Advances in interventional technology have enhanced the ability to safely sample deep-seated suspicious lesions by fine-needle aspiration procedures. These procedures often yield scant amounts of diagnostic material, yet there is an increasing demand for the performance of more ancillary tests, especially immunohistochemistry and, not infrequently, molecular assays, to increase diagnostic sensitivity and specificity. A systematic approach to conserving diagnostic material is the key, and our previously proposed algorithm can be applied aptly in this context.

Objective.—To elaborate a simple stepwise approach to the evaluation of cytology fine-needle aspiration specimens and small biopsy tissue specimens, illustrating the algorithmic application of small panels of immunohistochemical stains in providing an accurate diagnosis with scant amounts of tissue, including the potential pitfalls that may arise while using immunohistochemical staining on small quantities of tissue.

Data Sources.—The sources include literature (PubMed), the first Chinese American Pathologists Association Diagnostic Pathology Course material, and the review authors’ research data as well as practice experience. Seven examples selected from the CoPath database at Geisinger Medical Center (Danville, Pennsylvania) are illustrated.

Conclusions.—A stepwise approach to the evaluation of fine-needle aspiration and small biopsy tissue specimens in conjunction with a small panel of select immunohistochemical stains has been successful in accurately assessing the lineage/origin of the metastatic tumors of unknown primaries. The awareness of the common pitfalls of these biomarkers is essential in many instances.

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Application of Immunohistochemistry in Undifferentiated Neoplasms

A Practical Approach

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Application of Immunohistochemistry in Undifferentiated Neoplasms
The objectives of this paper are to (1) summarize the algorithms that discuss the approach and application of the most useful immunohistochemical panels in the accurate diagnoses of undifferentiated neoplasms/malignancies of unknown origin; (2) illustrate the utilities and pitfalls of frequently used and recently described diagnostic immunohistochemical markers; and (3) demonstrate the effectiveness of small IHC panels for the diagnosis of tumors of unknown origin by the presentation of 7 examples.

**GENERAL APPROACH AND PROPOSED ALGORITHMS**

At our institution, when adequate numbers of core biopsies are submitted, they are separated at the time of processing into at least 2 blocks to ensure that an adequate amount of tissue is available to perform IHC and molecular cytogenetic tests if indicated.

The general principles and strategies we use to work with cytologic smears and cell blocks containing cellular material or small tissue biopsies in cases with tumor of unknown primary are as follows: (1) formulate a differential diagnosis based on cytomorphology and cell block sections “blind” or “cold” without being aware of the clinical information; (2) review the patient’s chart, including the history, physical examination, and lab evaluations, including imaging; (3) formulate the differential diagnosis based on cytohistomorphology and clinical information; (4) follow the algorithm (see below) and perform the recommended small panel of immunohistochemical stains (a single stain is never recommended); (5) exclude certain diagnoses based on cytohistomorphology, IHC results, and clinical-radiologic findings; (6) be wary of and familiar with aberrant expression of tissue/organ-specific markers; (7) be cautious when the triple test (clinical, imaging, and cytohistomorphology) does not fit a specific entity; and (8) morphologic features and clinical findings are still fundamental.

As summarized above, the general approach begins with a morphologic evaluation of the adequately aspirated material, which usually helps to delineate the lineage of the tumor cells. The presence of cohesive, 3-dimensional clusters of cells with or without single cells in the background is suggestive of epithelial origin. Loosely cohesive clusters and numerous single cells with plasmacytoid or spindled appearance and a stippled nuclear chromatin pattern are suggestive of neuroendocrine differentiation. Spindle cells in general are suggestive of mesenchymal tumors. Small round blue cells with lympho-glandular bodies in the background are suggestive of a lymphoproliferative disorder. Individual plasmacytoid cells with prominent macronucleolus and granular cytoplasm, usually with some pigment, are suggestive of melanoma. If one is able to identify the lineage without too much difficulty, the neoplasm most likely is well to moderately differentiated. In these situations, organ-specific or tumor type–specific immunomarkers are indicated, and these are summarized in Table 1.

The poorly differentiated neoplasms are the entities that require more elaborate workup. Immunohistochemistry plays a critical role in identifying the cell lineage and probable site of origin. Given their undifferentiated nature, these tumors often lose the expression of tissue- or lineage-specific markers; therefore, a panel of immunohistochemical markers is usually recommended. By the same token, a lack of staining with 1 immunostain should not be regarded as conclusive evidence to exclude the corresponding specific tissue or lineage, because poorly differentiated/undifferentiated carcinomas frequently lose expression of site-specific antigens.

A screening panel consisting of cytokeratin (CK), S100, vimentin, and leukocyte common antigen (LCA) should be applied first as a general approach to a tumor of unknown origin, as illustrated in Figure 1.

When an epithelial origin is confirmed (CK+, S100−, vimentin−/−, LCA−), the basic panel of CK7 and CK20 will allow us to determine from which organ system the primary tumor could potentially be arising, as summarized in Table 2.

A CK cocktail consisting of AE1/3 and CAM 5.2 is an effective marker for identification of epithelial lineage. CAM 5.2 identifies the low–molecular weight CKs, including CK7 and CK8, whereas AE1/3 is a cocktail that identifies both low–molecular weight and high–molecular weight CKs (pankeratin); however, it does not appear to react well to CK8 in practice. AE1/3 by itself is insufficient to exclude an epithelial lineage because positive immunoreactivity for AE1/3 alone was also reported in 111 cases (28%) of hepatocellular carcinoma, 150 cases (66%) of clear cell renal cell carcinoma, 23 cases (48%) of adrenal cortical carcinomas, and 98 cases (88%) of pulmonary small cell carcinomas (SMCCs).

Other broad-spectrum CKs containing keratin 8 and keratin 18, such as the clones KL1, OSCAR, MAK6, and 5D3/LP3, are also popular choices as screening CKs. Because poorly differentiated carcinomas are known to be heterogeneous in their antigen expression, a combination of immunohistochemical stains may be essential to establish tumor lineage. A pitfall to keep in mind is that poorly fixed specimens may have an unpredictable pattern of staining for CK.

In general, carcinomas express CK, whereas mesenchymal tumors express vimentin. However, there are exceptions to this rule. There are carcinomas that show loss of CK expression, carcinomas that frequently coexpress vimentin, and carcinomas that rarely express both CK and vimentin. These entities are summarized in Table 3. Vice versa, mesenchymal tumors and hematopoietic neoplasms may express epithelial markers, and these are summarized in Table 4.

Positive staining with LCA (CD45) and/or vimentin, but negative staining for S100 and CK, indicates lymphoid lineage, with high sensitivity and specificity for lymphoid malignancies. However, a large percentage of lymphoblastic leukemias are frequently negative for LCA,10 with unpredictable and frequently negative staining patterns in plasma cell dyscrasias and anaplastic hematopoietic lesions. Rare cases of LCA staining in metastatic undifferentiated neuroendocrine carcinomas have also been reported.

