Spindle cell neoplasms of the gastrointestinal (GI) tract show considerable morphologic overlap. Thus, immunohistochemistry is an essential tool in the proper diagnosis of these lesions. Several immunohistochemical stains are particularly helpful in the evaluation of these spindle cell lesions, each with its own strengths and limitations. This review will serve as an outline of commonly used immunohistochemical markers helpful in the diagnosis of spindle cell neoplasms of the GI tract, as well as propose guidelines for their use. This review encompasses only spindle cell neoplasms of the GI tract that typically present as mural masses; discussion of spindle cell lesions that present as mucosal polyps are not included in this article.

DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS

Gastrointestinal stromal tumors (GISTs) compose most spindle cell neoplasms arising in the GI tract, followed by leiomyosarcomas and schwannomas. Less commonly encountered spindle cell neoplasms include solitary fibrous tumor, desmoid-type fibromatosis, and metastatic melanoma.

Tyrosine kinase receptor inhibitors such as imatinib mesylate (Gleevec, Novartis, East Hanover, New Jersey) and next generation agents such as sunitinib (Sutent, Pfizer, New York, New York) are efficacious in the treatment of GISTs; thus, the proper diagnosis of these tumors is crucial to ensure appropriate and effective therapy. For this reason, staining for c-kit has become an essential tool in evaluating GI tract spindle cell neoplasms. Positive staining with c-kit in a spindle cell lesion in the appropriate histologic context confirms the diagnosis of a GIST.1

However, 4% to 15% of GISTs do not stain with c-kit, and the morphology of these tumors often overlaps with other spindle cell tumors in the differential diagnosis; thus, immunohistochemistry is a vital diagnostic tool in evaluating spindle cell neoplasms of the GI tract.1,4

COMMONLY USED MARKERS

Most Useful Antibodies

c-kit (CD117).—Background.—c-kit is a transmembrane tyrosine kinase receptor involved in mitogenic signaling and has been assigned to the 117 cluster designation (CD) antigen group.2,3 In most GISTs, the c-kit gene acquires a gain of function mutation and is thus constitutively activated.5 Because of immunophenotypic and ultrastructural similarities, investigators2,5–8 have postulated that GISTs are derived from the interstitial cells of Cajal. Of note, c-kit is expressed in other normal tissues, including mast cells and certain hematopoietic precursor cells.

Advantages.—c-kit expression by immunohistochemistry is considered a key diagnostic feature in GISTs and is seen in most of these tumors.3,9,10 The pattern of c-kit expression in GISTs is typically strong and diffuse, showing a pan-cytoplasmic and sometimes membranous pattern (Figure 1). In addition, 46% of GISTs show a cytoplasmic “dotlike” immunostaining pattern; GISTs with a dotlike pattern of expression are more likely to be extraintestinal or show epithelioid morphology.4
Pitfalls in Diagnosis.—c-kit expression is not restricted to GISTs and can be seen in a variety of neoplasms including melanoma, renal cell carcinoma, and seminoma.\textsuperscript{11–13} Most relevant to this review is the variable expression of c-kit in desmoid-type fibromatosis; early reports describing the range of expression of c-kit by immunohistochemistry\textsuperscript{4,14} found that up to 75% of desmoid-type fibromatoses show weak, granular, cytoplasmic staining. However, a more recent article\textsuperscript{15} has shown that, with optimal dilution of the primary antibody, very few desmoid fibromatoses stain with c-kit. Other authors\textsuperscript{7,16,17} have addressed this issue, finding only minimal cytoplasmic staining in approximately 5% of desmoid-type fibromatoses. Miettinen\textsuperscript{17} further addressed this issue in 2003; he concluded that it is the choice of antibody and method used, specifically antibody dilution, which determines c-kit positivity in desmoid tumors. In 2003, Lucas et al\textsuperscript{15} published results which supported Miettinen’s findings, when they reported that only 1 of 19 cases stained for c-kit at the optimal dilution of 1:250 without antigen retrieval.

