Pretreatment neutrophil-to-lymphocyte ratio and cigarette smoking as prognostic factors in patients with advanced NSCLC treated with osimertinib

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Running title: Prognostic factors in NSCLC patients receiving osimertinib treatment
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All authors read and approved the final manuscript.

Keywords: non-small cell lung carcinoma; osimertinib; receptor, epidermal growth factor

Ethics approval

The Institutional Review Board of Hallym University Sacred Heart Hospital approved the study protocol and informed consent was waived owing to the retrospective nature of the study (HALLYM 2020-03-016-001). All methods were carried out in accordance with the approved guidelines and regulations (Declaration of Helsinki).

Data Sharing Statement

The dataset used and analysed during the present study is available from the corresponding author upon reasonable request.
Abstract
Background
The remarkable efficacy of osimertinib in non-small cell lung cancer (NSCLC) with acquired T790M mutation has widely been documented in clinical trials and real-world practice. However, some patients show primary resistance to the drug and even those patients who initially show a favorable response have inconsistent clinical outcomes. Therefore, this study aimed to identify additional clinical predictive factors for osimertinib efficacy.

Methods
We analyzed a prospective cohort of patients with acquired T790M positive stage IV lung adenocarcinoma treated with osimertinib salvage therapy in the Hallym University Medical Center.

Results
Sixty-one eligible patients were analyzed. The mean age was 63.3 years, 38 (62%) were women, and 39 (64%) never smoked. The median follow-up after treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) was 36.0 months (IQR 24.7–50.2); 45 (74%) patients were deceased. Based on univariate analysis, factors including low baseline neutrophil-to-lymphocyte ratios (NLR), age (≥50 years), never-smoking history, stage IVA at osimertinib initiation, and prolonged response to previous TKIs (≥10 months) were associated with a significantly longer progression-free survival (PFS). Multivariate analysis showed that never-smoking status (hazard ratio [HR], 0.54, 95% CI 0.30–0.98, \( p = 0.041 \)) and a baseline NLR less than or equal to 3.5 (HR 0.23, 95% CI 0.12–0.45, \( p < 0.001 \)) were independently associated with a prolonged PFS with osimertinib.

Conclusions
Smoking history and high NLR were independent negative predictors of osimertinib PFS in patients with advanced NSCLC developing EGFR T790M resistance after the initial EGFR-TKI treatment.
Introduction

The discovery of epidermal growth factor receptor (EGFR) mutations and EGFR tyrosine kinase inhibitors (TKIs) has changed the paradigm of non-small cell lung cancer (NSCLC) treatment. The efficacy of TKIs in EGFR mutation-positive NSCLC has been well established\(^1\),\(^2\). However, although some patients respond well to EGFR-TKIs, some exhibit little or no response, even when positive for EGFR-sensitizing mutations. Moreover, some patients show good long-term effects, whereas others develop resistance after a short-term response\(^3\). The prognosis for patients with EGFR mutations administered EGFR-TKI therapy could be partially predicted using clinical factors, such as female sex, non-smoker status, and Asian race, which were considered good predictors before the introduction of EGFR mutation testing\(^1\).

NSCLC cells become resistant to first- and second-generation EGFR-TKIs after approximately one year of treatment\(^4\). The most common underlying mechanism is the acquired EGFR T790M gatekeeper mutation in exon 20, accounting for approximately half of cases resistant to EGFR-TKIs. Osimertinib, a third-generation irreversible EGFR-TKI, selectively inhibits EGFR-TKI-sensitizing and T790M resistance mutations\(^5\). The AURA3 study demonstrated the efficacy of osimertinib in patients with acquired EGFR T790M mutation NSCLC over platinum plus pemetrexed chemotherapy\(^5\). Subsequently, this drug was approved in several countries, and it is used as salvage treatment in patients with the EGFR T790M mutation.

