

Neutrophil-to-lymphocyte Ratio Affects the Impact of Proton Pump Inhibitors on Efficacy of Immune Checkpoint Inhibitors in Patients with Non-Small-Cell Lung Cancer

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Abstract

Background The neutrophil-to-lymphocyte ratio (NLR) at the initiation of immune checkpoint inhibitor (ICI) therapy is a known predictor of prognosis. Proton pump inhibitors (PPIs) reportedly attenuate the therapeutic efficacy of ICIs. However, the attenuation effects are not consistently observed across all patients. This study aimed to evaluate whether NLR serves as a stratification factor to determine the impact of PPI on the efficacy of ICI.

Methods This retrospective study was conducted in patients with NSCLC treated with ICI monotherapy. Patients were stratified into two groups (higher NLR (≥ 4) and lower NLR (< 4)). PPI use was defined as the administration of PPIs within 30 days before or after ICI initiation. The primary outcome was progression-free survival (PFS) and the secondary outcome was overall survival (OS).

Results Among the 132 patients included, PPI users exhibited significantly shorter median PFS and OS than non-PPI users. In the higher NLR group ($n=61$), PPI users had a markedly shorter PFS and OS than non-PPI users (median PFS: 1.6 vs. 8.2 months; $p < 0.01$, median OS: 3.3 vs. 19.6 months; $p = 0.015$). Conversely, in the lower NLR group ($n=71$), no significant difference in PFS and OS was observed between PPI users and non-PPI users (median PFS: 2.8 vs. 7.3 months, $p = 0.83$, median OS: 17.6 vs. 24.4 months, $p = 0.40$).

Conclusion NLR may be a significant stratification factor for evaluating the impact of PPI on PFS and OS in patients with NSCLC undergoing ICI monotherapy.

Keywords: immune checkpoint inhibitor, neutrophil-to-lymphocyte ratio, non-small-cell lung cancer, proton pump inhibitor

Introduction

Lung cancer is among the most aggressive malignancies and remains a leading cause of cancer-related mortality worldwide. Non-small-cell lung cancer (NSCLC), which constitutes approximately 85% of all lung cancers, is the most prevalent subtype [1]. Immune checkpoint inhibitors (ICIs) targeting programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) have emerged as promising therapies for NSCLC [2]. With the growing use of ICIs, several studies have investigated the impact of concomitant medications on the ICI therapeutic efficacy. Proton pump inhibitors (PPIs), commonly prescribed for cancer patients to prevent gastric mucosal erosion caused by cancer treatments, are among the medications evaluated [3]. Meta-analyses suggest that PPI use may influence the outcomes of ICI therapy in patients with NSCLC [4, 5], although conflicting reports exist, with some indicating no significant effect on prognosis [6]. This discrepancy suggests the presence of stratifying factors that determine the impact of PPIs on ICI therapy. Alterations in the gut microbiome, which are influenced by PPIs, have been proposed as a mechanism affecting ICI efficacy [7]. Moreover, gut microbiome diversity is known to vary with the inflammatory status of patients [8].

Neutrophil-to-lymphocyte ratio (NLR) is an immune-inflammation-based indicator that predicts survival outcomes in various cancers [9], including NSCLC [10]. NLR, which is easily calculated from leukocyte counts, has been associated with poor prognosis in patients with NSCLC receiving immunotherapy [11, 12]. However, limited data exist on whether NLR can stratify the effect of PPIs in patients with NSCLC treated with ICIs. Therefore, this study aimed to evaluate NLR as a potential stratification factor for the prognostic impact of PPI use in patients with NSCLC undergoing ICI monotherapy.

Materials and Methods

Patients

This retrospective study included patients with pathologically confirmed advanced or recurrent NSCLC who received ICI monotherapy at the Nara Prefecture General Medical Center (Nara, Japan) between September 1, 2014, and December 31, 2020. The exclusion criteria were as follows: (a) patients participating in clinical trials involving drug-related interventions, (b) patients with incomplete prognosis records for ICI therapy, and (c) patients with missing characteristic data other than programmed death ligand 1 tumor proportion score (PD-L1 TPS). Peripheral NLR was calculated as the ratio of neutrophils

to lymphocytes using the automated hematology analyzer SIEMENS ADVIA2120i before ICI initiation. Based on prior studies, the NLR cut-off was set at 4 [12–14], categorizing patients into higher NLR ($\text{NLR} \geq 4$) and lower NLR ($\text{NLR} < 4$) groups. Targetable driver mutations were defined as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), c-ros oncogene 1 (ROS1), B-Raf proto-oncogene (BRAF), mesenchymal-epithelial transition factor (MET), rearranged during transfection (RET), which were detected using the cobas EGFR Mutation Test v2 and the AmoyDx assay. Concomitant use of steroids (daily prednisone-equivalent dose ≥ 5 mg) [15], antibiotics [16], and non-steroidal anti-inflammatory drugs (NSAIDs) [17], which may affect ICI efficacy, was also investigated. NSAID use was restricted to analgesics, excluding acetaminophen. Concomitant medication use, including PPIs, was defined as administration within 30 days before or after ICI initiation [18].

