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Infantile *NTRK*-associated Mesenchymal Tumors

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Abstract

Pediatric fibroblastic/myofibroblastic lesions are a relatively common group of tumors with varying morphologies, for which the molecular mechanisms are becoming increasingly well characterized. Congenital infantile fibrosarcoma (CIFS), perhaps the most well studied of these lesions is characterized by a recurrent *ETV6-NTRK3* gene fusion. However, a notable subset of locally aggressive congenital/infantile soft tissue lesions with similar morphologic features to CIFS, have not to-date, shown evidence of any canonical molecular aberration. We describe 6 patients with mesenchymal tumors composed of infiltrative fibroblastic/myofibroblastic tumor cells and showing a morphologic spectrum of features much analogous to that previously described in CIFS but without *ETV6* fusion transcripts. These tumors lacked a uniform immunoprofile, but showed variable expression of CD34, S100, smooth muscle actin, and CD30. All patients first developed a mass in infancy ($\leq$2 months of age). Using next-generation DNA sequencing, *TMP3-NTRK1* fusions were identified in 4 cases, an *LMNA-NTRK1* fusion in one case, and a variant *EML4-NTRK3* fusion in one case. Similar to infantile fibrosarcoma, these tumors were locally aggressive (with local recurrences if incompletely excised) and rarely metastasized (lung metastases in one patient). Proper identification of these tumors including investigation for *NTRK* family gene rearrangements is essential for diagnostic accuracy, as well as for clinical management decisions. Given the morbidity associated with radical resection of large soft tissue tumors, children with unresectable, recurrent, and/or metastatic disease may benefit from treatment with *NTRK* targeted therapies.

Keywords

infantile, fibrosarcoma, *NTRK1*, *NTRK3*, TRK, soft tissue tumor

Introduction

Infantile fibroblastic and myofibroblastic tumors, comprising a spectrum of diagnostic entities, are the second most common group of soft tissue neoplasms to occur in the first year of life.[1–4] In infancy, the majority of fibroblastic/myofibroblastic neoplasms are either benign or intermediate/low-grade malignant, with congenital infantile fibrosarcoma (CIFS) as the primary malignant concern.[1,2,4,5] CIFS is a locally aggressive tumor, occurring almost exclusively in children under the age of 2 years.[1,6,7] Morphologic overlap can exist between CIFS and a spectrum of benign or malignant infantile lesions, including myofibroma/myofibromatosis, lipofibromatosis and fibrous hamartoma of infancy, primitive myxoid mesenchymal tumor of infancy (PMMTI), and dermatofibrosarcoma protuberans (DFSP).[6,8–13] Molecular tests are useful in confirming the diagnosis of CIFS, particularly in morphologically challenging cases, as CIFS is characterized cytogenetically by non-random gains of chromosome 8, 11, 17, and 20, and more specifically by an *ETV6-NTRK3* gene fusion.[6,14–17] However, a subset of tumors occurring in infants may resemble CIFS, including shared

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non-random chromosomal gains, but lack the canonical *ETV6-NTRK3* fusion.

Herein, we describe a series of 6 infants who presented with locally aggressive soft tissue neoplasms demonstrating a spectrum of morphologic features reminiscent of those seen in CIFS but lacking *ETV6-NTRK3* fusions. In contrast, these tumors were found to have variant *NTRK1* or *NTRK3* gene fusions.

Materials and Methods

Since 2011, 6 patients (5 prospectively, 1 retrospectively) were identified at 2 institutions (SCH, n = 5 and UCSF, n = 1), with low-grade spindle cell tumors lacking evidence of *ETV6* fusion by fluorescence in situ hybridization (FISH). Hematoxylin and eosin (H&E) and immunohistochemical (IHC) stains performed at the time of diagnosis were reviewed, and these results were supplemented with additional IHC stains (see below). Clinical and follow-up data were obtained from the electronic medical record. The study was approved by the institutional review board.

Immunohistochemistry

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: 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-neurofilament (M0726, 1:400 dilution; Dako), mouse monoclonal anti-smooth muscle actin (SMA) antibody (M085101, 1:200; Dako), mouse monoclonal anti-muscle specific actin (HHF-35) antibody (M635, 1:400 dilution; Dako), mouse monoclonal anti-myogenin antibody (M-3559, 1:800; Dako), mouse monoclonal anti-desmin antibody (M0760, 1:800 dilution; Dako), mouse monoclonal anti-EMA antibody (247M-96, 1:100; Cell Marque), mouse monoclonal anti-CK7 antibody (M7018, 1:800 dilution; Dako), mouse monoclonal anti-CD99 (O13) antibody (SIG620-1000, 1:200 dilution; Covance), and mouse monoclonal anti-CD30 antibody (13M-96, 1:50 dilution; Cell Marque). Appropriate positive and negative controls were used for each antibody.

Electron Microscopy

For the initial biopsy material from case 2, multiple 1–2 mm fragments of tumor tissue were fixed in glutaraldehyde and embedded in resin for possible future examination. This material was retrieved to complete the ultrastructural examination.

Cytogenetics/Karyotype

Giemsa-trypsin-wright(GTW)-banded metaphase cells from 2 unstimulated cultures of tumor specimens (collagenase-disaggregated; harvested between days 6 and 10) were analyzed.

