Which patients should be considered for genetic testing?

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Chicago, IL
DISCLOSURE OF RELATIONSHIPS WITH INDUSTRY

Emily Y. Chu, M.D., Ph.D.

F032 – Practical Considerations for Patients with melanoma or Dysplastic Nevi

DISCLOSURES

[List companies, relevant relationships, compensation]
Germline genetic testing is distinct from somatic genetic profiling of cancer tissue to predict treatment response, diagnosis, or prognosis.
Types of testing

- Germline testing
- Somatic testing
Types of testing

• Germline testing
  – $CDKN2A$ mutation testing
  – Other considerations

• Somatic testing
Familial melanoma

- Approximately 5-10% of melanoma patients present in familial clusters
- *Cyclin-dependent kinase inhibitor 2A (CDKN2A)* germline mutations are found in melanoma-prone families
  - Found in 10% of families with 2 cases of melanoma
  - Found in 30-40% of families with 3+ cases of melanoma

CDKN2A locus

- CDKN2A encodes two different cell cycle regulatory proteins, p16INK4A, and p14ARF via alternative splicing

- p16 mutations lead to familial melanoma (40%), pancreatic tumors

- p14 mutations lead to familial melanoma (1%), neural tumors

* Aoude et al., Pigment Cell Melanoma Res 2014*
Tumors associated with *CDKN2A* mutations include:

- Melanoma
- Pancreatic cancer
- Neurofibroma
- Schwannoma
- GBM
- Malignant peripheral nerve sheath tumor
- Breast cancer
- Lung cancer
Who may be considered for \textit{CDKN2A} testing?

\begin{table}[h]
\centering
\begin{tabular}{|l|}
\hline
\textbf{Low melanoma incidence area/population} & \textbf{Moderate to high melanoma incidence area/population} \\
\hline
• Two (synchronous or metachronous) primary melanomas in an individual and/or & • Three (synchronous or metachronous) primary melanomas in an individual and/or \\
• Families with at least one invasive melanoma and one or more other diagnoses of melanoma and/or pancreatic cancers among first- or second-degree relatives on the same side of the family & • Families with at least one invasive melanoma and two or more other diagnoses of invasive melanoma and/or pancreatic cancer among first- or second-degree relatives on the same side of the family \\
\hline
\end{tabular}
\caption{Candidacy for consideration of genetic testing}
\end{table}

This table refers to pathologically confirmed invasive melanoma.

• Considerations which influence decision to test include UV exposure, age of diagnosis, ethnicity, skin type.

\textit{Leachman et al., JAAD 2009}
Who may be considered for **CDKN2A** testing?

### Table VI. Candidacy for consideration of genetic testing

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<tr>
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</tr>
</tbody>
</table>

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- Considerations which influence decision to test include UV exposure, age of diagnosis, ethnicity, skin type.

*Leachman et al., JAAD 2009*
Considerations for management in *CDKN2A* mutation-positive patients

- TBSE at least every 6 months
  - Lower threshold for skin biopsy?
- Adherence to SSE, sun protection/avoidance measures
- Rapid full body MRI to screen for tumors
  - used at Penn for other genetic syndromes associated with a high risk of cancer
  - Screening recommended beginning 10 years prior to the first onset of familial cancer
    - For instance, if pancreatic cancer diagnosed in patient’s father at age 45, screening to begin at age 35 in affected patient
  - Other screening modalities for pancreatic cancer include endoscopic ultrasound, CT
Other genes associated with familial melanoma

- CDK4
- BRCA2
- BAP1
- POT1
CDK4

• Only documented in a small number of melanoma families (17)
• There may be an increased incidence of pancreatic cancer in CDK4 families
BRCA2

- Breast and ovarian cancer
- Several studies suggest that *BRCA2* mutation carriers are 2.5-2.7 times more likely to develop melanoma compared to the general population

*Gumaste et al., BJD 2015*
**BAP1 (BRCA1 associated protein-1) tumor syndrome**

- Uveal melanoma
- Cutaneous melanoma
  - Only 15% of BAP1 mutation carriers have CMM, suggesting that it is a medium penetrance risk gene for CMM
- Mesothelioma
- Renal cell carcinoma
- BAPomas
- ? BCCs
“BAPoma”

- Indolent melanocytic lesions characterized by expression loss of the tumor suppressor \textit{BAP1}

