAS, EGFR, and ALK), which also guide therapeutic planning in NSCLC. Of our 36 patients, 10 (28%) patients harbored KRAS mutations, whereas 2 (6%) patients showed an EGFR mutation. None of our cases showed ALK rearrangements. The most common types of KRAS mutations are G12C, G12D, and G12V.[22] Of 10 cases with KRAS mutations in our study cohort, G12C was the most common mutation present in 6 (60%) patients. G12D, G12V, G12A, and G12S were all present in a single case.

Of the 10 patients presenting with KRAS mutation, 7 (70%) patients had PD-L1 expression either on initial tumor or NSCLC recurrence.

Discussion

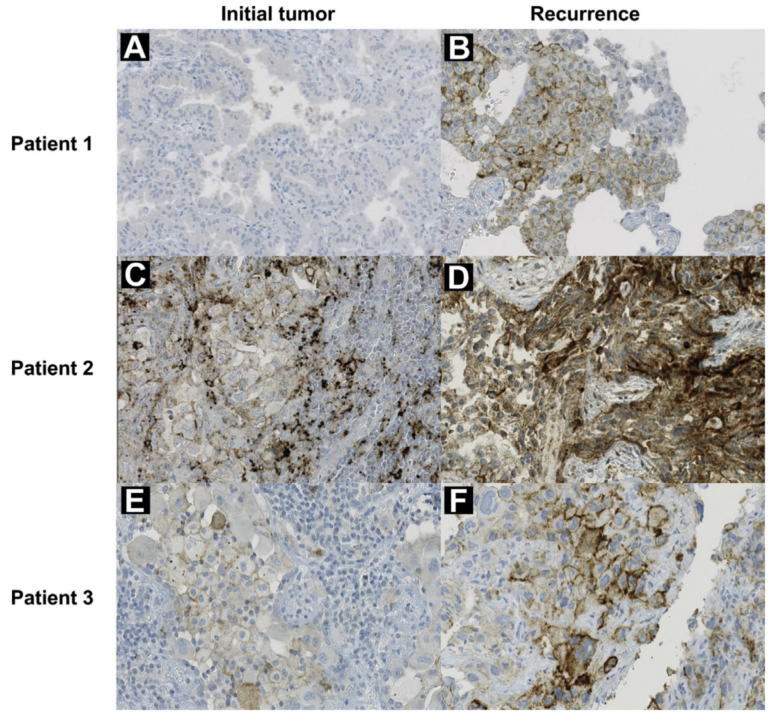
The aim of this study was to determine variation in PD-L1 expression from initial diagnosis to recurrence in NSCLC and its relation to chemotherapy. Recently published retrospective studies also investigating the effect of cytotoxic chemotherapy on PD-L1 expression indeed showed a trend towards higher PD-L1 expression after administration of platinum-based chemotherapy.[15,17] Interestingly, Sheng et al, as well as Rojko et al, reported a decrease in PD-L1 expression after chemotherapy.[14,16] However, the study cohort of Sheng et al included 11 (34.4%) of 32 patients harboring an EGFR mutation, with recent studies demonstrating that PD-L1 expression in EGFR mutated tumor samples are lower than in those of wild type for EGFR.[23] Secondly, chemotherapy regimens in the mentioned study included EGFR-tyrosine kinase inhibitors in approximately 25% of the cases, which have been reported to down-regulate PD-L1 expression in EGFR-mutated cell lines.[24] Furthermore, the study of Rojko et al included samples of not only NSCLC, which may explain the discrepancy in the change in PD-L1 expression compared with our study.

In our study, we demonstrated that platinum-based standard chemotherapy might increase PD-L1 expression in NSCLC. These findings support the use of such chemotherapies in combination with immune checkpoint inhibitors such as pembrolizumab and nivolumab. This new therapeutic approach has been evaluated in the Keynote-021 trial, which demonstrated that the combination of an immune checkpoint inhibitor (pembrolizumab) and a platinum-pemetrexed doublet increases progression-free survival compared with standard chemotherapy alone, even in the PD-L1 low-expression group.[25]

Moreover, in our study cohort, we have seen that PD-L1 expression might represent a dynamic biomarker, which can be expressed in NSCLC recurrences even if the initial tumor was PD-L1-negative (in 17% of our cases). Accordingly, re-biopsy at NSCLC recurrence should always be considered in order to assess the molecular profile and specific resistance mutations, which further guides therapy.

