Original Study

Soluble Programmed Cell Death Ligand 1 as a Novel Biomarker for Nivolumab Therapy for Non–Small-cell Lung Cancer

Yusuke Okuma,[1,2] Hiroshi Wakui,[3] Hirofumi Utsumi,[3] Yukiko Sagawa,[1] Yukio Hosomi,[2] Kazuyoshi Kuwano,[3] Sadamu Homma[1]

Abstract

Biomarkers for predicting the effect of anti–programmed cell death 1 monoclonal antibody therapy against non–small-cell lung cancer (NSCLC) are urgently required. We prospectively studied the baseline plasma soluble programmed cell death ligand 1 (sPD-L1) levels from 39 NSCLC patients as a predictive marker of nivolumab therapy. The clinical benefit from nivolumab therapy was significantly associated with the baseline plasma sPD-L1 levels. Plasma sPD-L1 levels could represent a novel predictive marker for nivolumab therapy against NSCLC.

Background: Biomarkers for predicting the effect of anti–programmed cell death 1 (PD-1) monoclonal antibody against non–small-cell lung cancer (NSCLC) are urgently required. Although it is known that the blood levels of soluble programmed cell death ligand 1 (sPD-L1) are elevated in various malignancies, the nature of sPD-L1 has not been thoroughly elucidated. We investigated the significance of plasma sPD-L1 levels as a biomarker for anti–PD-1 monoclonal antibody, nivolumab therapy. **Patients and Methods:** The present prospective study included 39 NSCLC patients. The patients were treated with nivolumab at the dose of 3 mg/kg every 2 weeks, and the effects of nivolumab on NSCLC were assessed according to the change in tumor size, time to treatment failure (TTF), and overall survival (OS). The baseline plasma sPD-L1 concentration was determined using an enzyme-linked immunosorbent assay. **Results:** The area under the curve of the receiver operating characteristic curve was 0.761. The calculated optimal cutoff point for sPD-L1 in the plasma samples was 3.357 ng/mL. Of the 39 patients, 59% with low plasma sPD-L1 levels achieved a complete response or partial response and 25% of those with high plasma sPD-L1 levels did so. In addition, 22% of the patients with low plasma sPD-L1 levels developed progressive disease compared with 75% of those with high plasma sPD-L1 levels. The TTF and OS were significantly longer for those patients with low plasma sPD-L1 levels compared with the TTF and OS for those with high plasma sPD-L1 levels. **Conclusion:** The clinical benefit from nivolumab therapy was significantly associated with the baseline plasma sPD-L1 levels. Plasma sPD-L1 levels might represent a novel biomarker for the prediction of the efficacy of nivolumab therapy against NSCLC.

Clinical Lung Cancer, Vol. 19, No. 5, 410-417
Keywords: Anti-programmed cell death 1 monoclonal antibody therapy, Biomarker, Nivolumab, NSCLC, sPD-L1

Introduction

Although anti-programmed cell death 1 (PD-1) monoclonal antibody (mAb) therapy has been established as one of the standard therapies for non–small-cell lung cancer (NSCLC),[1-4] many unknown factors remain to be resolved concerning the mechanism of anti–PD-1 mAb therapy. PD-1 is expressed on the cell surface of activated cytotoxic T lymphocytes (CTLs) that can recognize certain tumor antigens and acquire tumoricidal activity. Also, when PD-1 binds to programmed cell death ligand 1 (PD-L1) on tumor cells, a suppressive signal will be sent to CTLs, resulting in inactivation of

Y.O. and H.W. contributed equally to this report.

[1]Division of Oncology, Research Center for Medical Sciences, Jikei University School of Medicine, Tokyo, Japan
[2]Department of Thoracic Oncology and Respiratory Medicine, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan
[3]Division of Respiratory Diseases, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan

Submitted: Nov 21, 2017; Revised: Mar 6, 2018; Accepted: Apr 24, 2018; Epub: May 5, 2018

Address for correspondence: Sadamu Homma, MD, PhD, Division of Oncology, Research Center for Medical Sciences, Jikei University School of Medicine, Nishi-Shimbashi 3-25-8, Minato-ku, Tokyo 105-8461, Japan
E-mail contact: sahya@jikei.ac.jp

1525-7304/$ - see frontmatter
https://doi.org/10.1016/j.cllc.2018.04.014

CTLs and immune escape of the tumor cells.[5] Treatment with anti−PD-1 or anti−PD-L1 mAbs inhibits the interaction of PD-1 and PD-L1 and revitalizes the tumoricidal CTLs, providing significant antitumor activity, even to highly advanced malignant tumors.[6,7] Thus, PD-L1 expression in tumor tissue seemed to represent a promising prognostic factor for anti−PD-1 mAb therapy against NSCLC.[8] However, as it became clear that anti−PD-1 mAb therapy might be effective in lung cancer without PD-L1 expression,[9] PD-L1 expression in tumor tissue as a biomarker for anti−PD-1 mAb therapy should be reconsidered.[10,11] Thus, the mechanism of how anti−PD-1 mAb therapy is effective against PD-L1$^-$ lung cancer warrants further investigation. In addition to technical problems of immunohistochemical analysis of PD-L1 expression,[12,13] many unknown aspects in the immune checkpoint blockade therapy, including anti−PD-1 mAb therapy, remain to be determined.[14] Recently, it was suggested that blockade between PD-1 on T cells and PD-L1 on antigen-presenting cells (APCs) could induce antitumor immunity, even if tumor cells have no PD-L1 expression.[15]

