Expanding the Spectrum of Pediatric *NTRK*-rearranged Mesenchymal Tumors

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Abstract: Pediatric mesenchymal tumors harboring variant *NTRK* fusions (*ETV6*-negative) are being increasingly described; however, the histologic and clinical features of these variant *NTRK* tumors and their relationship to classic infantile fibrosarcoma are not well characterized. A better understanding of the clinicopathologic features of these tumors is necessary, and would aid in both early diagnosis and treatment. Therefore, the aim of this study was to characterize a series of pediatric *NTRK*-rearranged mesenchymal tumors, including classic *ETV6-NTRK3* fused tumors and tumors with variant (non-*ETV6*) *NTRK* fusions. The clinical features, morphology, immunophenotype, and genetics of 12 classic *ETV6-NTRK3* fused infantile fibrosarcoma and 18 variant *NTRK*-rearranged mesenchymal tumors were evaluated. For both classic and variant groups, the age at diagnosis ranged from birth to 15 years (median, 4 mo) with no sex predilection; the most common sites involved were the extremities and trunk. The rate of local recurrence and metastasis were not significantly different (recurrence rate: 11% classic, 40% variant; metastatic rate: 18% classic, 25% variant). Classic and variant *NTRK* tumors had an overlapping spectrum of histologic features, containing haphazardly arranged primitive cells in a myxoid background and/or spindle cells in long fascicles. Both groups showed diffuse pan-TRK expression by immunohistochemistry. Otherwise, the immunoprofile was nonspecific, but similar between both groups. No statistical difference was seen in any clinicopathologic feature between the classic *ETV6-NTRK3* and variant fusion cohorts. Pediatric *NTRK*-rearranged mesenchymal tumors with both classic and variant fusions likely represent a spectrum of disease with shared, recognizable cliniopathologic features.

Key Words: infantile fibrosarcoma, *NTRK*, TRK, soft tissue sarcoma, pediatric

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Infantile fibrosarcoma (IFS) is the most common soft tissue sarcoma of infancy, with nearly half of cases presenting at birth or antenatally.[1–5] IFS was first recognized as a distinct pathologic entity by Stout[1] in 1962 and further morphologically defined by Chung and Enzinger in 1976.[2] For several decades, the diagnosis was rendered based on clinical presentation and morphology.[3–6] Then in the late 1980s, nonrandom chromosomal gains in chromosomes 8, 11, 17, and 20 were noted in association with IFS, and karyotyping became common in concert with morphology as a complementary testing modality.[7–9] In 1998, a recurrent *ETV6-NTRK3* fusion was identified in IFS, and shortly thereafter the identical fusion was found

in the clinicopathologically similar kidney tumor, cellular congenital mesoblastic nephroma.[10–14] Fusions in both tumors were detected in 70% to 90% of cases by florescence in situ hybridization (FISH) or via reverse transcriptase-polymerase chain reaction (RT-PCR).[13–15] This genetic discovery precipitated a significant change in the standard method for rendering a diagnosis of IFS.[13–15] Many institutions now require molecular confirmation, by either *ETV6* breakapart FISH or *ETV6-NTRK3* RT-PCR, to render a diagnosis of IFS.

Recently, variant *NTRK* fusions that lack *ETV6* as a partner have been described in pediatric and adult soft tissue tumors.[16–23] Although several different histologic descriptors and diagnoses have been used for these tumors, many overlap with early, premolecular descriptions of IFS.[1–6,16–25] Given the advent of TRK-targeted therapies that have shown success in children with tumors harboring *NTRK* fusions, early recognition of tumors that harbor *NTRK*-rearrangements has become important for patient management.[16,26–29] Therefore, this study aimed to characterize a cohort of "*ETV6*-negative" *NTRK*-rearranged mesenchymal tumors, including their clinical demographics, morphology, and immunophenotype and to compare them with tumors harboring the canonical *ETV6-NTRK3* fusions.

METHODS

Case Selection

We queried the pathology databases of 2 institutions (UCSF and Seattle Children's Hospital) from 1990 to 2017 for pediatric and young adult tumors (from patients 0 to 25 y of age) diagnosed as IFS, morphologic mimics of IFS, tumors where IFS was listed in the comment section as a differential diagnosis, all tumors where *ETV6* FISH had been performed, and all unclassified/undifferentiated spindle cell sarcomas (Table 1 for original pathologic diagnoses). Search terms of morphologic mimics included *ALK*-negative inflammatory myofibroblastic tumor (IMT), myofibroma (tosis), and infantile fibromatoses including lipofibromatosis. Cases were eliminated from subsequent study if the original diagnosis was upheld on rereview by 2 pathologists (J.L.D.

