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Time-to-treatment failure and peripheral eosinophils in non-small cell lung cancer patients treated with immune checkpoint inhibitors

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Short title: TTF and eosinophils in ICPI-treated NSCLC patients

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What’s new?

With the advent of immune checkpoint inhibitors (ICPIs), the treatment of many carcinomas has made great strides. However, it is currently difficult to identify patients who will benefit from ICPI before or during treatment. As a biomarker for ICPI therapy, programmed cell death-ligand 1 (PD-L1) expression has been utilized. However, PD-L1 may show different immunostaining levels depending on the site where it was collected. In this study, we identified both “≥5% eosinophils within 6 weeks” and “≥330/μL eosinophils within 6 weeks” were significant favorable factors associated with time-to-treatment failure in patients receiving ICPI monotherapy. In patients treated with combination therapy of ICPI and chemotherapy, eosinophil variability will be further complicated by myelosuppression by antitumor drugs. However, fluctuations in peripheral eosinophils have been observed, and detailed analysis of this might reveal their usefulness as biomarkers. Our study suggests it may be possible to predict response to ICPI therapy from peripheral eosinophils.
Abstract

**Introduction:** There is an unmet clinical need to identify biomarkers to predict which non-small cell lung cancer (NSCLC) patients will benefit from immune checkpoint inhibitor (ICPI) treatment.

**Objectives:** The purpose of this study was to draw a detailed time-to-treatment failure (TTF) curve with information on peripheral eosinophils during ICPI treatment for NSCLC, and to clarify whether eosinophil information can predict prolonged TTF.

**Patients and methods:** In 259 patients with NSCLC treated with ICPI therapy, peripheral eosinophil counts and percentages at the time of each ICPI administration were evaluated in each patient from the beginning of ICPI treatment up to TTF. Univariable and multivariable analyses were performed to identify clinical factors that were associated with TTF.

**Results:** When 180 patients receiving ICPI monotherapy were divided into three groups – “TTF≤6 weeks”, “6 weeks<TTF≤24 weeks”, and “24 weeks<TTF” – the rate of “5% or more within 6 weeks” was significantly different among the three groups. By univariable and multivariable analyses, “PS 0-1” and “immune-related adverse event excluding ICPI discontinuation”, as well as “≥5% eosinophils within 6 weeks” and “≥330/μL eosinophils within 6 weeks”, were significant favorable factors for TTF. In 79 patients treated with combination therapy of ICPI and chemotherapy, the rate of “5% or more within 12 weeks” was significantly different between “TTF≤12 weeks”, and “12 weeks<TTF”. However, the
only significant favorable factor for TTF was “female gender”.

**Conclusions:** In NSCLC patients treated with ICPI therapy, especially ICPI monotherapy, eosinophil measurements during treatment might provide information useful in predicting prolonged TTF.
Introduction

Immune checkpoint inhibitors (ICPIs) have significantly changed the treatment of advanced non-small cell lung cancer (NSCLC) [1, 2]. In particular, the high ‘tail plateau’ on the survival curve is impressive, and the emergence of advanced NSCLC patients who can be cured is astonishing [1, 2]. However, not every patient will benefit from ICPI and be cured. When the results of progression-free survival (PFS) in clinical trials of ICPI monotherapy were examined in detail, patients could be divided into three groups; “no response group”, “short-term response group”, and “long-term response group” [3-8]. Clinical trials of combination therapy of ICPI and chemotherapy, however, found the “no response group” proportion decreased, leaving two primary groups; “short-term response group” and “long-term response group” [9, 10].

In ICPIs, programmed death-ligand-1 (PD-L1) is evaluated as the most common response-predicting biomarker [11, 12]. However, PD-L1 relies on immunostaining of pathological specimens, and the biopsy site may not fully represent the entire lung cancer [12]. Another issue is that PD-L1 results may change depending on where the surgically excised site is stained and evaluated [12]. Therefore, better biomarkers are needed in the clinical setting. A biomarker that does not require a complicated system or expensive equipment but, rather, is easy and inexpensive to evaluate, and, if possible, derived from standard clinical data, would be highly useful clinically. These factors are driving the search
for new biomarkers other than PD-L1 [13-31]. They include studies investigating whether neutrophils, lymphocytes and eosinophils could be used as biomarkers [18-31]. Although the detailed biological mechanism, either direct or indirect, is unknown, it seems that changes in peripheral blood cells are associated with ICPI treatment, and has been the focus of several studies [18, 20, 22, 23, 29, 31]. To the best of our knowledge, however, no investigation has been performed on the detailed changes of eosinophils during the clinical course of individual patients.

