



EGFR mutation types and abundance were associated with the overall survival of advanced lung adenocarcinoma patients receiving first-line tyrosine kinase inhibitors

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Background: Epidermal growth factor receptor tyrosine kinases inhibitors (EGFR-TKIs) are currently recognized as the standard treatment for advanced non-small cell lung cancer (NSCLC) patients with *EGFR* mutations. Clinically found patients with different *EGFR* mutational status have different prognosis.

Methods: A retrospective cohort study was performed to explore the relationship between *EGFR* mutations and abundance with patient survival by using patient data from the Affiliated Cancer Hospital of Zhengzhou University between January 2013 and November 2016. All patients involved in the present study had sensitive *EGFR* mutations [either exon 19 deletion (DEL) or exon 21 L858R] and treated by EGFR-TKIs. They were followed up every three months until lost or dead. Mutation abundance was calculated as the copies of *EGFR* mutation divided by copies of *EGFR* locus, and the cut-off values for 19DEL and L858R were 4.9% and 9.5%, respectively.

Results: Total of 236 patients were included, comprising 116 (49.2%) patients with 19DEL mutation and 120 (50.8%) patients with L858R mutation. The median follow-up duration was 23.2 months (95% CI: 14.9–26.7 months). Overall survival (OS) was significantly longer in patients with 19DEL mutation (20.9 months, 95% CI: 17.7–24.1 months versus 17.0 months, 95% CI: 14.4–19.6 months in patients with L858R; $P=0.008$) and in patients with high mutation abundance (20.9 months, 95% CI: 18.3–23.5 months versus 13.0 months, 95% CI: 10.3–15.7 months in patients with low mutation abundance; $P<0.001$). Multivariate Cox regression including age, performance status and tumor stage revealed that longer OS was independently associated with 19DEL mutation (HR: 0.48, 95% CI: 0.39–0.67, $P=0.033$) and high mutation abundance (HR: 0.62, 95% CI: 0.50–0.79, $P=0.027$).

Conclusions: *EGFR* mutation types and abundance was associated with the patients' survival which might be used to predict the efficacy of targeted therapy by EGFR-TKIs.

Keywords: Lung adenocarcinoma; epidermal growth factor receptor (*EGFR*) mutation abundance; EGFR-tyrosine kinase inhibitor (EGFR-TKI); overall survival (OS)

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Introduction

Epidermal growth factor receptor (*EGFR*) is a transmembrane receptor tyrosine kinase frequently over-expressed in non-small cell lung cancer (NSCLC) (1). Exon 19 deletions (19DEL) and exon 21 point mutation (L858R), accounting for up to 85–90% of all mutations (2–6), are hypersensitive to small molecule *EGFR* tyrosine kinase inhibitors (EGFR-TKIs) (7–10), leading to molecularly stratified therapy of NSCLC, including treatment with EGFR-TKIs (11).

EGFR-TKIs, which are anticancer agents that block overactive *EGFR* signaling in cancer cells (12), are currently recognized as the standard first-line treatment for advanced NSCLC patients with *EGFR* mutations (13). Several prospective trials have demonstrated remarkable efficacy of treatment with EGFR-TKIs, resulting in higher objective response rates (ORRs), longer progression-free survival (PFS), and few adverse effects when compared to chemotherapy in NSCLC patients harboring sensitive *EGFR* mutations (14–21). However, 2 exon 20 mutations including exon 20 point mutation (T790M) (22,23) and E20 insertions (24) confer primary resistance (T790M accounts for about 70%) (25) to the action of EGFR-TKIs on activating mutations by inducing a conformation change that re-activates the tyrosine kinase domain. The frequency of doublets overall is 6%, and T790M mutation is frequent in doublets but uncommon in singlets (26). With respect to echinoderm microtubule-associated protein-like 4 (*EML4*) anaplastic lymphoma kinase (*ALK*) translocation/*EGFR* co-alterations, Ulivi *et al.* conducted a study of 28 patients with *EGFR* mutation who underwent first-line EGFR-TKI treatment and demonstrated a clinical benefit on the disease control rate (DCR) in 81.8% of patients with *EGFR* mutations only, which was higher than the 67% of the 6 co-altered patients who received an EGFR-TKI (27). Recent studies (28,29) have demonstrated spatial heterogeneity in NSCLC, as on average, spatially heterogeneous mutations are present in 30–40% of a tumor mass. *EGFR* mutation status alone is not sufficient to predict outcome. Zhou *et al.* demonstrated that relatively high *EGFR* mutation abundance could predict which NSCLC patient subpopulation would have a PFS benefit from EGFR-TKI treatment (30) using direct sequencing and the amplification refractory mutation system (ARMS) assay (with different sensitivity). A qualitative detection method for *EGFR* mutations is not sufficient to guide precise targeted therapy in clinical practice. Zhao *et al.* demonstrated that the quantitative abundance of *EGFR*

mutations may be a potential predictor to select patients with acquired resistance to primary EGFR-TKIs that would benefit from EGFR-TKI re-administration (31). Li *et al.* (32) quantitatively evaluated the abundance of *EGFR* mutations for patients with advanced NSCLC and showed that the high abundance group had a median PFS benefit, and similar results were also observed in a study by Wang *et al.* (33). Plasma *EGFR* mutation abundance was also found to affect the clinical response to first-line EGFR-TKIs in patients with advanced NSCLC (34). Overall survival (OS) is an important index for evaluating antitumor therapy and could evaluate whether the treatment regimens or test indicators can be included in guidelines. However, these previous studies investigated PFS as the clinical outcome, while overall survival (OS) was not explored.