S100 is highly specific for melanocytic or neurogenic lesions, with both nuclear and cytoplasmic staining patterns. Sex-determining region Y box 10 (SOX10), melanoma-associated antigen recognized by T cells (Mart-1 [Melan-A]), and human melanoma black-45 (HMB-45) can all be performed to confirm melanocytic origin. Besides melanocytic lesions, positive staining with S100 with negative CK expression also suggests the possibilities of neurogenic tumors, chordoma, dendritic cell tumors, granular cell tumors, Langerhans cell tumors/histiocytosis, myoepithelial tumors, and some primary as well as metastatic carcinomas.
| Primary Site                          | IHC Markers                                                                 |
|--------------------------------------|------------------------------------------------------------------------------|
| Lung adenocarcinoma                  | TTF-1, Napsin A                                                              |
| Breast carcinoma                     | GATA3, ER, GCDFP-15                                                          |
| Urothelial carcinoma                 | GATA3, uroplakin II, S100P, CK5/6, p63, CK20                                 |
| Squamous cell carcinoma              | P40, CK5/6, p63, SOX2                                                         |
| Renal cell carcinoma, clear cell type| PAX-8/PAX-2, RCCma, vPHL, KIM-1                                              |
| Papillary renal cell carcinoma       | P504S, RCCma, vPHL, PAX 8, KIM-1                                              |
| Translocation renal cell carcinoma   | TFE3                                                                         |
| Hepatocellular carcinoma             | Arginase-1, glypican-3                                                       |
| Adrenal cortical neoplasm            | SF-1, Mart-1, inhibin-α, calretinin                                          |
| Melanoma                             | S100, Mart-1, HMB-45, MiTF, SOX10, PN2                                        |
| Merkel cell carcinoma                | CK20 (perinuclear dot staining), MCPyV                                       |
| Mesothelial origin                   | Calretinin, WT1, D2-40, CK5/6, mesothelin                                   |
| Neuroendocrine origin                | Chromogranin, synaptophysin, CD56                                            |
| Upper gastrointestinal tract         | CDH17, CDX2, CK20                                                            |
| Lower gastrointestinal tract         | SATB2, CDX2, CK20, CDH17                                                     |
| Intrahepatic cholangiocarcinoma      | pVHL, CAIX                                                                   |
| Pancreas, acinar cell carcinoma      | Glypican-3, antitrypsin, Bc10                                                |
| Pancreas, ductal adenocarcinoma      | MUC 5AC, CK17, maspin, S100P, IMP3                                            |
| Pancreas, neuroendocrine tumor       | PR, PAX-8, PDX1, CDH17, islet-1                                              |
| Pancreas, solid pseudopapillary tumor| Nuclear β-catenin, loss of E-cadherin, PR, CD10, vimentin, loss of chromogranin |
| Prostate, adenocarcinoma             | NXX3.1, PSA, PSAF, ER                                                         |
| Ovarian serous carcinoma             | PAX-8, ER, WT1                                                               |
| Ovarian clear cell carcinoma         | pVHL, HNF-1B, KIM-1, PAX-8                                                    |
| Endometrial adenocarcinoma           | PAX-8/PAX-2, ER, vimentin                                                     |
| Endocervical adenocarcinoma          | PAX-8, p16, CEA, HPV in situ hybridization, loss of PAX-2                    |
| Thyroid follicular cell origin        | TTF-1, PAX-8, thyroglobulin                                                  |
| Thyroid medullary carcinoma          | Calcitonin, TTF-1, CEA, chromogranin                                         |
| Salivary duct carcinoma              | GATA3, AR, GCDFP-15, HER2                                                    |
| Thymic origin                        | PAX-8, p63, CD5                                                              |
| Seminoma                             | SALL4, LIN28, OCT4, CD117, D2-40                                             |
| Yolk sac tumor                       | SALL4, LIN28, glypican-3, AFP                                                |
| Embryonal carcinoma                  | SALL4, LIN28, OCT4, NANOG, CD30, SOX2                                       |
| Chorionicarcinoma                    | GATA3, β-HCG, CD10                                                           |
| Sex cord stromal tumor               | SF-1, inhibin-α, calretinin, FOXL2                                           |
| Vascular tumor                       | ERG, CD31, CD34, Fli-1                                                       |
| Synovial sarcoma                     | TLE1, CK                                                                    |
| Chordoma                             | CK, S100, brachyury                                                          |
| Desmoplastic small round cell tumor  | CK, CD99, desmin, WT1 (N-terminus)                                           |
| Alveolar soft-part sarcoma           | TFE3                                                                        |
| Rhabdomyosarcoma                     | Myogenin, desmin, MyoD1                                                      |
| Smooth muscle tumor                  | SMA, MSA, desmin, calponin                                                   |
| Ewing sarcoma/PNET                   | NXX2.2, CD99, Fli-1                                                          |
| Myxoid and round cell liposarcoma    | NY-ESO-1                                                                    |
| Low-grade fibromyxoid sarcoma        | MUC4                                                                        |
| Epithelioid sarcoma                  | CD34, loss of INI1                                                            |
| Atypical lipomatous tumor            | MDM2 (MDM2 by FISH is a more sensitive and specific test), CDK4              |
| Histiocytosis X                      | CD1a, S100, langerin (CD207)                                                 |
| Angiomyolipoma                       | HMB-45, SMA, Mart-1                                                          |
| Gastrointestinal stromal tumor       | CD117, DOG1                                                                 |
| Solitary fibrous tumor               | STAT6, CD34, Bcl2, CD99                                                     |
| Myoepithelial carcinoma              | Cytokeratin and myoepithelial markers (may lose INI1)                        |
| Myoid sarcoma                        | CD43, CD34, MPO                                                              |
| Follicular dendritic cell tumor      | CD21, CD35                                                                  |
| Mast cell tumor                      | CD117, tryptase                                                              |

Abbreviations: AFP, α-fetoprotein; AR, androgen receptor; Bcl, B-cell lymphoma; β-HCG, β-human chorionic gonadotropin; CAIX, carbonic anhydrase IX; CDH17, cadherin 17; CK4, cyclin-dependent kinase 4; CDX2, caudal type homeobox 2; CEA, carinoembryonic antigen; CK, cytokeratin; D2-40, podoplanin; DOG1, discovered on GIST1; ER, estrogen receptor; ERG, ETS-related gene; FISH, fluorescence in situ hybridization; Fli-1, friend leukemia virus integration-1; FOXL2, forkhead box L2; GATA3, GATA-binding protein 3; GCDFP-15, gross cystic disease fluid protein 15; HER2, human epidermal receptor 2; HMB-45, human melanoma black 45; HNF-1B, hepatocyte nuclear factor 1B; HPV, human papilloma virus; IMP3, insulin-like growth factor 2 mRNA-binding protein 3; INI1, integrase interactor 1; KIM-1, kidney injury molecule 1; LIN28, abnormal cell lineage protein 28; Mart, melanoma antigen recognized by T cells; Maspin, mammary serine protease inhibitor; MCPyV, Merkel cell polyoma virus; MiTF, microphthalmia transcription factor; MPO, myeloperoxidase; MSA, muscle-specific actin; MUC, mucin; MyoD1, myogenic differentiation 1; NXX2.2, NK2 homeobox 2; NXX3.1, NK3 homeobox 1; NY-ESO-1, New York esophageal squamous cell carcinoma-1; OCT4, octamer-binding transcription factor 4; P504S, α-methylacyl-CoA racemase; PAX, paired box gene; PDX1, pancreatic and duodenal homeobox 1; PN2, melanoma-associated antigen; PR, progesterone receptor; PSA, prostate-specific antigen; PSAP, prostate-specific alkaline phosphatase; pVHL, von Hippel-Lindau tumor suppressor; RCCma, renal cell carcinoma marker; S100P, placent S100; SALL4, Sal-like protein 4; SATB2, special AT-rich sequence-binding protein 2; SF-1, steroidogenic factor 1; SMA, smooth muscle actin; SOX, sex-determining region Y box; STAT6, signal transducer and activator of transcription 6; TFE3, transcription factor E3; TLE1, transducin-like enhancer of split 1; TTF-1, thyroid transcription factor 1; WT1, Wilms tumor 1.
Vimentin is an intermediate filament characteristic of mesenchymal cells. It is found in almost all sarcomas (alveolar soft-part sarcoma tends to be negative for vimentin) and melanomas, and variably in lymphomas and even some carcinomas. It is usually part of a broad panel of immunohistochemical stains and plays a supportive role in conjunction with other immunomarkers. Because the antigenicity of the tissue is preserved on optimal alcohol fixation, it also serves as an indicator of appropriate tissue fixation when positive.