Outcomes

The primary outcome was progression-free survival (PFS), defined as the time from initiation to disease progression or death. Patients who had not completed ICI therapy by the data cut-off date were censored. Secondary outcomes included overall survival (OS), defined as the time from ICI initiation to death. Patients who had not died at the cut-off date were censored according to the last recorded date on which the patient was alive. Disease progression was assessed according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). Independent factors associated with PFS and OS were evaluated in the overall population and stratified by the NLR groups. We also performed exploratory analyses using NLR cut-offs of 3.55 [19], 5 [20], or the median NLR of this study. In addition, we conducted analyses excluding patients who were taking steroids or antibiotics, or who had a driver mutation. These factors were reported as poor prognostic indicators [16, 21, 22]. The observation period was 50 months from the initiation of ICI therapy, and the data cut-off was April 30, 2021.

Statistical analyses

Patient characteristics between PPI users and non-users were compared using the chi-square test. Kaplan–Meier methods were used to evaluate PFS and OS, with curves compared using the log-rank test. Cox proportional hazard models were employed for multivariate analysis to identify factors associated with PFS or OS, incorporating PPI use and patient characteristics affecting ICI therapy prognosis (age, sex, histology, smoking history, stage, PDL1-TPS status, targetable driver mutation,

treatment line, steroid use, antibiotic use, and NLR). Statistical significance was set at $p < 0.05$. Analyses were conducted using IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, New York).

Results

Patient characteristics

Of 143 patients with advanced or recurrent NSCLC who received ICI monotherapy, 11 were excluded due to missing concomitant medication information ($n = 1$), smoking history and NLR data ($n = 1$), and histological data ($n = 9$). Thus, 132 patients were included in the analysis. Table 1 summarizes the clinical characteristics of PPI users and non-users in the higher and lower NLR groups. The median NLR for the overall cohort ($n = 132$) was 3.79 (range: 0.70–39.35). Driver mutations were tested in 93 of the 132 patients. Of these patients, 20 showed positive for EGFR ($n=18$) or ROS1 ($n=2$) mutations, respectively. On one hand, 54 (40.9%) had a PD-L1 TPS $\geq 50\%$, 17 (12.9%) had a TPS of 1%–49%, 17 (12.9%) had a TPS $< 1\%$, and 44 (33.3%) were not evaluable. ICIs were administered as first-line therapy in 37 (28.0%) patients. Details of concomitant medication use within 30 days before and after ICI initiation are provided in Table 1. PPIs, steroids, antibiotics, and NSAIDs were used by 62 (47.0%), 56 (42.4%), 54 (40.9%), and 70 (53.0%) patients, respectively. In the higher NLR group, PPI users were more likely to use steroids than non-PPI users, while no significant differences were observed for other characteristics. Table 2 provides details on PPI administration routes and durations, with a median administration period of 60 days except for one patient receiving intravenous omeprazole.

Progression-free survival

The median PFS for the overall cohort was 3.5 months (95% confidence interval [95% CI], 1.8–5.1 months). PPI users ($n = 62$) had significantly shorter PFS than PPI non-users ($n = 70$; median PFS: 1.9 vs. 8.2 months, $p < 0.01$, Fig. 1a). In the higher NLR group (≥ 4), PPI use was associated with significantly decreased PFS (median PFS: 1.6 vs. 8.2 months, $p < 0.01$; Fig. 1b). In contrast, no significant difference in PFS was observed between PPI users and non-users in the lower NLR group (< 4) (median PFS: 2.8 vs. 7.3 months, $p = 0.83$; Fig. 1c). Similar results were observed in patients with NLR cut-off of 3.55 (Supplemental Fig. 1), the median NLR of this study of 3.79 (Supplemental Fig. 2) and 5 (Supplemental Fig. 3).

Overall survival

The median OS for the overall cohort was 17.3 months (95% CI, 13.3–21.4 months). PPI users had a significantly shorter OS than the non-PPI users (median OS: 8.2 vs. 19.6 months, $p < 0.01$; Fig. 1d). In the higher NLR group (≥ 4), PPI use was associated with significantly shorter OS (median OS: 3.3 vs. 19.6 months, $p = 0.015$; Fig. 1e). However, in the lower NLR group (< 4), PPI use was not significantly associated with OS (median OS: 17.6 vs. 24.4 months, $p = 0.40$; Fig. 1f). Similar results were observed in patients with NLR cut-off of 3.55 (Supplemental Fig. 1) and 3.79 (Supplemental Fig. 2). However, PPI use was not significantly associated with OS in patients with $\text{NLR} \geq 5$ (median OS: 3.3 vs. 19.6 months, $p = 0.09$; Supplemental Fig. 3c) or $\text{NLR} < 5$ (median OS: 16.8 vs. 24.4 months, $p = 0.10$; Supplemental Fig. 3d).