\textit{Busam et al., JAMA Derm 2013}
Multiple Cutaneous Melanomas and Clinically Atypical Moles in a Patient With a Novel Germline BAP1 Mutation

Pedram Gerami, MD; Oriol Yelamos, MD; Christina Y. Lee, BA; Roxana Obregon, BA; Pedram Yazdan, MD; Lauren M. Sholl, MS; Gerta E. Guitart; Ching-Ni Njauw, MS; Hensin Tsao, MD, PhD

**Importance** Several kindreds having germline BAP1 mutations with a propensity for uveal and cutaneous melanomas and other internal malignancies have been described in an autosomal dominant tumor predisposition syndrome. However, clinically atypical moles have not been previously recognized as a component of this syndrome, to our knowledge. We describe the first kindred to date with a germline mutation in BAP1 associated with multiple cutaneous melanomas and classic dysplastic nevus syndrome.

**Observations** We describe a 53-year-old man who was initially seen in 2003 with dysplastic nevus syndrome, multiple atypical melanocytic proliferations showing loss of immunostaining for BAP1, and 7 cutaneous melanomas. Germline testing was performed in the proband, his 16-year-old son, and his 13-year-old daughter, revealing a germline mutation in the BAP1 gene (c.592G>T, p.Glu198X) in the proband and in his 16-year-old son. CDKN2A and CDK4 genes were wild type. No members of this kindred reported a history of uveal melanoma.

**Conclusions and Relevance** To our knowledge, this is the first report of a patient with multiple melanomas, dysplastic nevus syndrome, and an inactivating germline BAP1 mutation. The coexistence of dysplastic nevus syndrome and a BAP1 germline mutation extends the spectrum of the BAP1 tumor predisposition syndrome and may confer a greater risk for cutaneous melanomas.

Published online July 8, 2015.

**Author Affiliations:** Department of Dermatology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois (Gerami, Yelamos, Lee, Obregon, Yazdan, Sholl, Guitart); Robert H. Lurie Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, Illinois (Gerami); Wellman Center for Photomedicine and Department of Dermatology, Massachusetts General Hospital, Harvard University, Boston (Njauw, Tsao); Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston (Njauw, Tsao).

**Corresponding Author:** Pedram Gerami, MD, Department of Dermatology, Feinberg School of Medicine, Northwestern University, 676 N St Clair St. Ste 1600, Chicago, IL 60611 (pgerami@nmifff.org).
Multiple Cutaneous Melanomas and Clinically Atypical Moles in a Patient With a Novel Germline BAP1 Mutation

Gerami et al., JAMA Derm 2015
Protection of telomeres (POT1)

• 13 families recently described to harbor mutations in POT1

Shi et al, Nature Genetics 2014
Robles-Espinoza et al., Nature Genetics 2014
Telomere dysregulation may be a key process associated with melanoma susceptibility

Aoude et al, Pigment Cell Melanoma Res 2014
Types of testing

• Germline testing
  – $CDKN2A$ mutation testing
  – Other considerations

• Somatic testing
Types of testing

• Germline testing

• Somatic testing, may guide
  – Treatment decisions
    • BRAF mutation testing, mutation panels
  – Diagnosis
    • Gene expression profiling, CGH, FISH
  – Prognosis
    • Gene expression profiling
Types of testing

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    • Gene expression profiling
Why is BRAF testing performed?

- 50-60% of all melanomas harbor mutations at codon 600
  - Valine (V) to glutamic acid (E) substitution most common mutation at position 600 = V600E (90%)
  - 2nd most common mutation is V600K (valine → lysine)
  - Mutation results in constitutive activation of the MAP kinase signaling pathway = dysregulated tumor growth
- V600E/K mutations confer increased sensitivity to BRAF inhibitors (vemurafenib, dabrafenib)
How is BRAF testing performed?

