Best Practices in Diagnostic Immunohistochemistry

Pleomorphic Cutaneous Spindle Cell Tumors

Andrew L. Folpe, MD; Kumarasen Cooper, MBChB, DPhil

Context.—Pleomorphic cutaneous spindle cell tumors can be difficult to distinguish solely on histologic grounds. The use of ancillary immunohistochemical studies can greatly assist in this differential diagnosis.

Objective.—To review histologic and immunohistochemical aspects of cutaneous spindle cell tumors and discuss a basic panel of markers to assist in the differential diagnosis.

Data Sources.—English-language literature published between 1981 and 2005.

Conclusions.—A basic immunohistochemistry panel for high-molecular-weight cytokeratin, melanocytic markers (S100 protein, HMB-45, Melan-A), smooth muscle actin, desmin, and endothelial markers (CD31, CD34) is effective in diagnosing most cutaneous spindle cell tumors.

This article focuses on the use of immunohistochemistry (IHC) in the differential diagnosis of a relatively common diagnostic scenario, the “histologically undifferentiated, pleomorphic spindle cell tumor involving the skin.” Applications of IHC in the diagnosis of monomorphic spindle cell tumors (eg, dermatofibrosarcoma protuberans) and histologically distinctive spindle cell tumors (eg, neurothekeoma) will be covered in subsequent articles in this series.

DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS

Most pleomorphic cutaneous spindle cell tumors occur in adults, and many involve sun-damaged skin. Thus, the typical differential diagnosis for these lesions is actually quite limited. The most common tumors encountered in this setting are sarcomatoid squamous cell carcinoma (SCC), sarcomatoid malignant melanoma, and atypical fibroxanthoma (AFX)/superficial malignant fibrous histiocytoma. Less common tumors that also need to be included in the differential diagnosis include leiomyosarcoma and spindle cell variants of angiosarcoma and very rarely unusual tumors such as rhabdomyosarcoma, pleomorphic liposarcoma, and so forth.

RECOMMENDED ANTIBODY PANEL

A panel of commonly evaluated antigens targeted to the differential diagnosis is outlined in Table 1. Issues specific to each antigen and to each potential diagnosis are discussed in the “Discussion of Antigens” and “Issues Specific to Particular Tumor Types” sections, respectively. A logical approach would be to choose a generic panel of antibodies in the first instance, followed by a secondary step composed of fewer, more specific antibodies based on the results of the initial panel. Such an algorithmic approach ensures maximum sensitivity and specificity of the antibody panel.

BASIC PRINCIPLES OF DIAGNOSTIC IHC AS APPLIED TO THE DIFFERENTIAL DIAGNOSIS OF CUTANEOUS PLEOMORPHIC SPINDLE CELL TUMORS

Careful Attention to Positive and Negative Internal Controls

Careful attention to positive and negative internal controls cannot be overemphasized. Every single IHC slide must be evaluated for appropriate staining of normal tissues for the antigen in question. Internal positive and negative controls completely “trump” external controls, whether those external controls are placed on the same glass slide or not. This is because specimen fixation is the single greatest variable in IHC. Fortunately for the pathologist, the skin is rich in normal internal positive controls for the majority of antigens (Table 2). Evaluation of negative controls simply means making sure that tissues that do not express an antigen are negative. Inappropriate staining of negative controls usually is the result of inappropriate antibody concentration and/or excessive epitope retrieval.
The cytokeratins are a family of 20 intermediate filament proteins, the expression of which is largely, but not entirely, restricted to epithelial cells. There are many different ways to approach the cytokeratins, as individual cytokeratins (eg, CK7, CK20), as acidic and basic cytokeratins pairs (eg, CK8/18), or as “low” and “high” molecular weight cytokeratins. With regard to the last category, it is important to realize that the division of the cytokeratins into low and high molecular weights is entirely arbitrary. It is more useful to think of the low-molecular-weight cytokeratins as those of simple epithelia, such as simple ductules, and the high-molecular-weight cytokeratins as those of complex epithelia, such as urothelium or skin.

