An accurate diagnosis of sarcoma requires a correlation of clinical and histological information with immunohistochemistry.

A CD117 and CD34 Immunoreactive Sarcoma Masquerading as a Gastrointestinal Stromal Tumor: Diagnostic Pitfalls of Ancillary Studies in Sarcoma

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**Background:** The immunohistochemical hallmarks of gastrointestinal stromal tumors (GISTs) are positivity for CD117 (c-kit) and CD34; however, CD117 is not specific for GISTs, and the list of CD117+ tumors/tissues is increasing. Also, MDM2 is known to be amplified in several types of mesenchymal tumors, including liposarcoma.

**Methods:** We report a spindle cell sarcoma arising in the mediastinum that morphologically and immunohistochemically mimicked GIST to illustrate the potential diagnostic pitfalls of ancillary studies in sarcoma and their appropriate use in conjunction with clinical content. Clinical information was obtained from electronic medical databases. Cytological, histological, and ancillary studies were retrieved from the archives of the Department of Anatomic Pathology at Moffitt Cancer Center. Literature of the last 20 years was reviewed. The role of biomarkers and their molecular testing in the prognosis and prediction of GIST is also discussed.

**Results:** A 75-year-old woman with a history of well-differentiated liposarcoma of the trunk/inguinal canal 5 years earlier developed a 5.5-cm heterogeneously enhancing mediastinal mass by computed tomography. Fine-needle aspiration biopsy revealed spindle cells with moderate pleomorphism and immunohistochemically reactive to CD117 and CD34 suggestive of GIST, but the clinical picture was unusual for GIST. Mutational analyses for KIT and platelet-derived growth factor receptor alpha (PDGFRα) were negative; DOG1 was not immunoactive, and this was believed to rule out GIST. An additional study of MDM2 by fluorescent in situ hybridization was positive, suggesting that this tumor was a dedifferentiated liposarcoma vs a spindle cell sarcoma not otherwise specified.

**Conclusions:** CD117+/CD34+ sarcoma is not diagnostic for GIST. KIT and PDGFRα mutational analyses are important in confirming a diagnosis of GIST and predicting its response to imatinib therapy. MDM2+ sarcoma is not diagnostic for liposarcoma. Although MDM2 is almost always positive in well-differentiated liposarcoma, which is useful in differentiating benign from atypical/well-differentiated lipomatous tumor, it should not be used in differentiating liposarcoma from other sarcomas.

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Introduction

As in many areas of oncology, tissue diagnosis is essential in planning the appropriate therapy in sarcoma treatment. Ancillary studies are essential in arriving at a definitive diagnosis and in subtyping sarcoma since traditional hematoxylin and eosin (H&E) stain has limitations.1,2 Ancillary studies include immunohistochemistry (IHC), electron microscopy, cytogenetics, and molecular analysis. IHC is currently the most commonly used and well-established method that studies the immunoreactivity of archived tissue. IHC is specific in its distribution in certain tissues that have characteristic patterns such as cytoplasmic, nuclear, membranous, or a combination thereof. The role of IHC in the workup of a mesenchymal lesion is threefold: to elucidate the lineage (eg, muscle, nerve, epithelial), to forecast the prognosis (eg, p53 mutation and increased Ki-67 are associated with poor prognosis), and to determine the response to targeted therapy (eg, CD117 positivity for response to imatinib therapy). To date, no IHC marker is available to differentiate benign from malignant mesenchymal tissue. Histopathology diagnosis by a pathologist using traditional criteria such as cellularity, nuclear pleomorphism, mitosis, and necrosis remains the gold standard in the diagnosis of a sarcoma. Electron microscopy looks into the ultrastructure of tissues and requires a specific fixative and special processing. For the most part, it is no longer used on a regular basis outside of kidney disorders and has been replaced with IHC and molecular studies.

Cytogenetics requires fresh tissue and cell culture to study the chromosomal structure of metaphased cells. Certain structural and/or numerical abnormalities are specific for some sarcomas. Most of the sarcomas have complex but not specific chromosomal abnormalities. The most commonly used molecular tests in sarcoma include fluorescent in situ hybridization (FISH) and mutational analysis, although polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) are also useful techniques for gene amplification or translocation detection. FISH can detect a specific translocation or amplification of interphased chromosomes on archived or fresh tissue. Because these studies are probe-specific, pathologists must be aware of the specific abnormality to look for when ordering the study. Mutational analysis studies mutation of genes using PCR and gene sequencing-based methodology.