Prognostic factors for PFS

Univariate analysis revealed that $\text{PDL1} \geq 50\%$ and first-line treatment were significant factors for longer PFS, while driver mutation positivity, PPI use, steroid use, antibiotic use and $\text{NLR} \geq 4$ were significant factors for shorter PFS in the overall cohort (Table 3). On one hand, multivariate analysis revealed that steroid use ($\text{HR} = 2.15$, 95% CI, 1.31–3.54, $p = 0.003$), antibiotic use ($\text{HR} = 2.04$; 95% CI, 1.20–3.47, $p = 0.009$) and $\text{NLR} \geq 4$ ($\text{HR} = 1.68$; 95% CI, 1.06–2.68, $p = 0.029$) were significant predictors of shorter PFS in the overall cohort. In the higher NLR group (≥ 4), steroid use ($\text{HR} = 2.99$; 95% CI, 1.18–7.58, $p = 0.021$) was significantly associated with shorter PFS. In the lower NLR group (< 4), first-line treatment ($\text{HR} = 0.25$; 95% CI, 0.07–0.96, $p = 0.043$) was a significant factor in achieving a longer PFS. Non-squamous ($\text{HR} = 6.09$; 95% CI, 1.12–33.16, $p = 0.037$), driver mutation positivity ($\text{HR} = 2.68$; 95% CI, 1.11–6.50, $p = 0.029$) and driver mutation status unknown ($\text{HR} = 10.72$; 95% CI, 1.89–60.78, $p = 0.007$) were significantly associated with shorter PFS.

Prognostic factors for OS

Univariate analysis revealed that the use of PPIs, steroids and antibiotics, and $\text{NLR} \geq 4$ were significant factors for shorter OS in the overall cohort (Table 4). On one hand, multivariate analysis revealed that the use of steroids ($\text{HR} = 2.01$; 95% CI, 1.15–3.53, $p = 0.015$) and $\text{NLR} \geq 4$ ($\text{HR} = 2.02$; 95% CI, 1.21–3.36, $p = 0.007$) were significant factors of shorter OS in the overall cohort. In the higher NLR group (≥ 4), no factor was significantly associated with shorter OS. In the lower NLR group (< 4),

PDL1 status unknown (HR = 0.19; 95% CI, 0.06–0.56, $p = 0.003$) was a significant factor for longer PFS.

Subgroup analysis excluding patients taking steroids or antibiotics

In the subgroup analysis that excluded patients who had used steroids, which were identified as factors significantly shortening PFS and OS in the multivariate analysis, PPI use was associated with a significantly decreased PFS (median PFS: 1.8 vs. 11.5 months, $p = 0.027$; Fig. 2a) in the higher NLR group (≥ 4). However, PPI use was not associated with a significantly decreased OS (median OS: 15.4 vs. 19.6 months, $p = 0.47$; Fig. 2e). In contrast, no significant difference in PFS or OS was observed between PPI users and non-users (median PFS: not reached vs. 10.4 months, $p = 0.56$; Fig. 2b, median OS: 17.6 vs. 24.9 months, $p = 0.97$; Fig. 2f) in the lower NLR group (< 4).

Similarly, the subgroup analysis that excluded patients who used antibiotics, which were also identified as factors significantly shortening PFS in the multivariate analysis, PPI use tended to prolong PFS (median PFS: 1.5 months vs. 11.5 months, $p = 0.060$; Fig. 2c), although the difference was not statistically significant. However, PPI use was associated with a significantly decreased OS (median PFS: 3.2 months vs. not reached, $p = 0.003$; Fig. 2g) in the higher NLR group (≥ 4). In contrast, no significant difference in PFS or OS was observed between PPI users and non-users in the lower NLR group (< 4) (median PFS: 7.0 vs. 12.5 months, $p = 0.81$; Fig. 2d, median OS: 17.6 vs. 33.4 months, $p = 0.59$; Fig. 2h).

Subgroup analysis excluding patients with driver mutation

In the subgroup analysis that excluded patients with driver mutation ($n=20$), similar to the overall population, PPI users ($n = 51$) had significantly shorter PFS and OS than PPI non-users ($n = 61$) (median PFS: 1.9 vs. 10.4 months, $p = 0.01$; Supplemental Fig. 4a, median OS: 9.4 vs. 18.7 months, $p = 0.009$; Supplemental Fig. 4d). In the higher NLR group (≥ 4), PPI use was associated with significantly decreased PFS and OS (median PFS: 1.6 vs. 11.5 months, $p < 0.001$; Supplemental Fig. 4b, median OS: 3.3 vs. 19.6 months, $p = 0.018$; Supplemental Fig. 4e). In contrast, no significant difference in PFS and OS was observed between PPI users and non-users in the lower NLR group (< 4) (median PFS: 14.9 vs. 10.4 months, $p = 0.83$; Supplemental Fig. 4c, median OS: 17.6 vs. 18.7 months, $p = 0.59$; Supplemental Fig. 4f).

