

Immune Checkpoint Inhibitor for Non-small Cell Lung Cancer With Negative or Low Tumor PD-L1 Expression

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Abstract. *Background/Aim:* We conducted a retrospective analysis of the survival durations of 25 patients diagnosed as having non-squamous cell non-small cell lung cancer with negative or low tumor programmed death-ligand 1 (PD-L1) expression treated with immune checkpoint inhibitor (ICI) monotherapy. *Patients and Methods:* The progression-free (PFS) and overall (OS) survival were calculated from the initiation of ICI monotherapy. The association between the patient characteristics and the PFS was analyzed using Cox proportional hazards model. *Results:* The median PFS was 2.6 months, and the 12-month PFS rate was 9.3%. The median OS was 5.5 months, and the 12-month OS rate was 39.8%. A Cox proportional hazards model identified the neutrophil/lymphocyte ratio and presence of liver metastasis as being significantly associated with PFS. *Conclusion:* Our findings suggest that a subset of patients with non-squamous cell non-small cell lung cancer who show negative or low tumor PD-L1 expression could benefit from ICI monotherapy.

Immune checkpoint inhibitor (ICI) monotherapy has been shown to yield a significantly longer survival period as compared to cytotoxic agents in patients with pre-treated

non-small cell lung cancer (NSCLC) (1, 2). However, in the CheckMate 057 trial, a phase III study conducted in patients with non-squamous cell NSCLC, ICI monotherapy was found to be less effective in patients with tumor programmed death ligand-1 (PD-L1) expression levels of <1% or <10% (hereinafter referred to as negative or low PD-L1 expression, respectively) as compared to patients with higher tumor PD-L1 expression (2). On the other hand, in the same trial, the progression-free survival (PFS) rate at 12 months was higher in patients who had received ICI monotherapy as compared to those who had received treatment with docetaxel (2), suggesting that a subset of patients with non-squamous cell NSCLC with negative or low tumor PD-L1 expression might benefit from ICI monotherapy. In this retrospective study, we attempted to examine the clinical course, after the initiation of ICI monotherapy, of patients with non-squamous cell NSCLC with negative or low tumor PD-L1 expression in clinical settings, and to conduct an exploratory investigation of the association between clinical parameters and PFS after the initiation of ICI monotherapy in these patients.

Patients and Methods

Clinical information. We conducted a retrospective analysis of patient data. The inclusion criteria were as follows: Patients with non-squamous cell NSCLC i) with wild-type epidermal growth factor receptor gene; ii) with non-squamous cell NSCLC with negative or low tumor expression of PD-L1 as evaluated using 22C3 antibody; iii) who had received ICI monotherapy between January 2016 and October 2020 at our Institution.

Clinical information was retrieved from the medical charts. Evaluation of tumor PD-L1 expression was commissioned to a commercial laboratory (BML, Tokyo, Japan), and was evaluated in formalin-fixed and paraffin-embedded tumor tissue specimens by immunohistochemistry using 22C3 antibody. History of radiation therapy within 180 days prior to the initiation of the ICI monotherapy was ascertained. Information on the serum levels of lactate dehydrogenase (LDH) and C-reactive protein (CRP), and the neutrophil/lymphocyte ratio (NLR) at the initiation of ICI

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monotherapy were retrieved from the medical records. Union for International Cancer Control stage (3) was evaluated based on the findings of imaging performed immediately before the start of the ICI monotherapy, and the presence/absence of brain, liver, adrenal, and bone metastasis at the initiation of ICI monotherapy was evaluated.

This study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Ministry of Health, Labour and Welfare, Japan). We disclosed the study information to the patients under the approval of the Ethics Committee, University of Toyama (approval number: R2020067).

Statistical analysis. The Kaplan-Meier method was used to determine the duration of PFS and overall survival (OS) from the initiation of ICI monotherapy. The PFS was calculated from the initiation of ICI monotherapy until the date of diagnosis of disease progression or occurrence of death from any cause, and censored at the last visit at which no disease progression was observed. The OS was calculated from the initiation of ICI monotherapy until occurrence of death, and censored at the last visit of the patient. The median and 95% confidence interval (95% CI) of the survival period and survival rate at 6 and 12 months were estimated. In addition, patients were subdivided according to categorical variables or median levels, and exploratory analysis to determine the association between the clinical background characteristics and the PFS was conducted using the log-rank test. In the analysis using a Cox proportional hazards model, variables that were identified as having an association with a *p*-value of less than 0.10 by univariate analysis were selected as independent variables.