TABLE 1. Clinicopathologic Features and Outcomes

Case	Fusion	Karyotype	Initial Diagnosis	Age (mo)	Sex	Tumor site	Margin Status	Recurrence
1	*TPM3-NTRK1*	Tri 8, 12, 17, 20	IFS	2	M	Arm	POS	2×
2	*TPM3-NTRK1*	Tri 8	LG spindle cell sarcoma	0	M	Thigh	POS	3×
3	*TPM3-NTRK1*	None	LG spindle cell sarcoma/HG at recurrence	0	M	Foot	POS	2×
4	*TPM3-NTRK1*	ND	Unclassified	0	M	Foot	NA	None
5	*TPM3-NTRK1*	Tri 17	IFS	18	M	Flank	POS	None
6	*TPM3-NTRK1*	ND	LG spindle cell tumor	36	F	Axilla	NA	None
7	*TPM3-NTRK1*	ND	Inflammatory spindle and round cell sarcoma	120	M	Pelvic	NA	None
8	*TPM3-NTRK1*	Tri 11	Inflammatory fibroid polyp	2	M	Gastric	POS	Unk
9	*TPM3-NTRK1*	ND	Spindle cell sarcoma	0	M	Pelvic	NA	None
10	*LMNA-NTRK1*	ND	Myxoid DFSP	2	F	Back	NEG	None
11	*LMNA-NTRK1*	ND	IFS	60	F	Shoulder	Unk	None
12	*LMNA-NTRK1*	ND	Cellular schwannoma	36	M	Leg	POS	1×
13	*MIR584F1-NTRK1*	ND	IFS	24	F	Paraspinal	POS	Unk
14	*SQSTM1-NTRK1*	None	Unclassified	2	F	Axilla	POS	1×
15	*TPR-NTRK1*	ND	IFS	5	M	Arm	POS	None
16	*NTRK1*	ND	IFS	10	M	Foot	POS	Unk
17	*STRN-NTRK2*	ND	Unclassified	132	F	Retroperitoneal	POS	1×
18	*EML4-NTRK3*	ND	LG spindle cell sarcoma	0	M	Axilla	POS	None
19	*ETV6-NTRK3**	ND	Unclassified	7	F	Thigh	NA	None
20	*ETV6-NTRK3**	ND	Spindle and round cell sarcoma	5	M	Retroperitoneal	NA	None
21	*ETV6-NTRK3**	ND	IFS	1	F	Hand	POS	None
22	*ETV6-NTRK3**	Tri 11, 17, 20	IFS	2	F	Abdominal wall	POS	None
23	*ETV6-NTRK3*	ND	IFS†	4	F	Dural	NA	None
24	*ETV6-NTRK3*	Tri 11, 15, 17	IFS	4	F	Shoulder	POS	None
25	*ETV6-NTRK3*	ND	LG spindle cell sarcoma	180	M	Lung	POS	1×
26	*ETV6+*	Tri 8, 11	IFS	5	M	Thigh	Unk	Unk
27	*ETV6+*	Tri 8, Tetra 11	IFS	0	F	Thigh	POS	None
28	*ETV6+*	ND	IFS	5	F	Ankle	POS	None
29	*ETV6+*	ND	IFS	0	M	Chest wall	POS	NA
30	*ETV6+*	Tri 8, 17, 10, Tetra 11	IFS	0	F	Foot	POS	None

LTF indicates lost to follow-up.

AWD indicates alive with disease; DFSP, dermatofibrosarcoma protuberans; DOD, dead of disease; F/U, follow-up; Laro, Larotrectinib; LG, low-grade; N, no; NA, not applicable; ND, not done; NED, no evidence of disease; NEG, negative; POS, positive; Tri, Trisomy; Tetra, Tetrosomy; Unk, unknown; Y, yes.

*Prior *ETV6* break-apart FISH = negative.

†NGS performed before diagnosis.

and E.R.R.). All cases where *ETV6* testing (FISH and/or RT-PCR) was negative or not performed were sequenced using next-generation sequencing (NGS) platforms. All classic IFS with *ETV6* rearrangements confirmed by FISH and/or PCR from the 2 primary institutions were included (n = 5). Seven additional known *NTRK*-rearranged cases were contributed from other institutions. Figure 1 details the case selection algorithm. Clinical, morphologic, cytogenetic/molecular, and immunohistochemical (IHC) data were ascertained.

Fluorescence In Situ Hybridization

Interphase FISH was performed on unstained formalin-fixed paraffin-embedded (FFPE) tissue sections or touch imprint slides, using dual-color, break-apart probes annealed to the *ETV6* gene region [*ETV6* (*TEL*) (12p13), cat. #07J77-001; Vysis Inc.].

High-throughput DNA Sequencing With Fusion Detection

All cases were sequenced by various clinically validated NGS platforms performed in CLIA approved laboratories. Twenty-four total cases from the 2 primary institutions were sequenced using either UW Oncoplex or UCSF500 Cancer Gene Panel; both are targeted DNA-hybrid capture based platforms that include *NTRK1*, *NTRK2* and *NTRK3* genes. Both UW Oncoplex and UCSF500 had primers for select introns of *NTRK1* and *NTRK2* to detect rearrangements. Primers for *EML4* and/or *ETV6* were included to detect *NTRK3* rearrangements. Because of gene size and use of a DNA-based capture approach, these tests did not include all introns of *NTRK3*.

Four tumors were sequenced by both panels. Six of the 7 additional cases submitted by other institutions were sequenced by platforms chosen locally, and 1 was confirmed by *ETV6* FISH.

Morphology Review

A centralized review of all cases was performed by 2 pediatric soft tissue pathologists (J.L.D. and E.R.R.). Morphologic features were scored on the initial diagnostic specimen. The morphologic features scored included number of mitoses in 10 high-power fields (HPF) and percent necrosis. All cases were also reviewed for various morphologic patterns, designated as present ($\geq 5\%$ of tumor) or absent ($< 5\%$ of tumor). The following morphologic patterns were based on early morphologic descriptions of IFS: fascicular/herringbone, haphazard primitive cells in myxoid stroma, hemangiopericytoma (HPC)-like vascular pattern, myoid/myofibromatosis-like, IMT-like, and infiltrative/fibromatosis-like.[1–6,24,25] Additional features were derived from morphologic patterns identified in prior published cases including: biphasic

TABLE 1. *(Continued)*

Tumor site	Margin Status	Mets	Treatment	Laro	Disease Status	F/U (y)
Arm	POS	None	Debulking, chemo	N	NED	2
Thigh	POS	None	Debulking, chemo	Y	AWD	2
Foot	POS	Yes (lung)	Amputation, chemo	Y	AWD	1
Foot	NA	None	Biopsy only	Y	NED	1
Flank	POS	None	Excision	N	NED	4
Axilla	NA	None	Biopsy only	Y	AWD	1.5
Pelvic	NA	None	Biopsy only	Y	NED	1.5
Gastric	POS	Unk	Excision	N	LTF	NA
Pelvic	NA	Yes (lung)	Biopsy only	Y	AWD	0.5
Back	NEG	None	Wide excision	N	NED	11
Shoulder	Unk	Yes (lung)	Surgery, chemo	Y	AWD	4
Leg	POS	None	Amputation, chemo	N	NED	10
Paraspinal	POS	Unk	Unk	N	LTF	NA
Axilla	POS	None	Debulking, chemo	Y	AWD	4
Arm	POS	None	Excision	N	NED	1
Foot	POS	None	Excision	N	NED	7
Retroperitoneal	POS	Yes (lung)	Debulking, chemo	Y	AWD	2.5
Axilla	POS	None	Excision	N	LTF	NA
Thigh	NA	None	Biopsy, chemo	Y	AWD	1
Retroperitoneal	NA	None	Biopsy only	N	DOD	NA
Hand	POS	None	Excision	N	NED	10
Abdominal wall	POS	None	Excision	N	NED	12
Dural	NA	None	Biopsy, chemo	Unk	AWD	6
Shoulder	POS	None	Excision	N	NED	7
Lung	POS	Yes (brain)	Unk	N	LTF	NA
Thigh	Unk	Unk	Unk	Unk	LTF	NA
Thigh	POS	None	Excision, chemo	N	NED	5
Ankle	POS	None	Excision, chemo	N	AWD	0.5
Chest wall	POS	Yes (lung)	Biopsy, chemo	N	DOD	NA
Foot	POS	None	Excision	N	NED	0.5

appearance with collagenized areas alternating with primitive myxoid component, hyalinized vessels/perivascular hyalinosis, rhabdoid-like cytomorphology, and nuclear palisading reminiscent of Verocay bodies.[21]

