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The Role of Molecular Testing in the Diagnosis of Cutaneous Soft Tissue Tumors

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A number of soft tissue tumors are characterized by recurring genetic abnormalities. The identification of these abnormalities has advanced our understanding of the biology of these tumors and has led to the development of molecular tests that are helpful diagnostically. This review will focus on the application of molecular diagnostic testing in select mesenchymal tumors of the dermis and subcutis.
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There have been great advances in recent years in the genetic characterization of cutaneous mesenchymal tumors. A growing number of mesenchymal neoplasms are being defined by recurring genetic events that make up a so-called genetic signature, most often in the form of chromosomal translocations that result in specific oncogenic fusion genes. Knowledge and identification of these recurrent molecular aberrations allow for more accurate diagnosis of mesenchymal tumors and are advancing our understanding of their underlying biology. The identification of these disease-defining genetic signatures is the basis for the development of targeted therapies. Molecular testing is thus gaining an increasingly important role in complementing histologic examination and immunohistochemistry. This review will focus on select cutaneous mesenchymal neoplasms with known molecular hallmarks that are useful diagnostically.

Dermatofibrosarcoma Protuberans

Dermatofibrosarcoma protuberans (DFSP) is a cutaneous fibrohistiocytic tumor of intermediate malignancy that most frequently affects young-to-middle–aged adults on the trunk or, less commonly, on the extremities as well as the head and neck. The typical presentation is of a nodule with slow but persistent growth, often over several years. DFSP has a propensity for local recurrence, but only rarely metastasizes. Management requires adequate margin control either by wide-local excision or Mohs surgery, the choice of which depends on individual tumor and patient characteristics as well as institutional experience.[1,2]

Histologically, DFSP is characterized by a tight storiform or cartwheel growth pattern of uniform and relatively bland spindled cells, with extensive infiltration of the dermis and subcutaneous fat (Fig. 1). The neoplastic cells are usually marked strongly and diffusely with CD34. Several histologic variants of DFSP exist and include pigmented DFSP (Bednar tumor), giant cell fibroblastoma, and rare granular cell and myxoid variants.[3-5] Fibrosarcomatous transformation of DFSP is heralded by fascicular growth of more atypical spindled cells with greater mitotic activity (Figs. 2 and 3). Fibrosarcomatous transformation is thought to represent tumor progression. Although some studies have not demonstrated an increased risk of metastasis associated with fibrosarcomatous transformation, there is a growing consensus that the risk of metastasis is significantly increased with this finding.[6-9] Fibrosarcomatous areas may overrun the DFSP component, and these areas often lose CD34 reactivity.

Translocations forming the fusion gene collagen type I alpha 1 (*COL1A1*)-platelet-derived growth factor (*PDGFβ*) are the molecular hallmark of DFSP. This oncogenic chimer results from fusion of *COL1A1* promoter on 17q22 to *PDGFβ* on 22q13. In one study of 27 patients, *COL1A1-PDGFβ* was demonstrated in nearly all cases of DFSP examined by multiplex reverse transcription polymerase chain reaction (RT-

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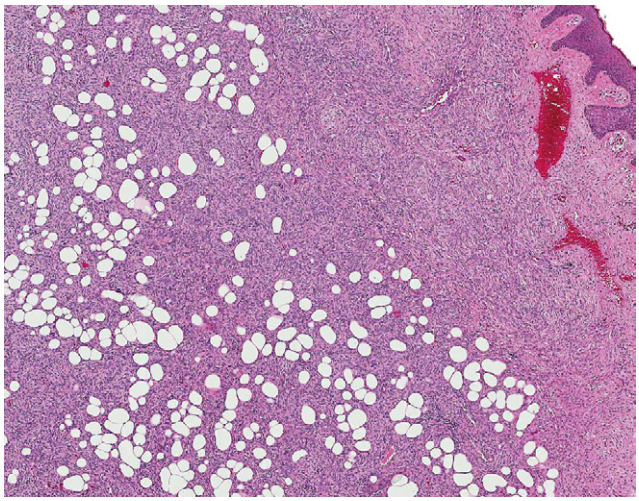

Figure 1 Dermatofibrosarcoma protuberans (DFSP). Diffuse infiltration through the subcutaneous fat imparts a characteristic "honeycomb" appearance.

PCR) and fluorescence RNA in situ hybridization (FISH).[10] *COL1A1-PDGFβ* is also seen in pigmented DFSP, giant-cell fibroblastoma, and myxoid and rare granular cell variants.[3-5] Fibrosarcomatous transformation of DFSP also demonstrates the same fusion.[11,12] The *COL1A1-PDGFβ* fusion gene most often results from a supernumerary ring chromosome 22 that contains low-level–amplified sequences from chromosomes 17q22ter and 22q10-q13.1.[13-15] Less commonly, *COL1A1-PDGFβ* results from an unbalanced translocation t(17;22)(q22;q13). There is significant breakpoint variability within the *COL1A1* gene, which can occur anywhere between exons 6 and 47.[16] Exon 2 of *PDGFβ* is consistently present in the *COL1A1-PDGFβ* fusion.

Tumorigenesis in DFSP is thought to result from an autocrine-loop mechanism involving platelet-derived growth factor receptors on cell membranes.[17] The *COL1A-PDGFβ* protein product is structurally similar to wild-type growth factor

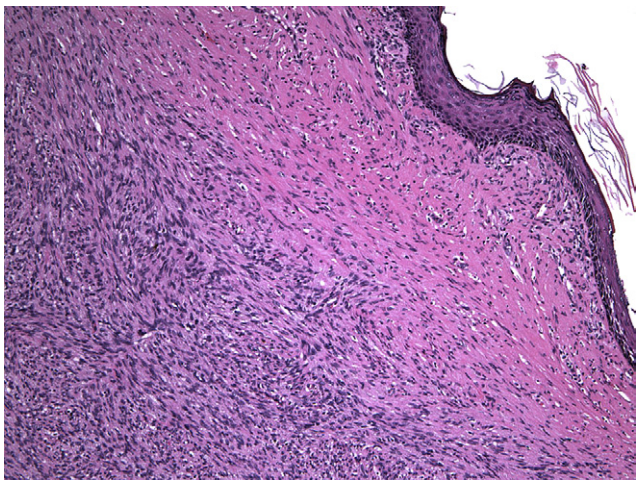

Figure 2 Fibrosarcomatous transformation in DFSP. The tumor is characterized by transition to densely cellular fascicles with significant cytologic atypia.

