

# Peripheral Blood Eosinophil Count Optimizes Pembrolizumab-Based Immunotherapy in The First-Line Setting of Advanced or Recurrent Non-Small Cell Lung Cancer

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## Research Article

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# Abstract

For cancer immunotherapy, the tumor proportion score for the programmed death-1 ligand is not a robust biomarker. The peripheral blood eosinophil count (PEC) is a potential alternative. However, it is not yet established. To test the efficacy of PEC-guided selection of pembrolizumab monotherapy (MONO) or pembrolizumab plus chemotherapy (COMBO), we retrospectively reviewed data of patients with advanced or recurrent non-small cell lung cancer in the first-line setting (April 2017 to April 2020). Among 137 patients enrolled, Kaplan–Meier analysis revealed no significant difference between the MONO ( $n = 84$ ) and COMBO ( $n = 53$ ) therapies. The influence of PEC before the second administration of each regimen (PEC<sub>2</sub>) was evaluated. The low PEC<sub>2</sub> subgroup ( $<150 \times 10^6/L$ ) receiving MONO had poorer survival rates than those receiving COMBO (median progression-free survival [mPFS]: 5.75 vs. 7.59 months and median overall survival [mOS]: 12.0 months vs. not reached [NR], respectively). In patients receiving MONO, the low PEC<sub>2</sub> showed worse prognosis compared with the high PEC<sub>2</sub> group (mPFS: 5.75 vs. 16.1 months and mOS: 12.0 months vs. NR, respectively). PEC<sub>2</sub> can be a potential predictive/prognostic biomarker for MONO, which encourages the switch from MONO to COMBO to avoid treatment failure.

## Introduction

Lung cancer is the leading cause of cancer deaths worldwide. Despite the introduction of new anticancer therapeutics, such as molecular targeted agents for driver gene-mutated non-small cell lung cancer (NSCLC) and immune checkpoint inhibitors (ICIs) including pembrolizumab and nivolumab, the prognosis of patients with advanced-stage NSCLC is poor. Pembrolizumab, which is an inhibitor of programmed death 1 (PD-1) that interrupts the interaction with its ligand, PD-L1, has been evaluated for both monotherapy and combination therapy with platinum-doublet chemotherapy for the first-line treatment of metastatic NSCLC [1–6]. Some patients exhibit a significant survival benefit from monotherapy or combination therapy using pembrolizumab; however, the PD-L1 tumor proportion score (TPS) is not always an accurate predictive/prognostic biomarker. Therefore, the methodology needed to identify the ICI-sensitive subpopulation has not been established. The appropriate biomarkers that guide the ICI treatment should reflect the anticancer immunity of each patient at the precise moment of testing with a simple sampling procedure at a low economical cost. Recent retrospective studies have indicated the potential use of peripheral white blood cell count as a predictive marker for ICIs, in particular, the peripheral blood eosinophil count (PEC) [7–9]. A PEC  $\geq 150 \times 10^6/L$  before the administration of nivolumab was associated with improved progression-free survival (PFS) and overall survival (OS) in advanced or recurrent NSCLC [7]. However, baseline PEC showed no significant association with the treatment response or survival to anti-PD-1 antibodies (nivolumab and pembrolizumab) [8]. Other groups found that baseline PEC was not statistically associated with the treatment response; however, a significant elevation of PEC after three cycles of pembrolizumab or four cycles of nivolumab was observed in patients who had complete or partial responses based on the Response Criteria for Solid Tumours version 1.1 [9]. PEC varies in healthy people and is affected by many factors including age, sex,

smoking, allergy, comorbidities, and medication [10]. Therefore, further studies are needed to determine the value of PEC as a practical biomarker for immunotherapy in NSCLC, especially for the establishment of PEC-guided optimization of the first-line therapy using pembrolizumab in advanced and recurrent NSCLC. We verified the appropriate timing to evaluate PEC as a practical biomarker to avoid treatment failure and established three clinical questions that should be answered to determine the usefulness of PEC as a biomarker: 1) Is PEC useful for selecting pembrolizumab monotherapy (MONO) versus pembrolizumab plus chemotherapy (COMBO); 2) Can PEC predict the subgroup that receives a survival benefit from MONO; and 3) Can PEC predict the subgroup that receives a survival benefit from COMBO?

We performed a retrospective study to evaluate the clinical utility of PEC to identify pembrolizumab-sensitive patients with advanced-stage NSCLC. We also determined whether PEC could guide the decision to choose MONO or COMBO in the first-line setting. We focused on these specific patients to establish a practical application of PEC as a biomarker for the pembrolizumab-based first-line therapy.

