Emerging Molecular Diagnostic Tests and Therapies for Melanoma: From Research Benches to Clinical Trenches

Aleodor (Doru) Andea, MD
Associate Professor of Pathology and Dermatology
Director of Dermatopathology Section
University of Alabama at Birmingham
Birmingham, USA
aandea@uab.edu
Disclosure information

I have no financial or industrial affiliation to disclose.

Aleodor A Andea
Overview

1. Problems in the diagnosis
2. Molecular alterations (with implications for diagnosis or treatment)
3. Diagnostic assays
4. New therapies
Melanoma diagnosis

<table>
<thead>
<tr>
<th>NEVI</th>
<th>MELANOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Symmetric</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>Well-circumscribed</td>
<td>Poorly-circumscribed</td>
</tr>
<tr>
<td>Not affected</td>
<td>Consumption, Ulceration</td>
</tr>
<tr>
<td>Predominantly nested</td>
<td>Predominantly single cells</td>
</tr>
<tr>
<td>Uniform</td>
<td>Irregular</td>
</tr>
<tr>
<td>No</td>
<td>Prominent</td>
</tr>
<tr>
<td>No</td>
<td>Present</td>
</tr>
<tr>
<td>Nested, Infiltrative</td>
<td>Sheetlike, Expansile</td>
</tr>
<tr>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Absent</td>
<td>Severe</td>
</tr>
<tr>
<td>Absent</td>
<td>Present, Atypical</td>
</tr>
</tbody>
</table>
Melanoma diagnosis

Factors that influence interpretation of histologic parameters

• Age
• Site
• History of trauma
2 y/o girl with lesion on the rt knee
Dermatopathology diagnosis

Spitz tumor of uncertain malignant potential
Dermatopathology diagnosis

Spitz tumor of uncertain malignant potential

<table>
<thead>
<tr>
<th>Diagnosis 1</th>
<th>Diagnosis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitotically active Spitz nevus</td>
<td>Spitzoid melanoma</td>
</tr>
</tbody>
</table>
• ~1-2 million bx/year in US to rule out melanoma

• ~3%-6% of bx are melanomas

• In most cases dx can be made on histopathology but a small proportion have ambiguous histology (Q.)

• **Ambiguous lesions with overlapping criteria**
  
  – Severely atypical/ dysplastic nevus vs LM or SSM
  – Nevi of special site vs LM or SSM
  – Spitz nevus vs spitzoid melanoma
  – Atypical blue nevi vs melanoma
  – Proliferative nodules vs melanoma
  – Nevvoid melanoma

• **Inter-observer variability**

Zembowicz and Scolyer. Arch of Pathol and Lab Med. 2011; 135
Melanocytic Tumors of Uncertain Malignant Potential

Results of a Tutorial Held at the XXIX Symposium of the International Society of Dermatopathology in Graz, October 2008

Lorenzo Cerroni, MD,* Raymond Barnhill, MD,† David Elder, MD,‡ Geoffrey Gottlieb, MD.§ Peter Heenan, MD,‖ Heinz Kutzner, MD,¶
Philip E. LeBoit, MD,† Martin Mihm, Jr., MD,** Juan Rosai, MD, †† and Helmut Kerl, MD*  

57 borderline lesion with clinical follow-up

• 17-benign
• 26-melanoma
• 14-borderline

Panel performance:
• 73% sensitivity
• 47% specificity
Need for a better test

- Prevent under/overtreatment of patients
- Reduce medical costs associated with unnecessary treatment
- Impact positively on patient care
- Most common reason for medical malpractice in pathology

Danube River, Romania
Molecular alterations in melanocytic neoplasms

- MAP kinase alterations
- Replicative senescence in melanocytic lesions
MAP Kinase

