MicroRNA, DNA-Methylation, RNA-expression: “beating the old dog with a new stick?”

brief discussion

Peter Dubsky, MD
Associate Professor of Surgery,
Medical University of Vienna
Disclosures

• No relevant financial disclosures

• Collaborator and co-author of Ivana Sestak in related projects of the trans-ATAC/ABCSG group (Abstract 200).
Applying new tools to old questions

• What am I learning in terms of (breast) cancer biology? How is this relevant to clinically oriented research?

• What is the potential clinical relevance?
  – Analytic and clinical validity
  – Clinical utility
  – Is the information gained complementary?
Molecular subtype of breast cancer metastases significantly influences patient post-relapse survival

Nick Tobin, PhD
Methods:

- prospective collection of 120 FNA biopsies from metastatic sites
- \( n = 111 \); ET vs. TEX, phase III trial in recurrent BC
- application of gene modules and PAM50 established in primary BC to metastatic samples
- Descriptive analysis of relapses according to RNA expression patterns and survival analysis to detect prognostic relevance
Main Findings

• Overrepresentation of:
  – low ER signaling, high proliferation, HER2 and angiogenic signaling
  – 25% basal, 32% HER2, 28% LUM B, 10% LUM A

• Both classifiers:
  – association with post-relapse overall survival
ER is unstable during disease progression.

Lindström L S et al. JCO 2012;30:2601-2608
Molecular subclasses of breast cancer: how do we define them?
The IMPAKT 2012 Working Group Statement

Summary

• Elegant proof of principle: individual types of breast cancer change during disease progression. This data clearly adds detailed biology to what we know from IHC.

• Uncertain clinical validity:
  – Unlikely to detect more “actionable changes” than IHC
  – Little potential to find new actionable targets
Evolutionary Patterns of microRNA expression through the Course of Disease and Treatment in Recurrent Breast Cancer

Dr. Maya Dadiani
Lab of Breast Cancer Translational Research
Cancer Research Center
Chaim Sheba Medical Center
Tel-Hashomer, Ramat Gan, ISRAEL
miRNAs interact with oncogenic pathways

Cancer Treatment Reviews, Serpicio et al. 2014
miRs are associated with subtype and therapy response

Table 3
miRNAs and breast cancer subtypes.

<table>
<thead>
<tr>
<th>Signature</th>
<th>Cancer subtype</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up: miR-150, -142-3p, -142-5p, 148a, -106a/b, -18a, -93, -155, -25, -187, -135b</td>
<td>Basal-like</td>
<td>[58]</td>
</tr>
<tr>
<td>Up: miR-150, -142-3p, -142-5p, -148a, -106b, -93, -155, -25, -187, -375 Down: miR-125a and b</td>
<td>HER2 positive</td>
<td>[58,59,62]</td>
</tr>
<tr>
<td>Up: miR-191, -26, -126, -136, -100, -99a, -145, -146b, -10a, -199a/b, -130a, -30a-3p, -30a-5p, -224, -214, let-7a/b/c/f, -342</td>
<td>Luminal A</td>
<td>[7,58,59]</td>
</tr>
<tr>
<td>Down: miR-206, -15b, -107, -103</td>
<td></td>
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<td>Up: miR-142-5p, -135b, -126, -136, -100, -99a, -145, -10a, -199a/b, -130a, -30a-3p, -214, -7a/c</td>
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Table 4
miRNAs involved in resistance/sensitivity to current breast cancer therapeutic strategies.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Drug</th>
<th>Anti-HER2</th>
<th>Mechanism</th>
<th>Type of evidence (Refs.)</th>
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<tbody>
<tr>
<td>miR-15a/16</td>
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<tr>
<td>miR-21</td>
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<td>miR-30c</td>
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<tr>
<td>miR-125b</td>
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<td>miR-128</td>
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<td>miR-205</td>
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<tr>
<td>miR-221/222</td>
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<tr>
<td>miR-301</td>
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<td>miR-326</td>
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<td>miR-451</td>
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<td>miR-548d-3p/559</td>
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Excellent rationale: miRs to predict response and prognosis...

