Introduction

Benign and malignant soft-tissue neoplasms are rare and have diverse clinical and biological behavior. This heterogeneous group of mesenchymal tumors are diagnosed and classified using criteria from the World Health Organization (WHO); more than 100 benign and malignant soft-tissue tumor entities have been defined. These tumors are predominantly classified by their histogenesis and incorporated cytogenetic and molecular genetic information. In practice, pathologists use a morphological pattern recognition approach to analyze the histological information. The tumors can be arbitrarily divided into different morphological groups such as round cell, spindle, epi-
thelioid/polygonal, pleomorphic, adipocytic, myxoid, and giant cell, among others. Each group consists of a collection of pertinent differential diagnoses from various histogenesis. Immunohistochemistry (IHC) is an important ancillary tool to aid in diagnosis because it can provide information about tumor histogenesis. 2

Cytogenetic and molecular genetic analyses of mesenchymal neoplasms have revealed 2 major groups: (1) soft-tissue neoplasms associated with complex karyotypes, and (2) soft-tissue neoplasms characterized by recurrent chromosomal structural abnormalities, gene amplification, mutations, or loss of heterozygosity. Although soft-tissue neoplasms with complex karyotypes do not demonstrate reproducible molecular alterations precluding the use of routine molecular testing for diagnostic purposes, the latter group can benefit from routine molecular testing to facilitate a definitive diagnosis.

With the continuous advancement in our understanding of the cytogenetic and molecular genetic pathology of soft-tissue neoplasms, pathologists evaluating soft-tissue tumors must rely on histopathology for diagnosis and integrate cytogenetic and molecular genetic information into diagnostic algorithms for accurate diagnoses. Fusion genes resulting from chromosomal rearrangements in soft-tissue neoplasms represent an important part of the pathologist’s diagnostic algorithm for tumor classification. 3 Typically, chromosomal translocations and fusion genes are present in the early phase of disease and persist in disease progression, metastatic development, or both, demonstrating that molecular testing for these types of genetic alterations would be diagnostically useful. Furthermore, the identification of particular soft-tissue tumoral genetic aberrations is important for therapy.

In this article, we provide an overview of the important recurrent or tumor-specific molecular aberrations associated with soft-tissue tumors, their differential diagnoses, the role of molecular testing in the clinical management of soft-tissue tumors, and potentially relevant therapeutic targets.

Round Cell Tumors
Small round blue cell tumors encompass a group of primitive-appearing neoplasms difficult to diagnosis on histopathology alone. The differential diagnoses include a neoplasm of epithelial, mesenchymal, hematopoietic, or melanocytic origin. IHC may be helpful in narrowing the differential, as it is particularly effective for identifying carcinoma, neuroendocrine carcinoma, lymphoma, plasmacytoma, and melanoma. However, IHC is often ineffective in distinguishing between different round cell sarcomas. 4 Most round blue cell tumors are associated with a tumor-specific chromosomal translocation, which is diagnostically valuable to pathologists. 5,6 Ewing sarcoma, the second most common sarcoma of bone in children and young adults, occurs in extraskeletal sites in about 10% to 20% of cases. 7 The histopathology and IHC profile may mimic poorly differentiated synovial sarcoma with round cell morphology. 8,9 Molecular testing is often necessary to establish an accurate diagnosis. Ewing sarcoma is known to demonstrate recurrent translocations. t(11;22)(q24;q13) is detected in approximately 85% of Ewing sarcomas and results in the fusion of EWSR1 on chromosome 22 to a member of the ETS gene family of transcription factors, FLI1, on chromosome 11. 11,12 Approximately 5% to 10% of Ewing sarcoma cases are associated with t(21;22)(q22;q12), which is a fusion of EWSR1 and ERG. 13 Rare cases (<1%) of Ewing sarcoma demonstrate FUS-ERG or FUS-PEF fusions, likely because the fused in sarcoma (FUS) protein amino-acid sequence is similar to Ewing sarcoma breakpoint region 1 (EWSR1) and is considered part of the tet methylcytosine dioxygenase (TET) family of proteins. 14 The EWSR1 gene rearrangement is shared by numerous sarcomas, including Ewing sarcoma, myxoid liposarcoma, angiomatoid fibrous histiocytoma, myoepithelioma, myoepithelial carcinoma, mixed tumor of soft tissue, clear cell sarcoma of soft tissue, extraskeletal myoid chondrosarcoma, malignant gastrointestinal neuroectodermal tumor (previously referred to as clear cell sarcoma-like gastrointestinal tumor), low-grade fibromyxoid sarcoma, and desmoplastic small round cell tumor. 5,6,15 Recently, the WHO classification described a group of small round cell sarcomas with features similar to Ewing sarcoma (“Ewing-like” sarcoma) but classified the group as undifferentiated round cell sarcomas because the tumors either demonstrated rearrangements of EWSR1 with non-ETS gene partners or lacked rearrangement of EWSR1 or other TET family members. 14,16-22 A small subset of these Ewing-like sarcomas (also known as undifferentiated round cell sarcomas) demonstrates CIC-DUX4 gene fusion resulting from t(4;19)(q35;q13) or t(10;19)(q26;q13) and is primarily described within the pediatric population. 19,23-25 Moreover, the CIC-FOXO4 gene fusion, t(X;19)(q13;q13.3), has also been identified in 2 cases of Ewing-like sarcoma. 26,27 In addition, an emerging group of Ewing-like sarcomas with the BCOR-CCNB3 fusion gene, arising from an X chromosome paracentric inversion, may represent a biologically distinct entity within undifferentiated round cell sarcomas. 28-30 Simple reverse transcription polymerase chain reaction (RT-PCR) assay in conjunction with cyclin B3 (CCNB3) IHC can be useful in diagnosing these tumors. Whether these Ewing-like sarcoma cases should be classified as Ewing sarcoma or represent separate tumor types is unknown, but they have similar treatment. 31 Further identification and clinical follow-up of Ewing-like sarcomas with...
distinct gene rearrangements are warranted to evaluate patient outcomes.