To illustrate the potential diagnostic pitfalls of ancillary studies in sarcoma, we report a CD117+/CD34+/MDM2+ sarcoma arising in the mediastinum that presented a diagnostic challenge. In addition, we review the benefits and pitfalls of ancillary studies, focusing on IHC and molecular analysis in the diagnosis of spindle cell mesenchymal neoplasms (particularly GIST), as well as the prognostic and predictive markers of GIST and the role of MDM2 among mesenchymal neoplasms.

Materials and Methods

Case Description

A 75-year-old woman with a history of well-differentiated, sclerosing variant liposarcoma of the trunk/inguinal region 5 years prior was being followed for known 6-cm low-grade residual disease in the inguinal canal and extending down to her right labia majora. Re-excision with a right inguinal hernia repair 2 years after initial resection was consistent with lipoma. She claimed that the labial mass was unchanged since the time of her resection, which was concordant with cross-sectional imaging. Contrast-enhanced computed tomography (CT) of the chest was obtained as part of a workup for gastroesophageal reflux disease with associated dysphagia, odynophagia, asthma, and chronic cough. CT identified a heterogeneously enhancing 5.5-cm mediastinal mass (Fig 1A-B). Physical examination demonstrated no signs of oral mucosal lesions, jugular venous distention, bruits, abnormal breath sounds, or murmurs. Her inguinal canal lesion was soft and mobile, and it extended to the right labia majora. An endoscopic, ultrasound-guided, fine-needle aspiration biopsy of the mediastinal lesion was obtained.
**Tissue Diagnosis and Ancillary Studies**

Cytology smears revealed spindle cells with moderate pleomorphism and myxoid background consistent with a spindle cell mesenchymal tumor (Fig 2A-B). A cell block was prepared from the rinse of the needle. H&E preparation of the cell block revealed spindle cells with rare mitosis and no necrosis (Fig 2C). These spindle cells were IHC positive for CD117 and CD34 (Fig 2D-E). IHC stains for additional markers were all negative: pankeratin and epithelial membrane antigen (EMA) to exclude carcinoma; S-100 to exclude neural-based lesions and melanoma; desmin, smooth muscle actin (SMA), and myoglobin to exclude muscle-derived tumors; and mesothelin and calretinin to exclude mesothelioma. The tumor was also positive for vimentin. These H&E and IHC findings were consistent with gastrointestinal stromal tumor (GIST). However, this clinical picture was unusual for GIST because greater than 95% of GISTs arise in the gastrointestinal tract, with approximately 60% occurring in the stomach, 30% in the small intestine, and 8% in the esophagus, colon, and rectum. GISTs can occur in extra-intestinal locations (< 5%); however, no mediastinal GIST has ever been reported.

Mutational analyses for \( KIT \) (CD117) and platelet-derived growth factor receptor alpha (PDGFR\( \alpha \)), both on chromosome 4, were sent to ARUP Laboratories in Salt Lake City, Utah. These two molecular marker mutations are mutually exclusive and together account for greater than 95% of GISTs. These tests are performed by utilizing melting curves wherein the mutant DNA has a lower

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**Fig 2A-B.** — Diff-Quik and Papanicolaou stains of the cytology smear corresponding to the cell block (400×).

**Fig 2C.** — Hematoxylin-eosin stain of the cell block showing pleomorphic spindled cells (600×).

**Fig 2D.** — CD117 immunostain showing strong, diffuse positivity within the cytoplasm of the tumor cells.

**Fig 2E.** — CD34 showing moderate cytoplasmic staining within the tumor cells.
melting point than the normal (wild-type) DNA. In the event of an atypical melting curve, direct sequence analysis of the questionable exons is performed to confirm or refute a mutation. KIT mutations account for approximately 85% of cases with a constitutionally active tyrosine kinase. The most common mutations are in exons 9 and 11, with exons 13 and 17 also being reported. Most of the remainder of GISTs shows a PDGFRα mutation involving exons 12, 14, or 18. Cases have been described that are KIT and PDGFRα wild-type, some studies claiming as many as 10% to 15%, and it is postulated that these cases harbor an as-of-yet unidentified mutation.3 The exon involved is important because approximately 10% of GISTs are resistant to imatinib (Gleevec) therapy, and these cases have been associated particularly with KIT exon 9 or PDGFRα exon 18. These apparently imatinib-resistant cases may respond to doses at twice the usual level or may achieve a better response to a second-line agent, sunitinib (Sutent). However, in our cases, these studies were uniformly negative (not shown). An IHC study of DOG1 was also negative. An additional analysis of MDM2 by FISH was performed at the University of Nebraska Molecular Diagnostic Laboratory and returned positive (Fig 3). The final pathologic differential diagnosis was metastatic dedifferentiated liposarcoma vs a new primary spindle cell sarcoma not otherwise specified.

