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REVIEW ARTICLE

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Fusion variants of the *ALK* gene — the key to modern therapy for patients with ALK-positive non-small-cell lung cancer

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ABSTRACT

Since the discovery of the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (*EML4-ALK*) fusion in non-small-cell lung cancer (NSCLC), followed by the identification of many different fusion variants, molecularly targeted therapy has revolutionized treatment for patients with ALK-positive lung cancer. Recent research has focused on understanding how specific variants may influence the biological and molecular behavior of cancer cells and how this knowledge can be used in routine clinical practice. This article explores the current understanding of *EML4-ALK* variants and highlights unanswered questions in the field.

Keywords: *EML4-ALK* Variant 1, *EML4-ALK* Variant 2, *EML4-ALK* Variant 3, NSCLC

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Introduction

Since the discovery of *ALK* gene rearrangement in non-small-cell lung cancer (NSCLC), many fusion variants of echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (*EML4-ALK*), categorized on the basis of *EML4* breakpoints, have been identified [1]. The *EML4-ALK* is the dominant fusion variant, accounting for approximately 80% of ALK-positive NSCLC cases [2]. It is also known that fusions occur with *KIF5B*, *TFG*, *KLC1*, *HIP1*, *TPR*, *SOCS5*, and *BIRC6* [3, 4]. Pathogenic *ALK* gene variants are observed in 2–9% of NSCLC patients [5]. Progress made in treating ALK-positive NSCLC patients has been documented by the approval of six ALK tyrosine kinase inhibitors (TKIs): 1st-generation crizotinib, 2nd-generation alectinib, brigatinib, ceritinib, ensartinib, and 3rd-generation lorlatinib. Therapeutic advances have been accompanied by significant progress in diagnostic methods. Initially, *ALK* gene rearrangement was detected through immunohistochemistry (IHC), which identifies the expression of abnormal ALK protein on the surface of cancer cells, or by using the fluorescence *in situ* hybridization (FISH) technique, which detects EML4/ALK translocation. However, these tests do not allow for the identification of fusion partners or the determination of the specific *EML4-ALK* fusion variants. This limitation was addressed by next-generation sequencing (NGS) technology. In Poland, all three methods are employed to qualify NSCLC patients for treatment, but access to NGS is available in few oncology centers.

EML4-ALK variants in NSCLC

The *EML4-ALK* rearrangement arises from an inversion on the short arm of chromosome 2, where both

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Table 1. The most common *EML4-ALK* fusion variants

variant	breakpoint location	Frequency	TAPE domain	References
Variant 1	E13 A20	33%	Partial TAPE	1, 14, 17
Variant 2	E20 A20	10%	Partial TAPE	1, 14, 17
Variant 3	E6 A20	29%	No TAPE	14, 17
Variant 4′	E14 A20	3%	Partial TAPE	17
Variant 5	E20 A20	2%	No TAPE	17

TAPE — tandem atypical beta-propeller

EML4 and *ALK* are located [6]. To date, 16 breakpoints have been identified within *EML4*: 2, 3, 6, 7, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, and 23 [7–13]. These breakpoints result in various *EML4-ALK* variants with differing lengths and protein stability [14]. The most common variant is v1 (v1), in which exon 13 of *EML4* fuses with exon 20 of *ALK* (33%), followed by variant 3a/3b (v3), where exon 6a or 6b of *EML4* fuses with exon 20 of *ALK* (29%), and v2, in which exon 20 of *EML4* fuses with exon 20 of *ALK* (10%) [14]. The full-length EML4 protein contains an N-terminal coiled-coil trimerization domain, followed by a tandem atypical beta-propeller (TAPE) domain that constitutes the rest of the EML4 protein [15]. The TAPE structure comprises a hydrophobic motif in the EML protein domain (HELP), which mediates tubulin binding, and nine tryptophan-aspartate (WD) repeats that facilitate protein-protein interactions. Functionally and clinically, *EML4-ALK* variants can generally be classified as "short" variants (v3a/b and v5a/b, which lack the TAPE domain) and "long" variants, which include parts of the TAPE domain, resulting in varying degrees of cellular protein stability [15, 16]. Detailed information on the most common *EML4-ALK* variants, their frequency, and the presence of the TAPE domain is presented in Table 1 [1, 14, 17].

