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Original Article

Mutation of the *epidermal growth factor receptor* gene and its impact on the efficacy of gefitinib in advanced non-small cell lung cance

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Abstract: Mutations in the *epidermal growth factor receptor* (*EGFR*) gene are associated with subsets of non-small cell lung cancer (NSCLC). Some patients with *EGFR* mutations are responsive to targeted therapy with the EGFR tyrosine kinase inhibitor gefitinib. Here, the mutation status of *EGFR* was assessed in advanced-stage NSCLC patients to determine how mutation status influences the clinical efficacy of gefitinib. The study included 106 patients with advanced NSCLC who were treated with gefitinib. Exons 19 and 21 of *EGFR* were sequenced from tumor tissues samples by PCR, and patient clinical characteristics, short-term outcomes (partial response, stable disease, progressive disease), and survival [overall survival (OS) and progression-free survival (PFS)] were compared. *EGFR* mutations in either exon 19 or exon 21 were detected in 54.7% of cases. The *EGFR* gene mutation rate was significantly different in patients with different pathological types (χ^2=6.612, P<0.05). The distribution of short-term outcomes differed significantly by *EGFR* gene mutation status, history of smoking, and bone metastasis (χ^2=6.481~35.938, P<0.05). Further, OS and PFS was significantly higher following gefitinib in patients with *EGFR* mutations than those without *EGFR* mutation (χ^2=19.135, 6.953, P<0.05). OS was also significantly higher in patients with an exon 19 deletion mutation than in those with the exon 21 point mutation (χ^2=8.575, P<0.05). Cox multivariate regression analysis indicated that OS was correlated with the pathological type of the tumor (HR=4.877), US Eastern Cooperative Oncology Group Physical Status (ECOG PS) score (HR=3.087), and *EGFR* mutation status (HR=1.876) (all P<0.05), while PFS was correlated with ECOG PS score (HR=2.218), cycles of chemotherapy (HR=1.829), and *EGFR* mutation status (HR=1.840) (all P<0.05). Only mild adverse events were reported during gefitinib treatment. The findings indicate that gefitinib treatment can improve the clinical outcomes of NSCLC patients with *EGFR* mutation, prolonging their survival time with only mild adverse events.

Keywords: Non-small cell lung cancer, epidermal growth factor receptor, gene mutation, gefitinib, survival analysis

Introduction

Non-small cell lung cancer (NSCLC) accounts for 80% to 90% of all lung cancer cases, and adenocarcinoma is the most common (38%) histological type of NSCLC [1]. This cancer typically has poor outcomes because more than 70% of patients have advanced disease at the time of diagnosis, thereby precluding the possibility of surgical resection; thus, chemotherapy is the main treatment option for most patients with NSCLC [2]. Although the two platinum-containing chemotherapy regimens are often used as the first-line treatment for NSCLC patients, these treatments do not significantly decrease the mortality of NSCLC patients: 5-year survival rate remains less than 20% [3-4]. Importantly, the therapeutic regimen is an independent risk factor that affects the survival time of patients with advanced NSCLC [5]. Further, although the disease of some patients will continue to progress after first-line therapy [6], establishing individualized treatment programs, on the basis of synthetically considering the patient's physical state, disease type, genetic status, and other factors, can offer a

reliable clinical benefit for those NSCLC patients whose condition has improved or is stable after first-line treatment [7].

These findings highlight the need for improved therapies that positively affect patient survival.

In recent years, targeted therapeutic drugs have been designed to treat NSCLC based on the mutational status of a patient's tumor. These targeted therapies offer an advance over traditional chemotherapy by prolonging survival time, improving the survival rate, and enhancing the therapeutic effect [8, 9]. The *epidermal growth factor receptor* (*EGFR)* gene commonly exhibits mutations in NSCLC tumors. When mutated, the EGFR receptor tyrosine kinase is overexpressed in NSCLC tumor tissue and promotes tumor cell proliferation and angiogenesis via its downstream signaling pathways, as well as inhibiting tumor cell apoptosis. EGFR tyrosine kinase inhibitors (TKIs) have been designed to selective inhibit the EGFR-mediated signaling pathway to combat NSCLC by preventing progression and/or inducing tumor regression [10].

