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ORIGINAL RESEARCH

Frequency, clinical features and differential response to therapy of concurrent *ALK/EGFR* alterations in Chinese lung cancer patients

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Purpose: *EGFR* and anaplastic lymphoma kinase (*ALK*) alterations have been regarded as oncogenic drivers and incorporated into clinical practices to manage nonsmall cell lung cancer (NSCLC). Alterations of these two genes were traditionally considered to be mutually exclusive, but recent studies have suggested that they can occur concomitantly. Here, we investigated the prevalence, clinical features and outcomes in response to the treatment of NSCLC patients who harbor *EGFR* and *ALK* co-alterations.

Methods: We reviewed the genomic profiles of 419 *ALK*-rearranged NSCLC patients with the intent of investigating the *EGFR* kinase domain (exon 18–21) and *ALK* co-alterations. The genomes of these patients were sequenced in a Clinical Laboratory Improvement Amendments-certified laboratory.

Results: The overall frequency of concomitant *EGFR* (exon 18–21) and *ALK* alterations was 5.01% (21/419) in *ALK*-rearranged NSCLC patients. The concomitant rate of *EGFR* alterations in patients with *EML4-ALK* co-alterations (3.06%, 11/359) was dramatically lower than that in patients with non-*EML4-ALK* co-alterations (16.67%, 10/60, $p<0.01$). *EML4-ALK*/*EGF R* co-alterations were more prone to occur in females than in males, and non-*EML4-ALK*/*EGFR* co-alterations were more common in males than in females ($p=0.02$). Before the detection of *EGFR-ALK* co-alterations, some patients were treated with EGFR-TKIs (n=16) according to previously detected *EGFR* alterations; Kaplan–Meier analysis revealed that *EML4-ALK*/*EGFR* co-altered patients (n=7) had a significantly shorter progression-free survival (PFS) after EGFR-TKI treatment than that of non-*EML4-ALK*/*EGFR* co-altered patients (n=8; mPFS, 6.0 vs 15.0 months, $p=0.046$). In addition, we demonstrated the subsequent clinical outcomes of co-altered patients after previous EGFR-TKI treatment. Five *EGFR*/*ALK* co-altered patients treated with single TKIs (EGFR-TKIs or ALK-TKIs) displayed diverse clinical outcomes. Three patients who received dual-TKI treatment (EGFR-TKI plus ALK-TKI) all achieved a PFS of more than 5 months (8.4 months, 8.6 months, >5.2 months).

Conclusion: *EML4-ALK/EGFR* and non-E*ML4-ALK/EGFR* co-alterations displayed distinct clinical features and responses to EGFR-TKIs, suggesting that non-*EML4-ALK* co-alterations are likely to occur as a resistance mechanism to EGFR-TKI. In addition, dual-TKI therapy might be a better choice than single-TKI treatments for these co-altered patients. To the best of our knowledge, this is the largest dual-positive *EGFR/ALK* cohort study in People's Republic of China.

Keywords: *EGFR* alteration, *ALK* rearrangement, nonsmall cell lung cancer, EML4-ALK, tyrosine kinase inhibitor

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Introduction

EGFR and anaplastic lymphoma kinase (*ALK*) gene alterations have both been regarded as oncogenic driver mutations and drug targets in nonsmall cell lung cancers (NSCLCs).[1–3] *EGFR* and *ALK* alterations were conventionally considered to be mutually exclusive[4–6] and as mutual causes of resistance to ALK-TKIs or EGFR-TKIs.[7,8] However, co-alterations of *EGFR* and *ALK* exist in a subset of NSCLCs and challenge the previous dogma.[9–11]

In *ALK*-rearranged NSCLC patients, echinoderm microtubule-associated protein-like 4 (*EML4*) is the main fusion partner of *ALK*. *EML4-ALK*, in which the N-terminal of EML4 is fused to the intracellular kinase domain of ALK, displays constitutive ALK activation and generates oncogenic activity.[4,5] In addition to *EML4*, other *ALK* 5′-partners have also been identified, including kinesin family member 5B, TRK-fused gene, and kinesin light chain 1.[12–14] The frequency of non-*EML4-ALK* alterations is approximately 10–20% in *ALK*-positive lung cancers,[15,16] and the clinical significance of these alterations still under investigation.

In previous studies about patients with *EGFR* and *ALK* co-alterations, researchers often combined patients with *EGFR/ALK* co-alterations as a single group, regardless of the *ALK* fusion partner, for clinical features or drug efficacy investigations. Little is known about the difference in clinical features and drug efficacy between the *EML4-ALK* /*EGFR* and non-*EML4-ALK*/*EGFR* co-alteration subgroups. Here, we interrogated the distinct concurrent alterations rate, clinical features, and clinical outcomes during EGFR-TKI treatment in both the *EML4-ALK* /*EGFR* and non-*EML4-ALK*/*EGFR* co-alteration subgroups. In addition, we sought to evaluate the clinical activity of these co-altered patients in response to single-TKI or dual-TKI treatments.