Discussion

This study demonstrated that the impact of PPI use on the efficacy of ICI therapy varies with NLR levels at the initiation of treatment. PPI use was associated with shorter PFS and OS in patients with $\text{NLR} \geq 4$, whereas no significant association was observed in patients with $\text{NLR} < 4$. To the best of our knowledge, this is the first study to assess the relationship between NLR and the effect of concomitant PPI use in patients with NSCLC treated with ICI monotherapy.

The different effect of PPI on the efficacy of ICI, depending on the level of NLR, may be explained by two primary factors. First, the patient's inflammatory status and its impact on the gut microbiome may play a role. Previous studies have shown that PPI use correlates with reduced ICI efficacy in patients with autoimmune diseases or viral hepatitis [3, 7]. In these diseases, NLR is elevated due to chronic inflammation [23–27]. NLR and gut microbiota are closely interlinked. Patients with higher NLR exhibit reduced gut flora diversity [8]. Specifically, in inflammatory diseases such as autoimmune diseases and viral hepatitis, the gut microbiota shows a reduction in *Firmicutes* and an increase in *Bacteroides* [28, 29]. Notably, PPI use has been associated with similar changes, including reduced *Firmicutes* and elevated *Bacteroides* levels [30]. Importantly, clinical evidence suggests that the efficacy of ICIs diminishes with a decrease in *Firmicutes* and an increase in *Bacteroides* [31]. Given the link between gut microbiota dysbiosis and inflammatory diseases [28, 29], the microbiome-modulating effects of PPIs could exacerbate gut dysbiosis in patients with pre-existing vulnerabilities. On the contrary, it is known that individuals with lower NLR have significantly higher phylogenetic diversity in their gut microbiota than those with higher NLR [8]. Although PPIs negatively impact on the gut microbiota and may reduce the therapeutic efficacy of ICI therapy, no attenuation effects of PPIs in ICI therapy have been observed in patients without inflammatory diseases, such as autoimmune diseases [3]. Therefore, the gut microbiota diversity is normal in patients with lower NLR and the reduction in therapeutic efficacy due to PPI use may be limited. Consequently, the patient's inflammatory status may be an important factor in determining the impact of PPIs on the prognosis of ICI therapy. However, this study did not include fecal analyses to directly evaluate gut microbiota changes, prospective studies are required to explore how bacterial community composition, which affects ICI efficacy, varies with NLR.

Another plausible explanation for the observed findings lies in the influence of PPIs on natural killer (NK) cells and lymphocytes. NK cells and lymphocytes, including regulatory T cells (T_{reg}), play essential roles in tumor immune defense [32, 33]. The previous report revealed that the median PFS was

significantly longer in the higher NK group than in the lower NK group [34]. Similar associations have been reported for lymphocytes [35]. Patients with $\text{NLR} \geq 4$ exhibit decreased NK cell activity, which strongly correlates with lower lymphocyte counts and impaired antitumor immunity [36]. Patients with higher NLR tend to exhibit relatively low lymphocyte counts. High levels of systemic inflammation are associated with diminished NK cell activity and reduced lymphocyte counts, which play a crucial role in antitumor immunity. Consequently, achieving an effective immune response to ICIs becomes challenging under these conditions. A pooled analysis of five randomized controlled trials investigating atezolizumab in advanced NSCLC (IMpower130 [37], IMpower131 [38], IMpower150 [39], OAK [40], and POPLAR [40]) consistently demonstrated that the pretreatment use of PPIs was prognostically associated with worse PFS and OS [41]. Notably, PPI use was associated with significantly lower expression levels of NK cells and lymphocytes prior to the initiation of ICI therapy compared with PPI non-users [41]. The attenuation effect of PPIs on the efficacy of ICI therapy, through reducing NK cell and lymphocyte expression, may be more pronounced in patients with higher NLR, in whom NK cells and lymphocytes are relatively decreased. In contrast, the impact of PPIs on the efficacy of ICIs may be limited in patients with lower NLR, in whom NK cells and lymphocytes are relatively maintained. This finding suggests that PPI-induced changes in the immune microenvironment might vary depending on the patient's inflammatory status, potentially influencing the efficacy of ICI therapy.

Although NLR 4 was adopted as the cut-off value for this study, similar results were obtained for NLR of 3.55 (Supplemental Fig.1) as suggested by the previous study [19] and the median value in this study of 3.79 (Supplemental Fig.2) for PFS and OS. However, when NLR cut-off value was set to 5 (Supplemental Fig.3c) as suggested by another previous study [20], no significant difference in overall survival was observed in patients with $\text{NLR} \geq 5$. Although, the analysis may not have been sufficiently robust due to the small number of patients with $\text{NLR} \geq 5$, NLR 5 as a cut-off value did not serve as an indicator for stratifying the effects of PPI. Based on these results, the appropriate NLR cut-off value for stratifying the impact of PPI was confirmed to be between 3.55 and 4.

