Pathologic analysis of melanocytic neoplasms
Jane L Messina, MD1,2 and Julie Gibbs, MD3

Abstract
Recent advances in techniques for pathologic evaluation of melanocytic neoplasms, updates in staging, and novel treatment and prognostic assays have brought pathologists to the forefront of the care of the melanoma patient. Specimen procurement, handling, and evaluation are all key to the production of a pathology report that guides the clinician to the proper treatment of the patient. Recent, relevant changes in the pathologic analysis of melanocytic neoplasms, highlighting the AJCC 8th edition guidelines, and pathologic changes related to therapy, are discussed herein. Also included is a discussion of molecular assays used to aid in diagnosis of challenging lesions, predict clinical outcome, and predict response to targeted therapy.

Semin Cutan Med Surg 37:88-100 © 2018 Frontline Medical Communications

Principles of biopsy and specimen handling
Accurate diagnosis of melanocytic neoplasms begins at the time of initial biopsy: specimen acquisition and accurate accompanying documentation are paramount. The pathology requisition should include, in addition to the required demographic information such as age and sex, a description of the size of the lesion, its duration, and any recent change. If the lesion is only partially biopsied, that should be clearly stated on the requisition. Those at the forefront of diagnosing melanoma, including dermatologists, primary care physicians, and surgeons, employ a variety of biopsy techniques. Excisional biopsies, shave biopsies, deep scallop shave biopsies, and punch biopsies are all employed in practice. All of these techniques have potential pitfalls that may lead to challenges in accurate diagnosis and staging, especially if they result in partial sampling and/or under-representation of the lesion.

Currently, the American Academy of Dermatology recommends excisional biopsy as the preferred method for biopsy of a skin lesion suspicious for melanoma,1,4 although in practice this is rarely performed. This may be due to time constraints in a busy clinic, as well as lack of clinical suspicion, considering that reported clinical sensitivity for recognition of melanoma is as low as 42% for general practitioners and up to 80% for dermatologists.5 There may be concern about the potential for alteration of the lymphatic drainage and compromise of the sentinel lymph node (SLN) mapping procedure by an excisional biopsy, but this risk is low.6 Conversely, specificity for clinical recognition of melanoma is less than 90%,7 so this practice may lead to a morbid excision for a lesion that turns out to be benign.8 Punch biopsy is still very commonly used in the initial assessment of cutaneous lesions suspicious for melanoma. While they are easy to perform under local anesthesia, biopsies larger than 3 mm require suture closure. Punch biopsies are limited in diameter, with a largest available diameter of 6 mm. Thus, while a punch can usually provide accurate assessment of dermal depth, its inability to encompass the entire periphery of the lesion puts the dermatopathologist at a disadvantage. This is because evaluation of overall size, circumscription, and symmetry is often key to distinguishing melanoma from its benign mimics. Additionally, in very large-diameter lesions that can only be partially sampled with a punch, there is a potential for inaccurate staging if other areas of the lesion have a thicker Breslow depth.4,8

In fact, 21% of patients whose melanomas were biopsied via incisional or punch biopsy are upstaged at the time of definitive excision.9 Proponents of the shave biopsy point to its time-saving nature and minimal morbidity, as well as a lack of need for suture placement.2,10 However, opponents point out that transection of the base of a lesion may lead to an underestimation of its true depth, leading to inaccurate staging and subsequent treatment decisions. Clearly, the ideal shave biopsy should include the full thickness of the melanoma for most accurate staging, and considering that the median tumor depth of melanoma at diagnosis is currently 0.60 mm,11 this should not be difficult. In practice, if a shave biopsy specimen measures at least 1 mm in depth, even if the melanoma is greater in thickness, the decision to perform a 2-cm-wide excision and sentinel node biopsy, as recommended by National Comprehensive Cancer Network guidelines, is still not compromised.1,12 In fact, in a recent large series, melanomas diagnosed by shave biopsy were upstaged in only 3% of subsequent wide excisions.13

Guidelines for the gross examination and sampling of therapeutic wide excisions vary among institutions. The rate of residual tumor in melanoma wide excisions in the United States,
where shave and punch biopsies are the most common initial diagnostic methods, is not well documented. While European studies showed minimal yield from examination of multiple sections, the initial method of diagnosis in these patients is excisional biopsy, which removes nearly the entire primary lesion.\textsuperscript{14,15} There are wide variety of protocols, and whichever is used must address the presence and extent of residual tumor and status of as much of the peripheral and deep margin as feasible, in order to facilitate accurate staging. Most centers employ variations of the “breadloaf” method, wherein specimens are serially sectioned and completely submitted after differential inking of margins. However, this is not feasible for excisions larger than 1 cm in diameter by 2 cm in length because of the large number of sections this would produce. Tangential sampling of all or most of the peripheral and deep margins, with enface sectioning of these cuts as well as submission of serial sections from the residual primary tumor and/or prior biopsy site, is an accepted and valid approach for large excisions.

