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# New fusion sarcomas: histopathology and clinical significance of selected entities☆

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**Summary** Many sarcomas contain gene fusions that can be pathogenetic mechanisms and diagnostic markers. In this article we review selected fusion sarcomas and techniques for their detection. *CIC-DUX4* fusion sarcoma is a round cell tumor now considered an entity separate from Ewing sarcoma with a more aggressive clinical course, occurrence in older age, and predilection to soft tissues. It is composed of larger cells than Ewing sarcoma and often has prominent necrosis. Nuclear DUX4 expression is a promising immuno histochemical marker. *BCOR-CCNB3* fusion sarcoma is cyclin B3–positive, usually occurs in bone or soft tissue of children, and may mimic a poorly differentiated synovial sarcoma. *EWSR1-NFATC2* sarcoma may present in bone or soft tissue. It is typically composed of small round cells in a trabecular pattern in a myxoid matrix resembling myoepithelioma. *ACTB-GLI1* fusion sarcoma may mimic a skin adnexal carcinoma, showing focal expression of epithelial markers and S100 protein. *NTRK*-fusion sarcomas include, in addition to infantile fibrosarcoma with *ETV6-NTRK3* fusion, *LMNA-NTRK1* fusion sarcoma, a low-grade spindle cell sarcoma seen in peripheral soft tissues in children and young adults. Methods to detect gene fusions include next-generation sequencing panels, anchored multiplex polymerase chain reaction systems to detect partner for a known fusion gene, and comprehensive RNA sequencing to detect virtually all gene fusions. In situ hybridization testing using probes for both fusion partners can be used as an alternative confirmation technique, especially in the absence of satisfactory RNA yield. In addition, fusion protein–related and other immunohistochemical markers can have a high specificity for fusion sarcomas.


## 1. Introduction

Gene fusions created by chromosomal rearrangements are associated with cancers of various types, especially sarcomas, and are often used as diagnostic markers. Among the first reported gene fusions in sarcomas was the *SS18-SSX*1/2 fusion resulting from the t(X;18) translocation, still believed to be specific to synovial sarcoma [1]. Other well-known gene fusions used as

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diagnostic markers in sarcomas include fusion of Ewing sarcoma gene (*EWSR1*) with *FLI1* or *ERG* in Ewing sarcoma and *PAX3* or *PAX7* fusions with *FOXO1* in alveolar rhabdomyosarcoma. Notably, *EWSR1* gene involvement is not specific for Ewing sarcoma, but occurs in many unrelated sarcomas [2,3].

Chimeric transcripts (chimeras, or fusion transcripts) originate from the fusion of two or more different gene transcripts. Traditionally, all chimeric RNAs were thought originate from gene fusions occurring due to chromosomal rearrangements, such as deletions, inversions, or translocations [4]. More recently, some fusion RNAs have been suggested to be products of either cis-splicing between neighboring genes, or a result from RNA trans-splicing, with both phenomena having detected in some non-neoplastic tissues [5,6].

With the recent advances in next-generation sequencing, numerous new chimeric RNAs have been discovered in sarcomas. In many cases, tumors harboring these fusions have emerged as new clinicopathologic entities with characteristic histological and immunophenotypic features. In this review, we present examples of such newly described entities regularly encountered in diagnostic pathology, based on our experience, and discuss their clinical significance and strategies for their detection.

## 2. Classic and newer fusion search strategies

The first fusion genes, such as those found in Ewing sarcoma and synovial sarcoma, were cloned and characterized following the cytogenetic detection of chromosome rearrangements by chromosome banding and fluorescence in situ hybridization (FISH) studies. However, this classical approach is limited by the requirement of fresh tumor tissue and obtaining metaphases for chromosome analysis. Therefore, it is now largely replaced by molecular genetic techniques.

In clinical practice, an interphase FISH employing probes for fusion genes is widely used to detect rearrangements [4]. However, this technique requires a strong diagnostic hypothesis and availability of commercial probes or a capability to manufacture probes from gene libraries. FISH is often unsuitable for analyzing structural rearrangements including inversions and interstitial deletions involving closely adjacent genes.