For practical purposes, it is simpler to consider cytokeratins in terms of the antibodies used to identify them. The most widely used pan-keratin antibody is the AE1/AE3 cocktail. AE1 recognizes the acidic cytokeratins 10, 14, 15, 16, and 19, whereas AE3 recognizes the basic cytokeratins 1, 2, 3, 4, 5, 6, and 8. Both antibodies recognize a mixture of high- and low-molecular-weight cytokeratins. So-called wide-spectrum cytokeratin antibodies, such as the OSCAR MAB and a variety of polyclonal antibodies, potentially have somewhat broader cytokeratin coverage than does the AE1/AE3 cocktail, although this may be laboratory dependent. The most widely used low-molecular-weight cytokeratin antibodies are CAM 5.2 (CK8, CK18, CK19) and 3B5/H11 (CK8 and CK18). Almost all laboratories use the high-molecular-weight cytokeratin antibody 34BE12 (CK1, CK5, CK10, CK14/15), also known as “cytokeratin 903.” Many other antibodies exist and one should always carefully read the product insert and any relevant literature before using them in the laboratory.

**Cytokeratin Expression in Normal Skin.**—The skin serves as a superb control tissue for the evaluation of cytokeratin expression, as each epithelial cell type present within the skin expresses unique cytokeratins, as detailed later (Table 3).

**Falsely Negative Basal Keratinocyte Staining With AE1/AE3 and Other Wide-Spectrum Cytokeratin Antibodies.**—Although these antibodies should react with basal keratinocytes (CK5 and CK14), these cells are frequently negative in many laboratories, for unknown reasons (Figure 1). This indicates that these high-molecular-weight keratins are not being identified. It is critical to realize that sarcomatoid SCCs may express almost exclusively high-molecular-weight cytokeratins such as CK5 and CK14. Therefore, the failure to appreciate negative basal cell staining with wide-spectrum cytokeratin antibodies may result in the misdiagnosis of sarcomatoid SCC.

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**Table 1. Recommended Panel for the Workup of Common Pleomorphic Cutaneous Spindle Cell Tumors, With Expected Immunophenotypes*\(^{1}\)**

<table>
<thead>
<tr>
<th>Cytokeratins (Wide-Spectrum, High MW, CK5/6)</th>
<th>S100 Protein</th>
<th>Melanocytic Markers (HMB-45, Melan-A)</th>
<th>Smooth Muscle Actin</th>
<th>Desmin</th>
<th>Endothelial Markers (CD31, CD34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcomatoid SCC</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Melanoma</td>
<td>–/+</td>
<td>+</td>
<td>–/+</td>
<td>–/–</td>
<td>–</td>
</tr>
<tr>
<td>AFX</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>–/+</td>
<td>–</td>
<td>–</td>
<td>+/+</td>
<td>–</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>–/+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

* MW indicates molecular weight; SCC, squamous cell carcinoma; AFX, atypical fibroxanthoma; +, positive in more than 90% of cases; –, negative; +/–, positive in 50% to 75% of cases; and –/+, usually negative but anomalous expression may be seen in up to 25% of cases.

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**Table 2. Commonly Evaluated Antigens and Their Normal Positive Internal Controls in the Skin**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Normal Positive Internal Control in Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan-cytokeratin and low-molecular-weight cytokeratins</td>
<td>Suprabasal keratinocytes and adnexae</td>
</tr>
<tr>
<td>High-molecular-weight cytokeratins</td>
<td>Basal keratinocytes</td>
</tr>
<tr>
<td>S100 protein</td>
<td>Melanocytes, Langerhans cells, nerves, myoepithelial cells</td>
</tr>
<tr>
<td>Melanocytic markers (eg, HMB-45, Melan-A, MiTF, tyrosinase)*</td>
<td>Dermal melanocytes (may be HMB-45 negative)</td>
</tr>
<tr>
<td>Smooth muscle actins</td>
<td>Pilar smooth muscle</td>
</tr>
<tr>
<td>Desmin</td>
<td>Pilar smooth muscle</td>
</tr>
<tr>
<td>Endothelial markers (eg, CD31, CD34, FLI-1 protein, von Willebrand protein)</td>
<td>Endothelial cells</td>
</tr>
</tbody>
</table>

* MiTF indicates microphthalmia transcription factor.

