Original Article

Efficacy of alectinib in lung adenocarcinoma patients with different anaplastic lymphoma kinase (*ALK*) rearrangements and co-existing alterations—a retrospective cohort study

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Background: Alectinib significantly improves survival of non-small cell lung cancer (NSCLC) patients with anaplastic lymphoma kinase (*ALK*)-rearrangement. In this study, we analyzed the effects of different *ALK* rearrangements and co-mutations on the efficacy of alectinib.

Methods: Using the electronic medical record system, we reviewed in terms of clinical and pathological features patients with advanced (IIIB/IV stage) *ALK*-rearranged NSCLC at Shanghai Chest Hospital between January 2018 and December 2021 who were treated with alectinib in first or second line and were assessed for objective response rate (ORR), disease control rate (DCR), and progression-free survival (PFS).

Results: A total of 66 patients were enrolled in the study, and 17 types of *ALK* rearrangements were detected, of which five types of *ALK* rearrangements responded well to alectinib. We classified *ALK*-rearrangements into four main types, namely echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* (E6:A20), *EML4-ALK* (E13:A20), *EML4-ALK* (E20:A20), and others. There was no significant difference in ORR and DCR of these types (ORR: 31.3% *vs.* 13.0% *vs.* 18.2% *vs.* 17.6%, P=0.575; DCR: 93.8% *vs.* 95.6% *vs.* 100.0% *vs.* 88.2%, P=0.627). The 3-year PFS rates were 25.0% (4/16) *vs.* 13.0% (3/23) *vs.* 27.3% (3/11) *vs.* 18.8% (3/16) for *EML4-ALK* (E6:A20), *EML4-ALK* (E13:A20), *EML4-ALK* (E20:A20), and others, respectively (P=0.725). The results of co-mutation analysis showed that the median PFS (mPFS) for patients with tumors harboring *TP53* mutations was 30.4 months, significantly shorter than that of patients with tumors without co-mutations and whose mPFS was not mature (P=0.026). *TSC1* co-mutation was also identified as a detrimental factor in outcome, with a DCR of 60% *vs.* 100% (P=0.031), mPFS of 30.4 months *vs.* not applicable (P=0.160) in patients with *vs.* those without this co-mutation, respectively. The efficacy of alectinib in patients with brain metastases is comparable to that in patients without distant organ metastases. There were two cases with specific fusion types that also responded to alectinib; namely, double *ALK*-rearrangements: *EML4-ALK* (E13:A20) and *MSH2-ALK* (M7:A20), and with a rare fusion partner, *SPECC1L-ALK* (S8:A20). Their PFS were 8.7 and 38.0 months, respectively.

Conclusions: In this study, the efficacy of alectinib in different types of *ALK*-rearrangements varied slightly. *TP53* and *TSC1* co-mutations were identified as detrimental factors affecting efficacy. This study

provides references for the response to alectinib in patients with different types of *ALK* rearrangements and co-mutation.

Keywords: Non-small cell lung cancer (NSCLC); anaplastic lymphoma kinase (*ALK*); chromosomal rearrangements; co-mutated genes; alectinib

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Introduction

Lung adenocarcinoma (LADC), the most frequent histological type of lung cancer, is often triggered by an aberration in a driver oncogene in tumor cells. Anaplastic lymphoma kinase (*ALK*) gene fusions define a molecular subtype of non-small cell lung cancer (NSCLC) and account for 4–6% of LADCs. The *ALK* gene is located on chromosome 2, and chromosomal rearrangements lead to the ectopic expression of the tyrosine kinase-containing part of *ALK* and its structural activation (1). *ALK* rearrangements lead to ligand-independent dimerization and hyperactivation of pro-mitogenic and anti-apoptotic signaling, including the RAS-mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase-protein kinase B (PI3K-AKT), and Janus kinase signal transducer activator of transcription (JAK-STAT) cascades (2-4). *ALK* rearrangement lung cancers show *ALK* dependence and are usually sensitive to tyrosine kinase inhibitor (TKIs).

Highlight box

Key findings

- Echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (*ALK*) (E20:A20) variant 2 fusion responds better to alectinib.
- *TP53* and *TSC1* co-mutations are detrimental factors affecting efficacy of alectinib.
- Some specific reported *ALK* fusions respond to alectinib therapy.

What is known and what is new?

- The ALEX study demonstrated that alectinib improves outcomes of patients with *ALK*-rearranged advanced non-small cell lung cancer.
- This retrospective study showed the influence of *ALK* fusion subtypes and co-mutations on the efficacy of alectinib.

What is the implication, and what should change now?

- More appropriate treatment strategies based on the specific type of *ALK* fusion, and co-alterations are needed.

So far, five kinds of ALK-TKIs have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of advanced *ALK*-rearrangement NSCLC, and more drugs are under clinical development (1). Since the initial report of *ALK*-rearrangements in NSCLC patients, more than 90 *ALK* fusion partners have been identified (5). Among the many types of *ALK*-rearrangements, one of the most common fusion partners is echinoderm microtubule-associated protein-like 4 (*EML4*), observed in nearly 80% of *ALK*-rearranged cases (6). Bulutay *et al.* showed that the *EML4-ALK* fusion was present in 3.8% of the total 251 LADC cases and it was associated with the solid pattern, signet ring cell morphology, and larger tumor size (7). Another study revealed that the rate of *EML4-ALK* fusion was 6.7% (6/90), and that it was not correlated with gender, smoking history, maximal tumor diameter, pleural invasion, lymphatic metastasis, or clinical staging, but was mainly associated with the predominant subtypes of acinar and solid tumors with mucin secretion (8). At least 15 *EML4-ALK* variants have been identified in patients with NSCLC (9). The most common variants are variant 1 (v1, E13:A20), variant 2 (v2, E20:A20), and variant 3 (v3, E6:A20) (10). Other rare non-EML4 fusion genes have also been found in patients with lung cancer, and the clinical significance of these fusion genes is still on study.

Crizotinib is the first targeted drug for treating *ALK* rearrangement NSCLC patients. It is also effective in the treatment of c-ros oncogene 1, receptor tyrosine kinase (*ROS-1*) and mesenchymal to epithelial transition (*MET*) factor mutations. A study included 149 patients with stage III or IV *ALK* rearrangement advanced NSCLC. Among the 143 assessable patients, the objective response rate (ORR) was 60.8%, the median progression-free survival (mPFS) was 9.7 months, and the continuous reaction time was 49.1 weeks, preliminary proof of the efficacy of crizotinib (11). The phase III randomized controlled trial of PROFILE1007 and the subsequent PROFILE1014 trial further confirmed the role of crizotinib in the treatment

of patients with advanced *ALK* rearrangement NSCLC, so crizotinib was recommended by FDA as first-line treatment for advanced *ALK* rearrangement NSCLC patients and second-line treatment for patients who had not received crizotinib before (12,13). Although crizotinib is effective in the treatment of *ALK* rearrangement patients, the limitation and drug resistance of brain metastases limit this effectiveness. Therefore, the research of the next generation of ALK inhibitors aims to overcome this deficiency of crizotinib. Alectinib is a new type of highly targeted second-generation ALK inhibitor. ALEX study and ALUR study show that alectinib has better efficacy and survival benefit than crizotinib and other chemotherapeutic drugs in treating advanced *ALK* rearrangement NSCLC patients, and has better permeability to the central nervous system (14). Especially in first-line treatment, alectinib effectively prolonged the survival of patients with advanced *ALK* rearrangement NSCLC compared with crizotinib.

