**BJMG** 25 (2), 2022 • 29-36
DOI: 10.2478/bjmg-2022-0015
sciendo
ORIGINAL ARTICLE

# EPIDERMAL GROWTH FACTOR RECEPTOR MUTATION STATUS AND THE IMPACT ON CLINICAL OUTCOMES IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

Huang HM[1], Wei Y[1], Wang JJ[1], Ran FY[1], Wen Y[2], Chen QH[3], Zhang BF[1,*]

[*]**Corresponding Author:** Dr. Bingfei Zhang, Sinopharm Dongfeng General Hospital, Hubei University of Medicine, No. 16 Daling Road, 442008, Shiyan, Hubei, China. Tel.: + 86-29-8272597, Email: dfzyysszx@163.com

## ABSTRACT

Epidermal growth factor receptor (EGFR) mutation status differs according to ethnicity, gender, smoking history, and histology types. The present study aimed to evaluate EGFR mutation status in patients with non-small cell lung cancer (NSCLC) and further explore its association with clinical characteristics and prognosis in advanced NSCLC patients (Stage IIIB-IV). 238 NSCLC patients were enrolled in this study from October 2016 through December 2019. Patient characteristics and clinical data including age, gender, smoking history, histology types, tumor stage, survival status, and time were collected via electronic medical record system or telephone. 21 somatic mutations which spanned exons 18-21 of EGFR were detected using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method, followed by analysis of links to clinical characteristics, progression-free survival (PFS) and overall survival (OS). 103 patients were detected harboring EGFR mutations among the 238 cases tested (43.3%), and exons 19 and 21 were the highest mutation frequencies, with 20.6% and 19.3% respectively. The EGFR mutation rate was much higher in female versus male (57.4% vs 31.5%, $p < 0.001$), in non-smokers compared to smokers (56.8% vs 25.9%, $p < 0.001$), and in those with adenocarcinoma than other histology types (48.3% vs 3.7%, $p < 0.001$). For patients in advanced stage, median PFS was 11 months in patients harboring EGFR mutations, versus 4 months in patients with wild type EGFR ($p < 0.001$); median OS was 24 versus 12 months ($p < 0.001$). Never smoking ($p = 0.042$) and adenocarcinoma ($p = 0.007$) were independent favorable factors for EGFR mutations. Our data strengthen the findings of high prevalence of EGFR mutations in Asian patients with NSCLC. Mutations are prevalent in those patients who are female, adenocarcinoma, and have never smoked. Moreover, advanced EGFR mutation-positive patients have better PFS and OS than those with wild type EGFR.

**Keywords**

Epidermal growth factor receptor; Non-small cell lung cancer; Mutations; Epidemiological characteristics

## INTRODUCTION

Lung cancer is the major cause of cancer-related death in the world. As reported by American Cancer Society, lung cancer caused more deaths than breast, prostate, colorectal, and brain cancers combined in 2017 [1]. Non-small cell lung cancer (NSCLC) consists of about 85% of all lung cancers and is usually diagnosed at advanced or metastatic stage [2]. Platinum-based regimens have been the mainly conventional chemotherapy for advanced NSCLC treatment with a poor benefit in survival. In the past decade, an increased understanding of the signaling pathways contributed to the development of target agents, and the addition of target therapy to the treatment protocols for NSCLC patients was a major breakthrough and had obtained clinically significant survival benefit [3]. The epidermal growth factor receptor (EGFR) mutations are predictive markers for response to target therapy in NSCLC patients [4]. EGFR is a transmembrane glycoprotein existing on the cell surface and plays an important role in tumor cell survival and proliferation. Tyrosine kinase inhibitors (TKIs) that specifically target EGFR have been

[1] Sinopharm Dongfeng General Hospital, Hubei University of Medicine, Shiyan, 442008, China

[2] School of Public Health, Xi'an Jiaotong University, Xi'an, 710003, China

[3] Department of Pharmacy, Shenzhen Baoan Authentic TCM Therapy Hospital, Shenzhen, 518101, China

used as first-line treatment in EGFR mutation-positive patients [5, 6]. Lindeman et al. [6] reported that the response to TKIs was approximately 68% in patients with activating EGFR mutations, while there was only an 11% response rate in patients with wild type EGFR. Given the benefit of EGFR-TKI therapy, EGFR mutation testing was recommended to NSCLC patients before initiation of first-line therapy, according to clinical practice guidelines [7].