GROWTH FACTORS

KIT, EGFR

RAS

H-Ras, K-Ras, N-Ras

RAF

A-Raf, B-Raf, C-Raf1

MEK

MEK-1, MEK-2

ERK

ERK-1, ERK-2

CYTOPLASM

NUCLEUS

CCND1

p16

CDK4/6

PROLIFERATION
KIT mutations in 14-19% of melanomas

GROWTH FACTORS

RAS

RAF

MEK

ERK

CCND1

CDK4/6

p16

PROLIFERATION

NRAS

RAF

MEK

ERK

GROWTH FACTORS

10-22% of melanomas
80% large congenital nevi

CCND1
CDK4/6

p16

PROLIFERATION

MAP Kinase pathway activation in melanocytic lesions

MAP Kinase pathway activation in melanocytic lesions

- HRAS: 27-29% of Spitz’s nevi
- RAF
- MEK
- ERK
- CCND1
- CDK4/6

Proliferation

CCND1: CDK4/6 

CCND1, CDK4/6, p16: Bastian B et al, Am J Pathol 2000
MAP Kinase pathway activation in melanocytic lesions

- **GROWTH FACTORS**
  - RAS
  - B-RAF
- **CYTOPLASM**
  - MEK
  - ERK
- **NUCLEUS**
  - CCND1
  - CDK4/6
  - p16
  - Proliferation

40-60% of melanomas (Q.)
80% of nevi

MAP Kinase pathway activation in melanocytic lesions

Growth factors

RAS

B-RAF

PKC

GNAQ

MEK

ERK

CYTOPLASM

NUCLEUS

CCND1

CDK4/6

p16

PROLIFERATION


Guanine nucleotide-binding protein G(q) subunit alpha

• Blue nevi: 80%
• Uveal melanoma: 50%
<table>
<thead>
<tr>
<th>Nevus type</th>
<th>KIT</th>
<th>NRAS</th>
<th>HRAS</th>
<th>KRAS</th>
<th>BRAF</th>
<th>GNAQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital</td>
<td>NA</td>
<td>70-81%</td>
<td>0</td>
<td>NA</td>
<td>0-88%</td>
<td>0</td>
</tr>
<tr>
<td>Acquired</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>70-88%</td>
<td>0</td>
</tr>
<tr>
<td>Clark’s</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>52-80%</td>
<td>NA</td>
</tr>
<tr>
<td>Spitz</td>
<td>NA</td>
<td>0%</td>
<td>27-29%</td>
<td>NA</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Blue nevus</td>
<td>NA</td>
<td>0-5%</td>
<td>NA</td>
<td>0-15%</td>
<td>0-12%</td>
<td>40-83%</td>
</tr>
</tbody>
</table>

Bastian B et al, Am J Pathol 2000
Saldanha et al. Int J Cancer 2004 Sep 20;111:705-10
# Genetic alterations in melanomas

<table>
<thead>
<tr>
<th>Melanoma type</th>
<th>KIT</th>
<th>NRAS</th>
<th>HRAS</th>
<th>KRAS</th>
<th>BRAF</th>
<th>GNAQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM on skin without chronic sun damage</td>
<td>0%</td>
<td>22%</td>
<td>0</td>
<td>0%</td>
<td>59%</td>
<td>0</td>
</tr>
<tr>
<td>MM on skin with chronic sun damage</td>
<td>16%</td>
<td>15%</td>
<td>0</td>
<td>0%</td>
<td>11%</td>
<td>15%</td>
</tr>
<tr>
<td>Acral</td>
<td>10-23%</td>
<td>10%</td>
<td>0</td>
<td>0%</td>
<td>23%</td>
<td>0%</td>
</tr>
<tr>
<td>Mucosal melanoma</td>
<td>15-21%</td>
<td>5%</td>
<td>0</td>
<td>0%</td>
<td>11%</td>
<td>0</td>
</tr>
<tr>
<td>Uveal melanoma</td>
<td>0%</td>
<td>0%</td>
<td>NA</td>
<td>0%</td>
<td>0%</td>
<td>50%</td>
</tr>
<tr>
<td>Malignant BN</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>46%</td>
</tr>
</tbody>
</table>

