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Research Article

On-treatment dNLR and metastatic burden predict survival in advanced non-small-cell lung cancer treated with nivolumab

Nivolumab ile tedavi edilen ileri evre küçük hücreli dışı akciğer kanserinde tedavi sırasındaki dNLR ve metastatik yük sağkalımı öngörür

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Abstract

Aim: Despite the survival benefit achieved with immune checkpoint inhibitors in advanced non–small-cell lung cancer (NSCLC), outcomes remain heterogeneous. While PD-L1 expression is an established biomarker, less is known about the prognostic relevance of on-treatment systemic inflammatory dynamics and the utility of endothelial stress–based indices such as the Endothelial Activation and Stress Index (EASIX) in patients receiving immunotherapy.

Material and Methods: We retrospectively evaluated patients with advanced NSCLC treated with nivolumab. PD-L1 expression, metastatic burden, baseline inflammatory parameters, and baseline EASIX were recorded. On-treatment inflammatory markers, including derived neutrophil-to-lymphocyte ratio (dNLR) at 3 months and longitudinal changes in NLR (ΔNLR), were analyzed. Overall survival (OS) and progression-free survival (PFS) were assessed using Kaplan–Meier and Cox regression models. To account for time-dependent bias, 3-month landmark analyses were performed.

Results: Eighty-one patients were included. High PD-L1 expression (≥50%) was associated with significantly improved OS but did not retain independent prognostic significance for PFS. A high metastatic burden, particularly involvement of four or more metastatic organs, was associated with inferior OS. In landmark-adjusted analyses, dNLR at 3 months was independently associated with both OS and PFS, while ΔNLR was associated with OS, indicating that favorable on-treatment immune–inflammatory changes correlated with improved survival. Baseline EASIX was not associated with either OS or PFS.

Conclusion: In nivolumab-treated advanced NSCLC, survival outcomes are primarily driven by tumor biology, disease burden, and immune–inflammatory dynamics evolving during therapy. On-treatment inflammatory markers, particularly dNLR and ΔNLR, provide more clinically relevant prognostic information than baseline indices, whereas baseline EASIX appears to have limited utility in this setting. These findings support a treatment-specific, time-dependent approach to biomarker-based risk stratification in immunotherapy.

Keywords: non-small-cell lung cancer, nivolumab, dNLR, immune checkpoint inhibitors, metastatic burden, easix, systemicinflammation, survival analysis, immunotherapy

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Öz

Amaç: İleri evre küçük hücreli dışı akciğer kanserinde (KHDAK) immün kontrol noktası inhibitörleri ile sağkalım avantajı elde edilmesine rağmen, sonuçlar heterojen kalmaktadır. PD-L1 ekspresyonu yerleşik bir biyobelirteç olsa da, tedavi sırasındaki sistemik inflamatuar dinamiklerin prognostik önemi ve immünoterapi alan hastalarda Endotel Aktivasyon ve Stres İndeksi (EASIX) gibi endotel stres tabanlı indekslerin kullanımı hakkında daha az bilgi mevcuttur.

Gereç ve Yöntemler: Nivolumab ile tedavi edilen ileri evre KHDAK'li hastalar retrospektif olarak değerlendirildi. PD-L1 ekspresyonu, metastatik yük, bazal inflamatuar parametreler ve bazal EASIX kaydedildi. 3. aydaki türetilmiş nötrofil-lenfosit oranı (dNLR) ve NLR'deki boylamsal değişimler (ΔNLR) dahil olmak üzere tedavi sırasındaki inflamatuar belirteçler analiz edildi. Genel sağkalım (OS) ve progresyonsuz sağkalım (PFS), Kaplan–Meier ve Cox regresyon modelleri kullanılarak değerlendirildi. Zaman bağımlı yanlılığı (time-dependent bias) önlemek amacıyla 3. ay landmark (sınır değer) analizleri yapıldı.

Bulgular: Çalışmaya 81 hasta dahil edildi. Yüksek PD-L1 ekspresyonu (≥%50), OS'de anlamlı iyileşme ile ilişkili bulundu ancak PFS için bağımsız prognostik önemi korunmadı. Özellikle dört veya daha fazla metastatik organ tutulumu başta olmak üzere yüksek metastatik yük, daha kötü OS ile ilişkilendirildi. Landmark düzeltmeli analizlerde, 3. aydaki dNLR hem OS hem de PFS ile bağımsız olarak ilişkili bulunurken; ΔNLR'nin OS ile ilişkili olması, tedavi sırasındaki olumlu immün-inflamatuar değişikliklerin sağkalım iyileşmesiyle korele olduğunu gösterdi. Bazal EASIX skorunun ise OS veya PFS ile ilişkisi saptanmadı.