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**DISCUSSION OF ANTIGENS**

**Cytokeratins**

**Background.**—The cytokeratins are a family of 20 intermediate filament proteins, the expression of which is largely, but not entirely, restricted to epithelial cells. There are many different ways to approach the cytokeratins, as individual cytokeratins (eg, CK7, CK20), as acidic and basic cytokeratins pairs (eg, CK8/18), or as “low” and “high” molecular weight cytokeratins. With regard to the last category, it is important to realize that the division of the cytokeratins into low and high molecular weights is entirely arbitrary. It is more useful to think of the low-molecular-weight cytokeratins as those of simple epithelia, such as simple ductules, and the high-molecular-weight cytokeratins as those of complex epithelia, such as urothelium or skin.

Careful evaluation of all pieces of tissue on the entire slide should be a routine exercise; however, many cases are simply missed because a small focus of cytokeratin-negative cells was overlooked in a sarcomatoid carcinoma or melanoma, respectively.

The use of a panel of immunostains, including both expected positives and negatives for all of the entities in the histologic differential diagnosis

A panel of immunostains, rather than single markers, should always be performed. This approach greatly eliminates the potential for misdiagnosis owing to anomalous expression of antigens (eg, cytokeratin expression in melanoma or angiosarcoma). The routine use of a small, carefully selected panel is also time and money effective.

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Figure 1. Normal skin, immunostained with AE1/AE3. Note the negative basal cell staining, reflecting the sometimes variable sensitivity of this antibody cocktail for high-molecular-weight cytokeratins. As sarcomatoid squamous cell carcinomas may express only high-molecular-weight cytokeratins, failure to appreciate this finding may result in a “false negative” study (original magnification ×100).

Figure 2. A, Sarcomatoid squamous cell carcinoma, arising from a basaloid squamous cell carcinoma (hematoxylin-eosin, original magnification ×100). B, The sarcomatoid component of this tumor, but not the basaloid component, shows strong vimentin expression (original magnification ×200).

Figure 3. Malignant melanoma, negative for S100 protein. Note the positive internal control, Langerhans cells (original magnification ×400).

Figure 4. Normal melanocytes, strongly positive with HMB-45. Positivity with this marker does not necessarily imply a malignant melanocytic lesion (original magnification ×100).

Figure 5. Angiosarcoma, diffusely positive for CD31. There are also a large number of CD31-positive histiocytes, showing somewhat fainter, more granular staining. Intratumoral CD31-positive histiocytes may be misinterpreted as positive tumor cells, leading to the erroneous diagnosis of “angiosarcoma” (original magnification ×200).
Vimentin

**Background.**—Vimentin is present in almost all embryonic cells, and most cells of any lineage re-express vimentin when grown in culture. This latter situation may be analogized to spindled change in neoplasms, inasmuch as essentially any spindled cell will express vimentin.5–9

**Diagnostic Uses.**—Vimentin is expressed by essentially all sarcomas, all melanomas, all spindled SCCs, all spindled angiosarcomas, and so forth (Figure 2, A and B). It is also expressed in almost all mesotheliomas, most gliomas, and a subset of nonspindled carcinomas. The diagnostic uses of vimentin are almost exclusively restricted to the subtyping of carcinomas, as certain carcinomas, such as endometroid carcinomas, frequently coexpress vimentin.10

**S100 Protein**

**Background.**—S100 protein is a 20-kd acidic calcium-binding protein, which gets its name from its solubility in 100% ammonium sulfate. S100 protein is composed of 2 subunits, with 3 isotypes—α (expressed in muscle), αβ (expressed in melanocytes, glia, chondrocytes, skin adnexae), and ββ (expressed in Langerhans cells, Schwann cells).1 The commonly used polyclonal antibodies recognize all of these isotypes.

**Diagnostic Uses.**—S100 protein is present in essentially 100% of normal melanocytes and nevi and in approximately 97% to 98% of melanomas.11–13 S100 protein is thus the single best screening marker for melanocytic neoplasms. It is important to realize that very rare melanomas are S100 protein negative (Figure 3). S100 protein is not, however, completely specific for melanocytic tumors and can be expressed in smooth muscle tumors (weakly), some carcinomas (but not SCC), myoepithelial tumors, peripheral nerve sheath tumors, and others.

