



Published in final edited form as:

Genes Chromosomes Cancer. 2019 November ; 58(11): 739–746. doi:10.1002/gcc.22767.

The histologic spectrum of soft tissue spindle cell tumors with *NTRK3* gene rearrangements

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Abstract

NTRK3-rearranged tumors other than infantile fibrosarcomas (IFSs) harboring the canonical *ETV6-NTRK3* fusions are uncommon, and include mainly inflammatory myofibroblastic tumors and gastrointestinal stromal tumors. Herein, we describe an additional subset of seven tumors sharing *NTRK3* gene rearrangements. The cohort included five females and two males (age range 1–67 years). Tumors were located in extremities, trunk, retroperitoneum, or intra-abdominal. In all tumors, fluorescence in situ hybridization (FISH) revealed rearrangements in *NTRK3* accompanied by *NTRK3* amplification in two cases. In three cases, RNA sequencing identified a fusion transcript composed of *NTRK3* exon 14 fused to *ETV6*, *TFG*, and *TPM4*, respectively, retaining the *NTRK3* kinase domain. All tumors were positive for pan-TRK by immunohistochemistry (IHC). Two cases showed low- to intermediate-grade histology composed of monomorphic spindle cells arranged in a patternless architecture, stromal bands, and perivascular rings of hyalinized collagen and coexpression of S100 and CD34. The remaining five cases were high-grade fascicular monomorphic spindle cell sarcomas, morphologically somewhat reminiscent of either malignant peripheral nerve sheath tumors (MPNSTs) or fibrosarcomas (FSs). Two high-grade *NTRK3* sarcomas showed aggressive clinical behavior with development of lung metastases. Identification of high-grade *NTRK3*-rearranged sarcomas is clinically important, since the development of selective *NTRK* inhibitors has opened new avenues for targeted therapy. Although IHC for pan-TRK can be applied as a screening tool, molecular studies are recommended for a conclusive diagnosis of *NTRK*-rearranged neoplasms.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Keywords

fibrosarcoma; fusion; malignant peripheral nerve sheath tumor; NTRK3; sarcoma

1 | INTRODUCTION

Neurotropic tropomyosin receptor kinase (*NTRK*) fusions were described recently in soft tissue tumors that are morphologically distinct from infantile fibrosarcoma (IFS).^{1,2} In particular, *NTRK1*-rearranged tumors are characterized by spindle cell morphology, presenting in patients of both sexes, with a wide range of age and anatomic locations, usually in extremities and trunk, and, more rarely, in intra-abdominal sites. These tumors show variable morphology and histologic grade, and frequently coexpress CD34 and S100 by immunohistochemistry (IHC), in addition to TRK-A and pan-TRK expression. Two histologic patterns have been described so far. First, in superficial locations, *NTRK1*-rearranged tumors infiltrate subcutaneous fat in a reticular fashion, a morphologic pattern reminiscent of lipofibromatosis (so-called lipofibromatosis-like neural tumor).³ The second group closely resembles malignant peripheral nerve sheath tumor (so-called MPNST-like tumors), spanning variable histologic grades. At one end of this morphologic spectrum are infiltrative MPNST-like tumors composed of haphazardly arranged monomorphic spindle cells with low mitotic activity and distinctive patterns of keloidal stromal and perivascular collagen deposition. At the other end of the spectrum, *NTRK1*-rearranged spindle cell sarcomas are composed of cellular fascicles of morphologically malignant spindle cells with a high mitotic rate (exceeding 10 mitotic figures [MFs]/10 high power fields [HPFs]) and these tumors are capable of developing (lung) metastases. In this study, we present a cohort of seven spindle cell sarcomas with *NTRK3* gene rearrangements, which also display a wide range of histologic grade and clinical behavior.

2 | MATERIALS AND METHODS

Seven *NTRK3*-rearranged soft tissue spindle cell tumors other than classic IFS were retrieved from the consultation files of two of the authors (C.R.A., C.D.F.). The case selection for molecular investigation was based on the initial reported observations that the morphologic spectrum of kinase fusion-positive sarcomas includes tumors resembling fibrosarcomas (FSs) or MPNSTs. A large collection of these various morphologies was available in our consultation files from prior molecular studies, which were previously screened and lacked abnormalities in *NTRK1/2*, *BRAF*, and *RAF1* genes.^{1,3} Clinical data, including age, gender, anatomic site, and gross features of tumors were retrieved from pathology reports. All tumors had been surgically removed and hematoxylin and eosin-stained slides from resection specimens were reviewed by two of us (A.S., C.R.A.). Tumors were graded according to the FNCLCC grading system. IHC for CD34, S100, SOX10, pan-TRK (TRK-ABC), and H3K27me3 was performed according to standardized procedures on automated platforms. Staining was performed on a Leica-Bond-3 (Leica, Buffalo Grove, Illinois) or a Ventana Benchmark (Ventana Medical Systems, Tucson, Arizona) automated immunostaining platform using a heat-based antigen retrieval method and high pH buffer.

