Review Article

Application of immunohistochemistry in diagnosis and management of malignant mesothelioma

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Abstract: Immunohistochemistry plays an indispensable role in accurate diagnosis of malignant mesothelioma, particularly in morphologically challenging cases and in biopsy and cytology specimens, where tumor architecture is difficult or impossible to evaluate. Application of a targeted panel of mesothelial- and epithelial-specific markers permits correct identification of tumor lineage in the vast majority of cases. An immunopanel including two mesothelial markers (calretinin, CK5/6, WT-1, or D2-40) and two epithelial markers (MOC-31 and claudin-4) offers good sensitivity and specificity, with adjustments as appropriate for the differential diagnosis. Once mesothelial lineage is established, malignancy-specific studies can help verify a diagnosis of malignant mesothelioma. BAP1 loss, *CDKN2A* homozygous deletion, and MTAP loss are highly specific markers of malignancy in a mesothelial lesion, and they attain acceptable diagnostic sensitivity when applied as a diagnostic panel. Novel markers of malignancy, such as 5-hmC loss and increased EZH2 expression, are promising, but have not yet achieved widespread clinical adoption. Some diagnostic markers also have prognostic significance, and PD-L1 immunohistochemistry may predict tumor response to immunotherapy. Application and interpretation of these immnuomarkers should always be guided by clinical history, radiographic findings, and above all histomorphology.

Keywords: Immunohistochemistry; mesothelioma; BAP1; peritoneum; pleura

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Introduction

A spectrum of hyperplastic and neoplastic mesothelial lesions arises from the serosal linings of the pleura, pericardium, and peritoneum, and tunica vaginalis. Mesothelial neoplasms range from benign localized tumors to aggressive diffuse malignancies that destructively infiltrate surrounding tissues and can metastasize to distant sites. Approximately 85% of malignant mesotheliomas arise in the pleural cavity, with most of the remainder arising in the peritoneum (1). Paratesticular and primary pericardial malignant mesotheliomas are well-documented but rare tumors. Malignant mesothelioma has a poor prognosis, with median survival of 16 months for pleural tumors in one recent large study (2). Accordingly, accurate distinction from benign mesothelial proliferations is crucial, to avoid both undue psychic injury and unnecessary surgical or systemic therapies.

Accurate diagnosis of mesothelial proliferations, however, is one of surgical pathology's persistent challenges. Malignant mesothelioma is a rare tumor, with approximately 2,500 new cases diagnosed annually in the United States (3). Furthermore, morphologic overlap between benign mesothelial proliferations and malignant mesothelioma

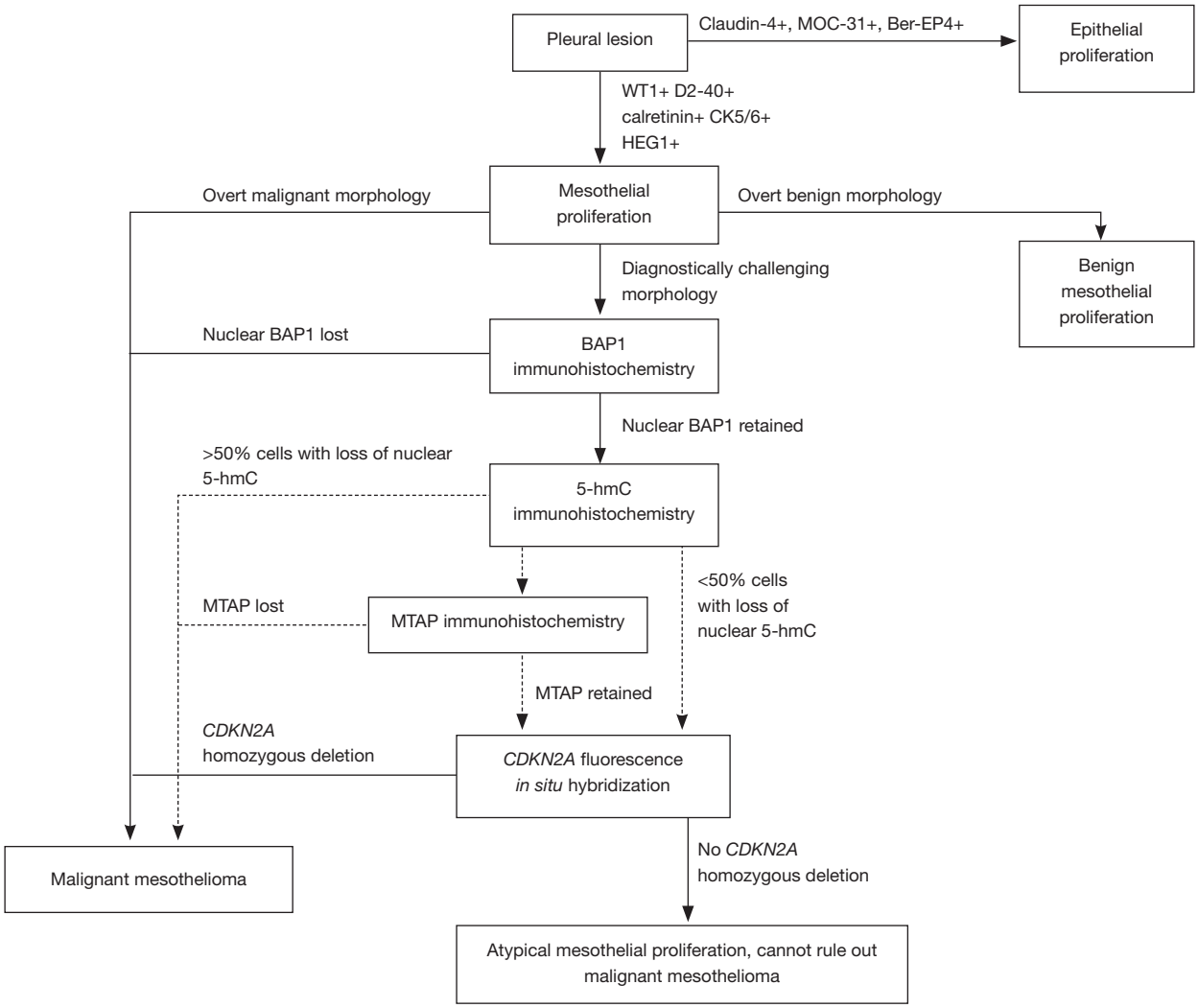

Figure 1 Algorithm for establishing a diagnosis of malignant mesothelioma. Dashed lines indicate provisional diagnostic markers not yet in widespread use. Adapted from Chapel DB, *et al. Mod Pathol* 2019;32:376-86.

complicates reliable distinction by histomorphology alone, particularly in small biopsy and cytopathology specimens. In consequence, ancillary diagnostic techniques, particularly immunohistochemical stains, have become indispensable for accurate diagnosis of mesothelioma. Nonetheless, it warrants emphasis that careful morphologic examination must precede application of ancillary diagnostic studies, and that the results of these studies must be interpreted in the context of the pathologist's morphologic impression.

The landscape of immunohistochemical studies for diagnosis of mesothelial proliferations has evolved rapidly in the last decade, including the introduction of some immunostains that can also be applied in evaluation of non-mesothelial tumors. Herein, we begin with a discussion of immunomarkers useful for establishing mesothelial lineage, followed by a detailed handling of markers useful for distinguishing benign from malignant mesothelial lesions. Prognostic and predictive markers are also addressed. We conclude with discussion of rare tumor sites and presentations.

Establishing mesothelial lineage

Establishing lineage is the first step in diagnosis of a serosa-based tumor (*Figure 1*). A carefully selected panel of mesothelial and epithelial immunomarkers permits

confident lineage identification in virtually all cases with sufficient tissue for evaluation, bearing in mind that rare non-epithelial tumors may also occur in the serosa and mimic mesothelioma (4). Recent guidelines recommend using mesothelial and epithelial markers with sensitivity and specificity of at least 80% (4), although in practice, markers with even higher specificity are available and are preferred.

Both our experience and the published literature suggest that markers of mesothelial and epithelial lineage show equivalent sensitivity in the pleura and peritoneum (4,5). However, because the differential diagnostic considerations in these two sites may differ substantially, mesothelial markers may show different specificity in the pleura versus peritoneum, due to cross-reactivity of certain markers in the most common carcinomas at each site. These caveats are handled in a recent review (6) and consensus guidelines (4), and are discussed alongside each marker below.

Panels of mesothelial- and epithelial-specific immunomarkers can be applied to large resections, small biopsies, and cell blocks. Current guidelines recommend that a cutoff of 10% staining should be used for cytoplasmic and membranous markers (4). It should be emphasized that (with the rare exceptions) the markers discussed in this section demonstrate mesothelial lineage only, and do not generally distinguish benign from malignant mesothelial lesions, which instead relies on clinical and radiologic correlation, careful morphologic assessment, and application of additional ancillary studies, as discussed below. Reported sensitivity and specificity statistics for the discussed markers are summarized in *Table 1*.

Markers of mesothelial lineage

Calretinin has long been considered a reliable marker for establishing mesothelial lineage. We encounter calretinin immunostains frequently in consultation and use this marker routinely in-house. Dual cytoplasmic and strong nuclear calretinin expression supports mesothelial lineage (*Figure 2*). Other staining patterns, especially lack of nuclear staining, have lower specificity and should be interpreted cautiously. When polyclonal antibodies against human recombinant calretinin are used, calretinin is 80–100% sensitive for epithelioid malignant mesothelioma (7-26), although variation in calretinin antibodies in clinical use may account for imperfect sensitivity in practice (12,27-29). Furthermore, sensitivity is somewhat lower in sarcomatoid mesotheliomas, with reported figures ranging from 5–88%, but generally in the range of 50–60% (11-13,27,28,30-34). Calretinin is not entirely specific for mesothelial cells, with expression reported in a substantial subset of breast carcinomas, squamous cell carcinomas, and serous ovarian carcinomas (8-10,35,36), and in 10–60% of sarcomatoid carcinomas (30,34,37,38). Calretinin is reported in only 0–10% of pulmonary adenocarcinomas and renal cell carcinomas (7,11,26,39). Other calretinin-positive lesions, such as ovarian sex cord-stromal tumors and adrenocortical tumors, may rarely enter the clinical and morphologic differential diagnosis for mesothelial proliferations of the peritoneum. Therefore, although generally reliable and widely used, calretinin is an imperfect mesothelial marker that should be interpreted in the context of a broader immunopanel.

