Original Article

Superior clinical outcomes in patients with non-small cell lung cancer harboring multiple *ALK* fusions treated with tyrosine kinase inhibitors

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Background: Patients with non-small cell lung cancer (NSCLC) harboring anaplastic lymphoma kinase (*ALK*) fusions may benefit from ALK-tyrosine kinase inhibitors (ALK-TKIs). However, few studies have analyzed the clinical outcome in patients harboring multiple *ALK* fusions, including double or triple *ALK* fusions. Here, our study aimed to analyze the impact of harboring multiple *ALK* fusions on the efficacy of receiving ALK-TKIs in NSCLC patients.

Methods: A total of 125 patients with *ALK*-rearranged NSCLC detected by targeted capture DNA-based next-generation sequencing (NGS) at West China Hospital were enrolled. The literature on patients harboring multiple *ALK* fusions was systematically reviewed. The clinical response to ALK-TKIs was evaluated according to *ALK* fusion patterns in 62 patients: 56 from our center and 6 from the literature.

Results: Among the 125 patients, a single canonical echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion was detected in 65.6% (82/125), a single non-*EML4*-*ALK* fusion was detected in 13.6% (17/125), and multiple *ALK* fusions were detected in 20.8% (26/125). Among the 62 patients with *ALK* fusion treated with ALK-TKIs, the median progression-free survival (PFS) was significantly longer in patients with multiple *ALK* fusions than in those with a single *ALK* fusion (26.9 *vs.* 11.2 months, P=0.009), irrespective of brain metastasis, type of TKI drug, and treatment lines. The multiple *ALK* fusion group also tended to have a longer overall survival (OS) (P=0.26). Multivariate Cox regression analysis revealed that harboring multiple *ALK* fusions had the potential to be an independent predictor of better PFS for *ALK*-positive NSCLC [hazard ratio (HR) =0.490; 95% confidence interval (CI): 0.229–1.049].

Conclusions: Harboring multiple *ALK* fusions could serve as an independent predictive marker of better clinical outcome for patients with NSCLC and *ALK* rearrangement who have received ALK-TKIs treatment.

Keywords: Multiple anaplastic lymphoma kinase fusions (multiple *ALK* fusions); *ALK*-rearranged; prognosis; single *ALK* fusion; distribution of *ALK* fusions

Submitted Jul 28, 2023. Accepted for publication Sep 08, 2023. Published online Sep 18, 2023.
doi: 10.21037/tlcr-23-484
View this article at: https://dx.doi.org/10.21037/tlcr-23-484

Introduction

Lung cancer is one of the most frequently diagnosed cancers and the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all diagnosed cases (1). Approximately 3–5% of NSCLC is driven by gene rearrangements in anaplastic lymphoma kinase (*ALK*) (1). Echinoderm microtubule-associated protein-like 4 (*EML4*) is the most common *ALK* fusion partner and is also referred to as canonical *ALK* fusion (2). To date, at least 15 different *EML4-ALK* fusion variants have been discovered, with variant 1 and variant 3 being the most common variants (3-5). Other noncanonical *ALK* fusions, such as *SLMAP-ALK*, *CMTR1-ALK*, *SDK1-ALK*, *HIVEP1-ALK*, and *STRN-ALK*, have been reported in patients with NSCLC, but their large-scale clinical data are still immature (6-11).

Crizotinib was the first tyrosine kinase inhibitor (TKI) to be approved by the US Food and Drug Administration for patients with *ALK*-rearranged NSCLC (12), and its efficacy is superior to standard chemotherapy, which has a significant longer progression-free survival (PFS), whether in previously treated *ALK*-positive NSCLC or previously untreated *ALK*-positive NSCLC patients (13,14). Moreover, second-generation ALK-TKIs, such as alectinib and ceritinib, have been developed for patients with *ALK*-positive NSCLC and have a higher potency compared to crizotinib (15-17). Patients with *ALK* fusion NSCLC have been effectively treated with ALK-TKIs, and the therapeutic outcome for canonical *EML4-ALK* fusion has been widely studied, including the efficacy on different *EML4-ALK* variants (18-20). Different *ALK* fusion variants might be associated with the development of resistance mutations and might be an important factor for the selection of ALK-TKIs (21,22). For patients with *ALK* rearrangements, more than one aberration in the *ALK* gene might be involved. A few case reports have described multiple *ALK* fusions, with some of these indicating that patients with NSCLC and multiple *ALK* fusions show a sensitivity to crizotinib or alectinib (23-25). However, few studies have analyzed the prognosis in patients with NSCLC and multiple *ALK* fusions, and data on efficacy of ALK-TKIs in this setting is currently lacking. Zhang *et al.* found that patients with dual *ALK* fusion partners have a significantly shorter median PFS than do single *ALK* fusion partners (26). However, Kang *et al.* found that complex *ALK* fusions are associated with a better prognosis in patients with advanced NSCLC (27). In the present study, we aimed to analyze the therapeutic outcome in patients with NSCLC and multiple *ALK* fusions who received ALK-TKIs to provide a reference for the treatment of this patient group. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-484/rc).

Highlight box

Key findings

- Patients with non-small cell lung cancer (NSCLC) harboring multiple anaplastic lymphoma kinase (*ALK*) fusions have better response to ALK-tyrosine kinase inhibitors (ALK-TKIs) than those with a single *ALK* fusion.

What is known and what is new?

- Few studies have analyzed the prognosis of patients with NSCLC and multiple *ALK* fusions, and there are no consistent opinions on the treatment of NSCLC patients with multiple *ALK* fusions and whether these patients with multiple *ALK* fusions significantly benefit from ALK-TKIs.
- The therapeutic outcome in patients with multiple *ALK* fusions was analyzed, revealing that those with multiple *ALK* fusions have a more favorable clinical response.

What is the implication, and what should change now?

- The presence of multiple *ALK* fusions is a predictive marker of better clinical outcome in patients with NSCLC treated with ALK-TKIs. Both crizotinib and second-generation ALK-TKIs can be used as the standard treatment for those with NSCLC and multiple *ALK* fusions.

