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ORIGINAL ARTICLE

The effect of PD-L1 status on survival outcomes in patients with stage II-III non-small cell lung cancer treated with chemotherapy

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Summary

Purpose: *There are conflicting results in the literature about the relationship between PD-1/PD-L1 expression and prognosis in non-small cell lung cancer (NSCLC). The purpose of this study was to identify the relationship between NSCLC patients' clinicopathologic characteristics and PD-1/PD-L1 expression.*

Methods: *Pathology specimens of eligible stage II-III NSCLC patients were immunohistochemically stained with PD-1 and PD-L1 antibodies. Patient files and digital records were retrospectively reviewed for demographic and clinical features such as age, gender, smoking status, Eastern Cooperative Oncology Group (ECOG) performance status (PS), histological tumor subtype, applied chemotherapeutic types and their dates and survival data. Statistical analyses were performed to evaluate prognostic effects of staining status of PD-L1 and PD-1 in tumor cells and PD-L1 in tumor infiltrating inflammatory cells.*

Results: *In a total 74 patients, 45.9% of them were positive for PD-L1 in tumor cells, 67.9% positive for PD-L1 in tumor infiltrating inflammatory cells and 83.8% positive for PD-1 in tumor cells (p>0.05). There was a statistically significant relationship between the positive staining of PD-L1 tumor cells and increased overall survival (OS) in univariate analysis (3-year OS; PD-L1(+) 76.6% vs PD-L1(-) 41%, p=0.031). In multivariate analysis only stage and ECOG PS were statistically significant.*

Conclusions: *PD-L1 positivity in tumor cells was a positive prognostic factor for OS in patients with stage II and III NSCLC.*

Key words: *lung cancer, NSCLC, immunotherapy, PD-1, PD-L1*

Introduction

Lung cancer is still the most common and one of the deadliest cancers with the low survival rate [1,2]. Historically, the response rate of standard platin based chemotherapy remains around 20% in non-small cell lung cancer (NSCLC) [3]. There have been improvements on survival with the developments in targeted therapies used in selected subgroups, which were put forward with genetic analysis, such as epidermal growth factor (EGFR), anaplastic lymphoma kinase (ALK) and ROS1 [4].

Understanding of the relationship between immune system and cancer provides new treatment opportunities. Programmed death receptor 1 (PD-1) that is expressed on active T- lymphocytes is a check-point that causes T- lymphocytes to be inhibited when stimulated and as a result downregulates the immune response. PD-1 generates the check-

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point inhibition mechanism in interaction with the ligand such as PD-L1 (CD274) and PD-L2 that are expressed on the surface of tumor cells in many cancer types [5]. In NSCLC, PD-1/PD-L1 pathway forms the basis of immunotherapeutic approaches. Nivolumab, pembrolizumab and atezolizumab are effective immunotherapeutics on PD-1/PD-L1 pathway and therefore results that hold promise are taken on NSCLC treatment [6-9].

There are many studies on the relationship between PD-1/PD-L1 expression that is determined immunohistochemically and prognosis in NSCLC. These studies demonstrate that PD-1/PD-L1 positivity has various effects in that it is good prognostic, bad prognostic or unrelated to prognosis. In one of the studies which includes mostly early stage NSCLC patients, the positive effect of PD-L1 expression on prognosis is identified in squamous carcinoma subgroup [10]. Another study that includes 458 patients in total with two different cohorts of mostly early stage patients shows good prognostic effect of PD-L1 positivity, independent of histology [11].

Another study, which includes 678 patients, positive prognostic effect of high density PD-L1 expression is identified in squamous cell subgroup [12]. In studies with mostly Asian patients, negative prognostic effect of PD-L1 expression on survival is identified especially in adenocarcinoma subgroup [13-16].

Nonetheless; the existing data conflicts with one another in the name of identifying the prognostic significance of the expression status of PD-1/PD-L1 pathway on NSCLC. The identification of PD-1/PD-L1 expression that has predictive biomarker potential on the emerging therapeutics, especially in the field of immunotherapy, will be more necessary when its prognostic significance is put forward. A true understanding of the prognostic value of this axis is also important to design and interpret the studies that search immunotherapeutic effects in the best possible way.

The purpose of this study was to identify the relationship between NSCLC patients' clinicopathologic characteristics and PD-1/PD-L1 expression that was determined with the immunostaining of archived tumor specimen and their overall survival.

Methods

The study was approved from the Local Ethics Committee.

