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ORIGINAL ARTICLE

Comparison of response and survival of first-line crizotinib therapy according to EML-4 ALK fusion variants in advanced non-small cell lung cancer patients: A Turkish Oncology Group study

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Summary

Purpose: *In the literature there are conflicting data about the treatment efficacy of anaplastic lymphoma kinase (ALK) translocation positive non-small cell lung cancer (NSCLC) patients according to ALK fusion variants. We aimed to study the impact of ALK fusion variants on the survival of first-line crizotinib-treated NSCLC patients.*

Methods: *101 locally advanced or metastatic ALK positive NSCLC patients treated with first-line crizotinib between January 2013 and December 2019 were retrospectively evaluated. We studied ALK fusion variants in 38 of those patients with adequate tumor tissue with reverse transcription polymerase chain reaction (RT PCR). Patients having ALK fusion variant 1 (v1) and non-variant 1 (non-v1) were compared for survival and response to crizotinib.*

Results: *Median age was 52.5 years (range 35-74), and 22 of 38 patients were male (57.9%). EML-4 ALK v1 was seen in 26 patients (68.4%) and 12 were non-v1 (variant 3a/b in 6, and non-EML-4 ALK variants in 6 patients). Objective response rate was 60.5% in all patients, whereas it was 61.5% in v1 and 58.3% in non-v1 group. Median progression-free survival (PFS) and overall survival (OS) were similar. Median PFS was 13.1 months in v1, and 12.4 months in non-v1 (p=0.232). Median OS was 23.2 months in v1, and 19.4 months non-v1 (p=0.493).*

Conclusion: *ALK v1 and non-v1 patients had the same OS and PFS after first-line crizotinib treatment, however there was a trend for v1 group for better OS.*

Key words: *non-small cell lung cancer, crizotinib, ALK inhibitor, EML4-ALK fusions*

Introduction

Rearrangement of anaplastic lymphoma kinase (ALK) gene has been identified in 3-7% of patients with advanced non-small cell lung cancer (NSCLC) [1]. ALK-tyrosine kinase inhibitors (TKI) have been shown to be superior to chemotherapy in the treatment of ALK-rearranged NSCLC patients. However, there is a lack of clear biomarkers to select patients who will benefit the most from targeted therapy [2-

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7]. Crizotinib is the first generation ALK-TKI and is the first approved agent for first-line therapy with superior progression-free survival times (PFS) and overall response rates (ORR) compared to chemotherapy [3]. ALK fusion variants are the most important genetic factors proposed to be predictive for treatment efficacy. To date, many EML4-ALK and non-EML4 fusion variants have been reported, most common being variant 1 (v1), variant 2 (v2), and variant 3a/3b (v3a/3b) [8-10]. Although many studies have reported response and survival data according to ALK fusion variants, data appears to be contradictory. Therefore, there are still no clear data showing efficacy of crizotinib treatment with respect to ALK fusion variants. In addition, although there are conflicting data relative to PFS, there are no clear OS data. In this study we aimed to compare the response and survival outcomes according to ALK fusion variants in advanced NSCLC patients on first-line crizotinib treatment.

Methods

Study design

Data of 101 metastatic lung adenocarcinoma patients who received first-line crizotinib therapy in 8 major medical oncology centers between January 2013 and December 2019 were retrospectively reviewed. Inclusion criteria were: Patients with NSCLC (adenocarcinoma and adenosquamous type histopathology) having ALK rearrangement by FISH method and who were treated with crizotinib (250 mg orally twice daily) until disease progression. Patients with no archived tumor tissue and whose follow-up information could not be obtained from their medical records were excluded from the study. Only 38 of 101 patients had adequate tumor tissue (Figure 1). Data on demographics and clinical outcome were retrospectively collected. Patients were grouped according to ALK variants as v1 and non-v1 to evaluate survival and response. The study was supported by the Turkish Oncology Group Association.

