Immunohistochemistry During Mohs Surgery

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Objectives:

- Review main indications for use of immunohistochemistry in Mohs surgery
- Discuss tips and pitfalls to increase utility in your Mohs lab and Mohs practice
- Present several illustrative cases to prompt discussion and facilitate troubleshooting

**I have no conflicts of interest**
Main Indications

- Lentigo maligna/melanoma in situ in critical locations where tissue sparing is important, and/or tumors are large, ill-defined, or recurrent
- High-risk squamous cell carcinoma, especially perineural invasion, moderate or poor differentiation, single cells and strands, or lots of background inflammation (CLL)
- Extramammary Paget’s Disease, which is notorious for subclinical extension and skip areas
Lentigo Maligna and LMM

• LM and LMM are approximately 10% of all MM, with rising incidence (Swetter JID 2005).

• A.K.A. Hutchinson’s melanotic freckle; melanoma-in-situ

• Estimated risk of progression of LM to LMM: 2-5% (Weinstock Br J Dermatol 1987)
True Confession: I perform Mohs for most facial LM. Why?

- **Phone Call**: “ENT here; got a dirty margin after excising a facial MM; can you help with the LM at the periphery?”

- **Path Report** after attempted staged excision of LM with horizontal paraffin sections: “>50% of the epidermis is absent for evaluation, so CPC recommended…” (Proper embedding difficult)

- **Patient Says**: “Why do I have to come back in 2 days for my next stage? How many times do I have to come back?”
Immunostains for LM

- MART-1 commonest stain utilized
- Not a panacea, but an adjunct
- Most useful in cases where freeze artifact occurs (pseudokoilocytes), pagetosis, nesting, and if adnexal tracking is occurring.
- Less helpful in my practice for lentiginous involvement
- Ultimately these stains do not predict biologic behavior or study subtle cytologic atypia
Tips for MART-1

• Cut unstained blocks in advance
• Helps to get a (+) control from your debulking
• Helps to get a (-) control from contralateral area
• Adds about 60 minutes per Mohs stage
• If your H&E shows obvious LM on Stage 1, go on to stage 2…
Nested LM + Dermal Inflammation or subtle LMM?
MART-1 Immunostaining

Note
No Staining

Background Stain not c/w H&E findings
Always helps to compare side-by-side, in parallel
Contralateral Sun-Damaged Skin ("Control")
Note Background “Noise” and DEJ increased hypermelanosis.
Side-by-side Comparison, H&E, MART-1
Recent Case Example

• 51 y.o. woman with pigmented lesion since 1998
• Biopsy 2004: MMIS/Lentigo Maligna
• Series of tangential and vertical excisions and partial excisions, possible superficial laser or chemical applications inferolaterally, 2004-2008
• Recurrent pigment 2010, closer to eyelid
Recurrent LM with Invasive MM; 2 sites MMIS, 2 sites invasive MM; invasive sites where small blue arrows point—note the one near lid margin has no pigment
Additional Scouting Biopsies...All negative except site “H”
Mohs Map and Mohs Defect
Repair Oculoplastics and Plastic Surgery
Postop 6 weeks frontal view
Additional Challenges with LM

• It is often multifocal, so “clear margins” are relative only

• Subtotal initial biopsies may miss invasive MM

• Many LM never progress to invasion, so why treat? If not treated, how does one monitor?

• What constitutes true MM-in-situ?
What Constitutes LM to me?

- **Nested or confluent** clearly atypical melanocytes +/- pagetosis. I need to see at least 3 together.

- Mart-1 stains and Melan-A stains may help distinguish melanocytes especially if freeze artifact present, but those stains DO NOT tell the viewer benign vs malignant. They can also stain melanosomes and melanophages...

- Overcalling sun-damaged melanocytes can lead to face-ectomies. “Control” samples may help for comparison.
MM Breslow 0.4mm depth: Excision; 1 cm margins
MM in situ: Mohs to conserve skin
MM in situ: Excision vs Mohs…
Invasive MM arising in MMIS: Now what?
73 y.o. woman with invasive Melanoma 2015, Breslow depth 0.81 mm, 1 mitosis. 
ENT excision, small flap, negative SLNB, Residual in situ melanoma at margins; opted for observation.
One year later, subtle pink and brown coloration; 2 biopsies showed scar; 1# showed in situ melanoma; 1* showed invasive MM 0.8 mm Breslow depth; Mohs Surgery to assist in tissue conservation around eye (plus minimal clinical signs)
4 stages of Mohs, lots of conjunctival involvement, eyelid sharing procedure for repair
Stage A1
H&E
4x
Stage A1
4x
Neg Ctrl
Stage A2
4x
Stage B1 4x
H&E
Mart-1
Easily Noted
MMIS
Stage B1 H&E 1x with challenges in viewing nests at lid margin when glandular tissue and freeze changes are mixed nearby
Stage B1 1x
H&E and Mart-1

Mart-1 Shows Transition From MMIS to background sun-exposed skin nicely
Stage B1 10x H&E with corresponding Mart-1, eyelid margin
Stage B3 H&E 1x with cells suspicious for MMIS
B3 4x
H&E
Mart-1

