Published in final edited form as:

Am J Surg Pathol. 2018 June ; 42(6): 791–798. doi:10.1097/PAS.0000000000001055.

***NTRK* Fusions Define a Novel Uterine Sarcoma Subtype with Features of Fibrosarcoma**

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Abstract

Tropomyosin receptor kinase (Trk) inhibitors have shown high response rates in patients with tumors harboring *NTRK* fusions. We identified four *NTRK* fusion-positive uterine sarcomas that should be distinguished from leiomyosarcoma and undifferentiated uterine sarcoma. *NTRK* rearrangements were detected by fluorescence in situ hybridization (FISH) and/or targeted RNA or DNA sequencing in four undifferentiated uterine sarcomas with spindle cell morphology. Due to histologic overlap with leiomyosarcoma, TrkA and pan-Trk immunohistochemistry was performed in 97 uterine leiomyosarcomas. *NTRK1* and *NTRK3* FISH was performed on tumors with TrkA or pan-Trk staining. We also performed whole transcriptome RNA sequencing of a leiomyosarcoma with TrkA expression, and targeted RNA sequencing of two additional undifferentiated uterine sarcomas. FISH and/or targeted RNA or DNA sequencing in the study group showed *TPM3-NTRK1*, *LMNA-NTRK1*, *RBPMS-NTRK3*, and *TPR-NTRK1* fusions. All tumors were composed of fascicles of spindle cells. Mitotic index was 7-30 mitotic figures per 10 high power fields; tumor necrosis was seen in two tumors. Desmin, estrogen receptor and progesterone receptor were negative in all tumors, while pan-Trk was expressed in all tumors with concurrent TrkA staining in three of them. TrkA and/or pan-Trk staining was also seen six leiomyosarcomas, but these tumors lacked *NTRK* fusions or alternative isoforms by FISH or whole transcriptome sequencing. No fusions were detected in two undifferentiated uterine sarcomas. *NTRK* fusion-

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positive uterine spindle cell sarcomas constitute a novel tumor type with features of fibrosarcoma; patients with these tumors may benefit from Trk inhibition. TrkA and pan-Trk expression in leiomyosarcomas is rare and does not correlate with *NTRK* rearrangement.

Keywords

NTRK; uterine sarcoma; fibrosarcoma

INTRODUCTION

Undifferentiated uterine sarcoma is a rare malignant mesenchymal neoplasm that is currently recognized as a subcategory of endometrial stromal tumors by the World Health Organization ¹. Although they were referred to as undifferentiated endometrial sarcomas in prior editions of the World Health Organization classification of endometrial stromal tumors ², they lack any histologic resemblance to proliferative phase endometrial stroma and may not involve or arise from the endometrium. Instead, they typically consist of sheets of tumor cells with significant cytologic atypia, brisk mitotic activity, tumor necrosis, and destructive myometrial invasion. Undifferentiated uterine sarcoma is considered a diagnosis of exclusion after ruling out more common uterine mesenchymal tumors (including leiomyosarcoma and high-grade endometrial stromal sarcoma) that have characteristic morphologic features, immunohistochemical profiles, or recurrent chromosomal translocations resulting in gene rearrangement.

Undifferentiated uterine sarcomas likely comprise a morphologically and genetically heterogeneous group of uterine mesenchymal neoplasms similar to undifferentiated pleomorphic sarcoma of the soft tissues³. While most if not all undifferentiated uterine sarcomas have a reportedly dismal prognosis, the scope of clinicopathologic studies describing the entity has been severely limited due to the rarity of this tumor type and likely contamination by morphologically high-grade endometrial stromal sarcomas harboring various genetic aberrations, particularly gene fusions that are distinct from low-grade endometrial stromal sarcomas ^{4,5}. Prior studies suggested that undifferentiated uterine sarcomas may be separable into monomorphic and pleomorphic types by morphologic assessment⁶; the former is characterized by isomorphic tumor cells, while the latter consists of neoplastic cells with marked nuclear pleomorphism. At least a subset of undifferentiated uterine sarcomas with monomorphic phenotype and less commonly with pleomorphic histology is in fact high-grade endometrial stromal sarcoma, such as those harboring *YWHAE*⁵, *BCOR*^{7,8}, or *SUZ12*⁹ gene rearrangements. However, only limited molecular data exist among undifferentiated uterine sarcomas, which appear to have complex karyotypes and harbor *TP53* mutations in approximately 30% of cases ^{6,10}.