A number of mutations in *EGFR* have been identified in tumors of patients with NSCLC. Most of these occur in exons 18- 21, with the highest rate of mutations detected in exons 19 and 21. Rarely, patients exhibit double mutations in exons 19 and 21 [8]. Mutations in exon 19 often involve a deletion of codons 746-753, particularly Del E746-A750. Exon 21 often exhibits the variation L858R [11]. *EGFR* mutations are more common in lung adenocarcinoma than in squamous cell lung carcinoma, and in female patients more than in male patients. In addition, *EGFR* is more commonly mutated in non-smoking NSCLC patients and those with a family history of lung cancer and other malignancies [12]. Despite these established patterns, at present no consensus has been reached in the relationship between *EGFR* mutation and tumor grade, staging, size, or metastasis [13]. Therefore, screening tumors and identifying *EGFR* mutation types has great significance in guiding EGFR-TKI targeted therapy for NSCLC patients [14].

One targeted treatment, the EGFR-TKI gefitinib, has shown some success in the clinic in treating EGFR-mutant NSCLC. Gefitinib competitively inhibits binding of ATP to the receptor region, preventing tyrosine kinase activation to exert its anti-tumor effect. In contrast to traditional chemotherapy drugs, gefitinib is able to regulate the pathogenesis of cancer at the molecular level of the cell receptors. Indeed, large-scale clinical studies have shown that gefitinib offers significant benefits for advanced NSCLC disease, improving clinical symptoms rapidly, ameliorating their quality of life, and significantly prolonging the survival time for EGFR mutation-positive patients [6, 12]. However, the effects of gefitinib are largely affected by EGFR mutation status; individual differences in gene mutation types lead to great differences in the prognosis of NSCLC patients treated with gefitinib [6]. To better understand the efficacy of gefitinib in EGFR-mutant NSCLC, this study analyzed the *EGFR* mutation status of patients with advanced NSCLC, and their clinical responses to gefitinib treatment.

Participants and methods

General information

The study selected 106 advanced-stage NSCLC patients who had been admitted to our hospital and treated with gefitinib between January 2011 and December 2011. Of the 106, 55 were males, 38 were smokers (current), and 60 patients were <60 years old (mean age was 54.6±8.9 years). The majority (79/106) of patients had a US Eastern Cooperative Oncology Group performance score (ECOG PS) of 0-1 point, while the remainder had an ECOG PS of ≥2 points. For treatment, 14 cases received as the first-line treatment, 92 cases as the second-line treatment. All enrolled patients were pathologically confirmed as having non-squamous NSCLC; 95 cases were adenocarcinoma. The study was approved by the ethics committee of our hospital, and all patients provided written informed content to participate in this research.

Observational index and methods

NSCLC tumor tissue specimens were obtained for final diagnosis and then processed for paraffin embedding. The restriction endonuclease method was used in combination with nested PCR to detect mutations in exons 19 and 21 of *EGFR* in tumor tissues. The primer sequences are shown in **Table 1**.

The following aspects of all patients were observed and analyzed: gender, age, smoking

Table 1. The mutant-enriched -PCR primers, annealing temperatures, and the lengths of the products

Exon	*Primer*	*Primer sequences*	*Tm (°C)*	Fragment
19	Outer	Forward: 5'–ATCCCAGAAGGTGAGAAAGATAAAATTC-3'	63.2	204 bp [WT]
		Reverse: 5'-ACATTTAGGATGTGGAGATGAGCAG-3'	62.8	
	Inner	Forward: 5'-AGGTGAGAAAGATAAAATTCCCGTC-3'	62.0	182 bp [WT]
		Reverse: 5'-GAGATGAGCAGGGTCTAGAGCAG-3'	61.9	
21	Outer	Forward: 5'-TCAGAGCCTGGCATGAACATGACCCTG-3'	74.6	297 bp
		Reverse: 5'-GGTCCCTGGTGTCAGGAAAATGCTGG-3	73.0	
	Inner	Forward: 5'-CAGCAGGGTCTTCTCTGTTTC-3'	59.1	213 bp
		Reverse: 5'-GAAAATGCTGGCTGACCTAAAG-3	60.3	

Table 2. The relationship between *EGFR* mutation status and clinical characteristics in patients with NSCLC (number, %)