In the current study, we determined the *EGFR* mutational status of advanced NSCLC patients receiving first-line EGFR-TKI treatment and investigated the correlation between *EGFR* mutation types, abundance, and the OS and PFS of these patients. We present the following article in accordance with the REMARK reporting checklist (available at <https://jtd.amegroups.com/article/view/10.21037/jtd-22-755/rc>).

Methods

Patients

This retrospective cohort study was conducted at the Affiliated Cancer Hospital of Zhengzhou University (Zhengzhou, China), and all the patients were admitted to our hospital between January 2013 and November 2016. Patients were eligible if: (I) they were ≥ 18 years of age; (II) they had an Eastern Cooperative Oncology Group performance status (ECOG-PS) score of 2 or less; (III) they underwent computed tomography (CT) and had a pathologically confirmed diagnosis of advanced lung adenocarcinoma with *EGFR* mutation (19DEL or L858R). Patients with severe comorbidities, tumors other than NSCLC, active infection and pulmonary fibrosis, or severe heart disease were excluded. They were followed up every three months until lost or dead.

The study protocol was approved by the Ethics Committee of the Affiliated Cancer Hospital of Zhengzhou University (No. 2019060) and the study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and Good Clinical Practice guidelines. Patient consent was not required because of the retrospective nature of the study. Patient data were anonymized.

Patient evaluation

The following clinical parameters were recorded from electronic records by retrospective collection: age, sex, smoking history, ECOG-PS, TNM stage without any treatment, the whole treatment process, *EGFR* mutation types, and abundance. Tumor stages were determined according to the 7th edition of the TNM classification (35). All patients were evaluated monthly with physical examination including ECOG-PS and routine laboratory investigations. Routine surveillance imaging included chest and abdomen CT and brain magnetic resonance imaging (MRI). Patients underwent bone scans when bone metastasis was suspected. The treatment response assessment was performed 1 month after the initiation of *EGFR*-TKI treatment and subsequently every 2 months according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1) (36) until they developed signs of disease progression or death from any cause. Additional assessment was performed at any time when signs or symptoms suggested disease progression.

EGFR mutation testing

EGFR mutations and abundance in tumor specimens of eligible NSCLC patients without any treatment were analyzed by the ARMS specific mutation quantitation (ARMS+) (32). All samples had sufficient tumor tissues for analysis. If the tumor content was observed to be <50%, the samples were trimmed to satisfy the criteria. Multiple cores per biopsy were analyzed. Microdissection was performed for the formalin-fixed paraffin-embedded (FFPE) blocks to reduce stroma/normal tissue/necrosis contamination. Extracted DNA was from the FFPE block core or slides. If extraction was performed from cores, multiple, spatially diverse cores were taken. If extraction was performed from slides, slides from multiple depths were used. Viable tumor cellularity was checked before performing DNA extraction. Genomic DNA was extracted from 1 FFPE block using a QIAmp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). DNA concentration was adjusted to 20–50 ng/mL. *EGFR* mutations including exon 19DEL, exon 21 L858R, exon 20 T790M, and exon 18 mutations (G719X) were detected using a Human *EGFR* Gene Mutations Quantitative Detection Kit (TB002, Beijing ACCB Biotech, Beijing, China). The assay was carried out according to the manufacturer's protocol with the MX3000P Real-Time PCR system (Stratagene; Agilent Technologies,

Inc., Santa Clara, CA, USA). Mutation abundance was calculated by copies of *EGFR* mutation divided by copies of *EGFR* locus and the cut-off values for 19DEL and L858R were 4.9% and 9.5%, respectively (32).

Treatments

Patients harboring sensitive *EGFR* mutations were treated with first-line *EGFR*-TKIs including icotinib (125 mg 3 times a day), erlotinib (150 mg once a day), and gefitinib (250 mg once a day). *EGFR*-TKIs were continued until disease progression as defined by RECIST guidelines (36) or death from any cause. Upon progression, patients received second- or further-line platinum-based chemotherapy, single-agent chemotherapy, first-generation *EGFR*-TKI if *EGFR* mutation testing performed on the re-biopsied tissue when they developed progression showed sensitive *EGFR* mutations (either 19DEL or L858R), or best supportive care (BSC).

Study outcomes

The primary outcome of the study was OS, which was calculated from the first day of first-line *EGFR*-TKI treatment to death from any cause or the last day of follow-up. The secondary outcomes included PFS and ORR. PFS was calculated from the first day of first-line *EGFR*-TKI therapy to disease progression or death from any cause. ORR was defined as the percentage of patients having either a complete response (CR) or a partial response (PR). DCR was defined as the percentage of patients with stable disease (SD), CR, or PR. The last follow-up was conducted in December 2017.

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences 18.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were analyzed using a χ^2 test or Fisher's exact test. OS and PFS were compared between the 2 groups using the Kaplan-Meier method, and 95% confidence intervals (CI) were presented. Univariate analysis was carried out and variables with $P < 0.1$ (age, performance status, tumor stage, tumor mutation types and abundance) were entered into multivariate Cox regression to evaluate prognostic factors. $P < 0.05$ was considered statistically significant.