Invention of the polymerase chain reaction (PCR) amplification revolutionized the search for fusion genes. PCR methods were developed for detection of specific fusion breakpoints at both DNA and RNA levels [7]. RACE (rapid amplification of cDNA ends), an RT (reverse-transcription)–PCR technique, was employed to amplify chimeric transcripts expressed by the fusion genes with only one known partner [8]. Also, gene expression profiling assays were successfully used to search for new fusion gene transcripts [9,10]. Oligo microarrays with known sarcoma fusion gene transcripts are also employed to detect exon-exon breakpoints [11,12]. Combined multiplex RT-PCR and microarray pipeline, and the GeneChip exon array screening were established to search for known, or new, fusion gene transcripts [13,14]. Additionally, digital expression profiling using NanoString Technologies nCounter Analysis System (NanoString Technologies, Seattle, WA) has proven efficient for detection of known fusion gene transcripts [5,15]. However, these methods are typically used in a research setting and not commonly applied in clinical practice.

The advent of next-generation sequencing (NGS) technologies opened a new era in the search for fusion genes and their chimeric transcripts. The whole-genome NGS of tumor DNA has been successfully used to identify new fusion genes, such as the *NAB2-STAT6* fusion in solitary fibrous tumor [16]. Yet, most NGS-based studies searching for unknown fusions have employed a transcriptome sequencing (RNA-seq) [1]. The advantage of the RNA-based NGS is the ability to identify fusion gene transcript variants as well as levels of fusion gene transcript expression. However, chromosomal rearrangements that cause the replacement of regulatory sequences cannot be detected using this method. In soft tissue tumors, fusions including upstream or promoter regulatory sequences represent a relatively small fraction of all known fusions. Moreover, chromosomal rearrangements silencing the involved genes may not be detected by the NGS RNA-seq [17]. In both methods, a search for fusions require well preserved nucleic acids, which are often difficult obtain from formalin-fixed, paraffin-embedded (FFPE) tissues. Because of this and high cost, the application of whole-genome sequencing or RNA-seq into diagnostic pathology has been limited. More recently, lower-cost methods for the efficient preparation of sequencing libraries (SMARTer-Stranded Total RNA-Seq Kit v2) from small (250 pg-10 ng) inputs of partially degraded FFPE RNA has been developed and commercialized by Takara Bio USA, Inc., (Mountain View, CA).

Recently, assays have been established for fusion gene transcripts detection combining multiplex anchored PCR amplification of specific targets with next-generation sequencing [18]. These amplicon-based assays employ anchored multiplex PCR to enrich chimeric transcripts in a low input of partially degraded RNA from FFPE tissue. While conventional PCR requires knowledge of both sequences flanking the area of interest, anchored PCR allows for detection of a target when only one sequence is known. In this technique, cDNA undergoes repair, adenylation and ligation with a universal adapter and subsequent amplification with a primer for the known gene and a primer complementary to the ligated universal adapter or ”anchor” [19,20]. Therefore, NGS libraries created with Anchored PCR may contain chimeric transcripts of both previously known and novel fusions. These assays, commercialized by ArcherDx, Inc, (Boulder, CO) and by NuGEN Technologies, Inc, (San Carlos, CA), are compatible with commonly used NGS platforms such as Illumina (Illumina, San Diego, CA) and Ion Torrent (Thermo Fisher Scientific, Waltham, MA), and their value in the evaluation of bone and soft tissue tumors has been well documented [21].

Increased application of fusion search techniques will likely lead to discovery of numerous novel gene fusions and improve our understanding of occurrence of known fusions.

Detection of larger numbers of specific fusion tumors will broaden our understanding of the clinicopathologic correlation of various fusion tumors.

## 3. *CIC* fusion sarcomas

Although initially included in the Ewing sarcoma family, *CIC-DUX4* fusion sarcoma is now considered an entity separate from Ewing sarcoma based on histologic and clinical differences, such as older patient average age and a more aggressive course than seen in Ewing sarcoma [22]. Originally identified from cytogenetic studies on tumors histologically often considered Ewing sarcoma [23,24], subsequent larger series have been identified by screening of *EWSR1* gene rearrangement-negative Ewing-like sarcomas [22,25-30]. In those studies, 22% to 68% of *EWSR1-FISH* negative tumors had a *CIC-DUX4* fusion, so that this fusion seems to be the most common one identified in *EWSR1* gene rearrangement negative Ewing-like sarcomas [26,28].