Results

Patient characteristics. Table I shows the patient characteristics. A total of 25 patients were included with non-squamous cell NSCLC; 16 (64.0%) had negative tumor PD-L1 expression and nine (36.0%) had low tumor PD-L1 expression. ICI monotherapy was initiated as second-line or later treatment in 22 (88.0%) patients. Seventeen (68.0%) patients had a Eastern Cooperative Oncology Group performance status (PS) of 0-1. Eight (32.0%) patients had a history of having received radiation therapy within 180 days prior to the initiation of ICI monotherapy.

Survival. Figure 1 shows the Kaplan–Meier curves for PFS and OS after the initiation of ICI monotherapy. The median (95% CI) PFS, 6-month PFS rate, and 12-month PFS rate were 2.6 (1.6-5.8) months, 28.0%, and 9.3%, respectively. The corresponding OS data were 5.5 (4.4-not estimated) months, 49.3%, and 39.8%, respectively.

Association between patient characteristics and PFS. Table II shows the PFS for patients subdivided according to different patient characteristics. The log-rank test identified the PS (*p*<0.001) and presence of liver metastasis (*p*<0.001) as being significantly inversely associated with PFS. In addition, the *p*-values of the association of the NLR and

Table I. Patient characteristics.

| Characteristic | Number (%) | |
|----------------------------|----------------------|------------|
| Age | <70 Years | 7 (28.0%) |
| | ≥70 Years | 18 (72.0%) |
| Gender | Male | 19 (76.0%) |
| | Female | 6 (24.0%) |
| Smoking history | Yes | 20 (80.0%) |
| | No | 5 (20.0%) |
| ECOG PS | 0-1 | 17 (68.0%) |
| | ≥2 | 8 (32.0%) |
| PD-L1 | <1% | 16 (64.0%) |
| | 1-10% | 9 (36.0%) |
| History of RT | Yes | 8 (32.0%) |
| | No | 17 (68.0%) |
| ICI | Nivolumab | 12 (48.0%) |
| | Pembrolizumab | 6 (24.0%) |
| | Atezolizumab | 7 (28.0%) |
| Treatment with ICI therapy | First-line | 3 (12.0%) |
| | Second-line | 13 (52.0%) |
| | Third-line or higher | 9 (36.0%) |
| LDH | <250 U/l | 13 (52.0%) |
| | ≥250 U/l | 12 (48.0%) |
| NLR | <5.0 | 14 (56.0%) |
| | ≥5.0 | 11 (44.0%) |
| CRP | <1.0 mg/l | 13 (52.0%) |
| | ≥1.0 mg/l | 12 (48.0%) |
| UICC Stage | 3A | 1 (4.0%) |
| | 4A | 9 (36.0%) |
| | 4B | 15 (60.0%) |
| Brain metastasis | Yes | 2 (8.0%) |
| | No | 23 (92.0%) |
| Liver metastasis | Yes | 4 (16.0%) |
| | No | 21 (84.0%) |
| Bone metastasis | Yes | 8 (32.0%) |
| | No | 17 (68.0%) |
| Adrenal gland metastasis | Yes | 9 (36.0%) |
| | No | 16 (64.0%) |

CRP: Serum C-reactive protein; ECOG PS: Eastern Cooperative Oncology Group performance status; ICI: immune checkpoint inhibitor; LDH: serum lactate dehydrogenase; NLR: neutrophil/lymphocyte ratio; PD-L1: programmed death 1 ligand; RT: radiation therapy; UICC: Union for International Cancer Control (3).

radiation therapy were less than 0.10 and were therefore also included in the Cox proportional hazards analysis.

Table III shows the results of the analysis using a Cox proportional hazards model. PS, liver metastasis, NLR, and radiation therapy were selected as independent variables. The analysis identified the NLR (*p*=0.025) and liver metastasis (*p*=0.013) as being significantly inversely associated with PFS.

Discussion

The results of the present study showed that ICI monotherapy exerted relatively low efficacy in patients with non-squamous cell NSCLC with negative or low tumor PD-L1 expression;