Fluorescence In Situ Hybridization

Interphase FISH was performed on touch imprint slides or unstained FFPE tissue sections using dual-color, break-apart probes for the *ETV6* gene region [ETV6 (TEL) (12p13), Vysis, Inc., cat. #07J77-001]. Interphase FISH to determine if there was an SS18 gene rearrangement and/or aneuploidy for chromosomes 8, 11, 17, and 20 was also performed on case 2 using the following probes: SS18 (18q11.2), Vysis Inc., chromosome 8 centromere (D8Z2), Cytocell Ltd, cat #LPE008G; MLL (11q23), Vysis Inc, cat #30-190083; chromosome 17 centromere (D17Z1), Vysis Inc, cat #32-131017; SYT (SS18, 18q11.2), Vysis Inc, cat #32-231018; and PTPRT/MYBL2 (20q12/20q13.12), Cytocell Ltd, cat #LPH020.

DNA Sequencing With Fusion Detection

UW-OncoPlex is a validated, clinical molecular diagnostic assay that can detect all classes of mutations as previously described.[18,19] Briefly, H&E-stained slides were used to direct tumor tissue microdissection from formalin-fixed, paraffin-embedded blocks. A minimum of 200 ng of genomic DNA was extracted, fragmented (Covaris, Woburn, MA), and used to prepare sequencing libraries (KAPA Hyper Prep, Kapa Biosystems, Wilmington, MA). Following custom hybrid capture (SureSelect; Agilent Technologies, Santa Clara, CA), targeted next-generation sequencing (NGS) (NetSeq; Illumina, San Diego, CA) was performed to yield >500× average coverage in 262 clinically relevant genes. A custom bioinformatics pipeline was used as previously described.[19] Structural rearrangements, including gene fusions, were identified using PINDEL version 0.2.5,[20] CREST version 1.0.1,[21] and BreakDancer version 1.4.4.[22]

Results

Clinical Characteristics

Patient 1 presented at 2 months of age with an enlarging 4 cm mass in the forearm (Table 1). The tumor was diagnosed as CIFS, although it lacked evidence of *ETV6* rearrangement by FISH. The patient was treated with

Table 1. Clinicopathologic Features.

Case #	Age at presentation/ Sex	Location	Size (cm)	Mitotic rate/10 HPF		Genetic abnormality	Follow-up			
				Primary	Recurrence		LR	Distant metastasis	Duration (months)	Status
1	2 mo/M	Forearm	4	3	6	*TPM3-NTRK1*	X1		30	NED
2	birth/M	Lower leg	6.3	2	7	*TPM3-NTRK1*	X2		63	AWD
3	2 mo/F	Back	2.6	<1	N/A	*LMNA-NTRK1*			50	NED
4	2 wks/M	Axilla	5	N/A	15	*EML4-NTRK3*			35	NED
5	birth/M	Foot	N/A	<1	10	*TPM3-NTRK1*		Lung	64	AWD
6	2 mo/M	Foot	1.7	3	N/A	*TPM3-NTRK1*			1	AWD

Note: AWD, alive with disease; cm, centimeters; F, female; HPF, high power fields; LR, local recurrence (number of); M, male; mo, months; N/A, not available; NED, no evidence of disease; wk, weeks.

9 weeks of neoadjuvant chemotherapy including vincristine and dactinomycin.[23] He then underwent a gross total surgical resection, with positive margins, but received no further chemotherapy. The tumor recurred 7 months later and a second gross total resection was performed, again with positive margins, after which he was managed with active surveillance. During this process, NGS sequencing was performed to inform future treatment options; however, he has received no further chemotherapy and remains disease free 27 months following his initial diagnosis.

Patient 2 presented at birth with a 6.3-cm mass involving the lower leg. The tumor was low grade, with a differential diagnosis of myofibromatosis versus CIFS. Again, the tumor lacked evidence of *ETV6* rearrangement. The patient was treated with neoadjuvant vinblastine and methotrexate for 1 year[24] with no significant change in the size of the mass. Ten months after completion of chemotherapy, a new enhancing lesion adjacent to the original tumor developed. The mass was surgically resected with positive margins, but he received no additional chemotherapy at that time. Twenty months later, the tumor again increased in size. Given the extensive involvement of the neurovascular bundle, a subtotal resection was performed. He was treated with sulindac for approximately 6 months with mild symptomatic improvement but no change on imaging. NGS testing was performed to inform possible treatment options, and subsequently he enrolled on a clinical trial investigating a pan-NTRK inhibitor.

Patient 3 presented at 2 months of age with a superficial mass on the lower back. A punch biopsy of skin showed a low-grade spindle cell lesion involving the dermis and subcutaneous tissues, which was subsequently widely resected. On resection, the lesion was diagnosed as a myxoid DFSP with negative margins. She has had no recurrence of disease and is now more than 6 years after definitive resection.

Patient 4 presented 2 weeks of age with a rapidly enlarging right axillary mass. An MRI at 8 months showed a 5-cm heterogeneous lesion thought to be compatible with a hemangioma. The patient underwent surgical resection at age 9 months with negative margins. The pathology specimen showed an unclassified spindle and round cell sarcoma, with the leading differential diagnosis including CIFS and PMMTI; however, the tumor lacked an *ETV6* rearrangement. No systemic therapy was given. The patient has had regular surveillance with no evidence of recurrence or metastases at 21 months post resection.