Alveolar rhabdomyosarcoma (ARMS), often seen in adolescents and young adults, carries 2 specific chromosomal translocations.32 t(2;13)(q22;q14) occurs in approximately 60% of cases, whereas t(1;13) (p36;q14) occurs in a smaller subset.33-36 These translocations involve FOXO1A on chromosome 13q14 and either PAX3 (2q35) or PAX7 (1p36).35 No specific chromosomal abnormality has been described in embryonal rhabdomyosarcoma.37 Thus, the identification of t(2;15) or t(1;13) is diagnostically valuable and prognostically significant because the PAX-FOXO1A fusion status imparts an unfavorable outcome for children with rhabdomyosarcoma.3 A report of event-free survival and overall survival rates at 5 years correlated histopathological subtype with PAX-FOXO1A status and showed that fusion-negative ARMS (lacking a detectable fusion of PAX3 or PAX7 with FOXO1A) had an outcome similar to embryonal rhabdomyosarcoma and superior event-free survival rates compared with ARMS with either fusions of PAX3 or PAX7 with FOXO1A when given therapy designed for children with intermediate-risk rhabdomyosarcoma.38,39 These findings support the incorporation of PAX-FOXO1A fusion status into risk stratification and treatment.40

Desmoplastic small round cell tumor primarily affects children and young adults and typically presents with widespread abdominal serosal involvement, exhibits polyphenotypic differentiation, and has a consistent association with t(11;22)(p13;q12) and fusion of EWSR1 and WT1.41-43 Because EWSR1 is shared by other sarcomas, identification of the partner gene is warranted for a specific diagnosis (Fig).

Extraskelatal myxoid chondrosarcoma (EMC) occurs in adults and, rarely, children, demonstrating no convincing evidence of cartilaginous differentiation; it is characterized by NR4A3 rearrangement.44-46 More than 90% of cases of EMC harbor either t(9;22) (q22;q12) or, less frequently, t(9;17)(q22;q11) resulting in the fusion of NR4A3 (9q22) to either EWSR1 (22q12) or TAF15 (17q12).47-50 IHC is not helpful in the diagnosis of EMC; therefore, molecular testing is essential. For example, EMC and mixed tumor of soft-tissue and myoepithelioma have overlapping histological and IHC features; thus, these entities present a diagnostic challenge. Considering the difference in therapies and outcomes of EMC and mixed tumor of soft-tissue and myoepithelioma, an accurate and definitive diagnosis is critical for the management of the condition. Because both tumors may show EWSR1 gene rearrangement and 75% of EMC cases and 45% of mixed tumor of soft-tissue and myoepithelioma cases exhibit this rearrangement,51 fluorescence in situ hybridization (FISH) testing of NR4A3 would be the ideal test to perform. Positive NR4A3 gene rearrangement, which represents a component of t(9;22) located on chromosome 9, would confirm the diagnosis of EMC. This is because NR4A3 fusions are unique or specific for EMC and are not present in other sarcomas; in fact, NR4A3 fusions are present in more than 90% of EMC cases.1,5

