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ORIGINAL RESEARCH ARTICLE

Clinical Management of Non-Small Cell Lung Cancer with Concomitant *EGFR* Mutations and *ALK* Rearrangements: Efficacy of EGFR Tyrosine Kinase Inhibitors and Crizotinib

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Abstract

Background Patients harboring concomitant *epidermal growth factor receptor* (*EGFR*) mutations and *anaplastic lymphoma kinase* (*ALK*) arrangements constitute a small subgroup of non-small-cell lung cancer (NSCLC) patients. The efficacy of EGFR tyrosine kinase inhibitors (TKIs) and the ALK-specific TKI crizotinib in these patients has not been well-established.

Objective This study investigated the efficacy of targeted therapies in these patients compared with patients with *EGFR* or *ALK* alterations alone.

Methods Patients were screened for *EGFR* mutation and *ALK* rearrangement at the Shanghai Chest Hospital (2011–2017). Progression-free survival (PFS), objective response rate (ORR), and overall survival (OS) were retrospectively analyzed.

Results A total of 5816 patients were screened, and 26 patients were identified as having concomitant *EGFR* mutations and *ALK* rearrangements; 22 patients were eligible for survival analysis. Additionally, 95 *EGFR*-mutant patients and 60 *ALK*-rearranged patients were randomly selected for analysis. The ORR to EGFR TKIs was 63.2% (12/19) for *EGFR/ALK* co-altered patients and 62.1% (59/95) for *EGFR*-mutant patients ($p = 0.93$) with a median PFS of 10.3 and 11.4 months, respectively (hazard ratio [HR] 0.96; 95% confidence interval [CI] 0.59–1.57; $p = 0.87$). The ORR to crizotinib was 66.7% (8/12) for double-positive patients and 65.0% (39/60) for *ALK*-rearranged patients ($p = 1.00$), with a median PFS of 11.1 and 12.5 months, respectively (HR 1.39; 95% CI 0.69–2.80; $p = 0.28$). OS was 27.1, 36.2, and 36.8 months for *EGFR*-mutant, *ALK*-rearranged, and *EGFR/ALK* co-altered patients, respectively, and the *EGFR/ALK* co-existing subgroup tended to have a longer survival period than *EGFR*-mutant cohorts, though no statistical difference was found ($p = 0.12$). The median PFS of crizotinib as a sequential therapy after failure of EGFR TKIs was 15.0 months, which exhibited no statistically significant difference compared with the median PFS of *ALK*-altered patients who received crizotinib ($p = 0.80$).

Conclusions Both first-generation EGFR TKIs and the ALK TKI crizotinib were effective in these patients. Sequential treatment with EGFR TKIs and crizotinib should be considered as a management option.

1 Introduction

Lung cancer is the most common malignancy and the leading cause of cancer-related death both in China and worldwide [1–3]. Treatment of non-small-cell lung cancer (NSCLC) has changed dramatically in recent years with the advent of targeted therapies for different oncogenic

Key Points

Previous experience with *epidermal growth factor receptor* (*EGFR*)/*anaplastic lymphoma kinase* (*ALK*) double-positive non-small-cell lung cancer (NSCLC) patients has been limited and inconsistent.

In our analysis, the efficacy of both first-generation EGFR tyrosine kinase inhibitors (TKIs) and the ALK TKI crizotinib was equal in double-positive NSCLC patients and in those with *EGFR* mutations or *ALK* rearrangements alone.

Sequential treatment with EGFR TKIs and crizotinib should be considered as a management option.

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drivers; hence, molecular analysis of NSCLC is routinely performed to screen for several major therapeutic targets, including activating mutations of the *epidermal growth factor receptor* (*EGFR*) and rearrangement involving the *anaplastic lymphoma kinase* (*ALK*) [4]. These two drivers have conventionally been considered to be mutually exclusive [5]. However, cumulative reports have revealed that concomitant occurrence of *EGFR* mutations and *ALK* rearrangement accounts for a small number of NSCLC cases, raising the issue of therapeutic strategies [6–16].

First-generation *EGFR* tyrosine kinase inhibitors (TKIs), including gefitinib, erlotinib, and icotinib, are effective in patients with *EGFR* mutations [17–19]. Crizotinib, a small-molecular TKI, also shows efficacy superior to that of conventional chemotherapy in *ALK*-positive patients [20]. Nevertheless, the efficacy of *EGFR* TKIs and crizotinib in *EGFR/ALK* double-positive patients has been controversial. Some reports have indicated that *EGFR* TKIs had a better response than *ALK* inhibitors in terms of objective response rate (ORR) and progression-free survival (PFS) [7, 9, 10], but others have come to the opposite conclusion [11, 14, 15, 21]. Moreover, the effectiveness of combination or sequential therapy remains to be elucidated because of the scant number of patients reporting with concomitant *EGFR* mutations and *ALK* rearrangements and receiving both TKIs. We performed a retrospective analysis of 22 patients harboring concomitant *EGFR* mutations and *ALK* rearrangements to analyze the therapeutic efficacy of first-generation *EGFR* TKIs and crizotinib in these patients compared with those with a single oncogenic driver and to highlight the effectiveness of sequential treatment with *EGFR* TKIs and crizotinib.