MMIS fairly obvious
Different area of Stage B3, with suspicious changes to the left, but where does the process end or transition to normal sun-exposed skin?
Stage B3
4x
H&E
Mart-1

MMIS and sun-exposed normal challenging but Mart-1 helps greatly
Stage C1 4x H&E

Mucosa conjunc. very difficult
C3 4x
H&E
Mart-1

Immunostain makes it easy to see there is no MMIS

Tumor cleared
74 y.o.m, high-grade SCC with intravascular involvement, extending focally to bone
Debulking tissue
2x
H&E

Obvious SCC
MCK Negative Control:

shows commonly observed background Staining (likely from granulocytes that in some Situations may be addressed with peroxidase)
Stage A3 H&E 4x  
SCC at deep margin in galea

Once again, NO staining noted with MCK, since unstained slides were not created concurrently when the H&E were cut!

The MCK were cut as an afterthought and SCC was then gone
Stage B1 H&E 10x Intravascular SCC
(NOT highlighted with MCK, since MCK stain not concurrent!)
Stage C2 2x
H&E and MCK

MCK can be trusted since 3rd stage was cut concurrently…
No tumor left
70 y.o.m with high-grade SCC and single-cell involvement with background CLL and many WBC in background on histology
Tumor Type: SCC  Location: Forearm  

<table>
<thead>
<tr>
<th>Initial Size (cm)</th>
<th>Final Defect (cm)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8 x 1.4</td>
<td>2.9 x 2.7</td>
<td></td>
</tr>
</tbody>
</table>

- SCC-G-3  
- NMM-311  
- 3-0V  
- 4-0M  
- 5-0N  

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Debulking 4x H&E with patchy SCC mixed with inflammation
Debulking 4x MCK with patchy SCC easily seen
Debulking MCK Negative Control
Debulking 4x H&E with MCK highlighting SCC comparison
Debulking
10x H&E
Debulking
10x MCK
Debulking 10 x H&E with MCK highlighting SCC in background of inflammation and CLL cells comparison
Stage A1 2x H&E showing no obvious SCC
Stage A1 2x MCK stain confirming no SCC. Follicles and eccrine coils highlight.
Stage A2 2x H&E showing no obvious SCC
Stage A2 2x MCK stain confirming no SCC. Follicles and eccrine coils highlight once again.
75 y.o. man with ill-defined Extramammary Paget’s Disease
No underlying internal malignancy. Scouting biopsies prior to Mohs. Specimens A, G, and anal verge +
Note sutures used to demarcate corresponding areas on map. Central island of tissue known obvious EMPD to be later resected en bloc. Nearby ulcers from scout bx’s
Debulking
EMPD 4x
H&E
CK7

Clearly evident tumor both stains
Debulking
CK7

Negative Control
A1 4x H&E and CK7  Note very subtle H&E but clear immuno findings
A4 4x H&E and CK7  Obvious involvement on both stains
A11 4x H&E and CK7  Much more subtle, CK7 helps greatly
B1 near anal opening 4x H&E and CK7: no tumor. Note background dermal staining
B2 4x H&E near anus, no obvious tumor
B2 4x CK7 near anus, clearly evident tumor on the left, background and Apocrine glandular staining present
A11 10x H&E with CK7 highlighting Paget cells
After all margins negative except intraanal areas that were inaccessible by Mohs, and those areas were more widely resected at time of repair next day.
Next day, anal speculum helps colorectal surgeon gain internal access.

Wedge resection of additional internal mucosa showed negative margins.

Sufficient tissue spared to allow reconstruction without a colostomy.
Large V to Y advancement flap repair
Key Tips for Immunostains

• Cut at about 5 microns; liquid nitrogen OK

• Make every other, or every third, cut an immuno cut and leave unstained if not sure you will need immunostains

• Remember to get a positive control from the same patient if possible—from the debulking or central clearly involved area, and a sun-exposed normal if MART-1

• Have several good reference controls on hand

• Surgeon should toggle back and forth between H&E and immuno—they are complimentary
Additional Tips

• Immunostaining and rush paraffin sectioning techniques have been developed in hopes of improving clearance rates, however their use results in increased cost ($30-$100) and procedure time (19-60+ min) both per slide.

• Mayo uses Leica kits though other brands available. We do a lot of volume so have an automated stainer.
Mohs for LM Background – Why send debulked central tissue?

- Although melanoma in situ carries nearly a 100% survival rate at 5 years, any level of invasion significantly worsens prognosis.

- Rates of an invasion found on debulk specimens initially thought to be LM: 5 to > 50% range in the literature; typical is 5-10% in larger series.

- **Some Mohs surgeons will process the entire specimen and not debulk; acceptable, though adds greatly to processing time.**
Upstaging of LM to MM at Mayo

• 1994-2012 cases reviewed
• 624 cases of “LM” with subtotal biopsy samples subsequently resected. Largest series to date
• 24 (4%) showed invasion
• Upstaging uncommon but possible, and less than average of pooled prior series

A Word on MiTF (Microphthalmia transcription factor) Immunostaining…

• A nuclear-staining antibody that may be more specific for atypical melanocytes than MART-1, HMB-45 and others

• How will it perform on frozen sections, and compare regarding cost, reliability, speed??