Spindle Cell Tumors

Clinically important, tumor-specific genetic abnormalities have been identified in spindle cell neoplasms. These genetic alterations are diagnostically helpful because the differential diagnoses of spindle cell neoplasms in soft tissue are diverse. Differentiation between tumor subtypes, such as monophasic synovial sarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor, fibrosarcoma, and gastrointestinal stromal tumor (GIST), may be challenging depending on specimen adequacy (quantity and quality), the immunostaining profile, and clinical presentation. Once the findings on the standard histopathological examination and IHC workup are inconclusive, molecular testing is a necessary adjunct. For example, synovial sarcoma can be difficult to discriminate from solitary fibrous tumor, malignant peripheral nerve sheath tumor, or sarcomatoid carcinoma. Given the ability to detect 95% of cases of synovial sarcoma through identification of its recurrent reciprocal t(X;18)(p11.2;q11.2), which fuses SYT (18q11) to 1 of the 3 homologous genes on Xp11 (SSX1, SSX2, or SSX4), molecular testing is essential for classification and treatment.1,52,53

Dermatofibrosarcoma protuberans, a low-grade spindle cell neoplasm, harbors either supernumerary ring chromosomes or unbalanced derivatives of t(17;22)(q22;q13). Both aberrations contain a chimeric gene that fuses COL1A1 with PDGFB commonly identified by multiplex RT-PCR or, preferably, FISH.54-59 Typically, dermatofibrosarcoma protuberans is treated

Fig. — Tumor positive for rearrangement of the EWSR1 (22q12) locus. This can be seen in DSRCT, but it is not specific because other sarcomas may share this marker. EWSR1-WT1 fusion (yellow signal) is detected by fluorescence in situ hybridization, which is specific and confirms the diagnosis of DSRCT. © Moffitt Cancer Center. Photographer: Julia A. Bridge, MD. DSCRT = desmoplastic small round cell tumor.
by wide local excision; however, in cases of unresectable and metastatic disease, targeted therapy with imatinib mesylate may be clinically useful.60,61 Molecular testing is important for identifying patients who may have a clinical response.62 A total of 10% to 15% of cases demonstrate fibrosarcomatous progression and may not show a response.1

A rare malignant fibroblastic neoplasm, low-grade fibromyxoid sarcoma, consistently has either FUS-CREB3L2 gene fusion or, less frequently, FUS-CREB3L1 gene fusion and results in t(7;16) or t(11;16), respectively.63,64 A small number of low-grade fibromyxoid sarcomas harbor EWSR1-CREB3L1 gene fusion.65 Sclerosing epithelioid fibrosarcoma (SEF), which is another rare malignant fibroblastic tumor with considerable morphological overlap with low-grade fibromyxoid sarcoma, harbors frequent EWSR1 gene rearrangements; a minority of cases exhibits FUS-CREB3L2 fusions.96 Both low-grade fibromyxoid sarcoma and SEF show mucin 4 expression by IHC.67 Thus, the presence of morphological features reminiscent of low-grade fibromyxoid sarcoma in SEFs as well as the presence of FUS rearrangements suggest that a possible relationship may exist between the 2 entities and that they are perhaps part of a disease spectrum.

Congenital or infantile fibrosarcoma, which is historically similar to adult fibrosarcoma, has a distinctive t(12;15)(p13;q26) resulting in the ETV6-NTRK3 fusion. This neoplasm occurs in children within the first year of life and the histological features may mimic other pediatric spindle cell neoplasms, such as infantile fibromatosis and infantile myofibromatosis or myofibroma.4,68,69 It is often difficult to cytogenetically identify the ETV6-NTRK3 fusion, so it is typically detected by FISH or PCR.70,71

Congenital or infantile spindle cell rhabdomyosarcoma is another diagnostic dilemma in this group. This tumor typically lacks PAX3-FOXO1 and PAX7-FOXO1 fusions but exhibits recurrent NCOA2 rearrangements.72

