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Original Research

The Evaluation of Neurotrophic Receptor Tyrosine Kinase (NTRK) Alterations in Neuroblastomas

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Abstract

Background: Neuroblastoma (NB) is the most common extracranial solid tumor among pediatric cancers and accounts for approximately 15% of childhood cancer-related deaths. Neurotrophic receptor tyrosine kinases (NTRKs) are genes that play critical roles in the development and function of the nervous system. Therefore, elucidating the role of NTRKs in NB is important for both understanding basic biological mechanisms and developing novel therapeutic approaches. Specifically, *NTRK* fusions are being investigated as potential biomarkers and therapeutic targets for targeted therapy strategies. The tumor-agnostic TRK inhibitors larotrectinib and entrectinib are used to treat advanced or metastatic solid tumors with *NTRK* gene fusions. Accordingly, this study aimed to investigate the clinical significance of *NTRK1*, *NTRK2*, and *NTRK3* point mutations, gene fusions, and protein expression, and to assess the effectiveness of these in guiding targeted therapy decisions in NB. **Methods**: This study investigated pan-TRK expression, point mutations, and fusions in the *NTRK1*, *NTRK2*, and *NTRK3* genes using next-generation sequencing (NGS) on paraffin-embedded blocks from 173 patients diagnosed with NB. Findings were analyzed in SPSS 29.0 using clinical data, *MYCN* amplification, and 11q deletion status, with Pearson correlation analysis applied at the $p < 0.05$ significance level. **Results**: Immunohistochemistry (IHC) for NTRK revealed that 67.9% of cases were NTRK-positive. NGS analysis identified *NTRK1* missense point mutations in 20 cases, *NTRK2* in 9 cases, and *NTRK3* in 9 cases. In addition, 5 fusions were detected in 4 of the 103 patients who underwent fusion analysis. **Conclusions**: Owing to the presence of neural tissue, NTRKs are highly positive in IHC, making these genes unsuitable as biomarkers for assessing NTRK inhibitor sensitivity and resistance, which are tissue-agnostic drugs. The observed low fusion rate is consistent with the literature, and the significance of the numerous point mutations identified as agnostic markers warrants further investigation. NTRK expression, fusion, and point mutations were not associated with clinical parameters or survival.

Keywords: neuroblastoma; neurotrophic receptor tyrosine kinase; gene fusion; point mutation

1. Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor among pediatric cancers and is responsible for 15% of childhood cancer deaths [1–3]. The process typically begins with primary tumors arising in the adrenal medulla or along the sympathetic nervous system. NB is notable for its biological heterogeneity, and the course of the disease can exhibit diverse clinical courses, including spontaneous regression, differentiation, or metastasis [4,5].

TRK proteins contain three main domains: an extracellular domain, a transmembrane domain, and an intracellular domain. The tyrosine kinase domain is the most important part of the receptor. The active kinase domain initiates biological responses by triggering various signaling pathways within the cell, including RAS/MAPK, PI3K/AKT, and PLC-γ [6].

Neurotrophic receptor tyrosine kinases (NTRKs) are genes that play critical roles in the development and function of the nervous system. *NTRK* genes (*NTRK1*, *NTRK2*, and *NTRK3*) encode transmembrane receptors with tyrosine kinase activity [7,8]. These receptors activate signaling pathways through interaction with neurotrophic factors, regulating biological processes such as cell proliferation, differentiation, and survival. However, abnormalities in these genes such as fusions, mutations, or overexpression, can affect tumor growth and metastasis, altering sensitivity to treatment [5,7].

The role of *NTRK* genes in NB is important both for elucidating the underlying biological mechanisms and for developing therapeutic approaches. *NTRK* fusions or aberrant expression are being evaluated as potential biomarkers and therapeutic targets for targeted therapy strategies [8,9]. In recent years, studies on the role of *NTRK* genes in NB have been an important step in understanding genetic heterogeneity.

Neurotrophic tyrosine receptor kinase (NTRK) inhibitors target abnormal tropomyosin receptor kinase (TRK) fusion proteins resulting from *NTRK* gene fusions,

blocking their tyrosine kinase activity and thereby inhibiting the growth of cancer cells [10,11]. Drugs such as larotrectinib and entrectinib bind with high affinity to TrkA, TrkB, and TrkC receptors, inhibiting their phosphorylation and thus downstream signaling pathways. This inhibition inhibits tumor growth by stopping cell proliferation and promoting apoptosis [12,13].

Accordingly, examining NTRK gene expression and genetic alterations in NB not only provides a better understanding of the disease's biology but also allows for the development of more effective treatment approaches. The potential of *NTRK* genes in targeted therapies, particularly the clinical success of tumor-agnostic TRK inhibitors, highlights the importance of *NTRK* fusions and other abnormalities as therapeutic targets. However, the rarity of *NTRK* fusions and factors such as genetic heterogeneity necessitate more comprehensive investigation of other alterations in these genes, particularly mutations and aberrant expression, to understand their clinical significance.

This study aimed to investigate neurotrophic tyrosine kinases at the genomic and protein levels in 173 patients diagnosed with NB, obtained from the Turkish Pediatric Oncology Group (TPOG) Association's completed TPOG Neuroblastoma 2009 Protocol and the ongoing TPOG Neuroblastoma 2020 Protocol. For this purpose, paraffin block samples from patients diagnosed with NB were used to investigate pan-tyrosine kinase protein expression by immunohistochemistry (IHC); *NTRK1*, *NTRK2*, and *NTRK3* gene fusions by RNA next generation sequencing (NGS) analysis; and single nucleotide variants (SNVs) in the *NTRK1*, *NTRK2*, and *NTRK3* genes by DNA NGS analysis.

2. Materials and Methods

2.1 Study Design and Patient Selection

Demographic characteristics and prognoses of 173 patients diagnosed with neuroblastoma, diagnosed, treated, and followed at our institution, were obtained from patient records. These cases were evaluated according to international neuroblastoma staging, risk classification, and treatment strategies and were evaluated within the scope of the Turkish Pediatric Oncology Group Association neuroblastoma protocol. Slide sections were taken from tumor samples obtained from archived paraffin tissues for immunohistochemistry. For Next generation sequencing (NGS), 100-micrometer sections were taken from paraffin blocks for DNA extraction, placed in two microtubes for point mutation and fusion determination from each case.

2.2 Immunohistochemical Examination

Sections were prepared on lysine-coated slides and stained with primary TrkA+B+C (5T6) monoclonal Antibody (BIOSS BSM-52715R) at 1/100 dilution. Pan NTRK was visualized by UltraView Universal DAB Detection Kit (REF 760-500, Ventana Medical Systems, Roche Group, Tucson, AZ, USA) and hematoxylin counterstaining. Samples were analyzed with a positive control and scored nuclear and cytoplasmic expression in percentage [14].

2.3 Next Generation Sequencing: DNA

The study was performed in accordance with the ONCO/Reveal™ Solid Tumor Panel guidelines. (REF: HDA-HS-1002-24, LOT: 23PB0318, Pillar Biosciences, Natick, MA, USA) *NTRK 1*, *NTRK2*, *NTRK3* genes are present in the panel. DNA isolation was performed by the spin column method (Roche® High Pure PCR Template Preparation Kit, version 23, LOT: 37696800, Roche Diagnostics, Indianapolis, IN, USA). DNA amount was measured in the Qubit device using Qubit-HS reagent (Qubit® fluorometer, Invitrogen, Thermo Fisher Scientific, MA, USA). After amplification by PCR, digestion was performed with exonucleases. Magnetic bead-based purification was performed. Indexing was performed by PCR and purified again. Quantification was performed again with Qubit. After library creation and normalization, the cartridge was loaded into the Illumina MiniSeq (Illumina©, Inc., San Diego, CA, USA) device. The data obtained from the device was evaluated with appropriate bioinformatics programs by taking "FASTQ" and the detection of variants was done according to the reference genome [15].