## Results

**Patient Characteristics:** A total of 137 patients with advanced or recurrent NSCLC who received MONO or COMBO as their first-line therapy were analyzed. The NSCLC staging was based on the eighth edition of the TNM classification for lung cancer from the International Association for the Study of Lung Cancer. None of the patients was known to have any treatable driver oncogenes, such as epidermal growth factor receptor gene mutations or anaplastic lymphoma kinase gene rearrangements. Among the included patients, 84 were treated with MONO and 53 were treated with COMBO (Table 1). The median follow-up was 374 days (range; 6 to 1,220) for the MONO group and 394 days (range; 78 to 689) for the COMBO group. Patient characteristics, including smoking status, allergy history, histology, and brain metastases, were similar between the two groups. A history of allergy including asthma, pollinosis, food, drug, and contrast agents was observed in 44 patients (32.1%) in the whole population. Compared with the group treated with COMBO, patients in the MONO group were older (median: 73 vs. 69 years,  $p < 0.001$ ) and had a poorer Eastern Cooperative Oncology Group (ECOG) performance status ( $p < 0.001$ ). PD-L1 TPS, which was evaluated using the 22C3 pharmDx assay (Dako), was significantly higher in the MONO group compared with the COMBO group ( $p < 0.001$ ). The patients with PD-L1 TPS  $\geq 50\%$  accounted for 84.7% in the MONO group and 17.0% in the COMBO group. However, because PD-L1 TPS was not always measured in the COMBO group, 12 patients (22.6%) had an unknown status. Compared to the COMBO group, prior anticancer therapies, such as surgery and radiation, were administered more frequently in the MONO group ( $p = 0.010$ ). Of note, 23 patients (27.4%) receiving MONO had undergone surgery. Discontinuation of the first-line therapy because of disease progression, AEs, or patient death occurred in 55 of 84 (65.5%) patients in the MONO group and 34 of 53 (64.2%) in the COMBO group ( $p = 1.000$ ). The second-line treatment after discontinuation of the first-line therapy was administered to 27 of 84 patients (32.1%) in the MONO group and 20 of 53 patients (37.7%) in the COMBO group, with no statistical difference. The frequency of adverse events (AEs), including immune-related AEs and interstitial lung pneumonitis, was not significantly different between the two groups. There was also no association

between the frequency of AEs and PEC before the first or second administration of each therapy (data not shown).

Table 1  
Patient characteristics

	Overall ( <i>n</i> = 137)	Pembrolizumab monotherapy group ( <i>n</i> = 84)	Pembrolizumab plus chemotherapy group ( <i>n</i> = 53)	<i>p</i> -value
Age—median (range)	70 (41– 88)	73 (46–88)	69 (41–76)	<0.001
Gender—male no. (%)	102 (74.5)	64 (76.2)	38 (71.7)	0.554
ECOG performance status score—no. (%)				
0	23 (16.8)	11 (13.1)	12 (22.6)	<0.001
1	82 (59.9)	42 (50.0)	40 (75.5)	
2	23 (16.8)	23 (27.4)	0 (0)	
3 or 4	9 (6.5)	8 (9.5)	1 (1.9)	
Smoking status—no. (%)				
Current	42 (30.7)	23 (27.4)	19 (35.8)	0.749
Former	80 (58.4)	51 (60.7)	29 (54.7)	
Never	14 (10.2)	9 (10.7)	5 (9.4)	
Unknown	1 (0.7)	1 (1.2)	0 (0)	
Allergic history—no. (%)	44 (32.1)	26 (31.0)	18 (34.0)	0.712
Histology—no. (%)				
Squamous	45 (32.8)	27 (32.1)	18 (34.0)	0.853
Nonsquamous	92 (67.2)	57 (67.9)	35 (66.0)	
Brain metastases—no. (%)	19 (13.9)	11 (13.1)	8 (15.1)	0.802

	Overall ( <i>n</i> = 137)	Pembrolizumab monotherapy group ( <i>n</i> = 84)	Pembrolizumab plus chemotherapy group ( <i>n</i> = 53)	<i>p</i> -value
PD-L1 TPS—no. (%)				
<1%	14 (10.2)	0 (0)	14 (26.4)	<0.001
1–49%	31 (22.6)	13 (15.3)	18 (34.0)	
≥50%	80 (58.4)	71 (84.7)	9 (17.0)	
Unknown	12 (8.8)	0 (0)	12 (22.6)	
Prior anticancer therapy—no. (%)				
None	96 (70.0)	51 (60.7)	45 (84.9)	0.010
Chemoradiation therapy	9 (6.6)	6 (7.1)	3 (5.7)	
Surgery	28 (20.5)	23 (27.4)	5 (9.4)	
Radiation	4 (2.9)	4 (4.8)	0 (0)	
Adverse events—no. (%)	32 (23.4)	20 (23.8)	12 (22.6)	1.000
Discontinuation due to disease progression, adverse events, or patient death—no. (%)	89 (65.0)	55 (65.5)	34 (64.2)	1.000
Administration of second-line treatment after discontinuation of first- line therapy—no. (%)	47 (34.3)	27 (32.1)	20 (37.7)	0.580
Peripheral eosinophil count—median (range) ( $\times 10^6/L$ )				
Eosinophil count before first administration of the treatment	160 (0– 2,840)	155 (0–2,840)	169 (20–690)	0.086
Eosinophil count before second administration of the treatment	150 (0– 1,590)	185 (0–1,590)	90 (0–670)	0.002

