Pretreatment Neutrophil-to-Lymphocyte Ratio and Smoking History as Prognostic Factors in Advanced Non–Small Cell Lung Cancer Patients Treated with Osimertinib

Ji Young Park, M.D.1,2, Seung Hun Jang, M.D., Ph.D.1,2, Chang Youl Lee, M.D., Ph.D.2,3, Tahee Kim, M.D.2,4, Soo Jie Chung, M.D.2,5, Ye Jin Lee, M.D.2,6, Hwan Il Kim, M.D.1,2, Joo-Hee Kim, M.D., Ph.D.1,2, Sungoon Park, M.D., Ph.D.1,2, Yong Il Hwang, M.D., Ph.D.1,2 and Ki-Suck Jung, M.D., Ph.D.1,2

*Author affiliations appear at the end of this article.

Background: The remarkable efficacy of osimertinib in non-small cell lung cancer (NSCLC) with acquired T790M mutation has been widely documented in clinical trials and real-world practice. However, some patients show primary resistance to this drug. Even patients who initially show a favorable response have inconsistent clinical outcomes later. Therefore, the aim of this study was to identify additional clinical predictive factors for osimertinib efficacy.

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

Results: Sixty-one eligible patients were analyzed, including 38 (62%) women and 39 (64%) who never smoked. Their mean age was 63.3 years. The median follow-up after treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) was 36.0 months (interquartile range, 24.7–50.2 months). The majority (n=45, 74%) of patients were deceased. Based on univariate analysis, low baseline neutrophil-to-lymphocyte ratios (NLR), age ≥50 years, never-smoking history, stage IV 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% confidence interval [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.

Conclusion: 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.

Keywords: Non–Small Cell Lung Carcinoma; Osimertinib; Receptor, Epidermal Growth Factor
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 they are 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 with EGFR-TKI therapy could be partially predicted using clinical factors such as female sex, non-smoker

| Table 1. Patient characteristics (n=61) |
|----------------------------------------|
| Characteristic                          | No. (%)                     |
| Age at diagnosis (mean±SD), yr         | 63.3±11.9                   |
| Female sex                             | 38 (62)                     |
| Performance at osimertinib initiation  |                             |
| ECOG 0–1                               | 43 (70)                     |
| ECOG 2–4                               | 18 (30)                     |
| Smoking status, never smoker           | 39 (64)                     |
| Stage at osimertinib initiation        |                             |
| IVA                                    | 17 (28)                     |
| IVB                                    | 44 (72)                     |
| CNS metastasis at osimertinib initiation |                             |
| Exon 19 deletion                       | 40 (66)                     |
| Exon 21 L858R/L861Q                    | 21 (34)                     |
| Detection methods of T790M             |                             |
| Re-biopsy tissue positive* and plasma negative | 7 (11)            |
| Re-biopsy tissue positive and plasma test not performed | 36 (59)   |
| Plasma positive and re-biopsy not performed | 12 (20)        |
| Plasma positive and re-biopsy tissue negative | 6 (10)        |
| Previous EGFR-TKI                      |                             |
| First-generation (gefitinib/erlotinib) | 42 (69)                     |
| Second-generation (afatinib)           | 19 (31)                     |
| PFS of previous EGFR-TKI, mo           |                             |
| <10 mo                                  | 23 (38)                     |
| ≥10 mo                                  | 38 (62)                     |
| Median PFS of previous TKIs (95% CI), mo | 12.0 (9.9–14.0) |
| Baseline NLR at osimertinib initiation (mean±SD) | 4.4±3.2          |
| Baseline NLR at osimertinib initiation >3.5 | 26 (43)       |
| Treatment line of osimertinib          |                             |
| Second line                            | 31 (51)                     |
| ≥Third line (3–8)                      | 30 (49)                     |
| Median PFS of osimertinib (95% CI), mo | 9.3 (6.7–11.9) |
| Median OS after osimertinib (95% CI), mo | 17.5 (13.4–21.5) |

*Tumor tissue investigation included solid tumor biopsy and cytological analysis of body fluids (e.g., pleural effusion).
ECOG: Eastern Cooperative Oncology Group; EGFR: epidermal growth factor receptor; TKI: tyrosine kinase inhibitor; CI: confidence interval; NLR: neutrophil-to-lymphocyte ratios; PFS: progression-free survival; OS: overall survival.
status, and Asian race, which are considered as good predictors before the introduction of EGFR mutation testing\(^7\).