Most cases contain a t(4;19)(q35;q13) corresponding to a rearrangement between the *CIC* and *DUX4* genes, and a smaller subset contains a t(10;19)(q26;q13), which corresponds to a fusion between *CIC* and the paralog gene *DUX4L* [27]. A small number of cases (currently 2) with *CIC-FOXO4* fusions corresponding to t(X;19) (q13;q13;3) translocation have been reported (discussed in the end of this section). Fluorescence in situ hybridization for *CIC* detects most cases, but some cases may be missed due to cryptic translocations [31].

*CIC* gene encodes for a morphogenetic transcription factor known from Drosophila as the morphogenetic Capicua transcription factor, a transcriptional repressor regulating kinase signaling. *CIC-DUX4* fusion up-regulates ETS transcription factors of PEA3 subfamily, especially ETV4 [32,33]. Murine model of induced *CIC-DUX4* fusion produced a round cell sarcoma like the human one supporting pathogenetic role of this fusion [34].

Nearly 200 cases of *CIC-DUX4* fusion sarcomas have been reported [22-30]. These sarcomas occur at a median age of approximately 30 years but with a wide age range from childhood to old age (6-81 years) with a mild male predominance. In contrast with Ewing sarcoma, they almost always present as soft tissue tumors occurring in the extremities, trunk wall, body cavities, and occasionally in bones or organ-based locations, such as kidney and brain. Tumors are usually large with a median size 9 to 10 cm with a grossly whitish appearance with frequent necrosis. Follow-up studies have noted poor overall survival with most patient succumbing with lung metastases in 1 to 2 years. Even small tumors have proven fatal. Median survival and responsiveness to Ewing sarcoma chemotherapy protocols is clearly inferior to typical Ewing sarcoma.

Histologically there is a round or ovoid cell tumor with occasional spindle cell change. Tumors often have extensive geographic necrosis showing a perivascular sparing pattern of cellularity (Fig. 1A). In addition to diffuse sheet-like histology, there is often myxoid matrix (Fig. 1B). The cells can have pale eosinophilic or clear cytoplasm. Nuclei are larger than in

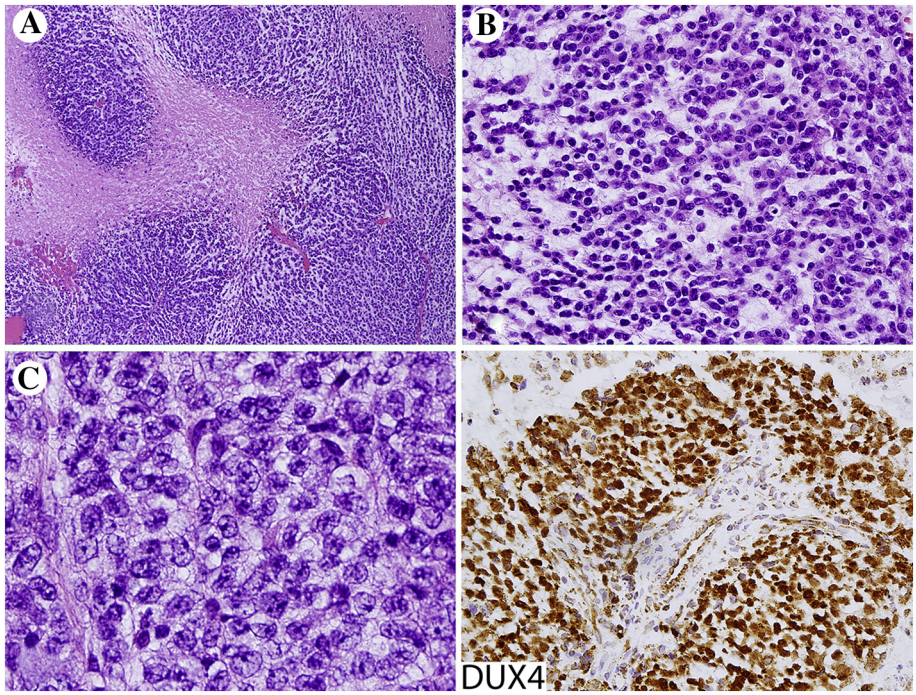


**Fig. 1** *CIC-DUX4* fusion sarcoma. A, Extensive tumor necrosis is common. B and C, Tumors are composed of round cells larger than typical Ewing sarcoma cells with prominent nucleoli. Tumor cells show nuclear positivity for DUX4, which is considered a useful marker for these fusion sarcomas.

