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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2026;15(1):370-6

NTRK gene fusions in non-small cell lung carcinomas and its correlation with other somatic mutations

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Received 28 December 2025; Accepted 09 February 2026
Available online 25 February 2026 with doi: 10.5455/medscience.2025.12.360

Abstract

NTRK gene fusions are rare but actionable oncogenic drivers of non-small cell lung cancer (NSCLC). Although receptor tyrosine kinase (TRK) inhibitors show high efficacy, the relationship between NTRK status, concurrent mutations, and immune biomarkers, such as PD-L1, remains to be fully elucidated. We retrospectively evaluated 413 NSCLC cases that underwent comprehensive profiling for NTRK fusions and other somatic alterations using targeted next-generation sequencing (NGS). PD-L1 expression was assessed using immunohistochemistry at 1%, 10%, and 50% cut-off levels. NTRK fusions were identified in 12 (2.9%) patients. The median age of the NTRK fusion-positive group was 65 years, with male predominance (83.3%). Adenocarcinoma was the most common histological type (50.0%). Genomic analysis revealed a high rate of co-occurring mutations, particularly in EGFR (25.0%) and TP53 (33.3%), whereas fusions in BRAF (8.3%) and FGFR (8.3%) were also detected. Notably, NTRK fusion-positive tumors showed a significantly lower rate of PD-L1 expression at the 1% cut-off compared to NTRK fusion-negative tumors (11.1% vs. 58.0%, p=0.006). Our study revealed that NTRK fusion-positive NSCLC frequently exhibits a complex molecular architecture characterized by concurrent alterations, most notably in EGFR and TP53. These findings align with the recent literature challenging the traditional “single-driver" model and suggest that such genomic complexity may mediate bypass signaling pathways. The presence of these co-occurring mutations underscores the clinical necessity of comprehensive NGS profiling to identify potential resistance mechanisms and guide personalized therapeutic strategies beyond solitary TRK inhibition.

Keywords: NTRK fusion, non-small cell lung carcinoma, somatic mutation, PD-L1

Introduction

As integral members of the receptor tyrosine kinase (TRK) family, TRKA, TRKB, and TRKC proteins are synthesized from the neurotrophic tyrosine receptor kinase 1 (NTRK1), NTRK2, and NTRK3 genetic sequences, respectively. These receptors are essential for the physiological growth, maintenance, and protection of the nervous system, and their activation triggers intracellular dimerization and autophosphorylation of tyrosine kinase domains [1]. NTRK fusions typically lead to constitutive activation of the TRK signaling pathway, promoting tumor cell proliferation and survival [2,3]. NTRK fusions are among five agnostic biomarkers, and treatment options include FDA-approved larotrectinib (a pan-TRK inhibitor) and entrectinib (a multikinase inhibitor targeting pan-TRK, ROS1, and ALK) [4–6].

Infantile fibrosarcoma, congenital mesoblastic nephroma, and secretory carcinomas of the breast and their analogs are rare malignancies in which NTRK fusions are highly prevalent, with an incidence of 90% or higher. However, it is quite rare in the most common tumors, such as lung tumors (<1%) [7]. Non-small cell lung carcinoma (NSCLC) is a major cause of cancer-related deaths globally, driving the need for effective molecular targets to improve patient outcomes. The treatment of NSCLC has recently evolved from standard chemotherapy to personalized medicine, largely due to the discovery of key mutations in genes such as EGFR, ALK, ROS1, and KRAS [8]. Among these, NTRK gene fusions involving NTRK1, NTRK2, or NTRK3 have emerged as

CITATION
Sagnak Yilmaz Z, Turkmen Usta Z, Teoman G, et al. NTRK gene fusions in non-small cell lung carcinomas and its correlation with other somatic mutations. Med Science. 2026;15(1):370-6.