Different *ALK*-rearrangements have a diverse impact on the treatment of LADC patients (15-17). Some studies showed that the *HIP1-ALK* rearrangement variant in LADC is resistant to crizotinib (18), but patients with *GHR-ALK* rearrangement gene had a limited response to crizotinib (19). Alectinib may show unsatisfactory therapeutic effects for *EML4-ALK* (E19:A20) fusion (20), but the *EML4-ALK* (E20:A20)-*BIRC6-ALK* double fusion variant in LADC confers sensitivity to alectinib (21). As highlighted by the abovementioned studies, it is of great clinical significance to identify *ALK*-rearrangement and their specific type as it may have impact on therapeutic choices, and subsequent development of a targeted drug (22,23). However, there is currently little data on the response of different types of *ALK* rearrangements to alectinib. For this study, a total of 66 LADC patients from our hospital were enrolled for analysis; all had *ALK*-rearrangement and were treated with alectinib as first- or second-line therapy. We aimed to explore the effect of *ALK*-rearrangement on alectinib therapeutic efficacy in the real-world, and provide references for the response to alectinib in patients with different types of *ALK* rearrangements. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-658/rc).

Methods

Study design

This retrospective cohort study was conducted to assess the efficacy of alectinib in different types of *ALK*-rearranged LADC. The medical records of patients with advanced *ALK*-rearranged NSCLC treated with alectinib at Shanghai Chest Hospital between January 2018 and December 2021 were reviewed. The inclusion criteria were as follows: (I) pathologically or cytologically confirmed NSCLC; (II) unresectable stage IIIB/IV according to the eighth edition of the tumor-node-metastasis (TNM) classification for lung cancer; (III) confirmed *ALK*-rearrangement detected by next-generation sequencing (NGS); (IV) the receipt of alectinib monotherapy as first-line or second-line treatment; and (V) an Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0–2. The exclusion criteria were as follows: (I) patients without *ALK*-rearrangement; (II) incomplete radiological records and images; and (III) patients lost to follow-up. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board of the Shanghai Chest Hospital (No. KS22002). The requirement for individual consent for this retrospective analysis was waived.

NGS

Formalin-fixed paraffin-embedded (FFPE) tissue of patients were subjected to DNA extraction and targeted sequencing, and these tests were performed in Burning Rock Biotech Ltd. (Guangzhou, China), a commercial clinical laboratory accredited by the College of American Pathologist (CAP) and certified by the Clinical Laboratory Improvement Amendments (CLIA). The tests were conducted according to the manufacturer's instructions; DNA of tissue samples were extracted by QIAamp DNA Kit (51306; Qiagen, Hilden, Germany), peripheral white blood cells (WBCs) were separated by centrifugation at 1,800 ×g for 10 minutes at 4 ℃ within 2 hours after blood collection, and genomic DNA was extracted from the WBCs as the germline control.

DNA fragmentation was performed using an M220 focused-ultrasonicator (Covaris, Woburn, MA, USA), followed by end repair, phosphorylation, and adaptor ligation. DNA fragments within 200–400 bp size were selected by magnetic bead (Agencourt AMPure XP Kit; Beckman Coulter, Brea, CA, USA), then subjected to hybridization with capture probes baits, hybrid selection with magnetic beads, and polymerase chain reaction (PCR) amplification. Then, the quality and size of the fragments were evaluated by a high-sensitivity DNA assay (Bioanalyzer

2100; Agilent Technologies, Santa Clara, CA, USA). Ultimately, indexed samples were sequenced on Nextseq500 sequencer (Illumina, Inc., San Diego, CA, USA) with pair-end reads and average sequencing depth of 1,000×. Genomic profiling was performed using a panel covering 68 lung cancer-related genes (Burning Rock Biotech Ltd.).

Sequence data analysis

The sequence data were mapped to the human genome (hg19) reference by Burrows-Wheeler Aligner version 0.7.10. Local alignment optimization, duplication marking, and variant calling were performed by Genome Analysis Tool Kit version 3.2 (Broad Institute, Cambridge, MA, USA), and VarScan version 2.4.3 (Washington University, St Louis, MO, USA). Tissue samples were compared against their own WBCs' control to identify somatic variants. Variants with population frequency over 0.1% in the ExAC, 1000 Genomes, database single nucleotide polymorphism (dbSNP), or ESP6500SI-V2 databases were grouped as SNPs and excluded from further analysis. Remaining variants were annotated with ANNOVAR (2016-02-01 release; Open Bioinformatics, Copenhagen, Denmark) and SnpEff version 3.6 (Washington University). DNA translocation analysis was performed using both Tophat2 (Johns Hopkins University, Baltimore, MD, USA) and Factera 1.4.3 (Stanford University, CA, USA).

Efficacy assessment and follow-up

Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 was used to evaluate tumor response. The first disease response assessment was performed at the end of two treatment cycles. ORR was defined as the percentage of patients who achieved a partial response (PR) or complete response (CR). Disease control rate (DCR) is defined as PR, CR and stable disease (SD) rate. PFS is defined as the time from initiation of alectinib to disease progression or death. The data deadline is March 2023, and patients with a sustained response at this time or at the last follow-up date are considered as censored.

Statistical analysis

One-way analysis of variance (ANOVA) was used for continuous variables and Cochran-Mantel-Haenszel test (CMH-χ^2) or Fisher's exact test was used for categorical variables in three or more group comparisons. Kaplan-Meier method was used to estimate PFS, and the log-rank test was used to assess survival difference between groups. All tests were two-sided and a P value of <0.05 was considered statistically significant. The statistical analyses were performed using SAS (version 3.1; SAS Institute, Cary, NC, USA), GraphPad Prism (version 8.0; GraphPad Software, San Diego, CA, USA) and R (version 4.0.4; the R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

A total of 66 patients with advanced (IIIB/IV) LADC patients with *ALK* rearrangement treated with alectinib were recruited from Shanghai Chest Hospital, from January 2018 to December 2021. The enrolled cases included 29 male and 37 female, with a median age of 53 years. Sixty-four patients were treated with alectinib in the first-line and two patients in the second-line. Most (66.7%) of the cases had no history of smoking. There were three main fusion types detected: *EML4-ALK* (E6:A20) (v3) (n=16), *EML4-ALK* (E13:A20) (v1) (n=23), and *EML4-ALK* (E20:A20) (v2) (n=11). The basic characteristics of the patients are shown in *Table 1*. *Figure 1* demonstrates the mutation landscape and the corresponding clinical features. No significant differences in clinical features between the different fusion types were noted.

Analysis of ALK fusion types and assessment of efficacy

In this analysis, among all 66 cases, 64 cases had simple *ALK*-rearrangement type, two cases had more than simple *ALK* rearrangement type (one double and one triple), and a total of 17 *ALK* rearrangement types were detected overall (*Table 2*, *Figure 2A*). After alectinib therapy, best overall response (BOR) was observed in five *ALK* rearrangement types, including *EML4-ALK* v3 (ORR: 5/16, 31.3%), *EML4-ALK* v1 (ORR: 3/23, 13.0%), *EML4-ALK* v2 (ORR: 2/11, 18.2%), *EML4-ALK* (E2:A20) variant 5 (v5) (ORR: 1/1, 100.0%), and *MSH2-ALK* (M7:A20) (ORR: 1/1, 100.0%). Disease progression after alectinib therapy occurred in patients with the following fusion types: *EML4-ALK* v3, *EML4-ALK* v1, *EML4-ALK* (K24:A20) variant, and *SPECC1L-ALK* (S8:A20) (*Table 2*). Comparative analysis revealed that after treatment with alectinib, the 3 major *ALK* rearrangement types (*EML4-ALK* v3, *EML4-ALK* v1,

Table 1 Baseline demographic and clinical characteristics of patients stratified by type of *ALK*-rearrangements

