The Prognostic Significance of Baseline Neutrophil-to-Lymphocyte Ratio in Melanoma Patients Receiving Immunotherapy

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Summary: Immunotherapy has revolutionized the treatment in metastatic melanoma, but alternative biomarkers that are economical, simple and reliable still need to be clarified. In this study, we aimed to comprehensively analyze the prognostic significance of baseline neutrophil-to-lymphocyte ratio (NLR) in melanoma patients with immunotherapy. We searched PubMed, Embase, and Cochrane Library, and conducted a systematic review and meta-analysis from April to September, 2020. Hazard ratio (HR) and 95% confidence intervals (CIs) were pooled to investigate the association of baseline NLR with overall survival (OS) and progression-free survival (PFS). Sensitivity analysis, subgroup analyses, publication bias assessment, and the Duval and Tweedie trim-and-fill method were used to evaluate the stability of results. A total of 18 studies including 2054 patients were included in our analysis. Pooled data demonstrated that higher baseline NLR was associated with a poorer OS (HR = 2.46, 95% CI = 1.77, 3.43) and PFS (HR = 2.38, 95% CI = 1.95, 2.89) of melanoma patients receiving immunotherapy. Subgroup analysis according to immunotherapy type showed that the prognostic effects of baseline NLR existed in all the subtypes of immunotherapy, including anticytotoxic T lymphocyte-associated protein 4 (CTLA4) therapy (OS HR = 2.26, 95% CI = 1.43, 3.59; PFS HR = 2.68, 95% CI = 1.79, 4.02), antiprogrammed cell death-1 therapy (OS HR = 3.08, 95% CI = 2.31, 4.27; PFS HR = 2.01, 95% CI = 1.64, 2.47), and combination therapy (OS HR = 1.75, 95% CI = 1.13, 2.72; PFS HR = 3.13, 95% CI = 1.63, 6.03). Conclusions were still consistent in subgroup analyses stratified by study year, region, study type, sample size, analysis of HR and cutoff of baseline NLR. Altogether, baseline NLR is a promising prognostic biomarker for melanoma patients receiving immunotherapy.

Key Words: neutrophil-to-lymphocyte ratio, melanoma, immunotherapy, biomarker, prognosis

Immunotherapy with antibodies targeting the programmed cell death-1 receptor (PD-1) or its ligand or the cytotoxic T lymphocyte-associated protein 4 (CTLA4) has revolutionized the treatment in metastatic melanoma.1,2 Recently, immunotherapy has been recommended as first-line treatment for advanced cutaneous melanoma.2 Clinical trials showed that 5-year overall survival was 44% in the nivolumab group and even 52% in the nivolumab-plus-ipilimumab group.3 However, some patients still have no response, and a subset of responding patients eventually deteriorate.4,5 Moreover, relative long response time for immunotherapy could cause patients with no clinical response to miss the optimal treatment window.6–8 Thus, it is imperative to investigate reliable markers to select the most suitable melanoma patients for immunotherapy.

Extensive research efforts have been undertaken to identify predictive biomarkers for the prognosis of melanoma patients receiving immunotherapy. Our team previously showed that some biomarkers, such as ADORA1 and P62, could be used to assess the clinical response of immunotherapy in melanoma.9,10 Other groups highlighted that the tumor programmed cell death ligand-1 expression level was an important biomarker for evaluating the efficacy of immunotherapy.11–13 Others markers like mutational burden and microsatellite instability, were also characterized in clinical practice.14–16 In addition, several clinical scoring systems have been proposed to predict the outcome of immunotherapy in melanoma patients.17–21 For example, Weide et al17 reported that a combination model including visceral involvement, lactate dehydrogenase-ratio, relative lymphocyte count and relative eosinophil count, could identify melanoma patients receiving immunotherapy with excellent prognosis. Berry et al18 developed the Astropsych platform via the analysis of multispectral imaging to predict the outcome and response of immunotherapy. However, these markers and clinical systems had limitations like high costs, complex procedures, and great heterogeneity.6 Therefore, alternative biomarkers that are economical, simple and reliable still need to be clarified.

Increasing studies demonstrated that neutrophil-to-lymphocyte ratio (NLR), a biomarker of systemic inflammation,
was associated with poor clinical prognosis in melanoma, a tumor that is highly associated with inflammation. Considering that tumor inflammation could predict the response of melanoma patients with immunotherapy, we wondered whether baseline NLR could be used as a biomarker for the assessment of clinical response to immunotherapy. Studies reported the associations, but conclusions were inconsistent. Therefore, in this study, we aimed to comprehensively analyze the prognostic significance of baseline NLR in melanoma patients with immunotherapy.

To our knowledge, this is the first meta-analysis to evaluate the association between baseline NLR and the prognosis of melanoma patients receiving immunotherapy. Our study will assist clinicians with patient counseling and clinical treatment guiding.