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DOI: 10.5455/medscience.2025.12.360

rare but highly significant therapeutic targets [9]. Although NTRK fusions occur in fewer than 1% of NSCLC cases, the remarkable clinical efficacy of TRK inhibitors (such as larotrectinib and entrectinib) underscores the need for routine molecular profiling in patients with advanced-stage disease [4,10].

These alterations are often reported to be mutually exclusive to other major driver mutations; however, recent studies using comprehensive next-generation sequencing (NGS) have occasionally identified co-occurring alterations [11]. Understanding the genomic landscape of NTRK fusion-positive NSCLC is crucial for predicting treatment resistance and clinical behavior.

Furthermore, the relationship between NTRK fusions and the tumor immune microenvironment, specifically programmed death ligand 1 (PD-L1) expression, remains an area of active investigation. Although immunotherapy has become a cornerstone of NSCLC treatment, its integration into the management of patients with rare targetable fusions remains complex. Preliminary data suggest that oncogene-driven tumors may exhibit distinct PD-L1 expression patterns, which could influence the choice between targeted therapy and immune check-point inhibitors [12].

In this study, we investigated the prevalence of NTRK gene fusions in a large cohort of 413 patients with NSCLC using targeted NGS. We aimed to elucidate the clinicopathological features and somatic mutation profiles associated with NTRK status. Additionally, we analyzed PD-L1 expression levels across multiple clinically relevant cut-offs to determine whether NTRK fusions correlated with high PD-L1 expression, providing insights into the potential role of immunotherapy in this unique patient subset.

Material and Methods

Case Selection and Study Design

In this study, 413 NSCLC cases that underwent NGS-based DNA and RNA mutation analyses between 2019 and 2023 were retrospectively evaluated. This study was approved by the Karadeniz Technical University Faculty of Medicine Research Ethics Committee (protocol number: 2023/255). The study cohort comprised specimens obtained through various clinical procedures, including lung core needle biopsy, endobronchial ultrasound-guided transbronchial needle aspiration, and surgical resection. Demographic parameters, including sex and mean age, were documented.

PD-L1 Immunohistochemical Analysis

PD-L1 expression (Ventana SP263 clone) was assessed using an automated immunostaining platform (Ventana Medical Systems, Inc.). PD-L1 analysis was performed in 402 cases, and the results were recorded as tumor proportion scores (TPS). PD-L1 expression was categorized based on established clinical cut-offs (1 %, 10 %, and 50 %) for membrane staining of neoplastic cells.

Molecular Profiling and Somatic Mutation Analysis

Somatic mutations were identified using NGS on the Illumina MiSeq platform, utilizing the QIAsec solid-custom microsatellite instability panel. The analysis of DNA mutations targeted a comprehensive set of genes: ALK, BCOR, BRAF, BRCA1, BRCA2, CDKN2A, CTNNB1, EGFR, ERBB2, ERBB3, FBXWY, FGFR1, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, PIK3R1, POLE, PTEN, ROS1, RPL22, SMAD4, TERT, TP53, and WNT1. To determine the microsatellite instability (MSI) status, nine specific loci were examined: BAT40(T)37, MONO-27(T)27, BAT26(A)27, NR24(T)23, BAT25(T)25, NR22(T)21, HSP110-T17(T)17, NR21(A)21, and BAT34C4(A)18. MSI status was determined in 379 patients using NGS.

The following genes were analyzed for RNA fusions via NGS: ALK, BAIAP2L1, BRAF, BRD4, CEP89, CLCN6, CLIP4, EGFR, EML4, ETV6, FGFR1, FGFR2, FGFR3, FN1, GATM, GNAI1, HIP1, KAP9, KIF5B, KLC1, LMNA, MET, NACC2, NPM1, NRG1, NTRK1, NTRK2, NTRK3, PLAG1, QKI, RANBP2, RET, ROS1, SEC31A, SLC45A3, SND1, TACC1, TCF3, TFG, TPM3, TPM4, TPR, TPR, VCL, WDCP, and ZNF703.