Characteristics	Total (n=66)	*EML4-ALK* (E6:A20) (n=16)	*EML4-ALK* (E13:A20) (n=23)	*EML4-ALK* (E20:A20) (n=11)	Others (n=16)	P value
Age (years)	53 [24, 77]	55 [41, 62]	67 [32, 73]	54 [32, 68]	64 [24, 77]	–
Gender						0.9280
Male	29 (43.9)	7 (43.8)	9 (39.1)	5 (45.5)	8 (50.0)	
Female	37 (56.1)	9 (56.3)	14 (60.9)	6 (54.5)	8 (50.0)	
Smoking status						0.7074
Current/former	22 (33.3)	4 (25.0)	7 (30.4)	4 (36.4)	7 (43.7)	
Never	44 (66.7)	12 (75.0)	16 (69.6)	7 (63.6)	9 (56.3)	
Stage						0.839
IIIB	21 (31.8)	4 (25.0)	8 (34.8)	3 (27.3)	6 (37.5)	
IV	45 (68.2)	12 (75.0)	15 (65.2)	8 (72.7)	10 (62.5)	
Genetic mutations						
TP53	18 (27.3)	6 (37.5)	6 (26.1)	2 (18.2)	4 (25.0)	0.74
TSC1	5 (7.6)	1 (6.3)	0 (0.0)	0 (0.0)	4 (25.0)	0.07
CDKN2A	4 (6.1)	1 (6.3)	1 (4.3)	1 (9.1)	1 (6.3)	0.877
ERBB1–4	8 (12.1)	1 (6.3)	3 (13.0)	1 (9.1)	3 (18.8)	0.77
PD-L1 status						0.357
<1%	18 (27.3)	3 (18.8)	10 (43.5)	3 (27.3)	2 (12.5)	
1–50%	17 (25.8)	3 (18.8)	6 (26.1)	3 (27.3)	5 (31.3)	
>50%	8 (12.1)	2 (12.5)	3 (13.0)	1 (9.1)	2 (12.5)	
Not examined	23 (34.8)	8 (50.0)	4 (17.4)	4 (36.4)	7 (43.7)	
Specific metastatic sites						0.606
Liver	3 (4.5)	1 (6.3)	0 (0.0)	0 (0.0)	2 (12.5)	
Bone	18 (27.3)	5 (31.3)	4 (17.4)	2 (18.2)	7 (43.7)	
Brain	9 (13.6)	3 (18.8)	4 (17.4)	0 (0.0)	2 (12.5)	
ECOG PS						0.694
0–1	64 (97.0)	16 (100.0)	22 (95.7)	11 (100.0)	15 (93.8)	
2	2 (3.0)	0 (0.0)	1 (4.3)	0 (0.0)	1 (6.2)	
Lines						0.694
First	64 (97.0)	16 (100.0)	22 (95.7)	11 (100.0)	15 (93.8)	
Second	2 (3.0)	0 (0.0)	1 (4.3)	0 (0.0)	1 (6.2)	

Data are presented as median [range] or n (%). *ALK*, anaplastic lymphoma kinase; *EML4*, echinoderm microtubule-associated protein-like 4; PD-L1, programmed cell death ligand 1; ECOG PS, Eastern Cooperative Oncology Group performance status.

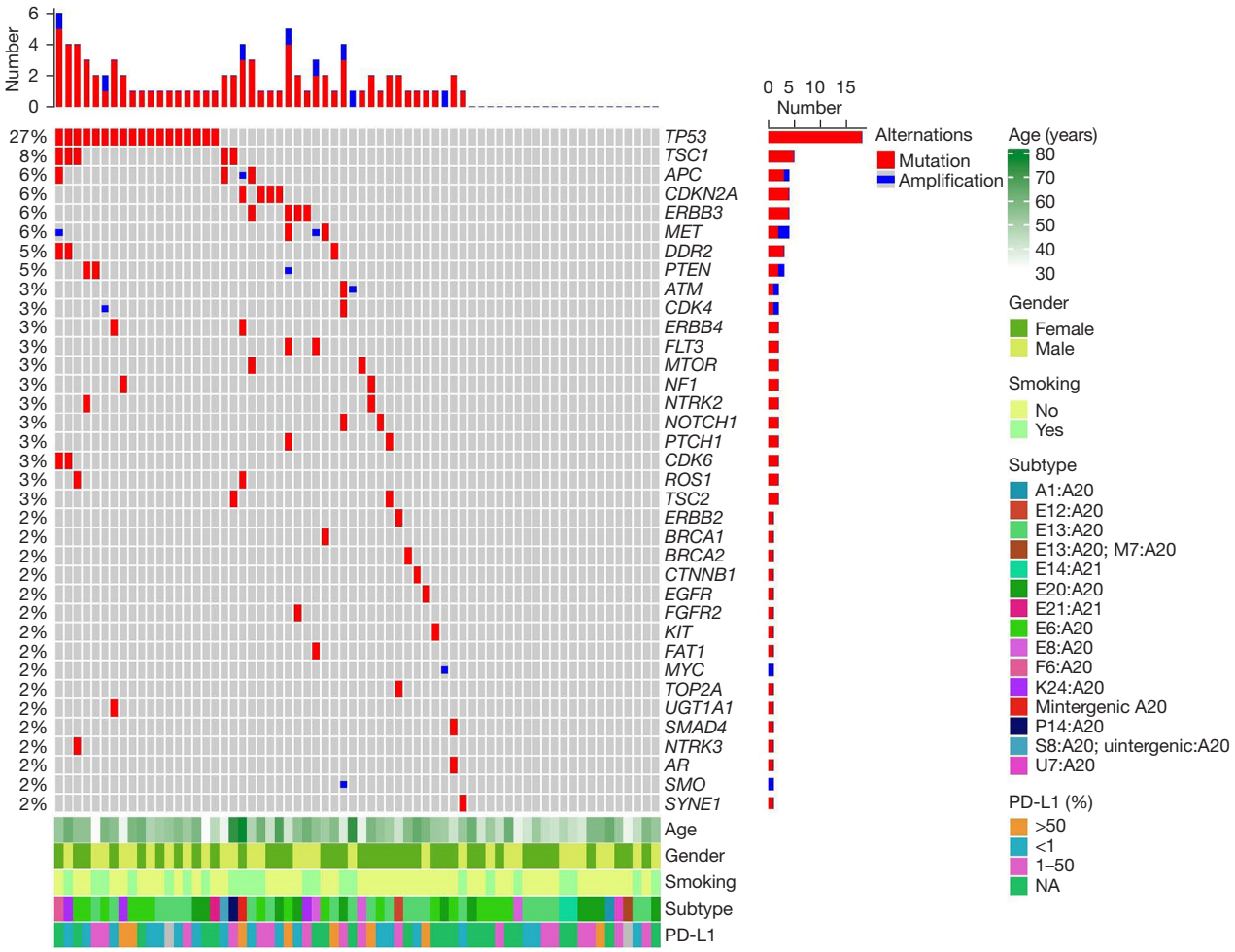

Figure 1 The OncoPrint of the somatic SNVs in 66 patients in our study. The genes are ranked by the frequency of the mutations across all samples. PD-L1, programmed cell death ligand 1; NA, not applicable; SNV, single nucleotide variant.

and *EML4-ALK* v2) had the following ORR: 31.3%, 13.0%, and 18.2%, respectively (P=0.378) and DCR: 93.8%, 95.6%, and 100.0%, respectively (P=0.720) (*Figure 2B*).

PFS analysis

The median follow-up time was 23.1 months (range, 2.9–58.7 months), the mPFS was not reached (*Figure 3A*), with a 1-year PFS rate of 80.3% (53/66), a 2-year PFS rate of 47.0% (31/66), and a 3-year PFS rate of 19.7% (13/66) (*Figure 3B*).

