Am J Cancer Res 2024;14(10):5095-5104
www.ajcr.us /ISSN:2156-6976/ajcr0159447

Original Article

The ascendancy of eosinophil counts in non-small cell lung cancer: a potential marker for predicting response and survival under nivolumab treatment

Mehmet Fatih Ozbay[1], Aysegul Merc Cetinkaya[2], Onur Yazdan Balcik[3], Yusuf Ilhan[4], Tugrul Burak Genc[5], Sema Sezgin Goksu[2]

[1]Department of Medical Oncology, Kirsehir Training and Research Hospital, Kirsehir 40100, Turkey; [2]Department of Medical Oncology, Faculty of Medicine, Akdeniz University, Antalya 07000, Turkiye; [3]Alanya Alaattin Keykubat University, Department of Medical Oncology, Antalya, Türkiye; [4]Antalya City Hospital, Department of Medical Oncology, Antalya, Türkiye; [5]Department of Medical Oncology, Mus State Hospital, Mus 49250, Turkey

Received July 25, 2024; Accepted October 4, 2024; Epub October 25, 2024; Published October 30, 2024

Abstract: Lung cancer is the leading cause of cancer-related death globally and is often diagnosed at an advanced stage. Nivolumab represents a significant advancement for treating advanced non-small cell lung cancer (NSCLC). However, the absence of reliable biomarkers predicting treatment response hinders personalized therapy. Eosinophils play a notable role in cancer biology, particularly when treated with immune checkpoint inhibitors. Eosinophils can infiltrate tumor tissues, directly interacting with tumor cells or modifying the tumor microenvironment. This study aims to assess the potential of PD-L1 expression and peripheral blood eosinophil count in predicting treatment response and patient survival. This retrospective cohort study was conducted in three major cancer centers in Turkey, including 174 advanced NSCLC patients who had progressed after chemotherapy between July 2019 and November 2023. Demographic and clinical data, PD-L1 levels, and eosinophil counts were analyzed using SPSS 27.0. Survival analyses were performed with Kaplan-Meier and Cox regression models. Increased peripheral blood eosinophil count was positively associated with response to Nivolumab treatment and overall survival. Among treatment responders, 54.1% had eosinophil levels between 100-499 cells/mm^3 before treatment, increasing to 70.8% post-treatment. In patients with high PD-L1 positivity (>50%), eosinophil levels averaged 266.0 cells/mm^3, with improved survival outcomes (mean survival: 24.06 months, median: 20.0 months). Non-responders had a mean survival of 19.05 months and a median survival of 15.2 months. Peripheral eosinophil count appears to be a potential biomarker for predicting response to Nivolumab treatment and survival in NSCLC patients. Combined evaluation of eosinophil count and PD-L1 expression may enhance personalized treatment strategies. Further validation in prospective, randomized studies is necessary.

Keywords: Eosinophil, NSCLC, nivolumab, biomarker, survival, treatment

Introduction

Lung cancer is the most common cause of death from cancer worldwide, and non-small cell lung cancer (NSCLC) accounts for 80 to 85% of all cases. The diagnosis of NSCLC often occurs in advanced stages, which is the main reason for dismal prognosis of patients [1]. Recent advances in the treatment of advanced NSCLC, such as nivolumab, a humanised IgG4 PD-1 immune checkpoint inhibitor antibody that potently restores antitumour immunity and is approved for squamous or non-squamous histologies based on CheckMate 017 (squamus) and 057 (non-squamus), highlight how therapeutic interventions can result into significant lengthening OS [2-4].

While these results are exciting, finding biomarkers is important to help predict in which patients nivolumab will work better than other treatment options can inspire its tailored use and so ensure long-term tumour control while avoiding severe side effects of the treatment in non-responding patients. Besides, most research in predicting ICIs response is limited to

https://doi.org/10.62347/KRTH2276

tumour-specific factors (such as PD-L1 status and Tumour mutation burden). Given that ICIs do not have direct anti-tumour effect, as they are meant to be immune-enhancers relying on the patient's pre-existing immuneresponse, solely tumour-based biomarkers may not apply here. Thus, patient-specific characteristics including gender, diet and diversity of gut microbiome as well the immune system are becoming essential for prediction ICI response. Hence, a composite biomarker predictive of ICI effects that captures both tumor-related features as well as host-specific characteristics may in greater detail predict the efficacy of ICIs [5-10, 29, 30].

In the context of immuno-oncology, the role of eosinophils has attracted attention due to their multifaceted interactions in the tumor microenvironment, particularly in cases treated with immune checkpoint inhibitors (ICPI) such as Nivolumab. Traditionally known for their role in allergic responses and parasitic infections, eosinophils are gaining attention as mediators in antitumor immunity.

Eosinophils can infiltrate tumor tissues and influence tumor progression either through direct interaction with tumor cells or indirectly by altering the tumor microenvironment. One critical function of eosinophils in this context is their ability to release various chemokines and cytokines that recruit specific immune cells to the tumor site, including CD8+ T cells, which are crucial for the antitumor immune response [5, 6, 10].