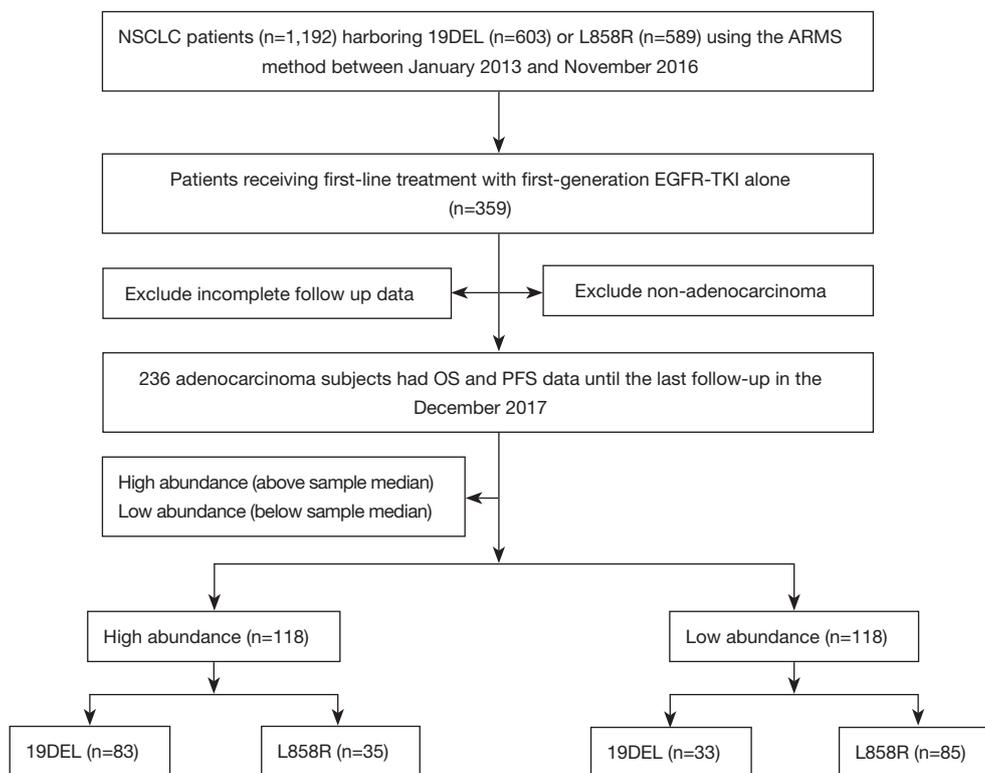


Figure 1 The study flow chart. NSCLC, non-small cell lung cancer; ARMS, amplification refractory mutation system; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; OS, overall survival; PFS, progression-free survival.

Results

Patient demographic and baseline characteristics

The study flow chart is shown in *Figure 1*. A total of 1,192 NSCLC patients were screened positive for 19DEL or L858R mutations. Among them, 833 patients who did not receive first-line EGFR-TKI therapy were excluded, 20 patients with NSCLC other than adenocarcinoma were not included, and 103 patients with incomplete follow-up data were also excluded. Finally, 236 EGFR-mutated lung adenocarcinoma patients who received first-line EGFR-TKI treatment were included in this retrospective cohort study. The demographic and baseline characteristics of the patients are shown in *Table 1*. More patients were female (58.1%) and the majority were never smokers (74.6%). Most patients had an ECOG PS score of 0 or 1 (84.7%) and 93.2% were TNM stage IV.

EGFR mutation testing revealed 19DEL mutation in 116 (49.2%) patients and L858R mutation in 120 (50.8%) patients. The high and low mutation abundance groups were comparable in terms of demographic and baseline

variables. EGFR mutation testing was performed on the re-biopsied tissue when patients developed progression. The results revealed 19DEL mutation in 47.5% (112/236) of patients, L858R mutation in 48.7% (115/236) of patients, 19DEL/T790M co-mutations in 0.8% (2/236) of patients, and L858R/T790M co-mutations in 1.7% (4/236) of patients, while T790M mutation was only present in 1.3% (3/236) of patients (*Table 1*).

Treatment characteristics

In terms of first-line treatment, 47 patients received erlotinib, 39 received icotinib, and 150 received gefitinib. As the second-line or further-line treatment, 25 (37.9%) and 80 (47.1%) patients received platinum-based combination chemotherapy in the low and the high abundance groups, respectively. Additionally, in the low and high abundance groups, respectively, 15 (22.7%) and 27 (15.9%) patients received single-agent chemotherapy, 21 (31.8%) and 48 (28.2%) patients received first-generation EGFR-TKI treatment, and 5 (7.6%) and 15 (8.8%) received BSC (*Table 1*).