Using a combination of next-generation targeted RNA and DNA sequencing as well as fluorescence in situ hybridization (FISH) screening in the clinical work-up of undifferentiated uterine sarcomas, we identified four spindle cell sarcomas harboring recurrent *NTRK* gene rearrangements and exhibiting unique morphologic and immunophenotypic features allowing their distinction from leiomyosarcoma and

undifferentiated uterine sarcoma. In this study, we define the clinicopathologic and molecular genetic features of this novel tumor type that expands the spectrum of fusion-positive uterine sarcomas as well as the variety of tumors harboring *NTRK* rearrangement. We also assess the frequency of TrkA and pan-Trk expression and *NTRK* gene rearrangement among uterine spindle cell leiomyosarcomas which represents the most important differential diagnosis in the assessment of these rare spindle cell sarcomas.

MATERIALS AND METHODS

Case Selection

From 2014 to 2017, four undifferentiated uterine sarcomas with spindle cell morphology and lacking known genetic abnormalities were prospectively identified on the Gynecologic Pathology Service at Memorial Sloan Kettering Cancer Center, New York, NY. Hematoxylin and eosin and immunohistochemical stained slides were reviewed to delineate the morphologic and immunophenotypic features by two pathologists with expertise in gynecologic and soft tissue pathology (S.C., C.R.A.). Clinical data were reviewed for demographics, presentation, treatment, and outcome. Unstained 5 µm slides of formalin-fixed, paraffin-embedded (FFPE) whole-tissue sections from each tumor were obtained for TrkA and pan-TRK immunohistochemistry, next-generation targeted RNA or DNA sequencing, and FISH.

Due to histologic overlap with leiomyosarcoma and undifferentiated uterine sarcoma, unstained 5 µm slides of triplicate 0.6 mm diameter core tissue microarrays of 97 FFPE uterine leiomyosarcomas and unstained 5 µm whole-tissue FFPE sections of two additional undifferentiated uterine sarcomas with spindle cell morphology and prominent myxoid stroma were obtained from Memorial Sloan Kettering Cancer Center and Massachusetts General Hospital, Boston, MA.

Immunohistochemistry

Immunohistochemical staining for TrkA, TrkB, and TrkC expression and TrkA only was performed on the study cohort and tissue microarrays of leiomyosarcoma using a commercially available pan-Trk monoclonal antibody, clone EPR17341 (Abcam, Cambridge, MA) at 6 µg/mL and a commercially available TrkA monoclonal antibody, clone (Abcam, Cambridge, MA) at 0.684 mg/mL, respectively. Staining was performed on a Leica-Bond-3 (Leica, Buffalo Grove, IL) automated immunostaining platform using a heat-based antigen retrieval method and high pH buffer solution (ER2, Leica) as previously reported¹¹. Normal testis, ganglia, and brain tissues were used as positive controls. Results were interpreted independently by three pathologists (S.C., J.F.H., A.A.J.). Intensity (strong, moderate, weak, and negative) and estimated percentage of positive tumor cells were evaluated. Pan-Trk and TrkA immunohistochemistry was repeated on 5-µm whole tissue FFPE sections of leiomyosarcomas that showed any staining on the tissue microarray.

Targeted Massively Parallel Sequencing

In all four undifferentiated uterine sarcomas of the study cohort and two additional undifferentiated uterine sarcomas with spindle cell morphology and myxoid stroma, RNA

was extracted from macrodissected FFPE tumor tissue mounted on charged glass slides and subjected to the Archer FusionPlex Custom Solid Panel, a next-generation targeted RNA-sequencing based assay utilizing the Anchored Multiplex PCR (AMP) technology¹². Unidirectional gene-specific primers were designed to several detect gene fusions and oncogenic isoforms in selected protein-coding exons of 62 genes and used in combination with adapter-specific primers to amplify known and novel fusion transcripts. Enriched amplicons were sequenced on an Illumina MiSeq instrument.