In that line, a rise in peripheral blood eosinophils has been proposed as an early biomarker of enhanced survival with Nivolumab treatment for NSCLC patients. Peripheral blood eosinophilia to more than 500/mm^3 is considered as for diagnosis, and there are reports in the literature indicating that an increase of peripheral blood eosinophil count result favorable survival. Nevertheless, additional studies are required on this topic [11-13].

In this study, we sought to explore the correlation of peripheral eosinophilia along with PD-L1 levels and their combined prognostic effects in patients. Here we investigate the kinetics of eosinopenia, how it associates with reduced levels of PD-L1 and whether these changes influence responses Nivolumab-treated patients. This study could represent a fundamental step to know more about the relationship between eosinophils and PD-L1 levels, giving other important information on their relevance in NSCLC treatment (and perhaps as biomarkers) [14].

The relationship between nivolumab-associated eosinophilia and NSCLC treatment outcomes is an important area of ongoing research to understand the complexity of immunotherapy and to develop potential therapeutic strategies. Better understanding the relationship between eosinophilia and PD-L1 levels is critical to optimise treatment decisions and improve patient outcomes in NSCLC [15].

Materials and methods research design and participants

This retrospective cohort study included patients with de novo metastatic or recurrent NSCLC after surgery and radiotherapy. The study was conducted in three major cancer treatment centres in Turkey, during the period from July 2019 to November 2023. The study group generally consisted of adult patients with disease progression after at least one line of chemotherapy and negative for driver mutations such as EGFR, ALK, ROS1, BRAF, MET. Patients included in the study were selected especially those who met the eligibility criteria for PD-1 inhibitors such as nivolumab. Patients who were not suitable for immunotherapy or required intensive steroid treatment were excluded from this study.

Nivolumab treatment protocols

The standard protocol is to give each patient 3 mg/kg nivolumab dissolved in 150 cc saline by one-hour infusion. Treatment was adjusted based on approved treatment protocols and patient response to treatment.

Data collection procedures

To ensure consistency and accuracy in data collection across all participating centers, standardized protocols were strictly followed for both eosinophil counts and PD-L1 measurements. Eosinophil counts were obtained from

routine complete blood counts (CBC), using fully automated hematology analyzers. Calibration of these analyzers was regularly performed, and all centers adhered to a unified schedule for CBCs, which were collected at baseline (prior to Nivolumab initiation) and monthly throughout the course of treatment.

PD-L1 expression levels were determined using Immunohistochemistry (IHC) staining techniques, employing FDA-approved assay (SP263 clones). PD-L1 staining was performed on formalin-fixed, paraffin-embedded (FFPE) tissue samples. Staining was classified into three distinct categories based on the percentage of tumor cells showing membrane staining: <1%, 1-50%, and >50%. The interpretation of PD-L1 expression levels was conducted by experienced pathologists at each center, and inter-center variability was minimized by employing standardized scoring criteria.

In addition to eosinophil counts and PD-L1 measurements, baseline levels of leukocytes, lymphocytes, lactate dehydrogenase (LDH), albumin, and C-reactive protein (CRP) were measured before the start of Nivolumab treatment. These laboratory parameters were recorded to assess their potential prognostic value and to monitor patient responses during treatment. Furthermore, detailed records of metastasis sites and previous chemotherapy regimens were maintained to provide a comprehensive understanding of each patient's disease progression.

To ensure uniformity across centers, staff were thoroughly trained on handling assays, data collection procedures, and PD-L1 scoring. Importantly, all data collected from the centers were centralized and transferred to a single data repository. Individual centers did not conduct their own statistical analyses, thereby avoiding inter-center variability in data interpretation. Biomarker thresholds, such as eosinophil counts (>100 cells/mm^3, >500 cells/mm^3), were predefined based on established clinical literature and their prognostic relevance.

Adverse events and eosinophilia monitoring

Adverse events in the study were defined as nivolumab-related side effects and classified according to CTCAE version 5.0 criteria. Eosinophilia was defined as a condition characterised by a peripheral blood eosinophil count above 500 cells/mm^3. The potential effects of adverse events and eosinophilia on treatment outcomes were carefully analysed.

Statistical analysis methods

SPSS 27.0 software was used in data analyses. During the analyses, frequencies and percentages were used for categorical data and mean, standard deviation, minimum, maximum and median statistics were used for numerical data. The data distribution was tested for normality and non-parametric tests were applied for data that did not show normal distribution. Mann Whitney U test, Kruskal Wallis test, Chi-Square test, Spearman correlation analysis, ROC analysis and Kaplan-Meier survival analysis were used for statistical comparisons.

Ethical approval

The study was designed and conducted in accordance with internationally recognised principles of Good Clinical Practice and the Declaration of Helsinki. Ethical approval for the study was obtained from Akdeniz University Clinical Research Ethics Committee.