CK5/6 is another widely used mesothelial immunomarker. Like calretinin, CK5/6 shows good but imperfect sensitivity in practice, ranging from 51–100% (8,9,11,15-18,20,21,23,24,26,29,35,38,40). In sarcomatoid mesothelioma, however, sensitivity is low, ranging from 13-29% (6,11,33,34,41). This marker is best studied in the differential diagnosis with adenocarcinoma of the lung, of which 0–20% (but generally fewer than 5%) are positive (24,38,40,42,43). CK5/6 is expressed by squamous cell carcinomas (including poorly differentiated squamous cell tumors lacking overt squamous morphology), ovarian serous carcinomas, breast carcinoma (particularly basal-like tumors), and sarcomatoid carcinomas of the lung (8,9,23,24,34,35,38,44). Accordingly, our use of CK5/6 is generally limited to cases where adenocarcinoma of the lung is effectively the only entity on the differential diagnosis.

WT-1 has reported sensitivity ranging from 70–100% for establishing mesothelial lineage (8,9,11,15-17,21,24,26,35,38), and just 10–45% for sarcomatoid mesothelioma (6,11,30,32-34). A clean nuclear staining pattern permits easy interpretation, and WT-1 expression is virtually zero in both squamous cell carcinoma and adenocarcinoma of the lung and in renal cell carcinoma (8,38,40,45). Although few studies report WT-1 expression in up to 37% of triple-negative breast carcinomas (43), the preponderance of evidence suggests that WT-1 is expressed in at most a few percent of breast cancers, and is typically weak and focal (26,35,46-48). Importantly, WT-1 is a classic marker of both low-grade and high-grade serous carcinomas of the uterine adnexa, which limits its usefulness in peritoneal tumors, where mesothelioma and serous carcinomas may also show considerable morphologic overlap.

Podoplanin (frequently termed “D2-40”, in reference

Table 1 Sensitivity and specificity of mesothelial- and epithelial-specific markers

Marker	Sensitivity	Specificity
Mesothelial markers		
Broad-spectrum cytokeratin	EMM: virtually 100%	Virtually 100% in carcinoma with epithelioid morphology
	SMM: 90%	Sarcomatoid carcinoma: 90%
Calretinin	EMM: 80–100%	Lung ACA: 0–10%; lung SCC: 35–40%; breast carcinoma: 13% (up to 38% of triple-negative breast carcinoma); HGSOC: 13–31%; RCC: 0–10%
	SMM: 50–60%	Sarcomatoid carcinoma: 10–60%
Cytokeratin 5/6	EMM: 51–100%	Lung ACA: 5%; lung SCC: 95–100%; breast carcinoma: 5%; HGSOC: 25–31%
	SMM: 13–29%	Sarcomatoid carcinoma: 15%
WT-1	EMM: 70–100%	Lung ACA: 0%; lung SCC: 0–2%; breast carcinoma: 5–8%; HGSOC: virtually 100%; RCC: 0%
	SMM: 10–45%	Sarcomatoid carcinoma: 25–35%
Podoplanin (D2-40)	EMM: 80–100%	Lung ACA: 0–3%; lung SCC: 15–42%; breast carcinoma: 0–3%; HGSOC: 15–65%
	SMM: 75–90%	Sarcomatoid carcinoma: 20%
HEG1	EMM: 92–99%	Lung ACA: 0%; lung SCC: 0%; breast cancer: 0%; HGSOC: 0%; RCC: 0% (only limited studies available to date)
	SMM: 64–78%	Sarcomatoid carcinoma: data not available
Epithelial markers		
MOC-31	Lung ACA, lung SCC, breast carcinoma, HGSOC: 85–100%; RCC: 50%	EMM: 0–10%
	Sarcomatoid carcinoma: 0–10%	SMM: 0–5%
Ber-EP4	Lung ACA, lung SCC, breast carcinoma, HGSOC: 85–100%; RCC: 50%	EMM: 0–10%
	Sarcomatoid carcinoma: 0–10%	SMM: 0–5%
Claudin-4	Lung ACA, lung SCC, breast carcinoma, HGSOC, RCC: 92–100%	EMM: 0–6% (rare granular cytoplasmic staining only)
	Sarcomatoid carcinoma: 45%	SMM: 0%
Peritoneal markers		
PAX8	HGSOC: 93–100%	Peritoneal mesothelioma: 6–18%
ER	HGSOC: 40–90%	Peritoneal mesothelioma: 0–2%
PR	HGSOC: 20–40%	Peritoneal mesothelioma: 0–7%

Where sufficient data were available to confidently identify outliers, they have been excluded from this table, but they are noted in the manuscript text. ACA, adenocarcinoma; Ca, carcinoma; EMM, epithelioid malignant mesothelioma; ER, estrogen receptor; HGSOC, high-grade serous ovarian carcinoma; PR, progesterone receptor; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; SMM, sarcomatoid malignant mesothelioma.

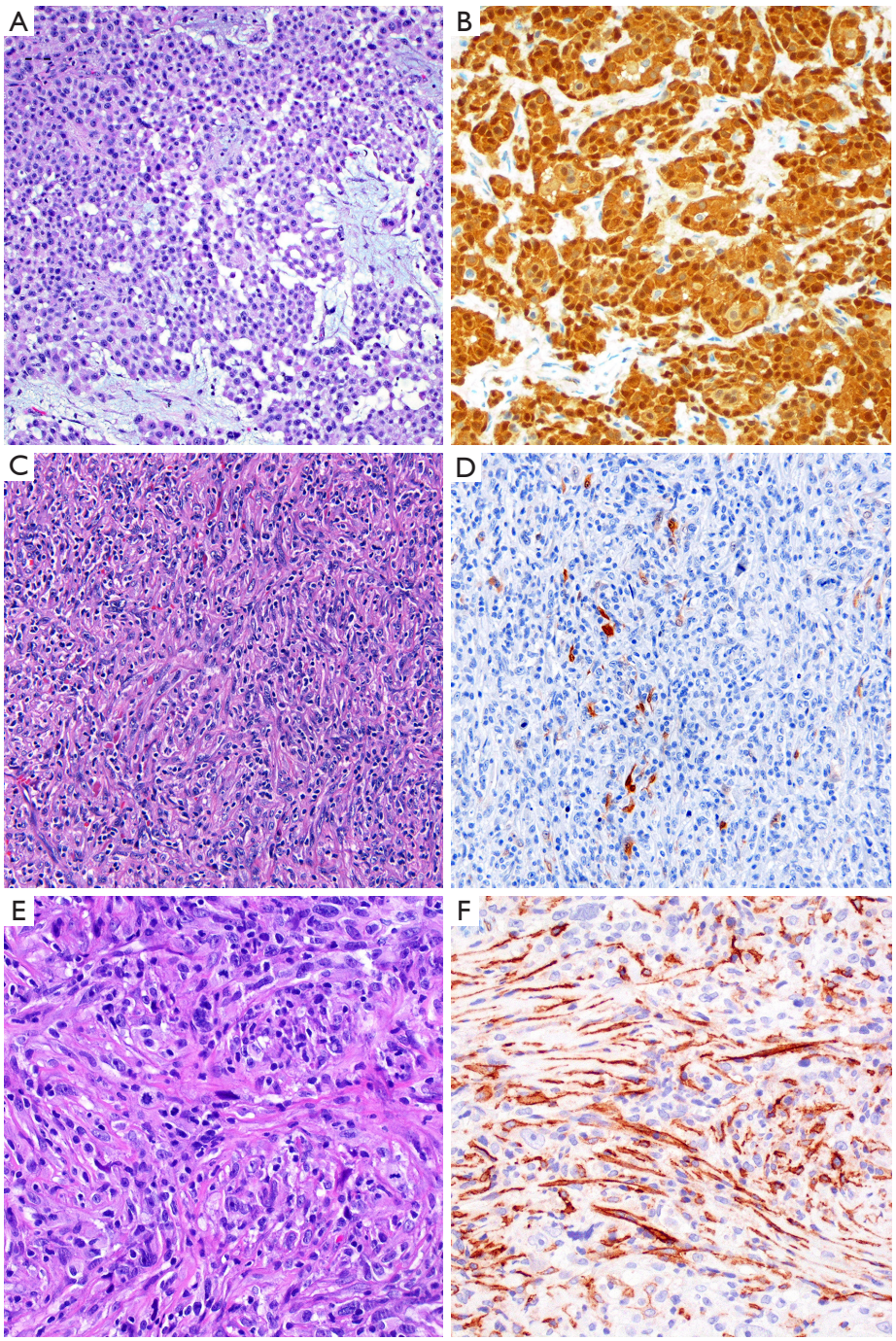

Figure 2 Mesothelial immunomarkers are useful for establishing tumor lineage. (A,B) Strong and diffuse cytoplasmic-and-nuclear calretinin staining in an epithelioid malignant mesothelioma (A, hematoxylin and eosin, original magnification ×100; B, calretinin immunostain, original magnification ×200). (C,D) Focal single-cell calretinin staining in a sarcomatoid malignant mesothelioma (C, hematoxylin and eosin, original magnification 200×; D, calretinin immunostain, original magnification ×200). (E,F) Diffuse membranous D2–40 (podoplanin) staining in a sarcomatoid malignant mesothelioma (E, hematoxylin and eosin; F, D2-40 immunostain; original magnification ×200).

to the most widely used anti-podoplanin clone) is a sensitive marker of epithelioid mesothelial lesions, showing membranous staining in 80–100% (8,9,15,16,19,24,26, 35,38,49,50) (*Figure 2*). Despite some discouraging older data, podoplanin also appears to be a sensitive marker for sarcomatoid mesothelioma, with staining in up to 90% of tumors (13,28,32,34,37,49,50), although careful examination is required, since D2-40 staining of lymphatic spaces may mimic tumor staining (4). Podoplanin is imperfectly specific for mesothelioma. It is expressed in 13–65% of serous ovarian carcinomas and approximately half of squamous cell carcinomas (8,9,44,50). True membranous staining is virtually always negative in conventional lung adenocarcinoma, but is reported to be seen in 25–30% of sarcomatoid carcinoma (32,34,49). Additionally, strong podoplanin is classic for certain non-mesothelial tumors, including seminoma and angiosarcoma, which may rarely enter into the differential diagnosis with mesothelial proliferations.

