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Complementary value of molecular analysis to expert review in refining classification of uncommon soft tissue tumors

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Abstract

The classification of many soft tissue tumors remains subjective due their rarity, significant overlap in microscopic features and often a non-specific immunohistochemical (IHC) profile. The application of molecular genetic tools, which leverage the underlying molecular pathogenesis of these neoplasms, have considerably improved the diagnostic abilities of pathologists and refined classification based on objective molecular markers. In this study, we describe the results of an international collaboration conducted over a 3-year period, assessing the added diagnostic value of applying molecular genetics to sarcoma expert pathologic review in a selected series of 84 uncommon, mostly unclassifiable mesenchymal tumors, 74 of which originated in soft tissues and 10 in bone. The case mix (71% historical, 29% contemporary) included mostly unusual and challenging soft tissue tumors, which remained unclassified even with the benefit of expert review and routine ancillary methods, including broad IHC panels and a limited number of commercially available fluorescence in situ hybridization (FISH) probes. All cases were further tested by FISH using a wide range of custom bacterial artificial chromosome probes covering most of known fusions in sarcomas, whereas targeted RNA sequencing was performed in 13 cases negative by FISH, for potential discovery of novel fusion genes. Tumor-defining molecular alterations were found in 48/84 tumors (57%). In 27 (32%) cases the tumor diagnosis was refined or revised by the additional molecular work-up, including five cases (6%), in which the updated diagnosis had clinical implications. Sarcoma classification is rapidly evolving due to an increased molecular characterization of these neoplasms, so unsurprisingly 17% of the tumors in this series harbored abnormalities only very recently described as defining novel molecularly defined soft tissue tumor subsets.

KEYWORDS
molecular pathology, next generation sequencing, soft tissue tumor

1 | INTRODUCTION

Accurate classification of soft tissue tumors remains difficult and challenging for several reasons. First, soft tissue tumors are rare and often require expert pathology review from tertiary referral hospitals and specialized centers, who can acquire sufficient exposure and expertise.[1–3] Second, soft tissue tumors have overlapping histologic features or undifferentiated phenotypes, which is why in a substantial number of cases even expert pathologists are unable to reach a conclusive diagnosis on morphologic grounds. Third, relatively few soft tissue tumors have a pathognomonic immunoprofile,[4] with panels of antibodies often being needed to narrow the differential diagnosis. Fortunately, a significant proportion of soft tissue tumors, particularly those occurring in children and young adults and displaying a monomorphic

phenotype, tend to harbor specific molecular genetic alterations, most of which are disease-defining reciprocal chromosomal translocations.[5] As of 2018, about 150 different fusion transcripts have been identified in one-third of soft tissue tumors, 10% of which being shared by bone tumors.[5] This number continues to grow due to the widespread routine application of next generation sequencing (NGS) methods in clinical practice. As a result of this complex molecular classification, the histopathologic diagnosis of soft tissue and bone tumors has become even more challenging, and often requires centralized review by expert pathologists who have access to molecular genetic testing.

In this study we describe the results of an international collaboration depicting the added diagnostic value of molecular genetics (fluorescence in situ hybridization [FISH], targeted RNA sequencing) to sarcoma expert pathologic review in a selected series of 84 uncommon, mostly unclassifiable soft tissue tumors, 10 of which originated in bone.