Analysis of fusion types showed that patients carrying *EML4-ALK* v3 had a mPFS of 33.2 months, the mPFS for other mutation types was not yet mature (*Figure 3C*). The 1-year PFS rates for *EML4-ALK* v3, *EML4-ALK* v1, *EML4-ALK* v2, and other were 81.3% (13/16), 78.3% (18/23), 72.7% (8/11), and 93.8% (15/16), respectively (P=0.511); the 2-year PFS rates were 50.0% (8/16), 39.1% (9/23), 45.5% (5/11), and 62.5% (10/16), respectively (P=0.555).

The 3-year PFS rates were 25.0% (4/16) *vs.* 13.0% (3/23) *vs.* 27.3% (3/11) *vs.* 18.8% (3/16) (P=0.725) (*Figure 3D*). *Figure 3E* shows the PFS for each patient.

Co-mutation analysis

We analyzed other gene mutations that coexisted with *ALK*-rearrangements, with *TP53*, *TSC1*, *CDKN2A*, and *ERBB* (including *ERBB*1–4) being the more frequent co-mutated genes in our study cohort (*Figure 4A*). The results showed that the mPFS of patients with concurrent *TP53*

Table 2 Analysis of *ALK*-rearrangements and BOR

Type of *ALK* rearrangement	BOR	Number of cases	ORR (%)	DCR (%)
EML4-ALK (E2:A20)	PR	1	100.0	100.0
EML4-ALK (E6:A20)	PR	5	31.3	93.8
	SD	10		
	PD	1		
EML4-ALK (E8:A20)	SD	2	0.0	100.0
EML4-ALK (E13:A19)	SD	1	0.0	100.0
EML4-ALK (E13:A20)	PR	3	13.0	95.6
	SD	19		
	PD	1		
EML4-ALK (E14:A21)	SD	1	0.0	100.0
EML4-ALK (E20:A20)	PR	2	18.2	100.0
	SD	9		
EML4-ALK (E21:A20)	SD	1	0.0	100.0
EML4-ALK (E21:A21)	SD	1	0.0	100.0
EML4-ALK (K24:A20)	SD	1	0.0	50.0
	PD	1		
FAM179A-ALK (F6:A20)	SD	1	0.0	100.0
KIF5B-ALK (K24:A20)	SD	1	0.0	100.0
UAP1L1-ALK (U77:A20)	SD	1	0.0	100.0
PRKCG-ALK (P14:A20)	SD	1	0.0	100.0
EML4-ALK (E13:A20), *MSH2-ALK* (M7:A20)	PR	1	100.0	100.0
ACOXL-ALK (A1:A20), *LOC729506-ALK* (L3:A20), *DCTN1-ALK* (D26:A20)	SD	1	100.0	100.0
SPECC1L-ALK (S8:A20)	PD	1	0.0	0.0

ALK, anaplastic lymphoma kinase; BOR, best overall response; ORR, objective response rate; DCR, disease control rate; *EML4*, echinoderm microtubule-associated protein-like 4; PR, partial response; SD, stable disease; PD, progressive disease.

mutation was 30.4 months, significantly shorter than those without *TP53* co-mutation, not applicable (NA) (P=0.026) (*Figure 4B*). Further the ORR was 17% and 19% (P=0.318) and the DCR was 83% and 100% (P=0.090) for patients with and without co-mutations, respectively. Our analysis found that *TSC1* co-mutation was also a detrimental factor in outcome. The mPFS of patients with *TSC1* co-mutation was also 30.4 months (*Figure 4C*). In patients with and without *TSC1* co-mutation, ORR were 20% and 19%, respectively (P=0.455), and DCR were 60% and 100%, respectively (P=0.031) (*Figure 4D*).

The effect of specific metastatic sites on the efficacy of alectinib

We analyzed the efficacy of alectinib in patients with bone metastases and brain metastases. The results found a trend towards more benefit for patients with brain metastases compared to those with bone metastases. The efficacy of alectinib in patients with brain metastases was comparable to

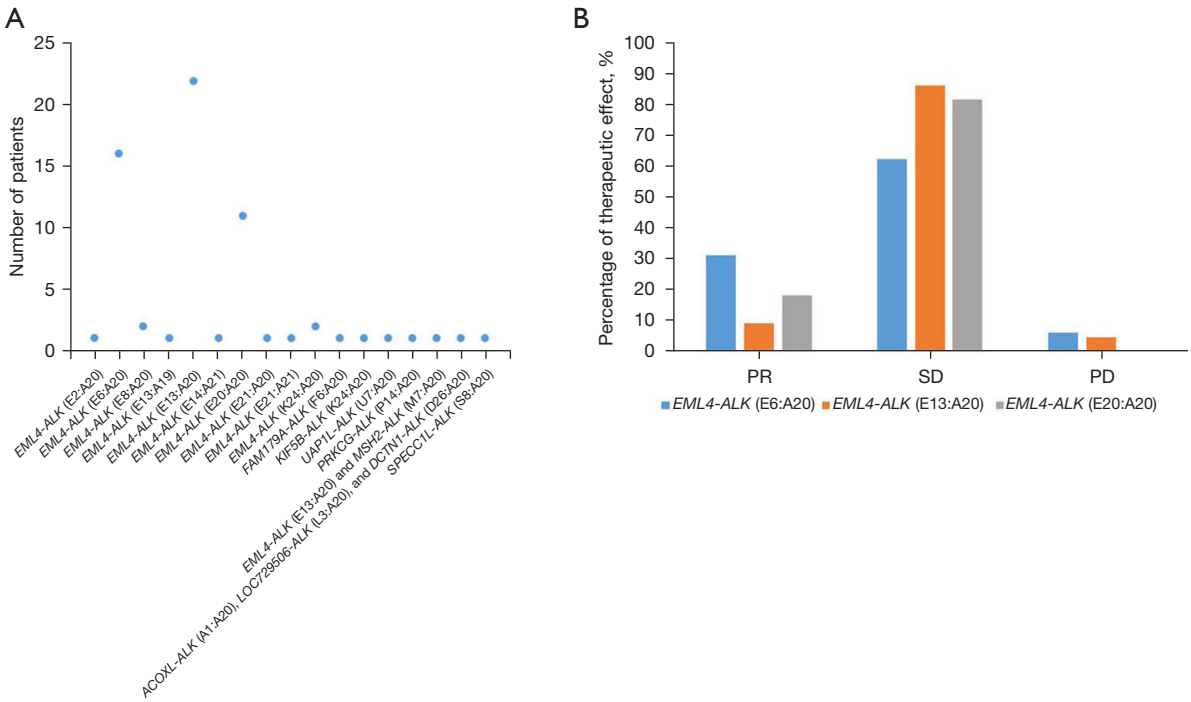

Figure 2 Analysis of *ALK*-rearrangement types. (A) Different *ALK*-rearrangement types in LADC patients. (B) BOR analysis on *EML4-ALK* (E6:A20), *EML4-ALK* (E13:A20), and *EML4-ALK* (E20:A20). *EML4*, echinoderm microtubule-associated protein-like 4; *ALK*, anaplastic lymphoma kinase; PR, partial response; SD, stable disease; PD, progressive disease; LADC, lung adenocarcinoma; BOR, best overall response.

that of patients without distant organ metastases (*Figure 5*).

Efficacy of alectinib for specific fusion types

We analyzed the efficacy of alectinib in two patients with specific rearrangements.

LADC patient 1, with double *ALK* rearrangements: *EML4-ALK* v1 and *MSH2-ALK* (M7:A20), achieved PR after alectinib therapy.

Patient 1, a 55-year-old man, was diagnosed as LADC in our hospital, on 8 July 2020. NGS detection revealed that this patient had a double *ALK*-rearrangement: *EML4-ALK* v1 and *MSH2-ALK* (M7:A20) rearrangement subtype. Alectinib (600 mg, twice a day) was given from 24 July 2020; he was followed-up until 13 April 2021; his PFS was 8.7 months and PR was the best response achieved (*Figure 6*).