Patients

Pathology specimens at the time of diagnosis were investigated of 225 NSCLC patients, who were diagnosed between the dates of January 2014 and October 2016 at the single center medical oncology outpatient clinic and followed at the same center afterwards. Pathology specimens at the time of diagnosis were identified to contain enough tumor tissue on 138 patients. Patient files and digital records were retrospectively analyzed to find out information on age, gender, smoking status, ECOG performance status, tumor histology, applied chemotherapeutics, chemotherapy start and end dates and responses, final situations and final evaluation dates of these patients, whose clinical information and follow-up periods were appropriate, and, who were diagnosed with stage 2 and stage 3 NSCLC. Pathology specimens of 74 patients, who were appropriate for evaluation, were stained. Pathology specimens at the time of diagnosis were stained with PD-1 and PD-L1 antibodies as immunohistochemically according to the manufacturer's instructions. PD-1 that were expressed on lymphocytes and PD-L1 percentages that were expressed on tumor cells were detected. Pathologists, who analyzed the pathology specimens at the time of staining and evaluation, did not have knowledge of patients' clinical conditions.

Immunohistochemistry

At the time of case selection, areas, which include tumor cells with highest density and tumor tissue with least necrosis, were selected through an analysis of hematoxylin and eosin stained tumor tissue slides of each case. PD-L1 immunohistochemical study were carried out on sections including selected areas.

Prepared slide sections, which were 4 μm thick, were stained with anti-PD-L1 (clone SP263, Ventana) rabbit monoclonal antibody and anti-PD-1 (clone EPR4877, 1: 50 dilutions, Abcam, Cambridge, UK) rabbit monoclonal antibody in a closed staining device of VentanaBench Mark platform (Ventana Medical Systems, Tucson, AZ). Tonsil tissue was used as positive control.

Determination of PD-1 and PD-L1 status

Immunohistochemistry stained slides were evaluated without knowing the clinical characteristics of the case. PD-L1 and PD-1 expression direction were scrutinized considering significant as membranous in tumor cells, cytoplasmic and membranous in inflammatory cell groups that accompany tumor.

Specimens, which showed staining in at least 1% of them immunohistochemically, were accepted as positive in tumor cells and inflammatory cell groups.

Statistics

Overall survival (OS) was defined as the period from patients' diagnosis date to any-reason-death date. Parameters, which were significant on OS in univariate analysis, were evaluated in multivariate analysis. The characteristics of each group were compared with chi square and Mann-Whitney U tests (whichever was more appropriate). For survival analysis, Kaplan-Meier curves and Log-rank test in univariate analysis and Cox regression in multivariate an alysis were used. P value < 0.05 was considered to denote statistical significance. All analyses were performed using SPSS 20.0 software (Armonk, NY: IBM Corp).

Results

In total, 74 patients, of whom sixty-six of them were men (89.2%) and 8 of them were women (10.8%), were included in the study. Patients, who were diagnosed as thirty-three of them in stage II (44.6%) and forty-one of them in stage III (55.6%), 45.9% of the patients (n=34) expressed PD-L1, while 54.1% of them (n=40) were not stained with PD-L1. While the positivity of PD-L1 in tumor infiltrating inflammatory cells were observed in 67.6% of the patients (n=50), it was negative in 32.4% of them (n=24). PD-1 was positive in 83.8% of the patients (n=62), but negative in 16.2% of them (n=12). 41.9% of the patients were diagnosed with adenocarcinoma (n=31) while 51.3% of them were diagnosed with squamous carcinoma and 5 patients were evaluated as NOS (not otherwise specified) (6.8%) histologically. While 57 of the patients had a smoking history (77%), 7 patients had no smoking history (9.5%). When the performance status of the patients was evaluated, 74.3% of the patients were identified as ECOG-0 (n=55) and 25.7% of them as ECOG-1 (n=19).

When PD-L1 positive and PD-1 positive staining of tumor cells and PD-L1 positive staining of tumor infiltrating inflammatory cells and patients' other characteristics were compared, statistically significant relationship was not detected with gender (p=0.719, p=0.115, p=0.709; respectively), smoking status (p=0.296, p=0.579, p=0.675; respectively), clinical stage (p=0.183, p=0.391, p=0.882; respectively), histopathology (adenocarcinoma; p=0.908, p=0.057, p=0.138, respectively) and squamous carcinoma (p=0.496, p=0.172, p=0.511, respectively) and ECOG performance status (p=0.885, p=0.352, p=0.927, respectively) (Table 1).

3-year OS was calculated as 57.7% for all the patients. There was a statistically significant relationship between the positive staining of PD-L1 tumor cells and increased survival in univariate analysis (3-year OS; PD-L1(+) 76.6% vs PD-L1(-) 41%, p=0.031). There was no statistically significant relationship between PD-1 expression and PD-L1 positivity in inflammatory cells on survival (p=0.413 and p=0.099, respectively).