• Another nuclear stain, SOX-10, may also show utility and should likely be investigated
MiTF Immunostaining

• Is a nuclear immunostain that may have more specificity than MART-1 for melanocytes, including atypical ones.

• Recent studies performed on fixed tissue


• Kim J et al. *J Cutan Pathol* 2011

  • Showed feasibility and utility of MiTF as an alternative to MART-1 in a pilot study comparing the 2 stains head-to-head in real time
Fig. 3. High-magnification image shows comparison of hematoxylin and eosin (A) and immunohistochemical melanocytic stains in melanoma in situ. Nuclear staining by MiTF (B) more clearly identifies intraepidermal melanocytes in melanoma in situ. Cytoplasmic Melan-A (C) and HMB-45 (D) highlight increased numbers of melanocytes and basal keratinocytes, even in the presence of Azure blue counterstain.
Melanocyte Counts, Mayo Pilot Study, showing improved specificity of MiTF over MART-1 especially for chronic sun-damaged skin used for comparison during Mohs, and for peripheral Mohs margins that were negative for residual MMIS.


**TABLE 3. Summary of Results**

<table>
<thead>
<tr>
<th>Stain</th>
<th>Control CSDS</th>
<th>Negative Margin</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MITF</td>
<td>Melan-A</td>
<td>MITF</td>
</tr>
<tr>
<td>Patients</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Mean</td>
<td>9.8*</td>
<td>13.7*</td>
<td>8.8*</td>
</tr>
<tr>
<td>SD</td>
<td>3.5</td>
<td>5.9</td>
<td>4.2</td>
</tr>
<tr>
<td>MAX</td>
<td>15.2</td>
<td>24.3</td>
<td>17.7</td>
</tr>
<tr>
<td>MIN</td>
<td>3.5</td>
<td>5.2</td>
<td>2.7</td>
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</tbody>
</table>

*Statistical significance ($p < .001$).
MAX, maximum; MIN, minimum.
Recent Literature Highlights

• Standard vs Mohs approach for over 400 cases of LM, retrospectively, over ~10 years.

• Similar cure rates with slight trend toward fewer recurrences with Mohs and narrower margins, especially in critical anatomic locations.

• Mirzoyev SA et al. J Am Acad Dermatol 2014;70:443-8

• Nosrati A et al. JAMA Dermatol 2017;Feb;epub
### Summary Slide for Treatment Approaches to LM/LMM


#### TABLE 2: Comparison of Modified Surgical Techniques Using Permanent Sections for the Management of Melanoma

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<thead>
<tr>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Section orientation</td>
<td>Horizontal</td>
<td>Horizontal</td>
<td>Horizontal</td>
<td>Vertical</td>
<td>Vertical</td>
<td>Vertical</td>
<td>Vertical</td>
</tr>
<tr>
<td>Description of technique</td>
<td>45° angled Mohs layers with permanent sections</td>
<td>MMS with rush permanent sections</td>
<td>Slow MMS with rush permanent sections</td>
<td>Square technique</td>
<td>Staged excision with radial cuts</td>
<td>Mapped serial excision</td>
<td>Perimeter technique</td>
</tr>
<tr>
<td>Margin evaluation</td>
<td>Complete</td>
<td>Complete</td>
<td>Complete</td>
<td>Partial</td>
<td>Partial</td>
<td>Complete</td>
<td>Complete</td>
</tr>
<tr>
<td>Tumor bulk assessment for invasion</td>
<td>With first stage</td>
<td>With first stage</td>
<td>With first stage</td>
<td>With final stage</td>
<td>With first stage</td>
<td>With first stage</td>
<td>With final stage</td>
</tr>
<tr>
<td>Diagram</td>
<td>2- to 3-mm margin</td>
<td>2- to 5-mm margin</td>
<td>5- to 10-mm margin</td>
<td>2- to 3-mm margin</td>
<td>5-mm margin</td>
<td>5-mm margin</td>
<td>5-mm margin</td>
</tr>
<tr>
<td>View from above</td>
<td>2-3 mm margin</td>
<td>2-5 mm margin</td>
<td>5-10 mm margin</td>
<td>2-3 mm margin</td>
<td>5 mm margin</td>
<td>5 mm margin</td>
<td>5 mm margin</td>
</tr>
<tr>
<td>View from side</td>
<td>2-3 mm margin</td>
<td>2-5 mm margin</td>
<td>5-10 mm margin</td>
<td>2-3 mm margin</td>
<td>5 mm margin</td>
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Conclusions

• There is no single best approach to LM and selected other challenging skin cancers

• Strongest indications when excising LM and EMPD; also recurrent SCC, high-grade SCC, and those close to critical structures or where lots of inflammation or CLL

• Be prepared for subclinical invasion and prepare in advance

• Don’t rely on immunostains as a panacea—they can be a helpful adjunct