Heart development protein with EGF-like domains 1 (HEG1) is a recently described mesothelial marker (5,33). Based on two clinically oriented studies of HEG1, cytoplasmic-membranous staining for HEG1 is reportedly 92–99% sensitive and 83–99% specific for mesothelial lineage overall, with HEG1 showing higher sensitivity and specificity than calretinin, D2-40 and WT-1 immunohistochemistry in both studies (5,33). Furthermore, HEG1 is reportedly 100% specific for epithelioid mesothelioma in the differential diagnosis with pulmonary adenocarcinoma (although 4 of 7 pleomorphic carcinomas were positive for HEG1), and 64–78% sensitive for sarcomatoid mesothelioma (5,33). As of this writing, the HEG1 antibody is currently commercially available only in Japan, although we anticipate that it will become more widely available in the near future.

Other markers that are reportedly useful for establishing mesothelial lineage include mesothelin, thrombomodulin, caveolin, tenascin-X, and type III collagen. We do not currently use these markers in our practice, but they have been recently reviewed elsewhere (6).

Markers of epithelial lineage

Epithelial markers should also be included alongside mesothelial markers when evaluating lineage of pleural or peritoneal lesions. Many markers are available, and the landscape of epithelial markers has, with few exceptions discussed here, remained largely unchanged over the last 20 years. Some older epithelial markers—such as CEA, CD15, BG8, and B72.8 (also termed TAG-72 or BRST3)—may seem somewhat old-fashioned, but they remain accurate tools for distinction of epithelial and mesothelial lesions (7,15-22,24,26,29,40,51,52), although sensitivity for sarcomatoid carcinoma is poor (32).

Two antibodies against the epithelial cell adhesion molecular (Ep-CAM) remain in widespread use: Ber-EP4 and MOC-31. Positive staining for these markers is cytoplasmic with membranous accentuation, although strong staining may simply appear cytoplasmic. Both MOC-31 and Ber-EP4 are generally reported to be 85–100% sensitive for adenocarcinoma and squamous cell carcinoma of the lung, adenocarcinoma of the breast, and serous ovarian carcinoma (7,8,14,19-21,23,24,26,40,52-55). Conversely, they mark only half of renal cell carcinomas, limiting their usefulness in this differential diagnosis (11). Furthermore, because Ep-CAM is lost in cells with sarcomatoid differentiation, MOC-31 and Ber-EP4 are poorly sensitive for sarcomatoid carcinoma, with reported sensitivity from 0–30% (though generally in the 0–10% range) (4,28,32,34).

Ber-EP4 shows 60–100% specificity for epithelial lineage in the differential diagnosis with mesothelial tumors, with reported figures typically in the range of 90–100% (7,8,14,15,18-21,23,25,52-56). Similarly, although occasional studies have reported up to 30% of mesotheliomas with some MOC-31 expression (28), MOC-31 is generally reported to be 90–100% specific for epithelial lineage (8,20,21,24,55), particularly when only strong and diffuse staining is considered to support epithelial lineage (20) (*Figure 3*). Still, we have occasionally encountered bona fide mesotheliomas with strong and diffuse MOC-31 and/or Ber-EP4 staining, highlighting the value of an immunopanel that also includes at least two mesothelial markers and claudin-4.

Indeed, there appears to be growing consensus that claudin-4 is the best epithelial marker currently in diagnostic use (4,53-57). Claudin-4 is a component of epithelial tight junctions, and membranous staining for claudin-4 is up to 92–100% sensitive and 94–100% specific for epithelial lineage in the differential diagnosis with mesothelial lesions (14,16,24,35,53-59) (*Figure 3*), and rare mesotheliomas with claudin-4 staining show only focal, granular cytoplasmic positivity (<10% of tumor cells) (16). Claudin-4 is highly accurate in effusion cytology specimens, as well (54,55,59). Although sensitivity of claudin-4 remains high in poorly differentiated adenocarcinoma of the

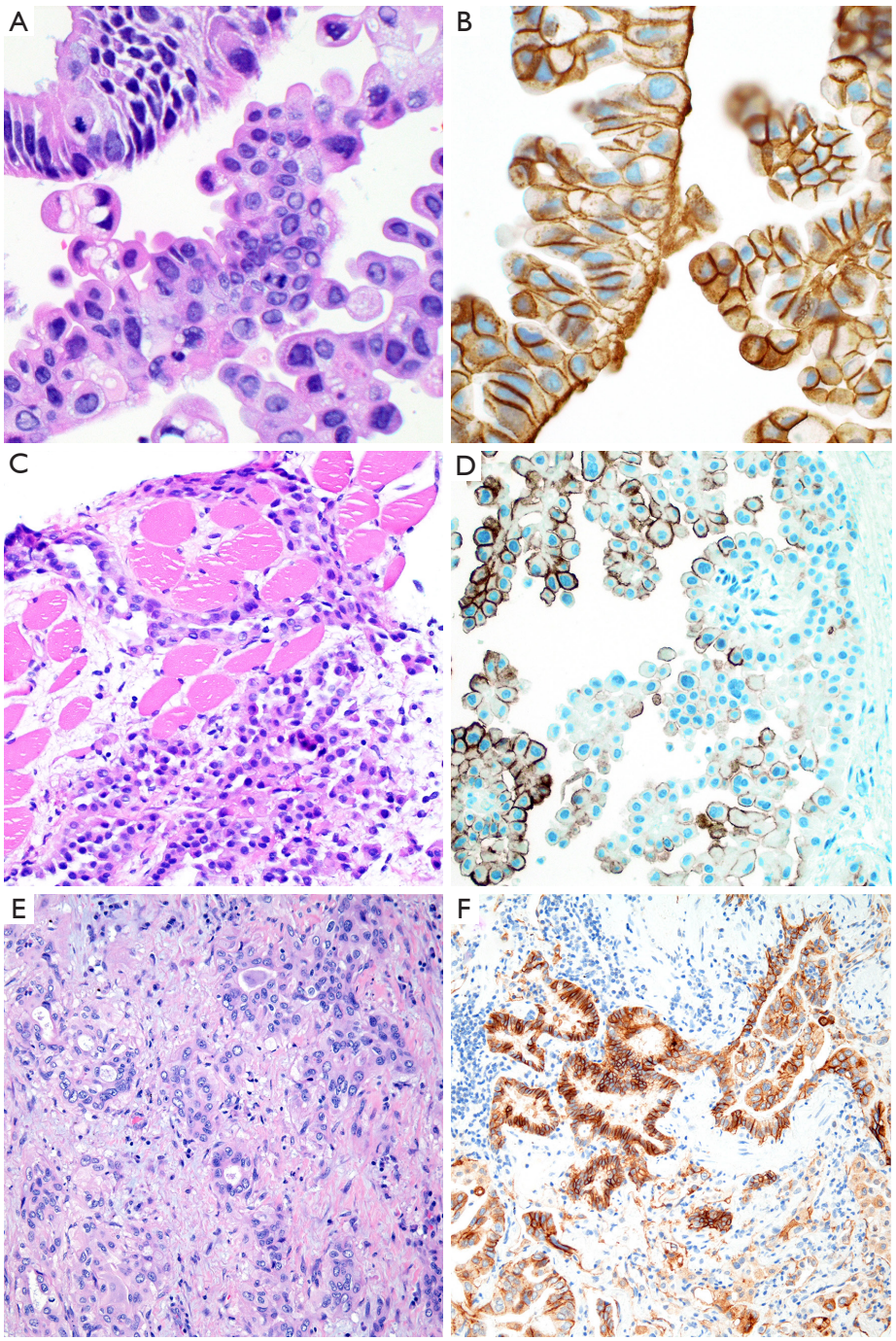

Figure 3 Epithelial immunomarkers are useful for establishing tumor lineage. (A,B) Strong and diffuse membranous MOC-31 staining in an adenocarcinoma of the lung (A, hematoxylin and eosin; B, MOC-31 immunostain; original magnification ×400). (C,D) Although most mesothelial lesions are negative for MOC-31, they may occasionally show patchy MOC-31 staining, as in this epithelioid malignant mesothelioma (C, hematoxylin and eosin; D, MOC-31 immunostain; original magnification ×200). (E,F) Strong and diffuse membranous claudin-4 staining in an adenocarcinoma of the lung (E, hematoxylin and eosin; F, claudin-4 immunostain; original magnification ×200).

lung (56), sensitivity is decreased in frankly sarcomatoid carcinomas, with one study reporting 45% sensitivity and 100% specificity of claudin-4 expression for sarcomatoid carcinoma of the lung (37).

In our diagnostic practice, we routinely employ a panel of two to three mesothelial and two epithelial markers, typically calretinin, WT-1 and/or D2-40, MOC-31, and claudin-4 (4). The first four markers are widely available. Although not all pathology practices have adopted the claudin-4 immunostain, evidence suggests that this excellent epithelial marker should be used in challenging cases, including by send-out testing or expert consultation. In our consultation practice, we review all epithelial and mesothelial markers ordered by the outside pathologist, and then routinely stain for claudin-4 (if not already done) to definitively exclude an epithelial tumor.