DNA/RNA Isolation and NGS Technical Specifications

Genomic material was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissues using the QIAGEN GeneRead DNA/RNA FFPE Kit. The DNA/RNA yield was quantified using a Qubit 4, ensuring sufficient material for the study. The genetic quality was verified using the Qiaxcel gel run method. NGS was performed on the Illumina NovaSeq system, with bioinformatic processing performed using the Qiagen Clinical Insight (QCI) Interpret and CLC Genomic Workbench platforms.

For FFPE-derived DNA NGS, the detection criteria included a read depth of 500X, an allele frequency of 5%, and a quality score (QUAL) > 200. Detected variants were categorized according to the Tier Classification; those of clinical importance were recorded as pathogenic or likely pathogenic. MSI status was defined using the following thresholds: MSI-H (>40% instability), MSI-L (15-40% instability), and MSS (<15% instability). RNA NGS studies are designed to detect fusions, including novel fusions involving clinically significant exons. Additionally, the panel evaluated genomic alterations characterized by exon skipping in the MET and EGFR genes.

Statistical Analysis

Associations between demographic data, PD-L1 expression, somatic mutations, RNA fusions, and MSI status were statistically compared between the NTRK1-, NTRK2-, or NTRK3-fusion-positive and NTRK1-, NTRK2-, or NTRK3-fusion-negative groups. Data analysis was conducted using SPSS version 31.0.

DOI: 10.5455/medscience.2025.12.360

Categorical variables are presented as numbers and percentages, whereas numerical variables are described using the median and 1st–3rd quartiles. Normality was assessed using the Kolmogorov-Smirnov test. Comparisons of continuous variables between the two independent groups were performed using the Student's t-test for normally distributed data and the Mann–Whitney U test for non-normal distributions. Categorical data were analyzed using the chi-square test. Statistical significance was set at $p<0.05$.

Result

Table 1 summarizes the comparative analysis of the clinicopathological and molecular characteristics of NTRK fusion-positive and NTRK fusion-negative patients. The evaluated parameters included demographic data (age and sex), histological subtypes, somatic DNA mutations, RNA fusions, MSI status, and PD-L1 expression levels across three distinct TPS cut-off thresholds.

Table 1. Clinicopathological characteristics, genomic landscape, and PD-L1 expression profile of NTRK fusion-positive vs. negative NSCLC patients