METHODS

Search Strategy

This meta-analysis was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. A systematic online search of PubMed, Embase, and Cochrane Library was performed to identify all relevant published literatures until September 16, 2020. Search strategies were as follows: (“Melanoma” OR “melanoma”) OR (“Skin Neoplasms” OR “malignant melanoma” OR “skin cancer”) AND (“Neutrophil-Lymphocyte ratio” OR “Neutrophil/Lymphocyte ratio” OR “NLR”) AND (“CTLA4” OR “cytotoxic T-lymphocyte-associated protein 4” OR “PD-1” OR “programmed death receptor 1” OR “immune checkpoint inhibitor” OR “ipilimumab” OR “tremelimumab” OR “nivolumab” OR “pembrolizumab”). We did not apply any restriction on language or study design. The references of eligible articles and main reviews were searched for further potentially relevant articles. The identifier of systematic review registration was PROSPERO CRD42021223932.

Inclusion and Exclusion Criteria

Studies were considered eligible if they met the following inclusion criteria: (1) advanced or metastatic melanoma patients receiving immunotherapy; (2) accessible survival outcomes between high and low baseline NLR groups. Exclusion criteria were: (1) studies without specifying the treatments or receiving other types of treatments; (2) studies including other types of tumors without performing subgroup analysis of melanoma; (3) duplicated studies with small sample size in the same institute or hospital; (4) review, case reports or meta-analysis.

Data Extraction and Quality Assessment

Two authors (F.Z. and Y.L.) independently scanned the initial search to exclude any duplicate and irrelevant studies. The following data were extracted from eligible studies: first authors, published year, region of study, type of study, cases, age, sex, cutoff value of baseline NLR, "Neutrophil to Lymphocyte ratio" OR “Neutrophil/Lymphocyte ratio” OR “NLR”) AND (“CTLA4” OR “cytotoxic T-lymphocyte-associated protein 4” OR “PD-1” OR “programmed death receptor 1” OR “immune checkpoint inhibitor” OR “ipilimumab” OR “tremelimumab” OR “nivolumab” OR “pembrolizumab”). We did not apply any restriction on language or study design. The references of eligible articles and main reviews were searched for further potentially relevant articles. The identifier of systematic review registration was PROSPERO CRD42021223932.

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Data Extraction and Quality Assessment

Two authors (F.Z. and Y.L.) independently scanned the initial search to exclude any duplicate and irrelevant studies. The following data were extracted from eligible studies: first authors, published year, region of study, type of study, cases, age, sex, cutoff value of baseline NLR,
immunotherapy type, hazard ratio (HR) of each study and corresponding 95% confidence interval (CI) for overall survival (OS) and progression-free survival (PFS). HR from multivariable analyses was preferentially retrieved. If studies did not report specified HR, HR was estimated from Kaplan-Meier curves between high and low baseline NLR groups according the previous methods. Studies quality was assessed using the Newcastle-Ottawa Scale with a total of 9 stars in 3 aspects: selection, comparability and outcome. The main characteristics of the included studies were summarized in Table 1. All the studies were published between 2015 and 2020, with 5 studies published before 2018. All the studies reported data related to OS and 12 studies presented data on PFS. About the regions, 8 studies were from Europe, 5 from America and 5 from Asia. As for therapy type, and 2 studies assessed combination therapy. The main characteristics of the included studies were summarized in Table 1. All the studies were published between 2015 and 2020, with 5 studies published before 2018. All the studies reported data related to OS and 12 studies presented data on PFS. About the regions, 8 studies were from Europe, 5 from America and 5 from Asia. As for study type and analysis of HR, 8 studies were multicenter and 13 studies were analyzed by multivariate analysis. Cutoffs of baseline NLR were not the same in these studies. Ten studies used 5 as cutoff to stratify high and low baseline NLR group. Regarding the immunotherapy types, 8 studies assessed anti-CTLA therapy, 7 studies evaluated anti-PD1 therapy, and 2 studies assessed combination therapy. The quality assessment of the selected studies was presented in Additional File 1 (Supplemental Digital Content 1, http://links.lww.com/JIT/A635).

### Statistical Analysis

All the statistical analyses were performed using STATA software (Version 12.0; STATA Corporation, College Station, TX). Statistical heterogeneity was assessed with $I^2$ and $P$-value. Random effect model was preferentially performed due to the heterogeneity in the comparisons. Fixed effect model was also adopted in all analyses to evaluate the stability of results. Sensitivity analysis was performed by omitting one study each time as previously described. Subgroup analyses were stratified by study year, region, study type, sample size, analysis of HR, cutoff of baseline NLR and immunotherapy type to test whether baseline NLR could predict survival outcomes in these circumstances. Publication bias was assessed using funnel plots and Egger tests. If publication bias existed, the Duval and Tweedie trim-and-fill method was implemented to adjust for this bias. $P < 0.05$ was considered statistically significant.

### RESULTS

#### Literature Search and Studies Characteristics

The detailed flowchart of our literature search was shown in Figure 1. In summary, a total of 175 abstracts and titles were initially identified, in which 36 studies were removed due to duplication. After abstract and title reviewing, 29 articles remained for full-text scanning. Eleven studies were excluded due to no relevant outcomes ($n = 8$), review and meta-analyses ($n = 2$), and duplicates ($n = 1$). Finally, 18 studies including 2054 patients were included in the meta-analysis.

The results showed that higher baseline NLR was associated with a shorter OS and PFS (Figs. 2A, B).

