Ancillary Diagnostic Tools in Dermatopathology

Nathan Cleaver D.O., FAOCD
Amy Spizuoco D.O., FAOCD
Disclosures

• Nathan Cleaver
  • Speaker, Castle Biosciences

• Amy Spizuoco
  • Speaker, Pfizer
  • Speaker, Celgene
Why is this important?

• To understand what tests are available to the medical dermatologist and the dermatopathologist
• To understand what tests help further define a diagnosis
• To understand which tests can predict prognosis
• To understand which tests are no longer utilized
Ancillary Testing

• Testing based on aberrations in cellular DNA material
  • Comparative Genomic Hybridization (CGH)
  • Fluorescent In-Situ Hybridization (FISH)
  • Genetic Testing for CDKN2A, BRAF, BAP1

• Testing based on Gene Expression
  • Melanoma Dx
  • DermTech
  • Myriad Test

• Reflectance Confocal Microscopy
Molecular Advancements

• “In the molecular era, there is precedent for analyzing genetic markers or patterns of gene expression (ie, gene signatures) in cancer to gain diagnostic or prognostic information that cannot be gleaned from histologic examination alone. “

• March et al. Practical applications of new technologies for melanoma. *JAAD* June 2015
Human Genome

- Human DNA has 6 million nucleotides packaged into 2 sets of 23 chromosomes
Mutations

• Large scale
  • Amplifications
  • Deletions
  • Translocations
  • Interstitial deletions
  • Inversions
  • Loss of heterozygosity

• Small scale
  • Point mutations
  • Insertions
  • Deletions
Copy Number Variation

• Copy-number variations
  • Alterations of the DNA of a genome that results in the cell having abnormal number of copies of one or more sections of the DNA

• Can result in large regions of the genome being deleted or duplicated on certain chromosomes

• Amplifications or deletions are most often cause of tumorigenesis

• Need to detect and map these aberations with a certain disease phenotype
Comparative Genomic Hybridization

• If a biopsy does not clearly indicate whether a lesion is a malignant melanoma, comparative genomic hybridization or fluorescent in situ hybridization may be helpful in determining a diagnosis. In both of these tests, doctors look for signs of melanoma as they compare the DNA in tumor cells to the DNA in normal tissue.

• Screens the entire genome for gains and losses in DNA material

• First described in 1993

• >= 3 abnormalities significant
Comparative Genomic Hybridization

• May be performed on formalin-fixed, paraffin-embedded tissues
• DNA from subject tissue and normal tissue (control) labeled with different tags
• Analyze regional differences of gains/losses compared to control tissue can identify the affected chromosome
• **Pro**: Can detect chromosomal abnormalities in affected tissue
• **Con**: False-negative if tumor cells are not adequately sampled
  : Unable to detect point mutations
CGH in Melanoma

- Common aberrations in melanoma are loss of 9q and 10, and gains in 7d
- Distinct genomic patterns are associated with particular melanoma subtypes
- Regions containing oncogenes (BRAF and MITF) are frequently amplified, while regions containing tumor suppressor genes (CDKN2A and PTEN) are frequently deleted
- BRAF and NRAS in 126 melanomas and found distinct patterns associated with melanomas from skin with chronic sun-induced damage, skin without such damage, and from acral and mucosal sites.

March et al. Practical applications of new technologies for melanoma. JAAD June 2015
CGH in Benign Nevi

• Compared to melanoma, most nevi lack or have isolated genomic aberrations
• Spitz nevi exhibit gains in chromosome 11p
Fluorescence In Situ Hybridization

- Molecular cytogenetic method for determining the copy number of specific regions or sequences of DNA
- Uses fluorescent probes to bind specific DNA segments in nuclei of cells
- Performed on formalin-fixed, paraffin-embedded tissues
- Can only detect genes and chromosomes targeted by specific probes
- **Pros**: Can detect single-point mutations
  - Technical expertise is less than with CGH
  - May distinguish from spitzoid melanoma and spitz nevi
- **Cons**: Can have false – positivity (27% in one study) due to tetraploidy
  - A fraction of dysplastic nevi are FISH positive
Gene Testing