Absolute PECs were assessed on the nearest day before the administration of each anticancer therapy. In the whole population, the median PEC was  $160 \times 10^6/L$  (range 0–2,840  $\times 10^6/L$ ) before the first

administration of MONO or COMBO and  $150 \times 10^6/L$  (range  $0-1,590 \times 10^6/L$ ) before the second administration. Therefore, we set  $150 \times 10^6/L$  as the cut-off to distinguish high and low PEC for further analyses. PEC before the first administration of therapy ( $PEC_1$ ) was  $155 \times 10^6/L$  (range  $0-2,840 \times 10^6/L$ ) in the MONO group and  $169 \times 10^6/L$  ( $20-690 \times 10^6/L$ ) in the COMBO group ( $p = 0.086$ ). Interestingly, PEC before the second administration ( $PEC_2$ ) was significantly higher in the MONO group (median:  $185 \times 10^6/L$  [ $0-1,590$ ]), compared with the COMBO group ( $90 \times 10^6/L$  [ $0-670$ ]) ( $p = 0.002$ ). The differences between  $PEC_1$  and  $PEC_2$  were determined in the two groups. Elevated, stable, or decreased PEC was observed in 41, 4, and 39 patients in the MONO group ( $p = 0.307$ ) and in 12, 4, and 37 patients ( $p = 0.006$ ) in the COMBO group, respectively. In other words,  $PEC_2$  in the COMBO group was significantly decreased compared with  $PEC_1$  ( $p = 0.002$ ).

**Analysis of Survival and Treatment Response in the Whole Population:** To test the efficacy of PEC as a biomarker for selecting MONO or COMBO treatment, we checked the survival status and treatment response in this dataset. Evaluation of the overall response rate (ORR) was based on the Response Evaluation Criteria in Solid Tumors, version 1.1: complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), or not evaluated (NE).

The Kaplan–Meier analysis revealed no significant differences in PFS (median: 10.8 months [95% confidence interval (CI): 6.44–16.1] vs. 8.41 months [5.95–19.6], Fig. 1a) or OS (median: 19.6 months [95% CI: 14.2–NR] vs. NR [15.7–NR], Fig. 1b) between the MONO and COMBO groups. However, the ORR was significantly higher in the COMBO group compared with the MONO group (62.3% vs. 46.5%,  $p = 0.041$ ) (Table 2).

Table 2  
Response for each regimen.

no. (%)	Pembrolizumab monotherapy group (n = 84)	Pembrolizumab plus chemotherapy group (n = 53)	p-value
CR+PR	39 (46.5)	33 (62.3)	0.041
CR+PR+SD	55 (65.5)	48 (90.6)	
CR	3 (3.60)	0 (0)	
PR	36 (42.9)	33 (62.3)	
SD	16 (19.0)	15 (28.3)	
PD	22 (26.2)	5 (9.4)	
NE	7 (8.30)	0 (0)	

**Is PEC Useful to Decide Whether to Choose Pembrolizumab Monotherapy or Combination Therapy Plus Chemotherapy?** We further investigated whether PEC can guide the selection of MONO or COMBO therapy for the first-line treatment of metastatic NSCLC. In patients with high or low  $PEC_1$ , the Kaplan–Meier analysis revealed no statistical differences between the MONO therapy and the COMBO therapy

(Supplementary Fig. 1). In the high PEC<sub>1</sub> subgroup, the median PFS was 13.6 months (95% CI: 8.87–NR,  $n = 46$ ) in the MONO group vs. 10.6 months (5.29–NR,  $n = 27$ ) in the COMBO group ( $p = 0.872$ ) (Supplementary Fig. 1a). The median OS was 21.1 months (95% CI: 12.1–NR) in the MONO group vs. NR (15.7–NR) in the COMBO group ( $p = 0.239$ , Supplementary Fig. 1b). In the low PEC<sub>1</sub> subgroup, the median PFS was 8.51 (95% CI: 1.71–11.6,  $n = 38$ ) in the MONO group vs. 7.03 months (4.01–19.6,  $n = 26$ ) in the COMBO group ( $p = 0.721$ , Supplementary Fig. 1c). The median OS was 16.8 months (95% CI: 10.5–25.4) in the MONO group vs. 16.3 months (14.9–NR) in the COMBO group ( $p = 0.506$ , Supplementary Fig. 1d).

Next, we evaluated PEC<sub>2</sub> with respect to survival analysis. The Kaplan–Meier results indicated that the high PEC<sub>2</sub> subgroup showed no statistical differences between the MONO ( $n = 53$ ) and COMBO ( $n = 17$ ) groups (Fig. 1c and 1d). The median PFS was 16.1 months (95% CI: 10.6–NR) in the MONO group vs. 10.1 months (3.68–NR) in the COMBO group ( $p = 0.462$ , Fig. 1c). The median OS was not reached in either the MONO (95% CI: 18.2–NR) or COMBO (8.84–NR) group ( $p = 0.885$ , Fig. 1d). However, survival analysis using low PEC<sub>2</sub> revealed that patients with low PEC<sub>2</sub> receiving the MONO therapy ( $n = 31$ ) had an unfavorable tendency of PFS (Fig. 1e) and a statistically shorter OS (Fig. 1f), compared with those receiving the COMBO therapy ( $n = 36$ ). In the low PEC<sub>2</sub> subgroup, the median PFS was 5.75 (95% CI: 1.64–10.3) in the MONO group vs. 7.59 months (5.95–NR) in the COMBO group ( $p = 0.054$ , Fig. 1e). The median OS was 12.0 (95% CI: 5.52–19.6) in the MONO group vs. NR (15.1–NR) in the COMBO group ( $p = 0.019$ , Fig. 1f). These results indicate that PEC<sub>2</sub> is a potential prognostic biomarker that warrants further analysis.