2.4 RNA Fusion Panel Processing Steps

The study was conducted in accordance with the ONCO/Reveal™ Multi-Cancer RNA Fusion v2 Panel (Part No.: HRA-HS-1002-24, İllumina, San Diego, CA, USA) Reference Guide. For the *NTRK1* gene: *TPM3*, *TFG*, *LMNA*, *SQSTM1*, *CHTOP*, *ARHGEF2*, *NFASC*, *IRF2BP2*, *PPL*, *BCAN*, *SCYL3*, *TP53*, *CD74*, *MPRIP*, and *TPR* fusions. For the *NTRK2* gene: *AFAP1*, *NACC2*, *BCR*, *TRIM24*, *QKI*, *PAN3*, and *SQSTM1* fusions. For the *NTRK3* gene: *ETV6*, *BTBD1*, *EML4*, *SQSTM1*, *TFG*, and *RBPMS* fusions. RNA isolation was performed using the spin column method (High Pure FFPET RNA Isolation Kit, Roche Diagnostics, Lot No. 89176700, Mannheim, Germany). cDNA Synthesis was performed using cDNA Master Mix and reverse transcription under thermal cycling. RNA concentration was determined using the Qubit RNA BR Assay kit (Life Sciences, Cat. Q10211 and Q10210; Quantitation range: 20–1000 ng). The lid was opened and the reaction was applied to a thermal cycler. After cDNA synthesis was completed, the reaction was diluted with nuclease-free water or low TE. Gene-Specific PCR Transcript Target Amplification, Gene-Specific Primer Annealing, and Purification were performed with AMPure XP beads. PCR indexing and re-purification were performed, and PCR products were quantified with Nanodrop. After indexing and library construction, sequencing was set up and initiated with the Illumina MiniSeq Instrument combined with the PhiX library control according to the "MiniSeq System User Guide" instructions. Fast Q data was acquired [15].

Table 1. Clinicopathological features of patients.

Category	Patient number (N) = 173			
	Min	Max	Mean ± STD	
Age (month)	1.00	204.00	39.33 ± 37.71	
Event free survival	1	131	27.97 ± 22.10	
Overall survival	1	131	19.41 ± 20.31	
Sex	94 (54.3%) Male		79 (45.7%) Female	
MYCN amplification status	36.4% Amplified		63.6% Non-amplified	
11q deletion status	33.5% Positive		62.4% Negative	4.1% No data
DNA index	74.0% DI = 1 (Diploid)		9.8% DI >1 (Higher than Diploid)	16.2% No data
Risk stratification (2020)	(4.6%) Too-low risk	(13.9%) Low risk	(11%) Medium risk	(70.5%) High risk
INRG stage	15.6% L1 stage	13.9% L2 stage	64.7% M stage	5.8% MS stage
Pathology	34.7% good		65.3% worse	

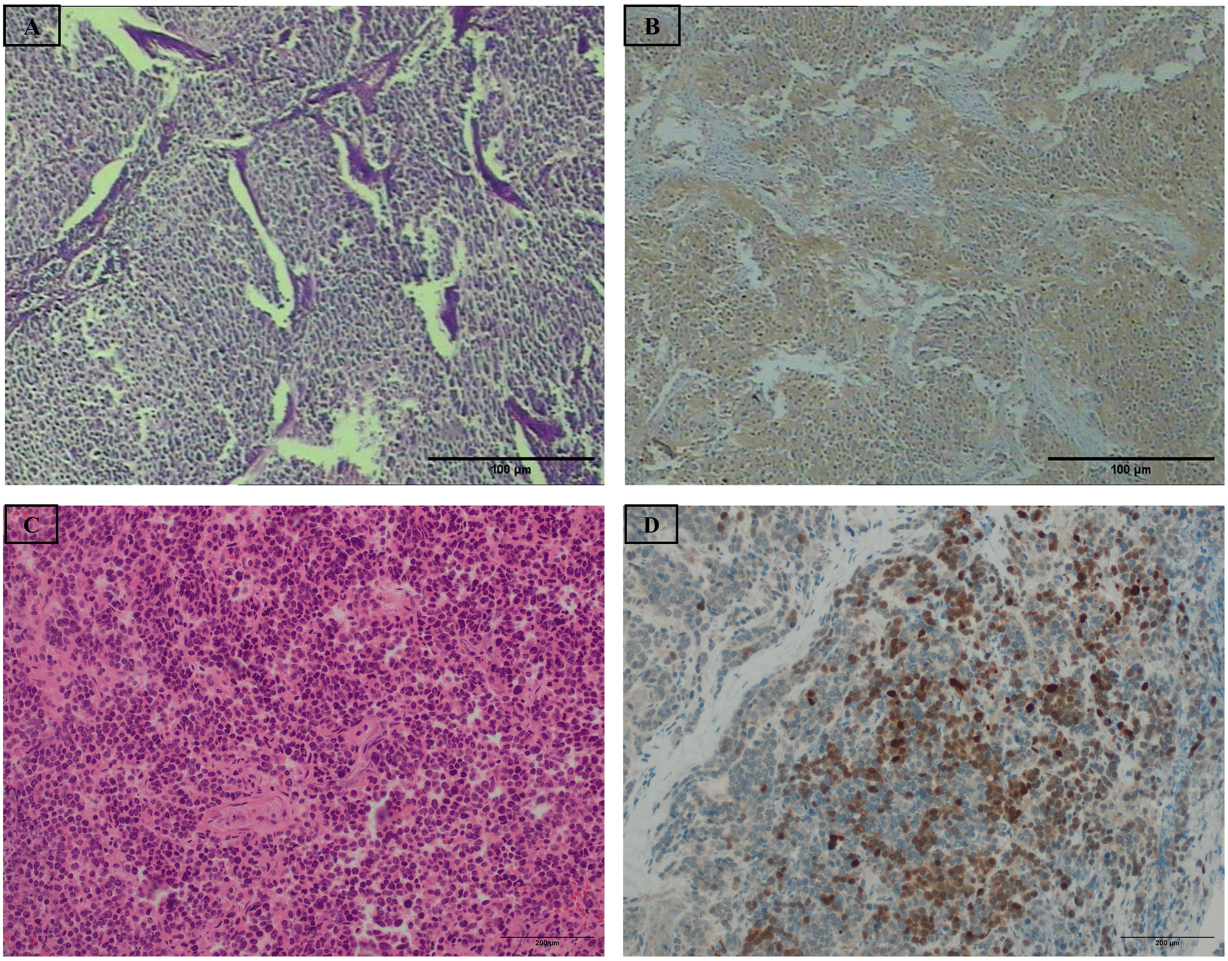

Fig. 1. The microscope images of hematoxylin & eosin (H&E) staining and immunohistochemical Pan-TRK expression. (A) 4 µm thick NB tumor slices stained with H&E at 40× objectives. Scale bars = 100 µm. (B) Pan-TRK diffuse cytoplasmic staining in 4 µm thick NB tumor slice at 40× objectives. Scale bars = 100 µm. (C) The comparison of 4 µm thick NB tumor slices stained with H&E at 200× objectives. Scale bars = 200 µm. (D) The comparison of 4 µm thick NB tumor slices stained with Pan-TRK antibody at 200× objectives. Scale bars = 200 µm. NB, Neuroblastoma; H&E, hematoxylin & eosin; TRK, tyrosine kinase.