Characteristic		*Total number*	*EGFR mutation status*		χ^2	P
			Negative	Positive		
Gender	Male	55	29 (52.7)	26 (47.3)	2.557	>0.05
	Female	51	19 (37.3)	32 (62.7)		
Age	<60	60	26 (43.3)	34 (56.7)	0.212	>0.05
	≥60	46	22 (47.8)	24 (52.2)		
Smoking history	Ever	38	18 (47.4)	20 (52.6)	0.104	>0.05
	Never	68	30 (44.1)	38 (55.9)		
Pathology	Adenocarcinoma	95	39 (41.1)	56 (58.9)	6.612	<0.05
	Non-adenocarcinoma	11	9 (81.8)	2 (18.2)		
ECOG PS	0-1	79	35 (44.3)	44 (55.7)	0.120	>0.05
	2-4	27	13 (48.1)	14 (51.9)		

Table 3. Clinical characteristics of NSCLC patients with different *EGFR* mutation types (number, %)

Characteristic		*Total number*	*EGFR mutation types*		χ^2	P
			Exon 19 (n=27)	Exon 21 (n=31)		
Gender	Male	26	15 (57.7)	11 (42.3)	2.351	>0.05
	Female	32	12 (37.5)	20 (62.5)		
Age	<60	34	17 (50.0)	17 (50.0)	0.393	>0.05
	≥60	24	10 (41.7)	14 (58.3)		
Smoking history	Ever	20	10 (50.0)	10 (50.0)	0.146	>0.05
	Never	38	17 (44.7)	21 (55.3)		
Pathology	Adenocarcinoma	56	26 (46.4)	30 (53.6)	0.010	>0.05
	Non-adenocarcinoma	2	1 (50.0)	1 (50.0)		
ECOG PS	0-1	44	23 (52.3)	21 (47.7)	2.398	>0.05
	2-4	14	4 (28.6)	10 (71.4)		

status, histological type of tumor, EOCG PS score, chemotherapy regimen, metastasis status, and pleural effusion. Evaluation and comparison were performed for the post-treatment short-term efficacy according to the Response Evaluation Criteria in Solid Tumors (RECIST), as well as performed on the adverse reactions during the chemotherapy period. All patients received follow-up with interview and telephone, and observation and comparison were performed on the patients' overall survival (OS) and progression-free survival (PFS).

Data analysis

The SPSS 13.0 statistical package was used to establish a database for all the data in this study and perform statistical analysis. Numerical data were compared using chi-square test, and the patients' predicted value

Table 4. The influence of clinical characteristics on short-term efficacy of gefitinib (number, %)

Characteristics		Total number	Short-term effects			χ^2	P
			PR (n=35)	SD (n=56)	PD (n=15)		
Gender	Male	55	4 (7.3)	38 (69.1)	13 (23.6)	35.938	<0.05
	Female	51	31 (60.8)	18 (35.3)	2 (3.9)		
Age	<60	60	20 (33.3)	32 (53.3)	8 (13.3)	0.076	>0.05
	≥60	46	15 (32.6)	24 (52.2)	7 (15.2)		
Smoking history	Ever	38	3 (7.9)	25 (65.8)	10 (26.3)	19.402	<0.05
	Never	68	32 (47.1)	31 (45.6)	5 (7.4)		
Pathology	Adenocarcinoma	95	32 (33.7)	50 (52.6)	13 (13.7)	0.270	>0.05
	Non-adenocarcinoma	11	3 (27.3)	6 (54.5)	2 (18.2)		
ECOG PS	0~1	79	25 (31.6)	43 (54.4)	11 (13.9)	0.339	>0.05
	2~4	27	10 (37.0)	13 (48.1)	4 (14.8)		
Chemotherapy cycles	≤1	75	26 (34.7)	41 (54.7)	8 (10.7)	2.575	>0.05
	≥2	31	9 (29.0)	15 (48.4)	7 (22.6)		
Bone metastases	Yes	28	15 (53.6)	11 (39.3)	2 (7.1)	7.510	<0.05
	No	78	20 (25.6)	45 (57.7)	13 (16.7)		
Brain metastases	Yes	16	6 (37.5)	8 (50.0)	2 (12.5)	0.179	>0.05
	No	90	29 (32.2)	48 (53.3)	13 (14.4)		
Liver metastases	Yes	9	3 (33.3)	5 (55.6)	1 (11.1)	0.078	>0.05
	No	97	32 (33.0)	51 (52.6)	14 (14.4)		
Other lobes metastases	Yes	64	21 (32.8)	34 (53.1)	9 (14.1)	0.006	>0.05
	No	42	14 (33.3)	22 (52.4)	6 (14.3)		
Pleural effusion	Yes	38	16 (42.1)	16 (42.1)	6 (15.8)	2.883	>0.05
	No	68	19 (27.9)	40 (58.8)	9 (13.2)		
EGFR mutation status	Positive	58	23 (39.7)	31 (53.4)	4 (6.9)	6.481	<0.05
	Negative	48	12 (25.0)	25 (52.1)	11 (22.9)		