Materials and methods

Patient information

We retrospectively reviewed the genomic profiling data of 7,661 lung cancer patients, whose tissue or plasma samples were sequenced in a Clinical Laboratory Improvement Amendments-certified clinical molecular diagnostic laboratory using next-generation sequencing (NGS) between September 2015 and January 2018. Among them, 419 patients were identified as harboring *ALK* rearrangements. The clinical characteristics of patients harboring dual-positive *EGFR* and *ALK* alterations were collected. All patients had a histologically confirmed diagnosis of advanced-stage NSCLC. Progression-free survival (PFS) after EGFR-TKI treatment and survival information were assessed for the cohorts. The study was approved by the institutional review board of Peking University Shenzhen Hospital. All other centers were covered by this protocol. All patients whose tissue and medical data were used in this research provided written informed consent, in accordance with the Declaration of Helsinki.

Tissue DNA and plasma cfDNA preparation

The tissue DNA was extracted from all tissue samples using the QIAamp DNA FFPE tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Circulating cfDNA was recovered from 4 to 5 mL plasma by using the QIAamp Circulating Nucleic Acid kit (Qiagen). DNA was quantified with the Qubit 2.0 fluorimeter (Thermo Fisher Scientific, Waltham, MA, USA).

Targeted DNA sequencing

The genomic DNA was profiled by using capture-based targeted sequencing panel that consisted of 8, 56, 168 or 295 cancer-related genes (Burning Rock Biotech, Guangzhou, People'sRepublic of China). Alterations of eight well-established driver genes, *EGFR, ERBB2, ALK, ROS1, RET, KRAS, BRAF* and *MET*, were all included in these sequencing panels. The NGS library was prepared as previously described.[17] In brief, DNA was sheared using Covaris M220 (Covaris, Inc., Woburn, MA, USA), which was then followed by end-repair, phosphorylation and adaptor ligation. Fragments that were 200–400 bp in size were selected by beads (Agencourt AMPure XP Kit, Beckman Coulter, Brea, CA, USA), followed by hybridization with capture probes or baits, hybrid selection with magnetic beads and PCR amplification. A high-sensitivity DNA assay was performed using a bioanalyzer to evaluate the DNA quality and size. Indexed samples were then sequenced on a NextSeq 500 (Illumina, Inc., San Diego, CA, USA) with pair-end reads.

The sequencing data were in a FASTQ format and mapped to the human genome (hg19) using BWA aligner 0.7.10.[18,19] Local alignment optimization, mark duplication and variant calling were performed using the Genome Analysis ToolKit 3.2,[20] Picard (http://picard.sourceforge.net/) and VarScan.[21] Gene translocations were called with FACTERA,[22] and the copy number variation was analyzed with an in-house algorithm based on sequencing depth.

Statistical analysis

Statistical analysis was conducted using R software version 3.3.3. Between the *EML4-ALK* and non-*EML4-ALK* subgroups, differences in sex and mutation rate were calculated and presented using Fisher's exact tests, while differences in age were calculated using paired, two-tailed Student's t-tests. For all statistical tests, $p<0.05$ was considered statistically significant.

Results

Patient characteristics

The genomic sequencing results of 419 *ALK*-rearranged NSCLC patients were retrospectively reviewed, including 359 (85.7%) *EML4-ALK* fusions and 60 (14.3%) non-*EML4-ALK* fusions. Among the 419 *ALK*-rearranged lung cancers, a total of 21 patients (5.01%) were detected to harbor concurrent *ALK* and *EGFR* (exon 18–21) genomic alterations. The concomitant rate of *EGFR* alterations in patients harboring *EML4-ALK* co-alterations (3.06%, 11/359) was dramatically lower than that in non-*EML4-ALK* co-altered patients (16.67%, 10/60, $p<0.01$, Fisher's exact test, Table 1).

All the 21 *ALK/EGFR* co-altered cases were diagnosed as adenocarcinomas. In the *EML4-ALK/EGFR* co-altered subgroup, 4 (36%) patients were male, and 7 (64%) patients were female. In contrast, the non-*EML4-ALK/EGFR* co-altered subgroup comprised 9 (90%) males and 1 (10%) female ($p=0.02$, Fisher's exact test). This difference suggested that *EML4-ALK/EGFR* co-alterations were more prone to occur in females than males, and non-*EML4-ALK /EGFR* co-alterations were more common in males than in females. The median age of the *EML4-ALK/EGFR* and non-*EML4-ALK/EGFR* co-altered subgroups were 53.0 and 59.5 years, respectively ($p=0.31$, *t*-test). The patient characteristics are summarized in Table 1.

