Update on Genetic Testing for Melanoma

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EMILY Y. CHU, MD PhD
U052: Melanoma and the Genes: Optimizing Patient Management in the Age of Molecular Testing
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 \textit{CDKN2A} mutations include:

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

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

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<th>Low melanoma incidence area/population</th>
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This table refers to pathologically confirmed invasive melanoma.

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

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

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*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
Multiple Cutaneous Melanomas and Clinically Atypical Moles in a Patient With a Novel Germline \textit{BAP1} Mutation

Pedram Gerami, MD; Oriol Yéramos, 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

\textbf{IMPORTANCE} Several kindreds having germline \textit{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 \textit{BAP1} associated with multiple cutaneous melanomas and classic dysplastic nevus syndrome.

\textbf{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 \textit{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 \textit{BAP1} gene (c.592G→T, p.Glu198X) in the proband and in his 16-year-old son. \textit{CDKN2A} and \textit{CDK4} genes were wild type. No members of this kindred reported a history of uveal melanoma.

\textbf{CONCLUSIONS AND RELEVANCE} To our knowledge, this is the first report of a patient with multiple melanomas, dysplastic nevus syndrome, and an inactivating germline \textit{BAP1} mutation. The coexistence of dysplastic nevus syndrome and a \textit{BAP1} germline mutation extends the spectrum of the \textit{BAP1} tumor predisposition syndrome and may confer a greater risk for cutaneous melanomas.

\textbf{Author Affiliations:} Department of Dermatology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois (Gerami, Yéramos, 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).

\textbf{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@nmhf.org).
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

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• Somatic testing, may guide
  – Treatment decisions
    • BRAF mutation testing, mutation panels
  – Diagnosis
    • Gene expression profiling, CGH, FISH
  – Prognosis
    • Gene expression profiling
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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%)
  - 2\textsuperscript{nd} 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 BRAF\textsuperscript{V600}: Clinical Mutation Panel Testing by Next-Generation Sequencing in Advanced Melanoma

Alan E. Siroy\textsuperscript{1}, Genevieve M. Boland\textsuperscript{2}, Denáí R Milton\textsuperscript{3}, Jason Roszik\textsuperscript{4}, Silva Frankian\textsuperscript{4}, Jared Malke\textsuperscript{2}, Lauren Haydu\textsuperscript{2}, Victor G. Prieto\textsuperscript{1,3}, Michael Tetzlaff\textsuperscript{1}, Doina Ivan\textsuperscript{1,3}, Wei-Lien Wang\textsuperscript{1}, Carlos Torres-Cabala\textsuperscript{1,5}, Jonathan Curry\textsuperscript{1}, Sinchita Roy-Chowdhuri\textsuperscript{1}, Russell Broaddus\textsuperscript{1}, Asif Rashid\textsuperscript{1}, John Stewart\textsuperscript{1}, Jeffrey E. Gershenwald\textsuperscript{2,6}, Rodabe N. Amaria\textsuperscript{4}, Sapna P. Patel\textsuperscript{4}, Nicholas E. Papadopoulos\textsuperscript{4}, Agop Bedikian\textsuperscript{4}, Wen-Jen Hwu\textsuperscript{4}, Patrick Hwu\textsuperscript{4}, Adi Diab\textsuperscript{4}, Scott E. Woodman\textsuperscript{4,7}, Kenneth D. Alldape\textsuperscript{1}, Rajyalakshmi Luthra\textsuperscript{8}, Keyur P. Patel\textsuperscript{8}, Kenna R. Shaw\textsuperscript{7}, Gordon B. Mills\textsuperscript{7}, John Mendelsohn\textsuperscript{9}, Funda Meric-Bernstam\textsuperscript{2,10}, Kevin B. Kim\textsuperscript{4}, Mark J. Routbort\textsuperscript{8}, Alexander J. Lazar\textsuperscript{1,5,11} and Michael A. Davies\textsuperscript{4,7,11}