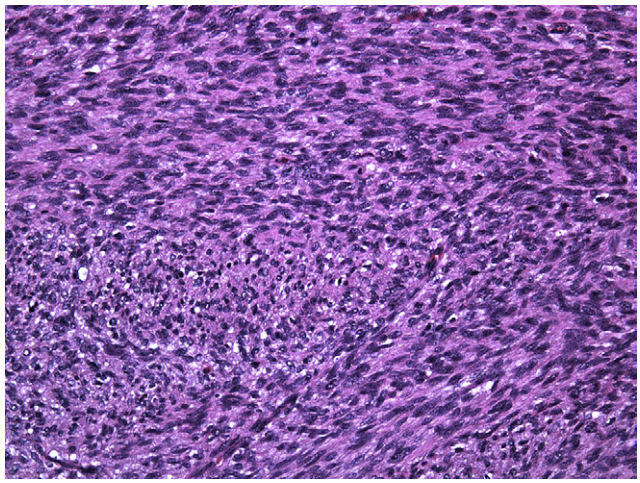

Figure 3 Higher magnification of fibrosarcomatous component of DFSP with significant nuclear atypia and prominent mitotic activity.

PDGF-BB that, in turn, acts via PDGFα and PDGFβ tyrosine kinase receptors.[17,18] Targeted therapy with the tyrosine kinase inhibitor, imatinib mesylate, has recently received Food and Drug Administration approval for unresectable, metastatic, or recurrent DFSP. Recent clinical trials have shown response rates of approximately 50% to this drug.[19] Neoadjuvant imatinib may also be of utility in reducing the surgical morbidity in large tumors in which wide margins may be difficult to achieve.

In practice, molecular testing in DFSP has utility both as a diagnostic aid in challenging cases and to guide therapy. Most cases of DFSP are easily diagnosed on the histopathologic features. That said, significant diagnostic challenges might arise in, for example, CD34-negative tumors that are superficially sampled or tumors with variant histology or unusual presentation. In circumstances where treatment with imatinib mesylate is being considered, molecular confirmation of *COL1A1-PDGFβ* is vital, as tumors lacking the fusion gene do not respond to this drug.[20,21] RT-PCR for detection of *COL1A1-PDGFβ* has been studied most extensively, and this method has a reported sensitivity between 74% and 96%, respectively.[22] To cover the breakpoint variability in *COL1A1*, a multiplex polymerase chain reaction (PCR) approach is often used.[10,22,23] FISH assays using both *PDGFβ* break-apart and *COL1A1/PDGFβ* dual-color dual-fusion probe techniques have also been used.[10,22,24,25] Although less studied, some research reports a greater sensitivity of FISH for DFSP than RT-PCR.[22,24]

Angiomatoid Fibrous Histiocytoma

Angiomatoid fibrous histiocytoma (AFH) is a rare fibrohistiocytic tumor of intermediate malignancy that most commonly affects children and young adults. The mean age of patients is 20 years, with a wide age range (from birth to 71 years).[26] Most cases arise in the subcutis or deep dermis of the extremities. AFH less commonly involves the trunk, head,

and neck.[27] Rarely, AFH can involve the deep soft tissue, viscera, and central nervous system.[28-30]

The usual presentation of AFH is of a slow-growing and clinically benign nodular or cystic mass, under 2 cm in diameter. Antecedent trauma is sometimes reported. A minority of patients develop constitutional symptoms, including fever, anemia, weight loss, and polyclonal gammopathy, thought to be a result of cytokine production by the neoplasm and resolved with excision.[27,30,31] Associated extensive lymphadenopathy-simulating Castleman disease has also been described.[32]

There has been much confusion over the behavior of AFH since its original description by Enzinger in 1979. In Enzinger's[30] original study, 5 of 24 patients developed metastases and 3 died of the disease. Larger studies have subsequently established the mostly indolent behavior of AFH. In a study by Costa and Weiss,[27] 4 of 107 cases developed regional lymph node metastases and 1 patient died of the disease. Regional metastatic rates as low as 1% have subsequently been reported.[31] Management is with wide excision and careful follow-up.

The classic histologic appearance of AFH is a nodular proliferation of bland histiocytoid tumor cells with blood-filled cystic spaces, surrounded by a dense, fibrous capsule and lymphocytic infiltrate (Figs. 4 and 5). However, these characteristic features are not always present in a biopsy specimen. Morphologic variants with striking atypia and mitoses may add to confusion with other atypical mesenchymal neoplasms with more aggressive behavior, such as pleomorphic undifferentiated sarcoma.[33] Other unusual morphologic features that may cause diagnostic difficulty include the presence of clear cells, rhabdomyoblast-like cells, and prominent myxoid change.[28,34] Immunohistochemistry has a limited supportive role in the diagnosis of AFH because the neoplasm lacks a consistent and specific immunophenotype. In about 50% of the cases, the tumor cells are positive for desmin, CD68, epithelial membrane antigen, and/or CD99.[31,35-37] In a pediatric patient, desmin immunoreactivity may cause diagnostic confusion with rhabdomyosarcoma.

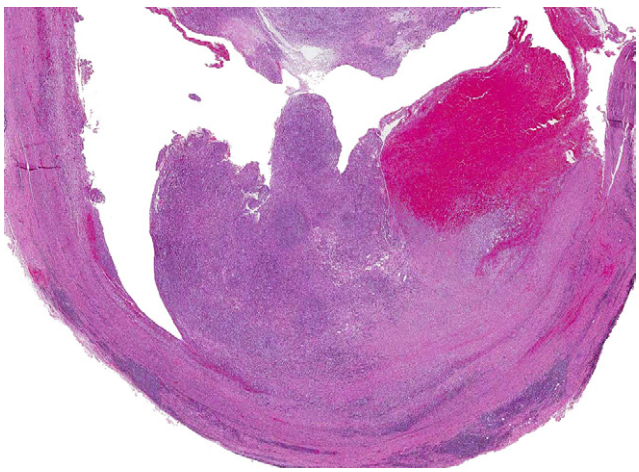

Figure 4 Angiomatoid fibrous histiocytoma. Classically, the tumor is surrounded by a fibrous capsule and lymphocytic infiltrate. Pseudovascular spaces filled with blood are commonly seen.