Caveats in lineage determination

Certain pitfalls should be kept in mind when interpreting immunomarkers considered to indicate a specific site of origin for carcinoma. First, although PAX8 has conventionally been considered as a highly specific marker for müllerian, thyroid, and renal carcinoma in the differential diagnosis with mesothelioma (57), more recent data indicate that PAX8 is expressed in 6–18% of malignant peritoneal mesotheliomas (including strong diffuse expression in some cases), up to 60% of well-differentiated papillary mesotheliomas, and up to one third of reactive mesothelial lesions in the peritoneum (15,25,60,61). Mesothelial PAX8 expression appears to be somewhat more common in women, which may point to some biological overlap with müllerian tumors, although this remains unproven (60). Usefully, expression of estrogen receptor and progesterone receptor is exceptionally rare in peritoneal mesotheliomas (0–2% and 0–7%, respectively, in published studies) and thus provides strong evidence against a diagnosis of mesothelioma (9,14,15,60,62).

The differential diagnosis between sarcomatoid carcinoma and sarcomatoid mesothelioma is notoriously difficult. Pan-cytokeratin immunohistochemistry is approximately 90% sensitive for both sarcomatoid mesothelioma and sarcomatoid carcinoma (34), but the sensitivity and specificity of other mesothelial and epithelial markers is poor in sarcomatoid tumors, as discussed above. Recent guidelines for diagnosis of sarcomatoid lesions provide practical guidance in these cases (34). A recent small study reported that nuclear GATA3 expression is 100% sensitive and 85% specific for sarcomatoid mesothelioma in the differential diagnosis with sarcomatoid carcinoma of the lung (63) (*Figure 4*). Similarly, MUC4 expression is reportedly 72% sensitive and 100% specific for diagnosis of sarcomatoid carcinoma of the lung, in the differential diagnosis with sarcomatoid mesothelioma (37). Both of these promising findings await validation in a larger, carefully designed multi-institutional study.

In the differential diagnosis with carcinoma of the lung or serous ovarian carcinoma, BAP1 loss is virtually 100% specific for mesothelioma (15,50,60,64,65). Accordingly, in these specific differentials, BAP1 loss serves as a marker of both mesothelial lineage and malignancy (see also below). However, BAP1 loss is common in other tumor types, including renal cell carcinoma, and is therefore not intrinsically diagnostic of malignant mesothelioma in all circumstances (66,67).

Rarely, the morphologic differential diagnosis for malignant mesothelioma includes non-epithelial tumors (including mesenchymal, hematologic, and melanocytic lesions, among others). In these rare cases, rote application of a panel of mesothelial and epithelial markers could prove confusing or frankly misleading. We reiterate that careful morphologic assessment remains the cornerstone of diagnosis, and should guide application of the appropriate diagnostic immunopanel (4,34).

Discriminating benign and malignant mesothelial lesions

In tumors with overtly malignant morphologic features, immunohistochemical confirmation of mesothelial lineage may be sufficient for diagnosis of mesothelioma. In cases without overtly malignant morphology, however, additional immunostains can assist in distinction of benign and malignant mesothelial proliferations. This is of great help in small biopsies and cytology preparations, in which the tumor's relationship to surrounding tissues may be altogether absent (65,68-73). None of these markers is without caveats, and interpretation in the context of morphology and other ancillary studies (see *Figure 1*) is imperative.

Numerous immunomarkers have been proposed to distinguish benign from malignant mesothelial proliferations, but few have stood the test of time. As noted in the introduction, specificity is paramount in markers of malignant mesothelioma, given the grave implications of this diagnosis. New immunomarkers with high specificity

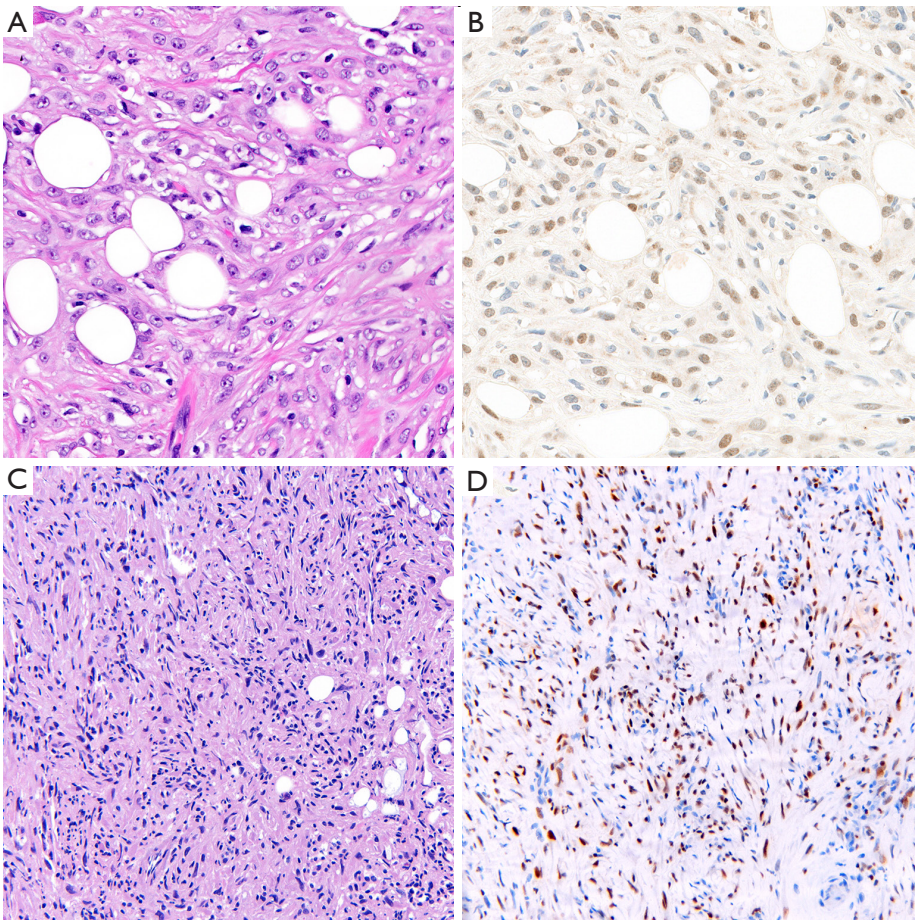

Figure 4 GATA3 expression is reportedly 72% sensitive and 100% specific for diagnosis of malignant mesothelioma, in the differential with adenocarcinoma of the lung. GATA3 staining intensity may be weak, as in B. (A,C, hematoxylin and eosin; B,D, GATA3 immunostain; original magnification ×200).

for malignancy in mesothelial lesions are therefore, quite appropriately, supplanting older, less specific markers in routine diagnostic practice. A recent publication provided a detailed review of five of these older mesothelioma markers, including desmin, EMA, GLUT-1, p53, and IMP-3 (74). The interested reader is referred there for further discussion of these markers, which we no longer recommend for routine diagnostic use. Reported sensitivity and specificity data for the discussed markers are summarized in *Table 2*.

BAP1

Immunohistochemical loss of nuclear BRCA-associated protein 1 (BAP1) has emerged in recent years as a highly specific marker of malignancy in mesothelial lesions (50,60,64,69-72,75-81). BAP1 loss in mesothelioma was originally described in the context of familial predisposition to mesothelioma in Cappadocia, Turkey, and the *BAP1* germline mutation has since been recognized as the most common germline event predisposing to pleural and peritoneal mesothelioma, particularly in young patients with no history of radiation or asbestos exposure (82-84). *BAP1* alterations are also an important event in sporadic pleural and peritoneal mesothelioma (75,85). In diagnostic immunohistochemistry, BAP1 is considered lost when tumor nuclear staining in absent in the presence of a positive internal control. Cytoplasmic-only staining (resulting from loss of BAP1's autodeubiquitination function) is seen in a subset of cases and is considered BAP1 loss for diagnostic purposes (86-88).

Loss of nuclear BAP1 staining by immunohistochemistry is highly concordant with BAP1 mutation (75,86,89),

Table 2 Sensitivity and specificity of malignant mesothelioma markers

Marker	Sensitivity for MM	Specificity for MM
BAP1 IHC	Pleural MM: 50–65%; EMM: 61–77%; BMM: 33–49%; SMM: 0–22%; peritoneal MM: 55–67%	Virtually 100% specific for malignancy in a mesothelial proliferation; 99–100% specific for MM in the differential diagnosis with lung adenocarcinoma and high-grade serous ovarian carcinoma
CDKN2A FISH	Pleural MM: 50–65%; SMM: 80–100%; peritoneal MM: 25–29%	Virtually 100% specific for malignancy in a mesothelial proliferation
MTAP IHC	For detection of CDKN2A HD: 65–88%; for diagnosis of pleural MM: 43–65%	For detection of CDKN2A HD: 96–100%; for diagnosis of malignancy in a pleural mesothelial proliferation: 96–100%
5-hmC IHC	Pleural MM: 92% (single report)	For diagnosis of malignancy in a pleural mesothelial proliferation: 100% (single report)
EZH2 IHC	Pleural MM: 45–66% (two reports)	For diagnosis of malignancy in a pleural mesothelial proliferation: 100% (two reports)
Panel: BAP1 IHC + CDKN2A FISH	Pleural MM: 85–93%	For diagnosis of malignancy in a pleural mesothelial proliferation: 100%
Panel: BAP1 IHC + MTAP IHC	Pleural MM: 74–90%	For diagnosis of malignancy in a pleural mesothelial proliferation: 96–100%

Except as noted for BAP1, specificity of these markers pertains to diagnosis of malignancy once mesothelial lineage has been established with lineage-specific markers. BMM, biphasic malignant mesothelioma; EMM, epithelioid malignant mesothelioma; FISH, fluorescent *in situ* hybridization; IHC, immunohistochemistry; MM, malignant mesothelioma; SMM, sarcomatoid malignant mesothelioma.

and BAP1 loss is virtually 100% specific for malignancy in mesothelial proliferations (72,78,81,90-92) (*Figure 5*). Unfortunately, BAP1 loss is a somewhat insensitive marker, occurring in approximately 50–65% of all pleural mesotheliomas, including 61–77% of epithelioid, 33–49% of biphasic, and 0–22% of sarcomatoid mesotheliomas (70-72,75-80,86,89,90,92-96). Sensitivity of BAP1 loss for diagnosis of peritoneal mesothelioma has been reported at 55–67% (15,60,64,70,97).