Patient 5 presented at birth with a right foot mass, and foot asymmetry had been detected on prenatal ultrasounds. The mass was biopsied at 9 days of age and showed a low-grade spindle cell lesion with a differential diagnosis of fibromatosis versus CIFS, but which lacked evidence of an *ETV6* rearrangement. At 3 years of age, he became symptomatic and a needle biopsy demonstrated tumor progression to a high-grade spindle cell sarcoma. He had no metastatic disease and was treated with 2 cycles of ifosfamide/doxorubicin followed by 2 cycles of ifosfamide alone. Subsequent imaging revealed local tumor progression and a right foot amputation was performed. Follow-up imaging 1 year later demonstrated multiple metastatic lung nodules, confirmed by biopsy. The original pathology was reviewed, and given the morphologic similarity to prior cases, NGS testing was performed. He is enrolled on a clinical trial investigating a pan-NTRK inhibitor.

Patient 6 developed a left heel mass at 2 months of age which enlarged rapidly. At 4 months of age, the lesion was biopsied. The tumor was diagnosed as a low-grade spindle cell lesion and, given the histologic similarity to the prior cases, tissue was sent for further molecular testing. Given the tumor proximity to the calcaneus and Achilles tendon, gross total resection was considered too morbid. Instead, he was enrolled on a clinical trial of a pan-NTRK inhibitor.

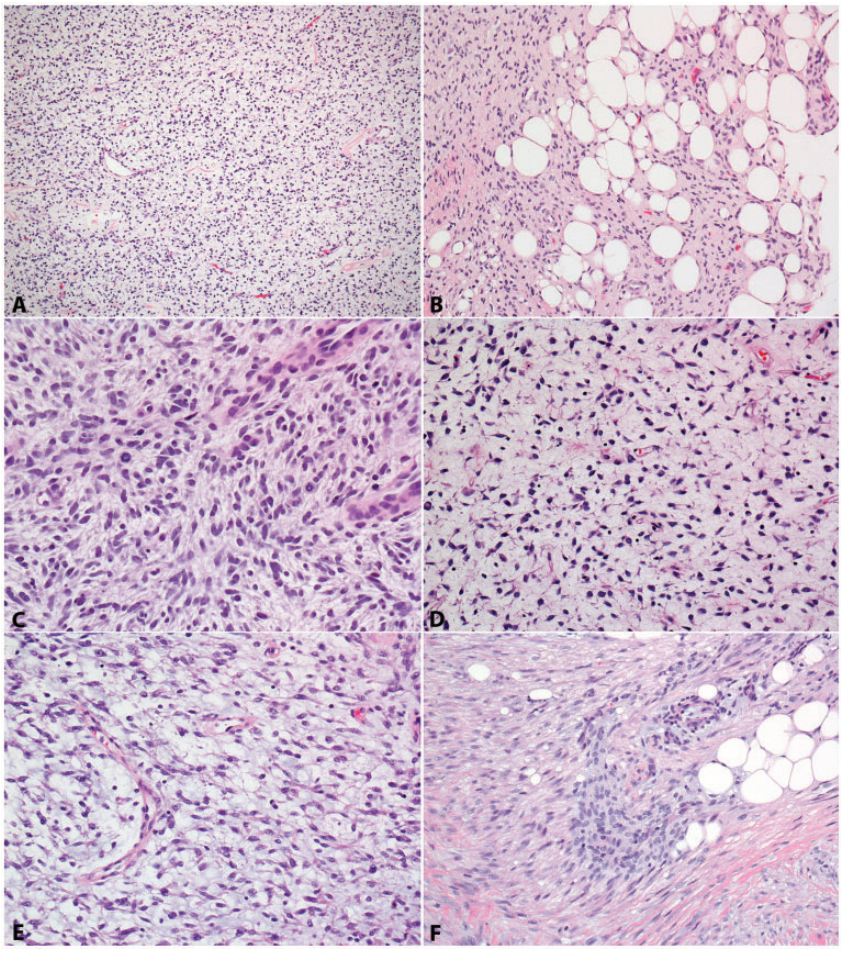

Figure 1. Morphologic appearance of tumors. All tumors showed areas resembling primitive mesenchyme with sheets of small ovoid cells with scant cytoplasm present within a myxoid matrix and variable areas with a fibroblastic appearance. (A) Patient 1, *TMP3-NTRK1* (H&E, 100×), (B) Patient 2, *TMP3-NTRK1* (H&E, 200×), (C) Patient 3, *LMNA-NTRK1* (H&E, 400×), (D) Patient 4, *EML4-NTRK3* (H&E, 400×), (E) Patient 5, *TMP3-NTRK1* (H&E, 400×), and (F) Patient 6, *TMP3-NTRK1* (H&E, 200×).

Microscopic Features and Immunohistochemistry

All 6 patients showed a low-grade spindle cell composed of a highly infiltrative, cellular, ovoid to spindled neoplasm. The 5 deep tumors showed extensive infiltration of both fat and skeletal muscle (Figure 1(A), (B), (D) to (F)). The neoplastic cells were occasionally arranged in sheets or broad fascicles, with a myxoid to collagenous stroma (Figure 2(B) to (C)). Areas resembling primitive mesenchymal cells with small ovoid cells with scant amphophilic cytoplasm were present in all 6 patients (Figure 1). The one superficial neoplasm (patient 3) involved subcutaneous adipose tissue with "honeycomb/pearls on a string" fat trapping (Figure 1(C)) with weak CD34 staining, raising the possibility of a myxoid DFSP. In all cases, individual cells were ovoid to spindled with vesicular nuclei and indistinct nucleoli. Mitoses on the original biopsies were low, ranging <1 to 3 per 10 high power fields; however, the recurrences had increased mitotic activity ranging from 6 to 15 per 10 high power fields (Table 1). No necrosis was seen.