## Practical implication for DX

<table>
<thead>
<tr>
<th></th>
<th>HRAS</th>
<th>NRAS</th>
<th>BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spitzoid melanoma</td>
<td>0</td>
<td>19%</td>
<td>64%</td>
</tr>
<tr>
<td>Atypical Spitz nevus</td>
<td>14%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Spitz nevus</td>
<td>29%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Orthodox Cathedral, Timisoara, Romania
Replicative senescence in melanocytic lesions

- Cells exit cell cycle after a number of divisions
- Chromosomes are protected by telomeres and the enzyme telomerase

Greider and Blackburn EH, Nature 1989
Szostak et al, Cell 1989
Chromosome

Mitosis

Mitosis

Cell cycle arrest (Senescence)

p53, RB, p16

Checkpoint
Chromosome

Mitosis

Mitosis

Mitosis

Mitosis

Mitosis

Cell Crisis

DNA breaks, fusions

Cell Death

P53, RB

p16

Gross chromosomal abnormalities
Increase apoptosis
Nevi

Cell cycle arrest ("Oncogene-Induced" Senescence)

No chromosomal aberrations

MAPK
-B-RAF
-N-RAS
-H-RAS
-GNAQ

p53, RB, p16

Mitosis

Chromosome

T T T T T

Mitosis

Chromosome

T T T T

Mitosis

Chromosome

T T
Melanoma

- Chromosome
- MAPK
  - B-RAF
  - N-RAS
  - H-RAS
  - GNAQ
- p53, RB
- p16 inactivation: ~50% of MM

Cell cycle arrest (Senescence)

- DNA breaks, fusions
  - Re-stabilize telomeres
  - Growth advantage

Cell Crisis

- Cell Death

Gross chromosomal abnormalities
Parliament House, Bucharest, Romania
Molecular tests differentiating melanoma from nevi

- IHC
- CGH
- FISH
Ki-67

Nevus
(<5%)

Melanoma (Q.)
(>5%)
HMB-45 problems

Combined Nevus

Melanoma
p16

Precursor → Nevus → Unchecked division/growth → Melanoma

- MAPK activation
- P16 inactivation (and others)

Reactive p16 overexpression

No p16 expression

~50% Cell crisis
Spitz Nevus

Melanoma

p16
p16

Spitz Nevus

Positive in 100% of Spitz nevi

Melanoma

Negative in 32-50% of melanomas

Comparative Genomic Hybridization

- Screens the entire genome for gains and losses in DNA material in one experiment (Q.)

- Variants:
  - Conventional CGH
  - Array based CGH
Conventional CGH
The ratio of green:red signals along each chromosome is determined
Array CGH

- Arrays of genomic bacterial artificial chromosome (BAC) clones or oligonucleotides
Classifying Melanocytic Tumors Based on DNA Copy Number Changes

Boris C. Bastian,*† Adam B. Olshen,†
Philip E. LeBoit,*† and Daniel Pinkel†

From the Departments of Pathology and Dermatology,*
Dermatopathology Section, and the University of California San Francisco Comprehensive Cancer Center,† University of California San Francisco, San Francisco, California

American Journal of Pathology, Vol. 163, No. 5, November 2003
CGH in Melanocytic lesions

- 54 benign nevi
  - 27 Spitz nevi
  - 19 Blue nevi
  - 7 Congenital nevi

- 132 MM
  - 22 Acral location
  - 108 non-Acral

Bastian B et al, Am J Pathol 2003
Gains: 6p
1q
7p
7q
8q
17q
20q

Losses:
9p
9q
10q
10p
6q
11q

Gains: 11p: 11%
7q: 2%

Losses: 0%

• The 7 cases were all Spitz nevi (no progression to MM at 7 yrs FU)