Sonuç: Nivolumab alan ileri evre KHDAK'de sağkalım sonuçları; temel olarak tümör biyolojisi, hastalık yükü ve tedavi sırasında gelişen immün-inflamatuar dinamikler tarafından yönlendirilmektedir. Tedavi sırasındaki inflamatuar belirteçler, özellikle dNLR ve ΔNLR, bazal indekslerden daha klinik olarak ilgili prognostik bilgiler sağlarken; bazal EASIX'in bu ortamda sınırlı kullanışlılığa sahip olduğu görülmektedir. Bu bulgular, immünoterapide biyobelirteç temelli risk sınıflamasında tedaviye özgü ve zamana bağımlı bir yaklaşımı desteklemektedir.

Anahtar Kelimeler: küçük hücreli dışı akciğer kanseri, nivolumab, dNLR, immün kontrol noktası inhibitörleri, metastatik yük, easix, sistemik inflamasyon, sağkalım analizi, immünoterapi

Introduction

The introduction of immune checkpoint inhibitors has reshaped the therapeutic landscape of advanced non–small-cell lung cancer (NSCLC). Despite clear survival gains at the population level, individual patient outcomes remain highly variable, with only a subset deriving durable clinical benefit from PD-1 blockade [1,2]. While a subset of patients achieves long-term disease control with PD-1 blockade, a considerable proportion experiences primary resistance or early disease progression despite treatment [1,3]. Tumor PD-L1 expression is currently the most widely used biomarker in clinical practice, yet its ability to consistently predict outcomes across treatment lines remains limited and does not fully account for host-related determinants of response [2,4]. These observations emphasize the need for additional biomarkers that reflect systemic and treatment-related biological processes to improve prognostic stratification in patients receiving immunotherapy [4].

Systemic inflammation plays a central role in modulating the efficacy of immune checkpoint inhibitors by shaping the interaction between tumor biology and host immune status [5]. Inflammation-based indices derived from routine blood parameters, particularly the neutrophil-to-lymphocyte ratio (NLR) and derived NLR (dNLR), have been shown to be associated with survival outcomes in patients with advanced non–small-cell lung cancer treated with PD-1 inhibitors [6]. Importantly, emerging evidence indicates that inflammatory markers assessed during treatment, rather than at baseline, provide more meaningful prognostic information by capturing early immune modulation induced by immunotherapy [7].

Despite increasing interest in host-related biomarkers, the comparative prognostic value of static baseline indices versus early on-treatment immune–inflammatory changes remains insufficiently defined in patients receiving immune checkpoint inhibitors [3]. In particular, it is unclear whether baseline composite scores reflecting systemic stress provide clinically meaningful prognostic information under PD-1 blockade, or whether biomarkers captured during treatment better reflect the evolving host–tumor interaction [8]. Therefore, the present study aimed to assess the prognostic relevance of baseline EASIX alongside key clinical characteristics and to compare baseline measures with on-treatment immune–inflammatory markers in patients with advanced non–small-cell lung cancer treated with nivolumab.

Material and Methods

This retrospective cohort study included patients with advanced or metastatic non–small-cell lung cancer who received nivolumab therapy at a single tertiary oncology center. Clinical, radiologic, pathologic, and laboratory data were retrospectively collected from electronic medical records. The index date ("time zero") for all survival analyses was defined as the date of nivolumab initiation, representing the onset of treatment-related biological effects relevant to outcome assessment. Patients without a confirmed nivolumab start date or with insufficient survival follow-up were excluded.

The initial study population consisted of 81 patients treated with nivolumab. For analyses incorporating on-treatment biomarkers, a predefined 3-month landmark approach was applied. Only patients who were alive and progression-free at 3 months after nivolumab initiation were included in these analyses to minimize time-dependent bias. Among these patients, analyses involving tumor PD-L1 expression were limited to cases with evaluable PD-L1 status. Tumor PD-L1 expression was assessed by immunohistochemistry on available formalin-fixed, paraffin-embedded tumor tissue samples using the VENTANA PD-L1 (SP263) assay (Roche Diagnostics), according to the manufacturer's instructions and institutional pathology protocols. PD-L1 expression was quantified using the tumor proportion score (TPS), defined as the percentage of viable tumor cells showing partial or complete membranous staining, irrespective of staining intensity. Only tumor cell membranous staining (either partial or diffuse) was considered for TPS calculation; cytoplasmic staining was not taken into account. PD-L1 expression on immune cells, including tumor-infiltrating lymphocytes and macrophages, was not evaluated. For analytical purposes, PD-L1 expression was categorized according to established clinical thresholds as TPS <1% (PD-L1 negative), TPS 1–49% (low/intermediate expression), and TPS ≥50% (high PD-L1 expression). In primary survival analyses, PD-L1 status was dichotomized using a cutoff of TPS ≥50% versus <50%, consistent with pivotal clinical trials of anti–PD-1 therapy.