Note abbreviations: PR, partial response; SD, stable disease; PD, progressive disease.

of OS and PFS were compared using Kaplan-Meier survival analysis method. A log-rank test was used to test statistical significance of the differences. The relevant factors affecting OS and PFS analysis were detected using Cox multivariate regression analysis. P<0.05 was considered statistically significant.

Results

EGFR gene mutations and their relationship with clinical features

Among 106 patients in this group, 58 (54.7%) exhibited an *EGFR* mutation in their lung tumors. Of these, 27 had an exon 19 deletion mutation, and 31 carried an exon 21 point mutation. **Table 2** summarizes the *EGFR* mutation status of patients by different clinical features. A statistically significant difference in the *EGFR* mutation rates was detected only for histological types of tumors (χ^2=6.612, P<0.05); no other clinical characteristics were associated with *EGFR* mutation rates (χ^2=0.104~2.557, P>0.05). Further, there was no statistically significant difference in the clinical characteristics of patients with different types of *EGFR* mutation (χ^2=0.010~2.398, P>0.05; **Table 3**).

Short-term efficacy of gefitinib and its relationship with clinical features

The short-term efficacy of gefitinib was determined at first three-month by assessing changes in NSCLC disease, categorized as partial

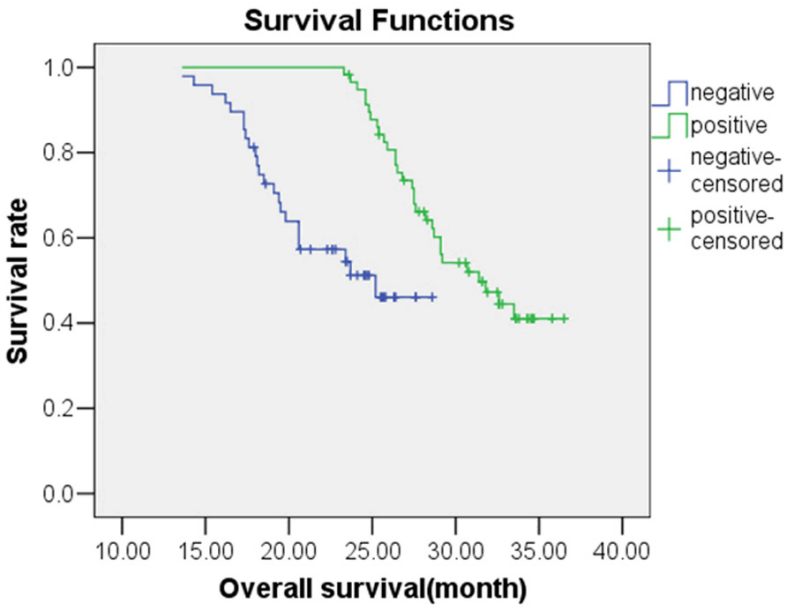

Figure 1. Kaplan-Meier survival analysis for overall survival for NSCLC patients who were either positive or negative for *EGFR* mutation.