Another patient with a rare *SPECC1L-ALK* (S8:A20) rearrangement displayed progressive disease (PD) after first-line alectinib treatment. Second-line treatment with pemetrexed + carboplatin + alectinib achieved a stable outcome.

Patient 2, a 37-year-old woman, was diagnosed as advanced LADC in our hospital on 28 December 2018. NGS detection revealed that this patient had *SPECC1L-ALK* (S8:A20) rearrangement subtype. On 7 January 2019, alectinib (600 mg, twice a day) was used for therapy. In November 2019, chest CT assessed a PR. Subsequently, radiotherapy was initiated for the first and second lumbar vertebrae metastases. On 24 December 2019, a bone scan showed new lesions in the skull. From 21 January 2020, pemetrexed + carboplatin regimen chemotherapy (pemetrexed 800 mg + carboplatin 500 mg) was used, and oral alectinib was continued, at the same time with lncadronate disodium to treat the bone metastases.

Efficacy assessed as SD on 16 July 2020. From August 14, 2020, the patient received pemetrexed single-agent 800 mg chemotherapy, lncadronate disodium treatment for bone metastases, and continued oral alectinib until 31 March 2023, achieving a best response of PR. Second-line treatment PFS was more than 38 months at last follow-up (*Figure 7*).

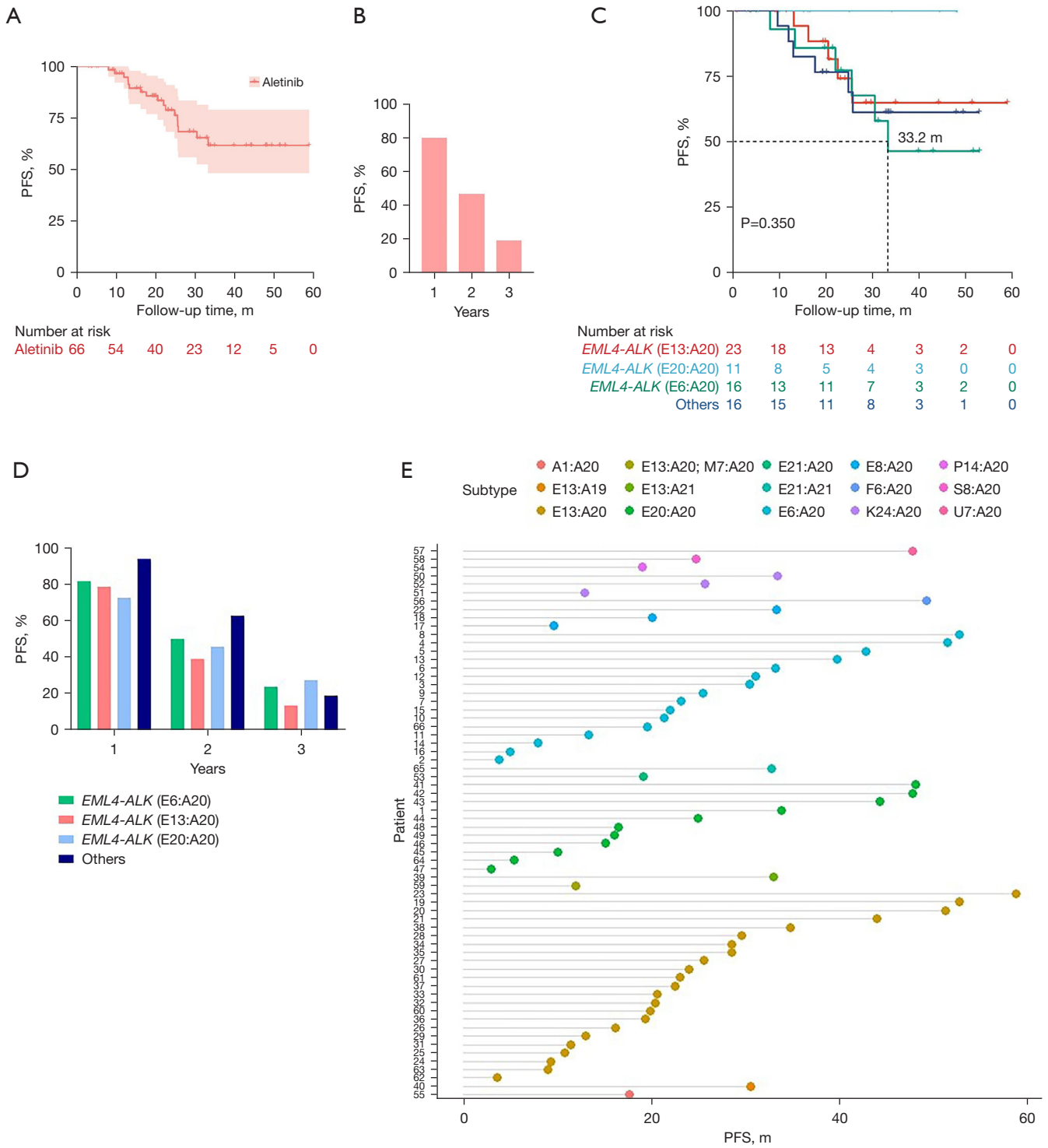

Figure 3 PFS analysis. (A) PFS for the whole cohort. (B) Three-year PFS rates for the entire cohort. (C) PFS of patients with different *ALK*-rearrangement types in LADC. (D) Three-year PFS rates for different fusion types. (E) PFS data for each patient. PFS, progression-free survival; m, months; *EML4*, echinoderm microtubule-associated protein-like 4; *ALK*, anaplastic lymphoma kinase; LADC, lung adenocarcinoma.