Findings participant profile

This study was performed on 174 patients with metastatic NSCLC who had progressed after at least one line of chemotherapy. The gender distribution of the study population was 86.8% male (n=151) and 13.2% female (n=23), showing trends parallel to general cancer demographics. Regarding the age distribution, 63.2% (n=110) were under 65 years of age and 36.8% (n=64) were 65 years of age or older, indicating that NSCLC is more common in the elderly population (**Table 1**).

Histological and clinical features

Adenocarcinoma accounted for 48.9% (n=85), squamous cell carcinoma 39.1% (n=68), unidentified origin (NOS) 11.5% (n=20) and mucinous carcinoma 0.6% (n=1). This histological distribution highlights the prevalence and diversity of adenocarcinoma in NSCLC patients. The most common localisation of metastasis was lung metastasis (60.3%, n=105), followed

Table 1. Distribution of demographic, clinical and adverse events

		n (%)
Gender	Erkek	151 (86.8)
	Female	23 (13.2)
Age	Under 65	110 (63.2)
	65 and over	64 (36.8)
Histology	Adenocarcinoma	85 (48.9)
	Squamous carcinoma	68 (39.1)
	Mucinous carcinoma	1 (0.6)
	NOS	20 (11.5)
ECOG	0	11 (6.3)
	1	129 (74.1)
	2	34 (19.5)
Hypothyroidism	No	151 (86.8)
	Yes	23 (13.2)
Hyperthyroidism	No	167 (96.5)
	Yes	6 (3.5)
Urticaria	No	160 (92.5)
	Yes	13 (7.5)
Colitis	No	170 (97.7)
	Yes	4 (2.3)
Pneumonitis	No	168 (96.6)
	Yes	6 (3.4)
Hepatitis	No	169 (97.7)
	Yes	4 (2.3)
Lung metastasis	No	69 (39.7)
	Yes	105 (60.3)
Bone metastasis	No	99 (56.9)
	Yes	75 (43.1)
Brain metastasis	No	132 (75.9)
	Yes	42 (24.1)
Liver metastasis	No	136 (78.2)
	Yes	38 (21.8)
PDL1	Negative	60 (34.5)
	1-50%	21 (12.1)
	Over 50%	11 (6.3)
	Unknown	82 (47.1)

by brain (24.1%, n=42), liver (21.8%, n=38) and bone (43.1%, n=75). These findings show how metastatic spread affects various organ systems. In the performance status assessment, the majority of patients (74.1%, n=129) were classified as ECOG performance status 1, indicating that patients started treatment in a generally good physical condition. The other performance status was ECOG 2 in 19.5% (n=34) and ECOG 0 in 6.3% (n=11) (**Table 1**).

Evaluation of the relationship between eosinophil levels and response

There is a statistically significant correlation between the response status of the patients and Eosinophil level (P=0.006<0.05) at the 6th month. The level is higher in patients with treatment response (75.0%). The relationship was not significant in other measurements (P>0.05) (**Table 2**).

Relationship between PDL1 status and eosinophil levels

PDL1 status was determined as unknown in 47.1% (n=82), negative in 34.5% (n=60), between 1-50% in 12.1% (n=21) and above 50% in 6.3% (n=11). There was a significant difference between the PDL1 status groups in terms of basal eosinophil measurement (P=0.036<0.05). Basal eosinophils (266.0 cells/mm^3) were found to be the highest in patients with PDL1 above 50%. Although the number of patients with PDL-1 level above 50% in the general cohort was 6.3%, it strongly suggests a correlation between basal eosinophilia and PDL-1 (**Table 3**).

Treatment related toxicities and eosinophil levels

The most common toxicity during nivolumab use was hypothyroidism (13.2%, n=23). Other toxicities such as hyperthyroidism, urticaria, colitis and pneumonitis were also recorded, with most toxicities being low grade (43.1% Grade 1, 27.6% Grade 2 and 13.8% Grade 3). Detailed analyses of eosinophil levels showed that baseline eosinophil levels were between 100-499 cells/mm^3 in 54.1% (n=94), less than 100 cells/mm^3 in 42.4% (n=74) and 500 cells/mm^3 or more in 3.5% (n=6). Starting from the first month, eosinophil levels generally increased and reached between 100-499 in 70.8% (n=123) patients at the 6th month. However, no correlation was observed between eosinophil levels and the frequency and severity of toxicity. When the change in eosinophil measurements of the patients over time is analysed, it shows a statistically significant difference (P=0.011). It is seen that eosinophils increased partially according to the measurement time (**Table 4**).