Soluble PD-L1 (sPD-L1) has been characterized. It has been demonstrated that the sPD-L1 level in the blood of various malignancies is elevated.[16-21] Many reports have shown that patients with a high blood sPD-L1 level have a poorer prognosis than those with a low level, suggesting that sPD-L1 might be a prognostic factor for various malignant tumors. We have demonstrated that the plasma level of sPD-L1 is a prognostic factor for lung cancer, showing that the patients with a high plasma sPD-L1 level experienced reduced survival.[21] Although Frigola et al[16] reported that the blood sPD-L1 level correlated with the tumor burden in renal cell carcinoma, our study showed that the sPD-L1 level is not associated with the stages of lung cancer.[21] Previous reports have demonstrated the immune suppressive activity of sPD-L1 and suggested that sPD-L1 is involved in immune suppression of tumor-bearing hosts.[16,22] Nevertheless, the biosynthesis and bioactivity of sPD-L1 in patients with malignant tumors have not been thoroughly elucidated to date. Recently, Kruger et al[23] reported that sPD-L1 might reflect the inflammatory activity in pancreatic cancer tissue. Furthermore, it has been reported that the sPD-L1 level could predict the antitumor effect of immune checkpoint blockade therapy in some populations of melanoma patients.[24]

Estimation of PD-L1 expression in tumor tissue is not easy, because the results will differ depending on the anti−PD-L1 mAb used.[25] Furthermore, PD-L1 expression in tumor tissue will be altered by chemotherapeutic drugs.[26] Because the number of NSCLC patients with a favorable response from anti-PD-1 mAb therapy is limited, establishment of reliable biomarkers for the prediction of the effect of anti−PD-1 mAb therapy is urgently required.[27,28] Although the nature of sPD-L1 is not fully understood, the shedding of membranous PD-L1 could possibly be involved.[22] We investigated the association between plasma sPD-L1 levels before anti−PD-1 mAb therapy with nivolumab and the clinical outcomes of nivolumab therapy in NSCLC patients.

Patients and Methods

Patients and Nivolumab Treatment

Blood samples at baseline from patients treated with nivolumab for NSCLC were prospectively obtained. The clinical information was collected from the patients at the Division of Respiratory Diseases, Department of Internal Medicine, Jikei University School of Medicine and Department of Thoracic Oncology and Respiratory Medicine, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital from May 2016 to April 2017 as part of the exploratory investigation. The patients were treated with nivolumab at the dose of 3 mg/kg every 2 weeks until disease progression or unacceptable toxicities in the clinical setting. The effects of nivolumab on NSCLC from each patient was assessed by the physicians and radiologists, according to Response Evaluation Criteria In Solid Tumors, version 1.1.[29] A complete response (CR), partial response (PR), and stable disease (SD) were defined as the clinical benefit received by the patients treated with nivolumab.

The ethics committees of Jikei University School of Medicine and Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital approved the study protocol (approval nos. 28-046 [8289] and 1744) and was conducted in accordance with the Declaration of Helsinki and the REMARK (reporting recommendations for tumour marker prognostic studies) guidelines.[30] The study was registered with University Hospital Medical Information Network Clinical Trials Registry (registry no. UMIN000021734/UMIN000023540).

Patient Data Acquisition

The clinical characteristics of NSCLC patients were obtained from the Registration Form, including age, gender, histologic subtype, clinical stages (Union for International Cancer Control, 8th edition), Eastern Cooperative Oncology Group (ECOG) performance status (PS), previous chemotherapy, cigarette smoking (> 400 by the Brinkman index), history of radiotherapy, oncogenic driver status (epidermal growth factor receptor mutations, anaplastic lymphoma kinase rearrangement, and ROS1), the use of steroids, and the clinical benefits of the tumor response. The time to treatment failure (TTF) was defined as the duration from the first nivolumab therapy to the first clinical evidence of progressive disease (PD), early discontinuation of treatment because of nivolumab toxicity, or death from any cause. In the present analysis, the suspension of nivolumab because of adverse events during the clinical setting was not considered the TTF. Overall survival (OS) was defined as the duration from enrollment to death or lost to follow-up.

Determination of Patients' Plasma sPD-L1 Levels

The plasma samples at baseline were collected from the NSCLC patients who were treated with nivolumab. Blood was collected into tubes containing potassium ethylenediaminetetraacetic acid (5 mL; Terumo Venogect II, Tokyo, Japan) and centrifuged at 1000 rpm at 4°C for 10 minutes within 30 minutes after taking the blood samples. Plasma samples were stored in 1000-μL aliquots at −80°C. The plasma sPD-L1 concentrations were measured using an enzyme-linked immunosorbent assay kit for PD-L1 (PDCD1LG1; Cloud-Clone Corp, Katy, TX) according to the manufacturer's protocol. The minimum detectable concentration of sPD-L1 was 0.117 ng/mL, and the quantitative range was 0.312 to 20 ng/mL. Each sample was analyzed in duplicate.