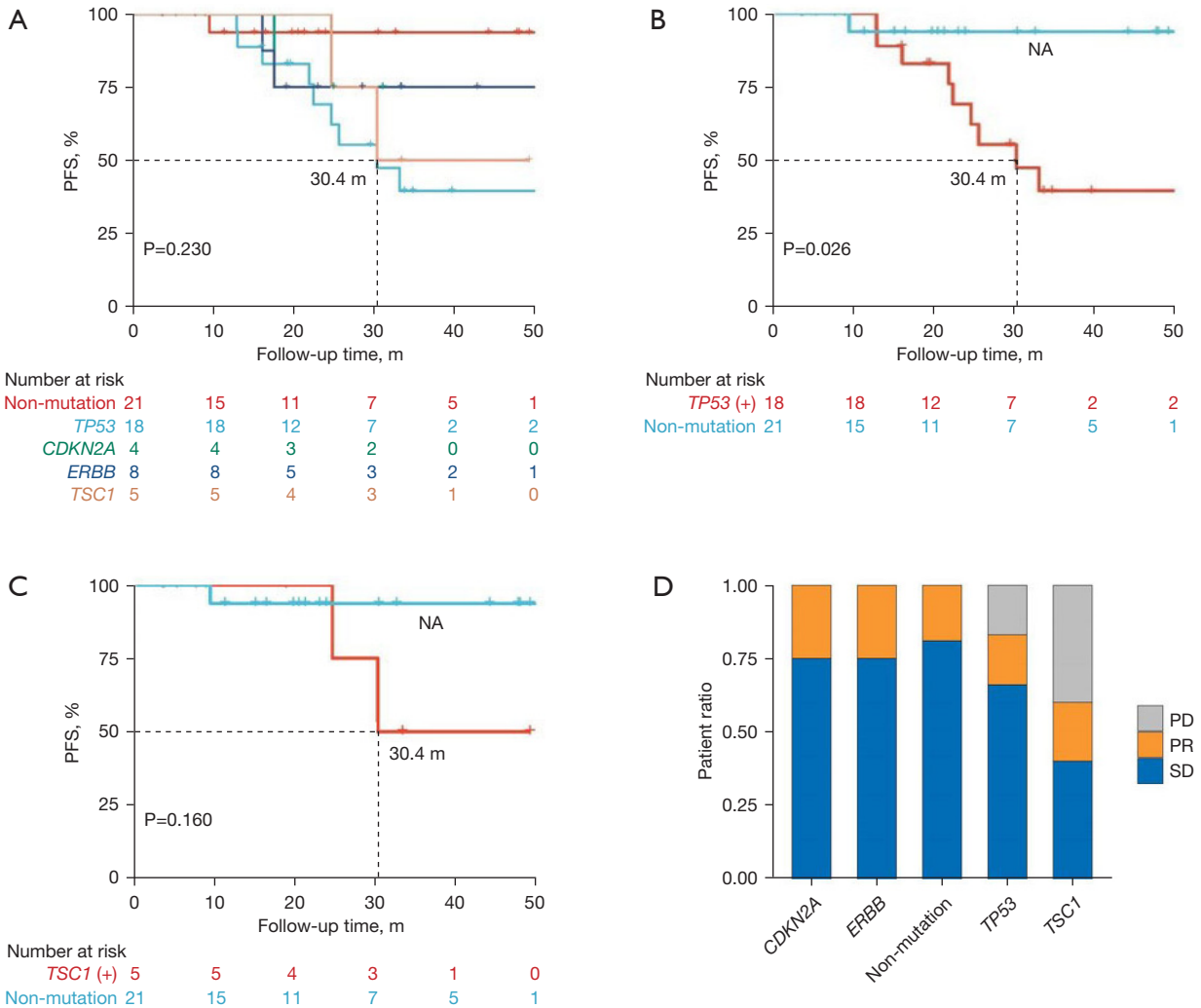

Figure 4 Co-mutation analysis. (A) Analysis of PFS carrying different types of co-mutations. (B) Analysis of PFS in patients with and without *TP53* co-mutations. (C) Analysis of PFS in patients with and without *TSC1* co-mutations. (D) Therapeutic effect (PD, PR, and SD) analysis on patients with different types of co-mutations. PFS, progression-free survival; m, months; NA, not applicable; PD, progressive disease; PR, partial response; SD, stable disease.

Discussion

Improving survival in patients with advanced NSCLC has been an area of intense research interest, and recent advances in targeted therapies have showed prolonged survival outcomes in NSCLC, particularly in patients carrying *EML4-ALK*-rearangements with median OS of 7 years (24,25). The ALEX study showed a significant improvement in PFS with alectinib compared to crizotinib in patients with naive *ALK*-rearranged NSCLC (26). Our previous study has analyzed the efficacy of alectinib in real-world *ALK*-rearranged patients (27). However, with the use and access to NGS technology, more and more new *ALK*-rearrangement types are being detected. There are currently few studies on the difference in efficacy of alectinib against different *ALK*-rearrangements. The use of individualized treatment for different fusion types is of great significance for patients. In this study, we retrospectively analyzed the efficacy of alectinib in different *ALK*-rearrangements with the aim of providing a reference for clinical treatment.

Among the 66 *ALK*-positive NSCLC cases we included, the top 3 rearrangement types were *EML4-ALK* v3, *EML4-ALK* v1, and *EML4-ALK* v2. Consistent with previous reports, the major partner of *ALK* rearrangements in

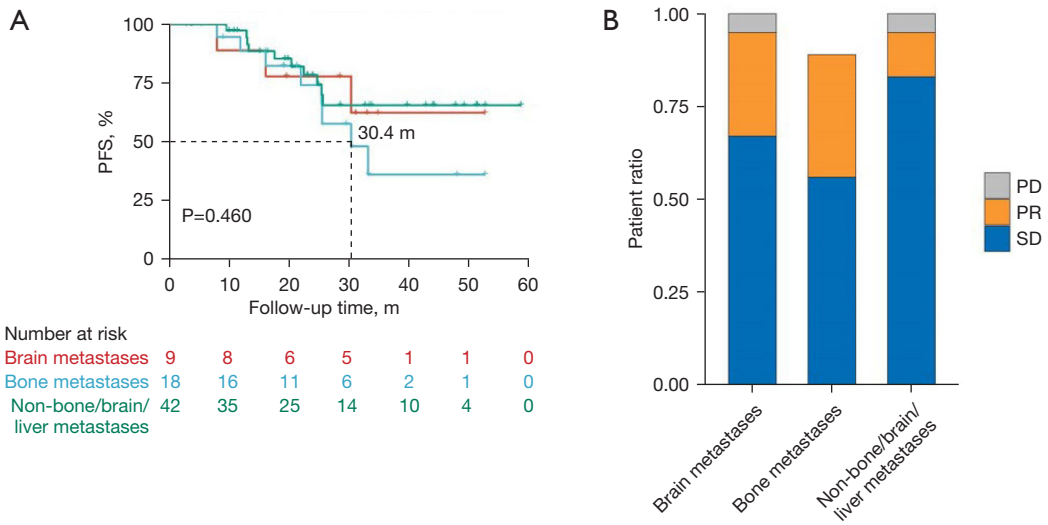

Figure 5 Efficacy of alectinib in patients with or without Specific metastasis site. (A) PFS of alectinib treatment in patients with different metastatic sites. (B) BOR of patients with different metastatic sites. PFS, progression-free survival; m, months; PD, progressive disease; PR, partial response; SD, stable disease; BOR, best overall response.

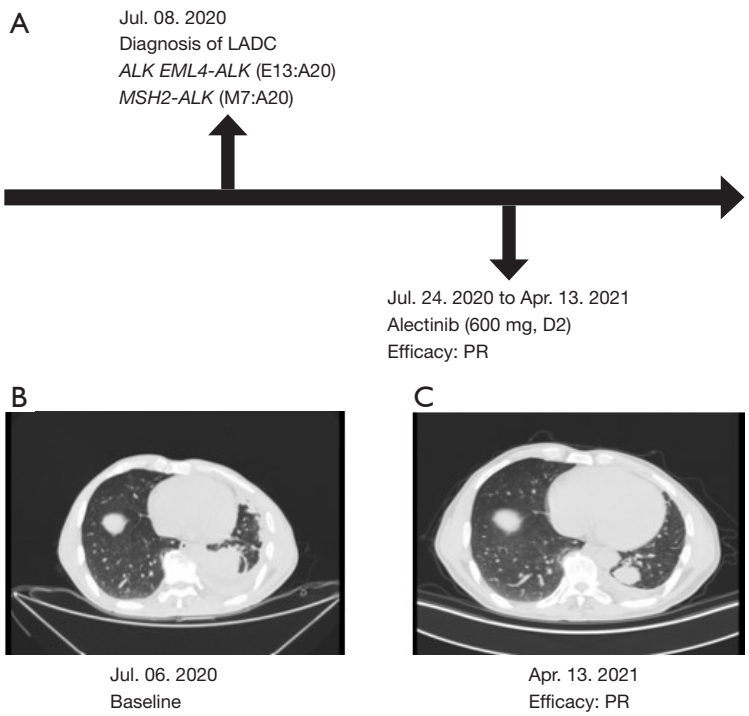

Figure 6 The therapeutic schedule of patient 1. (A) The details of therapeutic schedule. (B,C) CT images of patient 1. LADC, lung adenocarcinoma; *ALK*, anaplastic lymphoma kinase; *EML4*, echinoderm microtubule-associated protein-like 4; D2, twice a day; PR, partial response; CT, computed tomography.

