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Impact of EML4-ALK Variants and TP53 Status on the Efficacy of ALK Inhibitors in Patients With Non-small Cell Lung Cancer

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ABSTRACT

Background: The clinical implications of different EML4-ALK fusion variants remain poorly elucidated in the era of second-generation ALK inhibitors.

Methods: This was a retrospective cohort study, wherein patients diagnosed with locally advanced or metastatic non-small cell lung cancer harboring EML4-ALK fusion were stratified into two cohorts based on their first-line treatment: Cohort 1 received alectinib, while Cohort 2 received crizotinib. Statistical analysis was employed to investigate the impact of different EML4-ALK variants and TP53 status on the efficacy of first-line ALK-TKIs.

Results: Finally, 49 patients were enrolled in cohort 1 and 53 patients in cohort 2. In cohort 1, patients with long EML4-ALK fusion variants exhibited prolonged PFS (NR vs. 34.0 m, $p = 0.004$, HR = 0.30, 95% CI: 0.12–0.74) and an elevated 5-year OS rate (93.3% vs. 68.4%, $p = 0.020$, HR = 0.12, 95% CI: 0.02–0.62) compared to those with short variants. The median PFS was not reached in TP53-wt group and 47.0 m in TP53-mut group ($p = 0.087$, HR = 0.44, 95% CI: 0.17–1.17). The TP53-wt group exhibited a superior 5-year OS rate (100% vs. 77.8%, $p = 0.030$) compared to TP53-mut group. In cohort 2, the median PFS was 14.0 m in long variant group and 12.9 m in short variant group ($p = 0.094$, HR = 0.65, 95% CI: 0.37–1.13); the median OS was not reached in long variant group and 69.2 m in short variant group ($p = 0.254$, HR:0.62, 95% CI: 0.27–1.42). However, the efficacy of first-line crizotinib did not appear to be influenced by the TP53 status.

Conclusions: EML4-ALK short variants and TP53 mutations are both adverse factors for first-line alectinib efficacy, but they have little effect on first-line crizotinib.

Abbreviations: 2G ALK-TKIs, second-generation ALK-TKIs; ALK, anapastic lymphoma kinase; ALK+, ALK-positive; ALK-TKIs, ALK tyrosine kinase inhibitors; CI, confidence interval; CNS, central nervous system; EMR, Electronic Medical Records; HR, hazard ratio; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; RECIST 1.1, response evaluation criteria in solid tumors version 1.1; TP53-mut, TP53 mutant; TP53-wt, TP53 wild type.

Zihua Zou and Lige Wu contributed equally to this study.

1 | Introduction

Since the discovery of anaplastic lymphoma kinase (ALK) rearrangement in non-small cell lung cancer (NSCLC) in 2007 [1], significant advancements have been achieved in the treatment of ALK-positive (ALK^+) NSCLC, with multiple ALK tyrosine kinase inhibitors (ALK-TKIs) gaining approval as standard therapeutic options for responsive patients. Significant advancements in molecular diagnostic technology have facilitated the identification of a wide range of ALK rearrangements involving diverse fusion partners and breakpoints. Among these, the EML4-ALK fusion accounts for approximately 85% of ALK^+ NSCLC patients and can be further categorized into distinct fusion variants based on specific breakpoints within the exon of EML4 [2, 3].

Structurally, EML4-ALK fusion variants can be broadly classified into two forms: the short variants and the long variants (Figure 1). The breakpoint of EML4 in shorter variants (e.g., v3 and v5) is located at exon 6 and its upstream, accompanied by the presence of truncated or absent tandem atypical beta-propeller (TAPE) domains. In contrast, longer variants such as v1 and v2 often exhibit breakpoints downstream of exon 6 with relatively elongated TAPE domains [4]. The disparities in biochemical structure between short and long variants give rise to distinct properties. Compared to the long variants, the short variants exhibit enhanced protein stability, prolonged half-life, augmented oncogenic signaling, and diminished susceptibility to ALK-TKIs in vitro [5]. In terms of clinical aspects, several retrospective studies [6, 7] and the Japanese scholar Yoshida [8] have both demonstrated an association between longer variations and a more favorable progression-free survival (PFS) in crizotinib treatment. However, the impact of various fusion variants on the efficacy of second-generation ALK-TKIs remains uncertain. Several studies have presented conflicting findings: both the ALEX and BFAST studies reported no statistically significant difference in PFS among patients with different EML4-ALK fusion variants, while the ALTA-1 L study demonstrated a shorter PFS in v3 subgroup compared to v1 subgroup [9–11].

Despite significant advancements in this field, several unresolved questions still persist. Firstly, while alectinib consistently demonstrates improved PFS compared to crizotinib in numerous studies, it remains unknown whether this benefit varies across different ALK fusion variants. Secondly, the impact of ALK fusion variants with TP53 mutation status on the efficacy of ALK inhibitors has not been investigated in previous research. Thirdly, limited studies have explored the progression patterns and resistance mechanisms of ALK-TKIs associated with different ALK fusion variants. Based on these inquiries, we conducted a retrospective study to investigate the clinical outcomes of ALK inhibitors across different ALK fusion variants.