2 Patients and Methods

2.1 Patients

This study was a data-driven retrospective single institution clinical audit without a prospective protocol. We identified patients with NSCLC who underwent molecular analysis at the Shanghai Chest Hospital between January 2011 and March 2017. The aim of this study was to evaluate patients with advanced (stages IIIB–IV) disease with either *EGFR* mutations or *ALK* rearrangements and co-occurring *EGFR/ALK* aberrations who received first-generation *EGFR* TKIs, crizotinib, or both. Patients were excluded if they were diagnosed with early-stage IA–IIIA NSCLC, did not receive TKIs, or received next-generation TKIs first. Patients with *ALK* rearrangements that were weakly positive on immunohistochemistry (IHC) and not confirmed by fluorescent in-situ hybridization (FISH) were also excluded.

The primary outcome of this study was comparison of PFS in patients with *EGFR* mutations, *ALK* rearrangements, and *EGFR/ALK* co-alteration. Secondary outcomes included overall survival (OS) and ORR in these patients.

Positron emission tomography/computed tomography (PET/CT) or other radiological evaluation methods were used to confirm or exclude metastatic disease. Staging was performed according to the 7th edition of the tumor node metastasis (TNM) classification for NSCLC. The specimens used for molecular analyses were obtained from surgery, fine-needle small biopsies guided by CT or ultrasound, transbronchial biopsies, or malignant effusion cell blocks.

The study was approved by the institutional review board of the Shanghai Chest Hospital, and all patients signed written consent forms before invasive procedures and TKI treatments.

2.2 Molecular Analyses

EGFR mutations were evaluated with the direct sequence and amplification refractory mutation system (ARMS) according to the manufacturer's protocol. DNA was extracted from various specimens, amplified with polymerase chain reaction (PCR), and analyzed with standard sequencing. Details of the two methods are previously described [22, 23].

ALK rearrangements were identified by IHC or FISH. Samples were sent as formalin-fixed and paraffin-embedded (FFPE) biopsies, and FISH tests were performed on these tissues using a Vysis *ALK* break-apart FISH probe kit (Abbott Molecular, Des Plaines, IL, USA). Patients were diagnosed as *ALK* FISH positive when $\geq 15\%$ of the tumor cells showed split and/or isolated 3′ signals. *ALK* IHC was conducted with the anti-*ALK* (D5F3 Ventana) primary antibody combined with OptiView DAB IHC detection and OptiView amplification (Ventana Medical Systems, Inc., Tucson, AZ, USA). Patients were diagnosed as *ALK* IHC positive if strong granular cytoplasmic brown staining was present in tumor cells [24, 25].

2.3 Treatment and Follow-Up

Patients in three cohorts were treated with *EGFR* TKIs (gefitinib [Iressa, AstraZeneca Pharmaceuticals], erlotinib [Tarceva, Roche], or icotinib [Conmana, Betta]), crizotinib, or both. Clinical evaluation was performed every 4–6 weeks according to RECIST 1.1 (Response Evaluation Criteria in Solid Tumors) [26]. PFS was defined from the initiation of TKIs to radiographic or clinical progression or the last follow-up time. OS was calculated from pathological diagnosis of stage IIIB/IV NSCLC to death or last follow-up time. The median follow-up time was 33 months (range 4–100), and the last follow-up time was 23 January 2019.

2.4 Statistical Analyses

We eliminated the large difference in the numbers of patients among the *EGFR*-mutant or *ALK*-rearranged cohort, and the *ALK*/*EGFR* co-altered cohort. *EGFR*-mutant or *ALK*-rearranged patients included for survival analysis were randomly selected from those with a single oncogenic driver who received first-generation *EGFR* TKIs or crizotinib and had complete medical records. The ratio of selected patients to double-positive patients who received *EGFR* TKIs or crizotinib was 1:5. The randomization process using Excel 2013 (Microsoft, Redmond, WA, USA) was as follows: (1) every patient was given a random number using the RANDBETWEEN function, ranging from 0 to 1; (2) patients were ranked according to the assigned number; and (3) the top patients needed for analysis were selected. To test the representativeness of the selected patients, their baseline characteristics were compared with those of the whole population, including patients with missing data.

To compare baseline characteristics, we used the chi-squared test for categorical variables, Fisher's exact test for small samples, and the Wilcoxon rank sum test for continuous variables. Survival curves were generated to compare PFS and OS using Kaplan–Meier methods and further compared using the log-rank test. A p value <0.05 was considered statistically significant. All analyses were performed using the Statistical Package for Social Science (IBM; Armonk, NY, USA) version 22.0 for Windows. Figures were created using GraphPad Prism 7 (San Diego, CA, USA).