For one of the undifferentiated uterine sarcomas in our study cohort for which normal tissue was available, DNA was extracted from macrodissected tumor and normal samples using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's guidelines. Tumor and matched germline DNA was subjected to the Memorial Sloan Kettering – Integrated Mutational Profiling of Actionable Cancer Targets (MSK-IMPACT) sequencing assay targeting all exons and selected regulatory regions and introns of 468 key cancer genes on an Illumina HiSeq2500¹³.

Whole Transcriptome RNA Sequencing

One uterine leiomyosarcoma showing TrkA-positivity by immunohistochemistry, for which sufficient RNA was available, was subjected to RNA sequencing. Total RNA was extracted from frozen tumor tissue using RNeasy Plus Mini (Qiagen), followed by mRNA isolation with oligo(dT) magnetic beads and fragmentation by incubation at 94 °C in fragmentation buffer (Illumina) for 2.5 minutes. After gel size-selection (350 to 400 bp) and adapter ligation, the library was enriched by polymerase chain reaction for 15 cycles and purified. Paired-end RNA sequencing at read lengths of 50 or 51 bp was performed on an Illumina HiSeq2000¹⁴.

Fluorescence In Situ Hybridization

FISH was performed on interphase nuclei from FFPE tissue sections of three undifferentiated uterine sarcomas and all leiomyosarcomas with TrkA or pan-Trk expression. Custom probes using bacterial artificial chromosomes (BAC) flanking *NTRK1*, *NTRK3*, *LMNA*, *TPR*, and *TPM3* genes were chosen according to the UCSC genome browser (<http://genome.ucsc.edu>). BAC clones were retrieved from BACPAC sources of the Children's Hospital of Oakland Research Institute (CHORI, Oakland, CA) (<http://bacpac.chori.org>) (Supplementary Table 1). The DNA from individual BACs were isolated according to the manufacturer's recommendations, labeled with nick translation, and validated on normal metaphase chromosomes. Whole tissue 5 µm tumor sections mounted on charged slides were deparaffinized, pretreated, and hybridized with denatured probes overnight, followed by post-hybridization washes and counterstaining with DAPI. Slides were examined on a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany) using Isis 5 software (Metasystems). Two hundred tumor nuclei were counted, and gene rearrangement was confirmed if >20% of tumor nuclei demonstrated break-apart signals. As the *NTRK1* gene on 1q25 is located in close proximity to its gene partners (*LMNA*, *TPR* and *TPM3*) on 1q21-23, we also applied a two color-fusion assay which can document the fusion with more confidence, as the standard break-apart assay may not be definitive due to the small gaps detected. Thus *NTRK1* telomeric portion was labeled in green, while

centromeric *LMNA*, *TPR* and *TPM3* were labeled in red. A come-together signal (yellow), seen in >20% of tumor nuclei, was considered a positive result ¹⁴.

RESULTS

NTRK-fusion positive sarcomas affect premenopausal women with frequent cervical involvement

The four patients in our study cohort presented with a median age of 44 (range, 27-47) years and stage 1B disease using the 2009 International Federation of Gynecology and Obstetrics staging system for uterine sarcomas (Table 1). All patients presented with abnormal vaginal bleeding, three of whom had a large cervical mass upon pelvic examination. The remaining patient was found to have a fibroid uterus on pelvic ultrasound and a well-defined highly vascular intramural lesion without hemorrhage or necrosis on pelvic MRI. All four patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. One patient had pulmonary lesions suspicious for metastases by imaging at the time of hysterectomy and was subsequently treated with gemcitabine and docetaxel. Seven months later, the patient developed progression of disease with a recurrence superior to the vagina by imaging. Another patient developed lung metastasis 12 months after initial presentation. She was treated with gemcitabine and docetaxel followed by doxorubicin, but developed new lung, pancreas, and brain metastases, and ultimately died of disease 78 months after initial presentation. The two remaining patients had no evidence of disease 11 and 2 months after initial presentation. None of the patients had a history of neurofibromatosis or prior radiation therapy.