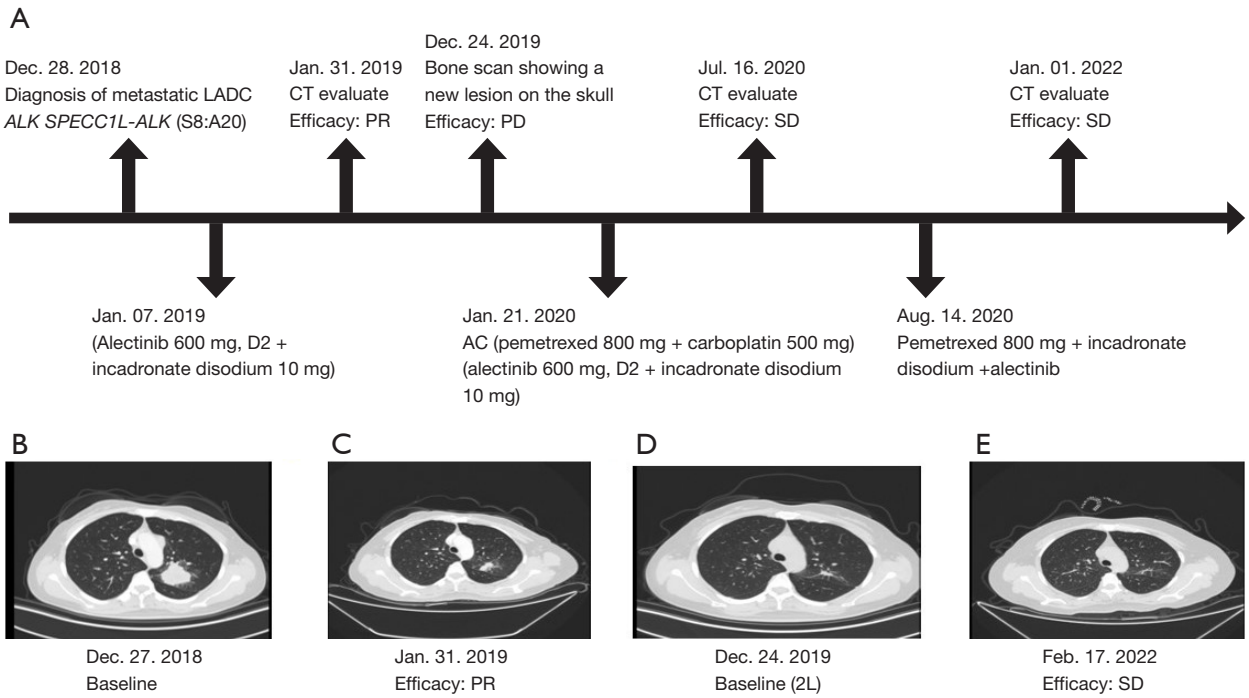

Figure 7 The therapeutic schedule of patient 2. (A) The details of therapeutic schedule. (B-E) CT images of patient 2. LADC, lung adenocarcinoma; *ALK*, anaplastic lymphoma kinase; CT, computed tomography; PR, partial response; PD, progressive disease; SD, stable disease; D2, twice a day; AC, pemetrexed/carboplatin.

NSCLC was the *EML4* gene (6). Over 15 *EML4-ALK* variants have been identified to date, the most common of which are v1 [exon 13 of *EML4* fused to exon 20 of *ALK* (E13:A20)] and v3a/b [exon 6a/b of *EML4* fused to exon 20 of *ALK* (E6a/b:A20)] (28,29). Previous studies of crizotinib have shown differences in patient response to crizotinib based on *ALK* variants. For example, v1 had a longer response to crizotinib compared to v3 (30,31). The other two studies found no difference in clinical response to crizotinib based on *ALK* variants (32,33). Furthermore, ALEX study showed that ORR of alectinib for *EML4-ALK* v1 was 90%, and for *EML4-ALK* v3 was 68% (34). Correspondingly, data from ALTA-1L study showed that ORR of brigatinib was 84% for *EML4-ALK* v1, and 91% for *EML4-ALK* v3 (35). Finally, *EML4-ALK* v3 showed the lowest sensitivity for crizotinib compared with other variants (36). This highlights the need for further research. In this study, we analyzed the response of *ALK* variants to alectinib and found that *EML4-ALK* v2 rearrangement had a higher DCR and longer PFS than other types, although the difference is not statistically significant. In our study, the ORR in *EML4-ALK* v3 was only 31%, while in ALEX study it was 68%, this might be due potentially to differences in the types of co-mutations carried by patients. We also investigated the effect of co-mutations on the efficacy of alectinib and determined the top four co-mutations in our included population. These were *TP53*, *TSC1*, *ERBB* (including *ERBB*1–4), and *CDKN2A*. Patients carrying *TP53* mutations had shorter PFS; patients harboring *TSC1* mutations had significantly lower DCR than those without co-mutations. In line with previous studies, alectinib remained effective in our study of patients with brain metastases. In addition, we identified for the first time two specific fusion types that also responded well to alectinib, namely double fusion of *EML4-ALK* (E13:A20) co-existing with *MSH2-ALK* (M7:A20), and *SPECC1L-ALK* (S8:A20), a rare fusion partner.

With the discovery of new drug targets and the continuous emergence of new combination therapies, how to maximize the benefit of patients is a problem that clinicians need to consider (37). Defining the best drug treatment scheme can not only prolong the survival time and improve the quality of life of NSCLC patients but also reduce the economic pressure on patients. Sequential

therapy and combined therapy have been put forward and put into practice. At present, the sequential sequence of chemotherapy and ALK inhibitors is still controversial and needs to be further studied in prospective large-sample trials. In the past 5 years, tumor immunotherapy has opened up a new field of treatment for NSCLC patients. In 2018, the American Society of Clinical Oncology announced the results of alectinib combined with atezolizumab in treating patients with stage I b *ALK* rearrangement NSCLC. The total ORR of 21 patients was 81%. The incidence of grade 3 adverse events was 62%. There were no serious adverse events above grade 4, and the overall effect was satisfactory (38). In the future, the problems of alectinib sequence and combined immunotherapy after the failure of chemotherapy and the progress of crizotinib treatment need to be further studied. Our study shows that different *ALK* rearrangement types and co-mutations respond differently to alectinib therapy. This suggests we need subgroup analyses of different *ALK* rearrangement types and co-mutations in future research to determine the best individualized treatment.

The interpretation of our findings may be limited by the retrospective nature of this study. The small sample sizes for some fusion types may have introduced bias. In addition, the overall survival results for groups were too early before the data cut-off and required further analyses. Prospective, randomized trials in larger populations are needed to confirm these findings and enable a more personal therapeutic approach.

Conclusions

Our study showed a slight difference in the efficacy of alectinib for different types of *ALK* rearrangements. *EML4-ALK* (E20:A20) had higher 3-year PFS rates and DCR among the four primary fusion types, although the difference is not significant. PFS was shorter in patients with *TP53* co-mutations; DCR was significantly lower in patients with *TSC1* co-mutations than in those without co-mutations. In addition, we found that two patients carrying specific *ALK*-rearrangements still responded better to treatment with alectinib.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-658/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-658/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-658/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-658/coif). Y.Z. and X.Z. are from 3D Medicines Inc., Shanghai, China. A.R. has received advisory board honoraria from AstraZeneca, MSD, Novartis, Pfizer, BMS, and Amgen; writing engagement honoraria from AstraZeneca, MSD, Roche and Novartis; speaker bureau from AstraZeneca and BMS. A.R. reports research funding from EMQN, GECP; consulting fees from AstraZeneca; payment or honoraria for lectures, presentations, speaker bureaus from Thermofisher Scientific, Illumina, Health in code; and travel expenses from Thermofisher Scientific, Bristol Myers Squibb Foundation, Takeda. She also serves as advisory board member in Takeda. E.M.U. reports honoraria for lectures from Amgen, AstraZeneca, Janssen, Novartis; support from AstraZeneca for participation at IASLC WCLC 2023, Singapore, and advisory board member in Pfizer, Roche, Takeda. 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). The study was approved by the Ethics Committees of Shanghai Chest Hospital (No. KS22002), and individual consent for this retrospective analysis was waived.