Table 1 Patient demographic and baseline characteristics

Characteristics	Low abundance, n (%)	High abundance, n (%)	Chi-square (χ^2) value	P value
Age (years)			0.623	0.430
≤60	30 (45.5)	87 (51.2)		
>60	36 (54.5)	83 (48.8)		
Sex			0.008	0.927
Female	38 (57.6)	99 (58.2)		
Male	28 (42.4)	71 (41.8)		
Smoking history			0.351	0.553
Yes	15 (22.7)	45 (26.5)		
No	51 (77.3)	125 (73.5)		
ECOG-PS			0.186	0.667
0–1	57 (86.4)	143 (84.1)		
2	9 (13.6)	27 (15.9)		
Mutation types prior to treatment			31.807	<0.001
19DEL	13 (19.7)	103 (60.6)		
L858R	53 (80.3)	67 (39.4)		
TNM stage			0.774	0.379
IIb	6 (9.1)	10 (5.9)		
IV	60 (90.9)	160 (94.1)		
First-line EGFR-TKI			1.438	0.487
Erlotinib	15 (22.7)	32 (18.8)		
Icotinib	13 (19.7)	26 (15.3)		
Gefitinib	38 (57.6)	112 (65.9)		
Second- or further-line treatment			2.448	0.485
Platinum-based chemotherapy	25 (37.9)	80 (47.1)		
Single-agent chemotherapy	15 (22.7)	27 (15.9)		
First-generation EGFR-TKI	21 (31.8)	48 (28.2)		
BSC	5 (7.6)	15 (8.8)		
Mutation types rebiopsy			34.732	<0.001
19DEL	12 (18.2)	100 (58.8)		
L858R	51 (77.3)	64 (37.6)		
19DEL/T790M	1 (1.5)	1 (0.6)		
L858R/T790M	1 (1.5)	3 (1.8)		
T790M	1 (1.5)	2 (1.2)		

Yes (smoking history): smokers are defined as current smokers smoking >100 cigarettes/lifetime, or smoking >100 cigarettes/lifetime but abstinence from smoking for less than 1 year on the day before the start of therapy. No (smoking history): no smokers are defined as smoking <100 cigarettes/lifetime. ECOG-PS, Eastern Cooperative Oncology Group performance status; TNM, tumor-node-metastasis; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; BSC, best supportive care; 19DEL/T790M, 19DEL/T790M co-mutations; L858R/T790M, L858R/T790M co-mutations.

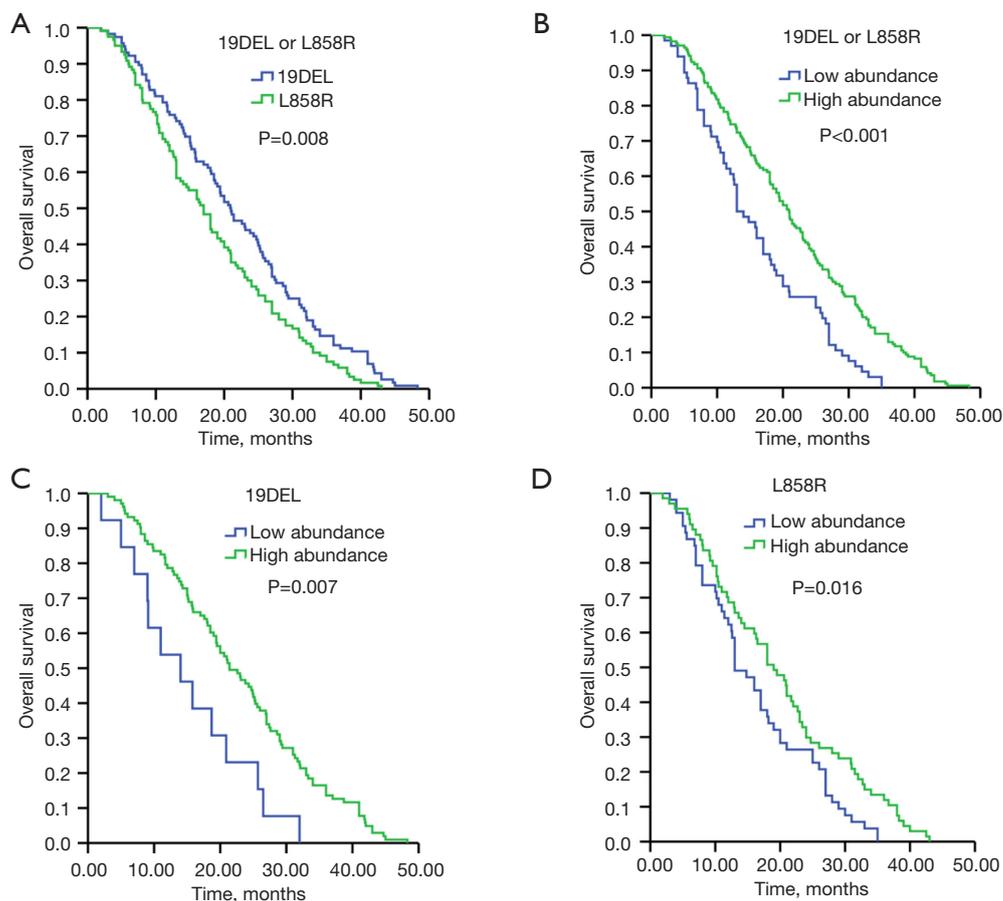


Figure 2 Kaplan-Meier curve of OS stratified by 19DEL versus L858R mutation (A), high versus low abundance of 19DEL or L858R mutation (B), high versus low abundance of 19DEL mutation only (C), and high versus low abundance of L858R mutation only (D). OS, overall survival.

