Dermatopathology plays a critical role in the study and practice of clinical dermatology. The latest research in dermatopathology may aid physicians in better understanding and diagnosing dermatologic conditions and thus allow them to take better care of their patients. Moreover, advances in dermatopathology help to shape the specialty by opening new avenues of inquiry and understanding of a host of dermatological conditions. Ahead, in this first of two articles, I will review and examine recent trends in dermatopathology, with a particular focus in this installment on malignant melanoma.

Melanoma Update
An area of recent interest concerns sentinel lymph node (SLN) evaluation in patients with a melanoma diagnosis. A 2006 article in *The New England Journal of Medicine* examined SLN biopsy and nodal observation in melanoma patients and found that SLN biopsy provides important prognostic information. However, it is important to note that overall five-year survival rates were similar in both the SLN and observation groups, and only subjects in the intermediate thickness melanoma group (1.2-3.5mm) showed benefit from SLN biopsy and immediate lymphadenectomy.

There are various ways of grossing a sentinel lymph node biopsy, the most effective of which, according to one study, is to cut the node into slices. Investigators examined two methods of slicing:

- **Multiple Slice Protocol**, in which the SLN is cut into 1-2mm thick slices, with 12 microtome sections from each slice stained; and
- **Bivalving Protocol**, in which the SLN is bivalved, followed by five consecutive series of microtome sections, with gaps of 50 microns prepared from each cut surface.

After review, investigators concluded that both methods are optimal first steps for examining sentinel lymph nodes. While multiple slice protocol often results in longer durations for embedding.

### Take-Home Tips
Advances in dermatopathology help to shape the specialty of dermatology by opening new avenues of inquiry and understanding of a host of dermatological conditions. Areas of recent development include: sentinel lymph node (SLN) evaluation in patients with a melanoma diagnosis, the use of fluorescence in situ hybridization (FISH) to distinguish intranodal nevus from metastatic melanoma, subclassification of desmoplastic melanoma, the D2-40 marker, and the CD99 Marker.
bivalving protocol was associated with a significantly longer processing period.

Location of SLN metastases is also an important factor that recently has garnered some attention. The design of current melanoma SLN histologic protocols is based on the premise that most metastases are found in the central parts of the nodes, however the evidence for this belief has not been thoroughly tested. Investigators in a 2009 study examined nodal location of metastases from 149 positive SLN patients and found that melanoma metastases are located throughout the nodes. They concluded that complete step sectioning of all SLNs will ensure both high metastasis detection rates and detection of all large metastases.

**Fluorescence in situ hybridization**

Another area of recent interest concerns encouraging feedback on the use of fluorescence in situ hybridization (FISH) to distinguish intranodal nevus from metastatic melanoma. FISH is a cytogenetic technique used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence similarity. In studies, researchers have used algorithm-using signal counts from a combination of four probes targeting chromosome 6p25, 6 centromere, 6q23, and 11q13. They correctly classified melanoma with 86.7 percent sensitivity and 95.4 percent specificity. It is a very new method, but FISH is nonetheless a useful adjunct tool to traditional methods in the diagnostic workup of deposits of melanocytes in lymph nodes that are histopathologically ambiguous.

Fluorescence in situ hybridization may also help physicians better understand the nature and distribution of residual melanoma in the skin surrounding primary tumors. In one study, researchers used FISH and array comparative genomic hybridization to detect and spatially map aberrations in the skin adjacent to acral melanomas. They found that on acral sites, melanoma field cells extend significantly into seemingly normal skin. These field cells provide a plausible explanation for the tendency for certain melanoma types to recur locally despite apparently having undergone complete excision.

**Desmoplastic melanoma**

A recent study examined subclassification of desmoplastic melanoma, in which researchers examined lymph node involvement in pure and mixed cases and found that mixed desmoplastic melanoma has a higher rate of lymph node metastasis.
The D2-40 Marker
Immunohistochemistry has proven valuable in the differentiation of particular conditions, despite the fact that few antibodies display absolute sensitivity or specificity. One of the more noteworthy antibodies is D2-40, a selective lymphatic marker that does not react with normal vascular endothelium but is specific for lymphatics. It is a mucin-type transmembrane glycoprotein highly expressed in lymphatic endothelium that may endow tumors with proliferative/invasive qualities.8-10

The D2-40 marker has been used to detect lymphatic invasion in malignant melanoma. In one 2005 study, researchers used D2-40 marker in 44 cases of MM with Breslow level greater than 0.75mm. All 44 cases of malignant melanoma were originally reported to have negative lymphovascular invasion. They observed seven cases of lymphatic invasion on D2-40 stain but determined there was no correlation between lymphatic invasion and other clinicopathological features.11

Another study found that lymphatic vessel density is significantly increased in melanoma using D2-40,12 while another found a higher number and area of peritumorous and intratumorous lymphatics in malignant melanoma metastatic to SLN.13 The findings of these studies and others suggest that lymphatic invasion detected by D2-40 does not predict SLN status in melanoma.14,15

However, another study did find a correlation between the D2-40 marker and sentinel lymph node invasion.16 In the study, researchers examined 74 cases of malignant melanoma with known SLN biopsy status. Results showed that 16 of the 74 cases (23 percent) had LVI with D2-40 stain. Overall and disease-specific survival was shorter for patients with LVI. In addition, 67 percent of patients with LVI-IHC and 19 percent without LVI-IHC had positive SLN.16

The CD99 Marker
CD99 is a transmembrane protein product of the MIC2 gene encoded by genes on X and Y chromosomes. It is involved in cell apoptosis, adhesion, extravasation, and transmigration, and it has shown to be expressed in malignant melanoma.17

D2-40 Linked to Other Dermatological Conditions
D2-40 has been linked to Kaposi’s Sarcoma (KS), however its specific role remains unclear. The origin of neoplastic cells in KS may be lymphatic or mixed lymphatic and vascular. This may be important if therapeutic options targeting vascular or lymphatic endothelial cells become available.16 Positive staining for D2-40 has been seen in several studies. For example, researchers in one study reported a patient with ALHE of the lip with positive staining with D2-40.22 (Fig. 4) Another report showed positive staining for D2-40 of a retiform hemangiendothelioma.23 (Fig. 5) New immunohistochemical stains such as these may lead to a re-classification of vascular neoplasms.
In one study, researchers examined expression of CD99 in 78 cases of invasive malignant melanoma, 60 percent of which had positive staining. In addition, no significant difference was observed in staining with depth of invasion.17 Another study compared the expression of CD99 in 27 cases of spitzoid MM and 58 cases of Spitz nevus, finding 56 percent positive staining for spitzoid malignant melanoma and just five percent positive staining for spitz nevus.18 (Figs. 6, 7)

**Conclusion**

In next month’s article, I will analyze the latest dermatopathology data on other types of tumors, such as merkel cell carcinoma, basosquamous carcinoma, basal cell carcinoma, and other adnexal tumors. I will also explore recent research and advances in inflammatory dermatoses and nephrogenic systemic fibrosis.

Dr. Goldenberg does not have any relevant relationships to industry.

**Gary Goldenberg, MD** is the Medical Director of the Dermatology Faculty Practice at The Mount Sinai Medical Center and is Assistant Professor of Dermatology and Pathology at The Mount Sinai School of Medicine.