**Key pathologic features of melanoma and its subtypes**

Accurate pathologic diagnosis of melanoma requires identification of a constellation of features that distinguish it from benign nevus. There is no minimum number of features required, rather systematic evaluation of the lesion’s growth pattern, cytomorphology, and alterations of the microenvironment such as inflammation and reactive sclerosis. Proper evaluation of a biopsy of an atypical pigmented lesion begins with a low-power examination of its architecture, assessing its size and symmetry. Deeper levels facilitate complete examination, especially if any portion of the biopsy shows incomplete sectioning. If the entire lesion has been sampled, attention should be paid to its border. Poor lateral circumscriptio is a hallmark of melanoma, as evidenced by a trailing edge of solitary, irregularly spaced melanocytes. Next, attention is paid to the junctional component of the lesion. The intraepidermal portion of a melanoma is characterized by irregular size, shape, and spacing of melanocytic nests (if present), as well as high cellularity evidenced by confluence of melanocytes. Single cells often predominate, but not always. These are often present in a suprabasal location, defined as the region above the hypothetical line drawn at the tips of the dermal papillae: so-called pagetoid spread. While portions of dysplastic nevi, acral nevi, and nevi with recent actinic exposure may show occasional single and even suprabasal melanocytes, their presence and/or predominance at the edges of a pigmented lesion is suspicious for melanoma.\textsuperscript{16,17} Consumption of the epidermis, defined as the presence of a thinned epidermis overlying expansile junctional nests of melanocytes, is found in 43\% of melanomas, compared to only 2.5\% of severely dysplastic nevi, and is absent in mildly dysplastic, congenital, and common nevi.\textsuperscript{18} Consumption may be a precursor to cutaneous ulceration.\textsuperscript{19} The dermal component of a melanoma is often markedly more cellular than benign counterparts. Sheet-like growth, with little or no intervening dermis between groups of cells, is characteristic of melanoma.

After the architecture of a lesion is closely examined, attention should be paid to its cytologic features. Often, classic malignant cytologic features such as marked pleomorphism, multinucleation, hyperchromasia, and atypical mitoses are prominent. Malignant melanocytes show epithelioid and/or spindle shapes and vary in size from small-cell variants to large, even ballooned cell types. While 1\% of nevi may demonstrate 1-2 superficial mitoses, the presence of mitoses near the base of the lesion is suspicious for melanoma.\textsuperscript{20} More subtle features include lack of maturation of the dermal component, with the presence of pleomorphic melanocytes at the base of the lesion the same size as the junctional component. Some lesions demonstrate pseudo-maturation, where the overall size of the cell diminishes because of loss of cytoplasm, but the high nuclear/cytoplasmic ratio is maintained.\textsuperscript{21} Melanoma typically elicits a distinctive dermal reaction with expanded and sclerotic papillary dermis, increased lymphocytic infiltrate, and irregular clumps of melanin-laden macrophages. Elastic fiber staining may help to distinguish the distinctive sclerotic peritumoral collagen of a melanoma from the unaffected stroma of a benign nevus.\textsuperscript{22}

The various histologic subtypes of melanoma show fairly characteristic morphologic features. The original four histologic subtypes of melanoma—superficial spreading, nodular, acral lentigious, and lentigo maligna melanoma—were first proposed by Clark and Elder in 1986.\textsuperscript{23} These variants show distinctive clinical presentations and cluster in certain ethnic subgroups and geographic locations. While they were originally thought to be distinctive prognostically, it is now known that tumor depth and the presence of ulceration are the major drivers of prognosis in primary melanoma. It is interesting to note that while they are no longer considered of prognostic importance, the most common melanoma subtypes have recently been shown to cluster with certain types of molecular abnormalities, especially with respect to the presence or absence of chronic sun damage.\textsuperscript{24,25}

Lentigo maligna melanoma occurs in heavily sun-damaged skin, so abundant solar elastosis and epidermal atrophy are often seen. The in situ growth phase (lentigo maligna) displays predominantly basally located growth of atypical melanocytes. Pagetoid involvement of the epidermis is typically a minor feature, but confluence of single melanocytes is characteristic. There is often quite prominent adnexal involvement. Occasionally, lesional cells may be small and nevoid, but confluent, broad growth is still a feature (Figure 1A).\textsuperscript{26} The invasive component is usually composed of spindled melanocytes,\textsuperscript{27} but epithelioid melanocytes may be observed. Downward displacement of the dense band of solar elastosis by the tumor nests may be found.\textsuperscript{28,29} A correlation between the
**Pathologic analysis of melanocytic neoplasms**

v-RAF murine sarcoma viral oncogene homolog B (BRAF) V600K mutation and melanomas on chronically sun-damaged skin has been observed. In a recent study, BRAF mutations were seen in 21% of the lentigo maligna melanoma lesions, and the BRAF V600K mutation predominated over the BRAF V600E mutation (10/61 [16%] and 3/61 [5%], respectively). Superficial spreading melanoma is the most common melanoma subtype, comprising approximately 70% of lesions. It is characterized by the presence of single and nested melanocytes at all levels within the epidermis, with marked variation in nesting, marked confluence, and abundant pagetoid spread (Figure 1B). Superficial adnexal epithelium may also be involved. Often, the abundant intraepidermal growth of melanocytes in superficial spreading melanoma leads to the formation of clefts from the epidermis because of lack of adhesive cellular junctions in melanocytes. The infiltrative component of superficial spreading melanoma may be sheet-like or fascicular in growth and composed of melanocytes with a variety of morphologies, including epithelioid, spindled, and nevoid. There is usually absence of maturation. BRAF mutation is most frequently in superficial spreading melanoma, present in about 40% to 60% of all lesions.

Nodular melanoma is characterized by development of vertical growth phase early in the evolution of the lesion, so that there is a minimal junctional component. The original definition of a nodular melanoma specifies that the junctional component should not extend 3 rete ridges beyond the invasive component (Figure 1C). The dermal component may show epithelioid or spindle morphology. Nodular melanomas are the variety most commonly ulcerated at presentation, and their average thickness at diagnosis is greater than the other subtypes. The Neuroblastoma RAS viral oncogene homolog (NRAS) mutation is most frequently found in this variant of melanoma.