Immunohistochemistry

A panel of IHC antibodies including panTRK, TRKA (NTRK1), S100, smooth muscle actin (SMA), CD34, and CD30 was applied when FFPE tissue was available. Not all cases had material available for staining. Immunohistochemistry was performed on 4-μm paraffin-embedded whole tissue sections using standard techniques. Detection and staining for all cases used a fully automated DAB antigen retrieval system (Benchmark ULTRA; Ventana Medical Systems, Tuscan, AZ). The following antibodies were utilized: monoclonal pan-TRK (EPR17341, 1:500 dilution; Abcam), rabbit monoclonal anti-NTRK1 (EP1058Y, 1:250 dilution; Epitomics), mouse monoclonal anti-CD34 antibody (MU-236-4C, 1:30 dilution; BioGenex), rabbit polyclonal anti-S100 (Z0311, 1:800 dilution; Dako), mouse monoclonal anti-SMA antibody (M085101, 1:200; Dako), and mouse monoclonal anti-CD30 antibody (13M-96, 1:50 dilution; Cell Marque). Appropriate positive and negative controls were used for each antibody.

PanTRK and TRKA IHC staining was assessed as reported previously.[30] The remainder of the IHC stains were scored as percent of cell staining as follows: 0, no staining; 1+, <5%; 2+, 5% to 25%; 3+, 26% to 50%; 4+, 51% to75%; 5+,76% to 100% cells. In addition, staining intensity was recorded as weak, moderate or strong. An overall assessment of positive staining required a minimum of 2+ moderate intensity staining.

RESULTS

Case Identification

The query of UCSF and Seattle Children's pathology institutional databases and pathologic review by authors J.L.D. and E.R.R. yielded 5 *ETV6*-positive IFS (FISH/RT-PCR), 11 *ETV6*-negative and 13 *ETV6*-unknown (total n = 29) pediatric/young adult soft tissue tumors. The 24 *ETV6*-negative or unknown tumors were then sequenced via NGS DNA platforms. Of these 24 cases: 18 tumors contained *NTRK* fusions, 1 case had insufficient tissue/DNA for analysis, and 5 tumors lacked *NTRK* rearrangements. Of the 5 tumors that lacked *NTRK* rearrangements: 2 had no identifiable genetic alteration via either UW Oncoplex or UCSF500, 2 harbored *BRAF* mutations, and 1 contained a homozygous *CDKN2A/p16* deletion (Fig. 1).

In total from the 2 primary institutions, 11 classic *ETV6-NTRK3* fusion tumors and 12 with variant *NTRK* fusions were identified. Four of the 11 (36%) of the classic *ETV6-NTK3* fusions were not-detected by conventional methods but were identified by NGS. Including the 7 additional cases contributed by other institutions, the final cohort consisted of 30 patients/cases including 12 *ETV6-NTRK3* tumors and 18 variant *NTRK* tumors (Fig. 1, Tables 1, 2). A subset of cases had tumor karyotype performed; in this relatively limited cohort both variant and classic fusion types demonstrated nonrandom gains in one or multiple chromosomes previously associated with IFS (8, 11, 17, and 20).[7–9]

Clinical Features

The cohort of 30 patients ranged from birth to 15 years (median, 4 mo) at diagnosis, with no sex predilection. Tumors

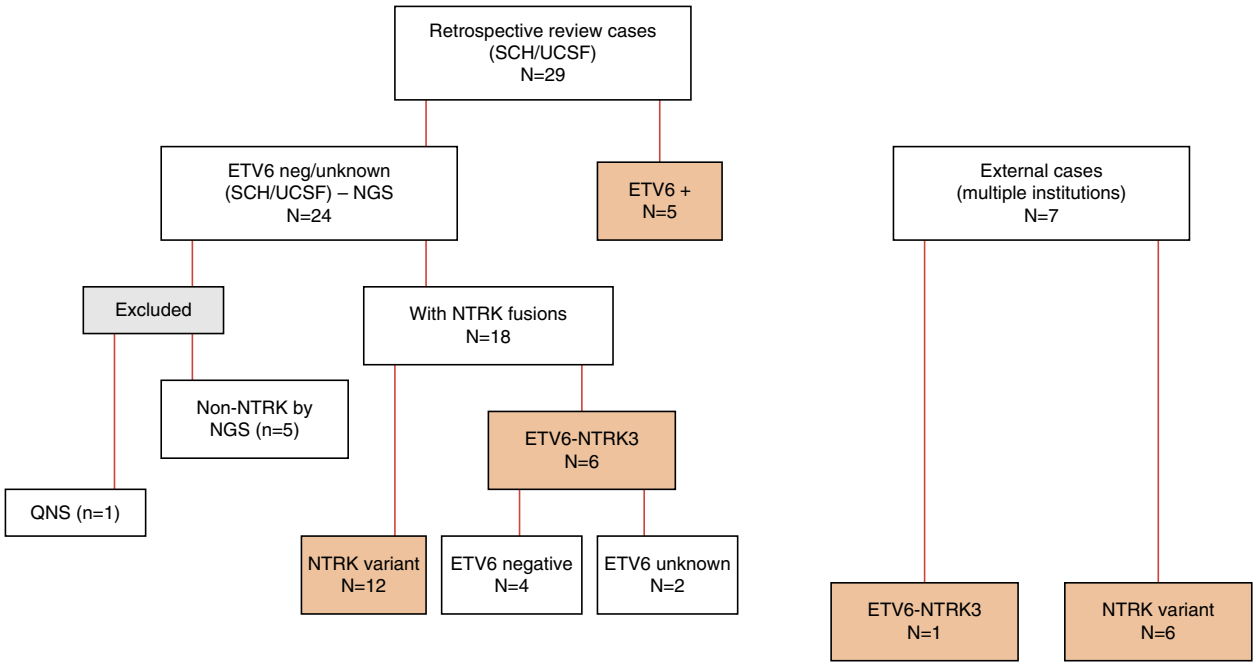

FIGURE 1. Case selection flow chart.