### Association Between Baseline NLR and OS

All the eligible studies with 2054 patients were chosen for the pooled analysis of the association between baseline NLR and OS. With great heterogeneity ($I^2 = 88.3\%$, $P < 0.001$), random effect model was adopted and results showed that higher baseline NLR was associated with a poorer OS (HR = 2.46, 95% CI = 1.77, 3.43, $P < 0.001$) (Fig. 2A). The fixed effect model and sensitivity analysis did not change the conclusion (Figs. 2A, B).

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**TABLE 1. Characteristics of Eligible Studies**

| References          | Region       | Study Type          | Cases | Age (y) | Sex (Male, %) | Cutoff | Immunotherapy Type          | Variables | NOS Scores |
|---------------------|--------------|---------------------|-------|---------|---------------|--------|----------------------------|-----------|------------|
| Ferrucci et al      | Italy        | Multicenter         | 187   | 60.6 ± 40.9 | 152 (81.3)  | 5      | Ipilimumab                 | OS*, PFS*  | 9          |
| Khoja et al         | Canada       | Single-center       | 183   | 56.9 ± 48.6 | 115 (62.8)  | 4      | Ipilimumab                 | OS*       | 8          |
| Zaragoza et al      | France       | Multicenter         | 58    | 54.7 ± 15.6 | 33 (56.9)   | 4      | Ipilimumab                 | OS*       | 8          |
| Araujo et al        | Brazil       | Single-center       | 74    | —        | —             | 5      | Nivolumab                  | OS, PFS   | 7          |
| Cassidy et al       | USA          | Single-center       | 197   | 50.0 ± 60.6 | 125 (63)    | 5      | Ipilimumab                 | OS*, PFS*  | 8          |
| Chow et al          | England      | Multicenter         | 86    | —        | —             | 3.1    | Ipilimumab                 | PFS*      | 8          |
| Jung et al          | Korea        | Multicenter         | 104   | 58.0 ± 12.0 | 51 (49)     | 5      | Ipilimumab                 | OS, PFS   | 7          |
| Capone et al        | Italy        | Single-center       | 97    | 55.4 ± 48.2 | 42 (43.2)   | 5      | Nivolumab                  | OS*, PFS*  | 8          |
| Garnier et al       | France       | Multicenter         | 101   | 66.8 ± 11.1 | 50 (49.5)   | 5      | Nivolumab/Ipilimumab       | OS*       | 8          |
| Minowa et al        | Japan        | Single-center       | 21    | 65.7 ± 45.3 | 11 (52.4)   | 3.4    | PD-1 blockade              | OS        | 7          |
| Rosner et al        | American     | Single-center       | 209   | 56.1 ± 48.1 | 124 (59.3)  | 4.73   | Nivolumab/Ipilimumab       | OS*       | 8          |
| Afzal et al         | Lebanon      | Single-center       | 120   | 63.35 ± 13.46 | 76 (63.5)  | 5      | Ipilimumab/nivolumab       | OS*, PFS*  | 9          |
| Lee et al           | Korea        | Single-center       | 152   | 54.3 ± 45.7 | 72 (47)     | 2.1    | Nivolumab/Ipilimumab       | OS*, PFS*  | 8          |
| Marconcini et al    | Italy        | Multicenter         | 48    | —        | —             | 0.7    | PD-1 blockade              | OS, PFS    | 7          |
| Martins et al       | Portugal     | Multicenter         | 85    | 64.24 ± 45.7 | —           | 3      | PD1 blockade               | PFS*      | 8          |
| Tsutsumida et al    | Japan        | Multicenter         | 61    | 64.4 ± 32.6 | 33 (54.1)   | 4      | Ipilimumab/nivolumab       | OS*, PFS*  | 8          |
| Balatoni et al      | Hungary      | Single-center       | 47    | 55.2 ± 43.6 | 27 (57)     | 4      | Ipilimumab                 | OS         | 7          |
| Bartlett et al      | USA          | Single-center       | 224   | 61.0 ± 52.2 | 147(66)     | 5      | Nivolumab/Ipilimumab       | OS*, PFS*  | 8          |

*Means their variables are calculated by multivariable analysis.
NOS indicates the Newcastle-Ottawa Scale; OS, overall survival; PD-1, programmed cell death-1; PFS, progression-free survival.
To explore whether heterogeneity affected the stability of results, we did the subgroup analyses stratified by study year, region, study type, sample size, analysis of HR, cut-off of baseline NLR and immunotherapy type. The conclusions were consistent in all the subgroup analyses (Table 2). Notably, subgroup analysis based on multivariate analysis demonstrated that elevated baseline NLR were correlated with inferior OS (HR = 2.13, 95% CI = 1.51, 2.99, P < 0.001). What’s more, the baseline NLR showed prognostic value either in a cut-off of 5 (HR = 2.65, 95% CI = 2.01, 3.49, P < 0.001) or less than 5 (HR = 2.15, 95% CI = 1.31, 3.52, P < 0.001). In addition, subgroup analysis according to immunotherapy type showed that the prognostic effects of baseline NLR existed in all the subtypes of immunotherapy, including anti-CTLA4 therapy (HR = 2.26, 95% CI = 1.43, 3.59, P < 0.001), anti-PD1 therapy (HR = 3.08, 95% CI = 2.21, 4.27, P < 0.001), and combination therapy (HR = 1.75, 95% CI = 1.13, 2.72, P < 0.001) (Table 2).