96% of cases

Bastian B et al, Am J Pathol 2003
Conclusion

- CGH will show multiple gains and losses of DNA material in the majority of cases (Q.).
- Potential diagnostic test for ambiguous melanocytic lesions.
- Sensitivity 96%
- Specificity 98%
Chromosomal Aberrations in Melanocytic Lesions

- Spitz nevi:
  - no abnormalities
  - gains on 11p (12-18%)
- Congenital nevi: no abnormalities
- Proliferative nodules: no abnormalities or whole chromosomal gains or losses
- Cellular blue nevi: no abnormalities

Bastian B et al, Am J Pathol 2000
Bastian B et al, J Invest Pathol 1999
Bastian B et al, Am J Pathol 2002
Disadvantages of CGH

- Requires 30-50% pure tumor cells
- Does not allow histologic correlation
- Cannot detect tumor subpopulations
Fluorescence In Situ Hybridization (FISH) as an Ancillary Diagnostic Tool in the Diagnosis of Melanoma

Pedram Gerami, MD,* Susan S. Jewell, PhD,† Larry E. Morrison, PhD,‡ Beth Blondin, BSc,† John Schulz, BSc,† Teresa Ruffalo, BSc,† Paul Matushek, IV, MS,† Mona Legator, BSc,† Kristine Jacobson, MS, MAJ,† Scott R. Dalton, MC,‡ Susan Charzan, MS,§ Nicholas A. Kolaitis, BS,§ Joan Guitart, MD,* Terakeith Lertsbarapa, MD,* Susan Boone, MD,* Philip E. LeBoit, MD,§ and Boris C. Bastian, MD§
CGH data from melanomas

- 1q31 (COX2)
- 4q12 (KIT)
- 7q34 (BRAF)
- 6p35 (RREB1)
- 6q23 (MYB1)
- 6 cen
- 7 cen
- 9p31 (p16)
- 10 cen
- 11q13 (CCND1)
- 17q25 (TK1)
- 17q21 (RARA)
- 17 cen
- 20q13 (ZNF217)

• Training cohort 301 melanocytic tumors
  – 148 melanomas
  – 153 nevi

• Validation cohort
  – Unequivocal lesions:
    • 83 melanomas
    • 86 nevi
  – 27 ambiguous cases with clinical follow-up
    • 6 cases developed metastases
    • 21 free of disease at > 5 years follow-up

<table>
<thead>
<tr>
<th>Probe Set (Specimens Tested)</th>
<th>Aqua</th>
<th>Green</th>
<th>Orange</th>
<th>Gold</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 175)</td>
<td>7q34 (BRAF)</td>
<td>6p25 (RREB1)</td>
<td>20q13 (ZNF217)</td>
<td>9p21 (p16)</td>
<td></td>
</tr>
<tr>
<td>II (n = 60)</td>
<td>Centromere 10</td>
<td>17q25 (TK1)</td>
<td>1q31 (COX2)</td>
<td>11q13 (CCND1)</td>
<td></td>
</tr>
<tr>
<td>III (n = 26)</td>
<td>6q23 (MYB1)</td>
<td>6p25 (RREB1)</td>
<td>Centromere 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV (n = 26)</td>
<td>4q12 (KIT)</td>
<td>17q21 (RARA)</td>
<td>Centromere 17</td>
<td>Centromere 7</td>
<td></td>
</tr>
<tr>
<td>V (n = 103)</td>
<td>Centromere 6</td>
<td>6p25 (RREB1)</td>
<td>6q23 (MYB1)</td>
<td>11q13 (CCND1)</td>
<td></td>
</tr>
<tr>
<td>VI (n = 105)</td>
<td>Centromere 6</td>
<td>Centromere 10</td>
<td>6q23 (MYB1)</td>
<td>6p25 (RREB1)</td>
<td></td>
</tr>
</tbody>
</table>