Recently, we reported the importance of eosinophil variability after the initiation of ICPI therapy [32]. Our study found that long-term time-to-treatment failure (TTF) of ICPI therapy was possible in patients with eosinophils of ‘5% or more’ and ‘330/μL or more’ at the time of 5 weeks after the initiation of the therapy [32]. However, we did not separately analyze peripheral eosinophils in patients treated with ICPI monotherapy and those in patients treated with combination therapy of ICPI and chemotherapy, although there was no significant difference in patient backgrounds between these two groups. Moreover, it was not possible to show in detail the changes in eosinophils during the patients’ clinical courses. As we considered that these data are important, and that a study to visualize the detailed TTF containing this information is absolutely necessary, we conducted the present study.

The purpose was to clarify the optimal information on peripheral eosinophils as a convenient and inexpensive biomarker that helps predict whether or not ICPI treatment should
be continued. In particular, we focused on findings that are useful for selecting patients who
could be administered ICPIs for a long period of time, and for those who should change from
ICPIs to other therapeutic agents.

Patients and Methods

Patients

We analyzed the medical records of all patients diagnosed with NSCLC in three tertiary
hospitals in Japan (Mito Medical Center, University of Tsukuba–Mito Kyodo General
Hospital, Ryugasaki Saiseikai Hospital, and Tsukuba University Hospital) between February
2016 and March 2021. Patients with NSCLC treated with ICPI monotherapy or combination
therapy of ICPI and chemotherapy during this period were included. NSCLC was diagnosed
based on the World Health Organization classification. Tumor node metastasis staging (TNM
Classification, 8th Edition) using head computed tomography or magnetic resonance imaging,
bone scans, and ultrasonography and/or computed tomography of the abdomen was
performed in all patients prior to ICPI therapy initiation. Patients with the following
comorbidities and with a history of treatment for these conditions were excluded; parasitic
infestations, allergic diseases, autoimmune diseases and hematologic malignancies. Patients
with chronic obstructive pulmonary disease and those with bronchial asthma and chronic
obstructive pulmonary disease overlap requiring systemic steroid use were also excluded.
Particular attention was paid to adrenal insufficiency as an immune related adverse event
Patients who developed eosinophilia associated with adrenal insufficiency as an irAE were excluded from this study. Patient demographic data, including age, sex, Eastern Cooperative Oncology Group score for performance status, histopathology, disease stage, PD-L1 expression, objective tumor response, and survival, were obtained from the patients’ medical charts. Tumor response was evaluated as complete response, partial response, stable disease, or progressive disease according to the Response Evaluation Criteria in Solid Tumors (Version 1.1).

Peripheral eosinophil count and percentage measurement

Eosinophil counts and percentages were measured at the same time as complete blood count measurements before and during ICPI therapy. Results were obtained from the medical records of each patient. Counts for leukocyte subpopulations were measured by routine clinical laboratory analysis using a Sysmex XN 3000 analyzer (Sysmex Co., Ltd. Kobe, Japan).

Measurements of eosinophils

In a previous study, we evaluated “eosinophils 5% or more” and “eosinophil count 330/μL or more” five weeks after the initiation of ICPI therapy [32]. However, current administration methods for immune checkpoint inhibitors are every two, three and six weeks [3-10]. Considering this, in the present study, we carried out analyses paying attention to “5% or more of eosinophils within 6 weeks after the initiation of treatment” and to “the number of
eosinophils is 330/μL or more within 6 weeks after the initiation of treatment” in patients treated with ICPI monotherapy. In patients treated with combination therapy of ICPI and chemotherapy, analyses paying attention to “5% or more of eosinophils within 12 weeks after the initiation of treatment” and to “the number of eosinophils is 330/μL or more within 12 weeks after the initiation of treatment” were performed.

**TTF and information on eosinophils during ICPI therapy**

Peripheral eosinophils were measured each time the immune checkpoint inhibitor was administered. Regarding the eosinophil percentage, a TTF curve was drawn by color-coding the period until the next administration according to “when the eosinophils were 5% or more” or not. Similarly, for the peripheral eosinophil count, a TTF curve was created by color-coding the period until the next administration depending on the presence or absence of “eosinophil count of 330/μL or more”. Next, we carried out a comparison of these groups paying attention to “5% or more of eosinophils within 6 weeks after the initiation of treatment” and to “the number of eosinophils is 330/μL or more within 6 weeks after the initiation of treatment” in patients treated with ICPI monotherapy. Similarly, in patients treated with ICPI combination therapy of ICPI and chemotherapy, we performed a comparison between groups II and III paying attention to “5% or more of eosinophils within 12 weeks after the initiation of treatment” and to “the number of eosinophils is 330/μL or more within 12 weeks after the initiation of treatment”.
For the purpose of investigating the characteristics of patients with long-term control from ICSI treatment, we also investigated eosinophil information in ICPI monotherapy patients with TTF of 120 weeks or longer. For those patients treated with combination therapy of ICPI and chemotherapy, information on eosinophils in patients with TTF of 60 weeks or longer were investigated.