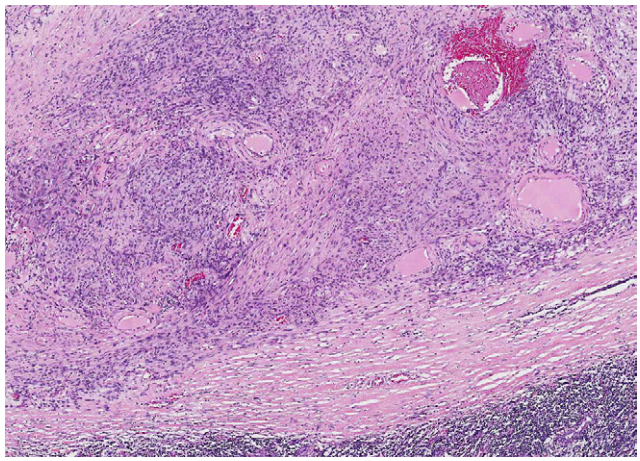

Figure 5 Angiomatoid fibrous histiocytoma. The histiocytoid tumor cells have minimal atypia.

AFH is characterized by recurrent translocations involving either Ewing sarcoma breakpoint region 1 (*EWSR1*) on 22q or its homologue fused in sarcoma (*FUS*) on 16p. The most common oncogenic-fusion gene is *EWSR1*-cyclic adenosine monophosphate responsive element-binding protein 1 (*CREB1*), from t(2;22)(q33;q12), which is present in about 70% or more of cases.[38,39] The other fusion genes identified in AFH include *FUS*-activating transcription factor 1 (*ATF1*), resulting from t(12;16)(q13;p11) (14,15), and *EWSR1-ATF1*, resulting from t(12;22)(q13;q12).[40,41]

EWSR1 and *FUS* belong to the ten-eleven-translocation (TET) family of RNA-binding proteins. *EWSR1* has been implicated in interactions with transcription factor IID (TFIID) and RNA polymerase II and may have a role in transcriptional regulation.[42,43] *ATF1* and *CREB1* are members of the *CREB* (cyclic adenosine monophosphate responsive element-binding protein) family of DNA-binding transcription factors. Activation of *CREB* and subsequent *CREB*-mediated transcriptional activation result from stimulation by hormones and neurotransmitters that raise intracellular cyclic adenosine monophosphate levels.[44,45] In *EWSR1-CREB* fusions, the *EWSR1* activation domain replaces the kinase-inducible domain of *CREB*. The result is a constitutively expressed transcriptional activator. The exact targets of *EWSR1-CREB* fusion genes are not known.

Molecular assays can be helpful in confirming the diagnosis of AFH. FISH assays with dual-color break-apart probes can be used to identify EWSR1 or FUS gene rearrangements, regardless of the translocation partner. In a study of 17 cases, the sensitivity of this FISH method was 76%.[46] FISH results need to be interpreted with caution. A negative result by FISH does not rule out the diagnosis of AFH. Possible explanations for a negative FISH result include variant rearrangements that are not detectable with the particular FISH probes used, or translocations with different chromosomes altogether. Of note, *EWSR1* rearrangements occur in several other soft tissue sarcomas, including the Ewing sarcoma fam-

ily of tumors (ESFT), desmoplastic small round-cell tumor, clear-cell sarcoma (CCS), extraskeletal myxoid chondrosarcoma, and a subset of myoepithelial tumors.[38,47-57] Correlation with the histologic and immunohistochemical findings remains paramount. RT-PCR is a sensitive and specific assay, but the practical utility of this technique is limited by the multiple primers to account for the various fusion transcripts described in AFH.

Clear-Cell Sarcoma (Melanoma of Soft Parts)

Clear-Cell Sarcoma (CCS) is a rare aggressive sarcoma with melanocytic differentiation that typically affects young adults. Most cases of CCS arise in the feet and hands and are associated with tendons or aponeuroses. Rarely, CCS can present in visceral and head-and-neck locations.[58,59] A subset of CCS arise as dermal-based primary cutaneous tumors,[60] where they may be confused with malignant melanoma. CCS usually presents as a slow-growing mass that may be associated with pain. The prognosis of CCS patients is generally poor and typified by recurrences, metastases to the lymph nodes, lung, and bone, and eventual death from disease.

CCS has overlapping histologic and immunohistochemical features with melanoma. It is characterized histologically by uniform nests of epithelioid to spindled cells with clear to eosinophilic cytoplasm and a central nucleus with a prominent nucleolus (Fig. 6). Uncommonly, nests of CCS cells may abut the epidermis and mimic junctional melanocytic nests that may be confused with melanoma. However, CCS usually lacks high mitotic activity and significant pleomorphism. Like melanoma, CCS cells may contain melanin pigment, and the tumor cells are immunoreactive for S-100 protein and melanocytic markers, HMB-45 and Melan-A. The histopathologic distinction of CCS from melanoma is difficult, and molecular testing may be required to objectively distinguish the 2.

Although CCS and melanoma overlap phenotypically, they are different entities at the molecular level. *EWSR1* rearrangements are characteristic of CCS, most commonly in the form of *EWSR1-ATF1* (from t[12;22][q13;q12]) or less commonly as *EWSR1-CREB1*. Melanoma has not demonstrated these translocations.[61] Activating mutations of *BRAF* and microsatellite instability are features commonly seen in melanoma and not in CCS.[62-66]

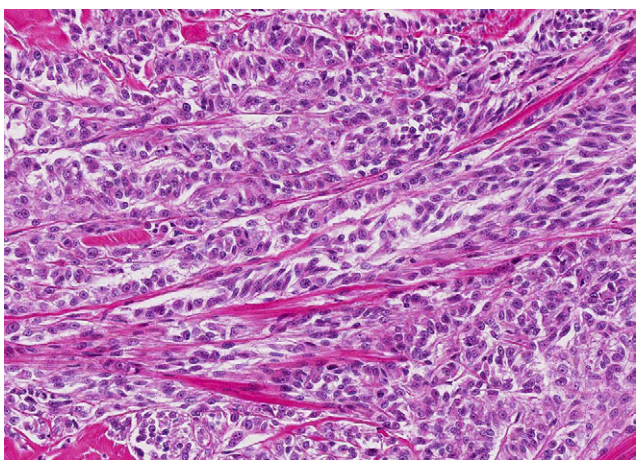

Figure 6 Clear cell sarcoma. The tumor is characterized by nests of epithelioid tumor cells divided by thin fibrous septa.