Table 5. Cox multivariate analysis of overall survival (OS)

Variable		*HR*	*Wald* χ^2	P
Gender	Male	1.789	1.468	>0.05
Age	<60	1.709	2.868	>0.05
Smoking history	Never	0.635	0.472	>0.05
Pathology	Non-adenocarcinoma	4.877	9.638	<0.05
ECOG PS	≥2	3.087	8.655	<0.05
Chemotherapy cycles	≥2	1.692	2.237	>0.05
Bone metastases	Yes	0.915	0.038	>0.05
Brain metastases	Yes	0.733	0.412	>0.05
Liver metastases	Yes	1.736	0.422	>0.05
Other lobes metastases	Yes	1.336	0.375	>0.05
Pleural effusion	Yes	0.785	0.607	>0.05
EGFR mutation status	Negative	1.876	4.277	<0.05

response, stable disease, or progressive disease (**Table 4**). There were statistically significant differences in efficacy by gender, smoking history, bone metastases, and *EGFR* mutation (χ^2=6.481~35.938, P<0.05). No relationship was detected between other clinical features and short-term efficacy of gefitinib (χ^2= 0.006~2.883, P>0.05).

Effect of EGFR mutations on survival time

Following treatment with gefitinib, the OS predictive value of patients with *EGFR* mutations was 31.36 months [95% confidence interval (CI), 30.10-32.63 months], while the OS predictive value of patients without *EGFR* mutations was 23.57 months (95% CI, 22.05-25.09 months); this difference was statistically significant (χ^2=19.135, P< 0.05). The Kaplan-Meier survival analysis curve for OS by *EGFR* mutation status is depicted in **Figure 1**. A Cox multivariate regression analysis showed that patient OS was correlated with histological type of tumor (HR=4.877), ECOG PS score (HR=3.087), and *EGFR* gene mutations (HR =1.876) (P<0.05), as shown in **Table 5**. The PFS predictive value of patients with *EGFR* mutations was 17.34 months (95% CI, 16.27-18.41 months), while that of patients without *EGFR* mutations was 16.22 months (95% CI, 13.87-18.56 months); this difference was statistically significant (χ^2=6.953, P<0.05). The Kaplan-Meier survival analysis curve for PFS by *EGFR* mutation status is provided in **Figure 2**. The Cox multivariate analysis showed that PFS was correlated with ECOG PS score (HR=2.218), chemotherapy cycle (HR=1.829), and *EGFR* mutation (HR=1.840) (P<0.05), as shown in **Table 6**. The OS predictive value of patients with exon 19 deletion mutation was 33.45 months (95% CI, 31.80-35.10 months), while that of patients without exon 19 deletion mutation was significantly lower at 29.04 months (95% CI, 27.61-30.47 months) (χ^2=8.575, P<0.05). **Figure 3** provides the Kaplan-Meier survival analysis curve for OS by exon 19 status. The PFS predictive value of patients with exon 19 deletion mutation was 17.29 months (95% CI, 16.72-17.86 months), while that of patients with exon 21 point mutation was 16.75 months (95% CI, 15.09-18.40 months); this difference was not statistically significant (χ^2=0.016, P>0.05);

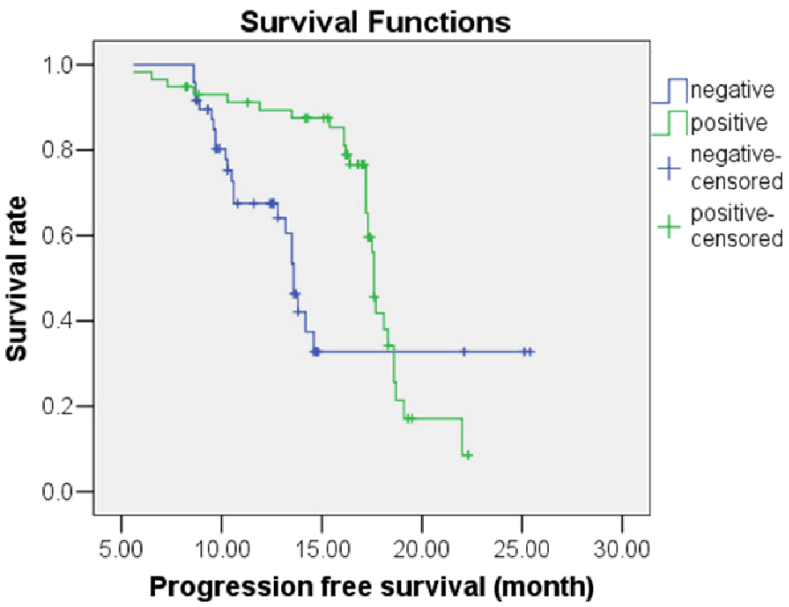

Figure 2. Kaplan-Meier survival analysis for progression-free survival of NSCLC patients who were positive or negative for *EGFR* mutation.