**Melanocytic Markers**

**HMB-45.**—**Background.**—HMB-45 is a monoclonal antibody that identifies a premelanosomal protein, gp100.14 HMB-45 is often negative in resting melanocytes and nevi, but may be expressed in “reactive” melanocytes (Figure 4). HMB-45 is organellar specific but not lineage specific, meaning that nonmelanocytic tumors that contain premelanosomes (eg, Bednar tumor, angiomylolipoma) will be HMB-45 positive.15–17 HMB-45 should not be used as a screening marker for melanoma; S100 protein should be used instead.

**Diagnostic Uses.**—Numerous studies have shown the sensitivity of HMB-45 in epithelioid melanomas to be approximately 85%, Sarcomatoid melanomas are less often positive (30%–50%), and true desmoplastic melanomas are essentially never HMB-45 positive in the spindled/desmoplastic zones.18

**Melan-A.**—**Background.**—Melan-A, the product of the MART-1 gene, is a 20- to 22-kd component of the premelanosomal membrane, of unknown function. Unlike HMB-45, Melan-A is expressed in resting melanocytes and nevi. As with HMB-45, Melan-A is organellar specific but not lineage specific.19–23 Melan-A should also not be used as a screening marker for melanoma. The most widely used antibody to Melan-A, A103, has a reproducible and diagnostically useful cross-reactivity with a cytoplasmic component of steroid-producing cells.22 This is helpful in the diagnosis of adrenal cortical neoplasms but is of no concern in the skin.

**Diagnostic Uses.**—Melan-A has essentially the same sensitivity and specificity as HMB-45. It is present in some HMB-45-negative melanomas and vice versa.

**Other Markers of Melanocytes.**—Other potential markers for melanocytes include microphthalmia transcription factor and tyrosinase. Microphthalmia transcription factor suffers from relatively low specificity and is not recommended.24,25 The sensitivity and specificity of tyrosinase are roughly equivalent to those of HMB-45 and Melan-A, and it is an acceptable alternative marker.19,26

**Markers of Endothelial Differentiation**

**CD31 (Platelet Endothelial Cell Adhesion Molecule 1).**—**Background.**—CD31 is a 130-kd transmembrane glycoprotein that is expressed on all endothelial cells, including lymphatic endothelial cells. CD31 is also routinely expressed by tissue macrophages and platelets.

**Diagnostic Uses.**—CD31 is the single most sensitive and specific marker of endothelial differentiation and is the single best endothelial marker to have in one’s laboratory, if one can have just one27–28 (Figure 5). CD31 is expressed by essentially all benign vascular tumors and by more than 90% of hemangioendotheliomas and angiosarcomas. CD31 expression in carcinomas is extraordinarily unusual, with only case reports in breast and thyroid carcinomas.29 Nonendothelial sarcomas, in particular epithelioid sarcoma, are CD31 negative. CD31-positive macrophages within nonendothelial tumors can be confused with positive tumor cells, leading to an erroneous diagnosis of an endothelial tumor.30

**CD34.**—**Background.**—CD34, a transmembrane glycoprotein of unknown function, is widely expressed on a number of normal tissues, including hematopoietic stem cells, interstitial cells of Cajal, endothelial cells, and dendritic interstitial cells in the skin and around nerves.31 CD34 is thus not a specific endothelial marker but is a useful marker in the appropriate histologic context.

**Diagnostic Uses.**—CD34 is a highly sensitive marker of benign, borderline, and malignant endothelial tumors, being expressed by more than 90% of cases.26 CD34 has also generally been regarded as the most sensitive marker for Kaposi sarcoma,32 although the Kaposi sarcoma–associated herpesvirus latency-associated nuclear antigen protein is probably the current marker of choice for Kaposi sarcoma.33 In the differential diagnosis of epithelioid angiosarcomas, it is important to remember that CD34 is expressed by up to 60% of epithelioid sarcomas but not by carcinomas.34–36 It is also expressed by dermatofibrosarcoma protuberans, gastrointestinal stromal tumors, leiomysarcomas, and solitary fibrous tumors.31

**FLI-1 Protein.**—**Background.**—FLI-1 protein is a member of the ETS family of nuclear transcription factors and is expressed in all mature endothelia and during the earliest stages of endothelial differentiation.37 FLI-1 is best known as the partner of EWS in the Ewing sarcoma–specific t(11;22)(q12;q24) (EWS-FLI-1) fusion gene. It is also routinely expressed by small lymphocytes (probably T cells). FLI-1 is the only nuclear marker of endothelium.