Baseline (pre-treatment) and 3-month on-treatment laboratory parameters were obtained from routine blood tests. Inflammation-based indices were calculated using standard definitions, including the neutrophil-to-lymphocyte ratio (NLR), derived NLR (dNLR), platelet-to-lymphocyte ratio (PLR), and systemic immune–inflammation index (SII). For landmark analyses, dNLR was dichotomized using a cutoff of 3.0, consistent with commonly applied thresholds in prior studies of immune checkpoint inhibitors. The prognostic nutritional index (PNI) was calculated using serum albumin concentration and peripheral lymphocyte count. The Endothelial Activation and Stress Index (EASIX) was calculated using the formula lactate dehydrogenase × serum creatinine / platelet count. Dynamic biomarkers (ΔNLR, ΔPNI, and ΔEASIX) were defined as the difference between 3-month and baseline values. Renal function at 3 months was expressed as the natural logarithm of the estimated glomerular filtration rate (ln[eGFR]). The number of metastatic organs present at nivolumab initiation was recorded as a measure of baseline metastatic burden.

Overall survival (OS) was defined as the interval from nivolumab initiation to death from any cause. Patients who were alive at the time of last clinical follow-up were censored. Progression-free survival (PFS) was defined as the time from nivolumab initiation to radiologic or clinical disease progression or death, whichever occurred first. Disease progression was determined according to routine clinical and radiologic assessments performed during follow-up.

The study protocol was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval number: E-60116787-020-776895, date: 10 November 2025). Due to the retrospective nature of the study, the requirement for informed consent was waived.

Statistical Analysis

Overall survival and progression-free survival were estimated using the Kaplan–Meier method and compared using the log-rank test. Cox proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals. Multivariable Cox models were constructed to assess the independent prognostic value of baseline clinical characteristics, metastatic burden, and selected inflammatory and nutritional biomarkers. For analyses incorporating on-treatment biomarkers, a 3-month landmark approach was applied, restricting the population to patients who were alive and progression-free at 3 months after nivolumab initiation. The proportional hazards assumption was evaluated using Schoenfeld residuals. Model performance was assessed using the concordance index and likelihood ratio statistics. All analyses were conducted using complete-case data, and statistical significance was defined as a two-sided p value <0.05.

Results

A total of 81 patients treated with nivolumab were included in the analysis. The cohort was predominantly male (73 patients, 90.1%), and most patients were ever smokers (73 patients, 90.1%), while 8 patients (9.9%) had never smoked. Histologically, 44 patients (54.3%) had squamous cell carcinoma and 36 patients (44.4%) had adenocarcinoma; 1 patient (1.2%) had another histologic subtype.

With respect to functional status, 64 patients (79.0%) had an ECOG performance status of 0–1, whereas 17 patients (21.0%) had ECOG ≥2. Baseline demographic and clinical characteristics of the study population are summarized in Table 1. PD-L1 expression was assessable in 56 of 81 patients (69.1%), while 25 patients (30.9%) did not have available PD-L1 results. Among patients with evaluable PD-L1 status, 24 patients (42.9%) demonstrated high PD-L1 expression (≥50%), whereas 32 patients (57.1%) had PD-L1 <50% or negative expression. The median follow-up duration was 13.1 months (399 days).

Table 1. Baseline demographic and clinical characteristics of the study population (n = 81)

Characteristic	Value
Age at diagnosis, years, median (IQR)	65.0 (58.4–69.3)
Sex, n (%)	
Male	73 (90.1)
Female	8 (9.9)
Smoking status, n (%)	
Never	8 (9.9)
Ever	73 (90.1)
Histology, n (%)	
Adenocarcinoma	36 (44.4)
Squamous cell carcinoma	44 (54.3)
Other	1 (1.2)
ECOG performance status, n (%)	
0–1	64 (79.0)
≥2	17 (21.0)
PD-L1 status, n (%)	
Assessed	56 (69.1)
Not assessed	25 (30.9)
PD-L1 expression among assessed patients (n = 56), n (%)	
≥50%	24 (42.9)
<50% or negative	32 (57.1)

Overall Survival According to PD-L1 Expression

Kaplan–Meier analysis revealed a significant association between PD-L1 expression and overall survival. Patients with PD-L1 ≥50% experienced significantly longer overall survival compared with those with PD-L1 <50% (log-rank p = 0.005, Figure 1). Median overall survival was 12.8 months (95% CI 12.5–23.0) in the PD-L1 <50% group, whereas the median overall survival was not reached in the PD-L1 ≥50% group due to a lower number of death events during follow-up. Survival probabilities differed markedly between the two groups. Twelve-month overall survival rates were 63.7% in patients with PD-L1 <50% and 90.0% in those with PD-L1 ≥50%. At 36 months, overall survival declined to 14.8% in the PD-L1 <50% group, while remaining 58.6% among patients with PD-L1 ≥50%, representing a more than four-fold difference in long-term survival probability (Figure 1).