2 | MATERIALS AND METHODS

2.1 | Patient selection and histologic diagnosis

Between May 2017 and June 2020, a total of 84 mesenchymal tumors of soft tissue ($n = 74$) and bone ($n = 10$) were shared between two of the authors (AJHS and CRA). All cases were drawn from the archives of the Department of Pathology and Medical Biology of the University Medical Hospital Groningen (UMCG). In this cohort, 24 cases (29%) were recent cases diagnosed between 2017 and 2020, whereas 60 cases (71%) were historical cases seen in earlier years (12 before the year 2000, 23 in the time period 2000–2009, and 25 during 2010–2016). The majority of retrieved cases shared a monomorphic but often undifferentiated phenotype, ranging from monomorphic spindle cell tumors, small blue round cell tumors, epithelioid neoplasms, and unusual vascular tumors. A hematoxylin and eosin-stained slide and a paraffin block or ample unstained slides were available in all cases. FISH was applied in all cases. All cases were evaluated by two co-authors (AJHS and CRA). A differential diagnosis was made after integration of clinical presentation, imaging studies, histology and immunohistochemical (IHC) staining, after which FISH probes considered relevant to the case were selected by one of us (CRA). Thirteen cases that were negative by FISH and potentially harboring novel fusion genes were selected for supplementary targeted RNA sequencing.

2.2 | Fluorescence in situ hybridization

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

2.3 | Targeted RNA sequencing

For the Illumina MiSeq platform RNA was extracted from formalin-fixed paraffin embedded (FFPE) tissue using Amsbio's ExpressArt FFPE Clear RNA Ready kit (Amsbio LLC, Cambridge, MA). Thirteen cases had adequate RNA quality and could be further processed and analyzed. RNA-seq libraries were prepared using 20–100 ng total RNA with the TruSight RNA Fusion Panel (Illumina, San Diego, CA). Targeted RNA sequencing was performed on an Illumina MiSeq platform, as previously described.[6] Reads were independently aligned with STAR (version 2.3) against the human reference genome (hg19) and analyzed by STAR-Fusion. The Illumina TruSight RNA fusion panel targets 507 known fusion-associated, cancer-related genes and is a much more comprehensive assay than the Archer panel, which covers 62 fusion-associated genes.

The detailed procedure for the two cases studied by the Archer Anchored Multiplex RNA sequencing assay has been previously described.[7] In short, unidirectional gene-specific primers were designed to target specific exons in 62 genes known to be involved in oncogenic fusions in solid tumors. RNA was extracted from FFPE specimens, followed by cDNA synthesis and library preparation. Anchored Multiplex polymerase chain reaction amplicons were sequenced on Illumina Miseq, and the data was analyzed using the Archer software.

2.4 | Other molecular testing methods

In two cases, PCR and Sanger sequencing was performed. In the first case, PCR for *BCOR* ITD was performed using genomic DNA isolated from archival paraffin tissue.[8] The sequencing results of the PCR products were compared to the NCBI human *BCOR* gene sequences. In the second case, targeted PCR was performed for the hot spot *MYOD1* exon 1 mutation and associated *PIK3CA* exons 9 and 20 mutations.[9] Direct sequencing of PCR products was performed and compared to the NCBI human *MYOD1* and *PIK3CA* gene sequences.

3 | RESULTS

A total of 84 challenging mesenchymal tumors were selected after expert review, including 74 primary soft tissue tumors and 10 originating in bone. By applying a combined molecular approach using FISH testing for a wide range of known gene rearrangements and supplemented when needed with targeted RNA sequencing (13 cases; 15%),

we identified specific gene fusions or rearrangements in 45 (54%) cases. Using the Illumina TruSight panel gene fusions were detected in 5 out of 13 cases (38%) including 5 cases with novel fusions (*PHF1*::*TFE3*, *SRF*::*ICA1L*, *MEIS1*::*NCOA2*, and *EWSR1*::*CREM*). In two additional cases, selective/targeted DNA PCR detected tumor-specific gene mutations (*BCOR* ITD and *MYOD1* mutation, one case each), while in an additional case we identified gene amplifications (by FISH for *MDM2*). Thus, molecular methods established disease-defining molecular alterations in 48/84 tumors (57%). The tumor diagnosis was refined or revised by this molecular work-up in 27/84 cases (32%), including 14 cases (17%) harboring novel fusion genes. The original histologic diagnosis was confirmed by molecular genetics in the 21/84 cases (25%). In 36 (43%) tumors no molecular abnormalities were detected for a more definitive subclassification.