Plasma samples were not collected from the NSCLC patients at surgery. Accordingly, a comparison between the tumor PD-L1

expression levels and the plasma sPD-L1 levels could not be conducted.

Statistical Analysis

The receiver operating characteristic (ROC) curves, area under the curve of the ROC, sensitivity, specificity, and likelihood ratios were calculated to determine the cutoff levels of sPD-L1 for clinical benefit (no PD [CR/PR/SD] vs. PD) for nivolumab. The level of sPD-L1 was calculated as the original level and numerically converted using a logarithmic conversion. However, the data were not normally distributed by the Kolmogorov-Smirnov test. Therefore, we calculated the median and interquartile range of the sPD-L1 level.

The Mann-Whitney *U* test was performed to determine the difference in the clinical characteristics of the patients stratified by the plasma PD-L1 levels. The association of tumor responses stratified by the Response Evaluation Criteria In Solid Tumors criteria and plasma PD-L1 levels at baseline was determined using the Cochran-Armitage test. Survival curves were plotted using the Kaplan-Meier method, and significant differences were determined using the log-rank test between the low or high level of sPD-L1. A Cox regression model was used to perform multivariate analyses that included all clinicopathologic features as covariates. All tests were 2-sided, and $P < .05$ was considered to indicate statistically significant. All statistical analyses were performed using JMP, version 11.2.1 (SAS Institute, Cary, NC). A biostatistician (M. Saito) reconfirmed these data using R, version 3.4.1.

Results

Clinical Characteristics of Study Population

The clinical characteristics of the 39 patients are summarized in Table 1. Of the 39 patients, 28 (71.8%) had a diagnosis of adenocarcinoma and 7 (17.9%), a diagnosis of squamous cell carcinoma. Of the 39 patients, 15 (38.5%) had ECOG PS of 0 or 1 and 24 (61.5%) had an ECOG of PS $\geq$ 2. For 32 patients (82.1%), nivolumab was given as second-line treatment. Of the 32 patients with nonsquamous cell carcinoma, 5 had EGFR mutations and 1 was positive for ROS-1 rearrangements. Two patients receiving steroids were included. One patient was treated with 4 mg/d of dexamethasone to palliate anorexia and fatigue for 3 days before and 2 days after nivolumab treatment. The second patient was treated with 2 mg/d of betamethasone to palliate anorexia and fatigue for 6 weeks before nivolumab and continuously received the same steroid therapy after nivolumab.

Plasma sPD-L1 Level and Clinical Features of NSCLC Patients Treated With Nivolumab

sPD-L1 was detected in all the plasma samples at a median concentration of 2.24 ng/mL (first quartile, 0.98; third quartile, 4.32). The associations between the plasma sPD-L1 levels and the clinicopathologic features of the patients are summarized in Figure 1 and Table 2. No correlations were noted between the plasma sPD-L1 levels and age, gender, ECOG PS, histologic subtype, driver mutation, clinical stage, smoking history, history of radiotherapy, or the use of steroids. The clinical benefits of achieving a CR, PR, or SD from nivolumab therapy were significantly associated with the plasma sPD-L1 level.

Table 1 Baseline Patient Characteristics (n = 39)

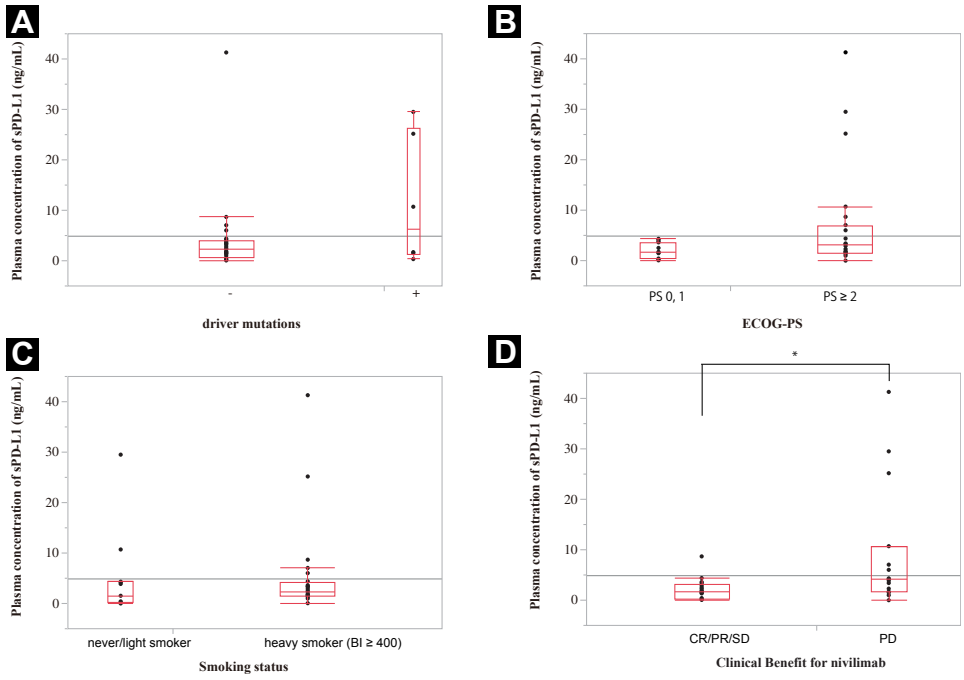
Characteristic	n (%)
Age, y	
Median	69
Range	50-88
Age group	
<75 y	32 (82.1)
≥ 75 y	7 (17.9)
Gender	
Male	29 (74.4)
Female	10 (25.6)
Histologic subtype	
Non–small-cell carcinoma	2 (5.1)
Adenocarcinoma	28 (71.8)
Squamous cell carcinoma	7 (17.9)
Large-cell carcinoma	2 (5.1)
Clinical stage (UICC classification 8th edition)	
IV	19 (48.7)
M1a	12
M1b	3
M1c	4
Recurrence	20 (51.3)
ECOG PS	
0-1	15 (38.5)
≥ 2	24 (61.5)
Chemotherapy line	
2	32 (82.1)
≥ 3	7 (17.9)
Complications, none	10 (25.6)
Cigarette smoking	
No/light (Brinkmann index <400)	11 (28.2)
Heavy smoker	28 (71.8)
History of radiotherapy	
Yes	24 (61.5)
No	15 (38.5)
Genetic status in nonsquamous cell carcinoma	32
EGFR-WT, ALK^- rearrangement	26 (81.3)
EGFR mutation	5 (15.6)
ROS1 rearrangement	1 (3.1)
Use of steroid	
Yes	2 (5.1)
No	37 (94.9)