3 Results

3.1 Patient Characteristics

Between January 2011 and March 2017, a total of 5816 patients underwent both *EGFR*-mutation and *ALK*-rearrangement analysis. In total, 2392 patients had *EGFR* mutations, 503 had *ALK* alterations, and 26 had concomitant *EGFR* mutations and *ALK* alterations. The frequency of concomitant *EGFR* mutations and *ALK* rearrangement was about 0.45% (26/5816) of the entire NSCLC patient cohort, 1.1% (26/2418) of *EGFR*-mutation patients, and 4.91% (26/529) of *ALK*-positive patients.

Excluding patients diagnosed with stage IA–IIIA, who had not received TKIs, or who had received next-generation TKIs first left 1328, 259, and 23 patients with advanced (stage IIIB–IV) NSCLC in the *EGFR*-mutant, *ALK*-rearranged, and *EGFR/ALK* co-altered cohorts, respectively. Table 1 presents the demographic profiles and clinico-pathological characteristics of the three groups. The median age at diagnosis of stage IIIB/IV NSCLC for *EGFR/ALK* co-altered, *EGFR*-mutant, and *ALK*-rearranged groups was 61, 60, and 56 years, respectively. Patients from the *ALK*-rearranged cohort were younger than those from the *EGFR*-mutant or *EGFR/ALK* cohorts ($p<0.01$). The specimens for diagnosis and molecular analysis were obtained in various ways ($p<0.01$). There was no difference between the three groups with respect to sex, smoking status, pathologic type, performance status, or stage at initiation of TKIs. The *EGFR*-mutation types also showed no difference between the *EGFR/ALK* co-altered and *EGFR*-mutant groups.

3.2 Response and Survival Analyses

In the *EGFR*-mutant, *ALK*-rearranged, and *EGFR/ALK* co-altered cohorts, respectively, 367, 56, and 1 patient with stage IIIB/IV NSCLC and missing data received TKIs. Using the abovementioned procedure, 95 patients with *EGFR* mutations and 60 with *ALK* rearrangements were randomly selected from those with complete medical records. The patient selection process is outlined in Fig. 1.

Finally, 95, 60, and 22 patients from the *EGFR*-mutant, *ALK*-rearranged, and *EGFR/ALK* co-altered groups were included for survival analysis. Among the *EGFR/ALK* co-altered cohort, ten patients received *EGFR* TKIs (gefitinib, erlotinib, icotinib) alone, three patients received crizotinib alone, and nine patients received both *EGFR* TKIs and crizotinib. Therefore, a total of 19 patients previously received *EGFR* TKIs, and 12 patients were treated with crizotinib. All patients in the *EGFR/ALK* co-altered cohort experienced disease progression as assessed by RECIST while receiving *EGFR* TKIs and crizotinib.

The baseline characteristics of the entire *EGFR*-mutant cohort (all patients with *EGFR*-mutant stage IIIB/IV NSCLC treated with *EGFR* TKIs), selected *EGFR*-mutant patients, and *EGFR/ALK* co-altered patients who received *EGFR* TKIs were well-balanced (Table 2). The clinicopathological characteristics of the entire *ALK*-rearranged cohort (all stage IIIB/IV NSCLC *ALK* patients treated with crizotinib), selected *ALK*-rearranged patients, and *EGFR/ALK* co-altered patients who received crizotinib were also well-balanced (Table 3). As a result, the randomly selected patients were considered to satisfactorily represent the entire *EGFR*-mutant and *ALK*-rearranged cohorts.

The ORR for patients treated with *EGFR* TKIs was 62.1% (59/95) for *EGFR*-mutant and 63.2% (12/19) for double-positive patients. The difference was not statistically significant ($p=0.93$). The ORR for patients treated with crizotinib was 65% (39/60) for *ALK*-rearranged and 66.7% (8/12) for *EGFR/ALK* double-positive patients. The difference was not statistically significant ($p=1.00$).

PFS was defined from the initiation of TKIs to radiographic or clinical progression or the last follow-up time. The median PFS of *EGFR/ALK* co-altered patients treated

Table 1 Baseline demographic profiles and clinico-pathological characteristics of patients with *EGFR* (+), *ALK* (+), and *EGFR/ALK* non-small-cell lung cancer