During the 3 years of UMCG-MSKCC consultations, only two cases had to be excluded from this study because FISH failed to render proper signals; one was a case out of Africa that had been stored in formalin for several weeks; the other case dated back to 1984 and in those years unbuffered formalin was used which probably resulted in nucleic acid fragmentation in the paraffin block over the years; overall, all other cases included in this study were eligible for FISH and only 1/14 cases proved unsuitable for RNA sequencing due to poor RNA quality, probably also due to the use of unbuffered formalin in the referring pathology laboratory.

3.1 | Molecular analysis refined or changed the initial diagnosis in 15% of the cases

As summarized in Table 1, the initial pathologic diagnosis was revised in 13 cases (15%) by applying further molecular investigations, including FISH in 10 cases, targeted PCR and Sanger sequencing in two cases (a primitive myxoid mesenchymal tumor of infancy with *BCOR ITD*, and a spindle rhabdomyosarcoma with a *MYOD1* p.L122R mutation), and targeted RNA sequencing in one case (a mesenchymal chondrosarcoma with *HEY1*::*NCOA2*).

In five cases, the updated diagnosis had clinical implications. In Case 1, which presented as a small subcutaneous tumor in the foot, with EMA and SMA co-expression by IHC, a malignant diagnosis (low-grade myofibroblastic sarcoma) was reversed to benign (syncytial myoepithelioma) based on a prototypical *EWSR1*::*PBX3* gene fusion. Cases 2 and 3 concerned two jawbone sarcomas presenting with an aggressive radiographic appearance, which were initially diagnosed as undifferentiated sarcoma and osteosarcoma, respectively, and subsequently reclassified as osseous spindle and epithelioid rhabdomyosarcomas with *EWSR1/FUS*::*TFCP2* fusions and expression of desmin, MyoD1 and/or myogenin by subsequent IHC. Case 4 was a bone sarcoma arising in the femur, initially diagnosed as an osteosarcoma, and subsequently reclassified as a primary sclerosing epithelioid fibrosarcoma of bone with the pathognomonic *EWSR1*::*CREB3L1* fusion and IHC positivity for MUC4. In Case 5, which was initially interpreted as juxtacortical myositis ossificans, the tumor was reclassified as a low-grade parosteal osteosarcoma of the proximal humerus after uncovering *MDM2* and *CDK4* gene amplifications by FISH analysis.

3.2 | Molecular investigation uncovered novel tumor entities and/or novel fusion genes

As listed in Table 2, in 14 (17%) cases molecular studies uncovered novel tumor entities and/or novel fusion genes: in eight cases by FISH, in five by targeted RNA sequencing, and in one case by Archer and FISH.

This group included eight spindle cell neoplasms with kinase gene fusions, six with *NTRK1* fusions and one each with *NTRK3* and *RAF1* rearrangement. There were two soft tissue tumors resembling

TABLE 1 Thirteen cases with revised diagnosis based on molecular testing.

Genetic finding	Original histological diagnosis	Revised histological diagnosis
EWSR1::*PBX3*	LG myofibroblastic sarcoma	Syncytial myoepithelioma
EWSR1::*TFCP2*	HG spindle cell sarcoma, bone	Rhabdomyosarcoma w/ *EWSR1-TFCP2*
FUS::*TFCP2*	Osteosarcoma	Rhabdomyosarcoma w/ *FUS-TFCP2*
EWSR1::*CREB3L1*	Osteosarcoma	Sclerosing epithelioid fibrosarcoma
MDM2 amplified	Juxtacortical myositis ossificans	LG parosteal osteosarcoma
NAB2::*STAT6*	Spindle cell sarcoma	Malignant solitary fibrous tumor
COL1A1::*PDGFB*	Spindle cell sarcoma	Fibrosarcoma ex DFSP
CIC rearranged	Small blue round cell tumor	CIC-rearranged sarcoma
BCOR::*CCNB3*	Spindle cell sarcoma	Sarcoma with *BCOR* genetic alterations
BCOR::*CCNB3*	Small blue round cell tumor	Sarcoma with *BCOR* genetic alterations
BCOR ITD	Myxoid spindle cell sarcoma	Sarcoma with *BCOR* genetic alterations (PMMTI)[a]
MYOD1 p.L122R	Spindle cell sarcoma	Spindle cell rhabdomyosarcoma
HEY1::*NCOA2*[b]	Synovial sarcoma	Mesenchymal chondrosarcoma