Abbreviations: ALK = anaplastic lymphoma kinase; ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor; PS = performance status; UICC = Union for International Cancer Control; WT = wild type.

Patients With Low Plasma sPD-L1 Levels Exhibited Favorable Responses to Nivolumab Compared With Those With High Plasma sPD-L1 Levels

Significant associations between the plasma sPD-L1 levels of the NSCLC patients and clinical benefit from nivolumab were observed (Figure 1). The ROC curve analysis was used, and the detected AUC was 0.761. The calculated optimal cutoff points for the

Figure 1 Association Between Plasma Levels of Soluble Programmed Cell Death Ligand 1 (sPD-L1) and Clinicopathologic Characteristics of Non–Small-cell Lung Cancer (NSCLC) Patients Treated With Nivolumab. (A) Driver Mutation Status, (B) Eastern Cooperative Oncology Group (ECOG) Performance Status (PS), (C) Smoking Status, and (D) Tumor Response Stratified by Nivolumab Therapy. The Results Are Shown as the Median, First Quartile, and Third Quartile of Programmed Cell Death 1 Concentrations (*$P < .05$)



Abbreviations: CR = complete response; PR = partial response; SD = stable disease.

sPD-L1 level in plasma samples was 3.357 ng/mL according to the clinical benefit (Youden index, 0.500; Supplemental Figure 1). The sensitivity was 66.7%, and the specificity was 83.3%. The survival analysis revealed that the patients with high sPD-L1 levels had a significantly shorter TTF (5.36 months vs. 1.48 months; $P = .032$) and OS (7.20 months vs. not reached; $P = .040$; than that of those with low sPD-L1 levels [Figure 2]). On multivariate analysis, the level of sPD-L1 was significantly associated with the TTF (Table 3). Even if the definition of a high level of sPD-L1 was set to > 7.32, as in our previous report,[21] the difference in TTF and OS was still statistically significant (TTF, $P = .025$; OS, $P = .031$).

Plasma sPD-L1 Level at Baseline Associated With Clinical Outcome of Nivolumab Therapy for NSCLC

The correlation between the basal plasma level of sPD-L1 and the clinical outcomes of nivolumab therapy for NSCLC was examined. The achievement of CR, PR, or SD was significantly greater for the patients with low plasma sPD-L1 levels than for those with high plasma sPD-L1 levels (Table 4; $P = .0069$, Cochran-Armitage test). In contrast, the rate of PD after nivolumab therapy was significantly greater in patients with high plasma sPD-L1 levels than in those with low plasma sPD-L1 levels. Of the patients with plasma sPD-L1 levels < 3.357 ng/mL, 59% achieved a CR or PR. In contrast, 25% of those with plasma sPD-L1 levels > 3.357 ng/mL achieved a CR or PR. Of the patients with plasma sPD-L1 levels < 3.357 ng/mL, 22% developed PD compared with 75% of those with levels > 3.357 ng/mL.