NSCLC cells can 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, which accounts for approximately half of cases resistant to EGFR-TKIs\(^5\). Osimertinib, a third-generation irreversible EGFR-TKI, can selectively inhibit EGFR-TKI-sensitizing and T790M resistance mutations\(^1\). The AURA3 study has demonstrated a better efficacy of osimertinib in patients with acquired EGFR T790M mutation NSCLC than platinum plus pemetrexed chemotherapy\(^5\). Subsequently, this drug was approved in several countries. It is currently used as salvage treatment in patients with the EGFR T790M mutation.

Patients with the acquired EGFR T790M mutation also respond differently to osimertinib treatment. Therefore, there is a need to elucidate the mechanism and analyze prognostic factors for different responses\(^6\). In addition, predictors for response to osimertinib other than the T790M mutation remain unclear. Therefore, the objective of this study was to analyze relationships between clinical factors and osimertinib efficacy in NSCLC patients with the acquired EGFR T790M mutation who were previously treated with EGFR-TKIs.

**Materials and Methods**

1. **Study population and design**

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 were analyzed. 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-generation EGFR-TKIs (gefitinib or erlotinib) or second-generation EGFR-TKIs (afatinib) with EGFR-sensitive mutation, (3) acquired EGFR T790M resistance mutation after EGFR-TKI treatment, and (4) osimertinib as a salvage treatment. Host-related factors were age at lung cancer diagnosis, sex, Eastern Cooperative Oncology Group (ECOG) performance status, and Asian race, which are considered as good predictors before the introduction of EGFR mutation testing\(^7\).

| Factors | Reference | HR, p-value |
|---------|-----------|-------------|
| Age at diagnosis, yr | Age >50 | HR=0.37, p=0.008 |
| Sex | Female | HR=0.61, p=0.091 |
| Smoking status | Never | HR=0.55, p=0.047 |
| ECOG at osimertinib initiation | 0–1 | HR=0.61, p=0.099 |
| NLR at osimertinib initiation | <3.5 | HR=0.24, p=0.001 |
| Stage at osimertinib initiation | IVB | HR=0.45, p=0.032 |
| CNS metastasis at osimertinib initiation | CNS m | HR=0.63, p=0.114 |
| EGFR mutation | 19del | HR=0.90, p=0.722 |
| Previous TKI generation | 2nd-gen. | HR=0.69, p=0.240 |
| PFS of previous TKI | <10 mo | HR=0.48, p=0.017 |
| Treatment line of osimertinib | 2nd line | HR=1.04, p=0.879 |

Figure 1. (A) Forest plot of univariate analysis of progression-free survival of those treated 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; NLR: neutrophil-to-lymphocyte ratio; CNS m: central nervous system metastasis; EGFR: epidermal growth factor receptor; TKI: tyrosine kinase inhibitors; PFS: progression-free survival.
(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. Blood test results used for the analysis were obtained immediately before osimertinib initiation without showing any symptoms of infection. Patients aged less than 50 years were included in the young-age group. This criterion was based on previous studies on young-age early-onset lung cancer. ECOG scores of 0 and 1 were defined as good performance status. Patients who had a previous EGFR-TKI treatment with progression-free survival (PFS) of less than ten months were defined as poor responders. We evaluated the optimal NLR cutoff 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 cutoff value as 3.5 with the best sensitivity (0.75) and specificity (0.67) based on similar values indicated in previous studies.