Table 2. Evaluation of the relationship between eosinophil levels and response

		Nivolumab best response		p
		None	Present	
Baseline eosinophil	<100	18 (58.1)	40 (37.7)	0.117
	100-499	12 (38.7)	62 (58.5)	
	≥500	1 (3.2)	4 (3.8)	
1st month eosinophil	<100	10 (38.5)	28 (27.5)	0.556
	100-499	15 (57.7)	70 (68.6)	
	≥500	1 (3.8)	4 (3.9)	
2nd month eosinophil	<100	10 (37)	28 (28)	0.655
	100-499	16 (59.3)	66 (66)	
	≥500	1 (3.7)	6 (6)	
3rd month eosinophil	<100	9 (47.4)	29 (29.3)	0.273
	100-499	10 (52.6)	67 (67.7)	
	≥500	0 (0)	3 (3)	
6th month eosinophil	<100	6 (75)	18 (20.5)	0.006*
	100-499	2 (25)	66 (75)	
	≥500	0 (0)	4 (4.5)	

Eosinophil levels are correlated with treatment response. *A statistically significant relationship was observed at the 6th month (P=0.006). Measurements in other months were not statistically significant (P>0.05).

ROC analysis results for response status

Basal Eosinophil measurement was a significant factor in predicting treatment response status (P=0.034). Cut-off value was calculated as 55 (ROC curve is given). According to this value, sensitivity was 81.1% and specificity was 41.9%. Eosinophil deltas calculated over the change of basal measurement and measurements in other months are not a significant factor in predicting treatment response status (P>0.05) (**Figure 1**).

Comparison of survival times and clinical measures

Significant positive correlations were found between baseline eosinophil levels and overall survival (OS) and progression-free survival (PFS) (OS for Responders: Mean 24.06 months (±16.31), Median 20.0 months Non-responders: Mean 19.05 months (±15.62), Median 15.2 months, *p* value =0.003). (For PFS, Responders: Mean 20.56 months (±9.99), Median 17.3 months Non-Responders: Mean 16.56 months (±16.95), Median 10.7 months). Significant differences were found in the number of immunotherapy (IO) cycles, basal lactate dehydrogenaz (LDH), basal albumin and monthly eosinophil levels between treatment responders and non-responders (lowest P= 0.000, highest P=0.048). In particular, the number of IO cycles was lower, basal LDH was higher and eosinophil levels showed less variability in patients who did not respond to treatment (**Table 4**).

Eosinophil basal levels were a statistically significant risk factor for treatment response (P=0.004). OS duration was highest in patients with eosinophil baseline levels of 100-499 (45.26 months). It was calculated as 29.04 months for <100 and 25.73 months for ≥500. The response rate was similarly highest in the 100-499 group (57.6%). It was calculated as 49.3% for <100 and 50.0% for ≥500 (**Figure 2**).

This large-scale data analysis allows us to gain an in-depth understanding of the side effect profile and efficacy of nivolumab treatment, as well as highlighting the role of potential biomarkers in predicting immunotherapy response in NSCLC patients.

Discussion

In the context of immuno-oncology, the role of eosinophils has attracted attention due to their multifaceted interactions in the tumour microenvironment, particularly in cases treated with immune checkpoint inhibitors (ICPI) such as Nivolumab. Traditionally known for their role in allergic responses and parasitic infections, eosinophils are gaining attention as mediators in antitumour immunity [16-19].

Eosinophils can infiltrate tumour tissues and influence tumour progression either through direct interaction with tumour cells or indirectly by altering the tumour microenvironment. One critical function of eosinophils in this context is their ability to release various chemokines and cytokines that recruit specific immune cells to the tumour site. More importantly, eosinophils can release chemical signals that specifically attract CD8+ T cells. These T cells are crucial for the antitumour immune response because

Table 3. Comparison of PDL-1 levels and eosinophil

	PDL1						p
	Negatif		1-50%		50% üstü		
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	
Baseline eosinophil	168.17±161.71	120.0	99.52±99.32	50.0	266±224.51	190.0	0.036*
1st month eosinophil	184.07±147.42	150.0	133.33±91.94	130.0	248.89±197.7	180.0	0.117
2nd month eosinophil	171.92±151.31	130.0	122.35±84.52	100.0	211±91.58	160.0	0.115
3rd month eosinophil	192.73±170.31	160.0	163.33±120.04	140.0	218.75±115.32	180.0	0.578
6th month eosinophil	240±179.07	210.0	208.18±152.7	220.0	218.33±123.84	225.0	0.845
Delta eosinophil 1-2	49.69±128.95	0.0	100.96±185.73	43.8	29.43±103.91	-5.9	0.748
Delta eosinophil 1-3	36.9±142.71	0.0	134.77±247.81	80.0	70.32±203.87	-6.1	0.278
Delta eosinophil 1-4	63.15±228.43	-11.7	142.11±265.17	27.3	121.63±242.36	6.3	0.420
Delta eosinophil 1-5	109.87±280.81	14.3	230.81±384.24	83.3	319.13±631.4	7.6	0.840

*P<0.05 significant difference, P>0.05 no significant difference; t/Mann Whitney test.