The genetic divergence of EGFR mutations according to ethnicity has been reported. Asian populations have the highest EGFR mutation frequency and it has become very common in clinical practice in some Asian countries to treat patients based on their EGFR status [8, 9]. However, most of the studies conducted on EGFR mutations in Asia were carried out in Korea or Japan, data available on EGFR mutations in China are limited. The present study was to evaluate the EGFR mutation status in Chinese NSCLC patients (Stage I-IV), explore its association with clinical characteristics, and further investigate differences in prognosis between advanced NSCLC patients (Stage IIIB-IV) with and without EGFR mutations.

## MATERIALS AND METHODS

### *Patients*

A total of 238 NSCLC patients are included in this study and Figure 1 shows the trial profile. Detailed clinical information of these patients was available on the Sinopharm Dongfeng Hospital medical record system. The gender ratio of these patients was 54.6% (n=130) males and 45.4% (n=108) females. 44.5% (n=106) of the 238 patients were aged over 65 years. Of the 227 patients with a known smoking history, 132 patients (58.1%) had never smoked (smoked <100 lifetime cigarettes), 37 patients (16.3%) were former smokers (≥1 year since quitting smoking), and 58 patients (25.6%) were current smokers (still smoking, or <1 year since quitting smoking). There were 211 NSCLC cases (88.7%) with adenocarcinoma, and other histology types were 27 cases (four adenosquamous carcinoma, five large cell carcinoma, and eighteen squamous cell carcinoma). 157 patients (66%) were diagnosed in advanced NSCLC (Stage IIIB-IV) (Table 1) .

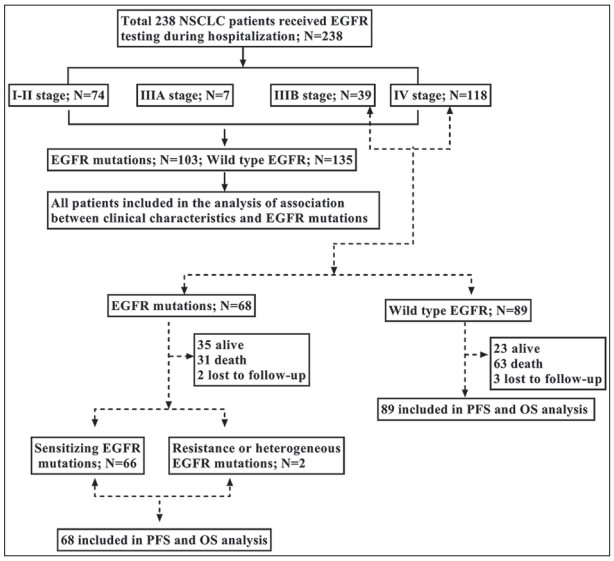


**Figure 1.** Screening and follow-up of patients
NSCLC: non-small cell lung cancer; EGFR: epidermal growth factor receptor; PFS: progression-free survival; OS: overall survival

### *Tumor specimens*

Tumor samples were obtained from 238 NSCLC patients in the daily clinical practice between October 2016 and December 2019 at Dongfeng Hospital. Tumor samples were fixed with 10% formalin, embedded in paraffin, and then 5μm thickness sections were cut. Tumor specimens were evaluated to confirm the NSCLC histology by experienced pathologists. All patients provided written informed consent before EGFR mutation testing. All procedures were supervised and approved by Sinopharm Dongfeng General Hospital Ethics Committee (Approval Number: LW-2021-21).