Identification of ALK fusion variant

All samples were collected from centers and studied at the same institute to design in house by an experienced pathologist. Formalin-fixed, paraffin-embedded (FFPE) tissues were analyzed by reverse transcriptase-quantitative PCR (RT-qPCR). RNA was extracted (Qiagen RNeasy FFPE Kit) from the same FFPE tissues. Primers used in RT-PCR covered the most frequent ALK translocations (v1 (e13;a20), v2 (e20;a20), v3a (e6;a20), v3b (e6;insa20), v4 (e14;(-49)(a20), v5a (e2;a20), v5b (e2;(+117)a20), v6 (e13; (+69)a20), v7 (e14; (-13)a20). One-step RT-qPCR assay uses a proprietary enzyme/buffer mix which enables first-strand cDNA synthesis (reverse transcription) and amplification of endogenous control and fusion genes/mutations in a single step (Primers were used from

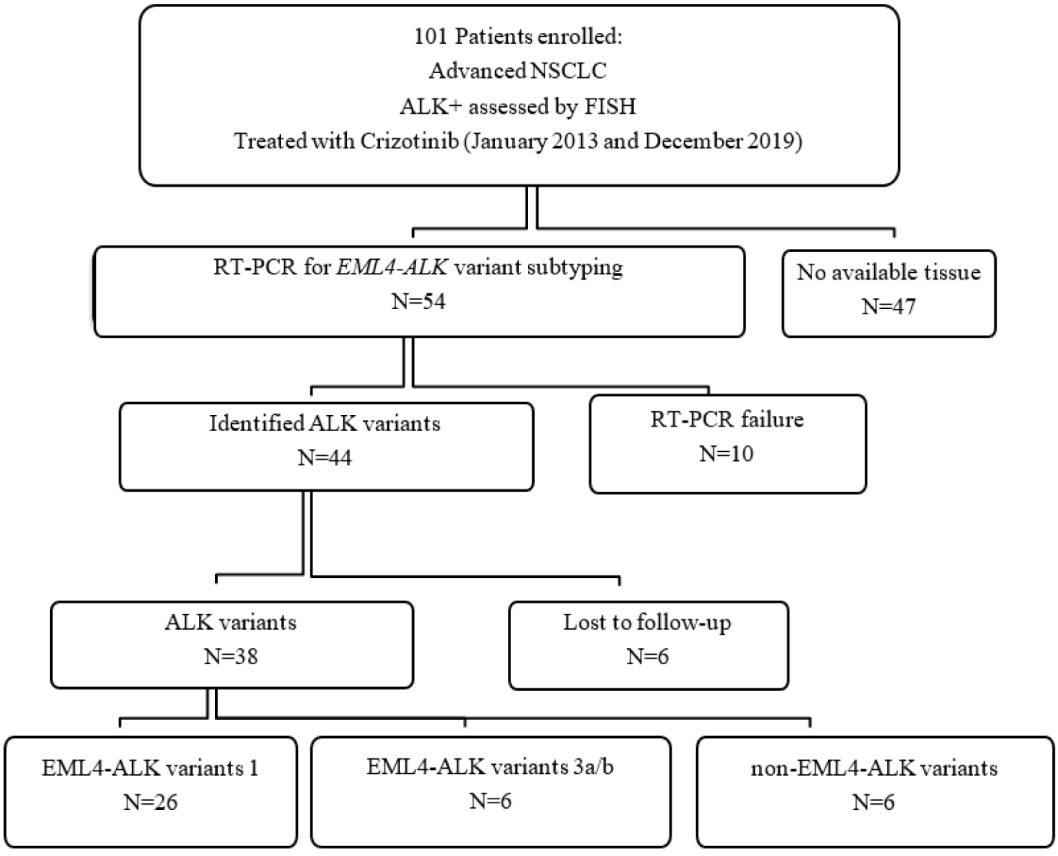

Figure 1. The CONSORT flow chart.

Thermo Fisher). The assay works by amplifying fusion gene and mutant-specific sequences in samples that contain a mixture of mutant and wild-type RNA, and relies on fluorescent probes for detection. Each reaction contains primer sets and probes for detection of the mutations, as well as an endogenous control gene. The endogenous control primers amplify an unrelated gene that is used to determine the condition of reagents and whether the reaction contains a sufficient amount of amplifiable RNA. The testing procedure involves three simple steps:

1. Isolation of RNA from FFPE tumor biopsies. After isolation, RNA concentration is measured using spectrophotometric or fluorometric analysis (i.e., Nanodrop, UV spectrophotometer, Qubit) and adjusted to 16 ng/μl. RNA concentration below 16 ng/μl might need to go through an RNA concentration step.

   This RNA concentration is based on fluorometric analysis of FFPE samples with a Qubit Fluorometer. Using this form of RNA quantification, the assay has been calculated to require approximately 640 ng of RNA i.e. 80 ng of sample per reaction. Because RNA concentrations quantified by spectrophotometric vs. fluorometric analysis will differ, each lab should evaluate the optimal RNA concentration for the assay.
2. Amplification of RNA using the provided reagents.
3. Data analysis and interpretation using real-time PCR software.