[a]PMMTI: primitive myxoid mesenchymal tumor of infancy.
[b]Targeted RNA sequencing; LG, low-grade.

TABLE 2 Fourteen uncommon or emerging soft tissue tumors with novel fusion genes.

Fusion gene	Original histological diagnosis	Revised histological diagnosis
LMNA::NTRK1	LG spindle cell sarcoma, MPNST-like	*NTRK*-rearranged spindle cell neoplasm
LMNA::NTRK1	LG spindle cell sarcoma, LPF-like	*NTRK*-rearranged spindle cell neoplasm
LMNA::NTRK1	HG spindle cell sarcoma, IFS-like	*NTRK*-rearranged spindle cell neoplasm
TPM3::NTRK1	HG spindle cell sarcoma, IFS-like	*NTRK*-rearranged spindle cell neoplasm
TPM3::NTRK1	HG spindle cell sarcoma, IFS-like, bone	*NTRK*-rearranged spindle cell neoplasm
TPR::NTRK1	LG spindle cell sarcoma, MPNST-like	*NTRK*-rearranged spindle cell neoplasm
NTRK3 rearranged[a]	HG spindle cell sarcoma, FS-like	*NTRK*-rearranged spindle cell neoplasm
RAF1 rearranged	LG spindle cell neoplasm, MPNST-like	*RAF1*-rearranged spindle cell neoplasm
PHF1::TFE3[b]	Ossifying fibromyxoid tumor (mOFMT)	mOFMT with *PHF1-TFE3* fusion
PHF1::TFE3[b]	Ossifying fibromyxoid tumor (mOFMT)	mOFMT with *PHF1-TFE3 fusion*
SRF::ICA1L[b]	LG spindle cell sarcoma NOS	Cellular myoid neoplasm w/ *SRF-ICA1L*
EWSR1::CREM[b]	Spindle cell sarcoma NOS	Epithelioid neoplasm w/ *EWSR1-CREM*
YAP1 rearranged	Retiform hemangioendothelioma	Retiform hemangioendothelioma
MEIS1::NCOA2[b]	Leiomyosarcoma	Spindle cell sarcoma w/ *MEIS1-NCOA2*

Abbreviations: HG, high-grade; IFS, infantile fibrosarcoma; LG, low-grade; LPF, lipofibromatosis; mOFMT, malignant ossifying fibromyxoid tumor; MPNST, malignant peripheral nerve sheath tumor.
[a]Archer Dx.
[b]Targeted RNA sequencing.

so-called lipofibromatosis-like neural tumor with haphazardly arranged monomorphic spindle cells infiltrating subcutaneous fat, showing co-expression of CD34 and S100, and harboring *LMNA::NTRK1* and *TPR::NTRK1*, respectively. Another subset of two cases consisted of spindle cell neoplasms with low-grade features resembling solitary fibrous tumor or low-grade MPNST, showing patternless growth, prominent stromal bands and perivascular rings of keloid-like collagen, and co-expression of CD34 and S100 (without SOX10 or STAT6). This subset included a jawbone (maxilla) tumor with *LMNA::NTRK1* and a soft tissue tumor with *RAF1* rearrangement. The third subgroup concerned tumors with a high-grade fibrosarcoma-like phenotype showing intersecting fascicles of CD34-positive and S100-negative monomorphic spindle cells with a high mitotic count. This subset included a bone sarcoma (F/22, sacrum) with *TPM3::NTRK1*, two pediatric soft tissue fibrosarcomas with *LMNA::NTRK1* and *TPM3::NTRK1*, respectively, and a colonic tumor (F/30) with *NTRK3* rearrangement.