TABLE 2. Clinical Demographics Summary

	Age (Birth-15 y)		Sex		Location				Size (cm)	
Fusion	Mean/Median (mo)	Range (y)	M	F	Extremity	Trunk	IntraAbd	Other*	Mean	Median
ETV6-NTRK3 (n = 12)	18/4	0-15	4	7	6	3	1	2	6	5
Variant fusions (n = 18)	25/3.5	0-11	12	7	8	8	1	1	5.7	4.8
TPM3-NTRK1 (n = 9)	20/2	0-10	8	1	4	4	0	1	5.4	4.5
LMNA-NTRK1 (n = 3)	33/36	0-5	1	2	2	1	0	0	4.3	4.3
Miscellaneous (n = 6)	29/7.5	0-11	3	3	2	3	1	0	6.7	5

*Other (n = 3): 2 mucosal based (1 gastric, 1 lung), 1 head and neck.
F indicates female; IntraAbd indicates intraabdominal; M, male.

were most commonly present in the extremities and trunk, with less frequent sites including intraabdominal/retroperitoneal (non-kidney based), mucosal based, and head and neck. The greatest dimension of the tumors of diagnosis ranged from 1.4 to 16 cm (median, 5 cm). No statistically significant difference was present in any clinical parameter between the *ETV6-NTRK3* group and the variant *NTRK* fusion cohort ($P > 0.05$, Students t test for age and size; Fisher exact test for sex; ANOVA for location) (Tables 1, 2).

Morphology Review

The most common morphologic features were haphazardly arranged primitive mesenchymal cells within a variable myxoid stroma, an infiltrative/fibromatosis-like growth, and fascicular/herringbone growth. This was followed by prominent chronic inflammation with cellular atypia in an IMT-like pattern, HPC-like vasculature and other rare morphologic patterns (Table 3, Supplemental Figure 1, Supplemental Digital Content 1, http://links.lww.com/PAS/A719). No statistical difference was demonstrated between classic and nonclassic fusion cohorts in the majority of morphologic patterns, with 2 exceptions: IMT-like growth was more common in *ETV6-NTRK3* tumors ($P = 0.045$); and biphasic morphology with alternating areas of primitive mesenchymal cells and densely collagenized stroma was more frequent in tumors with variant fusions ($P = 0.02$) (Table 3).

TABLE 3. Morphologic Patterns

	Fusion		
Histologic Feature	*ETV6-NTRK3* (% Tumors With Feature, Except Mitotic Rate) (n = 12)	Variant *NTRK* Fusions (% Tumors With Feature, Except Mitotic Rate) (n = 18)	*P*
Primitive cells in myxoid stroma*	83	75	1.0
Fascicular/herringbone*	42	33	0.712
Myoid*	8	39	0.099
IMT-like*	58	17	**0.045**
Infiltrative/fibromatosis-like	92	100	0.4
HPC-like vasculature	58	61	1.0
Biphasic pattern	8	50	**0.024**
Perivascular hyalinosis	17	39	0.223
Rhabdoid cytomorphology	8	11	1.0
Nuclear palisading	0	11	0.503
Necrosis (> 5% cells)	25	11	0.364
Mitotic rate, #/10 HPF (median/range)	11/< 1-78	3/< 1-15	**0.004**

*All tumors contained at least 1 of these 4 features. The top 2 features were seen in all but 2 cases.

All but 2 cases contained either a primitive spindle cell pattern, a fascicular/herringbone pattern, or both. The 2 exceptions included 1 *LMNA-NTRK1* rearranged case that was predominantly myoid in appearance and 1 *ETV6-NTRK3* tumor that demonstrated IMT-like features. The majority of cases, including variant *NTRK* fusions and classic *ETV6-NTRK3* fusions, demonstrated significant intratumoral heterogeneity with multiple morphologic patterns present within a single tumor (Figs. 2–4; Figs. 2 and 4 each represent 2 tumors; Fig. 3 represents 1 tumor); all but 3 cases (90%) contained ≥ 2 morphologic patterns. The 3 cases demonstrating a single morphologic pattern were small biopsies (including 2 needle cores).

Morphologically, *NTRK*-rearranged tumors demonstrated a monomorphic population of cells with plump oval to elongated nuclei. There was minimal to mild nuclear pleomorphism. Increased cytologic atypia was noted in association with inflammation/IMT-like areas; in these areas, variably prominent central nucleoli were present. In 3 cases (1 *ETV6* tumor and 2 variant tumors) rhabdoid-like cells with eccentric nuclei and large nucleoli were seen (Table 3). Necrosis (> 5% of cells) was seen in a subset of cases but showed no statistical difference between fusion cohorts (Table 3). Mitotic rate was higher in the *ETV6-NTRK3* rearranged cases ($P = 0.004$) (Table 3), but it ranged dramatically in both groups: <1 to 15 mitosis/10 HPF in the variant *NTRK* fusion group and <1 to 78/10 HPF in the *ETV6-NTRK3* group.