   Objective response rates (ORR) and progressive disease (PD) responses of the patients were retrospectively evaluated according to RECIST version 1.1 [11], using the imaging performed during routine follow-up. ORR was calculated as the total percentage of patients with a complete response (CR) or partial response (PR). Patients continued study treatment until they experienced unacceptable toxicity, or until PD was first detected. OS was defined as the time from the start of crizotinib treatment to death of any cause. For OS analysis, patients who did not have events, including those who dropped out or were lost to follow-up, were censored at the time of the last contact. PFS was defined as the time from the start of crizotinib treatment to the date of first documented disease progression or death of any cause. For PFS analysis, patients who did not have events were censored at the time of the last tumor assessment.

Statistics

Patients' demographics and baseline clinical characteristics were summarized using frequencies and percentages for categorical variables. The difference between v1 and non-v1 mutation was investigated using chi-square, Fisher exact and Student's t-tests, where appropriate. If p value was less than 0.05, the difference between the groups was considered statistically significant.

Median OS and PFS were estimated using the Kaplan–Meier method and the groups were compared by log-rank test. Statistical analyses were performed using SPSS 24 software.

Results

Clinicopathologic characteristics of patients

Patient demographics and baseline clinical characteristics are summarized in Table 1. Median age was 52.5 years (35-74), and 22 patients (57.9%) of the group were male. Twenty-eight patients (73.7%) had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1. Patients had stage IIIB or IV disease at the time of diagnosis, except one who was treated after postoperative relapse. Eighteen patients (47.4 %) were never smokers, 3 (7.9 %) were former, and 17 (44.7 %) were current smokers. Fourteen (36.8%) had brain metastasis at the time of diagnosis. Treatment responses were CR in 7 (18.4%), PR in 16 (42.1%), SD in 2 (5.3%), PD in 13 patients (34.2%). ORR of the entire patient group was 60.5%. ALK v1 was the most common variant seen in 26 patients (68.4%). The remaining 12 patients consisted of v3a/3b in 6 (15.8%), and non-EML ALK fusion variant in 6 (15.8%). Patients treated before 2018 could not use a second ALK TKI treatment. Only 13 patients were given second-line ALK TKI (11 had alectinib and 2 lorlatinib), and 11 had v1 and 2 non-v1.

Table 1. Patients, disease and treatment characteristics

Characteristics	*Patients n (%)*
Median age, years (range)	52.5 (34-75)
Sex	
Male	22 (57.9)
Female	16 (42.1)
PS	
0-1	28 (73.6)
2	8 (21.1)
3	2 (5.3)
Smoking status	
Never smoker	18 (47.4)
Former smoker	3 (7.9)
Current smoker	17 (44.7)
Stage	
Postoperative recurrence	1 (2.6)
IIIB	4 (10.5)
IV	33 (86.9)
Adenocarcinoma histology	38 (100)
Brain metastasis	
Yes	14 (36.8)
No	24 (63.2)
First-line crizotinib treatment	38 (100)
Second-line TKI	13 (34.2)
Second-line chemotherapy	4 (10.5)

PS: ECOG performance status, TKI: tyrosine kinase inhibitor

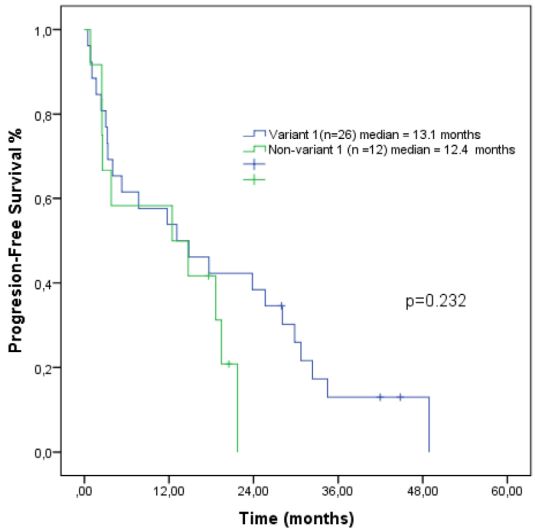

Figure 2. Kaplan-Meier curves for the progression-free survival in patients with ALK variant 1 (n=26) versus non-variant 1 (n=12).