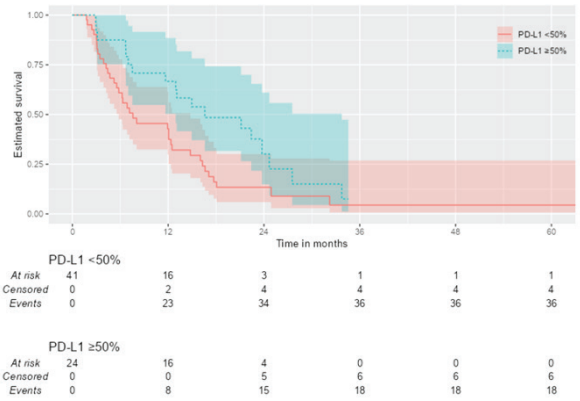

Figure 1. Overall Survival by Tumor PD-L1 Expression.

Kaplan–Meier curves showing overall survival (OS) stratified by tumor PD-L1 expression using a cutoff of 50% (PD-L1 <50% vs ≥50%). Overall survival was calculated from the initiation of nivolumab therapy to death from any cause. Patients with PD-L1 expression ≥50% demonstrated significantly longer overall survival compared with those with PD-L1 <50% (log-rank p = 0.005). Shaded areas represent 95% confidence intervals. Numbers at risk, censored observations, and events are displayed below the plot.

In univariable Cox proportional hazards analysis, high PD-L1 expression (≥50%) was associated with a significantly reduced risk of death (HR 0.33, 95% CI 0.14–0.75; p = 0.008). The model demonstrated moderate discriminatory ability (concordance index 0.628) and was statistically significant based on the likelihood ratio test ($\chi^2 = 8.48$, p = 0.004).

Multivariable Analysis of Overall Survival

When PD-L1 expression was evaluated alongside metastatic burden and immunologic and nutritional biomarkers in multivariable Cox models, several variables retained independent prognostic significance. Patients with involvement of four metastatic organs demonstrated substantially worse overall survival (HR 8.87, 95% CI 1.11–70.67; p = 0.039), whereas patients with one, two, or three metastatic

organs did not differ significantly from the reference group.

dNLR at 3 months emerged as a strong independent predictor of mortality (HR 1.88, 95% CI 1.30–2.71; p = 0.001). In contrast, baseline prognostic nutritional index (PNI) was independently associated with improved survival (p = 0.002). In addition, ΔNLR was independently associated with a reduced risk of death (HR 0.85, 95% CI 0.76–0.96; p = 0.007), suggesting that favorable immunologic remodeling during nivolumab therapy carries important prognostic relevance.

Baseline EASIX score was not significantly associated with overall survival in either univariable or multivariable Cox regression analyses. When analyzed as a continuous log-transformed variable, baseline EASIX showed no significant association with overall survival (HR 1.06, 95% CI 0.77–1.50; p = 0.741).

Other baseline inflammatory indices did not retain independent associations with overall survival after multivariable adjustment, supporting the dominance of on-treatment immune dynamics over static baseline measures. The final multivariable overall survival model included 71 events and demonstrated adequate discrimination (concordance index 0.673) with a statistically significant likelihood ratio test (p = 0.005).

Progression-Free Survival Analyses

PD-L1 expression did not retain independent prognostic significance for progression-free survival (Figure 2). Given the susceptibility of progression-free survival to misclassification in the setting of immunotherapy and the absence of a consistent association with PD-L1 expression, overall survival was selected as the primary PD-L1 outcome in this study. Accordingly, to appropriately evaluate the prognostic relevance of biomarkers measured during treatment and to minimize time-dependent bias, subsequent progression-free survival analyses were conducted using a landmark approach.

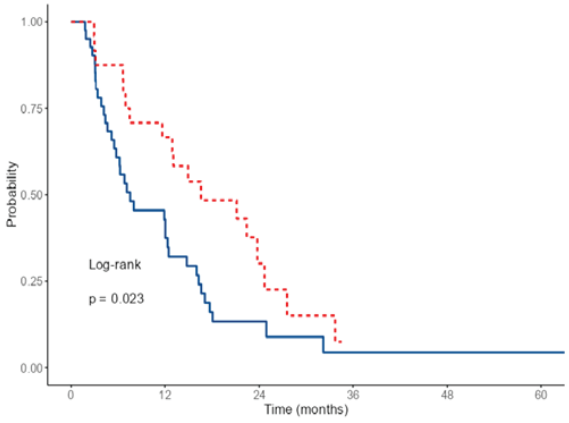

Figure 2. Progression-free survival according to PD-L1 expression status.