Retrospective single-center analysis

In 2016, Yoshida and colleagues [15], through a retrospective analysis of 35 patients with advanced NSCLC harboring *ALK* gene rearrangements, demonstrated differences in survival and response rates based on the fusion variant. The patients were treated with crizotinib in the first, second, or third line of therapy. The most common variant in the studied group was v1, identified in 19 patients (54%), followed by v2 (5 patients,14%), and variants 3a/3b (4 patients, 12%). Other variants were reported in the remaining patients. For the analysis of survival rates and objective response rates (ORR), the study population was divided into groups with and without v1. The ORR in the v1 group was 74% compared to 63% in the group without v1. A significantly higher proportion of patients with v1 achieved disease control compared to patients without v1 (95% *vs*. 63%). Similarly, median progression-free survival (PFS) was significantly longer in the v1 group (11.0 months) compared to the group without v1 (4.2 months). For patients with ALK-positive NSCLC, the most common site of progression was the CNS. In the studied population, there were no differences in the rate of intracranial progression between the analyzed groups [15].

Retrospective analyses of randomized prospective trials

Of the global phase III randomized trials conducted to verify an ALK TKIs values PROFILE1014 [18], ASCEND-4 [19], ALEX [20], ALTA-1L [21], eXalt3 [22], CROWN [23] additional survival analyses in relation to *EML4-ALK* variants were performed exclusively in the ALEX and ALTA-1L trials [24, 25].

In the ALEX trial, 303 patients were randomized 1:1 to receive either alectinib 600 mg twice daily or crizotinib 250 mg twice daily. Treatment was continued until disease progression or unacceptable toxicity. Patients with asymptomatic CNS metastases were eligible for the study. At the start of the trial, plasma and tumor tissue samples were collected for NGS analysis to assess the frequency of the most common *EML4-ALK* variants (v1, v2, and v3a/b), dividing the samples into plasma and tumor tissue subgroups [24]. Camidge and colleagues demonstrated that the most common fusion variants were v1 and v3a/b, with similar frequencies in plasma samples (v1: 37%, v3a/b: 36.3%) and tumor tissue samples (v1: 42.7%, v3a/b: 37.1%) [24]. In the plasma subgroup, median PFS for patients treated with alectinib compared to crizotinib was 34.8 *vs*. 7.4 months for patients with v1. For v2 patients, PFS was 24.8 *vs*. 8.8 months. For v3a/b patients, median PFS was 17.7 *vs*. 9.1 months [24]. In this additional analysis, also in the tumor tissue subgroup, median PFS for patients treated with alectinib compared to crizotinib was not reached (NR) for alectinib compared to 12.9 months for crizotinib in variant 1, 11.5 *vs*. 8.8 months in variant 2, and 34.9 *vs*. 14.6 months in variant 3a/b. Differences in PFS between variants 1, 2, and 3a/b were not significant in either treatment arm or sample type.

In the ALTA-1L trial, evaluating the efficacy of brigatinib versus crizotinib in the first-line treatment of ALK-positive NSCLC, additional analyses of efficacy in relation to fusion variants were also conducted [25]. During screening, blood samples were collected, and NGS analysis was performed to determine the fusion variant. The study included 124 patients in the brigatinib arm and 127 patients in the crizotinib arm. The three dominant *EML4-ALK* fusion variants (v1, v2, v3) were evenly distributed between arms, with v1 more commonly observed in patients without CNS metastases (47% *vs*. 36%). Sex and age did not influence the frequency of individual variants. Brigatinib demonstrated superiority in terms of ORR and median PFS compared to crizotinib across all variants; however, v3 patients had poorer PFS compared to those with v1 and v2, regardless of the treatment used.

Does the fusion variant have prognostic significance?

Christopoulos and colleagues analyzed a group of 67 patients with the most common fusion variants (v1, v2, and v3) and demonstrated that patients harboring v3 had a higher number of metastases than those with the other two variants (v3: 3.25 vs. v1: 1.88 vs. v2: 1.57) [26]. Both intrapulmonary metastases and extrathoracic metastases were more frequently reported in patients with v3. Additionally, metastases in atypical locations, such as the spleen or kidneys, were commonly observed. Patients with the v3 fusion variant in NSCLC had shorter median PFS when treated with both first- and second-generation TKI inhibitors (7.3 *vs*. 39.3 months for first-line treatment and 5.0 *vs*. 11.3 months for second-line treatment) [26].