NTRK-fusion positive sarcomas have unique spindle cell morphology and immunophenotype

Tumor size ranged from 2.6 to 16.3 cm, with a median of 11.7 cm. Three tumors were based in the cervix. Two of them were polypoid, tan-yellow, and necrotic lesions extending through the external os and confined to the cervix; the other was well-circumscribed, whorled, and tan-pink with limited extension into the lower uterine segment. The remaining tumor consisted of a tan-white whorled nodule within the myometrium and was confined to the corpus.

The tumor border was evaluable in two cases, and was pushing (n=1) and irregularly infiltrative (n=1). All four tumors consisted of haphazard fascicles of spindle cells with intermediate-sized nuclei, clumped chromatin, small nucleoli, and abundant eosinophilic cytoplasm (Table 2, Figure 1). One of the tumors also showed numerous foci of spindle cells with marked nuclear pleomorphism and smudgy chromatin (Figure 1). The stroma was edematous in one tumor and focally myxoid in another. Three of the tumors demonstrated delicate, thin-walled vasculature, while the remaining tumor with marked pleomorphism exhibited large, thick-walled blood vessels. Tumor necrosis was present in two of four tumors. Lymphovascular invasion was not seen in any of the four tumors. Mitotic activity was brisk in all tumors, ranging from 7 to 30 mitotic figures per 10 high power fields with a median of 13.5 mitotic figures per 10 high power fields.

All four tumors showed focal SMA staining, but absent desmin, estrogen receptor (ER) and progesterone receptor (PR) expression. S100 was focally positive in <10% of cells in all four tumors, but negative for SOX10 (Table 2). CD34 staining was negative, while H3K27me3 expression was retained in all tumors. CD10 was performed in three tumors and was focally positive in two and negative in one other. Pan-cytokeratin was also performed in three tumors and was negative.

Two tumors were initially classified as leiomyosarcoma despite the absence of desmin expression. The other two were diagnosed as undifferentiated uterine sarcoma.

Uterine sarcomas with *NTRK* rearrangement have various fusion partners and demonstrate TrkA and/or pan-Trk expression

RBPM3-NTRK3, *TPR-NTRK1*, *LMNA-NTRK1*, and *TPM3-NTRK1* gene fusions were detected in four tumors by targeted RNA sequencing (Table 2, Figure 2). The *TPR-NTRK1* fusion was also confirmed by targeted DNA sequencing in one tumor, while the other fusions in the three remaining tumors were confirmed by FISH (Figure 3). No fusions were detected in two additional undifferentiated uterine sarcomas with spindle cell morphology and prominent myxoid stroma.

TrkA expression was diffuse, defined as >95% of cells staining, with weakly cytoplasmic and strongly perinuclear staining in the tumor harboring *LMNA-NTRK1* fusion (Figure 4). There was strong and diffuse cytoplasmic TrkA staining in the tumor harboring *TPM3-NTRK1* fusion. Pan-Trk expression was strong and diffuse with mostly cytoplasmic staining and dot-like aggregates as well as rare nuclear staining and accentuation of the nuclear envelope in the tumor harboring *TPR-NTRK1* fusion. The tumor with *RBPM3-NTRK3* fusion showed diffuse moderate to strong cytoplasmic pan-Trk expression.

TrkA and pan-Trk expression may be seen in a subset of *NTRK* fusion-negative leiomyosarcomas

Among 97 uterine spindle cell leiomyosarcomas, only four showed weak and diffuse cytoplasmic TrkA staining, while two showed strong and diffuse cytoplasmic pan-Trk expression on both tissue microarray and whole tissue sections; the remaining tumors were negative for both TrkA and pan-Trk expression. No *NTRK* rearrangements were detected by FISH in any of the leiomyosarcomas demonstrating TrkA or pan-Trk immunoreactivity. In addition, no *NTRK* rearrangements or novel isoforms were detected by whole transcriptome sequencing of one of the TrkA-positive tumors for which frozen tumor tissue was available.