### *EGFR mutation analysis*

Genomic DNA was extracted using the TIANamp FFPE DNA Kit, according to the manufacturer's protocol, and DNA quality and purity was measured by Eppendorf Bio Photometer D30. We used commercially available EGFR kits to detect EGFR mutations in exons 18-21 via ARMS-PCR technology. The EGFR kit is able to detect 21 somatic mutation types, namely, 3 point mutations in exon 18 (G719A, G719C, and G719S , which are referred to as G719X); 11 deletions in exon 19 (which are referred to as 19-Del); 2 point mutations (S768I, T790M) and 3 insertion mutations (H773_V774insH; D770_N771insG; V769_D770insASV, which are referred to as 20-Ins) in exon 20; 2 point mutations in exon 21 (L858R and L861Q) (Table 2). The thermocycling conditions were used as following: 1 cycle of 95˚C for 3 min; 45 cycles of 94˚C for 15 sec and 60˚C for 35 sec. The results were analyzed according to the manufacturer's guideline.

### *Statistical analysis*

GraphPad Prism 7.0 and SPSS Statistics 22.0 were the software used for statistical analysis. The associations between EGFR mutation status and clinical characteristics, such as gender, age, smoking history were evaluated by Pearson's $\chi^2$ test or the Fisher exact test. PFS and OS were analyzed by the Kaplan-Meier method, and the differences were calculated by a log-rank test. Variables with a $p$ value less than 0.05 in univariate analysis were entered into a multivariate logistic regression analysis to analyze the fa-

**Table 1.** Patient characteristics

| Patient characteristics | Total N(%) | EGFR mutations N (%) | Wild type EGFR N (%) | *P*-value |
|---|---|---|---|---|
| **Gender** | | | | <0.001 |
| Female | 108(45.4%) | 62(57.4%) | 46(42.6%) | |
| Male | 130(54.6%) | 41(31.5%) | 89(68.5%) | |
| **Age** | | | | 0.308 |
| >65 | 106(44.5%) | 42(39.6%) | 64(60.4%) | |
| ≤65 | 132(55.5%) | 61(46.2%) | 71(53.8%) | |
| **Smoking history** | | | | <0.001 |
| Current smoker | 58(25.6%) | 15(25.9%) | 43(74.1%) | |
| Former smoker | 37(16.3%) | 11(29.7%) | 26(70.3%) | |
| Never smoker | 132(58.1%) | 75(56.8%) | 57(43.2%) | |
| **Histology types** | | | | <0.001 |
| Adenocarcinoma | 211(88.7%) | 102(48.3%) | 109(51.7%) | |
| Non-adenocarcinoma | 27(11.3%) | 1(3.7%) | 26(96.3%) | |
| **Stage classification** | | | | 0.990 |
| I-II | 74(31.1%) | 32(43.2%) | 42(56.8%) | |
| IIIA | 7(2.9%) | 3(42.9%) | 4(57.1%) | |
| IIIB | 39(16.4%) | 16(41.0%) | 23(59.0%) | |
| IV | 118(49.6%) | 52(44.1%) | 66(55.9%) | |

EGFR: epidermal growth factor receptor

**Table 2.** Mutations detected in exons 18-21 of EGFR

| Exon | EGFR mutation types |
|---|---|
| Exon 18 | G719A; G719C; G719S |
| Exon 19 | E746_A750del (Cosmic ID:6223); E746_T751>A; E746_S752>V; L747_A750>P; L747_E749del; L747_S752del; E746_A750del(Cosmic ID:6225); L747_A750>P; L747_P753>S; L747_T751del; L747_T751>P |
| Exon 20 | T790M; S768I; H773_V774insH; D770_N771insG; V769_D770insASV |
| Exon 21 | L858R; L861Q |

EGFR: epidermal growth factor receptor

vorable factors of EGFR mutations. $p<0.05$ was considered statistically significant.

## RESULTS

### *Clinical characteristics*

EGFR mutations were significantly more common in female than in male (57.4% vs 31.5%, $p<0.001$). Younger patients (≤ 65years) had a slightly higher mutation rate in comparison with patients >65years, but there was no statistical significance (46.2% vs 39.6%, $p = 0.308$). A higher frequency of EGFR mutation was observed in non-smokers versus smokers, and the difference was statistically significant (56.8% vs 29.7%, $p<0.001$). In addition, a noticeable increase of EGFR mutations was found in NSCLC patients with adenocarcinoma than those with non-adenocarcinoma (48.3% vs 3.7%, $p<0.001$) (Table 1).