Table 4. Comparison of survival times and clinical measures

	Nivolumab best response				p
	None		Present		
	Mean ± SD	Median	Mean ± SD	Median	
Age	62.45±9.27	64.0	60.95±8.87	61.0	0.401
OS (months)	19.05±15.62	15.2	24.06±16.31	20.0	0.003*
PFS (months)	16.56±16.95	10.7	20.56±9.99	17.3	0.000*
Diagnosis-nivolumab start time (months)	12.34±15.27	7.0	11.29±12.22	7.8	0.939
Number of nivolumab courses	7.48±8.3	6.0	21.02±14.34	18.0	0.000*
Baseline LDH	262.19±90.9	252.5	217.6±82.03	201.0	0.001*
Baseline albumin	4.02±0.38	4.1	4.1±0.44	4.1	0.356
Baseline corrected calcium	9.31±0.67	9.3	9.37±0.5	9.4	0.626
Baseline CRP	38.78±44.44	26.0	29.88±37	13.5	0.263
Baseline hemoglobin	11.62±1.42	11.5	11.79±1.77	11.5	0.609
Baseline WBC	9224.38±4022.14	8325.0	8320.84±3316.9	8060.0	0.201
Baseline lymphocyte	1406.25±623.91	1375.0	1890.89±995.4	1730.0	0.018*
Baseline neutrophil	6738.13±3706.77	5820.0	5512.34±2795.76	5000.0	0.055
Baseline platelet	279078.13±124627.58	258500.0	314876.64±135803.24	290000.0	0.119
Baseline eosinophil	134.52±161.3	90.0	179.43±147.69	140.0	0.034*
1st month eosinophil	205.77±173.23	180.0	188.09±137.14	160.0	0.632
2nd month eosinophil	164.07±142.75	120.0	209.25±221.65	160.0	0.283
3rd month eosinophil	144.21±133.55	100.0	185.66±152.52	150.0	0.184
6th month eosinophil	118.75±160.04	40.0	229.43±160.5	200.0	0.026*
Highest eosinophil	260.74±220.51	230.0	373.27±322.59	320.0	0.066

*P<0.05 significant difference, P>0.05 no significant difference; t/Mann Whitney test. A statistically significant relationship was found between baseline eosinophil levels and treatment response (P=0.034). Eosinophil levels measured at the 6th month were also significantly associated with treatment response (P=0.026).

of their ability to directly kill tumour cells [20-23].

The relationship between eosinophil increase and survival with the use of Nivolumab in non-small cell lung cancer is still under investigation. Several studies have shown that eosinophil levels may be a potential biomarker for predicting the efficacy of immune checkpoint inhibitor therapy, including Nivolumab [24-26].

Of particular interest are findings that non-small cell lung cancer (NSCLC) patients with higher eosinophil counts during immune checkpoint inhibitor (ICPI) therapy show better therapeutic efficacy and longer overall survival.

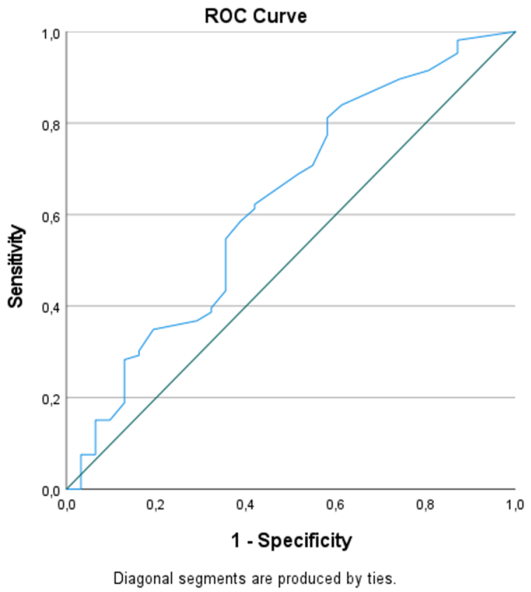

Figure 1. ROC analysis for response status.

Studies by Hu et al. (2023) and Carretero et al. (2015) indicate that eosinophil percentages above 5% during ICPI treatment are an important determinant of therapeutic efficacy. This suggests that eosinophils may play a critical role not only in the immune response against tumours, but also as potential biomarkers to measure the efficacy of treatment strategies [6, 14].

