Molecular breast pathology: what the diagnostic pathologist needs to know

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November 2013, Singapore
• Genetic classification of breast cancer
• Genetic tests for prognosis
• Prediction of response to systemic therapy (in advanced breast cancer and in the adjuvant setting)
• Whole genome sequence analysis of breast cancer
• Genetic classification of breast cancer
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• Whole genome sequence analysis of breast cancer
Analysis of gene expression in breast cancer

Scanned image of a flexjet 25,000 gene human microarray

Hybridized with mixture of 'red'-labeled cRNA of a tumor sample and 'green'-labeled reference cRNA
Unsupervised hierarchical cluster analysis
Classification based on immunohistochemistry

- ER+/PR+/HER2- 40%
- ER+/PR-/HER2- 30%
- ER+/PR+/HER2+ 3%
- ER+/PR-/HER2+ 2%
- ER-/PR-/HER2+ 10%
- ER-/PR-/HER2- 15%
• Genetic classification of breast cancer
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From: Polychemotherapy for early breast cancer: an overview of the randomised trials
Early Breast Cancer Trialists' Collaborative Group; Lancet, 1998
Decisions on adjuvant systemic treatment for breast cancer patients

• Who should be treated

• What treatment should be given
Supervised Classification for Prognosis

78 breast tumors
patients < 55 years
tumorsize < 5 cm
lymphnode negative (LN0)

Prognosis Reporter Genes

- distant metastasis < 5 years (n=34)
- no distant metastasis > 5 years (n=44)
Supervised Classification Prognosis

Leave-one-out cross-validation

70 significant prognosis genes

threshold set with 10% false negatives
91% sensitivity, 73% specificity
Validation series; n=295 (stage I and II)

Metastasis-free probability and overall survival for the whole cohort

![Graphs showing metastasis-free probability and overall survival over time for different profiles. The x-axis represents time in years, and the y-axis represents the probability. The graph shows two profiles: Good profile (115) and Poor profile (180). The survival curves are plotted for 12 years, with the numbers at the bottom indicating the number of observations at each time point.](image-url)
RASTER study

MicroRNA profiling in breast cancer

- Technology introduction program for 70 gene prognosis profile
- T1/2 N0 patients <60 years
- 16 participating hospitals
Standardizing methods to obtain tumor for gene expression profiling

RNA later
RASTER study

Patient inclusion: 2004-2006

• Patients included: 812 (100%)
  [mean age 48 yrs (range 27-60)]

• 70-gene expression profiles: 427 (53%)
  – Poor profile: 208 (49%)
  – Good profile: 219 (51%)

• Exclusion: 385 (47%)
Events after 5 years of follow-up

<table>
<thead>
<tr>
<th>Event</th>
<th>MP low risk (219)</th>
<th>MP high risk (208)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distant metastases</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Regional recurrence</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Contralat. Breast cancer</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Death</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>BC related death</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>
Overall Survival

MammaPrint

15% CT
85% CT

low risk: 98.3%
high risk: 96.6%
**MINDACT TRIAL DESIGN**

Registration & Screening Surgery

N= 6600

Clinical-Pathological (C) risk
(Adjuvant! Online)

Genomic (G) risk
(70-gene signature)

**Discordant cases**

C-HIGH / G-LOW or C-LOW / G-HIGH

1\textsuperscript{st} randomization to treatment
use Clinical vs. Genomic risk

Chemotherapy

Endocrine therapy

2\textsuperscript{nd} randomization
Anthracycline -based vs. Capecitabine-Docetaxel

No Chemotherapy

3\textsuperscript{rd} randomization
Tamoxifen 2y / Letrozole 5y vs. Letrozole 7y

**HR+**

**HR+**

**HR+**

**HR+**

**HR+**
Patient risk allocation

<table>
<thead>
<tr>
<th>Clinical-pathological risk</th>
<th>LOW</th>
<th>HIGH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Low</td>
<td>2586 (40)</td>
<td>1436 (22)</td>
<td>4022 (62)</td>
</tr>
<tr>
<td>High</td>
<td>678 (10)</td>
<td>1827 (28)</td>
<td>2505 (38)</td>
</tr>
<tr>
<td>Total</td>
<td>3264 (50)*</td>
<td>3263 (50)*</td>
<td>N=6527</td>
</tr>
</tbody>
</table>