Methods

Patients and methods

We retrospectively evaluated 2,231 patients with NSCLC who underwent next-generation sequencing (NGS) from 2016 to 2020 at West China Hospital. A total of 125 *ALK*-positive patients were included in the analysis. In this study, a cohort of 125 patients was established, all of whom were pathologically confirmed to have NSCLC and identified to harbor *ALK* rearrangements using NGS.

Out of the initial cohort, a subset of 56 patients who received ALK-TKIs for locally advanced or metastatic NSCLC were selected for further prognostic analysis. Inclusion criteria for this subgroup were: (I) the presence of advanced or metastatic NSCLC; (II) confirmed *ALK*-rearranged status; (III) treatment with ALK-TKIs as first- or second-line monotherapy; and (IV) availability of complete prognostic data. Patients were excluded from the subgroup analysis if they met any of the following criteria: (I) did not receive any treatment; (II) received only chemotherapy; (III) underwent surgery and/or postoperative maintenance treatment; (IV) were treated with a combination of ALK-TKIs and chemotherapy or bevacizumab; (V) received only immunotherapy; or (VI) were lost to follow-up within 1 month of initiating ALK-TKIs treatment. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of West China Hospital of Sichuan University (No. 2022-1085), and individual consent for this retrospective analysis was waived.

To supplement our in-house data, we conducted a comprehensive literature search of PubMed and Embase for additional case reports. Our search, which spanned until March 30, 2023, included case studies involving patients with NSCLC exhibiting multiple *ALK* fusions who had undergone ALK-TKIs treatment. Our search strategy utilized key terms such as "*ALK* double fusions", "coexistence/coexisting *ALK* fusion", "multiple *ALK* fusions", "complex *ALK* fusions", "triple *ALK* fusions", "dual *ALK* fusions", and "nonreciprocal/reciprocal *ALK* fusion". The studies selected from this search were required to meet the following criteria: (I) involve patients with advanced or metastatic NSCLC; (II) confirm the presence of multiple *ALK* fusions; (III) detail treatment with ALK-TKIs; and (IV) provide available PFS data. Our search yielded 23 potential cases. However, 17 of these were excluded due to a lack of necessary prognostic data (n=12), the administration of only postoperative maintenance treatment (n=3), or treatment with epidermal growth factor receptor (EGFR) TKIs (n=2) (Figure S1). This left us with 6 relevant cases from the literature of patients with NSCLC and multiple *ALK* fusions.

Consequently, a total of 62 patients with NSCLC and multiple *ALK* fusions who received ALK-TKIs treatment were analyzed in our study. This included 56 patients from our own data and an additional 6 from the literature.

NGS

Genomic DNA of NSCLC formalin-fixed, paraffin-embedded (FFPE) specimens was purified using a QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). Quantification of DNA obtained from FFPE tissues was assessed using a Qubit 2.0 fluorimeter with a double-stranded DNA high-sensitivity assay kit (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA). DNA was profiled with a panel targeting 56 lung cancer-associated genes (Burning Rock Biotech, Guangzhou, China). Sequence data were mapped to the human genome hg19 using Burrows-Wheeler Aligner software (version 0.7.10). Genome Analysis Toolkit GATK 3.2 software (RRID:SCR_001876; Broad Institute, Cambridge, MA, USA) was used for local alignment optimization. Variant calling was performed with MuTect software (Broad Institute).

Measurement of clinical outcomes

Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 was used to assess the response. The objective response rate (ORR) includes complete response (CR) and partial response (PR). The disease control rate (DCR) includes CR, PR, and stable disease (SD). PFS was measured from the date of initiation of ALK-TKI treatment until disease progression or death. Overall survival (OS) was calculated from the date of initiation of ALK-TKIs to death due to any causes or at the last follow-up time. The primary clinical outcomes were PFS and OS. The secondary clinical outcomes were ORR and DCR. For patients included from the literature search, the prognosis data were known.

Definition of multiple ALK fusions and single ALK fusion

In our retrospective study, multiple *ALK* fusions were defined as more than 1 (≥2) *ALK* fusion in patients with NSCLC, and a single *ALK* fusion was defined as only 1 *ALK* fusion, including a noncanonical *ALK* fusion, being detected.

Statistical analysis

The comparison of clinical characteristics between different *ALK* fusion groups was performed using the Fisher exact test. For survival data, Kaplan-Meier curves were analyzed using the log-rank test. Univariate and multivariate analyses were performed using the Cox regression model. All statistical analyses were conducted with SPSS (version 26; IBM Corp., Armonk, NY, USA) and R software (version

4.0.3 or version 4.2.2; The R Foundation for Statistical Computing, Vienna, Austria). Two-sided P values of less than 0.05 were considered statistically significant.

Results

ALK fusions identified in 125 patients with NSCLC

Among 2,231 patients with NSCLC who underwent NGS, *ALK* fusion was detected in 125 (125/2,231, 5.6%). We summarized the distribution of the *ALK* fusions in Table S1. Among the 125 *ALK*-positive patients, most (82/125, 65.6%) had single *EML4-ALK* fusion, in whom *EML4-ALK* variant 1 and *EML4-ALK* variant 3 were the most common *EML4-ALK* fusion; meanwhile, 17 (17/125, 13.6%) had single non-*EML4-ALK* fusion, 4 (4/17, 23.5%) of whom had an intergenic sequence *ALK* fusion, including *CENPA-ALK* (Cintergenic:A20), *CHRNA7-ALK* (Cintergenic:A20), *MEMO1-ALK* (Mintergenic:A20), and *PDCL3-ALK* (Pintergenic:A20). Except for a single *ALK* rearrangement, 26 (20.8%) had multiple *ALK* fusions, 23 patients harbored 2 coexisting fusions, and 3 had 3 coexisting fusions. Among these patients with multiple *ALK* fusions, 22 (22/26, 84.6%) had *EML4-ALK* fusion, and most had coexisting *EML4-ALK* variant 1 (8/26, 30.8%) or *EML4-ALK* variant 3 (9/26, 34.6%). Moreover, 22 (22/26, 84.6%) of these patients had a 3'-*ALK* fusion, and only 4 patients had both a 3'-*ALK* fusion and 5'-*ALK* fusion (Table S1).