Patients with the acquired EGFR T790M mutation also respond differently to osimertinib treatment, and they are no homogeneous group; thus, there is a need to elucidate the mechanism and analyze prognostic factors for different responses\(^6\),\(^7\). In addition, predictors for response to osimertinib other than the T790M mutation remain unclear. Therefore, in this study, we analyzed the relationships between clinical factors and osimertinib efficacy in patients with NSCLC with the acquired EGFR T790M mutation previously administered EGFR-TKIs.
Methods

Study population and design

We analyzed data of a cohort of patients with lung adenocarcinoma from the Lung Cancer Registry of Hallym University Medical Center, between January 2006 and August 2021. The inclusion criteria were as follows: 1) stage IV lung adenocarcinoma (according to the 8th edition of the American Joint Commission on Cancer TNM staging system), 2) previous treatment with first (gefitinib or erlotinib)- or second (afatinib)-generation EGFR-TKIs with EGFR-sensitive mutation, 3) acquired EGFR T790M resistance mutation after EGFR-TKI treatment, and 4) osimertinib as salvage treatment. Host-related factors were age at lung cancer diagnosis, sex, Eastern Cooperative Oncology Group (ECOG) performance status score at osimertinib initiation, and smoking status. Tumor-related factors were primary EGFR mutations, previous EGFR-TKIs, response to previous EGFR-TKIs, and serum neutrophil-to-lymphocyte ratio (NLR) at osimertinib initiation. The blood test result used for the analysis was obtained immediately before osimertinib initiation and must have shown no symptoms of infection. Patients aged less than 50 years were included in the young-age group. The criterion was based on previous studies for young-age early-onset lung cancer\textsuperscript{8, 9}. ECOG scores of 0 and 1 were defined as good performance status. Patients with previous EGFR-TKI treatment with progression-free survival (PFS) of less than ten months were defined as poor responders\textsuperscript{10}. We evaluated the optimal NLR cut-off value for PFS with osimertinib treatment via a receiver operating characteristic curve (area under the curve = 0.741, confidence interval 0.60–0.88). We selected the NLR cut-off value as 3.5, with the best sensitivity (0.75) and specificity (0.67), based on similar values being indicated in previous studies\textsuperscript{11, 12}.

We evaluated the tumor response and disease progression every eight weeks per cohort protocol. If necessary, radiologic evaluations were conducted to assess the response according to the clinician's judgment, even within this period. Evaluation of the response to EGFR-TKIs was performed by retrospective review of the radiologic images of the entire case by two experienced investigators, based on the RECIST v1.1 criteria. The attending physician's evaluation or decision was not included
in the efficacy evaluation. We defined PFS as the period from osimertinib initiation to the date of disease progression or death from any cause. Baseline clinical factors were analyzed according to favorable or unfavorable (primary resistant) efficacy groups, which were those with a PFS of greater than or equal to six and less than six months, respectively, with osimertinib treatment; this time was based on the AURA3 study, in which the lower quarter of patients showed a PFS of less than six months\(^5\). Overall survival (OS) was calculated as the time from osimertinib initiation to death from any cause or censorship at the last follow-up. If progression occurred after first-line EGFR-TKI treatment, the biopsy was repeated, if possible. The PNA-clamp EGFR mutation detection kit (Panagene, Daejeon, Korea), a peptide nucleic acid-mediated real-time PCR clamping technology, was used to detect acquired EGFR mutations\(^{13}\). When tissue-based assays were not feasible, plasma EGFR T790M mutation tests were performed (Cobas EGFR mutation test v2; Roche, Pleasanton, CA, USA). If T790M mutation was not detected by one method, another method was employed, but it was not mandatory. The Institutional Review Board approved the study protocol, and informed consent was waived owing to the study’s retrospective nature.

**Statistical analysis**

Categorical variables were statistically analyzed using Fisher's exact test. Continuous variables were analyzed using Student's \(t\)-tests or Mann-Whitney U-tests. We used Kaplan-Meier estimates to construct survival curves and calculate median PFS and OS. Cox regression methods (univariate and multivariate) were used to estimate prognostic factors for PFS and OS by adjusting baseline characteristics. All analyses were performed using SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA).
Results