Discussion

The significance of PD-L1 expression in tumor tissue as a predictive marker for anti-PD-1 mAb therapy is controversial for NSCLC.[25] The results of the present study have demonstrated that the clinical benefits (CR, PR, SD) were achieved more frequently in NSCLC patients with low plasma sPD-L1 levels than in those with high plasma sPD-L1 levels. Of the NSCLC patients with low plasma sPD-L1 levels, 59% exhibited a CR or PR with nivolumab therapy compared with only 25% of those with high plasma sPD-L1 levels. Furthermore, TTF and OS were significantly longer for the patients with low plasma sPD-L1 levels than for those with high plasma sPD-L1 levels. These results suggest that blood sPD-L1 levels could be a promising predictive biomarker for determining the efficacy of nivolumab therapy for NSCLC. However, as we reported previously,[21] sPD-L1 could serve as a prognostic marker for advanced lung cancer. Accordingly, sPD-L1 might be implicated as a sole prognostic marker in nivolumab treatment. We have had several NSCLC patients who showed a rapid and marked decrease in the sPD-L1 level with the tumor responses with nivolumab treatment. In some patients, the sPD-L1 level decreased to 0, although the tumor mass remained. These results suggest that the sPD-L1 level would be reflected by the status of antitumor

Table 2 Associations Between Plasma Concentrations of sPD-L1 and Clinicopathologic Variables in Advanced NSCLC Patients Treated With Nivolumab

Characteristic	Patients, n	Plasma sPD-L1 Level,[a] ng/dL	*P* Value
Age, y			.70
<75	32	2.05 (1.37–4.29)	
≥75	7	2.29 (0.22–4.39)	
Gender			.45
Male	29	2.29 (1.41-4.33)	
Female	10	1.58 (0. 26-5.15)	
ECOG PS			.11
0-1	15	1.64 (0.33-3.62)	
≥ 2	24	3.10 (1.39-6.79)	
Histologic subtype			.60
Nonsquamous NSCLC	32	2.27 (0.56-4.38)	
Squamous cell carcinoma	7	1.67 (1.34-3.87)	
Genetic status of driver mutation			.27
Positive	6	6.21 (1.23-26.3)	
Negative	33	2.24 (0.70-4.02)	
Clinical stage			.58
IV	19	1.87 (0.33-4.34)	
Recurrence	20	2.41 (1.54-4.11)	
Smoking history			.63
No/light (Brinkmann index <400)	11	1.53 (0.22-4.32)	
Heavy smoker	28	2.27 (1.50-4.16)	
History of radiotherapy			.44
Yes	24	2.41 (1.39-4.38)	
No	15	1.75 (0.42-3.62)	
Previous chemotherapy lines			.23
1	15	2.71 (1.38-4.34)	
≥ 2	24	1.67 (0.22-1.75)	
Use of steroids			.28
Yes	2	4.66 (3.29-6.02)	
No	37	1.87 (0.70-4.25)	
Clinical benefit			.0066[b]
CR/PR/SD	24	1.66 (0.244-3.19)	
PD	15	4.19 (1.71-10.71)	

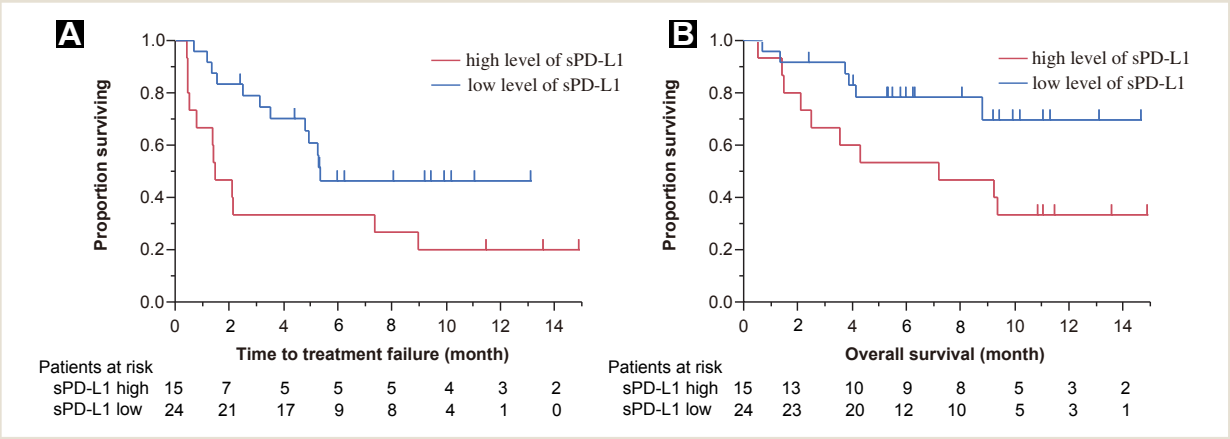
Abbreviations: ALK = anaplastic lymphoma kinase; CR = complete response; ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor; NSCLC = non–small-cell lung cancer; PS = performance status; PD = progressive disease; PR = partial response; SD = stable disease; sPD-L1 = soluble programmed cell death ligand 1.
[a]Data presented as median (first quartile-third quartile).
[b]$P < .05$.

immunity and not merely as a prognostic marker associated with NSCLC patients' responses to nivolumab treatment. Further study in a randomized trial would be helpful to better understand this.

The mechanism of generation and regulation of sPD-L1 and the bioactivity of sPD-L1 affecting antitumor immunity in NSCLC are largely unknown. Frigola et al[16] reported that sPD-L1 might be derived from tumor tissue in renal cell carcinoma because tumor size, advanced tumor stage, and tumor necrosis were associated with patients' blood sPD-L1 levels. The release of sPD-L1 was decreased by treatment of tumor cells with the inhibitor of matrix metalloproteinase in vitro, suggesting that sPD-L1 is generated by shedding of membranous PD-L1 on tumor cells through the proteolytic digestion by activated matrix metalloproteinase (MMP).[22] It was also shown that MMP expression is extremely high in NSCLC.[31] However, the present report, as well as our previous study,[21] showed that the plasma sPD-L1 level was not associated with the stages of NSCLC.