In this study, multivariate analysis identified steroid use, antibiotic use and $\text{NLR} \geq 4$ as independent factors associated with shortened PFS. Similarly, steroid use and $\text{NLR} \geq 4$ were found to be independent factors associated with shorter OS in overall patients. These findings align with previous reports [13, 15, 42]. In the univariate analysis, the use of PPIs was identified as a significant independent factor associated with shorter PFS. However, the multivariate analysis did not identify PPI as an

independent factor. Instead, it found that other concomitant medications, such as steroids and antibiotics, had a stronger impact on prognosis. In the overall population, the attenuation effect of PPIs on prognosis was found to be limited compared to that of these medications.

Additionally, a secondary analysis was conducted where patients who had used steroids or antibiotics were excluded, in order to adjust for the potential effects of these medications (Fig. 2). In subgroups of secondary analysis, the use of PPIs was associated with detrimental effects on PFS (Fig. 2a) and OS (Fig. 2g) in patients with $\text{NLR} \geq 4$. However, the use of PPIs did not have a detrimental effect on PFS and OS in patients with $\text{NLR} < 4$, which was generally consistent with the results observed in the overall population. The impact of PPI use on the therapeutic efficacy of ICI may therefore be stratified by NLR irrespective of steroid and antibiotic use. Similarly, driver mutation is one of the strong prognostic factors in patients treated with ICI therapy [22]. In order to exclude the effect of driver mutations on the efficacy of ICI therapy, we analyzed the data in which the patients with driver mutation-positive ($n=20$) were excluded. The results showed that PPIs shortened PFS and OS in patients with $\text{NLR} \geq 4$, while there was no association between PPIs and PFS or OS in patients with $\text{NLR} < 4$ (Supplemental Fig. 4). Therefore, driver mutation is unlikely to be a confounding factor in this study. On one hand, regarding the effects of the route of PPI administration (oral or intravenous) and the timing of PPI administration (Pre-ICI, Post-ICI and Pre and Post ICI) on the gut microbiota, we were unable to perform subgroup analyses because the number of patients receiving intravenous PPI administration and patients only with Pre-ICI or Post-ICI was small.

In the overall population, the median PFS and OS were 3.5 months (95% CI, 1.8–5.1 months) and 17.3 months (95% CI, 13.3–21.4 months), respectively. These outcomes are comparable to real-world data from 435 Japanese patients treated with ICI monotherapy (median PFS: 3.4 months; median OS: 13.0 months) [43]. The slightly better OS observed in this study may be attributed to a higher proportion of patients with high PD-L1 expression ($\text{TPS} \geq 50\%$) and those receiving ICIs as first-line therapy compared with previous reports [43]. While PPI use may be warranted in certain scenarios, such as preventing steroid- and NSAID-induced ulcers, careful consideration should be given to the necessity of PPI use during ICI therapy [44]. The stratification of patients by NLR, as demonstrated in this study, may hold clinical significance in this context.

This study has several limitations. First, it is a single-center retrospective analysis with a small sample size, and the findings cannot be considered definitive. Second, Eastern Cooperative Oncology

Group performance status (PS) was not included in the multivariate analysis due to incomplete medical records. However, there were no significant differences in patient characteristics such as age or presence of distant metastases—factors that may affect PS. Thus, the impact of missing PS data on the conclusions is likely limited. Third, this study focused solely on NLR at the initiation of ICI therapy, which may be influenced by factors such as infections or cancer-related complications. Fourth, a certain number of patients were prescribed PPIs by medical institutions other than our institution, which made it difficult to ascertain the purpose of the prescriptions. Therefore, we could not entirely eliminate the influence of the background factors for which the PPIs were prescribed. Fifth, the large number of explanatory factors for the number of PFS ($n = 90$) and OS ($n = 70$) events in the multivariate analysis potentially rendered the analysis insufficiently robust. Lastly, the retrospective design of the study precluded the investigation of gut microbiota distribution by NLR and the effects of PPI use using stool analyses.

In conclusion, NLR at the initiation of ICI therapy may serve as a stratifying factor for determining the impact of PPIs on PFS in patients with NSCLC receiving ICIs. In individuals with low NLR, the influence of PPI use on ICI efficacy appears to be minimal.

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Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Tomoki Hori. Study design and analysis were supervised by Kazuhiro Yamamoto. Tomoki Hori drafted the initial manuscript, and all authors provided feedback on earlier versions. All authors read and approved the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations**Conflict of interests**

Tomoki Hori, Kazuhiro Yamamoto, Takefumi Ito, Shigeki Ikushima, Tomohiro Omura, and Ikuko Yano declare no conflict of interest.