Baseline characteristics

Sixty-one patients with the acquired EGFR T790M mutation were enrolled. All patients were followed up until August 20, 2021; had histologically confirmed lung adenocarcinoma with EGFR-sensitizing mutations; and were treated with gefitinib, erlotinib, or afatinib until disease progression, at which point they were treated with osimertinib. The clinical characteristics of the study patients are summarized in Table 1. The mean age was 63.3 ± 11.9 years, 38 (62%) were women, and 39 (64%) never smoked. At osimertinib treatment initiation, 17 (28%) and 44 (72%) patients had disease stages IVA and IVB, respectively. Central nervous system metastasis was present in 24 (39%) patients at osimertinib treatment initiation. Forty (66%) patients initially had EGFR exon 19 deletions, and 21 (34%) had an EGFR exon 21 mutation (L858R, 20 patients; L861Q, one patient) before their first EGFR-TKI treatment, whereas none of the patients had de novo EGFR T790M mutation. The mean baseline NLR at osimertinib treatment was 4.4 ± 3.2; 26 (43%) patients had a high NLR (> 3.5).

Table 1. Patient characteristics (n = 61).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, mean ± SD</td>
<td>63.3 ± 11.9</td>
</tr>
<tr>
<td>Female</td>
<td>38 (62)</td>
</tr>
<tr>
<td>Performance at osimertinib initiation</td>
<td></td>
</tr>
<tr>
<td>ECOG 0–1</td>
<td>43 (70)</td>
</tr>
<tr>
<td>ECOG 2–4</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Smoking status, never smoker</td>
<td>39 (64)</td>
</tr>
<tr>
<td>Stage at osimertinib initiation</td>
<td></td>
</tr>
<tr>
<td>IVA</td>
<td>17 (28)</td>
</tr>
<tr>
<td>IVB</td>
<td>44 (72)</td>
</tr>
<tr>
<td>CNS metastasis at osimertinib initiation</td>
<td>24 (39)</td>
</tr>
<tr>
<td>EGFR co-mutation with T790M</td>
<td></td>
</tr>
<tr>
<td>Exon 19 deletion</td>
<td>40 (66)</td>
</tr>
<tr>
<td>Exon 21 L858R/L861Q</td>
<td>21 (34)</td>
</tr>
<tr>
<td>Detection methods of T790M</td>
<td></td>
</tr>
<tr>
<td>Re-biopsy tissue positive* &amp; plasma negative</td>
<td>7 (11)</td>
</tr>
<tr>
<td>Re-biopsy tissue positive &amp; plasma test not performed</td>
<td>36 (59)</td>
</tr>
<tr>
<td>Plasma positive &amp; re-biopsy not performed</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Plasma positive &amp; re-biopsy tissue negative</td>
<td>6 (10)</td>
</tr>
<tr>
<td>Previous EGFR-TKI</td>
<td></td>
</tr>
<tr>
<td>First-generation (gefitinib/erlotinib)</td>
<td>42 (69)</td>
</tr>
<tr>
<td>Second-generation (afatinib)</td>
<td>19 (31)</td>
</tr>
<tr>
<td>PFS of previous EGFR-TKI, months</td>
<td></td>
</tr>
<tr>
<td>&lt; 10 months</td>
<td>23 (38)</td>
</tr>
<tr>
<td>≥ 10 months</td>
<td>38 (62)</td>
</tr>
<tr>
<td>Median PFS of previous TKIs, months [95% CI]</td>
<td>12.0 [9.9-14.0]</td>
</tr>
<tr>
<td>Baseline NLR at osimertinib initiation, mean ± SD</td>
<td>4.4 ± 3.2</td>
</tr>
<tr>
<td>Baseline NLR at osimertinib initiation &gt; 3.5</td>
<td>26 (43)</td>
</tr>
<tr>
<td>Treatment line of osimertinib</td>
<td></td>
</tr>
<tr>
<td>Second line</td>
<td>31 (51)</td>
</tr>
<tr>
<td>≥ Third line (3–8)</td>
<td>30 (49)</td>
</tr>
<tr>
<td>Median PFS of osimertinib, months [95% CI]</td>
<td>9.3 [6.7–11.9]</td>
</tr>
<tr>
<td>Median OS after osimertinib, months [95% CI]</td>
<td>17.5 [13.4–21.5]</td>
</tr>
</tbody>
</table>

*Tumor tissue investigation included solid tumor biopsy and cytological analysis of body fluids (e.g., pleural effusion).