Immunohistochemistry

There was variable expression of S100, SMA, CD34, and CD30 across all fusion types, with no consistent immunophenotype with these antibodies (Table 4 and Figs. 2–4). Expression of panTRK in a cytoplasmic (*NTRK1/2*) or nuclear +/−cytoplasmic pattern (*NTRK3*) was seen in 100% cases evaluated (n = 22); NTRK1 antibody expression was positive in 90% of cases (Table 4, Figs. 2, 4); details of panTRK and NTRK1 immunohistochemistry expression and validation on these cases were previously published.[30]

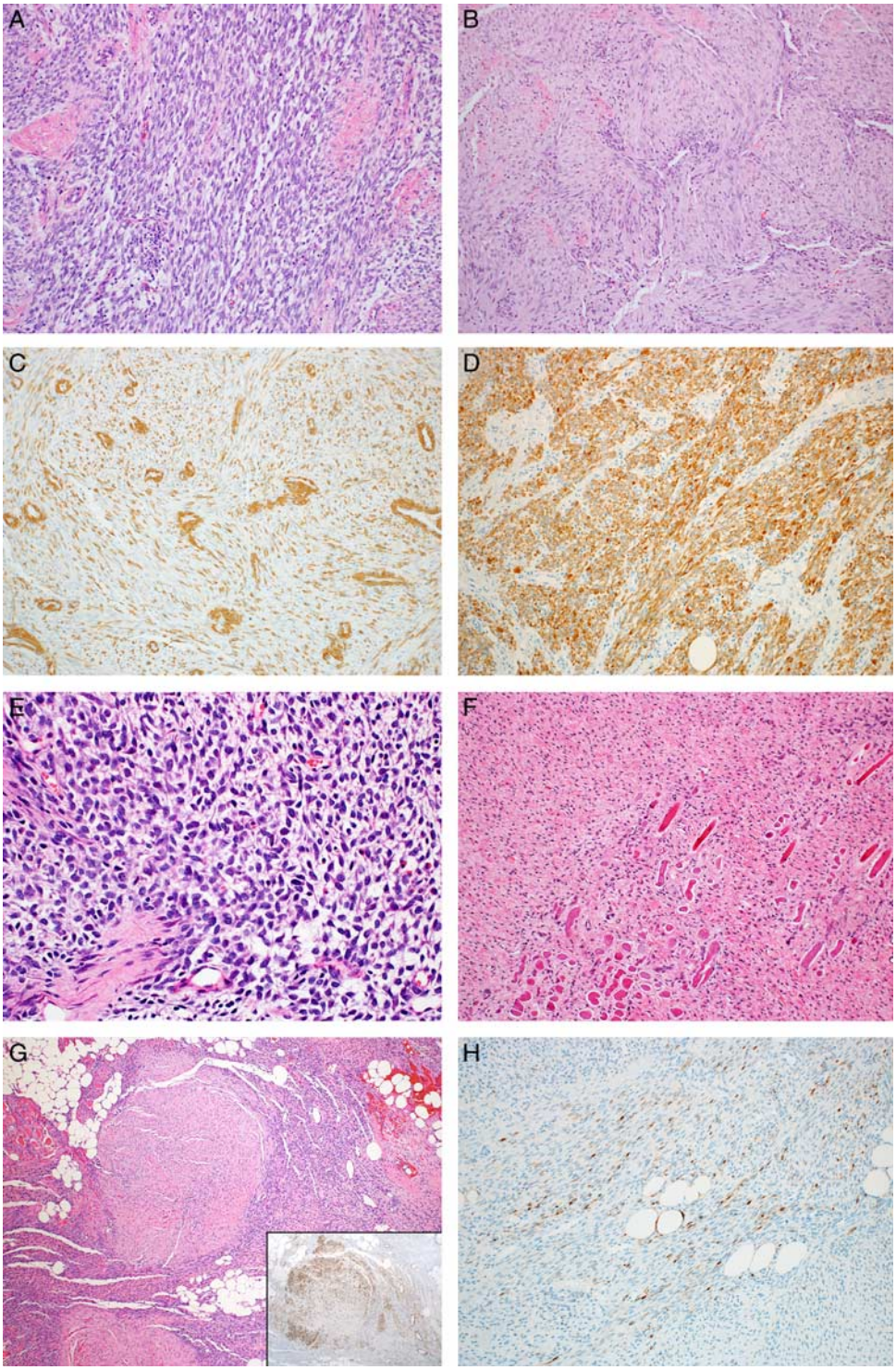

FIGURE 2. *NTRK1* fusion tumor examples. A–D, Patient 2, *TMP3-NTRK1*. A, Spindle cells arranged in fascicles. B, Prominent myoid features with HPC-like vasculature. C, Diffuse "checkboard" pattern of SMA expression. S100 and CD34 showed no expression. CD30 showed diffused staining (not shown). D, Diffuse strong cytoplasmic expression of panTrk antibody. E–H, Patient 15, *TPR-NTRK1*. E, Primitive cells in a myxoid matrix. F, Bland spindle cells with an infiltrative/fibromatosis-like pattern. G, Primitive cells juxtaposed to large myoid nodules. Inset—Patchy SMA immunohistochemical staining, most prominent in myoid nodules. H, S100 immunostain in area of most prominent staining (focally up to 5% of cells). CD34 was negative except in vessels in this case and CD30 showed patchy expression (not shown).