2.1 | Targeted RNA sequencing

One case was analyzed by targeted RNA sequencing (Table 1). RNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue using Amsbio's ExpressArt FFPE Clear RNA Ready kit (Amsbio LLC, Cambridge, Massachusetts). The fragment length was assessed with an RNA 6000 chip on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, California). RNAseq libraries were prepared using 20 to 100 ng total RNA with the TruSight RNA Fusion Panel (Illumina, San Diego, California). Targeted RNA sequencing was performed on an Illumina MiSeq platform. Reads were independently aligned with STAR (version 2.3) against the human reference genome (hg19) and analyzed by STAR-Fusion.

2.2 | Anchored multiplex RNA sequencing (Archer Dx)

Two cases were studied by anchored multiplex RNA sequencing assay, the detailed procedure has been previously described.⁴ In short, unidirectional gene-specific primers were designed to target specific exons in 62 genes known to be involved in oncogenic fusions in solid tumors. In brief, RNA was extracted from FFPE specimens, followed by cDNA synthesis and library preparation. Anchored multiplex polymerase chain reaction amplicons were sequenced on Illumina Miseq, and the data were analyzed using the Archer software.

2.3 | MSK-IMPACT assay

One case was investigated with the MSK-IMPACT assay.⁵ Briefly, MSK-IMPACT is a comprehensive molecular profiling assay that involves hybridization capture and deep sequencing of all exons and selected introns of up to 468 oncogenes and tumor-suppressor genes, allowing the detection of point mutations, small and large insertions or deletions, and rearrangements. In addition to capturing all coding regions of the genes, the assay also captures >1000 intergenic and intronic single-nucleotide polymorphisms (tiling probes), interspersed homogenously across the genome, aiding the accurate assessment of genome-wide copy number. In total, the probes target approximately 1.2 megabases of the human genome.

2.4 | Fluorescence in situ hybridization

All cases were tested by FISH for *NTRK3* gene abnormalities. Custom probes made by bacterial artificial chromosomes (BACs) clones flanking the genes of interest according to UCSC genome browser (<http://genome.ucsc.edu>) and obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (Oakland, California; <http://bacpac.chori.org>).⁶ DNA from each BAC was isolated according to the manufacturer's instructions. The BAC clones were labeled with fluorochromes (fluorescent-labeled dUTPs, Enzo Life Sciences, New York, New York) by nick translation and validated on normal metaphase chromosomes. The 4- μ m-thick FFPE slides were deparaffinized, pretreated, and hybridized with denatured probes. After overnight incubation, the slides were washed, stained with 4',6-diamidino-2-phenylindole, mounted with an antifade solution, and then examined on a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany) controlled by the Isis 5 software (Metasystems).

3 | RESULTS

The clinical presentation, gross and histologic features, IHC staining results, and molecular abnormalities of the seven *NTRK3*-rearranged spindle cell tumors are summarized in Table 1. The seven patients selected had a wide age range (1–67, median 21 years). Three tumors presented in children (range 1–11 years), one in a young adult (21 years) and three in older adults (range 49–67 years). Both genders were affected, including five women and two males. Anatomic sites were diverse with extremity, trunk, and retroperitoneal locations, whereas two high-grade sarcomas were located intra-abdominally, involving bowel wall. Tumor size ranged from 4 to 25 cm (mean 10 cm).

NTRK3-rearranged spindle cell tumors spanned a wide histologic spectrum. Two cases (cases 1 and 2) consisted of low- to intermediate-grade monomorphic spindle cell sarcomas with multifocal to diffuse expression of S100 protein. Both cases showed an infiltrative growth, with brisk mitotic activity in areas (>10 MF/10 HPFs) and lacked necrosis. Case 1 was superficial, while case 2 had a highly infiltrative pattern within skeletal muscle. The histology of both tumors displayed focally the presence of stromal hyalinization and perivascular collagen rings, reminiscent in part of the group of low-grade spindle cell neoplasms with *NTRK1*, *RAF*, and *BRAF* fusions recently described by our group¹ (Figure 1A,B). Case 2 in addition showed a highly infiltrative growth within subcutaneous fat, closely resembling lipofibromatosis-like neural tumor³ (Figure 1A). In fact, this case showed a multitude of architectural patterns, including sweeping herring bone-like fascicles, hemangiopericytoma-like (HPC-like) staghorn vessels and perivascular myopericytoma-like whorling (Figure 1C–E). Moreover, hypocellular areas with perivascular and stromal depositions of hyalinized collagen alternated with hypercellular areas with monomorphic hyperchromatic spindle cells showing brisk mitotic activity. This case also showed focal myxoid change and an area with marked nuclear pleo-morphism (Figure 1F). By IHC, both tumors showed multifocal coexpression of CD34 and S100, in addition to pan-TRK (Figure 1G–I), whereas Sox10 was negative and H3K27me3 was retained.