**Osimertinib treatment outcomes**

The EGFR T790M mutation was identified by tissue re-biopsy in 43 (plasma T790M negative: 7/43, plasma test not performed: 36/43) patients and by plasma sampling in 18 (re-biopsy tissue negative: 6/18, re-biopsy not performed: 12/18) patients. Thirty-one (51%) and 30 (49%) patients received osimertinib and cytotoxic chemotherapy as second-line therapy, respectively, followed by osimertinib as third- to eighth-line treatments. Thirty-nine (64%) achieved partial response (PR), 15 (25%) had stable disease (SD), and seven (11%) had progressive disease (PD) with osimertinib treatment. At data cut-off, 50 (82%) patients had PD. The overall median PFS following osimertinib treatment was 9.3 months (95% CI: 6.7–11.9). Table S1 presents a comparison of the clinical characteristics of patients with PFS with osimertinib treatment of less than six months (unfavorable group) and higher than or
equal to six months (favorable group). The baseline NLR at the commencement of osimertinib treatment was higher in the unfavorable than in the favorable group (5.8 ± 3.9 versus 3.5 ± 2.3, respectively; \( p = 0.016 \)). However, no significant differences were observed in other clinical factors among the predefined groups.

**Clinical factors associated with PFS with osimertinib**

Figure 1 shows the univariate analysis of clinical factors for PFS with osimertinib. The median PFS was significantly longer in patients with a low NLR at the start of osimertinib treatment than in those with a high NLR (≤3.5, 12.3 months versus >3.5, 5.7 months, respectively; \( p < 0.001 \)). In addition, age (≥50 years), non-smoking history, stage IVA at osimertinib initiation, and prolonged response to previous TKIs (≥10 months) induced a significantly longer PFS (\( p < 0.05 \)). Female sex and good performance (ECOG score, 0–1) also prolonged PFS trends, although the result was not significant (\( p < 0.1 \)). Corresponding to the T790M detection methods, the median PFS of osimertinib was 3.5 months in the tissue negative/plasma positive group, which was significantly shorter than other groups (tissue unknown/plasma positive, 9.4 months \( p = 0.034 \); tissue positive/plasma unknown, 9.3 months \( p = 0.009 \); tissue positive/plasma negative, 14.4 months \( p = 0.010 \)).

Table 2 shows the Cox proportional model of osimertinib PFS. Covariates of multivariate analysis were selected using log-rank tests for the Kaplan-Meier estimation of PFS (\( p < 0.1 \)). The analysis showed that the never-smoking status (hazard ratio [HR], 0.54, 95% CI 0.30–0.98, \( p = 0.041 \)) and a baseline NLR less than or equal to 3.5 (HR 0.23, 95% CI 0.12–0.45, \( p < 0.001 \)) were independent, good predictive factors for PFS after osimertinib treatment (Figure 2). However, there were no differences in clinical characteristics between the PR group and the SD/PD group (Table S2).

**Table 2.** Cox proportional hazard regression analysis of progression-free survival.

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>HR for progression</th>
<th>95% CI</th>
<th>( p ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never-smoking status</td>
<td>0.54</td>
<td>0.30–0.98</td>
<td>( p = 0.041 )</td>
</tr>
</tbody>
</table>
Never smoker 0.54 [0.30-0.98] 0.041
Ever smoker 1

Baseline NLR at osimertinib initiation
≤ 3.5 0.23 [0.12-0.45] <0.001
> 3.5 1

* Covariates of the multivariate analysis were selected using the log-rank test (p < 0.100) for the Kaplan-Meier estimation of PFS (age group, sex, smoking status, ECOG performance, baseline NLR, stage at osimertinib initiation, PFS with previous TKIs, and T790M detection methods).