**THE APPLICATION OF THE ALGORITHM BY EXAMPLES**

**Epithelial Tumors: Metastatic Papillary Renal Cell Carcinoma, Type II**

An 80-year-old man with shortness of breath was found to have mediastinal and lung masses as well as retroperitoneal lesions. Endoscopic ultrasound-guided FNA of the mediastinal mass was performed and revealed the following.

The smears revealed cohesive clusters and papillary groups of cells with enlarged round nuclei, prominent nucleoli, nuclear pleomorphism, and a moderate amount of eosinophilic, granular cytoplasm. The hematoxylin–eosin section of the cell block showed cohesive epithelial clusters of malignant cells with a moderate amount of dense eosinophilic cytoplasm on a fibrovascular core were forming papillae. Representative images are shown in Figure 2.

The assessment of cytomorphology and histomorphology suggested an epithelial neoplasm of the mediastinum that appeared to have a papillary architecture. The differential diagnosis includes primary tumors of this site, such as lung and thymic carcinomas, as well as metastases showing papillary architecture, such as thyroid, kidney, and urinary tract tumors.

The lack of nuclear grooving and pseudoinclusions of the tumor cells made thyroid papillary carcinoma less likely. The trabeculated appearance with polygonal cells and abundant cytoplasm also brought hepatocellular carcinoma and adrenal cortical carcinoma into the differential considerations. In this male patient, the presence of prominent nucleoli in the tumor cells raised the possibility of a metastatic prostate adenocarcinoma.

Immunohistochemistry studies revealed the tumor cells were positive for CK but lacked expression for CK7, CK20, and CK5/6; this CK expression phenotype made urinary and lung primaries less likely. In addition, immunostains for organ-specific immunomarkers revealed tumor cells that were positive for paired box gene 8 (PAX-8) and negative for thyroid transcription factor-1 (TTF-1) and NK3 homeobox 1 (NKX3.1). At this point, a metastatic kidney tumor was of the highest differential diagnosis consideration.

The immunostain phenotype showing negativity for CK7, CK20, CK5/6, and TTF-1 excluded lung, thyroid, and urinary tract as primary sites. Additional immunomarkers also revealed the tumor cells to be reactive to α-methylacyl-CoA racemase (P504S), patchy for von Hippel–Lindau tumor suppressor (pVHL), and focally weak for carbonic anhydrase IX (CA9). The cytohistomorphology in conjunction with the immunostaining profile was in favor of a metastatic papillary renal cell carcinoma (PRCC). Representative images are shown in Figure 3.

The final diagnosis was consistent with metastatic PRCC, type II.

Papillary renal cell carcinoma is the second most common subtype of kidney cancer and consists of approximately 10% to 16% of cases. These tumors may be bilateral or multifocal and are known to be less aggressive than RCC, clear cell type. The PRCC lesions are often red-brown and frequently have a well-demarcated pseudocapsule when small. At resection, these tumors can be either solid or have a solid and cystic appearance. They have papillary or tubulopapillary architecture and show variable proportions of papillae with frequent hemorrhage, which leads to...
necrosis and cystic degeneration. The papillae have a central fibrovascular core with aggregates of foamy macrophages and calcifications.\(^\text{15}\)

Papillary renal cell carcinomas have been subcategorized in to type I and type II based on morphology and immunostaining profile, as summarized in Table 5.\(^\text{16–20}\)

For the example above, we favor a type II PRCC, based on the morphologic features of the tumor cells, such as abundant eosinophilic cytoplasm and the prominent Fuhrman grade 3 to 4 nuclei, as well as the CK7 profile of this tumor.

Papillary renal cell carcinomas can be hereditary, and in these cases they are typically bilateral, multifocal, and frequently type I.\(^\text{21}\) Cytogenetic alterations are common in PRCC. Gains of chromosomes 7, 8q, 12q, 16p, 17, and 20, and loss of 9p are often reported. The type II PRCC tumors appear to frequently gain 8q and lose 1p and 9p.\(^\text{18,20}\)

**Small Round Blue Cell Tumor–Merkel Cell Carcinoma**

A 70-year-old man with a past history of colon cancer and shave biopsies for skin lesions was found to have liver masses on computed tomography scan, suspicious for metastases. An FNA of one of the liver masses was performed.

The Diff-Quik smear revealed a population of dyscohesive atypical cells with large nuclei with a fine chromatin pattern and small, inconspicuous nucleoli; a high nuclear to cytoplasmic ratio; and a very thin rim of basophilic cytoplasm in a clean background. The atypical cells were mostly single with focal nuclear molding. There were abundant mitoses and some single-cell necrosis. The Papanicolaou stain also revealed the atypical cells with a chromatin pattern suggestive of neuroendocrine differentiation. The cell block section showed clusters of small to medium-size cells with minimal cytoplasm, frequent mitoses, and apoptotic debris, similar to the findings on the smears. Representative images are shown in Figure 4.

The dyscohesive pattern of the tumor cells generated the differential considerations of a neuroendocrine neoplasm, a lymphoproliferative disorder, and melanoma. The neuroendocrine chromatin pattern and the apoptotic debris mandated the inclusion of SMcc; neuroendocrine carcinoma, large cell type; and Merkel cell carcinoma (MCc) in the differential diagnosis.

Immunohistochemical stains, including CK, CK7, CK20, LCA, vimentin, S100, synaptophysin, TTF-1, and MIB-1 (Ki-67), were performed. The LCA and S100 allowed us to screen for lymphoma and melanoma. If the synaptophysin was positive, then neuroendocrine neoplasms would be considered. A mitotic index (MIB-1) in this setting would help to stratify the neuroendocrine tumors.

The tumor cells were positive for CK20 in a perinuclear dot-like pattern, CK, and synaptophysin. LCA, CK7, vimentin, S100, and TTF-1 were all negative. The MIB-1 (Ki-67) showed a proliferative index of greater than 95%. Representative images are shown in Figure 4.

The final diagnosis of this case is a metastatic MCc. Merkel cell carcinoma and SMcc share many similarities, including small blue cell morphology and neuroendocrine appearance, as well as some similarities in the staining pattern on IHC. However, there are differences between the two both morphologically and immunohistochemically. The morphologic features that may suggest MCc are the slightly larger size of the cells and a less dense/more open chromatin pattern that imparts a blastlike appearance. Immunohistochemically, MCc shows characteristic staining for CK20 in a perinuclear dot-like pattern secondary to the paranuclear clumping of intermediate filaments.\(^\text{22}\)

CK20 is of critical value in the differentiation of MCc from SMcc. Studies reported CK20 positivity in 97% to 100% of MCcs, whereas only 1 case (8%) of SMcc was positive for CK20. TTF-1 is equally important in this differentiation. TTF-1 was reported to be positive in more than 85% of SMccs, and no staining was seen in MCcs.\(^\text{22–24}\) Similar findings were observed in comparison of the staining pattern in MCc and metastatic SMcc.\(^\text{24}\) In addition, neurofilaments were positive in 12 MCc cases (92%) and negative in all SMccs. Other immunomarkers, such as synaptophysin, chromogranin, CD56, CK7, and neuron-specific enolase, were expressed in both with nearly the same frequency.