2 | Methods

2.1 | Patient Selection

This study was approved by Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (19/096-1880). The present study is a retrospective cohort investigation that enrolled patients diagnosed with locally advanced or metastatic NSCLC harboring EML4-ALK fusion from two hospitals between July 2014 and March 2022. These patients were administered either alectinib or crizotinib as first-line therapy, and their EML4-ALK fusion variants were identified and classified using next-generation sequencing (NGS) technology. All enrolled patients must undergo a comprehensive baseline radiological examination prior to treatment and receive regular evaluations at designated medical institutions every 2–3 months. Patients with a second primary tumor other than non-small cell lung cancer were excluded from the study. Patients with EGFR mutations, ROS1 rearrangement, and other ALK fusions (excluding EML4-ALK) were excluded from the study. Cohort 1 comprised patients who underwent alectinib as their first-line treatment, whereas Cohort 2 encompassed individuals who received crizotinib as their initial therapy.

2.2 | Data Extraction

The demographic and clinical characteristics were meticulously documented. The ALK fusion variants were detected at baseline through NGS. The status and mutant subtypes of baseline TP53 were also meticulously documented. A comprehensive review of imaging data was conducted to assess treatment response, while

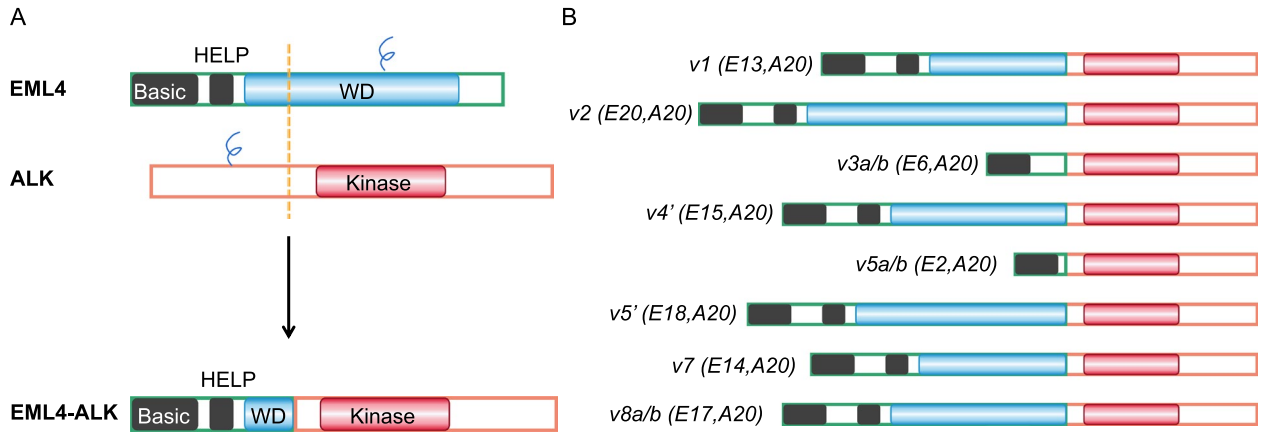

FIGURE 1 | EML4-ALK fusion diagram. (A) Reconstitution of the EML4-ALK fusion. (B) Common EML4-ALK fusion variants.

survival information was obtained through meticulous examination of clinical records or diligent follow-up via telephone by investigators. Repeated biopsies performed during ALK inhibitor treatment were also recorded. The cut-off date for this study was Sep 22, 2024. In case a patient was lost to follow-up on Sep 22, 2024, the most recent available follow-up data were considered as the designated cut-off date.

2.3 | Evaluation Criteria and Study Endpoints

In this study, the EML4-ALK fusion long variant was defined as having the EML4 break point located downstream of exon 6, whereas the short variant was defined as having the EML4 break point situated within exon 6 and its upstream region. Radiological assessment of intracranial and extracranial lesions was conducted following the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). The study endpoints included PFS, central nervous system time-to-progression (CNS-TTP), overall survival (OS), and objective response rate (ORR = PR + CR). PFS was defined as the duration from initiation of ALK-TKIs to initial radiological evidence of disease progression. CNS-TTP was defined as the period from initiation of ALK-TKIs to initial occurrence of CNS progression, encompassing patients with and without baseline CNS metastases. OS was defined as the time interval from initiation of ALK-TKIs to occurrence of death attributed to any cause. Additionally, this study investigated the pattern of disease progression and resistance mechanisms associated with ALK-TKIs, with a particular focus on frequency analysis for ALK resistance mutations. Oligo-progression was defined as disease progression occurring in no more than three lesions, while extensive-progression encompassed pulmonary lymphangitis, pleural/serous effusion or leptomeningeal metastases, etc.

2.4 | Statistical Analysis

The statistical analysis was conducted using SPSS 26.0 statistical software (Inc., Chicago, IL, USA). Descriptive statistics were employed to present the distribution of patients and baseline demographic/clinical characteristics. Categorical data were compared using Pearson's χ^2 test, while continuous data were compared using Student's *t*-tests. Survival curves were estimated utilizing the Kaplan–Meier method, and differences in variables were assessed with the log-rank test. Cox proportional hazards regression analysis was employed to adjust for potential confounders and determine the indicators associated with PFS. Statistical significance was determined by a two-sided *p*-value < 0.05.