3-year OS was determined as 65.2% on patients whose ECOG performance status was detected to be 0 and as 37.2% on ECOG 1 patients (p=0.037). There was no statistically significant relationship between gender, smoking status, adenocarcinoma histology, squamous carcinoma histology and stage on 3-year OS (p=0.946, p=0.841, p=0.845, p=0.59, p=0.059, respectively) (Table 2).

Table 1. Patient characteristics and staining status of PD-L1 and PD-1 in tumor cells and PD-L1 in tumor infiltrating inflammatory cells

		PD-L1 in Tumor			*PD-L1 in Inflammatory Cells*			*PD-1 in Tumor*		
	n (%)	*Positive n (%)*	*Negative n (%)*	*p*	*Positive n (%)*	*Negative n (%)*	*p*	*Positive n (%)*	*Negative n (%)*	*p*
Total	74 (100)	34 (45.9)	40 (54.1)	-	50 (67.6)	24 (32.4)	-	62 (83.8)	12 (16.2)	-
Gender				0.719			0.709			0.115
Male	66 (89.2)	31 (91.2)	35 (87.5)		45 (90.0)	21 (87.5)		57 (91.9)	9 (75.0)	
Female	8 (10.8)	3 (8.8)	5 (12.5)		5 (10.0)	3 (12.5)		5 (8.1)	3 (25.0)	
Smoking				0.296			0.675			0.579
Yes	57 (77.0)	29 (85.3)	28 (70.0)		40 (80.0)	17 (12.5)		49 (79.0)	8 (66.7)	
No	7 (9.5)	2 (5.9)	5 (12.5)		4 (8.0)	3 (70.8)		5 (8.1)	2 (16.7)	
Unknown	10 (13.5)	3 (8.8)	7 (17.5)		6 (12.0)	4 (16.7)		8 (12.9)	1 (16.7)	
Histology				0.908			0.138			0.057
Adenocarcinoma	31 (41.9)	14 (41.2)	17 (42.5)	0.496	18 (36.0)	13 (54.2)	0.511	23 (37.1)	8 (66.7)	0.172
Squamous carcinoma	38 (51.3)	16 (47.1)	22 (55.0)		27 (54.0)	11 (45.8)		34 (54.8)	4 (33.3)	
NOS	5 (6.8)									
Stage				0.183			0.882			0.391
Stage II	33 (44.6)	18 (52.9)	15 (37.5)		22 (44.0)	11 (45.8)		29 (46.8)	4 (8)	
Stage III	41 (55.4)	16 (47.1)	25 (62.5)		28 (56.0)	13 (54.2)		33 (53.2)	33.3 (66.7)	
ECOG				0.885			0.927			0.352
0	55 (74.3)	25 (73.5)	30 (75.0)		37 (74.0)	18 (75.0)		45 (72.6)	10 (83.3)	
1	19 (25.7)	9 (26.5)	10 (25.0)		13 (26.0)	6 (25.0)		17 (27.4)	2 (16.7)	

In multivariate analysis, significance was detected of stage (HR:3.59, 95% CI: 1.269-10.159; p=0.016) and ECOG performance status (HR: 4,489, 95% CI: 1.689-11.926; p=0.003) on 3-year OS, while there was a trend in the direction of significance on those with PD-L1 positive (HR: 0,405, 95% CI: 0.153, 1.074; p=0.069) (Table 2 and Figure 1).

Discussion

Clinical trials continue on the direction of investigating the effect of immunotherapeutics that target PD-1/PD-L1 axis such as nivolumab, pembrolizumab, atezolizumab and MEDI4736 in neoadjuvan and adjuvan settings in pursuit of