**Can PEC Predict the Subgroup with a Survival Benefit from the Pembrolizumab Monotherapy?** We investigated whether PEC<sub>2</sub> can predict the subgroup that has a favorable survival benefit from the MONO therapy. The Kaplan–Meier analysis demonstrated that patients with high PEC<sub>2</sub> had significantly better survival rates compared with those with low PEC<sub>2</sub>. The median PFS was 16.1 months (95% CI: 10.6–NR) in the high PEC<sub>2</sub> subgroup ( $n = 53$ ) vs. 5.75 months (1.64–10.3) in the low PEC<sub>2</sub> subgroup ( $n = 31$ ,  $p = 0.001$ , Fig. 2a). The median OS was NR (95% CI: 18.2–NR) in the high PEC<sub>2</sub> subgroup vs. 12.0 months (5.52–19.6) in the low PEC<sub>2</sub> subgroup ( $p = 0.005$ , Fig. 2b). The response rate for the MONO group was significantly higher in the high PEC<sub>2</sub> subgroup (56.6%) compared with the low PEC<sub>2</sub> subgroup (29.0%) ( $p = 0.024$ , Table 3).

Table 3  
Responses stratified according to PEC<sub>2</sub> for each regimen

<b>Pembrolizumab monotherapy</b> no. (%)	<b>High PEC<sub>2</sub> subgroup (n = 53)</b>	<b>Low PEC<sub>2</sub> subgroup (n = 31)</b>	<b>p-value</b>
CR+PR	30 (56.6)	9 (29.0)	0.024
CR+PR+SD	38 (71.7)	17 (54.8)	
CR	2 (3.8)	1 (3.2)	
PR	28 (52.8)	8 (25.8)	
SD	8 (15.1)	8 (25.8)	
PD	10 (18.9)	12 (38.7)	
NE	5 (9.4)	2 (6.5)	
<b>Pembrolizumab plus chemotherapy</b> no. (%)	<b>High PEC<sub>2</sub> subgroup (n = 17)</b>	<b>Low PEC<sub>2</sub> subgroup (n = 36)</b>	
CR+PR	9 (52.9)	24 (66.7)	0.375
CR+PR+SD	15 (88.2)	33 (91.7)	
CR	0 (0)	0 (0)	
PR	9 (52.9)	24 (66.7)	
SD	6 (35.3)	9 (25.0)	
PD	2 (11.8)	3 (8.3)	
NE	0 (0)	0 (0)	

A univariable Cox proportional hazard regression analysis for PFS revealed that the histology of squamous NSCLC ( $p = 0.048$ ), discontinuation of treatment ( $p < 0.001$ ), administration of the second-line treatment after discontinuation of the first-line therapy ( $p = 0.013$ ), and low PEC<sub>2</sub> ( $p = 0.002$ ) were significantly associated with poor PFS (Table 4). The univariable analysis for OS revealed that the histology of squamous NSCLC ( $p = 0.031$ ), discontinuation because of disease progression, AEs, death ( $p = 0.002$ ), and low PEC<sub>2</sub> ( $p = 0.006$ ) were significantly associated with shorter OS.

Table 4  
Univariable analysis of PFS and OS for pembrolizumab monotherapy

Variables	Category	PFS			OS		
		Hazard ratio	95% CI	<i>p</i> -value	Hazard ratio	95% CI	<i>p</i> -value
Age	≥75	1.48	0.79–2.77	0.222	1.80	0.97–3.33	0.061
Gender	male	0.96	0.45–2.01	0.904	1.03	0.49–2.15	0.941
ECOG-PS score	0–1	1.40	0.74–2.61	0.302	1.58	0.85–2.94	0.151
Smoking status	Yes	1.33	0.47–3.75	0.585	1.51	0.54–4.22	0.435
Allergic history	Yes	0.91	0.47–1.77	0.778	0.80	0.42–1.54	0.513
Histology	Squamous	1.88	1.01–3.51	0.048	1.94	1.06–3.53	0.031
Brain metastases	Yes	0.37	0.09–1.53	0.168	1.03	0.40–2.61	0.958
PD-L1 TPS	≥50	0.66	0.27–1.59	0.354	0.54	0.25–1.19	0.125
Prior anticancer therapy	Yes	1.26	0.68–2.35	0.461	1.32	0.72–2.40	0.367
Adverse events	Yes	0.59	0.28–1.27	0.180	0.98	0.48–1.99	0.955
Discontinuation due to disease progression, adverse events, or patient death	Yes	11.2	3.90–31.7	< 0.001	3.32	1.52–7.24	0.002
Administration of second-line treatment after discontinuation of the first-line therapy	Yes	2.12	1.17–3.85	0.013	1.18	0.64–2.18	0.600
Eosinophil count before first administration of the treatment	<150 ×10 <sup>6</sup> /L	1.70	0.91–3.18	0.094	1.35	0.74–2.46	0.326
Eosinophil count before second administration of the treatment (×10 <sup>6</sup> /L)	< 150 ×10 <sup>6</sup> /L	2.64	1.41–4.96	0.002	2.31	1.27–4.22	0.006