Clinical characteristics and the ALK fusion distribution in 62 patients who received ALK-TKIs treatment

Among the 62 patients with NSCLC harboring an *ALK* fusion treated with ALK-TKIs (*Table 1*), the median age was 50.4 years, 44 patients had a single *ALK* fusion, and the remaining 18 patients had multiple *ALK* fusions. In the single *ALK* fusion group, 8 (8/44, 18.2%) patients received alectinib, 34 (34/44, 77.3%) received crizotinib, and the remaining 2 patients received ensartinib and ceritinib, respectively. In the multiple *ALK* fusion group, 8 patients (8/18, 44.4%) accepted alectinib, and the remaining 10 (10/18, 55.6%) patients received crizotinib. The baseline characteristics between the multiple *ALK* fusion and single *ALK* fusion groups were compared, and no significant differences were observed in the baseline characteristics in these two groups except for contralateral lung metastasis (P=0.025) (*Table 1*).

We further analyzed the distribution of *ALK* fusions in 62 patients with NSCLC who received ALK-TKIs (*Figure 1*). Among these 62 patients, 71.0% (44/62) had a single *ALK* fusion, and among these patients, the most common *ALK* fusion was still *EML4-ALK* (36/44, 81.8%), and 8 patients (8/44, 18.2%), had a non-*EML4-ALK* fusion. The remaining 18 (18/62, 29.0%) patients had multiple *ALK* fusions, 6 of whom were included through literature retrieval (*Figure 1*).

Better prognosis in patients with NSCLC with multiple ALK fusions who received ALK-TKIs and subgroup analysis

Considering the lack of a standardized prognosis for NSCLC patients harboring multiple *ALK* fusions who undergo ALK-TKIs treatment, we initiated this study to discern whether these patients exhibit a superior or inferior prognosis compared to NSCLC patients harboring a solitary *ALK* fusion. We analyzed the response data of the different groups, and there was no significantly higher ORR or DCR between the single *ALK* fusion group and the multiple *ALK* fusion group, with P values of P=0.121 and P=1.000, respectively (*Figure 2A,2B*). Moreover, the median PFS was significantly increased in the patients with multiple *ALK* fusions (26.9 months) compared to those with a single *ALK* fusion (11.2 months, P=0.009; *Figure 3*). For OS, as shown in *Figure 4*, the multiple *ALK* fusion group tended to have a better OS than did the single *ALK* fusion group [hazard ratio (HR) =0.428; 95% confidence interval (CI): 0.095–1.928], although the P value was not statistically significant (P=0.26). In subgroup analyses of PFS, irrespective of age, gender, smoking status, types of TKIs drug, and treatment lines, the magnitude of the treatment effect was generally consistent across patients generally (*Figure 5*), but the magnitude of benefit was lower in the subgroup of patients receiving second generation ALK-TKIs therapy. Similar results were seen in the subgroup analyses of OS, but only a small number of patients had complete OS data (*Figure 6*).

Multiple ALK fusions was a predictive marker in patients with NSCLC treated with ALK-TKIs

Univariate Cox regression analysis of PFS revealed that ALK-TKIs and *ALK* fusion had prognostic value, with P values of P=0.004 and P=0.012, respectively (Table S2). We then conducted multivariate Cox regression analysis of PFS, and found that presence of multiple *ALK* fusions had

Table 1 Baseline characteristics of the 62 patients with ALK-rearranged NSCLC who received ALK-TKI therapy

Characteristics	All	Single *ALK* fusion	Multiple *ALK* fusions	P
No. of patients (%)	62 (100.0)	44 (71.0)	18 (29.0)	–
Age (years), median (range)	50.4 (42.0–60.1)	20.9 (42.4–64.5)	45.4 (41.8–56.8)	0.201
Age, n (%)				0.786
≤40 years	11 (17.7)	8 (18.2)	3 (16.7)	
41–55 years	28 (45.2)	18 (40.9)	10 (55.6)	
56–70 years	18 (29.0)	14 (31.8)	4 (22.2)	
>70 years	5 (8.1)	4 (9.1)	1 (5.6)	
Gender, n (%)				1.000
Male	28 (45.2)	20 (45.5)	8 (44.4)	
Female	34 (54.8)	24 (54.5)	10 (55.6)	
Smoking status, n (%)				0.729
Never	44 (71.0)	31 (70.5)	13 (72.2)	
Former/current	13 (21.0)	10 (22.7)	3 (16.7)	
Unknown	5 (8.1)	3 (6.8)	2 (11.1)	
ECOG PS, n (%)				<0.001
0	14 (22.6)	11 (25.0)	3 (16.7)	
1	30 (48.4)	23 (52.3)	7 (38.9)	
≥2	11 (17.7)	10 (22.7)	1 (5.6)	
Unknown	7 (11.3)	0 (0.0)	7 (38.9)	
Pathology, n (%)				1.000
Squamous cell carcinoma	1 (1.6)	1 (2.3)	0 (0.0)	
Adenocarcinoma	60 (96.8)	42 (95.4)	18 (100.0)	
Adenosquamous carcinoma	1 (1.6)	1 (2.3)	0 (0.0)	
Stage, n (%)				0.630
III	13 (21.0)	10 (22.7)	3 (16.7)	
IVA	22 (35.5)	14 (31.8)	8 (44.4)	
IVB	27 (43.5)	20 (45.5)	7 (38.9)	
Brain metastasis, n (%)				1.000
No	48 (77.4)	34 (77.3)	14 (77.8)	
Yes	14 (22.6)	10 (22.7)	4 (22.2)	
Liver metastasis, n (%)				0.152
No	51 (82.3)	34 (77.3)	17 (94.4)	
Yes	11 (17.7)	10 (22.7)	1 (5.6)	
Bone metastasis, n (%)				0.390
No	38 (61.3)	25 (56.8)	13 (72.2)	
Yes	24 (38.7)	19 (43.2)	5 (27.8)	