- Longitudinal observation to detect patterns of miR expression from FFPE matched samples
- on the nCounter miRNA expression assay
- n= 20 (10 with recurrence), pre/post NACT and metastasis.
- Example:

```
Expression pattern

Pre-treatment Post-treatment Recurrence
```
Main Findings I

• 21 miRs shared down/up/down pattern
• 13/21 miRs differentially expressed in BC
• This set of miRs enriched in cell cycle pathways
Main Findings II

• In each patient the ∧ pattern correlates with response to NACT, the absence of ∧ to resistance

• Recurrence invariably has the lowest expression of the 13miR set in comparison to pre and post-NACT sample

• Finally: absolute expression of the 13miR set at diagnosis is a prognostic marker
Biology

• **Pattern analysis** (as opposed to absolute expression levels) reveals miRs that are clearly informative about – a) response and b) prognosis
• This data helps direct miRNA research toward breast cancer biology
• This data reveals how miRNA interacts with known biologic pathways
Clinical validity

• Can this data add information to other models/markers of
  – response and prognosis
  – such as ER, Grading, RNA/DNA based markers?
  – or algorithms thereof

• Would a longitudinal miR approach like this work with liquid biopsy- and thus add prognostic or predictive information for post-treatment survival?
Analysis of multigene scores for the prediction of distant recurrence according to non-clinical baseline factors

Ivana Sestak
Mitch Dowsett, Sean Ferree, J. Wayne Cowens, Frederick L. Baehner, Jack Cuzick
on behalf of the ATAC/LATTE Trialists’ Group

Centre for Cancer Prevention, Queen Mary University of London, London, UK
Royal Marsden Hospital, London, UK
NanoString Technologies, Seattle, WA USA
Genomic Health, Redwood City, CA, USA
Do non-tumour derived factors influence prognostic scores?

• Methods:
  – 940 ER+ postmenopausal women- from ATAC
  – AI or TAM-treated in the absence of chemotherapy
  – primary endpoint: distant recurrence
  – HRs from Cox models to describe impact of:
    • Age, BMI, prior HRT, Smoking, Hysterectomy, RT, Mastectomy
  – ROR, RS, IHC4 as continuous variables and all adjusted for CTS
Dowsett M et al. JCO 2013;31:2783-2790

A

10-Year Predicted Risk of Distant Recurrence (%)

ROR Score

25th percentile

75th percentile

= 4x
Impact of age on scores for all patients
Hazard Ratios (95% CI)

**AGE**

**CTS**
- <59.8y: Hazard ratio 3.23 (2.22-4.69)
- 59.8-68.2y: Hazard ratio 1.76 (1.51-2.05)
- >68.2y: Hazard ratio 2.98 (2.23-3.97)

**ROR**
- <59.8y: Hazard ratio 2.07 (1.12-3.82)
- 59.8-68.2y: Hazard ratio 3.24 (2.02-5.20)
- >68.2y: Hazard ratio 1.33 (0.92-1.93)

**IHC4**
- <59.8y: Hazard ratio 2.23 (1.46-3.40)
- 59.8-68.2y: Hazard ratio 1.62 (1.17-2.24)
- >68.2y: Hazard ratio 1.55 (1.16-2.07)

**RS**
- <59.8y: Hazard ratio 1.78 (1.32-2.39)
- 59.8-68.2y: Hazard ratio 1.28 (1.04-1.57)
- >68.2y: Hazard ratio 1.26 (1.00-1.58)

*Adjusted for CTS*
Main Findings

- No detectable influence of HRT, Smoking, Hysterectomy, Radiotherapy, Mastectomy
- CTS and molecular scores work less well in women >68 years.
- Most prognostic information is added in normal and overweight women, but less in obese women
Biology...What could be behind age?

• Features of “Immunosenescence”\(^1\)
  – Impaired ability to respond to new antigens
  – Unsustained memory responses
  – Increasing incidence of immune disorder
  – Sustained, low-grade inflammation

• Angiogenesis/wound healing ?

• metabolic factors associated rather with age than BMI?

\(^1\) Goronzy and Weyand, Nat. Immunol. 2013
Clinical validity

• Retrospective unplanned analyses
• Validation in similar populations needed
• “Host factors” are often disregarded in MDT meetings
• Both tumor derived and patient derived factors are likely to influence the decision to a) order a molecular score b) decision-making concerning adjuvant therapy.
A novel methylation signature that reflects intratumoral lymphocyte infiltration in breast cancer and predicts for response to anthracycline treatment

J. Jeschke, M. Bizet, C. Desmedt, M. Defrance, S. Dedeurwaerder, E. Calonne, C. Sotiriou, F. Fuks
Immune infiltrates have IMPAKT

Fabrice Andre, Maria V. Dieci, Peter Dubsky, Christos Sotiriou, Giuseppe Curigliano, Carsten Denkert, and Sherene Loi.
Clin Cancer Res 2013;19:28-33
Interaction LPBC and Trastuzumab!!