Categorical variables	NTRK fusion-positive cases (n=12)	NTRK fusion-negative cases (n=401)	p-value
Gender (n, %)			
- Male	10/12 (83.3%)	333/401 (83.0%)	1.000
- Female	2/12 (16.7%)	68/401 (17.0%)	
Age (median)	65 (66-94)	66 (38-81)	
Histological Subtype (n, %)			
- Adenocarcinoma	6/12 (50.0%)	228/401 (56.9%)	0.840
- Squamous Cell Carcinoma	5/12 (41.7%)	128/401 (31.9%)	
- Other	1/12 (8.3%)	45/401 (11.2%)	
Somatic DNA Mutations (n,%)			
- TP53	4/12 (33.3%)	209/401 (52.1%)	0.248
- EGFR	3/12 (25.0%)	54/401 (13.5%)	0.222
- PIK3CA	1/12 (8.3%)	23/401 (5.7%)	1.000
- CDKN2A	1/12 (8.3%)	33/399 (8.3%)	1.000
- KRAS	0/12 (0.0%)	85/401 (21.2%)	0.138
- BRAF	0/12 (0.0%)	17/401 (4.2%)	1.000
- BRCA1	0/12 (0.0%)	8/400 (2.0%)	1.000
- BRCA2	0/12 (0.0%)	4/400 (1.0%)	1.000
- ERBB2	0/12 (0.0%)	8/400 (2.0%)	1.000
- PTEN	0/12 (0.0%)	9/400 (2.3%)	1.000
- MET	0/12 (0.0%)	2/400 (0.5%)	1.000
Microsatellite Instability			
- MSI-High	0	1/367 (0.3%)	1.000
- MSI-Low	1/12 (8.3%)	39/367 (10.6%)	1.000
- Microsatellite stable	11/12 (91.7%)	327/367 (89.1%)	1.000
RNA Fusions (n, %)			
- BRAF	1/12 (8.3%)	11/401 (2.7%)	0.301
- FGFR	1/12 (8.3%)	2/401 (0.5%)	0.085
- ALK	0/12 (0.0%)	3/401 (0.7%)	1.000
- ROS1	0/12 (0.0%)	4/401 (1.0%)	1.000
- RET	0/12 (0.0%)	3/401 (0.7%)	1.000
- JAK2	0/12 (0.0%)	1/401 (0.2%)	1.000
- MET	0/12 (0.0%)	4/401 (1.0%)	1.000
PD-L1 expression			
Cut off 1%			
- Positive (TPS ≥1%)	1/9 (11.1%)	228/393 (58.0%)	0.006
- Negative (TPS <1%)	8/9 (88.9%)	165/393 (42.0%)	
Cut off 10%			
- Positive (TPS ≥10%)	1/9 (11.1%)	145/393 (36.9%)	0.165
- Negative (TPS <10%)	8/9 (88.9%)	248/393 (63.1%)	
Cut off 50%			
- Positive (TPS ≥50%)	1/9 (11.1%)	80/393 (20.4%)	0.694
- Negative (TPS <50%)	8/9 (88.9%)	313/393 (79.6%)	

DOI: 10.5455/medscience.2025.12.360

Demographic and Clinicopathological Characteristics

We evaluated 413 patients with NSCLC, among whom 12 (2.9%) harbored NTRK gene fusions. The average age of the cohort was 64.95±9.31 years. There was no significant difference in age between the NTRK fusion-positive (median age, 65 years) and negative groups (median age, 66 years) (p=0.760). Regarding sex distribution, the NTRK fusion-positive group comprised 83.3% (n=10) men and 16.7% (n=2) women, whereas the NTRK fusion-negative group included 83.0% (n=333) men and 17.0% (n=68) women, showing no significant difference between the two groups (p=1.000). Histologically, fusions were detected in 50% (n=6) of adenocarcinomas and 41.7% (n=5) of squamous cell carcinomas. No significant predilection for histological subtypes was observed (p=0.840).

Genomic Landscape and Somatic Mutations

The molecular landscape of the 12 NTRK fusion-positive cases demonstrated significant diversity in both fusion partners and concurrent genomic alterations. Among the cohort, NTRK1 was the most prevalent fusion type, accounting for 50% of the cases (n=6), followed by NTRK2 (n=5, 41.7%) and NTRK3 (n=1, 8.3%). The NTRK fusion partners and co-occurring somatic mutations for each case are summarized in Table 2.

Table 2. Detailed genomic profiles of NTRK fusion-positive NSCLC cases: Fusion partners and concurrent somatic alterations

NTRK	Fusion Partner	Concurrent Mutations / Alterations
NTRK1	TPM3	FGFR fusion
NTRK1	LMNA	TP53 mutation
NTRK1	CAMTA1	EGFR and TP53 mutations
NTRK1	TPM3	None
NTRK1	ETV6	CDKN2A mutation
NTRK1	IRF2BP2	None
NTRK2	PDE3A	None
NTRK2	LPP	PIK3CA mutation
NTRK2	HFM1	EGFR and TP53 mutations
NTRK2	PLXNA2	EGFR and TP53 mutations
NTRK2	PK1B	None
NTRK3	TANC1	BRAF fusion