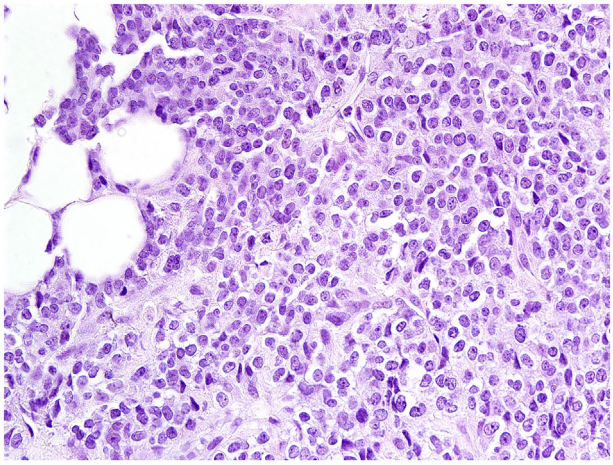

**Fig. 2** *CIC-FOXO1* fusion sarcoma resembles a classic Ewing sarcoma and is composed of uniform small round cells.

typical Ewing sarcoma, often showing prominent nucleoli (Fig. 1C).

Immunohistochemically *CIC-DUX* fusion sarcomas are variably CD99-positive and rarely show strong membrane staining typically seen in Ewing sarcoma. However, these tumors are distinctive for nuclear expression of DUX4, which seems to be the best immunohistochemical surrogate marker for tumors carrying this gene fusion (Fig. 1) [35]. While focal keratin and rarely desmin expression has been detected, expression of calretinin, ETV4, and WT1 also seem to be distinctive although less specific features [36-38].

Two patients with *CIC-FOXO4* fusion sarcomas have been reported, curiously both being intramuscular 2–3 cm tumors in the posterior neck: A 63 year-old man and a 13-year old boy [39,40]. Follow-up data of this molecular sub entity remains insufficient. Histologically these tumors may resemble classic Ewing sarcoma more than the *CIC-DUX4* sarcomas (Fig. 2).

## 4. *BCOR-CCNB3* fusion sarcoma

This tumor is defined as a spindle, oval, or round cell sarcoma containing a gene fusion involving *BCOR* (*BCL6* corepressor) and *CCNB3* (cyclin B3) genes. At the chromosomal level, there is a paracentric inversion in the X chromosome involving these two genes. The fusion causes overexpression of cyclin B3 protein [41].

*BCOR-CCNB3* fusion sarcomas occur from early childhood to middle age (2-44 years), with the median age of 15 years. The reported series have shown a marked male predominance, 9:1 with approximately an equal number of cases reported in soft tissue and bone [41-49]. The soft tissue examples have occurred in the extremities (40%), trunk wall (33%), abdominal cavity (17%), and head and neck (<10%), whereas in bone 40% they have arisen in long bones, pelvis (33%), and small bones (27%), with an apparent predilection for the calcaneus. Isolated cases have also been reported in the kidney, where this tumor can resemble clear cell sarcoma of the kidney. In fact, these tumors have also genetic overlap as clear cell sarcoma of the kidney can have the same fusion, although it more often contains an internal tandem duplication of the *BCOR* gene [49-52]. Tumor size has varied from 1.5 to 27 cm (median size, 10 cm). Tumors have been typically treated with Ewing sarcoma protocols, with similar treatment responses.