Acral lentiginous melanoma has a distinctive lentiginous pattern of intraepidermal growth, with single cells predominating over nests. The lentiginous melanocytes often have a perinuclear halo, or they may have heavily pigmented dendritic processes (Figure 1D). Pagetoid spread is less common in this variant compared to superficial spreading melanoma. Deep extension of intraepithelial melanocytes in eccrine epithelium is often found. The invasive component may be of epithelioid or spindle morphology, with approximately 15% of cases being amelanotic or even resembling nevus cells. There may be a desmoplastic stromal response. This subtype of melanoma has the highest incidence of KIT amplifications and mutations, found in 36% of acral lentiginous melanomas. Sarcomatous differentiation, typically osteosarcoma, while rare, is most commonly found in acral lentiginous melanoma.

The more unusual subtypes of melanoma deserve mention, as they are often more difficult to distinguish, both clinically and histologically, from benign nevus variants or even non-melanoma spindle lesions. Desmoplastic melanoma is a distinct subtype of melanoma, accounting for less than 4% of primary cutaneous melanomas, with an overall incidence rate of about 2 per million US persons, with a reported annual 4.6% increase. It is characterized by a subtle in situ component and paucicellular dermal growth of spindle cells.

**FIGURE 1.** (A) Lentigo maligna melanoma, with marked solar elastosis and growth of confluent melanocytes in a flattened epidermis and extending into adnexal epithelium. (B) Superficial spreading melanoma, showing confluent nests and single melanocytes with prominent pagetoid spread. (C) Nodular melanoma, showing invasive dermal component without lateral extension of melanoma in situ. (D) Acral lentiginous melanoma, with polygonal melanocytes containing pale cytoplasm exhibiting dermal-epidermal separation artifact because of confluence of single melanocytes. (A-D) H&E, original magnification 100x. Abbreviation: H&E, hematoxylin and eosin.
surrounded by abundant desmoplastic stromal change studded with lymphoid nodules. The mean Breslow thickness at presentation is 4 mm. Desmoplasia is defined as the presence of prominent dermal fibroblasts with abundant production of dermal mucin, mostly hyaluronic acid. Only about 70% of desmoplastic melanoma demonstrates an in situ component at initial diagnosis. These distinct histologic features contribute to the distinct clinical presentation of this lesion, which is often found in sun-damaged skin, rarely pigmented, and often scar-like at presentation. There are two subtypes of desmoplastic melanoma: pure and mixed. Pure desmoplastic melanoma is composed of >90% paucicellular dermal spindle cell growth. Mixed desmoplastic melanoma is diagnosed when >10% of the lesion shows higher cellularity with little intervening stroma, or >10% of the tumor has an epithelioid morphology. This distinction is important, since pure desmoplastic melanoma has a lower rate of sentinel node positivity than mixed and non-desmoplastic melanoma of similar thickness, and both subtypes have a higher rate of recurrence than nondesmoplastic subtypes. Mixed desmoplastic melanoma is associated with a 3.5-fold greater risk for metastasis or death, and a shorter time to local recurrence. Pure desmoplastic melanoma have a lower frequency of regional lymph node involvement (1%) as compared with mixed desmoplastic melanoma (10%) or as low as other melanoma subtypes (6%). Desmoplastic melanoma frequently demonstrates perineural invasion and/or small foci of collections of tumor cells resembling nerves, so-called neurotization. This subtype of melanoma is immunohistochemically distinctive: it almost always shows positive staining for S-100 and Sox-10 but by definition is negative for Melan-A and human melanoma black 45 (HMB-45). This morphology makes distinction from the malignant peripheral nerve sheath tumors (MPNST) difficult, but a recently described marker, H3K27me3, is retained in desmoplastic melanoma, lost in 69% of MPNST and in 95% of sporadic MPNST. Delineation of desmoplastic melanoma from spindle cell melanoma (ie, nodular or superficial spreading melanoma with a spindled dermal component) is often difficult. In contrast to desmoplastic melanoma, spindle cell melanoma is more likely to demonstrate Melan-A positivity, have a trichrome-negative stroma, and have more frequent (31% versus 5%) BRAF mutation.

There are a variety of other unusual subtypes of melanoma that are distinctive on the either the basis of histology or clinical presentation. These include nevoid melanoma, verrucous melanoma, balloon-cell melanoma, animal-type melanoma, signet ring-cell melanoma, Spitzoid melanoma, small cell melanoma, myxoid melanoma, and melanoma with divergent differentiation such as osteosarcoma and chondrosarcoma. Recognition of these variants is obviously important insofar as the accurate diagnosis and therefore treatment of melanoma is initiated, but these variants do not carry a different prognosis from the common varieties listed above when matched by stage.

The entity “primary dermal melanoma” deserves mention, as the proper staging of a patient who presents with melanoma in the dermis, devoid of an epidermal in situ component, is an occasional diagnostic dilemma. Whether these represent...
a primary melanoma arising in the dermis or a metastasis re-
 mains a matter of debate. The traditional Breslow depth of 
these lesions has been shown not to correlate well with pa-
 tient outcomes, and the majority of patients have a negative 
work-up for metastatic disease, including lymph node dissec-
tion.34,35 Many melanomas that present in the dermis are pri-
tary tumors that have lost the epidermal component be- 
cause of regression, prior biopsy, trauma (such as repeated scratch-
ing), or cryoablation. For example, traumatic removal of the 
epidermal component of a melanoma may be found in areas 
groomed by shaving, such as the legs in women and face in 
men. Melanomas presenting in the dermis and subcutaneous 
tissue behave more like thick, primary melanoma or local re-
currence than regional metastasis.36 Prior history of trauma or 
melanoma at another site must be sought, and the level of 
evaluating lesions of the nail matrix, where 70% 
clinical practice.57 While a thorough discussion of the 
benign mimickers of melanoma is beyond the scope of this 
work, some of the ad-
juvant tests used for diagnostic confirmation will be briefly 
discussed. A variety of terms have been promulgated to de-
scribe melanocytic neoplasms that demonstrate pathologic 
overlap with benign lesions, eg, atypical melanocytic neo-
plasm, melanocytic tumor of uncertain malignant potential, 
and superficial atypical melanocytic proliferations of uncertain 
significance. The most common challenging scenarios are 
when the lesion shows overlapping features with Spitz nevus, 
cellular blue nevus, or compound/congenital nevus. There 
have been numerous studies documenting the lack of histo-
logic consensus among experts in diagnosing these lesions, 
as well as the difficulty in predicting their biologic behavior 
from histology alone.72,73 Many of the patients affected by this 
dilemma are pediatric, with many of their lesions display- 