Identification of molecular patterns of co-altered ALK/EGFR

In the 11 *EML4-ALK/EGFR* co-altered patients, capture-based sequencing identified different *EML4-ALK* variants, including 6 with E13;A20 (V1), 3 with E6;A20 (V3), and 2 with E2;A20 (Figure 1). As for the 10 non-*EML4-ALK/EGFR* co-altered patients, 6 unique *ALK* fusion partners were detected. *STRN* was the most common fusion partner in non-*EML4-ALK* co-alterations and was identified in five patients (50%). Apart from those five patients, another two *STRN-ALK* positive patients were identified in the whole cohort of 419 *ALK*-rearranged NSCLC patients. One patient harbored a co-alteration of *STRN-ALK* and *EGFR* exon 15 V592I, and another patient was identified as being *STRN-ALK*-positive only. This observation suggested that co-alterations with *EGFR* were a common feature of *STRN-ALK* fusions. Before these five patients were detected to have *STRN-ALK /EGFR* (exon 18–21) co-alterations, all of them had previously been detected to have *EGFR* alterations and had been treated with EGFR-TKIs but not with ALK-TKIs, indicating that *STRN-ALK* might be one of the resistance mechanisms for EGFR-TKIs. In addition, five novel *ALK* fusion partners were identified including *WDR37-ALK, CEBPZ-ALK, KIDINS220-ALK*, *LOC284950-ALK* and *MFSD2B-ALK*.

Of the 21 patients who harbored co-altered *ALK/EGFR*, the following 11 patients with *EML4-ALK/EGFR* co-alterations were identified to have diverse *EGFR* variants: 7 patients had exon 19 deletions, 2 had exon 21 L858R point mutations, 1 had a P753S point mutation and 1 had a G863D point mutation. Meanwhile, the 10 non-*EML4-ALK/EGFR*-altered patients included 6 *EGFR* exon 19 deletions, 3 L858R point mutations and 1 V769M point mutation (Figure 2).

Is concomitant non-EML4-ALK co-alterations a de novo or acquired rearrangement?

The distribution of *EML4-ALK* and non-*EML4-ALK* co-alterations in the "*de novo ALK*", "acquired *ALK*" and "uncertain" groups are summarized in Table 2. Patients identified with co-altered *EGFR/ALK* at the time of the first diagnosis were classified as "*de novo ALK*" since they had not received TKI treatment before. This group contained 4 patients (4/5, 80%) with *EGFR/EML4-ALK* co-alterations and 1 patient (1/5, 20%) with an *EGFR*/non-*EML4-ALK* co-alteration. Patients who were identified as *EGFR* (+)/*ALK* (-) at a previous diagnosis and later detected to have co-altered *EGFR/ALK* after developing EGFR-TKI resistance were classified as "acquired *ALK*"; this group consisted of 1 patient with (1/4, 25%) an *EGFR/EML4-ALK* co-alteration and 3 patients (3/4, 75%) with *EGFR*/non-*EML4-ALK* co-alterations. In addition, patients who were detected to have *EGFR/ALK* co-alterations after developing EGFR-TKI resistance but whose previous *ALK* status was unavailable were defined as "uncertain"; this group included 6 patients with *EGFR/EML4-ALK* co-alterations and 6 patients with *EGFR*/non-*EML4-ALK* co-alterations. The observation that *EML4-ALK* co-alterations accounted for most of the rearrangements in the *de novo ALK* patients (80%) while non-*EML4-ALK* co-alterations accounted for most of the alterations in the acquired *ALK* patients (75%)

Table 1 Characteristics of patients harboring dual-positive *ALK*/*EGFR* alterations

Patient Characteristics	*EML4-ALK*	Non-*EML4-ALK*	*P*-value
Total	11	10	
Co-occurrence rate in *ALK*-rearranged NSCLC	3.06% (11/359)	16.67% (10/60)	$p<0.01$
Gender			$p=0.02$
Male	4	9	
Female	7	1	
Age (year)			$p=0.31$
Median	53	59.5	
Range	42–74	44–81	
Histological types			
Adenocarcinoma	11	10	

Abbreviations: ALK, *anaplastic lymphoma kinase*; NSCLC, nonsmall cell lung cancer; EML4, *echinoderm microtubule*-associated protein-like 4.

after EGFR-TKI treatment indicated that *EML4-ALK* co-alterations were likely to be *de novo* alterations and non-*EML4-ALK* co-alterations might be likely to appear as a drug resistance mechanism of EGFR-TKIs.

Next, for patients progressed on previous EGFR-TKI treatment ("acquired *ALK*" plus "uncertain" subgroups), we interrogated the correlation of PFS in response to EGFR-TKIs before the detection of *ALK* rearrangements and the distribution of *ALK* fusion partners after EGFR-TKI treatment. The Kaplan–Meier analysis revealed that after EGFR-TKI treatment, patients with *EML4-ALK*/*EGFR* co-alterations (n=7) commonly had a significantly shorter median PFS than that of patients with non-*EML4-ALK*/*EGFR* co-alterations (n=8, PFS information of one patient was unavailable; mPFS, 6.0 vs 15.0 months, $p=0.046$, Figure 3). The discrepancy in PFS between the two subgroups has never been reported before and could suggest that *EML4-ALK* co-alterations were likely to be induced in the early stages of EGFR-TKI treatment, whereas non-*EML4-ALK* co-alterations might be acquired at relatively late stages after EGFR-TKI treatment.