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<td></td>
</tr>
<tr>
<td>II (n = 60)</td>
<td>Centromere 10</td>
<td>17q25 (TK1)</td>
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<td></td>
</tr>
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<td>III (n = 26)</td>
<td>6q23 (MYB1)</td>
<td>6p25 (RREB1)</td>
<td>Centromere 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV (n = 26)</td>
<td>4q12 (KIT)</td>
<td>17q21 (RARA)</td>
<td></td>
<td>Centromere 17</td>
<td>Centromere 7</td>
</tr>
<tr>
<td>V (n = 103)</td>
<td>Centromere 6</td>
<td>6p25 (RREB1)</td>
<td>6q23 (MYB1)</td>
<td>11q13 (CCND1)</td>
<td></td>
</tr>
<tr>
<td>VI (n = 105)</td>
<td>Centromere 6</td>
<td>Centromere 10</td>
<td>6q23 (MYB1)</td>
<td>6p25 (RREB1)</td>
<td></td>
</tr>
<tr>
<td>Final (n = 305)</td>
<td>Centromere 6</td>
<td>11q13 (CCND1)</td>
<td>6q23 (MYB1)</td>
<td>6p25 (RREB1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gain</td>
<td>Gain</td>
<td>Loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>------------------------------</td>
<td>------------------------------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11q13</td>
<td>&gt;38% nuclei contain &gt;2 signals for 11q13</td>
<td>&gt;29% nuclei contain &gt;2 signals for 6p25</td>
<td>&gt;40% nuclei with &lt;1 ratio of 6q23 / 6cen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCND1</td>
<td>OR</td>
<td>OR</td>
<td>OR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• Validation cohort:
  – 72 of 83 melanomas FISH positive
    • 87% sensitivity
  – 82 of 86 nevi FISH negative
    • 95% specificity
    • 4 nevi were FISH positive

• 27 cases with indeterminate histology
  – 6 developed metastases, all FISH positive
    • 100% sensitivity
  – 21 disease free at > 5 years, 15 FISH negative
    • 71% specificity
RAPID COMMUNICATION

Diagnosis of cutaneous melanocytic tumours by four-colour fluorescence in situ hybridisation

ADRIENNE L. MOREY*, RAJMOHAN MURALIT†‡§, STANLEY W. MCCARTHY†‡§, GRAHAM J. MANN†∥ AND RICHARD A. SCOLYERT†‡§

*Department of Anatomical Pathology, St Vincent’s Hospital, Darlinghurst, †Sydney Melanoma Unit and Melanoma Institute Australia, Sydney, ‡Department of Anatomical Pathology, Royal Prince Alfred Hospital, Camperdown, §Discipline of Pathology, Faculty of Medicine, The University of Sydney, and ∥Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Westmead, NSW, Australia
CCND1 – green
RREB – red
MYB – gold
CEP6 – aqua

20 Nevi
19/20 negative
Specificity 95%

No alterations

20 Melanomas
18/20 positive
Sensitivity 90%

MYB loss

Morey et al, Pathology 2009
Sensitivity of FISH in melanoma subtypes

<table>
<thead>
<tr>
<th></th>
<th>Superficial Spreading N=70</th>
<th>Lentigo Maligna N=28</th>
<th>Nodular N=22</th>
<th>Acral lentiginous N=3</th>
<th>DM N=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>81%</td>
<td>82%</td>
<td>91%</td>
<td>100%</td>
<td>47%</td>
</tr>
<tr>
<td>Most common alteration</td>
<td>6p25 gain (RREB1) 73%</td>
<td>6p25 gain (RREB1) 68%</td>
<td>6p25 gain (RREB1) 82%</td>
<td>6p25 gain (RREB1) 100%</td>
<td>6p25 gain (RREB1) 86%</td>
</tr>
</tbody>
</table>

Gerami et al. Arch Dermatol 2010; 146:273-8
Classifying ambiguous melanocytic lesions with FISH and correlation with clinical long-term follow up

Timo Gaiser¹,², Heinz Kutzner³, Gabriele Palmedo³, Markus D Siegelin¹,⁴, Thomas Wiesner⁵, Thomas Bruckner⁶, Wolfgang Hartschuh⁷, Alexander H Enk⁷ and Maria R Becker⁷