We evaluated tumor response and disease progression every 8 weeks per cohort protocol. If necessary, radiologic evaluations were conducted to assess responses according to the clinician’s judgment, even within this period. Evaluation of responses to EGFR-TKIs was performed by retrospective review of radiologic images of the entire case by two experienced investigators based on the Response Evaluation Criteria in Solid Tumor (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 group. The favorable efficacy group was defined as those with a PFS of greater than or equal to 6 months with osimertinib treatment. The unfavorable efficacy group was defined as those with a PFS of less than 6 months with osimertinib treatment. The criterion of 6 months was based on the AURA3 study, in which the lower quarter of patients showed a PFS of less than 6 months.

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, then biopsy was repeated, if possible. The PNAClamp EGFR mutation detection kit (Panagene, Daejeon, Korea), a peptide nucleic acid-mediated real-time PCR clamping technology, was used to detect acquired EGFR mutations. When tissue-based assays were not feasible, plasma EGFR T790M mutation tests were performed using a Cobas EGFR mutation test v2 (Roche, Pleasanton, CA, USA). If T790M mutation was not detected by one method, another method was employed. However, this was not mandatory. 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.

2. 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

1. Baseline characteristics

Sixty-one patients with acquired EGFR T790M mutation were enrolled. All patients were followed up until August 20, 2021. They all had histologically confirmed lung adenocarcinoma.

### Table 2. Cox proportional hazard regression analysis of PFS

| Smoking status       | HR for progression | 95% CI      | p-value* |
|----------------------|--------------------|-------------|----------|
| Never smoker         | 0.54               | 0.30–0.98   | 0.041    |
| Ever smoker          | 1                  |             |          |

| Baseline NLR at osimertinib initiation | HR for progression | 95% CI      | p-value* |
|----------------------------------------|--------------------|-------------|----------|
| ≤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 progression-free survival (PFS) (age group, sex, smoking status, Eastern Cooperative Oncology Group performance, baseline neutrophil-to-lymphocyte ratio [NLR], stage at osimertinib initiation, PFS with previous tyrosine kinase inhibitors, and T790M detection methods). HR: hazard ratio; CI: confidence interval.
Prognostic factors in NSCLC patients receiving osimertinib treatment

Prognostic factors in NSCLC patients receiving osimertinib treatment

Tuberculosis and Respiratory Diseases

www.e-trd.org  https://doi.org/10.4046/trd.2021.0139

159

Prognostic factors in NSCLC patients receiving osimertinib treatment

Prognostic factors in NSCLC patients receiving osimertinib treatment

nominal with EGFR-sensitizing mutations. They were treated with gefitinib, erlotinib, or afatinib until disease progression, at which point they were treated with osimertinib. Clinical characteristics of the study patients are summarized in Table 1. Their mean age was 63.3±11.9 years. There were 38 women (62%) and 39 never-smokers (64%). 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 patients (39%) at osimertinib treatment initiation. Forty patients (66%) 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 these patients had a de novo EGFR T790M mutation. The mean baseline NLR at osimertinib treatment was 4.4±3.2. Twenty-six patients (43%) had a high NLR (>3.5).

2. Osimertinib treatment outcomes

The EGFR T790M mutation was identified in 43 patients by tissue re-biopsy (plasma T790M negative, 7/43; plasma test not performed, 36/43) and in 18 patients by plasma

A No. of patients Median PFS [95% CI]
Low NLR 35 12.3 mo [8.9–15.6]
High NLR 26 5.7 mo [2.2–9.3]

HR for disease progression or death: 0.24, p<0.001

B No. of patients Median PFS [95% CI]
Never smoker 39 9.8 mo [8.4–11.1]
Ever smoker 22 6.8 mo [4.4–9.1]

HR for disease progression or death: 0.55, p=0.047

C No. of patients Median OS [95% CI]
Low NLR 35 24.8 mo [19.5–30.2]
High NLR 26 8.3 mo [3.3–13.4]

HR for death: 0.19, p<0.001

D No. of patients Median OS [95% CI]
ECOG 0–1 43 20.2 mo [15.7–24.7]
ECOG 2–4 18 6.3 mo [0.8–11.7]