There was moderate variation in other histologic features seen in these cases (Figure 2). Three cases showed myoid/myofibroblastic differentiation with abundant eosinophilic cytoplasm (Figure 2(A)). In 3 cases (all *TPM3-NTRK1* fusions), these regions surrounded hypercellular islands of smaller cells having indistinct, amphophilic cytoplasm and a variably myxoid background creating a superficial resemblance to fibrous hamartoma of infancy (Figure 2(B) to (C)). Three cases showed prominent hemangiopericytomatous vasculature (Figure 2(B) and (C)), and one case showed focally prominent nuclear palisading (Figure 2(D)).

In 2 cases (patients 2 and 5), the tumor morphology changed on recurrence, with increased pleomorphism and cytologic atypia similar to that which has been described in recurrent/residual infantile fibrosarcoma. In these

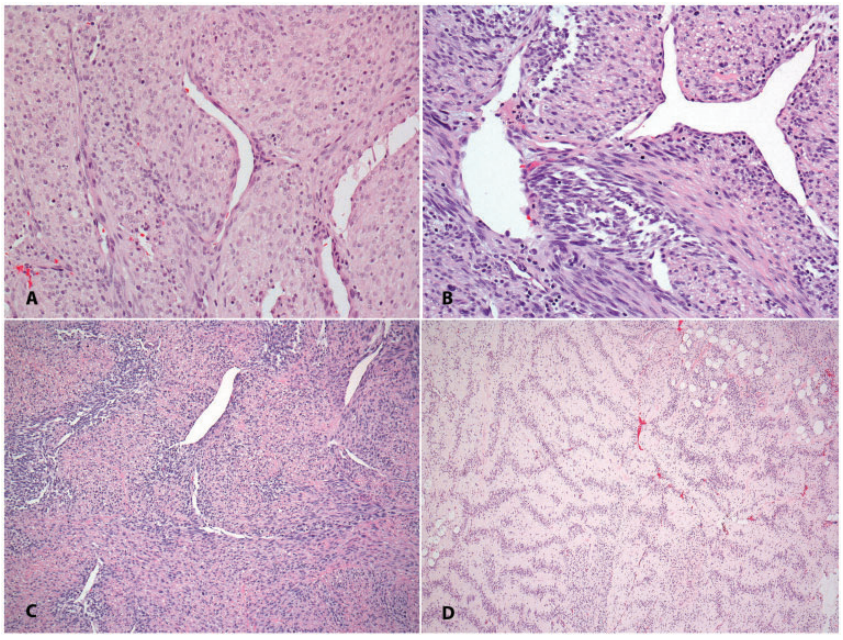

Figure 2. Variant morphologies included myoid/myofibroblastic features (A, patient 2) (H&E, 200×) with hemangiopericytomatous vasculature (B, patient 5) (H&E, 200×). This was often accompanied by hypercellular nodules of ovoid cells giving a biphasic appearance from low magnification (C-patient 5) (H&E, 100×). Rare cases showed nuclear palisading (D – patient 1) (H&E, 40×).

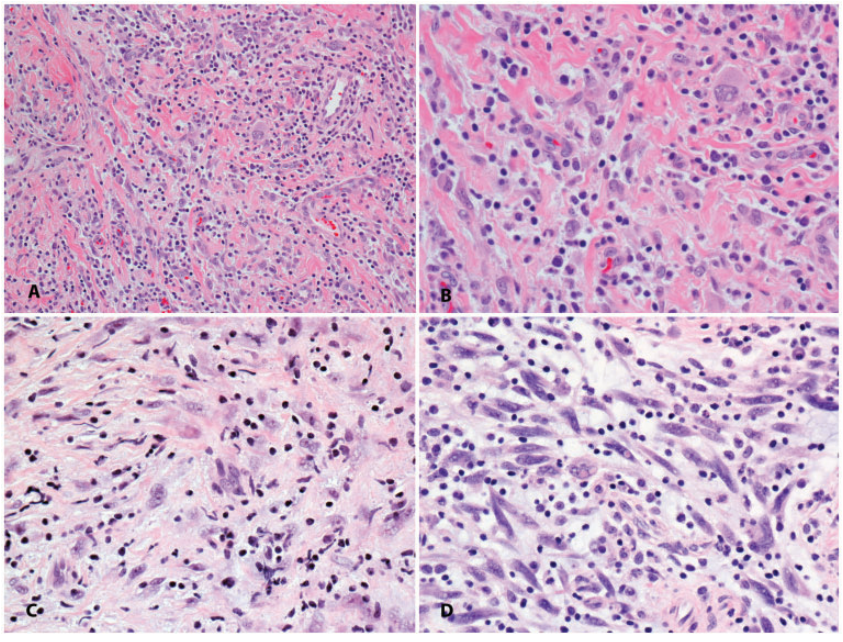

Figure 3. Two tumors showed morphologic transformation following chemotherapy. Patient 2 (A and B) and Patient 5 (C and D) showed sheets of dyshesive, epithelioid cells with abundant admixed inflammatory cells and large, pleomorphic nuclei with scattered nuclear pseudoinclusions (A, 200×; B to D, 400×).

specimens, both post chemotherapy, the cells became dyshesive and epithelioid with hyperchromatic nuclei showing scattered nuclear pseudoinclusions but inconspicuous nucleoli (Figure 3). There were abundant admixed inflammatory cells including lymphocytes and eosinophils, with resemblance to inflammatory myofibroblastic tumor (IMT) on these recurrence specimens (Figure 3).