3 | Results

3.1 | Baseline Characteristics

A total of 49 patients were enrolled in cohort 1 and 53 patients in cohort 2. Cohort 1 consisted of 30 patients with EML4-ALK fusion long variants and 19 patients with short variants; cohort 2 comprised of 26 patients with long variants and 27 patients with short variants. The distribution of EML4-ALK fusion variants was illustrated in Figure 2. The TP53 gene status was determined in 43 patients from cohort 1 (25 TP53-wild type and 18 TP53-mutant) and 39 patients from cohort 2 (29 TP53-wild type and 10 TP53-mutant) during the initial NGS analysis. And the TP53 gene mutation subtypes in the two cohorts were summarized in Table S1. The Chi-square test did not reveal any statistically significant differences between the two cohorts in terms of gender, age, pathological subtype, smoking history, ECOG PS score, tumor burden, and distant metastasis (Table 1).

3.2 | The Impact of EML4-ALK Fusion Variants on Initial ALK-TKI Efficacy

In cohort 1, the ORR was 70.0% (21/30) for the long variant group, while it was 68.4% (13/19) for the short variant group ($p = 0.907$). In cohort 2, the ORR was 76.9% (20/26) for the long variant group, and 70.4% (19/27) for the short variant group ($p = 0.589$). No statistical difference in ORR was observed between the long and short variants of EML4-ALK fusion in either cohort. For patients with long variants, the first-line treatment with alectinib or crizotinib did not yield a statistically significant difference in ORR (70.0% vs. 76.9%, $p = 0.560$). Similarly, for patients with short variants, no significant difference was found either (68.4%

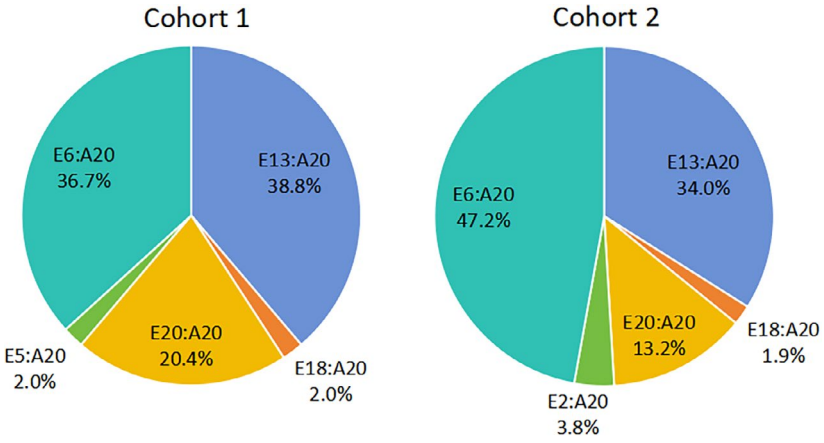

FIGURE 2 | Distribution of EML4-ALK fusion variants in cohort 1 and cohort 2. A total of 49 patients were enrolled in cohort 1 and 53 patients in cohort 2.

TABLE 1 | Baseline characteristics of patients in cohort 1 and cohort 2.

	Cohort 1			Cohort 2		
	Short variants $n=19$	**Long variants** $n=30$	***p*1**	**Short variants** $n=27$	**Long variants** $n=26$	***p*2**
Gender			1			1
Male	36.8%	40.0%		48.1%	50.0%	
Female	63.2%	60.0%		51.9%	50.0%	
Age			0.480			0.480
<65	73.7%	83.3%		88.9%	92.3%	
≥ 65	26.3%	16.7%		11.1%	7.7%	
Smoking history			0.720			0.766
Non-smoker	84.2%	76.7%		66.7%	73.1%	
Smoker	15.8%	23.3%		33.3%	26.9%	
Pathology			0.145			0.768
Adenocarcinoma	89.5%	100%		100%	100%	
Other	10.5%	0%		0%	0%	
ECOG			1			1
0–1	63.2%	63.3%		85.2%	88.5%	
≥ 2	36.8%	36.7%		14.8%	11.5%	
Distant metastases			0.053			0.610
Yes	84.2%	100%		88.9%	96.2%	
No	15.8%	0%		11.1%	3.8%	
Extrathoracic metastases			0.351			0.559
Yes	57.9%	73.3%		63.0%	73.1%	
No	42.1%	26.7%		37.0%	26.9%	
CNS metastases			0.49			0.704
Yes	15.8%	26.7%		11.1%	15.4%	
No	84.2%	73.3%		88.9%	84.6%	
Bone metastases			0.77			0.047
Yes	42.1%	50.0%		22.2%	50.0%	
No	57.9%	50.0%		77.8%	50.0%	
Liver metastases			1			1
Yes	10.5%	13.3%		18.5%	19.2%	
No	89.5%	86.7%		81.5%	80.8%	
Number of distant organ metastasis			1			0.175
≥ 3	31.6%	30.0%		11.1%	26.9%	
≤ 2	68.4%	70.0%		88.9%	73.1%	

Abbreviation: CNS, central nervous system.

vs. 70.4%, $p=0.887$). In summary, the classification of long and short fusion variants had minimal impact on ORR in the initial treatment with alectinib and crizotinib.