Subsequent TKI efficacy after previous EGFR-TKI treatment

The clinical data of eight patients with *EGFR/ALK* co-alterations who received subsequent single- or dual-TKI treatment after already receiving previous EGFR-TKI treatment were collected and are presented in Figure 4. Of the eight cases, six (75%) of them were detected to have the first-generation EGFR-TKI resistant mutation T790M, indicating that *ALK* rearrangements might occur concomitantly with EGFR-T790M after EGFR-TKI therapy. Five patients were treated with subsequent single TKIs (EGFR-TKI or ALK-TKI) after the detection of *EGFR/ALK* co-alterations, and the clinical efficacy of the single TKI varied greatly. Among those with the T90M mutation, three patients who received subsequent osimertinib treatment had a PFS of 2.5, 2.9 and >10.6 months. One patient received combined afatinib and

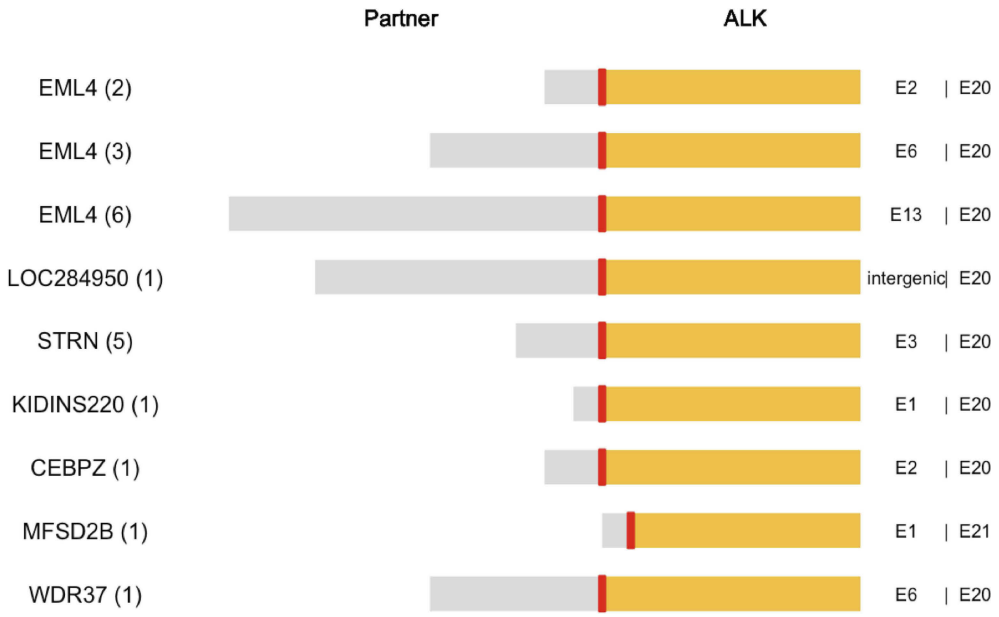

Figure 1 Structure and breakpoints of the 21 *ALK* fusions in *EGFR*-mutated nonsmall cell lung cancer patients identified by next-generation sequencing. E2:E20 indicates that exon 2 of *EML4* is fused to exon 20 of *ALK*. The number in the brackets indicates the number of patients with that respective fusion variant.
Abbreviations: ALK, *anaplastic lymphoma kinase*; EML4, *echinoderm microtubule*-associated protein-like 4.

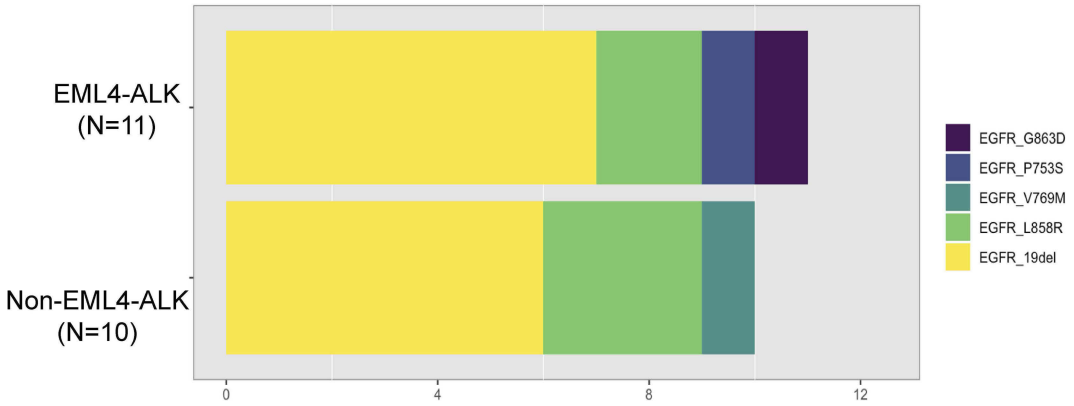

Figure 2 *EGFR* mutation variants in the 21 *EGFR/ALK* co-altered NSCLC patients. Different colors indicate different variants of *EGFR*.
Abbreviations: ALK, *anaplastic lymphoma kinase*; EML4, *echinoderm microtubule*-associated protein-like 4.