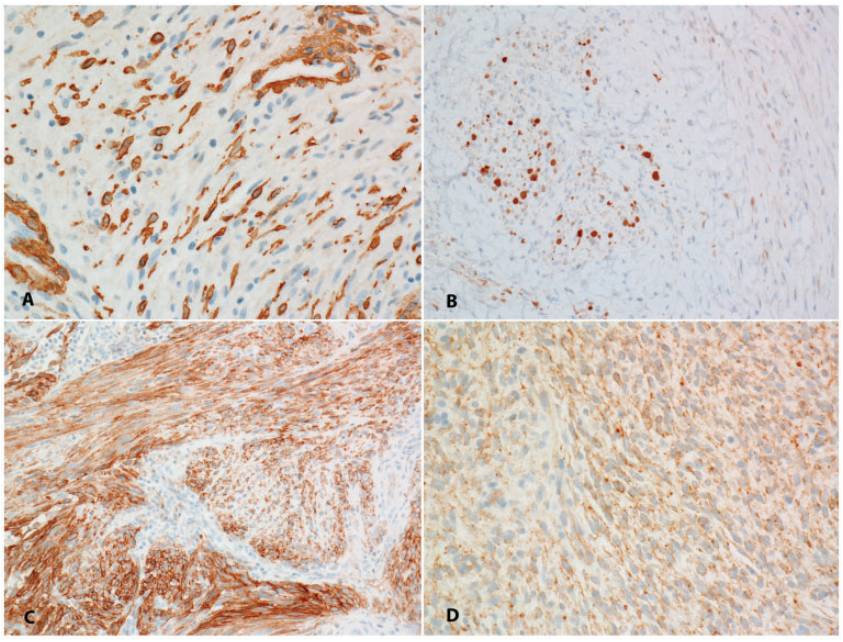

Figure 4. The immunohistochemical profile of these tumors was not uniform, but included variable expression of (A) smooth muscle actin (400×), (B) S100; image depicts the greatest degree (intensity and percent of cells) of all tumors examined (200×), (C) CD30 (100×) and CD34 (400×).

The IHC profile was non-specific but analogous to that seen in CIFS. CD34 and CD30 were the most commonly expressed antibodies; variable expression of SMA and S100 was also observed (Figure 4, Table 2). No cases had strong diffuse staining for S100; the most prominent S100 expression observed was focal and patchy (Figure 4(B)). All cases showed staining for NTRK1 (Figure 5, Table 2). The 4 patients with *TPM3-NTRK1* fusions showed strong, diffuse cytoplasmic staining, while the pattern associated with other fusions was more variable (Table 2). Upon local recurrence, patient 1 showed gain of S100 expression and patient 2 demonstrated new CD34 expression (Table 2); the meaning of these findings is unclear on this limited sample. NTRK1 expression remained unchanged in recurrence and metastasis.

G-Banded Chromosome Results

Patient 1: 52,XY,+2,+2,+8,+12,+17,+20[3]/46,XY[17]. An abnormal hyperdiploid clone was present. Seventeen cells exhibited a normal male karyotype. The remaining 3 cells displayed tetrasomy for chromosome 2 and trisomies for chromosomes 8, 12, 17, and 20.

Patient 2: The karyotype from the original biopsy was normal male, 46,XY[20]. The karyotype from the first tumor recurrence was 46,XY[19]. Nineteen cells exhibited a normal male karyotype, 46,XY. A single cell had 47 chromosomes, with the extra chromosome approximately the size of a C-group chromosome, could not be definitively identified due to poor morphology, but based on size and interphase FISH results, it was thought to represent a cell with trisomy 8 (see interphase FISH result below).

Patient 3: 46,XX[56]. This study was a limited sample due to poor growth in culture. Five of the 6 cells analyzed demonstrated a normal female karyotype. One of the 6 cells showed a non-clonal deletion of the long arm of one chromosome 9 at band q12, a finding seen in peripheral blood cultures attributable to an artifact of cell culture. One of the 6 cells also demonstrated a non-clonal deletion of the long arm of chromosome 1, with a breakpoint at band q11.

Patient 5: The karyotype from the initial biopsy was normal male, 46,XY[20].

Fluorescence In Situ Hybridization

Tumors from patients 1, 2, 4, 5, and 6 were negative for an *ETV6* (*TEL*) gene rearrangement by interphase FISH. Patient 1 demonstrated 3 copies of the ETV6 signal in 92% of the interphase nuclei, corresponding to trisomy 12 seen in the concurrent metaphase karyotype (ISCN: nuc ish(ETV6x3)[92/100]). The original biopsy from patient 2 showed no evidence of trisomy 8, 11, or 17. The first tumor recurrence from patient 2 demonstrated trisomy 8 in 66% of nuclei scored; it was negative for SS18 (SYT) gene rearrangement and there was no evidence of trisomies for chromosomes 11, 17, and 20; (ISCN: nuc ish (D8Z2x3,MLLx2) [66/100],(ETV6x2) [100], (D17Z1x2)[100],(SS18x2)[100],(PTPRT, MY

Table 2. Ancillary Studies.

