Lung Cancer 118 (2018) 36–40

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

PD-L1 expression in lung adenocarcinoma harboring *EGFR* mutations or *ALK* rearrangements

Yasuto Yoneshima[a], Kayo Ijichi[a,b], Satoshi Anai[a], Keiichi Ota[a], Kohei Otsubo[a], Eiji Iwama[a], Kentaro Tanaka[a], Yoshinao Oda[b], Yoichi Nakanishi[a,c], Isamu Okamoto[a,*]

[a] Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan
[b] Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan
[c] Center for Clinical and Translational Research, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan

ARTICLE INFO

Keywords:
Programmed cell death-ligand 1 (PD-L1)
Immunohistochemistry (IHC)
Epidermal growth factor receptor (EGFR)
Anaplastic lymphoma kinase (ALK)
22C3 pharmDx

ABSTRACT

Objectives: Expression of programmed cell death–ligand 1 (PD-L1) has been associated with clinical outcome of programmed cell death–1 (PD-1) pathway blockade in non–small cell lung cancer (NSCLC). The PD-L1 IHC 22C3 pharmDx assay, the only companion diagnostic for pembrolizumab therapy, has revealed that ~30% of all NSCLCs express PD-L1 at a high level. The frequency of high PD-L1 expression in NSCLCs with known driver oncogenes has remained unclear, however.

Materials and methods: We retrospectively evaluated PD-L1 expression with the 22C3 assay in tumor tissue of 80 lung adenocarcinoma patients including 71 with *EGFR* mutations and 9 with *ALK* rearrangements, all of whom were treated with corresponding tyrosine kinase inhibitors (TKIs).

Results: Of the 80 tumors analyzed, 26 (32.5%) had a PD-L1 tumor proportion score (TPS) of 1%–49% and 9 (11.3%) had a PD-L1 TPS of ≥50%; 35 (43.8%) thus had a PD-L1 TPS of ≥1%. Of the 71 tumors with *EGFR* mutations, 23 (32.4%) had a PD-L1 TPS of 1%–49% and 7 (9.9%) had a PD-L1 TPS of ≥50%. A PD-L1 TPS of ≥1% was not associated with any clinical characteristic examined. Progression-free survival on initial TKI treatment was significantly poorer for patients with a PD-L1 TPS of ≥1% than for those with a PD-L1 TPS of < 1% ($p = .016$).

Conclusions: A subset of patients with *EGFR* mutations or *ALK* rearrangements had a PD-L1 TPS of ≥50%. Prospective studies are thus warranted to examine the efficacy of PD-1/PD-L1 inhibitors in such patients.

1. Introduction

Management of advanced non–small cell lung cancer (NSCLC) has improved profoundly in recent years as a result of rapid advances in our understanding of its genetic drivers [1]. The presence of epidermal growth factor receptor gene (*EGFR*) mutations, anaplastic lymphoma kinase gene (*ALK*) rearrangements, or *ROS1* rearrangements is predictive of the therapeutic efficacy of corresponding oral tyrosine kinase inhibitors (TKIs), which are associated with more durable outcomes, less toxicity, and a better quality of life compared with conventional chemotherapy [2–4]. Immunotherapy is a new paradigm for the treatment of NSCLC, with immune-checkpoint inhibitors that target programmed cell death–1 (PD-1) or its ligand PD-L1 also conferring a survival benefit for patients with advanced disease when compared with conventional chemotherapy [5–9]. PD-L1 expression in tumor cells has been associated with an improved clinical outcome of PD-1 pathway blockade in NSCLC patients [6,10,11]. Although many antibodies are available for detection of PD-L1 expression by immunohistochemistry (IHC), the PD-L1 IHC 22C3 pharmDx assay is the only approved companion diagnostic for the treatment of NSCLC with the PD-1–targeted monoclonal antibody pembrolizumab.

On the basis of the results of the KEYNOTE-024 phase III trial, a randomized comparison of pembrolizumab versus platinum-based chemotherapy [8], pembrolizumab has become a standard option for first-line treatment of advanced NSCLC with a PD-L1 tumor proportion score (TPS) of at least 50%. Given that patients with *EGFR* mutations or *ALK* rearrangements were excluded from this study, the frequency of a high level of PD-L1 expression in NSCLC harboring these driver oncogenes has remained unclear. We have therefore now determined the PD-L1 TPS with the 22C3 pharmDx assay for lung adenocarcinoma patients with *EGFR* mutations or *ALK* rearrangements.

* Corresponding author.
E-mail address: okamotoi@kokyu.med.kyushu-u.ac.jp (I. Okamoto).

https://doi.org/10.1016/j.lungcan.2018.01.024
Received 2 December 2017; Received in revised form 8 January 2018; Accepted 29 January 2018

2. Materials and methods

2.1. Patients

In this retrospective study, we analyzed specimens from 80 patients with lung adenocarcinoma (71 with *EGFR* mutations, 9 with *ALK* rearrangements) who had been diagnosed and treated with TKIs at Kyushu University Hospital between January 2013 and November 2017. The clinical characteristics, pathological data, and tumor genotype for each patient were extracted by retrospective chart inspection. The progression-free survival (PFS) of patients on initial TKI treatment was also reviewed. This study was approved by the Ethics Committee of Kyushu University.