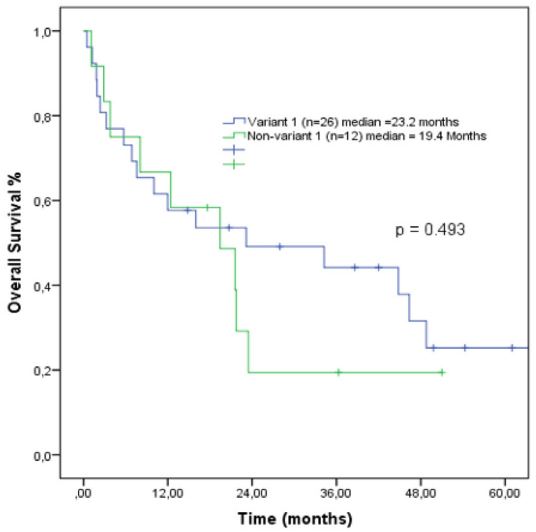

Figure 3. Kaplan-Meier curves for the overall survival in patients with ALK variant 1 (n=26) versus non-variant 1 (n=12).

Table 2. Clinical characteristics according to ALK variants

	Variant 1		*Non-variant 1*		*p*
	n	%	*n*	%	
Sex					0.970
Female	11	42.3	5	41.7	
Male	15	57.7	7	58.3	
Stage					0.321
I (relapse)	0	0.0	1	8.3	
IIIB	3	11.5	1	8.3	
IV	23	88.5	10	83.4	
Liver metastasis					0.972
No	24	92.3	11	91.7	
Yes	2	7.7	1	8.3	
Lung metastasis					0.367
No	9	34.6	6	50.0	
Yes	17	65.4	6	50.0	
Brain metastasis					0.062
No	19	73.1	5	41.7	
Yes	7	26.9	7	58.3	
Bone metastasis					0.851
No	16	61.5	7	58.3	
Yes	10	38.5	5	41.7	
Adrenal metastasis					0.402
No	24	92.3	10	83.4	
Yes	2	7.7	2	16.6	
LAP metastasis					0.252
No	16	61.5	5	41.7	
Yes	10	38.5	7	58.3	
Clinical Response					0.744
CR	5	19.2	2	16.6	
PR	11	42.3	5	41.7	
SD	2	7.7	0	0.0	
PD	8	30.8	5	41.7	

LAP: lymphadenopathy, CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease

Treatment responses and survival among patients with different EML4-ALK variants

Two groups, v1 (26 patients) and non-v1 (12 patients), were compared for treatment responses and survival. Demographic and clinical features between v1 and non-v1 are presented in Table 2. ORR with crizotinib were 61.5% and 58.3% in v1 and non–v1 group, respectively (p=0.744). There was no significant difference between the two groups in terms of treatment response rates, median PFS and OS. Median PFS in v1 group was 13.1 months (95% CI, 0.63 to 25.58), and in non-v1 it was 12.4 months (95% CI, 0.00 to 30.96) (p=0.232) (Figure 2). Median OS was 23.2 (95% CI, 0.00 to 55.39) months in v1, and 19.4 (95% CI, 5.78 to 29.65) months in non-v1 (p=0.493) (Figure 3). Although there were only 13 patients who also used second-line ALK TKI, it was found that median OS for second-line was not reached in patients with v1, while it was only 8 months in non-v1 (p<0.001).

Discussion

In this study, we evaluated the response and survival according to ALK fusion variants of patients using crizotinib in the first-line treatment. Our analysis showed that crizotinib was effective in the ALK-positive patients with any kind of ALK variants, and no difference was observed in ORR, PFS and OS between v1 patients and non-v1. Therefore, there might not be a correlation between crizotinib treatment efficacy compared to ALK fusion variants.

Although there are a lot of data in the literature regarding ALK fusion variants, we have very conflicting information about the effectiveness of crizotinib treatment compared to ALK fusion variants. One of the most important reasons for this may be that the studies consist of heterogeneous groups. These contradictory results may be caused by the common efficacy analysis of patients using tyrosine kinase inhibitor (TKI) treatment at different steps or receiving different TKI treatments according to variants. EML4-ALK fusion variant rates, ORR and survival outcomes of our current study and previously published studies are summarized in Table 3.