	Case 1			Case 2			Case 3		Case 4	Case 5			Case 6
	Bx	Res	LR1	Bx	LR1	LR2	Bx	Res	Res	Bx	Res	Met	Bx
NTRK1	Pos (diffuse)	ND	Pos (diffuse)	Pos (diffuse)	Pos (diffuse)	ND	ND	Pos (weak, diffuse)	Pos (patchy)	ND	Pos (diffuse)	Pos (diffuse)	Pos (diffuse)
CD34	Neg	Pos (focal)	Pos (focal)	Neg	Neg	Pos (diffuse)	Pos (weak)	Pos (patchy)	Pos (focal)	ND	Neg	ND	Neg
S-100	Neg	Neg	Pos (rare)	Pos (rare)	Pos (patchy)	ND	Neg	Pos (patchy)	Neg	ND	Pos (patchy)	ND	Pos (patchy)
SMA	Pos (rare)	ND	ND	Pos (patchy)	Neg	Neg	Neg	ND	ND	ND	Pos (diffuse)	ND	Pos (patchy)
CD30	ND	Pos (patchy)	Pos (patchy)	Pos (diffuse)	ND	Pos (diffuse)	ND	Pos (patchy)	ND	ND	Pos (diffuse)	ND	Pos (patchy)
Karyotype	Trisomy 2, 8, 12, 17, 20	ND	ND	46,XY [20]	Trisomy 8	ND	ND	del1q11	ND	46,XY [20]	ND	ND	ND
ETV6	Neg	ND	ND	Neg	Neg	ND	ND	ND	Neg	Neg	Neg	ND	Failed

Note: Bx, biopsy; LR, local recurrence; Met, metastasis; ND, not done; Pos, positive; Res, resection; SMA, smooth muscle actin.

BL2x2)[100]). Given the superficial location and original diagnosis of DFSP, FISH for ETV6 was not performed in patient 3.

DNA Sequencing With Fusion Detection

Tumors from patients 1, 2, 5, and 6 demonstrated *TPM3-NTRK1* fusions by clinical NGS. The translocations joined the tropomyosin-3 gene with *NTRK1* exon 9, which is upstream of the kinase domain, and are predicted to maintain the reading frame (Figure 6). Tumor from patient 3 was positive for a *LMNA-NTRK1* translocation that joins the lamin-A gene with *NTRK1* exon 11, which is also upstream of the kinase domain, and the reading frame is predicted to be maintained (Figure 6). Tumor from patient 4 demonstrated an *EML4-NTRK3* fusion that joins the *EML4* gene with *NTRK3* exon 14, which is upstream of the kinase domain, and the reading frame is predicted to be maintained (Figure 6).

Electron Microscopy

Ultrastructural examination, on the initial biopsy from patient 2, showed cells with mitochondria admixed with abundant smooth and rough endoplasmic reticulum and fine, filamentous extracellular matrix material. The filamentous material lacked organization into identifiable actin or collagen fibrils. There were scattered cell processes and occasional cells showed microvesicles, but no neurosecretory granules were identified. The cells lacked cell junctions and basal lamina. Overall, the ultrastructural features were those of undifferentiated mesenchymal cells; no neural or other differentiation was identified.

Discussion

CIFS is a tumor of intermediate malignant potential characterized by presentation below the age of 2 to 5 years, often an initial period of rapid growth, local recurrence following incomplete resection (17% to 43%) and uncommon metastatic spread (1% to 13%). CIFS harbors a canonical t(12;15)(p13;q25) translocation encoding an *ETV6-NTRK3* gene fusion, which is detected in approximately 85% of tumors.[23] We report a series of 6 *NTRK*-associated mesenchymal tumors, all originally presenting in infants less than 6 months of age, but lacking *ETV6* rearrangements. These tumors appear to share many clinicopathologic features with CIFS, and recognition of variant *NTRK* fusions in these otherwise undifferentiated mesenchymal tumors is important for accurate diagnosis and treatment.

NTRK1 (1q21-q22) is one member of a family of genes, along with *NTRK2* (9q21-22) and *NTRK3* (15q25), which encode for a series of tropomyosin-

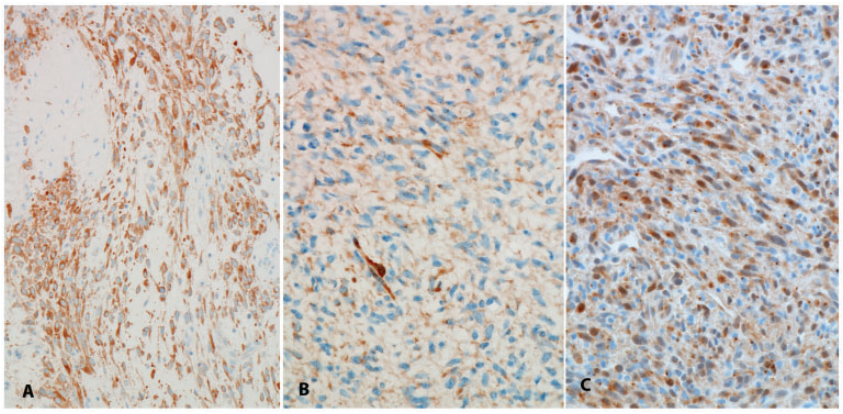

Figure 5. All tumors showed staining for NTRK1, although with a variable pattern including (A) strong diffuse cytoplasmic staining common to TMP3-NTRK1 fusions (Patient 2; 200×), (B) weak to moderate cytoplasmic staining (Patient 3; 400×) in the one LMNA-NTRK1 fusion, or (C) variable cytoplasmic with scattered perinuclear dot staining (Patient 4; 400×) showing cross reactivity in the EML4-NTRK3 fusion.

