MELANOMA VERSUS NEVUS: STRATEGIES FOR THE DIFFICULT DECISION

Jane L., Messina, MD
September 16, 2017
Conflicts

• I serve as a consultant to Castle Biosciences
Objectives

• Review classic features of melanoma
• AJCC update
• Discuss commonly encountered diagnostic dilemmas
The real thing

Asymmetry
Poor circumscription
Confluence, epidermal effacement
Sheet-like growth, atypia, mitoses
Primary tumor staging:

- Breslow depth to nearest 0.1 mm
- Ulceration (width measured!!)
- Microsatellites: “microscopic tumor foci within dermis or subQ adjacent to but discontinuous from primary”; no minimum size or distance
- Reporting of mitoses “recommended”
### AJCC 8th edition staging changes

<table>
<thead>
<tr>
<th>TX</th>
<th>Primary tumor thickness cannot be assessed (i.e. curettage specimen)</th>
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<tbody>
<tr>
<td>T0</td>
<td>Regressed melanoma or melanoma of unknown primary</td>
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<tr>
<td>T1a</td>
<td>0-0.8 mm</td>
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<tr>
<td>T1b</td>
<td>&gt;0.8 mm-1.0 mm or ulceration</td>
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<tr>
<td>T2a/b</td>
<td>1.1-2.0 mm w/ or w/o ulceration</td>
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<tr>
<td>T3a/b</td>
<td>2.1-4.0 mm w/ or w/o ulceration</td>
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<tr>
<td>T4a/b</td>
<td>&gt;4.0 mm w/ or w/o ulceration</td>
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### N1

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<tr>
<th>A-occult</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
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<tbody>
<tr>
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<td>2-3</td>
<td>&gt;4</td>
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### N2

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<th>B-clinical</th>
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<th>N3</th>
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<tr>
<td>2-3, at least 1 clinical+</td>
<td>&gt;4, at least 1 clinical+</td>
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### N3

<table>
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<th>C-microsatellites</th>
<th>N3</th>
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<tr>
<td>0 nodes+ microsatellite</td>
<td>At least 2 nodes+ microsatellite</td>
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Commonly encountered scenarios

• Lack of compunction at the junction
• Special sites
• Nevoid melanoma, dysplastic nevus, or both??
• Down and dirty in the dermis: spindled, pigmented, and ugly
• Spitzoid lesions
Lack of compunction at the junction
Melanoma in situ!
Immunohistochemistry in sun-damaged skin

- **S-100**
  - Polyclonal: cross reacts with pigmented AK
  - Monoclonal: diminished sensitivity in LM and ALM

- **HMB-45**: patchy/partial staining

- **Melan-A and Mart-1**: overstaining a problem

- **Sox-10 or MITF**: ideal
Quantification of melanocytes in sun-damaged skin (CSD)-what is normal?

- Confluence >3 melanocytes found in 16% of SDS specimens on H&E, 4% on Mart-1
- Deep follicular melanocytes in 6%, nested melanocytes in 1 BCC specimen
- Melanocyte density: LM >NMSC patients

Distinction of Melanoma In Situ From Solar Lentigo on Sun-Damaged Skin Using Morphometrics and MITF Immunohistochemistry

Will H. Black, MD,* Sumeet K. Thareja, BS,* Brett P. Blake, MD,|| Ren Chen, MD, MPH,‡
Basil S. Cherpelis, MD,*† and Lewis Frank Glass, MD*‡

- **Density**: number of melanocytes/200µm
- **Diameter**: mode of nuclear diameter of shortest axis of 3 melanocytes
- **Density x Diameter** >80 is 85% sensitive and 100% specific for MIS

Am J Dermatopathol 2011;33:573–578
Minimal diagnostic criteria for MIS (lentigo maligna type)

- H&E and Sox-10/MITF staining
- High number of melanocytes: >10 per 200 μm, especially if present over a broad front
- Nuclear enlargement >9μm diameter/pleomorphism
- In absence of nesting, confluence of ≥3 melanocytes
- Irregular distribution of melanocytes
- Descent of melanocytes far down adnexal structures
- Irregular distribution of pigment
- Melanocytes present above basal layer in significant numbers

Am J Dermatopathology; 18(6), December 1996, pp 560-566
Residual melanoma vs melanocytic hyperplasia?
Look at requisition!
“r/o residual BCC”
Diagnosis: No residual BCC (melanocytic hyperplasia at prior biopsy site)
Marked cytologic atypia
Myriad result: 3.1
Diagnosis: melanoma in situ, recurrent
Lesion diameter = 2 mm