Variant 1 is the most frequently reported ALK fusion variant with rates between 36% and 54.5% in studies [8,10,12-15,18,20]. In some studies, v3a/b has been reported as a common ALK fusion variant ranging from 33% to 42.7% [9,16,17,19]. The most common ALK fusion variant group in our study was determined as variant 1 patient (68.4%) group, which is consistent with the literature, but the highest rate reported so far is ours. Although the RT-PCR methods used in the present study were designed to detect 9 types of EML4-ALK rearrangements, only v1, v3a/b and non EML4-ALK were identified in our patients.

In our study, the objective response rate of patients receiving crizotinib treatment was 60.5%, while it was 61.5% in patients with v1, and 58.3% in non-v1. There was no significant difference between the two groups in terms of ORR. Similarly, Yoshida et al in their study, while the ORR was 74% in the v1 patient group, it was 63% in the non-v1, and the difference was not significant [8]. In another study, the ORR observed in the variant 1 and variant 3a/b groups of patients who received first-line crizotinib were 70.4% and 68%, respectively, and the difference was not significant [20]. Both studies supporting our study results showed that there was no difference in response rates of patients receiving crizotinib treatment compared to ALK fusion variants. In addition, none of the previous studies reported a difference in treatment response rates compared to ALK fusion variants in crizotinib treatment (Table 3).

Although all studies gave similar results in terms of treatment response rates, very different results were reported in survival data. In one of the first studies, Yoshida et al reported that the v1 group had a better PFS than the non-v1 group; however, their study included patients using crizotinib in 3 different steps [8]. Lei et al observed that there was no difference in patients receiving crizotinib treatment in PFS when they categorized patients as variant 1, variant 3a/b, and others in their study. In the study, it was reported that there was a significant difference between 10.5 vs 8.3 months (p=0.020) in the median PFS comparison of patients who received first-line therapy and patients who received second-line therapy [12]. Woo et al and Christopoulos et al compared patients on crizotinib with ALK v3a/b and non-v3, and both reported statistically significant difference for prolonged PFS in non-v3 group [9,17]. On the contrary, other 4 studies compared v1 versus others, and reported that crizotinib treatment did not differ in PFS compared to ALK fusion variants [14-16,18]. Update analysis of the phase 3 ALEX study, first-line treatment of ALK rearranged NSCLC with ALK-TKIs an showed alectinib to have better PFS than crizotinib in all ALK fusion variants (v1, v2, V3a / b) [20]. However, when crizotinib patients were analyzed, PFS and ORR were similar among ALK fusion variants. This was similar to our studys' patient population and findings.

ALK fusion variants' impact on OS was reported only in 3 studies prior to our study [17-19]. However, heterogeneous groups of patients were included, and in those studies different ALK TKIs

Table 3. Recent studies on ALK fusion variants: variant rates, crizotinib treatment response and survival outcomes