DISCUSSION

We report for the first time the presence of *NTRK* gene rearrangements in a subset of undifferentiated uterine sarcomas with fibrosarcoma-like morphology. All four tumors in our cohort occurred in premenopausal women with a median age of 44 years who presented with abnormal vaginal bleeding. All had FIGO stage 1B disease with three tumors arising in the cervix and one in the corpus; two patients recurred 7 and 12 months after initial presentation, one of whom died of disease. All four tumors shared distinct morphologic features of relatively monomorphic spindle cells with focal and moderate nuclear

pleomorphism arranged in long intersecting fascicles; one tumor also showed scattered foci of marked nuclear pleomorphism. Mitotic activity was brisk in all tumors with tumor necrosis present in two. While focal SMA expression was seen in all tumors, desmin, ER and PR were consistently negative in all. S100 was focally positive in all tumors, but all lacked SOX10 and CD34 expression; H3K27me3 expression was retained in all tumors. All tumors expressed either TrkA or pan-Trk by immunohistochemistry.

The tropomyosin receptor kinase (Trk) family includes TrkA, TrkB, and TrkC proteins which are encoded by *Neurotrophic Tyrosine Kinase Receptors NTRK1*, *NTRK2* and *NTRK3* genes, respectively^{15,16}. Binding of neurotrophins to Trk proteins induces receptor dimerization, phosphorylation, and activation of the downstream signaling cascades via PI3K, RAS/MAPK/ERK, and PLC-gamma¹⁷. Trk pathway aberrations, including gene fusions, protein overexpression, and single nucleotide alterations, have been implicated in the pathogenesis of many human cancers, with *NTRK* gene fusions being the most commonly validated oncogenic events to date. *NTRK* gene fusions are infrequent among most common cancers, such as lung and colorectal carcinomas in which the prevalence of the *NTRK* rearrangements is well below 5%¹⁸. However, certain rare tumor types are characterized by *NTRK* fusions. Among soft tissue sarcomas, *ETV6-NTRK3* fusions and *NTRK1* gene rearrangements appear to define the vast majority of infantile fibrosarcomas¹⁹ and lipofibromatosis-like neural tumors¹⁴, respectively. Interestingly, rare *NTRK3*-negative infantile fibrosarcomas have been reported to instead harbor *NTRK1* gene rearrangements^{20,21}, suggesting that oncogenic activation of various *NTRK* genes through chromosomal translocations are involved interchangeably in the pathogenesis of certain soft tissue sarcomas. This phenomenon has also been observed among our cohort of uterine sarcomas in which three tumors showed *NTRK1* fusion with various gene partners, while one tumor harbored an *NTRK3* gene rearrangement.

NTRK rearrangements appear to underpin a subset of high-grade uterine sarcomas with spindle cell morphology. We identified a novel *RBPMS-NTRK3* fusion in a uterine sarcoma that has not been previously reported in other tumor types with *NTRK* rearrangement. Among tumors with *NTRK1*-related fusions, there are numerous gene partners involved, with three in particular emerging as recurrent, such as *TPR*, *TMP3* and *LMNA*, all located on the long arm of chromosome 1 (1q21-q23), in the vicinity of *NTRK1*. Based on reported *NTRK* fusion-positive tumors to date, the *NTRK1* breakpoints include exon 9 (*TPM3-NTRK1* or *TPR-NTRK1*) or exon 12 (*LMNA-NTRK1*)¹⁴. Among the uterine sarcomas with *NTRK1* rearrangements, breakpoints include exon 10 (*TPR-NTRK1*) and exon 12 (*LMNA-NTRK1*). Despite the variability in genomic breakpoints and fusion transcripts, all reported cases to date, including our cohort of uterine sarcomas, retain the full-length coding sequence of the tyrosine kinase domain of *NTRK1* (exons 13-17) and *NTRK3* (exons 13-18). This is further confirmed in our study group by TrkA or pan-Trk protein overexpression in all tumors, regardless of the *NTRK1* and *NTRK3* breakpoint and gene partner, since the TrkA and pan-Trk antibodies bind to portions of TrkA and TrkC retained in the fusions. A recent study from our group suggested that pan-Trk immunoreactivity is associated with a fusion partner-specific pattern of staining, with cytoplasmic expression and nuclear membrane accentuation in tumors with *LMNA-NTRK1* fusions and cellular membrane accentuation in all *TPM3/4* fusions, while half of tumors with *ETV6-NTRK3*

fusions display nuclear staining ¹¹. While cytoplasmic and nuclear membrane TrkA staining was seen in one of our tumors with *LMNA-NTRK1* fusion, only cytoplasmic TrkA and pan-Trk expression was present in the tumors harboring *TPM3-NTRK1* and *RBPM3-NTRK3* fusions, respectively.