**Immunohistochemical and molecular analysis of diagnostically challenging melanocytic lesions**

Although immunohistochemical (IHC) analysis is not re-
quired in the workup of unequivocal melanoma, it is still com-
monly used in clinical practice.57 While the typical melanoma 
often displays many of the aforementioned features, many le-
sions only demonstrate a few abnormalities, and thus overlap 
with benign nevi. IHC analysis is often employed in this situ-
ation and can help evaluate a lesion’s circumscription, cellular-
ity, and depth. The most common stains employed for these 
purposes are Melan-A/Mart-1, Sox10, and MiTF (S-100 has 
mostly been replaced by Sox10 because of its lack of specific-
ity). Melan-A is a cytoplasmic stain that can sometimes give 
the impression that a lesion is more cellular than it actually 
is, especially in sun-damaged skin.58 Therefore, nuclear stains 
such as Sox10 and MiTF are often more helpful in examin-
ing biopsies on sun-damaged skin (Figure 2A). Sensitive stains 
such as Melan-A are very helpful in accurate assessment of 
melanoma Breslow depth, especially in heavily inflamed le-
sions (Figure 2C). One notable pitfall of S-100 is its lack of 
sensitivity in evaluating lesions of the nail matrix, where 70% 
of melanoma in situ was negative for S-100 in one study.59 The 
monoclonal antibody, HMB-45, is often utilized to identify 
the gp-100 tumor antigen that is often retained throughout the 
full thickness of the dermal component in melanoma (Figure 
2D). It may taper in intensity upon dermal depth in benign 
nevus, with the exception of blue nevi, deep penetrating nevi, 
and some Spitz nevi.60,61

Evaluation of proliferation with Ki-67 has both diagnostic 
and prognostic use. While many melanomas show a prolif-
eration index that overlaps with benign nevi, a Ki-67 index of 
>10% is almost always diagnostic of melanoma (Figure 
2D).62,63 Since Ki-67 is expressed in a high percentage of lym-
phocytes, which can lead to difficulty in interpreting heavily 
inflamed lesions, combining this marker with Melan-A using 
two chromogens (MelPro or K-Mart) is quite helpful.64 Phosphohistone H3 is a marker of cells in mitoses and can 
give an accurate assessment of the number of mitoses in a le-
sion. It does significantly correlate with manual assessments 
of mitotic rate and can be used to screen for “hot spots” of 
mitotic activity,65,66 though its use has decreased since mito-
ses are no longer part of the primary tumor staging schema. 
P16 expression is lost in up to 63% of melanomas67 and often 
retained in benign lesions. While complete loss of expression 
of p16 was originally thought to indicate homozygous dele-
tion of the CDKN2a gene encoding p16/INK4a and p14ARF 
(a hallmark of melanoma), it is now known that epigenetic 
silencing and heterozygous deletion can lead to absence of 
staining.68 Therefore, this stain cannot be used as a sole indi-
cator of the malignant potential of a lesion. In the rare lesion 
with Spitzoid morphology, IHC staining for BRCA1 associ-
ated protein (BAP-1) may be helpful. Loss of nuclear staining 
for this marker, indicating either sporadic or germline loss of 
the gene, has been described in a subset of lesions that are a 
marker for a family cancer syndrome; however, the location of 
the BAP1 mutation does not seem to have any bearing on the 
spectrum of types of cancers observed in these patients.69-71

While a thorough discussion of the benign mimickers of 
melanoma is beyond the scope of this work, some of the ad-
juvant tests used for diagnostic confirmation will be briefly 
discussed. A variety of terms have been promulgated to de-
scribe melanocytic neoplasms that demonstrate pathologic 
overlap with benign lesions, eg, atypical melanocytic neo-
plasm, melanocytic tumor of uncertain malignant potential, 
and superficial atypical melanocytic proliferations of uncertain 
significance. The most common challenging scenarios are 
when the lesion shows overlapping features with Spitz nevus, 
cellular blue nevus, or compound/congenital nevus. There 
have been numerous studies documenting the lack of histo-
logic consensus among experts in diagnosing these lesions, 
as well as the difficulty in predicting their biologic behavior 
from histology alone.72,73 Many of the patients affected by this 
dilemma are pediatric, with many of their lesions displaying 
worrisome clinical features.74 Accordingly, efforts have been 
made to develop tests to detect basic molecular differences 
between melanoma and its histologic mimics. One caveat in 
determining the utility of these tests is that they have been 
infrequently tested in ambiguous lesions using the gold stan-

---

**Seminars in Cutaneous Medicine and Surgery, Vol 37, June 2018**
Standard (long-term event-free survival or death from melanoma).