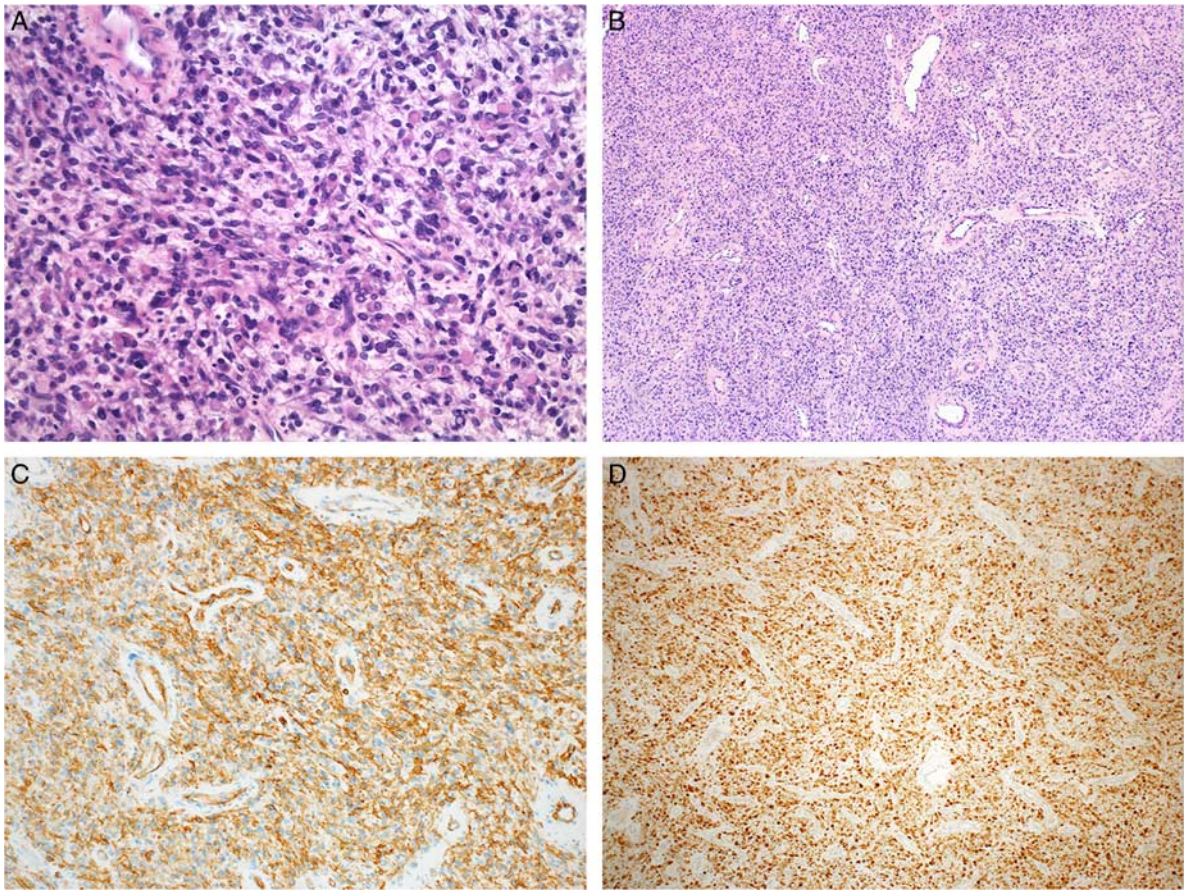

FIGURE 3. *NTRK2* fusion tumor. A–D, Patient 17, *STRN-NTRK2*. A, Primitive cells and scattered rhabdoid cells in a myxoid matrix. B, Myoid pattern with HPC-like vasculature. C, Strong diffuse CD34 immunostaining. D, Strong diffuse S100 immunostaining. SMA showed no expression and CD30 showed patchy immunostaining (not shown).

Clinical Outcomes

Data on clinical outcomes were available for 24 of 30 patients; with follow-up ranging from 6 months to 12 years (median: 2.5 y). No statistical significance in the rate of local recurrence or metastasis was observed between the classic and variant fusion groups ($P \geq 0.05$). Recurrence rates ranged from 11% (*ETV6*-positive) to 40% (*ETV6*-negative), with an overall rate of 29%. All patients with local recurrence, had positive margins on prior resection. Metastatic rates were 18% (*ETV6*-positive) to 25% (*ETV6*-negative), with an overall rate of 22% (Table 1). Of the 2 patients with metastatic *ETV6-NTRK3* fused tumors, 1 died of disease and 1 was lost to follow-up. One other patient with an *ETV6-NTRK3*-positive tumor died 2 weeks after diagnosis secondary to widespread local disease. Three patients with variant *NTRK* fusions had metastatic disease: 1 had multiple local recurrences with debulking surgery, chemotherapy and ultimately amputation, with subsequent development of lung metastases; the other 2 patients had metastatic disease at presentation. Treatment was similar between the 2 groups and included surgery, chemotherapy, or both; however, more of patients with variant fusions received targeted panTRK therapy in comparison to the *ETV6-NTRK3* cohort.[28] No correlation between risk of metastasis or recurrence was found with age, location, *NTRK* fusion partner, mitoses, or histologic pattern ($P > 0.05$ for all variables via logistic regression).

From the retrospective review of the data from the 2 primary institutions only, recurrence rates ranged from 13% (*ETV6*-positive) to 33% (*ETV6*-negative), with an overall rate of 24%. The metastatic rate ranged from 6% (*ETV6*-negative) to 20% (*ETV6*-positive), with an overall rate of 12%. No statistical significance in the rate of local recurrence or metastasis was observed between the classic and variant fusion groups ($P \geq 0.05$).

DISCUSSION

NTRK-rearrangements are being increasingly recognized in pediatric mesenchymal tumors, and favorable responses have been shown with the use of panTrk inhibitors for treating patients with locally advanced or metastatic disease.[28,31] However, studies detailing the clinicopathologic features of *NTRK*-rearranged soft tissue tumors in the pediatric population outside of classic IFS are limited to absent, and there is little to direct clinicians