In addition to the above kinase fusion positive spindle cell neoplasms, targeted RNA sequencing uncovered novel tumor entities and/or novel fusion genes in six other cases. Novel *PHF1::TFE3* fusions were found in two malignant ossifying fibromyxoid tumors with epithelioid neuroendocrine features and expression of cytokeratins and synaptophysin, by which they were initially confused with metastatic neuroendocrine carcinoma. A novel *SRF::ICA1L* fusion was detected in an unclassified low-grade spindle cell sarcoma with immature myoid cytomorphology and a smooth muscle-like immunophenotype. An *EWSR1::CREM* fusion was observed in a novel malignant epithelioid neoplasm with hybrid features between angiomatoid fibrous histiocytoma (cystic growth and lymphoid cuffing) and mesothelioma (serosal involvement, epithelioid phenotype, and cytokeratin and WT1 co-expression). A previously undescribed *YAP1* rearrangement was found in a retiform hemangioendothelioma and *MEIS1::NCOA2* fusion in a pelvic low-grade spindle cell sarcoma representing a novel tumor entity with predilection for genitourinary tract and gynecologic organs.

3.3 | Cases in which gene fusions confirmed the initial histological diagnosis

FISH studies confirmed the initial histological diagnosis in 21 cases (25%), as summarized in Table S1.

3.4 | Subset of soft tissue tumors in which no genetic alterations were found

In 36 (43%) soft tissue ($n = 32$) and bone ($n = 4$) tumors molecular testing was negative, as shown in Table S2. All 36 cases were studied by FISH, 6 by additional targeted (TruSight) RNA sequencing and one by additional Archer NGS.

4 | DISCUSSION

The complex histopathologic classification of soft tissue tumors has benefitted significantly from combining different scientific tools, particularly visualization of protein expression by IHC and detection of molecular genetic abnormalities by FISH or NGS methods. In this study, we investigated the added diagnostic value of molecular genetics to expert review in a series of uncommon mesenchymal tumors selected from the pathology archives of a tertiary sarcoma center. The retrospective case selection focused on rare and diagnostically challenging soft tissue tumors spanning over three decades, which were mostly under-recognized as representing new or emerging pathologic entities and characterized by recently described or novel fusion genes. During case selection we mainly included tumors with monomorphic spindle or round cell morphology in addition to cases with epithelioid features or vascular morphology, since it is generally appreciated that the large majority of tumors with balanced chromosomal translocations and gene fusions show uniform histologic features as opposed to tumors with unbalanced chromosomal aberrations and pleomorphic microscopic features. By a combined approach, using FISH (a wide range of custom BAC probes covering most known fusions) and auxiliary targeted RNA sequencing (performed in 15% of cases), specific gene fusions or rearrangements were detected in 48 of 84 cases (54%) and tumor-specific gene mutations or amplifications in three additional cases. Thus, disease-defining molecular alterations were encountered in 57% of tumors. Three cohorts with positive molecular tests could be discerned: cohort A (13 cases; 15%) in which molecular analysis refined or changed the diagnosis; cohort B (14 cases; 17%) with novel tumor types and/or novel fusion genes in tumor subsets; and cohort C (21 cases, 25%) where molecular tests confirmed the initial histological diagnosis.