The t(12;22) translocation has been demonstrated in 70% of cases of CCS by conventional cytogenetics, and the *EWSR1-ATF1* fusion transcripts have been demonstrated in > 90% of cases by RT-PCR.[51-54] Variant splicing results in 4 different *EWSR1-ATF1* fusion transcripts. The most-common fusion transcript is type 1 (34% of cases), involving *EWSR1* exon 8 fused to *ATF1* exon 4. The next most-common fusion (18% of cases) is type 2, involving *EWSR1* exon 7 fused to *ATF1* exon 5.[51-54] Some CCS may demonstrate mixed fusion types, most commonly a combination of types 1 and 2 (38% of cases). An association between type of transcript and prognosis has not been established.[51-54] A small subset of CCS harbors the *EWSR1-CREB1* fusion, most commonly seen in the gastrointestinal tract.[38,53,54] There have also been isolated reports of somatic soft tissue CCS that were positive for *EWSR1-CREB1*; 2 of these involved the hand and 1 was centered in the dermis.[53,54]

RT-PCR is the molecular assay most extensively studied for CCS, with reports of sensitivity between 93% and 100%.[67-69] RT-PCR allows determination of the *EWSR1* translocation partner (*ATF* vs *CREB*) and splice-site variations. The clinical significance of these variations remains to be studied extensively. The use of FISH break-apart probes to detect THE *EWSR1* rearrangement is a good alternative in specimens with inadequate or degraded RNA. It appears to correlate well with RT-PCR, and its sensitivity is reported from 70% to 100% in 2 studies.[54,70]

EWSR1-ATF1 is implicated in melanocytic differentiation in CCS, via activation of the microphthalmia-associated transcription factor (MITF).[67] Melanocyte-specific isoforms of MITF protein (MITF-M) and mRNA have been demonstrated in CCS.[67-69] Gastrointestinal CCS with the *EWSR1-CREB1* fusion has little or no expression of MITF-M transcripts, and these cases also tend to be negative for melanocytic markers.[38] However, *EWSR1-ATF1* is also present in AFH without the demonstration of MITF-M transcripts, suggesting that despite the presence of the same oncogenic fusions, other critical factors influence the eventual phenotype. MITF activates the tyrosine kinase c-Met. In vitro evidence of growth arrest of CCS with c-Met inhibitors lends promise of targeted molecular therapy for this aggressive sarcoma.[71]

Low-Grade Fibromyxoid Sarcoma

Low-grade fibromyxoid sarcoma (LGFMS) is a sarcoma of young-to-middle–aged adults. It typically involves the deep soft tissues of the proximal extremities. Less commonly involved sites include the trunk and head and neck. Isolated cases involving the retroperitoneum, abdominal cavity, and mediastinum have also been reported.[72-77] Approximately

20% cases of LGFMS arise as primary cutaneous neoplasms, usually centered in the dermis or subcutis. A disproportionate number of superficial LGFMS cases (40%) arise in children.[77] The typical presentation is of a slow-growing painless mass. Both deep and superficial LGFMS have a local recurrence rate of approximately 10%.[77,78] The risk of distant metastasis for deep-seated LGFMS is approximately 6%, but the risk appears to be lower for primary cutaneous cases. In 1 study of 19 cases of superficial LGFMS, no metastases developed in a mean follow-up period of 44 months.[77] However, because recurrence and metastasis may occur many years after diagnosis, patients with cutaneous LGFMS should still be followed indefinitely.

Histologically, LGFMS has a characteristic zonal pattern of alternating fibrous and myxoid areas (Fig. 7). The neoplastic cells are spindled and bland, with a swirling arrangement. A delicate curvilinear vascular pattern is commonly seen (Fig. 8). Hyalinizing spindle-cell tumor with giant rosettes was described in earlier reports as a separate tumor, but is now thought to be a morphologic variant of LGFMS, a relationship proven by genetic studies showing that the 2 tumors bear the same translocation.[78-80] Sclerosing epithelioid fibrosarcoma, a rare locally aggressive sarcoma of the deep soft tissues, also shows clinical and morphologic overlap with LGFMS, and some cases have demonstrated the same translocation, suggesting that a subset may be related to LGFMS.[81] Histologic evaluation of LGFMS can be challenging because of the tumor's variable morphology and deceptively bland appearance. Differential diagnoses of LGFMS are broad and also include several benign and malignant entities. In 1 study, 75% cases of superficial LGFMS were misdiagnosed as benign, and the most common misdiagnoses were nodular fasciitis and fibromatosis.[77] Myxofibrosarcoma and perineurioma are 2 of the most difficult neoplasms to distinguish from LGFMS on histologic grounds alone. Limited biopsies of these tumors may be indistinguishable. Immunohistochemistry is of little help as a diagnostic aid in LGFMS. Focal expression of smooth muscle actin, epithelial membrane antigen, and CD68 may be seen.

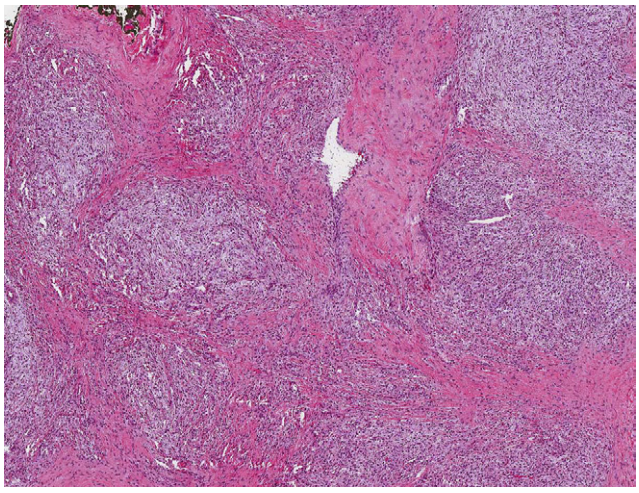

Figure 7 Low-grade fibromyxoid sarcoma (LGFMS). The tumor is characterized by alternating fibrous and myxoid zones at low power.