OS

The median follow-up duration was 23.2 months (95% CI: 14.9–26.7 months). Patients carrying 19DEL mutation had markedly longer OS (20.9 months, 95% CI: 17.7–24.1 months) than patients with L858R mutation (17.0 months, 95% CI: 14.4–19.6 months) ($P=0.008$) (Figure 2A). Patients with high abundance had significantly longer OS (20.9 months, 95% CI: 18.3–23.5 months) than those with low abundance (13.0 months, 95% CI: 10.3–15.7 months) ($P<0.001$) (Figure 2B). In the subgroup analysis that included 19DEL mutation only, patients with high mutation abundance had significantly longer OS (21.4 months, 95% CI: 17.4–25.3 months) than those with low mutation abundance (14.0 months, 95% CI: 6.1–21.9 months) ($P=0.007$) (Figure 2C). In the subgroup analysis that included L858R mutation only, patients

with high abundance also had significantly longer OS (19.0 months, 95% CI: 14.6–23.4 months) than those with low abundance (13.0 months, 95% CI: 10.0–16.0 months) ($P=0.016$) (Figure 2D). Further analysis of 19DEL mutation only showed that patients with low mutation abundance who received first-line EGFR-TKI treatment had significantly longer OS (14.0 months, 95% CI: 6.1–21.9 months) than patients who received first-line chemotherapy (11.6 months, 95% CI: 7.7–15.5 months), with no significant difference ($P=0.321$) (Figure 3A). In the subgroup analysis that included L858R mutation only, patients with low mutation abundance who received first-line EGFR-TKI treatment had significantly longer OS (13.0 months, 95% CI: 10.0–16.0 months) than patients who received first-line chemotherapy (11.0 months, 95% CI: 9.1–12.9 months), with no significant difference

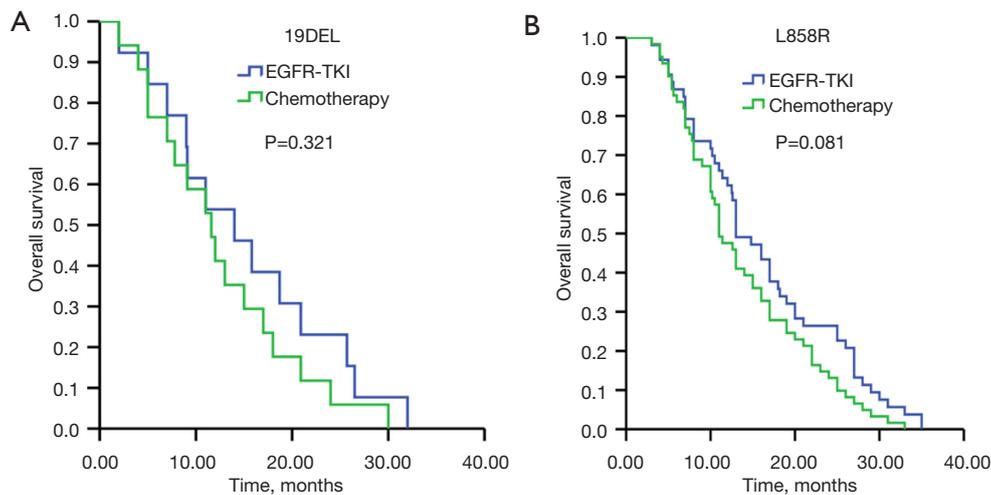


Figure 3 Kaplan-Meier curve of OS between patients with low mutation abundance who received first-line EGFR-TKI treatment or first-line chemotherapy that included 19DEL mutation only (A) or L858R mutation only (B). EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; OS, overall survival.

($P=0.081$) (Figure 3B).

Univariate analysis showed associations between OS and age (≤ 60) (HR: 0.51, 95% CI: 0.44–0.92, $P=0.003$), ECOG-PS (0–1) (HR: 0.49, 95% CI: 0.41–0.85, $P=0.038$), TNM stage (IIIB) (HR: 0.37, 95% CI: 0.21–0.54, $P=0.027$), 19DEL mutation (HR: 0.42, 95% CI: 0.31–0.69, $P=0.009$), high abundance (HR: 0.53, 95% CI: 0.41–0.77, $P=0.002$), DCR (HR: 0.75, 95% CI: 0.65–0.84, $P=0.036$), and subsequent therapy (chemoradiotherapy or BSC) (HR: 0.60, 95% CI: 0.52–0.76, $P=0.025$). In the multivariate Cox regression analysis, longer OS was independently associated with age (≤ 60 years) (HR: 0.64, 95% CI: 0.56–0.75, $P=0.032$), ECOG-PS (0–1) (HR: 0.50, 95% CI: 0.41–0.73, $P=0.041$), TNM stage (IIIB) (HR: 0.52, 95% CI: 0.44–0.62, $P=0.047$), 19DEL mutation (HR: 0.48, 95% CI: 0.39–0.67, $P=0.033$), high abundance (HR: 0.62, 95% CI: 0.50–0.79, $P=0.027$), and subsequent therapy (chemoradiotherapy or BSC) (HR: 0.72, 95% CI: 0.58–0.88, $P=0.042$) (Table 2).