2.2. Tumor genomic analysis

EGFR mutations were detected with the improved PNA (peptide nucleic acid)–LNA (locked nucleic acid) clamp method, with sequencing of exons 18–21 being performed at a commercial clinical laboratory (LSI Medience, Tokyo, Japan). *ALK* rearrangements were analyzed by IHC and fluorescence in situ hybridization at SRL (Tokyo, Japan).

2.3. Tumor PD-L1 analysis

PD-L1 expression was analyzed at SRL with the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies, Santa Clara, CA, USA). The PD-L1 TPS was calculated as the percentage of at least 100 viable tumor cells with complete or partial membrane staining. The pathologists of the commercial vendor provided the TPS interpretation.

2.4. Statistical analysis

The relation between the PD-L1 TPS in tumor specimens and other patient characteristics was examined with Fisher's exact test. PFS was measured from the onset of initial TKI treatment to the detection of disease progression, and it was evaluated with the Kaplan-Meier method. The log-rank test was applied to compare cumulative survival time between patient groups. Multivariate analysis was performed using a proportional hazards regression model incorporating age, sex, smoking history and PD-L1 expression. All p values shown are two-sided, and those of $< .05$ were considered statistically significant. All statistical analysis was performed with JMP software version 13 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Patient characteristics

The demographics and clinical characteristics of the study participants are shown in Table 1. The median age was 65 years (range, 24–91 years), 47 (58.8%) patients were female, 48 (60.0%) patients were never-smokers, and 63 (78.8%) patients had stage IV disease. Seventy-one patients had activating *EGFR* mutations and nine patients had *ALK* rearrangements. All patients had been treated with EGFR- or ALK-TKIs as appropriate.

3.2. PD-L1 TPS in lung adenocarcinoma with known driver oncogenes

Of the 80 lung adenocarcinoma specimens analyzed, 35 (43.8%) had a PD-L1 TPS of ≥1% (Fig. 1**A**). The PD-L1 TPS for patients with *EGFR* mutations or *ALK* rearrangements was depicted in Fig. 1**B** and **C**, respectively. A PD-L1 TPS of ≥1% was not associated with patient age, sex, smoking history, tumor stage, biopsy site, biopsy type, or *EGFR* or *ALK* status (Supplemental Table 1). Patients with a PD-L1 TPS of ≥1% were then divided into groups with a PD-L1 TPS of 1%–49% (n = 26, 32.5%) or ≥50% (n = 9, 11.3%) (Fig. 1**A**). However, there was still no association between patient clinicopathologic features and PD-L1 TPS stratified as < 1%, 1%–49%, or ≥50% (Table 2).

Table 1
Characteristics of the 80 study patients with lung adenocarcinoma and known oncogenic drivers.

Characteristic	No. of patients (%)
Age (years)	
Median	65
Range	24–91
Sex	
Female	47 (58.8)
Male	33 (41.3)
Smoking history	
Never-smoker	48 (60.0)
Smoker	32 (40.0)
Stage	
IV	63 (78.8)
III	4 (5.0)
Recurrent	13 (16.3)
Biopsy sample origin	
Lung	60 (75.0)
Lymph node	12 (15.0)
Pleura	3 (3.8)
Pleural effusion	2 (2.5)
Liver	1 (1.3)
Brain	1 (1.3)
Bone	1 (1.3)
Biopsy type	
TBLB	31 (38.8)
TBB	5 (6.3)
EBUS-TBNA	12 (15.0)
Small biopsy	18 (22.5)
Surgical resection	12 (15.0)
Effusion cell block	2 (2.5)
Oncogenic driver	
EGFR Ex19del	40 (50.0)
EGFR L858R	30 (37.5)
EGFR G719X and L861Q	1 (1.3)
EML4-ALK	9 (11.3)

TBLB, transbronchial lung biopsy; TBB, transbronchial biopsy; EBUS-TBNA, endobronchial ultrasound–guided transbronchial needle aspiration; Ex19del, exon-19 deletion.

3.3. PD-L1 TPS and outcome for patients treated with TKIs

Our data revealed that a substantial proportion of lung adenocarcinoma patients with *EGFR* mutations or *ALK* rearrangements had a PD-L1 TPS of ≥1%. To examine whether PD-L1 expression might be a determinant of TKI efficacy in patients with these driver oncogenes, we next evaluated PFS for initial treatment of all 80 patients with EGFR- or ALK-TKIs. Median PFS was 9 months (95% confidence interval, 6–13 months) for patients with a PD-L1 TPS of ≥1% and 14 months (95% confidence interval, 12–18 months) for those with a PD-L1 TPS of < 1% (Fig. 2**A**). Patients with a PD-L1 TPS of ≥1% thus had a significantly poorer PFS compared with those with a PD-L1 TPS of < 1% (p = .016). Patients with a PD-L1 TPS of ≥1% were then again divided into groups with a PD-L1 TPS of 1%–49% or ≥50%. Comparison of the three groups of patients stratified according to PD-L1 TPS revealed that PFS tended to decline as PD-L1 TPS increased (Fig. 2**B**). There was no significant difference in type of initial TKI administered between or among patients stratified according to PD-L1 TPS (Supplemental Table 2). When adjusted for age, sex and smoking history in the regression model, PD-L1 expression remained a significant adverse prognostic indicator (hazard ratio = 1.78, 95% confidence interval: 1.08–2.91, p = .03) (Table 3).

4. Discussion

We and others have previously shown that activation of EGFR and