It is notable that two of our tumors were initially considered high-grade leiomyosarcoma based on the spindle cell morphology and limited myogenic differentiation by immunohistochemistry. Since leiomyosarcoma is the most common uterine malignant mesenchymal tumor, this remains an important differential diagnostic consideration when assessing atypical spindle cell neoplasms of the uterus. All of our tumors showed morphologic features that were unusual for leiomyosarcoma; while there was brisk mitotic activity with more than 10 mitotic figures per 10 high power fields in three of our lesions, the cytologic atypia was only mild to moderate in most tumors, and tumor necrosis was seen in only two. Our tumors also consisted of haphazard fascicles of spindle cells, while the fascicles of leiomyosarcoma appear uniform, often crossing at 90-degrees. These unusual morphologic features prompted immunohistochemistry in all four sarcomas. All expressed only focal SMA without evidence of desmin expression. In addition, all of them lacked ER and PR expression which is found in approximately 40% of high grade uterine leiomyosarcomas ²². While immunohistochemistry is not routinely used for the diagnosis of uterine spindle cell leiomyosarcoma, our findings raise the possibility that some tumors diagnosed as leiomyosarcoma may in fact represent *NTRK* fusion-positive sarcomas. Although TrkA and/or pan-Trk expression was seen in only 6% of uterine spindle cell leiomyosarcomas, none of them harbored underlying genetic abnormalities involving *NTRK* genes. While TrkA and pan-Trk expression is present in normal smooth muscle and may account for the staining seen in leiomyosarcoma, it is unclear why only a small subset of tumors shows immunoreactivity. This highlights a potential pitfall in using TrkA or pan-Trk immunohistochemistry alone, and fusion status must be confirmed in all tumors with Trk expression. Our findings also suggest that in the absence of desmin expression in any uterine spindle cell sarcoma, the diagnosis of leiomyosarcoma should be reconsidered, and *NTRK* fusion-positive sarcoma must be clinically excluded by immunohistochemistry and if positive, FISH, targeted DNA or RNA sequencing, or RT-PCR confirmation of the fusion status.

Our tumors bear some morphologic and immunohistochemical resemblance to rare cervical lesions described as endocervical fibroblastic malignant peripheral nerve sheath tumor or neurofibrosarcoma that reportedly showed focal S100 and CD34 positivity, but lacked desmin, SOX10, ER, and PR expression ²³. Malignant peripheral nerve sheath tumor does not typically show the degree of cytologic atypia seen in one of our tumors and two of the previously reported neurofibrosarcomas. The absence of SOX10 staining along with retained H3K27me3 expression is also unusual, the latter particularly in sporadic and radiation-associated cases ²⁴. Based on the spindle cell morphology and immunophenotype, along with insufficient evidence for peripheral nerve sheath derivation, we propose classifying these tumors as *NTRK* fusion-positive uterine spindle cell sarcomas.

While our study cohort of *NTRK* fusion-positive uterine sarcomas is limited, it is notable that all four tumors arose in premenopausal women with an increased incidence of cervical

involvement. The trend towards disease presentation at a young age in our tumors contrasts with that of uterine leiomyosarcoma as well as low- and high-grade endometrial stromal sarcomas in which the average age at presentation is 55.4, 48, and 59 years^{25,26}. The clinical behavior of *NTRK* fusion-positive uterine sarcomas is uncertain based on only four cases in our small cohort; however, all patients presented to Memorial Sloan Kettering Cancer Center at the time of primary diagnosis, and two patients recurred despite optimal surgical resection at 7 and 12 months after initial presentation, one of whom died of disease, indicating the clinically aggressive behavior of these sarcomas. Several Trk-targeting compounds are currently in clinical development with two leading Trk inhibitors, larotrectinib and entrectinib, in ongoing clinical trials, with promising results²⁷. Patients with *NTRK* fusion-positive uterine sarcomas who develop progressive disease may benefit from enrollment in clinical trials with Trk inhibition. Since *NTRK* fusion-positive uterine sarcomas appear to affect premenopausal women, early identification of *NTRK* fusions in limited tissue samples also raises the possibility for neoadjuvant Trk inhibition in the setting of fertility preservation.