Funnel plot identified most of studies over the pseudo 95% CI (Additional File 2, Supplemental Digital Content 1, http://links.lww.com/JIT/A635), and Egger test was used to further detect the presence of publication bias (P < 0.001) (Additional File 2, Supplemental Digital Content 1, http://links.lww.com/JIT/A635). Thus, we applied the Duval and Tweedie trim-and-fill method to adjust for this bias. The results showed that no studies were trimmed and filled, suggesting that the conclusion was stable.

**Association Between Baseline NLR and PFS**

Twelve studies with 1435 patients were enrolled to analyze the correlation of baseline NLR and PFS. Due to significant heterogeneity (I² = 37.5%, P = 0.091), we used random effect model to analyze the pooled data and results suggested that higher baseline NLR was significantly
associated with poorer PFS (HR = 2.38, 95% CI = 1.95, 2.89, P < 0.001) (Fig. 3A). The fixed effect model and sensitivity analysis did not change the conclusion (Figs. 3A, B).

Subgroup analyses were used to evaluate the stability of results based on study year, region, study type, sample size, analysis of HR, cutoff of baseline NLR and immunotherapy type. The results showed that the trend of the pooled HR for all the subgroups were not changed. Noteworthily, stratified analysis by multivariate analysis suggested worse PFS in the low baseline NLR group (HR = 2.02, 95% CI = 1.73, 2.36, P < 0.001). Subgroup analysis according to baseline NLR cutoff showed that worse PFS was noted in high baseline NLR group with 5 as cutoff (HR = 2.32, 95% CI = 1.79, 3.01, P < 0.001) or cutoff less than 5 (HR = 2.44, 95% CI = 1.82, 3.27, P < 0.001). Moreover, subgroup analysis based on chemotherapy type demonstrated a consistent conclusion in anti-CTLA4 therapy (HR = 2.68, 95% CI = 1.79, 4.02, P < 0.001), anti-PD1 therapy (HR = 2.01, 95% CI = 1.64, 2.47, P < 0.001), and combination therapy (HR = 3.13, 95% CI = 1.63, 6.03, P < 0.001) (Table 3). The funnel plot was not symmetrical and Egger test detected the presence of publication bias (P = 0.012) (Additional File 3, Supplemental Digital Content 1, http://links.lww.com/JIT/A635). The Duval and Tweedie trim-and-fill method were then applied to adjust for this bias and 5 studies was filled, but the conclusion was consistent in both fixed effect model (HR = 1.97, 95% CI = 1.72, 2.25) and random effect model (HR = 2.01, 95% CI = 1.62, 2.51).

## DISCUSSION

Malignant melanoma is one of the most common, aggressive and lethal form of skin cancers. Its incidence has steadily increased by about 6.8% annually in the past 5 years, and the number of deaths has decreased from 10,130 to 6850 in 2016 to 2020. Notably, that decline reversed in 2021, with 7180 deaths in the United States. Immunotherapy plays a critical role in reducing mortality. However, a significant proportion of patients do not benefit from immunotherapy, which requires biomarkers to predict treatment outcomes and select the most appropriate patients.

NLR is a reflection of the alteration in peripheral blood cell composition, which is associated with systemic inflammation. Inflammation-induced cancer dedifferentiation has been reported to be highly associated with the acquired resistance to cancer immunotherapy. Increasing studies have reported the prognostic value of baseline NLR in melanoma patients receiving immunotherapy, 28,29,46,47 However, a comprehensive analysis is lacking and stronger evidence is needed to highlight the association between baseline NLR and the prognosis of melanoma patients receiving immunotherapy.

Through searching all the relevant studies, 18 studies including 2054 patients were finally enrolled in our study. Pooled data of these studies showed that higher baseline NLR was associated with a poorer OS and PFS. The conclusion was consistent in the fixed effect model, sensitivity analysis and subgroup analysis. Therefore, we concluded that higher baseline NLR is a poor prognostic biomarker for melanoma patients receiving immunotherapy.

The mechanisms underlying the association between high baseline NLR and poor prognosis of melanoma patients receiving immunotherapy are poorly known. Neutrophils have direct and indirect protumor and antitumor effects during the process of tumor initiation and growth. The phenotypic heterogeneity of neutrophils depends on the spatial-specific, temporal-specific, and disease-specific parameters. Moreover, several studies have identified several neutrophils subtypes associated with the protumor or antitumor function. We could reasonably speculate that these 2 types of neutrophils were increased in the melanoma patients with poor prognosis after receiving immunotherapy. Lymphocytes are considered as the primary effector cell in the immunotherapy, and tumor-infiltrating lymphocytes are prognostic as well as predictive of response to immunotherapy in multiple cancer types. Besides, less blood lymphocytes were reported to be associated with poor prognosis of melanoma patients receiving immunotherapy, because functional lymphocytes remain critically important for antitumor activity.66