Fluorescence in situ hybridization (FISH) is a commercially available assay to aid in the diagnosis of controversial melanocytic lesions. Melanomas display numerous chromosomal aberrations such as loss of chromosomes 6q, 8p, 9p, and 10q and copy number increases of chromosomes 1q, 6p, 7, 8q, 17q, and 20q, which are not found in nevi. Spitz nevi show an isolated copy number increase of chromosome 11p in a subset of cases, particularly the desmoplastic type, an aberration not found in melanoma. After extensive testing with probes for a number of chromosomal regions, a combination of four probes was demonstrated to have a sensitivity and specificity of 86.7% and 95.4%, respectively, for the diagnosis of melanoma.75

The first version of the FISH assay employed probes targeting 6p25 (RREB1), centromere 6, 6q23 (MYB), and 11q13 (CCND1) utilizing formalin-fixed paraffin-embedded (FFPE) tissues.76 In 2011, an assay employing new probes to 9p21 (CDKN2a), 11q13 (CCND1), 8q24 (MYC), and centromere 9, and maintaining probes for 6p25 (RREB1), was promulgated to increase the sensitivity in evaluating ambiguous Spitzoid neoplasms and to address the issue of false positivity in the setting of polyploidy. This test increased the sensitivity for detection of melanoma to 94% but is still hampered by lack of specificity in evaluation of atypical Spitzoid lesions, as low as 33%.78-81 Some authors suggest following the standard 4-probe method with either C-MYC or CDKN2A/centromere 9 to help minimize technical problems and assist in histopathologic diagnosis.82

### TABLE AJCC Cancer Staging, 8th Edition, T category for melanoma pathologic staging.

<table>
<thead>
<tr>
<th>T Category</th>
<th>Criteria/Thickness</th>
<th>Criteria/Declaration Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>No evidence of primary tumor (e.g., unknown primary or completely regressed melanomas)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Tis</td>
<td>Melanoma in situ</td>
<td>Not applicable</td>
</tr>
<tr>
<td>T1</td>
<td>≤1.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
<tr>
<td>T1a</td>
<td>&lt;0.8 mm</td>
<td>Without ulceration</td>
</tr>
<tr>
<td>T1b</td>
<td>&gt;0.8 mm</td>
<td>With ulceration</td>
</tr>
<tr>
<td>T1c</td>
<td>0.8–1.0 mm</td>
<td>With or without ulceration</td>
</tr>
<tr>
<td>T2</td>
<td>&gt;1.0–2.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
<tr>
<td>T3</td>
<td>&gt;2–4.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
<tr>
<td>T4</td>
<td>&gt;4.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
</tbody>
</table>

*Suffix of “a” for tumors with ulceration and suffix of “b” if without ulceration.

<table>
<thead>
<tr>
<th>N Category</th>
<th>Extent of regional lymph node and/or lymphatic metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional nodes not assessed (e.g., SUN biopsy not performed, regional nodes previously removed for another reason)</td>
</tr>
<tr>
<td>N0</td>
<td>No regional metastases detected</td>
</tr>
<tr>
<td>N1</td>
<td>One tumor-involved node or in-transit, satellite, and/or microsatellite metastases with no tumor-involved nodes</td>
</tr>
<tr>
<td>N1a</td>
<td>One clinically occult (e.g., detected by SUN biopsy)</td>
</tr>
<tr>
<td>N1b</td>
<td>One clinically detected</td>
</tr>
<tr>
<td>N1c</td>
<td>No regional lymph node disease</td>
</tr>
<tr>
<td>N2</td>
<td>Two or three tumor-involved nodes or in-transit, satellite, and/or microsatellite metastases with one tumor-involved node</td>
</tr>
<tr>
<td>N2a</td>
<td>Two or three clinically occult (e.g., detected by SUN biopsy)</td>
</tr>
<tr>
<td>N2b</td>
<td>Two or three, at least one of which was clinically detected</td>
</tr>
<tr>
<td>N2c</td>
<td>One clinically occult or clinically detected</td>
</tr>
<tr>
<td>N3</td>
<td>Four or more tumor-involved nodes or in-transit, satellite, and/or microsatellite metastases with two or more tumor-involved nodes or any number of matted nodes without or with in-transit, satellite, and/or microsatellite metastases</td>
</tr>
<tr>
<td>N3a</td>
<td>Four or more clinically occult (e.g., detected by SUN biopsy)</td>
</tr>
<tr>
<td>N3b</td>
<td>Four or more, at least one of which was clinically detected, or presence of any number of matted nodes</td>
</tr>
<tr>
<td>N3c</td>
<td>Two or more clinically occult or clinically detected and/or presence of any number of matted nodes</td>
</tr>
</tbody>
</table>

**Abbreviations:** AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase; SLN, sentinel lymph node.
In 2012, a commercial test offered by Myriad Genetics became available for use in formalin-fixed biopsies of diagnostically challenging lesions. The myPath test measures mRNA expression of 23 genes by quantitate RT-PCR. A weighting algorithm computes the expression of these genes, which are related to melanocyte differentiation, immune signaling, and others to produce a numerical score between −16 and +11. A negative score supports a benign lesion, while a positive score supports a diagnosis of melanoma, with a reported sensitivity of 90% and specificity of 91% in unequivocal lesions. A single report testing the utility of this assay in diagnostically challenging lesions has shown that the myPath score agreed with the histologic interpretation of a panel of experts in 64% of cases. This same study showed agreement of FISH result with histologic interpretation in 70% of cases.

Comparative genomic hybridization (CGH) is an assay that can detect losses or gains within portions of genomic material and map to their chromosomal regional location. This test uses FFPE tissue and has the advantage of evaluating the whole genome of the entire tissue sample rather than focusing on specific parts of the genome, such as with FISH. In CGH, the index lesion is compared to normal tissue, with the DNA from both samples allowed to compete for substrate and evaluated based upon the ratio of fluorescence intensity of tumor to normal tissue. Ninety-five percent of all melanomas harbor whole chromosomal gains and/or losses, especially in chromosome 9, followed by chromosome 10, 61, and 8p. In contrast, benign nevi typically show normal CGH and rarely isolated gains or losses of chromosomal regions in a pattern that does not overlap with melanoma. Spitz nevi can harbor an 11p or 7q gain. One drawback of this test is that it is not widely available, further complicated by issues of difficulties in test cost reimbursement by most major insurance carriers. As such, CGH is mostly employed as part of a comprehensive, expert consultation of a diagnostic lesion at major academic centers.