Secondary outcomes

PFS

Patients carrying 19DEL mutation had markedly longer PFS (10.2 months, 95% CI: 8.5–11.9 months) than patients with L858R mutation (8.3 months, 95% CI: 7.1–9.5 months) ($P=0.046$) (Figure 4A). PFS was significantly longer in the high mutation abundance group (11.0 months, 95% CI: 9.7–12.3 months) than the low abundance group (5.3 months, 95% CI: 3.6–7.0 months)

($P<0.001$) (Figure 4B). In the subgroup analysis that included 19DEL mutation only, patients with high mutation abundance had significantly longer PFS (11.0 months, 95% CI: 8.8–13.1 months) than those with low mutation abundance (5.5 months, 95% CI: 3.7–7.2 months) ($P=0.001$) (Figure 4C). In the subgroup analysis that included L858R mutation only, patients with high mutation abundance also had significantly longer PFS (10.1 months, 95% CI: 7.8–12.4 months) than those with low mutation abundance (5.3 months, 95% CI: 3.0–7.6 months) ($P=0.031$) (Figure 4D). Further analysis of 19DEL mutation only showed that patients with low mutation abundance who received first-line EGFR-TKI treatment had significantly longer PFS (5.5 months, 95% CI: 3.7–7.2 months) than patients who received first-line chemotherapy (4.2 months, 95% CI: 2.2–6.1 months) ($P=0.04$) (Figure 5A). Further analysis of L858R mutation only showed that patients with low mutation abundance who received first-line EGFR-TKI treatment had significantly longer PFS (5.3 months, 95% CI: 3.0–7.6 months) than patients who received first-line chemotherapy (4.6 months, 95% CI: 3.4–5.8 months) ($P=0.025$) (Figure 5B).

Univariate analysis revealed that 19DEL mutation (HR: 0.52, 95% CI: 0.39–0.89, $P=0.019$), high mutation abundance (HR: 0.60, 95% CI: 0.51–0.92, $P=0.022$), and DCR (HR: 0.85, 95% CI: 0.65–0.94, $P=0.039$) were prognostic determinants of PFS. Multivariate Cox regression analysis showed that 19DEL mutation (HR: 0.56, 95% CI: 0.42–0.60, $P=0.041$), high mutation abundance

Table 2 Univariate and multivariate Cox regression of patient variables for determinants of OS in the study population

Variables	Univariate Cox regression		Multivariate Cox regression	
	HR (95% CI)	P	HR (95% CI)	P
Age, years (≤ 60 , >60)	0.51 (0.44–0.92)	0.003	0.64 (0.56–0.75)	0.032
Sex (female, male)	0.74 (0.53–1.02)	0.17	–	–
Smoking history (yes, no)	0.68 (0.39–0.90)	0.45	–	–
ECOG-PS (0–1, 2)	0.49 (0.41–0.85)	0.038	0.50 (0.41–0.73)	0.041
TNM stage (IIIB, IV)	0.37 (0.21–0.54)	0.027	0.52 (0.44–0.62)	0.047
Mutation types (19DEL, L858R)	0.42 (0.31–0.69)	0.009	0.48 (0.39–0.67)	0.033
Abundance (high, low)	0.53 (0.41–0.77)	0.002	0.62 (0.50–0.79)	0.027
EGFR-TKI (erlotinib, icotinib, gefitinib)	0.67 (0.35–0.82)	0.059	–	–
Initial EGFR-TKI response (DCR, PD)	0.75 (0.65–0.84)	0.036	0.86 (0.68–0.89)	0.058
Subsequent therapy (chemoradiotherapy or BSC)	0.60 (0.52–0.76)	0.025	0.72 (0.58–0.88)	0.042

OS, overall survival; HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; TNM, tumor-node-metastasis; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; DCR, disease control rate; PD, progressive disease; BSC, best supportive care.

(HR: 0.77, 95% CI: 0.66–0.82, $P=0.037$), and DCR (HR: 0.68, 95% CI: 0.51–0.89, $P=0.045$) were independent prognostic determinants of PFS (Table 3).

ORR and DCR

Patients with high mutation abundance had a significantly higher ORR and DCR than those with low mutation abundance in subjects carrying 19DEL or L858R mutations (ORR: 67.10% versus 28.80%, $P<0.001$; DCR: 94.10% versus 71.20%, $P<0.001$, respectively). Similar results regarding ORR and DCR were observed in the subgroup analysis of patients carrying 19DEL and L858R mutations (Table 4).

Discussion

Delineation of gene mutations of NSCLC has led to molecularly stratified therapy for NSCLC and has improved lung adenocarcinoma management. The emergence of intratumor heterogeneity, including individual tumor biopsies and spatial separation between biopsies of the same tumor (37), may impact the tumor biopsy strategy, actionable targets, and individualized targeted treatment (37–40). In the present study, multiple cores per biopsy were analyzed. If extracted DNA was from the FFPE block cores, spatially diverse cores were taken. If extraction was from slides, slides from multiple depths were used. Tumor

sampling bias may arise due to differences between and within single biopsies or metastatic sites, and between the primary and metastatic sites (37). Heterogeneity is also dynamic and evolves over time (41). To some extent, the repeatability of mutation abundance measurement was not satisfactorily reproducible.

Both the LUX-Lung 3 and LUX-Lung 6 trials demonstrated that first-line afatinib significantly improved the OS of patients with 19DEL mutation, while those with L858R mutation did not show improved OS (42). Several retrospective studies (43–45) and a prospective study (46) also showed that 19DEL mutation was associated with better efficacy than L858R mutation in patients receiving first-line EGFR-TKIs. A previous study also showed that osimertinib was equally effective for NSCLC patients with various abundance of T790M mutation (47). The current study indicated that patients carrying 19DEL mutation had better OS and PFS than those with L858R mutation in response to first-line EGFR-TKI therapy. However, this was not observed in a retrospective cohort study which showed that the median OS of patients treated with a first-line EGFR-TKI with 19DEL and L858R mutation was 32.7 and 24.7 months, respectively (95% CI: 16.8–48.6 and 20.8–28.5 months, respectively, $P=0.174$) (48).