## TABLE 3. Subgroup Analysis of OS

| Subgroup | Cases | Effect Model | HR | Lower CI Limit | Upper CI Limit |
|----------|-------|--------------|----|----------------|---------------|
| Study year |       |              |    |                |               |
| Before 2018 | 8     | Random       | 2.58 | 1.55           | 4.29          |
|           |       | Fixed        | 1.06 | 1.03           | 1.1           |
| 2018 and beyond | 10 | Random | 2.32 | 1.86 | 2.88 |
|           |       | Fixed        | 2.3  | 1.87           | 2.84          |
| Region   |       |              |    |                |               |
| Europe   | 8     | Random       | 2.55 | 2.03           | 3.22          |
|           |       | Fixed        | 2.55 | 2.03           | 3.22          |
| America  | 5     | Random       | 1.99 | 1.2            | 3.29          |
|           |       | Fixed        | 1.06 | 1.02           | 1.09          |
| Asia     | 5     | Random       | 2.99 | 1.39           | 6.41          |
|           |       | Fixed        | 3.3  | 2.14           | 5.1           |
| Cases    |       |              |    |                |               |
| <100     | 10    | Random       | 2.59 | 2.02           | 3.31          |
|           |       | Fixed        | 2.52 | 2.04           | 3.1           |
| ≥100     | 8     | Random       | 2.2  | 1.37           | 3.54          |
|           |       | Fixed        | 1.06 | 1.03           | 1.1           |
| Analysis of HR |       |              |    |                |               |
| Univariate | 5    | Random       | 4.05 | 2.32           | 7.07          |
|           |       | Fixed        | 3.81 | 2.58           | 5.61          |
| Multivariate | 13  | Random       | 2.13 | 1.51           | 2.99          |
|           |       | Fixed        | 1.08 | 1.04           | 1.11          |
| Cutoff of NLR |       |              |    |                |               |
| <5       | 8     | Random       | 2.15 | 1.31           | 3.52          |
|           |       | Fixed        | 1.05 | 1.02           | 1.09          |
| 5        | 10    | Random       | 2.65 | 2.01           | 3.49          |
|           |       | Fixed        | 2.43 | 2.05           | 2.88          |
| Immunotherapy type | |              |    |                |               |
| Anti-CTLA4 | 8    | Random       | 2.26 | 1.43           | 3.59          |
|           |       | Fixed        | 1.06 | 1.03           | 1.1           |
| Anti-PD1  | 7     | Random       | 3.08 | 2.21           | 4.27          |
|           |       | Fixed        | 2.8  | 2.19           | 3.58          |
| Combination | 3    | Random       | 1.75 | 1.13           | 2.72          |
|           |       | Fixed        | 1.75 | 1.13           | 2.72          |

CI indicates confidence interval; CTLA4, cytotoxic T lymphocyte-associated protein 4; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PD-1, programmed cell death-1.
Nonetheless, lymphopenia still could not dampen the prognostic value of neutrophils for melanoma patients receiving immunotherapy, which highlights the better prognostic value of the combined indicator, NLR. NLR is calculated as the counts of neutrophil dividing by lymphocyte, which amplify their effects alone. As a systemic inflammation marker, NLR reflects the balance between the immunosuppressive protumor neutrophils and the adaptive antitumor lymphocytes. Therefore, NLR could be the prognostic biomarker for melanoma patients receiving immunotherapy, and more studies are needed to investigate the underlying mechanisms.

To the best of our knowledge, we are the first to comprehensively analyze the association between baseline NLR and the prognosis of melanoma patients receiving immunotherapy. Some other important strengths of our meta-analysis should be also addressed. For example, appropriate subgroup analyses were performed across studies, and almost consistent findings were obtained, despite the inter-study heterogeneity. Besides, multiple approaches, such as Duval and Tweedie trim-and-fill method, were applied to adjusted for the publication bias, further confirming the robustness of the results.

Admittedly, there are several limitations of our study. First, we found that considerable heterogeneity existed in the meta-analysis, though sensitivity analysis and subgroup analysis did not change the conclusion. Second, funnel plot asymmetry indicated the occurrence of publication bias for both OS and PFS, although the Duval and Tweedie trim-and-fill method indicated the same trend of the results. Third, some of the HRs were extracted from Kaplan-Meier curves for the unavailability of original data, which could lead to the imprecision of the HR. Fourth, we only validated the association between higher baseline NLR and poorer prognosis in melanoma patients receiving immunotherapy without exploring the detailed mechanism. Finally, NLR alone is insufficient to determine which patients are suitable for immunotherapy as it may exclude those patients with high baseline NLR who still benefit from immunotherapy, while our study provided a useful clinical prognostic indicator for the construction of other combined prognostic models for evaluating the efficacy of immunotherapy.