Histologically *BCOR-CCNB3* fusion sarcoma is highly cellular sarcoma composed of spindle or ovoid cells with a

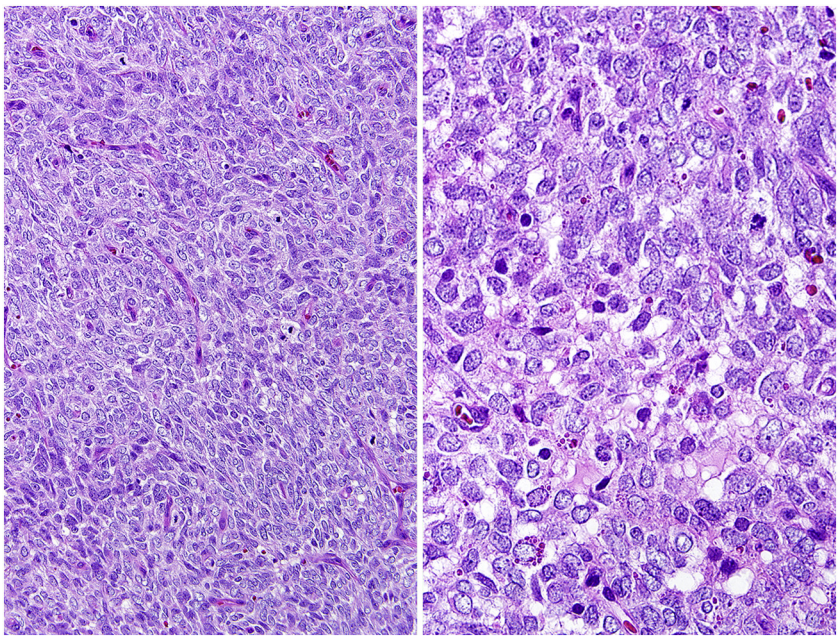

**Fig. 3** A *BCOR-CCNB3* fusion sarcoma is composed of spindled cells resembling a poorly differentiated synovial sarcoma.

prominent delicate capillary network (Fig. 3). The tumors composed of uniform, ovoid to spindled cells can resemble poorly differentiated synovial sarcoma, malignant peripheral nerve sheath tumor, or an undifferentiated sarcoma. Pleomorphic evolution has been seen in recurrent tumors [41-49].

Immunohistochemistry for cyclin B3, the protein product of the *CCNB3* gene, is positive in these fusion tumors, in contrast to classical Ewing sarcoma [45,53,54]. BCOR has also been suggested as a diagnostic marker due to overexpression of this gene; however, this protein is widely expressed in different tissues [55]. BCOR overexpression has also been reported in aggressive, newly described endometrial sarcomas containing other *BCOR* gene fusions [56].

## 5. *EWSR1-NFATC2* fusion sarcoma

This gene fusion corresponds to translocation t(20;22)(q13.2;q12.2) involving 2 genes encoding transcription factors, Ewing sarcoma protein and calcineurin-dependent nuclear factor of activated T-cells 2 (NFATC2 protein, also known as NFAT1) [57]. This transcription factor has homology to the NF-kappa B proteins and is involved in T-cell differentiation and transcriptional activation of cytokine genes. It also participates in osteoclast differentiation, cardiac valve morphogenesis, and regulates myosin heavy chain gene expression in skeletal muscle [58].

The frequency of occurrence of *EWSR1-NFATC2* fusion sarcoma is not precisely known, but 1 of 24 (4%) of tumors histologically compatible with bone fibrosarcoma was found to have this fusion [59], and there were 2 cases with this fusion among 32 Ewing sarcomas (6%) in another study [60]. Only 5 cases of *EWSR1-NFATC2* fusion sarcoma have been reported in detail. Histologically it is often characterized as an Ewing sarcoma–like tumor. Those tumors have occurred in male patients of ages 16–39 years (median, 25 years). All but one was a bone tumor involving long bones, 3 in the femur and 1 in the humerus. The tumor is clinically aggressive with potential for both local recurrence and metastasis [56,61]. Tumors with *FUS-NFATC2* fusion have also been recently reported [62].

*EWSR1-NFATC2* fusion sarcomas are small round cell tumors, often with corded patterns and myxoid matrix mimicking myoepithelioma of soft tissue (Fig. 4). When occurring in a bone, the tumor can resemble a poorly differentiated osteosarcoma by marked reactive sclerotic bone formation.