References	*Detection of ALK fusion*	*ALK TKI*	*Timing of ALK TKI*	*Patient number*	*Prominent ALK fusion variant*	*Comparison groups*	*Objective response rate*	*Median PFS months*	*Median OS months*
Yoshida et al. (8)	RT-PCR	Crizotinib	Mixed	35	V1 54%	V1 vs non -V1	74% vs 63% NS	11 vs 4.2 SS	NR
Woo et al. (9)	RT-PCR	Crizotinib	Mixed	51 (44*)	V3 44%	V3 vs non-V3*	75% vs 83.3%* NS	2 year 26.4% vs 76%* SS	NR
		Ceritinib							
		Alectinib							
Lin et al. (10)	RT-PCR	Crizotinib	First-line ALK TKI[a]	99[a]	V1 43%	V1 vs V3	NR	8.9 vs 6.9 NS	NR
	NGS		First-line[b]	55[b]					
Lei et al. (12)	RACE-coupled PCR	Crizotinib	Mixed	61	V1 36%	V1 vs V3 vs Others	72.7% vs 55.6 vs 81% NS	11 vs 10.9 vs 7.4 NS	NR
Cha et al. (14)	RT-PCR	Crizotinib	Mixed	52 (32*)	V1 38.5%	V1 vs V2 vs V3 vs non-EML4 *	30% vs 100% vs 50% vs 66.7%* NS	Not disclosed numbers[x]* NS	NR
		Ceritinib							
		Alectinib							
Lin et al. (15)	RT-PCR	Crizotinib	Mixed	54	V1 43%	V1 vs V2 vs V3 vs Others	43% vs 66% vs 60% vs 57% NS	6.1 vs 11 vs 7.3 vs 5.5 NS	NR
Li et al. (16)	NGS	Crizotinib	Mixed	60	V3 33%	V1 vs non-V1	46.1% vs 63% NS	12.3 vs 15.8 NS	
						V2 vs non-v2	66.6% vs 56.8% NS	34.5 vs 12.3 SS	NR
Christopoulos et al. (17)	RT-PCR	Crizotinib	Mixed	67 (21*)	V3 51%	V3 vs non-V3	NR	7.3 vs 39.3 SS	39.8 vs 59.6 SS
	NGS	Alectinib							
		Ceritinib							
Mitiuskina et al. (18)	RT-PCR	Crizotinib	Mixed	64 (23*)	V1 52%	V1 vs non -V1	75.6% vs 80.7% NS	Not disclosed numbers[x] NS	Not disclosed numbers[x] NS
		Alectinib							
		Ceritinib							
Su et al. (19)	NGS	Crizotinib	Mixed	110	V3 42.7%	EML4 vs nonEML4	NR	9.4 vs 14.5 NS	35.1 vs 35.5 NS
Camidge et al. (20)	NGS	Crizotinib	Firstline	203 (96*)	V1 42.7 %	V1 vs V2 Vs V3*	NR	12.9 vs 8.8 vs 14.6* NS	NR
	Plasma-Tissue	Alectinib		Tissue					
Current study	RT-PCR	Crizotinib	Firstline	38	V1 68.4%	V1 vs non-V1	61.5% vs 58.3% NS	13.1 vs 12.4 NS	23.2 vs 19.4 NS

NR: not reported, SS: statistically significant, NS: not significant, a: Patients who received crizotinib as first-line ALK TKI, b: Patients who received crizotinib as first-line treatment * patients who only used crizotinib; x only Kaplan-Meirer curves were available

were used in different lines of treatment. Christopoulos et al reported that the survival of patients in non-v3a/b group was significantly better than v3a/b [17]. Mitiuskina et al [18] and Su et al [19] reported that there was no OS difference with ALK TKI according to ALK fusion variants (as v1 versus non-v1, and EML4 versus non-EML4, respectively). We also found similar OS between ALK variants, although patients with v1 had numerically survived longer (23.2 months in v1 and 19.4 months in non-v1) on first-line crizotinib. To our knowledge, ALK variants' impact on OS data is the first study in first-line crizotinib-treated patients. This insignificant difference in OS analysis in our study may be related to the small number of our patients.

Although conducted in a selected patient group, our study has many limitations. It has a retrospective design and therefore only a limited number of patients could be included. It would also be better if ALK TKI resistance mutations could be looked at on progression as well as in primary ALK fusion variants. Although there were only 13 patients who got second-line ALK TKI on progression, patients with v1 seemed to live longer than non-v1 group. It was thought that the OS difference between patients with v1 and non-v1 in second-line TKI therapy may be due to the fact that resistance mutations that may develop after first-line crizotinib may differ according to ALK fusion variants. Lin et al reported that no ALK G1202r mutation was detected in v1 group, while it was seen in 32% of the v3a/b patients who had previously received ALK TKI treatment. They also reported that all ALK resistance mutations developed under ALK TKI treatment were 30% in v1 group and 57% in v3a/b group [10]. Differences in resistance mutations that may develop during treatment may also explain the OS difference of the v1 group.

Conclusions

Although we cannot select the group that will respond better with ALK fusion variants from the beginning of treatment, patients with ALK v1 might have better survival with multiple line ALK TKI treatments. We think that both ALK fusion variants at diagnosis and ALK resistance mutations at progression must be studied in depth to lead for treatment selection in the future.

Study approval

This study was approved by the instituonal review board of the Akdeniz University Hospital (EC:70904504/145-224;28.03.2018). Written informed consent was received from participants to inclusion in the study.

Author contributions

Conception and design: Ali Murat Tatli, Perran Fulden Yumuk.
Collection and assembly of data: All authors.
Data analysis and interpretation: Ali Murat Tatli, Perran Fulden Yumuk, Buge Oz, Saadettin Kilickap.
Manuscript writing: Ali Murat Tatli, Perran Fulden Yumuk, Buge Oz, Saadettin Kilickap.
Final approval of manuscript: All authors.

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Conflict of interests

The authors declare no conflict of interests.

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