The relationship between NTRK fusions and other somatic alterations was analyzed using a broad panel. While mutual exclusion was observed in most genes, co-mutation was observed in some genes. In the NTRK fusion-positive group, concurrent mutations were identified in EGFR (25%, n=3), TP53 (33.3%, n=4), PIK3CA (8.3%, n=1), and CDKN2A (8.3%, n=1) genes. One case each showed concurrent BRAF fusion (8.3%, n=1) and FGFR alterations (8.3%, n=1). Notably, several key oncogenic drivers were absent in the NTRK fusion-positive cohort. These included KRAS, RET, MET, BRAF, ERBB2, PTEN, BRCA1, and BRCA2 mutations, as well as ALK, ROS1, MET, and JAK2 fusions. No statistically significant difference was observed in the individual somatic mutations between the NTRK fusion-positive and negative groups (p>0.05) (Table 1). Neither group showed somatic DNA mutations in ALK, BCOR, CTNNB1, ERBB3, FBXWY, FGFR1, HRAS, IDH1, IDH2, KIT, MAP2K1, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3R1, POLE, ROS1, RPL22, SMAD4, TERT, and WNT1 genes.

PD-L1 Expression Analysis

The expression of PD-L1 was evaluated across three distinct clinically relevant cut-off thresholds: 1%, 10%, and 50% Tumor Proportion Score (TPS). A significant correlation was observed between NTRK fusion status and PD-L1 expression, with NTRK fusion-positive cases showing a marked tendency toward lower PD-L1 expression. When a 1% threshold was used to define high PD-L1 expression (TPS≥1%), only 11.1% (n=1/9) of the cases in the NTRK fusion-positive group exhibited high PD-L1 expression. In contrast, more than half of the NTRK fusion-negative group (58%, n=228/393) exhibited PD-L1 high expression at this level. This difference in expression at the 1% cut-off was statistically significant (p=0.006). The results at the 10% and 50% cut-offs did not reach statistical significance (p=0.165 and p=0.694, respectively) (Table 1).

Discussion

NTRK fusions are key therapeutic targets in many cancers; however, their specific roles and prevalence in NSCLC remain unclear. In our cohort of 413 patients, we identified NTRK fusions in 2.9% of the cases. In the literature, the incidence of NTRK fusion in NSCLC cases varies between 0.05% and 3.3%; however, in most cases, the rate is <1% [3,13–17]. This variation may be attributed to the high sensitivity of our NGS approach and potential enrichment within specific patient demographics. Our results confirm the rarity of these events but highlight that, within specific NSCLC populations, identifying these fusions is

DOI: 10.5455/medscience.2025.12.360

critical because they are highly effective therapeutic targets.

In NTRK fusion-positive NSCLC cases, the median age typically ranges between 48 and 63 years; however, our study reported a slightly higher median age of 65 years [15,19,20]. Although previous literature indicates a male predominance (51%–55%), our cohort demonstrated a distinct male majority of 83.3% [14,19]. Furthermore, while Xia et al.[17] and Cho et al. [19] reported adenocarcinoma rates of 83.3% and 86%, respectively, our findings showed a comparatively lower adenocarcinoma prevalence of 50%. This difference may be due to the small number of cases in the NTRK fusion-positive case series.

The diversity of fusion partners identified in this study underscores the significant genomic heterogeneity of NTRK alterations in NSCLC. Our results demonstrated high consistency with the most frequent genomic patterns reported in the literature. Specifically, Xu et al. [20] identified ETV6, LMNA, and TPM3 as the three most common NTRK fusion partners in a large-scale pan-cancer cohort. Our study mirrors this distribution; we detected TPM3 in two cases and LMNA in one case. Furthermore, although ETV6 is traditionally the most prevalent partner for NTRK3, we identified an ETV6-NTRK1 fusion, further illustrating the structural diversity of these rearrangements [20]. In addition to these classical drivers, the identification of several less common partners in our cohort, such as PDE3A, LPP, TANC1, HFM1, PLXNA2, PK1B, and IRF2BP2, is highly consistent with the real-world data of Westphalen et al. [21]. In their extensive analysis of over 295,000 patients, they reported 88 unique fusion pairs, 66% of which were previously undocumented. This striking prevalence of rare and novel rearrangements, as reflected in our findings, reinforces the clinical necessity of using comprehensive NGS panels rather than targeted assays. Such a broad molecular approach is essential to capture the full spectrum of both common and infrequent NTRK fusion partners, ensuring that no actionable alteration is overlooked in the complex molecular landscape of NSCLC.