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	All	Single *ALK* fusion	Multiple *ALK* fusions	P
Adrenal metastasis, n (%)				1.000
No	59 (95.2)	42 (95.5)	17 (94.4)	
Yes	3 (4.8)	2 (4.5)	1 (5.6)	
Pleural metastasis, n (%)				0.082
No	38 (61.3)	30 (68.2)	8 (44.4)	
Yes	24 (38.7)	14 (31.8)	10 (55.6)	
Contralateral lung metastasis, n (%)				0.025
No	46 (74.2)	29 (65.9)	17 (94.4)	
Yes	16 (25.8)	15 (34.1)	1 (5.6)	
Treatment lines, n (%)				0.427
First-line	53 (85.5)	39 (88.6)	14 (77.8)	
Second-line	9 (14.5)	5 (11.4)	4 (22.2)	
Disease progression, n (%)				0.179
No	23 (37.1)	14 (31.8)	9 (50.0)	
Yes	39 (62.9)	30 (68.2)	9 (50.0)	
ALK-TKI, n (%)				0.152
Alectinib	16 (25.8)	8 (18.2)	8 (44.4)	
Ensartinib	1 (1.6)	1 (2.3)	0 (0.0)	
Crizotinib	44 (71.0)	34 (77.3)	10 (55.6)	
Ceritinib	1 (1.6)	1 (2.3)	0 (0.0)	

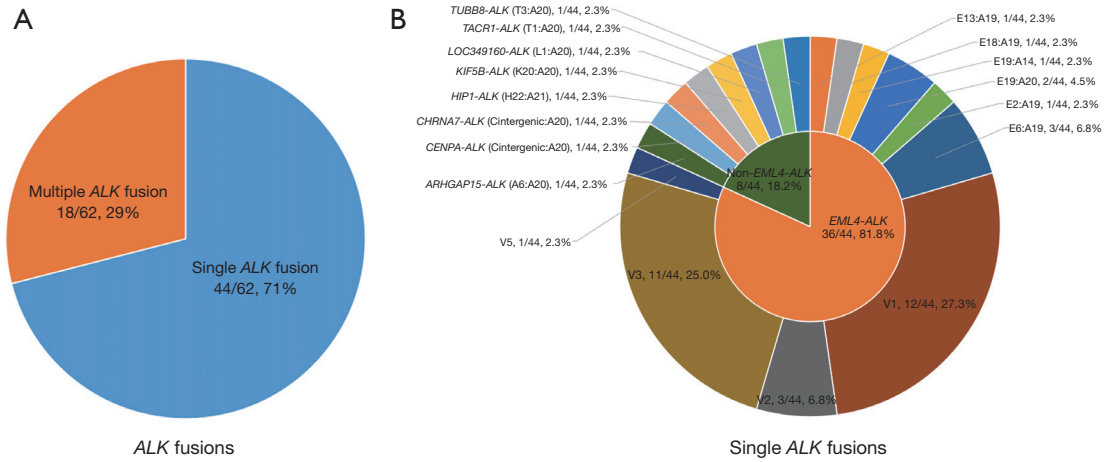
ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

the potential to serve as a predictive marker of better PFS for patients with *ALK*-rearranged NSCLC treated with ALK-TKIs (HR =0.490; 95% CI: 0.229–1.049) (Table S2). Due to the prognostic influence of the second-generation ALK-TKIs, to further demonstrate the predictive effect of multiple *ALK* fusions, we performed subgroup analysis in patients received different ALK-TKIs. Neither receiving first-generation or second-generation ALK-TKIs, patients who harbored multiple *ALK* fusions had a favorable PFS or OS than those harboring single *ALK* fusion, although there were no statistically significant differences in patients who received second-generation ALK-TKIs (Figure S2).

To reduce the data bias, we further analyzed the 56 patients came from our hospital. As shown in Table S3, no significant differences were observed in the baseline characteristics in these two groups. Univariate and multivariate Cox regression analyses of PFS revealed similar predictive value of multiple *ALK* fusions, although the P value was not statistically significant (P=0.091) (Table S4).

Prognosis in ALK-positive patients with brain metastases

In the present study, we investigated the incidence of brain metastases in patients with NSCLC harboring *ALK* fusion at baseline and analyzed the prognosis of patients with and without metastases: 22.7% (10/44) of single *ALK* fusion patients had brain metastases while 22.2% (4/18) of multiple *ALK* fusion patients had brain metastases (*Table 1*). Among the patients with brain metastases, there was no significant difference in PFS between the single *ALK* fusion group and multiple *ALK* fusion group (11.0 *vs.* 26.9 months, P=0.17) (Figure S3A), and there was also no significant difference in OS between these two groups (P=0.15) (Figure S3B). Moreover, for patients without brain



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Patients number	*ALK* fusions (n=18)
No. 1	*COX7A2L-ALK* (Cintergenic:A20); *LINCO1210-ALK* (Lintergenic:A20); *ATP13A4-ALK* (A9:A19)
No. 2	*DYSF-ALK* (Dintergenic:A20); *ITGAV-ALK* (I2:A20)
No. 3	*EML4-ALK* (V3); *CTNNA2-ALK* (C8:A19)
No. 4	*EML4-ALK* (E13:A19); *ALK-RBKS* (A19:E7)
No. 5	*EML4-ALK* (V1); *ALK-ACTN1* (A19:A18)
No. 6	*EML4-ALK* (V1); *CNTNAP5-ALK* (Cintergenic:A20)
No. 7	*EML4-ALK* (V1); *FSHR-ALK* (Fintergenic:A20)
No. 8	*EML4-ALK* (V1);*SOS1-ALK*(S10:A20)
No. 9	*EML4-ALK* (V1);*STK17B-ALK*(SNA:A20)
No. 10	*EML4-ALK* (V3); *LOC388942-ALK* (Lintergenic:A20)
No. 11	*EML_4-ALK* (V5); *RBM20-ALK* (R1:A20)
No. 12	*STRN-ALK* (S3:A20); *PDK1-ALK* (P7:A20)
No. 13	*DTN1-ALK* (D27:A20); *ALK-CLIP4* (A19:C12)*
No. 14	*EML_4-ALK* (E14:A21); *SETD2-ALK* (S12:A21)*
No. 15	*EML4-ALK* (V3); *CDK15-ALK* (C10:A19)*
No. 16	*HIP1-ALK* (H30:A20); *LTBP1-ALK* (L3:19)*
No. 17	*LNNR2-ALK* (L3:A19); *LTBP1-ALK* (L3:A19); *HIP1-ALK* (H30:A20)*
No. 18	*RGS18-ALK* (Rintergenic:A20); *THUMPD2-ALK* (T6:A20)*
*, cases included through literature retrieval	