2.5 Bioinformatics Analysis

Fast Q data was converted to BAM data. Data were obtained using the Pillar-Biosciences PiVAT (Pillar Variant Analysis Toolkit).

2.6 Statistical Analysis

SPSS 29.0 version (IBM Corp., Armonk, NY, USA) statistical analysis program was used to analyze the data. After performing descriptive statistics and normal distribu-

Table 2. Comparison of NTRK fusion analyses with IHC, stage and risk groups.

NTRK	Fusion status	NTRK IHC result	Stage	Risk stratification
NTRK1	*TFG*(*e4*):*NTRK1*(*e9*)	Positive	M	High risk
NTRK1	*TPR*(*e6*):*NTRK1*(*e5*)	Positive	M	High risk
NTRK2	-	-	-	-
NTRK3	*EML4*(*e14*):*NTRK3*(*e6*)	Positive	M	High risk
NTRK3	*EML4*(*e14*):*NTRK3*(*e6*), *TFG*(*e4*):*NTRK3*(*e6*)	Positive Positive	L1	Low risk

tion analysis, the relationship between the independent variable of the *NTRK* fusion in NB and *MYCN*, 11Q deletion, age, gender, EFS, and OS was examined using the Pearson correlation test. Parametric tests were used to examine the relationship between the groups. The Kaplan–Meier test was used for survival analyses, and the Log-Rank test was used to correlate survival analyses. $p < 0.05$ was considered statistically significant for all analyses.

3. Results

3.1 Patient Characteristics

This study included 173 patients diagnosed with neuroblastoma (NB). Of these patients, 54.3% were male and 45.7% were female. The patient ages ranged from 1 to 204 months. The mean patient age was 39.33 $\pm$ 37.71 months, and the median age was 30.00 months. The mean event-free survival (EFS) for all patients was 27.97 $\pm$ 22.10 months, and the mean overall survival (OS) was 19.41 $\pm$ 20.31 months (Table 1). Because the study included relapsed, refractory patients, the prevalence of high risk, metastatic disease, and *MYCN* amplification was higher than the general NB population.

3.2 Microscopic Evaluations of Immunohistochemical Stainings

After paraffin block sections of NB samples were taken, the suitability of tumors was determined using hematoxylin & eosin (H&E) staining. Immunohistochemical analysis was performed on 134 tissues identified by HE staining, as shown in Fig. 1. 67.9% were evaluated as NTRK positive and 32.08% as NTRK negative. Nuclear staining was observed in 16 (17.6%) of the positive patients. 75 (82.4%) of Pan-TRK positive NB samples demonstrated cytoplasmic staining.

The H&E staining of well-differentiated NB tumor tissue is seen in Fig. 1A. Pan-TRK is cytoplasmically expressed in 90% of NB tumor tissues in Fig. 1B. Poorly differentiated NB tumor tissue is stained with H&E (Fig. 1C). Pan-TRK is expressed in 70% of tumor tissues. NTRK expression is mainly nuclear or cytoplasmic (Fig. 1D).

3.3 Data From NGS SNV Mutation Analysis

Of the 115 patients who underwent SNV analysis, 20/115 (17.4%) had one or more clinically significant mutations in *NTRK1*, while 9/115 (7.83%) had clinically significant mutations in *NTRK2* and 9/115 (7.83%) in *NTRK3*. Among the patients with SNV mutations, *NTRK1* and *NTRK2* were observed together in 2 patients, *NTRK1* and *NTRK3* were observed in 2 patients, and *NTRK3* and *NTRK2* were observed in 2 patients. Only *NTRK1, NTRK2,* and *NTRK3* mutations were observed together in 3 patients. Mutation types observed in SNV analysis of patient tissues were categorized as synonymous, missense, stop-gained, and frameshift mutations. Missense, stop-gained, and frameshift mutations that were found as clinically significant in PIVAT program were chosen. According to SNV analysis, all of the single nucleotide variants detected in the *NTRK1*, *NTRK2* and *NTRK3* genes were seen in the tyrosine kinase domain of *NTRK1, NTRK2* and *NTRK3,* as seen in Fig. 2. The UniProt database (https://www.uniprot.org/uniprotkb?query=NTRK1) was used to determine the domain of the NTRK protein in which the detected *NTRK* somatic mutations were located. The data found are visualized in the Fig. 2.

3.4 Data From NGS RNA Fusion Analysis

NGS RNA fusion analysis was performed on 103 of 173 patients. 4 /103 (3.9%) patients had NTRK fusions. Whereas *NTRK1* fusions were detected in 2 patients, 2 patients had *NTRK3* fusions. Of the four fusions observed, the first patient had *TFG(e4):NTRK1(e9)*, the second had *TPR(e6):NTRK1(e5)*, the third patient had *EML4(e14):NTRK3(e6)*, and the fourth patient had both *EML4(e14):NTRK3(e6)* and *TFG(e4):NTRK3(e6)*. A total of five fusions were detected in four different patients. Except for the example with a double fusion, all fusions were observed in the metastatic stage (Table 2). Immunohistochemical staining types were evaluated as diffuse positive in the case with *TFG(e4):NTRK1(e9)* fusion, 40% cytoplasmic positive in the case with *TPR(e6):NTRK1(e5)* fusion, 50% cytoplasmic positive in the case with *EML4(e14):NTRK3(e6)* fusion, and 10% cytoplasmic positive in the case with *EML4(e14):NTRK3(e6), TFG(e4):NTRK3(e6)* fusions.

3.5 Statistical Analysis

The normal distribution of patient data was determined using the Kolmogorov-Smirnov Normality test. The data were found to be normally distributed, and parametric tests were performed. The relationship between NTRK IHC