receptor kinases (TRKs).[25–27] These TRK proteins play an integral role in central and peripheral nervous system development by means of cell cycle regulation, cellular proliferation, and cell differentiation primarily through activation of the RAS-RAF-MAPK pathway. However, TRK receptors are also expressed on a host of other non-neuronal tissues.[25,26,28]

Gene fusions involving *NTRK* family members have been implicated as driver mutations in a variety of tumors, and tumors with *NTRK* gene fusions are heavily weighted towards pediatrics/young adults.[25,29–33] Over the past 3 decades, *NTRK* gene fusions have been identified in pediatric gliomas (*NTRK1*, *NTRK2*, *NTRK3*), papillary thyroid carcinoma (*NTRK1*), spitzoid neoplasms (*NTRK1*), and infantile fibrosarcoma (*NTRK3*) among others.[14,25,33–37] *NTRK1* fusions have only recently been described in soft tissue neoplasms/sarcomas.[38–42]

The first case of a spindle cell sarcoma with an *LMNA-NTRK1* gene fusion arising in an infant was reported by Wong et al.[38] This patient presented at 1 month of age, and ultimately developed lung metastases which responded to targeted therapy (Crizotinib). Subsequently, Haller et al.[39] described a series of 2 adult and 2 pediatric (less than 2 years of age) soft tissue sarcomas with a myopericytic/hemangiopericytic pattern and *NTRK1* rearrangements, including *LMNA-NTRK1* and *TPM3-NTRK1*. Doebele et al.[40] reported a similar tumor in an adult patient with lung metastases and confirmed an *LMNA-NTRK1* fusion. Variant *NTRK3* fusions have also rarely been reported in CIFS, including one report of a novel *EML4-NTRK3* fusion in a patient who developed lung metastases.[41] More recently, genomic profiling studies have found a variety of *NTRK* fusions in adolescent and pediatric tumors; however, the morphology and clinical course of these tumor are not described.[42] Although we also encountered one patient with metastatic disease, the number of metastatic tumors with *NTRK1* rearrangements may be over-represented in these early case reports given that NGS platforms are often offered only to patients who have failed prior therapy.

Finally, Agaram et al.[43] recently described a series of locally aggressive soft tissue tumors occurring in patients ranging 4 to 38 years in age, which demonstrated predominantly fusions of *LMNA-NTRK1*. The morphology of these tumors was described as lipofibromatosis-like, with extensive infiltration into fat, and they showed consistent strong, diffuse S100 expression.[43] Given the reported strong, diffuse expression of S100 and the role of the *NTRK* family of genes in neurodevelopment, the classification "lipofibromatosis-like neural tumor" was suggested.[43] It is not yet clear whether lipofibromatosis-like neural tumor represents a unique tumor type, with more diffuse S100 expression and perhaps with preferential occurrence in an older patient population than seen in our series of infantile *NTRK* tumors, or whether these tumors characterized by a superficial location and are simply more amenable to resection and therefore better outcomes.

Although all the cases of *NTRK*-associated soft tissue tumors reported in the literature share many histologic features, it has been challenging to draw definitive morphologic correlates from limited case series and case reports. Our series of 6 patients, all presenting at 2 months of age or under, demonstrate tumors with significant overlap in the morphologic spectrum of features seen in CIFS. The morphologic intratumoral heterogeneity in our series lead to initial differential diagnoses of CIFS, PPMTI, myofibromatosis, and DFSP, which is further complicated by the significant