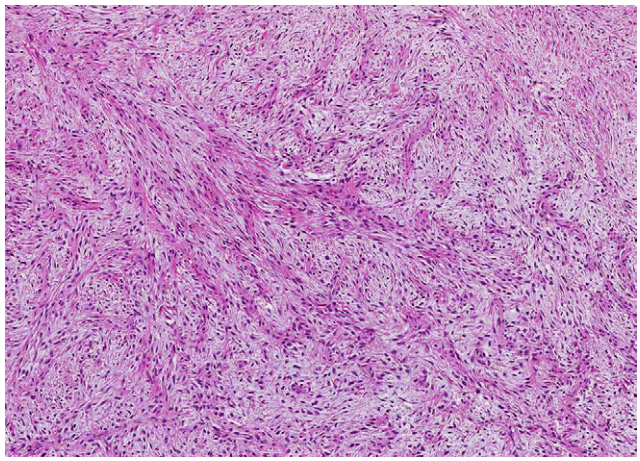

Figure 8 LGFMS. The tumor cells are arranged in a swirling pattern, and thin curvilinear vessels are conspicuous.

LGFMS is characterized by rearrangements of *FUS* on chromosome 16p. Up to 95% of LGFMS studied harbor the *FUS*-CREB protein-like 2 (*CREBL2*) fusion gene, formed by t(7;16)(q34;p11). The less common *FUS*-CREB protein-like 1 (*CREB3L1*) variant, formed by t(11;16)(p11;p11), is found in 5% of cases.[82]

FUS is a homologue of *EWSR1* from the large *TET* family of RNA-binding proteins (see section on Angiomatoid Fibrous Histiocytoma). *FUS* rearrangements are also seen in myxoid liposarcoma, where it is paired with DNA damage inducible transcript 3 (*DDIT3*). The role of *FUS-CREB* in neoplasia is not known, but the fusion brings *CREB* under the control of the *FUS* transcriptional activator, resulting in an aberrant transcription factor with more potent activity than wild-type CREB.[83,84]

Molecular testing plays an important complementary role in the diagnosis of LGFMS. RT-PCR results have led to reclassification of a significant number of cases previously diagnosed as LGFMS in 2 studies.[81,85] RT-PCR assays performed on formalin-fixed paraffin-embedded (FFPE) tissues is reasonably sensitive (81%-88%).[81,86] FISH testing for *FUS* gene rearrangement is less sensitive (approximately 70%), but is a good alternative to PCR, particularly in paraffin blocks with poor-quality RNA.[87,88] (Fig. 9). Negative FISH results may be accounted for by variant breakpoints within the *FUS* gene, different translocations, or truly fusion-negative cases of LGFMS. *FUS* gene rearrangements have not been demonstrated in fibromatosis, myofibromatosis and perineuriomas.[82,85]

Postradiation Angiosarcoma

Cutaneous angiosarcoma arises in 3 major clinical settings: (1) sporadic forms that usually affect elderly patients on the head and neck, (2) chronic lymphedema-associated angiosarcoma, and (3) postradiation angiosarcoma. Postradiation angiosarcoma usually arises in the field of radiation for breast carcinoma and is the rarest of the 3 forms of angiosarcoma.

However, as greater emphasis is placed on breast-conserving surgery, it is becoming an increasing problem. The latency for postradiation angiosarcoma of the breast is short (median 6 years), but as many as 20% of cases may develop in under 3 years.[89] Classic lymphedema-associated angiosarcoma (Stuart–Treves syndrome) develops on the upper extremities of women who have had a mastectomy and axillary lymph node dissection. Angiosarcoma can also develop in other settings of chronic lymphedema, including elephantiasis, massive localized lymphedema of the morbidly obese,[90] and in congenital lymphedema.[91] Both secondary and sporadic forms of angiosarcoma have a grim prognosis, with rapid dissemination and, in most instances, death from disease.

All clinical variants of angiosarcoma have a similar microscopic appearance of infiltrative neoplastic vessels ramifying through the dermis, ranging from well-differentiated cases with subtle or focal atypia to cases with very pleomorphic endothelial cells with poorly recognizable vessels (Fig. 10). In the postradiotherapy setting, distinguishing well-differentiated postradiation angiosarcoma from benign radiation-associated atypical vascular lesions (AVL) can be difficult (Fig. 11). There is significant clinical and morphologic overlap between the 2 entities; however, in contrast to angiosarcoma, AVL do not show aggressive behavior.[92-95] Notably, AVL may coexist with postradiation angiosarcoma, and some cases show transitional areas of AVL with significant cytologic atypia, suggesting that some cases of AVL may be a potential precursor to angiosarcoma.[95] Immunohistochemistry is unhelpful in this distinction, as both AVL and angiosarcoma will express endothelial markers, such as CD31, CD34, factor VIII, and D2-40.

High-level amplification of the *MYC* oncogene is a recurring feature of radiation and lymphedema-associated angiosarcoma, and is not seen in AVL or sporadic angiosarcoma.

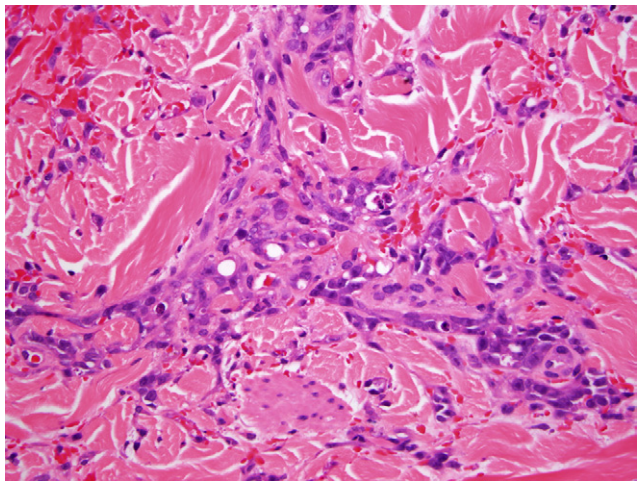

Figure 10 Postradiation angiosarcoma. The tumor is characterized by infiltrating vessels with significant atypia and multilayering of the endothelial cells.