Table 2 Distribution of *EML4-ALK* and non-*EML4-ALK* in de novo and acquired *ALK* rearrangements

ALK fusions	NGS before baseline[a]	Treatment before baseline	*EML4-ALK*	Non-*EML4-ALK*
De novo ALK (n=5)	N/A	Treatment-naive	4/5 (80%)	1/5 (20%)
Acquired ALK (n=4)	EGFR (+), ALK (-)	EGFR-TKI	1/4 (25%)	3/4 (75%)
Uncertain (n=12)	EGFR (+), ALK (unknown)	EGFR-TKI	6/12 (50%)	6/12 (50%)

Notes: [a]The timepoint of positive-detection of co-altered EGFR/ALK was defined as baseline.
Abbreviations: ALK, *anaplastic lymphoma kinase*; N/A, not applicable; NGS, next generation sequencing; EML4, *echinoderm microtubule*-associated protein-like 4.

osimertinib and had a PFS of more than 13.6 months. One patient was treated with osimertinib plus chemotherapy and only had a PFS of 1.9 months, and one patient received crizotinib and achieved a PFS of 3.7 months. In addition, three patients received subsequent dual-TKI treatment (EGFR-TKI plus ALK-TKI), and the PFS of all three patients were longer than 5 months (8.4 months, 8.6 months, >5.2 months), indicating that patients might benefit from the combination therapy of ALK-TKIs and EGFR-TKIs.

Discussion

EGFR mutations and *ALK* rearrangements were commonly considered to be mutually exclusive in previous studies. However, there is increasing evidence to support the notion that they can be concomitantly mutated in cancers. In this study, we retrospectively reviewed the genomic profiling data of 419 *ALK*-positive Chinese NSCLC patients and identified 21 patients who harbored concurrent *EGFR* and *ALK* gene alterations. We investigated and compared the prevalence, clinical features, and different responses to tyrosine kinase inhibitors in subgroups of patients harbored *EML4-ALK /EGFR* or non-*EML4-ALK/EGFR* co-alterations. We found that *EML4-ALK/EGFR* and non-E*ML4-ALK/EGFR* co-alterations showed different clinical characteristics and clinical outcomes to EGFR-TKIs, suggesting that non-*EML4-ALK* co-alterations might be likely to emerge as a drug resistance mechanism for EGFR-TKIs. In addition, dual-TKI therapy might be a better choice for patients with co-altered *ALK/ EGFR* than single-TKI therapy. To the best of our knowledge, this is the largest cohort study of *ALK* rearrangements for dual *EGFR/ALK* co-alterations in People'sRepublic of China. In addition, this is the largest series of *ALK*-positive patients reported, and five novel *ALK* fusion partners were identified in this study.

There were inconsistencies about the clinical features of *EGFR/ALK* co-mutated patients. Yang et al reported that the median age in *EGFR/ALK* co-altered patients was 59 years, and the number of patients who were female and male were 8 (62%) and 5 (38%), respectively.[9] A European study revealed that there were two males and one female in their study of three *EGFR/ALK* co-altered patients.[23] We also interrogated the clinical demographics of *EGFR/ALK* comutated patients in this study. We found that the median age of the co-altered patients was 58 years old. Among them, 8 (38.1%) patients were female and 13 (61.9%) were male. This difference in sex was probably induced by different factors and the limited cohort size of each study. Moreover, none of the previous studies compared the clinical characteristics between patients