In recent studies, it was demonstrated that PD-L1 expression might correlate with genetic driver alterations.[26] Chen and colleagues reported that KRAS mutations induce upregulation of PD-L1 expression in lung adenocarcinoma.[26] Further, Sumimoto and colleagues showed that KRAS mutation contributes to enhanced PD-L1 expression in human lung cancer.[27] Our results show that 70% of KRAS-mutated patients express PD-L1 either in the initial tumor sample or at recurrence. Thus, our data suggests that blockade of the PD-1/PD-L1 pathway might be a promising therapeutic option for KRAS-mutated NSCLC. Recent clinical trials demonstrated a benefit for immune checkpoint inhibitors in

Figure 1 Changes in Programmed Death-ligand 1 (PD-L1) Expression From Initial Stage to Recurrence in Non–small cell Lung Cancer. Four Immunohistochemistry Score Groups Were Defined: TC0 < 1%, TC1 ≥ 1% and < 5%, TC2 ≥ 5% and < 50%, and TC3 ≥ 50%. Upregulation of PD-L1 Expression From Initial Tumor Sample (=TC0) (A) and Biopsy (=TC3) (B) after Adjuvant Therapy. Increase in PD-L1 Expression From Initial Tumor Sample (=TC1) (C) and Biopsy (=TC3) (D) After Adjuvant Therapy. Change in PD-L1 Expression in the Initial Tumor Sample (=TC2) (E) and Biopsy (=TC3) (F) at Recurrence With No Adjuvant Chemotherapy



patients with KRAS mutations towards standard chemotherapy in second-line treatment.[28] More recently, Jeanson et al showed that for patients with KRAS-mutant NSCLC, the efficacy of immune checkpoint inhibitors is similar to that for patients with other types of NSCLC, yet PD-L1 expression seems to be more relevant for predicting the efficacy of immune checkpoint inhibitors in KRAS-mutant NSCLC.[29]

The major limitations of our study are the small sample size and its retrospective and exploratory nature. Despite considerable efforts, we were only able to retrieve 72 tissue samples that matched initial tumor samples and recurrences. The main reason was the lack of a biopsy at NSCLC recurrence. Secondly, often only fine needle biopsy was performed at tumor recurrence, which yields limited tumor material unsuited for PD-L1 analysis. In addition, harmonization studies regarding PD-L1 analysis on biopsy and resection specimens are still lacking up to this date. Intratumoral heterogeneity of PD-L1 expression in NSCLC has been verified in recent studies, underlining the fact that small tissue samples such as biopsy specimens may cause false-negative results and may not be representative of the entire tumor specimen.[30]