| Carcinomas That Frequently Express Both | Carcinomas That Rarely Express Both | Mesenchymal Tumors That Frequently Express Both |
|----------------------------------------|----------------------------------|-----------------------------------------------|
| Renal cell carcinoma                   | Breast carcinoma                 | Synovial sarcoma                               |
| Anaplastic thyroid carcinoma           | Ovarian carcinoma                | Desmoplastic small round blue cell tumor        |
| Endometrial carcinoma                  | Gastrointestinal carcinoma       | Epithelioid sarcoma                             |
| Thyroid carcinoma                      | Lung small cell carcinoma        | Epithelioid angiosarcoma                       |
| Sarcomatoid carcinoma                  | Lung non-small cell carcinoma    | Malignant rhabdoid tumor                       |
| Mesothelioma                           | Prostate carcinoma               | Leiomyosarcoma                                 |
| Myoepithelial carcinoma                |                                  | Chordoma                                       |
| Metaplastic breast carcinoma           |                                  | Adamantinoma                                   |

**Table 4. Mesenchymal Tumors and Hematopoietic Neoplasms that Express Cytokeratins**

| Mesenchymal tumors that frequently express both | hematopoietic tumors that express cytokeratins |
|-------------------------------------------------|-----------------------------------------------|
| Synovial sarcoma                                 | Plasmacytoma                                  |
| Desmoplastic small round blue cell tumor         | Diffuse large B-cell lymphomas                |
| Epithelioid sarcoma                              | Anaplastic large cell lymphoma                 |
| Epithelioid angiosarcoma                         |                                              |
| Pleomorphic undifferentiated sarcomas            |                                              |
| Rhabdomyosarcomas                               |                                              |
| Clear cell sarcomas                              |                                              |
| Malignant rhabdoid tumor                        |                                              |
| Leiomyosarcoma                                  |                                              |
| Chordoma                                        |                                              |
| Adamantinoma                                    |                                              |
| Myoepithelial carcinomas                        |                                              |
| Hematopoietic tumors that express cytokeratin    |                                              |

Application of IHC in Undifferentiated Neoplasms—Kandukuri et al
Mammalian or human achaete-scute complex homolog-1 (MASH1/HASH1) was reported to be a useful adjunct marker for the diagnosis of SMcC of the lung. In this study, 49 SMcC cases (83%) expressed MASH1 with a nuclear staining pattern; among those tumors, 43 cases (73%) also expressed TTF-1 along with staining of the crushed cells. It was observed that none of the 30 McCs expressed MASH1, and only 1 of the 30 McCs expressed TTF-1.

Merkel cell polyomavirus (MCPyV) was identified in January 2008 by Feng et al in tumor tissue from McC patients, proving clonal integration of the virus DNA into the host genome and finding prevalence of MCPyV DNA in McCs to be as high as 80% (n = 10). Since then, several studies have investigated the correlation between infection with MCPyV and clinical and prognostic factors.

Studies suggested that CK19 protein expression in tumor cells could represent functional MCPyV DNA status. Andres et al found CK19 expression in 11 of 32 specimens (34%), including a CK20- McC. This expression of CK19 was seen twice as often in MCPyV DNA-negative McCs compared with MCPyV DNA-positive ones, which renders CK19 an especially useful marker in CK20- McCs.

Unusual positivity was reported with such markers as mammary serine protease inhibitor (maspin), TTF-1, PAX-5, and terminal deoxynucleotidyl transferase (TdT). High-grade lymphomas, such as diffuse large B-cell lymphoma or Burkitt lymphoma, are often in the differential diagnosis because they share common cytologic or histologic features. The lymphoma cells are usually large in size, with large vesicular nuclei and prominent nucleoli that can be multiple. They usually have a moderate amount of cytoplasm that can be clear to eosinophilic to basophilic with lymphoglandular bodies in the background. A negative flow cytometry study showing a population of CD56+/CD57-/CD45- cells should raise concern for a malignancy of neuroendocrine origin, such as SMcC or McC.

Anaplastic T-Cell Lymphoma

A 55-year-old man with a history of esophageal adenocarcinoma presented with a left axillary solid, tender, nontender, ill-defined mass with a diameter of 6 cm. The mass was firm to palpation and fixed to the chest wall. The patient had no history of hepatitis or immunosuppression.

Figure 2. Representative images of metastatic papillary renal cell carcinoma, type II. A, Cohesive clusters of tumor cells with enlarged pleomorphic nuclei, prominent nucleoli, and a moderate amount of cytoplasm. B, Papillary group of tumor cells with fibrovascular cores. C and D, Cell block sections showing cohesive groups of tumor cells with significant nuclear atypia and a moderate amount of eosinophilic and granular cytoplasm (Diff-Quik, original magnification ×400 [A]; Papanicolaou stain, original magnification ×400 [B]; hematoxylin-eosin, original magnification ×400 [C and D]).

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that was greater than 5 cm in dimension. An FNA of
the mass was performed.

The cellular smears were composed of a population of
highly atypical cells with markedly enlarged pleomorphic
nuclei and abundant eosinophilic cytoplasm. Many bizarre
cells were seen showing binucleation or multinucleation,
some resembling “wreath cells” or “horseshoe” or “donut-
shaped” cells in the background of abundant lymphocytes,
as shown in Figure 5. Frequent mitoses were also identified,
whereas significant lymphoglandular bodies were not
appreciated.

The presence of large, multinucleated atypical cells
triggered a differential diagnosis of Hodgkin lymphoma,
poorly differentiated carcinoma, anaplastic T-cell lympho-
ma, and pleomorphic undifferentiated sarcoma.

Immunostains with a screening panel consisting of CK,
S100, vimentin, and LCA were performed and revealed
the atypical cells were positive for LCA and vimentin and

| Table 5. Differential Histomorphology and Immunoprofile of Papillary Renal Cell Carcinoma (PRCC) |
|--------------------------------------------------|--------------------------------------------------|
| Type I PRCC                                      | Type II PRCC                                     |
| Small basophilic cells with little cytoplasm     | Large eosinophilic cells with abundant granular cytoplasm |
| Thin papillae lined by tumor cells in a single layer surrounding the basal membrane | Thicker papillae lined by tumor cells in pseudostratified layers |
| Nuclear grade usually Fuhrman grades 1–2        | Nuclear grade usually Fuhrman grades 3–4         |
| These tumors have better prognosis and longer survival | These tumors are more commonly larger, present at higher T stage and grade, and frequently have nodal or distant metastatic disease |
| Immunohistochemical staining is usually positive for CK7, P504S, KIM-1, and CD10 | Immunohistochemical staining is frequently negative for CK7, strongly positive for P504S and CD10, and can be focally positive for CK20 |

Abbreviations: CK, cytokeratin; KIM-1, kidney injury molecule-1; P504S, α-methylacyl-CoA racemase.
Figure 4. Representative images of Merkel cell carcinoma. A, A population of dyscohesive atypical cells with large nuclei, high nuclear to cytoplasmic ratio, and rim of basophilic cytoplasm. B, The large nuclei with a fine chromatin pattern give an almost blastlike or neuroendocrine appearance, as well as small, inconspicuous nucleoli. C, Cell block showing clusters of medium-size cells with minimal cytoplasm, abundant nuclear apoptotic debris, and mitoses. D, Cytokeratin 20 positivity in a perinuclear dot-like pattern. E, Synaptophysin showing cytoplasmic positivity. F, MIB-1 proliferative index is higher than 95%. (Diff-Quik, original magnification ×600 [A]; Papanicolaou stain, original magnification ×600 [B]; hematoxylin-eosin, original magnification ×600 [C]; immunohistochemistry, original magnifications ×400 [D and E] and ×600 [F]).
negative for CK and S100. This phenotype indicated the lymphoid nature of this tumor. Additional immunomarkers were applied, revealing tumor cells that were positive for CD4, CD30, epithelial membrane antigen, and anaplastic lymphoma kinase (ALK); rare for CD3 and CD2; and negative for CD20, CD10, CD5, and PAX-5. The MIB-1 was estimated at 80%. Representative images are shown in Figure 6. Flow cytometric studies were performed but were uninformative because of the lack of adequate viable cells.