• PCR-based BRAF V600 mutation test
  – Used on formalin-fixed, paraffin-embedded tissue, so biopsy specimens sent for routine histopathology are used
  – Sensitive detection of the BRAF V600E mutation
  – May also detect other mutations such as V600D, V600K, V600R
Additional options for BRAF testing

- Targeted next generation sequencing panels
  - Ability to assay for mutations in multiple oncogenes
  - Used on formalin-fixed, paraffin-embedded tissue

http://www.pennmedicine.org/personalized-diagnostics/services.html
Beyond $\text{BRAF}^{V600}$: Clinical Mutation Panel Testing by Next-Generation Sequencing in Advanced Melanoma


The management of melanoma has evolved owing to improved understanding of its molecular drivers. To augment the current understanding of the prevalence, patterns, and associations of mutations in this disease, the results of clinical testing of 699 advanced melanoma patients using a pan-cancer next-generation sequencing (NGS) panel of hotspot regions in 46 genes were reviewed. Mutations were identified in 43 of the 46 genes on the panel. The most common mutations were $\text{BRAF}^{V600}$ (36%), $\text{NRAS}$ (21%), $\text{TP53}$ (16%), $\text{BRAF}^{\text{Non-V600}}$ (6%), and $\text{KIT}$ (4%). Approximately one-third of melanomas had >1 mutation detected, and the number of mutations per tumor was associated with melanoma subtype. Concurrent $\text{TP53}$ mutations were the most frequent events in tumors with $\text{BRAF}^{V600}$ and $\text{NRAS}$ mutations. Melanomas with $\text{BRAF}^{\text{Non-V600}}$ mutations frequently harbored concurrent $\text{NRAS}$ mutations (18%), which were rare in tumors with $\text{BRAF}^{V600}$ mutations (1.6%). The prevalence of $\text{BRAF}^{V600}$ and $\text{KIT}$ mutations were significantly associated with melanoma subtypes, and $\text{BRAF}^{V600}$ and $\text{TP53}$ mutations were significantly associated with cutaneous primary tumor location. Multiple potential therapeutic targets were identified in metastatic unknown primary and cutaneous melanomas that lacked $\text{BRAF}^{V600}$ and $\text{NRAS}$ mutations. These results enrich our understanding of the patterns and clinical associations of oncogenic mutations in melanoma.

*Journal of Investigative Dermatology (2015) 135, 508–515; doi:10.1038/jid.2014.366; published online 25 September 2014*
Prevalence of detected gene mutations by melanoma subtype.

Panels show the rate of gene mutations observed in (a) cutaneous melanomas ($n=484$); (b) unknown primary melanomas ($n=104$); (c) acral melanomas ($n=54$); and (d) mucosal melanoma ($n=43$).
Actionable mutations in metastatic melanoma

- $BRAF$ V600E/K $\rightarrow$ BRAF and/or MEK inhibitor
- $NRAS$ $\rightarrow$ MEK inhibitor
- $KIT$ $\rightarrow$ imatinib, dasatinib
**NF1** is the third most frequently mutated gene in melanoma after **BRAF** and **NRAS**

Exome sequencing identifies recurrent mutations in **NF1** and RASopathy genes in sun-exposed melanomas

Michael Krauthammer1,2, Yong Kong3, Antonella Bacchiocchi4, Perry Evans1, Natapol Pornputtapong2, Cen Wu5, James P McCusker2, Shuangge Ma5, Elaine Cheng4, Robert Straub4, Merdan Serin4, Marcus Bosenberg2,4, Stephan Ariyan6, Deepak Narayan6, Mario Sznol7, Harriet M Kluger7, Shrikant Mane8,9, Joseph Schlessinger10, Richard P Lifton9,11 & Ruth Halaban4

We report on whole-exome sequencing (WES) of 213 melanomas. Our analysis established **NF1**, encoding a negative regulator of RAS, as the third most frequently mutated gene in melanoma, after **BRAF** and **NRAS**. Inactivating **NF1** mutations were present in 46% of melanomas expressing wild-type **BRAF** and **RAS**, occurred in older patients and showed a distinct pattern of co-mutation with other RASopathy genes, particularly **RASA2**. Functional studies showed that **NF1** suppression led to increased RAS activation in most, but not all, melanoma cases. In addition, loss of **NF1** did not predict sensitivity to MEK or ERK inhibitors. The rebound pathway, as seen by the induction of phosphorylated MEK, occurred in cells both sensitive and resistant to the studied drugs. We conclude that **NF1** is a key tumor suppressor lost in melanomas, and that concurrent RASopathy gene mutations may enhance its role in melanomagenesis.