The status of EGFR mutation types is not well studied, quantification of EGFR mutations for molecular stratification of NSCLC patients has only recently drawn

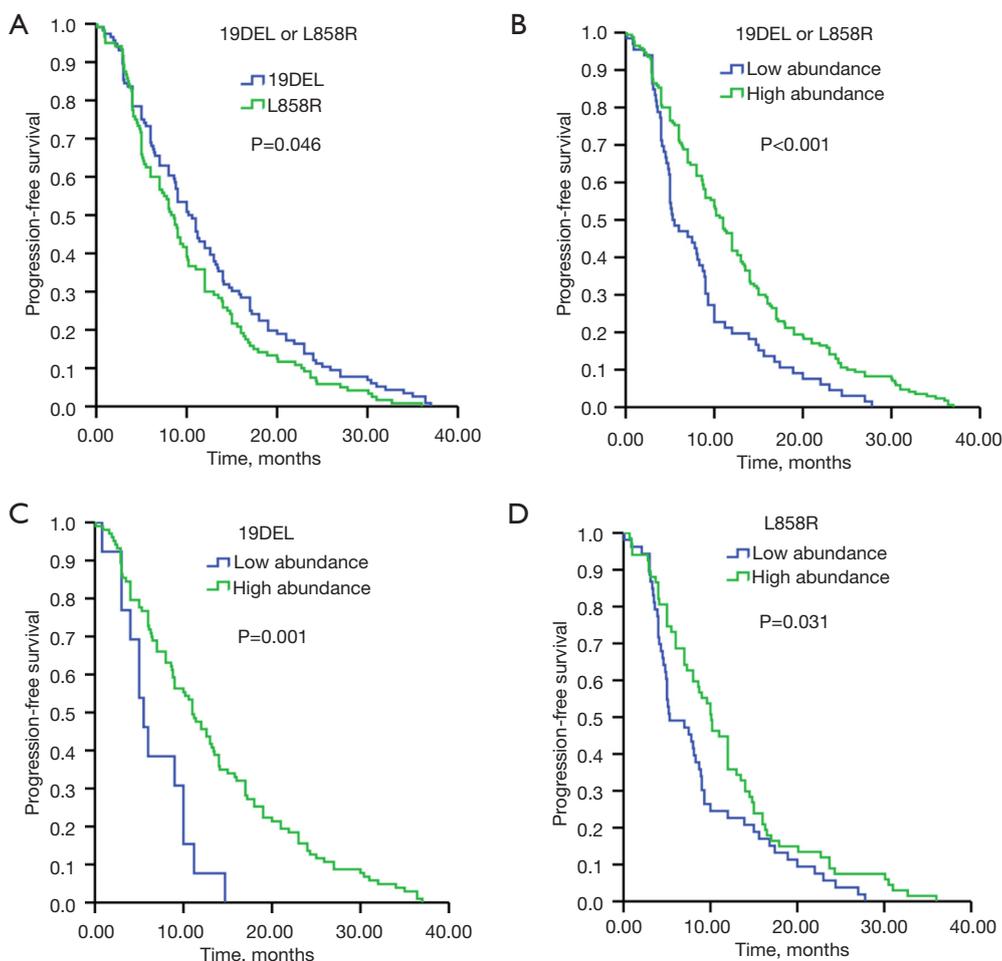


Figure 4 Kaplan-Meier curve of PFS stratified by 19DEL versus L858R mutation (A), high versus low abundance of 19DEL or L858R mutation (B), high versus low abundance of 19DEL mutation only (C), and high versus low abundance of L858R mutation only (D). PFS, progression-free survival.

the attention of clinical investigators. In the present study, we carried out quantitative analysis of 19DEL and L858R mutations in advanced lung adenocarcinoma patients on EGFR-TKI therapy. Our study demonstrated that high *EGFR* mutation abundance correlated with the clinical outcomes of NSCLC patients receiving EGFR-TKI therapy. In addition, our study showed that 88.8% (103/116) of patients with 19DEL mutation exhibited high mutation abundance, while 55.8% (67/120) of patients with L858R mutation exhibited high mutation abundance. The OS benefit of patients with 19DEL mutation may be due to the higher proportion of high mutation abundance in patients with L858R mutation.

Patients with high *EGFR* mutation abundance had significantly longer OS and PFS versus those with low

EGFR mutation abundance of 19DEL or L858R, and similar results were obtained from our subgroup analysis in patients with high mutation abundance carrying 19DEL only or L858R only mutations. These findings indicate that *EGFR* mutation abundance could be used to help stratify NSCLC patients for EGFR-TKI therapy. We further observed that *EGFR* mutation abundance was significantly associated with response to EGFR-TKIs. ORR and DCR were significantly higher in patients with high mutation abundance than those with low mutation abundance, as well as with 19DEL or L858R mutation. Our observations were similar to a previous study (32). Our study also showed that patients with low mutation abundance who received first-line EGFR-TKIs had significantly longer PFS than patients who received first-line standard chemotherapy.