Characteristics	Total ($n=1610$)	*EGFR* (+) ($n=1328$)	*ALK* (+) ($n=259$)	*EGFR/ALK* ($n=23$)	p value
Median age (range)	58 (24–84)	60 (25–84)	56 (24–82)	61 (30–75)	< 0.01
Sex					0.83
Male	681 (42)	557 (42)	114 (44)	10 (43)	
Female	929 (58)	771 (58)	145 (56)	13 (57)	
Smoking					0.46
Non-smoker	1185 (74)	969 (73)	198 (76)	18 (78)	
Smoker	425 (26)	359 (27)	61 (24)	5 (22)	
Pathology					0.50
Adenocarcinoma	1468 (91)	1206 (91)	241 (93)	21 (91)	
Non-adenocarcinoma	142 (9)	122 (9)	18 (7)	2 (9)	
PS					0.32
0–1	1493 (93)	1235 (93)	238 (92)	20 (87)	
2–3	117 (7)	93 (7)	21 (8)	3 (13)	
Stage					0.58
IIIB	132 (8)	113 (9)	17 (7)	2 (9)	
IV	1478 (92)	1215 (91)	242 (93)	21 (91)	
EGFR mutation type					1.0
19del		651 (49)	–	12 (52)	
21L858R		571 (43)	–	10 (43)	
Others		106 (8)	–	1 (5)	
Test for ALK alteration					0.78
IHC		–	211 (81)	20 (87)	
FISH		–	48 (19)	3 (13)	
Specimen					< 0.01
Small biopsy	1045 (65)	822 (62)	207 (80)	16 (70)	
Operation	449 (28)	412 (31)	31 (12)	6 (26)	
Cell block	116 (7)	94 (7)	21 (8)	1 (4)	

Data are presented as *n* (%) unless otherwise indicated

ALK anaplastic lymphoma kinase, *EGFR* epidermal growth factor receptor, *FISH* fluorescent in-situ hybridization, *IHC* immunohistochemistry, *PS* performance status

with *EGFR* TKIs was 10.3 months, which was not statistically different from that of *EGFR*-mutant patients (11.4 months; hazard ratio [HR] 0.96; 95% confidence interval [CI] 0.59–1.57; $p=0.87$) (Fig. 2a). Additionally, the median PFS of crizotinib was 11.1 months and 12.5 months for *EGFR/ALK* double-positive and *ALK*-rearranged patients, respectively. No statistically significant difference was found between these two groups (HR 1.39; 95% CI 0.69–2.80; $p=0.28$) (Fig. 2b).

OS was calculated from pathological diagnosis of stage IIIB/IV NSCLC to death or last follow-up date, with 68% (15/22), 72% (68/95), and 63.3% (38/60) of patients known to have died at the data cut-off date in the *EGFR/ALK* co-alteration, *EGFR*-mutation, and *ALK*-rearrangement groups, respectively. Median OS was 36.8, 27.1, and 36.2 months ($p=0.034$) (Fig. 3). Although no statistically significant difference was found between the *EGFR/ALK* co-existing group and the selected *EGFR*-mutant group (HR 0.66; 95% CI 0.40–1.08), there was a trend toward increased long-term survival for double-positive patients.

Among a group of patients who were treated with both *EGFR* TKIs and crizotinib, only one patient received crizotinib before first-generation *EGFR* TKIs, and eight patients received crizotinib after failure of *EGFR* TKIs. In this subgroup, the ORR was 55.6% (5/9) and 66.7% (6/9), respectively, for *EGFR* TKIs and crizotinib. The median PFS of crizotinib as a sequential therapy after failure in *EGFR* TKIs was 15.0 months, which exhibited no statistically significant difference from that of *ALK*-altered patients who received crizotinib (12.5 months; HR 1.10; 95% CI 0.51–2.37; $p=0.80$). Four patients received crizotinib as first-line therapy (three only received crizotinib; one received both crizotinib and an EGFR TKI), and the ORR for crizotinib in this subgroup was 75.0% (3/4).