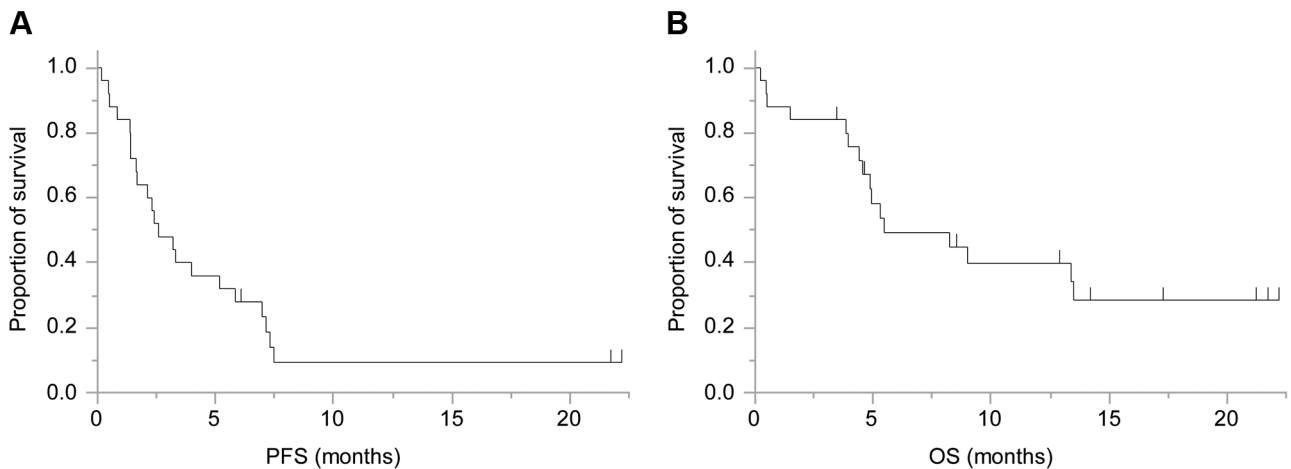


Figure 1. Kaplan–Meier curves for progression-free and overall survival of patients diagnosed as having non-squamous cell non-small cell lung cancer with negative or low tumor programmed death-ligand 1 expression after the initiation of immune check point inhibitor monotherapy. The median (95% confidence interval) progression-free survival was 2.6 months (1.6-5.8) months and overall survival was 5.5 months (4.4-not estimated) months.

the median (95% CI) PFS was only 2.6 (1.6-5.8) months, consistent with the corresponding result reported by the CheckMate 057 trial; median PFS was reported as 2.1 months in patients with PD-L1 expression levels of <1% and <10% (2). On the other hand, in the present study the 6- and 12-month PFS rates were 28.0% and 9.3%, respectively. The CheckMate 057 trial also demonstrated that the 12-month PFS rate was higher in patients with non-squamous cell NSCLC who received ICI monotherapy than in those who received treatment with docetaxel.

Furthermore, combination therapies, including ICI, are expected to improve the prognosis of patients with NSCLC with negative or low tumor PD-L1 expression. In the KEYNOTE 189 trial, pembrolizumab plus cytotoxic agents were associated with a higher survival rate across all PD-L1 categories as compared to cytotoxic agents (4). CheckMate 9LA trial also showed the survival benefit of combination therapy with nivolumab plus ipilimumab combined with two cycles of cytotoxic agents, regardless of PD-L1 expression (5).

The serum levels of LDH and CRP, and the NLR have been shown to be associated with the survival period in patients with NSCLC initiated on treatment with ICIs (6-8). The present study suggests that the NLR might serve as a predictor of PFS in patients with non-squamous cell NSCLC with negative or low tumor PD-L1 expression; on the other hand, serum LDH and CRP showed no association with PFS. These parameters might not be associated with the survival period in patients with non-squamous cell NSCLC with negative or low tumor PD-L1 expression. However, it is possible that the association was not detected due to the small sample size and lack of statistical power of the analysis.

The association of the PFS with the presence of liver metastasis, and the PFS after the initiation of ICI monotherapy were identified. The presence of liver metastasis was identified as a poor prognostic factor in patients with stage IIIB-IV NSCLC who were treated by chemotherapy or palliative therapy (9). Furthermore, the presence of liver metastasis has been reported to be associated with a shorter PFS in patients with NSCLC treated with ICI monotherapy (10), and ICI monotherapy has been shown to be less effective against metastatic lesions in the liver than against metastatic lesions in other organs in patients with malignant melanoma (11). The liver is a hypervascular organ and shows increased production of vascular endothelial growth factor (VEGF) (12). VEGF inhibits dendritic cell maturation and immune cell migration, and suppresses immunity (12). It has also been reported that tumor VEGF expression was a predictor of liver metastasis in patients with pancreatic cancer (13), and colorectal cancer (14). Although the precise underlying mechanism remains unclear, liver metastasis might be associated with resistance to immune therapy through VEGF overexpression.

The present study included only 25 patients, and the small sample size was a major limitation of the study. Random error may have influenced the analysis and confounding factors might not have been fully adjusted for, even though multivariate analysis was performed for analyzing the association between PFS and patient characteristics.

