Molecular Profiling in Cutaneous Melanoma: Perspectives from a Lab Rat

Alexander Meves, M.D.
Conflict of Interest

• I report no conflicts of interest

• I have patents pending:
  - WO 2014/077915 A1
  - WO 2016/025717 A1
A New Revolutionary Era in Molecular Techniques

Next-Gen Sequencing

Proteomics

Microfluidics

Chemistry

Automation

Computation
The Diagnostic Mainstream in Dermatopathology (2016)
Towards Molecular Diagnostics in Cutaneous Oncology

$\$$ $\$$ $\$$
What’s Needed to Develop a Clinical Test

- Highly reproducible standardized methods
- Right to use
- Money
- A defined set of clinical specimens
- Clinical history of patients
- Thousands of unique patients
- An innovative idea that is so good that it outperforms the standard of care (and is biologically sound)
- Statistics to show that your test is better than existing methods
It’s Hard to Change the World to the Better

CLIA Certified
It's Hard to Change the World to the Better
How have we done so far?

‘difficult’ to interpret pigmented lesion

RREB – MYB – CCND1 – MYC – CDKN2A

benign

malignant

Goldstandard: Histopathology Expert Opinion
It’s Hard to Change the World to the Better

How have we done so far?

‘difficult’ to interpret pigmented lesion

RREB – MYB – CCND1 – MYC – CDKN2A

Change Diagnosis?

yes  no
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How have we done so far?

‘difficult’ to interpret pigmented lesion

FISH positive → univarietly associated with SLN positivity?

* Sominidi-Damodaran et al.; paper under review
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How have we done so far?

- FISH ➔ $1500
- CGH ➔ $2500
- QPCR ➔ $1500 (MYRIAD MyPATH)
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Our own contribution

High-Risk?  

- yes  ➔  WLE+SLNB  
- no  ➔  WLE
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Our own contribution

Microfluidic qRT-PCR

Calculate Predicted Probability of SLN positivity

SLN biopsy indicated?

yes  no
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ROC Curve (Area)
- Clinicopathologic model (0.7809)
- Clinicopathologic + molecular model (0.8901)

DEVELOPMENT COHORT

VALIDATION COHORT

ROC Curve (Area)
- Clinicopathologic model (0.6771)
- Clinicopathologic + molecular model (0.9270)
It’s Hard to Change the World to the Better

Our own contribution

Tumor Cell Adhesion As a Risk Factor for Sentinel Lymph Node Metastasis in Primary Cutaneous Melanoma

Alexander Meves, Ekaterina Nikolova, Joel B. Heim, Edwin J. Squirewell, Mark A. Cappel, Mark R. Pittelkow, Clark C. Otley, Nille Behrendt, Ditte M. Saunte, Jørgen Lock-Andersen, Louis A. Schenck, Amy L. Weaver, and Vera J. Suman
How to Develop a Prognostic Gene Expression Signature?

*It’s easy! Just simulate the real world!*
How to Develop a Prognostic Gene Expression Signature?

*It’s easy! Just simulate the real world!*

What is the decision point in clinical care that the test wants to help with?

*In our case:* SLN biopsy (yes/no); outcome of interest: SLN status

Who would likely be considered for molecular testing in real life?

Set inclusion / exclusion criteria

Develop test in a real-life cohort of patients (consecutive patients)
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

What is the decision point in clinical care that the test wants to help with?

I want to address multiple decision points by identifying all melanomas that metastasize, be it regional or distant; outcome of interest: any kind of metastasis. More frequent follow-up, referrals to specialists (e.g., oncologists), adjuvant therapy (yes/no); e.g., immunotherapy.
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

**Problem:** Now I try to develop one model / test for very different outcomes

**Regional Metastasis**
- affected by patient age
- mitotic rate *not* univariately associated

**Distant Metastasis**
- not affected by patient age
- mitotic rate *is* univariately associated
How to Develop a Prognostic Gene Expression Signature?

*How not to do it...*

Who would likely be considered for molecular testing in real life?

Any type of melanoma (biggest market)
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

16% in-situ melanoma

Table 2. Clinical characteristics by metastasis status and class prediction for the training and test set

<table>
<thead>
<tr>
<th></th>
<th>Nonmetastasis (n=97)</th>
<th>Metastasis (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up time (y), median (range)</td>
<td>6.8 (0.06-13.7)</td>
<td>15.0 (7.0-7.8)</td>
</tr>
<tr>
<td>Time to metastasis (y), median (range)</td>
<td>47 (23-87)</td>
<td>68 (23-89)</td>
</tr>
<tr>
<td>Age (y), median (range)</td>
<td>73.5 (64-82)</td>
<td>72.2 (58-91)</td>
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<tr>
<td>AJCC stage</td>
<td></td>
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<tr>
<td>I</td>
<td>15</td>
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<td>IA</td>
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<td>IB</td>
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<td>II</td>
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<tr>
<td>Breslow thickness (mm)</td>
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<tr>
<td>Median (range)</td>
<td>1.0 (0.3-10.4)</td>
<td>2.8 (0.15-16)</td>
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<tr>
<td>&lt;1 mm</td>
<td>38</td>
<td>6</td>
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<td>1.1-1.99 mm</td>
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NOTE: P values reflect differences in class prediction and were determined by x