The remaining five cases consisted of high-grade spindle cell sarcomas showing increased mitotic activity and geographic areas of necrosis. Three tumors (cases 3–5) also showed focal S100 positivity. These tumors consisted of cellular fascicles of monomorphic hyperchromatic spindle cells with a variable amount of pale eosinophilic cytoplasm (Figure 2A). Most cases showed relatively monomorphic cytomorphology, except for one case, which showed increased nuclear pleomorphism. The overall histologic features of this subset were somewhat reminiscent of high-grade MPNST showing multifocal coexpression of S100 (Figure 2B) and CD34. However, IHC for Sox10 was negative and nuclear H3K27me3 staining was retained. All three tumors were diffusely positive for pan-TRK, showing a cytoplasmic pattern (Figure 2C). The latter two cases (cases 6–7) showed a non-specific immunoprofile and had a fascicular growth with areas of necrosis (Figure 3A). The tumor cells had variably eosinophilic cytoplasm and case 7 showed patchy nuclear pleomorphism (Figure 3B). The two lesions showed CD34 and pan-TRK coexpression (Figure 3C), while S100 protein and SOX10 were negative. H3K27me3 expression was retained.

3.1 | Molecular pathology

As shown in Table 1, four different *NTRK3* fusion partners were identified, including *ETV6-NTRK3* (detected by FISH), *TFG-NTRK3* and *TPM4-NTRK3* (by Archer Fusionplex RNA sequencing), and *EML4-NTRK3* (by targeted RNA sequencing). In all three fusion transcripts detected by RNA sequencing, *NTRK3* breakpoints were in exon 14 (fused with *TFG* exon 5, *TPM4* exon 6, and *EML4* exon 2), which retained the NTRK kinase domain in the projected fusion oncoprotein (Figure 4). By RNA sequencing, one case showed *NTRK3* mRNA overexpression in contrast to other sarcoma types. All cases were confirmed by FISH to harbor *NTRK3* gene rearrangements (Figure S1A); however, in two cases *NTRK3* showed gene amplifications in addition to the break-apart signal (Figure S1B). By MSK-IMPACT, the intermediate-grade tumor with focal areas of anaplasia (case 2, *TFG-NTRK3* fusion) contained two p53 mutations and *CDKN2A/B* deletions.

3.2 | Clinical follow-up

Follow-up information is summarized in Table 1. Given the limited number of cases with clinical follow-up and relatively short follow-up time, accurate prediction of prognosis of *NTRK3*-rearranged adult tumors is not feasible. Nevertheless, two tumors with high-grade morphology (cases 4 and 5) showed aggressive clinical behavior with metastatic spread to the lung. In case 4, lung metastases were excised and this patient has no evidence of disease after 3 years.

4 | DISCUSSION

In recent years it has become evident that a small subset of spindle cell sarcomas (other than IFS with canonical *ETV6-NTRK3* fusions) harbor *NTRK* fusions.^{1,6-9} Most of the reported tumors show *NTRK1* gene fusions, with various partners, such as *LMNA*, *TPM3*, or *TPR*, whereas *NTRK2* and *NTRK3* rearrangements are relatively rare.^{1,10,11} *NTRK1*-positive spindle cell sarcomas span a wide clinical spectrum, with highly variable histology and tumor grade. Based on microscopic features, *NTRK1*-rearranged soft tissue sarcomas have been divided into low-grade tumors with haphazardly arranged monomorphic spindle cells and distinctive hyalinization and high-grade tumors with cellular fascicles of mitotically active hyperchromatic spindle cells, often with necrosis. Based on their additional immunoprofile, these tumors have been designated as FS-like (showing a nonspecific immunoprofile of vimentin or CD34 expression only)² or MPNST-like (showing coexpression of S100 and CD34).¹ In addition, a group of likely benign tumors with a highly infiltrative growth within fat, mimicking lipofibromatosis have also been described, harboring exclusively to date *NTRK1* gene rearrangement.³