This finding suggests that secondary angiosarcoma arises from different genetic mechanisms. *MYC* gene amplification can be detected by FISH, and high-level amplification is defined as > 8 or 9 *MYC* gene signals or tight clusters of signals.[96-99] (Fig. 12). This method has an overall sensitivity of 80% for the diagnosis of postradiation and lymphedema-related angiosarcoma.[96-99] However, the reported sensitivity has ranged widely from 55% in the initial study[96] to 100% in subsequent studies.[97-99] Although most cases studied were postradiation angiosarcoma in the setting of treatment of breast carcinoma, secondary angiosarcoma of other sites were also *MYC* amplified, suggesting that this molecular feature is not a site-specific phenomenon. By Western blot, it

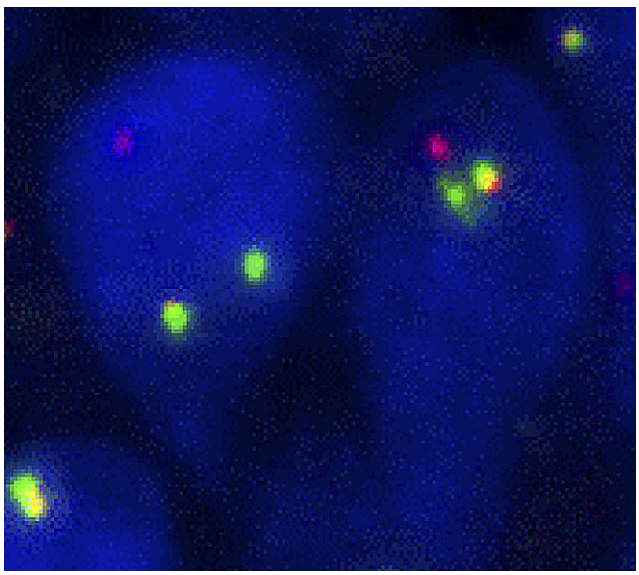

Figure 9 LGFMS. Detection of FUS rearrangement by fluorescence RNA in situ hybridization. Using dual-color break-apart probes, separated red and green signals indicate rearrangement of FUS gene. A fused yellow signal indicates intact FUS.

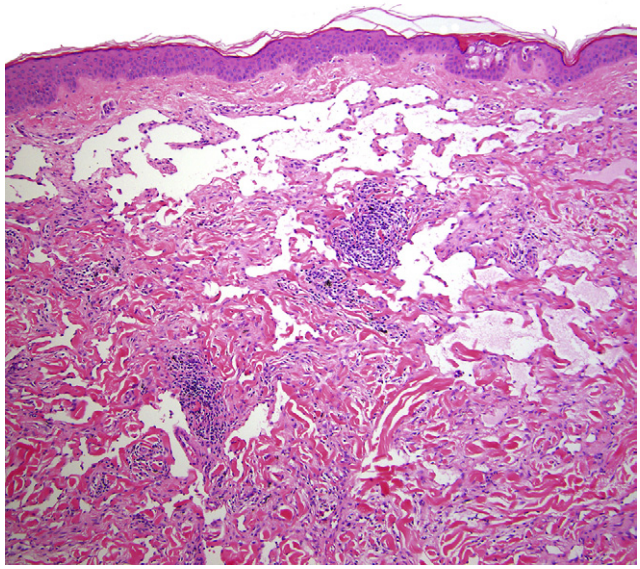

Figure 11 Atypical vascular lesion. Ramifying vascular channels are seen dissecting through the dermal collagen, without endothelial multilayering. Limited biopsies of atypical vascular lesions may be difficult to distinguish from well-differentiated angiosarcoma.

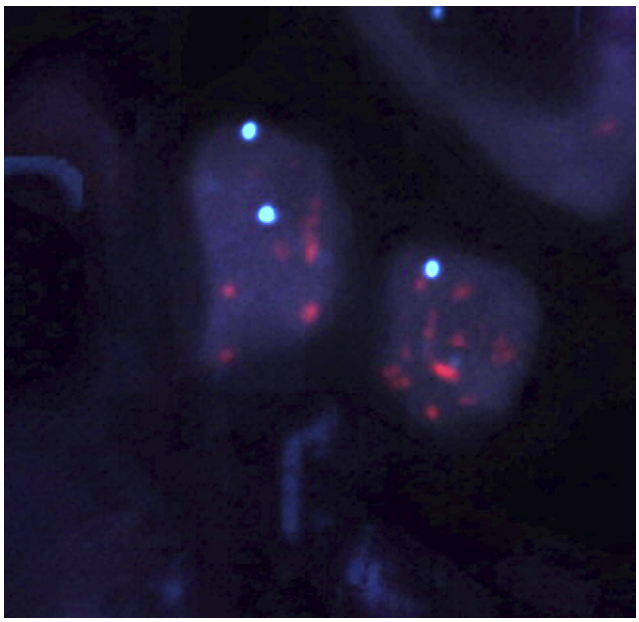

Figure 12 Postradiation angiosarcoma. Fluorescence RNA in situ hybridization demonstrating that numerous copies of *MYC* (red signal) are present relative to the centromere probe (blue signal).

appears that the *MYC-II* isoform is expressed by secondary angiosarcoma and not by primary angiosarcoma.[98]

Immunohistochemistry for *MYC* overexpression appears to correlate with FISH in the limited reports thus far.[97,99] Given the inherent difficulty in discriminating well-differentiated postradiation angiosarcoma from AVL, *MYC* immunohistochemistry may be a useful diagnostic aid in determining the extent of angiosarcoma in resection specimens and in the setting of small biopsy specimens. However, further studies on the accuracy of this marker as a surrogate for *MYC* FISH are needed.

The exact role of *MYC* in the pathogenesis of secondary angiosarcoma is yet to be defined. *MYC* has diverse regulatory roles in cell growth, proliferation, metabolism, differentiation, and apoptosis, and its gene targets are broad.[100] *MYC* overexpression is seen in many other malignancies, including carcinomas, melanoma, and various sarcomas.[101-104] It is also linked with adverse prognosis in several cancers.[105-107]

Epithelioid Hemangioendothelioma

Epithelioid hemangioendothelioma (EHE) is a rare and poorly understood vascular tumor that has been dogged by confusion since its original description by Weiss and Enzinger in 1982.[108] Adults are most commonly affected with a wide age range. The most frequently involved sites include somatic soft tissues, liver, lung, and bone. Multicentric presentation is common, particularly in the liver and skeleton. EHE is commonly associated with pain, and cutaneous tumors may present with ulcers.[109,110] It was originally thought that EHE was of borderline malignancy, with behavior between that of hemangioma and angiosarcoma. However, the behavior of EHE can be unpredictable, and a subset (approximately 20%) has been seen to behave aggressively, with dissemination to the liver, lung, and bone, and can cause death.[111,112] According to the World Health Organization, EHE is currently classified as a fully malignant neoplasm.[26]