Multiple *ALK* fusions

Figure 1 Distribution of *ALK* fusions in 62 patients who received ALK-TKIs. (A) *ALK* fusions were subdivided into multiple *ALK* fusions and single *ALK* fusion. (B) The frequency of *EML4-ALK* fusion and non-*EML4-ALK* fusion in single *ALK* fusion patients. (C) *ALK* fusion information in 18 patients. *ALK*, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitor; EML4, echinoderm microtubule-associated protein-like 4.

metastases, we found a significantly better PFS in the multiple *ALK* fusion group (26.0 *vs.* 15.6 months, P=0.028) (Figure S3C), but no significant difference in OS was reported between these two groups (P=0.76) (Figure S3D).

Discussion

In this retrospective study, we investigated the incidence of multiple *ALK* fusions in patients with NSCLC harboring *ALK* rearrangement and analyzed the therapeutic outcome in different *ALK* fusion groups who received ALK-TKIs. We found that approximately 20% of patients with NSCLC and *ALK* rearrangement had multiple *ALK* fusions, and in comparison to the single *ALK* fusion group, the multiple *ALK* fusion group had a better response to ALK-TKIs.

An increasing number of cases of complex *ALK* fusions or coexisting *ALK* fusions have been reported. Moreover, with the widespread use of NGS, some new *ALK* rearrangements have been detected. In this retrospective study, we investigated the distribution of *ALK* fusions in a large Chinese NSCLC cohort. We identified that the most common *ALK* fusion was *EML4-ALK*, and among the single *EML4-ALK* fusions, variant 3 and variant 1 were the most frequently occurring; meanwhile, 13.6%

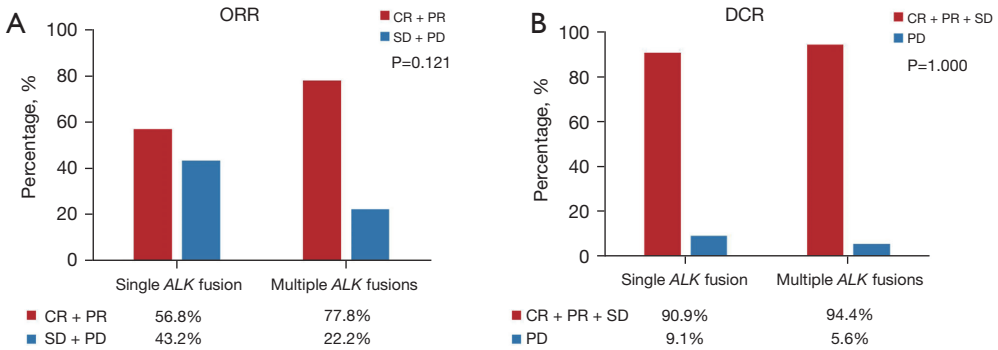

Figure 2 Different clinical outcomes in multiple *ALK* fusion group and single *ALK* fusion group. (A) Histogram showing the proportions of patients who achieved an ORR in the multiple *ALK* fusion group and single *ALK* fusion group. (B) Histogram showing the proportions of patients with controlled disease in the multiple *ALK* fusion group and single *ALK* fusion group. ORR, objective response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; *ALK*, anaplastic lymphoma kinase; DCR, disease control rate.

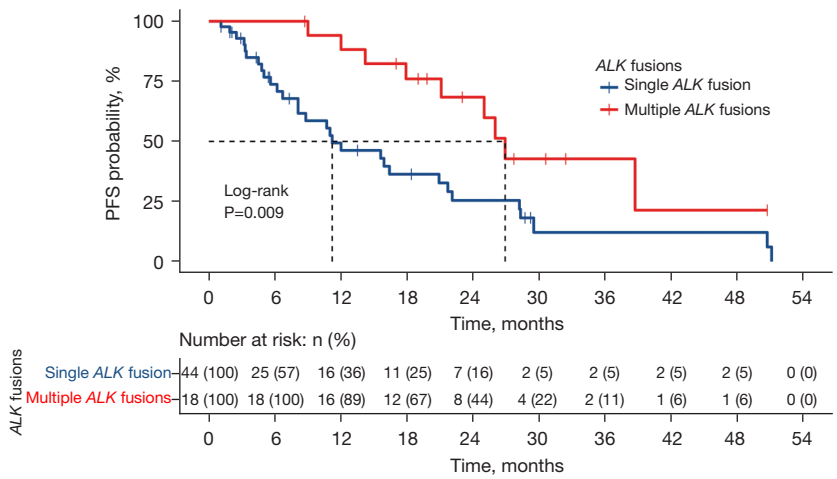

Figure 3 Clinical outcome in the different *ALK* fusion groups. Kaplan-Meier curve of PFS of ALK-TKI treatment in 62 patients. *ALK*, anaplastic lymphoma kinase; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