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 \textbf{BRAF\textsuperscript{V600}} (36%), \textbf{NRAS} (21%), \textbf{TP53} (16%), \textbf{BRAF\textsuperscript{Non-V600}} (6%), and \textbf{KIT} (4%). Approximately one-third of melanomas had > 1 mutation detected, and the number of mutations per tumor was associated with melanoma subtype. Concurrent \textbf{TP53} mutations were the most frequent events in tumors with \textbf{BRAF\textsuperscript{V600}} and \textbf{NRAS} mutations. Melanomas with \textbf{BRAF\textsuperscript{Non-V600}} mutations frequently harbored concurrent \textbf{NRAS} mutations (18%), which were rare in tumors with \textbf{BRAF\textsuperscript{V600}} mutations (1.6%). The prevalence of \textbf{BRAF\textsuperscript{V600}} and \textbf{KIT} mutations were significantly associated with melanoma subtypes, and \textbf{BRAF\textsuperscript{V600}} and \textbf{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 \textbf{BRAF\textsuperscript{V600}} and \textbf{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** → BRAF and/or MEK inhibitor
- **NRAS** → MEK inhibitor
- **KIT** → imatinib, dasatinib
A 61 year old healthy woman presents for skin cancer screening, prompted by her mother’s recent diagnosis of metastatic melanoma. Her mother’s melanoma was found to harbor a $NF1$ mutation. The patient’s skin exam is unremarkable. The patient wants to know if she (the patient) has neurofibromatosis.
Our patient

- Her mother’s melanoma metastasis specimen was tested using a clinical mutation panel, which revealed a somatic mutation in *NF1*
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 Pornputtapon², 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>
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<tbody>
<tr>
<td>PRAME*</td>
<td>S100A9</td>
<td>CCL5</td>
<td></td>
</tr>
<tr>
<td>S100A7†</td>
<td>CD38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S100A8‡</td>
<td>CXCL10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S100A12†</td>
<td>CXCL9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI3†</td>
<td>IRF1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LCP2</td>
<td>PTPRC</td>
<td>SELL</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.

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KEYWORDS: clinical validation, gene expression, melanoma, molecular diagnosis, reverse transcription-polymerase chain reaction.
How do we use ancillary diagnostic molecular testing?

• Ancillary testing (CGH, FISH, MyPath) must be interpreted in the context of histopathologic and clinical information

• Should be used as a tool rather than a crutch
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>Germ Line</th>
<th>Benign Lesion</th>
<th>Intermediate Lesion, Probably Benign</th>
<th>Intermediate Lesion, Probably Malignant</th>
<th>Melanoma In Situ</th>
<th>Invasive Melanoma</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>Metastatic Melanoma</th>
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<tr>
<td>3 11 13 9</td>
<td>BRF VQDS</td>
<td>BRF VQDS</td>
<td>BRF VQDS</td>
<td>BRF VQDS</td>
<td>BRF VQDS</td>
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<tr>
<td>2 27 16 23 32 15 31 1 29 17 14 12 26 30 35 24 33 10 6 37 4 19 28 5 20 25 11 34 36 7 8</td>
<td>BRF VQDS</td>
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<td>BRF VQDS</td>
</tr>
</tbody>
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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
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    • 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\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP5</td>
<td>BRCA\textsubscript{1}-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>S100A8</td>
<td>S100 calcium-binding protein A8</td>
<td>Down</td>
<td>0.031</td>
</tr>
<tr>
<td>B2G1</td>
<td>B-cell translocation gene 1, antiproliferative</td>
<td>Down</td>
<td>0.024</td>
</tr>
<tr>
<td>SAP130</td>
<td>Sin3A-associated protein, 130 kDa</td>
<td>Down</td>
<td>0.024</td>
</tr>
<tr>
<td>ARGI</td>
<td>Arginase 1</td>
<td>Down</td>
<td>1.05e-08</td>
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<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>
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<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>TYRP1</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>Leukothriene A4 hydrolase</td>
<td>Down</td>
<td>0.0001</td>
</tr>
<tr>
<td>CST6</td>
<td>Cystatin E/M</td>
<td>Down</td>
<td>1.02 e-08</td>
</tr>
</tbody>
</table>

\textsuperscript{a}P value reflects t-test analysis of ΔC\textsubscript{T} values from nonmetastatic cases compared with metastatic cases within the 268 sample cohort.

\textsuperscript{b}Two assays for BAPI were included to target both the E' and C' regions of the gene.

Gerami et al, Clin Cancer Research 2015
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>IIB</td>
<td>42</td>
<td>14</td>
<td>6 (43%)</td>
<td>28</td>
<td>27 (96%)</td>
</tr>
<tr>
<td>IIC</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
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
Thank you!

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