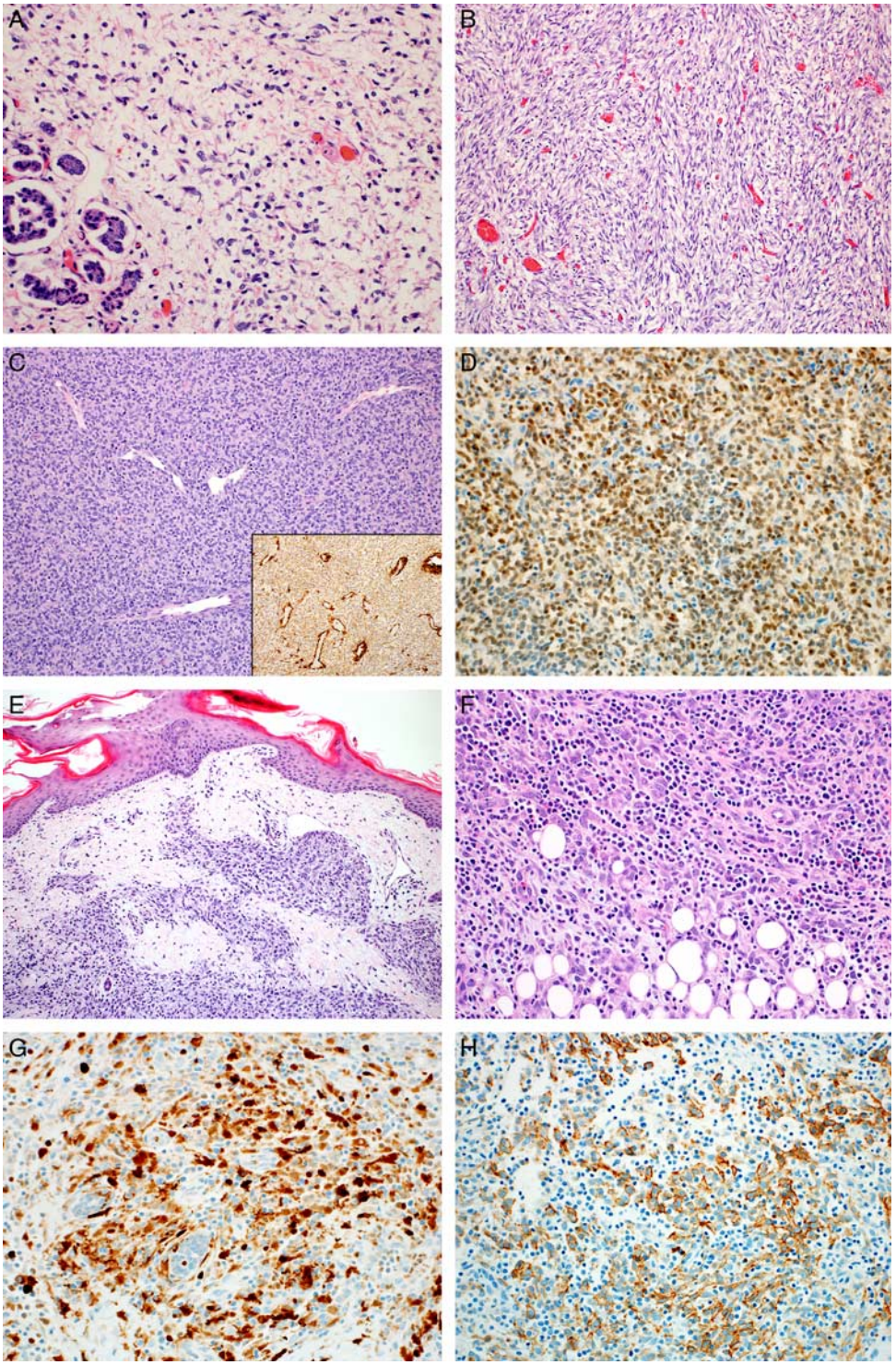

FIGURE 4. *ETV6-NTRK3* fusion tumor examples. A–D, Patient 20. A, Hypocellular primitive cells in myxoid stroma involving/invading the pancreas. B, Elongate spindle cells in fascicles. C, Hypercellular primitive cells with HPC-like vasculature. Inset—diffuse SMA immunoexpression. No immunoexpression of S100 or CD34 was present; diffuse weak expression of CD30 was present (not shown). D, Diffuse nuclear panTrk immunohistochemical staining. E–H, Patient 21. Superficial tumor of the hand. E, Primitive cells in myxoid stroma in nodules within the dermis. F, More plump to pleomorphic cells with marked mixed inflammation (IMT-like pattern). G, S100 immunostain with ~25% to 50% of cells staining. H, CD30 showed diffuse staining. SMA was negative and CD34 was patchy 5% to 25% of cells (not shown).

TABLE 4. Immunohistochemical Staining Pattern

	Fusion (#Tumors Positive*/#Tumors Tested)		
Antibody	***ETV6-NTRK3***	**Variant *NTRK* Fusions**	***P***
panTrk	7/7	14/14	1.0
TrkA	3/5	13/13	0.0654
S100	3/10	9/16	0.248
SMA	5/10	10/16	0.689
CD34	3/8	10/15	0.221
CD30	4/7	9/12	0.6

*Positive = minimum of 2+ staining (5% to 25% of cells) with moderate intensity.

and pathologists to the early recognition of these cases. In this study, we provide the largest and most detailed clinicopathologic description of pediatric soft tissue tumors harboring variant *NTRK* fusions and compare this group with a synchronous cohort of patients with classic IFS harboring the canonical *ETV6-NTRK3* fusion. Our data demonstrate that this subset of pediatric *NTRK*-rearranged mesenchymal tumors share similar clinicopathologic features and morphology analogous to early descriptions of IFS, recognition of which may aid in early diagnosis and treatment.

In our population, both classic and variant *NTRK* fusion tumors occurred most commonly in children of age 2 years and below but extended into the adolescent age group as well. Both groups of tumors occurred in similar locations, most commonly the extremities and trunk but also in the viscera. In a limited number of cases but across both fusion cohorts, nonrandom chromosomal gains were observed, as historically utilized as a diagnostic aid for IFS.[7–9]

Classic and variant *NTRK*-rearranged mesenchymal tumors harbor a recognizable morphologic pattern, with nearly all tumors (93%) demonstrating haphazardly arranged primitive cells in a variably myxoid stroma and/or spindled cells arranged in fascicles (dominant features). Often, these features were seen in conjunction with one or multiple secondary morphologic patterns such as HPC-like vessels, infiltrative growth, myoid appearance, and IMT-like areas. Tumoral heterogeneity itself was a useful diagnostic finding, as 90% of cases demonstrated multiple histologic patterns. The few that did not were small biopsy samples; suggesting this feature requires adequate sampling and may not be detected with needle core biopsies (Figs. 2–4). In these instances, recognition of a secondary morphologic pattern (such as a myoid or IMT-like appearance) may aid in diagnosis.

Common IHC stains for cellular differentiation are of limited utility for pediatric *NTRK*-rearranged mesenchymal tumors. Specifically, 50% of cases expressed S100, SMA, and/or CD34 which may lead to initial diagnosis of a neural tumor (S100 and SOX10-positive), myofibroma (tosis) (SMA-positive; *PDGFRB* mutations), or dermatofibrosarcoma protuberans ($CD34^+$; *COL1A1-PDGFB* fusions) in a subset of cases. The only consistent IHC antibody expressed in all *NTRK*-rearranged tumors was panTRK,[30] and thus this may be a useful diagnostic adjunct.[30,32] Fusion subtypes showed distinctive TRK IHC staining patterns, with *NTRK1/2* fusions demonstrating cytoplasmic staining and *NTRK3* fusions nuclear +/− weak cytoplasmic staining,[22,30,32,33] which may help direct subsequent molecular testing.

While traditionally *ETV6*-based tests have been considered the first line molecular testing methodology for these tumors, this testing methodology will fail to detect variant *NTRK* fusions and may fail significant number of cases with a classic *ETV6-NTRK3* rearrangements (36% in this series). Therefore, a negative result by FISH/PCR may require additional testing, and the panTRK immunostaining pattern may guide both test selection and interpretation of molecular analysis.