Ethical approval

This retrospective study involving human participants was conducted in compliance with the ethical standards of the institutional and national research committees and the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical guidelines. The Ethics Committee of Nara Prefecture General Medical Center approved this study (Approval No. 706). Informed consent was obtained by allowing patients to opt-out of the study at any time by reviewing a summary of the study summary published on the institution's website.

Consent to Participate

Informed consent for participation was obtained using an opt-out approach.

Consent for Publish

Consent for publication was obtained from patients using an opt-out approach.

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Figure caption

Fig. 1 Association of PPI with PFS and OS according to NLR levels. The impact of PPI was analyzed for the overall cohort (a, d), patients with $\text{NLR} \geq 4$ (b, e), and patients with $\text{NLR} < 4$ (c, f) NLR, neutrophil-to-lymphocyte ratio; NR, not reached; OS, overall survival; PFS, progression-free survival; PPI, proton pump inhibitor

Fig. 2 Association of PPI with PFS and OS according to NLR levels in patients not receiving steroids or antibiotics: (a,e) patients not receiving steroids with $\text{NLR} \geq 4$, (b,f) patients not receiving steroids with $\text{NLR} < 4$, (c,g) patients not receiving antibiotics with $\text{NLR} \geq 4$ and (d,h) patients not receiving antibiotics with $\text{NLR} < 4$

NLR, neutrophil-to-lymphocyte ratio; NR, not reached; PFS, progression-free survival; PPI, proton pump inhibitor

Supplemental Fig. 1 Association of PPI with PFS and OS when stratified by NLR of 3.55: (a, c) patients with $\text{NLR} \geq 3.55$ and (b, d) patients with $\text{NLR} < 3.55$

NLR, neutrophil-to-lymphocyte ratio; NR, not reached; OS, overall survival; PFS, progression-free survival; PPI, proton pump inhibitor

Supplemental Fig. 2 Association of PPI with PFS and OS when stratified by NLR of 3.79: (a, c) patients with $\text{NLR} \geq 3.79$ and (b, d) patients with $\text{NLR} < 3.79$

NLR, neutrophil-to-lymphocyte ratio; NR, not reached; OS, overall survival; PFS, progression-free survival; PPI, proton pump inhibitor

Supplemental Fig. 3 Association of PPI with PFS and OS when stratified by NLR of 5: (a, c) patients with $\text{NLR} \geq 5$ and (b, d) patients with $\text{NLR} < 5$

NLR, neutrophil-to-lymphocyte ratio; NR, not reached; OS, overall survival; PFS, progression-free survival; PPI, proton pump inhibitor

Supplemental Fig. 4 Association of PPI with PFS and OS according to NLR levels in patients excluding driver mutation positive (n=20): (a, d) overall population, (b, e) patients with $\text{NLR} \geq 4$ and (c, f) patients with $\text{NLR} < 4$

NLR, neutrophil-to-lymphocyte ratio; NR, not reached; OS, overall survival; PFS, progression-free