Krauthammer et al., Nature Genetics 2015
Types of testing

• Germline testing
• Somatic testing, may guide
  – Treatment decisions
    • BRAF mutation testing, mutation panels
  – Diagnosis
    • Gene expression profiling, CGH, FISH
  – Prognosis
    • Gene expression profiling
Evaluating benign nevi and melanomas using a gene expression signature

- Candidate biomarker genes identified, based on differential expression in benign vs primary malignant melanocytic lesions reported in the literature or observed in practice.
- Using a training set of 464 melanocytic lesions, a 23 gene signature yields an area under the curve of (AUC) 96%.
- RT-PCR of RNA from FFPE tissue.

Clarke et al, Journal of Cutaneous Pathology 2015
Evaluating benign nevi and melanomas using a gene expression signature

Table 2. List of genes included in the final multivariate signature.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRAME*†</td>
<td>S100A9</td>
<td>CCL5</td>
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</tr>
<tr>
<td>S100A7†</td>
<td>CD38</td>
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<td></td>
</tr>
<tr>
<td>S100A8‡</td>
<td>CXCL10</td>
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<td></td>
</tr>
<tr>
<td>S100A12†</td>
<td>CXCL9</td>
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</tr>
<tr>
<td>P13†</td>
<td>IRF1</td>
<td></td>
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<tr>
<td></td>
<td>LCP2</td>
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</tr>
<tr>
<td></td>
<td>PTPRC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SELL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* PRAME gene expression represents the average of two amplicon measurements.
† These genes were added to the gene expression signature after evaluation of the signature with the training cohort.
‡ These eight immune genes were evaluated as an averaged group in the multivariate signature.
Housekeeping genes included: CLTC, MRFAP1, PPP2CA, PSMA1, RPL13A, RPL8, RPS29, SLC25A3, and TXNL1.
Evaluating benign nevi and melanomas using a gene expression signature

Figure 2. Distribution of diagnostic scores in the clinical validation cohort.

Clarke et al, Journal of Cutaneous Pathology 2015
Evaluating benign nevi and melanomas using a gene expression signature

- Histopathologically ambiguous lesions
  - All 9 cases deemed to be malignant upon review by expert dermatopathologists were classified as malignant by the gene signature
  - 4/8 cases deemed to be benign were classified as benign by gene signature
  - Role for an indeterminate score?

- Metastatic lesions excluded from this study

*Clarke et al, Journal of Cutaneous Pathology 2015*
An Independent Validation of a Gene Expression Signature to Differentiate Malignant Melanoma From Benign Melanocytic Nevi

Loren E. Clarke, MD1; Darl D. Flake II, MS2; Klaus Busam, MD3; Clay Cockerell, MD4; Klaus Helm, MD5; Jennifer McNiff, MD6; Jon Reed, MD7; Jaime Tschen, MD8; Jinah Kim, MD9; Raymond Barnhill, MD10; Rosalie Elenitsas, MD11; Victor G. Prieto, MD12; Jonathan Nelson, BS2; Hillary Kimbrell, MD1; Kathryn A. Kolquist, MD1; Krystal L. Brown, PhD2; M. Bryan Warf, PhD1; Benjamin B. Roa, PhD1; and Richard J. Wenstrup, MD2

BACKGROUND: Recently, a 23-gene signature was developed to produce a melanoma diagnostic score capable of differentiating malignant and benign melanocytic lesions. The primary objective of this study was to independently assess the ability of the gene signature to differentiate melanoma from benign nevi in clinically relevant lesions. METHODS: A set of 1400 melanocytic lesions was selected from samples prospectively submitted for gene expression testing at a clinical laboratory. Each sample was tested and subjected to an independent histopathologic evaluation by 3 experienced dermatopathologists. A primary diagnosis (benign or malignant) was assigned to each sample, and diagnostic concordance among the 3 dermatopathologists was required for inclusion in analyses. The sensitivity and specificity of the score in differentiating benign and malignant melanocytic lesions were calculated to assess the association between the score and the pathologic diagnosis. RESULTS: The gene expression signature differentiated benign nevi from malignant melanoma with a sensitivity of 91.5% and a specificity of 92.5%. CONCLUSIONS: These results reflect the performance of the gene signature in a diverse array of samples encountered in routine clinical practice. Cancer 2017;123:617-28. © 2016 Myriad Genetics, Inc. Cancer published by Wiley Periodicals, Inc. on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEYWORDS: clinical validation, gene expression, melanoma, molecular diagnosis, reverse transcription-polymerase chain reaction.
B) Thigh, right distal posterior: MALIGNANT MELANOMA, 0.37MM THICK, SEE NOTE

B) Thigh, right distal posterior - Multiple sections of this biopsy specimen were cut and examined. The histologic features seen in this biopsy specimen are those of invasive melanoma. The epidermis demonstrates a proliferation of atypical melanocytes. Severe cytologic atypia was noted. Prominent pleomorphism was seen. Large nests were present at the dermal-epidermal junction. Areas of pagetoid spread were noted. Focally, there is invasion of the dermis by the atypical enlarged melanocytes. A mild lymphocytic infiltrate was seen.