**OS after osimertinib treatment**

Forty-five (74%) patients were deceased by the end of the study, whereas the median OS in all patients was 17.5 months (95% CI, 13.4–21.5) after osimertinib treatment. The univariate analysis showed that patients with a good performance status (ECOG 0–1), low baseline NLR (≤3.5), and stage IVA at osimertinib initiation had a significantly longer OS (Figure 1). Corresponding to the T790M detection methods, the median OS was 4.8 months in the tissue negative/plasma positive group, which was significantly shorter than other groups (tissue positive/plasma unknown, 17.5 months [p = 0.005]; tissue positive/plasma negative, the median OS was not reached). Multivariate Cox regression analysis revealed performance status and NLR as independent prognostic factors for OS (ECOG 0–1: HR 0.35, 95% CI 0.18–0.67, p = 0.002; low NLR: HR 0.17, 95% CI 0.09–0.34, p < 0.001) (Table 3).

**Table 3.** Cox proportional hazard regression analysis of overall survival after osimertinib treatment according to clinical factors.

<table>
<thead>
<tr>
<th></th>
<th>HR for progression</th>
<th>95% CI</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance at osimertinib initiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG 0–1</td>
<td>0.35</td>
<td>[0.18-0.67]</td>
<td>0.002</td>
</tr>
<tr>
<td>ECOG 2–4 (reference)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline NLR at osimertinib initiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 3.5</td>
<td>0.17</td>
<td>[0.09-0.34]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt; 3.5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Covariates of the multivariate analysis were selected using the log-rank test ($p < 0.100$) for the Kaplan-Meier estimation of overall survival (ECOG performance, baseline NLR, stage, CNS metastasis at osimertinib initiation and PFS with previous TKIs, and T790M detection methods).
Discussion

This real-world study showed that osimertinib PFS in patients with acquired T790M mutation after primary EGFR-TKI treatment was shortened with smoking history and a high baseline NLR. In addition, NLR was also an independent prognostic factor for OS after osimertinib treatment together with ECOG performance status.

Finding EGFR mutations in advanced stage NSCLC is a powerful marker for screening patients with the corresponding driving mutation and predicting the efficacy of EGFR-TKI. On the other hand, among patients with EGFR mutations, 20–30% develop primary resistance to EGFR-TKI therapy. Therefore, additional factors that may determine the prognosis of EGFR-TKIs are continuously being studied. Recently, as the accessibility of genetic profiling has increased, some studies suggested that co-mutations in genes other than EGFR are essential in determining the therapeutic response. Furthermore, the authors reported that the EGFR-TKIs efficacy decreases when there is a concomitant mutation, particularly with more mutations or a specific type of mutation (e.g., an oncogene mutation) related to a poor outcome. Therefore, determining which clinical characteristics in these groups might provide clues to elucidate the complicated carcinogenesis of EGFR lung cancer and select these patients for more individualized treatment is essential.

The results of studies on clinical factors related to first- and second-generation EGFR-TKIs efficacy are as follows. A subgroup analysis of randomized clinical trials (RCTs) that compared EGFR-TKIs to cytotoxic chemotherapy indirectly revealed prognostic factors of the efficacy of EGFR-TKI treatment in EGFR mutation-positive NSCLC. In the IPASS study (gefitinib vs. carboplatin-paclitaxel), gefitinib prolonged the PFS in the older subgroup (≥ 65 years) more significantly than in the younger subgroup. In the LUX-lung 3 trial (afatinib vs. pemetrexed plus cisplatin), a significantly longer PFS and OS with afatinib were observed in patients with the EGFR 19del mutation than in those with the L858R mutation. A meta-analysis of seven RCTs, including this trial, confirmed the benefit of EGFR-TKI, regardless of past smoking history, but showed a more significant prolongation of PFS in
never-smokers by meta-regression analysis\textsuperscript{17}. However, because these were results from a subgroup analysis of the TKIs efficacy compared to cytotoxic agents, the prognostic evaluation based on specific clinical factors has limitations. On the other hand, previous real-world studies analyzing patients receiving EGFR-TKIs only did not show consistent prognostic factors. Although there was a problem with the small number of subjects, other reasons were the inclusion of cases with unconfirmed EGFR mutations, the omission of important prognostic factors, or the inconsistent EGFR test methods or treatment processes within one study\textsuperscript{18, 19}. However, well-designed recent studies have reported that smoking history affects the efficacy of first- and 2nd-generation TKIs through multivariate analysis\textsuperscript{20-22}.