Recently, mutations in the promoter region of the telomerase reverse transcriptase (TERT) gene, which regulates the activity of telomerase, have been described in melanomas in both adults and children. In a recent study of 56 atypical Spitz tumors and Spitzoid melanomas, the presence of a TERT promotion mutation was the most significant predictor of distant metastatic spread and death. Three of the 4 patients who died had initially been diagnosed with an atypical Spitzoid lesion.

**Reporting and staging of primary melanoma**

Once a diagnosis of melanoma has been established, examination of the lesion for a variety of pathologic features that are essential for accurate staging is necessary. While the most recent 8th edition of the AJCC staging criteria only employs a handful of staging features of primary melanoma, a host of other features have been shown to have prognostic or predictive ability either in univariate or, occasionally, multivariate analysis. Several of these are required by AJCC to be assessed and recorded, though not used for final staging.

Correct AJCC staging of primary melanoma requires assessment and reporting of tumor depth in millimeters, presence or absence of ulceration, and presence or absence of microsatellite metastases in the biopsy and wide excision. The Breslow depth of the tumor, calculated by measuring from the granular layer of the epidermis to the deepest portion of the invasive component, was first described by Alexander Breslow in 1970 and is the most important prognostic feature of a primary melanoma. Tumor thickness can only be evaluated accurately in sections cut perpendicular to the epidermis. When ulceration is present, the Breslow depth is measured from the base of the ulcer. In the most recent 8th edition of the AJCC staging system (Table 1), tumor depth is reported to the closest 0.1 mm. While most ocular micrometers have the ability to read to the closest 0.01 mm, the reading would be rounded to the nearest 0.1 mm, rounding down for decimal values ending in 1 to 4 and rounding up for 5 to 9. Foci of neurotropism or lymphovascular invasion should not be included in the measurement of tumor depth. It is also generally accepted that melanoma growing within adnexal epithelium is not considered in the measurement of Breslow depth unless it is the only focus of invasion. The 8th edition T1–4 categories (Table 1) continue to be defined by whole number integers (T1: 0-1.0 mm; T2: >1.0-2.0 mm; T3: >2.0-4.0 mm; T4: >4.0 mm). The T1 category is subdivided as follows: T1a: 0-0.8 mm; T1b: >0.8-1 mm. If tumor thickness cannot be evaluated, the tumor is staged as TX. Melanoma in situ is staged as Tis. Patients with completely regressed melanoma or melanoma of unknown primary are staged as T0.

Tumor ulceration continues to be part of the T-staging, with its presence upstaging a tumor from T stage (a) to (b) at any thickness. Ulceration is defined as absence of the full thickness of the epidermis and is accompanied by reactive change such as fibrin deposition and presence of neutrophils. Mere thinning of the epidermis, the presence of scale crust, or narrow, sharp ulceration consistent with traumatic excoriation does not constitute ulceration. In one recent study of 4,661 patients, the 5-year melanoma-specific survival was significantly impacted in patients with an ulcer diameter <5 mm compared to those with an extensively ulcerated (>5 mm) melanoma. Therefore, the 8th edition staging mandates that the width of an ulcer be measured in millimeters using an ocular micrometer and reported.

While mitotic rate is no longer considered in the staging of a lesion, the AJCC recommends measuring and recording number of mitoses per square millimeter. This is accomplished using the “hot spot” method, wherein the region containing the most mitoses is first identified. After counting mitoses in the initial high-power field, the count is extended to adjacent non-overlapping fields until an area of 1 mm² is counted. The pa...
The pathologist must know the field diameter of their microscope in order to determine how many high-power fields comprise this area, using the formula $r^2$. The mitotic rate is recorded as a whole integer. If the invasive component measures <1 mm$^2$, the number of mitoses found should still be reported as for a tumor with 1 mm$^2$ of dermal component. Conversely, if only 1 mitosis was found in the entire dermal component, then it is recorded as the absolute number. The revisions in recording of tumor depth, ulceration, and mitotic rate will cause some differences in staging of thin melanomas in the revised AJCC staging. For instance, in the 7th edition, a mitotically active lesion measuring 0.70 mm would be recorded as T1b, while in the 8th edition, it would be classified as a T1a melanoma. Conversely, a 0.90 mm melanoma with 0 mitoses/mm$^2$ previously classified as T1a would be T1b in the updated staging system.

Microsatellite metastases are defined as microscopic tumor foci within the dermis or subcutaneous fat adjacent to, but discontinuous from, the primary melanoma. Microsatellites must be separated from the primary tumor by normal dermis or fat, not fibrosis or inflammation. These were previously strictly defined as measuring >0.05 mm in size and located at least 0.3 mm from the main primary tumor mass. However, the 8th edition does not delineate a minimum size or distance. Thus, to avoid a potential for “overcall” of microsatellites, it is recommended to examine multiple tissue levels to distinguish these from peri-adnexal extension, especially in tumors with a poorly circumscribed interface with the surrounding dermis. The presence of microsatellites is an adverse prognostic factor in primary melanoma. Patients with microsatellites are classified as Stage III disease and staged as N1c, N2c, or N3c according to the number of positive regional lymph nodes present.