Kaplan–Meier curves illustrating progression-free survival stratified by PD-L1 expression (≥50% vs <50%) in patients treated with nivolumab. Patients with PD-L1 ≥50% showed longer progression-free survival compared with those with PD-L1 <50% (log-rank p = 0.023). Despite the observed univariable separation of curves, PD-L1 expression did not retain independent prognostic significance for PFS in multivariable Cox regression analysis.

Landmark-Adjusted Progression-Free Survival

In 3-month landmark-adjusted analyses, dNLR at 3 months was significantly associated with progression-free survival. Patients with elevated dNLR at the landmark time point demonstrated earlier disease progression compared with those with lower dNLR values (Figure 3).

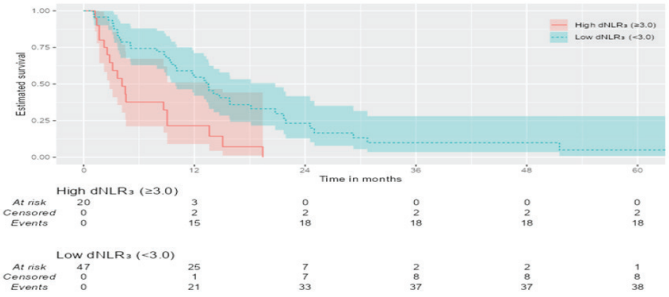

Figure 3. Landmark progression-free survival according to dNLR at 3 months.

Kaplan–Meier curves showing landmark-adjusted progression-free survival stratified by dNLR at 3 months (≥3.0 vs <3.0) in patients treated with nivolumab. Patients with high dNLR at 3 months (≥3.0) experienced significantly shorter progression-free survival compared with those with low dNLR (<3.0). These findings indicate that early on-treatment systemic inflammatory status is strongly associated with disease progression under immunotherapy.

Baseline EASIX score did not demonstrate a significant association with progression-free survival in landmark-adjusted analyses.

Multivariable Landmark Analysis of Progression-Free Survival

In landmark-adjusted multivariable Cox regression analyses, dNLR at 3 months remained an independent predictor of disease progression. In addition, renal function at 3 months, expressed as ln-transformed eGFR, was strongly associated with shorter progression-free survival (HR 6.86, 95% CI 2.48–18.99; p < 0.001). Baseline metastatic burden (≤2 vs >2 organs) did not demonstrate an independent association with

progression-free survival after adjustment (HR 0.94; p = 0.853). The results of the multivariable landmark model are illustrated in Figure 4. In contrast, neither baseline nor 3-month EASIX, analyzed as continuous log-transformed variables, showed a significant association with landmark-adjusted progression-free survival (baseline EASIX: HR 1.06, 95% CI 0.77–1.50, p = 0.741; 3-month EASIX: HR 0.90, 95% CI 0.66–1.20, p = 0.497).

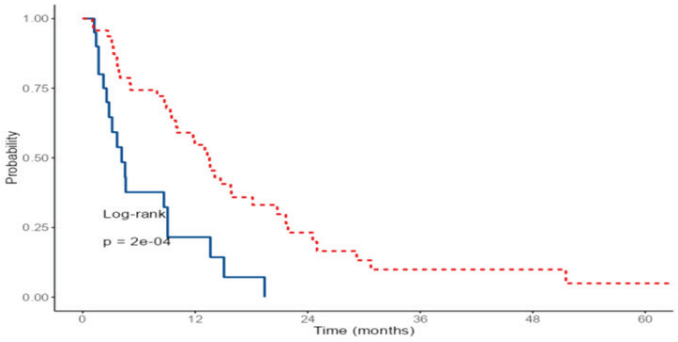

Figure 4. Multivariable landmark Cox regression model for progression-free survival. The forest plot displays adjusted hazard ratios for 3-month dNLR, ln(eGFR$_3$), and metastatic burden among patients who were alive and progression-free at the 3-month landmark time point.