The patients in cohort 1 initially received alectinib as the first-line treatment. At the time of data cut-off, the median follow-up duration was 46.3 months (range: 8.8–77.5 months) for the short

variant group and 45.4 months (range 9.9–66.6 months) for the long variant group. Compared to short variant group, patients with long variants exhibited prolonged PFS (NR vs. 34.0 m, $p = 0.004$, HR = 0.30, 95% CI: 0.12–0.74) in response to alectinib treatment, along with an elevated 5-year OS rate (93.3% vs. 68.4%, $p = 0.020$, HR = 0.12, 95% CI: 0.02–0.62) (Figure 3A,B). The findings suggest that the classification of long and short fusion variants can serve as a predictive tool for assessing the efficacy of first-line alectinib. The PFS between the EML4-ALK fusion v1 and v2 were subsequently compared. The two curves diverged at the outset and converged at 48 months, implying that patients harboring EML4-ALK fusion v2 may exhibit a superior median PFS compared to those with v1 within the first 48 months of alectinib therapy (Figure 3C).

The patients in cohort 2 were initially administered crizotinib as the first-line therapy. At the time of data cut-off, the median follow-up duration was 70.8 months (range: 6.5–99.3 months) for the short version group and 83.6 months (range:14.8–127.0 months) for the long version group. The median PFS was 14.0 months in the long version group and 12.9 months in the short version group, and no statistically significant difference was observed between the two groups ($p = 0.094$). These findings are in line with the outcomes of our previous multi-center retrospective study [12]. However, the divergence of the two groups' curves became evident after 1 year of follow-up, indicating a potential association between long fusion variants and prolonged survival in crizotinib-treated patients (Figure 3D). The median OS was not reached in the long version group and 69.2 months in the short version group ($p = 0.254$, HR:0.62, 95% CI: 0.27–1.42). The subsequent divergence of the OS curves between the two groups may be attributed to second-generation ALK-TKIs treatment following disease progression (Figure 3E). For the median PFS of v1 and v2, the two curves initially intersected and did not demonstrate superior PFS in the v2 group until 21.5 months of follow-up (Figure 3F).

Therefore, alectinib demonstrated superior efficacy in patients harboring EML4-ALK long variants compared to those with short variants; however, the predictive value of classifying EML4-ALK fusion variants as long or short for crizotinib efficacy was limited. Furthermore, it is worth noting that the observed differences in PFS between EML4-ALK fusions v1 and v2 seem to be associated with the duration of follow-up; however, substantiating this claim would necessitate a larger sample size and an extended follow-up period.

3.3 | The Impact of TP53 Status on Initial ALK-TKI Efficacy

The mutation rate of TP53 in NSCLC ranges from 50% to 60%, but the impact of TP53 status on ALK-TKI efficacy remains unknown. For this part, we investigated the impact of baseline