In conclusion, baseline NLR was identified as an independent predictor for the prognosis of melanoma patients receiving immunotherapy. Baseline NLR is a simple, cost-efficient and readily available biomarker that could be used to help predict response to immunotherapy in patients with metastatic or advanced melanoma. Future clinical trials are advocated to determine the association...
TABLE 3. Subgroup Analysis of PFS

| Subgroup | Cases | Effect Model | Lower CI Limit | Upper CI Limit |
|----------|-------|--------------|----------------|----------------|
| Study year | | | | |
| Before 2018 | 5 | Random | 2.72 | 1.92 | 3.85 |
| Fixed | 2.48 | 2.02 | 3.04 |
| 2018 and beyond | 7 | Random | 2.03 | 1.65 | 2.49 |
| Fixed | 2.03 | 1.65 | 2.49 |
| Region | | | | |
| Europe | 5 | Random | 2.49 | 1.92 | 3.25 |
| Fixed | 2.49 | 1.92 | 3.25 |
| America | 3 | Random | 1.88 | 1.49 | 2.37 |
| Fixed | 1.87 | 1.51 | 2.31 |
| Asia | 4 | Random | 2.96 | 1.74 | 5.05 |
| Fixed | 2.76 | 2.06 | 3.71 |
| Study type | | | | |
| Single-center | 6 | Random | 1.91 | 1.6 | 2.29 |
| Fixed | 1.91 | 1.6 | 2.29 |
| Multicenter | 6 | Random | 3 | 2.35 | 3.83 |
| Fixed | 3 | 2.35 | 3.83 |
| Cases < 100 | 8 | Random | 2.26 | 1.87 | 2.74 |
| Fixed | 2.26 | 1.87 | 2.74 |
| ≥ 100 | 4 | Random | 2.55 | 1.55 | 4.22 |
| Fixed | 2.21 | 1.77 | 2.76 |
| Analysis of HR | | | | |
| Univariate | 3 | Random | 3.97 | 2.74 | 5.76 |
| Fixed | 3.97 | 2.74 | 5.76 |
| Multivariate | 9 | Random | 2.02 | 1.73 | 2.36 |
| Fixed | 2.02 | 1.73 | 2.36 |
| Cutoff of NLR < 5 | 5 | Random | 2.32 | 1.79 | 3.01 |
| Fixed | 2.32 | 1.79 | 3.01 |
| 5 | 7 | Random | 2.44 | 1.82 | 3.27 |
| Fixed | 2.21 | 1.85 | 2.62 |
| Immunotherapy type | | | | |
| Anti-CTLA4 | 4 | Random | 2.68 | 1.79 | 4.02 |
| Fixed | 2.43 | 1.96 | 3.01 |
| Anti-PD1 | 6 | Random | 2.01 | 1.64 | 2.47 |
| Fixed | 2.01 | 1.64 | 2.47 |
| Combination | 2 | Random | 3.13 | 1.63 | 6.03 |
| Fixed | 3.13 | 1.63 | 6.03 |

CI indicates confidence interval; CTLA4, cytotoxic T lymphocyte-associated protein 4; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; PD-1, programmed cell death-1; PFS, progression-free survival.

between baseline NLR and the outcomes of immunotherapy, as well as the optimal cutoff of baseline NLR, to select the suitable population for immunotherapy.

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Conflicts of Interest/Financial Disclosures

None reported. All authors have declared there are no financial conflicts of interest with regard to this work.