Other nonstaging prognostic features required by AJCC and College of American Pathologists to be recorded as a primary invasive tumor characteristic include lymphovascular invasion, regression, and neurotropism. While one may debate the necessity of evaluating and reporting other non-AJCC required features, such as tumor-infiltrating lymphocytes (TILs) and anatomic (Clark) level, most academic centers continue to report the majority of these, in the spirit of recording this data for possible use in future predictive models. The level of invasion, first described by Wallace Clark, is a surrogate for tumor depth. Defined as penetration of tumor relative to anatomic landmarks of the dermis, its relative lack of reproducibility is the likely reason for its prognostic significance in univariate analyses, but not in most multivariate analyses.

Lymphocytic infiltration of the primary melanoma tumor is a favorable prognostic factor and may predict lower rates of sentinel node positivity. TILs are defined as lymphocytes that surround and extend into nests and individual tumor cells of a melanoma. The tripartite grading scheme most commonly used is absent, non-brisk, and brisk TILs. To classify a tumor as having brisk TILs, the lymphocytes must infiltrate either the entire base of the tumor or diffusely permeate it. Lymphovascular invasion occurs when melanoma tumor cells infiltrate either blood vessels or lymphatics; it is recorded as present or absent. A double immunostain for melanoma and lymphatic endothelium has been proposed as a method to augment its recognition, and this phenomenon was demonstrated to be a factor predicting metastasis in patients with clinical stage IB and IIA melanoma.

Melanoma regression occurs when the host immune response results in destruction of all or a portion of a primary melanoma. It is characterized by the absence of melanoma, associated with superficial dermal fibrosis/sclerosis, accompanied by lymphocytes, melanophages, and epidermal effacement. The mere presence of fibrosis, inflammation, and/or melanophages in the vicinity of viable tumor does not equate to regression—this requires complete absence of tumor. Regression is scored as present or absent. The prognostic significance of regression is a matter of debate. While it has been shown to portend a worse prognosis in thin melanomas, a systematic review and meta-analysis of 14 studies comprising 10,098 patients showed a lower incidence of sentinel node positivity in tumors with regression compared to those without it. The varied results of these studies may stem in part from lack of a standardized definition or criteria for the diagnosis of regression, as well as poor interobserver reproducibility. Neurotropism is defined as the presence of melanoma cells abutting, often circumferentially, a nerve sheath (perineural) or within nerves (intraneural). The periphery of the tumor is the best location to evaluate for neurotropism, since “entrainment” of nerves within the expanding tumor is not considered neurotropism. Additional prognostic parameters often reported include growth phase (radial or vertical) and presence or absence of a coexisting nevus.

**Pathologic evaluation of regional lymph nodes**

Accurate pathologic diagnosis of the sentinel node is central to proper staging, optimal treatment decisions, and precise prognostication of patients with melanoma. Intraoperative handling, gross dissection, and histologic/IHC evaluation techniques are all key components of this process. Although not currently part of routine handling, newer molecular techniques may potentially add to the valuable information gained from evaluating sentinel node specimens.

Gross examination of the SLN includes reporting the anatomic location, SLN number, presence or absence of blue dye, and radioactivity counts. After proper fixation, any gross lesion, such as pigment or visible tumor, should be described and measured. Unless voluminous, the perinodal fat should not be dissected away, taking care to not to disturb the lymph node capsule. If small (<4 mm), the node can be submitted in its entirety without sectioning. Larger SLN should be serially sectioned at 2- to 3-mm intervals along the longest axis and completely embedded for histopathologic evaluation.
Pathologic analysis of melanocytic neoplasms

Rapid intraoperative identification of SLN metastases may allow the patient to avoid second surgery and treatment delays. However, frozen section analysis of melanoma SLN has low sensitivity, with reported rates ranging from 47% to 59%. While one group found a false negative rate as low as 5.3%, this has not been repeated by others and has not gained widespread acceptance. Because of these data, as well as concerns about tissue exhaustion and cryostat contamination, currently SLN frozen section analysis is not advised in the detection of melanoma. Intraoperative imprint cytology (IIC) gained widespread interest in the early 2000s as a rapid alternative to frozen section analysis that can sample a broader area of tissue with no concerns for tissue usage. After touch imprinting one to several lymph node surfaces to a glass slide and rapid staining, IIC has been shown to have a sensitivity of 33% to 61%, with a negligible false positive rate. The sensitivity of IIC increases with tumor stage, as high as 47% in patients with T4 lesions, and rises to 62% when metastases >2 cm in size are present. Thus, IIC may be considered when metastasis is strongly suspected on gross examination and is preferable to frozen section of SLN.

While standardized lymph node examination protocols have been in place in Europe for a number of years, there is no standard protocol mandated in the United States, and several major institutions have developed their own sets of guidelines. The most widely utilized protocol is that of Cochrane and colleagues, who advocate cutting 10 serial sections from each lymph node section, staining sections 1, 3, 5, and 10 with hematoxylin and eosin (H&E), 4 with HMB-45, and 6 with Mart-1, saving 4 for possible additional studies. This approach identifies metastatic melanoma in 16% to 20% of specimens. Numerous studies have demonstrated that extended histopathologic examination of the SLN can improve diagnostic yield. Current and past AJCC guidelines recognize 2 categories of SLN involvement in melanoma: clinically occult micrometastasis and clinically apparent macrometastasis. Micrometastatic melanoma is defined as the presence of morphologically malignant cells positive for at least 1 IHC marker (S-100, HMB-45, Melan-A/Mart1, Sox10) and/or melanoma detectable on H&E staining alone. S-100 is the most sensitive marker (~99%), and Melan-A approaches this in one study (97%). More recently, Sox10 has a sensitivity equivalent to S-100, with added specificity because, unlike S-100, this nuclear stain is negative in nodal dendritic cells and macrophages. S-100 and Melan-A also highlight benign nodal nevi, present in the capsule, trabeculae, and rarely the parenchyma in up to 28% of lymph nodes, but these are typically HMB-45-negative.