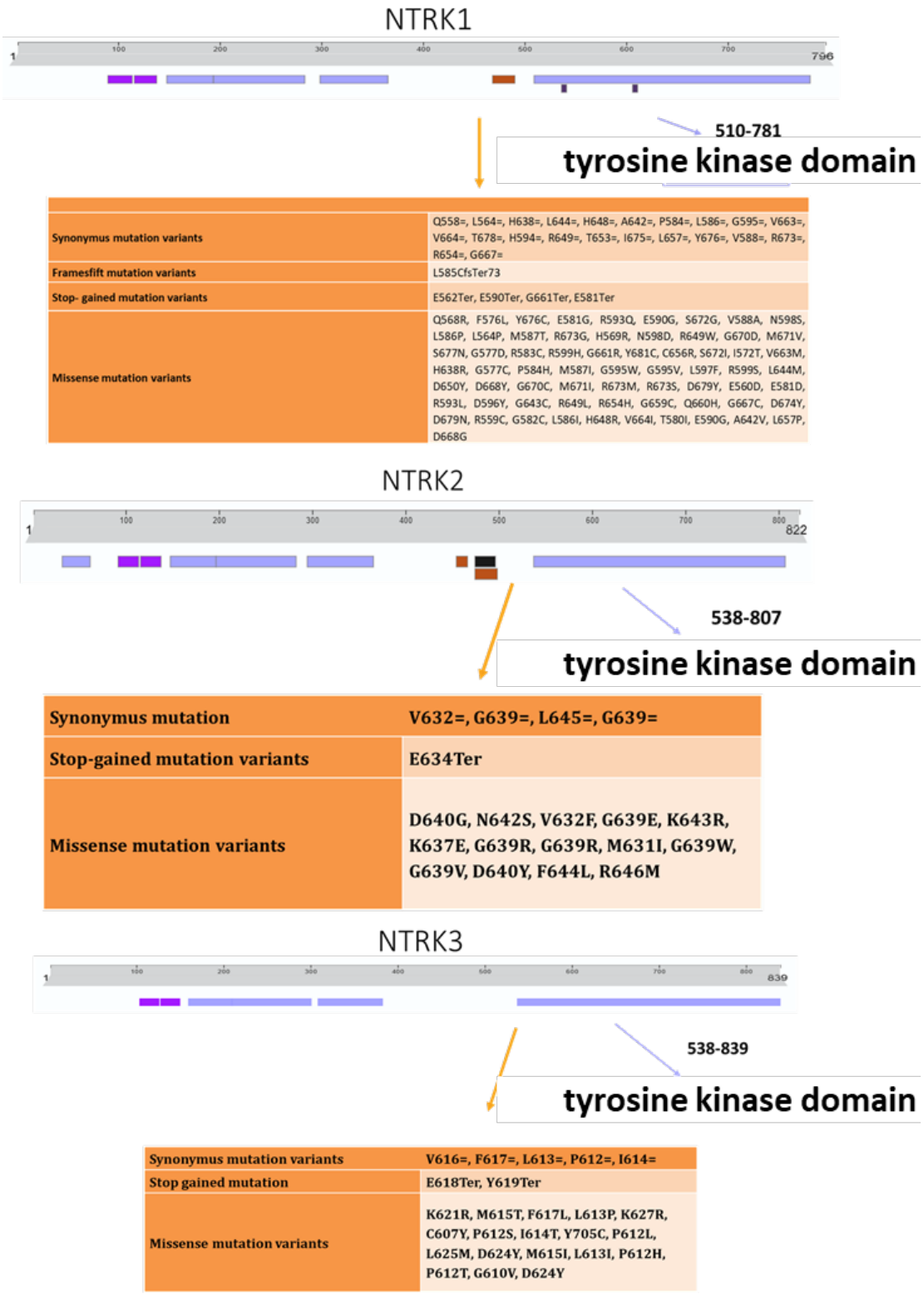

Fig. 2. Schematic representation of NTRK 1, 2 and 3 protein structures. Synonymous, missense, frame-shift and stop-gained mutations were observed in the NTRK1, 2 and 3 tyrosine kinase domains. NTRK1, 2 and 3, Neutrophic tyrosine kinase 1, 2 and 3.

results and other parameters was investigated using the ordinal logistic regression test and Pearson's chi-square tests. Ordinal logistic regression analysis indicates that NTRK IHC positivity was significantly associated with predicting increasing neuroblastoma risk classes ($\chi^2 = 4.39$, $p = 0.036$). The model fit was quite good (Pearson $p = 0.911$; Deviance $p = 0.905$). NTRK IHC positivity significantly reduced the probability of being in the high-risk group ($\beta =$

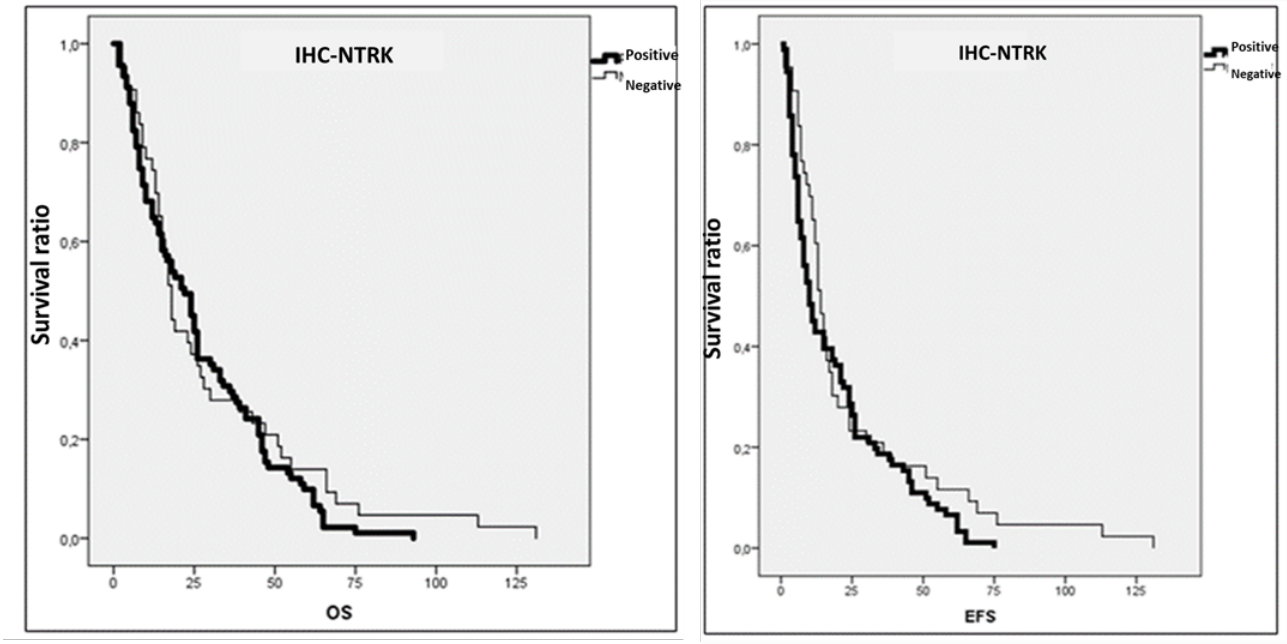

Fig. 3. The Kaplan-Meier survival analysis of neuroblastoma patients in respect to the presence of NTRK status. OS and EFS times (month) in Pan-TRK positive and negative NB pateints were analyzed by Log-rank test. $p < 0.05$. EFS, Event-free survival; OS, Overall survival; IHC-NTRK, Pan-TRK staining.