REFERENCES

1. Grasso CS, Tsoi J, Onyshchenko M, et al. Conserved interferon-gamma signaling drives clinical response to immune checkpoint blockade therapy in melanoma. Cancer Cell. 2020;28:500.e1–515.e3.
2. Sullivan RJ, Flaherty KT. Immunotherapy: anti-PD-1 therapies—a first-line option in advanced melanoma. Nat Rev Clin Oncol. 2015;12:625–626.
3. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2019;381:1535–1546.
4. Baruch EN, Youngster I, Ben-Betzele R, et al. Fetal micro-biota transplant promotes response in immunotherapy-refractory melanoma patients. Science. 2020;371:602–609.
5. Ascieri PA, Long GV, Robert C, et al. Survival outcomes in patients with previously untreated BRAF wild-type advanced melanoma treated with nivolumab therapy: three-year follow-up of a randomized phase 3 trial. JAMA Oncol. 2019;5:187–194.
6. Qi Y, Liao D, Mei D, et al. Elevated neutrophil-to-lymphocyte ratio is associated with poor outcomes for melanoma patients treated with PD-1 inhibitor or chemotherapy in a Chinese population. Front Oncol. 2020;10:1752.
7. Deng G, Zeng F, Su J, et al. BET inhibitor suppresses melanoma progression via the noncanonical NF-kappaB/SPP1 pathway. Theranostics. 2020;10:11428–11443. doi:10.7150/thno.47432.
8. Olson DJ, Eroglu Z, Brockstein B, et al. Pembrolizumab plus ipilimumab following anti-PD-1/L1 failure in melanoma. J Clin Oncol. 2021;39:2647–2655.
9. Liu H, Kuang X, Zhang Y, et al. ADORA1 inhibition promotes tumor immune evasion by regulating the ATF3-PD-L1 axis. Cancer Cell. 2020;37:324:e1–339.e8.
10. Li H, Kuang X, Liang L, et al. The beneficial role of sunitinib in tumor immune surveillance by regulating tumor PD-L1. Adv Sci (Weinh). 2020;8:2001969.
11. Chen Y, Liu Q, Chen Z, et al. PD-L1 expression and tumor mutational burden status for prediction of response to chemotherpay and targeted therapy in non-small cell lung cancer. J Exp Clin Cancer Res. 2019;38:193.
12. Cerezo M, Guemiri R, Drulienne S, et al. Translational control of tumor immune escape via the eIF4F-STAT1-PD-L1 axis in melanoma. Nat Med. 2018;24:1877–1886.
13. Cai S, Chen Z, Wang Y, et al. Reducing PD-L1 expression with a self-assembled nanodrug: an alternative to PD-L1 antibody for enhanced chemo-immunotherapy. Theranostics. 2021;11:1970–1981.
14. Passaro A, Stenzinger A, Peters S. Tumor mutational burden as a pan-cancer biomarker for immunotherapy: the limits and potential for convergence. Cancer Cell. 2020;38:624–625.
15. Sha D, Jin Z, Budeczies J, et al. Tumor mutational burden as a predictive biomarker in solid tumors. Cancer Discov. 2020;10:1808–1825.
16. Tan KT, Yeh CN, Chang YC, et al. PRKDC: new biomarker and drug target for checkpoint blockade immunotherapy. J Immunother Cancer. 2020;8:e000485.
17. Weide B, Martens A, Hassel JC, et al. Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. Clin Cancer Res. 2016;22:5487–5496.
18. Berry S, Giraldo NA, Green BF, et al. Analysis of multispectral imaging with the AstroPath platform informs efficacy of PD-1 blockade. Science. 2021;372:eaba2609.
19. Al Darazi G, Martin E, Delord JP, et al. Improving patient selection for immune-oncology phase 1 trials: external validation of six prognostic scores in a French Cancer Center. Int J Cancer. 2020. doi:10.1002/ijc.34309.
20. Stein JE, Lipson EJ, Cottrell TR, et al. Pan-tumor pathologic scoring of response to PD-(L)1 blockade. Clin Cancer Res. 2020;26:545–551.
21. Liu D, Schilling B, Liu D, et al. Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma. Nat Med. 2019;25:1916–1927.
22. Cohen JT, Miner TJ, Vezeridis MP. Is the neutrophil-to-lymphocyte ratio a useful prognostic indicator in melanoma patients? Melanoma Manag. 2020;7:M1747.
23. Cocorocchio E, Martinoli C, Gandini S, et al. Baseline neutrophil-to-lymphocyte ratio (NLR) is associated with outcome of patients treated with BRAF inhibitors. Clin Transl Oncol. 2020;22:1818–1824.
24. Kanatsios S, Melanoma Project M, Li Wai Suen CSN, et al. Neutrophil to lymphocyte ratio is an independent predictor of outcome for patients undergoing definitive resection for stage IV melanoma. J Surg Oncol. 2018;118:915–921.
25. Ding Y, Zhang S, Qiao J. Prognostic value of neutrophil-to-lymphocyte ratio in melanoma: evidence from a PRISMA-compliant meta-analysis. *Medicine (Baltimore)*. 2018;97:e11446.

26. Bartok O, Pataška A, Nagel R, et al. Anti-tumour immunity induces aberrant peptide presentation in melanoma. *Nature*. 2020;590:332–337.

27. Afzal MZ, Sarwar T, Shirai K. Prognostic significance of hematological indices in malignant melanoma treated with immune checkpoint inhibitors. *J Immunother*. 2019;42:251–264.

28. Balaton T, Ladányi A, Fröhlich G, et al. Biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Pathol Oncol Res*. 2020;26:317–325.

29. Bartlett EK, Flynn JR, Panageas KS, et al. High neutrophil-to-lymphocyte ratio (NLR) is associated with treatment failure and death in patients who have melanoma treated with PD-1 inhibitor monotherapy. *Cancer*. 2020;126:76–85.

30. Minowa T, Kato J, Hida T, et al. Prognostic role of neutrophil to lymphocyte ratio in advanced melanoma treated with anti-programmed death-1 therapy. *J Dermal*. 2018;45:e250–e251.

31. Tsutsuimada A, Fukushima S, Yokota K, et al. Japanese real-world study of sequential nivolumab and ipilimumab treatment in melanoma. *J Dermal*. 2019;46:947–955.

32. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ*. 2009;339:b2535.

33. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*. 2009;339:b2700.

34. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*. 1998;17:2815–2834.

35. Tierney JF, Stewart LA, Gherzi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*. 2007;8:16.

36. Zeng F, Huang Y, Guo Y, et al. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. *Int J Infect Dis*. 2020;96:467–474.

37. Zeng F, Li L, Zeng J, et al. Can we predict the severity of coronavirus disease 2019 with a routine blood test? *Pol Arch Intern Med*. 2020;130:400–406.

38. Araujo DV, De Moraes RV, Sousa VAR, et al. Prognostic relevance of neutrophil to lymphocyte ratio (NLR) before anti-PD1 therapy in metastatic melanoma patients. *J Clin Oncol*. 2017;35(suppl 15):e21045.

39. Garber K, Pandini G, Baracchi P, et al. Baseline neutrophil-to-lymphocyte ratio (NLR) and derived NLR could predict overall survival in patients with advanced melanoma treated with nivolumab. *J Immunother Cancer*. 2018;6:74.

40. Cassidy MR, Wolchok RE, Zheng J, et al. Neutrophil to lymphocyte ratio is associated with outcome during ipilimumab treatment. *EBioMedicine*. 2017;18:56–61.