FISH positive

- Sensitivity: 60%
- Specificity: 50%

22 total lesions

12 ambiguous lesions

FISH worked in 8 cases

- 5 malignant (3 FISH positive)
- 3 benign (1 FISH negative)
## Summary of sensitivity/specificity estimates

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study sample</strong></td>
<td>169</td>
<td>233</td>
<td>40</td>
<td>43</td>
<td>10</td>
<td>324</td>
<td>324</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>87%</td>
<td>83%</td>
<td>90%</td>
<td>85%</td>
<td>42%</td>
<td>84%</td>
<td>64%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>95%</td>
<td>94%</td>
<td>95%</td>
<td>90%</td>
<td>100%</td>
<td>95%</td>
<td>98%</td>
</tr>
</tbody>
</table>

3. Morey et al, Pathology 2009
4. Vergier et al. Mod Pathol 2011;24:613-23
5. Gaiser et al, Mod Pathol 2010; 23:413-9
6. NeoGenomics validation set
7. NeoGenomics validation set with original cutoff criteria
## Summary of sensitivity estimates on ambiguous lesions

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study sample</td>
<td>27</td>
<td>113</td>
<td>8</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>43%</td>
<td>60%</td>
</tr>
<tr>
<td>Specificity</td>
<td>71%</td>
<td>80%</td>
<td>33%</td>
</tr>
</tbody>
</table>

2. Vergier et al. Mod Pathol 2011;24:613-23
3. Gaiser et al, Mod Pathol 2010; 23:413-9
Reasons for False Positives/Negative

- Technical problems
- Lack of experience
- Tetraploidy in Spitz nevi (5-10%)
- Detection of non-significant clones
- Signal cutoffs

<table>
<thead>
<tr>
<th>Probe</th>
<th>Criterion</th>
<th>Original Cutoff</th>
<th>Neo Genomics Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>RREB1</td>
<td>&gt;2 RREB1</td>
<td>&gt;29</td>
<td>&gt;16</td>
</tr>
<tr>
<td>RREB1</td>
<td>RREB1&gt;CEP6</td>
<td>&gt;55</td>
<td>&gt;53</td>
</tr>
<tr>
<td>MYB</td>
<td>MYB&lt;CEP6</td>
<td>&gt;40</td>
<td>&gt;42</td>
</tr>
<tr>
<td>CCND1</td>
<td>&gt;2 CCND1</td>
<td>&gt;38</td>
<td>&gt;19</td>
</tr>
</tbody>
</table>
Other potential applications

• “Nevoid” melanomas vs “Mitotically active” nevi
Mitotically active nevus
N=10

10/10 cases FISH negative

Nevoid melanoma
N=10

10/10 cases FISH positive

Other potential applications

• “Nevoid” melanomas vs “Mitotically active” nevi

• Intranodal nevus vs Metastatic Melanoma
<table>
<thead>
<tr>
<th></th>
<th>Metastatic melanoma N=24</th>
<th>Nodal nevus N=17</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH status</td>
<td>20 cases FISH positive</td>
<td>16 cases FISH negative</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>83%</td>
<td>Specificity 94%</td>
</tr>
</tbody>
</table>

Other potential applications

- “Nevoid” melanomas vs “Mitotically active” nevi
- Intranodal nevus vs Metastatic Melanoma
- Blue nevus vs Blue nevus-like metastatic melanoma
<table>
<thead>
<tr>
<th></th>
<th>Blue nevus-like metastatic melanoma N=10</th>
<th>Blue nevus N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH status</td>
<td>9 cases FISH positive</td>
<td>10 cases FISH negative</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>90%</td>
<td>Specificity 100%</td>
</tr>
</tbody>
</table>


6p25 gains
Conclusion

• FISH provide additional criteria to help diagnose histologically ambiguous cases