survival; PPI, proton pump inhibitor

Tables

Table 1 Patient characteristics

		NLR ≥ 4 (n=61)			NLR < 4 (n=71)		
Features		PPI + (n=36)	PPI - (n=25)	<i>P</i>	PPI + (n=26)	PPI - (n=45)	<i>P</i>
Age, n (%)	≥ 75	14 (38.9)	11 (44.0)	0.79	20 (76.9)	28 (62.2)	0.29
Sex, n (%)	Female	10 (27.8)	8 (32.0)	0.78	7 (26.9)	12 (26.7)	1
Smoking history, n (%)	Never Smoker	11 (30.6)	10 (40.0)	0.59	7 (26.9)	10 (22.2)	0.77
	Current and Ex-Smoker	25 (69.4)	15 (60.0)		19 (73.1)	35 (77.8)	
Histology, n (%)	Non-Squamous	19 (52.8)	16 (64.0)	0.44	15 (57.7)	32 (71.1)	0.30
	Squamous	17 (47.2)	9 (36.0)		11 (42.3)	13 (28.9)	
Stage, n (%)	Recurrence	18 (50.0)	3 (12.0)	0.002	12 (46.2)	12 (26.7)	0.094
	Advanced	18 (50.0)	22 (88.0)		14 (53.8)	33 (73.3)	
Targetable driver mutation, n (%)	Yes	7 (19.4)	3 (12.0)	0.23	4 (15.4)	6 (13.3)	0.54
	<i>EGFR</i>	6	3		4	5	
	<i>ROS1</i>	1	0		0	1	
	No	16	17		12	28	
	Unknown	13	5		10	11	
PD-L1 TPS, n (%)	$\geq 50\%$	13 (36.1)	13 (52.0)	0.10	8 (30.8)	20 (44.4)	0.15
	$\geq 1\%, < 50\%$	2 (5.6)	6 (24.0)		6 (23.1)	3 (6.7)	
	$< 1\%$	5 (13.9)	1 (4.0)		4 (15.4)	7 (15.6)	
	Unknown	16 (44.4)	5 (20.0)		8 (30.8)	15 (33.3)	
Brain metastasis, n (%)	Yes	11 (30.6)	4 (16.0)	0.24	6 (23.1)	11 (24.4)	0.79
Liver metastasis, n (%)	Yes	8 (22.2)	6 (24.0)	1	4 (15.4)	5 (11.1)	0.72
Bone metastasis, n (%)	Yes	20 (55.6)	10 (40.0)	0.24	5 (19.2)	12 (26.7)	0.73
Number of prior therapy lines, n (%)	0	8 (22.2)	13 (52.0)	0.062	4 (15.4)	12 (26.7)	0.34
	1	10 (27.8)	7 (28.0)		10 (38.5)	17 (37.8)	
	2	10 (27.8)	3 (12.0)		7 (26.9)	5 (11.1)	
	≥ 3	8 (22.2)	2 (8.0)		5 (19.2)	11 (24.4)	
Treatment, n (%)	Nivolumab	18 (50)	9 (36.0)	0.52	14 (53.8)	24 (53.3)	0.47
	Pembrolizumab	16 (44.4)	15 (60.0)		9 (34.6)	19 (42.2)	
	Atezolizumab	2 (5.6)	1 (4.0)		3 (11.5)	2 (4.4)	
Concomitant medication, n (%)	Steroids	22 (61.1)	7 (28.0)	0.011	11 (42.3)	16 (35.6)	0.62
	Antibiotics	20 (55.6)	11 (44.0)	0.44	12 (46.2)	11 (24.4)	0.060
	NSAIDs	23 (63.9)	15 (60.0)	0.79	10 (38.5)	22 (48.9)	0.46

EGFR, epidermal growth factor receptor; NLR, neutrophil-to-lymphocyte ratio; NSAIDs, nonsteroidal

anti-inflammatory drugs; PD-L1 TPS, programmed death ligand 1 tumor proportion score; PPI, proton pump inhibitor; ROS1; c-ros oncogene 1

Table 2 Administration route and duration of proton pump inhibitors of each patient ($n = 62$)

PPIs	Route	n (%)	Administration period of PPIs around the initiation ICI (days; median (range))
Esomeprazole	Oral	31 (50.0)	60 (3–60)
Rabeprazole	Oral	6 (9.7)	60 (27–60)
Lansoprazole	Oral	19 (30.6)	60 (6–60)
Omeprazole	IV	1 (1.6)	16
Vonoprazan	Oral	3 (4.8)	60 (26–60)
Omeprazole →Esomeprazole	IV → Oral	1 (1.6)	60
Lansoprazole →Esomeprazole →Rabeprazole	IV → Oral → Oral	1 (1.6)	60

IV, intravenous; ICI, immune checkpoint inhibitor; PPI, proton pump inhibitor

Table 3 Univariate and multivariate Cox proportional hazard ratio of the prognostic score for progression-free survival

		Overall (n=132)				NLR $\geq$ 4 (n=61)		NLR < 4 (n=71)	
		Univariate analysis		Multivariate analysis		Multivariate analysis		Multivariate analysis	
		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	$\geq$ 75	0.68 (0.43-1.08)	0.10	0.85 (0.51-1.44)	0.55	0.67 (0.29-1.52)	0.34	1.03 (0.40-2.66)	0.94
Sex	Female	1.37 (0.87-2.17)	0.18	0.91 (0.50-1.65)	0.74	0.81 (0.34-1.92)	0.63	1.61 (0.61-4.23)	0.33
Histology	Non-Squamous	1.26 (0.82-1.95)	0.30	2.58 (0.95-7.00)	0.063	1.42 (0.37-5.46)	0.61	6.09 (1.12-33.16)	0.037
Smoking history	Current and Ex-Smoker	0.68 (0.44-1.06)	0.088	0.75 (0.44-1.29)	0.30	0.48 (0.19-1.20)	0.12	0.86 (0.36-2.08)	0.74
Stage	Recurrence	0.91 (0.57-1.43)	0.68	0.96 (0.56-1.63)	0.96	0.94 (0.37-2.35)	0.89	0.79 (0.34-1.84)	0.58
PDL1 TPS	< 50%	Ref		Ref		Ref		Ref	
	$\geq$ 50%	0.51 (0.29-0.88)	0.017	0.71 (0.36-1.39)	0.32	0.79 (0.28-2.22)	0.65	0.72 (0.24-2.11)	0.55
	Unknown	0.97 (0.57-1.66)	0.92	1.20 (0.68-2.13)	0.53	1.80 (0.73-4.41)	0.20	0.64 (0.24-1.68)	0.36
Targetable driver mutation	Negative	Ref		Ref		Ref		Ref	
	Positive	2.41 (1.34-4.33)	0.003	1.67 (0.87-3.21)	0.12	1.04 (0.28-3.93)	0.95	2.68 (1.11-6.50)	0.029
	Unknown	1.27 (0.79-2.04)	0.32	2.59 (0.94-7.14)	0.067	0.93 (0.25-3.41)	0.91	10.72 (1.89-60.78)	0.007
Treatment line	First-line	0.52 (0.31-0.88)	0.015	0.93 (0.43-1.98)	0.84	2.32 (0.80-6.72)	0.12	0.25 (0.07-0.96)	0.043
PPI use	Yes	1.83 (1.18-2.82)	0.006	1.35 (0.81-2.23)	0.25	2.48 (0.95-6.49)	0.065	1.07 (0.52-2.20)	0.85
Steroid use	Yes	2.34 (1.54-3.57)	<0.001	2.15 (1.31-3.54)	0.003	2.99 (1.18-7.58)	0.021	1.73 (0.86-3.50)	0.13
Antibiotic use	Yes	2.34 (1.49-3.66)	<0.001	2.04 (1.20-3.47)	0.009	1.83 (0.90-3.70)	0.095	2.21 (0.85-5.75)	0.11
NLR	NLR $\geq$ 4	1.81 (1.18-2.76)	0.006	1.68 (1.06-2.68)	0.029				