The preponderance of evidence indicates that BAP1 immunohistochemistry can be accurately applied to cell block specimens (70-72,90), although one study showed specificity of 86% for diagnosis of mesothelioma (98). Accordingly, caution is warranted in interpretation of BAP1 immunohistochemistry on cytology specimens, especially when rendering a primary diagnosis of malignant mesothelioma.

In the differential diagnosis with carcinoma of the lung, breast, and stomach, BAP1 loss is highly specific for malignant mesothelioma, with one study showing BAP1 loss in just 1% of lung cancers (79,90,99,100). Multiple studies have also indicated that BAP1 loss is virtually 100% specific for mesothelioma in the differential diagnosis with ovarian carcinoma (60,64,90). However, as noted above, multiple other malignancies (including renal cell carcinoma and melanoma) also frequently show BAP1 loss (66,67,79). These tumors may occasionally metastasize to the serosal surfaces, so careful consideration of the clinical and radiographic data, tumor morphology, and lineage-specific markers is critical when interpreting BAP1 immunohistochemistry.

In addition to its diagnostic role, multiple studies indicate that BAP1 loss is a favorable prognostic marker in sporadic mesothelioma (95,96,101,102), and mesotheliomas arising in the context of germline *BAP1* mutation have a relatively favorable prognosis (82). This is discussed in greater detail below.

CDKN2A and MTAP

In mesothelial proliferations without clear-cut morphology or BAP1 loss, fluorescence *in situ* hybridization (FISH) to detect homozygous deletion of *CDKN2A* (which encodes p16 protein) remains the next best diagnostic study (see *Figure 1*). Like BAP1 loss, *CDKN2A* homozygous deletion is virtually 100% specific for malignancy in mesothelial proliferations, but only 48–88% sensitive for diagnosis of pleural mesothelioma, with most figures in the range of 50–65% (77,81,92,103-105), and up to 80–100% sensitivity for diagnosis of sarcomatoid mesothelioma, specifically

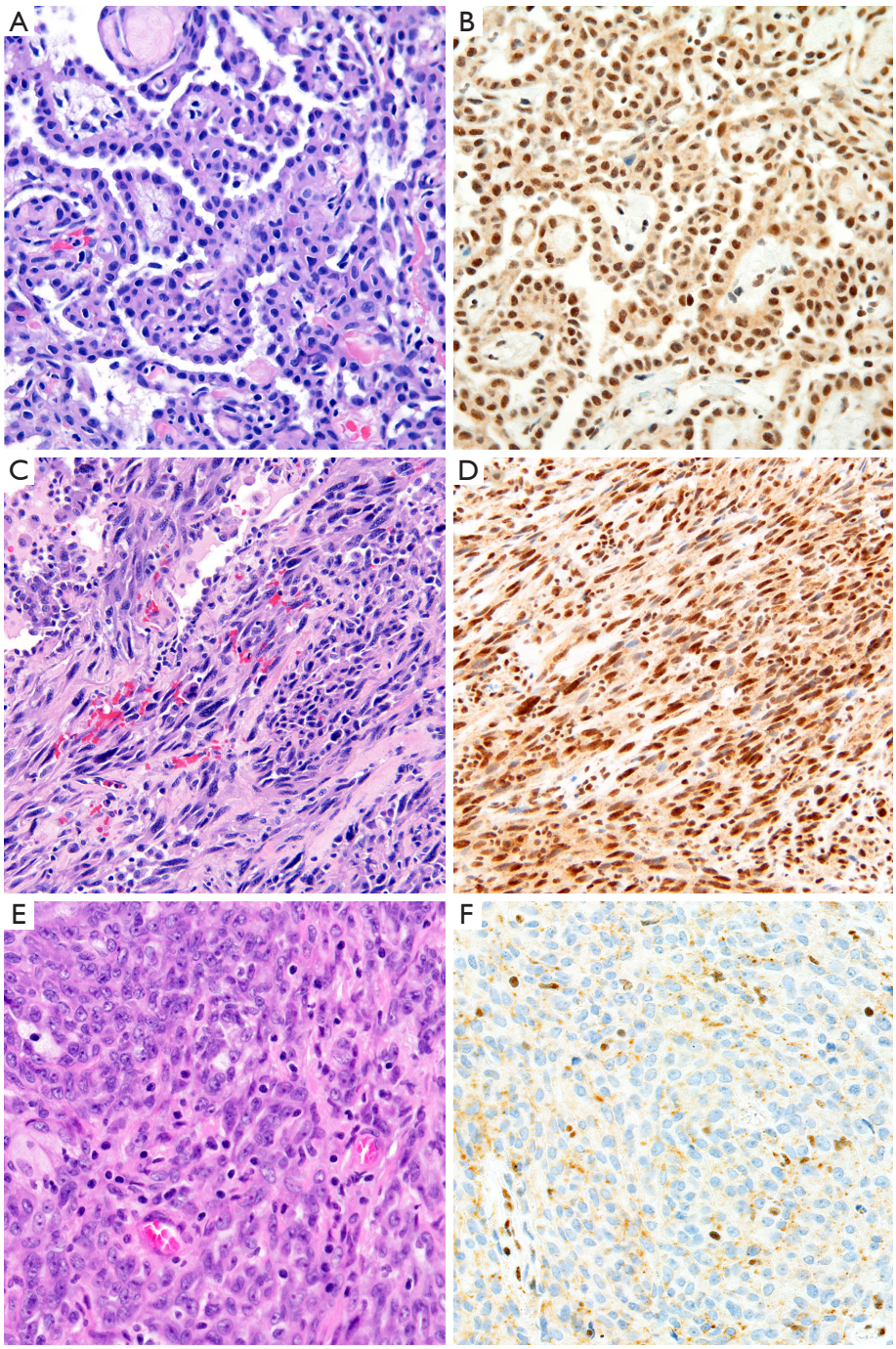

Figure 5 BAP1 loss is highly specific for malignancy in a mesothelial lesion. (A,B) BAP1 retention in papillary mesothelial hyperplasia (A, hematoxylin and eosin; B, BAP1 immunostain; original magnification ×400). (C,D) BAP1 retention in a sarcomatoid malignant mesothelioma. BAP1 loss is seen in only some 15% of sarcomatoid mesothelioma. (E,F) BAP1 loss in an epithelioid malignant mesothelioma. Approximately 70% of epithelioid malignant mesotheliomas show BAP1 loss. (C,E, hematoxylin and eosin; D,F, BAP1 immunostain; original magnification ×200). BAP1, BRCA-associated protein 1.

(76,94,106). In the peritoneum (where sarcomatoid morphology is significantly rarer), sensitivity of *CDKN2A* FISH is lower, at 25–29% (97,105).

Because immunohistochemistry is more widely available and less expensive than FISH, there has been interest in identifying an immunohistochemical surrogate for *CDKN2A* FISH; p16 immunohistochemistry, however, has proved unreliable (78). Nearly twenty years ago, cytogenetic studies revealed frequent co-deletion of the neighboring *MTAP* gene (encoding methylthioadenosine phosphorylase, involved in adenosine salvage) with *CDKN2A* at the 9p21 locus (107-109), raising the potential for MTAP immunohistochemistry as a surrogate for *CDKN2A* deletion. One early study of MTAP immunohistochemistry showed poor concordance with *CDKN2A* FISH (110), but multiple more recent studies have shown that loss of cytoplasmic MTAP staining is 65–88% sensitive and 96–100% specific for *CDKN2A* homozygous deletion, and 43–65% sensitive and up to 100% specific for diagnosis of mesothelioma (72,73,78,91,92,111), including in sarcomatoid mesothelioma (112) (*Figure 6*). The literature currently contains only one example of a mesothelioma with MTAP loss by immunohistochemistry but normal *CDKN2A* copy number by FISH (111), and no reports of MTAP loss in a benign mesothelial lesion. MTAP immunohistochemistry is relatively easy to interpret, shows excellent interobserver reproducibility (111), and has been validated for application to cell block specimens (72,73). Although the published case of MTAP loss in a tumor with normal *CDKN2A* copy number raises some concern about specificity, we have begun using MTAP immunohistochemistry in routine clinical diagnosis, in close correlation with histomorphology and BAP1 immunohistochemistry, and reserving the use of confirmatory *CDKN2A* FISH for challenging cases.

Thorough validation of these highly specific markers of malignant mesothelioma has led to the introduction of diagnostic panels into routine practice, combining different markers to enhance sensitivity. A diagnostic panel including both BAP1 immunohistochemistry and *CDKN2A* FISH achieves a sensitivity of 58–93%, with most reported figures in the 85–93% range (72,77,78,81,92,94). By comparison, an immunopanel of BAP1 and MTAP is 74-90% sensitive for diagnosis of malignant mesothelioma (72,78,91,92), in keeping with the imperfect sensitivity of MTAP loss for *CDKN2A* deletion.