### *EGFR mutations*

We identified 103 cases with EGFR mutations among the 238 NSCLC patients, and the total EGFR mutation rate was 43.3%. A single mutation was found in 102 patients and 1 patient had multiple exon mutations (19-Del mutation and T790M mutation). Therefore, a total of 104 mutations were detected in 103 patients. Exons 19 and 21 were the highest mutation frequencies, with 20.6% and 19.3%, respectively. The mutation rate of exon 18 was 2.5% and it was 0.4% for exon 20. An overview of detected mutations is shown in Figure 2.

### *Distribution of exons 18-21*

Table 3 shows the distribution of exons 18-21 in 103 EGFR mutation-positive patients. 60.2% of the patients with mutations were female, and exons 18 and 21 mutations more commonly occurred in female patients (83.3% and

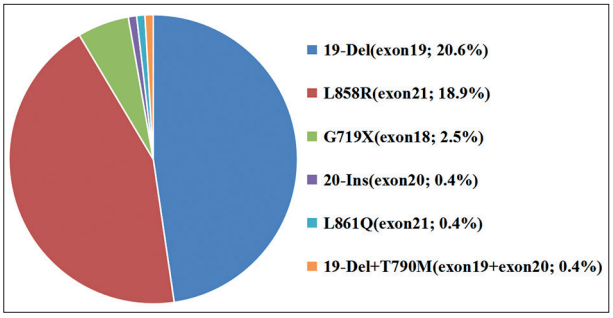


**Figure 2.** The contribution of EGFR mutation types

60.9%, respectively). 59.2% of the patients with mutations were under 65 years of age, and exons 18 and 19 mutations mostly occurred in younger patients (100.0% and 62.0%, respectively), however exon 20 mutations mainly occurred in patients of advanced age (>65 years, 100%). 74.3% of the patients with mutations were in never smokers and exons 18, 19, and 21 were the major mutant sites (83.3%, 77.1% and 69.6%, respectively). Patients with adenocarcinoma accounted for 99.0% of all EGFR mutations.

***Independent favorable factors for EGFR mutations***

According to the results of univariate analysis, variables significantly associated with EGFR mutations were gender ($p$ <0.001), smoking history ($p$ <0.001), and histology types ($p$ <0.001). Table 4 shows the details. We defined the EGFR mutation status as a dependent variable, the statistically significant independent variables in the univariate analysis were entered into the multivariate logistic regression analysis. Multivariate logistic regression identified never smoker (OR = 2.76, 95% CI = 1.04 -7.34, $p$ =0.042) and adenocarcinoma (OR = 17.07, 95% CI = 2.21-132.04, $p$ =0.007) to be independent favorable factors for EGFR mutations. When adjusted by smoking history and histology types, gender was no longer found to be significant ($p$ = 0.498).

***Progression-free survival and overall survival***

Figure 3 shows the hazard ratio (HR) for the risk of progression in 157 advanced NSCLC patients (Stage IIIB-IV), with and without EGFR mutations. The most common EGFR mutations identified among advanced patients were 19-Del mutation and L858R mutation, comprising 44% (n=30) and 46% (n=31) of the mutation positive cases, respectively. The remaining mutations (four G719X, one L861Q, one 20-Ins, one T790M/19-Del) accounted for 10% (data not shown). Patients with any type of EGFR mutations were enrolled in analysis of the PFS and OS in the present study. At the time of analysis, 31 of 68 patients with EGFR mutations (46%) and 63 of 89 patients with wild type EGFR (71%) had died. The median follow-up was 17 months (range 1-52 months). The median PFS was 11 months (95% CI 7.6-14.4) in patients with EGFR mutations versus 4 months (95% CI 3.0-5.0) in patients with wild type EGFR (Figure 3A). The median OS in patients with EGFR mutations was 24 months (95% CI 20.5-27.5), and it was 12 months (95% CI 8.0-16.0) in patients with wild type EGFR (Figure 3B). Compared to patients with EGFR mutations, patients with wild type EGFR had 2.24 times the risk of progression (HR 2.24, 95% CI 1.62-3.10; p <0.0001) and 2.28 times the risk of death (HR 2.28, 95% CI 1.52-3.42; p <0.0001).