Table 6. Cox multivariate analysis of progression-free survival (PFS)

Variables		*HR*	*Wald* χ^2	P
Gender	Male	1.772	2.629	>0.05
Age	<60	1.031	0.018	>0.05
Smoking history	Never	0.783	0.455	>0.05
Pathology	Non-adenocarcinoma	1.538	1.278	>0.05
ECOG PS	≥2	2.128	5.828	<0.05
Chemotherapy cycles	≥2	1.829	4.125	<0.05
Bone metastases	Yes	0.833	0.202	>0.05
Brain metastases	Yes	0.527	1.658	>0.05
Liver metastases	Yes	1.836	0.645	>0.05
Other lobes metastases	Yes	1.041	0.021	>0.05
Pleural effusion	Yes	1.306	0.585	>0.05
EGFR mutation status	Negative	1.840	4.787	<0.05

Figure 4 provides the Kaplan-Meier survival analysis curve for PFS by mutation location.

Adverse reactions during treatment period

The main adverse reactions during gefitinib treatment were rash and diarrhea; all reactions were mild. No patient discontinued treatment due to adverse reactions. There was no statistically significant difference in overall incidence of adverse reactions between patients with and without *EGFR* mutations, or in incidence between various types of adverse reactions (χ^2=0.001~0.751, P>0.05), as shown in **Table 7**.

Discussion

The clinical efficacy of gefitinib in treating NSCLC has been widely recognized; however, some studies have confirmed that gefitinib is likely to develop drug resistance, adverse reactions, and secondary resistance [8]. Resistance of NSCLC to gefitinib and other targeted drugs is closely related to the occurrence of exon 20 point mutation.Hypermethylation of the *EGFR* promoter region can down-regulate the expression of *EGFR*, which decreases NSCLC sensitivity to gefitinib [15]. Secondary resistance to gefitinib is correlated with a secondary mutation of EGFR gene. Further, gefitinib resistance is correlated with drug transport, amplification of the *EGFR/Met* gene, and change of signaling pathways [16]. *EGFR* mutations affect PFS, OS, and survival time of NSCLC patients treated with gefitinib [17]. In addition, there is great difference in the sensitivity of different NSCLC cell lines to gefitinib. Some studies have shown that HCC827 cells with the exon 19 deletion mutation are most sensitive to gefitinib, while H1650 cells with exon 19 deletion mutation are not sensitive; the sensitivity of wild-type H358 cells to gefitinib is even higher compared to the H1650 cells with exon 19 deletion mutation, while the EGFR wild-type H1299 and A549 cells are not as sensitive to gefitinib as the wild-type H358 cells are [18]. The demonstrated success of gefitinib in subsets of patients with NSCLS, combined with mild adverse reactions like rash, pruritus, and diarrhea, gefitinib is consider safer and more effective than conventional chemotherapeutic agents in treating NSCLC.

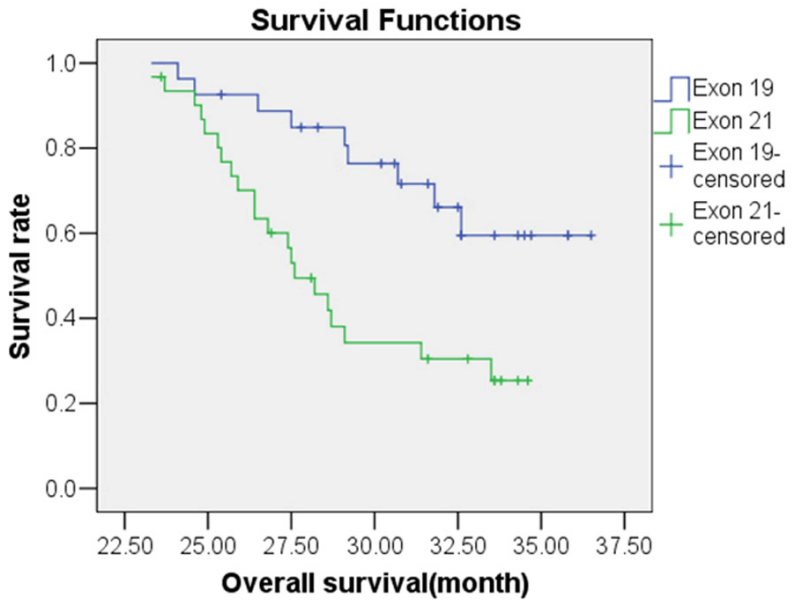

Figure 3. Kaplan-Meier survival analysis of overall survival of NSCLC patients with Exon 19 or Exon 21 *EGFR* mutation.