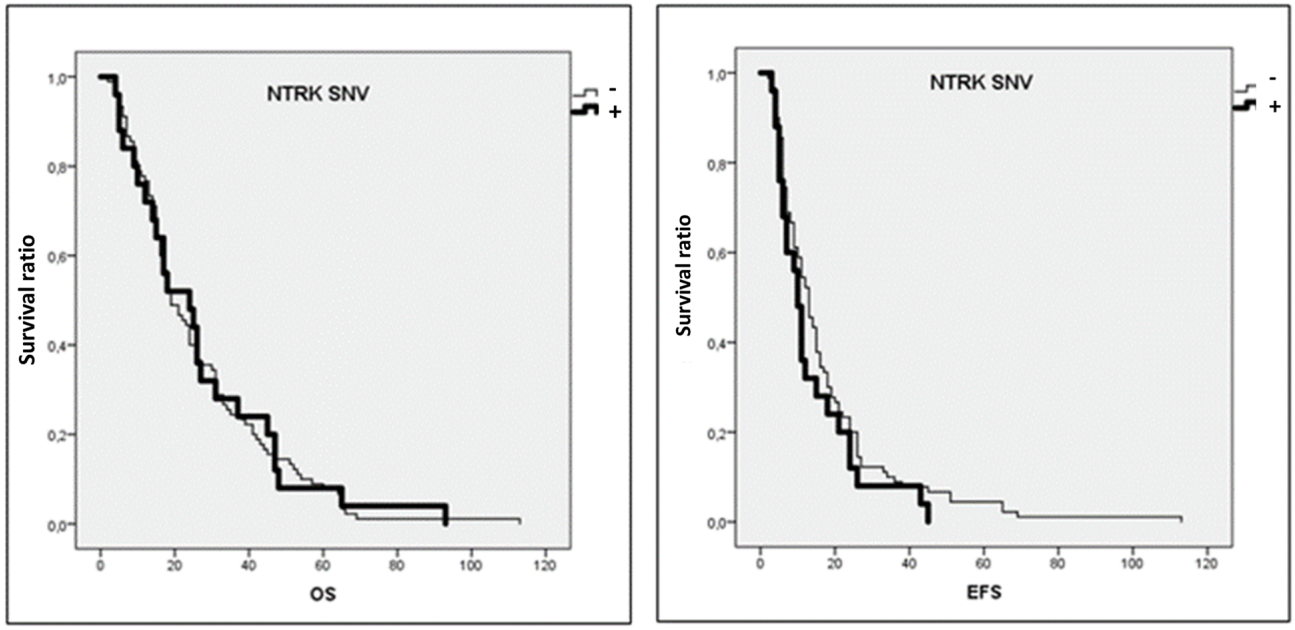

Fig. 4. The Kaplan-Meier survival analysis of neuroblastoma patients in respect to the presence of SNV. OS and EFS times (month) in NTRK-SNV positive and negative NB pateints were analyzed by Log-rank test. $p < 0.05$. EFS, Event-free survival; OS, Overall survival; NTRK-SNV, NTRK single nucleotide variation.

–0.834, $p = 0.044$). The calculated OR = 0.43 demonstrates that a positive IHC reduced the probability of being in the high-risk group by approximately 57%.

To further evaluate the relationship between NTRK IHC status and INRG stage, we also performed ordinal logistic regression using INRG staging (L1–L2–M–MS) as ordinal input. The model showed a significantly better fit compared to the intercept-only model ($\chi^2 = 4.24$, $p = 0.039$) and excellent goodness of fit (Pearson $p = 0.755$; Deviation $p = 0.754$). According to this, NTRK IHC positivity remained a significant predictor of stage distribution and was associated with a lower probability of belonging to a more

advanced INRG stage (β = –0.765, p = 0.044). The corresponding odds ratio (OR = 0.46, 95% CI: 0.22–0.98) indicates that IHC-positive tumors were approximately 54% less likely to be classified into higher INRG stages compared to IHC-negative tumors. These findings confirm the robustness of our results.

The association between NTRK immunohistochemistry and *NTRK* SNV status was assessed using both Pearson's chi-square test (χ^2 = 6.28, p = 0.012) and Fisher's exact test (p = 0.015) that were indicated a statistically significant association between the two variables. NB patients with *NTRK* SNV were almost as likely to be IHC positive (84%) as they were to be IHC negative (16%). There was a consistent association between IHC expression and the presence of the mutation.

A significant relationship was observed between NTRK IHC positivity and the presence of *NTRK1* mutations (Pearson χ^2 = 6.47, p = 0.011; Fisher's exact test p = 0.011). 87.5% of cases harboring clinically significant *NTRK1* mutations were IHC positive, compared to 52.5% of mutation-negative cases. The effect size (phi = 0.29) indicated a modest but significant consistency between IHC expression and *NTRK1* genomic alterations.

No statistically significant association was observed between *NTRK2* mutations and NTRK IHC positivity (Pearson χ^2 = 2.86, p = 0.091; Fisher's exact p = 0.134). Although a trend toward higher IHC positivity was seen in mutation-positive cases, the very small number of tumors with *NTRK2* mutations limited statistical power. This result is consistent with the rarity and biological heterogeneity of *NTRK2* alterations.

There was no statistically significant association observed between *NTRK3* mutations and NTRK IHC positivity (Pearson χ^2 = 2.86, p = 0.091; Fisher's exact p = 0.134). Although a significant trend toward higher IHC positivity was shown in mutation-positive tumors, the small number of cases with *NTRK3* mutations limited statistical power.

No significant association was observed between NTRK fusion status and NTRK IHC expression (Pearson χ^2 = 2.13, p = 0.144; Fisher's exact p = 0.267). All fusion-positive tumors were IHC-positive; however, the very limited number of fusion-positive cases (n = 3) resulted in insufficient statistical power.

When we examined the effects of NTRK protein expression positivity on EFS and OS times of NB patients, we used Kaplan-Meier survival curve with Log-Rank test as shown in Fig. 3. As a result, EFS and OS were longer in NB patients with negative NTRK expression than in NTRK-positive patients. Although NTRK positivity had an effect on EFS and OS, this was not statistically significant (EFS p = 0.153, OS p = 0.368).

As shown in Fig. 4, *NTRK* SNV demonstrated clinically significant mutations. When we investigated whether the presence of *NTRK* SNV in the patient affected the patient's EFS and OS duration, the EFS and OS survival times of patients with *NTRK* SNV were found to be shorter. However, this finding was not found to be statistically significant in the Log-rank test (OS p = 0.904, EFS p = 0.230).

When we examined the risk classification groups, we observed that EFS and OS times were shorter in the very low-risk group, while EFS and OS times were longer in the low-risk group (Fig. 5). However, the Log-rank test showed that this finding was not statistically significant (EFS p = 0.079, OS p = 0.132).

In the INRG staging system, EFS time was shorter in the metastatic stage and OS time was shorter in the L2 stage. EFS and OS times were longer in the L1 stage than in the other stages (Fig. 6). In this case, Log-rank analysis was found to be statistically significant in EFS periods (EFS p = 0.044, OS p = 0.476).

To examine whether IHC-based NTRK expression is an independent prognostic factor, multivariable Cox proportional hazards regression analyses were applied for both event-free survival (EFS) and overall survival (OS), adjusting for clinical and biological risk factors, including *MYCN* amplification, 11q deletion, DNA ploidy, risk stratification and INRG stage. In a multivariable Cox model for EFS, including IHC-based NTRK expression demonstrated a trend toward improved EFS (HR = 0.574), although this was not statistically significant (p = 0.074). Risk stratification remained an independent prognostic factor for EFS. In the multivariate Cox analysis for OS, IHC-based NTRK expression was independent of OS status (death/alive), whereas 11q deletion was found to be an independent negative prognostic factor (HR = 3.704, p = 0.038). Cox multivariate analysis with *NTRK* SNV and clinical factors showed that *NTRK* SNVs have a non-significant trend toward poorer event-free survival (HR = 1.77, p = 0.059), whereas there was no association between NTRK SNVs and overall survival (HR $\approx$ 0, p = 0.967). Detailed results of the multivariable Cox regression analyses are provided in **Supplementary Table 1**.

4. Discussion

This study investigated the distribution and clinical significance of NTRKs in 173 NB cases using point mutations in the *NTRK1*, *NTRK2*, and *NTRK3* genes from DNA samples, fusion analyses from RNA samples, and IHC expression rates. The study results revealed a high percentage of immunohistochemical expression of NTRKs (67.9%) and *NTRK* point mutations (78.26%) in NB, while the percentage of *NTRK* fusions (3.88%) was low. *NTRK* missense mutations were commonly seen in tyrosine kinase domains of NTRKs. A study has determined whether the c.1810C>T mutation is commonly found in neuroblastoma patients younger than 18 months of age with *NTRK1* non-MYCN amplification and is associated with an unfavorable prognosis [16].