It is also uncertain whether sPD-L1 elicits immunosuppressive activity. According to the previous report, the PD-1 binding domain (IgV)[32] was identified in the sPD-L1 molecule using liquid chromatography/mass spectrometry/mass spectrometry analysis.[16] It was also reported that sPD-L1 could induce apoptosis of activated $CD4^+$ T cells but not $CD8^+$ T cells.[16] Recently, it was reported that PD-L1 was capable of binding to the CD80 molecule.[33] If blood sPD-L1 could bind to CD80 on APCs and prevent the interaction between CD80 on APCs and CD28 on T cells, it would

Figure 2 Kaplan-Meier Curves of Nivolumab-treated Non–Small-cell Lung Cancer Patients With Baseline Soluble Programmed Cell Death Ligand 1 (sPD-L1) Level < 3.357 ng/mL (Blue) or Baseline sPD-L1 Level ≥ 3.357 ng/mL (Red). (A) Time to Treatment Failure and (B) Overall Survival



inhibit the induction of effective antitumor immunity, leading to failure of anti–PD-1 mAb therapy.

The generation of sPD-L1 in tumor-bearing hosts is not that simple and seems to be more complicated. Recently, Kruger et al[23] reported that the blood sPD-L1 levels reflect the inflammatory activity of the tumor tissue in advanced pancreatic cancer. As is well-known, pancreatic cancer is 1 of the representative tumors that generates a potent immunosuppressive tumor microenvironment.[34,35] Accordingly, it is worth investigating whether the inflammatory response in pancreatic cancer tissue is associated with the induction of antitumor immunity. Because matured dendritic cells (DCs), but not immature DCs or T cells, have been reported to produce sPD-L1 in vitro, functional DCs in tumor-bearing hosts might be 1 of the sources of sPD-L1.[36] DCs express high levels of MMP for the migration into the tissue.[37,38] Considerable sPD-L1 could be released by shedding from DCs through the digestion of membranous PD-L1 by MMP expressed in DCs. It is conceivable that the generation of sPD-L1 from DCs is regulated by cytokines released from T cells that are stimulated by DCs. If a high amount of sPD-L1 is released from DCs when immunosuppressive cytokines are released from T cells stimulated with DCs, high blood sPD-L1 levels should indicate impaired antitumor immunity, possibly resulting in a poor prognosis for these patients and resistance to nivolumab therapy. It was reported that interleukin-6 stimulates MMP-9 and MMP-2 expression in human lymphoid cell lines,[39] possibly leading to digestion of membranous PD-L1 and release of sPD-L1. In contrast, if interferon-γ could suppress sPD-L1 production from DCs by inhibition of MMP, low blood sPD-L1 levels could indicate the activation of antitumor immunity. Expression of MMP-9 in macrophages is strongly suppressed by treatment with interferon-γ.[40]

For successful results of immune checkpoint blockade therapy, such as nivolumab therapy, spontaneous induction of T-cell–mediated pre-existing antitumor immunity would be essential.[41,42] The target antigens of pre-existing antitumor immunity are thought to be immunogenic neo-antigens generated by mutation of the genes in tumor cells.[43,44] If immunosuppressive cytokines are induced by priming of T cells with tumor antigens and if sPD-L1 is subsequently released from DCs, induction of pre-existing antitumor immunity would be difficult, resulting in failure of anti–PD-1 mAb therapy.

Table 3 Multivariate Analysis of Clinical Variables Associated With Time to Treatment Failure in Patients With Advanced NSCLC Treated With Nivolumab

Variant	HR	95% CI	*P* Value
Age (<75 vs. ≥ 75)	0.82	0.23-2.36	.73
ECOG PS (good vs. poor)	0.88	0.28-2.58	.82
Driver mutation (positive vs. negative)	1.93	0.56-5.96	.28
sPD-L1 (low vs. high)	0.37	0.13-0.96	.041[a]

Abbreviations: CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; HR = hazard ratio; NSCLC = non–small-cell lung cancer; sPD-L1 = soluble programmed cell death ligand 1; PS = performance status.
[a]$P < .05$.

Table 4 Correlation Between Plasma Level of sPD-L1 at Baseline and Results of Nivolumab Therapy[a]

Plasma sPD-L1 (ng/mL)	CR/PR	SD	PD	Total
<3.357	16 (59)	5 (19)	6 (22)	27 (100)
≥3.357	3 (25)	0 (0)	9 (75)	12 (100)

Data presented as n (%); the patients who were not evaluated using Response Evaluation Criteria In Solid Tumors because of death were classified as having PD.
Abbreviations: CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease; sPD-L1 = soluble programmed cell death ligand 1.
[a]Cochran-Armitage test, $P = .0069$.

Conclusion

Baseline plasma sPD-L1 levels could represent a novel biomarker for the prediction of the efficacy of nivolumab therapy against NSCLC.