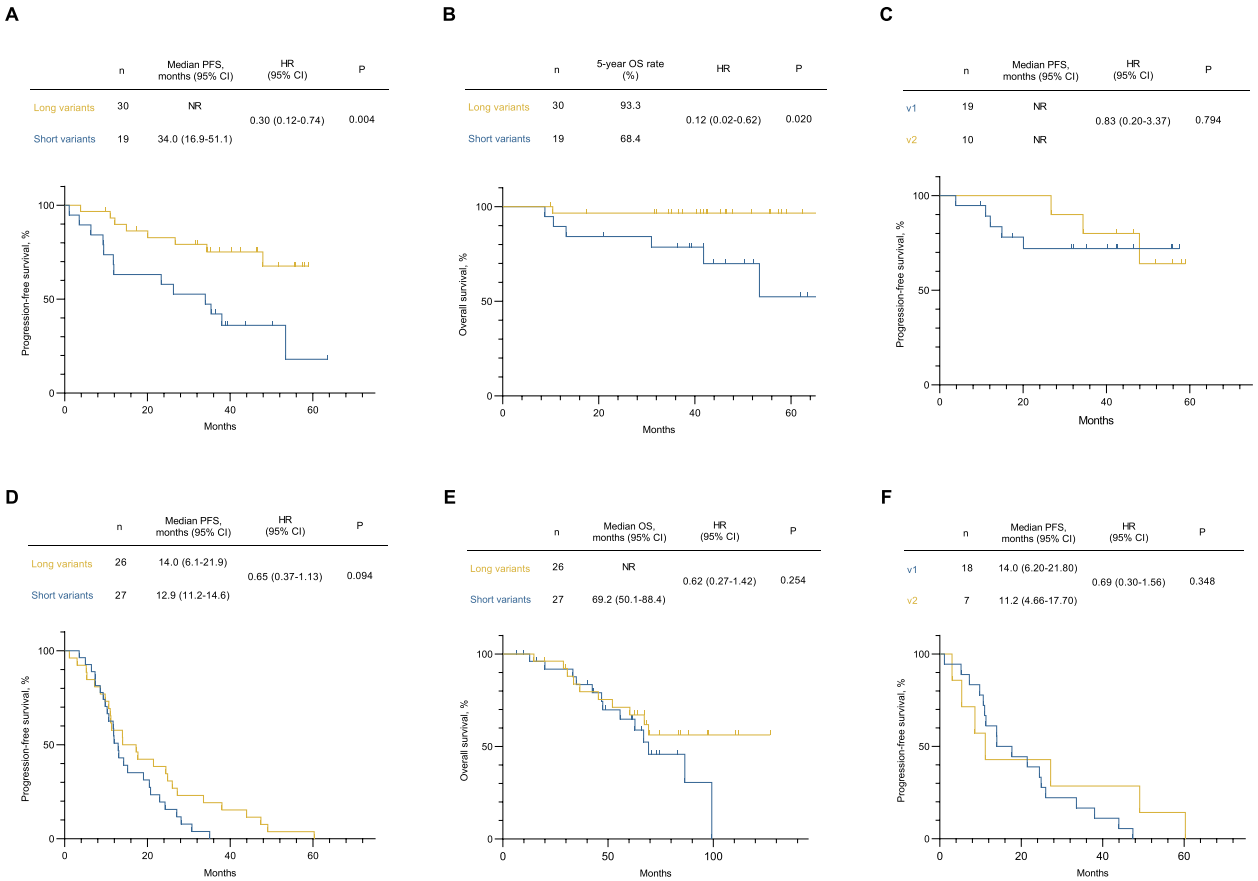

FIGURE 3 | The impact of different EML4-ALK fusion variants on the efficacy of first-line ALK-TKI treatment. (A, B) PFS and OS in patients receiving alectinib as first-line treatment (cohort 1). (C) PFS comparison of EML4-ALK fusion variants 1 and 2 in cohort 1. (D, E) PFS and OS in patients receiving crizotinib as first-line treatment (cohort 2). (F) PFS comparison of EML4-ALK fusion variants 1 and 2 in cohort 2.

TP53 gene status on the efficacy of first-line ALK-TKIs. In cohort 1, the median PFS was not reached in the TP53-wt group, while it was 47.0 months in the TP53-mut group ($p = 0.087$, HR:0.44, 95% CI: 0.17–1.17). After a follow-up period of 11 months, patients with TP53 wild-type exhibited superior PFS in response to alectinib treatment (Figure 4A). Compared to TP53-mut group, the TP53-wt group exhibited a superior 5-year OS rate (100% vs. 77.8%, $p = 0.030$) (Figure 4B). We subsequently observed a significant disparity in the median PFS between the Long variants+TP53-wt group and the Short variants+TP53-mut group (Figure 4C). Our findings suggest that TP53 mutations can impact the effectiveness of first-line alectinib treatment, with Short variants and TP53 mutations being contributing factors to its limited efficacy.

In cohort 2, there was no significant difference in median PFS between the TP53-wt and TP53-mut groups (17.7 m vs. 14.0 m, $p = 0.948$, HR = 0.98, 95% CI: 0.48–2.00) (Figure 4D). The median OS was 99.3 months in TP53-wt group and 60.4 months in TP53-mut group ($p = 0.174$, HR: 0.49, 95% CI: 0.14–1.75). The divergence observed between the two curves during late follow-up period may be attributed to subsequent administration of second-generation ALK-TKIs following resistance to crizotinib treatment (Figure 4E). The PFS analysis also failed to demonstrate a statistically significant distinction between the Long variants+TP53-wt group and the Short variants+TP53-mut group (Figure 4F). The findings suggest that the effectiveness of first-line crizotinib is not impacted by TP53 mutations.

The main types of p53 mutations encompass missense, truncation, in-frame, and splicing mutations, with missense mutations constituting approximately 80% [13]. The TP53 missense mutations encompass both conformational and DNA contact mutations. Unfortunately, due to the limited sample size of this study, it is not feasible to further elucidate the impact of a specific TP53 mutation subtype on ALK-TKI treatment.

3.4 | COX Analysis of Factors Associated With PFS in NSCLC Patients Treated With First-Line Alectinib

The above findings indicate that different EML4-ALK fusion variants and TP53 mutant statuses are associated with the efficacy of first-line alectinib, but not with crizotinib. To address the limitations of single-factor survival analysis and to better control for and estimate the impact of confounding factors, a COX proportional hazards regression analysis was conducted on potential factors influencing the PFS of first-line alectinib (Table 2). In the univariate Cox regression analysis, smoking history and EML4-ALK fusion variant affected the PFS of first-line alectinib. Then, we included variables with a P-value of