41. Chow J, Alrifai D, Shields A, et al. The search for viable biochemical and clinical prognostic markers for patients with inoperable melanoma being treated with Anti CTLA-4 therapy. *Ann Oncol*. 2017;28:x115.

42. Ferrucci PF, Gandini S, Battaglia A, et al. Baseline neutrophil-to-lymphocyte ratio is associated with outcome of ipilimumab-treated metastatic melanoma patients. *Br J Cancer*. 2015;112:1904–1910.

43. Garnier M, Zaraeuddin D, Mallardo D, et al. Baseline neutrophil-to-lymphocyte ratio (NLR) and derived NLR could predict overall survival in patients with advanced melanoma treated with nivolumab. *J Immunother Cancer*. 2018;6:74.

44. Jung M, Lee J, Kim TM, et al. Ipilimumab real-world efficacy and safety in Korean melanoma patients from the Korean named-patient program cohort. *Cancer Res Treat*. 2017;49:44–53.

45. Khazaie K, Jr., Alrifai D, Shields A, et al. High neutrophil-to-lymphocyte ratio before starting anti-programmed death 1 immunotherapy predicts poor outcome in patients with metastatic melanoma. *J Am Acad Dermal*. 2018;79:165.e1–167.e2.

46. Lee J, Lee SJ, Kim K, et al. Comprehensive molecular and clinical characterization of Asian melanoma patients treated with anti-PD-1 antibody. *BMC Cancer*. 2019;19:805.

47. Marconcini R, Nuzzo A, Manacorda S, et al. Prognostic factors for efficacy of Ipilimumab used after anti-PD1 and/or BRAF + MEK inhibitors in melanoma patients: An Italian melanoma intergroup study. *Ann Oncol*. 2019;30:v551.

48. Martins SL, Miguelsmedo P, Martinsbranco DA, et al. Hematological profile: a prognosis tool in melanoma patients treated with immunotherapy. *J Clin Oncol*. 2019;37:2298–2310.

49. Rosner S, Kwong E, Shoushtari AN, et al. Peripheral blood clinical laboratory variables associated with outcomes following combination nivolumab and ipilimumab immunotherapy in melanoma. *Cancer Med*. 2018;7:690–697.

50. Zaragoza J, Caille A, Beneton N, et al. High neutrophil to lymphocyte ratio measured before starting ipilimumab treatment is associated with reduced overall survival in patients with melanoma. *Br J Dermal*. 2016;174:146–151.

51. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66:6–30.

52. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:7–30.

53. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68:7–30.

54. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69:e7–34.

55. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70:7–30.

56. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71:7–33.

57. Mehta A, Kim YJ, Robert L, et al. Immunotherapy resistance by inflammation-induced dedifferentiation. *Cancer Discov*. 2018;8:935–943.

58. Hedrick CC, Malanchi I. Neutrophils in cancer: heterogeneous and multifaceted. *Nat Rev Immunol*. 2021. doi: 10.1038/s41577-021-00571-6.

59. Zhu YP, Padgett L, Dinh HQ, et al. Identification of an early unipotent neutrophil progenitor with pro-tumoral activity in mouse and human bone marrow. *Cell Rep*. 2018;24:2329.e1–2341.e8.

60. Evrard M, Kwok IWH, Chong SZ, et al. Developmental analysis of bone marrow neutrophil progenitors reveals populations specialized in expansion, trafficking, and effector functions. *Immunity*. 2018;48:364.e1–379.e8.

61. Pillay J, Kamp VM, van Hofven E, et al. A subset of neutrophils in human systemic inflammation inhabits T cell responses through Mac-1. *J Exp Med*. 2017;214:322–336.

62. Marin O, Costa S, Bevilacqua D, et al. Mature CD10(+) and immature CD10(-) neutrophils present in G-CSF-treated donors display opposite effects on T cells. *Blood*. 2017;129:1343–1356.

63. Sagiv JY, Michaeli J, Assi S, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. *Cell Rep*. 2015;10:562–573.

64. Zhu YP, Eggert T, Araujo DJ, et al. CyTOF mass cytometry reveals phenotypically distinct human blood neutrophil populations differentially correlated with melanoma stage. *J Immunother Cancer*. 2020;8:e000473.

65. Garber K. Pursuit of tumor-infiltrating lymphocyte immunotherapy speeds up. *Nat Biotechnol*. 2019;37:969–971.

66. Friedes C, Chakrabarti T, Olson S, et al. Association of severe lymphopenia and disease progression in unresectable locally advanced non-small cell lung cancer treated with definitive chemo-radiation and immunotherapy. *Lung Cancer*. 2021;154:36–43.

67. Ferrucci PF, Ascierto PA, Pigossio J, et al. Baseline neutrophils and derived neutrophil-to-lymphocyte ratio: prognostic relevance in metastatic melanoma patients receiving ipilimumab. *Ann Oncol*. 2018;29:524.

68. Wang SJ, Khu Lee S, Khu T, et al. Effect of cyclo-oxygenase inhibitor use during checkpoint blockade immunotherapy in patients with metastatic melanoma and non-small cell lung cancer. *J Immunother Cancer*. 2020;8:e000889.