Validation in GeparQuatro, SABCS 2013!
## SABCS 2013- Level I Evidence

<table>
<thead>
<tr>
<th></th>
<th>Loi et al</th>
<th>Adams et al</th>
</tr>
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<tbody>
<tr>
<td><strong>Randomized Ph III trial</strong></td>
<td>BIG 02-98; 8-year f/u, 256 TNBC, all LN pos</td>
<td>E2197, E1199; 10.6- year f/u, 481 TNBC, 59% LN pos</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td>REMARK &amp; pre-specified analysis, H&amp;E full section, 2 pathologists independently, Analyzed in 10% increments + binary</td>
<td>REMARK &amp; pre-specified analysis, H&amp;E full section, 2 pathologists jointly, Analyzed in 10% increments + binary</td>
</tr>
<tr>
<td><strong>TIL%</strong></td>
<td>Median: 20 sTIL, 5 iTIL, LPBC: 10.6</td>
<td>Median:10 sTIL, 0 iTIL, LPBC: 4.4</td>
</tr>
<tr>
<td><strong>LPBC vs &lt;50% TIL</strong></td>
<td>HR 0.31 (p=0.02, DFS)</td>
<td>HR 0.58 (p=0.18, DFS)</td>
</tr>
<tr>
<td><strong>Intraepi TIL, 10% increase</strong></td>
<td>HR 0.83 (p=0.1, DFS)</td>
<td>HR 0.72 (p=0.06)</td>
</tr>
<tr>
<td></td>
<td>HR 0.73 (p=0.03, OS)</td>
<td>HR 0.64 (p=0.08)</td>
</tr>
<tr>
<td><strong>Stromal TIL, 10% increase</strong></td>
<td>HR 0.84 (p=0.02, DFS)</td>
<td>HR 0.86 (p=0.02, DFS)</td>
</tr>
<tr>
<td></td>
<td>HR 0.82 (p=0.02, OS)</td>
<td>HR 0.81 (p=0.01, OS)</td>
</tr>
<tr>
<td></td>
<td>HR 0.85 (p=0.02, DFS multivariate)</td>
<td>HR 0.84 (p=0.005, DFS multivariate)</td>
</tr>
<tr>
<td></td>
<td>HR 0.83 (p=0.02, OS multivariate)</td>
<td>HR 0.79 (p=0.003, OS multivariate)</td>
</tr>
</tbody>
</table>
DNA methylation profiling is a sensitive tool to capture BC infiltration of immune cells.

Specifically T-cell marker genes correlate with clinical outcomes.

Dedeurwaerderder and Fuks, Oncoimmunology 2013

Improvement of prognosis and prediction to treatment response
Methods and Findings

- MeT signature derived from breast cancer cell lines versus T-cell lines
- Validated in the TOP cohort
- Optimized in the same cohort

AUC: 88%
Sens: 100%
Spec: 73%
PPV: 32%
NPV: 100%

Coeff: -1.97
SE: 0.766
P: 0.0099
HR: 6.29
P: 0.0122
Clinical Impact: Anthracyline response

• Analytic validity:
  – H&E staining of TILs vs. Methylation arrays?

• Clinical validity:
  – H&E measure of sTILs is validated in TNBC. How would a methylation array perform? Is the information complementary?

• Clinical utility:
  – Potentially this technology could be combined with IHC of CEP17 and TOP2A to predict benefit from Anthracylines...
Enriching classic Immunology using new biotech...

Banchereau and Steinman 1998
Biology

• Epigenetic activation and silencing is complementary to the current understanding of cancer immunology
• Epigenetics clearly add a layer of complexity to the interaction of T-cell activation via APC
• What are the links between the biochemistry of methylation and the (co)-stimulatory activation via dendritic cells? This is relevant to therapies involving costimulatory blockade but also to mAb targeting HER2, EGFR etc.
Thank You