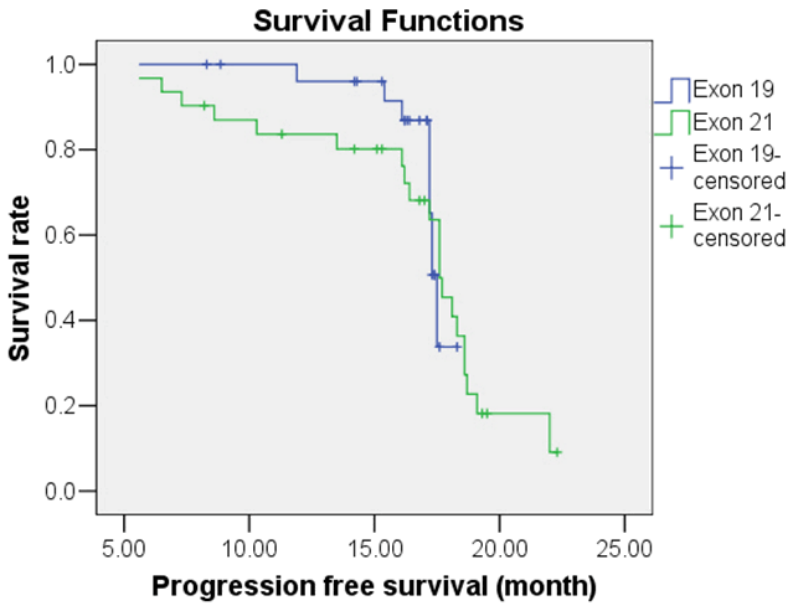

Figure 4. Kaplan-Meier survival analysis of progression-free survival for NSCLC patients with Exon 19 or Exon 21 mutation.

The results of this study confirmed that a subset of NSCLC cases is *EGFR* mutation-positive for exons 19 and 21. *EGFR* mutations were correlated with the histological type of tumor. Short-term efficacy differed by gender, smoking history, bone metastases, and *EGFR* mutation status ($P<0.05$), suggesting that certain subgroups may have better short-term outcomes following gefitinib treatment. Patients with *EGFR* mutations in exons 19 or 21, in general, had better survival outcomes following gefitinib treatment than patients without those mutations, and patients with exon 19 mutations had better outcomes than patients with exon 21 mutations. These findings support previous indications that gefitinib can significantly prolong survival time of patients with *EGFR* mutations, particularly the exon 19 deletion mutation. OS also appeared related to histological type of tumor and ECOG PS score, while PFS was correlated with ECOG PS score and chemotherapy cycle. Thus, in addition to *EGFR* mutations, the effect of gefitinib on NSCLC patient survival depends on tumor type, treatment cycle, physical condition of the patient, and other factors. The findings of mild adverse reactions during treatment of patients in this study confirm that gefitinib is safe as a treatment for advanced NSCLC, and *EGFR* mutations had no significant effect on the incidence of adverse reactions.

In summary, *EGFR* mutation rate in NSCLC appears to be correlated with the histological type of tumors. The efficacy of gefitinib is enhanced for *EGFR* mutation-positive NSCLC patients, prolonging their survival time with mild adverse reactions and high safety.

Disclosure of conflict of interest

None.

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Table 7. Rate of adverse events

Adverse events	*EGFR mutation status*		χ^2	P
	Negative (n=48)	Negative (n=58)		
Rash	23 (47.9)	28 (48.3)	0.001	>0.05
Diarrhea	9 (18.8)	11 (19.0)	0.001	>0.05
Loss of appetite	3 (6.3)	2 (3.4)	0.459	>0.05
Elevated transaminase	3 (6.3)	2 (3.4)	0.459	>0.05
Nausea/vomiting	1 (2.1)	2 (3.4)	0.178	>0.05
Total number	31 (64.6)	42 (72.4)	0.751	>0.05

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