## DISCUSSION

Many studies have been carried out to estimate EGFR mutation status among NSCLC patients in different regions and populations in order to evaluate the benefits from EGFR-TKI. Results showed that EGFR mutation frequency possesses variability based on ethnicity and regional differences, with 36.3% of positivity in Korea [10], 13.6% in Spain [3], 10.6% in Poland [11], 15.7% in Greece [12], 36.7% in Iran [13], 11.9% in Lebanon [14], and greater in Japan with 53.9% [15]. The prevalence of

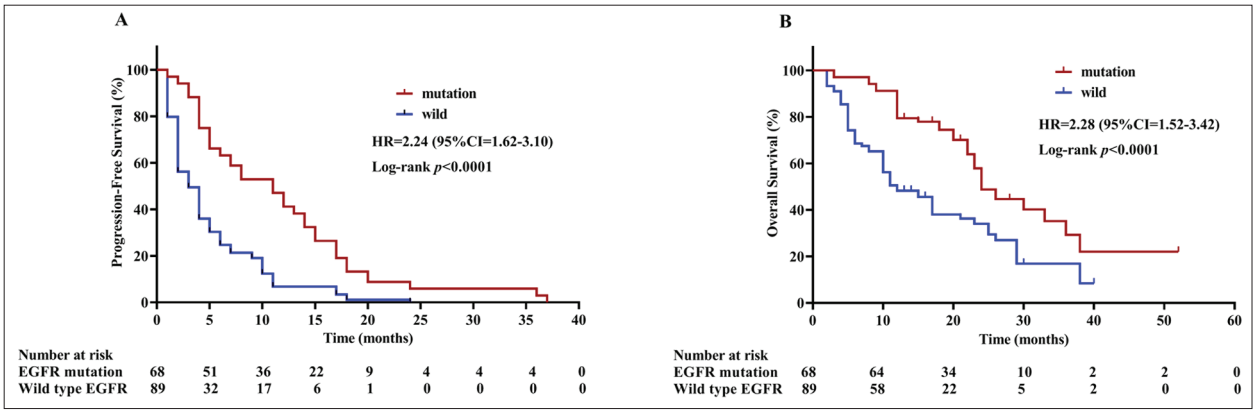


**Figure 3.** Kaplan - Meier curve of progression-free survival (A) and overall survival (B) in advanced NSCLC patients with EGFR mutations or wild type EGFR.

**Table 3.** Distribution of exons 18-21

| Patient characteristics | EGFR mutations N (%) | Exon 18 | Exon 19 | Exon 20 | Exon 21 |
|---|---|---|---|---|---|
| **Gender** | | | | | |
| Female | 62(60.2%) | 5(83.3%) | 28(56.0%) | 1(50.0%) | 28(60.9%) |
| Male | 41(39.8%) | 1(16.7%) | 22(44.0%) | 1(50.0%) | 18(39.1%) |
| **Age** | | | | | |
| >65 | 42(40.8%) | 0(0.0%) | 19(38.0%) | 2(100.0%) | 22(47.8%) |
| ≤65 | 61(59.2%) | 6(100.0%) | 31(62.0%) | 0(0.0%) | 24(52.2%) |
| **Smoking status** | | | | | |
| Current smoker | 15(14.9%) | 1(16.7%) | 6(12.5%) | 0(0.0%) | 8(17.4%) |
| Former smoker | 11(10.9%) | 0(0.0%) | 5(10.4%) | 1(50.0%) | 6(13.0%) |
| Never smoker | 75(74.3%) | 5(83.3%) | 37(77.1%) | 1(50.0%) | 32(69.6%) |
| **Histology types** | | | | | |
| Adenocarcinoma | 102(99.0%) | 6(100.0%) | 50(100.0%) | 2(100.0%) | 45(97.8%) |
| Non-adenocarcinoma | 1(1.0%) | 0(0.0%) | 0(0.0%) | 0(0.0%) | 1(2.2%) |
| **Stage classification** | | | | | |
| I-II | 32(31.1%) | 2(33.3%) | 17(34.0%) | 0(0.0%) | 13(28.3%) |
| IIIA | 3(2.9%) | 0(0.0%) | 2(4.0%) | 0(0.0%) | 1(2.2%) |
| IIIB | 16(15.5%) | 1(16.7%) | 8(16.0%) | 0(0.0%) | 7(15.2%) |
| IV | 52(50.5%) | 3(50.0%) | 23(46.0%) | 2(100.0%) | 25(54.3%) |