Our study reports the peripheral blood eosinophilia level, its relationship with PDL-1 level and survival analyses in non-small cell lung cancer patients receiving Nivolumab. In the literature, 500 cells/mm^3 is frequently used to define eosinophilia. In a previous study including 909 patients receiving immunotherapy, the incidence of peripheral eosinophilia was reported as 2.8% [9, 25]. This is consistent with the rate of 3.5% reported by us. However, the patients in this study included many advanced cancer types. The patient group with peripheral eosinophilia also consisted of melanoma and renal cell carcinoma patients. The patients in our study included non-small cell lung cancer patients. Analysing data from the French pharmacovigilance database, Scanvion et al. found that 37 (2.4%) of 1546 patients treated with ICIs had eosinophilia (≥1000) [26]. In studies involving a similar patient group, it is possible to see the peripheral eosinophil rate as 3%-27.3%. When we look at the study by Alves et al., which found the rate of peripheral eosinophilia to be 27.3%, we see that approximately 30% of these patients were treatment naive and the immunotherapy agents used were different (Pembrolizumab, Atezolizumab and Nivolumab). In our population, all patients had received at least one line of chemotherapy and nivolumab was used in all patients [15, 26]. We think that this difference in the rate of eosinophilia may be due to the lower production capacity in our patient group receiving chemotherapy due to the bone marrow suppressive effect of chemotherapy. At the same time, it should be taken into consideration that different ICIs used may have different effects on eosinophil levels.

In our study, a statistically significant positive correlation was found between basal eosinophil levels and treatment response in our population. A statistically significant correlation was also found between eosinophil levels measured at the sixth month and treatment response (P=0.006). In patients who responded to treatment, this level was recorded to be 75% higher.

In addition, significant differences were observed in OS, PFS, number of IO cures, baseline LDH, baseline and six-month eosinophil levels between patients with and without treatment response. Statistical analyses showed that OS, PFS, number of IO cycles, baseline lymphocyte and eosinophil levels were higher in treatment responders compared to non-responders; however, baseline LDH levels were lower. In particular, the mean OS was 24. months versus 19. months, PFS was 20.5 months versus 16.5 months, and OS cure rate was 21 versus 7.4 in patients with treatment response.

The results are consistent with the existing literature; however, we did not find a relationship between increased eosinophilia and adverse events. Previous studies have reported that increased side effects, particularly pruritus and hypothyroidism, were associated with survival. We believe that this discrepancy may be due to the retrospective nature of our study.

Baseline eosinophil measurement was a significant factor in predicting treatment response status (P=0.034). ROC curve analysis showed that the cut-off values for patients with controlled disease and patients with progressive disease were 55/cell/mm^3 eosinophil count. According to this value, sensitivity was 81.1% and specificity was 41.9%. Eosinophil deltas calculated from the change between the basal

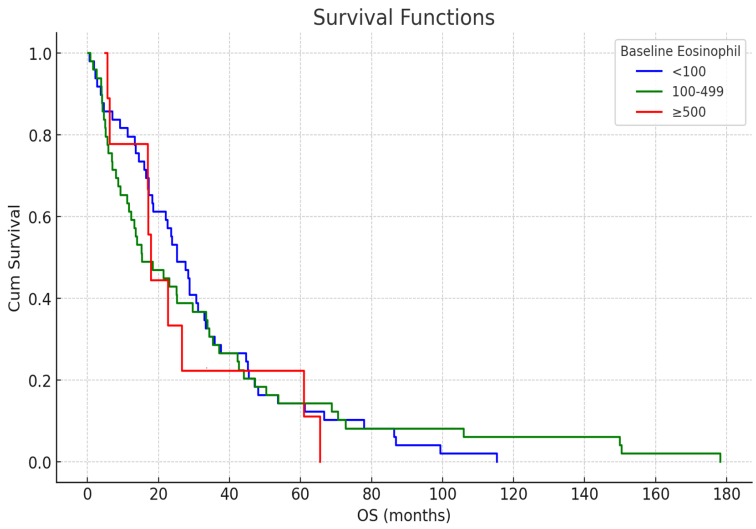

Figure 2. Kaplan Meier test results.

measurement and the measurements in other months were not a significant factor in predicting the treatment response status (P>0.05). Eosinophil increases, especially in the 2nd and 3rd months, were significantly higher than the baseline values. In their study, Okauchi et al. determined the maximum level as the 5th week after starting ICPI treatment. This period seems to be similar to our study. In the aforementioned study, they obtained 330/cell/mm^3 as eosinophil cut-off value, but when we look at the study cohort, we see that the patients used different ICPIs and did not receive chemotherapy before, which may explain the better bone marrow response and higher eosinophil levels [12, 24, 27, 28].

Our study also focused on the relationship between eosinophil levels and PDL-1, which is different from previous similar studies. Interestingly, there was a significant difference between the PDL1 status groups in terms of basal eosinophil (P=0.036<0.05) measurement. Basal eosinophil (266.0) measurement was higher in patients with PDL1 above 50%. This suggests that evaluation of peripheral basal eosinophil level together with PDL-1 level may be a predictive marker and may better predict the treatment outcome in these patients.

Our findings underscore the importance of eosinophil counts as potential biomarkers for predicting treatment response and survival outcomes in non-small cell lung cancer (NSCLC) patients undergoing Nivolumab treatment. The observed association between eosinophil increase and improved survival with immune checkpoint inhibitors (ICPI) suggests that eosinophils may serve as a reliable predictive marker. However, as this association is still under investigation, prospective validation studies are needed to confirm the role of baseline eosinophil counts in predicting responses to Nivolumab, across different cancer types and stages. Such studies should not only focus on verifying this predictive role but also investigate the kinetics of eosinophil levels at multiple treatment stages.