Immunohistochemically the tumors are usually positive for vimentin and CD99 showing membrane expression for the latter as seen in typical Ewing sarcoma, while the reported tumors have been negative for desmin, EMA, keratins, SMA, and S100 protein [56,61]. Weak FLI1 and ERG expression has been noted [4]. With its transcriptome differing from Ewing sarcoma, *CIC-DUX4* fusion sarcoma and *BCOR-CCNB3* fusion sarcoma, *EWSR1-NFATC2* fusion sarcoma should be considered a separate tumor entity [63].

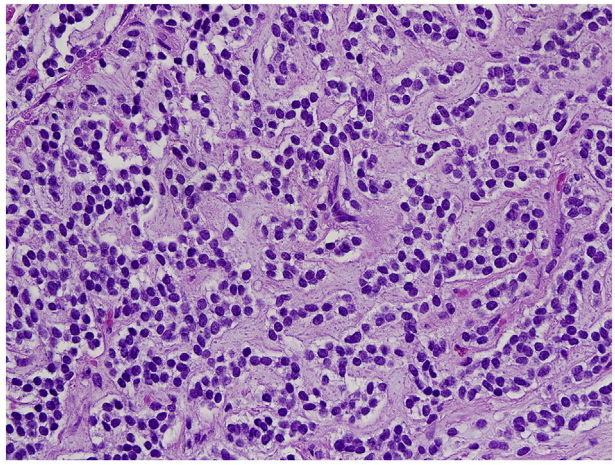

**Fig. 4** *EWSR1-NFATC2* fusion sarcoma has often a corded cellular pattern with myxoid matrix between the cellular cords composed of uniform round cells.

## 6. *GLI1* fusion sarcomas

*GLI1* gene encodes glioma-associated oncogene 1 which is involved in the sonic hedgehog signaling pathway. It is a morphogenetic transcription factor with zinc finger elements known to be important in the development of the nervous system. Recently, 3 different fusions involving this gene were reported in tumors mostly occurring in soft tissues. There were 4 tumors with fusions involving the beta-actin gene: *ACTB-GLI1* and 1 each of fusions involving patched 1 gene: *PTCH1-GLI1*, and a third fusion with the *MALAT1* gene encoding metastasis associated lung adenocarcinoma transcript [64]. The latter fusion was previously detected in plexiform fibromyxoma of the stomach [65] and gastroblastoma, a rare biphasic epithelial-mesenchymal neoplasm of stomach [66]. In the latter reports, immunohistochemical nuclear and cytoplasmic positivity for GLI1 was detected suggesting its value as surrogate immunohistochemical marker for *GLI1* fusion tumors.

*ACTB-GLI1* fusion tumors occur in various soft tissue locations and have a predilection for female adults <40 years of age. Histologically it is an epithelioid neoplasm with a nested or trabecular pattern differing from Ewing sarcoma family tumors. Mitotic activity has been reported as low, <5/10 HPFs, and Ki67-labeling as 5% [64]. In our experience, these tumors can histologically mimic mixed tumor/myoepithelioma of soft tissue when composed of epithelioid nests with myxoid matrix (Fig. 5A and B). Immunohistochemically these tumors are often positive for S100 protein (Fig. 5) and sometimes focally for keratins. However, in contrast with myoepitheliomas, they are negative for SOX10 [64]. Like Ewing sarcomas, they can have membrane positivity for CD99 (Fig. 5).