Smooth Muscle Actin.—Background.—The actins are an important family of cytoskeletal proteins that are involved in contractility and cell movement. There are several antibodies used to detect actins; however, anti–smooth muscle actin remains the most widely used, as this antibody specifically recognizes the smooth muscle isoform. Although this isoform was originally thought to be specific for cells that show smooth muscle differentiation, myofibroblasts and myoepithelial cells also express smooth muscle actin (SMA).\textsuperscript{18}

Advantages.—One of the main entities in the differential diagnosis of spindle cell lesions of the GI tract is leiomyosarcoma. Depending on the series, SMA positivity is reported in 63% to 100% of leiomyosarcomas; the degree of SMA expression is often related to the grade of the tumor, with high-grade lesions showing a lower degree of expression.\textsuperscript{4,19–21} Furthermore, leiomyosarcomas typically do not express c-kit, CD34, S100, or β-catenin. Also, solitary fibrous tumors do not typically stain with SMA.\textsuperscript{4,22–24}

Pitfalls in Diagnosis.—In addition to leiomyosarcoma, 10% to 47% of GISTs show expression of SMA. The likelihood of positive staining of a GIST with SMA appears to depend on its location within the GI tract. Up to 47% of small-bowel GISTs and 10% to 13% of rectal and esophageal GISTs demonstrate positive staining for SMA.\textsuperscript{7,25} This finding is typically not a diagnostic issue since leiomyosarcomas are negative for c-kit.

Desmin.—Background.—Desmin is a 53-kDa intermediate filament protein that is characteristically found in cardiac, smooth, and striated muscle cells. The clones D33, DER-11, and DEB-5 are the 3 most used monoclonal antibodies to desmin.\textsuperscript{26,27}

Advantages.—Reports of gastrointestinal leiomyosarcoma show that 33% to 100% of cases demonstrate positive staining with desmin; like SMA, the degree of staining is related to the grade of the tumor, with higher-grade leiomyosarcomas less likely to be positive.\textsuperscript{4,23,26,29} Additionally, studies addressing the immunophenotype of leiomyosarcoma are small, ranging from 3 to 18 cases, a fact that also accounts for the wide variation in desmin expression. It is also important to note that some of the cases that were considered desmin positive showed only focal staining in 5% to 10% of tumor cells; this may be problematic for needle biopsies or small samples, which may not represent the entire lesion.\textsuperscript{28,30}

Pitfalls in Diagnosis.—Although desmin is a sensitive marker for myogenic differentiation, it may also stain tumors that show myofibroblastic differentiation such as desmoid-type fibromatosis. At least 1 report\textsuperscript{4} has stated that up to 5% of desmoid-type fibromatoses stain with desmin. Furthermore, approximately 5% to 10% of GISTs stain with desmin. However, positivity is seen more commonly in tumors with an epithelioid morphology (10%) as opposed to tumors with spindle-shaped cells (2.5%).\textsuperscript{27,17,30} Of note, other markers of smooth muscle differentiation, such as h-caldesmon, commonly stain GISTs.\textsuperscript{31}

S100 Protein.—Background.—S100 protein was first dis-
covered in glial cells from brain extracts and belongs to a family of calcium-binding proteins. Its name is derived from the fact that it is soluble in 100% ammonium sulfate solution. Although it was originally isolated from brain extracts, many tissues express S100 protein, including melanocytes, chondrocytes, Schwann cells, Langerhans histiocytes, and various epithelia such as those from the breast, salivary gland, and female reproductive tract. Despite this wide expression profile, S100 expression remains important in identifying tumors of schwannian, melanocytic, and chondrocytic differentiation.32–34

Advantages.—The utility of S100 protein expression in spindle cell neoplasms of the GI tract is its ability to identify schwannomas. Schwannomas show strong and uniform nuclear and cytoplasmic staining for S100 protein in 100% of cases in reported series.22,35,36