Figure 5. Representative images of anaplastic T-cell lymphoma. A, Abundant lymphocytes with large atypical cells. B, The atypical cells with markedly enlarged “wreath cells.” C and D, Some large, bilobed atypical pleomorphic cells. E and F, Cell block showing atypical multilobated giant cells that appear like “wreath cells” (Diff-Quik, original magnifications ×600 [A] and ×1000 [B]; Papanicolaou stain, original magnifications ×600 [C] and ×1000 [D]; hematoxylin-eosin, original magnifications ×400 [E] and ×1000 [F]).
These findings were consistent with ALK+ anaplastic T-cell lymphoma.

Anaplastic large cell lymphomas (ALCLs), initially described in 1985, are a subset of non-Hodgkin lymphomas that were previously called “Ki-1 lymphomas” and are characterized by large CD30+ (Ki-1+) anaplastic cells. The identification of the translocation of the chimeric fusion protein, a tyrosine kinase gene, NPM-ALK, involving ALK gene on chromosome 2p23 and the nucleophosmin (NPM) gene on chromosome 5q35, led to subsequent studies that revealed and subsequently confirmed that ALK+ ALCLs had a favorable prognosis.36,37

Peripheral T-cell lymphomas are not commonly seen, and ALCL is one of the most common subtypes, accounting for 25% of peripheral T-cell lymphomas in Western countries. The WHO classification currently recognizes 3 entities, including the ALK+ ALCL and ALK− ALCL (a provisional entity in the WHO 2008 classification), which are systemic diseases, and the primary cutaneous ALCL.38

The cytologic features of ALK+ ALCL are quite variable; however, the characteristic cells known as “hallmark” or “wreath” cells are present in all cases. They are large cells with abundant cytoplasm, and the nuclei are often horseshoe- or donut-shaped or lobated. The hallmark cells can range from abundant to scant depending on the histologic variant.

The WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue36 describes several histologic variants, including the common variant, lymphohistiocytic variant, small cell variant, Hodgkin-like variant, and composite variant. The common type (described in the example above) shows sheets of large lymphoid cells featuring hallmark cells. The lymphohistiocytic variant accounts for 10% of ALK+ ALCLs and reveals abundant reactive histiocytes in addition to anaplastic tumor cells. The small cell variant accounts for 5% to 10% of ALCLs and is frequently considered a peripheral T-cell lymphoma. Both the lymphohistiocytic and small cell variants are more common in children and can often be misdiagnosed as benign infiltrates. The Hodgkin-like pattern (3%) may resemble nodular-sclerosing subtype of classical Hodgkin lymphoma with tumor nodules surrounded by fibrous bands. Immunohistochemically, ALK-positive ALCL expresses both CD30 and ALK, and the strong correlation between immunohistochemical positivity for ALK and the presence

Figure 6. Immunophenotype of anaplastic T-cell lymphoma. A, Leukocyte common antigen decorate both the large atypical cells as well as lymphocytes in the background. B, CD30+ tumor cells. C, CD4+ tumor cells. D, Anaplastic lymphoma kinase–positive tumor cells (original magnification ×600).
of an ALK rearrangement has led to the use of immuno-histochemistry to diagnose this entity.39–46

Anaplastic large cell lymphoma must be differentiated from other hematopoietic entities, like ALK+ large B-cell lymphoma. The former is negative for CD20 and expresses CD30 (hallmark cells) in addition to ALK. ALK+ large B-cell lymphomas are generally of immunoblastic/plasmablastic morphology, with large central nuclei. They are typically negative for CD20 and CD30 immunostains and positive for CD138, epithelial membrane antigen, and ALK, and show light chain restriction with occasional multiple myeloma oncogene 1 (MUM1) positivity.

One must be aware of the pitfalls of ALK expression in other nonhematopoietic entities. ALK gene rearrangements and/or immunohistochemical expression is seen in lung adenocarcinomas, inflammatory myofibroblastic tumors, rhabdomyosarcomas, spindle cell/pleomorphic lipoma, intramuscular lipoma, Ewing sarcoma/peripheral primitive neuroectodermal tumors, malignant fibrous histiocytomas, and leiomyosarcomas.44,46 MUM1, also called interferon regulatory factor 4 (IRF4), immunohistochemical staining is seen in a significant number of ALLCs that are ALK+ (16 of 17; 94%) and ALK− (20 of 21; 91%).47

**Melanoma**

An 81-year-old man with prior history of squamous cell carcinoma of the skin presented with dizziness and difficulty breathing. A computed tomography image of the chest revealed multiple lung masses and lymphadenopathy. A possible brain lesion was also suspected because he complained of dizzy spells. The hilar lymph nodes were aspirated via endoscopic ultrasound guidance.

The smears were highly cellular, containing numerous single and loose clusters of spindled to plump cells with necrosis in the background. The atypical cells showed hyperchromatic nuclei without discernible nucleoli. Some rosettelike structures and scattered pigment-laden macrophages were evident. The cell block showed minute cores of tumor cells with spindled morphology, palisading around vessels. Representative images are shown in Figure 7, A through C.

The cellular findings, such as high cellularity with loosely cohesive and predominant single-cell pattern as well as the nuclear chromatin pattern, suggested a neuroendocrine neoplasm. However, the presence of dirty pigments in macrophages raised the possibility of a spindle cell melanoma. The palisading and rosettelike structures also brought neurogenic tumors, like malignant peripheral nerve sheath tumor (MPNST) and schwannoma, into the differential diagnosis considerations. The more frequently occurring sarcomatoid carcinoma was also considered.

A screening IHC panel to include CK, S100, synaptophysin, and vimentin was performed. The tumor cells were diffusely and strongly positive for S100 and vimentin, whereas CK and synaptophysin were negative. The profile strongly suggested a spindle cell melanoma or a neurogenic tumor, such as an MPNST, and, less likely, a schwannoma, given the presence of high-grade features. Additional immunohistochemical markers were applied and revealed tumor cell positivity for SOX10, Mart-1, and HMB-45. Representative images are illustrated in Figure 7, D through F.

The final diagnosis was consistent with metastatic melanoma, spindle cell morphology.

SOX10 is a member of the sex-determining region Y-related HMG-box family of transcription factors. This neural crest transcription factor is critical in the development and maintenance of Schwann cells and melanocytes, which allows for specific immunohistochemical characterization of tumors of melanocytic and Schwann cell lineage. The SOX10 protein is widely expressed in human benign tissues, including melanocytes, breast tissue, myoepithelial cells of salivary glands, cranial ganglia, dorsal root ganglia, and the otic vesicle. Therefore, it is also expressed in and identified on IHC in malignant tumors, such as melanoma, breast carcinoma, myoepithelioma, gliomas, and benign tumors, such as schwannomas.48,49 Somatic mutations in the SOX10 gene contribute to the development and progression of malignant melanoma and breast carcinoma.50,51

According to Nonaka et al,52 SOX10 and S100 show nearly the same specificity and sensitivity for melanoma and neural tumors, such as schwannoma and neurofibroma. However, they show lower sensitivity for MPNST: only 49% (38 of 77) for SOX10 and 30% (23 of 77) for S100, as summarized in Table 6.