Overall, the findings present in this biopsy specimen are of the type seen in invasive melanoma. In the sections reviewed, the lesion extends to both lateral margins and close to the deep margin. A re-excision to achieve complete removal is recommended.

TUMOR CHARACTERISTICS:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Superficial spreading type</td>
</tr>
<tr>
<td>Radial Growth Phase</td>
<td>Present</td>
</tr>
<tr>
<td>Vertical Growth Phase</td>
<td>Present</td>
</tr>
<tr>
<td>Level of Invasion</td>
<td>Level III</td>
</tr>
<tr>
<td>Greatest Thickness</td>
<td>0.37mm</td>
</tr>
<tr>
<td>Site</td>
<td>Thigh</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
</tr>
<tr>
<td>Mitotic Count</td>
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<tr>
<td>Tumor Infiltrating</td>
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<tr>
<td>Lymphocytes</td>
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<tr>
<td>Regression</td>
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<tr>
<td>Precursor Lesion</td>
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<tr>
<td>Vascular Invasion</td>
<td>Not identified</td>
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<tr>
<td>Perineural Invasion</td>
<td>Not identified</td>
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<tr>
<td>Tumor Stage</td>
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</tr>
</tbody>
</table>

The diagnosis was called in to 'Christina' at Dr. K. Evans' office on 11-5-2015 (2:28p.m.) by 'hem'. The diagnosis was read back and verified. JTS/hem ADDENDUM 11/12/15: Sections from this biopsy specimen were sent to Myriad Genetics for gene expression analysis. The gene expression analysis using the MyPath methodology confirmed that this lesion is likely to represent a melanoma. JTS/dh
RESULT DESCRIPTION:
Myriad myPath Melanoma utilizes a molecular signature measured by qRT-PCR that would classify a sample as malignant, benign or indeterminate. This graph shows your patient’s Score relative to Myriad myPath Melanoma Scores according to the range of benign and malignant lesions in the independent validation cohort with a threshold of “0”.
- For Scores from -16.7 to -2.1 the gene signature classification is benign; for Scores from -2.0 to -0.1 the gene signature classification is indeterminate (“less than 10% of the cases); for Scores from 0 to +11.1 the gene signature classification is malignant melanoma.
- A Score range of -16.7 and +11.1 was established in the validation study and Scores within this range will be reported. Scores outside of the validated range may lead to test cancelation or follow-up with the ordering physician.
- Individual lesions may or may not be representative of this cohort.

INTENDED USE:
This assay is intended for the in vitro analysis of melanocytic lesions to aid in the diagnosis of the lesion as being benign or malignant. This is an adjunctive assay and should be used in conjunction with clinical data and histopathological features.

Myriad myPath Melanoma has not been validated on metastatic melanomas, re-excision specimens, non-melanocytic neoplasms, or biopsies from a patient receiving immunosuppressant therapy or radiation treatment. Analysis of these samples may result in incorrect test interpretation; therefore these specimens are not suitable for testing and will be canceled.
Myriad myPath® Melanoma Score: 1.7

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How do we use ancillary diagnostic molecular testing?

- Ancillary testing (CGH, FISH, MyPath) must be interpreted in the context of histopathologic and clinical information.
The Genetic Evolution of Melanoma from Precursor Lesions

A. Hunter Shain, Ph.D., Iwei Yeh, M.D., Ph.D., Ivanka Kovalyshyn, D.O., Aravindhan Sriharan, M.D., Eric Talevich, Ph.D., Alexander Gagnon, B.A., Reinhard Dummer, M.D., Jeffrey North, M.D., Laura Pincus, M.D., Beth Ruben, M.D., William Rickaby, M.B., Ch.B., Corrado D’Arrigo, M.B., Ch.B., Ph.D., Alistair Robson, F.R.C.Path., and Boris C. Bastian, M.D.
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Intermediate Lesion, Probably Benign</th>
<th>Intermediate Lesion, Probably Malignant</th>
<th>Melanoma In Situ</th>
<th>Invasive Melanoma</th>
<th>Metastatic Melanoma</th>
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<tbody>
<tr>
<td>3</td>
<td>BRAF V600E</td>
<td>TEST promoter</td>
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<tr>
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</tr>
</tbody>
</table>

Shain et al., NEJM 2015
Patterns of Mutations and Mutation Burden at Each Stage of Progression

Lesions with intermediate histopathologic features also show an intermediate number of genetic alterations.