Patient age: 8
Diagnosis: Pagetoid Spitz nevus
Pagetoid Spitz nevus

- 5-10% of all Spitz nevi
- Largest series (12): all female, extremity predominant, median age 34 years
- <5 mm diameter
- Nests <1/3 overall cellularity; single cells confined to lower ½ of epidermis in 75%
- Molecular/IHC analysis not extremely helpful

Junctional melanocytic neoplasm on the leg of a woman
Diagnosis: Junctional melanocytic nevus ("lentiginous" type)
“Leg-type” nevi

- Described on thigh, below knee, and ankle
- “Epithelioid cell melanocytic nevus”, “melanocytic nevi of ankle with atypical features”, “dysplastic nevi of leg of women”
- Small (4-5 mm)
- Single cells=nests
- Low pagetoid spread


FIGURE 2

**FIGURE 2.** Intraepidermal melanocytic proliferation with epithelioid appearance (hematoxylin and eosin stain, original magnification x10).

*The Melanocytic Epithelioid Cell Nevus of the Thigh of Woman: A Simulator of Melanoma.*
Donati, Pietro; Muscardin, Luca; Cota, Carlo; Panetta, Chiara; Paolino, Giovanni

DOI: 10.1097/DAD.0b013e31824d4f86
Every site is SPECIAL in its own way

**Acral skin**

- Lentiginous\textgreater nested growth
- Pagetoid spread in 36\% (MANIAC lesion!)
- Banal, patchy dermal component
Genitalia

- Large (up to 1 cm) and well circumscribed
- High cellularity of junctional nests
- Bridging and fibroplasia
- Dermal mitotic activity in 7%
- LsEtA background: epidermal effacement and single cell confluence possible
Genital nevus with LSetA from Sangueza et al.
Ear

• Poor circumscription
• Irregular nesting, bridging, fibroplasia
• Cytologic atypia less common

from Sangueza et al.
Scalp

- Features overlap with dysplastic nevi
  - Asymmetry, poor circumscription, irregular nesting, bridging, fibrosis, inflammation

- Most commonly, but not exclusively, found in adolescents

Flexural sites
- milk line
- antecubital/popliteal fossae
- breast

• Nests at sides/between rete
• Dyscohesive nests and single cells, lentiginous growth, pagetoid spread, and adnexal epithelial involvement
• Atypia mild to absent
• Umbilicus: fibroplasia
• Breast: atypia, high cellularity more common
Breast nevi

from Sangueza et al.
Nevoid melanoma, dysplastic nevus, or both?
Useful immunohistochemical tools

• Proliferation markers: Ki-67, phosphohistone H3
• Maturation marker: HMB-45
• P16

OR

?
Ki-67: the good, the bad, and the ugly
Melan-A/Ki-67 (MelPro) in nevoid melanoma

Nevi: <5%
Melanoma: >10%
MelPro in a nevus
Phosphohistone H3 for mitoses

- Compound nevus: MR 0.06/sq mm
- Spitz nevus: mean MR 0.5 mitoses/10 HPF (range 0-2) or MR 0.325/sq mm
- Melanoma: mean MR 24.7 (range 2-75)

HMB-45 staining in nevus versus nevoid melanoma

Nevus

Melanoma

Pitfalls
- Blue nevus, DPN, traumatized nevus express HMB-45
- 10% of melanomas show gradient expression
p16 and malignant potential

Retained expression: favors benign

Expression lost in 50-98% of melanomas

p16 caveats

- Many melanomas still express p16, and benign nevi may not express it
- Loss of p16 staining does not equate to homozygous deletion of 9p21
  - It can be positive even in 9p21 deleted lesions (15%)
  - It can be negative in lesions with heterozygous loss, epigenetic silencing, or even normal 9p21

Gray-Schopfer VC et al. Br J Cancer 2006; 95:496-505
Nevoid melanoma

- a/k/a/ minimal deviation or small cell, ~3% of melanomas
- Non-uniform criteria (Spitzoid lesions?)
  - 55% plaque-like: parallel theques
  - 45% polypoid: resemble compound nevi
  - “pseudomaturation”: cell size shrinks, but nucleus remains same
  - DE junction most helpful
  - Deep mitoses

Idriss et al. JAAD 2015 Nov; 73(5):836-42
Dermal melanoma vs. pre-existing nevus
Melanoma!
Pre-existing nevus!
37 y/o male, back lesion, “r/o atypia”
Sox10
Pagetoid melanocytes in dysplastic nevi

- 43 dysplastic nevi
- Pagetoid melanocytes seen in 63% of cases on IHC, 11% on H&E

*Am J Dermatopathol 2014;36:340–343*
Down and dirty in the dermis
S-100
P75-NGFR
Sox-10
MiTF negative (usually)
HMB-45 negative
Melan-A negative
**Desmoplastic melanoma**

- Pure v mixed: > or < 90% paucicellular dermal growth
  - Hypercellular component may be spindle or epithelioid