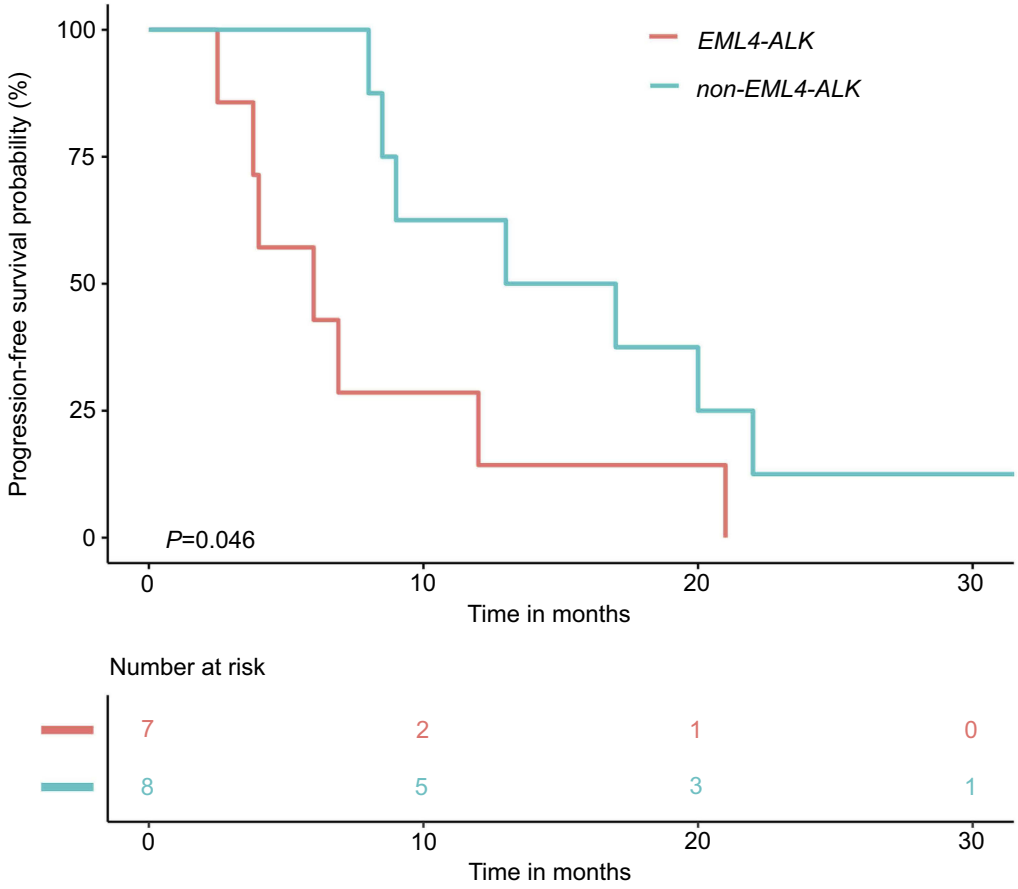

Figure 3 Kaplan–Meier curve demonstrates the correlation of PFS in response to EGFR-TKIs before *ALK* detection and the distribution of *ALK* fusion partners after EGFR-TKI treatment. The risk table (below) illustrates the number of patients included per time point.
Abbreviations: ALK, *anaplastic lymphoma kinase*; EML4, *echinoderm microtubule*-associated protein-like 4; PFS, progression-free survival.

with *EML4-ALK/EGFR* and non-*EML4-ALK/EGFR* co-alterations. We found that *EML4-ALK/EGFR* co-alterations were more prone to occur in females than in males, and non-*EML4-ALK/EGFR* co-alterations were more likely to occur in males than in females (p=0.02). The median age of *EML4-ALK/EGFR* co-altered patients was slightly younger than that of non-*EML4-ALK/EGFR* co-altered patients (53.0 vs 59.5 years, p=0.31). This clinically relevant observation may be important for further investigations of treatment strategies.

Several previous studies have reported that acquired *ALK* rearrangements could occur as a resistance mechanism after EGFR-TKI treatment. For example, Liang et al reported that *ALK* fusions were detected in the new metastatic lesion of an *EGFR*-mutated NSCLC patient.[8] In our study, we found that after EGFR-TKI treatments *EML4-ALK* co-alterations accounted for most of the 5 *de novo ALK*-rearranged patients (80%, 4/5), while the non-*EML4-ALK* co-alterations accounted for most of the 4 acquired *ALK*-positive patients (75%, 3/4). We also observed that *EML4-ALK/EGFR* co-altered patients had a significantly inferior median PFS in response to EGFR-TKI compared to that of non-*EML4-ALK/EGFR* co-altered patients (mPFS, 6.0 vs 15.0 months, p=0.046). These results indicated that *EML4-ALK* co-alterations were likely to be a *de novo* alteration and non-*EML4-ALK* co-alterations might be an acquired resistance mechanism induced by EGFR-TKIs. Moreover, *EML4-ALK* co-alterations were likely to be induced in the early stages during EGFR-TKI treatment, and non-*EML4-ALK* co-alterations might emerge at a relatively late stage. Although the evidence of non-*EML4/ALK* co-alterations as a resistance mechanism to EGFR-TKIs has not been solidified, our study has greatly expanded the current knowledge of the underlying association of *EGFR* and *ALK* alterations and might be helpful for understanding the co-alteration mechanism and guiding clinical decision-making. In addition, we proposed that for patients developing first-line EGFR-TKI treatment resistance, genomic profiling and sequencing analysis should be performed rather than