Shain et al. NEJM 2015
Types of testing

• Germline testing

• Somatic testing, may guide
  – Treatment decisions
    • BRAF mutation testing, mutation panels
  – Diagnosis
    • Gene expression profiling, CGH, FISH
  – Prognosis
    • Gene expression profiling
Prognostic gene expression profiling (GEP) test

- 31 gene signature identified from microarray analysis: primary cutaneous melanoma vs. metastasis
- RT-PCR of RNA extracted from melanoma FFPE tissue

Table 1. Discriminant genes included in the prognostic genetic signature for cutaneous melanoma metastatic risk

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene title</th>
<th>Direction of regulation in class 2</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP5P</td>
<td>BRCA1-associated protein-1</td>
<td>Down</td>
<td>0.007</td>
</tr>
<tr>
<td>MGP</td>
<td>Matrix Gla protein</td>
<td>Down</td>
<td>0.486</td>
</tr>
<tr>
<td>SPP1</td>
<td>Secreted phosphoprotein 1</td>
<td>Up</td>
<td>6.08 e-16</td>
</tr>
<tr>
<td>CXCL14</td>
<td>Chemokine (C-X-C motif) ligand 14</td>
<td>Down</td>
<td>3.31 e-12</td>
</tr>
<tr>
<td>CLCA2</td>
<td>Chloride channel accessory 2</td>
<td>Down</td>
<td>1.02 e-08</td>
</tr>
<tr>
<td>S100A9</td>
<td>S100 calcium-binding protein A8</td>
<td>Down</td>
<td>0.031</td>
</tr>
<tr>
<td>BTG1</td>
<td>B-cell translocation gene 1, antiproliferative</td>
<td>Down</td>
<td>0.024</td>
</tr>
<tr>
<td>SAPI3O</td>
<td>Sin3A-associated protein, 150 kDa</td>
<td>Down</td>
<td>0.024</td>
</tr>
<tr>
<td>ARG1</td>
<td>Arginase 1</td>
<td>Down</td>
<td>1.05e-08</td>
</tr>
<tr>
<td>KRT6B</td>
<td>Keratin 6B</td>
<td>Up</td>
<td>0.160</td>
</tr>
<tr>
<td>GJA1</td>
<td>Gap junction protein, alpha 1, 43 kDa</td>
<td>Down</td>
<td>0.034</td>
</tr>
<tr>
<td>ID2</td>
<td>Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein</td>
<td>Down</td>
<td>3.91 e-06</td>
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<tr>
<td>EIFB</td>
<td>Eukaryotic translation initiation factor 1B</td>
<td>Up</td>
<td>0.024</td>
</tr>
<tr>
<td>S100A9</td>
<td>S100 calcium-binding protein A9</td>
<td>Down</td>
<td>0.012</td>
</tr>
<tr>
<td>CRABP2</td>
<td>Cellular retinoic acid binding protein 2</td>
<td>Down</td>
<td>0.0006</td>
</tr>
<tr>
<td>KRT14</td>
<td>Keratin 14</td>
<td>Down</td>
<td>1.75 e-05</td>
</tr>
<tr>
<td>ROBO1</td>
<td>Roundabout, axon guidance receptor, homolog 1 (Drosophila)</td>
<td>Down</td>
<td>0.0004</td>
</tr>
<tr>
<td>RBM23</td>
<td>RNA-binding motif protein 23</td>
<td>Down</td>
<td>0.018</td>
</tr>
<tr>
<td>TACSTD2</td>
<td>Tumor-associated calcium signal transducer 2</td>
<td>Down</td>
<td>0.037</td>
</tr>
<tr>
<td>DSC1</td>
<td>Desmocollin 1</td>
<td>Down</td>
<td>7.00 e-09</td>
</tr>
<tr>
<td>SPRR1B</td>
<td>Small proline-rich protein 1B</td>
<td>Down</td>
<td>0.001</td>
</tr>
<tr>
<td>TRIM29</td>
<td>Tripartite motif containing 29</td>
<td>Down</td>
<td>2.34 e-09</td>
</tr>
<tr>
<td>AQP3</td>
<td>Aquaporin 3 (Gill blood group)</td>
<td>Down</td>
<td>5.08 e-06</td>
</tr>
<tr>
<td>TYRPI</td>
<td>Tyrosinase-related protein 1</td>
<td>Down</td>
<td>2.41 e-06</td>
</tr>
<tr>
<td>PPL</td>
<td>Periplakin</td>
<td>Down</td>
<td>5.59 e-11</td>
</tr>
<tr>
<td>LTA4H</td>
<td>Leukotriene A4 hydrolase</td>
<td>Down</td>
<td>0.0001</td>
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<tr>
<td>CST6</td>
<td>Cystatin E/M</td>
<td>Down</td>
<td>1.02 e-08</td>
</tr>
</tbody>
</table>