Univariable and multivariable analyses

By univariable analysis, we investigated the association between patient background factors (gender, PS, age, pathology, stage, driver genes, PD-L1, and irAE) and TTF. The association between TTF was investigated for “5% or more eosinophils within 6 weeks of the start of treatment” and “330/μL or more of eosinophils within 6 weeks of the start of treatment” in patients treated with ICPI monotherapy. Similarly, the association between TTF was investigated for “5% or more eosinophils within 12 weeks of the start of treatment” and “330/μL or more of eosinophils within 12 weeks of the start of treatment” in patients treated with combination therapy of ICPI and chemotherapy. When the factors were statistically significant by univariable analysis, multivariable analysis was then performed. These analyses were performed separately for patients receiving ICPIs alone and those receiving ICPI and chemotherapy combination.

Statistical analysis

The χ² test was used to compare nominal variables. We used the nonparametric Mann–
Whitney test to compare values with unknown population variance. We adopted the definition of TTF that is commonly used in cancer treatment; the interval from initiation of therapy with ICPIs to treatment discontinuation or the last follow-up visit. TTF failure was estimated by the Kaplan–Meier method and compared using the log rank test. We used the Cox proportional hazards model and forward backward stepwise method to determine the independent variables used in the final model. In this study, multivariable analyses were performed using only variables where P < 0.10 by univariable analysis. TTF was the dependent variable in that model. All statistical analyses were conducted using SPSS version 23 (IBM Corporation, Armonk, New York). A P-value less than 0.05 was considered significant.

Ethics

This study conformed to the Ethical Guidelines for Clinical Studies issued by the Ministry of Health, Labor, and Welfare of Japan. Written informed consent for a non-interventional retrospective study was obtained from each patient. The analysis of the medical records of patients with lung cancer was approved by the ethics committee of Mito Medical Center–University of Tsukuba Hospital (NO 20-57).

Results

Patient characteristics

We analyzed the clinical characteristics of 259 patients who met all inclusion criteria within the study period. Detailed data of the study patients are shown in Table 1. Of the 259
patients enrolled, 180 were treated with ICPI monotherapy, and 79 were treated with combination therapy of ICPI and chemotherapy. In the 180 patients treated with ICPI monotherapy, median TTF was 12 weeks (range, 3-217 weeks; 20 had ongoing treatment). In 180 patients treated with ICPI monotherapy, 71 (39.4%) had eosinophil≥5%, with a median 7.9% (range, 5.0%-53.0%). Eighty-five (47.2%) had eosinophils≥330/μL, with a median of 598/μL (range, 330 /μL -6413 /μL). Eight of the 180 patients had TTF of 120 weeks or more. Seven out of these eight patients had eosinophil 5% or more several times over the course of treatment.

In the 79 patients treated with combination therapy of ICPI and chemotherapy, median TTF was 23 weeks (range, 9-93 weeks; 25 had ongoing treatment). In 79 patients treated with combination with ICPI and chemotherapy, 37 (46.8%) had eosinophil≥5%, with a median 8.2% (range, 5.2%-33.0%). Thirty-three (41.8%) had eosinophils≥330/μL, with a median of 616/μL (range, 381 /μL -5742 /μL). Seven of the 79 patients had a TTF of 60 weeks or more. Four of these had eosinophil 5% or more several times.

**TTF curves and patient grouping by information on peripheral eosinophils**

Figure 1 shows the TTF curves of 180 patients who received ICPI monotherapy. Figure 1A is a TTF curve with a cutoff of 5% of peripheral blood eosinophils, and Figure 1B, a TTF curve with a cutoff of 330/μL of eosinophils. Based on the TTF curves, ICPI monotherapy-administered patients were divided into three groups; “no response group (group I: TTF≤6
weeks”), “short-term response group (group II: 6 weeks<TTF≤24 weeks)”, and “long-term response group (group III: 24 weeks<TTF).” First, we examined patient background factors (gender, PS, age, pathology, stage, driver genes, and PD-L1) of the groups, and confirmed that there was no difference among groups I, II, and III (Table 2A).

Figure 2 shows the TTF curves of 79 patients who received combination therapy of ICPI with chemotherapy. Figure 2A is a TTF curve with a cutoff of 5% of peripheral blood eosinophils, and Figure 2B, a TTF curve with a cutoff of 330/μL of eosinophils. Based on the TTF curve, patients who received combination therapy of ICPI and chemotherapy were divided into two groups, a “group IV (TTF≤12 weeks)” and a “group V (12 weeks<TTF).” No difference in patient background factors was found between group IV and group V (Table 2A).