A multivariable analysis for PFS and OS was performed with parameters that had *p* values < 0.1 in the univariable analysis to identify independent parameters (Table 5). The multivariable analysis for PFS

revealed that low PEC<sub>2</sub> ( $p = 0.043$ ), discontinuation because of disease progression, AEs, or death ( $p < 0.001$ ) were significantly associated with unfavorable PFS. The multivariable analysis for OS revealed that discontinuation of treatment ( $p = 0.002$ ) was significantly associated with unfavorable OS; however, PEC<sub>2</sub> did not reach statistical significance ( $p = 0.061$ ).

Table 5  
Multivariable analysis of PFS and OS for pembrolizumab monotherapy

Variables	Category	PFS			OS		
		Hazard ratio	95% CI	$p$ -value	Hazard Ratio	95% CI	$p$ -value
Age	$\geq 75$				1.84	0.98–3.46	0.058
Histology	Squamous	1.22	0.67–2.21	0.523	1.31	0.70–2.45	0.398
Discontinuation due to disease progression, adverse events, or patient death	Yes	10.0	3.48–28.8	$<0.001$	3.32	1.53–7.24	0.002
Administration of second-line treatment after discontinuation of the first-line therapy	Yes	0.89	0.48–1.65	0.714			
Eosinophil count before the first administration of the treatment	$<150 \times 10^6/L$	1.10	0.47–2.58	0.822			
Eosinophil count before the second administration of the treatment	$<150 \times 10^6/L$	1.84	1.02–3.32	0.043	1.82	0.97–3.40	0.061

**Can PEC Identify the Subgroup with a Survival Benefit from Pembrolizumab Plus Chemotherapy?** We determined whether PEC<sub>2</sub> could identify the subgroup with improved survival by the COMBO therapy. The Kaplan–Meier analysis revealed no statistical differences between the high and low PEC<sub>2</sub> subgroups. The median PFS was 10.1 months (95% CI: 3.68–NR) in the high PEC<sub>2</sub> subgroup ( $n = 17$ ) vs. 7.59 months (5.95–19.6) in the low PEC<sub>2</sub> subgroup ( $n = 36$ ,  $p = 0.916$ , Fig. 2c). The median OS was not reached in either subgroup (95% CI: 8.84–NR in the high PEC<sub>2</sub> subgroup vs. 15.1–NR in the low PEC<sub>2</sub> subgroup,  $p = 0.892$ , Fig. 2d). No relationship was observed between PEC<sub>2</sub> and the ORR when comparing high and low PEC<sub>2</sub> subgroups. The ORRs were 52.9% and 66.7%, respectively ( $p = 0.375$ , Table 3).

## Discussion

We conducted a retrospective study to determine whether PEC is a prognostic or predictive biomarker capable of predicting the outcome for advanced/recurrent NSCLC patients receiving MONO or COMBO in the first-line setting. Our study focused on the first-line treatment with pembrolizumab for metastatic NSCLC, although the confounding factors inevitable in a retrospective analysis should be avoided as much as possible. The importance of both MONO and COMBO was weighed as the first-line treatment of advanced/recurrent NSCLC. To our knowledge, this is the first study to show that low PEC before the second administration of therapy (PEC<sub>2</sub>) predicts the poor survival rates in patients receiving the MONO therapy compared with those receiving the COMBO therapy. PEC<sub>2</sub> was also associated with significant differences in PFS, OS, and ORR between patients with high and low PEC<sub>2</sub> receiving the MONO therapy.

We set  $150 \times 10^6/L$  as the cut-off for the classification of high and low PEC<sub>2</sub> subgroups, which was the median value of PEC<sub>2</sub> in the patient population. There was no relationship between PD-L1 TPS and PEC (both PEC<sub>1</sub> and PEC<sub>2</sub>) and the frequency of AEs was not statistically different between the high and low PEC groups (data not shown). The cut-off value of PEC was generally consistent with that observed in previous studies: 125 [11], 150 [7], 150, and 300 [12]  $\times 10^6/L$ . This cut-off value is less than the  $500 \times 10^6/L$  value, which represents the threshold of eosinophilia. Peripheral blood eosinophilia was also reported to improve the response and prognosis to the ICI monotherapy [13]. The true cut-off value of the PEC should be verified in a well-designed, large-scale, prospective study, and the difference in the immuno-oncological significance between PEC elevation and eosinophilia are unresolved. The results of our study warrant further investigation into PEC as a prognostic biomarker and the role of eosinophils in lung cancer immunotherapy.