The T790M mutation is confirmed in about half of cases where resistance develops after EGFR-TKI treatment. Based on the results of the AURA3 trial, 6.5\% of patients with the acquired EGFR T790M mutation showed rapid progression after osimertinib treatment\textsuperscript{5}. In a subgroup analysis, no specific clinical factors were associated with osimertinib efficacy. However, the AURA3 trial did not represent all real-world patients (only patients with an ECOG score of 0–1 were enrolled; 96\% of patients were treated with osimertinib as second-line after first-line TKIs). A retrospective real-world study by Kato et al. reported that an older age and an ECOG score of 0–1 were good predictors for PFS after osimertinib treatment in 30 patients with the acquired EGFR T790M mutation\textsuperscript{6}. Yoshimura et al. reported that a prolonged PFS history with previous EGFR-TKIs is a predictive factor for subsequent osimertinib treatment among 27 patients\textsuperscript{7}. Similar to previous generation TKI studies, each study showed different results. Therefore, similar to previous studies, the leading cause of the heterogeneity was the small case number. However, it is worth noting that the factors that were non-significant in our multivariate analysis but tended to be prognostic factors in univariate analysis were consistent with those previously reported. Interestingly, Chang et al. recently reported that osimertinib efficacy was diminished in a group of patients who had a complex mutation apart from the T790M mutation\textsuperscript{23}. 
EGFR mutations are more frequently identified in NSCLC in non-smokers, but approximately 30% of mutation-positive patients are reported to have a smoking history. In our study, the low efficacy of osimertinib in patients with a history of smoking may indicate that inhibition of EGFR activation alone does not entirely block the carcinogenesis pathway. Smoking induces various genetic alterations in lung cancer, and the most frequently observed co-occurring alteration with EGFR mutations is TP53 mutations, which are highly related to smoking. The effect of TP53 mutations on the EGFR-TKI therapy has been demonstrated in several clinical studies and preclinical studies. Recently, Kim et al. reported a significantly reduced PFS and a worse OS of osimertinib treatment in patients with acquired T790M mutation with a TP53 accompanying mutation. A preclinical study reported that cigarette smoke extract (CSE) and tobacco smoke-derived carcinogen upregulate the c-MET pathway, which induces resistance to EGFR-TKI in 19del mutation cell lines. Ahn et al. showed that c-MET amplification in re-biopsy histology is associated with previous smoking history in the case of progression after EGFR-TKI treatment. Li et al. reported that CSE inhibits the effect of EGFR-TKIs through Src activation and epithelial to mesenchymal transition. The above results are also consistent with next-generation sequencing data showing that a higher tumor mutation burden leads to lower EGFR-TKI responses.

Additional studies have identified relationships between systemic inflammation and tumorigenesis factors such as tumor angiogenesis, invasion, and metastasis. Tumor-related inflammation correlates with neutrophilia, lymphopenia, or both in peripheral blood, and is associated with a poor prognosis in patients with various carcinomas. In addition, neutrophils play a role as a metastasis promoter by trapping and migrating tumor cells using extracellular traps. Moreover, tumor-associated neutrophils may support tumor angiogenesis and invasion by producing matrix metalloproteinase-9 and vascular endothelial growth factors. Previous reports suggest that an elevated NLR is a useful prognostic biomarker in early, locally advanced, and advanced stages of lung cancer, and also for various treatment modalities in lung cancer patients. In a small number of studies, a high NLR is also an independent poor prognostic marker for PFS or OS after first- or second-
generation EGFR-TKI treatment\textsuperscript{12, 38}. To the best of our knowledge, our study is the first to report that NLR elevation correlates with poor osimertinib efficacy in patients with acquired T790M mutation.