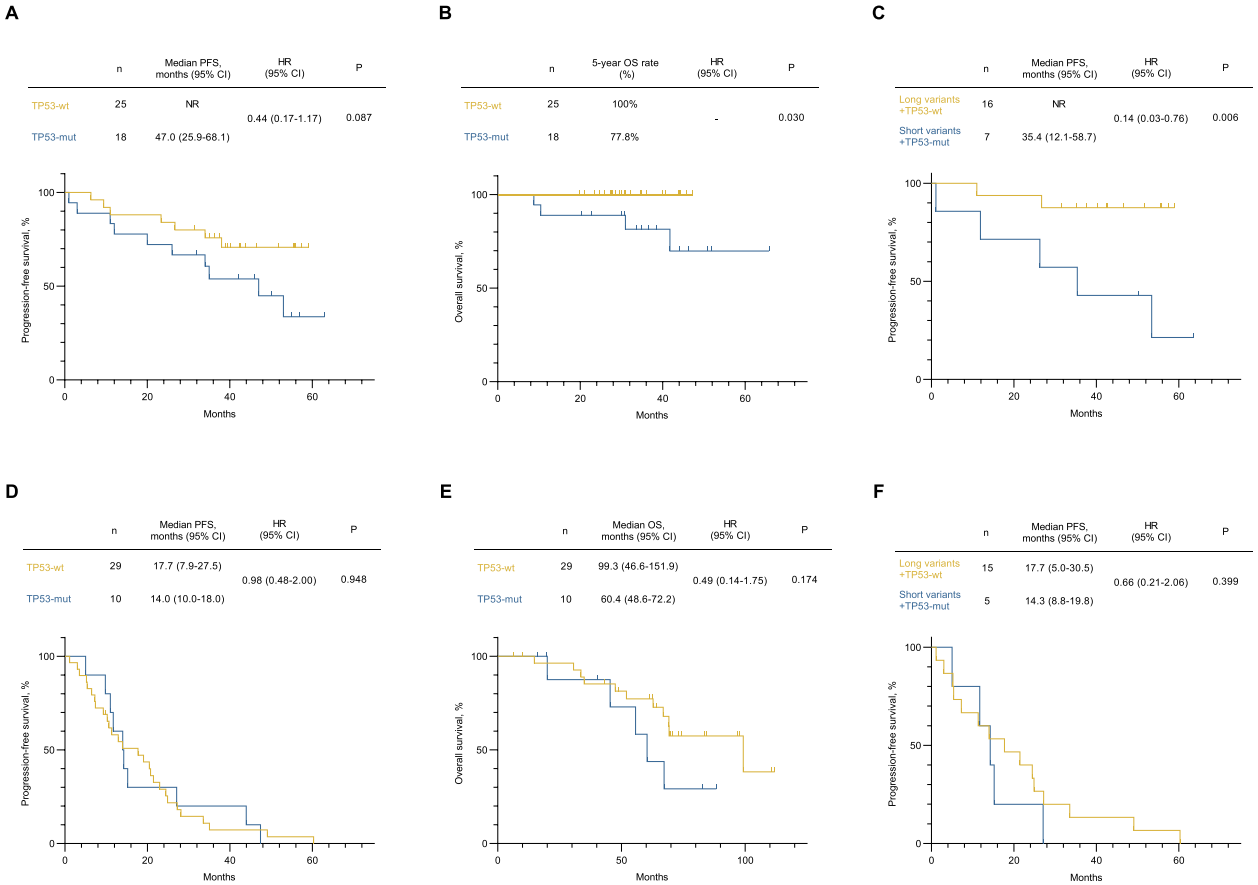

FIGURE 4 | The impact of baseline TP53 gene status on the efficacy of initial alectinib and crizotinib treatment. (A, B) PFS and OS in patients receiving alectinib as first-line treatment. (C) The combined impact of EML4-ALK fusion variants and TP53 status on the efficacy of first-line alectinib treatment. (D, E) PFS and OS in patients receiving crizotinib as first-line treatment. (F) The combined impact of EML4-ALK fusion variants and TP53 status on the efficacy of first-line crizotinib treatment. wt, wild-type; mut, mutant.

TABLE 2 | Univariate and multivariate COX analysis of factors associated with PFS in NSCLC patients treated with first-line alectinib.

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age	0.470	0.138–1.601	0.227			
Sex	0.525	0.222–1.237	0.140			
Smoking history (vs. never smoker)	**3.674**	**1.484–9.096**	**0.005**	**2.884**	**1.012–8.214**	**0.047**
Histology	1.496	0.199–11.244	0.696			
ECOG	1.529	0.643–3.637	0.337			
Distant metastases	1.847	0.247–13.813	0.550			
Extrathoracic metastases (vs. no extrathoracic metastases)	**2.507**	**0.843–7.459**	**0.099**	1.584	0.267–9.416	0.613
CNS metastases	1.289	0.470–3.533	0.622			
Bone metastases (vs. no bone metastases)	**2.263**	**0.934–5.485**	**0.071**	3.463	0.567–21.148	0.179
Liver metastases	1.094	0.318–3.764	0.886			
Number of distant organ metastasis	1.794	0.742–4.337	0.195			
EML4-ALK variant (vs. short fusion variants)	**0.294**	**0.121–0.712**	**0.007**	**0.204**	**0.068–0.611**	**0.005**
TP53 mutant (vs. TP53 wild-type)	**2.225**	**0.846–5.855**	**0.105**	**3.280**	**1.031–10.434**	**0.044**

Note: Bold indicates statistically significancant value ($p < 0.05$).

less than 0.1, such as smoking history, extrathoracic metastases, bone metastases, EML4-ALK variant, and TP53 mutant, in a multivariate COX regression analysis. It was determined that smoking history (HR = 2.884, $p = 0.047$), EML4-ALK variant (HR = 0.204, $p = 0.005$), and TP53 mutant (HR = 3.280, $p = 0.044$) are independent prognostic factors for PFS of first-line alectinib. The association between TP53 and smoking exposure may be one of the important confounding factors [14].

3.5 | Progression Patterns and Resistant Mechanisms in Patients With Different EML4-ALK Fusion Variants

In Cohort 1, at the time of data cut-off, a total of 21 patients exhibited disease progression subsequent to initial treatment with alectinib (13 in short variant group and 8 in long variant group). The incidence of CNS progression (0% vs. 15.4%, $p = 0.688$) and oligo-progression (25.0% vs. 15.4%, $p = 1$) exhibited comparable rates between the long and short variant groups. In Cohort 2, a total of 52 patients were identified with disease progression at the time of data cut-off, evenly distributed between the short version group and long version group (26 patients each). There was no statistically significant difference observed in the incidence of CNS progression (53.8% vs. 50.0%, $p = 0.781$) or oligo-progression (26.9% vs. 34.6%, $p = 0.548$) between the long and short variant groups (Table 2). Additionally, patients harboring long fusion variants who received initial crizotinib treatment exhibited comparable CNS-TTP outcomes to those with short variants (26.0 months vs. 19.1 months, $p = 0.272$, HR = 0.67, 95% CI: 0.31–1.46) (Figure S1).