HR for death: 0.38, p=0.003

Figure 2. Kaplan-Meier survival curves for progression-free survival (PFS) of patients who received osimertinib as salvage treatment stratified by baseline neutrophil-to-lymphocyte ratio (NLR) (A) and smoking status (B). Kaplan-Meier survival curves for overall survival (OS) of patients who received osimertinib as salvage treatment stratified by baseline NLR (C) and Eastern Cooperative Oncology Group (ECOG) performance scores (D). CI: confidence interval; HR: hazard ratio.
sampling (re-biopsy tissue negative, 6/18; re-biopsy not performed, 12/18). Thirty-one (51%) and 30 (49%) patients received osimertinib and cytotoxic chemotherapy as second- to eighth-line therapy. With osimertinib treatment, 39 (64%) achieved partial response (PR), 15 (25%) had stable disease (SD), and seven (11%) had progressive disease (PD). At data cutoff, 50 patients (82%) had PD. The overall median PFS following osimertinib treatment was 9.3 months (95% confidence interval [CI], 6.7–11.9 months). Supplementary Table S1 presents a comparison of clinical characteristics of patients with PFS with osimertinib treatment for less than 6 months (unfavorable group) or higher than or equal to six months (favorable group). The baseline NLR at the commencement of osimertinib treatment was higher in the unfavorable group than in the favorable group (5.8±3.9 vs. 3.5±2.3, p=0.016). However, no significant differences in other clinical factors were observed among predefined groups.

3. Clinical factors associated with PFS with osimertinib

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

Table 2 shows results of analysis using 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 a 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 and 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 (Supplementary Table S2).

4. OS after osimertinib treatment

Forty-five patients (74%) were deceased by the end of the study. The median OS of all patients was 17.5 months (95% CI, 13.4–21.5 months) after osimertinib treatment. The univariate analysis showed that patients with a good performance status (ECOG 0–1), low baseline NLR (≤3.5), and stage IV at osimertinib initiation had significantly longer OS (Figure 1). Corresponding to T790M detection methods, the median OS was 4.8 months in the tissue negative/plasma positive group, which was significantly shorter than that in 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 that performance status and NLR were 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).

Discussion

This real-world study showed that osimertinib PFS in patients with acquired T790M mutation after primary EGFR-TKI treatment was shortened by a smoking history and a high

| Table 3. Cox proportional hazard regression analysis of overall survival after osimertinib treatment according to clinical factors |
|---------------------------------------------------------------|
| **HR for progression** | **95% CI** | **p-value*** |
| Performance at osimertinib initiation | | |
| ECOG 0–1 | 0.35 | 0.18–0.67 | 0.002 |
| ECOG 2–4 | 1 | | |
| Baseline NLR at osimertinib initiation | | |
| ≤3.5 | 0.17 | 0.09–0.34 | <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 overall survival (Eastern Cooperative Oncology Group [ECOG] performance, baseline neutrophil-to-lymphocyte ratio [NLR], stage, central nervous system metastasis at osimertinib initiation and progression-free survival with previous tyrosine kinase inhibitors, and T790M detection methods). HR: hazard ratio; CI: confidence interval.
Prognostic factors in NSCLC patients receiving osimertinib treatment

Baseline NLR. In addition, NLR was an independent prognostic factor for OS after osimertinib treatment together with ECOG performance status.

EGFR mutations in advanced stage NSCLC are powerful markers 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 have suggested that co-mutations in genes other than EGFR are essential for determining the therapeutic response. Furthermore, the authors have 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.

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) comparing EGFR-TKIs to cytotoxic chemotherapy indirectly has 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), 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. However, because these were results from a subgroup analysis of TKIs efficacy compared to cytotoxic agents, the prognostic evaluation based on specific clinical factors had 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, and inconsistent EGFR test methods or treatment processes within one study. However, well-designed recent studies have reported that smoking history can affect the efficacy of first- and 2nd-generation TKIs through multivariate analysis.