Future multicenter studies with larger sample sizes are essential to explore the interplay between eosinophil counts and other biomarkers, such as PD-L1 levels, to further clarify their combined effect on immunotherapy outcomes. Furthermore, research into the molecular mechanisms behind eosinophil activation during ICPI therapy could provide insights into novel therapeutic targets.

Beyond merely validating eosinophil counts as a biomarker, there is potential for investigating therapeutic interventions aimed at modulating eosinophil levels, either through targeted therapies or combination regimens with immune checkpoint inhibitors. Such studies could reveal new strategies for enhancing the efficacy of immunotherapy in NSCLC patients. Additionally, integrating eosinophil counts into clinical decision-making tools could improve personalized treatment plans by allowing clinicians to stratify patients based on their likelihood of response to immunotherapy, thereby optimizing outcomes.

While our study offers valuable insights into the role of eosinophils as biomarkers, several limitations should be noted. As the study was retrospective, causal associations are difficult to confirm due to the possibility of bias and confounding factors inherent in pre-planned data collection and intervention strategies. The small sample size may also limit the generalizability of the findings, and the complex nature of immunotherapy response variability may not

be fully explained by simple biomarkers like eosinophils. Therefore, future prospective, multicenter studies with larger cohorts are crucial to overcome these limitations and better understand how eosinophil counts can guide clinical decision-making in NSCLC patients.

In summary, our study suggests that the increase in eosinophil counts during Nivolumab treatment holds significant promise as a predictive biomarker in NSCLC patients. The potential of eosinophils as a biomarker is further enhanced when combined with PD-L1 levels, providing a more robust framework for predicting therapeutic response and overall survival. However, further extensive studies are required to establish the reproducibility and reliability of these findings. Additionally, prospective research should focus on integrating eosinophil levels into clinical decision-making tools, improving personalized treatment strategies, and investigating therapeutic options to modulate eosinophil levels for better immunotherapy outcomes.

Disclosure of conflict of interest

None.

Address correspondence to: Mehmet Fatih Ozbay, Department of Medical Oncology, Kirsehir Training and Research Hospital, Kirsehir 40100, Turkey. E-mail: mfozbay@hotmail.com

References

[1] Adam T, Becker TM, Chua W, Bray V and Roberts TL. The multiple potential biomarkers for predicting immunotherapy response-finding the needle in the haystack. Cancers (Basel) 2021; 13: 277.

[2] Basudan AM. The role of immune checkpoint inhibitors in cancer therapy. Clin Pract 2022; 13: 22-40.

[3] Bernard-Tessier A, Jeanville P, Champiat S, Lazarovici J, Voisin AL, Mateus C, Lambotte O, Annereau M and Michot JM. Immune-related eosinophilia induced by anti-programmed death 1 or death-ligand 1 antibodies. Eur J Cancer 2017; 81: 135-137.

[4] Conforti F, Pala L, Bagnardi V, De Pas T, Martinetti M, Viale G, Gelber RD and Goldhirsch A. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. Lancet Oncol 2018; 19: 737-746.

[5] Carbone DP, Gandara DR, Antonia SJ, Zielinski C and Paz-Ares L. Non-small-cell lung cancer: role of the immune system and potential for immunotherapy. J Thorac Oncol 2015; 10: 974-984.

[6] Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P and Hämmerling GJ. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. Nat Immunol 2015; 16: 609-617.

[7] Cortinovis DL, Canova S, Abbate M, Colonese F and Bidoli P. Focus on nivolumab in NSCLC. Front Med (Lausanne) 2016; 3: 67.

[8] Davis BP and Rothenberg ME. Eosinophils and cancer. Cancer Immunol Res 2014; 2: 1-8.

[9] Eosinophil. In: Encyclopedia of Immunology. 2nd edition. Springer; 2008. pp. 995-1000.

[10] O'Sullivan JA and Bochner BS. Eosinophils and eosinophil-associated diseases: an update. J Allergy Clin Immunol 2018; 141: 505-517.

[11] Landolina NA and Levi-Schaffer F. Eosinophils as a pharmacological target for the treatment of allergic diseases. Curr Opin Pharmacol 2014; 17: 71-80.

[12] Facchinetti F, Veneziani M, Buti S, Gelsomino F, Squadrilli A, Bordi P, Bersanelli M, Cosenza A, Ferri L, Rapacchi E, Mazzaschi G, Leonardi F, Quaini F, Ardizzoni A, Missale G and Tiseo M. Clinical and hematologic parameters address the outcomes of non-small-cell lung cancer patients treated with nivolumab. Immunotherapy 2018; 10: 681-694.

[13] Ghaffari S and Rezaei N. Eosinophils in the tumor microenvironment: implications for cancer immunotherapy. J Transl Med 2023; 21: 551.