Table 3 shows patient grouping and information of peripheral eosinophils. In patients treated with ICPI monotherapy, the rate of “5% or more within 6 weeks” was significantly different among the three groups (P=0.0027). A similar analysis was performed with 330/μL eosinophils as the cutoff, but there was no significant difference in the ratio among the three groups in patients receiving ICPI monotherapy (P=0.0994). Similarly, in patients treated with combination therapy with ICPI and chemotherapy, the rate of “5% or more within 12 weeks” was significantly different between the two groups (P=0.0231). Using this cutoff for patients treated with combination therapy of ICPI and chemotherapy, there was no difference between
the two groups (P=0.2683).

Univariable and multivariable analyses

Table 2B shows a comparison of the patient background factors. We confirmed that there was no difference among them. Table 4A shows the results of univariable and multivariable analyses in patients treated with ICPI monotherapy. “PS 0-1”, “IrAE excluding ICPI discontinuation” and “≥5% eosinophils within 6 weeks” were significant favorable factors for TTF by multivariable analysis. “PD-L1 25% or more” was not a significant favorable factor for TTF by univariable and multivariable analyses. Table 4B shows that “≥330/μL eosinophils within 6 weeks” was a significant favorable factor for TTF by univariable and multivariable analyses.

In patients treated with combination therapy with ICPI and chemotherapy, only “female gender” was a significant favorable factor for TTF by univariable analysis (P=0.0186).

Discussion

Based on the Kaplan-Meier curves of PFS in several clinical trials of immune checkpoint inhibitor monotherapy for NSCLC, patients could be divided into three groups, “no response group”, “short-term response group”, and “long-term response group” [3-8]. On the other hand, patients who received combination therapy of ICPI and chemotherapy could be divided into two groups, “short-term response group”, and “long-term response group” [9, 10]. Assuming these response groups, the relationship between information on peripheral
eosinophils and TTF was investigated. We first visualized Kaplan-Meier curves for TTF with eosinophil information measured at the time of each ICPI administration during the clinical course of each patient. As a result of the analysis of this data, the following matters were clarified. When patients receiving ICPI monotherapy were divided into three groups – “TTF≤6 weeks (group I)”, “6 weeks<TTF≤24 weeks (group II)”, and “24 weeks<TTF (group III)” – the rate of “5% or more within 6 weeks” was significantly different among the three groups. By univariable and multivariable analyses, not only “PS 0-1” and “IrAE excluding ICPI discontinuation”, but also “≥5% eosinophils within 6 weeks” and “≥330/μL eosinophils within 6 weeks”, were significant favorable factors for TTF. In patients treated with combination therapy of ICPI and chemotherapy, the rate of “5% or more within 12 weeks” was significantly different between group IV and group V (P=0.0231). However, by univariable analysis, the only significant favorable factor for TTF was “female gender”. Therefore, in this study, favorable factors for TTF in patients treated with combination therapy of ICPI and chemotherapy could not be clarified.

With regard to the factors, “≥5% eosinophils” and “≥330/μL eosinophils”, the cutoff was 6 weeks for patients receiving ICPI monotherapy, and 12 weeks for patients receiving combination therapy of ICPI and chemotherapy. This was to reflect as much patient information as possible, but it remains arguable whether this grouping cutoff was optimal.

There have been reports suggesting the involvement of eosinophils in immunity at
cancerous lesions [33-38]. However, it is unlikely that the increased number of eosinophils in the peripheral blood directly reflects the immune status of the cancerous lesions. Indeed, some patients in the “long-term response group” in the present study had an increase in the absolute number of 1000/μL eosinophils and a high proportion of eosinophils in excess of 20%. However, in the majority of patients, no such increase in numbers or high percentages were observed. From these results, in peripheral blood, the relative variability of eosinophils linked to the fluctuation of other blood cells might be more important. It might also be consistent with the observation that the percentage of eosinophils was more useful than the increase in the absolute number of eosinophils.

Elucidation of the biological role of eosinophils in cancer immunity is likely to be an area of future research. At the same time, our understanding of the changes in peripheral blood cells during ICPI therapy will increase. Such advances will clarify the role of eosinophils as a biomarker for response to ICPI. This study did not find clear eosinophil variability in TTF patients treated with combination therapy of ICPI and chemotherapy. Myelosuppression by antitumor drugs causes neutropenia, which is presumed to further complicate the movement of peripheral blood cells. As such, this area will also benefit from future research.

This study had several limitations. First, we did not elucidate the relationship between changes in eosinophils following ICPI therapy and the biological role of eosinophils. Second,
it was a retrospective study that included patients with various baseline characteristics. Third, it involved a limited number of patients with a short follow-up period, and the number of patients required was not preset on a statistical basis. Among 79 patients treated with combination therapy of ICPI and chemotherapy, 25 patients were currently on treatment, and this might have influenced the results. Fourth, we focused on the indications for patients who should change from ICPI to other treatments, and for patients who can continue ICPI for a long period of time. Therefore, the analysis was conducted with the intention of providing useful information as to whether treatment could be continued at 2-3 courses of ICPI monotherapy and at 3-4 courses of combination therapy of ICPI and chemotherapy.