Histologically, EHE is characterized by infiltrative cords or nests of polygonal cells embedded within a myxohyaline stroma (Fig. 13). Up to half of the cases arise from a blood vessel, most commonly a vein. Intracytoplasmic vacuoles, representing abortive vascular lumina, occasionally containing erythrocytes, are a characteristic feature of the tumor cells. Well-formed vascular channels are usually not prominent. Overlapping morphology and terminology add significant confusion to the distinction from other epithelioid vascular proliferations, including epithelioid hemangioma, epithelioid angiosarcoma, and epithelioid sarcoma-like hemangioendothelioma. In cases where the vasoformative nature is not appreciated, there may be confusion with epithelioid sarcoma, extraskeletal myxoid chondrosarcoma, mixed tumor, or even metastatic adenocarcinoma. By immunohistochemistry, EHE is positive for vascular markers CD31, CD34, D2-40, and ETS related gene (ERG)[113]; it frequently expresses cytokeratins and smooth muscle actin.[113] However, this immunoprofile does not allow distinction from other epithelioid vascular proliferations.

EHE is characterized by a t(1;3)(p36;q25) translocation resulting in the WW domain-containing transcription factor 1 (WWTR1)-calmodulin-binding transcription factor 1 (CAMTA1) fusion gene.[114,115] This translocation was initially demonstrated in a report of 2 cases of EHE in 2001.[116] The same gene rearrangements were subsequently demonstrated either by FISH or RT-PCR in 59 cases of EHE of various

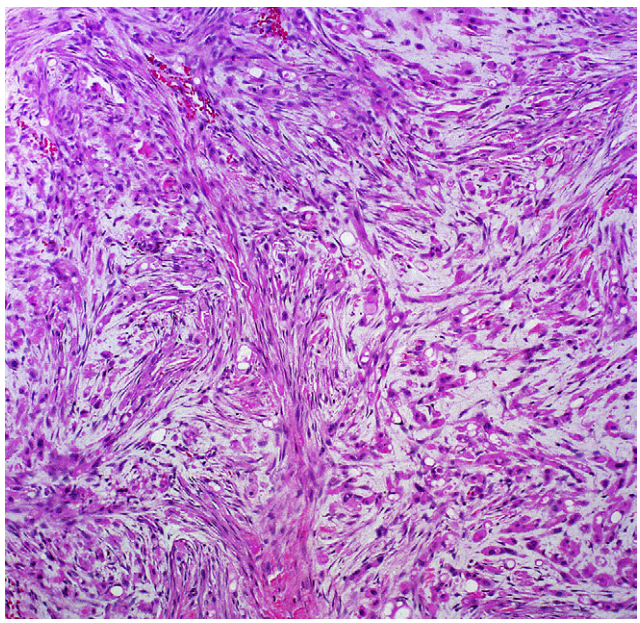

Figure 13 Epithelioid hemangioendothelioma. Infiltrative cords of epithelioid tumor cells are embedded within a myxohyaline stroma. Intracytoplasmic vacuoles containing erythrocytes are present within individual cells.

sites.[114,115] Epithelioid hemangioma, epithelioid angiosarcoma, and other vascular neoplasms have not displayed this translocation. *WWTR1*, located on chromosome 3q, functions as a transcriptional coactivator and has been implicated in differentiation of mesenchymal stem cells.[117] *WWTR1* overexpression is seen in breast, thyroid, and nonsmall-cell lung carcinoma.[118-120] *CAMTA1*, located on chromosome 1p, is a calmodulin-binding transcription factor usually expressed in the brain. In the context of oligodendroglioma and neuroblastoma, which are central nervous system neoplasms that show chromosome 1p losses, *CAMTA1* has been characterized as a tumor suppressor gene.[121,122] It appears more likely that, when paired with *WWTR1* in EHE, *CAMTA1* functions as an oncogenic transcription factor. The exact function of *WWTR1-CAMTA1* fusion protein is not yet known. FISH break-apart probes can be used to detect either *WWTR1* or *CAMTA1* rearrangements, with equivalent sensitivity (87%-89%) and specificity (100%) for EHE.[1] The discovery of a genetic hallmark in EHE should allow for more objective and accurate diagnosis of this rare tumor and help us better understand its biology.

Ewing Sarcoma Family of Tumors

Ewing sarcoma is traditionally regarded as an aggressive pediatric small-round-cell tumor of the bone or deep soft tissue. Rarely, Ewing sarcoma can arise as a primary cutaneous neoplasm. In the limited reports to date, most cutaneous ESFT were small, localized dermal, or subcutaneous nodules in the extremities or trunk, often associated with pain. The median age is 17 years. In contrast to primary bone and deep soft tissue ESFT that tend to be metastatic at presentation, superficial ESFT appear to behave more indolently. Approximately 10% of cutaneous ESFT reported in the literature developed metastasis. Overall survival also appears to be greater for superficial ESFT (91%).[123-128]

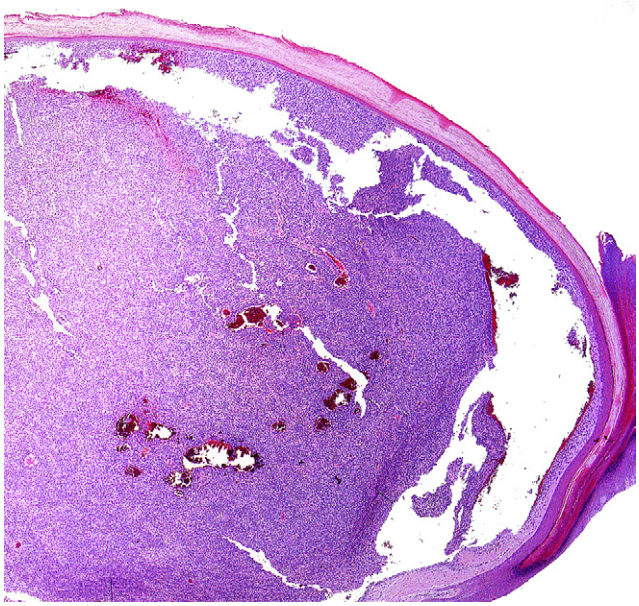

Figure 14 Cutaneous Ewing sarcoma. The tumor is characterized by a circumscribed nodular proliferation of "small blue cells" within the dermis with significant hemorrhage.