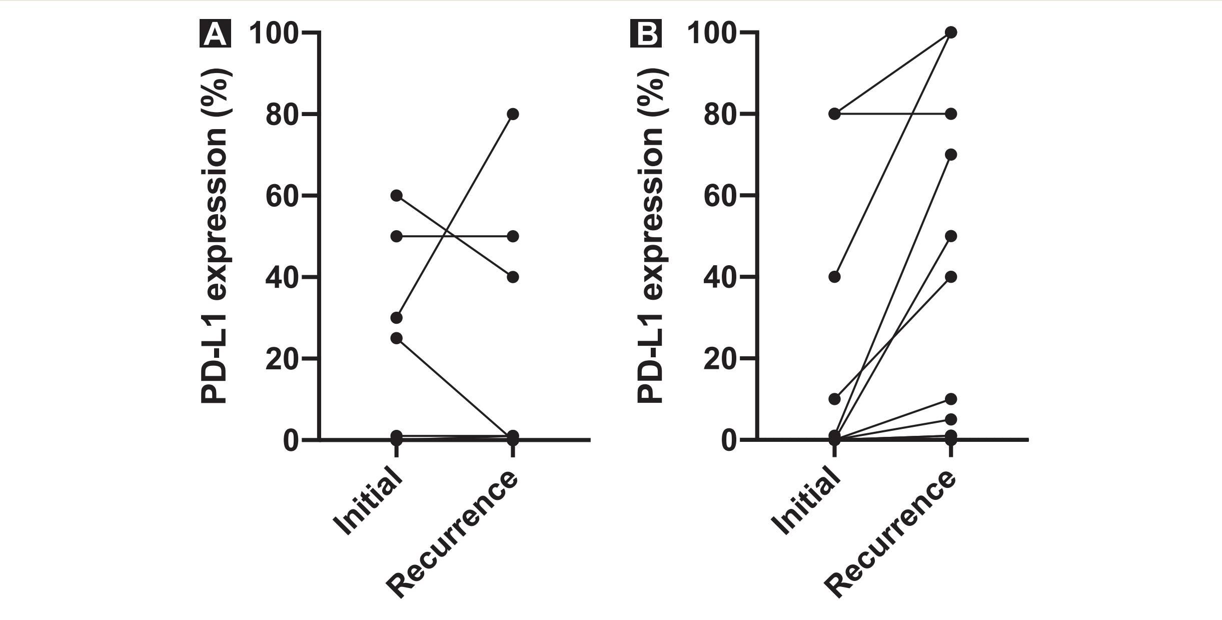
However, despite the small sample size of our study, we were able to create 2 homogenous study groups consisting of all patients with the diagnosis of adenocarcinoma who underwent initial surgical resection followed or not by adjuvant chemotherapy with no previous exposure to radiotherapy. Our study strongly suggests the impact of chemotherapy on PD-L1 expression and the consequences thereon.

Table 2 Upregulation in PD-L1 Expression for the Indicated Cases After Adjuvant Chemotherapy and Correlation With Oncogenic Driver Mutations: PD-L1 Expression (TC Score)

	EGFR	ALK	KRAS	Pre, %	Post, %
Case 1	WT	WT	Mutated (pG12A)	0 (TC0)	50 (TC3)
Case 2	WT	WT	Mutated (pG12C)	0 (TC0)	1 (TC1)
Case 3	WT	WT	Mutated (pG13S)	0 (TC0)	10 (TC2)
Case 4	WT	WT	WT	1 (TC1)	70 (TC3)
Case 5	WT	WT	WT	0 (TC0)	5 (TC2)
Case 6	WT	WT	WT	0 (TC0)	40 (TC2)
Case 7	WT	WT	WT	0 (TC0)	1 (TC1)

Abbreviations: ALK = anaplastic lymphoma kinase; EGFR = epidermal growth factor receptor; PD-L1 = programmed death-ligand 1; WT = wild type.

Figure 2 (A) Changes in PD-L1 Expression From Initial Tumor to Recurrence Without Adjuvant Chemotherapy. (B) Upregulation in PD-L1 Expression From Initial Tumor to Recurrence after Adjuvant Chemotherapy



Abbreviation: PD-L1 = programmed death-ligand 1.

Conclusion

We have demonstrated the variability in PD-L1 expression between primary NSCLC and tumor recurrence and the importance of re-biopsy at tumor relapse.

Chemotherapy might induce PD-L1 expression in NSCLC, which supports the idea that combination therapy of standard chemotherapy with immune checkpoint inhibitors such as PD-1/PD-L1 inhibitors can have good anti-tumoral effect in patients with NSCLC.

Given the small sample size of our preliminary data, future studies with larger number of patients are necessary to confirm our promising data and eventually improve the clinical outcome of patients with NSCLC.

Clinical Practice Points

- Platinum-based chemotherapy might increase PD-L1 expression in NSCLC specimens, supporting the addition of immunotherapy such as PD-1/PD-L1 inhibitors as a new therapeutic strategy for advanced or metastatic NSCLC.
- PD-L1 expression is a dynamic biomarker: even if the initial tumor is PD-L1-negative, the tumor recurrence might be PD-L1-positive, possibly justifying re-biopsy at recurrence.
- More patients than expected might benefit from immunotherapy at relapse.
- KRAS alterations correlate with PD-L1 expression in lung adenocarcinoma; therefore, blockade of the PD-1/PD-L1 pathway by immune checkpoint inhibitors might be a promising therapeutic option for KRAS-mutated NSCLC.

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Disclosure

The authors have stated that they have no conflicts of interest.

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