Several other studies53–55 that investigated the staining pattern of SOX10 in melanocytic lesions showed 95% to 100% staining in conventional malignant melanomas, 100% staining in spindle cell melanomas and desmoplastic melanomas, and 97% to 100% staining in metastatic melanomas. Fortuitously, the entities that are usually considered in the differential diagnosis with MPNST, like the spindle cell monophasic synovial sarcomas, do not express SOX10.56–58

It seems imperative that a spindle cell lesion with SOX10 positivity be further worked up with melanocytic markers, like HMB-45 and Melan-A, to include or exclude melanocytic lesion. Benign and dysplastic nevi showed a 100% expression for SOX10; therefore, it is not useful in separating benign from malignant melanocytic proliferations. A small percentage (12%) of breast carcinomas (invasive ductal carcinomas and not lobular carcinomas) and all benign breast tissue (myoepithelial and lobular cells) were found to express SOX10.52 In salivary gland tissues and tumors, SOX10 is a marker of acinus and intercalated duct differentiation. Acinic cell carcinomas, adenoid cystic carcinomas, epithelial-myepithelial carcinomas, myoepithelial carcinomas, myoepitheliomas, and pleomorphic adenomas were reported to express SOX10.59 Other tumors, such as ovarian (serous, clear, and poorly differentiated) carcinomas, endometrial (endometrioid and serous) carcinomas, lung (adenocarcinoma, squamous, poorly differentiated, and adenosquamous) carcinomas, and colon (well-differentiated and poorly differentiated) carcinomas showed no expression of SOX10.60 Spindled fibroblasts and histiocytes, including epithelioid histiocytes as seen in scar tissue, showed weak to no staining with SOX10.61

**Epithelioid Gastrointestinal Stromal Tumor**

A 70-year-old woman from a mental health facility presented to the hospital with shortness of breath, altered mental status, and bone pain. The past medical records were fragmented; the caregiver had no relevant information except that she was diabetic and had a remote history of abdominal surgery. Imaging revealed multiple hepatic and pulmonary nodules and lymphadenopathy. An FNA of the mediastinal lymph node was performed under endoscopic ultrasound guidance.
Figure 7. Representative images of melanoma. A, Stain shows highly cellular smears with single and loose clusters of spindled to plump cells in a necrotic background. B, The tumor cells showing elongated nuclei without discernible nucleoli; in addition, dirty pigments in macrophages are demonstrated. C, The cell block showed cores of malignant spindle cells, with a focal palisading arrangement forming rosettelike structures, as well as focal areas of necrosis. D, S100 showing strong, diffuse nuclear and cytoplasmic staining. E, Nuclear staining for sex-determining region Y box 10 (SOX10). F, Tumor cells positive for vimentin (Diff-Quik, original magnification ×400 [A]; Papanicolaou stain, original magnification ×400 [B]; hematoxylin-eosin, original magnification ×400 [C]; original magnification ×400 [D through F]).
The FNA smears were highly cellular, composed of large dyscohesive cells with round to oval, eccentrically located nuclei, discernible to prominent nucleoli, and moderate eosinophilic cytoplasm in a background of focal necrosis. There were occasional binucleated or multinucleated cells and rare intranuclear inclusions; few demonstrated rosetting and acini formation reminiscent of adenocarcinoma. The cell block revealed a few intact vascular, myxoid tissue fragments containing nests of spindled to plump cells showing similar morphology. Only rare mitotic figures were seen. Representative images are illustrated in Figure 8, A through C.

Based on the cytomorphology and histomorphology, the differential diagnosis generated included sarcomatoid carcinoma, melanoma, neuroendocrine neoplasm, plasmacytic neoplasm, and mesenchymal spindle cell tumors, including gastrointestinal stromal tumor (GIST) of the epithelioid subtype.

A screening panel of IHC consisting of CK, S100, LCA, synaptophysin, and vimentin was applied. It revealed tumor cells that were strongly positive for vimentin and negative for CK, S100, synaptophysin, and LCA. This phenotype led to a further workup to categorize this mesenchymal tumor. The additional IHC studies showed that the tumor cells were positive for CD117, discovered on GIST1 (DOG1), CD34, and smooth muscle actin stain, and negative for desmin. Representative images are illustrated in Figure 8, D through F.

The final diagnosis was consistent with GIST, epithelioid subtype.

Since the introduction of the term GIST by Mazur and Clark in 1983,62 tremendous work at the subcellular and molecular levels has identified the interstitial cell of Cajal, the pacemaker cell responsible for controlling motility, to be the cell of origin for the GIST.63 The discovery of c-kit proto-oncogene mutations in GISTs allowed for accurate segregation of GISTs from other mesenchymal lesions in the gastrointestinal tract.64 CD117 (or c-kit), a transmembrane tyrosine kinase growth factor receptor involved in cellular differentiation, is expressed by the interstitial cells of Cajal, and therefore in most GISTs.

Morphologically, GISTs usually are spindle cell neoplasms composed of uniform spindle cells arranged in whorls or short intersecting fascicles. Nuclear palisading and extensive stromal hyalinization are also seen. An epithelioid morphology can also be seen, as shown in the index case above. Several other subtypes have been described.65,66 Currently the prognosis of GIST is based on the size of tumor, mitotic count, and location of the tumor, as elucidated by Fletcher et al.67

About 80% of GISTs have c-kit mutations in the receptor tyrosine kinase (RTK) genes, which are positive for CD117 by immunohistochemistry. A small subset of GISTS, approximately 5% to 8%, has a platelet-derived growth factor receptor α (PDGFRA) mutation, which also causes activation of tyrosine kinase. This group of tumors is usually epithelioid in morphology, like in the example above. About 12% to 15% of GISTs lack the mutations for both genes and are known as wild-type GISTS. DOG1, a hypothetical protein encoded by gene FLJ10261, is a novel marker that is specifically expressed in GISTs expressing both the c-kit and PDGFRA mutations. The predominant staining pattern of DOG1 in epithelioid GIST is membranous, whereas it is membranous and cytoplasmic in spindle cell GIST.68,69

The importance of accurately identifying the presence of the mutation is critical because effective targeted therapies are now available and continue to be developed.70,71 Although most GISTs are easily diagnosed based on clinical type, morphologic type, and immunophenotype, the small proportion of c-kit+ (4%–15%) GISTS will certainly benefit from identification by positive DOG1 staining, and thus further workup by screening for the mutations.

In the study by Espinosa et al,72 DOG1 reactivity was seen in 570 GIST cases (87%), whereas the expression of KIT and CD34 on IHC was found in 317 cases (74%) and 254 cases (59%) of GISTs, respectively. These data suggest that the DOG1 antibody is more specific than c-kit because DOG1 can identify a small subset of c-kit+ GISTs that may be amenable to kinase inhibitor therapy. The expression of DOG1, c-kit, and CD34 in GISTS with different mutations is summarized in Table 7.

DOG1 expression was relatively specific for GISTS among the mesenchymal tumors seen in the abdomen. In a study that included various mesenchymal and nonmesenchymal tumors, only rare cases of leiomyosarcoma (1 of 326; 0.3%), synovial sarcoma (1 of 39; 2.5%), and desmoplastic melanoma (1 of 10; 10%) were positive for DOG1. In contrast, CD117 expression was identified in 3 cases (0.9%) of leiomyosarcomas, 1 case (1.1%) of undifferentiated sarcomas, and occasional carcinomas of liver, pancreas, kidney, bladder, endometrial, and seminomas. Similar to DOG1, 1 case (10%) of desmoplastic melanoma was also reactive for CD117.72 West et al73 observed 4 cases (0.9%) of non-GIST tumors to be immunoreactive to DOG1 antiserum: 1 case (5%) of synovial sarcoma, 1 case (2.5%) of leiomyosarcoma, 1 case (25%) of fibrosarcoma, and 1 case (11%) of Ewing sarcoma/PNET.