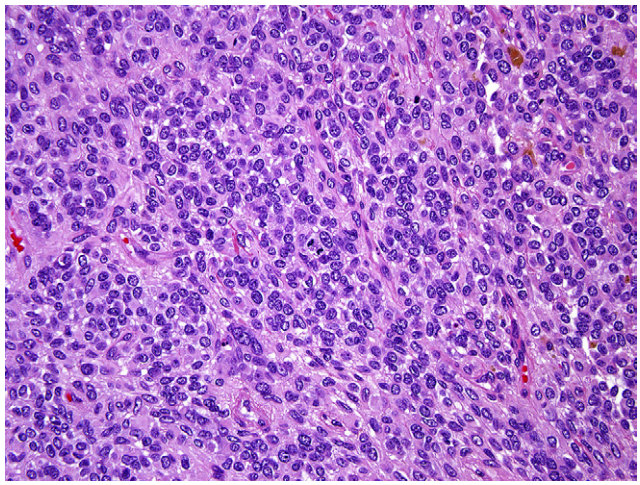

Figure 15 Cutaneous Ewing sarcoma. Higher magnification of a solid sheet of tumor cells with scant clear to eosinophilic cytoplasm, coarsely stippled chromatin, and occasionally prominent nucleolus and prominent mitotic activity.

Cutaneous ESFT is characterized histologically by circumscribed dermal or subcutaneous nodules of undifferentiated "small round blue cells," with stippled chromatin and prominent mitotic activity (Figs. 14 and 15). The histologic differential diagnosis is broad and includes other round-cell tumors that may occur in superficial locations. In pediatric patients, rhabdomyosarcoma and neuroblastoma need to be considered. Other differential diagnoses include lymphoblastic lymphoma, carcinomas of skin appendage origin, Merkel carcinoma, small-cell melanoma, and metastasis. By immunohistochemistry, ESFT show strong membranous expression of the MIC2 gene product, CD99 (a feature that is sensitive, but not specific). Positive nuclear expression for Friend leukemia integration 1 transcription factor (FLI1) is also characteristic, but may be variable. ESFT may also express cytokeratin, neuroendocrine markers, and S100 protein.

EWSR1 rearrangements are the genetic hallmark of ESFT. Usually, this involves oncogenic fusions with various members of the E-twenty-six (*ETS*) family of transcription factors, of which there are > 30 members.[129] Although *EWSR1* can be translocated to various other transcription factors in other soft-tissue tumors, its partnership with *ETS* genes appear to be specific for ESFT. *EWSR1-FLI1*, resulting from t(11;22)(q24;q12), is the most common fusion gene in ESFT, seen in > 85% of cases. Approximately 10% of cases harbor the *EWSR1-ERG* fusion gene, resulting from t(21;22)(q22;q12). Many other ETS genes have been implicated in EWSR1 fusions, including ETS translocation variant 1 (ETV1), ETV4, fifth Ewing Sarcoma variant (FEV), and others.[130-138]

EWSR1-ETS fusion genes are thought to drive tumorigenesis and phenotype in ESFT. They have been shown to induce small round-cell morphology and ESFT-like immuno-

phenotype in mesenchymal stem cells.[139] Though the *EWSR1-ETS* genes have broad and complex functions; they are best characterized by their role as aberrant transcription factors.[140-143] There are a multitude of proteins implicated as downstream targets of *EWSR1-ETS* fusions, with various roles in cell survival, proliferation, and differentiation.[129,144] It is not known which of these targets is critical for oncogenesis in ESFT.

Molecular diagnostics plays a fundamental role in the distinction of ESFT from other small round cell tumors. RT-PCR and FISH on FFPE tissue are both highly sensitive and specific in this regard.[145-151] However, there is significant molecular heterogeneity in ESFT, so RT-PCR assays require multiple primers to cover the spectrum of fusion transcripts identified in this tumor. Molecular heterogeneity in ESFT results from breakpoint variability within *EWSR1* and *ETS* genes, different partnerships with *EWSR1*, and substitution of *EWSR1* itself by its homologue, *FUS*.[134-136] In practice, routine testing covers *EWSR1-FLI1* and *EWSR1-ERG* transcripts, as these account for > 95% of cases. However, even within the most common fusion gene (*EWSR1-FLI1*), *EWSR1* breakpoints can occur anywhere between introns 7 and 9, and FLI1 breakpoints can occur between introns 3 and 9.[152] Two major types of *EWSR1-FLI1* transcripts have been described: type 1 (*EWSR1* exon 7 fused to *FLI* exon 6) in 60% of cases, and type 2 (*EWSR1* exon 7 fused to *FLI1* exon 5) in 25% of cases. No prognostic difference between fusion types has been shown by recent large prospective studies.[153,154] Multiplex PCR technique is commonly used, as this allows for the simultaneous amplification of multiple targets in one reaction. Quantification of fusion transcripts can be done with real-time PCR technology, which combines amplification with simultaneous detection of amplified product. This method has been proposed as a means to monitor minimal residual disease, but its clinical utility needs to be evaluated further.

EWSR1 rearrangement may also be detected by commercial dual-color break-apart FISH assays, without determination of the exact translocation present. FISH appears to be as sensitive as RT-PCR on FFPE tissue. One advantage of using the break-apart *EWSR1* FISH technique is that it has utility in the diagnosis of other *EWSR1*-rearranged tumors, such as AFH and desmoplastic small round-cell tumor, among others. However, as *EWSR1* rearrangements are not specific, interpretation of FISH results in isolation of the whole clinical and histopathologic picture can lead to potential error. Confirmation of EWSR1 rearrangement is more likely to be helpful if the suspicion of ESFT is already high. Given the molecular heterogeneity in ESFT, failure to demonstrate *EWSR1* rearrangement also does not rule out the diagnosis of ESFT. Atypical cases may require multiple molecular techniques to establish the diagnosis.

Conclusions

Specific genetic signatures characterize a growing number of soft-tissue tumors that affect the skin. Molecular testing on FFPE complements histology and immunohistochemistry in the diagnosis of these tumors, especially in challenging cases with atypical morphology, nonspecific immunophenotype, and/or limited sampling. Molecular diagnostics also has implications for more accurate classification and prognostication of poorly understood entities. Importantly, molecular testing lays the foundation for the development of targeted molecular therapies, and with that, there is a potential scope to expand the application of molecular assays to the detection of minimal residual disease and response to these therapies. That said, molecular assays do have limitations and, like all ancillary tests, their results require interpretation in the context of the full clinical and histologic picture.

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