<sup>a</sup>P value reflects t-test analysis of ΔC<sub>T</sub> values from nonmetastatic cases compared with metastatic cases within the 268 sample cohort.

<sup>b</sup>Two assays for BAPI were included to target both the 5’ and 3’ regions of the gene.
Prognostic gene expression profiling (GEP) test

- Class 1: low metastatic risk
- Class 2: high metastatic risk
Prognostic gene expression profiling (GEP) test

Table 4. Accuracy of class prediction for stage I and II cutaneous melanoma subgroups

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total cases</th>
<th>Cases without documented metastasis</th>
<th>Cases called class 1</th>
<th>Cases with documented metastasis</th>
<th>Cases called class 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/IA/IB</td>
<td>119</td>
<td>110</td>
<td>104 (95%)</td>
<td>9</td>
<td>5 (56%)</td>
</tr>
<tr>
<td>IIA</td>
<td>45</td>
<td>24</td>
<td>16 (67%)</td>
<td>21</td>
<td>19 (90%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>42</td>
<td>14</td>
<td>6 (43%)</td>
<td>28</td>
<td>27 (96%)</td>
</tr>
<tr>
<td>IIIC</td>
<td>14</td>
<td>3</td>
<td>1 (33%)</td>
<td>11</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>

- Median follow up time without evidence of LN involvement or distant metastasis = 7.6 years

Gerami et al, Clin Cancer Research 2015
Likewise, similar results for Stage I melanoma in an independent cohort of patients undergoing GEP testing analysis.

Table 2 Distant metastasis according to stage and molecular class in the stage I and II patients

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total cases</th>
<th>No Distant Metastasis</th>
<th>With Distant Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Class 1</td>
<td>Class 2</td>
</tr>
<tr>
<td>Ia/IA/IB</td>
<td>264</td>
<td>251</td>
<td>216</td>
</tr>
<tr>
<td>IIa</td>
<td>15</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>IIA</td>
<td>35</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>IIB</td>
<td>26</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>IIC</td>
<td>17</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>357</td>
<td>314</td>
<td>244</td>
</tr>
</tbody>
</table>

*aSubstage unknown

96% vs. 69%, and 100% vs. 88% 5-year RFS, DMFS, and MSS rates, respectively. SLN-positive/Class 1A vs. SLN-positive/Class 2B cases had 60% vs. 32%, 76% vs 38%, and 97% vs. 59% 5-year RFS, DMFS, and MSS rates respectively.
Types of testing

• Germline testing – CDKN2A, other
• Somatic testing, may guide
  – Treatment decisions
    • BRAF mutation testing, mutation panels
  – Diagnosis
    • Gene expression profiling, CGH, FISH
  – Prognosis
    • Gene expression profiling
Penn Multidisciplinary Melanoma Program

**Medical Dermatology**
- Michael Ming
- Rose Elenitsas
- Brian Capell

**Dermatopathology**
- Rose Elenitsas
- David Elder
- George Xu

**Medical Genetics**
- Kate Nathanson

**Medical Oncology**
- Lynn Schuchter
- Ravi Amaravadi
- Tara Gangadhar

**Surgical Oncology**
- Giorgos Karakousis

**Derm Surgery**
- Chris Miller
- Joseph Sobanko
- Thuzar Shin
- Jeremy Etzkorn
Thank you!

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