CI, confidence interval; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; PD-L1 TPS, programmed death ligand 1 tumor proportion score; PFS, progression-free survival; PPI, proton pump inhibitor

Table 4 Univariate and multivariate Cox proportional hazard ratio of the prognostic score for overall survival

		Overall (n=132)				NLR $\geq$ 4 (n=61)		NLR < 4 (n=71)	
		Univariate analysis		Multivariate analysis		Multivariate analysis		Multivariate analysis	
		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	$\geq$ 75	1.15 (0.70-1.89)	0.58	1.27 (0.71-2.27)	0.42	1.33 (0.58-3.07)	0.50	1.66 (0.56-4.90)	0.36
Sex	Female	1.22 (0.73-2.04)	0.45	0.86 (0.44-1.67)	0.65	0.64 (0.24-1.69)	0.37	1.35 (0.47-3.94)	0.58
Histology	Non-Squamous	0.99 (0.61-1.60)	0.95	1.03 (0.47-2.28)	0.94	1.09 (0.36-3.34)	0.88	2.47 (0.49-12.46)	0.27
Smoking history	Current and Ex-Smoker	0.87 (0.52-1.44)	0.58	1.05 (0.58-1.90)	0.88	1.40 (0.53-3.70)	0.50	0.50 (0.18-1.36)	0.17
Stage	Recurrence	1.08 (0.66-1.79)	0.76	0.95 (0.53-1.70)	0.86	0.69 (0.28-1.69)	0.42	1.63 (0.58-4.61)	0.35
PDL1 TPS	< 50%	Ref		Ref		Ref		Ref	
	$\geq$ 50%	0.60 (0.34-1.06)	0.078	0.52 (0.24-1.13)	0.096	0.56 (0.19-1.70)	0.31	0.44 (0.16-1.24)	0.12
	Unknown	0.68 (0.37-1.23)	0.20	0.74 (0.40-1.39)	0.35	1.35 (0.51-3.60)	0.55	0.19 (0.06-0.56)	0.003
Targetable driver mutation	Negative	Ref		Ref		Ref		Ref	
	Positive	1.21 (0.64-2.28)	0.55	1.02 (0.47-2.20)	0.97	1.59 (0.36-6.90)	0.54	1.79 (0.60-5.36)	0.30
	Unknown	1.02 (0.59-1.77)	0.95	0.87 (0.36-2.11)	0.76	0.90 (0.27-3.00)	0.86	2.53 (0.40-15.92)	0.32
Treatment line	First-line	0.86 (0.50-1.49)	0.59	1.15 (0.50-2.61)	0.74	1.83 (0.64-5.25)	0.26	0.84 (0.21-3.33)	0.80
PPI use	yes	1.93 (1.20-3.11)	0.007	1.52 (0.88-2.62)	0.14	2.04 (0.81-5.17)	0.13	1.16 (0.53-2.55)	0.71
Steroid use	yes	1.92 (1.20-3.07)	0.007	2.01 (1.15-3.53)	0.015	2.10 (0.88-5.03)	0.095	1.99 (0.78-5.07)	0.15
Antibiotic use	yes	2.10 (1.30-3.41)	0.003	1.54 (0.89-2.65)	0.12	1.50 (0.72-3.11)	0.28	0.90 (0.29-2.76)	0.85
NLR	NLR $\geq$ 4	1.93 (1.21-3.10)	0.006	2.02 (1.21-3.36)	0.007				

CI, confidence interval; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival;

PD-L1 TPS, programmed death ligand 1 tumor proportion score; PPI, proton pump inhibitor