To date *NTRK3* gene fusions have been described in only five sarcomas thought to represent fibrosarcomas (so-called FS-like sarcomas). Their clinicopathologic features, molecular genetics, and clinical follow-up being summarized in Table 2. Olson et al.¹⁰ found an *EML4-NTRK3* fusion in a regional metastasis of an aggressive superficial spindle cell sarcoma occurring in the lower back of a 50-year-old female. Initially, this tumor was considered to represent fibrosarcomatous transformation in dermatofibrosarcoma protuberans (FS-DFSP). Yamazaki et al.¹¹ detected a *STRN3-NTRK3* fusion (in addition to

a *CDKN2A* homozygous deletion) in a FS originating in the thigh of a 26-year-old man and a *STRN-NTRK3* fusion in a low-grade FS originating in the radius. Moreover, two rare uterine FS-like sarcomas with *EML4-NTRK3*¹² and *RBPMS-NTRK3* fusion have been reported.⁶ *ETV6-NTRK3* fusions have also been described in rare examples of adult pulmonary inflammatory myofibroblastic tumors,^{13,14} as well as in rare gastrointestinal stromal tumors lacking genetic abnormalities in the *KIT/PDGFR α /SDH α /BRAF* pathways.^{15,16}

In this study, we investigate seven additional soft tissue spindle cell sarcomas with *NTRK3* fusions, with morphology reminiscent of either MPNST or FS. Similar to *NTRK1/2*-rearranged tumors, those with *NTRK3* fusions had a wide age distribution and occurred in children and (young) adults, as well as in elderly patients. With respect to tumor location, in addition to originating in extremities or trunk, a significant number of *NTRK3* rearranged sarcomas presented in abdominal locations. In contrast to *NTRK1* fusion-positive tumors, none of the cases in this cohort showed benign or pure low-grade morphology. Although two cases showed focal areas of a lower grade component, the large majority of *NTRK3*-positive tumors in this series and those reported in the literature were high-grade sarcomas, and this translated into metastatic behavior (lung metastases) and poor clinical outcome (Tables 1 and 2).

In all three cases analyzed with RNA sequencing the breakpoint in *NTRK3* was consistent in exon 14, resulting in a preserved tyrosine kinase domain. By RNA expression analysis, overexpression of *NTRK3* was documented in one case. At the protein level, cytoplasmic pan-TRK was seen in all cases. However, pan-TRK immunoreactivity is not entirely specific,¹⁷ and therefore, additional molecular genetic testing is recommended for a conclusive diagnosis of *NTRK*-fused sarcomas. This has become particularly relevant, as these advanced stage tumors are potentially amenable to TRK inhibition, such as with Larotrectinib, a selective TRK inhibitor, which may reduce tumor volume as a preoperative strategy or improve survival in advanced stage disease.^{18,19}

The mutational burden of sarcomas with *NTRK* fusions has not been studied in detail. However, one of our cases studied showed simultaneous *p53* mutations and *CDKN2A/B* deletions. Interestingly, in two of five *NTRK3*-rearranged sarcomas described in the literature, *CDKN2A* deletions have been described^{11,12} (Table 2). Furthermore, two of the cases showed unbalanced *NTRK3* gene rearrangements associated with *NTRK3* amplification. The impact of these additional secondary genetic events of *NTRK3*-fusion-positive tumors on pathogenesis (tumor initiation and progression), histologic grade (mainly high grade), and aggressive outcome remains to be established.

Given the morphologic spectrum and nonspecific immunostaining for CD34 and S100, the differential diagnosis of *NTRK3*-rearranged sarcomas includes MPNST, FS, including FS-DFSP, solitary fibrous tumor, synovial sarcoma, among others.

Low-grade MPNST is often associated with neurofibromatosis type I (NF1) and usually, represents malignant transformation of a pre-existent neurofibroma. Low-grade MPNST contains numerous S100-positive and SOX10-positive Schwann cells, while losing most of

the intermixed CD34-positive fibroblastic framework stroma, which is typically retained in the pre-existent neurofibroma component. In contrast, *NTRK*-rearranged tumors of low- to intermediate-grade often show coexpression of S100 and CD34, and consistently lack SOX10 reactivity. High-grade MPNST can be either sporadic, NF1-associated or arise in association with prior radiation. Approximately 30 to 40% of high-grade MPNSTs show focal expression of the nerve sheath markers S100, GFAP, and SOX10. Moreover, diffuse loss of trimethylation of H3K27me3 is observed in the large majority of high-grade MPNSTs, which currently is the most sensitive and specific IHC marker.^{20,21} Notably, *NTRK*-rearranged sarcomas (including *NTRK3*-fused high-grade sarcomas) are not associated with NF1 or prior radiotherapy. Although these tumors may show reactivity for S100, they are always negative for SOX10 and retain H3K27me3 expression.

In superficial locations, CD34-positive *NTRK*-rearranged neoplasms showing a higher grade morphology may closely resemble FS ex-DFSP. However, the typical DFSP component usually has a repetitive storiform architecture and stains diffusely for CD34, whereas S100 is negative. CD34 may be lost in areas of fibrosarcomatous change. Importantly, the typical COL1A1-PDGFB fusion gene is retained in FS ex-DFSP.