Although the contribution of ICPIs to prolonging survival in many carcinoma patients was significant, ICPIs can cause irAEs in organs throughout the body, and irAEs range from controllable to life-threatening [39]. Therefore, we clinicians should be on the alert for the onset of irAEs. On the other hand, however, an association between the appearance of controllable irAEs and prolongation of survival has been reported [40, 41]. When unfortunately an irAE develops, it should first be determined whether it is controllable. When considering whether to continue ICPI treatment after the determination, changes in peripheral eosinophils associated with ICPI treatment might provide useful information.

With regard to “≥5% eosinophils” and “≥330/μL eosinophils”, it was 6 weeks for patients receiving ICPI monotherapy, and 12 weeks for patients treated with combination
therapy of ICPI and chemotherapy. These were set to reflect as much patient information as possible. This was to reflect as much patient information as possible. It is arguable whether this grouping cutoff was optimal.

**Contribution statement:** HO and HS designed the study, SO, TS, KM, YS, GO, KK, SS, TK, and HS collected the data. HO, SO, KN, RN, HS and NH analyzed the data and prepared the manuscript. All authors approved the final version of the article.

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References

1. Remon J, Passiglia F, Ahn MJ, et al. Immune checkpoint inhibitors in thoracic malignancies: review of the existing evidence by an IASLC Expert Panel and recommendations. J Thorac Oncol. 2020; 15: 914-947.

2. Qiu Z, Chen Z, Zhang C, Zhong W. Achievements and futures of immune checkpoint inhibitors in non-small cell lung cancer. Exp Hematol Oncol. 2019; 8:

3. Rizvi NA, Mazières J, Planchard D, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol. 2015; 16: 257-265.

4. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015; 373: 123-135.

5. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015; 373: 1627-1639.

6. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015; 372: 2018-2028.

7. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016; 387: 1540-1550.

8. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients
with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016; 387: 1837-1846.

9. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med. 2018; 378: 2078-2092.

10. West H, McCleod M, Hussein M, et al. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. Lancet Oncol. 2019; 20: 924-937.

11. Lantuejoul S, Sound Tsao M, Cooper WA, et al. PD-L1 testing for lung cancer in 2019: perspective from the IASLC pathology committee. J Thorac Oncol. 2020; 15: 499-519.

12. Williams JB, Li S, Higgs EF, et al. Tumor heterogeneity and clonal cooperation influence the immune selection of IFN-γ signaling mutant cancer cells. Nat Commun. 2020; 11: 602.

13. Simon SCS, Hu X, Panten J, et al. Eosinophil accumulation predicts response to melanoma treatment with immune checkpoint inhibitors. Oncoimmunology. 2020; 9: 1727116.

14. Rigoni A, Colombo MP, Pucillo C. Mast cells, basophils and eosinophils: From allergy to cancer. Semin Immunol. 2018; 35: 29-34.

15. Simon SCS, Utikal J, Umansky V. Opposing roles of eosinophils in cancer. Cancer Immunol Immunother. 2019; 68: 823-833.
16. Wang X, Zhang B, Chen X, et al. Lactate dehydrogenase and baseline markers associated with clinical outcomes of advanced esophageal squamous cell carcinoma patients treated with camrelizumab (SHR-1210), a novel anti-PD-1 antibody. Thorac Cancer. 2019; 10: 1395-1401.

17. Benitez JC, Recondo G, Rassy E, Mezquita L. The LIPI score and inflammatory biomarkers for selection of patients with solid tumors treated with checkpoint inhibitors. Q J Nucl Med Mol Imaging. 2020; 64: 162-174.

18. Delyon J, Mateus C, Lefeuvre D, et al. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol. 2013; 24: 1697-1703.

19. Umansky V, Utikal J, Gebhardt C. Predictive immune markers in advanced melanoma patients treated with ipilimumab. Oncoimmunology. 2016; 5: e1158901.

20. Moreira A, Leisgang W, Schuler G, Heinzerling L. Eosinophilic count as a biomarker for prognosis of melanoma patients and its importance in the response to immunotherapy. Immunotherapy. 2017; 9: 115-121.

21. Buder-Bakhaya K, Hassel JC. Biomarkers for clinical benefit of immune checkpoint inhibitor treatment-A review from the melanoma perspective and beyond. Front Immunol. 2018; 9: 1474.