A breakthrough in the molecular pathology of solitary fibrous tumor (SFT) is the identification of the recurrent NAB2-STAT6 fusion by integrative sequencing.73 NAB2-STAT6 has been established as the defining driver mutation of SFT. Fusion variants NAB2ex4-STAT6ex2/3 and NAB2ex6-STAT6ex16/17 have been identified; the former fusion occurs in pleuropulmonary SFTs and mostly exhibits a benign behavior, whereas the latter fusion occurs in deep-seated extrapleural SFTs and has more aggressive behavior.74 STAT6 IHC can be a useful adjunct tool in the diagnosis of SFT, particularly in cases with aberrant morphology and limited material.75,76 Of note, STAT6 amplification has been described in a small subset of dedifferentiated liposarcomas resulting in STAT6 IHC expression, which can be a potential pitfall, particularly in retroperitoneal masses for which the differential diagnosis also includes SFT.77

GIST is a mesenchymal tumor characterized by activating oncogenic mutations rather than specific translocations or fusion genes and has a clinical spectrum that ranges from benign to malignant.1,78 Most GISTs harbor a mutation in 1 of 3 sites: KIT exon 9, KIT exon 11, or PDGFRα exon 18.79,80 Tyrosine kinase inhibitors (eg, imatinib mesylate, sunitinib malate) have been used to successfully treat GISTs.81 Mutation analysis has become essential to evaluate patient prognosis, make treatment decisions, predict treatment response, and select appropriate dosages.78,82-84 Most laboratories utilize PCR to amplify the most commonly mutated exons with subsequent direct sequencing analysis of the amplified exon.78 Recently, BRAFV600E mutation was identified in patients with GIST lacking KIT and PDGFRα mutations.85,86 In addition, cases of GIST are typically associated with the Carney triad and Carney–Stratakis syndrome when they show mutations in SDH-related genes.87,88 These tumors have a distinct morphology.

**Lipomatous Tumors**

A few genetic alterations exist that involve lipomatous neoplasms and aid in discriminating tumor subtypes with similar morphological features. Myxoid/round cell liposarcoma may be challenging to diagnose when the differential diagnosis includes other myxoid neoplasms such as EMC or myxofibrosarcoma — this is particularly true on biopsy specimens. However, approximately 95% of cases of myxoid liposarcoma demonstrate the FUS-DDIT3 chimeric gene due to reciprocal t(12;16)(q13;p11).43,89,90 Incorporating molecular testing with histopathology is useful in these cases because myxoid liposarcomas are sensitive to radiation therapy and select patients receive neoadjuvant therapy.91

Another diagnostic challenge is the differentiation between lipoma and atypical lipomatous tumor (ALT)/well-differentiated liposarcoma (WDL). Lipoma is a mass-forming tumor with mature-appearing fat; however, differently from normal fat, lipoma exhibits a HMGA2 translocation.92 The diagnostic atypical cells in fibrous septa can be scarce, the atypia may be cytologically subtle, or the ALT/WDL may demonstrate a lipoma-like morphological pattern. ALT/WDL is likely to recur and carries the risk of dedifferentiation, which results in a poor prognosis depending on the anatomical location. For clinical purposes, molecular testing is useful in discriminating between lipoma and ALT/WDL, and this is particularly true if clinicoradiological information is inconclusive. Furthermore, dedifferentiated liposarcoma with predominant, high-grade dedifferentiated areas may be difficult
to discriminate from other high-grade pleomorphic sarcomas. Molecular testing that identifies supernumerary ring chromosomes and/or giant-marker chromosomes corresponding to amplification of the 12q13-15 band support a diagnosis of ALT/WDL or dedifferentiated liposarcoma depending on histopathology.3,5 Of note, some lipomas have gains of the mouse double minute 2 homolog (MDM2) and some other mesenchymal tumors can have MDM2 amplification or expression (Table 1).23,93

Tumor of Uncertain Histogenesis

Tumors of uncertain histogenesis have a range of morphological features from round cells to spindle cells to epithelioid/polygonal cells. Some have characteristic translocations as well as unique and recognizable histopathology and clinical features. However, significant histopathological and IHC overlap exists between clear cell sarcoma (malignant melanoma of soft parts) and conventional melanoma. Clear cell sarcomas possess a recurrent EWSR1-ATF1 fusion in more than 90% of cases from a reciprocal t(12;22) (q13;q12).94,95 A related variant, t(2;22)(q32.3;q12), the EWSR-CREB1 fusion, has been reported in a small subset of clear cell sarcomas.96,97 Molecular testing is necessary to establish an unequivocal diagnosis of clear cell sarcoma.98 In addition, rare clear cell sarcomas have been reported to contain BRAF mutations, possibly representing a therapeutic target.99,100