The typical histologic features of solitary fibrous tumor are alternating hypocellular and hypercellular areas, HPC-like vessels, and fibromyxoid stroma containing haphazardly arranged primitive mesenchymal spindle cells. Some of these features overlap closely with those seen in *NTRK*-rearranged spindle cell neoplasm at the low-grade end of the spectrum. However, the molecular genetic hallmark of solitary fibrous tumor is *NAB2-STAT6* gene fusion and the diffuse nuclear STAT6 staining can be used as a surrogate marker.^{22,23}

By histology, typical features of monophasic synovial sarcoma are cellular fascicles of delicate monomorphic blue spindle cells with focal deposition of collagen and HPC-like vasculature. Tumors may focally express S100, but the majority are at least focally positive for epithelial membrane antigen and cytokeratins. An accurate diagnosis of monophasic synovial sarcoma is possible by molecular genetic techniques that detect the fusion genes *SS18-SSX1/2*.²⁴

In conclusion, the seven *NTRK3*-rearranged soft tissue spindle cell sarcomas reported herein represent a morphologically diverse group of tumors with variable expression of CD34 and S100. Although *NTRK3*-rearranged lesions show histologic overlap with *NTRK1* fusion-positive tumors, none of the cases were benign, being mostly at the high-grade end of the spectrum. To assist the practicing pathologist in a better pattern recognition, we have artificially divided these lesions in two groups, based on histologic features, grade and coexpression of S100 and CD34. First, tumors with low- to intermediate-grade histology were composed of monomorphic spindle cells haphazardly arranged and associated with hyalinized collagen in stromal bands and perivascular rings, a hallmark feature that may help pathologists to recognize these heterogeneous *NTRK*-rearranged mesenchymal neoplasms. Moreover, by IHC, these *NTRK*-fused tumors typically show coexpression of S100 and CD34, in the absence of SOX10. At the other end of the spectrum were *NTRK3*-rearranged sarcomas with high-grade morphology, consisting of cellular fascicles with predominantly monomorphic hyperchromatic spindle cells (fascicular monomorphic blue spindle cell

sarcomas). Some of the cases in this subset showed S100 positivity, while others had a nonspecific immunoprofile. This latter group resembled tumors that otherwise probably would have been classified either as high-grade MPNSTs or adult (or infantile) FSs. However, given the lack of specific diagnostic criteria for adult FS, we propose to classify these tumors simply as “*NTRK3*-rearranged spindle cell sarcomas”. Unlike classic high-grade MPNSTs, sarcomas with *NTRK* fusions are not associated with NF1 or a history of prior irradiation and, overall, IHC staining for SOX10 is negative, whereas H3K27me3 is retained. The clinical presentation of *NTRK3*-rearranged tumors (age and sex distribution and anatomic location) matches that of other *NTRK*-rearranged neoplasms and includes retroperitoneal, intra-abdominal, and uterine sites. Although selection bias cannot be ruled out, it appears that this group of *NTRK3*-rearranged tumors, compared to *NTRK1*-fused neoplasms, more often display high-grade morphology, which is often accompanied by aggressive clinical behavior and development of lung metastases. As highly effective targeted therapies with selective *NTRK* inhibitors are now available for clinical use, it is becoming important to correctly diagnose these high-grade *NTRK*-rearranged sarcomas. Although not entirely specific, IHC for pan-TRK remains very helpful in recognizing *NTRK*-rearranged sarcomas, which are otherwise misdiagnosed as MPNST, FS, or solitary fibrous tumor. Clearly, molecular studies are sometimes critical for a definitive diagnosis of *NTRK*-rearranged mesenchymal neoplasms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