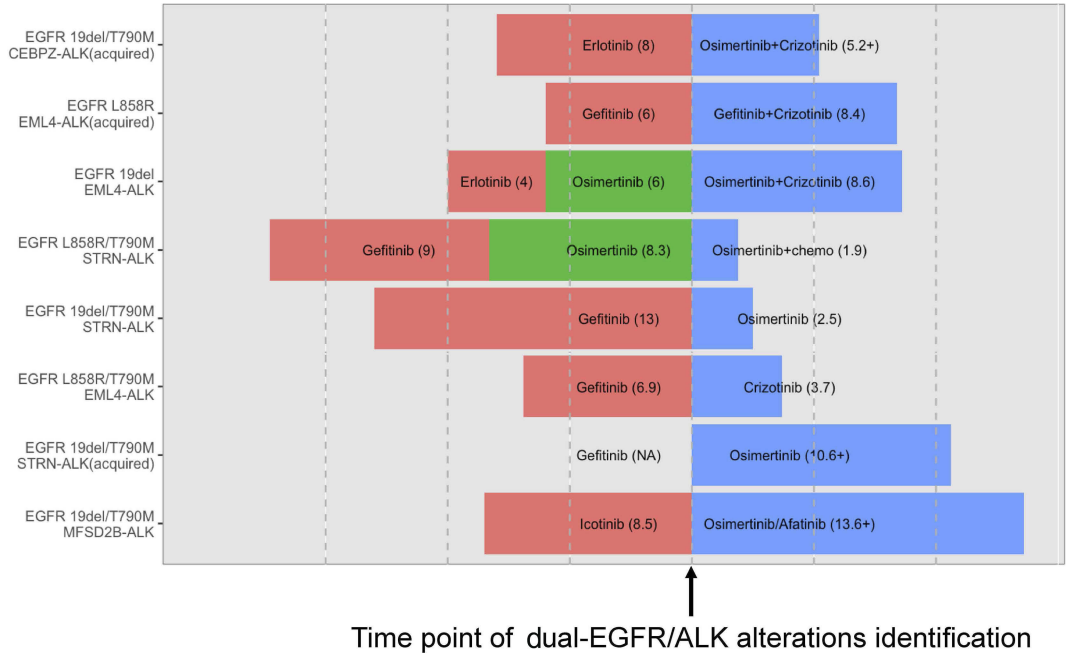

Figure 4 Presentation of the detailed treatment history (before and after baseline) and subsequent clinical outcomes (after baseline) of eight patients with *EGFR/ALK* co-alterations. The timepoint of the positive detection of the *EGFR/ALK* co-alteration was defined as baseline. After baseline, five patients were treated with subsequent single TKIs (EGFR-TKIs or ALK-TKIs), and three patients received subsequent dual-TKI treatment (EGFR-TKI plus ALK-TKI).
Abbreviation: ALK, *anaplastic lymphoma kinase*.

testing for *EGFR*-T790M solely since the *EGFR*-T790M mutation and *ALK* rearrangements may be detected simultaneously.

A limited number of studies have reported about the clinical efficacy of TKIs in patients who harbor *EGFR/ALK* co-alterations and have reached diverse conclusions. A Caucasian patient with lung adenosquamous carcinoma who harbored such a co-alteration displayed resistance to erlotinib treatment.[24] Ten NSCLC patients in a Chinese cohort achieved a response rate of 80% to the first-line EGFR-TKIs.[9] In a Korean cohort with 14 co-altered patients, 3 patients who were treated with gefitinib showed poor response, and 8 patients who received *ALK* inhibitors showed good response with a response rate of 87.5%.[11] Previous studies with targeted therapy on *EGFR/ALK* co-altered patients commonly focused on single TKIs or the sequential use of EGFR- and ALK-TKIs. No studies have compared the clinical outcomes of single TKIs with combinatorial therapies of EGFR-TKIs and ALK-TKIs in E*GFR/ALK* co-altered patients. Here, we demonstrated the clinical activities of single TKIs and dual TKIs in E*GFR/ALK* co-altered NSCLC patients who progressed after EGFR-TKIs. We found that the clinical efficacy of single TKIs in five patients varied greatly. We also observed that patients who harbor dual alterations might benefit from the combination therapy of ALK-TKIs and EGFR-TKIs, and the PFS of all three cases with dual alterations in this study were longer than 5 months.

However, there were several limitations in this study. First, 21 (1.2%) NSCLC patients were identified to have *EGFR/ALK* co-alterations from a total of 419 *ALK*-positive patients. Based on the small number of patients, it was difficult to draw clear conclusions on the clinical characteristics and therapeutic outcomes of the patients. Second, since not all samples were collected at baseline and since there was previously identified targetable mutations and treatment records were lacking, we proposed several concepts from our analysis, and further investigations are still needed to validate our ideas.