Importantly, the available literature on MTAP immunohistochemistry for diagnosis of mesothelioma pertains only to mesothelial proliferations of the pleura. There are no published data on MTAP immunohistochemistry in peritoneal mesothelial lesions, although we anticipate that the number of such lesions showing MTAP loss would be low, as *CDKN2A* homozygous deletion is a relatively rare phenomenon in peritoneal mesothelioma. Indeed, in a cohort of 33 peritoneal mesotheliomas subjected to MTAP immunohistochemistry in our lab, only 1 showed MTAP loss (unpublished data). It should also be emphasized that *CDKN2A* homozygous deletion is seen in a broad spectrum of malignant tumors, and that *CDKN2A* deletion (or, by extension, MTAP loss) warrants a diagnosis of malignant mesothelioma only after mesothelial lineage has been firmly established.

5-hydroxymethylcytosine (5-hmC)

5-hmC is a modified nucleotide, produced from 5-methylcytosine by the TET family of DNA hydroxylases as the first step of DNA demethylation (113-116). Discovery of 5-hmC coincided with the larger realization that DNA methylation is, in fact, a reversible epigenetic phenomenon. Decreased levels of 5-hmC have been reported in a broad range of malignancies, including in seminal studies of acute myeloid leukemia, glioblastoma, and melanoma (117,118). There have been few reports on the significance of 5-hmC in mesothelioma. Early work using rat and human cell lines implicated decreased TET expression and decreased global 5-hmC in mesothelioma tumorigenesis (119). In a study of 49 mesotheliomas and 23 reactive mesothelial proliferations, our group found that loss of 5-hmC staining in greater than 50% of tumor nuclei was 100% specific and 92% sensitive for diagnosis of malignancy (120) (*Figure 7*). These promising results await validation in a larger multi-institutional study, using more recently available monoclonal antibodies. Application of 5-hmC immunohistochemistry has not yet been studied in small biopsies or cytology cell blocks, where quantification of tumor 5-hmC loss may present additional challenges.

As with MTAP immunohistochemistry, data on 5-hmC immunohistochemistry have only been reported for mesothelial lesions of the pleura (120). Of 89 malignant peritoneal mesotheliomas stained in our laboratory for 5-hmC, only 52 (58%) showed loss of 5-hmC in greater than 50% of tumor nuclei (unpublished data), suggesting that sensitivity of 5-hmC immunohistochemistry for diagnosis of peritoneal mesothelioma may be lower than for pleural mesothelioma.

Molecular assays for 5-hmC have been developed

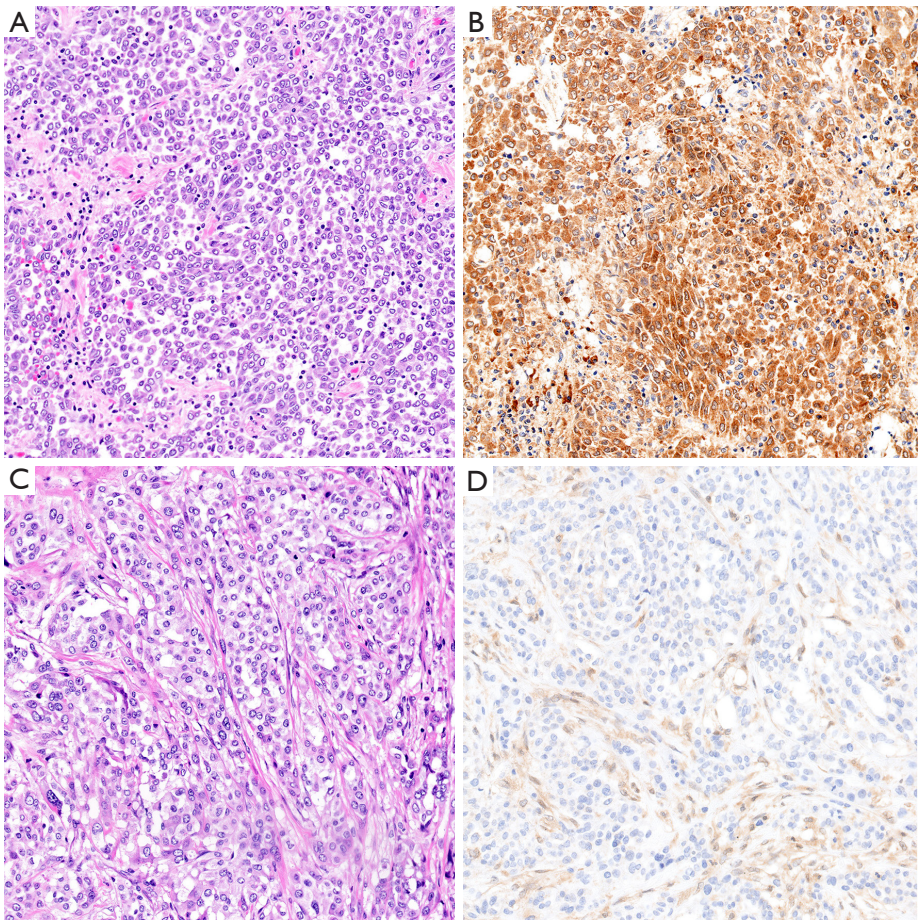

Figure 6 MTAP loss is a surrogate for CDKN2A homozygous deletion and is highly specific for malignancy in a mesothelial lesion. (A,B) MTAP retention in an epithelioid malignant mesothelioma. (A, hematoxylin and eosin; B, MTAP immunostain; original magnification ×100). (C,D) MTAP loss in an epithelioid malignant mesothelioma. MTAP loss is approximately 80% sensitive for detecting CDKN2A homozygous deletion and 60% sensitive for diagnosis of malignant mesothelioma. (C, hematoxylin and eosin; D, MTAP immunostain; original magnification ×100). MTAP, methylthioadenosine phosphorylase.

recently, and provide substantially more detailed information on 5-hydroxymethylation patterns than can be achieved with immunohistochemistry. Molecular studies investigating the tumor-specific 5-hmC profile in mesothelioma are underway.

EZH2

Enhancer of zeste homolog 2 (EZH2) is a component of polycomb repressive complex 2 (PRC2) and plays a central role in epigenetic suppression of gene expression through trimethylation of a critical lysine residue in histone 3 (H3K27) (121). Overexpression of EZH2 is reported in a range of malignancies, and a link between *BAP1* loss and overexpression of EZH2 suggests a role for the PRC2 pathway and H3K27 hypermethylation in mesothelioma development and progression (122). Early work demonstrated significantly higher EZH2 expression in malignant mesothelioma compared to reactive mesothelial lesions, whereas knockdown of EZH2 inhibits mesothelioma tumorigenesis (5,123). In two recent studies of mesothelial proliferations, expression of EZH2 in >50% of lesional cells was found to be 45–66% sensitive and 100% specific for malignant mesothelioma, with sensitivity increasing to 74–90% for an immunopanel including BAP1 and EZH2 (80,92).

Importantly, as with loss of MTAP and 5-hmC, overexpression of EZH2 is not specific for mesothelioma in

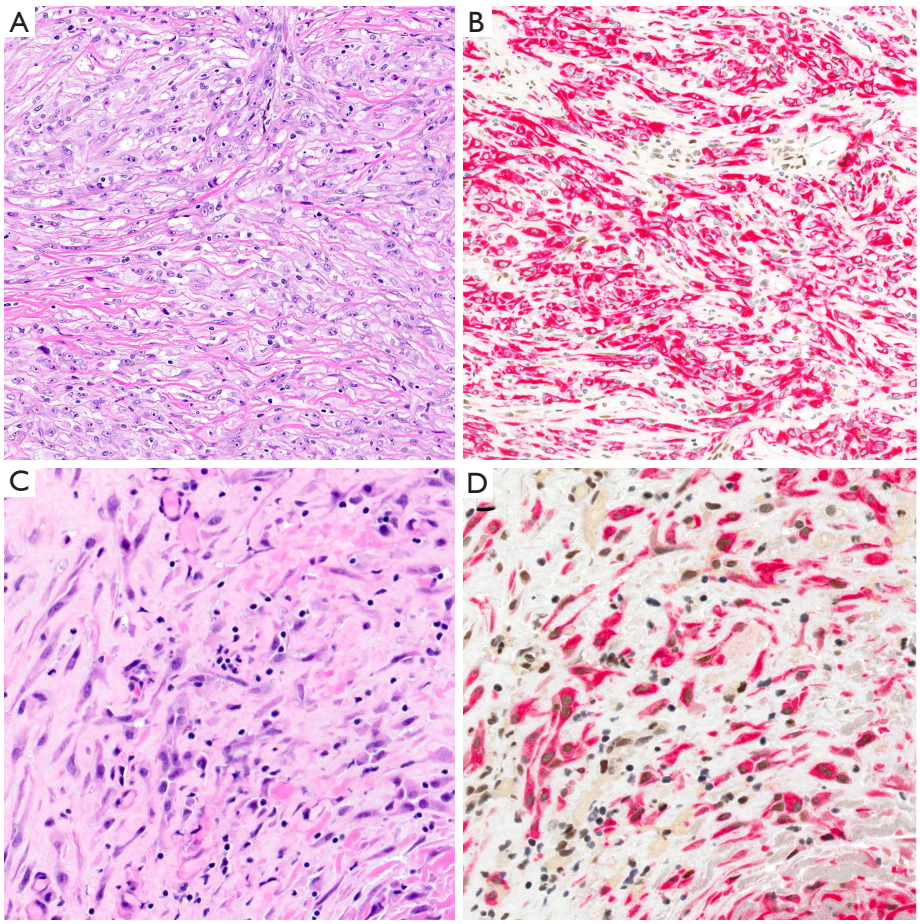

Figure 7 Loss of nuclear 5-hydroxymethylcytosine (5-hmC) staining is sensitive and specific for malignancy in a mesothelial lesion. (A,B) Extensive 5-hmC loss in a sarcomatoid malignant mesothelioma [A, hematoxylin and eosin; B, CAM5.2/5-hmC double immunostain (CAM5.2 in red, 5-hmC in brown); original magnification ×100]. (C,D) Nuclear 5-hmC is largely retained in this reactive spindled mesothelial proliferation [C, hematoxylin and eosin; D, CAM5.2/5-hmC double immunostain (CAM5.2 in red, 5-hmC in brown); original magnification ×200].

the differential diagnosis with other malignant tumors (124), and should only be used to support this diagnosis once mesothelial lineage has been confidently established. Although EZH2 is a promising marker of malignant mesothelioma, we are awaiting validation in larger multi-institutional studies before adopting this marker in our routine diagnostic practice.