In this study, all the crizotinib-resistant patients were subsequently administered second-generation ALK-TKIs (2G ALK-TKIs). At the time of 2G ALK-TKI resistance, a total of 10 patients in cohort 1 and 22 patients in cohort 2 underwent secondary biopsies and next-generation sequencing (NGS). Patients with short variants exhibited a higher prevalence of resistance mutation in ALK kinase domain compared to those with long variants (77.8% vs. 42.9%, $p = 0.043$). Interestingly, we also observed a higher incidence of I1171N secondary mutations in patients with long fusion variants, while patients with short variants were more prone to developing G1202R mutations (Table 3). The comprehensive details regarding the secondary mutation in ALK kinase domain was described in Table S2.

4 | Discussion

Significant advancements have been made in the management of advanced ALK$^+$ NSCLC, leading to substantial survival benefits for these patients attributed to successive generations of ALK-TKIs. However, previous studies have consistently demonstrated heterogeneous responses to ALK inhibitors among patients with ALK$^+$ NSCLC, highlighting substantial tumor heterogeneity. The EML4-ALK fusion variants have consistently emerged as a focal point when discussing influential factors impacting the efficacy of ALK inhibitors. Previous in vitro and in vivo research has generally indicated that short fusion variants exhibit enhanced protein stability and demonstrate reduced sensitivity to ALK inhibitors compared to their longer counterparts. The application of these results, however, requires further validation in real-world scenarios.

Our study presents several noteworthy findings that can provide valuable insights for clinical practice. Firstly, in real-world cohorts, we observed that the efficacy of first-line crizotinib remained unaffected by the type of EML4-ALK fusion variant. The effectiveness of first-line alectinib, however, demonstrated superior survival outcomes in patients with long fusion variants compared to those with short fusion variants. Furthermore, among patients with long fusion variants, alectinib demonstrated

TABLE 3 | Progression patterns and resistance mechanisms in patients with short and long variants.

	Long variants	Short variants	*p*
CNS progression at the resistance of first-line alectinib	0% (0/8)	15.4% (2/13)	0.688
Oligo-progression at the resistance of first-line alectinib	25.0% (2/8)	15.4% (2/13)	1
CNS progression at the resistance of first-line crizotinib	53.8% (14/26)	50.0% (13/26)	0.781
Oligo-progression at the resistance of first-line crizotinib	26.9% (7/26)	34.6% (9/26)	0.548
Resistance mutation in ALK kinase domain at the resistance of 2G ALK-TKIs	42.9% (6/14)	77.8% (14/18)	0.043
G1202R mutation	0 (0/6)	85.7% (12/14)	0.002
I1171N mutation	83.3% (5/6)	7.1% (1/14)	0.004

Abbreviation: 2G ALK-TKIs, Second generation anaplastic lymphoma kinase tyrosine kinase inhibitors.

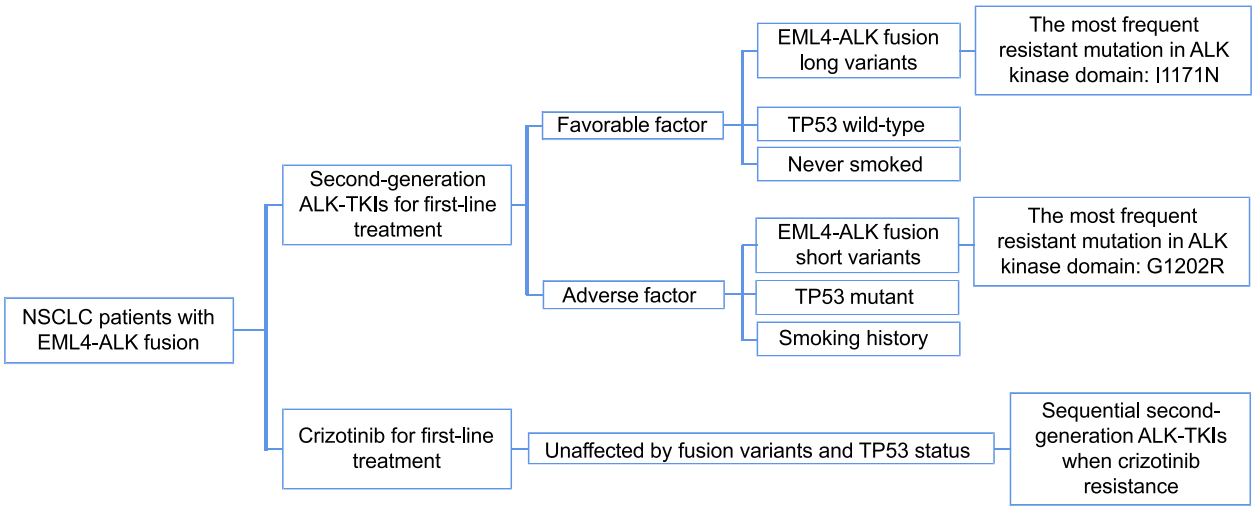

FIGURE 5 | Prediction of efficacy and drug resistance for initial ALK-TKI treatment in patients with EML4-ALK fusion NSCLC.