Conclusion

In summary, we demonstrated that NSCLC patients with *EML4-ALK/EGFR* and non-*EML4-ALK/EGFR* co-alterations displayed distinct rates of concurrent alterations, clinical demographicsand survival in response to EGFR-TKI treatment. We proposed that non-*EML4-ALK* alterations are likely to be an acquired resistance mechanism later during EGFR-TKI treatment. The underlying

molecular mechanisms of the two alterations and potential of combination treatments with dual TKIs require further investigation with this co-altered subgroup.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med*. 2010;363(18):1693–1703. doi:10.1056/NEJMoa1006448
2. Croegaert K, Kolesar JM. Role of anaplastic lymphoma kinase inhibition in the treatment of non-small-cell lung cancer. *Am J Health Syst Pharm*. 2015;72(17):1456–1462. doi:10.2146/ajhp140836
3. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2005;352(8):786–792. doi:10.1056/NEJMoa044238
4. Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol*. 2009;27(26):4247–4253. doi:10.1200/JCO.2009.22.6993
5. 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(7153):561–566. doi:10.1038/nature05945
6. Horn L, Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. *J Clin Oncol*. 2009;27(26):4232–4235. doi:10.1200/JCO.2009.23.6661
7. Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. *Cancer Res*. 2011;71(18):6051–6060. doi:10.1158/0008-5472.CAN-11-1340
8. Liang W, He Q, Chen Y, et al. Metastatic EML4-ALK fusion detected by circulating DNA genotyping in an EGFR-mutated NSCLC patient and successful management by adding ALK inhibitors: a case report. *BMC Cancer*. 2016;16:62. doi:10.1186/s12885-016-2088-5
9. Yang JJ, Zhang XC, Su J, et al. Lung cancers with concomitant EGFR mutations and ALK rearrangements: diverse responses to EGFR-TKI and crizotinib in relation to diverse receptors phosphorylation. *Clin Cancer Res*. 2014;20(5):1383–1392. doi:10.1158/1078-0432.CCR-13-0699
10. Ulivi P, Chiadini E, Dazzi C, et al. Nonsquamous, non-small-cell lung cancer patients who carry a double mutation of EGFR, EML4-ALK or KRAS: frequency, clinical-pathological characteristics, and response to therapy. *Clin Lung Cancer*. 2016;17(5):384–390. doi:10.1016/j.cllc.2015.11.004
11. Won JK, Keam B, Koh J, et al. Concomitant ALK translocation and EGFR mutation in lung cancer: a comparison of direct sequencing and sensitive assays and the impact on responsiveness to tyrosine kinase inhibitor. *Ann Oncol*. 2015;26(2):348–354. doi:10.1093/annonc/mdu530
12. Togashi Y, Soda M, Sakata S, et al. KLC1-ALK: a novel fusion in lung cancer identified using a formalin-fixed paraffin-embedded tissue only. *PLoS One*. 2012;7(2):e31323. doi:10.1371/journal.pone.0031323
13. Choi YL, Lira ME, Hong M, et al. A novel fusion of TPR and ALK in lung adenocarcinoma. *J Thorac Oncol*. 2014;9(4):563–566. doi:10.1097/JTO.0000000000000093
14. Shan L, Jiang P, Xu F, et al. BIRC6-ALK, a novel fusion gene in ALK break-apart FISH-negative lung adenocarcinoma, responds to crizotinib. *J Thorac Oncol*. 2015;10(6):e37–e39. doi:10.1097/JTO.0000000000000467
15. Li Y, Zhang T, Zhang J, et al. Response to crizotinib in advanced ALK-rearranged non-small cell lung cancers with different ALK-fusion variants. *Lung Cancer*. 2018;118:128–133. doi:10.1016/j.lungcan.2018.01.026
16. Ou S-HI, Schrock AB, Gowen K, et al. Association of ALK resistance mutations by EML4-ALK variant (v3 vs. non-v3) in ALK+ non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2017;35(15_suppl):9010. doi:10.1200/JCO.2017.35.15_suppl.9010
17. Mao X, Zhang Z, Zheng X, et al. Capture-based targeted ultradeep sequencing in paired tissue and plasma samples demonstrates differential subclonal ctDNA-releasing capability in advanced lung cancer. *J Thorac Oncol*. 2017;12(4):663–672. doi:10.1016/j.jtho.2016.11.2235
18. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114–2120. doi:10.1093/bioinformatics/btu170
19. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754–1760. doi:10.1093/bioinformatics/btp324
20. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297–1303. doi:10.1101/gr.107524.110
21. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res*. 2012;22(3):568–576. doi:10.1101/gr.129684.111
22. Newman AM, Bratman SV, Stehr H, et al. FACTERA: a practical method for the discovery of genomic rearrangements at breakpoint resolution. *Bioinformatics*. 2014;30(23):3390–3393. doi:10.1093/bioinformatics/btu549
23. Lee T, Lee B, Choi YL, Han J, Ahn MJ, Um SW. Non-small cell lung cancer with concomitant EGFR, KRAS, and ALK mutation: clinicopathologic features of 12 cases. *J Pathol Transl Med*. 2016;50(3):197–203. doi:10.4132/jptm.2016.03.09
24. Tiseo M, Gelsomino F, Boggiani D, et al. EGFR and EML4-ALK gene mutations in NSCLC: a case report of erlotinib-resistant patient with both concomitant mutations. *Lung Cancer*. 2011;71(2):241–243. doi:10.1016/j.lungcan.2010.11.014