Discussion

In this real-world cohort of 81 patients with advanced non–small cell lung cancer treated with second-line nivolumab, overall survival was shaped by a combination of tumor-related factors, disease burden, and host immune–inflammatory dynamics during treatment. High tumor PD-L1 expression (≥50%) was associated with significantly prolonged overall survival, underscoring the prognostic relevance of tumor immune phenotype at baseline. In addition, advanced disease burden, reflected by extensive metastatic involvement, was linked to inferior survival outcomes. Notably, however, the strongest prognostic signals were derived from on-treatment inflammatory markers rather than baseline composite indices. dNLR measured at 3 months and longitudinal changes in NLR (ΔNLR) showed consistent and robust associations with both overall and progression-free survival in landmark-adjusted analyses, whereas baseline EASIX did not demonstrate prognostic value. These findings indicate that in nivolumab-treated patients, early systemic immune–inflammatory remodeling during therapy provides more clinically relevant prognostic information than static baseline scores alone.

The observation that high PD-L1 expression (≥50%) was strongly associated with overall survival but did not retain independent prognostic significance for progression-free survival in our cohort is consistent with evidence from pivotal immune checkpoint inhibitor trials and long-term follow-up analyses. In the CheckMate 017 and 057 studies, nivolumab produced a sustained overall survival benefit compared with docetaxel in previously treated NSCLC, whereas effects on progression-free survival were modest and less consistent across PD-L1 subgroups [9,10]. Similarly, long-term follow-up of trials enrolling patients with high PD-L1 tumor proportion scores, such as KEYNOTE-024 with pembrolizumab, has demonstrated that PD-L1 expression is more closely associated with durable survival benefit than with early radiographic progression events [11]. The discordance observed between overall survival and progression-free survival is now widely acknowledged in immunotherapy trials and is generally attributed to limitations inherent to PFS as an endpoint rather than to biological inconsistency. Delayed treatment effects, treatment beyond RECIST-defined progression, and atypical response patterns—including pseudoprogression—may attenuate the ability of PFS to capture long-term benefit [12]. Real-world cohorts of nivolumab-treated NSCLC patients further support this concept, showing that radiographic progression does not uniformly translate into poor long-term outcomes, particularly in patients with favorable tumor immune characteristics [13]. Within this context, our findings reinforce the appropriateness of prioritizing overall survival as the primary endpoint for assessing the prognostic relevance of PD-L1 expression in patients treated with nivolumab.

In our cohort, the most informative prognostic signal was derived from inflammatory markers assessed during treatment rather than at baseline. dNLR measured at 3 months and longitudinal changes in NLR (ΔNLR) were consistently associated with both overall and progression-free survival in landmark-adjusted analyses, whereas baseline inflammatory indices showed weaker or inconsistent associations. This finding suggests that the systemic immune–inflammatory balance that evolves under nivolumab therapy better reflects subsequent clinical outcomes than pretreatment inflammatory status alone. These results are in line with prior studies indicating that early post-treatment NLR assessments provide superior prognostic information compared with baseline measurements in patients receiving immune checkpoint inhibitors. In nivolumab-treated NSCLC cohorts, NLR evaluated within the first weeks after treatment initiation has been shown to correlate with survival outcomes, while pretreatment NLR often fails to retain independent prognostic significance. Collectively, these findings indicate

that NLR-based indices — whether assessed at baseline or during treatment — carry consistent prognostic relevance in nivolumab-treated NSCLC [6,7,14]. By applying a landmark approach, our analysis minimizes immortal time bias and strengthens the interpretability of on-treatment biomarkers. The persistence of 3-month dNLR as a prognostic factor within this framework reinforces its role as a practical indicator of immune–host interaction during PD-1 blockade, rather than a surrogate of baseline disease severity.

In our cohort, metastatic burden was a strong determinant of overall survival, with extensive systemic dissemination conferring a marked survival disadvantage despite nivolumab therapy. Patients with involvement of four or more metastatic organs experienced substantially worse outcomes, indicating that extreme disease burden exerts a dominant negative effect on survival. This finding suggests that beyond a certain threshold of tumor spread, the potential benefit of immune checkpoint inhibition may be limited by aggressive disease biology and compromised host reserve. Consistent with our observations, real-world studies of nivolumab-treated NSCLC have shown that indicators of advanced disease extent, including widespread metastatic involvement and unfavorable metastatic patterns, are associated with inferior survival outcomes [15,16]. Although the number of metastatic organs represents a simplified measure of tumor burden, it provides a pragmatic and reproducible surrogate in retrospective settings where volumetric assessments are not routinely available. Within this framework, our results indicate that very high metastatic burden may attenuate the prognostic impact of favorable tumor immune features or on-treatment inflammatory dynamics, highlighting disease extent as a critical contextual factor in immunotherapy outcomes.