• **BRAF**
  - encodes a protein belonging to the RAF family of serine/threonine protein kinases
  - V600E
  - plays a role in regulating the MAP kinase/ERK signaling pathway, which affects cell division, differentiation, and secretion
  - mutations in this gene, most commonly the V600E mutation, are the most frequently identified cancer-causing mutations in melanoma
  - non-Hodgkin lymphoma, colorectal cancer, thyroid carcinoma, non-small cell lung carcinoma, hairy cell leukemia and adenocarcinoma of lung
  - also associated with cardiofaciocutaneous, Noonan, and Costello syndromes

• **CDK2NA**
  - generates several transcript variants which differ in their first exons
  - function as inhibitors of CDK4 kinase
  - frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene
BRAF

- ½ advanced mm cases have mutation
- V600E most common
- Many different tests in USA
- MAPK pathway- signal transduction
  - BRAF-kinase mutation 40-60% cases
- Vemurafenib/Dabrafenib
  - selective inhibitors of BRAF
- Trametanib/Cobimetanib
  - inhibitors of MEK kinase
CDK2NA

- Small portion of melanomas
- 9p21.3
- Strong family history of melanoma
- FAMMM syndrome
### Diagnostic criteria for Familial Atypical Multiple Mole Melanoma syndrome

1. **Malignant melanoma in one or more first- or second-degree relatives**

2. **High total body nevi count (often >50) including some of which are clinically atypical**
   - Asymmetric, raised, color variegation present, of variable sizes

3. **Nevi with certain histologic features on microscopy**
   - Architectural disorder with asymmetry, subepidermal fibroplasia, and lentiginous melanocytic hyperplasia with spindle or epithelioid melanocytes gathering in nests of variable size and fusing with adjacent rete ridges to form bridges; variable dermal lymphocyte infiltration and the "shouldering" phenomenon wherein intraepidermal melanocytes extend alone or in groups beyond the main dermal component may also be present

---

All three criteria are needed to make a diagnosis
Melanoma Dx

- 31 gene expression profile test
- Quantifies expression from primary tumor using reverse transcriptase PCR
- Classifies patients as high risk or low risk
- Utilizes formalin-fixed, paraffin embedded tissue
- Must have 40% tumor density to perform the test
- Cannot be performed on melanoma in situ
Results

• Will give you the following possible results based on probability score
  • Class 1a – 92% RFS at 5 years, 96% DFMS at 5 years
  • Class 1b – 90% RFS at 5 years, 90% DFMS at 5 years
  • Class 2a – 77% RFS at 5 years, 80% DFMS at 5 years
  • Class 2b – 48% RFS at 5 years, 65% DFMS at 5 years
Clinical validity and prognostic value of DecisionDx-Melanoma have been demonstrated in 690 Stage I-III patients.

Leachman et al. Soc Melanoma Res Meeting 2017

### GEP Class

<table>
<thead>
<tr>
<th>Class</th>
<th>5-year RFS (95% CI)</th>
<th>Events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>90% (87-93%)</td>
<td>37 (12%)</td>
</tr>
<tr>
<td>1B</td>
<td>81% (73-90%)</td>
<td>18 (23%)</td>
</tr>
<tr>
<td>2A</td>
<td>68% (58-79%)</td>
<td>32 (38%)</td>
</tr>
<tr>
<td>2B</td>
<td>37% (31-44%)</td>
<td>130 (61%)</td>
</tr>
</tbody>
</table>

### 5-year DMFS (95% CI) Events (%)

<table>
<thead>
<tr>
<th>Class</th>
<th>5-year DMFS (95% CI)</th>
<th>Events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>94% (91-97%)</td>
<td>24 (8%)</td>
</tr>
<tr>
<td>1B</td>
<td>85% (77-93%)</td>
<td>15 (19%)</td>
</tr>
<tr>
<td>2A</td>
<td>75% (66-85%)</td>
<td>25 (30%)</td>
</tr>
<tr>
<td>2B</td>
<td>50% (43-58%)</td>
<td>100 (47%)</td>
</tr>
</tbody>
</table>

### 5-year MSS (95% CI) Events (%)

<table>
<thead>
<tr>
<th>Class</th>
<th>5-year MSS (95% CI)</th>
<th>Events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>99% (97-100%)</td>
<td>4 (1%)</td>
</tr>
<tr>
<td>1B</td>
<td>95% (90-100%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>2A</td>
<td>91% (85-98%)</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>2B</td>
<td>75% (69-83%)</td>
<td>42 (20%)</td>
</tr>
</tbody>
</table>
Sentinel Lymph Node Guidance?