Discordant cases (10 + 22 = 32%) match protocol hypothesis

The **absolute difference** between C-HIGH / G-LOW and C-LOW / G-HIGH is **11.6%**

*The 50-50 split is coincidental*
Preregister

Oncotype DX assay

Register
Specimen banking

Secondary study group 1
RS < 11

ARM A
Hormonal therapy alone

Primary study group
RS 11-25

Randomly Assigned
Stratification factors:
Tumor size, menopausal status, planned chemo, planned radiation

Secondary study group 2
RS > 25

ARM D
Chemotherapy + hormonal therapy

ARM B
Hormonal therapy alone

ARM C
Chemotherapy + hormonal therapy
Commercially available prognostic gene expression based tests for breast cancer

• Oncotype DX®
• MammaPrint®
• ‘Intrinsic’ gene molecular classification/ PAM50
• MapQuant DX®
• EndoPredict®
• Breast Cancer Index℠ (HoxB13:IL17BR/ MGI)
<table>
<thead>
<tr>
<th>Gene Expression test</th>
<th>Oncotype DX®</th>
<th>MammaPrint®</th>
<th>‘Intrinsic’ gene molecular classification/ PAM50</th>
<th>MapQuant DX®</th>
<th>EndoPredict®</th>
<th>Breast Cancer Index&lt;sup&gt;SM&lt;/sup&gt; (HoxB13:IL17BR/ MGI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provider</td>
<td>Genomic Health</td>
<td>Agendia BV</td>
<td>ARUP laboratories</td>
<td>Qiagen (formerly Ipsogen Inc); still available?</td>
<td>Sividion Diagnostics</td>
<td>Biotheranostic</td>
</tr>
<tr>
<td>Assay</td>
<td>21-gene Recurrence Score</td>
<td>70-gene signature</td>
<td>“intrinsic gene” list or 50-gene PCR</td>
<td>97-gene signature or 8-gene qRT-PCR</td>
<td>qRT PCR 8 prognostic genes, 3 normalization gene</td>
<td>2-gene HOXB13:IL17R/ molecular-grade index</td>
</tr>
<tr>
<td>RNA isolated from</td>
<td>Formalin fixed paraffin embedded</td>
<td>Frozen or formalin fixed paraffin embedded</td>
<td>Frozen or formalin fixed paraffin embedded</td>
<td>Frozen or formalin fixed paraffin embedded</td>
<td>Formalin fixed paraffin embedded</td>
<td>Formalin fixed paraffin embedded</td>
</tr>
<tr>
<td>Outcome</td>
<td>Disease-free relapse at 10 years</td>
<td>Distant metastasis at 5 years</td>
<td>Disease-free, distant metastasis-free and overall survival</td>
<td>Good (GGI I) or Poor (GGI III) prognosis</td>
<td>Distant metastasis at 10 years</td>
<td>Relapse-free and overall survival</td>
</tr>
<tr>
<td>Clinical application</td>
<td>Prediction of recurrence risk in ER+ BC treated with tamoxifen</td>
<td>Prognosis of NO, &lt; 5 cm diameter, stage I/II BC</td>
<td>Classification of invasive breast cancers</td>
<td>Molecular grading, for ER+, histological grade II BC</td>
<td>Prognosis of endocrine treated BC</td>
<td>Prognostic in ER+ BC, prediction of response to tamoxifen</td>
</tr>
<tr>
<td>Risk groups identified</td>
<td>Three risk groups based on recurrence score</td>
<td>Dichotomous; good or poor prognosis</td>
<td>Classification of tumors into luminal A, luminal B, HER2 and basal-like</td>
<td>Dichotomous, GGI I or GGI III</td>
<td>Dichotomous, Low risk or high risk</td>
<td>Continuous variable; risk of recurrence score</td>
</tr>
</tbody>
</table>
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New targets in breast cancer - the role of the pathologist

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What % of high level and low level HER2 amplified cases can be expected?