EGFR: epidermal growth factor receptor

**Table 4.** Univariate analysis and multivariate logistic regression analysis for EGFR mutations

| Variable | Univariate analysis | | Multivariate analysis | | |
|---|---|---|---|---|---|
| | *P*-value | | OR | 95% CI | *P*-value |
| **Gender**[a] | **<0.001** | | | | |
| Male | | | 1 | References | |
| Female | | | 1.35 | 0.57-3.19 | 0.498 |
| **Age** | 0.308 | | | | |
| >65 | | | | | |
| ≤65 | | | | | |
| **Smoking status**[a] | **<0.001** | | | | |
| Current smoker | | | 1 | References | |
| Former smoker | | | 1.32 | 0.51-3.41 | 0.568 |
| Never smoker | | | 2.76 | 1.04-7.34 | **0.042** |
| **Histology types**[a] | **<0.001** | | | | |
| Non-adenocarcinoma | | | 1 | References | |
| Adenocarcinoma | | | 17.07 | 2.21-132.04 | **0.007** |
| **Stage classification** | 0.990 | | | | |
| I-II | | | | | |
| IIIA | | | | | |
| IIIB | | | | | |
| IV | | | | | |

EGFR: epidermal growth factor receptor; OR: odds ratio; CI: confidence interval
[a]Included in multivariate analysis

the EGFR mutations in our study is 43.3%, lower than that reported in a large Asian study including Chinese patients with NSCLC (50.2%) [16], however, it is higher than that which was reported in a multi-center diagnostic survey carried out in the Asia Pacific Region (38.1%) [17]. In general, the frequencies of EGFR mutations in patients from Asian countries are quite high, even up to 68.5% in female non-smokers with adenocarcinoma [18]. The most common mutations detected in the present study are exons19 and 21 (21.0% and 19.3%, respectively). 19-Del mutation and L858R mutation were the most common mutation types for exons 19 and 21, which was in line with previous literature [19].

The median PFS and OS for patients with EGFR mutations were 11 months and 24 months, respectively, while patients with wild type EGFR demonstrated a median 4-month PFS and 12-month OS. The results show prominent benefits in patients with EGFR mutations compared to those with wild type EGFR. Correlations between EGFR mutations and improved PFS and survival in EGFR mutation-positive patients with administration of EGFR-TKI have been reported, while PFS and survival differ due to exon mutation sites. Exons 19 and 21 mutations were associated with sensitivity to EGFR-TKI and it has been reported that the 19-Del mutation was associated with a better response than the L858R mutation when patients were treated with TKI [20, 21]. Mutations in exon 18, including G719A, G719C, and G719S, were also drug sensitizing mutations, however, T790M and 20-Ins mutations had been demonstrated to confer resistance to EGFR-TKI. In the present study, one patient with 20-Ins mutation was observed by the authors. When compared with sensitizing EGFR mutations, the 20-Ins mutation case failed to respond to a combination of pemetrexed and cisplatin and demonstrated a poor prognosis, with only a 3-month survival. The inclusion of patient with 20-Ins mutation resulted in a lower PFS and OS compared to patients with sensitizing EGFR mutations, however this inclusion did not seem to adversely affect overall PFS or OS due to the limited numbers of patients included. Studies reported that T790M was the most common mutant type in exon 20 [22], and in the present study, T790M point mutation was detected in one sample together with 19-Del mutation. Namely, the patient had a rare combination of exon 19 sensitizing mutation and T790M resistance mutation. The mutations are heterogeneous and the EGFR-TKI efficacy in patients with heterogeneous mutations requires individual assessment [23, 24]. Limited to the present study, the patient with heterogeneous mutations received icotinib therapy and demonstrated similar treatment outcomes, compared to sensitizing EGFR mutations, with a 14-month PFS and 23-month survival.