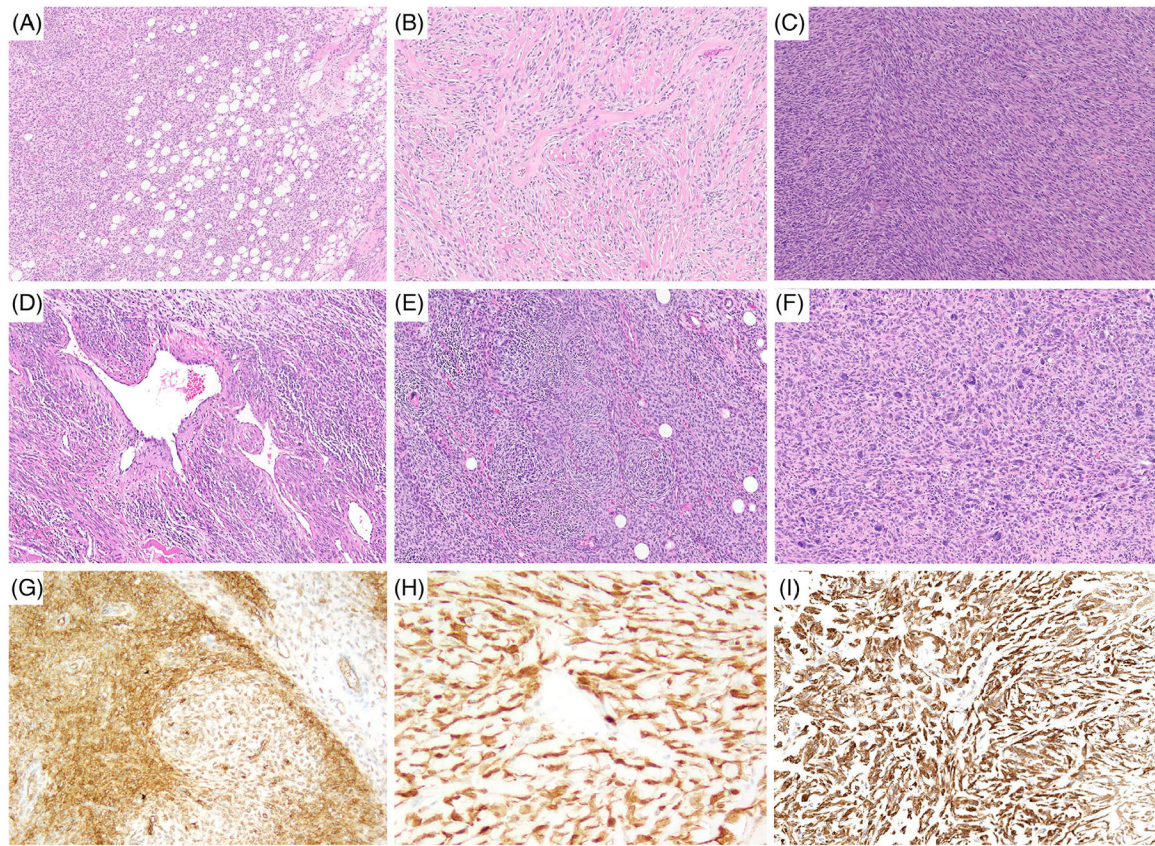
Funding information

Kristin Ann Carr Foundation; Cycle for Survival; NIH Clinical Center, Grant/Award Numbers: P30 CA008748, P50 CA217694, P50 CA 140146-01

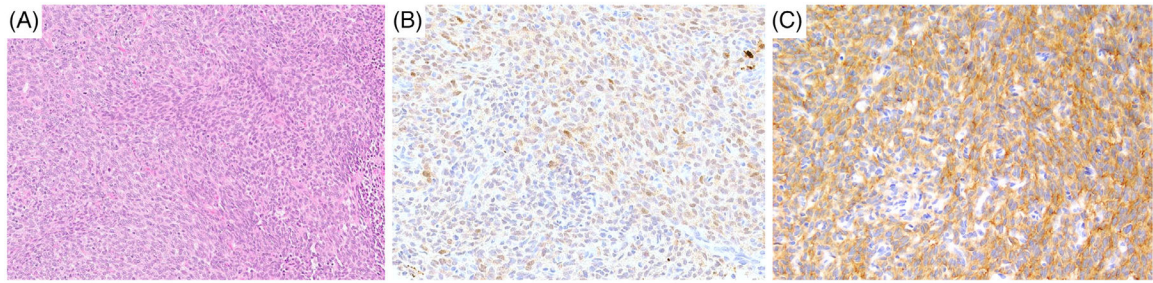
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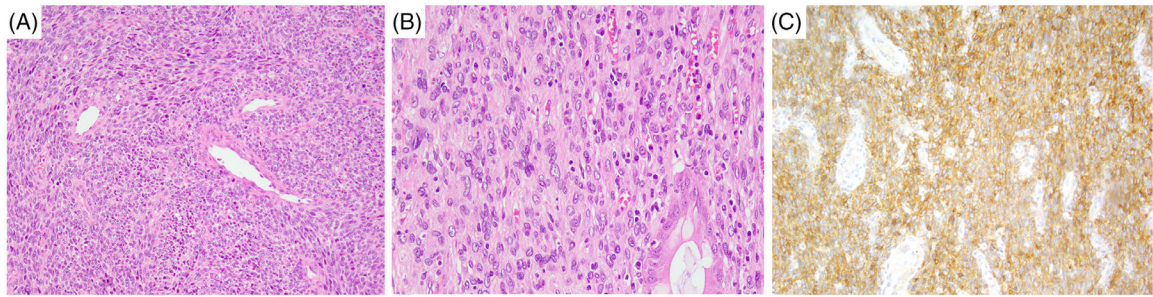
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**FIGURE 1.**