Co-occurring mutations

The *TP53* gene mutation is the most common genetic alteration in NSCLC and may co-occur with driver mutations, such as *EGFR* mutations or *ALK* gene rearrangements. Previous studies have shown that the presence of a TP53 mutation is an unfavorable prognostic factor for survival rates [27]. However, no differences were observed in the frequency of *TP53* mutations across the most common *EML4-ALK* fusion variants (v1, v2, and v3) [28].

EML4-ALK fusion variants in resectable NSCLC

Hong Tao and colleagues [29] reported findings from a study of 55 patients with resectable NSCLC in clinical stages I–III. The most common variant in the studied population was v 1 (45.5%), followed by v3 (34.5%) and v2 (14.5%). The frequency of v3, associated with poorer prognosis, was lower in earlier stages of disease compared to stage III (29.0% *vs*. 41.7%). However, no clinically significant correlation was observed between the frequency of individual variants and the clinical stage of the disease. The median disease-free survival (DFS) was 22.1 months. Multivariate analysis showed that patients with stage T1 disease and variants other than v3 had longer DFS than patients with stage T2-T4 disease [hazard ratio (HR) = 0.350; 95% confidence interval (CI) 0.45–0.845; p = 0.020] and v3 (HR = 0.249; 95% CI 0.076–0.823; p = 0.023) [29].

Discussion

The identification of *EML4-ALK* fusion variants has significantly contributed to our understanding of ALK-positive NSCLC and its therapeutic implications. As demonstrated in multiple studies, a specific fusion variant may influence treatment response, disease progression, and survival outcomes. A retrospective analysis conducted by Yoshida et al. [15] revealed that v1 was the most prevalent fusion type and was associated with better treatment outcomes in response to crizotinib compared to other variants. Patients harboring v1 had higher ORR and longer PFS than those without v1. This aligns with findings from randomized trials, such as ALEX and ALTA-1L, which further demonstrated that PFS differed among patients with various fusion variants but did not show statistically significant differences in overall survival (OS) [24, 25]. Superior PFS was observed in v1 patients, which suggests that this variant may be more sensitive to ALK TKI therapy, particularly with second-generation inhibitors like alectinib and brigatinib.

Conversely, patients harboring v3 often have poorer prognosis. Christopoulos et al. [26] reported that the v3 fusion variant was associated with higher metastatic burden and shorter median PFS when treated with first- and second-generation ALK TKIs. This suggests that structural differences in the fusion protein may impact its stability and oncogenic potential, influencing the tumor's aggressiveness. The increased presence of metastases in atypical locations further underscores the potential of v3 as a marker of aggressive disease.

The presence of co-occurring TP53 mutations has also been explored as a factor influencing prognosis in ALK-positive NSCLC. While TP53 mutations are generally considered unfavorable prognostic factors in NSCLC, studies have not demonstrated significant differences in their frequency among *EML4-ALK* variants [27, 28].

In the context of resectable NSCLC, studies indicate that the frequency of v3 increases with disease

progression, although no significant correlation between fusion variants and disease stage has been established [29]. Notably, patients with non-v3 variants and lower tumor stages had significantly longer DFS, highlighting the potential prognostic value of fusion variant profiling in early-stage disease management.

Despite these insights, several questions remain. While *EML4-ALK* variants exhibit differential responses to ALK TKIs, the exact biological mechanisms underlying these differences are not fully understood. Further research is needed to elucidate how specific structural features of the fusion protein influence kinase activity, drug binding, and resistance mechanisms. Additionally, the role of emerging biomarkers, such as circulating tumor DNA (ctDNA), in refining treatment strategies warrants further investigation.

Access to NGS remains a challenge in certain regions, including Poland, limiting the ability to comprehensively profile ALK fusion variants in clinical practice. Expanding access to NGS and integrating variant-specific treatment strategies could enhance personalized therapeutic approach es, optimizing patient outcomes.

Conclusions

In conclusion, *EML4-ALK* fusion variants play a crucial role in determining NSCLC prognosis and treatment response. While v1 is associated with better outcomes with current ALK TKIs, v3 is associated with a more aggressive disease course. Future research should focus on improving variant-specific treatment strategies, identifying additional prognostic biomarkers, and expanding the use of comprehensive molecular profiling in routine clinical practice.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

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