These clinicopathologic features suggest that, at least in pediatric patients, tumor harboring classic or variant *NTRK* fusions are more similar than not. Some features were more typical of classic *ETV6-NTRK3* fusion tumors, such as IMT-like morphology, high mitotic activity and trisomy 11. However, classic tumors also often lacked any or all of these features and were indistinguishable from variant *NTRK* fusion tumors. Combined with the overlapping clinical features, our data suggest that mesenchymal tumors harboring classic or variant *NTRK* fusions are related entities. Further study is needed to determine whether there is a difference in prognosis between these various groups, and, if different, whether this stems from tumor size, location (superficial vs. deep vs. visceral), patient age, and/or underlying tumor biology.

A limitation of our study is the lack of representation of adult patients. Previous reports of mesenchymal tumors with variant *NTRK* fusion have also shown heterogeneous but overlapping morphologies with IFS, but with various diagnostic interpretations. Haller et al evaluated 4 sarcomas (2 pediatric and 2 adult) with *NTRK1* fusions and containing myopericytic-like areas[18] and Chiang and colleagues described 4 young adult women with novel uterine *NTRK*-rearranged sarcomas (3 *NTRK1* and 1 *NTRK3* fusions) with features of fibrosarcoma.[22] Agaram and colleagues describe the largest prior series of 11 (6 pediatric and 5 adult) patients with superficial, subcutaneous masses described as Lipofibromatosis-like and harboring frequent *NTRK1* gene rearrangments.[19] It is unclear if all these descriptions should be considered separate entities, but all of these reports include many of the morphologic findings detailed in this series and original descriptions of IFS.[2,6,18,19,22,24] Further research is needed to evaluate the pathologic relationship (if any) between those *NTRK*-rearranged mesenchymal tumors in the adult versus the pediatric populations.[18,19,22] As well as on whether tumor site (eg, superficial vs. deep) influences the histologic appearance.

Additional study on the clinical outcome and prognosis of these various cohorts of patients with *NTRK*-rearranged mesenchymal tumor is also needed. A recent study of 54 patents with morphologically defined IFS (a subset of 13 cases had *ETV6* FISH performed; 9 cases demonstrated *ETV6* gene rearrangements)[23] demonstrated an 89% 5-year

overall survival and an 81% event-free survival rate in patients presenting with localized disease, with the majority of patients receiving neoadjuvant chemotherapy and surgery. Multiple case reports and small case series of metastatic *NTRK*-rearranged mesenchymal tumors have also been published[16,18,21,34]; however, no large series with clinical outcome data has examined the natural history of these tumors with concurrent evaluation of genetic findings. Outside of the current study, the 11 subcutaneous lipofibromatous-like neural tumors with confirmed *NTRK1* rearrangements represent the largest reported series with clinical follow-up. These superficial tumors demonstrated a ~40% recurrence rate with infrequent (~8%) metastasis.[19] In contrast, 1 of 4 patients with *NTRK*-rearranged uterine sarcomas with features of fibrosarcoma died of disease.[22] In our series, there was an overall recurrence rate of ~29% and metastatic rate of 22% (including classic and variant *NTRK* fusions).

In addition to *NTRK1* mesenchymal tumors reported in various locations; *NTRK3* tumors have also been described in other mesenchymal tumors, particularly IMT.[35–37] Rare IMT have been reported with confirmed *ETV6-NTRK3* fusions; however, many of these cases were noted to share morphologic overlap with IFS including two cases having an initial diagnosis of IFS.[35–37] In addition, one of these cases occurred in the uterus and lacked any muscle or myofibroblastic differentiation via IHC[37]; raising the possibility of *NTRK*-rearranged uterine sarcoma. In these reports, a diagnosis of IMT was rendered based on older patient age, visceral location, and presence of admixed plasma cells. Given that IFS and IMT can occur along a spectrum of ages, locations, and have significant morphologic overlap, these tumor may be better considered as IFS. In addition, this highlights the biologic overlap between these 2 tumor types, with shared oncogenesis secondary to relatively homologous tyrosine kinase fusions seen in both IFS and IMT. Regardless, given that both IFS and IMT have intermediate malignant potential, the putative clinicopathologic diagnosis may be of lesser importance than recognition of general morphologic pattern and association with *NTRK* fusions.

With regard to treatment, even amongst patients with classic IFS, management is variable.[38] Some patients with tumors amenable to primary surgical resection may never require additional therapy.[38] However, tumors with both classic and variant *NTRK* fusions do harbor a low rate of metastasis, and many patients experience significant morbidity, with 2 of our patients requiring amputation (Table 1). With the advent of TRK targeted therapy, many of the recently diagnosed patients in our study were enrolled on pan-TRK inhibitor (larotrectinib) clinical trials,[28,31] including 1 patient with a classic *ETV6-NTRK3* fusion and 9 of the patients with variant *NTRK* fusions (Table 1). Given the published 93% objective response to larotrectinib[28,31] this may have altered the natural history of this disease. A significant limitation of our study is therefore the unbalanced enrollment of the patients with variant *NTRK* fusions treated with targeted therapy, which may favorably bias the outcome data of the variant *NTRK* fusion tumors. In the *ETV6-NTRK3* cohort, largely comprised of patients treated before the advent of TRK targeted therapy, 2 of 10 patients with clinical follow-up died of disease, similar to the mortality rate reported in other series.[2–5,22]

In conclusion, our data expands the spectrum of pediatric *NTRK*-rearranged mesenchymal tumors and provides the largest clinicopathologic evaluation of molecularly confirmed pediatric *NTRK* tumors to date. In our population of pediatric patients, the morphologic features of the tumors with both classic and variant *NTRK* rearrangements resembled early descriptions of IFS. While age is a barrier for diagnosis of IFS outside of the pediatric population, this study suggests that "infantile fibrosarcoma" may occur outside of infancy. Regardless of fusion type, tumors can be recognized morphologically and confirmed by pan-TRK immunohistochemistry.[30] Molecular or cytogenetic confirmation of *NTRK* rearrangements may remain a criterion for drug eligibility; however, the clinical, pathologic, and genetic features described here may expedite diagnosis of these tumors and guide directed molecular testing.

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