In the 8th edition AJCC schema, the designation of nodal stage of melanoma (Table 2) depends on the number of involved nodes, presence of in-transit, satellite, or micrometastases, and whether tumor was detected by sentinel node staging (a) or clinically (b). Several studies have suggested that the pattern and burden of SLN tumor involvement may be linked to non-SLN positivity and clinical outcome measures. The AJCC advocates measuring the largest tumor diameter in the SLN. The terms “microscopic” and “macroscopic” have been replaced with “clinically occult” and “clinically detected,” referring to whether or not the deposit was identified by sentinel node biopsy. The presence of gross nodal extension is no longer considered in the N criterion, while microsatellites and in transit metastases are now stratified by N category according to the number of lymph nodes involved.

Starz et al developed a 3-stage schema based on the number of metastases and depth of tumor invasion from the interior of the SLN capsule in millimeters, showing that this schema was predictive of distant metastases and long-term survival. Cochran demonstrated that the 2-dimension percentage of involved nodal area could independently predict non-SLN positivity. Dewar demonstrated that an SLN with metastases in the subcapsular region had a lower rate of non-SLN positivity that those with multifocal, extensive, or intraparenchymal disease. The Rotterdam criteria, based on work by van Akkooi et al., demonstrated that the maximum diameter of the largest metastasis had a significant influence on non-SLN involvement and survival. While none of these criteria have yet to be fully validated in a multicenter or prospective study, reporting is recommended. The ongoing European Minitub trial comparing outcome in patients with minimal SLN tumor burden managed by observation versus a complete lymph node dissection (CLND) will likely answer this question.

Pathologic evaluation of the CLND is similarly important for accurate staging. Clearly, this requires submission of at least 1 section from each grossly identified lymph node. However, there is controversy over the necessity for submission of each lymph node in its entirety versus a single representative section. Similar to the controversy surrounding optimal methods for evaluation of sentinel nodes, there is debate concerning the necessity for IHC evaluation of non-SLNs. Until recently, immediate CLND was usually recommended for patients with sentinel-node melanoma metastases; however, evidence of the overall efficacy of this protocol is lacking. The procedure carries a risk of significant morbidity, and although it may provide prognostic information and improve regional disease control, evidence now suggests that it is not more effective at improving melanoma-specific survival in patients with positive sentinel-node metastases when compared to observation.

Distant metastatic melanoma

In the 8th edition AJCC schema, any of the clinical M category classifications, cM0, cM1, or pM, may be used in the designation of the M category (Table 3) for pathological stage grouping. The terms pM0 and MX are no longer considered valid categories, and a suffix of (0) or (1) should be included to designate whether or not a patient’s lactate dehydrogenase
(LDH) levels are elevated, since elevated LDH levels have been shown to be of prognostic value for patients with advanced melanoma.

Melanoma metastases may be resected as part of the initial diagnostic workup in a patient with a previously undetected or unknown primary, or as a palliative and/or therapeutic excision in a patient with oligometastatic disease. In patients without a prior history of melanoma, a thorough diagnostic workup often includes the IHC markers previously discussed. The most useful markers include a combination of at least 1 highly sensitive marker such as S-100 or Sox-10 and 1 highly specific marker such as Melan-A or HMB-45. There are several potential pitfalls to this approach: desmoplastic melanoma may be missed, and tumor of perivascular epithelioid cells (PEComa) and clear cell sarcoma may have a similar immunoprofile. PEComa is a family of tumors comprising angio-myolipoma, lymphangiomylomatosis, clear cell sugar tumor of lung, and most importantly in the differential diagnosis of MPNST.51

Clear cell sarcoma is a tumor of young adults that chiefly arises in the soft tissue of acral locations. It displays compact nests or fascicles of oval cells with clear cytoplasm in a delicate connective tissue framework. S-100 is almost always positive for Sox-10 and S-100, and thus demonstra-

Finally, the emergence of therapies targeted to specific gene mutations present in melanoma has also changed our evaluation of metastatic melanoma tumors. It is now customary to evaluate metastatic disease at the time of diagnosis for the presence of actionable gene mutations. There are a number of methods employed, such as pyrosequencing, Sanger sequencing of individual genes, or next-generation sequencing that target a panel of genes. While mutations in BRAF and KIT are most commonly evaluated, there are also therapies and/or therapeutic trials available for patients with mutations of a variety of other genes, including NRAS, MET, EGFR, ALK, ROS1, PIK3CA, mTOR, PTEN, nF1, AKT, and NTRK fusions. Approximately 60% of cutaneous melanomas harbor mutations in BRAF or NRAS. Identification of these mutations is useful for selection of patients who may benefit from BRAF and MEK inhibitor targeted therapies. HIC stains are an efficient and cost-effective method useful in identifying specific mutations. Kakavand et al recently demonstrated that BRAF(V600E) and NRAS(Q61R) antibodies have high sensitivity and specificity and may also be useful in identifying undifferentiated metastatic melanomas that no longer express the usual melanoma antigens when evaluated by currently available immunoperoxidase methods.

In summary, recent knowledge gained from exhaustive mining of clinical and molecular data has revolutionized the ways in which tissue from melanocytic lesions is analyzed and reported. From pathologic microstaging of localized and regionally metastatic disease to submission of tumor tissue for molecular testing to stratify patients for treatment, pathologists and oncologists must be aware of these advances in order to avail their patients of the most relevant treatment options.

References

17. Walters RF, Groben PA, Busam K, et al. Consumption of the epidermis: a cri-
Pathological analysis of melanocytic neoplasms


31. Price NM, Rywlin AM, Ackerman AB. Histologic criteria for the diagnosis of melanoma: a diagnostic criterion in the differential diagnosis of melanoma and dysplastic nevi that is as-...


Pathologic analysis of melanocytic neoplasms