We found that low PEC<sub>2</sub>, but not PEC<sub>1</sub>, was associated with worse survival outcomes of patients receiving the MONO therapy compared with that of the COMBO group. However, the timing of when to measure PEC remains controversial. PEC<sub>1</sub> was associated with better survival with nivolumab for the second-line or later treatment [7] and the ICI monotherapy or the combination with an anti-angiogenesis agent or chemotherapy for the first-line or later [11]. In contrast, PEC<sub>1</sub> was not associated with treatment response and survival with nivolumab or pembrolizumab as the first-line or later therapy [8]. Elevation of PEC after the administration of three cycles of pembrolizumab or four cycles of nivolumab was confirmed in patients who achieved a better treatment response [9]. Another group also reported that the maximum value of PEC during ICI with or without chemotherapy was observed 5 weeks after the initiation of each therapy in patients who obtained disease control and at 2 weeks for those who exhibited uncontrolled diseases [12]. However, we did not confirm elevated PEC after the administration of pembrolizumab with or without chemotherapy in this study. Instead, we demonstrated the importance of PEC<sub>2</sub> in the pembrolizumab-based therapy for predicting treatment response and prognosis. Considering these findings, PEC<sub>2</sub>, which was evaluated 3 weeks after the initiation of the pembrolizumab-based therapy, is a convenient biomarker that may be routinely evaluated in clinical practice to predict the treatment response.

We found that PEC<sub>2</sub> was significantly associated with improved survival and treatment response. The reason why PEC<sub>2</sub> may predict a more favorable outcome in the MONO therapy remains unclear. Inhibition of the PD-1/PD-L1 interaction by the MONO therapy did not result in significant changes in PEC, but the high PEC<sub>2</sub> subgroup was associated with better prognosis and response compared with the low PEC<sub>2</sub> subgroup. These findings indicate that PEC<sub>2</sub> may reflect tumor response to PD-1 inhibition. Surprisingly, our study revealed that COMBO did not always result in a survival benefit compared with MONO, and patients with low PEC<sub>2</sub> showed significantly poorer survival with the MONO therapy. PEC dropped significantly after the first administration of COMBO and PEC<sub>2</sub> was not associated with prognosis. This phenomenon may be explained, in part, by antiemetic treatment with steroids during the COMBO therapy. In patients with metastatic NSCLC treated with ICIs as the first-line and later treatment, early use of steroids was associated with worse clinical outcomes because of the modulation of peripheral blood immune cells, resulting in PEC suppression and decreased activation of antitumor immunity [14]. The combination therapy using COMBO is expected to enhance the antitumor effect; however, the use of steroids for antiemesis may inhibit eosinophils from acting not only as a prognostic biomarker, but also as a mediator of antitumor activity. These findings indicate that the choice of MONO or COMBO in the first-line settings for advanced or recurrent NSCLC is controversial. However, low PEC<sub>2</sub> in MONO may be sufficient to select the COMBO treatment.

There are several limitations to the present study. First, this is a retrospective study with a relatively short follow-up period at a single hospital. However, this study provides evidence to support a larger prospective study with an adequate sample size to determine an accurate cut-off value and interpretation of PEC. To clarify the usefulness of PEC as a biomarker, we designed our study to focus on the first-line therapy for metastatic or recurrent NSCLC in which the regimen only contained pembrolizumab for immunotherapy. Second, we did not improve our understanding of the precise role of eosinophils in cancer immunity through the administration of pembrolizumab. Eosinophils are known to infiltrate tumors, exert an antitumor response in the tumor microenvironment [15] and inhibit metastatic tumor development via interleukin (IL)-33 [16, 17]. IL-33 expression in tumor cells increases the immunogenicity and promotes the type 1 antitumor immune responses through CD8-positive T cells and natural killer cells, whereas IL-33 in the tumor stroma and serum facilitates immunosuppression via regulatory T cells and myeloid-derived suppressor cells [18]. IL-33 combined with a PD-1 blockade was reported to enhance antitumor immunity in a melanoma model [19]. These findings support the hypothesis that peripheral blood eosinophils do not always function as antitumor effectors, but once activated, they migrate into the tumor microenvironment and exert antitumor effects along with CD8-positive T cells and inhibit the PD-1/PD-L1 interaction.

In conclusion, PEC<sub>2</sub> in the pembrolizumab monotherapy is a potential predictive and prognostic biomarker for the first-line treatment of advanced or recurrent NSCLC. Patients with low PEC<sub>2</sub> are less likely to receive a survival benefit from the monotherapy compared with high PEC<sub>2</sub> patients, which may encourage a switch to the combination of pembrolizumab plus chemotherapy. The role of eosinophils in lung cancer and the effect of ICI treatments on eosinophils remain unclear. Further investigation is

needed to develop a PEC-guided therapeutic strategy and a thorough understanding of eosinophils in immunotherapy for NSCLC.

