qRT-PCR Based Methods for Prognosis of Melanoma

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Disclosures:
I have been a consultant to Abbott Molecular, Neogenomics, Castle Biosciences, Myriad Genomics, and Derm Tech Int.

True or False?

- Nearly twice as many SLNB negative compared to SLNB positive patients will ultimately die of metastatic melanoma.
  - Answer: True

- Less than half of N1a patients will die from melanoma by 5 years.
  - Answer: True

MSLT-1: Twice as many node-negative patients died compared to node-positive
Initial Report - 2006  
Final Report - 2014
Where we started: Gene expression profiling in uveal melanoma

- 15-gene expression profile test
- Prospective clinical validation study
- Strong separation of metastatic risk by class

- Adopted by >95% of ocular oncologists as standard of care
- Achieved a market penetration of 70%
- Transformed care by enabling personalized treatment planning

1 Onken, 2012, Ophthalmology
2 Aaberg, 2014, Clinical Ophthalmology

Clinical Need in Early Stage Melanoma

- All newer therapies and regional interventions are effective in metastatic melanoma
- Within Stage IV use and resected Stage III disease, early intervention is consistently shown to be a significant (or the most significant) predictor of response

- While AJCC clinicopathologic factors are good → majority of deaths occur in early stage disease

- Prognostic accuracy needs to be improved as it has direct implications on patient follow up

Interrogating the Genome

DNA analysis:
1) CGH or FISH for gene copy number changes
2) Next Gen Seq for Mutational Status
mRNA expression profiling

Immunohistochemistry and now Proteomics to analyze Proteins
Cellular functions represented in GEP signature

54 genes initially assessed and then narrowed to 28 with additional controls

- Migration/chemotaxis
  - Metastasis
  - CXCL14
  - SPP1
  - CLCA2
  - S100A9
  - S100A8

- Differentiation/proliferation
  - CRABP2
  - SPRRIB

- Proliferation
  - ROC1

- Cell surface receptors
  - TACSTD2
  - CLCA2
  - ROBO1

- Angiogenesis regulator
  - CXCL14

- Chemokine/secreted molecules
  - CCL14
  - MGP
  - SPP1

- Cell surface receptors
  - TACSTD2
  - CLCA2
  - ROBO1

- Structural proteins
  - MGP
  - SPP1
  - CST6

- Gap junction/cellular adhesion
  - GJA1
  - DSC1
  - PPL

1st intended use: Identify the node negative patients who have aggressive disease

- Quantifies expression of 31 genes from primary tumor
- Applies a validation algorithm
- Classifies patients as low vs high risk with strong accuracy

 Patients with Stage I and II melanoma

Class 1 test result: Low risk of metastasis within 5 years

Class 2 test result: High risk of metastasis within 5 years

Identification of high risk patients allows them the opportunity to access further evaluation, treatment, and monitoring with the goal of improving long-term survival

Validation Study #1: Background demographics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Training Set (n = 164)</th>
<th>Validation Set (n = 104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median yrs (range)</td>
<td>61 (23-89)</td>
<td>58 (18-94)</td>
</tr>
<tr>
<td>Follow-up, median yrs (range)</td>
<td>4.9 (0.0-13.7)</td>
<td>5.7 (0.0-11.9)</td>
</tr>
<tr>
<td>AJCC Stage n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15 (9%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>I</td>
<td>63 (38%)</td>
<td>56 (54%)</td>
</tr>
<tr>
<td>II</td>
<td>67 (41%)</td>
<td>34 (33%)</td>
</tr>
<tr>
<td>III</td>
<td>18 (11%)</td>
<td>12 (11%)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (1%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Breslow Thickness Median mm (range)</td>
<td>1.86 (0.15-16.0)</td>
<td>1.4 (0.1-14.0)</td>
</tr>
<tr>
<td>≤ 1mm</td>
<td>46 (28%)</td>
<td>45 (43%)</td>
</tr>
<tr>
<td>&gt; 1mm</td>
<td>101 (62%)</td>
<td>58 (56%)</td>
</tr>
<tr>
<td>Mitotic Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1/mm</td>
<td>2 (26%)</td>
<td>2 (28%)</td>
</tr>
<tr>
<td>&gt; 1/mm</td>
<td>82 (50%)</td>
<td>53 (51%)</td>
</tr>
<tr>
<td>Ulceration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>104 (63%)</td>
<td>65 (63%)</td>
</tr>
<tr>
<td>Present</td>
<td>46 (28%)</td>
<td>38 (36%)</td>
</tr>
<tr>
<td>Growth Pattern</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial spreading</td>
<td>75 (46%)</td>
<td>56 (54%)</td>
</tr>
<tr>
<td>Nodular</td>
<td>47 (29%)</td>
<td>25 (24%)</td>
</tr>
<tr>
<td>Desmoplastic/lentigo maligna/acral lentiginous</td>
<td>25 (15%)</td>
<td>10 (10%)</td>
</tr>
</tbody>
</table>