[14] Hu WT, Zhang Q, Zhang Z, He X, Zhou M, Guo Y and Wang X. Eosinophil and IFN-γ associated with immune-related adverse events as prognostic markers in patients with non-small cell lung cancer treated with immunotherapy. Front Immunol 2023; 14: 1112409.

[15] Jung CY and Antonia SJ. Tumor immunology and immune checkpoint inhibitors in non-small cell lung cancer. Tuberc Respir Dis (Seoul) 2018; 81: 29-41.

[16] Jung YJ, Woo SY, Jang MH, Miyasaka M, Ryu KH, Park HK and Seoh JY. Human eosinophils show chemotaxis to lymphoid chemokines and exhibit antigen-presenting-cell-like properties upon stimulation with IFN-γ, IL-3 and GM-CSF. Int Arch Allergy Immunol 2008; 146: 227-234.

[17] Kalantari Khandani N, Ghahremanloo A and Hashemy SI. Role of tumor microenvironment in the regulation of PD-L1: a novel role in resistance to cancer immunotherapy. J Cell Physiol 2020; 235: 6496-6506.

[18] Legrand F, Driss V, Delbeke M, Loiseau S, Hermann E, Dombrowicz D and Capron M. Human eosinophils exert TNF-α and granzyme a-mediated tumoricidal activity toward colon carcinoma cells. J Immunol 2010; 185: 7443-7451.

[19] Leiter A, Veluswamy RR and Wisnivesky JP. The global burden of lung cancer: current status and future trends. Nat Rev Clin Oncol 2023; 20: 624-639.
[20] Lim JS and Soo RA. Nivolumab in the treatment of metastatic squamous non-small cell lung cancer: a review of the evidence. Ther Adv Respir Dis 2016; 10: 444-454.
[21] de Mello RA, Veloso AF, Esrom Catarina P, Nadine S and Antoniou G. Potential role of immunotherapy in advanced non-small-cell lung cancer. Onco Targets Ther 2016; 10: 21-30.
[22] Minton K. Granulocytes: eosinophils enable the antitumour T cell response. Nat Rev Immunol 2015; 15: 333.
[23] Nishikawa D, Suzuki H, Beppu S, Terada H, Sawabe M, Kadowaki S, Sone M and Hanai N. Eosinophil prognostic scores for patients with head and neck squamous cell carcinoma treated with nivolumab. Cancer Sci 2021; 112: 339-346.
[24] Okauchi S, Shiozawa T, Miyazaki K, Nishino K, Sasatani Y, Ohara G, Kagohashi K, Sato S, Kodama T, Satoh H and Hizawa N. 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.
[25] Prizment AE, Vierkant RA, Smyrk TC, Tillmans LS, Lee JJ, Sriramarao P, Nelson HH, Lynch CF, Thibodeau SN, Church TR, Cerhan JR, Anderson KE and Limburg PJ. Tumor eosinophil infiltration and improved survival of colorectal cancer patients: Iowa women's health study. Mod Pathol 2016; 29: 516-527.
[26] Scanvion Q, Béné J, Gautier S, Grandvuillemin A, Le Beller C, Chenaf C, Etienne N, Brousseau S, Cortot AB, Mortier L, Staumont-Sallé D, Morschhauser F, Forestier A, Groh M, Launay D, Hachulla É, Labalette M, Kahn JE and Lefèvre G. Moderate-to-severe eosinophilia induced by treatment with immune checkpoint inhibitors: 37 cases from a national reference center for hypereosinophilic syndromes and the French pharmacovigilance database. Oncoimmunology 2020; 9: 1722022.
[27] Sundar R, Cho BC, Brahmer JR and Soo RA. Nivolumab in NSCLC: Latest evidence and clinical potential. Ther Adv Med Oncol 2015; 7: 85-96.
[28] Tanizaki J, Haratani K, Hayashi H, Chiba Y, Nakamura Y, Yonesaka K, Kudo K, Kaneda H, Hasegawa Y, Tanaka K, Takeda M, Ito A and Nakagawa K. Peripheral blood biomarkers associated with clinical outcome in non-small cell lung cancer patients treated with nivolumab. J Thorac Oncol 2018; 13: 97-105.
[29] Wendlinger S, Wohlfarth J, Kreft S, Siedel C, Kilian T, Dischinger U, Heppt MV, Wistuba-Hamprecht K, Meier F, Goebeler M, Schadendorf D, Gesierich A, Kosnopfel C and Schilling B. Blood eosinophils are associated with efficacy of targeted therapy in patients with advanced melanoma. Cancers (Basel) 2022; 14: 2294.
[30] Yang G, Jian SQ, Cao H, Wen C, Hu B, Peng M, Li P, Yuan J and Liang L. Changes in microbiota along the intestine of grass carp (ctenopharyngodon idella): community, interspecific interactions, and functions. Aquaculture 2019; 504: 290-302.