Differential diagnosis

• **Scar**: Sox-10 negative

• **Desmoplastic (Spitz) nevus**: p16 NOT helpful (strong in 75% pure DM)

• **Neurofibroma**: “fingerprint” pattern CD34 positivity

• **MPNST**: S-100 and Sox-10 usually weak/patchy, BRAF usually negative, loss of H3K27me3
H3K27me3 expression can distinguish between MPNST and desmoplastic melanoma

- Loss of staining using C36B11 antibody (Cell Signaling Technology) seen in 69% of MPNST
- However, in sporadic and radiation-related MPNST, absent staining seen in 95% and 91%
- TMA containing 55 desmoplastic melanomas all showed retained staining

Retained expression of H3K27me3 in desmoplastic/spindle cell melanoma
Loss of H3K27me3 in MPNST arising in neurofibroma
Feeling blue

- Epithelioid blue nevus
- Pigmented epithelioid melanocytoma
- Animal-type melanoma
Epithelioid blue nevus

• Carney triad

• Loss of PRKAR1α
Pigmented epithelioid melanocytoma

- Epidermal hyperplasia
- Perinuclear pigment clearing
- <<5% proliferation rate
  - 40% SLN+
- All cases negative f/u thus far
Epithelioid and fusiform blue nevus

CSD skin, no Carney triad, PRKAR1α retained

Clonal ("inverted type A") nevus
MBAIT (Melanocytic BAP-1 loss associated intradermal tumor)

- Loss of nuclear staining by BAP1
- Screen for uveal>cutaneous melanoma, mesothelioma
MELANOMAS OF CHILDHOOD

Sophie Spitz, M.D.

(From the Pathology Laboratories of the Memorial Hospital, New York, N.Y.)

It has become apparent over a period of years that even when a histologic diagnosis of malignant melanoma has been made in children the clinical behavior rarely has been that of a malignant tumor. The disparity in behavior of the melanomas of adults and children, despite the histologic similarity of the lesions occurring in the different age groups, is obviously a matter of fundamental importance and the following questions immediately arise: Does the histologically malignant melanoma of children differ in any structural detail from that of adults? Can the clinical behavior of these lesions be predicted from their histologic structure? What, if any, are the factors known to influence the clinical behavior? Should the melanomas of children be treated any differently from the melanomas of adults?
Incidence (%)

- BAP-1 loss
- HRAS
- NTRK
- ALK
- ROS-1
- BRAF
- RET
- Unknown

34% unknown

Wiesner et al. *Nat Commun* 2014;5:3116
Significance of kinase fusions

- Found in lesions across Spitzoid spectrum
- Can be identified by immunohistochemistry
- Associated AST’s may have distinctive histology
- Confer possible sensitivity to targeted therapy
  - Crizotinib, cabozantanib, vandetinib used to treat ALK and ROS-1+ lung tumors of lung and RET+ thyroid tumors
Moffitt 5-tiered nomenclature for melanocytic neoplasms

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<thead>
<tr>
<th>CATEGORY</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>B-BLUE NEVUS LIKE</td>
<td>BENIGN</td>
<td>ATYPICAL, FAVOR BENIGN</td>
<td>UNCERTAIN MALIGNANT POTENTIAL</td>
<td>ATYPICAL, FAVOR MALIGNANT</td>
<td>MALIGNANT</td>
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<tr>
<td>C-CONGENITAL</td>
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<tr>
<td>D-DYSPLASTIC</td>
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<tr>
<td>S-SPITZOID</td>
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- CLINICAL HISTORY AND APPEARANCE
- HISTOLOGIC AND IMMUNOHISTOCHEMICAL FINDINGS
- MOLECULAR TESTING IF NECESSARY
Putting it all together

S1  Benign Spitz nevus

S2  Atypical, favor benign

S3  Atypical, uncertain biologic potential

S4  Atypical, favor malignant

S5  Spitzoid melanoma

HRAS amplification
11p or 7p gain

6p25 gain
11q13 gain

9p21 deletion or abn. CGH

6q23 gain
Customizing treatment

S1
Benign Spitz nevus

S2
Atypical, favor benign

S3
Atypical, uncertain biologic potential

S4
Atypical, favor malignant

S5
Spitzoid melanoma

Re-excision 0.5-1 cm

Re-excision 1 cm with SLNB
Atypical Spitz tumours and sentinel lymph node biopsy: a systematic review


Let’s do the numbers....