Clinical Practice Points

- Because patients showing successful responses to anti−PD-1 mAb therapy have been limited, biomarkers for predicting the effect of anti−PD-1 mAb are urgently required.
- PD-L1 expression in tumor tissue as a predictive marker for anti−PD-1 mAb therapy has not been thoroughly elucidated to date.
- We investigated the significance of baseline plasma sPD-L1 levels as a predictive marker for anti−PD-1 mAb nivolumab therapy.
- We included 39 NSCLC patients in the present prospective study, 28 of whom had adenocarcinoma and 7 of whom had squamous cell carcinoma.
- The patients were treated with nivolumab at a dose of 3 mg/kg every 2 weeks.
- No correlations were observed between the plasma sPD-L1 level and age, gender, ECOG PS, histologic subtype, driver mutation, clinical stage, smoking history, history of radiotherapy, or the use of steroids.
- The clinical benefit achieving CR/PR by nivolumab therapy was significantly associated with the baseline plasma sPD-L1 levels.
- Of the patients with plasma sPD-L1 levels < 3.357 ng/mL, 59% a CR or PR compared with 25% of those with levels > 3.357 ng/mL.
- In contrast, 22% of the patients with plasma sPD-L1 levels $<$ 3.357 ng/mL developed PD compared with 75% of those with levels > 3.357 ng/mL.
- The TTF and OS were significantly longer for the patients with low plasma sPD-L1 levels than for those with high plasma sPD-L1 levels.
- The baseline plasma sPD-L1 levels could represent a novel predictive marker for nivolumab therapy against NSCLC.

Acknowledgments

The authors thank Dr Makoto Saito, Senior Biostatistician, Office for Clinical Research Support, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, for statistical advice, and the American Journal Experts (available at: www.aje.com) for the English language review.

Disclosure

The authors declare that they have no competing interests.

Supplemental Data

The supplemental data accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2018.04.014.

References

1. Giroux Leprieur E, Dumenil C, Julie C, et al. Immunotherapy revolutionises non-small-cell lung cancer therapy: results, perspectives and new challenges. *Eur J Cancer* 2017; 78:16-23.
2. Valecha GK, Vennepureddy A, Ibrahim U, et al. Anti-PD-1/PD-L1 antibodies in non-small cell lung cancer: the era of immunotherapy. *Expert Rev Anticancer Ther* 2017; 17:47-59.
3. Ellis PM, Vella ET, Ung YC. Immune checkpoint inhibitors for patients with advanced non-small-cell lung cancer: a systematic review. *Clin Lung Cancer* 2017; 18:444-59.
4. Khanna P, Blais N, Gaudreau PO, et al. Immunotherapy comes of age in lung cancer. *Clin Lung Cancer* 2017; 18:13-22.
5. Frydenlund N, Mahalingam M. PD-L1 and immune escape—insights from melanoma and other lineage-unrelated malignancies. *Hum Pathol* 2017; 66:13-33.
6. Wang J, Yuan R, Song W, et al. PD-1, PD-L1 (B7-H1) and tumor-site immune modulation therapy: the historical perspective. *J Hematol Oncol* 2017; 10:34.
7. Qin A, Coffey DG, Warren EH, et al. Mechanisms of immune evasion and current status of checkpoint inhibitors in non-small cell lung cancer. *Cancer Med* 2016; 5: 2567-78.
8. Gandini S, Massi D, Mandala M. PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: a systematic review and meta-analysis. *Crit Rev Oncol Hematol* 2016; 100:88-98.
9. Aguiar PN Jr, Santoro IL, Tadokoro H, et al. A pooled analysis of nivolumab for the treatment of advanced non-small-cell lung cancer and the role of PD-L1 as a predictive biomarker. *Immunotherapy* 2016; 8:1011-9.
10. Grigg C, Rizvi NA. PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? *J Immunother Cancer* 2016; 4:48.
11. Chae YK, Pan A, Davis AA, et al. Biomarkers for PD-1/PD-L1 blockade therapy in non-small-cell lung cancer: is PD-L1 expression a good marker for patient selection? *Clin Lung Cancer* 2016; 17:350-61.
12. Wang X, Teng F, Kong L, et al. PD-L1 expression in human cancers and its association with clinical outcomes. *Onco Targets Ther* 2016; 9:5023-39.
13. Ilie M, Hofman V, Dietel M, et al. Assessment of the PD-L1 status by immunohistochemistry: challenges and perspectives for therapeutic strategies in lung cancer patients. *Virchow Arch* 2016; 468:511-25.
14. Pitt JM, Vetizou M, Daillere R, et al. Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors. *Immunity* 2016; 44:1255-69.
15. Kleinovink JW, Marijt KA, Schoonderwoerd MJA, et al. PD-L1 expression on malignant cells is no prerequisite for checkpoint therapy. *Oncoimmunology* 2017; 6: e1294299.
16. Frigola X, Inman BA, Lohse CM, et al. Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma. *Clin Cancer Res* 2011; 17:1915-23.
17. Rossille D, Gressier M, Damotte D, et al. High level of soluble programmed cell death ligand 1 in blood impacts overall survival in aggressive diffuse large B-cell lymphoma: results from a French multicenter clinical trial. *Leukemia* 2014; 28: 2367-75.
18. Wang L, Wang H, Chen H, et al. Serum levels of soluble programmed death ligand 1 predict treatment response and progression free survival in multiple myeloma. *Oncotarget* 2015; 6:41228-36.
19. Finkelmeier F, Canli O, Tal A, et al. High levels of the soluble programmed death-ligand (sPD-L1) identify hepatocellular carcinoma patients with a poor prognosis. *Eur J Cancer* 2016; 59:152-9.
20. Takahashi N, Iwasa S, Sasaki Y, et al. Serum levels of soluble programmed cell death ligand 1 as a prognostic factor on the first-line treatment of metastatic or recurrent gastric cancer. *J Cancer Res Clin Oncol* 2016; 142:1727-38.
21. Okuma Y, Hosomi Y, Nakahara Y, et al. High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer. *Lung Cancer* 2017; 104:1-6.
22. Chen Y, Wang Q, Shi B, et al. Development of a sandwich ELISA for evaluating soluble PD-L1 (CD274) in human sera of different ages as well as supernatants of PD-L1+ cell lines. *Cytokine* 2011; 56:231-8.
23. Kruger S, Legenstein ML, Rosgen V, et al. Serum levels of soluble programmed death protein 1 (sPD-1) and soluble programmed death ligand 1 (sPD-L1) in advanced pancreatic cancer. *Oncoimmunology* 2017; 6:e1310358..
24. Zhou J, Mahoney KM, Giobbie-Hurder A, et al. Soluble PD-L1 as a biomarker in malignant melanoma treated with checkpoint blockade. *Cancer Immunol Res* 2017; 5:480-92.
25. Yu H, Boyle TA, Zhou C, et al. PD-L1 expression in lung cancer. *J Thorac Oncol* 2016; 11:964-75.
26. Peng J, Hamanishi J, Matsumura N, et al. Chemotherapy induces programmed cell death-ligand 1 overexpression via the nuclear factor-kappaB to foster an immunosuppressive tumor microenvironment in ovarian cancer. *Cancer Res* 2015; 75:5034-45.
27. Topalian SL, Taube JM, Anders RA, et al. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* 2016; 16:275-87.
28. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol* 2016; 17:e542-51.
29. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; 45: 228-47.
30. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumour MARKer prognostic studies (REMARK). *Br J Cancer* 2005; 93:387-91.
31. Merchant N, Nagaraju GP, Rajitha B, et al. Matrix metalloproteinases: their functional role in lung cancer. *Carcinogenesis* 2017; 38:766-80.
32. Wang S, Bajorath J, Flies DB, et al. Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. *J Exp Med* 2003; 197:1083-91.