Follow-up
Multidisciplinary discussion regarding the imaging findings and pathology deemed the lesions unresectable due to the proximity/involvement of major vascular and pulmonary structures within the mediastinum. The patient was treated with four cycles of doxorubicin (Adriamycin) at a cumulative dose of 300 mg/m² and followed with repeat cross-sectional imaging. CT of the chest at the conclusion of systemic therapy demonstrated disease progression with worsening displacement of the previously involved mediastinal structures. She died of her disease 7 months after diagnosis of the mediastinal lesion.

Discussion
Spindle cell mesenchymal neoplasms are a common diagnostic challenge for even the most experienced pathologist. The differential diagnosis is dependent on location, but it may include smooth muscle tumors, neural tumors, desmoids tumors, monophasic synovial sarcoma, dermatofibrosarcoma protuberans, inflammatory myofibroblastic tumor, fibrosarcoma, spindle cell sarcoma not otherwise specified, and GIST. In these cases, a well-chosen panel of IHC will help elucidate the correct diagnosis (Fig 4). The IHC hallmark of GISTs is positivity for CD117 (c-kit) and CD34. However, neither CD117 nor
CD34 is specific for GISTs, and the list of CD117+ and/or CD34+ tumors is increasing (melanoma, seminoma, adenoid cystic carcinoma, small cell lung carcinoma, follicular thyroid carcinoma, Ewing sarcoma, Kaposi’s sarcoma, oncocytoma and chromophobe carcinoma of the kidney, thymic lesions, angiosarcoma, and certain leukemias/lymphomas).3

GISTs are mesenchymal neoplasms arising primarily in the gastrointestinal tract (95%) with approximately 60% occurring in the stomach, 30% in the small intestine and the remainder in the esophagus, colon, and rectum.4 Rare cases have been reported in the omentum and mesentry.5,6 As noted above, no mediastinal GIST has ever been reported. They are believed to derive from the interstitial cells of Cajal, and their pathogenesis involves activation of the KIT signaling pathway, making a constitutionally active tyrosine kinase.7-9 Most GISTs arise sporadically, but a minority occur as part of a syndrome, either in a familial setting with heritable germline KIT or PDGFRA mutation, in patients with neurofibromatosis type 1 syndrome (NF-1), or in the Carney triad (GISTs, pulmonary chondroma, and paraganglioma, occurring more often in young women).10,11 GISTs usually develop in the fifth or sixth decade and are rarely diagnosed in patients younger than 40 years of age. They rarely have been reported in children, the occurrence rate being under 1% and usually developing in the second decade, with a predilection for females, a gastric localization, and the epithelioid variant.12,13 No sex-related predilection has been reliably demonstrated (although some data indicate a predominance in males), nor have differences been reported based on race, ethnicity, occupation, or specific geographic distribution.3

Histologically, GISTs vary from spindle cell tumors to epithelioid and pleomorphic tumors. Most GISTs (95%) express CD117 (c-kit), CD34 (70%), and heavy caldesmon (80%), and 25% are positive for smooth muscle actin.

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**Fig 4.** — IHC approach to spindle cell mesenchymal neoplasms. SMA = smooth muscle actin, EMA = epithelial membrane antigen.
and < 5% for desmin. Despite histological overlap with smooth muscle neoplasms, GISTs are usually readily distinguishable from other mesenchymal tumors by their typical IHC profile. As described above, approximately 95% of GISTs express the kit receptor tyrosine kinase CD117, the most specific marker for these lesions. CD34 is also a useful but less specific finding (described below). C-kit, however, is not specific for GIST. Positivity is also seen in mast cells and also in melanocytes, which may be confusing in spindle cell melanomas that also have a similar histology to GISTs. Other tumors that have been shown to have some c-kit positivity include renal cell carcinomas, seminoma, dedifferentiated liposarcoma, Kaposi's sarcoma, angiosarcoma, Ewing sarcoma and extramedullary myeloid tumors, and lymphoid tumors. CD117 positivity in GIST is indicative of a mutation in the KIT gene on chromosome 4, a proto-oncogene encoding a type III transmembrane tyrosine kinase receptor. The most common exons involved are 9 and 11, with 13 and 17 also being reported. In GISTS, KIT gain-of-function mutations lead to constitutive activation of KIT; this overexpression is the target of the novel antityrosine kinase therapy with imatinib. Exons 9 and 13 are associated with resistance to imatinib; in these cases, patients may benefit from double the usual dose of imatinib or switching to a second-line agent such as sunitinib.