In our study, *NTRK* mutations were found at a high frequency in neuroblastoma patients. According to the lit-

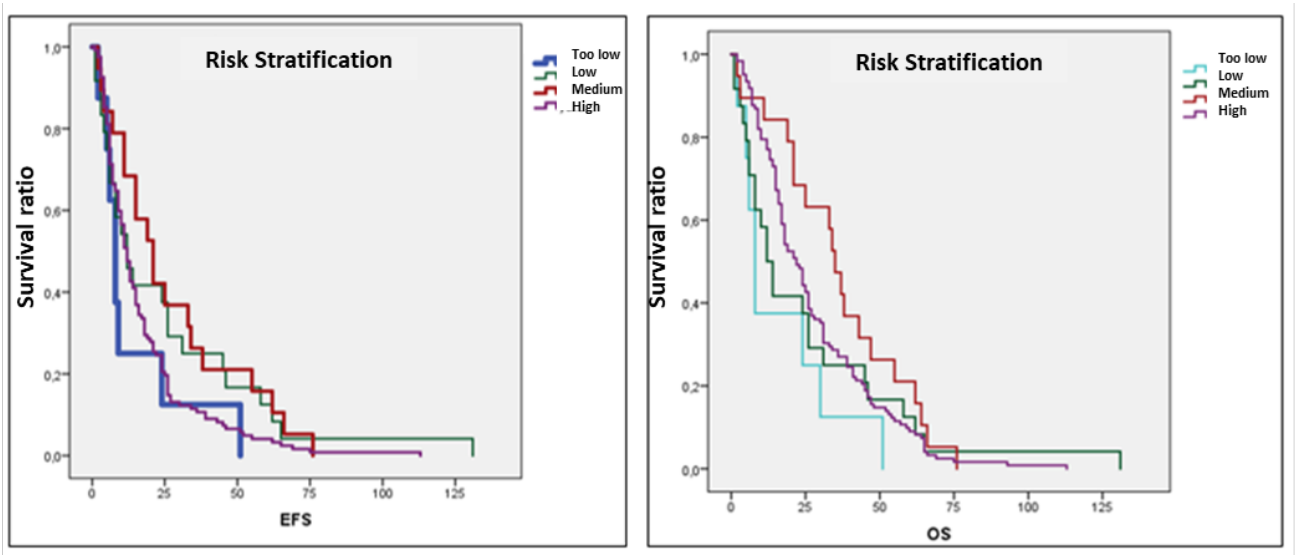

Fig. 5. The Kaplan-Meier survival analysis in respect to risk stratification of NB patients. OS and EFS times (months) were analyzed by Log-rank test. $p < 0.05$. EFS, Event-free survival; OS, Overall survival.

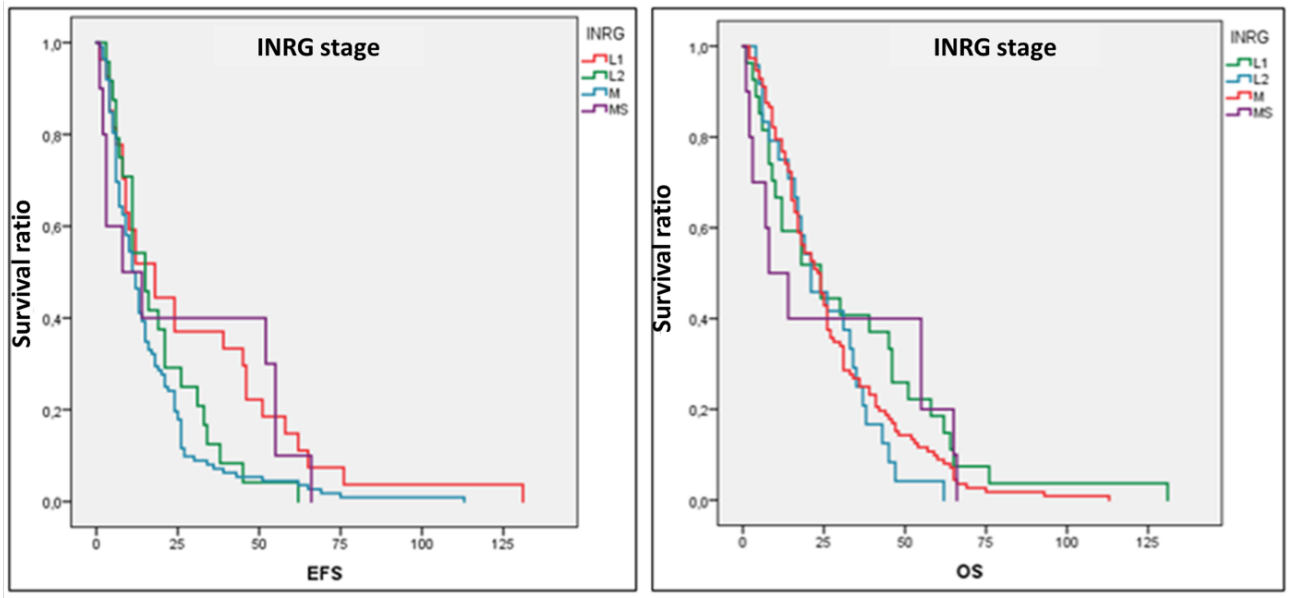

Fig. 6. The Kaplan-Meier survival analysis in respect to INRG stage of NB patients. OS and EFS times (months) were analyzed by Log-rank test. $p < 0.05$. EFS, Event-free survival; OS, Overall survival; INRG, International Neuroblastoma risk group.

erature, *NTRK* mutations are rare and, while most are polymorphisms, are rarely seen in cancers. Furthermore, studies have reported that *NTRK* point mutations (especially found in tyrosine kinase domain) have oncogenic potential and can cause TRK inhibitor resistance [17,18]. *NTRK1* L564H, V573M and G667C mutations or *NTRK3* F617L, G623E, G623R and G696A were detected by NGS and resistant to Cabozantinib, Larotrectinib and Entrectinib that are TRK inhibitors [17–19]. Joshi *et al.* [20] identified somatic *NTRK2* and *NTRK3* mutations in hematological neoplasms and functionally demonstrated to be transforming/oncogenic that *NTRK2* and *NTRK3* point mutations. *NTRK1* p.G595R and p.G667C mutations have been associated with entrectinib/larotrectinib resistance in tumors with *NTRK* fusions (e.g., colorectal cancer). These mutations were generally identified as resistance mutations located within the ATP-binding pocket of the TRK fusion protein [21]. In our study, we found *NTRK1* p.G667C and *NTRK3* F617L mutations.

NTRK gene fusions were first identified in a colorectal adenocarcinoma cell line in 1982 [22]. The concept of tumor-agnostic markers refers to markers used as therapeutic targets in tumors with specific genetic or molecular characteristics (e.g., a specific biomarker or mutation), regard-

less of cancer type. This concept represents modern oncological approaches, where cancer treatment is based on the molecular profile of the tumor rather than its location [23]. The approval of larotrectinib as a tumor-agnostic treatment by the FDA in 2018 and by the European Medicines Agency (EMA) in 2019 has provided access to treatment for patients with *NTRK* gene fusion regardless of age and tumor type [9,24,25].

In our cohort, *NTRK* fusions were identified in 4 of 103 tested patients (3.9%), comprising 2 patients having *NTRK1* and 2 patients having *NTRK3* fusions. This detection ratio is concordant with the low prevalence of *NTRK* alterations reported across solid tumors, yet it is notably higher than the 8.7% fusion prevalence observed by Mohamed *et al*. [26] in CNS tumors when performing a combined NGS and Pan-TRK IHC workflow. Importantly, while Mohamed *et al*. [26] demonstrated that Pan-TRK expression lacked specificity—detecting fusion–negative tumors in 82% of Pan-TRK–positive CNS cases—our study relied exclusively on RNA-based NGS for fusion detection, thereby avoiding false-positive immunoreactivity and ensuring high analytical accuracy. The absence of *NTRK2* fusions in our cohort also contrasts with their study, where both confirmed fusions were *NTRK2*-driven, underscoring potential biological differences between tumor types and patient populations [26]. Collectively, these findings highlight the substantial variability of *NTRK* fusion across tumor groups and strengthen RNA-based NGS as a more trusted diagnostic method compared with IHC, particularly in settings where physiologic TRK expression or tumor heterogeneity may confound IHC interpretation.