The genomic landscape of NTRK fusion-positive NSCLC is typically characterized by strong mutual exclusivity with other canonical driver mutations, such as KRAS, ALK, ROS1, and RET. Our study corroborates this trend, as none of the NTRK fusion-positive cases harbored concurrent alterations in KRAS, RET, MET, BRAF, ERBB2, PTEN, BRCA1, BRCA2, ALK, ROS1, or JAK2 genes. This finding aligns with the prevailing "single-driver" model of lung adenocarcinoma, in which a single dominant oncogenic fusion typically drives tumorigenesis [11].

Our study revealed remarkable complexity in the NTRK fusion-positive group, characterized by concurrent mutations in EGFR (25%) and TP53 (33.3%). While NTRK fusions are traditionally viewed as mutually exclusive with other drivers, Gatalica et al. [3] reported that 71% of their NTRK fusion cases harbored at least one other pathogenic genomic alteration. Consistent with our findings, they identified TP53, PTEN, and PIK3CA as the most frequent concurrent mutations [3]. Furthermore, the detection of targetable co-alterations, such as EGFR and MET amplifications, mirrors our identification of EGFR and BRAF co-occurrences in our study. These findings challenge the "single-driver" paradigm and suggest that NTRK fusions often exist within a complex molecular architecture that may influence clinical responses and resistance mechanisms. However, the emergence of concurrent EGFR and NTRK alterations is increasingly recognized in the literature, often documented as a secondary resistance mechanism in patients previously treated with EGFR tyrosine kinase inhibitors (TKIs). Specifically, NTRK fusions have been reported to mediate 'bypass signaling, which allows tumor survival despite EGFR inhibition [17].

In our study, a notable finding was the coexistence of NTRK fusions and EGFR mutations in three cases. While some studies have suggested that NTRK fusions can emerge as an acquired resistance mechanism following EGFR-TKI therapy, the clinical treatment histories of these specific cases could not be retrieved, as they were consultation cases referred to our center primarily for molecular profiling. Consequently, it remains unclear whether these alterations were present de novo or developed as a consequence of prior treatment. Although longitudinal clinical data are unavailable, the detection of these concurrent alterations highlights the complex genomic landscape of NSCLC.

The molecular architecture of our NTRK fusion-positive cohort was characterized by a high frequency of concomitant alterations, most notably TP53 (33.3%). This finding is strongly corroborated by the data of Westphalen et al. [21], who conducted a large-scale analysis of over 295,000 patients and identified TP53 as the most prevalent co-occurring mutation, present in 50.1% of NTRK fusion-positive solid tumors. In addition to TP53, they reported significant co-occurrences with cell cycle regulators, such as CDKN2A (23.6%) and CDKN2B (16.9%), as well as other key oncogenic drivers, such as KRAS (10.7%) and PIK3CA (8.2%) [22]. The presence of these diverse co-mutations, as reflected in our results, underscores that NTRK fusions frequently do not exist in isolation but rather within a complex genomic context that may influence tumor progression and therapeutic response.