33. Ni X, Song Q, Cassady K, et al. PD-L1 interacts with CD80 to regulate graft-versus-leukemia activity of donor CD8+ T cells. *J Clin Invest* 2017; 127:1960-77.
34. Seo YD, Pillarisetty VG. T-cell programming in pancreatic adenocarcinoma: a review. *Cancer Gene Ther* 2017; 24:106-13.
35. Seton-Rogers S. Pancreatic cancer: dodging immunosuppression. *Nat Rev Cancer* 2016; 16:480-1.
36. Frigola X, Inman BA, Krco CJ, et al. Soluble B7-H1: differences in production between dendritic cells and T cells. *Immunol Lett* 2012; 142:78-82.
37. Yen JH, Khayrullina T, Ganea D. PGE_2-induced metalloproteinase-9 is essential for dendritic cell migration. *Blood* 2008; 111:260-70.
38. Kis-Toth K, Bacskai I, Gogolak P, et al. Monocyte-derived dendritic cell subpopulations use different types of matrix metalloproteinases inhibited by GM6001. *Immunobiology* 2013; 218:1361-9.
39. Kossakowska AE, Edwards DR, Prusinkiewicz C, et al. Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin's lymphomas. *Blood* 1999; 94:2080-9.
40. Shapiro SD, Campbell EJ, Kobayashi DK, et al. Immune modulation of metalloproteinase production in human macrophages: selective pretranslational suppression of interstitial collagenase and stromelysin biosynthesis by interferon-gamma. *J Clin Invest* 1990; 86:1204-10.
41. Pico de Coana Y, Choudhury A, Kiessling R. Checkpoint blockade for cancer therapy: revitalizing a suppressed immune system. *Trends Mol Med* 2015; 21:482-91.
42. Aerts JG, Hegmans JP. Tumor-specific cytotoxic T cells are crucial for efficacy of immunomodulatory antibodies in patients with lung cancer. *Cancer Res* 2013; 73:2381-8.
43. Gubin MM, Zhang X, Schuster H, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* 2014; 515:577-81.
44. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* 2015; 348:69-74.

Supplemental Figure 1 Receiver Operating Characteristic (ROC) Curve Analysis. ROC Curve Analysis Determining Cutoff Point for Plasma Soluble Programmed Cell Death Ligand 1 With Optimal Sensitivity and Specificity in Cohort of 39 Patients With Non–Small-cell Lung Cancer Patients Treated With Nivolumab. The Area Under the ROC Curve Was 0.761 (95% Confidence Interval, 0.595-0.927 [The DeLong Test Was Used As a Predicting Model of ROC])