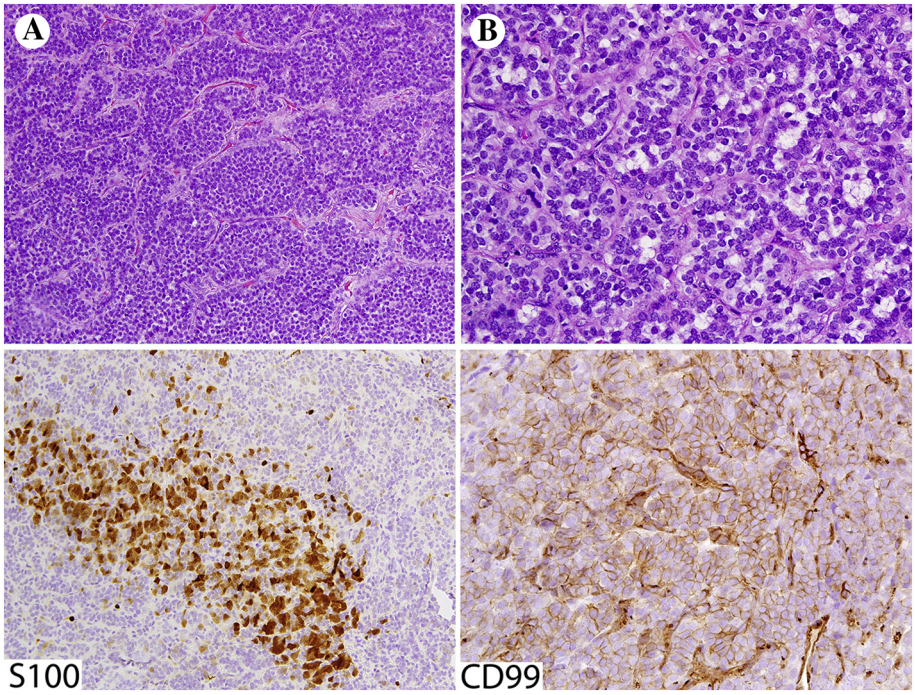


**Fig. 5** A and B, *ACTB-GLI1* fusion sarcoma shows histologic resemblance to skin adnexal tumors such as myoepithelioma. Tumor cells show variable positivity for S100 protein and CD99.

The *ACTB-GLI1* fusion tumors were originally identified by cytogenetic studies showing the corresponding t(7;12)(p22;q13) translocation and subsequently demonstrated to harbor the *ACTB-GLI1* fusion. These tumors were designated pericytomas based on histological perivascular patterns and variable smooth muscle actin expression. Three of those tumors originated in the tongue, and one each in the calf and stomach [67]. One example was subsequently reported in the talus bone [68]. Clinical follow-up of *ACTB-GLI1* fusion tumors have shown only regional lymph node metastases with no evidence for distant metastases, but follow-up data is scant necessitating caution in prognostication [64,67,68].

## 7. *NTRK1/2/3* gene-involving fusions

Neurotrophic tyrosine kinases (NTRK1, NTRK2, and NTRK3), also known as tropomyosin receptor kinases and high-affinity nerve growth factor receptors, are a family of receptor tyrosine kinases involved in the development, differentiation, and metabolism of neural and other tissues. Their activation may lead to activation of at least MAP kinase and PIK3CA downstream pathways. The clinical significance in recognizing *NTRK* gene fusions lies in the utilization of NTRK inhibitors as a targeted oncologic therapy [69-71].

*ETV6-NTRK3* fusions were originally reported in infantile fibrosarcoma and cellular mesoblastic nephroma as recurrent genetic events [72,73]. A similar fusion was subsequently described in secretory carcinoma of the breast [74], and secretory carcinoma analog tumors in the salivary glands [75]. Here we discuss newly described sarcomas with *NTRK* fusions.

### 7.1. *LMNA-NTRK1* fusion sarcoma with a spindle cell pattern

This fusion corresponds to t(1;1)(q22;q23) intrachromosomal inversion translocation. It involves genes *LMNA* encoding nuclear envelope protein lamin A/C and *NTRK1* encoding neurotrophic receptor tyrosine kinase 1 in the MAP kinase signaling pathway. The fusion protein contains an active tyrosine kinase domain of the NTRK1 protein. Tumors with fusions *TPM3-NTRK1* may have similar histologic and clinicopathologic features [76-79].

*LMNA-NTRK1* fusion sarcoma with a spindle cell pattern occurs in children from early infancy and young adults in a wide variety of peripheral soft tissue locations. This tumor has significant potential for local recurrence, but so far, no metastases have been rare. In tumors that are inoperable or metastasize, NTRK inhibitor therapy could be applicable. Histologically these tumors are infiltrative, non-pleomorphic spindle cell sarcomas with low-grade morphology showing mitotic rate <5/10 HPFs (Fig. 6A-C).