In a study by Cocco *et al*. [7], TRKA and TRKC expression in NB patients was generally a good prognostic indicator, while TRKB was primarily expressed in high-grade tumors that also harbor *MYCN* amplification and was associated with a poor prognosis. This study used a pan-TRK antibody containing all three proteins. In the study by Vingiani *et al*. [14], they detected *NTRK* fusion in only 11 of 30 (37%) cases with positive pan-TRK IHC and in 1 of 87 (1.1%) cases with negative pan-TRK IHC. This study included a cohort of 124 cancer patients (carcinomas, 93 cases; soft tissue sarcomas, 19; primary central nervous system tumors, 10; and neuroblastomas, only 2 cases and negative for fusion). Based on this information, IHC sensitivity was calculated as 91.7% and specificity as 81.9% [14]. However, when we examined the positive predictive value, it was low at 36.7%, while the negative predictive value was high at 98.8%. This indicates that there are many false-positive results. When we considered IHC results according to histological types, higher concordance was seen in carcinomas (94.2%), while the concordance rate was low in nervous system tumors and neuroblastomas (18.2%). It has been reported that IHC expression patterns of the detected *TPR-NTRK1* fusion showed cytoplasmic and membranous staining patterns [14]. Compared to these data, in our study, of all cases showing positive IHC, only 44 had fusion studies and 46 had point mutation studies. Percentage-wise, fusions were observed in 6.81% of cases with immunohistochemical expression. When point mutations were evaluated, clinically significant mutations were detected in 34.78% of positive cases, while no mutations were detected in 65.21%. The high *NTRK* rate in our study was associated with the fact that a large proportion of the patients included were advanced-stage, relapsed-refractory patients.

In another study, Zhao *et al*. [27] investigated the frequency of *NTRK* fusions in pediatric tumors. In a cohort of 1347 pediatric patients, *NTRK* fusions were detected in 2.22% of all tumors and 3.08% of solid tumors. *NTRK1* and *NTRK3* fusions were commonly observed in papillary thyroid carcinomas, while *NTRK2* fusions were more common in central nervous system tumors, and these tumors were mostly low-grade gliomas. In soft tissue tumors, fusions such as *ETV6-NTRK3* were found in rare tumors such as spindle cell tumors and infantile fibrosarcoma. TRK inhibitors have emerged as an effective method to support surgery in these types of tumors. No *NTRK* fusion was detected in any of the 79 NB cases examined in the study [27].

In Cox model analysis, NTRK positivity was not independently associated with overall survival, but it showed a non-significant trend toward improved event-free survival. *NTRK* single-nucleotide variants were not independently associated with either event-free or overall survival, although a trend toward poorer event-free survival that was not significant. The lack of statistical significance may result from the low frequency of NTRK expression and the limited number of events.

Light *et al*.'s study [28] evaluated the impact of *NTRK* gene family expression levels on the clinical features and outcomes of NB patients. 814 NB patient samples were analyzed using Quantitative Real-Time Reverse Transcriptase PCR (qRT-PCR). *NTRK1* expression was associated with all established risk factors; Statistically significant differences were found in patients younger than 18 months, with low stage, *MYCN* amplification negative, hyperploidy, and good histology. In patients with high *NTRK1* expression, 5-year EFS and OS rates were significantly higher than in patients with low expression levels ($p < 0.0001$). *NTRK2* was found to be significantly associated with age, *MYCN*, and ploidy status, but not with survival outcomes. However, *NTRK3* was not significantly associated with risk factors but was associated with worse OS [28]. Our own research findings indicate that NTRK-IHC negative patients had longer EFS and OS times than positive patients. Although NTRK positivity had an effect on EFS and OS, this was not found to be statistically significant.

As suggested by Märkl *et al*. [29], in cases with a high NTRK splicing rate, such as congenital fibrosarcoma, FISH, RT-PCR, and NGS are recommended for direct or positive results after NTRK IHC. In cases with low fre-

quency, direct or panTRK-IHC tests were recommended if they included multiple gene assessments, and FISH and NGS were recommended for positive results. For negative results, no other analyses were required [29].

Tyrosine kinases are tumor-agnostic markers, and targeted therapies are available for them. Detecting the presence of *NTRK* fusions in cancers is crucial for initiating treatment. However, the importance of point mutations, gain-of-function mutations, and missense mutations for the application of NTRK-targeted therapy in NB has not been fully elucidated. In this study, IHC staining of NTRK showed NTRK expression in the majority of NB patients. Therefore, it was concluded that it cannot be a leading biomarker for fusion.

In this study, some samples were not suitable for IHC or NGS analyses due to lack of tissue in the paraffin block, inadequate tissue follow-up, or limited sample size. Furthermore, budget constraints precluded the application of NGS to all samples. However, the relatively large sample size increased statistical power. Tumor mutational burden assessment or broad copy-number analysis are the limitations of our study in terms of NGS panel that we used in this study. One of the limitations of this study is that the detected SNVs were previously interpreted and classified in clinical databases and did not include functional validation. The significant heterogeneity of neuroblastomas is another limitation that must be considered in the interpretation of the findings.

5. Conclusions

In conclusion, evaluation for TRK inhibition is needed in all cases of NB, a tumor of neural origin. NTRK fusions and NTRK point mutations or other SNVs should be both evaluated to determine possible resistance to NTRK inhibitors. Our data supported that Pan-NTRK immunohistochemistry is not a good biomarker for the selection of further NGS studies. In this study, no concordance was found with nuclear immunohistochemical NTRK expression in the four cases in which fusions were detected.

NTRK fusions are rare in NB, as confirmed in our study and the literature. As an agnostic therapeutic target, RNA fusion and multigene DNA panels containing NTRK genes should be evaluated using NGS in relapsed, refractory, high-risk, poor clinical conditions, and chemotherapy-resistant NB cases.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

SA and TÇA designed the study. DK, NI, SMÖ, AE, and ÖEG collected the patient samples and data. NI, SMÖ, AE, and ÖEG conducted the research and laboratory experiments. SA, TÇA, and SMÖ performed the statistical analysis of the data. All authors participated in the writing and editing of the manuscript. All authors read and approved the final version of the manuscript. All authors participate in the study, taking responsibility for the adequacy of the content and contributing to the accurate and complete completion of the work.

Ethics Approval and Consent to Participate

Ethical Approval was taken from Dokuz Eylul University Non-Interventional Research Ethics Committee on 07.05.2023 with decision number 2023/22-14. Approval was obtained by having the patients' parents sign Informed Consent forms. The study was carried out in accordance with the guidelines of the Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBS46963.

References

[1] Maris JM. Recent advances in neuroblastoma. The New England Journal of Medicine. 2010; 362: 2202–2211. https://doi.org/10.1056/NEJMra0804577.

[2] Colon NC, Chung DH. Neuroblastoma. Advances in Pediatrics. 2011; 58: 297–311. https://doi.org/10.1016/j.yapd.2011.03.011.

[3] Yue C, Zhang Q, Sun F, Pan Q. Global, regional and national burden of neuroblastoma and other peripheral nervous system tumors, 1990 to 2021 and predictions to 2035: visualizing epidemiological characteristics based on GBD 2021. Neoplasia (New York, N.Y.). 2025; 60: 101122. https://doi.org/10.1016/j.neo.2025.101122.