• This test should be performed in conjunction with standard histopathologic evaluation
<table>
<thead>
<tr>
<th><strong>CGH</strong></th>
<th>Vs.</th>
<th><strong>FISH</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Technically difficult</td>
<td>• Technically easier</td>
<td></td>
</tr>
<tr>
<td>• Needs tumor enrichment (30-50% tumor purity)</td>
<td>• Does not need tumor enrichment</td>
<td></td>
</tr>
<tr>
<td>• Cannot be used in thin lesions</td>
<td>• Can be used in thin lesions</td>
<td></td>
</tr>
<tr>
<td>• No histologic correlations</td>
<td>• Allows some histologic correlations</td>
<td></td>
</tr>
<tr>
<td>• Can miss changes in subpopulations (low sensitivity)</td>
<td>• Will detect focal changes (high sensitivity)</td>
<td></td>
</tr>
<tr>
<td>• Scans entire genome (improve sensitivity)</td>
<td>• Evaluates only 3 loci (decrease sensitivity)</td>
<td></td>
</tr>
<tr>
<td>• Positive aberrations are likely significant (high specificity)</td>
<td>• Higher probability of false positive (lower specificity)</td>
<td></td>
</tr>
</tbody>
</table>

- Lack of experience in counting
- Tetraploidy
- Different cutoff points
Ambiguous lesion

Favor benign

FISH -
Nevus

FISH +
R/O False Positive
• Tetraploidy
• Focal changes
Yes
Nevus
No
Ambiguous

Favor malignant

FISH -
Ambiguous
(20-25% false negative)

FISH +
Melanoma
Is this final?

• Sensitivity not great
• Interpretation is labor intensive
• Technically challenging
• Expensive
• More probes/cutoff points need to be evaluated
Melanoma treatment
FDA approved therapy

- High-dose interleukin-2
- Dacarbazine

- Response rates: 7-20%
- No improvement in survival
New FDA approved drugs

- Ipilimumab (YERVOY)
  - FDA approval: 3/25/2011
- Vemurafenib (ZELBORAF)
  - FDA approval: 8/17/2011
Ipilimumab

• Blocks cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) and promoted antitumor immune response(Q.)
Ipilimumab phase 3 trial

Hodi et al. N Eng J Med, 2010,

Med survival

Ipi alone: 10.1 months
Ipi+gp100: 10 months
gp100: 6.4 months
Ipilimumab phase 3 trial

**Med survival**

- Ipi+dacarbazine: 11.2 months
- Dacarbazine: 9.1 months

Robert et al. N Eng J Med, 2011,
Vemurafenib

• BRAF V600E inhibitor (Q.)
MAP Kinase pathway activation in melanocytic lesions

GROWTH FACTORS → RAS → B-RAF

40-60% of melanomas, 90% is V600E

B-RAF → MEK → ERK

PROLIFERATION
MAP Kinase pathway activation in melanocytic lesions

GROWTH FACTORS

RAS

B-RAF

MEK

ERK

Vemurafenib

PROLIFERATION

NUCLEUS

CYTOPLASM
PLX4032 (Vemurafenib)

- Phase III (BRIM3):
  - 675 patients
  - Vemurafenib vs Dacarbazine
  - 63% reduction in risk of death
  - 74% reduction in risk of death and disease progression

Vemurafenib phase 3 trial

6-month survival

Vemurafenib: 84%
Dacarbazine: 64%
Side effects

- Rash
- Photosensitivity
- Hair loss
- Joint pain
- Liver problems
- Arrhythmias
- Allergic reactions
- Cutaneous SCC (26%) (Q.)
Cobas 4800 BRAF V600E Mutation Test

- FDA approved companion diagnostic test
- Detects BRAF V600E mutation
- Real time PCR (Q.)
QUESTIONS?
References

Molecular alterations in melanoma


References

CGH/ FISH in melanoma

References

CGH/ FISH in melanoma


References

Ipilimumab


References

Vemurafenib


References

Other