Pitfalls in Diagnosis.—Many series have shown a lack of S100 protein reactivity in GISTs; however, several authors2,7,9,25 have reported cytoplasmic and nuclear positivity for S100 in approximately 5% to 10% of GISTs. Additionally, Miettinen et al25 found that S100 protein expression in GISTs is restricted to the small intestine, whereas GISTs derived from other sites along the GI tract are typically S100 negative. Gastrointestinal stromal tumors that express S100 were formerly classified as gastrointestinal autonomic nerve tumors37; however, this nomenclature has been abandoned since there is no clinical utility in separating gastrointestinal autonomic nerve tumors from GISTs. In the case of a S100-positive GIST, the differential diagnosis would include schwannoma; however, the distinguishing stain in this case is c-kit since schwannomas are uniformly negative for this marker.3,38 Additionally, 4% of solitary fibrous tumors express S100; rare cells in desmoid-type fibromatosis may be positive for S100.39

β-Catenin.—Background.—β-Catenin is involved in the Wnt and E-cadherin signaling pathways, which play a role in tumorigenesis. In certain neoplasms, β-catenin accumulates in the cytoplasm and aberrantly translocates to the nucleus when there is dysregulation of these pathways.40,41 As β-catenin accumulates in the nucleus, it activates oncogenes. Detection of nuclear accumulation of β-catenin can be accomplished by immunohistochemistry.42

Advantages.—The literature43–45 reports positive nuclear immunostaining of desmoid-type fibromatosis with β-catenin in the range of 71% to 100%. The largest of these studies44 included 30 desmoid fibromatoses, 80% of which showed positive nuclear staining. It is important to note that only nuclear expression of β-catenin is considered positive; only cytoplasmic staining should be regarded as negative (Figure 2).43

Pitfalls in Diagnosis.—The focal positivity of β-catenin in desmoid-type fibromatoses can be problematic in needle core biopsies and may result in a false-negative interpretation. In addition to desmoid-type fibromatoses, up to 24% of solitary fibrous tumors show positive staining for β-catenin. However, solitary fibrous tumors and desmoid-type fibromatoses are morphologically distinct.4

Potentially Useful Antibodies

DOG1.—Background.—DOG1 (Discovered On GIST-1) is a 960-amino acid protein with 8 transmembrane domains. Its specific function is unknown; however, its large number of transmembrane domains suggests that it may function in ion trafficking. DOG1 was discovered using gene expression profiling on GISTs.1,46

Advantages.—A recent tissue microarray study examined 447 GISTs and found that DOG1 stained 87% of GISTs, whereas c-kit stained 74% of GISTs. Positive staining in spindle cell GISTs showed membranous and cytoplasmic staining, whereas epithelioid GISTs showed predominantly membranous staining. Immunohistochemistry for DOG1 shows promising results with improved sensitivity and specificity as compared with c-kit; DOG1 is highly expressed in both kit-mutant and platelet-derived growth factor receptor α (PDGFRα)–mutant GISTs.1

Pitfalls in Diagnosis.—Since this is a relatively new antibody, limited data exist with regard to its performance in human tumors. However, the studies that are available seem to imply that DOG1 is not expressed in most other tumors. One recent study1 showed rare staining for DOG1 in 2.5% of synovial sarcomas, 0.3% of leiomyosarcomas, and 10% of desmoplastic melanomas. However, this antibody should be used with caution as its performance needs to be further studied.

Less Useful Antibodies

CD34.—Background.—CD34 is an approximately 110-kDa, heavily glycosylated transmembrane protein whose function is not yet fully understood. CD34 is normally expressed in embryonic cells of the hematopoietic system as well as in endothelial cells. In addition, CD34 is expressed in a wide variety of tumors including solitary fibrous tumors, epithelioid sarcomas, dermatofibrosarcoma protubersans, myofibroblastomas, and spindle cell lipomas.24,47,48

Advantages.—CD34 stains nearly 100% of solitary fibrous tumors and does not typically stain leiomyomas or desmoid-type fibromatoses.5

Pitfalls in Diagnosis.—Most GISTs (70%) show expression of CD34. The percentage of GISTs that are positive for CD34 varies by location, as 47% of small-bowel GISTs, 96% of rectal GISTs, and 100% of esophageal GISTs stain with CD34.4,24,25 Solitary fibrous tumors strongly stain with CD34 but are uniformly negative for c-kit.