22. Simon SCS, Hu X, Panten J, et al. Eosinophil accumulation predicts response to melanoma treatment with immune checkpoint inhibitors. Oncoimmunology. 2020; 9:
23. Tanizaki J, Haratani K, Hayashi H, et al. Peripheral blood biomarkers associated with clinical outcome in non-small cell lung cancer patients treated with nivolumab. J Thorac Oncol. 2018; 13: 97-105.

24. Facchinetti F, Veneziani M, Buti S, et al. Clinical and hematologic parameters address the outcomes of non-small-cell lung cancer patients treated with nivolumab. Immunotherapy. 2018; 10: 681-694.

25. Fujimoto S, Fujita A, Minato K, et al. Complete response of a patient with lung squamous cell carcinoma after only three administrations of nivolumab. Jpn J Lung Cancer (Haigan). 2018; 58: 292-297 [in Japanese].

26. Inomata M, Kado T, Okazawa S, et al. Peripheral PD1-positive CD4 T-lymphocyte count can predict progression-free survival in patients with non-small cell lung cancer receiving immune checkpoint inhibitor. Anticancer Res. 2019; 39: 6887-6893.

27. Soda H, Ogawara D, Fukuda Y, et al. Dynamics of blood neutrophil-related indices during nivolumab treatment may be associated with response to salvage chemotherapy for non-small cell lung cancer: A hypothesis-generating study. Thorac Cancer. 2019; 10: 341-346.

28. Lou Y, Marin-Acevedo JA, Vishnu P, et al. Hypereosinophilia in a patient with metastatic non-small-cell lung cancer treated with antiprogrammed cell death 1 (anti-PD-1) therapy. Immunotherapy. 2019; 11: 577-584.
29. Alves A, Sucena I, Dias M, et al. Eosinophilia in lung cancer patients treated with immunotherapy. Eur Respir J. 2019; 54 (suppl 63) PA4664;

30. Singh N, Lubana SS, Constantinou G, Leaf AN. Immunocheckpoint inhibitor-(Nivolumab-) associated hypereosinophilia in non-small-cell lung carcinoma. Case Rep Oncol Med. 2020; 2020: 7492634.

31. Hude I, Sasse S, Bröckelmann PJ et al. Leucocyte and eosinophil counts predict progression-free survival in relapsed or refractory classical Hodgkin lymphoma patients treated with PD1 inhibition. Br J Haematol. 2018; 181: 837-840.

32. Okauchi S, Shiozawa T, Miyazaki K, et al. Association between peripheral eosinophils and clinical outcomes in patients with non-small cell lung cancer treated with immune checkpoint inhibitors. Pol Arch Intern Med. 2021; 131: 152-160.

33. Sawyers CL, Golde DW, Quan S, Nimer SD. Production of granulocyte-macrophage colony-stimulating factor in two patients with lung cancer, leukocytosis, and eosinophilia. Cancer. 1992; 69: 1342-1346.

34. Matsumoto S, Tamai T, Yanagisawa K, Kawamura S, Fujita S. Lung cancer with eosinophilia in the peripheral blood and the pleural fluid. Intern Med. 1992; 31: 525-529.

35. Pandit R, Scholnik A, Wulfekuhler L, Dimitrov N. Non-small-cell lung cancer associated with excessive eosinophilia and secretion of interleukin-5 as a paraneoplastic syndrome. Am J Hematol. 2007; 82: 234-237.
36. El-Osta H, El-Haddad P, Nabbout N. Lung carcinoma associated with excessive eosinophilia. J Clin Oncol. 2008; 26: 3456-3457.

37. Rigoni A, Colombo MP, Pucillo C. Mast cells, basophils and eosinophils: From allergy to cancer. Semin Immunol. 2018; 35: 29-34.

38. Simon SCS, Utikal J, Umansky V. Opposing roles of eosinophils in cancer. Cancer Immunol Immunother. 2019; 68: 823-833.

39. Domagała-Kulawik J, Leszek P, Owczarek W, et al. Immunotherapy of solid tumors: safety of treatment. Pol Arch Intern Med. 2020; 130: 766-778.

40. Fan Y, Xie W, Huang H, et al. Association of Immune Related Adverse Events With Efficacy of Immune Checkpoint Inhibitors and Overall Survival in Cancers: A Systemic Review and Meta-analysis. Front Oncol. 2021; 11: 633032.