Morphologic spectrum of *NTRK3* rearranged tumors with low- to intermediate-grade MPNST-like histological features. A-I, Intermediate-grade sarcoma with *TGF-NTRK3* fusion presenting as a paraspinal tumor in a 21-year-old female (case 2); A, haphazardly arranged monomorphic spindle cell proliferation with reticular infiltration of fat morphologically resembling lipofibromatosis-like neural tumor; B, stromal and perivascular deposition of hyalinized collagen; C, cellular fascicular areas with monomorphic spindle cell sarcoma; D, hemangiopericytoma-like vasculature; E, concentric perivascular whorling as in myopericytoma; F, area with pleomorphic tumor; G-I, positive IHC staining for CD34, S100, and pan-TRK, respectively. IHC, immunohistochemistry; MPNST, malignant peripheral nerve sheath tumor [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 2.**

Morphologic spectrum of *NTRK3* rearranged sarcomas with a high-grade MPNST-like phenotype and S100 expression. A-C, High-grade MPNST-like sarcoma with *EML4-NTRK3* fusion (case 3); A, cellular fascicular monomorphic spindle cell sarcoma; B, multifocal S100 expression; C, diffuse staining for pan-TRK. MPNST, malignant peripheral nerve sheath tumor [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 3.**

Morphologic spectrum of *NTRK3* rearranged high-grade sarcomas with a nonspecific immunophenotype. A-C, High-grade FS-like sarcoma with *NTRK3* rearrangement and amplification presenting as a huge pelvic tumor in a 49-year-old female (case 7); A, cellular fascicular spindle cell sarcoma; B, tumor infiltrating colon; C, IHC expression of pan-TRK. FS, fibrosarcoma; IHC, immunohistochemistry [Color figure can be viewed at wileyonlinelibrary.com]