Although CD34+ cells have been reported in a small percentage of colorectal schwannomas (2 of 9 cases), the staining is focal and not the diffuse pattern seen in solitary fibrous tumors or CD34+ GISTs.35 Additionally, Kaposi sarcoma, which may present as a mural lesion in the GI tract, especially in the population with human immunodeficiency virus/acquired immunodeficiency syndrome, is known to stain strongly for CD34. In a recent report,39 Kaposi sarcoma has been shown to express c-kit in a minority of cases. This potential overlap could present some potential diagnostic difficulties, especially in core biopsy material.

Platelet-Derived Growth Factor Receptor, α-Polyepitope.—Background.—Platelet-derived growth factor receptor (PDGFR) shares some similarities to the c-kit protein in that it is a type III tyrosine kinase, and, as the name implies, binds platelet-derived growth factor. The combination of receptor and ligand form 2 isoforms (αα or ββ) or heterodimers (αβ). Importantly, receptor tyrosine kinase inhibitors, such as Gleevec, also block PDGFR, making them important therapeutic agents.30 Many GISTs that are negative for c-kit by immunohistochemistry have mutations in the PDGFR gene53,55; despite the lack of a c-kit mutation, these
**Table: Immunohistochemical Approach to the Differential Diagnosis of Spindle Cell Lesions of the Gastrointestinal Tract**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Most Useful Antibodies</th>
<th>Typical Profile and Comments</th>
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</thead>
<tbody>
<tr>
<td><strong>GIST</strong></td>
<td>c-kit</td>
<td>Uniformly and strongly positive for c-kit in ~95% of cases. GISTS can be positive for SMA, desmin, and S100. DOG1/PDGFR-α may be useful for c-kit-negative GISTS (further studies needed).</td>
</tr>
<tr>
<td><strong>Leiomyosarcoma</strong></td>
<td>SMA, desmin</td>
<td>SMA positive in ~75% of cases. Desmin positive in 33%–100% of cases. Cytokeratin positive in up to 50% of cases. c-kit negative.</td>
</tr>
<tr>
<td><strong>Schwannoma</strong></td>
<td>S100</td>
<td>S100 strongly and uniformly positive in 100% of cases. c-kit negative.</td>
</tr>
<tr>
<td><strong>Desmoid-type fibromatosis</strong></td>
<td>β-catenin (nuclear stain only)</td>
<td>Nuclear staining is rather specific for desmoid-type fibromatosis. Up to 25% of SFTs may stain. Helps confirm SFT. 70% of GISTS are positive for CD34. c-kit negative.</td>
</tr>
<tr>
<td><strong>SFT</strong></td>
<td>CD34</td>
<td></td>
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</tbody>
</table>

**Abbreviations:** DOG1, discovered on GIST-1; GIST, gastrointestinal stromal tumor; PDGFR, platelet-derived growth factor; SMA, smooth muscle actin; SFT, solitary fibrous tumor.

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Tumors may still respond to receptor tyrosine kinase inhibitors. Histologically, c-kit-negative GISTS may have the classical spindle cell appearance, but they are more likely to have an epithelioid morphology and are more likely to arise from omental/peritoneal surfaces.

**Advantages.**—Approximately 4% to 5% of GISTS are c-kit negative. Antibodies against both PDGFR-α and PDGFR-β exist, though results of assays run with these antibodies can be difficult to interpret. Platelet-derived growth factor receptor, α polypeptide is more sensitive, staining 100% of c-kit-negative GISTS in a small series, whereas PDGFR-β only stained 2 of 8 c-kit-negative GISTS. In a more recent series, PDGFR-α was expressed in a significant number of c-kit-negative GISTS.