- 541 patients with AST
  - 303 (56%) SLNB
  - 238 (44%) No SLNB
    - 119 (39%) +SLN
    - 184 (61%) -SLN
      - CLND 97 (82%)
      - No CLND 22 (18%)
      - +CLND 18 (19%)
      - 11 (5%) REGIONAL RECURRENCE
      - 5 (1%) DEATHS

- 24 studies, 2002-2013
- Average age: 24 years
- Median f/u: 59.3 months
Molecular tests for accurate identification of melanoma

- Fluorescence in situ hybridization
  - Frequently used in the evaluation of diagnostically challenging melanocytic proliferations
    - Atypical Spitzoid proliferations (AST), atypical blue nevus-like proliferations
- Comparative genomic hybridization
- Myriad myPATH
### Fluorescence in situ hybridization - NeoSITE® melanoma

#### Old assay
- **6q23**
- **CEP6**
- **6p25, 11q13**

#### New assay
- **8q24**
- **9p21**

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<th>Unequivocal melanoma</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<td>87%</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>94%</td>
<td>98%</td>
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- Neogenomics Laboratories (Ft. Myers, FL): $1500
- What about borderline lesions such as this?
FISH in histologically ambiguous Spitzoid lesions (AST)

<table>
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<tr>
<th>Probe set</th>
<th>AST w/o recurrence</th>
<th>“AST” with poor outcome (melanoma)</th>
<th>Typical Spitz</th>
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<tr>
<td>OLD sensitivity</td>
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<td>50-100% 6,1,4</td>
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<tr>
<td>OLD specificity</td>
<td>57-91% 4,1</td>
<td></td>
<td>75-100% 5,1</td>
</tr>
<tr>
<td>NEW sensitivity</td>
<td></td>
<td>50-100% 3,2</td>
<td></td>
</tr>
<tr>
<td>NEW specificity</td>
<td>74-87% 2,3</td>
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5 Dika et al *Mel Research* 2015; 25(4): 295-301
6 Vergier et al. *Modern Pathology* 2011; 24: 613-623
Specific FISH abnormalities associated with outcome in borderline lesions (AST)

- **Homozygous deletion of 9p21**: predicts local recurrence and only feature predictive of death
- **Gain of 6p25 and/or 11q13**: predicts SLN involvement but not recurrence or death
- **Heterozygous 9p21 loss**: significant sentinel node involvement but no spread beyond the regional nodes
- **6q23 (MYB) loss**: associated with good long term outcome

Comparative genomic hybridization

• Assesses chromosomal copy number changes across tissue, but misses individual cell abnormalities
• 95% of melanomas harbor numerous chromosomal gains and losses
• Nevi rarely show aberrations
• **Exceptions:**
  – 12-26% of Spitz nevi (esp. recurrent) have 11p or 7q gain
  – Some AST have abnormal CGH (45%, most common gain of 1p)
    • Does not predict SLN involvement
    • Proliferative nodules in congenital nevi may have whole chromosomal gains

Comparative genomic hybridization

• Most commonly used to distinguish pediatric atypical Spitz tumor from melanoma

• Not widely available, insurance coverage varies

• Typical out-of-pocket cost $2500
Myriad MyPath-diagnostic aid for equivocal lesions

• 23-gene expression signature that can help differentiate nevi from melanoma - utilizes 5 recut slides from paraffin block
• Training and validation cohorts of 900+ unequivocal melanoma and nevi: sensitivity 90% specificity 91%
• Currently undergoing large-scale testing of clinical utility with melEval\textsuperscript{PRO}

Clarke et al. \textit{J Cutan Pathol} 2015; 42:244-252
Independent validation of myPath

• 1400 lesions independently reviewed by 3 experts
  – Triple concordance: 993 lesions (70.9%)
  – Excluded indeterminate scores: 860 lesions (24% malignant, 76% benign)
  – Excluded lesions with <10% tumor volume: 763 lesions

• Sensitivity 91.5% -- Specificity 92.5%
  – False negatives-lentigo maligna and desmoplastic melanoma variants
  – False positives-dysplastic nevi

Clarke et al., *Cancer*, October 2016
Clarke, ASDP October 2016
29 year old pregnant female with changing lesion on back

Junctional dysplastic nevus with moderate to severe atypia
**MyPath and diagnostically challenging lesions**

- 39 unequivocal lesions: 62% sensitivity, 95% specificity
- 78 challenging lesions with expert consensus (27 favor malignant, 30 favor benign, 21 ambiguous)
- myPath score agreed with histology in 64%, agreed with FISH 70% of time
  - Limited by lack of clinical outcome

**SUMMARY**: myPath score can increase diagnostic certainty and influence treatment recommendations, BUT performance not proven in ambiguous lesions

Molecular tests for melanoma diagnosis

Summary

• FISH and myPath sensitivity and specificity ~90% in unequivocal melanomas and nevi
• FISH sensitivity drops to 50-100% and specificity to 74-87% in AST
• CGH can aid in atypical cases with negative FISH, but hampered by limited availability and reimbursement