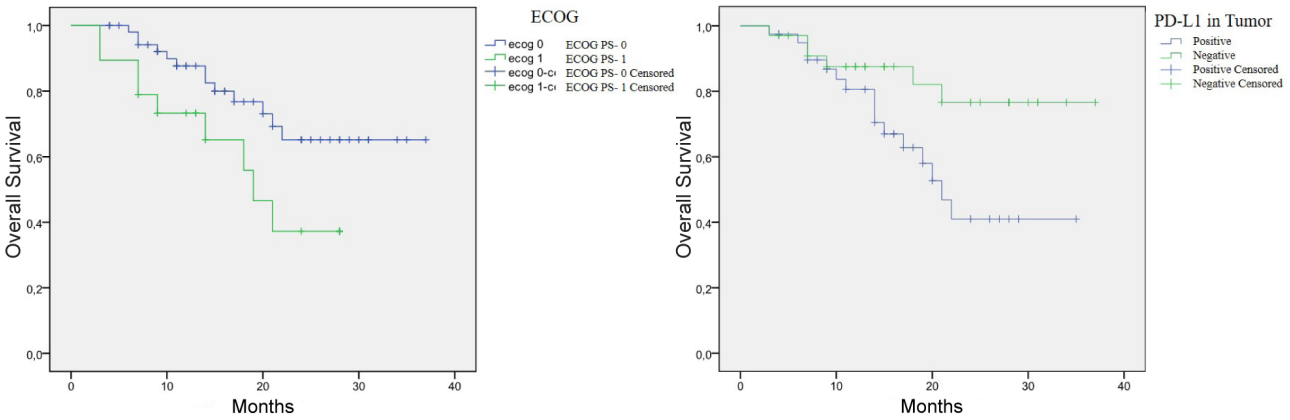

Figure 1. Kaplan-Meier survival according to ECOG performance status (p=0.003) and PD-L1 in tumor (p=0.069) for 3-year OS.

Table 2. Results of univariate and multivariate analysis on 3-year OS

Variables	*Univariate*		*Multivariate*		
	3-year OS %	*p*	*HR*	*95% CI*	*p*
Total	57.7				
Gender		0.946			
Male	59.0				
Female	47.6				
Smoking status		0.841			
Yes	55.6				
No	61.4				
Histology		0.845			
Adenocarcinoma	53.8	0.590			
Squamous carcinoma	55.8				
Stage		0.059	3.590	1.269-10.159	0.016
Stage II	67.1				
Stage III	49.9				
ECOG PS		0.037	4.489	1.689-11.926	0.003
0	65.2				
1	37.2				
PD-L1 in tumor		0.031	0.405	0.153-1.074	0.069
Positive	76.6				
Negative	41.0				
PD-L1 in inflammatory cells		0.099			
Positive	65.4				
Negative	41.5				
PD-1 in tumor		0.413			
Positive	55.4				
Negative	67.9				

shown effect and routinely use of immunotherapy in advanced stage and metastatic NSCLC diseases. (NCT03081689, NCT02818920, NCT02994576, NCT02504372, NCT02273375) The existence of PD-1/PD-L1 axis, which is shown through immunostaining methods, holds a predictive value for check-point inhibitor immunotherapeutics towards this axis [5].

The prognostic effect of immunohistochemically showing the existence of PD-1/PD-L1 axis in tumor specimens on survival and recurrence has been researched in many studies [10-12,27,28]. These studies put forward positive prognostic effects. There are many asserted reasons why there are so many different results on various cohorts. While it might be because patient characteristics such as race, stage, histology are heterogeneous, it might also be because the use of various PD-L1 antibodies as generating discordance is considered to be an effective factor [29]. Different scoring systems and positivity cut-offs may affect the study results.

When it is analyzed on molecular level, showing PD-1/PD-L1 pathway as immunohistochemically-that is its being active- it enables tumor cells to escape from host immunity by downregulating T cells and causes immune tolerance. Therefore, it is expected to be associated with poor prognosis. Interferons, which are known to increase anti-tumoral immune response, make cells express interferon-inducible immune suppressive factors including PD-L1 as compensatory [30]. PD-L1 expression in tumor cells was detected to be interferon-inducible expression rather than a basal value [31]. T-lymphocytes create self-inhibition by secreting cytokines that drive PD-L1 expression [32]. Besides, this phenomenon can be explained to be created to evade intense immune pressure as adaptive mechanism of PD-1/PD-L1 axis by tumor cells and to actually show the strength of host immunity. The emerging of clinical response might be aimed by damaging balance against immune system through check-point inhibitors breaking this adaptive mechanism. In addition, it might also be told that tumor micro environment is shaped like mixed immune cell infiltrate, which is comprised of cytotoxic and regulatory T cells, and that treatments on PD-1/PD-L1 axis will increase the domination of cytotoxic response [10]. Differentiating the emerging axis as adaptive mechanism and as tumor aggressiveness with future studies will increase the use of axis from a predictive perspective.

In this current study, PD-L1 positivity was detected to be a positive prognostic factor on survival on stage II and III patients receiving chemotherapy. In this study, PD-L1 prognostic value was evaluated in a more homogeneous group as patients followed-up in single center with similar treatment and follow-up periods.

PD-L1 expression in fresh tumor samples is a more sensitive prognostic marker than that in archived specimens [33]. In this study, the use of archived specimens in immunohistochemical analysis because of retrospective design is one of the limitations. The other limitations are limited number of patients, retrospective design and single-center attendance.

In conclusion, PD-1/PD-L1 axis should be considered in the design of studies, which are done through check-point inhibitors, especially, this axis, where it might play a role as a prognostic factor all by itself. Particularly, controversies on this topic toward future might be eliminated through randomizing control groups from the point of PD-1/PD-L1 in phase II/III check-point inhibitors studies with high level of participation from many centers.

Conflict of interests

The authors declare no conflict of interests.

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