**Pitfalls in Diagnosis.**—Because there is not a uniformly reliable antibody for PDGFR-α for use in paraffin-embedded tissue, data are scarce regarding its staining profile for various spindle cell tumors, including reports of positive staining in cases of desmoid-type fibromatosis. Because of this, PDGFR-α is not routinely used in practice to diagnose c-kit-negative GISTS.

**USE OF IMMUNOHISTOCHEMISTRY FOR DIFFERENTIAL DIAGNOSIS: PROPOSED GUIDELINES FOR SPECIFIC CLINICAL SITUATIONS**

When faced with a spindle cell lesion of the gastrointestinal tract, there are 2 panels of antibodies that are particularly useful. The first panel consists of SMA, desmin, c-kit, and S100. This panel should be used most commonly in daily practice to aid in identification of gastrointestinal stromal tumor, leiomyosarcoma, and schwannoma. The second panel, composed of β-catenin and CD34, can be used when desmoid-type fibromatosis and solitary fibrous tumor are in the differential diagnosis (see Table).

**Group 1**

- **c-kit Positive (+/− SMA, Desmin, and S100 Expression).**—**Diagnosis: Gastrointestinal Stromal Tumor.**—c-kit expression, in the appropriate histologic context, is diagnostic of GIST. Gastrointestinal stromal tumors stain variably for the other antigens in the basic panel: 10% to 47% are positive for SMA, 5% to 10% are positive for desmin, and 5% to 10% are positive for S100.

**Group 2**

- **c-kit Negative, CD34+.**—**Diagnosis: Solitary Fibrous Tumor Versus c-kit–Negative GIST.**—Less than 5% of GISTS are negative for c-kit. In the case of a c-kit-negative and CD34+ tumor, the histologic differential diagnosis includes solitary fibrous tumor and c-kit–negative GIST. Fortunately, these 2 lesions are histologically distinct. Solitary fibrous tumor shows a “patternless pattern” of growth, dense collagen bands, and hemangiopericytoma-like/“staghorn” vessels. The pattern of growth in GISTS is more uniform in appearance. Gastrointestinal stromal tumors can contain skeinoid fibers, but they lack the dense collagen fibers and staghorn vessels seen in solitary fibrous tumors. These key histologic features are helpful in rare cases where a c-kit–negative GIST and solitary fibrous tumor are under consideration. As of this writing, there is no definitive positive diagnostic marker of c-kit–negative GIST, though DOG1, and possibly PDGFR-α, may hold promise (see above).

**Group 3**

- **SMA Positive, β-Catenin Positive (Nuclear), c-kit Negative, CD34++.**—**Diagnosis: Desmoid-type Fibromatosis.**—In this immunophenotypic group, the key finding is positive nuclear staining with β-catenin. Although it has been reported that up to 24% of solitary fibrous tumors can show nuclear positivity for β-catenin, solitary fibrous tumor and desmoid-type fibromatosis are morphologically distinct.

**Group 4**

- **SMA Positive, Desmin Positive, S100 Negative, c-kit Negative, CD34++.**—**Diagnosis: Leiomyosarcoma.**—Although CD34 can be positive in leiomyosarcomas, expression of SMA and/or desmin support the diagnosis of leiomyosarcoma. Additionally, cytokeratins can be positive in up to 50% of leiomyosarcomas, with the epithelioid subtype being more likely to be keratin positive. Leiomyoma of the retroperitoneum is a described entity that would stain identically to leiomyosarcoma. However, the precise classification of these bland smooth muscle tumors is controversial and depends on features such as a lack of nuclear atypia and a very low mitotic rate.

**Group 5**

- **SMA Negative, Desmin Negative, S100 Positive, c-kit Negative.**—**Diagnosis: Schwannoma (Consider Metastatic Melanoma).**—An S100-positive spindle cell lesion, in the appropriate histologic context, is diagnostic of schwannoma. It always bears mentioning that the small intestine is a common metastatic site for malignant melanoma. In cases in which melanoma is a consideration, testing for HMB-45 and/or MART-1 expression would be helpful. Also, c-kit is known to stain a minority of melanomas.

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References