Of note, alveolar soft-part sarcoma, which is a rare sarcoma composed of nests of tumor cells lined by sinusoidal vascular channels that may have eosinophilic, granular-to-clear cytoplasm, may be mistaken for renal cell carcinoma or perivascular epithelioid cell tumor.1 On IHC, alveolar soft-part sarcoma is negative for keratin, epithelial membrane antigen, and HMB-45. Molecular testing is diagnostically helpful to confirm the diagnosis. Alveolar soft-part sarcoma is defined by recurrent, unbalanced der(17)t(X;17) (p11;q25) involving the fusion of TFE3 (Xp11) and ASPSCR1 (17q25).101,102 Although ASPSCR1-TFE3 appears specific for alveolar soft-part sarcoma, this same gene fusion has been identified in a small subset of renal cell carcinomas.103,104

Soft-tissue angiofibroma is a relatively new histologic entity characterized as a benign, fibrovascular soft-tissue tumor of uncertain cellular origin. This tumor is characterized by an AHRR-NCOA2 fusion resulting from t(5;8) (p15;q15).105,106 The evaluation of NCOA2 rearrangements using FISH is a practical method to confirm a diagnosis of soft-tissue angiofibroma.105

Myxoid Sarcomas

Myxoid tumors do not represent a standalone group of tumors per the WHO criteria.2 However, this type of tumor is commonly encountered in clinical practice. The tumor cells may have various morphological features and they produce abundant myxoid stroma. The common genetic change of this group of tumor is summarized in Table 2. Among this group, myxoinflammatory fibroblastic sarcoma exhibits t(1;10) involving TGFB3 and MGEA5.107,108

Conclusions

Soft-tissue tumors form a heterogeneous and complex group with diverse morphology and, at times, a non-specific immunohistochemical profile. The availability of tumor-specific genetic markers has modified the routine diagnostic workup of soft-tissue neoplasms. The cytogenetic and molecular identification of chromosomal translocations and their associated gene fusions, loss, and gains of specific chromosomal regions and activating or inactivating mutations of select oncogenes or tumor-suppressor genes have contributed to therapeutic and prognostic assessments and the classification of soft-tissue neoplasms. The integration of morphology, immunohistochemistry, and molecular pathology is essential for appropriate and accurate diagnostic algorithms. A better understanding of how to apply the molecular pathology of soft-tissue neoplasms will provide new roles for pathologists in the diagnostics and targeted therapy of these tumors.3,109

<table>
<thead>
<tr>
<th>Tumor Type: angiofibroma</th>
<th>MDM2 by FISH</th>
<th>MDM2 by IHC</th>
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<tbody>
<tr>
<td>Lipoma</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Atypical lipomatous tumor/well-differentiated liposarcoma</td>
<td>+</td>
<td>+ (nuclear)</td>
</tr>
<tr>
<td>Dedifferentiated liposarcoma</td>
<td>+</td>
<td>+ (diffuse, nuclear)</td>
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<tr>
<td>Intimal sarcoma</td>
<td>+</td>
<td>+ (≤ 70%)</td>
</tr>
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FISH = fluorescein in situ hybridization, IHC = immunohistochemistry, MDM2 = mouse double minute 2 homolog.

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Table 2. — Genetics of Myxoid Sarcomas

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Defect</th>
<th>Gene</th>
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<tbody>
<tr>
<td>Myxoma</td>
<td>Activating Gs-α mutations</td>
<td>GNAS</td>
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<tr>
<td>Low-grade fibromyxoid sarcoma/hyalinizing spindle cell tumor with giant rosettes</td>
<td>t(7;16)(q33;p11)</td>
<td>CREBL2-FUS</td>
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<tr>
<td>Myxoid liposarcoma</td>
<td>t(12;16)(q13;p11)</td>
<td>DDIT3-FUS</td>
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<tr>
<td>Extrapleural myxoid chondrosarcoma</td>
<td>t(9;22)(q22;q12)</td>
<td>NRI4A3-EWSR1</td>
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<tr>
<td>Myxofibrosarcoma</td>
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<td>None</td>
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<tr>
<td>Myxoinflammatory fibroblastic sarcoma</td>
<td>t(1;10)</td>
<td>TGFB3-MGEA5</td>
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References