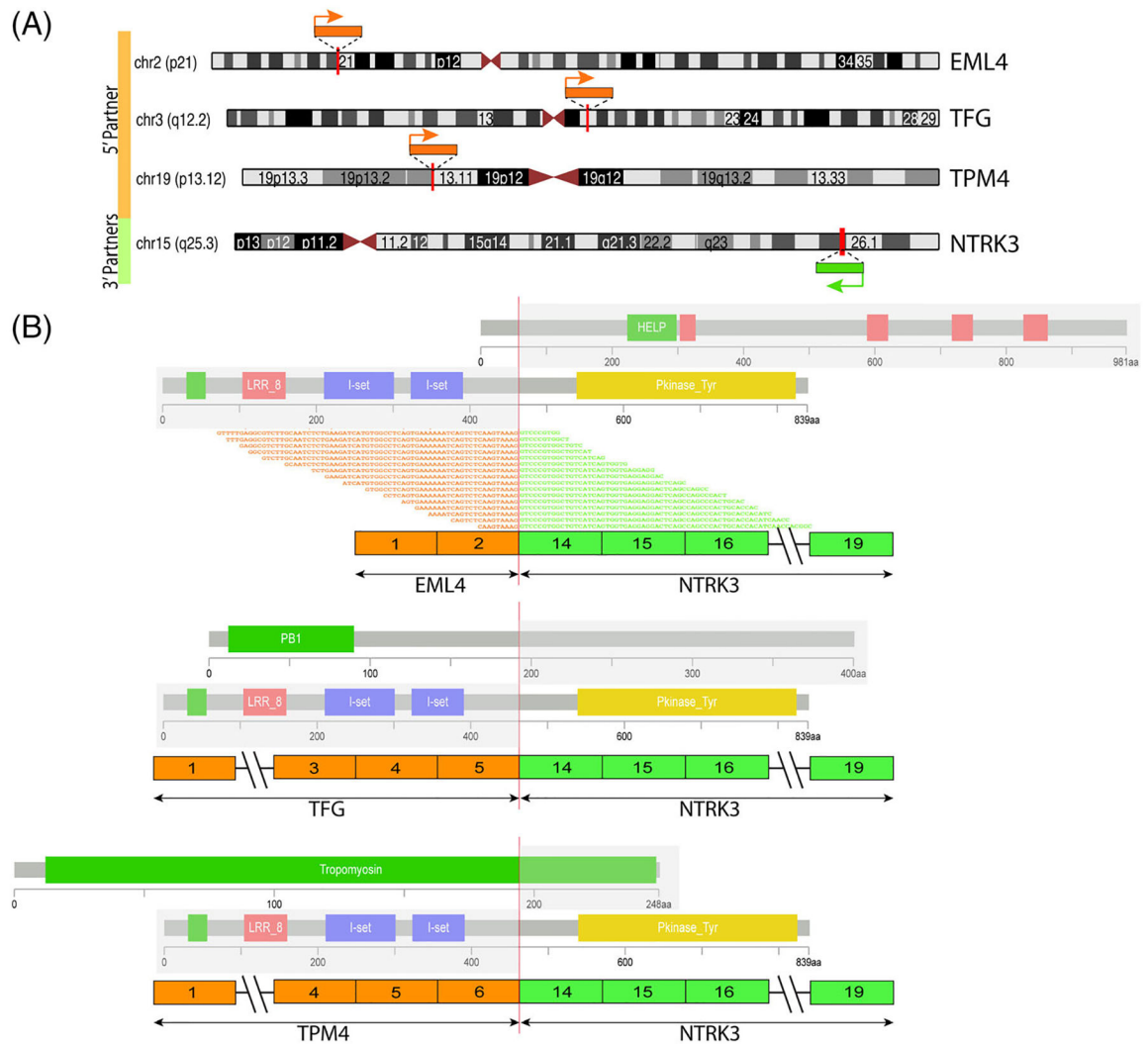


FIGURE 4.

Diagrammatic representation of three *NTRK3* fusion-positive sarcomas studied with RNA sequencing. A, Chromosomal localization of *NTRK3* on 15q25.3 and the three different gene partners: *EML4*, on 2p21; *TFG*, on 3q12.2; and *TPM4*, on 19p13.12; red vertical lines depict the genomic breakpoints. Arrows show the direction of transcription of each gene. B, Each of the three different *NTRK3* fusion transcripts are depicted in relationship to their exonic composition. For the *EML4-NTRK3* fusion detected by TruSight RNAseq, supporting fusion junction reads are also illustrated. In all cases, the fusion transcript included the 3' of *NTRK3* gene, starting from exon 14, thus retaining the kinase domain in the putative fusion oncoprotein. The protein domains of each of the genes involved are also schematically depicted. [Correction added on June 14, 2019 after first online publication: Figure 4 has been revised. The revised version is included here.] [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Clinicopathologic and molecular findings of tumors with *NTRK3* fusions

Patient	Location	cm	G	MC	NE	S100	CD34	Sox10	H3	TRK	Molecular	Clinical FU
1	67/F	Thigh	4	IG	14	-	pos	neg	pos	pos	<i>ETV6-NTRK3^a</i>	NA
2	21/F	Paraspinal	8	IG	11	-	pos	neg	pos	pos	<i>TFG-NTRK3^b</i>	NED 5 mo
3	1/M	Foot	4	HG	13	+	pos	NA	NA	pos	<i>EML4-NTRK3^c</i>	NA
4	60/F	Popliteal	8	HG	6	+	pos	neg	pos	neg	<i>TPM4-NTRK3^b</i>	NED 36 mo
5	11/M	Mesentery	11	HG	28	+	pos	NA	NA	pos	<i>NTRK3 b/a^a</i>	DOD 1 mo
6	6/F	Retropert	11	HG	10	+	neg	neg	pos	pos	<i>NTRK3 b/a +^a</i>	AWD 4 mo
7	49/F	Abdomino-pelvis	25	HG	28	+	neg	neg	pos	pos	<i>NTRK3 b/a +^a</i>	NED 11 mo

Abbreviations: cm, size in cm; G, grade; H3, H3K27me3; HG, high grade; IG, intermediate grade; MC, mitotic count/10 HPFs; NE, necrosis.

^aFISH; b/a, break-apart; +, amplification.^bArcher FusionPlex assay.^cTargeted RNA sequencing; FU, follow-up; NA, not available; NED, no evidence of disease; AWD, alive with disease; DOD, dead of disease; mo, months.

TABLE 2

Literature review of five *NTRK3*-rearranged adult fibrosarcomas

Patient	Location	cm	Grade	MC	NE	S100	CD34	TRK	Molecular	Follow-up
1	Thigh	11	HG	12	+	neg	pos	pos	<i>STRN3-NTRK3</i> ^a	DOD 29 mo ¹¹
2	Back	3	HG	15	-	neg	pos	pos	<i>EML4-NTRK3</i>	LN met 60 mo ¹⁰
3	Radius	9	LG	3	-	neg	pos	pos	<i>STRN-NTRK3</i>	AWD 113 mo ¹¹
4	Cervix	12	HG	3	+	pos	pos	pos	<i>EML4-NTRK3</i> ^a	AWD 52 mo ¹²
5	Cervix	14	HG	12	+	pos	neg	pos	<i>RBPMS-NTRK3</i>	DOD 79 mo ⁶

Abbreviations: cm, size in cm; HG, high grade; MC, mitotic count/10 HPFs; NE, necrosis; TRK, pan-TRK IHC.

^aAdditional homozygous *CDKN2A* deletion; mo; months; DOD, dead of disease; LN, lymph node; met, metastasis; AWD, alive with disease.