(17/125) had non-*EML4-ALK* fusion alone, and 20.8% (26/125) had multiple *ALK* fusions. Previous work has reported a frequency of 79.78% for *EML4-ALK* fusions and 20.22% (18/89) for non-*EML4-ALK* fusions, with 17.98% (16/89) showing more than 1 *ALK* rearrangement (28). Another study reported that 69.3% (104/150) had a single *EML4-ALK* rearrangement, 12.0% (18/150) had a non-*EML4-ALK* rearrangement alone, and 18.7% (28/150) had nonreciprocal/reciprocal *ALK* rearrangements (29). Possible reasons for the observed inconsistency in frequency may include variations in cohort size and the employment of diverse detection tools. ALK-TKIs have dramatically expanded the therapeutic landscape of *ALK*-positive NSCLC. For patients with NSCLC and multiple *ALK* fusions, there is no consensus concerning the treatment outcome of these patients. For patients with *ALK* fusions who received first-line crizotinib, a previous study found that patients with nonreciprocal/reciprocal *ALK* fusions had a poor prognosis compared to patients with 3'-*ALK* fusion alone or *EML4-ALK* fusion alone (29). However, some studies have reported different results. In their study, Kang *et al.* discovered that patients with NSCLC harboring complex *ALK* fusions exhibited a more favorable OS compared to those with either pure canonical *EML4-ALK* fusion or pure noncanonical *ALK* fusion (27). Xia *et al.* identified a prolonged yet statistically insignificant

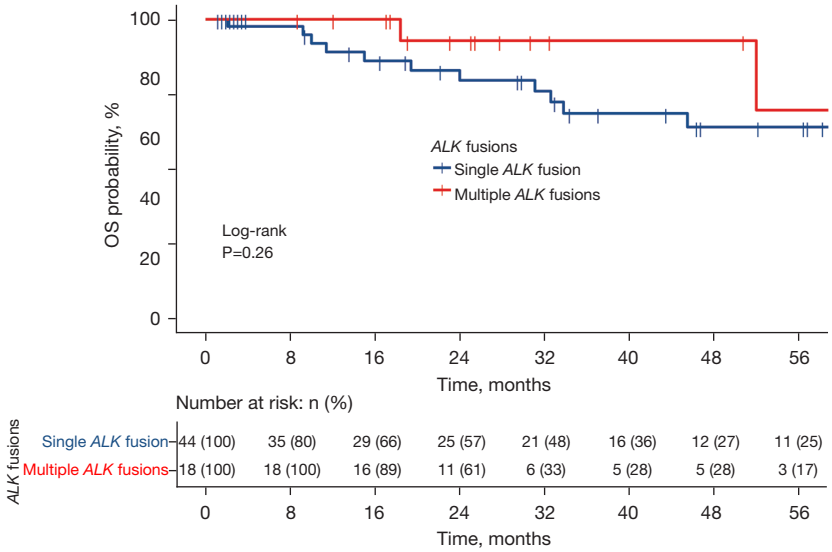

Figure 4 Clinical outcome in the different *ALK* fusion groups. Kaplan-Meier curve of OS of ALK-TKI treatment in 62 patients. *ALK*, anaplastic lymphoma kinase; OS, overall survival; TKI, tyrosine kinase inhibitor.

difference in PFS between patients with canonical *ALK* fusions and those with complex *ALK* fusions (30). In contrast, our study demonstrated that patients with multiple *ALK* fusions presented with a superior PFS than those with a single *ALK* fusion. The discrepant therapeutic outcomes in these clinical studies may be due to the different inclusion criteria of multiple *ALK* fusions and concomitant mutation status. The definition of multiple *ALK* fusions in our study were more extensive in comparison to those of the nonreciprocal/reciprocal *ALK* fusion, which was defined as harboring concurrent *ALK* fusions with at least one 3'-*ALK* fusion and one 5'-*ALK* fusion. A different study suggested that concurrent 5'-*ALK* fusion is associated with poor prognosis (29). Regarding the influence of concomitant mutation on prognosis, one study found that concomitant *TP53* or *PIK3R2* alteration was predictive of poor survival (31), which was associated with poor prognosis in *ALK*-rearranged patients. In our present study, the mutation status was not included in analyses because of a lack of complete mutation data. Moreover, in our study, we further demonstrated the superior clinical outcome in patients with NSCLC and multiple *ALK* fusions, and further confirmed that multiple *ALK* fusions have the potential to be an independent predictive marker of better PFS in patients with NSCLC treated with ALK-TKIs. For *ALK*-rearranged NSCLC, genomic heterogeneity has been demonstrated, and which could account for differences in treatment response with ALK-TKIs (32). Among multiple *ALK* fusions, there are higher rates for the intergenic sequence-*ALK* and non-*EML4-ALK* fusions in patients (28), and most have one *EML4-ALK* fusion, and the other *ALK* fusion could be a promising target for subsequent ALK-TKIs treatment (27). Zhang *et al*. found that patients harboring dual *ALK* fusions with concurrent 5'-*ALK* fusions had a poor response to ALK-TKIs targeted therapy, and then also identified that specific combinations of *ALK* fusions in multiple *ALK* fusions bring about different treatment responses (29). In our study, there were only 3 patients harboring multiple *ALK* fusions with concurrent 5'-*ALK* fusions, and most of them harbored two or three coexisting 3'-*ALK* fusions, which might account for the different prognoses compared to patients harboring nonreciprocal/reciprocal *ALK* fusions. Furthermore, there were 6 patients harboring multiple *ALK* fusions coexisting with intergenic *ALK* fusions, which might also predict good response to ALK-TKIs therapy, because previous study found that intergenic-breakpoint rearrangement of *ALK* had favorable clinical outcomes and there may be a complicated splicing mechanism which could transcribe intergenic-breakpoint rearrangements into functional chimeric RNAs (33). However, the mechanism of the better response in patients with multiple *ALK* fusions is still unknown, and tumors with multiple *ALK* fusions are likely to be more reliant on the *ALK* signaling pathway, thus ALK-TKIs would be