41. Corsello SM, Barnabei A, Marchetti P, De Vecchis L, Salvatori R, Torino F. Endocrine side effects induced by immune checkpoint inhibitors. J Clin Endocrinol Metab. 2013; 98: 1361-1375.
| Characteristic                                    | ICPI monotherapy | Combination therapy of ICPI and chemotherapy |
|--------------------------------------------------|-------------------|---------------------------------------------|
| No. of patients                                  | 180               | 79                                          |
| Age, median (range), years                       | 69 (29-87)        | 69 (29-80)                                  |
| Gender, female/male                              | 39/141            | 20/59                                       |
| PS, 0-1 / 2-                                     | 152/28            | 77/2                                        |
| Pathology, AD/others                             | 114/66            | 49/30                                       |
| Stage, IIIA-C/IVA-B                              | 49/131            | 15/64                                       |
| Driver genes, absent/present                     | 161/19            | 5/74                                        |
| PD-L1, ≥25% : <25%                               | 71/109            | 19/60                                       |
| ICPI, P/A/N/D/N+Ipi                              | 55/23/102/0/0     | 61/11/0/6/1                                |
| Response, CR/PR/SD/PD                            | 5/54/66/55        | 0/48/25/6                                  |
| irAE excluding discontinuation of ICPI, present/absent | 24/156            | 11/68                                       |
| TTF median (range), weeks                        | 12 (3-217)        | 23 (9-93)                                   |
| Treatment ongoing                                | 20                | 25                                          |

A: atezolizumab, AD: adenocarcinoma, CR: complete response, D: durvalumab, ICPI: immune checkpoint inhibitor, Ipi: Ipilimumab, irAE: immune-related adverse event, N: nivolumab, NSCLC: non-small cell lung cancer, P: pembrolizumab, PD: progressive disease, PD-L1: programmed death-ligand-1, PR: partial response, SD: stable disease, SQ: squamous cell carcinoma, TTF: time-to-treatment failure
Table 2. Comparison of patient backgrounds by patient group and that by information of peripheral eosinophils

(A) Comparison of patient backgrounds by patient group

|                          | Group I | Group II | Group III | P-value | Group IV | Group V | P-value |
|--------------------------|---------|----------|-----------|---------|----------|---------|---------|
| Number of patients       | 58      | 54       | 68        |         | 19       | 60      |         |
| Gender, female/male      | 16/42   | 13/41    | 10/58     | 0.19    | 6/13     | 14/46   | 0.47    |
| PS (ECOG), 0-1/2 ≥       | 45/13   | 47/7     | 60/8      | 0.21    | 18/1     | 59/1    | 0.38    |
| Age (years), <70/70 ≥    | 32/26   | 27/27    | 40/28     | 0.62    | 9/10     | 35/25   | 0.40    |
| Pathology, AD/others     | 43/15   | 31/23    | 41/27     | 0.14    | 11/8     | 38/22   | 0.67    |
| Stage, IIIA-C/VIA-B      | 15/43   | 14/40    | 20/48     | 0.88    | 2/17     | 13/47   | 0.29    |
| Driver genes, absent/present | 48/10 | 51/3     | 62/6      | 0.11    | 17/2     | 57/3    | 0.39    |
| PD-L1, 25% ≤/<25%        | 20/38   | 18/36    | 33/35     | 0.15    | 7/12     | 12/48   | 0.13    |

(B) Comparison of patient backgrounds by information of peripheral eosinophils

|                          | Present | Absent | P-value | Present | Absent | P-value |
|--------------------------|---------|--------|---------|---------|--------|---------|
| Number of patients       | 40      | 140    | 0.77    | 25      | 54     |         |
| Gender, female/male      | 8/32    | 31/109 | 0.27    | 4/21    | 16/38  | 0.2     |
| PS (ECOG), 0-1/2 ≥       | 36/4    | 116/24 | 0.28    | 15/10   | 29/25  | 0.63    |
| Age (years), <70/70 ≥    | 25/15   | 74/66  | 0.09    | 14/11   | 35/19  | 0.47    |
| Pathology, AD/others     | 21/19   | 94/46  | 0.96    | 6/19    | 9/45   | 0.54    |
| Stage, IIIA-C/VIA-B      | 11/29   | 38/102 | 0.48    | 5/25    | 18/28  | 0.17    |
| Driver genes             | 3/37    | 16/124 | 0.62    | 2/23    | 9/45   | 0.3     |
| PD-L1, 25% ≤/<25%        | 18/22   | 53/87  | 0.42    | 8/17    | 11/43  | 0.27    |
| irAE                     | 7/33    | 17/123 | 0.38    | 9/45    | 11/43  | 0.3     |

|                          | Present | Absent | P-value | Present | Absent | P-value |
|--------------------------|---------|--------|---------|---------|--------|---------|
| Number of patients       | 53      | 127    | 0.23    | 35      | 31/20 | 0.99    |
| Gender, female/male      | 8/45    | 31/96  | 0.99    | 45/8    | 107/20 | 0.99    |
| PS (ECOG), 0-1/2 ≥       | 35/18   | 64/63  | 0.06    | 31/22   | 84/43 | 0.33    |
| Age (years), <70/70 ≥    | 12/41   | 37/90  | 0.46    | 5/18    | 10/46 | 0.76    |
| Driver genes | 3/50 | 16/111 | 0.2 | Driver genes | 0/23 | 5/51 | 0.31 |
|--------------|------|--------|-----|--------------|------|------|------|
| PD-L1, 25%/<25% | 24/29 | 47/80 | 0.32 | PD-L1, 25%/<25% | 9/14 | 10/46 | 0.08 |
| irAE          | 9/44  | 15/112 | 0.35 | irAE         | 3/20 | 8/48  | 0.85 |