Our results reveal a novel subset of high-grade uterine sarcomas with features reminiscent of soft tissue fibrosarcoma and characterized by oncogenic activation of Trk through recurrent *NTRK* gene fusions, certifying these lesions as a unique clinicopathologic entity. Based on their morphologic features, we propose classifying these tumors as *NTRK*-fusion positive uterine spindle cell sarcomas, distinct from more common malignant mesenchymal neoplasms such as leiomyosarcoma. Evaluation of Trk expression and *NTRK* fusion status in these tumors may allow patients to benefit from enrollment in clinical trials for selective inhibition of Trk signaling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Morphologic features of four *NTRK* fusion-positive uterine sarcomas. A) Haphazard fascicles of spindle cells arranged in a storiform pattern and associated with scattered lymphocytes. Herringbone pattern of spindle cells with mild nuclear atypia in one tumor (B) and rare pleomorphic cells in another (C). D) Spindle cells with mild to moderate nuclear atypia forming fascicles (right) adjacent to cells with marked pleomorphism and multinucleation.

Figure 2.
Confirmation of *RBPMS-NTRK3* fusion in a uterine sarcoma by targeted RNA sequencing.

Figure 3.
LMNA-NTRK1 gene fusion demonstrated by FISH. A two-color fusion assay was applied showing the *NTRK1* (green, telomeric) and *LMNA* (red, centromeric) signals come together as a yellow fused signal (white arrows).

Figure 4.

Trk expression patterns in uterine sarcomas with *NTRK* gene rearrangement. A and B) Diffuse cytoplasmic TrkA and pan-Trk expression in tumors with *TPM3-NTRK1* and *RBPM5-NTRK3* fusions, respectively. C) Weakly cytoplasmic and strongly perinuclear staining in the tumor harboring *LMNA-NTRK1* fusion. D) Strong and diffuse pan-Trk expression with mostly cytoplasmic and dot-like aggregates (top inset) as well as rare nuclear staining accentuation of the nuclear envelope (bottom inset) in the tumor harboring *TPR-NTRK1* fusion.

Table 1.

Clinical characteristics of the cohort.

Case	Age, y	Signs and Symptoms	Disease Site	Stage	Treatment	Outcome, mo
1	46	Vaginal bleeding	Cervix	1B	Surgery, chemotherapy	Alive with disease, 7
2	27	Fibroid uterus	Corpus	1B	Surgery	No evidence of disease, 11
3	47	Vaginal bleeding	Cervix	1B	Surgery, chemotherapy	Died of disease, 78
4	42	Vaginal bleeding	Cervix	1B	Surgery	No evidence of disease, 2

Table 2.

Gross, morphologic, and immunophenotypic features of the cohort.

Gross and Morphologic features					Immunohistochemical profile								
Case	Size, cm	Atypia	Necrosis	LVI	MI	Desmin	SMA	ER	PR	CD34	S100	SOX10	H3K27me3
1	9.3	Moderate	Present	Absent	15	Neg	Pos (F)	Neg	Neg	Neg	Pos (R)	Neg	Pos
2	16.3	Moderate	Absent	Absent	7	Neg	Pos (F)	Neg	Neg	Neg	Pos (R)	Neg	Pos
3	14.0	Moderate	Present	Absent	12	Neg	Pos(F)	Neg	Neg	Neg	Pos (R)	Neg	Pos
4	2.6	Severe	Absent	Absent	30	Neg	Pos (F)	Neg	Neg	Neg	Pos (R)	Neg	Pos

ER indicates estrogen receptor; LVI, lymphovascular invasion; MI, mitotic index (# of mitotic figures/10 high power fields); PR, progesterone receptor; SMA, smooth muscle actin.