We found osimertinib to have inconsistent efficacy in the univariate analysis, varying according to different T790M detection methods. Previously, in the post-hoc analysis of plasma samples from the phase I AURA trial, the PFS of osimertinib in the tissue T790M negative/plasma T790M positive group was shorter than that in the tissue positive/plasma positive group (4.2 months vs. 9.3 months, \( p = 0.002 \))\textsuperscript{39}. In another retrospective study, the tissue negative/plasma positive group showed a poor objective response rate of only 7.6\%\textsuperscript{40}. Tumor heterogeneity may explain this discrepancy and it could be assumed that different resistance mechanisms for previous EGFR-TKIs occurred in multiple metastatic tumors in the same patient\textsuperscript{41}. On the other hand, the tissue positive/plasma negative group showed the most prolonged PFS in our study, although without statistical significance. In the AURA3 trial (all subjects must have confirmation of re-biopsy tissue T790M mutation), the PFS in tissue- and plasma circulating tumor DNA (ctDNA)- T790M positive subgroup was approximately two months shorter than patients in the intention-to-treat population (8.2 months vs. 10.1 months)\textsuperscript{5}. Hong et al. also reported a better efficacy in the tissue positive/plasma negative group\textsuperscript{40}. The absence of plasma ctDNA of T790M mutation in systemic circulation may mean that metastasis is indolent\textsuperscript{40}. Interestingly, in our study, the high NLR was 1/7 (14.3\%) in the tissue positive/plasma negative group, and 4/6 (66.7\%) in the tissue negative/plasma positive group. Further studies are needed on the relationship of ctDNA and peripheral blood NLR in lung cancer.

Regarding the effect of age on PFS with subsequent osimertinib treatment, Kato et al.\textsuperscript{6} showed that an older age is a good independent prognostic factor. In our study, the age factor showed a difference in univariate analysis. No known biological or other mechanism explains the difference in the effects of EGFR-TKIs on age groups. In a previous report, uncommon EGFR mutations, which have lower EGFR-TKI response rates, are more prevalent at a young age\textsuperscript{42}. However, this hypothesis cannot be applied to the study by Kato et al., in which there were two uncommon mutations at old age.
The limitations of our study are its retrospective nature and the relatively limited number of patients. However, we could reduce some bias and minimize missing data because the same protocol was used for diagnostic testing and treatment within prospective lung cancer cohorts. Moreover, the observation period was sufficiently long: we were able to analyze survival data, which were highly maturated. During the response evaluation of EGFR-TKIs, the investigators were not blinded to other clinical features and laboratory results. To overcome this limitation, independent investigators, other than an individual patient's treating physician, reviewed the radiologic images of all cases and re-evaluated the response strictly according to the RECIST criteria. Lastly, there are limitations in interpreting the results of prognosis according to T790M detection methods because several confounding factors act in selecting the T790M test methods, and both tissue biopsy and plasma tests are not routinely performed on all patients.

**Conclusion**

Smoking history and a low NLR were found to be associated with good outcomes with osimertinib treatment in patients with NSCLC developing EGFR T790M resistance after the initial EGFR-TKI treatment. Furthermore, a poor performance status and high NLR were poor prognostic factors for OS in these patients. Additional large-scale studies are necessary to validate these results.
Competing interests: The authors have declared that no competing interests exist.

Acknowledgments: None

Funding: No funding was obtained for this study

Supporting information

Table S1. Patient characteristics in osimertinib response groups by six-month progression-free survival.

Table S2. Patients characteristics according to osimertinib responsiveness.
Figure legends.

Figure 1. (A) Forest plot of univariate analysis of progression-free survival with osimertinib and clinical factors. (B) Forest plot of univariate analysis of overall survival after osimertinib treatment and clinical factors. ECOG, Eastern Cooperative Oncology Group; CNS m, Central Nervous System metastasis; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitors; PFS, progression-free survival; NLR, neutrophil-to-lymphocyte ratio; PR, partial response; SD, stable disease; PD, progressive disease.
**Figure 2.** Kaplan-Meier survival curves for progression-free survival of patients who received osimertinib as salvage treatment stratified by baseline NLR (A) and smoking status (B). Kaplan-Meier survival curves for overall survival of patients who received osimertinib as salvage treatment stratified by baseline NLR (C) and ECOG performance scores (D). NLR, neutrophil-to-lymphocyte ratio.
REFERENCES