The T790M mutation was confirmed in about half of cases where resistance developed after EGFR-TKI treatment. Based on results of the AURA3 trial, 6.5% of patients with the acquired EGFR T790M mutation showed rapid progression after osimertinib treatment. 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. have reported that an older age and an ECOG score of 0–1 are good predictors for PFS after osimertinib treatment in 30 patients with the acquired EGFR T790M mutation. Yoshimura et al. have reported that a prolonged PFS history with previous EGFR-TKIs is a predictive factor for subsequent osimertinib treatment among 27 patients. 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, factors that were non-significant in our multivariate analysis tended to be prognostic factors in univariate analysis, consistent with those previous reports. Interestingly, Chang et al. have recently reported that osimertinib efficacy is diminished in a group of patients who have a complex mutation apart from the T790M mutation.

EGFR mutations are more frequently identified in NSCLC in non-smokers. However, 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 might indicate that inhibition of EGFR activation alone does not entirely block the carcinogenesis pathway. Smoking can induce various genetic alterations in lung cancer. The most frequently observed co-occurring alteration with EGFR mutations is TP53 mutation, which is highly related to smoking. The effect of TP53 mutation on the EGFR-TKI therapy has been demonstrated in several clinical studies and preclinical studies. Recently, Kim et al. have 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 has reported that cigarette smoke extract (CSE) and tobacco smoke-derived carcinogen can upregulate the c-MET pathway, which induces resistance to EGFR-TKI in 19del mutation cell lines. Ahn et al. have shown that c-MET amplification in pre-biopsy histology is associated with previous smoking history in the case of progression after EGFR-TKI treatment. Li et al. have reported that CSE can inhibit 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 can lead 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 is correlated with neutrophilia, lymphopenia, or both in peripheral blood. It is also associated with a poor prognosis in patients with various carcinomas. In addition, neu-
trophils 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 have suggested that an elevated NLR is a useful prognostic biomarker in early, locally advanced, and advanced stages of lung cancer, as well as 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. To the best of our knowledge, our study is the first to report that NLR elevation is correlated 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). In another retrospective study, the tissue negative/plasma positive group showed a poor objective response rate of only 7.6%. Tumor heterogeneity might explain this discrepancy. It could be assumed that different resistance mechanisms for previous EGFR-TKIs occurred in multiple metastatic tumors in the same patient. On the other hand, the tissue positive/plasma negative group showed the most prolonged PFS in our study, although such result did not reach 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 that of patients in the intention-to-treat population (8.2 months vs. 10.1 months). Hong et al. have also reported a better efficacy in the tissue positive/plasma negative group. The absence of plasma ctDNA of T790M mutation in systemic circulation may mean that metastasis is indolent. Interestingly, in our study, the high NLR was found in one of seven patients (14.3%) in the tissue positive/plasma negative group and four of six patients (66.7%) in the tissue negative/plasma positive group. Further studies are needed to determine the relationship of ctDNA with peripheral blood NLR in lung cancer.

Regarding the effect of age on PFS with subsequent osimertinib treatment, Kato et al. have found 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 explained the difference in the effect of EGFR-TKIs depending on age groups. In a previous report, uncommon EGFR mutations, which have lower EGFR-TKI response rates, are more prevalent in a young-age group. However, this hypothesis could not be applied to the study by Kato et al., in which there were two uncommon mutations at old age.

Limitations of our study include 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 a prospective lung cancer cohort. 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 or 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 results of prognosis according to T790M detection methods because several confounding factors acted in selecting T790M test methods. In addition, both tissue biopsy and plasma tests were not routinely performed for all patients.

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 needed to validate these results.