In this study, we found that baseline EASIX was not significantly associated with either overall survival or progression-free survival in patients with advanced NSCLC treated with nivolumab. This finding indicates that a static index reflecting endothelial activation and systemic stress at treatment initiation does not adequately capture the determinants of outcome under PD-1 blockade in this population. In contrast, previous studies have demonstrated a prognostic role for EASIX in disease settings characterized by aggressive biology and treatment paradigms dominated by cytotoxic chemotherapy. Specifically, elevated EASIX has been associated with inferior survival in diffuse large B-cell lymphoma [17], small cell lung cancer [18], metastatic pancreatic cancer [19], and upper tract urothelial carcinoma [20]. In these malignancies, endothelial dysfunction, coagulation imbalance, and systemic stress responses represent major biological drivers of disease progression and treatment resistance. Our negative finding therefore appears biologically plausible when interpreted in the context of immunotherapy-treated NSCLC. Unlike cytotoxic therapy, the clinical efficacy of nivolumab primarily depends on effective immune activation and sustained antitumor immune surveillance rather than baseline endothelial stress. In line with this mechanism, our results demonstrated that dynamic immune–inflammatory markers assessed during treatment, such as dNLR at 3 months and ΔNLR, were strongly associated with survival outcomes, whereas EASIX was not. Collectively, these findings suggest that the prognostic utility of EASIX is highly context-dependent and may be limited in NSCLC patients receiving immune checkpoint inhibitors, highlighting the importance of treatment-specific and mechanism-driven biomarker selection.

In this study, we observed that the prognostic associations of on-treatment inflammatory markers, particularly dNLR at 3 months and ΔNLR, became evident only when survival analyses were conditioned on patients who remained alive and at risk at the predefined landmark time point. This finding indicates that the clinical relevance of these biomarkers emerges during nivolumab therapy rather than at baseline and highlights the importance of appropriate handling of time-dependent variables. To address this issue, we applied a 3-month landmark analysis, a well-established methodological approach designed to minimize immortal time bias when evaluating variables measured during follow-up. Without landmark conditioning, early deaths occurring before biomarker assessment may lead to biased or spurious associations with survival outcomes [21,22]. By restricting analyses to patients who reached the landmark time point, we ensured that observed associations reflected true on-treatment biological processes rather than differences in follow-up duration. Within this methodological framework, our findings support the interpretation that changes in dNLR and ΔNLR during nivolumab therapy represent biologically meaningful immune–inflammatory dynamics associated with survival, rather than analytical artifacts driven by early events.

Limitations of the Study

This study has several limitations. Its retrospective, single-center design and the modest cohort size may have affected the precision of some multivariable estimates, as reflected by wider

confidence intervals in selected analyses, and may limit the generalizability of the results. Although the number of observed events permitted adjusted modeling, the overall sample size may have influenced the stability of certain estimates. Accordingly, the findings should be interpreted with appropriate caution and validated in larger, independent cohorts. Biomarkers were assessed at predefined time points, and more frequent longitudinal sampling could have provided a more detailed characterization of immune–inflammatory dynamics during nivolumab therapy. Although adjustment was performed for key clinical and laboratory factors, residual confounding cannot be excluded in an observational setting. PD-L1 expression was not available for all patients, reflecting real-world testing practices during the study period. This may have limited the interpretability of PD-L1–based subgroup analyses and introduced additional residual confounding. In addition, EASIX was evaluated only at baseline, as serial measurements of its individual components were not uniformly available at standardized follow-up time points across the entire cohort. This limitation precluded a consistent longitudinal analysis comparable to that performed for dNLR and ΔNLR. It is therefore possible that time-dependent changes in endothelial stress during immunotherapy were not fully captured in the present analysis. Prospective studies incorporating systematic serial biomarker assessments may help clarify the dynamic role of endothelial stress indices in patients receiving immune checkpoint inhibitors.

In conclusion, this study demonstrates that in patients with advanced NSCLC treated with nivolumab, prognostic information is driven predominantly by treatment-specific and time-dependent factors rather than static baseline indices. While tumor PD-L1 expression and extreme metastatic burden remain relevant baseline determinants of overall survival, early on-treatment immune–inflammatory dynamics, particularly dNLR at 3 months and longitudinal changes in NLR, provide more robust and clinically meaningful prognostic stratification. In contrast, baseline EASIX did not confer prognostic value in this immunotherapy-treated cohort, underscoring the context-dependent utility of endothelial stress–based indices. These findings support the integration of dynamic, readily available inflammatory biomarkers into prognostic assessment during immune checkpoint inhibition and highlight the need for prospective validation in independent cohorts.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Ethics approval

This study was approved by Pamukkale University Non-Interventional Clinical Research Ethics Committee (ID: E-60116787-020-776895; Nov 10, 2025).