In a study by Farago et al. [14], 11 NSCLC patients with NTRK1 fusion mutations had other mutations detected in six patients. However, these genes were not KRAS, EGFR, ALK, or ROS1. The genes showing mutations together with NTRK fusion were ATM, SMARCB1, TP53, CCND2, RICTOR, FGF6, FGF23, CTNNB1, CDKN21, and ARID1A [14]. In our study, compared with the literature, mutations were observed in the TP53 and CDKN2A genes in NTRK fusion-positive cases, but not in CTNNB1. The other genes mentioned were not included in our panels. In Xia et al. [17] study, NTRK fusion was detected in 12 NSCLC cases, and in these cases, mutations were observed in the TP53 (58%), EGFR (50%), RB1 (25%), ARID2 (17%), ARID1A (17%), PIK3CA (17%), KMT2C (17%), SMARCA4 (17%), KMT2B (17%), SMAD4 (17%), and NF1 genes (17%). In one case with a positive NTRK fusion, no other mutations were detected [17].

DOI: 10.5455/medscience.2025.12.360

Furthermore, the presence of TP53 mutations, which are often associated with chromosomal instability and a more aggressive clinical course, underscores the fact that NTRK fusion-positive NSCLC is not a monolithic genomic entity and may harbor secondary mutations that can influence the duration of response to TRK inhibitors. The covariates observed in a portion of the NTRK fusion-positive cases in our study further demonstrated the indispensable nature of comprehensive NGS profiling in capturing the full spectrum of the tumor's molecular architecture. This is because these bypass pathways can potentially mediate primary or acquired resistance to targeted therapies.

A significant finding of this study was the observed association between NTRK fusion status and PD-L1 expression. Our results demonstrated that NTRK fusion-positive tumors are predominantly PD-L1-negative, with only 11.1% showing high expression at the 1% cut-off (p=0.006). Gatalica et al. [3] observed higher PD-L1 expression in only 23% of NTRK fusion-positive cases. Their study utilized NTRK fusion-positive tumors that frequently exhibit low PD-L1 levels. The biological implications of this "cold" immune microenvironment suggest that while immune check-point inhibitors (ICIs) are the standard of care in NSCLC, they may be less effective in patients harboring NTRK fusions. As reported by Dudnik et al. [18], patients with NSCLC and rare drivers, such as NTRK, often have low tumor mutational burden and microsatellite-stable status. However, PD-L1 expression in these cases is highly variable. In a study by Dudnik et al. [18], in one case with an NTRK fusion mutation, PD-L1 expression was above 50%. In larger case series of NTRK fusion mutants, PD-L1 expression status must be investigated. Our study highlights that NTRK fusion-positive NSCLC represents a unique molecular subset with generally suppressed PD-L1 expression. These results reinforce the need to prioritize targeted therapies over immunotherapy for patients harboring these rare yet actionable genomic alterations. Given the exceptional response rates to TRK inhibitors, such as larotrectinib and entrectinib, identifying these rare fusions is critical, especially when the likelihood of benefit from immunotherapy appears limited due to low PD-L1 expression [4,10].

Limitations

The small sample size of the NTRK fusion-positive cohort limited the statistical power. In cases with concurrent EGFR mutations and NTRK fusions, the precise clinical context—whether these alterations existed de novo or emerged as acquired resistance after prior TKI therapy—was not available for all patients. PD-L1 analysis was performed in only 402 of 413 cases, and the small number of NTRK fusion-positive samples with available PD-L1 analysis (n=9) may limit the generalizability of the observed correlation between NTRK status and low PD-L1 expression.

Conclusion

Our findings suggest that NTRK fusions may be more common in specific patient subgroups than previously estimated. Furthermore, the identification of concurrent alterations, most notably the frequent co-occurrence of TP53 and EGFR mutations, challenges the conventional "single-driver" paradigm and highlights the genomic complexity of NTRK fusion-positive NSCLC. A key observation from our study is the significant association between NTRK fusions and low PD-L1 expression, suggesting a predominantly "cold" immune microenvironment in these tumors. These results highlight the importance of comprehensive molecular profiling in improving our understanding of the complex mutational landscape and immune profile of NTRK fusion-positive lung carcinomas.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
This study was approved by the Karadeniz Technical University Faculty of Medicine Research Ethics Committee (12.12.2023; Approval No: 2023/255).

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