*Author Affiliations

1Department of Pulmonary, Allergy and Critical Care Medicine, Hallym University Sacred Heart Hospital, Anyang, 2Lung Research Institute of Hallym University College of Medicine, Chuncheon, 3Department of Pulmonary, Allergy and Critical Care Medicine, Chuncheon Sacred Heart Hospital, Hallym University College of Medicine, Chuncheon, 4Department of Pulmonary, Allergy and Critical Care Medicine, Kangnam Sacred Heart Hospital, Hallym University College of Medicine, Seoul, 5Department of Pulmonary, Allergy and Critical Care Medicine, Dongtan Sacred Heart Hospital, Hallym University College of Medicine, Hwaseong, 6Department of Pulmonary, Allergy and Critical Care Medicine, Kangdong Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Republic of Korea

Authors’ Contribution

Conceptualization: Park JY, Jang SH. Formal analysis: Lee CY, Kim T, Chung SJ, Lee YJ. Data curation: Park JY, Jang SH, Lee CY, Kim T, Chung SJ, Lee YJ, Kim HJ, Kim JH, Park S. Hwang YI, Jung KS. Validation: Kim HJ, Kim JH, Park S. Investigation: Lee CY, Kim T, Chung SJ, Lee YJ. Supervision: Jang SH, Hwang YI, Jung KS. Writing - original draft: Park JY, Jang SH.
Prognostic factors in NSCLC patients receiving osimertinib treatment

Writing - review and editing: Park JY, Jang SH, Lee CY, Kim T, Chung SJ, Lee YJ, Kim HI, Kim JH, Park S, Hwang YI, Jung KS. Approval of final manuscript: all authors.

Conflicts of Interest
Ki-Suck Jung serves as editor-in-chief of the Tuberculosis of Respiratory Diseases, but has no role in the decision to publish this article. All remaining authors have declared no conflicts of interest.

Funding
No funding to declare.

Supplementary Material
Supplementary material can be found in the journal homepage (http://www.e-trd.org).
Supplementary Table S1. Patient characteristics in the osimertinib response group by six-month progression-free survival.
Supplementary Table S2. Patient characteristics according to osimertinib responsiveness.

References
1. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
2. Sequist LV, Yang JC, Yamamoto N, O’Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013;31:3327-34.
3. Chang YS, Choi CM, Lee JC. Mechanisms of epidermal growth factor receptor tyrosine kinase inhibitor resistance and strategies to overcome resistance in lung adenocarcinoma. Tuberc Respir Dis 2016;79:248-56.
4. Huang YH, Hsu KH, Tseng JS, Chen KC, Hsu CH, Su KY, et al. The association of acquired T790M mutation with clinical characteristics after resistance to first-line epidermal growth factor receptor tyrosine kinase inhibitor in lung adenocarcinoma. Cancer Res Treat 2018;50:1294-303.
5. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 2017;376:629-40.
6. Kato Y, Hosomi Y, Watanabe K, Yomota M, Kawai S, Okuma Y, et al. Impact of clinical features on the efficacy of osimertinib therapy in patients with T790M-positive non-small cell lung cancer and acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors. J Thorac Dis 2019;11:2350-60.
7. Yoshimura A, Yamada T, Okura N, Takeda T, Hirose K, Kubota Y, et al. Clinical characteristics of osimertinib responder in non-small cell lung cancer patients with EGFR-T790M mutation. Cancers (Basel) 2019;11:365.
8. Suidan AM, Roisman L, Belilovsky Rozenblum A, Ilozue M, Dudnik E, Zer A, et al. Lung cancer in young patients: higher rate of driver mutations and brain involvement, but better survival. J Glob Oncol 2019;5:1-8.
9. Lara MS, Brunson A, Wun T, Tomlinson B, Qi L, Cress R, et al. Predictors of survival for younger patients less than 50 years of age with non-small cell lung cancer (NSCLC): a California Cancer Registry analysis. Lung Cancer 2014;83:264-9.
10. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380-8.
11. Yu Y, Qian L, Cui J. Value of neutrophil-to-lymphocyte ratio for predicting lung cancer prognosis: a meta-analysis of 7,219 patients. Mol Clin Oncol 2017;7:498-506.
12. Ono T, Iwaga S, Kuraorchashi S, Okuma Y, Sugimoto A, Kusuhara S, et al. Impact of neutrophil-to-lymphocyte ratio in patients with EGFR-mutant NSCLC treated with tyrosine kinase inhibitors. Invest New Drugs 2020;38:865-93.
13. Park JY, Jang SH, Kim HI, Kim JH, Park S, Hwang YI, et al. Thyroid transcription factor-1 as a prognostic indicator for stage IV lung adenocarcinoma with and without EGFR-sensitizing mutations. BMC Cancer 2019;19:574.
14. Guo Y, Song J, Wang Y, Huang L, Sun L, Zhao J, et al. Concurrent genetic alterations and other biomarkers predict treatment efficacy of EGFR-TKIs in EGFR-mutant non-small cell lung cancer: a review. Front Oncol 2020;10:610923.
15. Kim Y, Lee B, Shim JH, Lee SH, Park WY, Choi YL, et al. Concurrent genetic alterations predict the progression to target therapy in EGFR-mutated advanced NSCLC. J Thorac Oncol 2019;14:193-202.
16. Yang JC, Wu YL, Schuler M, Sebastian M, Popat S, Yamamoto N, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol 2015;16:141-51.
17. Hasegawa Y, Ando M, Maemondo M, Yamamoto M, Inoue A, Sasa S, Saka H, et al. The role of smoking status on the progression-free survival of non-small cell lung cancer patients harboring activating epidermal growth factor receptor (EGFR) mutations receiving first-line EGFR tyrosine kinase inhibitor versus platinum doublet chemotherapy: a meta-analysis of prospective randomized trials. Oncologist 2015;20:141-51.
18. Lin JH, Lin D, Xu L, Wang Q, Hu HH, Xu HP, et al. The association between clinical prognostic factors and epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI)
efficacy in advanced non-small-cell lung cancer patients: a retrospective assessment of 94 cases with EGFR mutations. Oncotarget 2017;8:3412-21.