Immunohistochemically, the tumors are often positive for S100 protein, which could raise the differential diagnosis of low-grade MPNST (Fig. 6). However, these tumors do

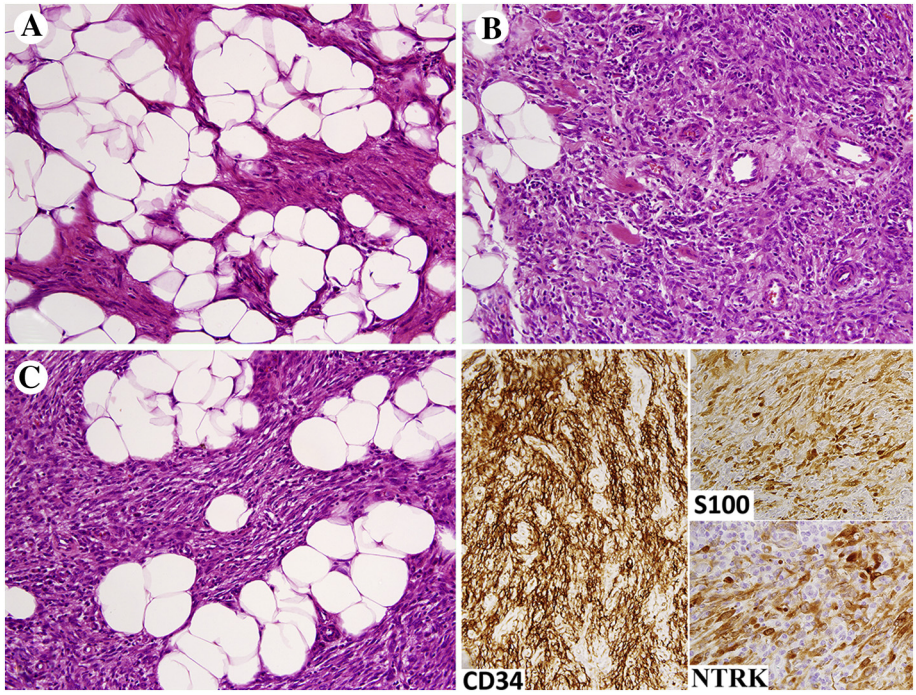


**Fig 6** A-C, *LMNA-NTRK1* fusion sarcoma shown here grows permeatively in subcutaneous fat and is composed of uniform spindled to ovoid cells with low mitotic activity. The tumor cells are immunohistochemically positive for S100 protein and often CD34. Immunoreactivity for NTRK is a consistent although not totally specific feature.

not have true neuroectodermal differentiation and are SOX10-negative. The tumors are typically positive for CD34 (Fig. 6) and may also be positive for SMA while negative for desmin. A pan-NTRK antibody recognizing NTRKs 1–3 has been reported useful in screening *NTRK* gene-rearranged sarcomas [80,81]. This antibody typically strongly labels the membranes/cytoplasm (Fig. 6). However, potential positivity for NTRK antibody in other sarcomas, such as rhabdomyosarcoma, and physiological positivity in some endothelial and skeletal muscle cells, should be considered in the differential.

Some low-grade *LMNA-NTRK1* fusion sarcomas have been reported with myopericytoma-like features [77]. The same fusion has also been detected in unrelated malignancies of various lineages, such as uterine spindle cell sarcoma, generalized eruptive histiocytosis, and colonic adenocarcinoma [82-84].

### 7.2. *ETV6-NTRK3* fusions in sarcomas other than infantile fibrosarcoma

*ETV6-NTRK3* fusions have been recently in *KIT/PDGRA* and other known GIST driver mutation-negative (wild-type) gastrointestinal stromal tumors. These tumors occurred in middle-aged men in the small intestine and rectum [85,86]. These tumors were high-risk GISTs with one of them metastatic to liver and other containing high mitotic activity. One of the tumors had epithelioid cytology.

Recognition of an *ETV6-NTRK3* fusion GIST ultimately requires genomic sequencing, but application of pan NTRK immunohistochemistry could also be useful [80].

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