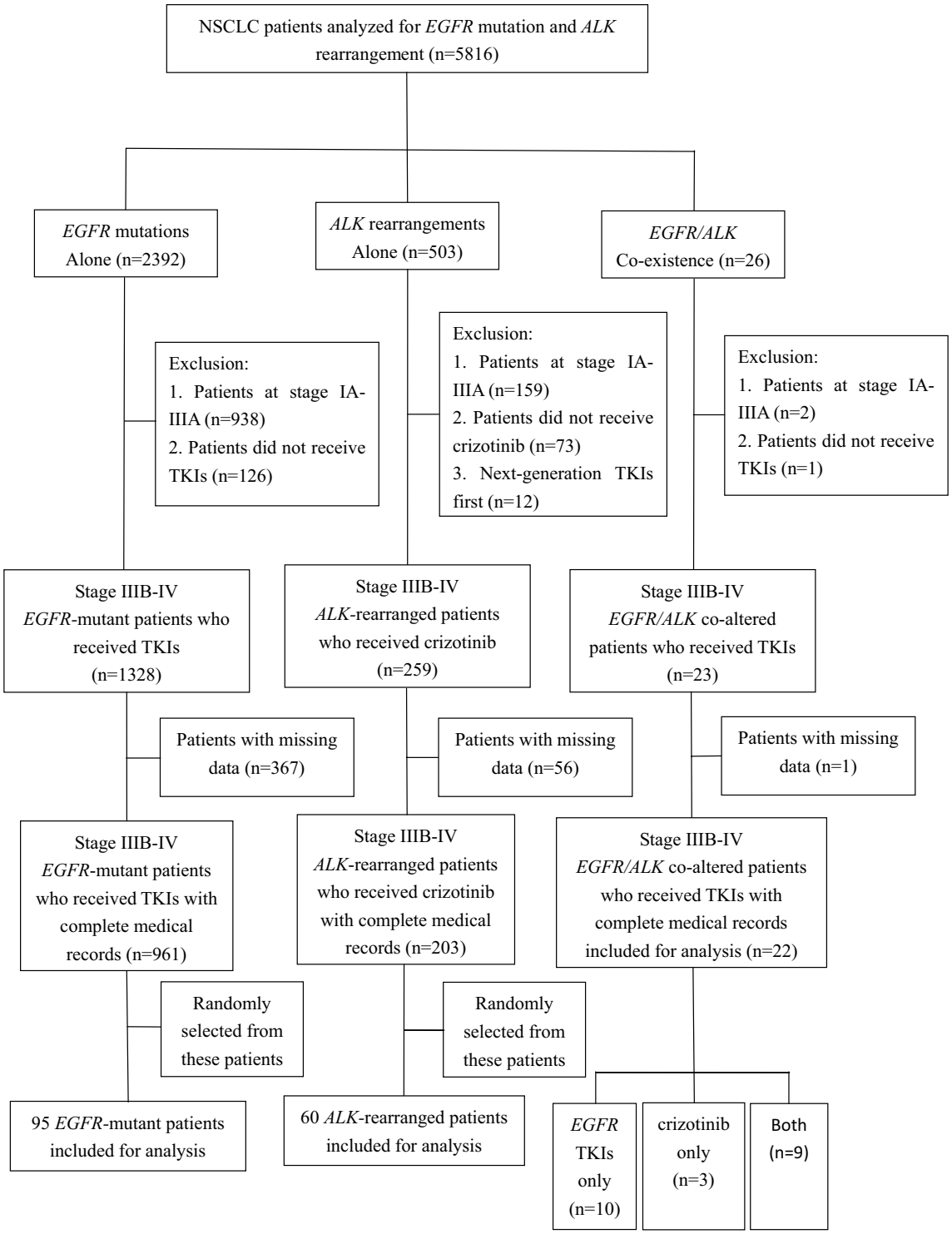

Fig. 1 Flow diagram outlining the patient selection process. *ALK* anaplastic lymphoma kinase, *EGFR* epidermal growth factor receptor, *NSCLC* non-small-cell lung cancer, *TKI* tyrosine kinase inhibitor

4 Discussion

Our study suggests that the coexistence of *EGFR* mutations and *ALK* rearrangement accounts for about 0.45% (95% CI 0.28–0.62) of NSCLC, 1.1% (26/2418) of *EGFR*-mutant disease, and 4.91% (26/529) of *ALK*-positive disease. First-generation *EGFR* TKIs and crizotinib in patients with concomitant *EGFR* mutations and *ALK* rearrangement were

Table 2 Baseline characteristics of the entire *EGFR*-mutant cohort, selected *EGFR*-mutant patients, and *EGFR/ALK* co-altered patients who received *EGFR* TKIs

Characteristics	Entire *EGFR* (+) ($n=1328$)	Selected *EGFR* (+) ($n=95$)	*EGRF/ALK* (+) ($n=19$)	p value
Median age (range)	60 (25–84)	62 (27–82)	64 (30–75)	0.74
Sex				0.66
Male	557 (42)	44 (46)	9 (47)	
Female	771 (58)	51 (54)	10 (53)	
Smoking				0.54
Non-smoker	969 (73)	74 (78)	15 (79)	
Smoker	359 (27)	21 (22)	4 (21)	
Pathology				0.64
Adenocarcinoma	1206 (91)	84 (88)	17 (89)	
Non-adenocarcinoma	122 (9)	11 (12)	2 (11)	
PS				0.43
0–1	1235 (93)	86 (91)	17 (89)	
2–3	93 (7)	9 (9)	2 (11)	
EGFR mutation type				0.99
19del	651 (49)	45 (47)	10 (53)	
21L858R	571 (43)	43 (45)	8 (42)	
Others	106 (8)	7 (8)	1 (5)	
First-generation TKIs				0.70
Gefitinib	596 (45)	48 (51)	9 (48)	
Erlotinib	372 (28)	21 (22)	4 (21)	
Icotinib	360 (27)	26 (27)	6 (31)	
Stage				0.64
IIIB	113 (9)	10 (10)	2 (11)	
IV	1215 (91)	85 (90)	17 (89)	

Data are presented as *n* (%) unless otherwise indicated

ALK anaplastic lymphoma kinase, *EGFR* epidermal growth factor receptor, *FISH* fluorescent in-situ hybridization, *IHC* immunohistochemistry, *PS* performance status, *TKI* tyrosine kinase inhibitor

equally efficacious as in patients with single-gene alterations. *EGFR/ALK* co-altered patients also appeared to have longer OS than patients with *EGFR*-mutant disease.