0 (negative)

1+ (negative)

2+ (equivocal)

3+ (positive)
The benefit of using diagnostic tests in clinical trials: Herceptin in MBC

Simulation of effect of Herceptin in unscreened population

Herceptin combination pivotal trial: overall survival (IHC 3+)

MBC = metastatic breast cancer
Predicting response to chemotherapy

Chemotherapy:

- Neoadjuvant
- Adjuvant
- In metatastatic disease
Therapy response predicting profiles

• Are mostly analyzed in neoadjuvant studies
• Are harder to find than prognosis profiles
• Core needle biopsy prior to neoadjuvant chemotherapy

- Freeze biopsy
- Isolate RNA
- Perform gene expression profiling
Response to neoadjuvant chemotherapy

before CT

after 2 courses of CT

unfavourable response

after 6 courses of CT

favourable response (also pCR)
IHC Subtype and Pathol. CR
(ddAC for HER2-; PTC for HER2+ tumors)

- ER+ HER2- (N=207)
  - pCR (breast & axilla): 6%
  - pCR (breast only): 12%

- TN (N=92)
  - pCR (breast & axilla): 34%
  - pCR (breast only): 40%

- HER2+ (N=95)
  - pCR (breast & axilla): 46%
  - pCR (breast only): 57%

Update Jan 2011
Supervised classification for response

breast tumors from patients who underwent neoadjuvant treatment

Response Reporter Genes

- Response
- No response

- To date: no very strong response predicting profiles have been identified
- Explanation: probably various independent mechanisms for drug resistance
# Chemosensitivity Signatures

<table>
<thead>
<tr>
<th>Study</th>
<th>N total (learning set/validation set)</th>
<th>Chemotherapy treatment</th>
<th>Surrogate for chemosensitivity</th>
<th>Signature identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang et al. (2003)</td>
<td>30 (24/6)</td>
<td>Docetaxel</td>
<td>Clinical response</td>
<td>Yes (92 probesets)</td>
</tr>
<tr>
<td>Ayers et al. (2004)</td>
<td>42 (24/18)</td>
<td>P -&gt; FAC</td>
<td>pCR</td>
<td>Yes (74 probesets)</td>
</tr>
<tr>
<td>Iwao-Koizumi et al. (2005)</td>
<td>70 (44/26)</td>
<td>Docetaxel</td>
<td>Clinical response</td>
<td>Yes (85 genes)</td>
</tr>
<tr>
<td>Hanneman et al. (2005)</td>
<td>46 (46/-)</td>
<td>AC or AD</td>
<td>pCR</td>
<td>No</td>
</tr>
<tr>
<td>Gianni et al. (2005)</td>
<td>171 (89/82)</td>
<td>AP-&gt;P</td>
<td>pCR</td>
<td>Yes (86 genes)</td>
</tr>
<tr>
<td>Hess et al. (2006)</td>
<td>133 (82/51)</td>
<td>P-&gt;FAC</td>
<td>pCR</td>
<td>Yes (30 probesets)</td>
</tr>
<tr>
<td>Thuerigen et al. (2006)</td>
<td>100 (52/48)</td>
<td>GE-&gt;Doc or GEDoc</td>
<td>pCR</td>
<td>Yes (512 probesets)</td>
</tr>
<tr>
<td>Cleator et al. (2006)</td>
<td>40 (40/0)</td>
<td>AC</td>
<td>Clinical response</td>
<td>Yes</td>
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<tr>
<td>Potti et al. (2006)</td>
<td>51 (-/51)</td>
<td>T-&gt;FAC</td>
<td>pCR</td>
<td>Yes</td>
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<tr>
<td>Bonnefoi et al. (2007)</td>
<td>125 (-/125)</td>
<td>FEC</td>
<td>pCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Chang et al. (2008)</td>
<td>66</td>
<td>FEC</td>
<td>Clinical response</td>
<td>Yes</td>
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<tr>
<td>Farmer et al. (2009)</td>
<td>59</td>
<td>T-&gt;ET</td>
<td>pCR</td>
<td>Yes</td>
</tr>
</tbody>
</table>

_Bonnefoi et al, Eur J Cancer 2009, 45: 1733-43_
Identification of therapy response predicting tests will require a combination of *in vitro* and clinical studies.
Clinical anti-HER2 receptor therapies

• monoclonal antibodies
  Trastuzumab (HER2)
  Pertuzumab (HER2)

• tyrosine kinase inhibitors
  Lapatinib (HER1+2)
Herceptin clinical results

Addition of Herceptin to chemotherapy was associated with:

**In metastatic setting**
- longer time to disease progression (7.4 vs 4.6 months)
- higher rate of objective response (50% vs 32%)
- increased survival (25.1 vs 20.3 months)

**In adjuvant setting**
- reduction of recurrence risk with 52%
- after 2 yr follow-up 33% reduction in risk of death
Herceptin resistance loss-of-function barcode screen in BT-474 cells

- Herceptin 2 µg/ml

BT-474

RNAi library

Infect

Split /select

PCR recovery

ULS labeling

59-mer oligo

spot DNA micro-array

array hybridization
Only PTEN knockdown confers resistance to Herceptin

RS-GFP

RS-PTEN

RS-Nki-library
HER2 downstream signalling

Validation of predictive value of PTEN in Herceptin treated patient cohort

55 patients treated for metastatic breast cancer with Herceptin + chemotherapy

34 at the Netherlands Cancer Institute
21 at MD Anderson Cancer Center

PTEN scoring by IHC or reverse phase protein lysate array
Superior prediction of Herceptin response by PI3K pathway activation analysis

![Graph showing time to progression in months and probability of tumor progression with different activation statuses of the PI3K pathway. The red line represents 'yes' activation status, and the blue line represents 'no' activation status. The p-value is 0.0022.]
ESR1 ligand-binding domain mutations in hormone-resistant breast cancer

- Weiyi Toy, Yang Shen, Helen Won, Bradley Green, Rita A Sakr, Marie Will, Zhiqiang Li, Kinisha Gala, Sean Fanning, Tari A King, Clifford Hudis, David Chen, Tetiana Taran, Gabriel Hortobagyi, Geoffrey Greene, Michael Berger, José Baselga & Sarat Chandarlapaty

- 80 patients with ER-positive metastatic breast cancer
- Comprehensive genetic analysis: 14 tumors harbour ESR1 gene mutations in the ligand-binding domain (LBD)
Activating $ESR1$ mutations in hormone-resistant metastatic breast cancer


- 11 patients with ER-positive metastatic breast cancer
- Whole-exome and transcriptome analysis: 6 cases harbored mutations of $ESR1$ affecting its ligand-binding domain (LBD)
• Genetic classification of breast cancer
• Genetic tests for prognosis
• Prediction of response to systemic therapy (in advanced breast cancer and in the adjuvant setting)
• Whole genome sequence analysis of breast cancer
Massively parallel sequencing (next-generation sequencing)

“Old way” 500bp

454
100,000,000 (100M) bp

Illumina/Solexa
1,000,000,000 (1G) bp

AB/Solid
3Gbp

Progress in the generation of DNA sequence 1980-2010
BASIS project  
(2010-2014)

- 500 ER+/HER2- breast carcinomas
  - Sequence analysis
  - mRNA/miRNA gene expression profiling
  - Genome wide methylation
The landscape of driver mutations in breast cancer.

sequence analysis in breast cancer

- Shah et al, Nature 2009, 1 metastasis of invasive lobular carcinoma, *whole exome sequence*
- Ding et al, Nature 2010, 1 basal like breast carcinoma, *whole genome sequence*
- Stephens et al, Nature 2012, 100 primary breast carcinomas, *whole exome sequence*
- The Cancer Genome Atlas Network, Nature 2012, 507 breast carcinomas, *whole exome sequence*
- Nik-Zainal et al, Cell 2012, 21 primary breast carcinomas, *whole genome sequence*
- Shah et al, Nature 2012, 104 triple negative breast carcinomas, *RNA/whole exome sequence*
- Ellis et al, Nature 2012, 77 ER+ breast carcinomas, *whole genome (46), exome (31) sequence*
- Banerji et al, Nature 2012, 103 breast carcinomas, *whole exome sequence*
Driver mutations in >10% of breast carcinomas

- TP53
- PIK3CA
- GATA3
Amplifications from Nik-Zainal et al. (Cell, 2012)

- ERBB2
- CCND1
- MYC
- MDM2
- ZNF217
- ZNF703
The way forward will be to integrate

- Histopathological features
- Genetic alterations (full genome sequence)
- Gene expression profiles
- Expression of non-coding RNA’s
- Epigenetic changes
- Protein expression and modification
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