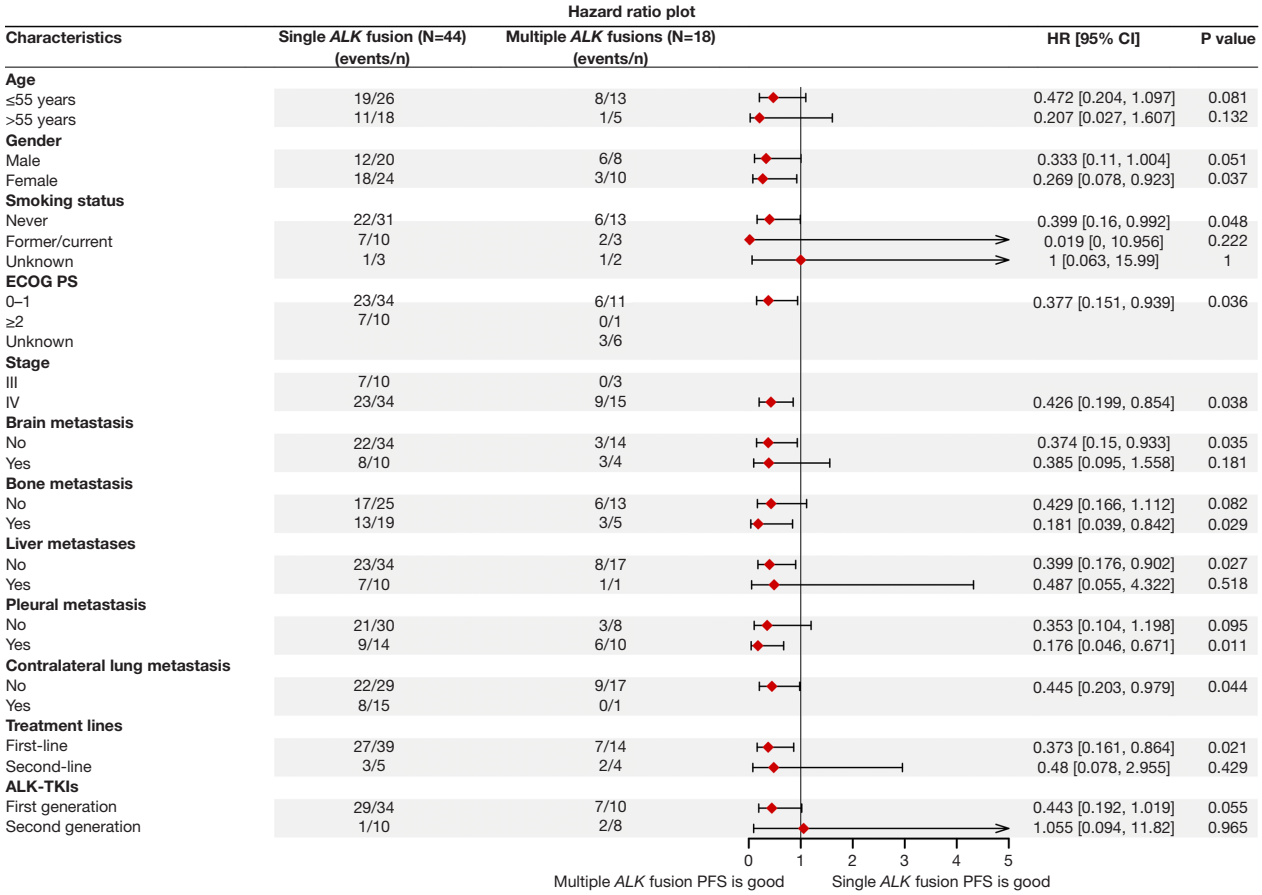

Figure 5 Univariate analyses of PFS in patients with NSCLC who received ALK-TKIs. ALK, anaplastic lymphoma kinase; HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; TKI, tyrosine kinase inhibitor; PFS, progression-free survival; NSCLC, non-small cell lung cancer.

more effective in this condition (27). Thus, to clarify the mechanism of prolonged survival in those with multiple *ALK* fusions, preclinical studies to explain prolonged survival in patient with NSCLC harboring multiple ALK fusions and studies on the clinical outcome of a larger cohort of patients with multiple *ALK* fusions are warranted.

Furthermore, the prognosis in patients with brain metastases was unfavorable. Clinical trials with TKI treatment for patients with NSCLC and brain metastases have shown prolonged PFS, a high percentage of objective response, and improved quality of life (34). A previous study found that patients with NSCLC and brain metastases have a significantly higher rate of possessing several targetable genomic alterations, including *ALK* fusions (35). To further determine whether multiple *ALK* fusions could affect the prognosis of patients with brain metastases, we conducted an analysis of this patient group. We found that patients with brain metastases harboring multiple *ALK* fusions had favorable PFS, although there was no statistically significant difference in PFS between the single *ALK* fusion group and the multiple *ALK* fusions group, which was consistent with the clinical outcome in the patients overall. This likely demonstrates that any improvement in prognosis with multiple *ALK* fusions is overturned by the higher risk conferred by the presence of central nervous system (CNS) disease.

Limitations

This retrospective study is subject to several inherent limitations that warrant consideration. The primary concern is that a proportion of patients in the multiple *ALK*-fusion