Remarkable progress has been made in the treatment of NSCLC due to the discovery of several critical activating pathways. Among these pathways, *EGFR*-activating mutations and *ALK* rearrangements are routinely screened for and clinically relevant [27]. Although these two driver alterations were previously considered mutually exclusive [5], recent reports have shown that *EGFR/ALK* double-positive patients account for a small proportion of NSCLC [6–16]; however, previous data has been inconsistent with regard to the frequency of double-positive patients in NSCLC, ranging from 0.1 to 1.6% [9, 15, 21, 28]. The prevalence of double-positive patients in our study is within this range when using ARMS and IHC/FISH techniques to detect *EGFR* mutations and *ALK* rearrangement. Won et al. [15] reported the frequency of *EGFR* and *ALK* coexistence in *ALK*-positive patients to be 4.4% when using Sanger sequencing, but this increased to 15% with high-sensitivity next-generation sequencing (NGS). Therefore, as more advanced techniques emerge, the proportion of double-positive patients could be much higher than previously expected, which will have implications for the treatment of these patients.

First-generation *EGFR* TKIs such as gefitinib, erlotinib, and icotinib provide survival benefits over conventional chemotherapy and have revolutionized the therapy of patients with NSCLC with *EGFR*-activating mutations [17–19], as has crizotinib, a TKI targeting *ALK* in activating rearrangements, for ALK-positive patients [20]. These TKIs play irreplaceable roles in managing NSCLC with a single oncogenic driver, but there is no consensus on their effects in double-positive patients because of the limited number of reported cases. Lou et al. showed that the median PFS was 11.2 months for *EGFR/ALK* co-altered patients treated with *EGFR* TKIs and 13.2 months for *EGFR*-mutant patients. A less favorable result for crizotinib was found in double-positive patients, with a median PFS of 1.9 months compared with 6.9 months in *ALK*-rearranged patients, although no statistical significance

Table 3 Baseline characteristics of the entire *ALK*-rearranged cohort, selected *ALK*-rearranged patients, and *EGFR/ALK* co-altered patients who received crizotinib

Characteristics	Entire *ALK* (+) ($n=259$)	Selected *ALK* (+) ($n=60$)	*EGRF/ALK* (+) ($n=12$)	p value
Median age (range)	56 (24–82)	59 (27–77)	56 (35–75)	0.12
Sex				1.0
Male	114 (44)	28 (47)	6 (50)	
Female	145 (56)	32 (53)	6 (50)	
Smoking				0.33
Non-smoker	198 (76)	51 (85)	10 (83)	
Smoker	61 (24)	9 (15)	2 (17)	
Pathology				0.82
Adenocarcinoma	241 (93)	55 (92)	12 (100)	
Non-adenocarcinoma	18 (7)	5 (8)	0 (0)	
PS				0.61
0–1	238 (92)	53 (88)	11 (92)	
2–3	21 (8)	7 (12)	1 (8)	
Test for ALK alteration				0.16
IHC	211 (81)	54 (90)	9 (75)	
FISH	48 (19)	6 (10)	3 (25)	
Stage				0.80
IIIB	17 (7)	3 (5)	1 (8)	
IV	242 (93)	57 (95)	11 (92)	

Data are presented as *n* (%) unless otherwise indicated

ALK anaplastic lymphoma kinase, *EGFR* epidermal growth factor receptor, *FISH* fluorescent in-situ hybridization, *IHC* immunohistochemistry, *PS* performance status

was demonstrated because of the small number of patients included in this analysis [9]. Sweis et al. [12] illustrated a poor response to both *EGFR* and *ALK* TKIs with a disease control rate (DCR) of 33.3% (1/3) and 25% (1/4), respectively, among four patients with coexisting *EGFR* mutations and *ALK* rearrangement. However, some reports showed encouraging responses when *EGFR* and *ALK* TKIs were given to these patients. Baldi et al. [6] reported a double-positive long-term survivor achieving 3 years of stable disease using erlotinib and a partial response to crizotinib afterwards. Lo Russo et al. [8] conducted a literature review of 100 cases, among which 51 cases received *EGFR* TKIs and 37 received *ALK* TKIs. Disease control or disease response were reported as best response in 69.8% and 43.4% compared with 79.5% and 51.3% of reviewed cases treated with *EGFR* and *ALK* TKIs, respectively. It is hard to draw firm conclusions from these results, partly because most of these patients were presented in single case reports, and no large-scale study was conducted. In our study, the ORR for *EGFR* TKIs and crizotinib for dual-positive patients was 63.2% (12/19) and 66.7% (8/12), and the median PFS was 10.3 months and 11.1 months, respectively, which showed no statistically significant difference from patients with single gene drivers. Thus, we may conclude that both *EGFR* and *ALK* TKIs were effective in the treatment of *EGFR/ALK* double-positive patients.