Studies have shown that female, no-smoking status, adenocarcinoma histology, and Asian ethnicity are all favorable factors for EGFR mutations [25, 26]. Similar results were observed in the present study. The statistical analysis showed that the EGFR mutation rate was much higher in female than in male. The reasons for the effect of gender on EGFR mutation rate remained incompletely understood. Differential smoking habits and sex hormones might contribute to the effect [27]. There were more EGFR mutations in positive cases than in never smokers compared with current smokers or former smokers. In the present study, the EGFR mutations were found in 56.8% (75/132) patients who had never smoked and in 25.9% (15/58) current smokers ($p$ <0.001). Likewise, the difference in EGFR mutation rate between NSCLC patients with adenocarcinoma and non-adenocarcinoma was significant ($p$ <0.001). Indeed, molecular testing guidelines recommend EGFR testing to all advanced patients with adenocarcinoma to guide selection of EGFR-TKI therapy, regardless of gender, race, smoking status, or other clinical risk factors [28].

In conclusion, the EGFR mutation rate is 43.3% among all NSCLC patients and the mutations are more frequently observed in exons 19 and 21. EGFR mutations are prevalent in patients who are female, have adenocarcinoma, and have never smoked. Advanced EGFR mutation-positive patients have longer PFS and OS than those with wild type EGFR. But this study has some limitations such as a relatively small number of selected cases and having been a single-center study. Therefore, a larger sample size and multi-center investigations are necessary to make the research results more comprehensive and convincing.

**Declaration of Interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

## REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020; 70(1): 7-30.
2. Duma N, Santana-Davila R, Molina JR. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. Mayo Clin Proc. 2019; 94(8): 1623-1640.
3. Vázquez S, Casal J, Afonso Afonso FJ, Fírvida JL, Santomé L, Barón F, et al. EGFR testing and clinical management of advanced NSCLC: a Galician Lung Cancer Group study (GGCP 048- 10). Cancer Manag Res. 2016; 8(11-20.
4. Rebuzzi SE, Zullo L, Rossi G, Grassi M, Murianni V, Tagliamento M, et al. Novel Emerging Molecular

Targets in Non-Small Cell Lung Cancer. Int J Mol Sci. 2021; 22(5):.