Use of immunomarkers to subtype malignant mesothelioma

Once a diagnosis of malignant mesothelioma is made, the tumor should be designated as epithelioid, biphasic, or sarcomatoid histotype. This classification has significant prognostic and therapeutic implications, but not all lesions can be easily classified morphologically. A recent study found only moderate agreement among pathologists with mesothelioma expertise in assignment of biphasic histotype (125). Here we discuss two problematic areas where immunohistochemistry may be useful. First, cytokeratin immunostaining may help to distinguish sarcomatoid mesothelioma from epithelioid mesothelioma in challenging cases. Second, application of malignancy-specific markers may help distinguish biphasic mesothelioma from epithelioid mesothelioma associated with reactive spindled mesothelial cells.

In challenging cases, a cytokeratin immunostain may

assist in distinguishing sarcomatoid and epithelioid patterns by highlighting greater cell-cell cohesion in the latter (*Figure 8*). This approach is sometimes used as an adjunct in diagnosis of biphasic mesothelioma. In the recent study of interobserver agreement in diagnosis of biphasic mesothelioma referenced above, participating pathologists reported that a cytokeratin immunostain aided histotype classification in 77% of cases (125). However, there are no objective criteria for interpreting cytokeratin immunostains in this context, and the diagnosis is ultimately a subjective evaluation by the pathologist. In future, this application may also help delineate foci of transitional mesothelioma, although the immunohistochemical characteristics of this provisional morphologic subtype are not yet fully elucidated.

Use of immunohistochemistry to distinguish reactive from malignant spindled cells—and thus to distinguish true biphasic mesothelioma from epithelioid mesothelioma with adjacent reactive spindled mesothelial cells—remains an area of controversy. In one study of 43 biphasic mesotheliomas, all 21 tumors with BAP1 loss showed concordant loss in both the epithelioid and sarcomatoid components (95). A subsequent study found BAP1 loss in 5 of 13 biphasic mesotheliomas, with concordant BAP1 staining between the epithelioid and sarcomatoid elements in all 13 cases (76). These studies suggest that, when associated with an epithelioid mesothelioma with BAP1 loss, spindled mesothelial cells with intact BAP1 are likely reactive. In contrast, however, a recent study of 42 biphasic mesotheliomas found that only 11 of 21 cases with BAP1 loss showed concordant loss in both tumor elements (125) (Schulte, unpublished data). Similarly, in one recent study of 13 tumors diagnosed as biphasic mesothelioma on the basis of morphology, the authors suggested that 5 tumors be reclassified as epithelioid mesothelioma on the basis of BAP1 loss in the epithelioid component and BAP1 retention in the sarcomatoid component (86). Considering these studies together, it seems that their differing conclusions likely stem, at least in part, from lack of an objective gold standard method for definitively classifying challenging cases as epithelioid or biphasic, thus introducing the potential for circular reasoning. In our practice, we presently regard BAP1 loss in spindled mesothelial cells as specific but not sensitive for confirming a diagnosis of biphasic mesothelioma. That is, retention of BAP1 in a spindled mesothelial population does not, in this context, make it benign.

Analogously, *CDKN2A* FISH or MTAP immunohistochemistry could also be used to distinguish true biphasic mesothelioma from epithelioid mesothelioma with an associated reactive spindle component. In fact, one might intuit that this approach would be more sensitive than BAP1 immunohistochemistry, given the higher incidence of *CDKN2A* homozygous deletion in sarcomatoid mesothelioma. Wu and colleagues found that the epithelioid and sarcomatoid components of a biphasic mesothelioma show concordant *CDKN2A* status by FISH in 29 of 29 cases (76). This suggests that *CDKN2A* FISH could be both sensitive and specific for confirming a diagnosis of biphasic mesothelioma in tumors with *CDKN2A* homozygous deletion in the epithelioid element, although this may prove burdensome, if FISH must be performed twice to capture both tumor elements. MTAP immunohistochemistry, as a surrogate for *CDKN2A* FISH, could be a more practical approach, but this application has not yet been formally studied.

Our group also found that extensive 5-hmC loss in a spindled mesothelial proliferation is characteristic of malignancy and may warrant diagnosis of biphasic mesothelioma when located adjacent to a malignant epithelioid component (120) (*Figure 9*). New data suggest that molecular-based epigenetic profiling can accurately identify mesothelioma histotype (unpublished data, presented USCAP 2019, National Harbor, MD, USA), although this technique is not yet in clinical use.

Prognostic and predictive markers

Beyond their role in mesothelioma diagnosis, immunomarkers can also provide clinically valuable prognostic and predictive information. Although numerous markers are reportedly related to prognosis in mesothelioma, we will highlight only those markers currently used in routine diagnostic practice.

The preponderance of prognostic studies supports a better outcome in mesothelioma with BAP1 loss (80,89,93,95,96,101,102,126), although one study found that BAP1 loss portends a poorer prognosis and suggests that reports of improved prognosis may reflect confounding (127). Published data on BAP1 loss in peritoneal mesothelioma also suggest improved survival (85), although some have found no significant prognostic effect (97). Importantly, these studies have not generally discriminated between germline and sporadic BAP1 loss.

Studies specific to BAP1 loss secondary to germline *BAP1* mutation have found a significantly better prognosis

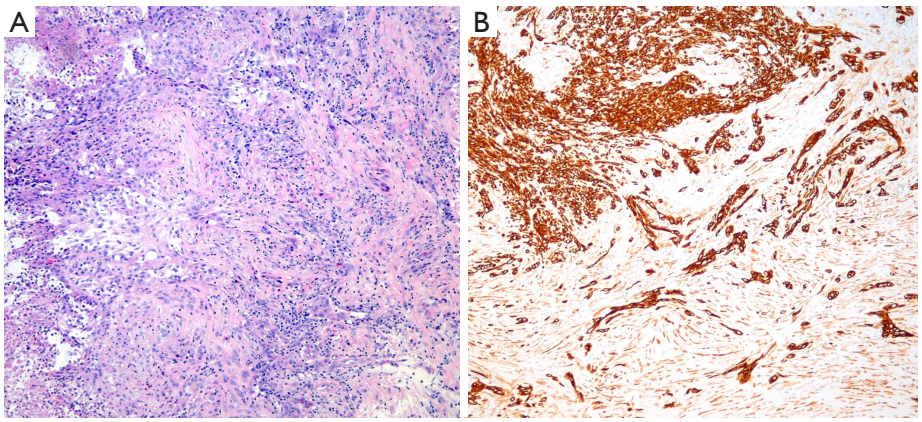

Figure 8 A cytokeratin immunostain on a biphasic malignant mesothelioma contrasts cohesive epithelioid growth (upper left) from spindled single-cell sarcomatoid growth (lower right) (A, hematoxylin and eosin; B, CAM5.2 immunostain; original magnification ×100).

compared to non-germline-mutated mesothelioma, irrespective of tumor site, with 5-year overall survival of 47%, and 7%, respectively (82,128). Among patients with *BAP1* germline mutation, peritoneal mesothelioma is associated with significantly longer overall survival than pleural mesothelioma (median 10 and 2 years, respectively, versus 9 months for patients with non-germline-mutated pleural or peritoneal mesothelioma) (82). Although improved outcomes in mesothelioma with germline *BAP1* mutation may be partly attributable to active surveillance and earlier detection going forward, better survival in these patients predates the discovery of germline *BAP1* syndrome, suggesting that differences in tumor biology also play a role (82). We are still awaiting a single unifying study, comparing outcomes in mesotheliomas with retained BAP1, sporadic BAP1 loss, and germline *BAP1* mutation, which may permit further prognostic stratification of patients.

Multiple studies have shown that homozygous deletion of *CDKN2A* is associated with shorter survival in both pleural and peritoneal mesothelioma (97,101,109,129,130). An analogous prognostic function for MTAP immunohistochemistry is logical, but has not yet been validated. The prognostic role of EZH2 immunohistochemistry has been examined in only a limited capacity, with one study showing poorer outcomes in patients with high EZH2 expression (92) and a second study showing no association between EZH2 expression and outcome (80). Together, these two studies had only 80 patients, and larger studies are needed to establish the prognostic significance of EZH2 expression.

Programmed death-ligand 1 (PD-L1) expression in mesothelioma has been associated with shorter survival, compared to mesotheliomas without PD-L1 expression (131-137). Interaction between PD-L1 on tumor cells and PD-1 on tumor infiltrating lymphocytes downregulates the anti-tumor immune response, and therapeutic targeting of this interaction represents a major advance in clinical oncology. Positive PD-L1 expression (>1% tumor cell staining) is reported in 11–72% of mesotheliomas, with substantial variation between different laboratories and different anti-PD-L1 antibody clones (131-138). Pembrolizumab (an anti-PD-1 monoclonal antibody) is now accepted as salvage therapy for mesothelioma with PD-L1 expression by immunohistochemistry, and nivolumab (another anti-PD-1 agent) is accepted as salvage therapy regardless of PD-L1 immunohistochemistry findings. This highlights the uncertain value of PD-L1 immunohistochemistry as a predictive marker for immunotherapy response, with conflicting results for different agents in a rapidly evolving therapeutic landscape. Notwithstanding, we perform PD-L1 immunostaining on all mesotheliomas at our institution, and PD-L1 immunohistochemistry is the subject of multiple ongoing clinical trials. The interested reader is referred to a recent excellent review for an in-depth discussion from the clinical perspective (139).