superior clinical outcomes when compared to crizotinib, as evidenced by significant early divergence of the two survival curves during follow-up (Figure S2A,B). Conversely, in patients with short fusion variants, the survival curves of first-line alectinib and crizotinib initially converged but later diverged after 1 year, suggesting limited efficacy of alectinib in addressing early resistance to short fusion variants (Figure S2C,D). These findings indicate that regardless of the length of EML4-ALK fusion variants, both groups should receive first-line alectinib treatment; and patients with short fusion variants may require more aggressive management strategies during the initial medication phase. Additionally, brigatinib—another second-generation ALK-TKI—also exhibited improved ORR and median PFS compared to crizotinib in both short and long variant populations [15].

The aforementioned findings are supported by a previous in vitro experiment [16], which demonstrated that alectinib exhibited the highest fold decrease in inhibitory activity against v3 compared to v1, with a ratio of 21. In contrast, crizotinib only showed a ratio of 8. However, the difference in cellular IC50 of crizotinib between v1 and v3 was not as significant as observed with alectinib, suggesting that other confounding factors may play a role in determining the efficacy of crizotinib in different ALK fusion variants. Furthermore, all patients enrolled in our study received second-generation ALK-TKIs upon progression on crizotinib treatment, and a substantial proportion of patients opted for lorlatinib as their subsequent line of therapy following resistance to second-generation ALK-TKIs. Therefore, it is possible for patients with short fusion variants to achieve favorable clinical outcomes through sequential treatment with multiple generations of ALK inhibitors. The CROWN study reported that among patients receiving first-line lorlatinib treatment, median PFS was not reached for v1 and v2 and was 33.3 months for V3. In comparison, among patients receiving first-line crizotinib treatment, median PFS was 7.4 months for v1, not achieved for v2, and 5.5 months for v3. Overall, lorlatinib reduces the risk of disease progression and death compared to crizotinib in both long or short ALK fusion variants [17].

Secondly, our findings indicated that TP53 gene mutations confer a detrimental impact on the efficacy of first-line alectinib therapy, while exerting minimal influence on the effectiveness of first-line crizotinib treatment. Subgroup analysis revealed that the long variants + TP53-wt group exhibited markedly superior efficacy in first-line alectinib treatment compared to the short variants + TP53-mut group. Regrettably, neither the EML4-ALK fusion variant type nor the TP53 gene status can serve as predictive factors for the efficacy of first-line crizotinib. Multivariate COX analysis revealed that the EML4-ALK short

variant, TP53 mutation, and smoking history were independent risk factors for first-line alectinib efficacy.

Lastly, we also investigated the progression patterns and resistant mechanisms in diverse ALK fusion variants. Our findings suggest that the incidence of CNS progression and oligo-progression is comparable between the long and short version groups following initial treatment with alectinib or crizotinib. Patients carrying short fusion variants are more susceptible to secondary mutations in the ALK kinase domain. The most prevalent secondary mutation observed in the long variant group was I1171N, whereas G1202R was predominantly found in the short variant group. These findings imply that distinct sequential treatment strategies may be necessary for different EML4-ALK fusion variants. Here, we summarized the findings of this study in a flowchart (Figure 5).

Our research also had some limitations. Firstly, it is important to note that the sample size in this study is relatively small, which may introduce a degree of selection bias. Secondly, longer follow-up periods are necessary to evaluate the long-term efficacy and survival outcomes of different ALK fusion variants, particularly in cohort 1. Additionally, due to the limited number of patients in the crizotinib cohort with identified TP53 gene status at baseline, we were unable to make a more precise categorization. Lastly, the utilization of diverse tumor samples and NGS panels in this study may introduce heterogeneity and potentially compromise the accuracy of our findings.

5 | Conclusions

Patients harboring long and short EML4-ALK fusion variants exhibit distinct responses to ALK-TKIs. Subgroup analysis considering these variants should be incorporated into clinical trials in the future. The first-line alectinib should be recommended for NSCLC patients with EML4-ALK fusion variants regardless of the length of the fusion variant. Patients carrying short fusion variants are more prone to acquiring secondary mutations within the ALK kinase domain. The presence of TP53 gene mutations may impact the long-term efficacy of alectinib, necessitating consideration of more aggressive treatment strategies for patients harboring this mutation at baseline.

Author Contributions

Z.H.Z., J.L.L., and P.Y.X. were responsible for study conception and design. Z.H.Z., L.G.W., X.Z.H., Y.L., L.L., Y.C.G., J.M.Y. collected the data. L.G.W., Z.H.Z., P.Y.X., and J.L.L. assembled the data. Z.H.Z. and L.G.W. analyzed the data. Z.H.Z. and L.G.W. drafted the report. L.G.W. drew illustrations. All authors critically reviewed drafts of the manuscript and read and approved the final manuscript.

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Ethics Statement

This study was approved by Ethics Committee of National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (19/096–1880).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated and analyzed during this study are available from the corresponding authors on reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.