5-yr DFS

Training Set

Class 1 = 97%
Class 2 = 31%

Validation Set

Class 1 = 95%
Class 2 = 34%

Sensitivity = 89%

Accuracy = 86%

GEP Accuracy: Disease-free survival prediction - all cases

SLNB vs. GEP - 1st and 2nd Validation Studies with SLNB procedure

SLN Results

- SLN positive = 59
- SLN negative = 59
- Met = 37
- Non = 21
- PPV = 66%
- NPV = 56%
- Sensitivity = 89%
- Specificity = 79%

GEP Results

- Class 1 = 141
- Class 2 = 76
- Met = 91
- Non = 50
- PPV = 65%
- NPV = 79%

Gerami, Clin Cancer Res 2015
Censor Date: May 2013

Gerami, Clin Cancer Res 2013
Censor Date: May 2013
**DecisionDx-Melanoma Improves Prediction Over SLNB**

Negative Status for Distant Metastasis-Free Survival

![Graph showing DMFS and SLNB](image)

**Cox Regression Analysis of 523 Patients**

<table>
<thead>
<tr>
<th></th>
<th>RFS (p&lt;0.0001)</th>
<th>HR (95% CI)</th>
<th>p value</th>
<th>SLN (p&lt;0.0001)</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.3</td>
<td>0.2-1.3</td>
<td>&lt;0.001</td>
<td>0.3</td>
<td>0.2-1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Melanoma rate</td>
<td>0.3</td>
<td>0.3-3.7</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3-3.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>4.3</td>
<td>3.4-5.3</td>
<td>&lt;0.001</td>
<td>2.4</td>
<td>1.2-4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SN positive</td>
<td>2.5</td>
<td>2.1-4.1</td>
<td>&lt;0.001</td>
<td>2.1</td>
<td>1.7-2.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Comparison of GEP and SLN in 523 patients**

<table>
<thead>
<tr>
<th></th>
<th>GEP Class (%)</th>
<th>SLN Status (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFS</td>
<td>Sensitivity</td>
<td>70% (62-78%)</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>71% (63-79%)</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>41% (33-55%)</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>87% (62-92%)</td>
</tr>
<tr>
<td>DMFS</td>
<td>Sensitivity</td>
<td>73% (66-80%)</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>69% (62-76%)</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>40% (33-47%)</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>97% (67-94%)</td>
</tr>
<tr>
<td>MS</td>
<td>Sensitivity</td>
<td>85% (72-94%)</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>64% (60-83%)</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>19% (14-25%)</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>80% (75-99%)</td>
</tr>
</tbody>
</table>

**GEP plus SLN in combination**

<table>
<thead>
<tr>
<th></th>
<th>RFS (p=0.0001)</th>
<th>DMFS (p=0.0001)</th>
<th>MSS (p=0.0001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1 (n=159)</td>
<td>79% (73-85%)</td>
<td>87% (84-91%)</td>
<td>97% (94-100%)</td>
</tr>
<tr>
<td>Class 2 (n=178)</td>
<td>52% (48-57%)</td>
<td>59% (54-64%)</td>
<td>78% (70-85%)</td>
</tr>
<tr>
<td>SLN- (n=186)</td>
<td>79% (73-85%)</td>
<td>85% (80-91%)</td>
<td>95% (92-99%)</td>
</tr>
<tr>
<td>SLN+ (n=147)</td>
<td>47% (42-54%)</td>
<td>53% (46-61%)</td>
<td>75% (66-84%)</td>
</tr>
<tr>
<td>Class 1/SLN- (n=103)</td>
<td>87% (85-90%)</td>
<td>93% (88-98%)</td>
<td>98% (92-100%)</td>
</tr>
<tr>
<td>Class 1/SLN+ (n=67)</td>
<td>61% (52-70%)</td>
<td>75% (65-84%)</td>
<td>93% (86-100%)</td>
</tr>
<tr>
<td>Class 2/SLN- (n=77)</td>
<td>76% (68-85%)</td>
<td>78% (65-88%)</td>
<td>92% (85-98%)</td>
</tr>
<tr>
<td>Class 2/SLN+ (n=101)</td>
<td>87% (84-89%)</td>
<td>84% (71-96%)</td>
<td>88% (81-93%)</td>
</tr>
</tbody>
</table>

**Independent Validation of 357 Previously Unreported Stage I and II patients**

![Graph showing DMFS and GEP](image)
RFS in validation cohort of 264 previously unreported stage I patients

Independent cohort of 93 previously unreported stage II cases

166 previously unreported stage III patients

DecisionDx-Melanoma Identifies ~70% of SLNB Negative Patients who had Distant Metastasis

DecisionDx in SLNB- Patients

Independent, Prospective Single-Center Study Confirms Previous Studies

- Class 2A/2B patients were 22 times more likely to develop metastatic disease
- 76.9% of metastatic patients were correctly identified as high risk

University Affiliated Private Dermatology Practice

Independent, Prospective, Single-Center Study

- Independent single-center study
- Used prospectively-collected melanoma registry with chart review

Total of 375 patients were consented

256 successfully tested

214 (84%) Class 1
193 Class 1A
21 Class 1B

42 (16%) Class 2
16 Class 2A
26 Class 2B

319 eliminated (lack adequate residual tumor tissue, exhausted FFPE blocks)

159 consecutive patients underwent SLNB mapping and successful DecisionDX-Melanoma testing (November 2013 through September 2015).