5. Qi WX, Shen Z, Lin F, Sun YJ, Min DL, Tang LN, et al. Comparison of the efficacy and safety of EFGR tyrosine kinase inhibitor monotherapy with standard second-line chemotherapy in previously treated advanced non-small-cell lung cancer: a systematic review and meta-analysis. Asian Pac J Cancer Prev. 2012; 13(10): 5177-5182.
6. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol. 2013; 8(7): 823-859.
7. Hanna N, Johnson D, Temin S, Baker S, Brahmer J, Ellis PM, et al. Systemic Therapy for Stage IV Non-Small-Cell Lung Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J Clin Oncol. 2017; 35(30): 3484-3515.
8. Midha A, Dearden S, McCormack R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: a systematic revie w and global map by ethnicity (mutMapII). Am J Cancer Res. 2015; 5(9): 2892-2911.
9. Lee DH, Srimuninnimit V, Cheng R, Wang X, Orlando M. Epidermal Growth Factor Receptor Mutation Status in the Treatment of Non-small Cell Lung Cancer: Less ons Learned. Cancer Res Treat. 2015; 47(4): 549-554.
10. Choi YL, Sun JM, Cho J, Rampal S, Han J, Parasuraman B, et al. EGFR mutation testing in patients with advanced non-small cell lung cancer: a comprehensive evaluation of real-world practice in an East Asian tertiary hospital. Plos One. 2013; 8(2): e56011.
11. Szumera-Cie Kiewicz A, Olszewski WT, Tysarowski A, Kowalski DM, G Ogowski M, Krzakowski M, et al. EGFR mutation testing on cytological and histological samples in non-small cell lung cancer: a Polish , single institution study and systematic review of European incidence. Int J Clin Exp Pathol. 2013; 6(12): 2800-2812.
12. Syrigos KN, Georgoulias V, Zarogoulidis K, Makrantonakis P, Charpidou A, Christodoulou C. Epidemiological Characteristics, EGFR Status and Management Patterns of Advanced Non-small Cell Lung Cancer Patients: The Greek REASON Observational Registry Study. Anticancer Res. 2018; 38(6): 3735-3744.
13. Haghgoo SM, Khosravi A, Mortaz E, Pourabdollah-Toutkaboni M, Seifi S, Sabour S, et al. Prognostic value of rare and complex mutations in EGFR and serum levels of soluble EGFR and its ligan ds in non-small cell lung carcinoma patients. Clin Biochem. 2017; 50(6): 293-300.
14. Naderi S, Ghorra C, Haddad F, Kourie HR, Rassy M, El Karak F, et al. EGFR mutation status in Middle Eastern patients with non-squamous non-small cell lung carcinoma: A si ngle institution experience. Cancer Epidemiol. 2015; 39(6): 1099-1102.
15. Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, Kawakami F, et al. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. J Thorac Oncol. 2013; 8(1): 52-61.
16. Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J Thorac Oncol. 2014; 9(2): 154-162.
17. Yatabe Y, Kerr KM, Utomo A, Rajadurai P, Tran VK, Du X, et al. EGFR mutation testing practices within the Asia Pacific region: results of a multicenter diagnostic survey. J Thorac Oncol. 2015; 10(3): 438-445.
18. Sun PL, Seol H, Lee HJ, Yoo SB, Kim H, Xu X, et al. High incidence of EGFR mutations in Korean men smokers with no intratumoral heterogeneity of lung adenocarcinomas: correlation with histologic subtypes, EGFR/TTF-1 expressions, and clinical features. J Thorac Oncol. 2012; 7(2): 323-330.
19. Tu HY, Ke EE, Yang JJ, Sun YL, Yan HH, Zheng MY, et al. A comprehensive review of uncommon EGFR mutations in patients with non-small cell lung cancer. Lung Cancer. 2017; 114(96-102.
20. Jackman DM, Miller VA, Cioffredi LA, Yeap BY, J Nne PA, Riely GJ, et al. Impact of epidermal growth factor receptor and KRAS mutations on clinical outcomes in previously untr eated non-small cell lung cancer patients: results of an online tumor registry of clinical trials. Clin Cancer Res. 2009; 15(16): 5267-5273.
21. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 2009; 361(10): 958-967.
22. Massarelli E, Johnson FM, Erickson HS, Wistuba II, Papadimitrakopoulou V. Uncommon epidermal growth factor receptor mutations in non-small cell

lung cancer and their mechanism s of EGFR tyrosine kinase inhibitors sensitivity and resistance. Lung Cancer. 2013; 80(3): 235-241.

23. Esteban E, Majem M, Martinez Aguillo M, Martinez Banaclocha N, Dómine M, Gómez Aldaravi L, et al. Prevalence of EGFR mutations in newly diagnosed locally advanced or metastatic non-small cell lung cancer Spanish patients and its association with histological subtypes and clinical features: The Spanish REASON study. Cancer Epidemiol. 2015; 39(3): 291-297.
24. Beau-Faller M, Prim N, Ruppert AM, Nanni-Metéllus I, Lacave R, Lacroix L, et al. Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicent re observational study by the French ERMETIC-IFCT network. Ann Oncol. 2014; 25(1): 126-131.
25. Lee B, Lee T, Lee SH, Choi YL, Han J. Clinicopathologic characteristics of EGFR, KRAS, and ALK alterations in 6,595 lung cancers. Oncotarget. 2016; 7(17): 23874-23884.
26. Calibasi-Kocal G, Amirfallah A, Sever T, Umit Unal O, Gurel D, Oztop I, et al. EGFR mutation status in a series of Turkish non-small cell lung cancer patients. Biomed Rep. 2020; 13(2): 2.
27. Gahr S, Stoehr R, Geissinger E, Ficker JH, Brueckl WM, Gschwendtner A, et al. EGFR mutational status in a large series of Caucasian European NSCLC patients: data from daily practice. Br J Cancer. 2013; 109(7): 1821-1828.
28. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol. 2018; 13(3): 323-358.