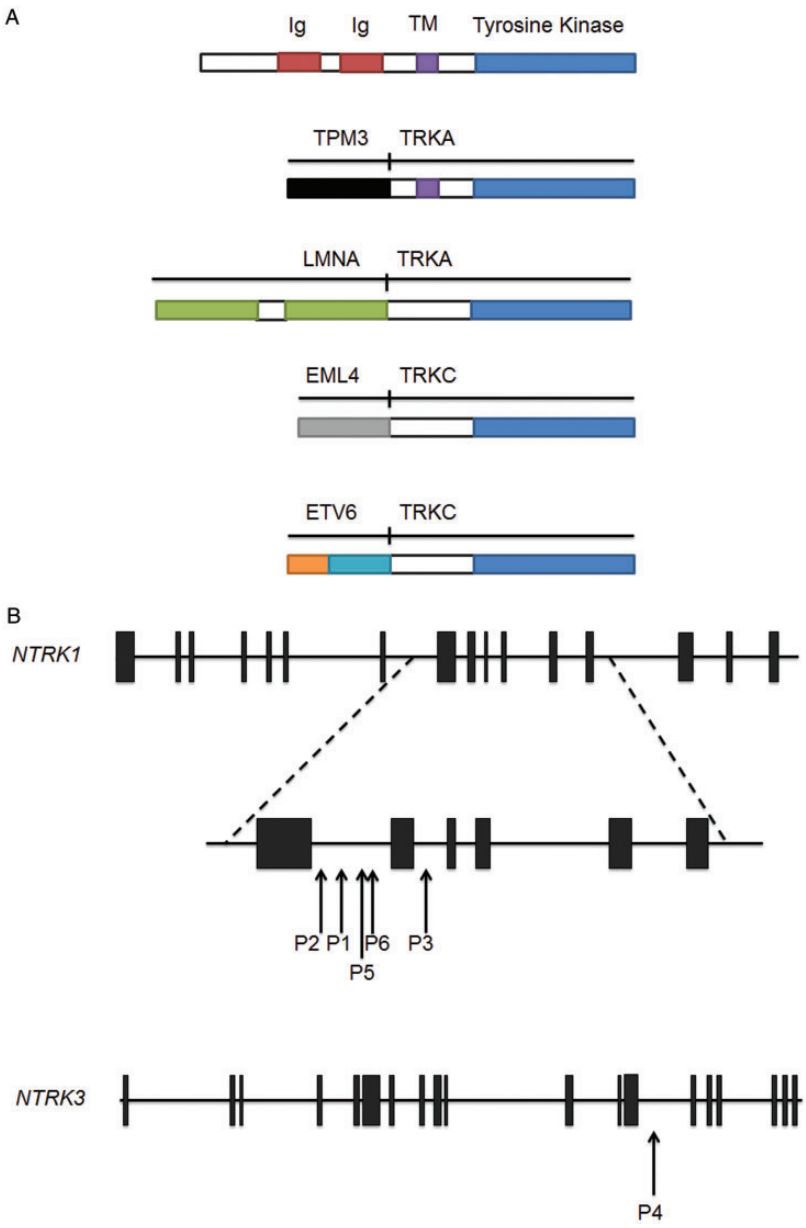

Figure 6. *NTRK* gene fusion partners. A, schematic of a generalized TRK protein, as well as TRKA and TRKC oncogenic protein fusions. Shown are the TPM3-TRKA fusion (patients 1, 2, 5, 6), the LMNA-TRKA fusion (patient 3), and the EML4-TRKC fusion (patient 4). The ETV6-TRKC fusion, classic to CIFS is shown for reference, although it was not present in this series. B, Genomic structure of the *NTRK1* and *NTRK3* genes with exons indicated by boxes. Each arrow represents a patient case with an *NTRK1* or *NTRK3* fusion.

histologic overlap which may be present between these diagnostic entities. For instance, an overlap between *ETV6-NTRK3* positive CIFS and infantile myofibromatosis has previously been described and it was hypothesized that CIFS has the potential to differentiate along a myopericytic lineage.[8] Additionally, focal Hemangiopericytoma(HPC)-like growth has been described in CIFS.[7,8] DFSP, characterized by recurrent *COL1A1-PDGFB* rearrangements, may show a variety of histologic subtypes, including myxoid and/or myoid differentiation.[13,44,45] The presence of CD34 positivity in an infiltrative, superficial spindle cell lesion should prompt consideration of this diagnosis, but the *NTRK*-associated tumors had a more fascicular and less storiform architecture overall with weak or patchy CD34 expression. DFSP is also distinctly uncommon under the age of 2 years. The presence of primitive ovoid cells in a markedly myxoid matrix may also raise the consideration, particularly in an infant, of PMMTI, characterized by internal tandem duplication repeats in *BCOR* or *YWHEA* gene rearrangements.[12,46] However, morphologically, the *NTRK*-associated tumors typically show more fascicular

growth and lack the chicken-wire type vascular pattern typical of PMMTI.

The most characteristic morphologic feature of these *NTRK*-associated mesenchymal tumors is a highly infiltrative, cellular proliferation of primitive ovoid cells, with a pale basophilic myxoid to eosinophilic collagenized stroma, much analogous to that seen in CIFS. Additionally, similar to CIFS, these variant *NTRK*-associated tumors show no consistent immunophenotype with variable expression of several proteins including CD34, S100, CD30, and SMA (Table 2), and by cytogenetics, there are non-random chromosomal gains.[6,7,11,15–17]

Overall, histologic identification of these *NTRK*-associated mesenchymal tumors may be challenging and may lead to misdiagnosis as a variety of other entities or lack of a definitive diagnosis. However, awareness that *NTRK* rearrangements occur in a subset of soft tissue tumors is of increasing importance for clinical treatment and management. Like both ''lipofibromatosis-like neural tumor'' and CIFS, this spectrum of *NTRK*-associated mesenchymal tumors seems to be predominantly locally aggressive due to their highly infiltrative growth, but they may not require additional therapy if completely excised. In patients for whom a complete resection would be too morbid (ie, requiring amputation for negative margins), pan-TRK inhibitors are now available as therapeutic options. Early studies have shown responses to treatment with Ertrectinib (RXDX-101) in metastatic colorectal carcinomas and *LMNA-NTRK1* soft tissue tumors,[38,47,48] as well as with Afatinib (LOXO-101) in CIFS and in the single adult case of *LMNA-NTRK1* soft tissue sarcoma.[39,49] Given the tremendous morbidity associated with resection of large deep soft tissue tumors in young infants particularly, wide resection is often not favored as a first-line therapeutic approach, and the addition of these targeted therapies may improve outcome.

Together, our experience suggests that *NTRK*-associated mesenchymal tumors in infants show morphologic overlap and clinical behavior analogous to that seen in CIFS, but with variant *NTRK* fusions. We suggest referring to these neoplasms as *NTRK*-associated mesenchymal tumors to emphasize the common molecular features and potential role of targeted therapies for patients with locally aggressive disease.

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