Although we found no difference between *ALK*-rearranged and *EGFR/ALK* co-altered patients with respect to OS, there was a tendency towards longer OS for double-positive patients compared with those with *EGFR* mutations alone. This was probably related to their effective response to both EGFR and ALK TKIs. In contrast to our results, Lou et al. reported that double-positive patients had relatively shorter OS than those with either an *EGFR* mutation or an *ALK* rearrangement [9], possibly because of the smaller sample size.

Among the 22 patients included for analysis in our study, nine patients received both *EGFR* TKIs and crizotinib. *EGFR* TKIs were administered before crizotinib in the majority of cases (eight patients). In this subgroup, we found that the ORRs were 55.6% (5/9) and 66.7% (6/9) for *EGFR* TKIs and crizotinib, respectively. The median PFS for crizotinib as sequential therapy after failure in *EGFR* TKIs was 15.0 months, which exhibited no statistical significance compared with that of patients with an *ALK* rearrangement who only received crizotinib ($p=0.80$). This all suggests that sequential treatment with *EGFR* and *ALK* TKIs is effective in treating double-positive patients. Furthermore, *EGFR* TKIs did not influence the efficacy of crizotinib as a

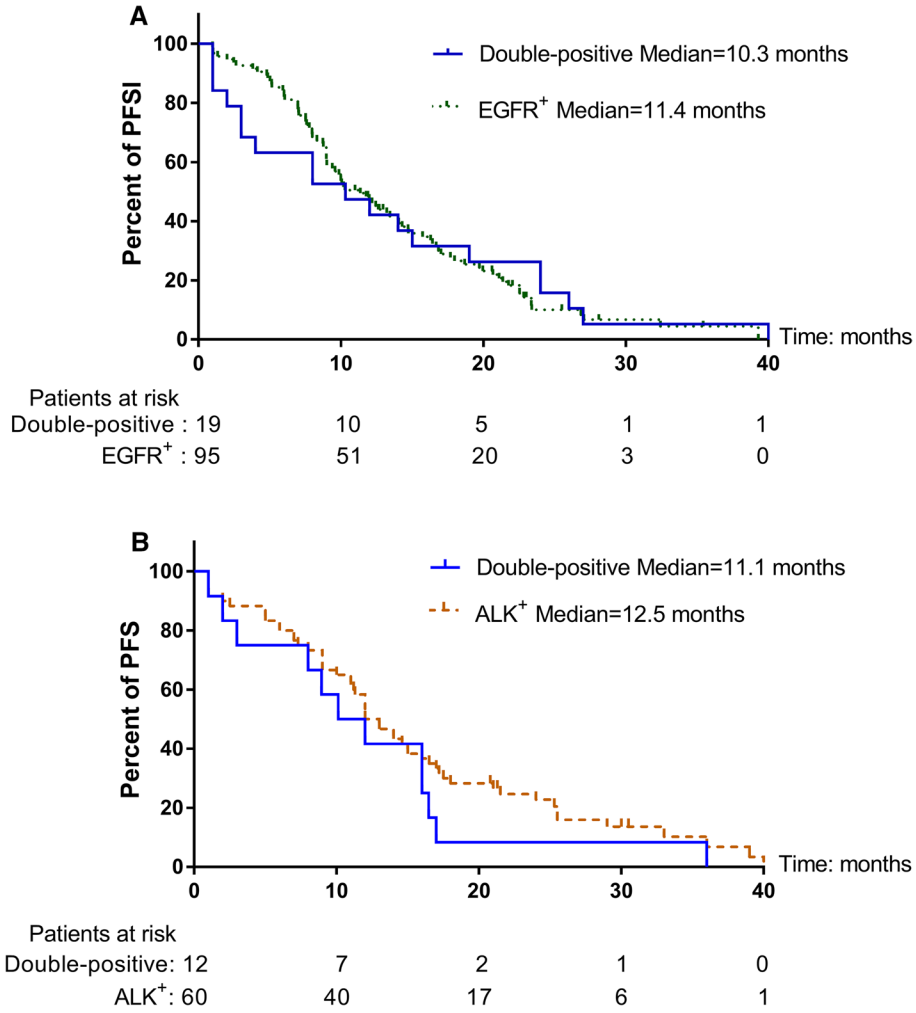

Fig. 2 **a** Kaplan–Meier curves for progression-free survival of selected *EGFR*-mutant patients and *EGFR/ALK* double-positive patients treated with *EGFR* TKIs. **b** Kaplan–Meier curves for progression-free survival of selected *ALK*-rearranged patients and *EGFR/ALK* double-positive patients treated with crizotinib. *ALK* anaplastic lymphoma kinase, *EGFR* epidermal growth factor receptor