The incremental analytical value of molecular diagnostics in soft tissue tumor pathology has been addressed by other investigators. For instance, in an expert diagnostic setting, a large European multicenter study showed that molecular tests (FISH and/or RT-PCR) confirmed a probable or possible (but uncertain) pathologic diagnosis in 26% and 12% of translocation-associated sarcomas (including synovial sarcoma, Ewing sarcoma, alveolar rhabdomyosarcoma, and myxoid liposarcoma), and 31% and 19% of atypical lipomatous tumors with *MDM2* amplification, respectively.[10]

More recently, sophisticated NGS with broader coverage, such as targeted RNA sequencing, have become available.[11,12] In contrast to FISH and RT-PCR, targeted RNA sequencing methods enable the simultaneous detection of multiple gene fusions and have the advantage to identify both known and unknown gene fusions, albeit at the expense of higher costs.[12] Recently, clinical applications of targeted RNA sequencing methods for sarcoma diagnosis have been reported in the literature.[11,13–16] As an example, Zhu et al.[15] applied an anchored multiplex RNA sequencing assay that targets 62 specific genes involved in recurrent rearrangements in solid tumors including sarcomas (MSK-Fusion Solid) on 175 soft tissue tumors and 9 osteosarcomas and found fusion transcripts in 43% of cases, including 6 cases (3%) with novel fusion partners.

With the advent of the advanced molecular genetic methods described above, the histopathologic classification of soft tissue tumors has become more objective and refined, as reflected by the appearance of several new entities and subtypes in the 2020 WHO classification blue book. Not surprisingly, many of these newly described tumor types and subtypes were not recognized before 2020 and, therefore, mistaken for other tumors. Among the 13 cases (15%) in which molecular genetic analysis altered the initial diagnosis, clinical management and/or tumor prognosis was affected in 5 cases (6%), 4 of them being discussed here.

The presence of an *EWSR1::PBX3* fusion reversed a diagnosis of low-grade myofibroblastic sarcoma to a syncytial myoepithelioma. First described in 2013, syncytial myoepithelioma represents a benign skin tumor with uniform spindle cell morphology that may recur when incompletely excised.[17,18]

Two cases harbored *EWSR1/FUS::TFCP2* gene fusions, which are characteristic of a subset of recently described intraosseous rhabdomyosarcomas.[19,20] As detected by Xu et al.,[20] head and neck rhabdomyosarcomas with *TFCP2* fusions show a peculiar immunoprofile, with co-expression of myogenic markers, epithelial markers, and ALK. These molecularly defined tumors pursued a very aggressive clinical course with an estimated 2-year-survival of 35%.

Another pitfall includes tumors that have an unusual clinical presentation, as exemplified by the case of sclerosing epithelioid fibrosarcoma (SEF) presenting in bone. In this patient, SEF was mistaken for a high-grade skeletal osteosarcoma due to presence of dense collagenous matrix that simulated osteoid matrix. However, MUC4 expression by IHC initiated additional molecular genetic analysis, which rendered a correct diagnosis. Tsuda et al.[21] summarized the literature of 21 cases, including this case, and reported that bone SEF showed an aggressive clinical course with metastasis developing in about half of cases with available follow-up. Importantly, skeletal SEF does not seem to benefit from chemotherapy regimens administered for osteosarcoma or Ewing sarcoma.

The novel tumor entities and/or novel fusion genes encompassed in this study comprised 14 cases (17%). Eight cases belonged to a morphologic spectrum of distinct spindle cell tumors with frequent co-expression of S100 and CD34 and harboring various kinase fusions, including *NTRK1* and *RAF1*. Two cases represented lipofibromatosis-like neural tumor (LP-FNT), a neoplasm first described by Agaram in 2016.[22] LP-FNT closely resembles lipofibromatosis and may be confused with dermatofibrosarcoma protuberans or neurofibroma, due to uniform spindle cell tumors infiltrating

subcutaneous fat in a reticular fashion. The other six cases in this morphologic spectrum were either fascicular spindle cell tumors with uniform morphology and low mitotic rate, often misinterpreted as low-grade MPNST and solitary fibrous tumor, or fascicular spindle cell tumors with a malignant phenotype resembling pediatric or adult fibrosarcoma. Although not entirely specific, tumors with *NTRK1-3* fusions may be identified using Pan-TRK immunohistochemistry. The biologic behavior of these spindle cell neoplasms with kinase fusions appears to correlate with histologic grade. Tumors with low-grade morphology (low cellularity and mitotic activity) show an indolent course and favorable outcome, while tumors with fascicular growth, increased cellularity, and mitotic activity are prone to distant metastases and death from disease. Identification of *NTRK* rearranged spindle cell sarcomas is clinically relevant since the development of selective TRK inhibitors has opened new avenues for targeted therapy. Further details of these neoplasms are described in the original publications by our group.[6,23–25]