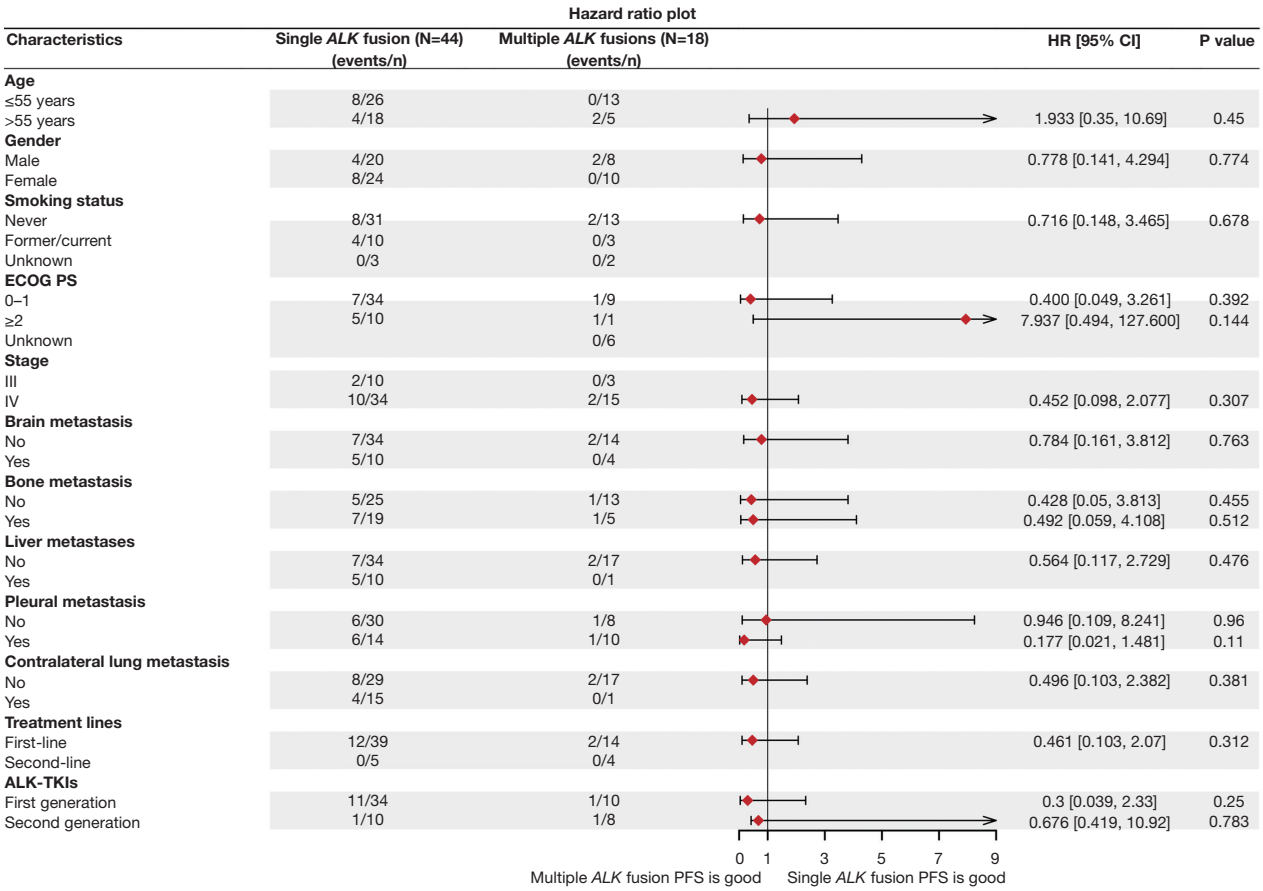

Figure 6 Univariate analyses of OS in patients with NSCLC who received ALK-TKIs. ALK, anaplastic lymphoma kinase; HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; TKI, tyrosine kinase inhibitor; OS, overall survival; NSCLC, non-small cell lung cancer.

group were sourced through a literature search, a method that may not necessarily capture the full scope and detail of the patient characteristics, as compared to original case data obtained from clinical or hospital databases. Furthermore, the nature of the available data from these literature-sourced patients may be prone to publication or reporting bias, particularly as studies with negative results are less likely to be published. This potential bias could skew our findings and interpretations.

Additionally, a significant limitation is that some of the OS data of the patients included from the literature search were not mature at the time of our analysis. This lack of mature OS data creates a challenge when trying to accurately analyze survival outcomes. The absence of comprehensive and mature OS data precludes our ability to draw definitive conclusions about the long-term survival impact of multiple *ALK*-fusions in this population. This limitation is compounded by the potential for lead-time bias, given that survival measurements began from the time of diagnosis, which may vary widely among patients.

We acknowledge that these limitations may affect the robustness of our results and the strength of our conclusions. Therefore, the findings of this study should be interpreted cautiously and further validated in prospective studies with comprehensive and mature OS data.

Conclusions

Our study highlights the fact that multiple *ALK* fusions are not a rarity among patients with *ALK*-rearranged NSCLC. These findings challenge the conventional perception of the frequency of multiple *ALK* fusions and emphasize the importance of comprehensive genomic profiling to accurately capture the complex mutational landscape of NSCLC. This

information underscores the necessity for more nuanced and detailed diagnostic testing that can recognize and differentiate between these multiple *ALK* fusions.

Most importantly, our results indicate a noteworthy association between multiple *ALK* fusions and improved PFS in NSCLC patients undergoing treatment with ALK-TKIs. This statistically significant correlation suggests a prognostic advantage for patients harboring multiple *ALK* fusions and reinforces the therapeutic value of ALK-TKIs in this molecularly defined subset of NSCLC.

Consequently, these insights pave the way for the potential use of multiple *ALK* fusions as predictive biomarkers for patients with NSCLC treated with ALK-TKIs. The identification of multiple *ALK* fusions may guide the selection of appropriate therapeutic strategies and help to predict patient response, potentially leading to improved patient outcomes.

Acknowledgments

Funding: This work was supported by the National Natural Science Foundation of China (Nos. 82072598, 81871890, and 91859203), the 1-3-5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (No. ZYJC21052), the Science and Technology Program of Sichuan, China (No. 2020YFS0572), the Major Science and Technology Innovation Project of Chengdu City (No. 2020-YF08-00080-GX), the Central Guide Place-Free Exploration Project, Sichuan Provincial Department of Science and Technology (No. 2020ZYD005), and the Fundamental Research Funds for the Central Universities (No. SCU2022D025).

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-484/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-484/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-484/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-484/coif). AD is an advisory board member in Sanofi, Amgen, and Foundation Medicine. The other authors have no conflicts of interest to declare.

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 was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethics Committee of West China Hospital of Sichuan University (No. 2022-1085). Individual consent for this retrospective analysis was waived.

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Cite this article as: Wei Q, Zhang Y, Wang Y, Desai A, Tan S, Huang Q, Pu X, Tian P, Li Y. Superior clinical outcomes in patients with non-small cell lung cancer harboring multiple *ALK* fusions treated with tyrosine kinase inhibitors. Transl Lung Cancer Res 2023;12(9):1935-1948. doi: 10.21037/tlcr-23-484