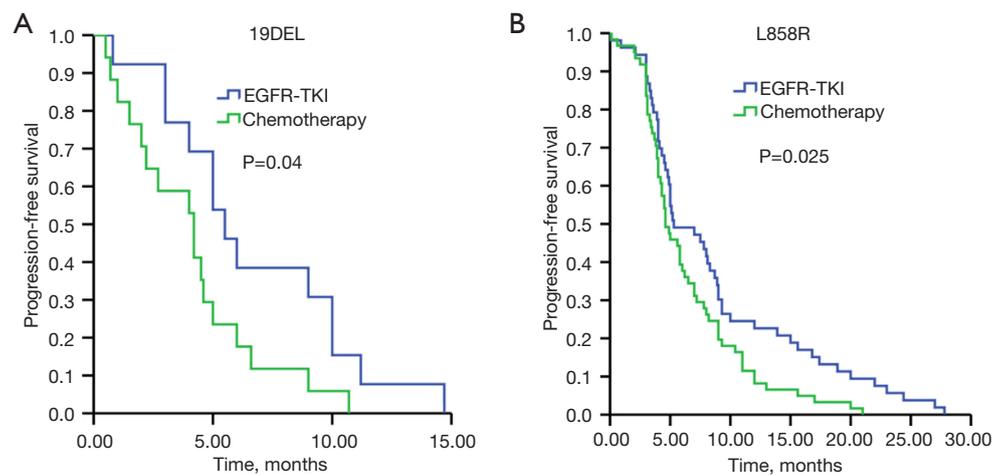


Figure 5 Kaplan-Meier curve of PFS between patients with low mutation abundance who received first-line EGFR-TKI treatment or first-line chemotherapy that included 19DEL only (A) or L858R mutation only (B). EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PFS, progression-free survival.

Table 3 Univariate and multivariate Cox regression of patient variables for determinants of PFS in the study population

Variables	Univariate Cox regression		Multivariate Cox regression	
	HR (95% CI)	P	HR (95% CI)	P
Age, years (≤ 60 , >60)	0.78 (0.64–0.92)	0.21	–	–
Sex (female, male)	0.74 (0.53–0.86)	0.50	–	–
Smoking history (yes, no)	0.69 (0.49–0.81)	0.36	–	–
ECOG-PS (0–1, 2)	0.75 (0.52–0.83)	0.075	0.87 (0.74–0.93)	0.064
TNM stage (IIIB, IV)	0.43 (0.35–0.59)	0.051	0.53 (0.45–0.61)	0.059
Mutation types (19DEL, L858R)	0.52 (0.39–0.89)	0.019	0.56 (0.42–0.60)	0.041
Abundance (high, low)	0.60 (0.51–0.92)	0.022	0.77 (0.66–0.82)	0.037
EGFR-TKI (erlotinib, icotinib, gefitinib)	0.37 (0.28–0.45)	0.44	–	–
Initial EGFR-TKI response (DCR, PD)	0.85 (0.65–0.94)	0.039	0.68 (0.51–0.89)	0.045

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group performance status; TNM, tumor-node-metastasis; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; DCR, disease control rate; PD, progressive disease.

These results were similar to those of the blind independent central review (BICR) (49), but there was no significant difference in OS, as confounding factors such as subsequent treatment were present.

Hence, both *EGFR* mutation types and mutation abundance are associated with outcome of EGFR-TKI treatment for advanced lung adenocarcinoma. These observations warrant confirmation in a large prospective study in the future. Exploration of the biological mechanisms of the differences between high and low

mutation abundance should be undertaken. With respect to spatial diversity in genomic instability processes, evidence suggests that opportunities to exploit such mechanisms therapeutically may be limited for patients with NSCLC (50).

Our study has several limitations. First, our study is a single center study, with the majority of patients being Chinese. This limits the generalizability of our findings. Second, our study was a retrospective cohort study and could not establish a causal relationship. The mutation

Table 4 ORR and DCR of the study population stratified by *EGFR* mutation abundance

Efficacy	19DEL or L858R (n=236)				19DEL (n=116)				L858R (n=120)			
	Low abundance	High abundance	χ^2	P	Low abundance	High abundance	χ^2	P	Low abundance	High abundance	χ^2	P
PR	19	114			4	72			15	42		
SD	28	46			6	27			22	19		
PD	19	10			3	4			16	6		
ORR	28.80%	67.10%	28.311	<0.001	30.80%	69.90%	7.825	0.01	28.30%	62.70%	14.030	<0.001
DCR	71.20%	94.10%	23.143	<0.001	76.90%	96.10%	7.499	0.03	69.80%	91.00%	8.911	0.003

ORR, overall response rate; DCR, disease control rate; *EGFR*, epidermal growth factor receptor; PR, partial response; SD, stable disease; PD, progressive disease;

abundance described in the present study was based on ARMS plus technology. Droplet digital PCR (ddPCR) has become a well-developed method for rapidly and quantitatively assessing *EGFR* mutations with higher specificity and sensitivity (51-53).

In conclusion, both *EGFR* mutation types and mutation abundance may be prognostic determinants of lung adenocarcinoma patients receiving first-line *EGFR*-TKIs, and 19DEL and high *EGFR* mutation abundance may predict significantly longer OS and PFS of stage IIIB/IV lung adenocarcinoma patients receiving first-line *EGFR*-TKI therapy.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at <https://jtd.amegroups.com/article/view/10.21037/jtd-22-755/rc>

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study protocol was approved by the Ethics Committee of the Affiliated Cancer Hospital of Zhengzhou University (No. 2019060) and the study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and Good Clinical Practice guidelines. Patient consent was not required because of the retrospective nature of the study.

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