## Methods

**Patients and Data Collection:** All medical records at the Department of Thoracic Oncology and Respiratory Medicine, Tokyo Metropolitan Cancer and Infectious Diseases Center of Komagome Hospital from April 2017 to April 2020 were reviewed. Clinical information was collected after approval from the institutional ethics review board (approval no. 2612) in accordance with the Declaration of Helsinki. A total of 140 patients were enrolled after excluding three patients from analysis owing to insufficient PEC data. The final data review was completed in January 2021. Informed consent for the use of pathological samples to evaluate the driver gene mutation profiles and the PD-L1 TPS was obtained from every patient in a clinical setting.

**Treatment Regimen:** Pembrolizumab monotherapy or pembrolizumab plus chemotherapy was administered every 3 weeks concordant with the clinical trials [1–6]. For antiemesis, dexamethasone was administered intravenously on day 1 of the cisplatin/carboplatin plus pemetrexed regimen and carboplatin plus paclitaxel regimen and on days 1, 8, and 15 of the carboplatin plus nanoparticle albumin-bound paclitaxel regimen. Oral dexamethasone was administered on days 2, 3, and 4 of the cisplatin-based regimen.

**Statistical Analysis:** Comparisons between patient characteristics were performed using  $\chi^2$  or Fisher's exact test for discrete variables and unpaired *t*-test, Wilcoxon signed-rank test, or analysis of variance for continuous variables. Survival analyses were performed using the Kaplan–Meier method and the log-rank test. All *p* values <0.05 were considered statistically significant. The hazard ratio and 95% CI were calculated using the univariable Cox proportional hazard model. Parameters with a *p* value less than 0.1 in the univariable analysis were selected for inclusion in the multivariable analyses. All statistical analyses were performed using the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) with a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [20].

## Declarations

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### Author contributions

M. U performed data collection and curation, investigation, and writing of the original draft. K. N conceptualized this study and performed project administration, investigation, writing of the original draft, review, and editing. T. S participated in data collection and curation. Y. H performed supervision and review. All authors approved the final version of this manuscript for submission.

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## Competing interests

The authors declare no competing interests.

## Data availability

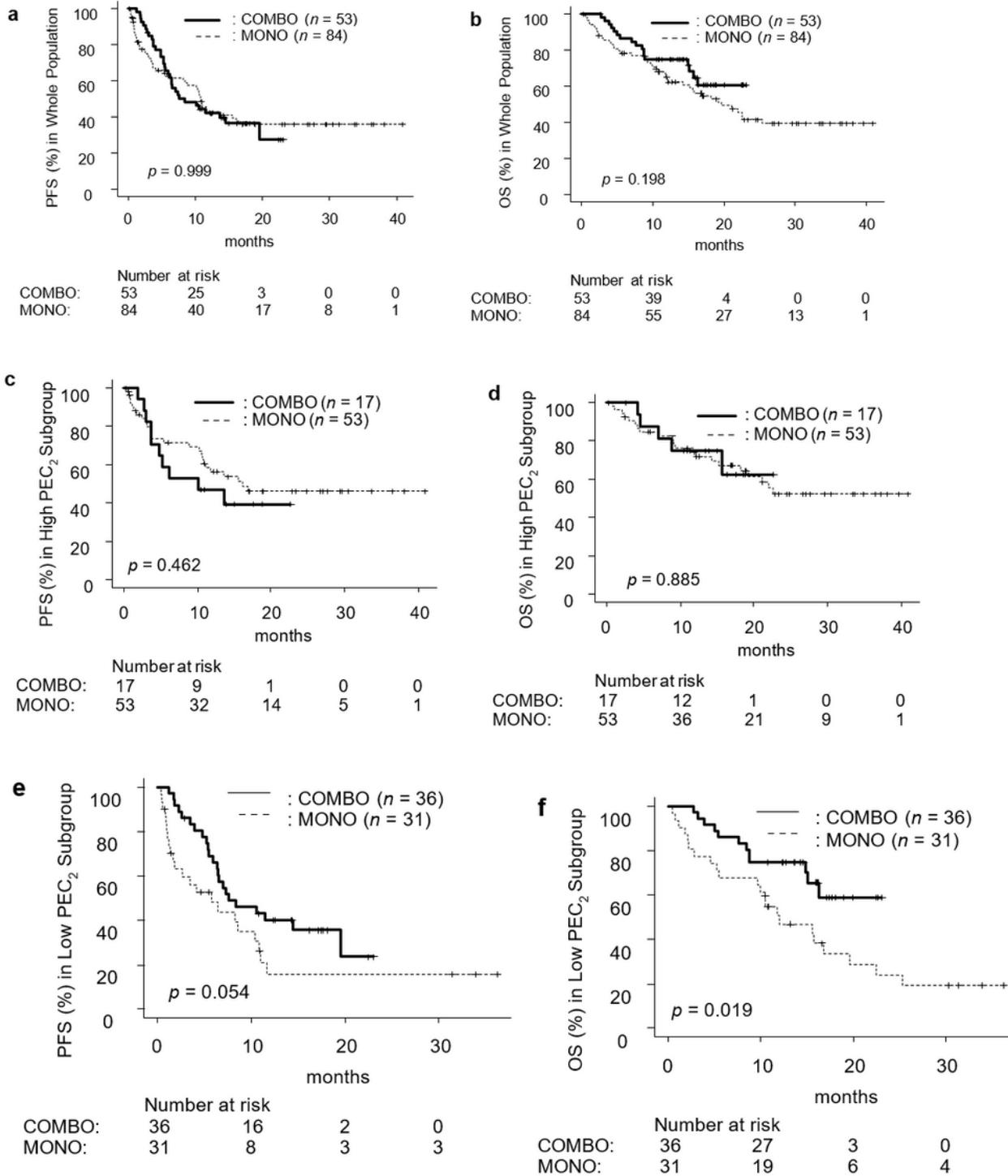
All data described in this article are preserved by M. U. and K. N. Data were generated from the information contained in the electronic medical records after ethics approval by the Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital. The datasets generated during this study are included in this published article and its supplementary information files. Further information is available from the corresponding author upon reasonable request.