Rare sites and unusual tumors

Mesothelioma in situ

The existence of an *in situ* precursor to mesothelioma has long been postulated, but the concept had previously

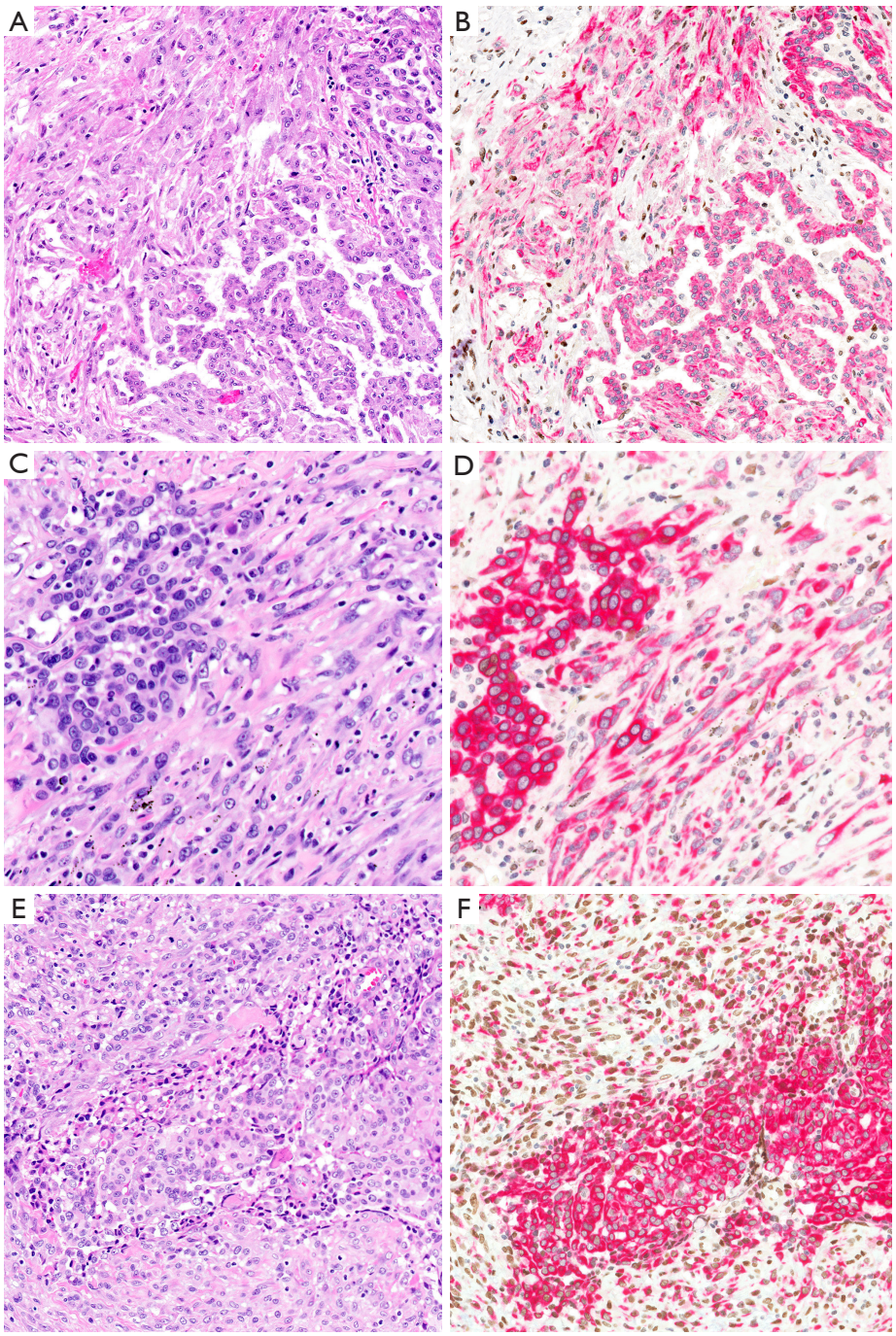

Figure 9 Immunohistochemistry for 5-hydroxymethylcytosine may aid in mesothelioma subtyping. (A,B) In true biphasic malignant mesothelioma, both the epithelioid and sarcomatoid elements show extensive loss of 5-hydroxymethylcytosine (5-hmC) [A, hematoxylin and eosin; B, CAM5.2/5-hmC double immunostain (CAM5.2 in red, 5-hmC in brown); original magnification ×100]. (C,D) Biphasic malignant mesothelioma with extensive loss of 5-hmC in both epithelioid and sarcomatoid tumor elements [C, hematoxylin and eosin; D, CAM5.2/5-hmC double immunostain (CAM5.2 in red, 5-hmC in brown); original magnification ×200]. (E,F) An epithelioid malignant mesothelioma with an associated reactive spindled mesothelial population. The epithelioid mesothelioma shows extensive 5-hmC loss, whereas the adjacent reactive spindled component shows extensive nuclear 5-hmC staining [E, hematoxylin and eosin; F, CAM5.2/5-hmC double immunostain (CAM5.2 in red, 5-hmC in brown); original magnification ×100].

gained little credibility, due to difficulties both in definition and in detection (140,141). Two recent related series characterizing a total of 12 cases of apparent mesothelioma *in situ* (11 pleural, 1 peritoneal) have prompted a renewed interest in the concept (142,143). Churg and colleagues propose a narrow and rigorous definition of malignant mesothelioma *in situ*: (I) a single layer of surface mesothelial cells, (II) immunohistochemical loss of BAP1, and (III) no radiographic or clinical evidence of a tumor. (To improve specificity, their studies also excluded any patients who developed invasive mesothelioma within 1 year of the *in situ* diagnosis). Note that ancillary diagnostic studies (BAP1 loss or, in principle, MTAP loss or *CDKN2A* homozygous deletion) are necessary to diagnose mesothelioma *in situ*, because its constituent cells overlap cytologically with reactive mesothelium, The precise significance of incidentally discovered mesothelioma *in situ* is unclear, although the recent series reported progression to mesothelioma in 7 of 10 patients, over the course of 1–8 years (143). Given the clinical and diagnostic uncertainty that continues to surround malignant mesothelioma *in situ*, we would recommend that this diagnosis be made only in consultation with a pathologist with expertise in mesothelioma.

Localized malignant mesothelioma

Localized malignant mesothelioma is a rare malignant neoplasm that may arise in the pleura or peritoneum and accounts for approximately 1% of all malignant mesothelioma diagnoses. In contrast to more typical diffuse malignant mesothelioma, localized malignant mesothelioma is defined by sharp circumscription and relatively good prognosis, with reported median survival ranging from 29–134 months in three studies (144,145) (Marchevsky *et al.*, in press, Modern Pathology, 2019). Despite these clinical differences, the histomorphology and immunophenotype of localized and diffuse mesothelioma are identical, including the same relative frequency of epithelioid, biphasic, and sarcomatoid histotype in localized and diffuse tumors. Localized mesothelioma is also associated with asbestos exposure in approximately one-third of cases. Although studies of localized malignant mesothelioma are limited, recent data by Marchevsky and colleagues (in press, *Modern Pathology*, 2019) indicate that the ancillary studies discussed in this review, including immunohistochemical loss of BAP1, can be applied to diagnosis of localized malignant mesothelioma. These studies may be particularly useful in this setting, as localized malignant mesothelioma is a rare tumor that can closely mimic non-mesothelial tumors and benign localized mesothelial proliferations. For a thorough and current review of available data on localized mesothelioma, the interested reader is referred to the above-cited forthcoming manuscript by Marchevsky and colleagues.

Tumors at rare sites: paratesticular and pericardial mesothelioma

Paratesticular mesothelioma is an exceptionally rare tumor, accounting for <1% of mesothelioma diagnoses in men. Because it arises from the tunica vaginalis, paratesticular mesothelioma would seem developmentally related to peritoneal mesothelioma. However, three published series, with a total of 49 cases, point to morphological and clinical differences (146-148). Some studies suggest that biphasic morphology may be more common in mesothelioma of the tunica vaginalis (146,147), and clinical outcomes are poor. In one study of 6 patients, 5 had died of disease over 8–74 months (146). Although immunohistochemical testing was limited in two studies by retrospective review, mesothelial and epithelial markers appear to show the same sensitivity and specificity for paratesticular mesothelioma as at other sites (146-148). Markers of mesothelial malignancy have not yet been studied in paratesticular mesothelioma, to our knowledge.

Pericardial mesothelioma is similarly rare, comprising <1% of all mesothelioma diagnoses. Pericardial mesothelioma appears to be clinically, morphologically, and immunophenotypically similar to pleural mesothelioma. In a recent large series of 61 pericardial mesotheliomas (reported to date in abstract form only), 82% of tumors were epithelioid, 13% biphasic, and 5% sarcomatoid, and clinical outcomes were poor, with median survival of 13 months and only 11% 24-month survival. Immunohistochemistry for cytokeratin AE1/AE3, calretinin, CK5/6, and WT-1 showed sensitivity of 100%, 94%, 90%, and 81%, respectively (149). We are awaiting publication of a full manuscript for details on application of malignancy-specific immunomarkers to pericardial mesothelioma, but we anticipate that they will be as useful in this setting as in pleural mesothelioma.

Conclusions

Immunohistochemistry plays an ever-larger role in accurate diagnosis of mesothelial lesions. Although mesothelial-

and epithelial-specific markers have long been used to confirm mesothelial lineage, novel markers, such as claudin-4, continue to increase the accuracy of this determination. Multiple markers of malignancy (including immunohistochemistry for BAP1 and, more recently, MTAP) have entered routine diagnostic use in the last decade, and promising new markers are the subject of ongoing study. Beyond their diagnostic use, immunomarkers are also assuming a novel prognostic and predictive role, which will likely grow as targeted therapies are increasingly deployed in treatment of malignant mesothelioma.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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