19. Zhao D, Chen X, Qin N, Su D, Zhou L, Zhang Q, et al. The prognostic role of EGFR-TKIs for patients with advanced non-small cell lung cancer. Sci Rep 2017;7:40374.

20. Kim IA, Lee JS, Kim HJ, Kim WS, Lee KY. Cumulative smoking dose affects the clinical outcomes of EGFR-mutated lung adenocarcinoma patients treated with EGFR-TKIs: a retrospective study. BMC Cancer 2018;18:768.

21. Cha YK, Lee HY, Ahn MJ, Park K, Ahn JS, Sun JM, et al. The impact of smoking status on radiologic tumor progression patterns and response to epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors in lung adenocarcinoma with activating EGFR mutations. J Thorac Dis 2016;8:3175-86.

22. Kim MH, Kim HR, Cho BC, Bae MK, Kim EY, Lee CY, et al. Impact of cigarette smoking on response to epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors in lung adenocarcinoma with activating EGFR mutations. Lung Cancer 2014;84:196-202.

23. Chang N, Duan J, Wang L, Dong Z, Liu Z. Patients with advanced non-small cell lung cancer with EGFR mutations in addition to complex mutations treated with osimertinib have a poor clinical outcome: A real-world data analysis. Oncol Lett 2020;20:2266-72.

24. Qin K, Hou H, Liang Y, Zhang X. Prognostic value of TP53 concurrent mutations for EGFR-TKIs and ALK-TKIs based targeted therapy in advanced non-small cell lung cancer: a meta-analysis. BMC Cancer 2020;20:328.

25. Tu CY, Cheng FJ, Chen CM, Wang SL, Hsiao YC, Chen CH, et al. Cigarette smoke enhances oncogene addiction to c-MET and desensitizes EGFR-expressing non-small cell lung cancer to EGFR TKIs. Mol Oncol 2018;12:705-23.

26. Ahn BC, Lee JH, Kim MH, Pyo KH, Lee CK, Lim SM, et al. Distinct characteristics and clinical outcomes to predict the emergence of MET amplification in patients with non-small cell lung cancer who developed resistance after treatment with epidermal growth factor receptor tyrosine kinase inhibitors. Cancers (Basel) 2021;13:3096.