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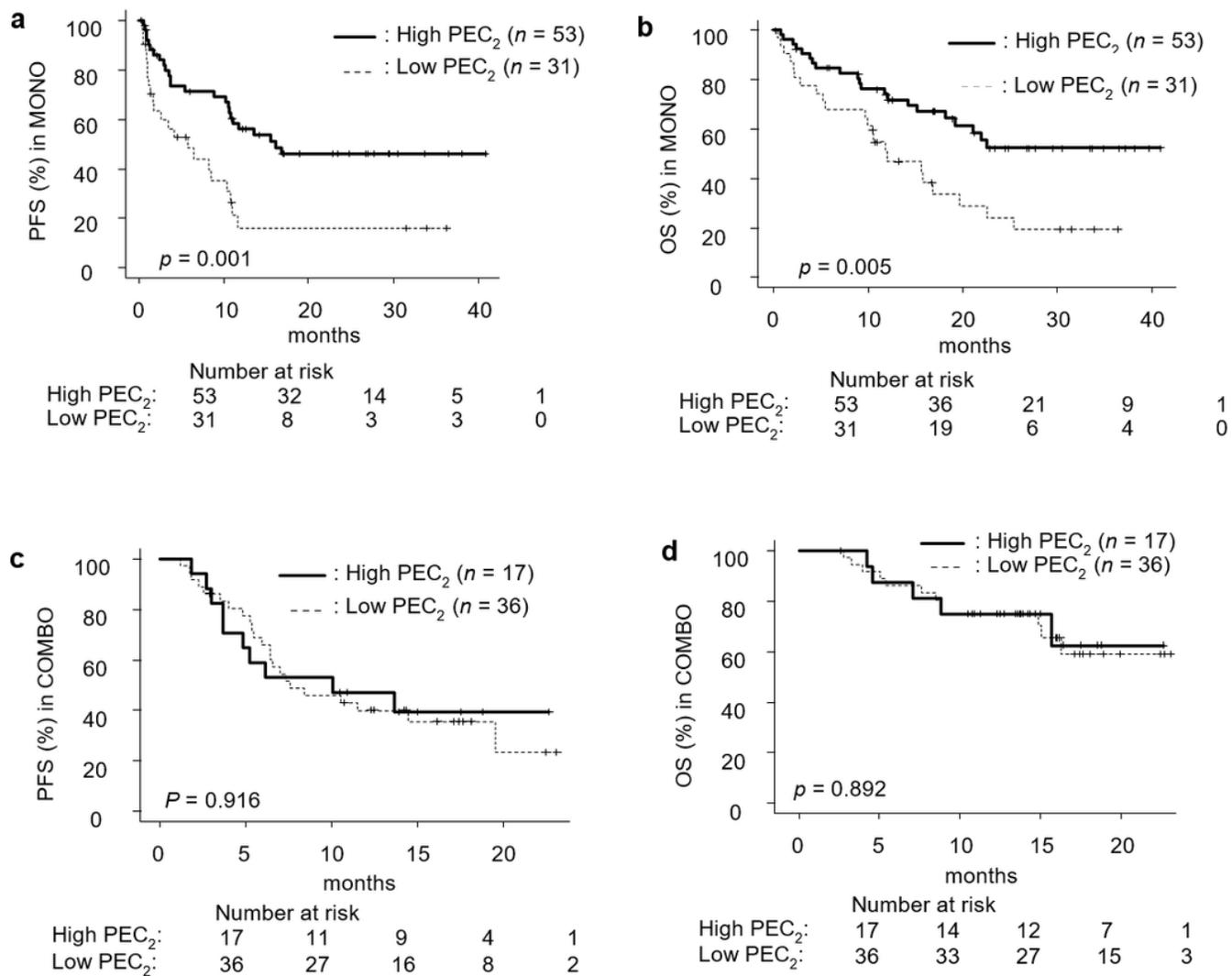
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# Figures



**Figure 1**

Survival outcomes for each regimen in the whole population and high or low PEC2 subgroup. a. PFS and b. OS in the whole population. c. PFS and d. OS in the high PEC2 subgroup. e. PFS and f. OS in the low PEC2 subgroup



**Figure 2**

Survival outcomes stratified by PEC2 for each regimen. a. PFS and b. OS in pembrolizumab monotherapy. c. PFS and d. OS in pembrolizumab plus chemotherapy.

## Supplementary Files

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