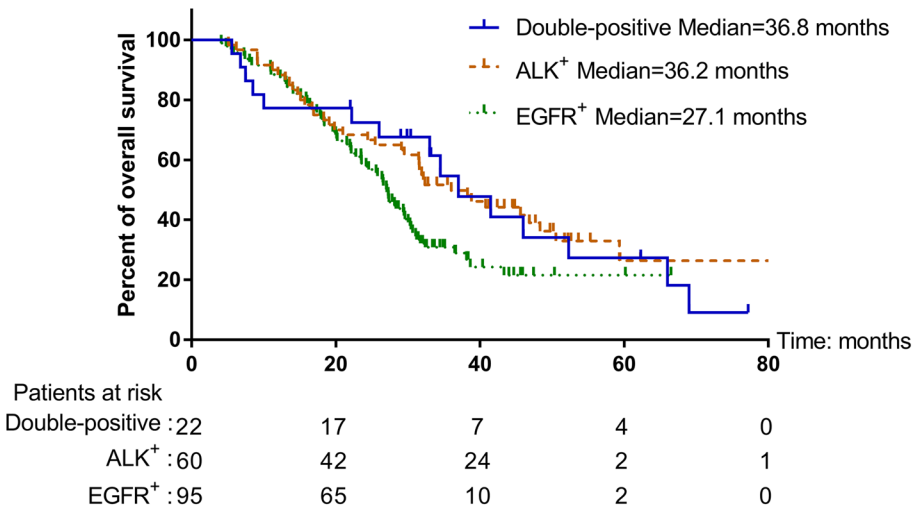

Fig. 3 Kaplan–Meier curves for overall survival of selected *EGFR*-mutant, selected *ALK*-rearranged, and *EGFR/ALK* double-positive patients. *ALK* anaplastic lymphoma kinase, *EGFR* epidermal growth factor receptor

subsequent therapy after progression on *EGFR* TKIs. Four patients received crizotinib as first-line treatment, with an ORR of 75% (3/4). Given the limited number of patients who received crizotinib before *EGFR* TKIs in our study, it was infeasible to determine the optimal sequence of administration.

Finding biomarkers to predict the efficacy of *EGFR* and *ALK* TKIs is another challenge. The burden of *EGFR*

mutations in the double-positive patients may be a predictor of response to *EGFR* TKIs. Meanwhile, Yang et al. [16] proposed that relative phospho-*ALK* and phospho-*EGFR* levels, as well as the level of phosphorylation of downstream proteins in the signaling pathway, could predict the efficacy of *EGFR* TKIs and crizotinib.

Different theories exist concerning the coexistence of *EGFR* mutations and *ALK* rearrangement in NSCLC. Cai et al. [29] demonstrated that the two gene alterations were not from the same tumor cell and that coexistence was due to intratumor heterogeneity. However, Yang et al. [16] found mutant *EGFR* proteins co-expressed with an *ALK* fusion protein in the same cell population using IHC, and Sasaki et al. [30] showed that co-activation of *EGFR* signaling occurred in an *ALK*-rearranged cell line.

As mentioned, *EGFR/ALK* co-altered patients can benefit from sequential treatment with both TKIs and have long-term survival, so a more effective way to find these co-altered patients is needed. NGS is now widely available, and more *EGFR/ALK* patients can be identified using highly sensitive NGS [15]. Therefore, we recommend that NGS would be better performed in all patients with advanced NSCLC to find all targetable gene alterations; additionally, in areas with limited access to NGS, patients should also be analyzed for both *EGFR* mutations and *ALK* rearrangements using conventional methods.

This was a retrospective single-center study, which cannot accurately reflect the entire double-positive patient population. Although the study comprised the highest number of patients with concomitant *EGFR* mutations and *ALK* rearrangements in one institution, to our knowledge, the sample size was still relatively small. Additionally, chemotherapy and radiation therapy could have been used in different lines of treatment, which might interfere with the result.

5 Conclusions

EGFR/*ALK* double-positive patients accounted for 0.45% (26/5816) of all patients with NSCLC, 1.1% (26/2418) of *EGFR*-mutant patients, and 4.91% (26/529) of *ALK*-positive patients. Both first-generation *EGFR* TKIs, including gefitinib, erlotinib, and icotinib, as well as crizotinib, were effective in these patients, which showed no statistically significant difference from patients with a single gene driver in terms of PFS and ORR. The sequential treatment with *EGFR* TKIs and crizotinib appears promising, and *EGFR* TKIs did not influence the efficacy of crizotinib as a subsequent treatment in these patients.

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Compliance with Ethical Standards

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Conflict of interest Yiming Zhao, Shuyuan Wang, Bo Zhang, Rong Qiao, Jianlin Xu, Lele Zhang, Yanwei Zhang and Baohui Han have no conflicts of interest that might be relevant to the contents of this manuscript.

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