[4] Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. Nature Reviews. Cancer. 2003; 3: 203–216. https://doi.org/10.1038/nrc1014.

[5] Vaishnavi A, Le AT, Doebele RC. TRKing down an old oncogene in a new era of targeted therapy. Cancer Discovery. 2015; 5: 25–34. https://doi.org/10.1158/2159-8290.CD-14-0765.

[6] Homma S, Shimada T, Hikake T, Yaginuma H. Expression pattern of LRR and Ig domain-containing protein (LRRIG protein)

in the early mouse embryo. Gene Expression Patterns: GEP. 2009; 9: 1–26. https://doi.org/10.1016/j.gep.2008.09.004.

[7] Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nature Reviews. Clinical Oncology. 2018; 15: 731–747. https://doi.org/10.1038/s41571-018-0113-0.

[8] Amatu A, Sartore-Bianchi A, Bencardino K, Pizzutilo EG, Tosi F, Siena S. Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2019; 30: viii5–viii15. https://doi.org/10.1093/annonc/mdz383.

[9] Dunn DB. Larotrectinib and Entrectinib: TRK Inhibitors for the Treatment of Pediatric and Adult Patients With *NTRK* Gene Fusion. Journal of the Advanced Practitioner in Oncology. 2020; 11: 418–423. https://doi.org/10.6004/jadpro.2020.11.4.9.

[10] Liu D, Offin M, Harnicar S, Li BT, Drilon A. Entrectinib: an orally available, selective tyrosine kinase inhibitor for the treatment of *NTRK*, *ROS1*, and *ALK* fusion-positive solid tumors. Therapeutics and Clinical Risk Management. 2018; 14: 1247–1252. https://doi.org/10.2147/TCRM.S147381.

[11] Shulman DS, DuBois SG. The Evolving Diagnostic and Treatment Landscape of NTRK-Fusion-Driven Pediatric Cancers. Paediatric Drugs. 2020; 22: 189–197. https://doi.org/10.1007/s40272-020-00380-9.

[12] Moreira DC, Mikkelsen M, Robinson GW. Current Landscape of NTRK Inhibition for Pediatric CNS Tumors. CNS Drugs. 2024; 38: 841–849. https://doi.org/10.1007/s40263-024-01121-z.

[13] Pacenta HL, Macy ME. Entrectinib and other ALK/TRK inhibitors for the treatment of neuroblastoma. Drug Design, Development and Therapy. 2018; 12: 3549–3561. https://doi.org/10.2147/DDDT.S147384.

[14] Vingiani A, Lorenzini D, Conca E, Volpi CC, Trupia DV, Gloghini A, *et al*. Pan-TRK immunohistochemistry as screening tool for NTRK fusions: A diagnostic workflow for the identification of positive patients in clinical practice. Cancer Biomarkers: Section a of Disease Markers. 2023; 38: 301–309. https://doi.org/10.3233/CBM-220357.

[15] Aktas T, Kızmazoglu D, Aktas S, Erol A, Serinan E, Gokbayrak O, *et al*. Unraveling the Mystery: Next Generation Sequencing Sheds Light on Neuroblastoma Pathogenesis and Targeted Therapies. Frontiers in Bioscience (Landmark Edition). 2023; 28: 171. https://doi.org/10.31083/j.fbl2808171.

[16] Lipska BS, Drozynska E, Scaruffi P, Tonini GP, Izycka-Swieszewska E, Zietkiewicz S, *et al*. c.1810C>T polymorphism of NTRK1 gene is associated with reduced survival in neuroblastoma patients. BMC Cancer. 2009; 9: 436. https://doi.org/10.1186/1471-2407-9-436.

[17] Rogers C, Morrissette JJD, Sussman RT. NTRK point mutations and their functional consequences. Cancer Genetics. 2022; 262-263: 5–15. https://doi.org/10.1016/j.cancergen.2021.12.002.

[18] Drilon A, Nagasubramanian R, Blake JF, Ku N, Tuch BB, Ebata K, *et al*. A Next-Generation TRK Kinase Inhibitor Overcomes Acquired Resistance to Prior TRK Kinase Inhibition in Patients with TRK Fusion-Positive Solid Tumors. Cancer Discovery. 2017; 7: 963–972. https://doi.org/10.1158/2159-8290.CD-17-0507.

[19] Fuse MJ, Okada K, Oh-Hara T, Ogura H, Fujita N, Katayama R. Mechanisms of Resistance to NTRK Inhibitors and Therapeutic Strategies in NTRK1-Rearranged Cancers. Molecular Cancer Therapeutics. 2017; 16: 2130–2143. https://doi.org/10.1158/1535-7163.MCT-16-0909.

[20] Joshi SK, Qian K, Bisson WH, Watanabe-Smith K, Huang A, Bottomly D, *et al*. Discovery and characterization of targetable NTRK point mutations in hematologic neoplasms. Blood. 2020; 135: 2159–2170. https://doi.org/10.1182/blood.2019003691.

[21] Kheder ES, Hong DS. Emerging Targeted Therapy for Tumors with *NTRK* Fusion Proteins. Clinical Cancer Research. 2018; 24: 5807–5814. https://doi.org/10.1158/1078-0432.CCR-18-1156.

[22] "UniProt," UniProt. [Online]. Available at: https://www.uniprot.org/uniprotkb/X5D2R1/entry (Accessed: 24 September 2025).

[23] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr, Kinzler KW. Cancer genome landscapes. Science (New York, N.Y.). 2013; 339: 1546–1558. https://doi.org/10.1126/science.1235122.

[24] Awada A, Berghmans T, Clement PM, Cuppens K, De Wilde B, Machiels JP, *et al*. Belgian expert consensus for tumor-agnostic treatment of NTRK gene fusion-driven solid tumors with larotrectinib. Critical Reviews in Oncology/hematology. 2022; 169: 103564. https://doi.org/10.1016/j.critrevonc.2021.103564.

[25] Mascarenhas L, DuBois SG, Albert CM, Bielack S, Orbach D, Federman N, *et al*. Elective Discontinuation of Larotrectinib in Pediatric Patients With TRK Fusion Sarcomas and Related Mesenchymal Tumors. Journal of Clinical Oncology. 2025; 43: 1180–1187. https://doi.org/10.1200/JCO.24.00848.

[26] Mohamed F, Kurdi M, Baeesa S, Sabbagh AJ, Hakamy S, Maghrabi Y, *et al*. The Diagnostic Value of Pan-Trk Expression to Detect Neurotrophic Tyrosine Receptor Kinase (NTRK) Gene Fusion in CNS Tumours: A Study Using Next-Generation Sequencing Platform. Pathology Oncology Research: POR. 2022; 28: 1610233. https://doi.org/10.3389/pore.2022.1610233.

[27] Zhao X, Kotch C, Fox E, Surrey LF, Wertheim GB, Baloch ZW, *et al*. NTRK Fusions Identified in Pediatric Tumors: The Frequency, Fusion Partners, and Clinical Outcome. JCO Precision Oncology. 2021; 1: PO.20.00250. https://doi.org/10.1200/PO.20.00250.

[28] Light JE, Koyama H, Minturn JE, Ho R, Simpson AM, Iyer R, *et al*. Clinical significance of NTRK family gene expression in neuroblastomas. Pediatric Blood & Cancer. 2012; 59: 226–232. https://doi.org/10.1002/pbc.23343.

[29] Märkl B, Hirschbühl K, Dhillon C. NTRK-Fusions - A new kid on the block. Pathology, Research and Practice. 2019; 215: 152572. https://doi.org/10.1016/j.prp.2019.152572.