- Median follow-up time 18 months

- Baseline thickness 0.94 mm (0.17 – 1.32 mm)
- Ulceration status: Absent 76%, Present 24%
- Tumor Status: Negative 87%, Positive 13%
- DecisionDX-Melanoma Class:
  - Class 1 (low risk): 74% (n=117)
  - Class 2 (high risk): 26% (n=42)

- Rate of recurrence in Class 2, SLN negative patients was consistent with previous studies (Gerami 2015, Zager.2016)

- 8% (16/18) of all recurrences were identified as Class 2:
  - 10 node negative patients had recurrences, 8 were Class 2
  - 8 node positive patients had recurrences, 8 were Class 2
  - NVP for Class 1 = 98%

- Earlier detection with lower tumor burden is associated with better response to therapy

Hsueh Independent, Prospective, Single-Center Study

<table>
<thead>
<tr>
<th>Author</th>
<th>Therapy Investigated</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mendes et al, 2014</td>
<td>Dabrafenib + trametinib</td>
<td>Better overall response (ORR) was significantly smaller (except those in sub-group).</td>
</tr>
<tr>
<td>Buinman et al, 2014</td>
<td>Isolated limb infusion</td>
<td>Class 2.2b, isolated limb infusion, lower tumor burden, volume was significant predictor of absence.</td>
</tr>
<tr>
<td>Joseph et al, 2014</td>
<td>Pembrolizumab</td>
<td>Pembrolizumab, invasive melanoma, clinical (imaging) and pathological stage, metastasis.</td>
</tr>
<tr>
<td>Mehta et al, 2014</td>
<td>Bephalumab + dacarbazine</td>
<td>Underwent, clinical melanoma, invasive melanoma, significantly associated with lower tumor burden.</td>
</tr>
<tr>
<td>Deming et al, 2015</td>
<td>Ipilimumab</td>
<td>Melanomas, invasive melanoma, metastasis, with melanoma, significantly associated with lower tumor burden.</td>
</tr>
<tr>
<td>Reddy et al, 2010</td>
<td>Ipilimumab</td>
<td>Melanomas, invasive melanoma, metastasis, with melanoma, significantly associated with lower tumor burden.</td>
</tr>
<tr>
<td>Job et al, 2014</td>
<td>Pembrolizumab</td>
<td>Melanomas, invasive melanoma, metastasis, with melanoma, significantly associated with lower tumor burden.</td>
</tr>
<tr>
<td>Eapman et al, 2015</td>
<td>T-VAC</td>
<td>No significant correlation was present, but OS, durable response, and overall survival (OS).</td>
</tr>
<tr>
<td>Sun et al, 2015</td>
<td>Pembrolizumab</td>
<td>No significant correlation was present, but OS, durable response, and overall survival (OS).</td>
</tr>
<tr>
<td>Hsueh et al, 2016</td>
<td>Pembrolizumab</td>
<td>No significant correlation was present, but OS, durable response, and overall survival (OS).</td>
</tr>
</tbody>
</table>

SLNB+/– trends are similar in thin melanomas (≤1.0mm)

- Combined results from Nine Studies on Thin Melanomas**

<table>
<thead>
<tr>
<th>Events</th>
<th>SLNB+</th>
<th>SLNB-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>137</td>
<td>33</td>
</tr>
<tr>
<td>Positive</td>
<td>104 (76%)</td>
<td>33 (24%)</td>
</tr>
</tbody>
</table>

74 y/o male with a 0.7 mm melanoma with 2 mitoses/mm2

- Negative SLNB
- t1bN0Mx
- Castle Class II

- Negative baseline imaging and negative repeat imaging at 6 months
Current and Near Term Collaborative Centers

- Emory University (Lawson, Delman, Russell)
- UMMC (Lyle)
- Kelsey-Seybold Foundation (Greisinger, Jackson)
- UC Denver (Gonzalez, Amaria)
- Northwestern (Gerami)
- St. Thomas Path (Robbins)
- Baylor College of Medicine (Rosen)
- FL Hosp Mem Med Ctr (Windham)
- University of Arizona (Cramner)
- Moffit (Sondak, Zager)
- UPMC (Ferris)
- Cleveland Clinic (Gastman)
- Others at contract / IRB stage