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References

1. Schneider JL, Lin JJ, Shaw AT. ALK-positive lung cancer: a moving target. Nat Cancer 2023;4:330-43.
2. Hrustanovic G, Olivas V, Pazarentzos E, et al. RAS-MAPK dependence underlies a rational polytherapy strategy in EML4-ALK-positive lung cancer. Nat Med 2015;21:1038-47.
3. Yang L, Li G, Zhao L, et al. Blocking the PI3K pathway enhances the efficacy of ALK-targeted therapy in EML4-ALK-positive nonsmall-cell lung cancer. Tumour Biol 2014;35:9759-67.
4. Li Y, Li Y, Zhang H, et al. EML4-ALK-mediated activation of the JAK2-STAT pathway is critical for non-small cell lung cancer transformation. BMC Pulm Med 2021;21:190.
5. Cooper AJ, Sequist LV, Lin JJ. Third-generation EGFR and ALK inhibitors: mechanisms of resistance and management. Nat Rev Clin Oncol 2022;19:499-514.
6. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
7. Bulutay P, AkyÜrek N, MemiŞ L. Clinicopathological and Prognostic Significance of the EML4-ALK Translocation and IGFR1, TTF1, Napsin A Expression in Patients with Lung Adenocarcinoma. Turk Patoloji Derg 2021;37:7-17.
8. Wang H, Zhang W, Wang K, et al. Correlation between EML4-ALK, EGFR and clinicopathological features based on IASLC/ATS/ERS classification of lung adenocarcinoma. Medicine (Baltimore) 2018;97:e11116.
9. Sabir SR, Yeoh S, Jackson G, et al. EML4-ALK Variants: Biological and Molecular Properties, and the Implications for Patients. Cancers (Basel) 2017;9:118.
10. Noh KW, Lee MS, Lee SE, et al. Molecular breakdown: a comprehensive view of anaplastic lymphoma kinase (ALK)-rearranged non-small cell lung cancer. J Pathol 2017;243:307-19.
11. Gridelli C, Peters S, Sgambato A, et al. ALK inhibitors in the treatment of advanced NSCLC. Cancer Treat Rev 2014;40:300-6.
12. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2017;377:829-38.
13. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 2014;371:2167-77.
14. Hida T. Anaplastic lymphoma kinase inhibitor development: enhanced delivery to the central nervous system. Transl Lung Cancer Res 2023;12:1822-5.
15. Zhang SS, Nagasaka M, Zhu VW, et al. Going beneath the tip of the iceberg. Identifying and understanding EML4-ALK variants and TP53 mutations to optimize treatment of ALK fusion positive (ALK+) NSCLC. Lung Cancer 2021;158:126-36.
16. Tabbò F, Muscarella LA, Gobbini E, et al. Detection of ALK fusion variants by RNA-based NGS and clinical outcome correlation in NSCLC patients treated with ALK-TKI sequences. Eur J Cancer 2022;174:200-11.
17. Long X, Wu H, Yang C, et al. Complex genetic alterations contribute to rapid disease progression in an ALK rearrangement lung adenocarcinoma patient: a case report. Transl Cancer Res 2021;10:3081-6.
18. Li M, Tang Q, Chen S, et al. A novel HIP1-ALK fusion variant in lung adenocarcinoma showing resistance to Crizotinib. Lung Cancer 2021;151:98-100.
19. Pan X, Zhong A, Xing Y, et al. A novel GHR-ALK fusion gene in a patient with metastatic lung adenocarcinoma and its response to crizotinib: a case report. J Int Med Res 2021;49:3000605211044652.
20. Song P, Zhang J, Shang C, et al. Alectinib treatment response in lung adenocarcinoma patient with novel EML4-ALK variant. Thorac Cancer 2018;9:1327-32.
21. Zhong JM, Zhang GF, Lin L, et al. A novel EML4-ALK BIRC6-ALK double fusion variant in lung adenocarcinoma confers sensitivity to alectinib. Lung Cancer 2020;145:211-2.
22. Sasaki T, Yoshida R, Nitanai K, et al. Detection of resistance mutations in patients with anaplastic lymphoma kinase-rearranged lung cancer through liquid biopsy. Transl Lung Cancer Res 2023;12:1445-53.
23. Zhou S, Sun G, Wang J, et al. Anaplastic lymphoma kinase (ALK) rearrangement in adult renal cell carcinoma with lung metastasis: a case report and literature review. Transl Androl Urol 2020;9:2855-61.
24. Pacheco JM, Gao D, Smith D, et al. Natural History and Factors Associated with Overall Survival in Stage IV ALK-Rearranged Non-Small Cell Lung Cancer. J Thorac Oncol

2019;14:691-700.
25. Pacheco JM, Camidge DR. Is long-term survival possible for patients with stage IV ALK+ non-small cell lung cancer? Expert Rev Respir Med 2019;13:399-401.
26. Mok T, Camidge DR, Gadgeel SM, et al. Updated overall survival and final progression-free survival data for patients with treatment-naive advanced ALK-positive non-small-cell lung cancer in the ALEX study. Ann Oncol 2020;31:1056-64.
27. Su C, Zhou J, Qiang H, et al. Special issue "The advance of solid tumor research in China": Real-world clinical outcomes of alectinib for advanced nonsmall-cell lung cancer patients with ALK fusion in China. Int J Cancer 2023;152:15-23.
28. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008;14:4275-83.
29. Sasaki T, Rodig SJ, Chirieac LR, et al. The biology and treatment of EML4-ALK non-small cell lung cancer. Eur J Cancer 2010;46:1773-80.
30. Yoshida T, Oya Y, Tanaka K, et al. Differential Crizotinib Response Duration Among ALK Fusion Variants in ALK-Positive Non-Small-Cell Lung Cancer. J Clin Oncol 2016;34:3383-9.
31. Woo CG, Seo S, Kim SW, et al. Differential protein stability and clinical responses of EML4-ALK fusion variants to various ALK inhibitors in advanced ALK-rearranged non-small cell lung cancer. Ann Oncol 2017;28:791-7.
32. Cha YJ, Kim HR, Shim HS. Clinical outcomes in ALK-rearranged lung adenocarcinomas according to ALK fusion variants. J Transl Med 2016;14:296.
33. Lei YY, Yang JJ, Zhang XC, et al. Anaplastic Lymphoma Kinase Variants and the Percentage of ALK-Positive Tumor Cells and the Efficacy of Crizotinib in Advanced NSCLC. Clin Lung Cancer 2016;17:223-31.
34. Camidge DR, Dziadziuszko R, Peters S, et al. Updated Efficacy and Safety Data and Impact of the EML4-ALK Fusion Variant on the Efficacy of Alectinib in Untreated ALK-Positive Advanced Non-Small Cell Lung Cancer in the Global Phase III ALEX Study. J Thorac Oncol 2019;14:1233-43.
35. Camidge DR, Kim HR, Ahn MJ, et al. Brigatinib Versus Crizotinib in ALK Inhibitor-Naive Advanced ALK-Positive NSCLC: Final Results of Phase 3 ALTA-1L Trial. J Thorac Oncol 2021;16:2091-108.
36. Heuckmann JM, Balke-Want H, Malchers F, et al. Differential protein stability and ALK inhibitor sensitivity of EML4-ALK fusion variants. Clin Cancer Res 2012;18:4682-90.
37. Chen MF, Chaft JE. Early-stage anaplastic lymphoma kinase (ALK)-positive lung cancer: a narrative review. Transl Lung Cancer Res 2023;12:337-45.
38. Kim DW, Gadgeel S, Gettinger SN, et al. Brief Report: Safety and Antitumor Activity of Alectinib Plus Atezolizumab From a Phase 1b Study in Advanced ALK-Positive NSCLC. JTO Clin Res Rep 2022;3:100367.

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