**Diagnostic Uses.**—FLI-1 is a highly sensitive marker of endothelial neoplasms, including hemangiomias, hemangioendotheliomas, angiosarcomas, and Kaposi sarcoma.37,38 FLI-1 is not expressed by epithelioid sarcomas, melanomas, or carcinomas. It is important not to mistake intratumoral FLI-1–positive endothelial cells and lymphocytes for positive tumor cells.
von Willebrand Factor (Factor 8–Related Protein).—

Background.—von Willebrand factor is a clotting factor that is theoretically present only in the Weibel-Palade body of endothelium and in platelets and that should be in theory the most specific marker of endothelial differentiation.59 Unfortunately, von Willebrand factor is secreted into the serum, and the inevitable high background staining seen with this marker greatly reduces its “real world” utility. von Willebrand factor staining in serum may be seen only around the outer surface of cells and closely mimics true membranous expression (Figure 6).

Diagnostic Uses.—von Willebrand factor is the least sensitive endothelial marker, particularly in angiosarcomas.32 Given the technical issues discussed previously, there is arguably no real role for von Willebrand factor in the era of CD31, CD34, and FLI-1.

Muscle Markers

Smooth Muscle Actin.—Background.—The actins are a ubiquitously distributed family of intracellular proteins that may be broadly divided into muscle and nonmuscle isoforms. The muscle-specific isoforms can be divided again into smooth, skeletal, and cardiac isoforms.30–33 From a practical perspective, the single most useful actin antibody is MAb 1A4, which recognizes only smooth muscle isoforms. In the skin, smooth muscle actin is routinely expressed by pilar and vascular smooth muscle, re- active myofibroblasts, a subset of pericytes, and myoepi- thelium.41

Diagnostic Uses.—Smooth muscle actin is a highly sensitive marker of smooth muscle and myofibroblastic tumors in the skin (Figure 7).

Desmin.—Background.—Desmin is an intermediate filament protein that is expressed by muscle cells of all types, as well as submesothelial fibroblasts, a subset of lymph node dendritic cells, and endometrial stromal cells.44–48 Desmin expression is uncommon in myofibroblasts.

Diagnostic Uses.—Desmin is best used as a screening marker for tumors of skeletal muscle differentiation, which are extraordinarily rare in the skin. Although desmin expression is almost always present in pilar smooth muscle tumors, it may be absent in smooth muscle tumors of vascular smooth muscle origin (vascular smooth muscle less commonly expresses desmin, for unknown reasons). Smooth muscle actin is a much better screening marker for smooth muscle tumors. When the differential diagnosis includes true smooth muscle and myofibroblastic tumors, strong desmin expression supports true smooth muscle differentiation. Anomalous desmin expression may be seen in melanoma, schwannoma, tenosynovial giant cell tumor, Ewing sarcoma, and angiomyoid (malignant) fibrous histiocytoma, among others.49–59

ISSUES SPECIFIC TO PARTICULAR TUMOR TYPES

Squamous Cell Carcinoma

Extremely Focal or Absent Cytokeratin Expression in Sarcomatoid SCC—Sarcomatoid SCCs may show only extremely focal cytokeratin expression, may entirely lose cytokeratin expression in spindled areas, or may express only high-molecular-weight cytokeratins (Figure 8). The most sensitive markers of sarcomatoid SCC are 34BE12 and CK5/6.7–4 It is also important to recognize that essentially all sarcomatoid SCCs will express vimentin, as discussed previously in “Discussion of Antigens.”

Melanoma

Cytokeratin and/or Desmin Expression.—Melanomas are notorious for anomalous expression of intermediate filament proteins, with some series documenting anomalous expression in more than 30% of cases.50 Cytokeratin is the most common anomalously expressed intermediate filament in melanoma, obviously raising significant potential for misdiagnosis of cytokeratin-positive melanomas as carcinoma. Use of a panel of immunostains, including S100 protein, should greatly alleviate this problem. In general, anomalous intermediate filament expression tends to be focal in nature, although it may rarely be diffuse. We have seen desmin-positive melanomas misdiagnosed as rhabdomyosarcoma and leiomyosarcoma; additional IHC with markers such as HMB-45, myogenin, and muscle actins is necessary for arriving at the correct diagnosis in these unusual cases (Figure 9).