Not simulating reality (not real-life cases)

melanoma with distant metastasis at testing

Not simulating reality (not real-life cases)
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

Gene expression profiles not just a function of malignant potential, also affected by:

- Anatomic structure
- Histologic type
- Spatial context
- Immune status
- Patient age... Etc...
How to Develop a Prognostic Gene Expression Signature?

*How not to do it...*

Set inclusion / exclusion criteria to define the type of patient you want tested in real-life

“Developing tests from sample types that will not be tested in clinically relevant circumstances makes it uncertain that these tests will perform as predicted for future patients at risk.”*

*Sominidi-Damodaran et al.; Mayo Clinic Proc (2016)*
It’s Hard to Change the World to the Better

*Take your time!*
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

Metastasis = includes regional mets

Lymph node status unknown for some of these: mis-categorized cases?
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

Metastasis = includes regional mets

Who got SLNB +/- CLND and who didn’t? Influenced outcome?
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

Numbers don’t add up.
How to Develop a Prognostic Gene Expression Signature?

*How not to do it...*

Not a real-life cohort of consecutive patients; hand selected cases?

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**NOTE:** P values reflect differences in class prediction and were determined by x

*Biological Human Tumors*

**Development of a Prognostic Genetic Signature to Predict the Metastatic Risk Associated with Cutaneous Melanoma**

Podram Garami¹, Robert W. Cook², Jeff Wilkinson³, Maria C. Russell⁴, Havneet Dhillion⁵, Rodabe N. Amara⁶, Rene Gonzalez⁷, Stephen Lyle⁸, Clare E. Johnson⁹, Kristin M. Oelschlager⁰, Gilchrist L. Jackson¹, Anthony J. Greisinger¹, Derek Maetzold¹, Keith A. Delman¹, David H. Lawson¹, and John P. Stone¹
How to Develop a Prognostic Gene Expression Signature?

How not to do it...

Model development + validation based on an unknown number of partial samples

Performing tests on partial samples, i.e., as obtained by wide reexcision surgical procedures, is inappropriate for multiple reasons. First, an unknown amount of tumor will have been removed by the preceding biopsy, including an unknown number of high-risk tumor cells (Figure). Second, the diagnostic biopsy induces a wound healing reaction that can lead to changes in gene expression akin to what is observed in cancer (“tumors are wounds that do not heal”).*

* Sominidi-Damodaran et al.; Mayo Clinic Proc (2016)
How to Develop a Prognostic Gene Expression Signature?

...versus the standard of care

2016 ASCO Annual Meeting (Poster Session (Board #186))
Zager SJ et al. (Castle funded)

test not developed and validated to identify patients with positive SLN

→ not superior to SLN for predicting distant metastasis (DM)
How to Develop a Prognostic Gene Expression Signature?

<table>
<thead>
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<tr>
<td>Addresses a well-defined clinical decision point</td>
<td>x</td>
</tr>
<tr>
<td>Developed from a well-defined group of melanoma patients that will likely benefit from testing</td>
<td>x</td>
</tr>
<tr>
<td>Developed from a ‘real-life’ cohort of consecutive patients</td>
<td>x</td>
</tr>
<tr>
<td>Outperforms the standard of care</td>
<td>x</td>
</tr>
<tr>
<td>Developed using diagnostic biopsy tissue only</td>
<td>x</td>
</tr>
<tr>
<td>Ready for the mainstream of patient care</td>
<td>x</td>
</tr>
<tr>
<td>Interesting research</td>
<td>✓</td>
</tr>
</tbody>
</table>
Conclusion

The way forward…

• Need academia to organize large melanoma registries
• Share tissue, share succes, validate tests on large # of cases
• Work in teams: private practice physicians, academic physicians, molecular biologists, bioinformaticians, statisticians
• Get the need for short-term profit-making out of the equation
• Take time, don’t play on the fears of cancer patients
Letter to the Editor

Gene Expression Profiling in Cutaneous Melanoma: Caveats for Clinicians

Sindhuja Sominidi-Damodaran, MD, Mark R. Pittelkow, MD, Alexander Meves, MD

http://dx.doi.org/10.1016/j.mayocp.2016.05.012
Molecular Profiling in Cutaneous Melanoma

Andrew L. Ji, MD\textsuperscript{a,b}; Christopher K. Bichakjian, MD\textsuperscript{c}; and Susan M. Swetter, MD\textsuperscript{a,b}

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