- Looked at the results of the test, age, and NCCN guidelines for SLNB.
- Current SLNB guidelines:

<table>
<thead>
<tr>
<th>Guideline</th>
<th>SLN+ (positivity) rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discuss and offer</td>
<td>&gt;10%</td>
</tr>
<tr>
<td>Discuss and consider</td>
<td>5% to 10%</td>
</tr>
<tr>
<td>Do not recommend</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>
SLNB positivity risk for patients with T1-T2 tumors and inform SLNB guidance

Thresholds based on NCCN Guidelines (v2.2018)

n=1,421

SLN+ probability in T1-T2 patients:

- Is below the 5% threshold established by guidelines in those ≥55 years old with a Class 1A result
- Is above the 10% threshold established by guidelines in all age groups with a Class 2B result

NCCN Recommendations for SLNB (v2.2018)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>SLN Positivity Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;55</td>
<td>Discuss and Offer</td>
</tr>
<tr>
<td>55-64</td>
<td>Discuss and Consider</td>
</tr>
<tr>
<td>≥65</td>
<td>Do not Recommend</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DecisionDx-Melanoma result</th>
<th>Probability of a Positive Sentinel Lymph Node for T1-T2 Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1A</td>
<td>&lt;55 years</td>
</tr>
<tr>
<td></td>
<td>7.6%</td>
</tr>
<tr>
<td>Class 1B/2A</td>
<td>19.6%</td>
</tr>
<tr>
<td>Class 2B</td>
<td>24.0%</td>
</tr>
</tbody>
</table>
Thoughts

• Pros – Can provide some clinical guidance on prognosis
  Non-invasive test
  Cost – currently free to patient, they will receive an EOB
  that many patients think is a bill
  May be able to provide SLNB supplemental guidance in
  older patient populations

• Cons – More work for your staff
  Cost – insurance is still often paying something for the test,
  and will ultimately need to collect on the test to stay in
  business
  Not considered standard of care
  Will it ultimately change your management?
  • Maybe? Lower threshold for imaging
  • If no, then does patient prognostic education validate ordering the test?
Myriad MyPath

- When to use: cannot distinguish nevi from melanoma histologically
- Ordered by dermatopathologist
- Varies from CHG and FISH by analysis of gene expression, rather than genetic aberrations
- Measures the expression of 23 genes by reverse transcriptase-PCR methodology
- Look at genes involved in cell differentiation and immune signaling: PRAME, S100A7, S100A8, S100A9, S100A12, P13, CCL5, CD38, CXCL10, CSCL9, IRF1, LCP2, PTPRC, SELL
Myriad MyPath

- Sensitivity of 90-94%
- Specificity of 91-96%
- Result is a single numerical score
Thoughts

• 10-15% of biopsied melanocytic lesions may be histopathologically ambiguous and may help define a melanocytic lesion
• May also be helpful with disagreement
Reflectance Confocal Microscopy and Dysplastic Nevi

• Weinstock et al\textsuperscript{23}
  • Characteristics of melanocytes at the DE junction
  • Grades dysplasia
  • Substantial to low agreement
Reflectance Confocal Microscopy and Dysplastic Nevi
HISTOLOGICAL CONTROVERSY

• Weinstock et al\textsuperscript{23}
  • Characteristics of melanocytes at the DE junction
  • Grades dysplasia
  • Substantial to low agreement

Nevo-Melanocytic Industrial Complex

• Term describing what may be perceived as an increasing tendency to over-biopsy and over-treat dysplastic nevi
• Reflectance Confocal Microscopy may be an alternative
What is Reflectance Confocal Microscopy?