In conclusion, the present study suggested that a subset of patients with non-squamous cell NSCLC with negative or low tumor PD-L1 expression may benefit from ICI monotherapy, and patient variables such as the NLR and liver metastasis may be associated with PFS in this population.

Table II. Association between patient background characteristics and progression free survival after the initiation of treatment with immune checkpoint inhibitor (ICI) (log-rank test).

| Factor | Median PFS (95% CI) | p-Value |
|----------------------------------|---------------------|------------------|
| Age | | |
| <70 Years | 3.2 (0.8-7.3) | 0.560 |
| ≥70 Years | 2.4 (1.4-5.2) | |
| Gender | | |
| Male | 3.2 (1.4-5.8) | 0.694 |
| Female | 2.0 (0.5-7.5) | |
| Smoking history | | |
| Yes | 2.9 (1.4-7.0) | 0.618 |
| No | 1.7 (0.5-7.5) | |
| ECOG PS | | |
| 0-1 | 5.2 (1.7-7.1) | <0.001 |
| ≥2 | 1.5 (0.2-2.4) | |
| PD-L1 | | |
| <1% | 2.9 (1.4-5.8) | 0.882 |
| 1-10% | 2.4 (0.2-7.3) | |
| History of RT | | |
| Yes | 5.5 (2.6-7.5) | 0.084 |
| No | 1.7 (0.8-5.2) | |
| ICI | | |
| Nivolumab | 1.7 (0.5-7.0) | 0.405 |
| Pembrolizumab | 3.8 (1.4-NE) | |
| Atezolizumab | 3.3 (2.1-7.3) | |
| Treatment line of ICI therapy | | |
| 1 st /2 nd | 2.9 (1.4-7.0) | 0.579 |
| ≥3 rd | 2.3 (0.5-7.5) | |
| LDH | | |
| <250 U/l | 5.2 (1.4-7.1) | 0.106 |
| ≥250 U/l | 2.2 (0.5-4.0) | |
| NLR | | |
| <5.0 | 3.3 (1.4-7.5) | 0.088 |
| ≥5.0 | 1.7 (0.5-5.2) | |
| CRP | | |
| <1.0 mg/l | 3.3 (1.4-7.1) | 0.309 |
| ≥1.0 mg/l | 2.5 (0.5-7.0) | |
| UICC Stage | | |
| 3/4A | 4.6 (1.4-7.1) | 0.335 |
| 4B | 1.7 (0.5-3.3) | |
| Brain metastasis | | |
| Yes | 3.9 (0.8-7.0) | 0.710 |
| No | 2.6 (1.6-5.2) | |
| Liver metastasis | | |
| Yes | 0.7 (0.2-1.4) | <0.001 |
| No | 3.3 (2.1-7.0) | |
| Bone metastasis | | |
| Yes | 2.1 (0.5-3.3) | 0.172 |
| No | 4.0 (1.4-7.1) | |
| Adrenal gland metastasis | | |
| Yes | 2.6 (0.2-7.0) | 0.539 |
| No | 2.9 (1.6-5.8) | |

CRP: Serum C-reactive protein; ECOG PS: Eastern Cooperative Oncology Group performance status; LDH: serum lactate dehydrogenase; NLR: neutrophil/lymphocyte ratio; PD-L1: programmed death 1 ligand; RT: radiation therapy; UICC: Union for International Cancer Control (3). Statistically significant p-values are shown in bold.

Table III. Association between patient background characteristics and progression free survival after the initiation of treatment with immune checkpoint inhibitor (Cox proportional hazards model).

| Factor | HR (95% CI) | p-Value |
|------------------|------------------|--------------|
| ECOG PS | | |
| 0-1 | 0.33 (0.08-1.34) | 0.122 |
| ≥2 | 1.00 | |
| History of RT | | |
| Yes | 0.48 (0.17-1.36) | 0.168 |
| No | 1.00 | |
| NLR | | |
| <5 | 0.33 (0.12-0.87) | 0.025 |
| ≥5 | 1.00 | |
| Liver metastasis | | |
| No | 0.10 (0.02-0.61) | 0.013 |
| Yes | 1.00 | |

NLR: Neutrophil/lymphocyte ratio; ECOG PS: Eastern Cooperative Oncology Group performance status; RT: radiation therapy. Statistically significant p-values are shown in bold.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization: MI; data collection, MI, NK, IM, KA, KH, Kotaro Tokui, CT, SO, KK, SI, TM, and RH; original draft preparation: MI; review and editing: NT, IM, KA, KH, Kotaro Tokui, CT, SO, KK, SI, TM, RH, and SM; supervision, Kazuyuki Tobe.

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