27. Li D, Zhang L, Zhou J, Chen H. Cigarette smoke extract exposure induces EGFR-TKI resistance in EGFR-mutated NSCLC via mediating Src activation and EMT. Lung Cancer 2016;93:35-42.

28. Ollín M, Rizvi H, Tenet M, Ni A, Sanchez-Vega F, Li BT, et al. Tumor mutation burden and efficacy of EGFR-tyrosine kinase inhibitors in patients with EGFR-mutant lung cancers. Clin Cancer Res 2019;25:1063-9.

29. Elnav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumors, immune cells and microorganisms. Nat Rev Cancer 2013;13:759-71.

30. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-7.

31. Proctor MJ, Morrison DS, Talwar D, Balmer SM, Fletcher CD, O’Reilly DS, et al. A comparison of inflammation-based prognostic scores in patients with cancer: a Glasgow inflammation outcome study. Eur J Cancer 2011;47:2633-41.

32. Mizuno R, Kawada K, Itatani Y, Ogawa R, Kiyasu Y, Sakai Y. The role of tumor-associated neutrophils in colorectal cancer. Int J Mol Sci 2019;20:529.

33. Pinato DJ, Shiner RJ, Seckl MJ, Stebbing J, Sharma R, Mauri FA. Prognostic performance of inflammation-based prognostic indices in primary operable non-small cell lung cancer. Br J Cancer 2014;110:1930-5.

34. Scilla KA, Bentzen SM, Lam VK, Mohindra P, Nichols EM, Vyphuis MA, et al. Neutrophil-lymphocyte ratio is a prognostic marker in patients with locally advanced (stage IIIA and IIIB) non-small cell lung cancer treated with combined modality therapy. Oncologist 2017;22:737-42.

35. Cedres S, Torrejon D, Martinez A, Martinez P, Navarro A, Zamora E, et al. Neutrophil to lymphocyte ratio (NLR) as an indicator of poor prognosis in stage IV non-small cell lung cancer. Clin Transl Oncol 2012;14:864-9.

36. Li Y, Zhang Z, Hu Y, Yan X, Song Q, Wang G, et al. Pretreatment neutrophil-to-lymphocyte ratio (NLR) may predict the outcomes of advanced non-small-cell lung cancer (NSCLC) patients treated with immune checkpoint inhibitors (ICIs). Front Oncol 2020;10:654.

37. Sebastian N, Wu T, Bazan J, Driscoll E, Willers H, Yegga-Raman N, et al. Pre-treatment neutrophil-lymphocyte ratio is associated with overall mortality in localized non-small cell lung cancer treated with stereotactic body radiotherapy. Radiother Oncol 2019;134:151-7.

38. Xu C, Yao X, Li T, Wang J, An B, Wang J, et al. Pretreatment neutrophil-to-lymphocyte ratio is a predictive biomarker for EGFR TKI-treated patients with advanced EGFR-mutant Non-small cell lung cancer. Transl Cancer Res 2020;9:2875-83.

39. Oxnard GR, Thress KS, Alden RS, Lawrance R, Paweletz CP, Cantarini M, et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol 2016;34:3375-82.

40. Hong MH, Kim HR, Ahn BC, Heo SJ, Kim JH, Cho BC. Real-world analysis of the efficacy of rebiopsy and EGFR mutation test of tissue and plasma samples in drug-resistant non-small cell lung cancer. Yonsei Med J 2019;60:525-34.

41. Thress KS, Brant R, Carr TH, Dearden S, Jenkins S, Brown H, et al. EGFR mutation detection in ctDNA from NSCLC patient plasma: a cross-platform comparison of leading technologies to support the clinical development of AZD9291. Lung Cancer 2015;83:509-15.

42. Wu SG, Chang YL, Yu CJ, Yang PC, Shih JY. Lung adenocarcinoma patients of young age have lower EGFR mutation rate and poorer efficacy of EGFR tyrosine kinase inhibitors. ERJ Open Res 2017;3:00092-2016.