Angiosarcoma

A 45-year-old morbidly obese man with a history of ulcerative colitis and type 2 diabetes presented with right upper quadrant pain. An ultrasound of the abdomen revealed a hepatic mass measuring 6.5 × 5.0 × 4.0 cm with adjacent fluid, suggesting hemorrhage. An ultrasound-guided FNA of the mass was performed.

The Diff-Quik–stained smear showed a few clusters of spindled to plump atypical cells with enlarged, hyperchromatic, and pleomorphic nuclei, some with prominent nucleoli in a bloody background. The cell block preparation

### Table 6. Sex-Determining Region Y Box 10 (SOX10) and S100 Staining in Malignant Melanoma and Neurogenic Tumors

| Tumors            | SOX10, No. (%) | S100, No. (%) |
|-------------------|----------------|---------------|
| Malignant melanoma |                |               |
| Conventional      | 41/43 (95)     | 37/43 (86)    |
| Spindle cell      | 7/7 (100)      | 7/7 (100)     |
| Desmoplastic      | 28/28 (100)    | 27/28 (96)    |
| Neurofibroma      |                |               |
| Cutaneous localized | 12/12 (100)   | 12/12 (100)  |
| Cutaneous diffuse  | 13/13 (100)    | 13/13 (100)  |
| Plexiform         | 26/27 (96)     | 25/27 (93)    |
| Schwannoma        |                |               |
| Conventional      | 26/26 (100)    | 26/26 (100)   |
| Cellular          | 7/7 (100)      | 7/7 (100)     |
| MPNST             | 38/77 (49)     | 38/77 (50)    |

Abbreviation: MPNST, malignant peripheral nerve sheath tumor.

* Data derived from Noma & et al.74
Figure 8. Representative images of epithelioid gastrointestinal stromal tumor. A, Highly cellular smear composed of a population of large, dyscohesive, plasmacytoid cells. B, Round to oval nuclei with fine chromatin pattern and small nucleoli. C, The cell block section shows tissue fragments containing atypical cells in myxoid background. D, Tumor cells positive for CD117. E, Tumor cells positive for discovered on GIST1 (DOG1). F, Tumor cells negative for S100 (Diff-Quik, original magnification x200 [A]; Papanicolaou stain, original magnification x400 [B]; hematoxylin-eosin, original magnification x600 [C]; original magnification x600 [D through F]).
revealed a minute tissue fragment composed of highly atypical spindled to plump cells with hyperchromatic and pleomorphic nuclei lining thin slitlike spaces with focal fibrinous material, as illustrated in Figure 9, A through C.

The cytomorphology and histomorphology suggest a spindle cell lesion. A screening panel of IHC to include CK, S100, vimentin, desmin, CD31, and CD34 was performed, revealing the tumor cells to be positive for vimentin, CD31, and CD34, and negative for CK, S100, and desmin. An ETS-related gene (ERG) immunostain highlighted the lining malignant cells, as illustrated in Figure 9, D.

The positive CD31, CD34, and ERG immunostaining confirmed the vascular origin of the tumor. The final diagnosis was consistent with angiosarcoma.

ERG immunostain, a member of the erythroblast transformation–specific (ETS) family of transcription factors, is responsible for regulating endothelial cell differentiation, angiogenesis, and expression of several endothelial-specific antigens. ERG overexpression was first described in prostatic adenocarcinomas resulting from the transmembrane protease serine 2 (TMPRSS2)-ERG oncogene translocations.74–76 ERG overexpression was first described in prostatic adenocarcinomas resulting from the transmembrane protease serine 2 (TMPRSS2)-ERG oncogene translocations.74–76

Our previous study80 on ERG expression by IHC evaluation in prostate carcinoma, high-grade prostatic intraepithelial neoplasia, and other benign conditions revealed that overexpression of ERG is specific for prostate carcinoma, albeit with a relatively low sensitivity. Only 44% (40 of 90) of low-grade prostatic adenocarcinomas (Gleason scores 3 + 3 or 3 + 4) and 22% (8 of 36) of high-grade prostatic adenocarcinomas (Gleason scores 4 + 4 or above) showed staining with ERG immunostain. Premalignant lesions, such as high-grade prostatic intraepithelial neopla-

| Mutations     | DOG1, No. (%) | c-kit (CD117), No. (%) |
|---------------|--------------|------------------------|
| c-kit         | 200/218 (92) | 180/221 (81)           |
| PDGFRa        | 23/29 (79)   | 3/32 (9)               |
| Wild-type GISTs | 33/37 (89)  | 29/35 (83)             |
| Total GISTs   | 370/425 (87) | 317/428 (74)           |

Abbreviation: PDGFRα, platelet-derived growth factor receptor α.

Table 7. Comparison of Immunoreactivity of Discovered on GIST1 (DOG1) and c-kit in Wild-Type Gastrointestinal Stromal Tumors (GISTs) and GISTs With Mutations

Figure 9. Representative images of angiosarcoma. A and B, Clusters of spindled to plump atypical cells with enlarged, hyperchromatic nuclei with conspicuous nucleoli. C, The tissue fragment in the cell block shows atypical spindle cells with hyperchromatic nuclei lining thin slitlike spaces; in addition, fibrin deposits are noted. D, The tumor cells were positive for ETS-related gene protein (ERG) (Diff-Quik, original magnification ×600 [A and B]; hematoxylin-eosin, original magnification ×600 [C]; original magnification ×600 [D]).
sia, showed a positive rate of 22% (4 of 18). Of the 4 cases that were positive, 3 were associated with prostatic adenocarcinoma, and the other was associated with atypical small acinar proliferation. Benign conditions, like radiation atypia (n = 20) and atrophic glands (n = 18), showed no reactivity to ERG staining. Normal prostatic tissue and seminal vesicles were also nonreactive. A large series of nonprostate carcinomas/neoplasms were nonreactive as well.

Other studies have found that ERG is an immunohistochemical marker for vascular tumors, including lymphangiomas and hemangiomas of various subtypes, and is seen in a nuclear staining pattern. In addition, 7 cases (70%) of blastic extramedullary myeloid tumors (acute myeloid leukemia tissue infiltrates) and 2 cases (7%) of Ewing sarcomas expressed ERG, likely secondary to the TLS/FUS-ERG fusion transcripts and Ewing sarcoma breakpoint region 1 (EWSR1)-ERG rearrangement, respectively. Other nonvascular and nonepithelial tumors, such as mesenchymal, neuroectodermal, and hematopoietic tumors, were nonreactive to ERG.

ERG was expressed in 41 cases (38%) of epithelioid sarcomas, usually with a uniform nuclear staining similar to that seen in angiosarcomas, whereas other endothelial markers, like CD31, were negative. However, all epithelioid sarcomas were negative for ERG gene rearrangement, indicating that ERG expression in epithelioid sarcoma is not likely related to ERG-involved translocations.

The lack of ERG reactivity in adenocarcinomas of the breast, colon, lung, stomach, and epithelial malignancies that frequently metastasize proves ERG to be a suitable marker of possible prostatic origin.

**Dysgerminoma**

A 23-year-old woman with no significant past medical history presented with lower abdominal discomfort. Imaging revealed a large retroperitoneal mass and an enlarged periaortic lymph node measuring 3 cm. An FNA of the lymph node was performed.