Authors' contributions

T.D.: Conceptualization, methodology, formal analysis, investigation, data curation, writing - original draft, visualization, supervision, project administration. S.T.: Methodology, investigation, resources, data curation, writing - review & editing. T.G.K.: Investigation, resources, data curation, writing - review & editing. E.İ.: Methodology, validation, resources, data curation. E.H.: Investigation, data curation, writing - review & editing. M.Ö.: Investigation, data curation, writing - review & editing. F.B.: Methodology, investigation, resources, data curation (pathology). B.Y.T.: Methodology, validation, writing - review & editing, supervision. A.G.D.: Methodology, formal analysis, writing - review & editing, supervision. A.Y.: Resources, writing - review & editing, supervision. G.G.D.: Conceptualization, methodology, resources, writing - review & editing, supervision

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References

1. Zimmermann S, Peters S, Owinokoko T, Gadgeel SM. Immune checkpoint inhibitors in the management of lung cancer. Am Soc Clin Oncol Educ Book 2018; 38: 682-95.
2. Borghaei H, Gettinger S, Vokes EE, Chow LQM, Burgio MA, de Castro Carpeno J et al. Five-Year Outcomes From the Randomized, Phase III Trials CheckMate 017 and 057: Nivolumab Versus Docetaxel in Previously Treated Non-Small-Cell Lung Cancer. J Clin Oncol 2021; 39: 723-33.
3. Schoenfeld AJ, Hellmann MD. Acquired resistance to immune checkpoint inhibitors. Cancer Cell 2020; 37: 443-55.
4. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer 2019; 19: 133-50.
5. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 2010; 140: 883-99.

6. Nakaya A, Kurata T, Yoshioka H, Takeyasu Y, Niki M, Kibata K et al. Neutrophil-to-lymphocyte ratio as an early marker of outcomes in patients with advanced non-small-cell lung cancer treated with nivolumab. Int J Clin Oncol 2018; 23: 634-40.
7. Takeda T, Takeuchi M, Saitoh M, Takeda S. Neutrophil-to-lymphocyte ratio after four weeks of nivolumab administration as a predictive marker in patients with pretreated non-small-cell lung cancer. Thorac Cancer 2018; 9: 1291-9.
8. Liu D, Schilling B, Liu D, Sucker A, Livingstone E, Jerby-Arnon L et al. Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma. Nat Med 2019; 25: 1916-27.
9. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015; 373: 123-35.
10. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med 2015; 373: 1627-39.
11. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2016; 375: 1823-33.
12. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol 2017; 18: e143-52.
13. Gettinger S, Horn L, Jackman D, Spigel D, Antonia S, Hellmann M et al. Five-Year Follow-Up of Nivolumab in Previously Treated Advanced Non-Small-Cell Lung Cancer: Results From the CA209-003 Study. J Clin Oncol 2018; 36: 1675-84.
14. Bagley SJ, Kothari S, Aggarwal C, Bauml JM, Alley EW, Evans TL et al. Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. Lung Cancer 2017; 106: 1-7.
15. Dudnik E, Moskovitz M, Daher S, Shamai S, Hanovich E, Grubstein A et al. Effectiveness and safety of nivolumab in advanced non-small cell lung cancer: The real-life data. Lung Cancer 2018; 126: 217-23.
16. Vokes EE, Ready N, Felip E, Horn L, Burgio MA, Antonia SJ et al. Nivolumab versus docetaxel in previously treated advanced non-small-cell lung cancer (CheckMate 017 and CheckMate 057): 3-year update and outcomes in patients with liver metastases. Ann Oncol 2018; 29: 959-65.
17. Park S, Go SI, Lee GW. The Endothelial Activation and Stress Index (EASIX) score is an independent prognostic factor in patients with diffuse large B-cell lymphoma. BMC Cancer 2022; 22: 816.
18. Go SI, Park S, Kang MH, Kim HG, Kang JH, Kim JH et al. Endothelial activation and stress index (EASIX) as a predictive biomarker in small cell lung cancer. Cancer Biomark 2022; 35: 217-25.
19. Cavdar VC, Gokmen Y, Aric M, Altunkaya T, Erdem CG, Gulturk I et al. The Prognostic Value of the EASIX Score in Patients with Metastatic Pancreatic Cancer. Diagnostics (Basel) 2025; 15: 1740.
20. Gu JS, Ryu JW, Yu SH, Chung HS, Hwang JE, Bae WK et al. Prognostic value of the endothelial activation and stress index in patients with upper tract urothelial cancer undergoing radical nephroureterectomy. Investig Clin Urol 2022; 63: 623-30.
21. Anderson JR, Cain KC, Gelber RD. Analysis of survival by tumor response. J Clin Oncol 1983; 1: 710-19.
22. Suissa S. Immortal time bias in pharmacoepidemiology. Am J Epidemiol 2008; 167: 492-9.