CD68 Expression.—CD68 (KP1) is a relatively nonspecific marker of lysosomes, rather than a lineage-specific histiocytic marker.61 CD68-positive melanomas may be mistaken for AFXs, if one is using this marker in an attempt to confirm “fibrohistiocytic differentiation.” CD68 has a very limited role in the diagnosis of cutaneous spindle cell tumors, as it may also be expressed in angiosarcomas, carcinomas, and leiomyosarcomas, among others.62–65 (Figure 10, A and B).

Entrapped Smooth Muscle Actin—Positive Myofibroblasts.—Myofibroblasts are frequently found intimately associated with the neoplastic cells of desmoplastic melanoma.18 We have seen such cases misdiagnosed as leiomyosarcoma or myofibroblastic lesions such as nodular fascitis or fibromatosis, based on this staining. An S100 immunostain is invaluable here to highlight the neoplastic melanocytes.

Angiosarcoma

Cytokeratin Expression.—Approximately 10% to 30% of angiosarcomas express cytokeratins (low molecular weight only).64–69 Failure to appreciate this may result in an erroneous diagnosis of carcinoma. Technically this is not considered anomalous expression, as normal endothelial cells also express cytokeratins at low levels.

CD68 Expression.—As in melanoma (see previous), angiosarcomas may express CD68, particularly when they have granular cell features (so-called granular cell angiosarcoma).

Leiomyosarcoma

Cytokeratin Expression.—A minority of leiomyosarcomas (<25%) will contain cytokeratin-positive cells, sometimes numerous. Cytokeratin-positive smooth muscle tumors express only low-molecular-weight isoforms, unlike sarcomatoid SCCs.71–73 Application of a panel of immunostains, including those to smooth muscle actin and desmin, should allow for confident separation of these 2 entities.

AFX/Superficial Malignant Fibrous Histiocytoma

Diagnostic Criteria.—Atypical fibroxanthoma typically presents as a rapidly growing mass in a sun-exposed region of an older adult. Histologically, most AFXs are undifferentiated, pleomorphic spindle cells tumors, although relatively monomorphic variants do exist. The diagnosis of AFX should be reserved for small (<1–1.5 cm) lesions
Figure 6. Conventional (clear cell) renal cell carcinoma, showing artifactual strong “membranous expression” of von Willebrand factor (vWF). This common artifact is because of the presence of circulating vWF in the serum and may be extremely difficult to distinguish from true vWF expression (original magnification ×100).

Figure 7. Apparent smooth muscle actin expression in desmoplastic/sarcomatoid malignant melanoma. Careful inspection shows the smooth muscle actin–positive cells to represent entrapped myofibroblasts (smooth muscle actin, 1A4, original magnification ×200).

Figure 8. Sarcomatoid squamous cell carcinoma, negative for cytokeratins (AE1/AE3, original magnification ×100).

Figure 9. Anomalous desmin expression in malignant melanoma (original magnification ×200).

Figure 10. A, Nodular fasciitis (hematoxylin-eosin, original magnification ×100). B, Intense expression of CD68 (original magnification ×100). Partially on the basis of this finding, this case was submitted in consultation with a suggested diagnosis of “malignant fibrous histiocytoma.” CD68 expression is very common in a variety of neoplasms and does not imply histiocytic or “fibrohistiocytic” differentiation.
that are confined to the dermis and that are completely visualized.\textsuperscript{74}

**Immunohistochemistry.**—Atypical fibroxanthoma is a histologic and immunohistochemical diagnosis of exclusion. There are no markers or combinations of markers that establish the diagnosis of AFX/malignant fibrous histiocytoma. The lesional cells of AFX must be negative for cytokeratins and S100 protein; a small amount of actin is expressed. There are no markers or combinations of markers to specifically identify melanoma. There are no markers to specifically identify melanoma cells, and these must be rigorously identified as nonlesional cells. CD68 expression does not support or exclude the diagnosis of AFX. CD99 (MIC2 glycoprotein, O13, HBA71) expression is not at all specific for the diagnosis of AFX and should have no role in this diagnosis.\textsuperscript{75}

**References**

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