Reflectance Confocal Microscopy (RCM) create images by illuminating the skin with a low power laser diode and reflecting the light back though the system, utilizing customized optics to display the final image computer screen.
Procedure

Start to Finish: 5 to 10 Minutes
## Classification

<table>
<thead>
<tr>
<th>Normal Features</th>
<th>Atypical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Ringed pattern</td>
<td>• Atypical Cells at DEJ</td>
</tr>
<tr>
<td>• Meshwork pattern</td>
<td>• Irregular junctional nests</td>
</tr>
<tr>
<td>• Clod patterns</td>
<td>• Non-edged papilla</td>
</tr>
<tr>
<td>• Edged papilla</td>
<td></td>
</tr>
</tbody>
</table>
RCM produce Horizontal Sections

Stratum Corneum
Granular layer
Spinous layer
Basal layer
Papillary dermis
Superficial reticular dermis
Histology & Confocal: Dermo-epidermal Junction

- Dark areas w/ microcirculation
- Capillary loops and collagen bundles
- Ring of basal keratinocytes
Normal DEJ

Small polygonal cells surrounding papillae and forming rings of variable reflectivity depending on the skin prototype.

RINGED PATTERN
Predominance of edged papillae at DEJ, corresponding to dermal papillae surrounded by a rim of small bright cells, appearing as bright rings sharply contrasting with the dark background.
Predominance of junctional thickenings corresponding to enlargements of the inter-papillary space formed by aggregated cells and/or clusters bulging within the dermal papilla in contiguity with the basal layer.
Dense compact clusters of melanocytes within dermal papillae.
NON-EDGED PAPILLAE
ATYPICAL DENDRITIC CELLS
Dysplastic Nevi

- On RCM, dysplastic nevi often have a primarily ring-meshwork pattern with 1-2 atypical features
- Benign nevi usually have no atypical features
- Melanoma often has greater than 2 atypical features on RCM
Epidermal Genetic Information Retrieval (EGIR)

- Adhesive tape “tape-stripping”
- Obtains RNA from stratum corneum
- 2-gene classification algorithm
DermTech, Inc

- Pigmented Lesion Assay (PLA)
- LINC00518/PRAME gene expression
- Noninvasive adhesive patch biopsy
LINC50018/PRAME

- Long intergenic non-protein coding RNA 518 gene
- Preferentially expressed antigen in melanoma gene
Detection of the genes scored 1-100

- Higher score malignant disease
- Lesion biology rather than visual features of lesion
- Sens/Spec 92%/69%
Medicare Guidelines

• Originally stated by company representatives that you could charge for biopsy CPT code for obtaining the sample

• New 2019 CPT codes, specifically state that this will not be a covered biopsy procedure
MelaFIND (MELA Sciences Inc, Irvington, New York)

- multispectral computer vision system
- additional information on melanocytic lesions
- objectively assessing their three-dimensional morphology
- less than 6mm
- 10 different spectral bands
  - blue (430nm) to near infrared (950nm)
MelaFIND

• Higher sens/spec than clinician
• Binary output
  • positive= consider biopsy
  • negative= observe
Multispectral images obtained by MelaFind
Sensor hardware

Automatic diagnosis

Lesion benign

Expert software analysis

Lesion malignant

Courtesy IBM
How Does MelaFind Work?

Hardware:
- MelaFind acquires multi-spectral data
- Uses light in 10 wavelengths from 430 nm (blue) to 950 nm (near infrared)
- Depth up to 2.5 mm into the skin
- 20 micron-resolution (sees clusters of 3 melanocytes)

Software:
- Sophisticated proprietary automatic algorithms analyze data
- Provides MelaFind output – High or Low Disorganization
Independent Evaluation

- Independent study of 360 pigmented
- Melafind scores >2 were found in 147 lesions (suspicious of malignancy)
- Of 107 excised, 3 were melanoma
- Among all lesions biopsied, gave sensitivity of 100%, specificity of 5.5%
- Authors concluded “overall specificity and benign-to-malignant ratio of excised lesions were acceptable”