As described above, approximately 15% of GISTS will be c-kit negative. These cases almost always harbor a mutation in PDGFR. PDGFR is a tyrosine kinase like c-kit whose gene is also located on chromosome 4. Mutations occur within exon 12 (1%), 14 (6%), or 18 (80%). Mutations on exon 14 are associated with a worse prognosis. Since PDGFR is a tyrosine kinase, some of these cases have also been shown to respond to imatinib. However, a subset of cases, specifically those with the exon 18 mutation, will show resistance to imatinib and, similar to the resistant c-kit mutations, these patients may benefit from higher doses or second-line treatment.

CD34 is a monomeric transmembrane glycoprotein that is expressed on hematopoietic progenitor cells and endothelial cells. It is positive in early precursor leukemias, vascular tumors, and some mesenchymal tumors. CD34 has a role as an adhesion molecule in the bone marrow, and it is postulated to have a role in presenting carbohydrate ligands to selectins and in regulating adhesion to stromal cells. CD34 may react with a variety of mesenchymal lesions, such as nerve sheath tumors, dermatofibrosarcoma protubersans, and solitary fibrous tumors. Extensive membrane and cytoplasmic staining for CD34 is also a consistent feature of spindle cell lipomas. In addition, CD34 immunoreactivity identifies a sparse network of dendritic spindle cells in a variety of lipomatous tumors, including angiolipomas, myxoid liposarcomas, and atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLS). For ALT/WDLS, staining of the scattered atypical stellate cells may be variable, and positivity is usually widespread when present in the malignant spindle cell population of dedifferentiated liposarcoma. The utility of CD34 immunoactivity in distinguishing GIST from a lipomatous neoplasm has not been established and requires other immunostains or ancillary studies.

The DOG1 gene (discovered on GIST1) encodes a hypothetical protein specifically expressed in GISTs and is involved in the regulation of cell adhesion. DOG1 is proposed for use with CD117 as antibodies as choice for the diagnosis of GIST.
liposarcoma that is now dedifferentiated. However, due to its mediastinum location, the possibility of a new primary sarcoma cannot be excluded.

**MDM2 codes for the MDM2 protein.** The key target of MDM2 is the p53 tumor suppressor gene where it represses p53 transcriptional activity by blocking the N-terminal transactivation domain. MDM2 is also upregulated by p53, wherein its transcription can be activated by p53. Thus, when p53 is stabilized, the transcription of MDM2 is also induced, resulting in higher MDM2 protein levels. In addition, MDM2 acts as a ligase, targeting both itself and p53 for degradation by proteosomes. Its link to p53 suppression substantiates its oncogenic potential. Further supporting this role is its increased level in several tumors, including soft tissue sarcomas, osteosarcomas, and some breast tumors. Well-differentiated and dedifferentiated liposarcomas (WDLPS/DDLPS) contain amplified sequences from the 12q13-15 region, including the MDM2 gene.42-43 The detection of MDM2 overexpression (by IHC) or amplification (by FISH) was originally proposed as a reliable tool for distinguishing benign adipose tissue tumors from WDLPS and distinguishing DDLPS from other poorly differentiated tumors.44 However, recent studies, including this article, have shown that other tumors can be positive for MDM2 including chordomas, leiomyosarcomas, intimal sarcomas of the pulmonary artery, and focally, chondrosarcomas.45,46 MDM2 positivity is still a reliable marker to differentiate lipoma from an atypical lipomatous tumor/well-differentiated liposarcoma.

**Conclusions**
Ancillary studies are an integral part of pathologic diagnostics; however, these studies need to be used in conjunction with clinical and histological information. Pathologists must be aware that though several tumors have hallmark IHC profiles, much overlap exists between tumors. We used the example of a sarcoma initially thought to be a GIST to elucidate this as it pertains to CD117, CD34, and MDM2. It is important to note that a CD117+/CD34+ spindle cell neoplasm is not necessarily a GIST, and a MDM2+ sarcoma is not necessarily a liposarcoma. Awareness of the potential diagnostic pitfalls of ancillary studies, as well as their appropriate applications, is critical.

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