The smears revealed few clusters of pleomorphic cells containing prominent nuclei and abundant cytoplasm admixed with scattered lymphocytes. The background showed a tiger-stripe pattern, as illustrated in Figure 10, A. The cell block preparation revealed rare tissue fragments containing foci of hyperchromatic, large polygonal cells with centrally placed pleomorphic nuclei and admixed with lymphocytes in the tiger-stripe background, were suggestive of a germ cell tumor (GCT)—that is, dysgerminoma.

An IHC screening panel to include CK, LCA, S100, and vimentin, as well as germ cell markers (ie, Sal-like protein 4 [SALL4], octamer-binding transcription factor 3/4 [OCT3/4], NANOG, and CD117), was performed. The tumor cells were positive for CK, SALL4, OCT3/4, NANOG, and CD117, and negative for LCA, S100, and vimentin, as illustrated in Figure 10, C through F.

These findings are consistent with metastatic dysgerminoma.

Germ cell tumors account for greater than 95% of testicular neoplasms and less than 1% of ovarian malignancies. The distinction of seminomatous from nonseminomatous GCTs is critical for management and predicting prognostic outcomes. However, this distinction can be challenging in cytology specimens, given the paucity of diagnostic material and the lack of histomorphology. In the setting of lymph node metastasis, the lack of the history of a GCT adds another level of complexity to the diagnosis on a scant FNA specimen. Picking up on subtle cytologic clues, such as tiger-stripe background and biphasic population of cells, plays a key role in initiating the workup for GCTs.

The newer embryonic stem cell transcription factors, OCT3/4 (OCT4), NANOG, and SOX2, play an important role in stem cell growth and differentiation. Immunohistochemically, different expression patterns were noted in various subtypes of GCTs, like seminoma, embryonal carcinoma, yolk sac tumor, teratoma and choriocarcinoma, in primary and metastatic lesions.

The study by Santagata et al including 21 cases of pure primary testicular GCTs (14 seminoma, 3 embryonal carcinoma, and 4 teratoma), 20 cases of mixed GCTs (foci of 15 embryonal carcinoma, 6 seminoma, 15 teratoma, 6 yolk sac tumor, and 5 choriocarcinoma), and 43 cases of retroperitoneal metastatic GCTs (with components of 8 seminoma, 21 embryonal carcinoma, 16 teratoma, 6 yolk sac tumor, and 2 choriocarcinoma) reported the expression of NANOG and OCT3/4 in both primary and metastatic seminomas as well as in embryonal carcinoma. However, these immunomarkers were negative in yolk sac tumors and choriocarcinomas. The immunomarker SOX2 was positive for embryonal carcinoma but negative in seminomas, yolk sac tumors, and choriocarcinomas. The study results are summarized in Table 8.

A study performed at our institution confirmed NANOG expression in 17 cases (100%) of testicular intraepithelial germ cell neoplasm, 23 cases (96%) of testicular classic seminomas, and 16 cases (94%) of testicular embryonal carcinomas. It was observed that none of the testicular yolk sac tumors or other testicular tumors, such as spermatocytic seminoma and Leydig cell tumor, stained positively. Other epithelial neoplasms, including urothelial carcinoma (n = 31), RCC (n = 46), hepatocellular carcinoma and cholangiocarcinomas (n = 22), gastrointestinal adenocarcinoma (n = 64), gynecologic (uterine and ovarian) carcinomas (n = 66), prostate adenocarcinoma (n = 124), lung carcinoma (small and non–small cell carcinoma; n = 206), pancreatic adenocarcinoma (n = 60), breast carcinoma (n = 171), and thyroid carcinoma (n = 72), were all nonreactive for NANOG.

In other studies performed at our institution (F.L., unpublished data, March 2012), SOX2 expression in 1020 cases of tumors from various organs revealed positive SOX2 staining in 16 cases (63%) of embryonal carcinomas and 7 cases (57%) of yolk sac tumors, whereas no staining was observed in the seminomas (n = 21). In a study comparing the IHC patterns of placental alkaline phosphatase and NANOG in identifying central nervous system germinomas, NANOG was found to be a better marker than placental alkaline phosphatase because of its strong nuclear signal. Placental alkaline phosphatase, a reasonably sensitive diagnostic marker for GCTs, often showed weak and patchy cytoplasmic staining, and therefore was considered less reliable. Other central nervous system tumor types, such as pineoblastomas, medulloblastomas, high-grade gliomas, pituitary adenomas, supratentorial primitive neuroectodermal tumors, central neurocytomas, Langherans cell histiocytosis, atypical teratoid/rhabdoid tumors, and non–Hodgkin lymphomas, were all negative for NANOG.
Although negative results for NANOG in breast carcinomas (n = 171) have been seen in our experience, expression of NANOG protein was found in an unknown number of samples of the Michigan Cancer Foundation-7 (MCF-7) breast carcinoma cell line. This study found that NANOG was expressed in breast carcinoma but not in normal breast tissue, and this should be a potential pitfall of which one must be aware.

Figure 10. Representative images of dysgerminoma. A, A few cohesive clusters of large polygonal tumor cells with pleomorphic nuclei containing prominent nucleoli and abundant vacuolated cytoplasm admixed with lymphocytes; also noted is the diagnostic “tiger-stripe” pattern in the background. B, The cell block section showed tissue cores with a small cluster of atypical cells with hyperchromatic, irregular nuclei interspersed with lymphocytes. C, Nuclear staining for NANOG. D, Nuclear staining for Sal-like protein 4 (SALL4). E, Positive for CD117. F, Negative for CD30 (Diff-Quik, original magnification ×600 [A]; hematoxylin-eosin, original magnification ×200 [B]; original magnification ×400 [C through F]).
CONCLUSIONS

In the expanding knowledge on tumor genomics with accompanied targeted therapy, the role of cytopathologists is expanding. Lending an accurate diagnosis to each specimen is only a part of the service provided to patients. The preservation of limited diagnostic material for potential molecular and other ancillary testing, as well as the selection of those assays, is critical, and these are some of the newer responsibilities of the “guardians of the tissue.” A systematic approach along every step of the process, beginning with rapid on-site evaluation and including the cytomorphologic or histomorphologic evaluation, the algorithmic application of a small and effective panel of immunomarkers, and the precise selection of molecular or other ancillary tests, is required. This allows for the most efficient use of the limited material available on the cell block, and hence allows for better patient care.

Through this paper we have emphasized the key role of cytopathology or histomorphology in generating appropriate differential diagnoses and ultimately arriving at an accurate diagnosis. An algorithmic approach to working up tumors of unknown primary origin and effective small panels of IHC were proposed, and discussions of the potential pitfalls were elaborated. The application of the algorithms and IHC panels was illustrated through 7 selected presentations, which were entities commonly seen in tumors of unknown primary origin. The small screening panel consisting of CK, S100, vimentin, and LCA allows for lineage specification and provides a direction for further workup. The organ- or tumor-specific immunomarkers, including some newer generations of immunomarkers, help to achieve a specific diagnosis. By exercising a systematic approach for working up tumors of unknown primary origin, not only can an accurate specific diagnosis be reached, but also the preservation of tissue for potential molecular or other ancillary testing can be achieved in most cases of daily practice.

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Table 8. Immunostaining Pattern of Testicular Germ Cell Tumors

| Tumors                  | NANO G | OCT3/4 | SOX2 |
|-------------------------|--------|--------|------|
| Seminoma                | +      | +      | -    |
| Embryonal carcinoma     | +      | +      | -    |
| Yolk sac tumor          | -      | +      | -    |
| Choriocarcinoma         | -      | -      | -    |

Abbreviations: OCT3/4, octamer-binding transcription factor 3/4; SOX2, sex-determining region Y box 2.
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