**Table 3.** Patient grouping and information of peripheral eosinophils

(A) 180 patients treated with ICPI monotherapy

| Patient group | I | II | III | P-value |
|---------------|---|----|-----|---------|
| Number of patients | 58 | 54 | 68 | |
| 5% or more within 6 weeks for the initiation of ICPI therapy | | | | |
| Present : Absent | 4/54 | 15/39 | 21/47 | 0.003 |
| 330/μL or more within 6 weeks for the initiation of ICPI therapy | | | | |
| Present : Absent | 12/46 | 15/39 | 26/42 | 0.099 |

(B) 79 patients treated with combination therapy with ICPI and chemotherapy

| Patient group | VI | V | P-value |
|---------------|----|--|---------|
| Number of patients | 19 | 60 | |
| 5% or more within 12 weeks for the initiation of ICPI therapy | | | |
| Present : Absent | 2/17 | 23/37 | 0.023 |
| 330/μL or more within 12 weeks for the initiation of ICPI therapy | | | |
| Present : Absent | 3/16 | 20/40 | 0.268 |

ICPI: immune checkpoint inhibitor
**Table 4.** Results of univariable and multivariable analysis in patients treated with ICPI monotherapy

|                          | (A) Eosinophil 5%≥within 6 weeks | (B) Eosinophil 330/μL≥within 6 weeks |
|--------------------------|-----------------------------------|-------------------------------------|
|                          | univariable analysis              | multivariable analysis              |
|                          | P-value                           | hazard ratio                        | 95% CI                | P-value |
| Gender: female           | 0.17                              | 1.837                               | 1.242 - 2.717         | 0.002   |
| Performance status (ECOG), 0-1 | 0.077                             | 1.559                               | 1.007 - 2.412         | 0.047   |
| Age, 70 years or younger | 0.56                              | 1.722                               | 1.115 - 2.660         | 0.014   |
| Pathology, adenocarcinoma | 0.42                              | 1.303                               | 0.929 - 1.826         | 0.125   |
| Stage, IIIA-C            | 0.41                              | 2.826                               | 1.680 - 4.754         | <0.001  |
| Driver genes, absent     | 0.12                              | 1.234                               | 0.930 - 1.831         | 0.12    |
| PD-L1, 25% or more       | 0.04                              | 1.234                               | 0.930 - 1.831         | 0.12    |
| irAE excluding ICPI discontinuation, present | <0.001                             | 2.826                               | 1.680 - 4.754         | <0.001  |
| Eosinophil 5%≥within 6 weeks | 0.003                             | 1.837                               | 1.242 - 2.717         | 0.002   |
| Eosinophil 330/μL≥within 6 weeks | 0.061                             | 1.471                               | 1.038 - 2.085         | 0.03    |

ICPI: immune checkpoint inhibitor, ECOG: Eastern Cooperative Oncology Group, PD-L1: programmed death-ligand-1, irAE: immune-related Adverse Event
Figure 1. TTF curves of 180 patients who received ICPI monotherapy. TTF curve with a cutoff of 5% of peripheral blood eosinophils (A), TTF curve with a cutoff of 330/μL of eosinophils (B). TTF curve was drawn by color-coding the period until the next administration according to “when the eosinophils were 5% or more (dark blue)” or not (gray). For the peripheral eosinophil count, TTF curve was created by color-coding the period until the next administration depending on the presence (dark blue) or absence (gray) of “eosinophil count of 330/μL or more”. Patients were divided into three groups; “no response group (group I: TTF≤6 weeks)”, “short-term response group (group II: 6 weeks<TTF≤24 weeks)”, and “long-term response group (group III: 24 weeks<TTF)”.
Figure 2. TTF curves of 79 patients who received combination therapy with ICPI and chemotherapy. TTF curve with a cutoff of 5% of peripheral blood eosinophils (A), TTF curve with a cutoff of 330/μL of eosinophils (B). Patients were divided into two groups, a “group IV (TTF ≤12 weeks)” and a “group V (12 weeks<TTF).” TTF curve was drawn by color-coding the period until the next administration according to “when the eosinophils were 5% or more (dark blue)” or not (gray). For the peripheral eosinophil count, TTF curve was created by color-coding the period until the next administration depending on the presence (dark blue) or absence (gray) of “eosinophil count of 330/μL or more”.