In six cases novel gene fusions (two cases with *PHF1*::*TFE3*, and one case each with *SRF*::*ICA1L*, *EWSR1*::*CREM*, *YAP* rearrangement, and *MEIS1*::*NCOA2*) were encountered in known tumor entities, as recently described by us in previous articles, and summarized here. Novel recurrent *PHF1*::*TFE3* fusions were reported in a subset of five cases of OFMT.[26] The histologic spectrum of these tumors resembled that of OFMT cases with other *PHF1* fusions. However, none of the tumors showed areas of peripheral ossification and most were negative for S100. Two tumors displayed neuroendocrine features by which these were initially confused with metastatic neuroendocrine carcinoma. Overexpression of TFE3 protein may serve as a reliable IHC screening tool. Three tumors showed aggressive clinical behavior.

Novel *SRF*::*ICA1L* fusions were found in a small distinct group of cellular myoid spindle cell tumors with incomplete smooth muscle cell differentiation, originally thought to be low-grade leiomyosarcomas.[27] These tumors were composed of cellular fascicles of uniform eosinophilic spindle cells often with increased mitotic activity embedded in densely hyalinized stroma. These neoplasms are probably histogenetically related to cellular perivascular myoid tumors with *SRF-RELA* fusions.[28]

The clinicopathologic spectrum of soft tissue tumors with fusions between *EWSR1* or *FUS* and one of the genes encoding CREB-transcription factors, has recently been expanded by a unique series of malignant epithelioid neoplasms with *EWSR1*::*CREM* (and less often *FUS*::*CREM* or *EWSR1*::*ATF1*) fusions. Microscopically, these tumors show hybrid features between angiomatoid fibrous histiocytoma (cystic growth and lymphoid cuffing) and mesothelioma (peritoneal/pleural involvement, epithelioid phenotype, and cytokeratin and WT1 co-expression). More than half (7/13) of the patients in this first series presented with or developed metastases indicative of malignant biologic behavior.[29]

Retiform and composite hemangioendotheliomas (RHE and CHE) are very rare vascular tumors characterized by the presence of arborizing vascular channels lined by endothelial cells with a hobnail morphology. These vascular neoplasms are locally infiltrative and rarely metastasize. Our group recently identified novel *YAP1* rearrangements in a subset of these tumors.[30]

MEIS::*NCOA2* fusions have been reported in rare and previously unrecognized undifferentiated spindle cell sarcomas that have a predilection for the genitourinary tract and gynecologic organs.[31,32] The morphologic spectrum exhibits a wide range of appearances. In addition to spindle cells with whorling or storiform growth patterns, rare tumors show sheets of ovoid cells, microcystic change, and increased nuclear pleomorphism. The immunoprofile of this tumor is nonspecific. Clinical follow-up suggests low-grade malignant behavior with multiple local recurrences, sometimes many years after initial diagnosis.[31]

In a subset of 36 tumors (43%) molecular testing was negative. These cases were studied with FISH, whereas RNA sequencing was applied in seven cases. It cannot be excluded that a more comprehensive molecular testing might have detected molecular genetic alterations.

In conclusion, in this retrospective and historical review, we have shown that advanced molecular genetic analysis of mostly unclassifiable soft tissue tumors unraveled new gene fusions that defined novel and rare but distinct tumor entities or tumor subsets in a significant amount (17%) of cases shared by international expert pathologists. Moreover, molecular genetics refined tumor diagnosis in 15% of assorted diagnostically challenging cases in which the initial clinicopathologic diagnosis was uncertain.

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SUPPORTING INFORMATION

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

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